

Regulation of Anaphase in Mammalian Meiosis



Ahmed Anwer Ali Rattani

Department of Biochemistry

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Trinity 2012

I would like to dedicate my thesis to my loving parents, Anwer Ali
Hassan Ali Rattani and Malek Sultana Rattani.

Acknowledgements

I would like to thank my supervisors Bela Novak and Kim Nasmyth for their guidance, support and encouragement over the last four years. This has been an excellent experience to work in such a stimulating environment.

I owe special thanks to Magda Wolna and Wolfgang Helmhart for all their help and support during my DPhil period. I would like to thank Jonathan Godwin for providing technical support in many different projects, and Ajay Mishra, with whom I had numerous cups of tea and whose help I could not have done without during my first year in Kim's Lab. Thanks to Elwy Okaz whose suggestions have undoubtedly improved my research, Maurici Brunet Roig for many helpful discussions and for helping with the H1 kinase assay, Mickael Ploquin for his help with Sgol2 purification and for the Sgol2 dimerisation assay, Lysie Champion and Kikue Tachibana-Konwalski for providing the pRNA-CENPB-GFP vector, Jan Ellenberg for sharing the pGEMHE-EB3-mEGFP construct, Iain Cheeseman, Arshad Desai, Duane A. Compton, J.L. Barbero, Jason Swedlow, Yoshi Watanabe, Katja Wassmann and Linda Wordeman for providing antibodies, Bernd Mayer and Olaf Stemmann for the Far Western experiment, Seamus Morrone and Wenqing Xu for the PP2A interaction assay, Chris Preece and Ben Davies for valuable micro-injection training, and to the animal care staff, especially Denise Jelfs and Sian Polley, at the BSB Facility, Dept. of Biomedical Services. Lastly, I would like to thank Vinod Palakkad Krishnanunni and James Rhodes for reviewing my thesis and am very grateful to all members of the Nasmyth and Novak Group, past and present, who made these labs such

an enjoyable place to work.

This work would not have been possible without the love, support and dedication of my parents. I am extremely grateful to my brother, Aziz Rattani, for his support and for encouraging me to pursue my fullest potential. To my sister, Munira Hassan Ali Tharwani, thank you for your love and support. I am grateful to my wife, Nudrat Noor, for her understanding, patience, and unfaltering support. She gave me courage and strength to continue on with my endeavours regardless of circumstances. I cannot thank her enough for always being there.

Finally, I am thankful to the Aga Khan Foundation and the Higher Education Commission, Pakistan for supporting my studies at the Doctoral Training Centre, and the Boehringer Ingelheim Fonds for their generous scholarship for my doctoral research at the Department of Biochemistry.

Abstract

Missegregation of chromosomes during meiosis leads to formation of aneuploid eggs. Estimates suggest that in humans, about 10-30% of fertilised eggs and one-third of all miscarriages are aneuploid. Accurate chromosome segregation depends on the coordination between stepwise cohesion resolution and attachments of homologous chromosomes through kinetochores to microtubules, emanating from opposite poles of the cell. The Spindle Assembly Checkpoint (SAC) monitors microtubule-kinetochore attachments and prevents resolution of cohesin complexes by inhibiting the ubiquitin ligase APC/C^{Cdc20} until all aberrant microtubule-kinetochore attachments have been rectified by an Aurora Kinase-dependent error correction machinery. During meiosis, these pathways work in seamless coordination to achieve balanced segregation of the genome at the first meiotic division.

The cross-talk between different cell cycle pathways requires members with shared affiliations. During my DPhil studies, I worked on understanding the role of two such proteins, namely Bub1 (budding uninhibited by benzimidazoles 1) and Sgol2 (Shugoshin-like protein 2) in mouse oocytes.

During the first meiotic division, Bub1 maintains the SAC, and through its kinase activity, Bub1 recruits Sgol2 to kinetochores to protect centromeric cohesion. This recruitment is essential for two rounds of chromosomes segregation in meiosis. Thus, Bub1 localisation at kinetochores can coordinate the timing of anaphase with the centromeric cohesion protection.

During the first meiotic division, Sgol2 protects centromeric cohesion by recruiting PP2A to kinetochores, accelerates cohesin resolution by silencing the SAC through its interaction with PP2A and Mad2, and also promotes biorientation and congression of homologous chromosomes by its interaction with MCAK and through dephosphorylation of Aurora B/C kinase substrates at kinetochores.

This research revealed that Bub1 and Sgol2 can regulate anaphase by linking multiple cell cycle pathways that work together to achieve faithful chromosomes segregation in mammalian meiosis.

Contents

Contents	vi
Nomenclature	viii
1 Introduction	1
1.1 The Functional Unit Of Life	1
1.2 Information Transfer In Living Organisms	2
1.3 The Cell Cycle	4
1.3.1 Linking the Physical Process of Cell Division to the Bio- chemical Machinery of the Cell	6
1.4 The Cohesin Complex	10
1.4.1 Loading, Establishment, Maintenance, and Nonproteolytic Removal of Sister Chromatid Cohesion	15
1.5 Ensuring Faithful Transmission of Information	16
1.5.1 Spindle Assembly Checkpoint	18
1.6 Meiosis	23
1.6.1 Creation of Bivalent Chromosomes	23
1.6.2 Meiosis-Specific Cohesion	25
1.6.3 Mono-orientation	26
1.6.4 Protection of Centromeric Cohesion	28
1.6.4.1 Shugoshin-The Guardian Spirit	29
1.6.5 One Round of Replication Followed by Two Rounds of Seg- regation	31

2	Bub1 Maintains the Spindle Assembly Checkpoint and Protects Centromeric Cohesion by Recruiting Sgol2 to Kinetochores	33
2.1	INTRODUCTION	33
2.2	RESULTS	36
2.2.1	Kinase Domain of BUB1 Is Dispensable for the Spindle Assembly Checkpoint (SAC) in Oocytes	36
2.2.2	Kinase Activity of Bub1 Is Required for the Recruitment of Shugoshin Like Protein 2 (Sgol2) to Kinetochores	38
2.2.3	Centromeric Protection at the First Meiotic Division Requires Kinase Activity of Bub1	40
2.3	DISCUSSION	42
2.3.1	SAC and the Kinase Activity of Bub1	42
2.3.2	Sgol2 Recruitment and Cohesion Protection	43
2.4	EXPERIMENTAL PROCEDURES	45
2.4.1	Mouse Strains	45
2.4.2	Oocyte Culture and Microinjection	45
2.4.3	Chromosome Spreads	45
3	Shugoshin-Like Protein 2 (SGOL2) With Its Interacting Partners Protects Centromeric Cohesion, Promotes and Corrects Microtubule-Kinetochore Interactions, Maintains Inter-Kinetochore Distances, and Silences the Spindle Assembly Checkpoint In Mouse Oocytes	47
3.1	INTRODUCTION	47
3.2	RESULTS	51
3.2.1	Sgol2 Interacts With PP2A Through Conserved Residues in Its Putative Coiled Coil Domain	51
3.2.2	Sgol2 Protects Centromeric Cohesion by Recruiting PP2A to Kinetochores	54
3.2.3	Conserved MIM Domain Mediates Sgol2 Interaction With Mad2	57
3.2.4	Sgol2 and Its Interactions With PP2A and Mad2 Are Required for Silencing the Spindle Assembly Checkpoint	57

CONTENTS

3.2.5	Sgol2, by Recruiting MCAK to Kinetochore, Maintains Inter-Kinetochore Distances to Protect Chiasmata	61
3.2.6	Sgol2 Promotes Biorientation of Homologous Chromosomes on the Meiotic Spindle	65
3.2.7	Sgol2 Promotes Microtubule-Kinetochore Interactions . . .	70
3.2.8	Sgol2 Recruitment Inhibits Aurora B/C Kinase Activity At Kinetochore	73
3.3	DISCUSSION	76
3.3.1	Centromeric Cohesion Protection	76
3.3.2	Timing of Anaphase and Sgol2	77
3.3.3	K Fibre Synthesis and Sgol2	79
3.4	EXPERIMENTAL PROCEDURES	82
3.4.1	Generation of Mouse Strains	82
3.4.2	Isolation and Culture of Oocytes	82
3.4.3	Preparation of mRNAs	82
3.4.4	Microinjection of mRNA	83
3.4.5	Live Cell Confocal Microscopy	83
3.4.6	Chromosome Spreads	83
3.4.7	Immunofluorescence	84
3.4.8	Protein Expression and Purification	84
3.4.9	PP2A-Sgol2 in-Vitro Binding Assay	85
3.4.10	Co-Immunoprecipitation of Sgol2 Coiled-Coil Domains . .	86
	Appendix	87
	Contributions	95
	References	96

Chapter 1

Introduction

1.1 The Functional Unit Of Life

In 1665 with the newly invented microscope, the English physicist and natural philosopher, Robert Hooke observed that slices of cork are made up of lots of tiny compartments fitted closely together. To Robert Hook the inner structure of cork resembled the walled compartments of a monastery and possibly due to this resemblance, he called these structures cells [1]. A contemporary of Hooke, Anton van Leeuwenhoek (1632-1723), was the first person to observe living microorganisms such as bacteria and protozoa [2] using a monocular microscope of his own design [3]. Extending on these observations, the English naturalist Robert Brown in 1831 made the fundamental discovery that morphologically different types of plants when observed under a microscope are all made of cells. He further observed that inside each plant cell, there is a dark circular opaque structure, which he called nucleus [4]. Upon learning of Graham's observations a few years later, in 1839, two German scientists Theodor Schwann and Matthias Schleiden observed that like plants, animal tissues are composed of cells and that both plant and animal cells have a nucleus. The realisation that the cell is the structure that unites all life forms was codified by Schwann and Schleiden [5, 6, 7].

By the mid-nineteen hundreds, the cell had been accepted as the structural unit of life. Yet, to be accepted as the structural and functional building block of life,

a cell had to show certain characteristics unique to the living organism, one of them being the ability to reproduce. Through the works of Robert Remak and Rudolph Carl Virchow, it was established that cells propagate by division. This idea was popularised by the epigraph *Omnis cellula e cellula* (“every cell originates from another existing cell like it.”) coined by Virchow in 1858. Their work finally established the cell as the structural and functional unit of life [8, 6, 7].

A momentous discovery in the history of cell biology, namely the cell cycle, came in the year 1881 with the publication of a book by the German Zoologist Walther Flemming [9]. Flemming for the first time described the process of cell division in great detail using samples from tadpole tails, as he was aware that the tail of a tadpole divides quite rapidly. With the help of a powerful new microscope and novel dye staining techniques, he identified structures, which he called chromosomes. He observed that during cell division, chromosomes undergo dynamic changes and that they divide into two identical parts, one for each daughter cell. Flemming called this process mitosis [9].

1.2 Information Transfer In Living Organisms

From early times, it has been appreciated that “Like begets like”. However, the mechanism responsible for the transmission of traits or characteristics from one generation to another remained unclear. A landmark discovery by an Austrian friar, Gregor Johann Mendel, provided a mathematical understanding of the passage of traits. In the middle of the 19th century, he performed his famous experiments with the garden pea, *Pisum sativum*, in his abbey in Brno, in today's Czech Republic. Mendel's success relied on two key factors. Firstly, he selected well-defined and easily recognisable characteristics (traits or phenotypes), and secondly, he used careful quantifications on thousands of inbred peas that he meticulously tracked over many generations. After years of observation and analysis, he came to the conclusion that genetic information is present in two copies and that during reproduction one and only one copy (allele) from each parent is transmitted randomly to the offspring, giving it two copies. His results,

published in 1866, went largely unnoticed until their rediscovery by Carl Correns, Hugo de Vries, and Erich Tschermak at the beginning of the 20th century [10].

At the time of the rediscovery of Mendel's work, Oscar Hertwig and Hermann Fol had already established that fertilisation resulted from the union of two gametes, sperm and eggs [11]. What was unclear was why these cells are the only cells in the body that are capable of forming new life. The answer came in 1883 from the work of the Belgian zoologist Edouard Van Beneden. While studying a species of round worm, he observed that all its cells contained four chromosomes; the only exceptions being the gametes which had only two. He further demonstrated that when two gametes came together, a full set of four chromosomes appeared in the fertilised egg [11]. But the question remained, why do only germ cells have half the number of chromosomes compared to other somatic cells?

In 1890, while studying the behaviour of chromosomes German biologist August Weismann noted that two cell divisions were necessary to reduce the number of chromosomes in germ cells. He found that at the first round of division, the two homologous chromosomes pair up, and unlike in mitosis, these chromosomes do not split but instead the homologs separate at the first division [12]. Later it was observed that the reduction in chromosome number in germ cells is achieved by one round of DNA replication followed by two rounds of DNA segregation. In 1905, Farmer and Moore termed this specialised form of reductional cell division meiosis (Greek word meaning "reduction") [11]. The discovery of this reductional division provided the key insight into how only one copy of Mendel's factors is transmitted during reproduction.

The observations of Van Beneden and August Weismann suggested that Mendel's traits could be transmitted by the chromosomes. However, the proof came from the elegant experiments performed in 1889 by Theodor Boveri. He discovered that when he fertilised enucleated eggs (without chromosomes) of one sea urchin species with sperm from another sea urchin species, the resulting embryos only resembled the species from which the sperm was obtained [13]. This provided clear experimental evidence that genetic information (or Mendelian factors) resides

within the nucleus, and not the cytoplasm. Independently, in 1903 an American scientist and physician, Walter Sutton, pointed out the parallelism between the behaviour of chromosomes and Mendelian factors [14]. He also explained that the random distribution of traits (traits are passed with a certain probability according to Mendel's theory) can be accounted for by the fact that different homologous chromosome pairs orient at random on the meiotic spindle. The insight provided by the works of Boveri and Sutton was aptly named by E.B Wilson as the "Sutton-Boveri theory of chromosomal inheritance" [8].

1.3 The Cell Cycle

Following the discovery of the cell as the fundamental unit of life and chromosomes as carriers of genetic information, microscopists dedicated their efforts on the details of the mechanics by which the cell duplicates its contents and then distributes them into the daughter cells. The description of cell replication, summarised below, led to our current understanding of the cell division cycle. Most of the discussion below will be focused on mitosis and at the end of this section, I will point to a few important differences between the two chromosome segregation mechanisms in mitosis and meiosis.

The mitotic cell cycle, the series of events by which a somatic cell duplicates itself, is divided into distinct phases that are defined on the basis of chromosome duplication [15]. S phase is the period during which chromosomes duplicate, and during M phase those duplicated chromosomes, or sister chromatids, are segregated to opposite poles of the cell and then packaged into genetically identical daughter cells. Throughout the cell cycle, not only the chromosomes, but all essential components of the cell duplicate. In most cases, by the time a cell divides at the end of M phase, the resulting daughter cells are about the same size as the parental cell [16].

M phase is a particularly eventful phase of the cell cycle during which chromosomes are segregated (mitosis) and distributed by cell division (cytokinesis). The process of chromosome segregation depends on a dynamic molecular ma-

chine, called the mitotic spindle, a bipolar array of microtubule polymers that forms as the cell enters mitosis. During mitosis microtubules emanating from opposite poles of the cell becomes attached to duplicated chromosome pairs. The attachment of microtubules to chromosomes is mediated by kinetochores, large protein complexes that provide microtubule binding sites on the surface of the chromosomes [16].

M phase is further divided into four distinct phases on the basis of chromosome condensation and arrangement. These are prophase, metaphase, anaphase, and telophase. Prophase is distinguished from the previous cell cycle state by two major landmarks. Firstly, chromosomes inside the cell nucleus begin to condense from their normally disperse state, into compact, rod-like structures. Secondly, the two microtubule-organising centres move apart from one another along the surface of the nucleus to begin the formation of a mitotic spindle. At the end of prophase, the nuclear envelope dissolves, allowing the microtubules emerging from the organising centres to gain access to the duplicated sister chromatid pairs inside the nucleus. This mechanism, with support from an error-correction machinery (discussed below), results in the formation of stable associations between microtubules and kinetochores on the surface of chromosomes. When the cell reaches metaphase, paired sister chromatid are attached to the mitotic spindle, in a bi-oriented fashion whereby corresponding sister chromatids attach to microtubules emanating from opposite poles. After the formation of stable microtubule-kinetochore attachments, chromosome segregation occurs in which the cohesion between sister chromatids is removed resulting in the separation of sister chromatids and their movement to opposite poles of the mitotic spindle. At telophase, sister chromatids are packaged into individual daughter nuclei, and this is followed by cytokinesis that partitions the genetic information into two daughter cells [16].

1.3.1 Linking the Physical Process of Cell Division to the Biochemical Machinery of the Cell

Microscopic description of the cell cycle revealed that its events always occur in a particular order with appropriate timing and coordination. This was quite puzzling for biologists as this implied that there must be a regulatory system(s) that guides the cell through the stages of the division cycle.

Early cell-fusion experiments with mammalian cells and the slime mold *Physarum polycephalum*, clearly demonstrated that G2 cells can be accelerated into mitosis if fused with M phase cells while G1 cells will progress into DNA replication upon fusion with S phase cells. These experiments suggested the existence of factors, named M phase-promoting factor (or MPF) and S phase-promoting factor (or SPF), that accumulate in cells at appropriate cell cycle stages. Furthermore, MPF activity was capable of initiating mitosis when injected into non-mitotic cells [17, 18, 19, 20]. Such experiments indicated the existence of factors present in cells at specific cell cycle stages that would drive cells through different stages of the cycle. However, the identity of these factors could not be revealed using cell fusion techniques [15].

Major discoveries about the regulatory system that controls and guides the cell through the cell cycle came from researchers studying very simple eukaryotic organisms: the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. These yeasts offered a number of experimental advantages including ease of culture and their short generation times. Most importantly, researchers were able to apply methods of classical genetics to these organisms to isolate mutant genes that allowed the analysis of the principles and the components involved in cell cycle control [15].

Budding yeast, as the name implies, divides by budding. At the end of G1 phase when this cell enters the cell cycle, it starts growing a bud on the side of the mother cell. As the cell progresses through the cell cycle, the bud increases in size. Therefore, in budding yeast, the size serves as a good indicator of the position

of a cell in the cell cycle. This morphological characteristic allowed the isolation of mutants that arrested at specific cell cycle stages. Using this approach, Lee Hartwell and his colleagues in the late 1960s and early 1970s isolated a number of mutants in various genes that arrested cells at a particular cell cycle stage [21, 22, 23, 24]. These mutants were conditional temperature-sensitive mutants, i.e. at room temperature their gene products were functional, but at high temperature, 37°C, the mutant gene products were inactivated and as a consequence the cell arrested at a specific cell cycle stage when that gene product was required in the cell cycle [21].

By isolation and characterising a large number of *cdc* mutants, yeast geneticists working in budding yeast and also in fission yeast built the foundations of the understanding of cell cycle control. The budding yeast group identified a protein kinase called Cdc28 to be required for entry into the cell cycle. In fission yeast, a highly related protein kinase called Cdc2 was identified as an important kinase for the progression into mitosis [21, 22, 23, 24, 25, 26]. These two protein kinases, Cdc2 and Cdc28, were later found to be orthologs (reviewed in [15]).

The other major discovery about cell cycle control came from a completely different line of inquiry, i.e. protein synthesis and degradation, and from a very different model system, namely the sea urchin. Following fertilisation, the eggs from sea urchins divide rapidly and synchronously. Using fertilised sea urchin eggs, Tim Hunt identified a protein called Cyclin whose levels oscillate during the cell cycle. He noticed that this protein rises during mitosis and falls thereafter, suggesting it might be an important regulatory molecule in cell cycle control [27]. Evidence from yeast genetics and protein dynamics in sea urchin merged in the 1980s when it was realised that molecules discovered in these three different organisms by multiple approaches are part of MPF, the elusive molecule responsible for driving the cell cycle. It was found that MPF is, in fact, a serine/threonine protein kinase composed of Cdc28 (budding yeast) or Cdc2 (fission yeast), and cyclins. Hence, this kinase, called Cyclin-Dependent Kinase or CDK, was discovered to be the essential molecule that guides the cell through different stages of the cell cycle [15].

It was soon established that Cdks are inactive until they associate with their cyclin regulatory partners, resulting in the activation of the Cdk-cyclin complex. Active Cdk-cyclin complexes phosphorylate a large number of substrates in the cell, which initiates specific cell cycle events. Cdk-cyclin complexes phosphorylate and thereby regulate the activities of various proteins involved in DNA replication, chromosome segregation, and cell division. We now know that there are different types of Cdk/cyclin complexes each of which is responsible for driving specific events of the cell division cycle. For instance, the complex of Cdk with an S-phase cyclin forms in late G1, and this complex triggers the onset of DNA replication. Likewise, during mitotic M-phase, Cdk-cyclin activity initiates mitotic entry. Therefore, through this series of Cdk-cyclin complexes, the events of the cell cycle are triggered in an appropriate order and at the right time [16]. Furthermore, it has been demonstrated recently that the temporal ordering of cell-cycle events can be imposed by manipulating the activity of a single Cdk-cyclin complex, arguing that the events of the cell cycle depend not on the specific regulatory cyclins, but on the level of activity of Cdk-cyclin complexes [28].

Since the discovery of Cdks, it has become clear that the regulation of Cdks is more complex than just the association of the cyclin regulatory subunits. It has emerged that the Cdk-cyclin complexes are regulated by phosphorylation on the Cdk1 subunit and also by inhibitory proteins that associate with Cdks at certain cell cycle stages to restrain their activity. Hence, the up and down regulation of Cdk activity during the cell cycle depends on a wide variety of regulatory mechanisms [16].

The discovery of cyclins by Tim Hunt clearly showed that as a cell exits from M phase, cyclin levels drop precipitously [29]. Furthermore, cells expressing a version of cyclins that cannot be degraded, failed to get out of anaphase and to exit into the following G1 phase, arguing that cyclin destruction is required for exit from mitosis [30]. This led to a quest for the mechanism responsible for cyclin destruction. Biochemical assays and yeast genetics identified a large protein complex, now known to be composed of 15 different subunits, that is responsible for the destruction of cyclins [29]. This protein complex was named Anaphase

Promoting Complex or Cyclosome (APC/C). APC/C is a ubiquitin ligase (E3 ligase), which catalyses the attachment of a small protein, called ubiquitin, onto its substrates. By attaching a large number of ubiquitins, APC/C targets the substrates for proteolysis by the proteasome. APC/C is, therefore, capable of triggering the destruction of its target proteins. During M phase, the activity of APC/C rises at the metaphase-to-anaphase transition and this results in degradation of key cell cycle proteins, including the cyclins. (reviewed in [29, 31, 32]).

During mitosis the ubiquitylation activity of APC/C requires its association with one of two activators, Cdh1 and Cdc20 [33]. The association of these activators with the APC/C core at specific cell cycle stages results in the formation of the active APC/C^{Cdc20} or APC/C^{Cdh1} holoenzyme. The association of the APC/C core with the cofactor is thought to direct APC/C's ubiquitylation activity towards specific substrates that possess short recognition motifs, called the KEN- or the D-box [29, 31, 32]. It has been proposed that activators, Cdc20 and Cdh1, recognise these destruction boxes on the substrates and this promotes substrate recruitment to the APC/C holoenzyme [34, 35, 36]. The two activators have different substrate specificities: Cdc20 can target few substrates to the core complex, whereas, Cdh1 can direct a diverse set of substrates for degradation. The differential recognition of substrates by APC/C cofactors is presumed to be due to subtle differences in D-box and KEN sequences, or sequence context, which could influence substrate recognition by cofactors of APC/C [29, 31, 32].

The binding of APC/C to its cofactors is regulated by phosphorylation of the core complex and the cofactors by Cdk1 and polo kinase. The Cdk1 phosphorylation of APC/C subunits during mitosis promotes the association of Cdc20. By contrast, Cdh1 can only associate with the APC/C core when the kinase activity of Cdk1 has subsided [31]. APC/C^{Cdc20} decreases the Cdk1 activity by initiating cyclin destruction and this results in a dephosphorylation of Cdk1 substrates, including Cdh1 and the APC/C core complex. The other major target of the APC/C is a protein called securin. Securin is a tight binding inhibitor of separase. When securin is destroyed in mitosis by APC/C^{Cdc20}, it releases separase from its inhibitory complex with securin. The active separase then cleaves a sin-

gle subunit of a protein complex called cohesin, which holds sister chromatids together. Through simultaneous destruction of cohesin and Cdk, APC/C^{Cdc20} brings about sister chromatid disjunction at anaphase (reviewed by [37, 29]) (Figure 1.2). At telophase, the dephosphorylated APC/C and Cdh1 can associate to form APC/C^{Cdh1}, which can then ubiquitinate a wide range of cell cycle targets, including cyclins and proteins that are not recognised by Cdc20, such as Aurora kinases A and B [37].

In summary, the cell cycle control system is a series of biochemical switches made up of cyclin-dependent kinases that turn-on multiple events of the cell cycle, and through APC/C phosphorylation prepares for its own destruction and exit from mitosis. As the cell reaches metaphase with chromosomes aligned on the mitotic spindle, proteolytic activity of APC/C closes the cycles and prepares the cell for the next round of division.

1.4 The Cohesin Complex

For the balanced segregation of the genome, the replicated sister chromatids must resist the pulling forces generated by the microtubule-kinetochore attachments, until all chromosomes have aligned on the metaphase plate [38]. The cohesion between sister chromatids was thought to provide the counteracting force required to balance the pulling forces. This idea also implied that the molecule/mechanism that holds replicated chromatids together must be removed at anaphase, as sister chromatids move to opposite poles during this phase of the cell cycle [39]. One of the proposed mechanisms for the cohesion between sisters was the intercatenation of DNA, since sister chromatids were known to be connected by DNA concatamers, which are formed as a byproduct of replication [40]. In this model, the resolution of sister chromatid cohesion at anaphase was brought about by the type II topoisomerase [41, 42]. This model proposed an interesting mechanism for the establishment of cohesion by coupling it to replication and suggested that sisters would be joined as soon as they are formed. Nevertheless, this model could not explain how the concatenation between sisters is only removed at anaphase,

since the activity of type II topoisomerase is not cell cycle regulated. Thus, despite a fairly accurate description of the requirement of sister chromatid cohesion, the nature of the glue, as well as the molecular basis of the machinery that removes cohesion at anaphase, remained unclear.

By the late 1990's it was already known that APC/C is responsible for sister chromatid separation at anaphase and in *S. cerevisiae* this function of APC/C is independent of cyclin degradation. This finding suggested that the glue holding sister chromatids together might be a protein, which is targeted for degradation by the APC/C at anaphase. The break-through finally came in 1997 when two groups independently reported components of sister chromatid cohesion by using genetic screening in *S. cerevisiae*. They identified four genes, namely, Scc1 (Sister chromatid cohesion 1, also called Mcd1 and Rad21), Scc2, Scc3, Smc1 and Smc3 (Structural Maintenance of Chromosomes 1 and 3), that were essential for sister chromatid cohesion and together these proteins were called cohesins [43, 44]. Four of the identified proteins, namely, Smc1, Smc3, Scc1, and Scc3, were purified as stable 14S cohesion complex from *Xenopus laevis* egg extracts [45, 46], and in yeast these subunits were reported to associate with DNA from S-phase until the onset of anaphase [47]. Therefore, Smc1, Smc3, Scc1, and Scc3 were called "core" cohesin components.

Smc1 and Smc3 belong to the Structural Maintenance of Chromosomes (SMC) superfamily of proteins. Member of the SMC family of proteins play an important role in different aspects of chromosome structure and dynamics, including cohesion (Smc1 and Smc3), chromosome condensation (Smc2 and Smc4), and double strand break repair (Smc5 and Smc6). These SMC proteins share a characteristic five-domain structure; the N- and C-terminal globular domains contain a canonical nucleotide-binding motifs, known as the Walker A and Walker B motifs. The two terminal domains are separated by a long alpha-helical stretch. Between these long helices, there is an additional globular domain, the hinge domain. The N- and C-globular domains fold back on themselves, this aligns the antiparallel intramolecular coiled-coil domains. In its final conformation, the N- and C- terminal globular domains are in close proximity, such that the N-terminal

Walker A motif and the C-terminal Walker B together form an ABC-like (ATP-binding cassette) ATPase domain. This structure is called the SMC head. The two SMC molecules (in the case of cohesin; Smc1 and Smc3) bind to each other through interactions at their hinge domains, forming a V-shaped structure with the ATPase heads located at the apices. The two heads of the dimers are bridged by proteins of the kleisin superfamily, and in the case of cohesin, the N- and C-terminal domains of the α -kleisin protein Scc1 interact with the Smc3 and Smc1 heads, respectively [48, 49, 50]. The interactions between these three subunits form a tripartite ring-like structure, with Scc1 and hinge domains joining Smc1 and Smc3. [51, 52]. The fourth subunit, Scc3, associates with the complex through its interaction with the Scc1 subunit [52] (Figure 1.1).

The finding that the cohesin complex forms a ring-like structure suggested an interesting model in which cohesin holds sister chromatids together by topologically entrapping them within its ring. According to this model, the ring opens up at anaphase to allow sister chromatid segregation. However, the first, and probably the most convincing, *in vivo* proof of cohesin's role in providing sister chromatid cohesion came from experiments, in which endogenous Scc1 was replaced with a modified version that carried recognition sites for the exogenous site-specific Tobacco Etch mosaic Virus (TEV) protease. It showed that cleavage of Scc1 by TEV protease was sufficient to trigger sister chromatid separation and anaphase chromosome movements in yeast without APC/C activity [53]. These results have now been confirmed in *Drosophila* embryos and mouse oocytes [54, 55].

The TEV-induced separation of chromatids provided direct evidence that cohesin cleavage is sufficient to trigger sister chromatid separation. But how this complex is disassembled (or destroyed) during a normal cell cycle at anaphase remained unclear. However, it was expected that the machinery responsible for the cohesin cleavage had to be cell cycle-regulated. Biochemical assays in budding yeast revealed that Scc1 was proteolytically destroyed at the metaphase-to-anaphase transition by the cell-cycle regulated thiol-protease Esp1, now most commonly called separase [56]. Biochemical assays combined with genetics showed that the protease separase is kept inactive in a complex with its inhibitor Pds1 (also

called securin) until the metaphase-to-anaphase transition [57]. At the end of metaphase, Cdc20-activated APC/C ubiquitinates securin and thereby targets it for degradation [58, 59]. Destruction of securin relieves the inhibition of separase, which then cleaves the Scc1 subunit of the cohesin complex. Cleavage of Scc1 destroys the cohesin ring and this allows sister chromatids to separate [56]. The discovery of cohesin and its proteolytic destruction by separase at the metaphase-to-anaphase transition have explained how sister chromatids are initially tightly linked to each other and how sister chromatid cohesion is suddenly and synchronously removed at a specific stage of the cell cycle. In addition, it also provided insight into the mechanism that ensures that each daughter inherits a complete set of chromosomes.

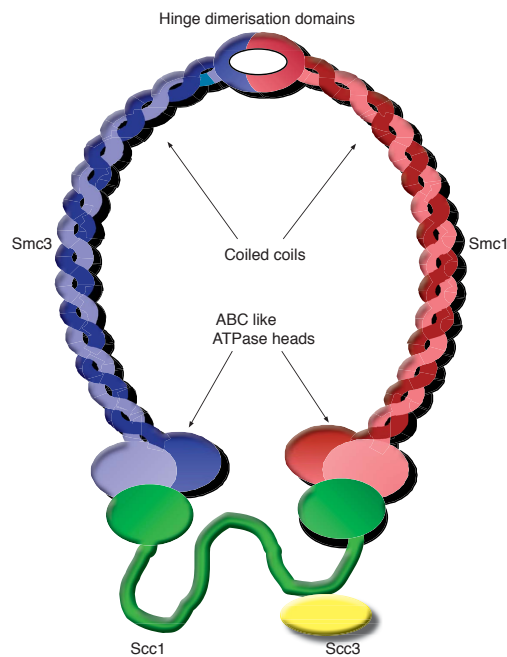


Figure 1.1: Molecular architecture of the cohesin complex. *Hinge domains on Smc1 and Smc3 fold back forming antiparallel intramolecular coiled-coil domains. This brings together the N- and C-termini to form ABC-like ATPase head domains. Scc3/SA binds to the C-terminal part of Scc1.*

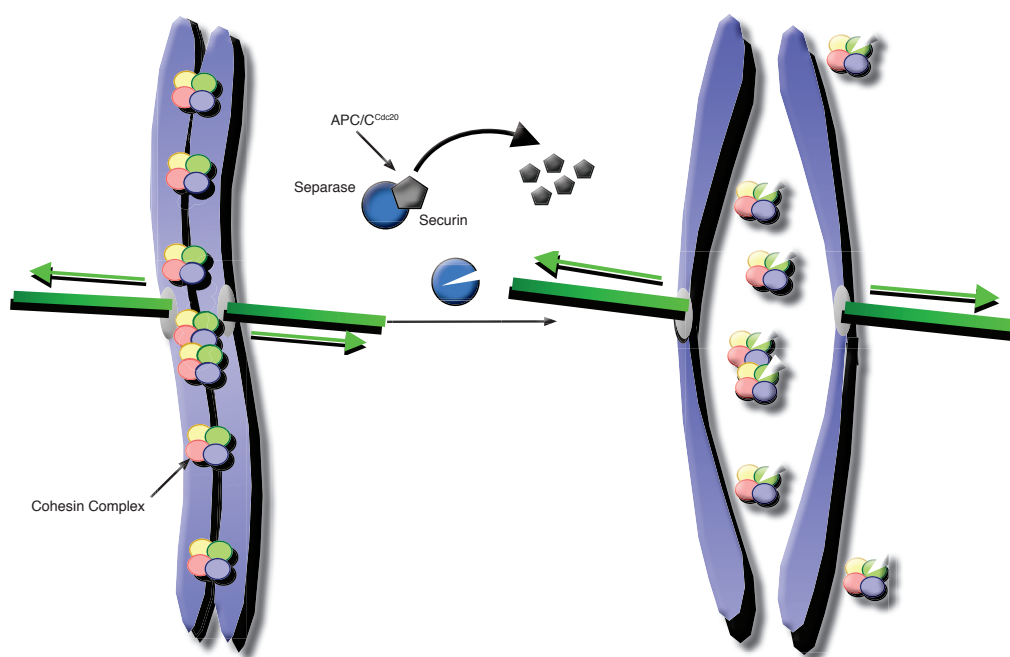


Figure 1.2: Sister chromatid separation during mitosis. *Counteracting forces generated by microtubule-kinetochore attachments and sister chromatid cohesion align the chromosomes. At the onset of anaphase, APC/C destroys securin thereby liberating separase from its inhibitory complex; active separase then cleaves the Scc1 subunit of cohesin, bringing about sister chromatid separation.*

1.4.1 Loading, Establishment, Maintenance, and Nonproteolytic Removal of Sister Chromatid Cohesion

Cohesin is loaded onto the chromatin by a conserved Scc2/Scc4 loading complex in an ATP hydrolysis-dependent reaction [43, 60, 61, 62, 63]. In *Saccharomyces cerevisiae*, the cohesin rings bind to DNA in late G1 phase [43, 61], while in vertebrates, cohesin loading commences in telophase of the preceding cell division [64, 65, 66, 67]. *In vivo* studies have shown that all subunits of the cohesin complex are required for its association with DNA, suggesting that the entire ring might be loaded as a preassembled complex onto chromatin [43, 47]. These cohesin complexes do not associate homogeneously along the length of chromosomes but are preferentially loaded at pericentromeric regions of the DNA, at least in budding yeast cells [68, 69].

The cohesin complexes loaded during the G1 phase of the cell cycle provide sister chromatid cohesion after DNA replication. Fluorescence recovery after photobleaching (FRAP) experiments using fluorescently tagged cohesin subunits demonstrated that its association with unreplicated chromatin (in G1 phase) is highly dynamic, whereas about half of chromatin-associated cohesin remains stably bound to chromatin after DNA replication [66]; this suggests that replication somehow modifies cohesin's association with the chromatin. Consistent with this hypothesis, in unperturbed yeast cells, cohesin molecules loaded after DNA replication, during the G2 phase, fail to provide sister chromatid cohesion [70, 71]. Experimental work has shown that replication is associated with the acetylation of two conserved lysine residues in the Smc3 head domain. This modification requires the activity of an acetyl-transferase, called Eco1 (establishment of cohesion) [72, 73, 74]. Interestingly, acetylation activity of Eco1 is required during DNA replication, but not in G2 phase, when sister chromatid cohesion has been established [75, 76, 47]. Further evidence suggests that Eco1, through acetylation of lysine residues in the Smc3 head domain, locks the Smc3-Scc1 interface, which allows cohesin molecules to physically entrap the DNA strands [72, 73, 77, 74, 78].

In yeast, cohesion established between sister chromatids during S-phase holds

chromosomes together until anaphase at which point separase cleaves the Scc1 subunit, which is both necessary and sufficient for sister chromatid separation [56]. Analogous to yeast, in most eukaryotic cells, separase is necessary for sister chromatid separation at anaphase. However, unlike in yeast where separase removes most of the cohesin, in most other eukaryotes, separase removes only about 10% of the cohesin from the centromeres at anaphase and the majority of cohesin loaded onto the arms of chromosomes is removed during prophase/prometaphase in a separase-independent pathway. Additionally, this removal does not even require cleavage of Scc1. This cleavage-independent release of cohesin, named prophase dissociation pathway [46, 79, 80, 81], requires the Wapl protein [82, 83], the activity of Polo-like kinase, and Aurora B [80, 79], and involves Scc3/SA and Scc1 phosphorylation. Apart from the components of the prophase pathway, the molecular details of the non-proteolytic dissociation of cohesin from chromatin and the interplay between different anti-establishment factors working in the prophase pathway remains unclear. However, it is well established that the persistence of cohesin at centromeres until anaphase depends on a protein called shugoshin (Japanese for guardian spirit) [84]. Shugoshin physically interacts with the protein phosphatase PP2A and recruits it to kinetochores. This interaction is thought to counteract cohesin phosphorylation [85, 86], and hence protects centromeric cohesion until anaphase when separase cleaves the Scc1 subunit.

1.5 Ensuring Faithful Transmission of Information

Characterisation of the role of Cdk-cyclins, APC/C, and cohesin complexes in the cell cycle linked the physical (microscopic) details of the cell cycle with the biochemical events in the cell. These findings, nevertheless, could not explain how the cell ensures faithful segregation of the sister chromatids. In other words, does the cell cycle engine always function in a precise order or does it need help from a feedback mechanism that prevents error propagation?

The early studies from yeast genetics and frog embryonic cells led to opposing views regarding the cell cycle control. Analysis of various *cdc* mutants showed that if a *cdc* mutant arrested at a specific cell cycle stage, then in many cases it also blocked progression through subsequent stages of the mitotic cycle. For instance, a *cdc* mutant that arrested cells prior to DNA replication also prevented the onset of M phase. These results, therefore, argued that there are dependency relationships between the events of the cell division cycle, such that preceding events must be completed in order for later events to begin. This implied that the cell has an intrinsic program: the Cdk-cyclin kinase guides the cell through mitosis, but some events in the cell cycle can signal back and block the program to prevent further progression [16].

An alternative view came from studies with early embryos of the frog. In frog embryos, it was observed that if DNA replication was blocked, or even if the nucleus was completely removed from the cell, the following events of the cell cycle occurred normally. Therefore, the results from early embryos indicated that the cell cycle was controlled by a program that initiates cell cycle events at a certain time irrespective of whether preceding events were completed. Crucially, the program in this case was found to be independent of the events of the cell cycle [16].

Later work reconciled the two models by showing that both the model were at least partially correct. There is, indeed, an intrinsic biochemical program (operated by Cdk-cyclin kinase), in all eukaryotic cells that guides the cell through cell division cycle, but in many cells the events of the cell cycle can block onward progression and adjust the program of cell cycle or arrest progression through certain stages, if necessary [16].

There are three major transitions or so-called checkpoints in the cell cycle where the cell cycle can be arrested if conditions are not appropriate. For example, if the cell initiates S phase but for some reason DNA synthesis fails and a chromosome fails to duplicate, then this will send a message to block progression through the G2/M checkpoint, and will prevent entry into mitosis. Likewise, there are similar mechanisms in operation at the metaphase-anaphase transition and at

the start of the cell cycle [16]. At all of these checkpoints or transition points, the onward progression of the cell cycle is blocked if preceding events have not been completed successfully. The key questions in the cell cycle field has been to understand the molecular details of cell cycle checkpoints and how the relevant proteins are assembled together into a functional regulatory system. Below, I will discuss one such checkpoint, which ensures that each daughter cell receives an equal number of chromosomes at cell division.

1.5.1 Spindle Assembly Checkpoint

Discovery of cell division as an essential process of life and chromosomes as its messenger led to the realisation that the process of cell division must be carried out with great fidelity. Insightfully, Theodor Boveri, while studying sea urchin embryos fertilised by multiple sperms, suggested that abnormal chromosome segregation might lead to uncontrolled proliferation of cells, resulting in cancer formation [87, 88]. However, the mechanism by which a cell ensures accurate transmission of genetic information remained unclear.

In the early 20th century, it was observed that drugs such as colchicine and cold treatment can arrest cells in mitosis. It was later found that the target of these chemicals are the spindle microtubules, a polymer of α - and β -tubulins. Cytological studies combined with chemical treatments showed that the attachment and movement of chromosomes along the spindle depend on the attachment of microtubules to a layer of proteinaceous material built on the centromeric DNA, called the kinetochore. This microtubule-kinetochore attachment forms a key part of the functional spindle. These attachments are essential for the proper alignment of chromosomes to the metaphase plate, and, therefore, for accurate segregation of chromosomes at cell division [89].

Using high resolution microscopes and injecting fluorescently labelled tubulin into live cells, biologists showed that the attachment of microtubules to kinetochores is a somewhat random process. However, just before anaphase, cells

can form stable bioriented microtubule-kinetochore attachments; corresponding kinetochores on each chromosome attached to microtubules emanating from the opposite poles [90].

By the late 1950s, it was thought, but not tested, that microtubules can attach to kinetochores at random but only the bioriented attachments of the sister chromatids generate tension between the two kinetochores and this tension leads to a stabilisation of the bipolar attachments [91]. In 1969, elegant experiments by Bruce Nicklas using the newly designed micro-manipulator, showed that chromosomes (specifically meiotic chromosomes) could detach artificially generated unipolar attachments, and kinetochores with unipolar attachment can reorient into the bipolar configuration within several minutes. Furthermore, Nicklas' experiments also showed that the experimentally produced unipolar attachment can be stabilised by pulling on the incorrectly attached chromosome, using a glass needle. He demonstrated that as a result of the externally applied tension, the improperly attached chromosomes remained in a unipolar orientation for many hours, and did not achieve the correct bipolar orientation [92]. Nicklas' results convincingly showed that cells are able to detect and correct improper microtubule-kinetochore attachments and that tension stabilises the attachments. Yet the molecular machinery responsible for this error correction remained elusive for about thirty years.

A hunt for the molecular mechanism responsible for gain and loss of chromosomes in budding yeast identified the Ipl1 (increase-in-ploidy) kinase [93, 94]. Subsequent research showed that Ipl1 promotes the turnover of microtubule-kinetochore attachments in the absence of tension, suggesting that it might function in the error correction machinery observed in Nicklas' experiment. Crucially, in yeast, Ipl1 kinase can generate unattached kinetochores in response to lack of tension, which is an essential requirement for correcting the lateral and unipolar attachments [95, 96]. Furthermore, in vertebrates, inactivation of the Ipl1-ortholog, Aurora B kinase, using small molecules or inhibitory antibodies led to stabilisation of incorrect attachments and removing the inhibitor resulted in correction of malattached microtubule-kinetochore interactions, possibly by selective destabilisation

of the non-bipolar attachments [97, 98]. Together, these studies demonstrate that Ipl1/Aurora B can provide biochemical insight into the error correction mechanism.

Applying force across kinetochores suggested that the error correction machinery is able to sense tension. Therefore, based on this assumption, Aurora kinase should, either directly or indirectly, respond to tension at kinetochores. Indeed, recent experiments have suggested that the ability of Aurora Kinase to phosphorylate its substrates at kinetochore depends on the interkinetochore stretch. A FRET-based reporter assay by placing the biosensor close to the Aurora Kinase, i.e. at inner kinetochores, showed that the Aurora Kinase is constitutively active at that site and that the kinase activity per se does not respond to tension. However, if positioned at the outer kinetochores, i.e. more distant from the kinase and closer to the microtubule-kinetochore binding site, the FRET biosensor started to show tension-dependent dephosphorylation [99]. Indeed, endogenous Aurora B substrates at the outer kinetochore are also dephosphorylated when kinetochores bi-orient [100, 101]. Moreover, if Aurora B is artificially targeted to the outer kinetochore, the outer kinetochore substrates of Aurora are constitutively phosphorylated, and microtubule kinetochore attachments cannot be stabilised [99]. Together, these findings suggest that the tension generated at kinetochores changes the localisation of Aurora B relative to its substrates at the outer kinetochore and this increased distance stabilises the bipolar attachments.

Aurora kinase functions as an enzymatic component of the chromosome passenger complex (CPC), which also includes the inner centromere protein (INCENP), Survivin, and Borealin (also known as Dasra or CSC-1). Together, these proteins are thought to mediate the tension sensing and error correction machinery by modulating the phosphorylation of various kinetochore substrates [102]. Recent work has shown that Aurora kinase is recruited to kinetochores by the conserved phosphorylation of histone H3 at threonine-3 (H3T3) by the Haspin kinase [103, 104, 105].

The discovery of error correction and tension sensing mechanisms partly explained

how cells ensure balanced segregation of the genome. What it did not account for was how the cell cycle machinery can as a whole respond to incorrect attachments. Studies using chemicals, which destabilise microtubules, clearly showed that cells treated with such chemicals can not only delay separation of sister chromatids but can also block inactivation of Cdk1/cyclin B kinases. However, the mechanism that blocked anaphase in the presence of spindle toxins remained unclear. The breakthrough finally came when two independent genetic screens isolated mutants that escaped cell cycle arrests induced by the spindle poisons benomyl and benzimidazole. These screens identified the three MAD (mitotic arrest deficient) genes and the three BUB (budding uninhibited by benzimidazole) genes [106, 107]. Later studies also added the protein kinases Mps1 to the list [108, 109]. These genes collectively form the Spindle Assembly Checkpoint (SAC).

In an unperturbed yeast cell cycle, the components of the SAC are not essential for survival. Their functions are nevertheless required to prevent an increased rate of chromosome loss [106, 107]. By contrast, in mammals, SAC components, namely Mad1, Mad2, BubR1 (Mad3), Bub1, and Bub3, are essential for viability [110, 111].

After the identification of components of the SAC, biochemical studies addressed the mechanism by which this surveillance machinery can detect and prevent error propagation. It is now established that checkpoint proteins can actively bind to unattached kinetochores, which provide a platform for the assembly of checkpoint components. Two of the checkpoint proteins, Mad2 and BubR1 (Mad3), interact directly with APC/C^{Cdc20} and form the Mitotic Checkpoint Complex (MCC). This interaction inhibits the ubiquitylation activity of APC/C^{Cdc20} by interfering with substrate recognition [112, 113, 114, 115, 116, 117, 118]. Structural studies combined with biochemical assays have shown that Mad2 can exist in two conformational states, either as a closed form (C-Mad2) or in an open conformation (O-Mad2). In its closed conformation (C-Mad2), the C-terminal domain of Mad2, referred to as the safety belt, can bind to either Mad1 or Cdc20, and in its open conformation (O-Mad2) Mad2 is not engaged with either of its binding partners [119, 120]. The unattached kinetochore promotes the formation of a

Mad1-Mad2 complex, and this complex at kinetochores functions as a template for the assembly of further complexes of Mad2 with APC/C^{Cdc20} [121, 120]. It is proposed that Mad2-Cdc20 complexes could further amplify the SAC signal via a positive feedback loop that generates further complexes [120]. This model suggests an interesting mechanism by which a signal generated by a single unattached kinetochore can be amplified to delay sister chromatid separation until the error correction machinery rectifies incorrect attachments.

Early *in vitro* biochemical assays suggested that MCC sequesters Cdc20, preventing its binding to the APC/C. This model was supported by observations that binding of checkpoint components reduced the affinity of Cdc20 for the APC/C [117, 118]. Nevertheless, it could not explain how certain proteins such as cyclin A and Nek2A are degraded by APC/C^{Cdc20} as soon as the nuclear envelope disintegrates, i.e. when the SAC is bound to Cdc20 [122, 123, 124, 125]. Recent studies have been able to explain these observations by showing that SAC components interact with Cdc20 bound to APC/C and this interaction of SAC with APC/C^{Cdc20} blocks access of certain substrates such as Cyclin B and Securin to the core complex and hence preventing their degradation until all chromosomes have aligned on the metaphase plate [126]. Additionally, recent evidence has pushed APC/C^{Cdc20} from its passive role in the SAC, in which SAC dictates the terms of inhibition, to an active participant in which its ubiquitylation activity can disassemble the MCC [127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137]. This suggests a dynamic system with a double negative feedback loop, in which both SAC and APC/C can mutually inhibit each other. This double negative feedback loop, possibly with assistance from the Mad2-binding protein, p31comet [138, 139, 140, 130, 141, 142, 133, 143, 144, 145, 126], could explain how checkpoint can be rapidly inactivated when all microtubule kinetochore interactions have passed the quality assurance monitored by the SAC and the error correction machinery.

Overall, the emerging picture appears to suggest that an incorrect or unattached kinetochore, probably through perturbed inter-kinetochore tension, activates Aurora Kinase, which then works locally by destabilising incorrect attachments

through phosphorylation of substrates at microtubule-kinetochore attachments. The activated Aurora, either by itself or by detaching erroneously attached kinetochores, generates a signal that results in Mad1-Mad2 complex formation, and consequently SAC activation. Together error correction and SAC pathway ensure faithful segregation of the genome.

1.6 Meiosis

To produce haploid gametes, with half the number of chromosomes from diploid germ cells, there are certain key features that are unique to meiosis. Most importantly, meiosis involves two consecutive rounds of chromosome segregation, meiosis I and meiosis II, preceded by only one round of DNA replication. During meiosis I, homologous chromosomes of maternal and paternal origins segregate, while in meiosis II sister chromatids segregate to opposite poles (Figure 1.3). To achieve this reductional division at meiosis I, the following modifications to the mitotic mechanism are essential [11].

1.6.1 Creation of Bivalent Chromosomes

The proper alignment and segregation of homologous chromosomes during the first meiotic division depends on a physical linkage between maternal and paternal homologous. This linkage begins with the introduction of DNA double-strand breaks (DSBs), generated by the topo-like transesterase sporulation protein (Spo11) [146]. The DSBs are generated after pre-meiotic S-phase [147, 148], at which time cohesin is loaded onto the chromosome and links the sister chromatids together, supporting interhomolog interactions [149, 150, 151, 70].

The DSBs generated during meiotic prophase are repaired using the homologous, non-sister chromatid as a template [152]. This repair, at least in budding yeast and mammals, requires recruitment of meiosis-specific proteins onto the chromosome, resulting in the formation of a structure called the synaptonemal

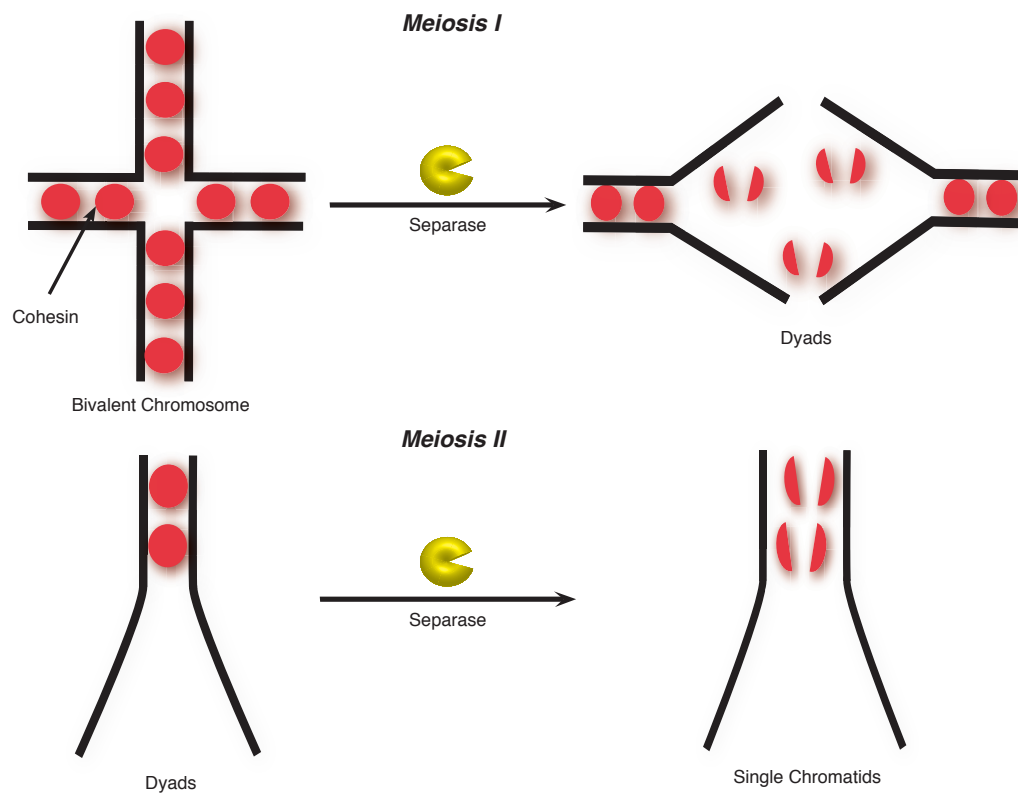


Figure 1.3: Chromosome segregation during meiosis. Homologous chromosomes are held together by sister chromatid cohesion distributed along arms and centromeres. At the first meiotic division, separase selectively cleaves cohesin complexes along chromosome arms. This leads to resolution of bivalents into a pair of dyads, with sister centromeres linked by centromeric cohesion. Meiosis I is followed by the second nuclear division, meiosis II. During the second round of division, separase cleaves centromeric cohesin producing single chromatids.

complex (SC). Some DSBs can generate reciprocal crossovers between the non-sister homologous chromatids, at least one event per chromosome. This results in the formation of bivalent chromosomes (bivalents) that are connected at the site of crossovers (cytologically known as chiasmata) and at the arm, distal to the chiasmata, by meiosis-specific cohesin complexes [152, 11].

1.6.2 Meiosis-Specific Cohesion

As in mitosis, sister chromatids of bivalent chromosomes are held together by cohesin complexes. However, several subunits of the canonical cohesin complex are replaced by their meiotic counterparts. The most conserved modification of the meiotic complex is the substitution of the α -kleisin subunit, Scc1/Rad21, by its meiotic counterpart, Rec8 [153, 154, 155]. The presence of this meiosis-specific α -kleisin is essential for the successful completion of meiosis, as its deletion or replacement with its mitotic counterpart results in defects in homologous recombination, co-orientation (in fission yeast), and a failure to protect centromeric cohesion at the first meiotic division [156, 157, 158, 159, 160, 152].

In several organisms, there are a number of different cohesin subunits which are specific to meiosis. In mice, for example, Smc1 β is expressed solely in the germ cells. This subunit replaces Smc1 α , and is essential for both male and female fertility [161, 162]. Similarly, SA1/ SA2 (Scc3 homologues) are replaced by their meiotic counterpart, STAG3, which in spermatocytes immunoprecipitates with Smc1 β and Smc3 [163]. Recently, in addition to Rec8, a new meiosis specific α -kleisin subunit has been identified in mammalian germ cells, called RAD21L [164, 165, 166, 167, 168]. Similar variations have been reported in *Caenorhabditis elegans* germ cell in which two meiosis-specific α -kleisin subunits, namely COH-3 and COH-4, have been identified [169]. The coexistence of multiple cohesin complexes, in some cases, has been found to be essential for meiosis-specific functions along chromosomes. In the fission yeast, for example, Rec11, a meiosis-specific Scc3 subunit, is required for the arm cohesion and recombination, whereas canonical Scc3 is required for centromeric cohesion and not for recombination

[170]. Thus, additional subunits may diversify cohesin functions along meiotic chromosomes.

1.6.3 Mono-orientation

Unlike in mitosis and meiosis II where sister kinetochores attach to microtubules from opposite poles, sister kinetochores in meiosis I attach to microtubules from the same pole. This monopolar attachment of sister kinetochores ensures their co-segregation at the first meiotic division [11].

Classical studies by Paliulis and Nicklas demonstrated that homologous pairs from an oocyte if transferred, by micromanupulation, into an egg (cell in meiosis II), can still establish monopolar attachments and segregate as in meiosis I, indicating that monopolar attachment is a property of chromosomes and is not dependent on any specific features of microtubules or of the cell cycle state [171]. Microscopic studies have shown that sister kinetochores are fused into a single unit by sister chromatid cohesion during meiosis I [172, 173]. Therefore, not surprisingly, it has been reported that sister chromatid cohesion is essential for the co-orientation of sister kinetochores in both budding and fission yeast. In the absence of REC8, homologous chromosomes fail to co-orient and sister chromatids segregate randomly at the first meiotic division [150, 153].

The molecular machinery responsible for this important modification in kinetochore orientation is, perhaps, best understood in budding yeast, where molecules responsible for the monopolar alignment are best characterised. Identified through genetic screens, Mam1 (monopolar microtubule attachment during meiosis I), Hrr25 (casein kinase 1 δ/ϵ of budding yeast), Csm1 (chromosome segregation in meiosis I), and Lrs4 (loss of rDNA silencing-4), together form a complex, called monopolin [174, 175, 176]. In budding yeast, the monopolin complex is required for sister kinetochore mono-orientation and in the absence of any one of the subunits of monopolin, sister kinetochores attach to microtubules in a bipolar manner in meiosis I [174, 175]. Consistent with its role in kinetochore mono-orientation, Mam1 localises to kinetochores in the first meiotic division, but dissociates at the

onset of anaphase I [174]. Csm1 and Lrs4, unlike Mam1, are not meiosis-specific and these subunits are also present during the mitotic cell cycle, where they reside in the nucleolus [175]. Only during the meiotic cell cycle, Csm1 and Lrs4 leave the nucleolus and associate with kinetochores, where they interact with Mam1 [175]. Structural and microscopic studies have shown that monopolin complex, by cross-linking kinetochore components, is able to suppress the biorientation of sister kinetochores [177].

The presence of these factors in the mitotic cell, though trapped in the nucleolus, raises a question of why they function only during meiosis I. Studies in budding yeast showed that Cdc5, together with DDK, is required for the association of monopolin complex with kinetochores and for the efficient phosphorylation of monopolin subunits [178, 179, 180], Cdc5 activity is also required for the release of Lrs4 and Csm1 from the nucleolus. This shows that during meiosis I, phosphorylations by Cdc5 and DDK regulate the function of the monopolin complex [178, 179].

Molecular details of monopolar orientation in higher eukaryotes remains poorly understood. Even among yeasts (the budding yeast and the fission yeast) it seems that the pathway is not fully conserved. In fission yeast, Pcs1 (homolog of Csm1) and Mde4 (homolog of Lrs4) are dispensable for the monoorientation of sister kinetochores, and the fission yeast homologue of casein kinase 1 is not required for the mono-orientation pathway [181, 175]. Moreover, the mono-orientation of homologous chromosomes in fission yeast depends on Rec8-mediated sister chromatid cohesion, and if substituted by the mitotic kleisin Rad21/Sccl, sisters fail to co-orient [182, 183]. In budding yeast, however, both Rec8- and Sccl- containing cohesin complexes can support mono-orientation in meiosis I [156]. These results suggest that the mono-orientation pathway might have diverged along different pathways in higher eukaryotes.

1.6.4 Protection of Centromeric Cohesion

At anaphase I, APC^{Cdc20} activates separase, which cleaves cohesin complexes loaded during DNA replication. In meiosis, there is no intervening round of DNA replication between the two meiotic divisions; therefore, cohesin complexes unloaded at the first meiotic division cannot reestablish cohesion in the second meiotic division. To achieve the two rounds of DNA segregation without an intervening round of DNA replication, meiotic cells have evolved a mechanism, which ensures that cohesin complexes holding meiotic chromosomes together are lost in a stepwise manner. At the first meiotic division, homologous chromosomes linked by arm cohesion and crossovers, are segregated by cleavage of cohesin along the arms of chromosomes. This selective separase-mediated cleavage of the cohesin subunit resolves chiasmata and, therefore, abolishes the linkage between homologous pairs and allows them to segregate to opposite poles of the meiosis I spindle. Cohesion between sister chromatids is, however, protected from the proteolytic activity of separase during meiosis I [184, 11]. The preservation of centromeric cohesion is essential for the alignment of chromosomes on the meiosis II spindle and hence for the two rounds of division.

There are two possibilities for how cohesin complexes could be differentially regulated during the two rounds of division, without a need for a special protection machinery. Firstly, there could be different α -kleisin subunits on arms and centromeres. However, immunolocalisation studies demonstrated that Rec8 is localised along the entire chromosome and is selectively cleaved from the arms at the first meiotic division [149, 150, 151, 185, 186]. Furthermore, Rec8 is essential for linking homologs as well as sisters [154, 55]. Crucially, in mouse strains that express REC8 with target sites for the tobacco etch virus (TEV) protease, microinjection of TEV protease induces separation of both homologs and sister chromatids, showing that Rec8 cleavage is sufficient for sister chromatid separation in meiosis [55]. Another possible mechanism to allow for differential loss of cohesin complexes from arms during the first meiotic would be to restrict the proteolytic activity of separase to the second meiotic division, and to remove cohesin from the arms through non-proteolytic mechanisms, as in the prophase pathway

of mitotic cells [79, 80]. However, convincing experiments in yeast and mice have shown that the chromosome segregation at the first meiotic division depends of separase-mediated cleavage of Rec8 [155, 154, 187].

The fact that both rounds of meiotic division are brought about by the separase-dependent cleavage of Rec8 led to a hunt for a molecular mechanism that could confer the protection to cohesin complexes around centromeres. Importantly, studies in yeasts showed that the substitution of Rec8 with Rad21 leads to the loss of cohesins along the entire length of chromosomes at the first meiotic division [174, 183]. This complete loss of cohesion could not be explained by defects in monoorientation, since at least in budding yeast, Rad21 did not perturb mono-orientation. Therefore, it was thought that Rec8 might be unique in its ability to interact with protein(s) at kinetochores that might be important to protect the centromeric Rec8 from separase activity in anaphase I.

1.6.4.1 Shugoshin-The Guardian Spirit

Genetic screens in yeast and fruitfly (*Drosophila melanogaster*) uncovered Bub1, a component of SAC, and the *Drosophila* mei-S332 gene as protectors of centromeric cohesion [188, 189]. Mutations in these genes caused premature separation of sister chromatids in meiosis I. Bub1 was already known to be a conserved component of the Spindle Assembly Checkpoint. However, due to poor sequence conservation, the homologs of MEI-S332 could not be identified by sequence comparisons. Later, three different genetic screens in yeast discovered a coiled-coil protein, shugoshin (Japanese word for Guardian Spirit) as a centromeric cohesion protector in meiosis [190, 191, 192, 193]. These proteins were identified as fungal orthologs of MEI-S332. Sequence alignment of these proteins with Mei-S332 revealed that although these proteins have little sequence homology, they share a conserved N terminal homodimeric coiled-coil domain and also a C terminal domain rich in basic amino acids [191]. Based on these characteristics, Shugoshin homologues have now been identified in almost all eukaryotes. However, not all the members are involved in centromeric cohesion protection in meiosis [194, 195, 196]. For instance, in *Schizosaccharomyces pombe* there are

two orthologs of the shugoshin family, namely Sgo1 and Sgo2, but only Sgo1 protects centromeric cohesion against proteolytic activity of separase in meiosis I [191, 193]. Likewise, mammals also have two Sgo-like proteins, Sgol1 and Sgol2, but only Sgol2 protects centromeric cohesion during meiosis I. Sgol1 protects centromeric cohesion from the prophase pathway during mitosis (discussed above) [194, 195]. After the discovery of shugoshins, it was soon established that Bub1 protects centromeric cohesion by recruiting shugoshins to kinetochores [191, 197]. Recent work in fission yeast has shown that this recruitment depends on the kinase activity of Bub1, which phosphorylates histone H2A at a conserved residue [198].

Following the discovery of shugoshins as a functionally conserved family of proteins, efforts were directed to work out the molecular details of the centromeric protection pathway. In other words, how do shugoshins protect sister chromatid cohesion at centromeres? Initial mutagenesis studies in *Drosophila* MEI-S332 showed that a specific mutation in the homodimeric coiled-coil domain abolishes MEI-S332's ability to protect centromeric cohesin [199]. Studies, mainly from yeast, indicated that shugoshin can stably associate with protein phosphatase 2A (PP2A) with a regulatory subunit of the B' type (Par1 in the fission yeast and Rts1 in the budding yeast) and recruits it to kinetochores [85, 86]. The interaction between Sgo1 and PP2A depends on the conserved N-terminal coiled-coil domain of Sgo1. Crystal structure of the human Sgol1 revealed that the conserved N-terminal domain forms a parallel homodimeric coiled coil whose N- and C-terminal ends interact with the C and B' subunits of PP2A, respectively [200]. Importantly, a budding yeast shugoshin mutant defective solely in its ability to bind to PP2A, precociously segregated sister chromatids at the first meiotic division [200]. Consistent with these findings, genetically engineered yeast cells lacking the Rts1 (PP2A B' subunit) subunit fail to protect centromeric cohesion at meiosis I [86]. Together, these results show that shugoshin, through its interaction with PP2A, protects centromeric Rec8 from the proteolytic activity of separase.

The above mentioned results, firstly demonstrate that Rec8, and not Scc1, is

able to resist the proteolytic activity of separase in meiosis and secondly, that shugoshin's ability to protect Rec8 requires its interaction with PP2A. This led to the suggestion that phosphorylation of the Rec8 subunit of cohesin may be required for its cleavage by separase [86], and by recruiting PP2A shugoshin promotes the dephosphorylation of Rec8 thereby preventing its cleavage by separase. Consistent with this model, Rec8 is indeed phosphorylated at multiple residues during meiosis I by casein kinase 1 (CK1) and Dbf4-dependent Cdc7 kinase [201, 202]. Critically, substitution of the serines and threonines on Rec8 by alanine greatly reduces its cleavage by separase and blocks chiasmata resolution, whereas substitution with aspartate, mimicking phosphorylation, causes premature loss of sister chromatid cohesion [201]. These observations suggest that shugoshin, by recruiting PP2A, protects centromeric cohesion by opposing CK δ/ϵ - and DDK-dependent phosphorylation of Rec8.

1.6.5 One Round of Replication Followed by Two Rounds of Segregation

Meiosis creates haploid gametes from diploid precursor cells. This is achieved by the two rounds of chromosome segregation without an intervening round of DNA replication. To ensure that meiosis I is not followed by DNA replication, cells must exit meiosis I without fully inactivating Cdks activity; doing so would result in assembly of pre-Replication Complexes (pre-RCs) that would permit another round of DNA replication [152]. There are two possible scenarios as to how germ cell can prevent complete inactivation of Cdks at anaphase I: Firstly, cells could partially inhibit cyclin-B degradation upon exit from meiosis I, and secondly, cells could increase synthesis of cyclin B upon entry into meiosis II. The first scenario seems to be valid in fission yeast, where Mes1, a small protein, competes with the Cdks for binding to the WD40 domain of Cdc20 to prevent the complete degradation of cyclins during exit from meiosis [203]. This might be important for retaining Cdks activity between the two meiotic divisions in fission yeast. Since, in the absence of Mes1 yeasts cells complete meiosis I but fail to enter meiosis II [204]. This *mes1* deletion phenotype resembles the budding yeast cells that lack

the B-type cyclins, Clb1 and Clb4 [205, 206], suggesting Mes1 prevents complete CDK inactivation during the meiosis I-meiosis II transition.

Vertebrate eggs are arrested during the second meiotic division by the activity of cytostatic factor (CSF), which maintains partial APC/C activity against Cdk1 [207]. The establishment, but not the maintenance, of CSF activity depends on a signalling pathway that involves the Mos kinase [208]. By promoting the activity of Emi2, an APC/C inhibitor, the Mos kinase pathway prevents the activation of APC/C and thereby anaphase and exit from the second meiotic division [209, 210, 211, 212]. A transient increase in intracellular calcium levels at fertilisation activates calmodulin-dependent kinase II, which then phosphorylates Emi2 and promotes its degradation [213, 214]. Subsequent activation of APC/C^{Cdc20} results in sister chromatid disjunction and cells enter the first mitotic cycle.

Chapter 2

Bub1 Maintains the Spindle Assembly Checkpoint and Protects Centromeric Cohesion by Recruiting Sgol2 to Kinetochores

2.1 INTRODUCTION

Accurate chromosome segregation in eukaryotic cells depends on attachment of kinetochores to microtubules emanating from opposite poles of the cell. Aberrant segregation of chromosomes during cell division leads to genomic instability in mitosis, whereas in meiosis, it leads to formation of aneuploid eggs. Estimates suggest that in humans about 10-30% of fertilised eggs and one-third of all miscarriages are aneuploid [215]. To ensure faithful transmission of genetic material, cells have evolved a surveillance and an error correction mechanism. Together, these ensure that cohesion between replicated chromosomes is preserved until all chromosomes have successfully established bipolar microtubule-kinetochore attachments. The surveillance system, called the Spindle Assembly Checkpoint (SAC), monitors the microtubule-kinetochore attachments and inhibits the pro-

teolytic activity of an ubiquitin ligase, called the anaphase-promoting complex or cyclosome (APC/C) until all aberrant attachments have been rectified by the error correction pathway. The SAC, therefore, connects the spatial/physical signal i.e. microtubule-kinetochore attachments to the chemical signal which, blocks entry into anaphase by inhibiting the activity of APC/C. Activation of APC/C at anaphase triggers the cleavage of the α -kleisin subunit of cohesin by a highly regulated protease called separase.

In yeast, the SAC is not essential for unperturbed mitotic divisions and the components of SAC, such as Mad1, Mad2, Bub1, and BubR1, were only identified in genetic screens in *S. cerevisiae* using microtubule-depolymerising drugs [106, 107]. By contrast, in mammalian cells, loss of SAC leads to massive chromosomal missegregation and embryonic lethality [110, 111]. In mammalian tissue culture cells, a single unattached kinetochore results in a SAC-induced delay in APC/C activation [216]. However, in contrast to somatic cells, mouse oocytes with single X chromosomes can pass through the first meiotic division in the presence of an unaligned chromosome [217]. This observation together with the high rate of aneuploidy observed in mammalian meiosis suggested that the SAC might not be functional during the first meiotic division.

The speculation that the SAC prevents aneuploidy in oocytes has been challenged in a number of findings. Firstly, microtubule-depolymerising drugs, such as nocodazole, clearly block the first polar body extrusion (PBE) [218, 219] and the activation of APC/C [220, 221]. Furthermore, knockdown of Mad2, a component of the SAC, by morpholino anti-sense oligonucleotides allows a fraction of oocytes to undergo polar body extrusion (PBE) in the presence of nocodazole [220, 221]. The Mad2 knockdown also induces modest chromosome missegregation in meiosis I [221]. Conclusively, the conditional knockout of *BUB1* in mouse oocytes showed that SAC is required to restrain APC/C activity in mouse oocytes and it also prevents chromosome missegregation at the first meiotic division. Together, these findings argue against the notion that SAC might not be able to restrain APC/C^{Cdc20} in mouse oocytes.

Apart from a loss in SAC function, *BUB1* knockout led to chromosome alignment and centromeric cohesion defects at the second meiotic division. Importantly, inactivation of SAC by knocking down other components of SAC in mouse oocytes did not show similar phenotypes [220, 221]. In other words, chromosome alignment and centromeric cohesion defects in the second meiotic division cannot simply be ascribed to defective SAC. In meiosis, cohesion at the centromeres is protected by members of a conserved protein family, called shugoshins. Shugoshin, by recruiting protein phosphatase 2A (PP2A) to kinetochores, makes centromeric cohesin refractory to separase cleavage during meiosis I [85, 86]. Persistence of centromeric cohesion is required for the alignment of dyads, sister chromatids attached at centromeres by meiotic specific cohesin, to the metaphase II spindle. In fission yeast, Bub1 recruits shugoshin (abbreviated as Sgo1) to kinetochores to protect centromeric cohesion during meiosis I [85, 197]). Recently it has been shown that this recruitment is mediated by phosphorylation of a conserved residue in H2A by the kinase activity of Bub1.

Mice have two orthologs of shugoshin, Shugoshin-like protein 1 (Sgo1) and Shugoshin-like protein 2 (Sgo2). Deletion of Sgo2 leads to precocious segregation of sister chromatids at meiosis I and *SGOL2* knockout mice, although completely viable, are infertile [222]. However, it has not been established whether the centromeric cohesion protection conferred by Bub1 is through the recruitment of Sgo2 in mouse oocytes. Interestingly, the ability of Bub1 to restrain APC/C activity during the first meiotic division has been reported to be independent of its kinase domain, arguing that the kinase activity of Bub1 might be dispensable for the SAC signalling in mouse oocytes [223]. Therefore, the physiological role of the kinase activity of Bub1 in mammalian meiosis remains unclear.

In this section, I report that Bub1, through its kinase activity, recruits Sgo2 to kinetochores. By comparing wild type Bub1 with a mutant Bub1 completely lacking the kinase domain (kinase dead Bub1), I have found that in mouse oocytes, centromeric cohesion protection at the first meiotic division is dependent on Bub1's kinase activity. Therefore, this work shows that Bub1 through its role in the SAC can coordinate the timing of anaphase with centromeric cohe-

sion protection, by recruiting Sgol2 to kinetochores. Both functions of Bub1 are essential to ensure balanced segregation of chromosomes in mammalian meiosis.

2.2 RESULTS

2.2.1 Kinase Domain of BUB1 Is Dispensable for the Spindle Assembly Checkpoint (SAC) in Oocytes

A female germ cell specific knockout of *BUB1* results in infertility in female mice [223]. *In vitro* cultured oocytes from the conditional *BUB1* knockout mice are unable to restrain APC/C^{Cdc20} activity in the presence of unattached kinetochores resulting in severe defects in chromosome alignments and segregation defects at the first meiotic division. Premature activation of APC/C^{Cdc20} in the *BUB1* knockout accelerates the chiasmata resolution and the polar body extrusion. Critically, the ability of Bub1 to restrain APC/C^{Cdc20} until metaphase alignment does not require its kinase domain. This became apparent as microinjection of mutant Bub1 mRNA completely lacking the kinase domain is able to restore the timing of anaphase [223]. These results, therefore, suggest that the kinase activity of Bub1 might not be required for the SAC response in mouse oocytes. To confirm that the kinase domain of Bub1 is dispensable for the SAC response, control (*BUB1*^{F/F}), *bub1*^{Δ/Δ}, *bub1*^{Δ/Δ} injected with wild type Bub1 mRNA and *bub1*^{Δ/Δ} injected with mutant Bub1 mRNA lacking the kinase domain were cultured from GV stage in M16 with 5μM nocodazole (a microtubule destabilising drug that would activate the SAC). Securin-EGFP was used as a marker of APC/C activation and hence SAC response. Compared to *bub1*^{Δ/Δ} oocytes, which destroyed Securin-EGFP, *BUB1*^{F/F}, *bub1*^{Δ/Δ} expressing wild type Bub1, and *bub1*^{Δ/Δ} microinjected with kinase dead Bub1 mRNA maintained high levels of Securin-EGFP in the presence 5μ M nocodazole (Figure 2.1). This result clearly demonstrates that kinase activity of Bub1 is not required to restore SAC signalling in *BUB1* knockout oocytes.

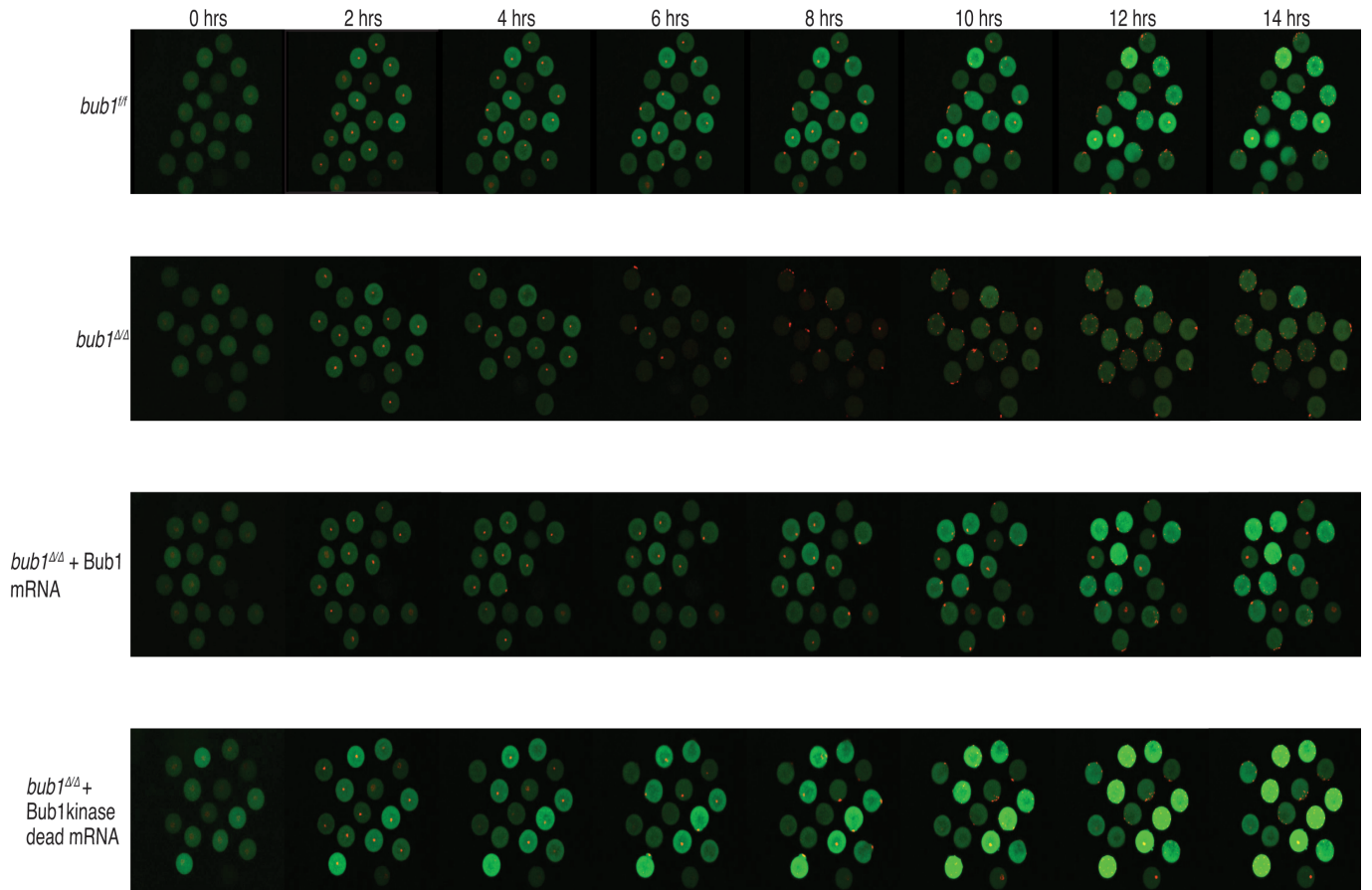


Figure 2.1: Kinase domain of BUB1 is not required for the meiotic arrest induced by nocodazole. Time lapse confocal images showing chromosome movements and Securin-EGFP levels in *BUB1^{f/f}*, *bub1^{Δ/Δ}*, *bub1^{Δ/Δ}* oocytes microinjected with wild type *Bub1* mRNA and *bub1^{Δ/Δ}* oocytes microinjected with Kinase-Dead *Bub1* mRNA, cultured in M16 supplemented with 5 μ M nocodazole.

2.2.2 Kinase Activity of Bub1 Is Required for the Recruitment of Shugoshin Like Protein 2 (Sgol2) to Kinetochores

Bub1-deleted mouse oocytes, apart from checkpoint dysfunction, were not able to maintain centromeric cohesion at the first meiotic division and a significant fraction of *BUB1* knockout oocytes resolved sister chromatids at this point [223]. Similarly, *SGOL2* knockout oocytes completely resolve sister chromatids at the first meiotic division [222]. Therefore, to check whether this precocious resolution of sister chromatids observed in *bub1*^{Δ/Δ} oocytes is due to defects in Sgol2 localisation at kinetochores, oocytes were harvested at GV stage in M2 media supplemented with IBMX from littermate controls (*BUB1*^{F/F}) and *BUB1*^{f/f}-*Zp3Cre* mice. Microinjections of wild type and kinase dead mutant Bub1 mRNA into *bub1*^{Δ/Δ} were performed at GV stage in M2 media supplemented with IBMX. Oocytes were then cultured in IBMX-free medium for about 3-4 h and metaphase I stage chromosome spreads from each group were stained with DAPI (blue), Bub1 (green) and Sgol2 (Red). It was noted that unlike control oocytes in which Sgol2 localised to the inner kinetochores in meiosis I, Sgol2 localisation to kinetochores was abolished in *bub1*^{Δ/Δ} oocytes (Figure 2.2). Furthermore, the kinetochore localisation of Sgol2 was restored by injecting conditional knockout oocytes with wild type Bub1 mRNA. Crucially, the microinjection of mutant Bub1 mRNA lacking the kinase domain into conditional *BUB1* knockout oocytes failed to recruit Sgol2 to kinetochores at metaphase I. Importantly, the kinase mutant of Bub1 localised to the outer kinetochores (Figure 2.2). These results show that Bub1, through its kinase activity, recruits Sgol2 to kinetochores.

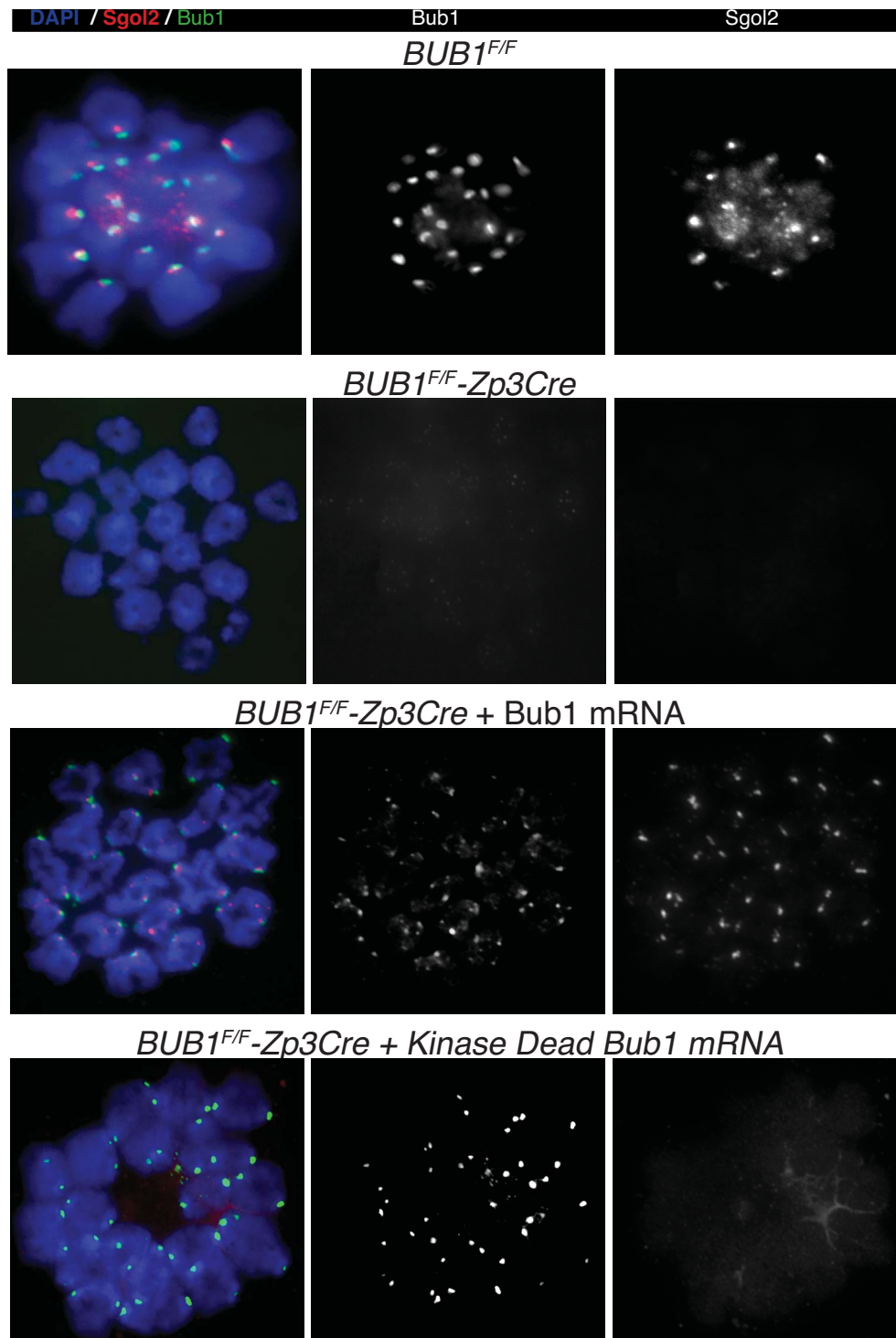


Figure 2.2: Kinase Activity of Bub1 is Required to Recruit Sgol2 to Kinetochores. Chromosome spreads from individual oocytes from indicated groups were prepared at 3-4 hrs post-GVBD. Slides were stained with DAPI (blue), Bub1 (green) and Sgol2 (red).

2.2.3 Centromeric Protection at the First Meiotic Division Requires Kinase Activity of Bub1

Having found that the kinase activity of Bub1 is required for Sgol2 recruitment, I wanted to check whether Bub1's ability to confer centromeric cohesion protection is dependent on its kinase activity. Oocytes were harvested and microinjected at GV stage in M2 media supplemented with IBMX. After washing in IBMX free media, oocytes were matured *in vitro* for up to 24 hr in M16 media at 37°C and 5%CO₂. Chromosome spreads were prepared from oocytes that extruded the first polar body and were stained with DAPI, Sgol2, and CREST. Compared to chromosomes from controls, which were dyads after the first meiotic division, *BUB1* knockout oocytes showed a significant fraction of chromosomes that had resolved sister chromatids at the first meiotic division (Figure 2.3). Microinjection of wild type Bub1 mRNA into *bub1*^{Δ/Δ} oocytes fully rescued the centromeric cohesion protection defects observed in the knockout oocytes (Figure 2.3). Most importantly, *BUB1* knockout oocytes expressing kinase-dead Bub1 failed to protect centromeric cohesion at the first meiotic division (Figure 2.3). Moreover, the microinjection of mutant Bub1 mRNA further exacerbated the cohesion defects observed in the *BUB1* knockout oocytes resulting in precocious loss of centromeric cohesion from all chromosomes. These results clearly demonstrate that the kinase activity of Bub1 is required to recruit Sgol2 to kinetochores and also to protect the centromeric cohesion at the first meiotic division. Together, these findings imply that Bub1, by recruiting Sgol2 to kinetochores, confers centromeric cohesion protection at the first meiotic division.

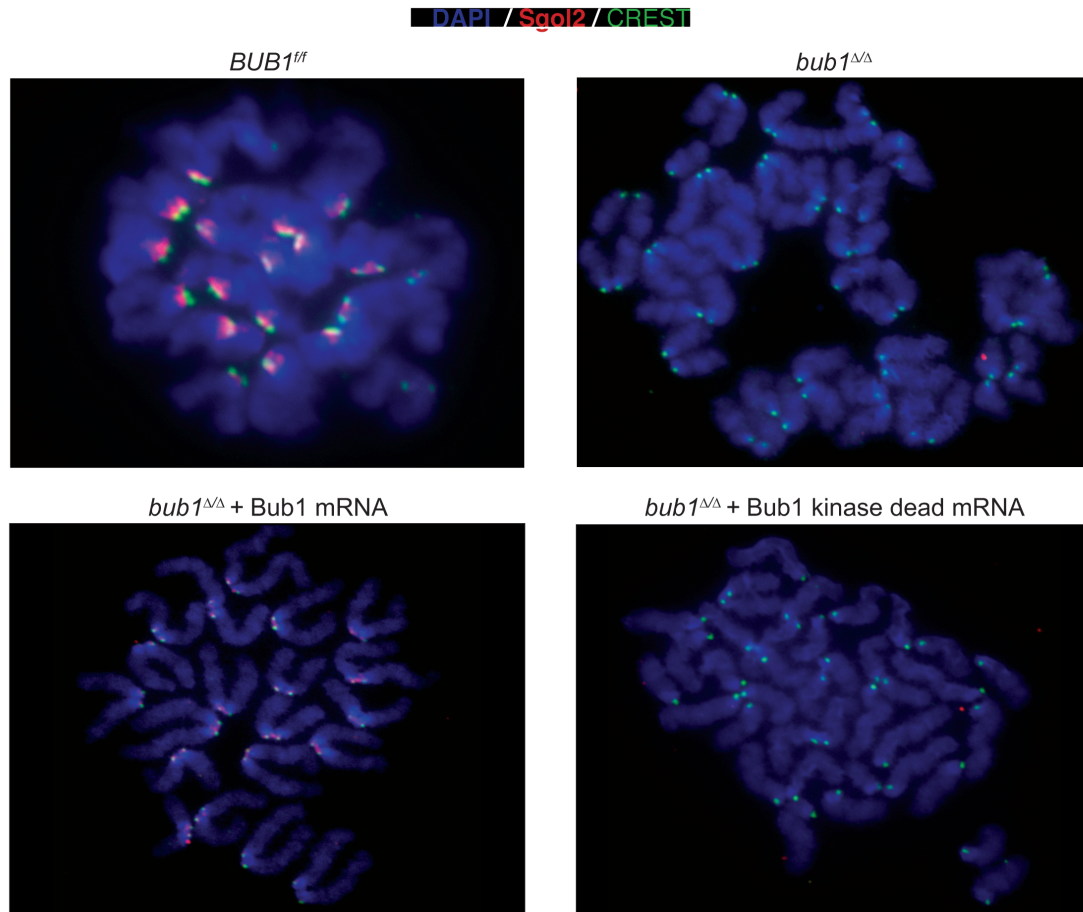


Figure 2.3: Centromeric Cohesion Protection Requires Kinase Activity of *Bub1*. Chromosome spreads from metaphase II-arrested individual oocytes were stained with DAPI (blue), CREST (green) and Sgo2 (red). Representative spreads from control (*BUB1^{F/F}*), *bub1^{Δ/Δ}*, *bub1^{Δ/Δ}* groups expressing wild type and kinase dead *Bub1* are shown.

2.3 DISCUSSION

Coordination between the spindle assembly checkpoint and the centromeric cohesion protection mechanism is essential to ensure balanced segregation of the genome during the two rounds of segregation in meiosis. From the results presented in this part of the thesis, it can be inferred that Bub1 mediates the connection between the surveillance machinery that operates to ensure proper chromosome segregation, and the centromeric cohesion protection by recruiting Sgol2 to the kinetochore. Both functions of Bub1 are required for the second round of chromosome segregation. By expressing wild type and kinase dead Bub1 in *bub1 Δ/Δ* oocytes, this work has been able to uncouple the two functions of Bub1 in the first meiotic division in mouse oocytes.

2.3.1 SAC and the Kinase Activity of Bub1

Since the discovery of Bub1 as a component of the SAC, the precise role of its kinase activity in the SAC response has remained controversial. Initially, in budding yeast a mutation in the kinase domain (*bub1-K733R*) failed to complement the benomyl-sensitivity exhibited by a *bub1* deletion strain [224], suggesting that the functioning of SAC requires the kinase activity of Bub1. However, later it was found that the K733R mutation in the kinase domain destabilises Bub1 in budding yeast [225]. Therefore, the whole protein and not just the kinase domain is affected by this mutation. Subsequently, a budding yeast mutant entirely lacking the kinase domain of Bub1 was shown to mount a proper SAC response to spindle poisons [226]. Therefore, it was concluded that in budding yeast, the kinase activity of Bub1 is not required for the proper functioning of the SAC. In contrast to budding yeast, in fission yeast, both a point mutation in the kinase domain, *bub1-K762R*, (corresponding to *bub1-K733R* in budding) and a mutant lacking the entire kinase domain failed to activate the SAC in response to the microtubule toxin carbendazim, arguing that Bub1 kinase activity is required for checkpoint function [227]. Importantly, in fission yeast Bub1-K762R is a stable protein. However, later work showed that a fission yeast strain with *bub1-K762M* mutation was benomyl resistant [228], arguing, on the contrary, that the kinase

activity of Bub1 is dispensable for its role in the SAC response. Similarly, in *Xenopus* egg extracts, a kinase-inactive Bub1 (with K872R mutation) was able to mount a checkpoint response in the presence of high numbers of sperm nuclei [229]. However, a mutant lacking the entire kinase domain was less efficient in maintaining the checkpoint when low numbers of nuclei were added [230], suggesting that the catalytic activity of Bub1 might amplify a weak signal initiated by few unattached kinetochores. Thus, on the whole, it was thought that kinase activity might not be required for SAC, but the evidence regarding the role of Bub1's kinase activity in establishing or maintaining the SAC was ambivalent.

The present study clearly shows that in mouse oocytes, the Bub1 protein is required to mount a SAC response to spindle poisons, but this function clearly does not depend on the kinase activity of Bub1, as a mutant Bub1 lacking the entire kinase domain can support the SAC response to nocodazole. Together with the finding that a kinase mutant of Bub1 can restore the APC/C activity in the knockout oocytes [223], the results presented above clearly argue that the efficient SAC signalling in mouse oocytes depends on Bub1, but not on its kinase activity. However, previous work measuring securin destruction timing, as a marker of APC/C^{Cdc20} activity, showed a small but consistent difference in APC/C^{Cdc20} activation time between *bub1*^{Δ/Δ} expressing the wild-type and the kinase mutant of Bub1 [223]. This may indicate that for the amplification of the SAC signal in response to a single or few unattached kinetochores, the cell might require the kinase activity of Bub1. Nevertheless, this cannot be tested using a chemical such as nocodazole, which effects the entire meiotic spindle.

2.3.2 Sgo2 Recruitment and Cohesion Protection

The question that emerged from previous research was that if the catalytic activity of Bub1 is not required for SAC function, what is the physiological role of this activity? In a number of organisms, Bub1 knockout resulted in chromosome alignment and segregation defects that could not be explained by the loss of SAC function [188, 231, 232, 225]. In *S. pombe*, Bub1 by phosphorylating H2A at a conserved residue recruits shugoshins (Sgo1 and Sgo2) to centromeres to ensure that

sister chromatids do not prematurely separate during meiosis I [198, 191, 197]. Similarly in budding yeast, the kinase activity of Bub1 is required to recruit Sgo1 to centromeres [226]. In mammalian somatic cells, Bub1 is required for the centromeric localisation of Sgo1 [233, 234, 235, 236]. However, in *BUB1*-null mouse embryonic fibroblasts Sgo1 was able to bind to heterochromatin in G2 and in early prophase, and this binding maintained centromeric cohesion until anaphase. This called into question the physiological role of the kinase domain in protecting centromeric cohesion from the prophase pathway in somatic cells and also suggested that there might be an alternate pathway that could recruit shugoshins to the chromatin.

The results presented in this chapter clearly show that Bub1 through its kinase activity recruits Sgo2 to inner kinetochores and this activity is essential in protecting centromeric cohesion at the first meiotic division. However, when comparing the metaphase II chromosome spreads from *BUB1* and *SGOL2* knockout oocytes, it becomes immediately apparent that most chromosomes from the conditional knockout of *BUB1* oocytes still retained the centromeric cohesion at the second meiotic division, whereas in the case of *SGOL2* knockout, all bivalents resolved into single chromatids by one round of separase activity (??). This suggests that there might be an alternate pathway by which Sgo2 could localise to kinetochores during first meiotic division and the staining techniques employed was able to detect the remaining Sgo2 at kinetochores. However, strikingly *bub1*^{Δ/Δ} expressing kinase-dead form of Bub1 increased the fraction of single chromatids observed on metaphase II chromosome spreads, compared to the chromosome spreads from *bub1*^{Δ/Δ} oocytes. This suggests that the kinase-dead mutant behaves as the dominant negative form of Bub1, and the conditional knockout of *BUB1* might still have some remaining Bub1 activity which could be sufficient to confer, at least, partial protection to the centromeric cohesion at the first meiotic division.

2.4 EXPERIMENTAL PROCEDURES

2.4.1 Mouse Strains

Bub1 protein was depleted specifically in growing oocytes by crossing *BUB1^{f/f}* [111] mice to a transgenic mouse line expressing Cre recombinase from the Zona pellucida 3 promoter (Zp3-Cre) [237] as previously described [223].

2.4.2 Oocyte Culture and Microinjection

Fully grown mouse Germinal Vesicle (GV) oocytes were isolated by physical disaggregation of ovaries from 8-10 weeks old adult mice in M2 medium (Sigma-Aldrich) supplemented with 200 μ M 3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich) at 37°C. Oocytes were cultured in M16 medium (40-50mL drops) (Millipore) covered with mineral oil (Sigma) at 37°C and 5% CO₂.

Fully grown oocytes were injected with 5-10 pL of mRNA at a final concentration of 0.1 mg/mL in RNase-free water (Ambion) using a Pneumatic PicoPump (World Precision Instruments) [187, 238]. To allow for protein expression, following microinjection of mRNA, GV oocytes were cultured for 12 h in M16 supplemented with IBMX. Oocytes were then washed in inhibitor-free M16 and thereafter cultured at 37°C and 5%CO₂.

2.4.3 Chromosome Spreads

Chromosome spreads from mouse oocytes were prepared as previously described [239, 240]. Briefly, zona pellucida was removed by 10 mg/ml Pronase (Sigma-Aldrich) at 37°C for 5-10min. Zona-free oocytes were treated by hypotonic solution (50% FCS in deionized water) for 10 min at 37°C. Oocytes were then fixed in paraformaldehyde solution (1% paraformaldehyde, 0.15% Triton-X 100, 3 mM dithiothreitol, adjusted to pH 9.2 with NaOH) in a 15-well multichamber glass slide (MP Biomedicals) overnight at room temperature in a humidified chamber.

After drying, slides were washed twice in 0.4% Photoflo/H₂O (Kodak) and PBS for 5 min, at room temperature. Slides were then processed for immunostaining with mouse anti-Bub1 (1:200; abcam-ab54893) or CREST (1:500; Davis Lab) and rabbit anti-Sgo2 serum (1:50; gift from J.L. Barbero [241]) and were mounted in Vectashield with DAPI.

Chapter 3

Shugoshin-Like Protein 2 (SGOL2) With Its Interacting Partners Protects Centromeric Cohesion, Promotes and Corrects Microtubule-Kinetochore Interactions, Maintains Inter-Kinetochore Distances, and Silences the Spindle Assembly Checkpoint In Mouse Oocytes

3.1 INTRODUCTION

Accurate segregation of eukaryotic chromosomes during cell division relies on the coordination between cohesion resolution and attachment of chromosomes through kinetochores to spindle microtubules emanating from opposite spindle

poles. The cohesion between the replicated sister chromatids, mediated by the cohesin complex, holds sister DNAs together from DNA replication until anaphase by trapping it inside a tripartite ring formed by its Smc1, Smc3, and α -kleisin subunits [242, 243, 244]. The cell cycle machinery ensures that the cohesin complex is not destroyed until all chromosomes have established bipolar microtubule-kinetochore attachments. This is achieved partly by error correction machinery that senses tension, generated by the pulling of microtubules and counteracting adhesion provided by cohesion complexes, at kinetochores. The disrupting effects of the conserved Aurora kinase selectively destabilises the incorrect attachments [102]. Additionally, signals generated at kinetochores, collectively known as the Spindle Assembly Checkpoint, can prevent cohesin cleavage by blocking the ubiquitinylation activity of Anaphase-Promoting Complex or Cyclosome (APC/C) until all chromosomes have aligned to the metaphase plate [120]. Together the error correction machinery and the spindle assembly checkpoint, orchestrated by kinetochores, ensure that chromosome segregation is only brought about when all chromosomes have established proper connections with the segregation machinery via microtubule-kinetochore attachments [184].

At the onset of anaphase, sister chromatid disjunction is brought about by opening of the cohesin ring by cleavage of its α -kleisin subunit by a thiol protease called separase, which is kept inactive through most of the cell cycle by the binding of an inhibitory chaperone called securin and phosphorylation by cyclin B/Cdk1 kinase [245]. At the metaphase-to-anaphase transition, SAC dissociates from the APC/C^{Cdc20} complex, which allows it to destroy securin and cyclin B. The APC/C^{Cdc20}, therefore, activates separase which releases the sister chromatids [56]. However, the process by which a cell coordinates cohesin cleavage with activities of error correction machinery and SAC remains unclear.

Like mitotic chromosomes, homologous chromosomes in meiosis are entrapped by the cohesin complex [242]. However, unlike mitosis where the sister chromatids segregate to opposite poles during anaphase, sister chromatids remain attached at the first meiotic division, while homologous chromosomes of maternal and paternal origins are partitioned. The segregation of homologous chromosomes at

the first meiotic division requires the sister kinetochores to attach to microtubules emanating from the same pole, such that the maternal sister centromeres align in the opposite direction of their paternal homologs. This is supported, in part, by the reciprocal recombination between homologous non-sister chromatids, which creates structures called chiasmata. Together with cohesin complexes, recombination links the maternal and paternal chromosomes to produce a structure called a bivalent chromosome. During the meiotic division, bivalent chromosomes attach to microtubules such that the tension that stabilises microtubule-kinetochore interactions is generated by the traction of maternal sister centromeres in the opposite direction of their paternal homologs [11].

During meiosis, two rounds of chromosome segregation (meiosis I and II) without an intervening round of DNA replication is required to produce haploid gametes from diploid progenitors. The two-step division requires the sister chromatids to remain attached at the centromeres by the cohesin complex after the first meiotic division [152]. This persistence of centromeric cohesion is essential for the seamless transition from segregation of homologs to alignment of sister chromatids on the second meiotic spindle [223, 222]. This is achieved by the stepwise removal of cohesin from the homologous chromosomes. At the first division, the homologous chromosomes are resolved into dyads by cleavage of cohesin at arms, but cohesin complexes at centromeres remain protected. Then, in a second round of division, each chromosome is split apart into its component chromatids, by cleavage of centromeric cohesion. At both steps, the partners to be separated are initially held together by cohesin and then split apart when separase cleaves cohesin [152].

The persistence of cohesin at centromeres after the first meiotic division requires an ortholog of the *Drosophila* MEI-S332 protein, called shugoshin [246, 190, 191, 192, 193]. Members of the shugoshin family of proteins contain an N-terminal homodimeric parallel coiled-coil, and a conserved C-terminal basic region. The N terminus provides docking onto protein phosphatase 2A's (PP2A) C and B' subunits [200]. In yeast, the interaction between shugoshin and PP2A recruits PP2A to centromeres and protects centromeric Rec8 from separase [85, 86]. In budding yeast, a mutant shugoshin defective in the binding of PP2A fails to con-

fer protection to centromeric cohesion in meiosis I [200]. Phosphatase activity of PP2A is thought to protect centromeric cohesin by opposing Rec8s phosphorylation by CK δ/ϵ and DDK [201, 202].

Mammals have two members of the conserved shugoshin family: Shugoshin-like protein 1 (Sgol1), and Shugoshin-like protein 2 (Sgol2). Sgol1 protects centromeric cohesion against the non-proteolytic anti-establishment activity of the prophase pathway during the mitotic cell cycle [84]. Sgol2, on the other hand, protects centromeric cohesion from the proteolytic activity of separase at the first meiotic division [222, 247]. Deletion of *SGOL2* in mice results in infertility [222]. Cytological examination of the germ line cells from *SGOL2* knockout or knockdown showed complete loss of cohesion at meiosis I [222, 247]. It has been reported that Sgol2, through its coiled coil domain, can bind *in vitro* to PP2A [200]. However, it has not been established whether centromeric cohesion protection in mammalian germ cells requires Sgol2s interaction with PP2A. In mammalian cells, Sgol2 can interact with MCAK [248], a motor protein implicated in the error correction machinery, and has also been recently shown to bind to Mad2, an essential component of the SAC [249]. Based on its interactions, Sgol2 can be linked to cohesion protection, SAC, and error correction pathways. However, the physiological significance of its multiple interactions remains unclear.

This study shows that during the first meiotic division Sgol2 promotes coordination between cohesion protection, bi-orientation of homologous chromosomes, corrections of microtubule-kinetochore attachments, SAC inactivation, and, hence APC/C^{Cdc20} activation. It protects centromeric cohesion by recruiting PP2A to kinetochores, accelerates the release of APC/C^{Cdc20} from the spindle assembly checkpoint by its interaction with PP2A and Mad2, and also maintains inter-kinetochore distances, promotes bi-orientation and congression of homologous chromosomes by increasing kinetochore microtubule interactions possibly by recruiting MCAK and by inactivating Aurora B/C kinase activity at kinetochores. These pathways linked by Sgol2 together ensure proper segregation of chromosomes in mouse oocytes.

3.2 RESULTS

3.2.1 Sgol2 Interacts With PP2A Through Conserved Residues in Its Putative Coiled Coil Domain

An essential feature of the shugoshin family of proteins is the conserved N-terminal homodimeric coiled-coil domain (Figure 3.1A). Structural analysis revealed that the N-terminal homodimeric coiled-coil domain of hSgol1 directly interacts with the B' and C subunits of PP2A. The binding interface between human Sgol1 (hSgo1) and PP2A is brought together by a pair of hydrogen bonds between conserved arginine residues in the middle of the coiled-coil interface of Sgol2, which allows the surrounding residues to form the hydrogen bonds and salt bridges with PP2A [200].

Like hSgol1, the coiled-coil domain of mouse Sgol2 (mSgol2cc) is sufficient for its interaction with PP2A. However, in contrast to hSgol1, which can only interact with the holoenzyme (complex of A, B', and C subunits), the coiled-coil domain in Sgol2 can also form a stable association with the core complex (A and C subunit) [200].

With the intention to mutate Sgol2 such that the mutant protein is defective solely in its ability to interact with PP2A, conserved residues (namely L51, N54, R56, and E82) on mSgol2 mapping onto the interaction surfaces between PP2A and hSgol1 were substituted with alanine. To reverse the charge at position 82, glutamic acid was mutated to lysine (E82K). Additionally, a triple mutant with L51A/N54A/R56A (3A) was prepared. Furthermore, to disrupt the binding interface of Sgol2-PP2A interaction, the conserved asparagine residue (N55 on mSgol2) aligning to N61 in hSgol1, which forms a hydrogen bond that stabilises the binding interface in the middle of coiled coil, was changed to isoleucine (Figure 3.1B).

To check whether mutations of conserved residues in the coiled-coil domain of Sgol2 can affect the homodimerisation of the coiled-coil domain, equal amounts of purified Flag- and His- tagged MBP-Sgol2cc (31-117) with the same substitutions were mixed, and proteins were pulled down using the Talon beads. The relative abundance of each form of mSgol2cc was assessed by western blotting. Mutants, except for the 3A, showed no effect of amino acid substitutions on coiled-coil dimerisation. With this assay, 3A showed a reduction in dimer formation (Figure 8B). Therefore, to verify whether substituting these residues hinders coiled-coil domains dimerisation, Flag- and His- tagged mSgol2cc from either wild type or 3A mutant were co-expressed. After His pull down the relative abundance of each form was analysed by western blot using anti-Flag and anti-His antibodies. The co-IP failed to detect any difference in dimerisation of coiled-coil domains from the wild type and the 3A mutant Sgol2 (Figure 8C), suggesting that the reduction observed by mixing purified proteins might be an *in vitro* effect.

To assess whether the coiled-coil residues in mSgol2 mapping onto the binding interface of hSgol1 and PP2A are conserved in their interaction with PP2A, GST-tagged PP2A A subunit (PP2A-A) was incubated with excessive untagged A, B', and C subunits, and MBP-Sgol2-Flag. The GST pull down showed that all mutations in conserved residues in the coiled-coil domain reduce Sgol2cc ability to interact with PP2A. However, these mutations varied considerably in their ability to disrupt the Sgol2-PP2A interaction. Substitution of Sgol2's N55 to isoleucine, which is predicted to disrupt the binding interface, abolishes Sgol2cc binding to PP2A. While N54A greatly reduces the interaction, substitution of a conserved arginine (R56) to alanine has a moderate effect. Mutation of individual residues L51A and E82A have little effect on PP2A binding, though E82K causes a modest reduction. The triple substitution L51A/N54A/R56A (3A) abolishes PP2A binding under these experimental conditions (Figure 3.1C).

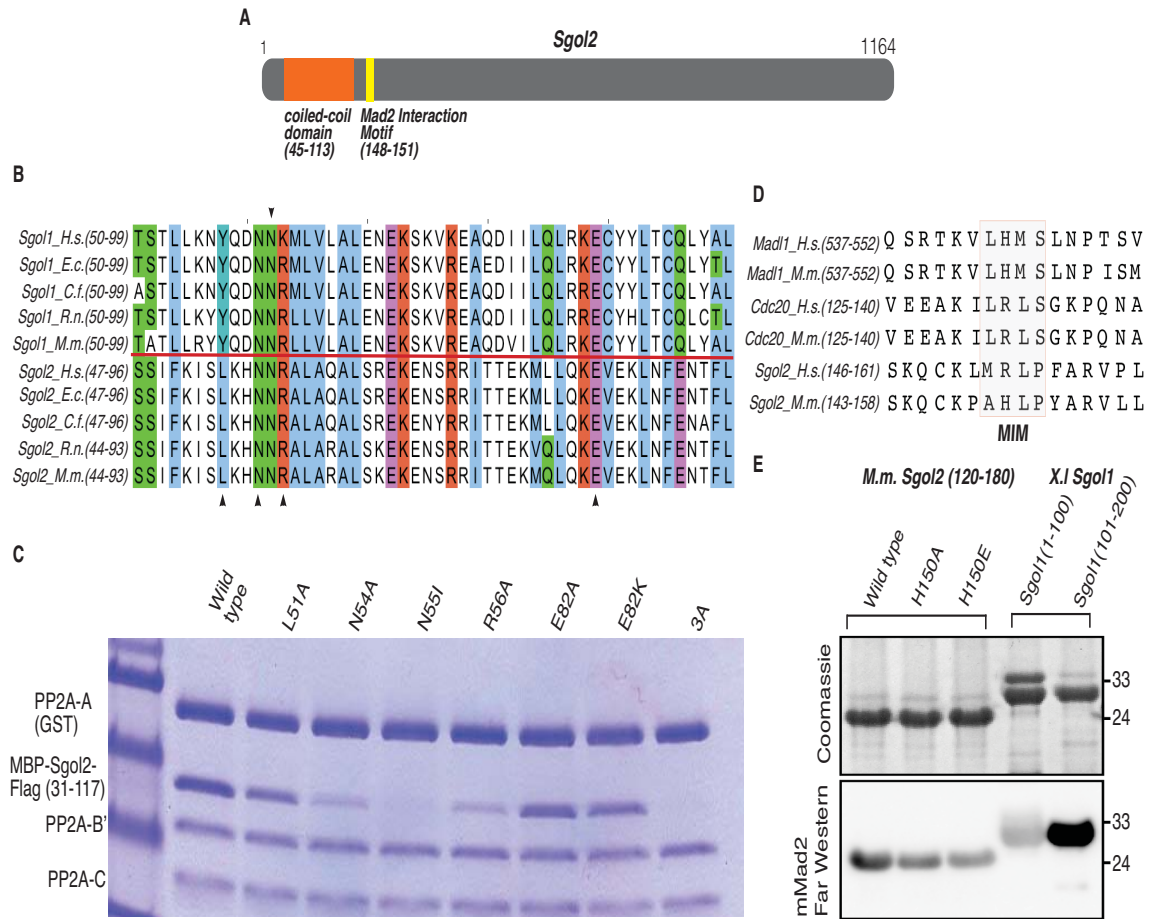


Figure 3.1: *Sgol2* binds to PP2A and Mad2 through coiled coil domain and Mad2 Binding Motif (MIM), respectively. (A) Schematic diagram indicating relative positions of the coiled-coil domain and the MIM sequence in *mSgol2* (not drawn to scale). (B) Sequence alignment of the coiled coil domains of *Sgol1* and *Sgol2* from different vertebrate species. Residues (L51, N54, R56 and E82) in *mSgol2* mapping onto binding sites between PP2A and h*Sgol1* are indicated by arrowheads below the alignment. The conserved asparagine residue (N55 in *mSgol2*), important for maintaining the interaction surface of the coiled-coil domain, is marked by an arrowhead above the alignment. (C) The *in vitro* binding assay of PP2A A(GST-tagged), B', and C subunits with wild type and various mutants of MBP-*mSgol2cc*-Flag. After GST pull down, eluates were resolved by SDS-PAGE (Experiment performed by Seamus Morrone). (D) Sequence alignment of conserved MIM (Mad2 Interacting Motif) in *Sgol2*, *Mad1*, and *Cdc20* from human and mouse origin. (E) *mSgol2* and Mad2 binding assay. *mSgol2* fragment spanning amino-acid (aa) residues 120-180 with wild type and mutant MIM sequences were assessed for Mad2 interaction by far western blot. Fragments from *Xenopus Sgo1*, with and without MIM domain, were used as positive and negative controls (Experiment performed by Dr. Bernd Mayer).

3.2.2 Sgol2 Protects Centromeric Cohesion by Recruiting PP2A to Kinetochores

Sgol2 and PP2A co-localise to inner kinetochores at metaphase I on bivalent chromosomes from wild type control oocytes (Figure 3.2A). By contrast, the kinetochore localisation of Sgol2 and PP2A was completely abolished in *sgol2*^{Δ/Δ} oocytes (n=26). Moreover, microinjection of wild type mSgol2 mRNA in *sgol2*^{Δ/Δ} oocytes restored Sgol2 and PP2A localisation at inner kinetochores in meiosis I. This demonstrates that in mouse oocytes, mSgol2 is required for the recruitment of PP2A to kinetochores.

To test whether the conserved residues in Sgol2, which either abolished or greatly reduced the *in vitro* interaction between the coiled-coil domain of Sgol2 and PP2A, are required for the *in vivo* interaction between full length Sgol2 and PP2A, *sgol2*^{Δ/Δ} oocytes were injected with *in vitro* transcribed RNA from Sgol2 bearing N54A, N55I, and 3A mutations. In contrast to the wild type Sgol2 mRNA, these Sgol2 mutants failed to rescue the PP2A localisation (Figure 3.2A). Crucially, these mutations did not effect Sgol2 localisation at inner kinetochores during the first meiotic division (Figure 3.2A). Thus, these findings further confirm that Sgol2, through its coiled coil domain, interacts with PP2A and recruits it to kinetochores.

To address the role of Sgol2-PP2A interaction in centromeric cohesion protection, chromosome spreads were prepared from metaphase II-arrested oocytes from wild type, *sgol2*^{Δ/Δ}, and *sgol2*^{Δ/Δ} microinjected with various forms of Sgol2. As previously reported [222], *SGOL2* knockout oocytes fail to protect centromeric cohesion and all sister chromatids were disjoined at the first meiotic division. This phenotype was fully rescued with all sister chromatids attached at centromeres (dyads) by injection of wild type mSgol2 mRNA into knockout oocytes. Strikingly, the ability of the mutants to rescue sister chromatid disjunction corresponded quite well with their *in vitro* interaction with PP2A. For instance, injection of Sgol2 mRNA with L51A (n=16) and E82A (n=18) mutations that minimally disrupts the interaction fully rescued the precocious segregation of sis-

ter chromatids in *sgol2 Δ/Δ* oocytes (Figure 3.2B). Furthermore, microinjection of equal concentrations of R56A (n=18) and E82K (n=25) mutants that modestly affected the Sgol2cc-PP2A interaction, showed asymmetric distributions of sister chromatids in each oocyte, with frequent appearance of individual chromatids amid dyads (Figure 3.2B). Mutants N54A (n=48), N55I (n=12), and 3A (n=26), which either greatly reduced or fully abolished the interaction, failed to rescue the centromeric cohesion defects in *sgol2 Δ/Δ* oocytes (Figure 3.2B). In addition, injection of the triple mutant produced centromeric cohesion defects in wild type oocytes at the first meiotic division (Figure 9A and B). These results clearly demonstrate that the interaction between PP2A and Sgol2 is essential for centromeric cohesion protection at the first meiotic division and hence for the two rounds of chromosomes segregation in meiosis.

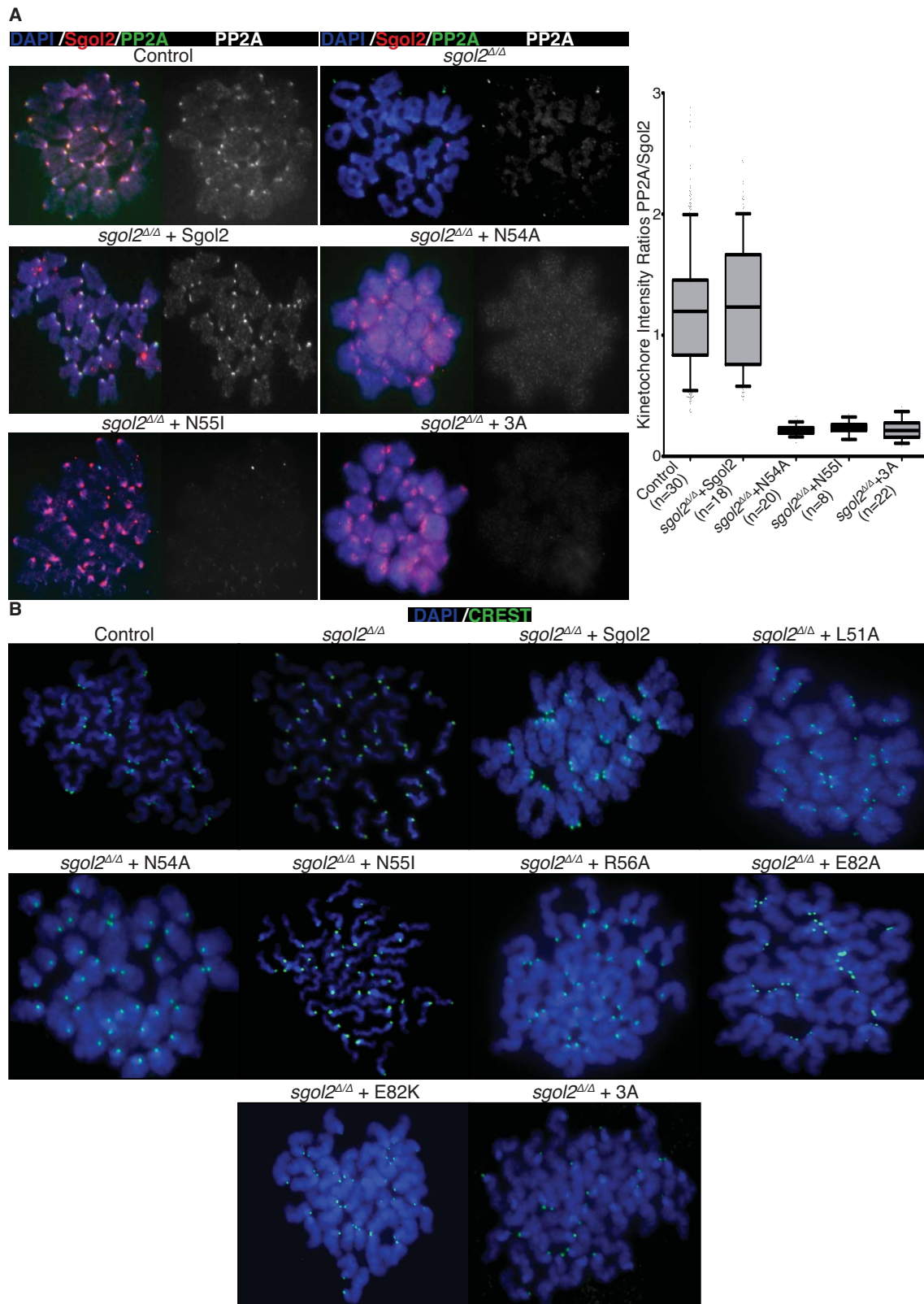


Figure 3.2: *Sgol2*-*PP2A* interaction is required to recruit *PP2A* to kinetochores and protect centromeric cohesion at the first meiotic division. (A) GV oocytes were harvested in the presence of IBMX. Microinjections were performed at GV stage in M2 medium supplemented with IBMX. Oocytes were then cultured in IBMX-free medium for about 6hrs, corresponding to metaphase I stage. Chromosome spreads were prepared and stained with DAPI (blue), *Sgol2* (red), and *PP2A*-C (green). Box and whisker plot shows fluorescence intensity ratios of *PP2A* and *Sgol2* at kinetochores. Upper and lower bars indicate 95th and 5th percentiles, respectively. Numbers of oocytes examined are indicated (n). (B) Oocytes were harvested at GV stage and matured in vitro for up to 24 hr. Chromosome spreads prepared from oocytes that had extruded the first polar body were stained with DAPI (blue) to visualise DNA and CREST (green) to mark centromeres.

3.2.3 Conserved MIM Domain Mediates Sgol2 Interaction With Mad2

Spindle Assembly Checkpoint's ability to inhibit APC^{Cdc20} activity against securin and cyclins relies on the interactions of Mad2 with Mad1 and Cdc20. This interaction is mediated by a conserved Mad2 Interacting Motif (MIM) with a basic amino acid at its center surrounded by hydrophobic residues on either side in Mad1 and Cdc20. Recently, it was discovered that, like Mad1 and Cdc20, shugoshins (Sgol2 from human and Sgo1 from *Xenopus*) can interact with Mad2 through the conserved MIM sequence in Sgol2 [249]. This motif is conserved in mouse Sgol2 (Figure 3.1D). Therefore, to check whether this domain is able to mediate the interaction between mSgol2 and Mad2, purified mSgol2 and mouse Mad2 was analysed by far-western blots. A fragment of Sgol2 with the MIM sequence at its center associated with Mad2, and the interaction was disrupted by substituting the basic amino acid at the center of MIM with alanine (H150A) or glutamic acid (H150E), a mutation to reverse the charge (Figure 3.1E). This suggests that the binding with Mad2 is a conserved feature of vertebrate shugoshins possessing the interaction motif.

3.2.4 Sgol2 and Its Interactions With PP2A and Mad2 Are Required for Silencing the Spindle Assembly Checkpoint

The time-course DIC images of the first meiotic division of *in vitro* maturing oocytes showed that compared to the litter-mate control (wild type) oocytes *sgol2*^{Δ/Δ} oocytes were delayed on average by about 2hrs in extruding the first polar body. Moreover, only 61% of the *sgol2*^{Δ/Δ} oocytes extruded the first polar body, in contrast to the 88% of wild type control oocytes (Figure 3.3A). This suggested that *sgol2*^{Δ/Δ} oocytes are defective either in their ability to extrude a polar body or they fail to activate the APC/C^{Cdc20}, i.e. they are arrested in metaphase I.

To further investigate the role of Sgol2 in meiotic cell cycle progression, fully grown germinal vesicle (GV)-stage oocytes harvested from wild-type littermate control and *SGOL2* knockout mice were microinjected with mRNA encoding histone H2B-mCherry to mark chromosomes and Securin-EGFP to measure APC/C^{Cdc20} activity (Figure 3.3B). Similar to the frequency of polar body extrusion, on average 40% of the oocytes from *sgol2*^{Δ/Δ} mice failed to activate the APC/C^{Cdc20}, compared to only 12% of the controls (Figure 3.3C). Furthermore, the oocytes that degraded Securin-EGFP were delayed on average by about 2hrs in APC/C^{Cdc20} activation. These defects in APC/C^{Cdc20} activation were rescued by microinjection of wild type Sgol2 mRNA (Figure 3D and E) and 89% of the oocytes from this group destroyed Securin-EGFP (Figure 3.3D). Moreover, microinjection of Sgol2 mRNA in *sgol2*^{Δ/Δ} oocytes accelerated APC/C^{Cdc20} activation and Securin-EGFP was destroyed on average about 2hrs before the wild type control oocytes (Figure 3.3E). Also, microinjection of Sgol2 mRNA accelerated the APC/C^{Cdc20} activation in wild type oocytes, suggesting that the ability of Sgol2 to accelerate APC/C^{Cdc20} is dose-dependent (Figure 10F). These results argue that Sgol2 is important for the activation of APC/C^{Cdc20} in mouse oocytes.

It has previously been reported that after nuclear envelope breakdown, APC/C^{Cdc20} activity is restrained by the activity of SAC [221, 223, 238]. Therefore, to address whether the defects in APC/C^{Cdc20} activation observed in *SGOL2* knockout oocytes are due to the SAC-dependent arrest, *sgol2*^{Δ/Δ} oocytes were microinjected with a dominant-negative version of the APC/C activator Cdc20 (Cdc20-R132A), with a mutant MIM sequence, which cannot be bound by Mad2 (Figure 3.1D). Microinjection of wild type Cdc20 mRNA in *sgol2*^{Δ/Δ} oocytes did not increase the fraction of oocytes that activate APC/C^{Cdc20}. By contrast, Cdc20-R132A mRNA microinjection resulted in securin destruction in all *sgol2*^{Δ/Δ} oocytes (Figure 3.3C). This demonstrates that *SGOL2* knockout results in SAC-dependent inhibition of APC/C^{Cdc20} activity. Therefore, together with the observation that microinjection of Sgol2 mRNA results in advancement of APC/C^{Cdc20} activation, these data suggest that Sgol2 is involved in silencing the SAC in mouse oocytes.

To investigate if the silencing of the SAC by Sgol2 requires an interaction with PP2A and Mad2, *sgol2*^{Δ/Δ} oocytes were microinjected with mutant Sgol2 mRNA with a point mutation in its coiled-coil domain (N54A) or MIM sequence (H150E). Of the *sgol2*^{Δ/Δ} oocytes microinjected with N54A mutant Sgol2 mRNA, that severely effects its interaction with PP2A and fails to rescue centromeric cohesion defects, 69% destroyed Securin-EGFP and 71% destabilised Securin-EGFP in *SGOL2* knockout microinjected with H150E Sgol2 mRNA (Figure 3D). Furthermore, the fraction of *SGOL2* knockout oocytes microinjected with PP2A or Mad2 binding mutant Sgol2 mRNA that destroyed securin, was delayed in APC/C^{Cdc20} activation to a similar extent as the *SGOL2* knockout oocytes (Figure 3.3E and Figure 10B and C). Similar results were observed in *sgol2*^{Δ/Δ} oocytes expressing triple alanine (PP2A mutant with L51A/N54A/R56A) and H150A (basic residue in MIM domain mutated to alanine) mutant (Figure 10D and E). Crucially, a Mad2 binding mutant of Sgol2 did localise to the kinetochore, recruited PP2A, and protected the sister chromatid cohesion at the first meiotic division (Figure 9C and D). Furthermore, the Sgol2 mutant defective in binding to PP2A and Mad2 can recruit MCAK to kinetochores (Figure 3.4A), suggesting that the SAC-dependent arrest or delay observed in the *SGOL2* knockout cannot be ascribed to a defect in MCAK recruitment to kinetochores. Therefore, these results indicate that Sgol2, through its interactions with PP2A and Mad2, can silence SAC activity during the first meiotic division in mouse oocytes.

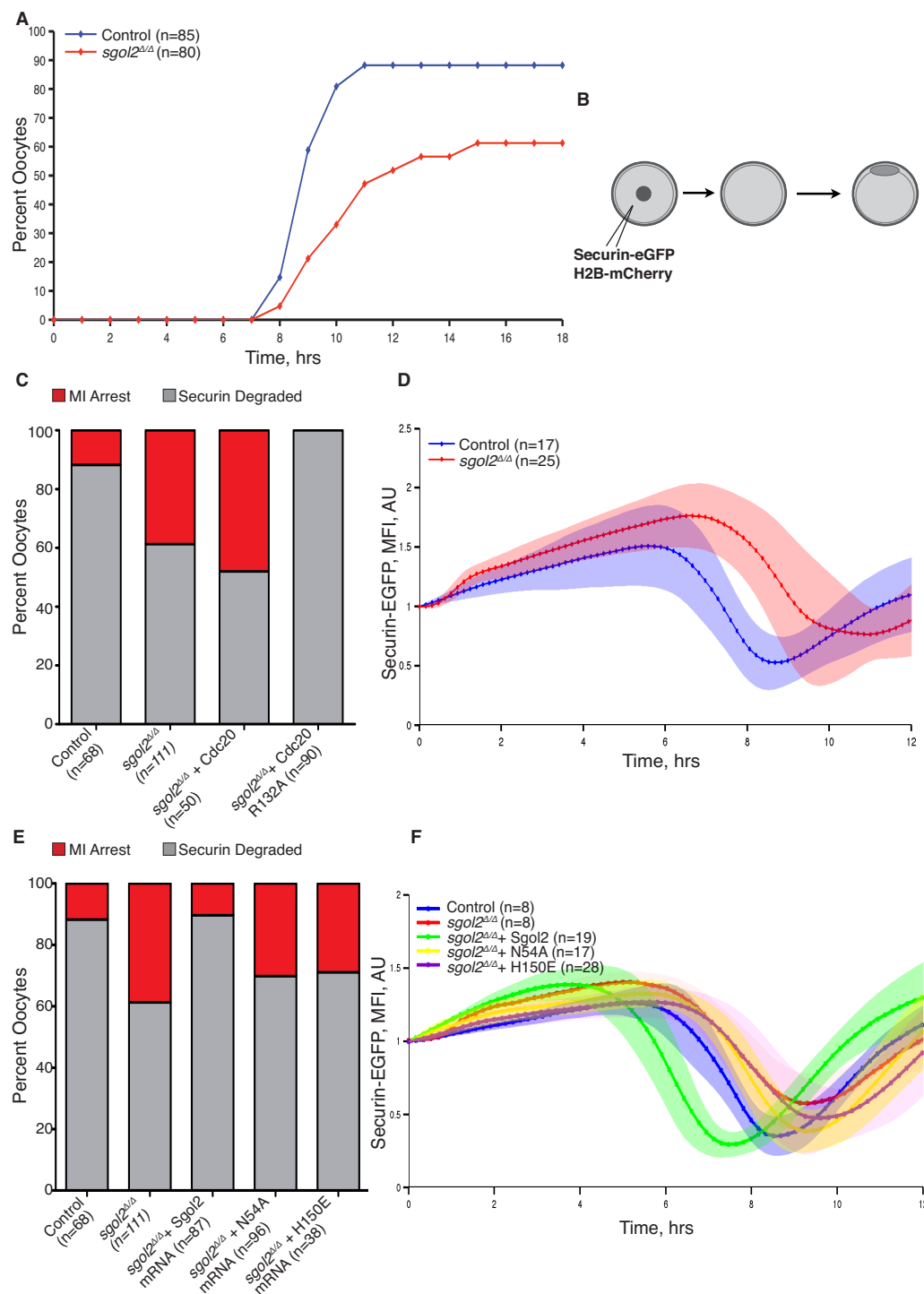


Figure 3.3: *Sgol2* Silences Spindle Assembly Checkpoint through its Interactions with PP2A and Mad2. (A) Kinetics of the first polar body extrusion (PBE) of oocytes cultured *in vitro*. Numbers of oocytes examined are indicated (n). (B) Oocytes were microinjected with the indicated mRNAs at the GV stage. Chromosome movements and kinetics of Securin-EGFP were visualised by time-lapse confocal microscopy. (C) Frequencies of oocytes that destroyed Securin-EGFP, hence activated APC/*C^{Cdc20}*, at the metaphase-anaphase transition are shown. SAC was inactivated by expressing a dominant-negative version of the APC/C activator *Cdc20* (*Cdc20R132A*) that cannot be bound by Mad2. (D) Data represent the means and standard deviations of Securin-EGFP levels in *sgol2^{Δ/Δ}* (red) and litter mate control (blue) oocytes at each time point. Values from individual oocytes were normalised relative to that at GVBD (0 hr), and mean and standard deviations are plotted in arbitrary units, (AU), against time. Securin-EGFP curves from individual oocytes are shown in Figure 10A. (E) Securin-EGFP destruction was used as a marker for APC/C activation. Control and *SGOL2* knockout are compared to *sgol2^{Δ/Δ}* oocytes microinjected with indicated *Sgol2* mRNA. (F) GV-normalised mean and standard deviations of Securin-EGFP levels are plotted against time for the indicated groups of oocytes. Securin-EGFP curves from individual oocytes are displayed in Figure 10B and C.

3.2.5 Sgol2, by Recruiting MCAK to Kinetochores, Maintains Inter-Kinetochores Distances to Protect Chiasmata

Unlike bivalents from wild type oocytes that were attached at arms and centromeres and formed X-shaped chiasmata, bivalent chromosomes from *sgol2*^{Δ/Δ} oocytes often displayed distally associated homologs without a visible chiasmata (Figure 3.4A). Since arm cohesin is required to stabilise chiasmata, I asked whether *SGOL2* deletion could result in premature loss of cohesion from the arms of homologous chromosomes. To check the localisation of cohesin on bivalents from *sgol2*^{Δ/Δ} oocytes, *SGOL2*-targeted mice were crossed to mice expressing tagged Rec8-Myc from a BAC transgene, (Tg)Rec8-Myc [187]. Chromosome spreads prepared from littermate controls and *sgol2*^{Δ/Δ} oocytes expressing myc tagged Rec8 at 6-7hrs post-GVBD detected Rec8-Myc along arms and around centromeres on bivalents from both groups, and no appreciable difference was observed in the amount of myc-tagged Rec8 on bivalent chromosomes from control and knockout groups (Figure 3.4B). Importantly, the distally associated homologous chromosomes from *sgol2*^{Δ/Δ} oocytes retained Rec8 staining (Figure 3.4B).

To investigate the frequent appearance of distally associated homologous chromosomes on metaphase I stage chromosome spreads from *sgol2*^{Δ/Δ} oocytes, live-cell confocal imaging was performed on oocytes microinjected at GV stage with H2B-mCherry and GFP-MCAK. Strikingly, live-cell confocal images revealed that metaphase I-aligned chromosomes in *sgol2*^{Δ/Δ} oocytes stretched much further than the homologous chromosomes in wild type oocytes (t-test, $p < 0.0001$), resulting in increased inter-kinetochore distances (Figure 3.4C). The confocal images suggested that increased inter-kinetochore stretch and distances of metaphase aligned chromosomes in *sgol2*^{Δ/Δ} oocytes could explain the distally associated chromosomes observed on metaphase I-stage chromosome spreads from *sgol2*^{Δ/Δ} oocytes. Importantly, no appreciable difference was observed in the length of the metaphase I spindle (Anova, $p = 0.97$; Figure 3.4D). Furthermore, chromosomes in *sgol2*^{Δ/Δ} oocytes did align in a bipolar manner on the metaphase plate (Figure 3.4C, D, Figure 3.5, Figure 13). These observations suggest that Sgol2 maintains

inter-kinetochore stretch and distance to protect chiasmata.

To further probe the role of Sgol2 in maintaining inter-kinetochore distances during the first meiotic division, *sgol2*^{Δ/Δ} oocytes were microinjected at GV stage with *in vitro* transcribed mRNA from wild type and mutant Sgol2. Chromosome spreads from the rescue group (*SGOL2* knockout oocytes microinjected with wild type Sgol2 mRNA) did not display the distally attached bivalent chromosomes (Figure 3.4A). Furthermore, live-cell confocal imaging showed that *SGOL2* knockout oocytes expressing wild type Sgol2 not only rescues the increased inter-kinetochore stretch and distances, but reduced the inter-kinetochore distances even further than the wild type oocytes (Figure 3.4C, t-test $p < 0.0001$). Critically, microinjection of wild type Sgol2 mRNA did not alter the length of the meiotic spindle (Figure 3.4D). Similar reductions in inter-kinetochore distances were observed in *sgol2*^{Δ/Δ} oocytes microinjected with mutant Sgol2 mRNA, defective in PP2A (N54A) or Mad2 (H150E) interactions (Figure 3.4C, t-test $p < 0.0001$). Since Sgol2-PP2A interaction is required to protect cohesin complexes (Figure 3.2B), this further confirms that distally attached bivalents cannot be explained by a premature loss of cohesin complexes.

Live cell confocal imaging clearly indicated that inter-kinetochore distances between homologous chromosomes correlated inversely with the GFP-MCAK signal at kinetochores. This strongly suggested that Sgol2 maintains inter-kinetochore distances by recruiting the motor protein MCAK to kinetochores. Aurora phosphorylation on Sgol2 has recently been shown to be important for the binding of Sgol2 to MCAK [250]. Indeed, in mouse oocytes, Aurora Kinase activity is required to recruit MCAK at kinetochores, but not to the arms of homologous chromosomes (Figure 11B); crucially, during the first meiotic division Aurora B/C kinase phosphorylates Sgol2 at T521 (Figure 12B). Microinjection of the T521A T600A mutant Sgol2 mRNA in *sgol2*^{Δ/Δ} oocytes did recruit MCAK to arms of homologous chromosomes, but it failed to rescue the kinetochore localisation of MCAK (Figure 11B). Importantly, the T521A T600A mutant Sgol2 did localise to kinetochores and was able to rescue the premature segregation of sister chromatids in *sgol2*^{Δ/Δ} oocytes (Figure 9B and C). Microinjection of T521A

T600A Sgol2 mRNA in *sgol2 Δ/Δ* oocytes reduced the inter-kinetochores distances compared to *sgol2 Δ/Δ* oocytes (t-test, $p < 0.0001$), but had higher inter-kinetochore distances compared to wild type oocytes (Figure 3.4A and C, t-test $p = 0.027$). Taken together, these results suggest that Sgol2, through MCAK recruitment, maintains inter-kinetochore distances and stretch during metaphase of the first meiotic division.

I asked if the localisation of MCAK at kinetochores/centromeres is sufficient to maintain inter-kinetochore distances and protect chiasmata, then the artificial targeting of MCAK to centromeres should rescue the increased inter-kinetochore distances and stretch observed between homologous chromosomes from *sgol2 Δ/Δ* oocytes. Indeed, bivalent chromosomes from *SGOL2* knockout oocytes microinjected with MCAK fused to the C-terminus of CENPB-GFP maintained the normal X-shaped chiasmata (Figure 3.4A). Moreover, compared to *sgol2 Δ/Δ* oocytes microinjected with CENPB-GFP, which reproduced the increased inter-kinetochore distances, microinjection of CENPB-GFP-MCAK in *sgol2 Δ/Δ* oocytes rescued the abnormal stretching of metaphase-aligned homologous chromosomes (Figure 3.4C and Figure 11A, t-test $p < 0.0001$). These results convincingly demonstrate that MCAK at kinetochores can balance the inter-kinetochore stretch between homologous chromosomes during the first meiotic division.

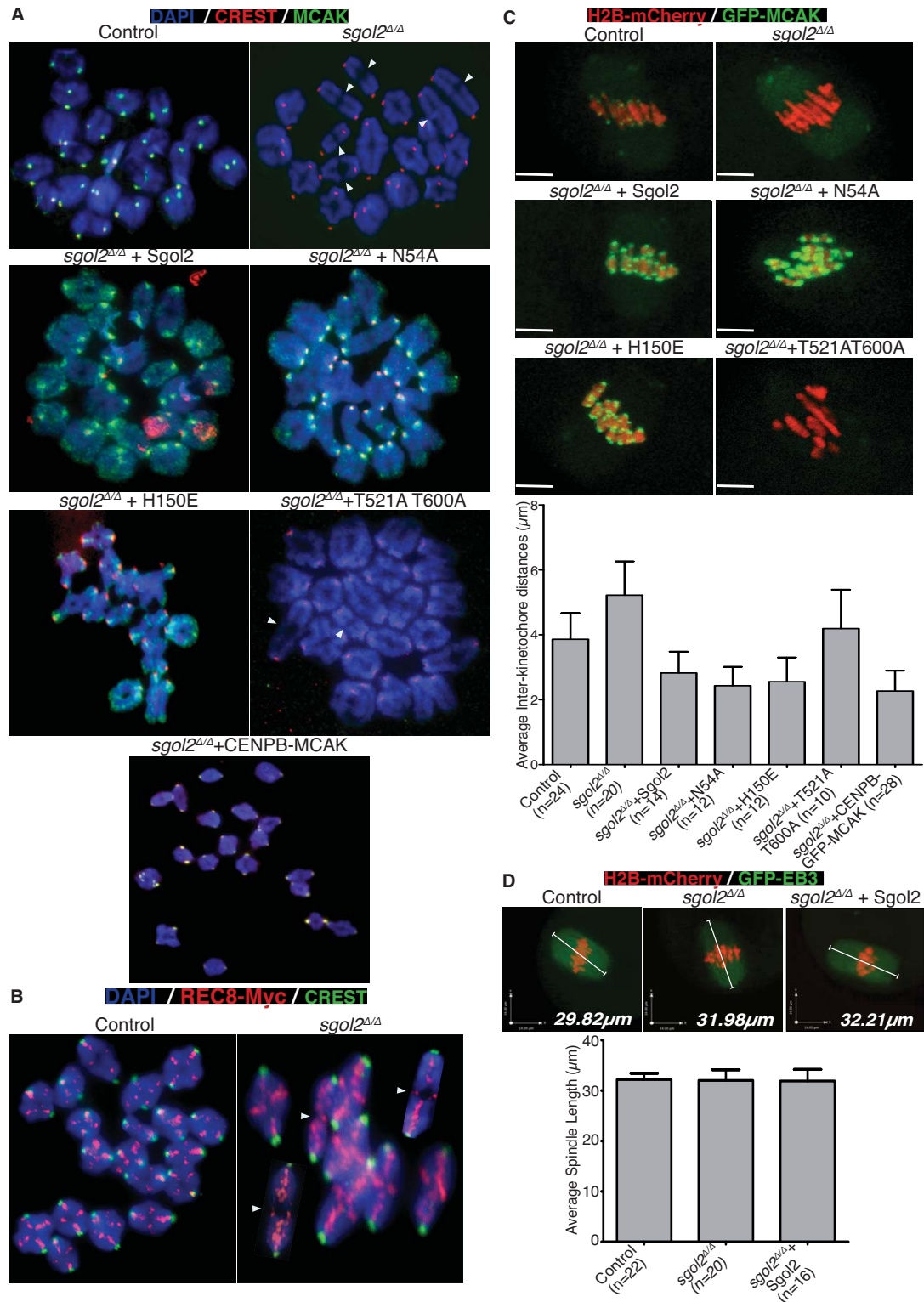


Figure 3.4: *Sgol2*, by recruiting MCAK to kinetochores, maintains inter-kinetochore distances between homologous chromosomes. (A) GV stage oocytes were harvested and microinjected in M2 medium supplemented with IBMX. Release from IBMX resulted in GVBD and at 6-7hrs post GVBD chromosome spreads were prepared. Slides were stained with DAPI (blue), CREST (Red), and MCAK (green). Arrowheads indicate distally attached bivalent chromosomes. (B) *SGOL2* targeted mice were crossed to (Tg)Rec8-Myc females to generate control and *sgol2^{Δ/Δ}* expressing the Rec8-myc transgene. Chromosome spreads were prepared at 6-7hrs post-GVBD and were stained with DAPI (blue), CREST (green), and c-Myc (Red). (C) Representative Z-projected time-lapse confocal microscopy images and average (+ SDs) inter-kinetochore distance measurements from metaphase I stage oocytes expressing H2B-mCherry (chromosomes, red) and GFP-MCAK. Scale bars represent 5 μm. (D) Z-projected (12 slices 1.5 μm apart) live-cell confocal images and spindle length measurements (+ SDs) from metaphase I stage oocytes expressing EB3-GFP (microtubule plus ends, green) and H2B-mCherry (chromosomes, red). Scale bar is 14 μm.

3.2.6 Sgol2 Promotes Biorientation of Homologous Chromosomes on the Meiotic Spindle

Detailed microscopic analysis of spindle shape and the arrangement of chromosomes on the meiotic spindle suggests that the time between Germinal Vesicle Breakdown (GVBD) and anaphase onset can be divided into four functionally distinct phases. After GVBD, multiple microtubule-organising centres (MTOCs) form a ball of microtubules that continuously polymerise with individual chromosomes distributed as single objects on the surface of the spherical ball (phase 1). After laterally sliding along the surface, chromosomes congress on the surface of the ball, forming the prometaphase belt (phase 2). Thereafter, both chromosomes and microtubules concomitantly go through major changes. Chromosomes slowly start invading the cluster of microtubules and the ball of microtubules elongates perpendicular to the belt of chromosomes to form the barrel-shaped bipolar spindle. This highly dynamic phase culminates when homologous chromosomes align to the metaphase plate on the bipolar spindle (Phase 3). Finally, before segregation at anaphase, metaphase plate-aligned chromosomes oscillate with low amplitudes (Phase 4) [251, 252].

Live-cell confocal imaging of mouse oocytes microinjected with H2B-mCherry and GFP-MCAK showed that in wild type control oocytes after GVBD, MCAK localises to MTOCs and to the cluster of microtubules forming the ball-shaped spindle (Figure 3.5). At about 4hrs post-GVBD, as the concentration of Sgol2 increases at the kinetochore and Aurora Kinase phosphorylates Sgol2 at T521 (Figure 12); the GFP-MCAK signals appear at kinetochores (Figure 3.5). Chromosomes start to congress on the surface of the ball of microtubules at the transition between phase 1 and 2. Its signal at kinetochores gradually increases as homologous chromosomes start to invade the interior of the spindle, and the concentration of MCAK at kinetochores peaks as the meiotic chromosomes bi-orient and congress on the metaphase plate (Figure 3.5).

In *sgol2*^{Δ/Δ} oocytes, GFP-MCAK did not localise to kinetochores, confirming observations from chromosome spreads (Figure 3.5 and 3.4A), but it did bind

to MTOCs and microtubules (Figure 3.5). After GVBD, individual homologous chromosomes in *sgol2 Δ/Δ* oocytes distributed on the surface of the ball-shaped spindle; however, compared to chromosomes in wild type control oocytes at a similar stage, homologous chromosomes in *sgol2 Δ/Δ* oocytes were distributed more peripherally on the ball of microtubules (Figure 3.5, Figure 3.6A and Figure 13). Furthermore, homologous chromosomes in *sgol2 Δ/Δ* oocytes were stretched while they were aligning to form the prometaphase belt on the ball-shaped spindle. Chromosomes were stretched even further as soon as the spindle achieved bipolar shape (Figure 3.5 and Figure 13), as reported above (Figure 3.4C). In summary, the time-course confocal images showed that in *sgol2 Δ/Δ* oocytes, phase 1 was more elaborate with chromosomes decorating the ball from more peripheral locations than the wild type oocytes and there was no clear demarcation between phases 2 and 3. From the analysis of time-course confocal images, it seems as if the coordination between the spindle structure and chromosome movements on the spindle is lost in Sgol2-depleted oocytes.

To confirm whether the elaborate distribution of chromosomes on the ball-shaped spindle in *sgol2 Δ/Δ* oocytes is, indeed, due to the absence of Sgol2 on kinetochores, *SGOL2* oocytes were microinjected with Sgol2 mRNA. Strikingly, in this group after GVBD, homologous chromosomes did not localise to the periphery and stayed inside the ball-shaped spindle (Figure 3.6A and Figure 13). Moreover, the chromosomes bi-oriented and congressed on the ball-shaped spindle much before the bipolar orientation of the spindles (Figure 3.5, 3.6A and Figure 13). This clearly suggests that the relative distribution of chromosomes on the ball of microtubules at the transition of phase 1 and 2 depends on Sgol2, and possibly the Aurora Kinase-dependent phosphorylation of Sgol2 at T521 and MCAK localisation at the kinetochore. It further shows that Sgol2 expression promotes the invasion of chromosomes into the spindle microtubules at phase 3 and assists in aligning the chromosomes on the metaphase plate. Furthermore, this role of Sgol2 does not depend on its interactions with PP2A and Mad2, since *sgol2 Δ/Δ* oocytes expressing PP2A or Mad2 binding mutants of Sgol2 accelerated bi-orientation of homologous chromosomes (Figure 3.5). However, microinjection of PP2A and Mad2 mutant Sgol2 in *SGOL2* knockout oocytes failed to rescue the delay in

APC/C^{Cdc20} activation (Figure 3.3F) and, therefore, bi-oriented chromosomes remained aligned to the metaphase plate for a prolonged period of time, compared to *sgol2*^{Δ/Δ} oocytes expressing wild type Sgol2, which accelerated both the bi-orientation and the APC/C^{Cdc20} activation (Figure 3.5 and 3.3F). Together, these findings argue that bi-orientation is a separate function of Sgol2 in the first meiotic division.

To address whether the ability of Sgol2 to promote bi-orientation requires MCAK recruitment to kinetochores, *sgol2*^{Δ/Δ} oocytes were microinjected with Aurora phosphorylation site (T521 and T600) mutated Sgol2. Knockout oocytes expressing T521A T600A mutant Sgol2 that failed to recruit MCAK to kinetochores (Figure 3.4A, C and 3.5) did not accelerate bi-orientation. Similar to *sgol2*^{Δ/Δ} oocytes, chromosomes in *SGOL2* knockout oocytes expressing T521A T600A Sgol2 mRNA distributed peripherally on the ball-shaped spindle during phases 1 and 2 (Figure 3.6A and Figure 13). Additionally, microinjection of T521A T600A Sgol2 mRNA resulted in alignment defects in the *SGOL2* knockout oocytes (Figure 3.4C, 3.5 and Figure 13), possibly due to increased MCAK binding to the arms of bivalent chromosomes (Figure 11B). These results demonstrate that Sgol2, possibly through its interaction with MCAK, can promote biorientation and congression of homologous chromosomes. Importantly, microinjection of T521A T600A mRNA in *sgol2*^{Δ/Δ} oocytes advanced polar body extrusion (Figure 3.5), further confirming that Sgol2 mediates homologous chromosome bi-orientation and SAC silencing through different interactions.

Based on the evidence presented above, I asked if the ability of Sgol2 to accelerate bi-orientation of homologs depends solely on kinetochore localisation of MCAK. To test this hypothesis I injected in vitro-transcribed CENPB-GFP-MCAK mRNA in *sgol2*^{Δ/Δ} oocytes at GV stage. However, compared to oocytes expressing increased amounts of Sgol2, *sgol2*^{Δ/Δ} oocytes injected with CENPB-GFP-MCAK did not accelerate the bi-orientation of homologous chromosomes on the meiotic spindle (data not shown). This suggests that either MCAK localisation is not sufficient for the proper bi-orientation of homologous chromosomes and possibly the sites that recruit MCAK at kinetochores also perform some

other functions or maybe MCAK might not be able to perform this function if it's targeted to the centromeres.

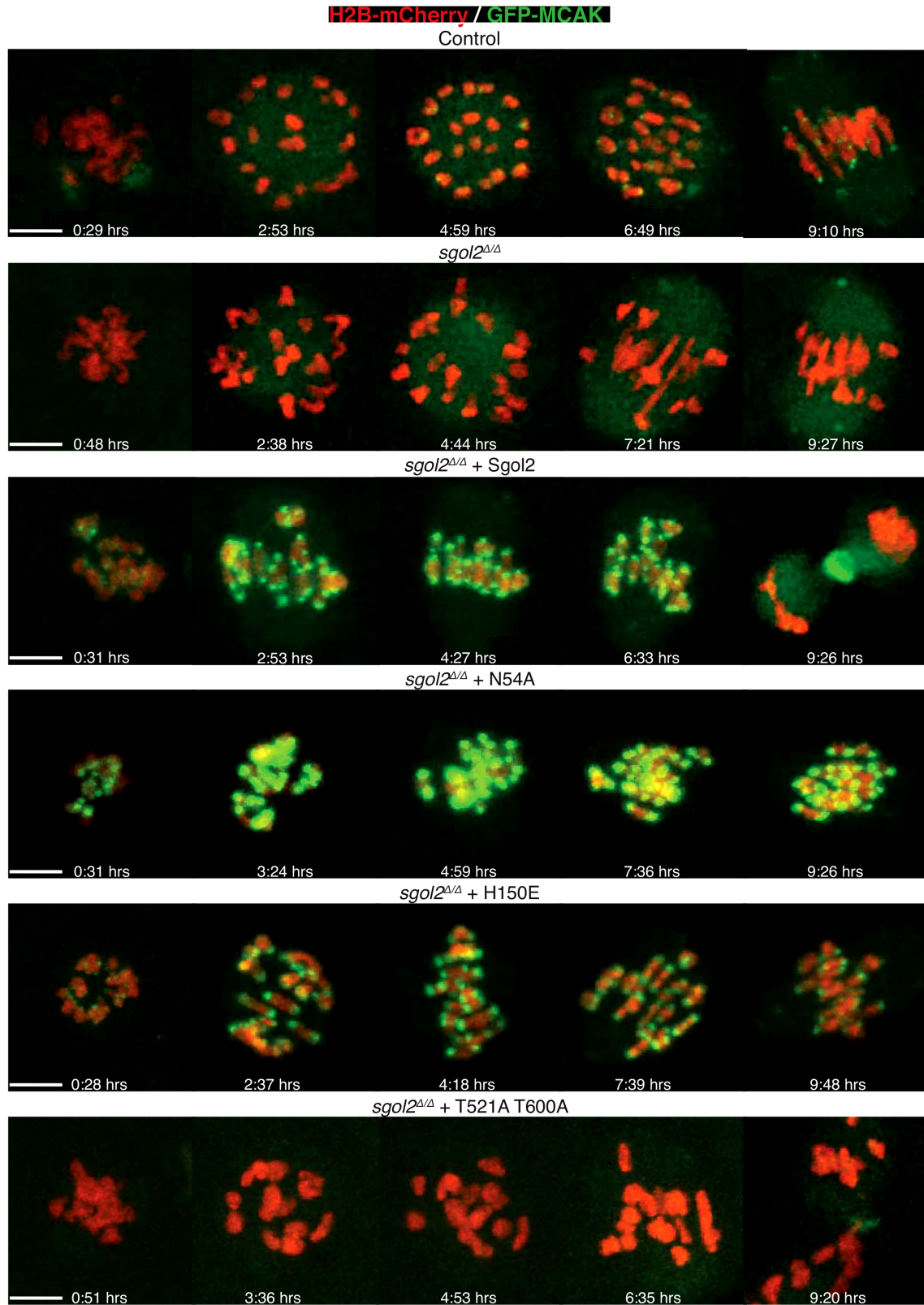


Figure 3.5: *Sgol2* assists in the biorientation of bivalent chromosomes on the metaphase plate. Time-lapse confocal microscopy images from oocytes expressing H2B-mCherry and GFP-MCAK. Representative images from littermate control, *sgol2^{Δ/Δ}*, *sgol2^{Δ/Δ}* microinjected with wild type, N54A, H150E, and T521A T600A *Sgol2* mRNA are aligned to show the dynamics of MCAK localisation and chromosome movements along the meiotic spindle. GVBD normalised time (in hrs) is indicated on each frame. Scale bars represent 5 μ m.

3.2.7 Sgol2 Promotes Microtubule-Kinetochores Interactions

Previous studies have shown that the bi-orientation of homologous chromosomes correlates with the appearance of microtubule-kinetochore bundles [251]. Results presented above indicate that Sgol2 might promote microtubule-kinetochores interactions (K fibre formation/stabilisation). Therefore, to directly visualise kinetochore-microtubule attachments, oocytes were briefly treated with calcium-containing buffer to destabilise dynamic non-kinetochore microtubules. Cells were then fixed in 1% PFA. This allowed direct investigation of microtubule-kinetochore attachments. The analysis was performed on oocytes fixed at 6hrs post-GVBD, when most kinetochores in wild type oocytes have established end-on attachments with microtubules, and chromosomes have achieved bi-orientation.

Comparison between control and *SGOL2* knockout oocytes showed two important differences. Firstly, compared to wild type, where multiple K-fibres attached to a single kinetochore, the K fibres in *SGOL2* knockout oocytes were relatively thin and sparse. Secondly, most *SGOL2* knockout oocytes at 6-7hrs post-GVBD retained lateral microtubule-kinetochore attachments and increased fractions of these oocytes had kinetochores with stretched CREST signals and microtubules entered kinetochores from the side, not in an end-on manner (Figure 3.6B). Most convincingly, knockout oocytes expressing wild type Sgol2 not only rescued the lateral attachments and the stretch on kinetochores, but the microinjection of Sgol2 mRNA resulted in increased amounts of K fibres attaching to each kinetochore. The CREST signals were compressed on the surface of chromosomes (Figure 3.6B).

Microinjection of T521A T600A mutant Sgol2 in *sgol2*^{Δ/Δ} oocytes failed to rescue the paucity of K fibres. Also, these oocytes failed to correct lateral attachments and displayed frequent side attachments with stretched CREST signals. This suggests that Sgol2, possibly by recruiting MCAK to kinetochores, corrects lateral and side attachments. These results also show that Sgol2 localisation at kinetochores promotes microtubule-kinetochore interactions. They also indicate

that Sgol2, by promoting K fibre formation/stabilisation assists in the biorientation of homologous chromosomes on the meiotic spindle. Since, Aurora B/C Kinase phosphorylates Sgol2 on T521 these results, therefore, show that Aurora B/C kinase activity at kinetochores through Sgol2 phosphorylation promotes and corrects aberrant interactions.

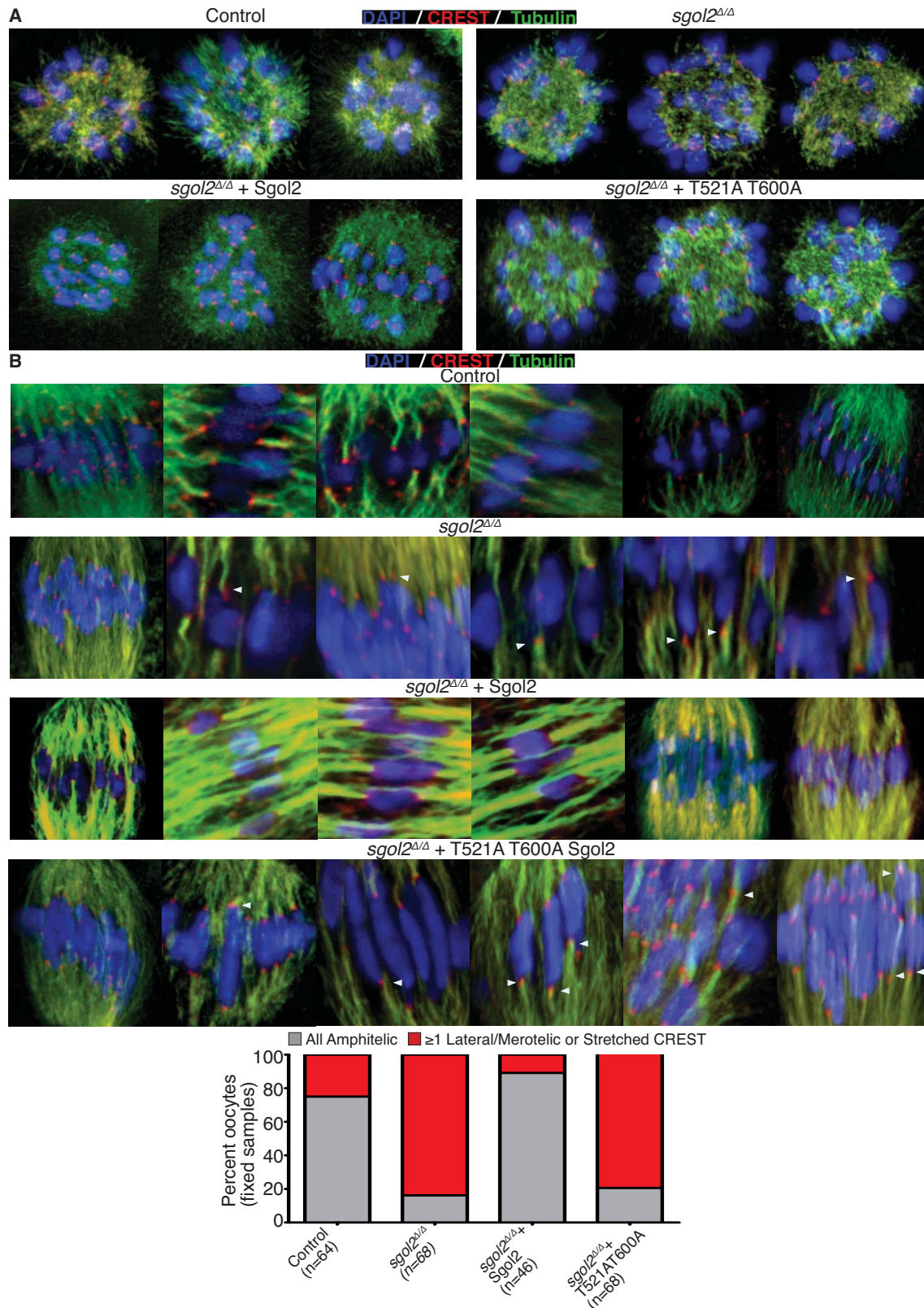


Figure 3.6: *Sgol2* promotes biorientation of homologous chromosomes by increasing microtubule-kinetochore interactions. (A) To study the relative distributions of chromosomes on the ball shaped spindle, at 2-3hrs post-GVBD, oocytes were treated with Ca^{+2} buffer for 90s and then fixed in 1% PFA. Cells were stained with anti-Tubulin (microtubules, green), CREST (kinetochores, red), and DAPI (chromosomes, blue). (B) Confocal images of oocytes fixed at 6-7hrs post-GVBD. Lateral/Merotelic and side attachments with abnormally stretched CREST signals are indicated by arrowheads. Fraction of metaphase I stage oocytes with lateral attachments and stretched CREST signals in each group were quantified.

3.2.8 Sgol2 Recruitment Inhibits Aurora B/C Kinase Activity At Kinetochores

The above findings showed that Sgol2 localisation at kinetochores promotes biorientation of homologous chromosomes by increasing K-fibre synthesis. In a mitotic cell, Aurora B Kinase activity at kinetochores destabilises microtubule-kinetochore attachments. Recently, it has been shown that by phosphorylating substrates in the KMN network, Aurora B Kinase decreases the microtubule-binding affinity of kinetochores [100]. Therefore, I asked whether Sgol2 can influence phosphorylation of KMN network substrates. Phosphospecific (Ser24p) Knl1 antibody was used to immunostain chromosome spreads prepared at 6hrs post-GVBD. Compared to the homologous chromosomes from littermate control oocytes, the phosphorylation of Knl1 at Ser24 was significantly higher in *SGOL2* knockout oocytes (Figure 3.7A). Furthermore, the phosphospecific antibody signal almost completely disappeared in knockout oocytes microinjected with Sgol2 mRNA. Crucially, the de-phosphorylation of Ser24 on Knl1 by Sgol2 was not due to PP2A recruitment to kinetochores (Figure 3.7A), since microinjection of the N54A mutant had a similar effect. However, expression of T521A T600A failed to rescue the increased phosphorylation of Ser24 on Knl1 in *sgol2*^{Δ/Δ} oocytes. Similar results were observed by using phosphospecific antibodies against Ser100 on Dsn1 (data not shown). These results show that in mouse oocytes, Sgol2, independent of PP2A interaction, is able to counteract the phosphorylation of Aurora B/C Kinase substrates at kinetochores. (Figure 3.7A).

To further investigate whether the dephosphorylation of Aurora B/C substrates at kinetochores by Sgol2 is due to its effect on Aurora B/C activity at kinetochores, I used a phosphospecific antibody that detects auto-phosphorylation of Aurora A/B/C at Thr288, Thr232, or Thr198, respectively. The immune-staining of chromosome spreads showed that Aurora kinase activity at kinetochores was higher in Sgol2 depleted oocytes and expression of wild type or PP2A mutant Sgol2 abolished the auto-phosphorylation of Aurora kinase at kinetochores (Figure 3.7B). However, the levels of auto-phosphorylation of Aurora B/C on the arms of homologous chromosomes were not altered. The T521A T600A Sgol2 mutant

failed to rescue increased auto-phosphorylation levels of Aurora B/C kinase at kinetochores (Figure 3.7B). Crucially, oocytes microinjected with wild type or PP2A binding mutant Sgol2 mRNA still retained Aurora C at kinetochores (Figure 14A and B). These results show that Sgol2 localisation at inner kinetochores inhibits the activity of Aurora B/C at kinetochores. Furthermore, since Aurora phosphorylation sites on Sgol2 are required for the inhibition of Aurora B/C kinase activity by Sgol2, this suggests that Aurora B/C, by phosphorylating Sgol2, activates its own inhibitor at kinetochores, creating a negative feedback loop.

The data presented above show that during the first meiotic division after GVBD, the concentration of Sgol2 gradually increases at kinetochores and after GVBD Aurora B/C kinase phosphorylates Sgol2 on T521, and possibly on T600 (Figure 12A and B). It suggests that once Sgol2 is phosphorylated at T521 and T600, by Aurora B/C kinase, Sgol2 in turn inhibits the activity of Aurora B/C kinase at kinetochores. Therefore, to check if increasing concentration of Sgol2 inversely correlates with the Aurora B/C activity at kinetochore, chromosome spreads were prepared at 3 and 6 hrs post-GVBD from wild type control oocytes and were stained for Ser100 on Dsn1. Indeed, in wild type oocytes, Aurora B/C activity at kinetochores decreases as the concentration of Sgol2 builds up at inner kinetochores (Figure 14C).

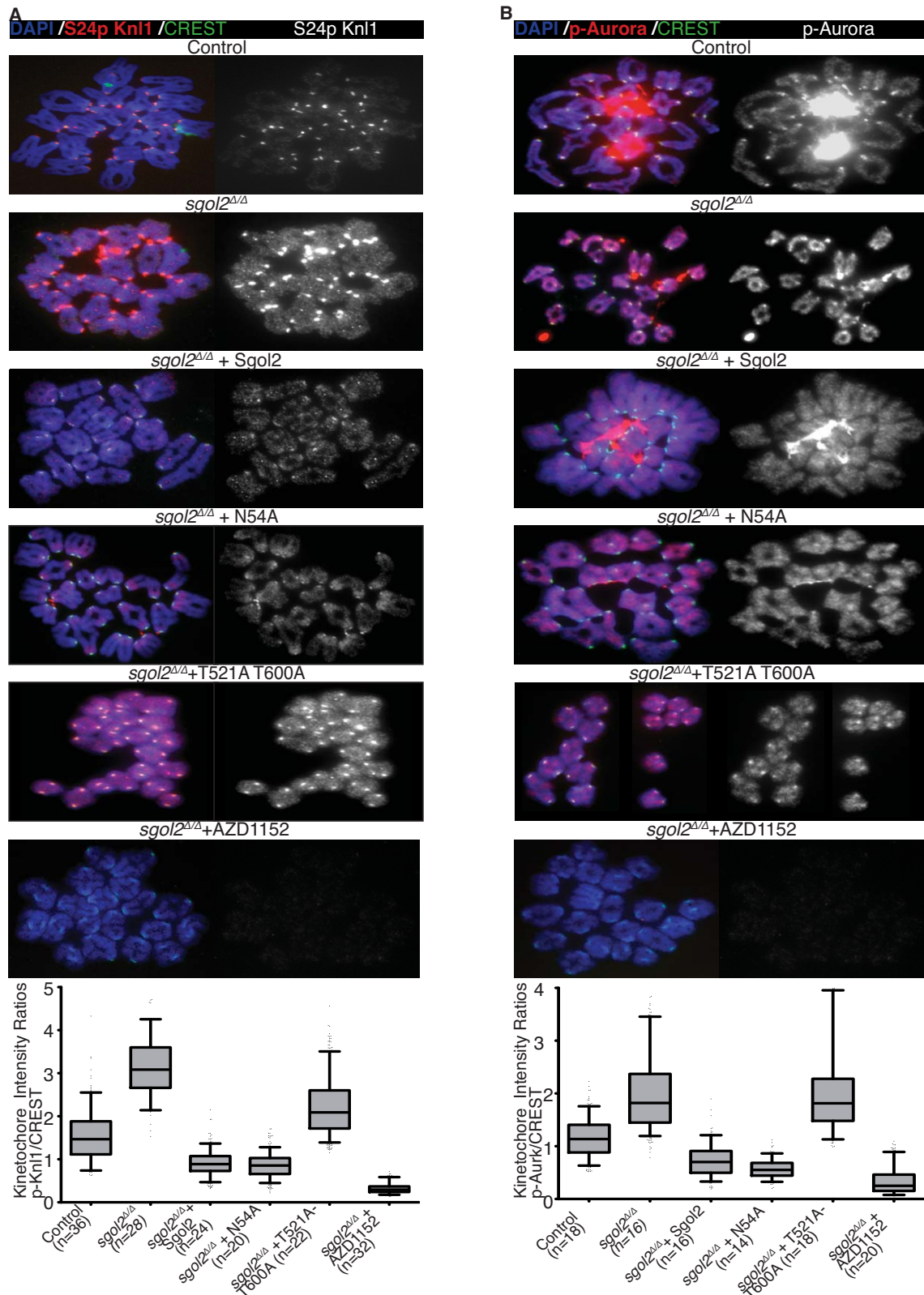


Figure 3.7: *Sgol2* inhibits Aurora B/C Kinase activity at kinetochores. (A) Chromosome spreads were prepared from indicated groups at 4hrs post GVBD. Slides were stained with DAPI (blue), anti-pSer24-Knl1 (red) and CREST (green). Kinetochores intensities of anti-pSer24-Knl1 and CREST signals were quantified and the intensity ratios of anti-pSer24-Knl1 and CREST signals are shown. Upper and lower bars indicate 95th and 5th percentiles, respectively. (B) Chromosome spreads prepared at 4hrs post GVBD were stained with DAPI (blue), anti-pAurora A/B/C (red) and CREST (green). Kinetochores fluorescence intensity ratios of anti-pAurora A/B/C and CREST are displayed.

3.3 DISCUSSION

Balanced segregation of the genome during the cell cycle relies on two key processes. Firstly, sister chromatid cohesion holds duplicated sister chromatids together until their segregation at anaphase and secondly, through bipolar microtubule-kinetochore attachments, sister chromatids establish connections with the segregation machinery. Both of these processes are monitored by surveillance mechanisms in the cell, formed by error correction machinery that can destabilise incorrect attachment and the SAC, which inhibits APC/C^{Cdc20} until all chromosomes have established bipolar microtubule-kinetochore attachments. This work shows that in mouse oocytes during the first meiotic division, Sgol2 can protect centromeric cohesion and, by promoting microtubule-kinetochore attachments, links the chromosomes to the segregation machinery. Furthermore, Sgol2 at kinetochores can signal to inactivate the Aurora B/C kinase, an essential component of the error-correction machinery and silences the SAC to activate APC/C^{Cdc20} so that when oocytes have protected centromeric cohesion and achieved bi-orientation, meiotic cells can segregate homologous chromosomes. This work, therefore, shows that during meiosis, Sgol2 can coordinate multiple cell cycle pathways that ensure faithful segregation of the genome.

3.3.1 Centromeric Cohesion Protection

Due to its stepwise removal, sister chromatid cohesion established during pre-meiotic S phase is capable of mediating two rounds of chromosome segregation without an intervening round of DNA replication. Separase activation at meiosis I resolve bivalent chromosomes and create dyads, which are held together at centromeres by cohesin until their resolution into single chromatids by a second burst of separase activity in meiosis II. The finding that in mammals, protection of centromeric cohesin from separase at the first meiotic division requires Sgol2 protein [222] raised the question whether this protein, as its yeast counterpart, also requires PP2A interaction to confer protection to centromeric cohesin. This work shows that like Sgo1 in budding and fission yeast [86, 85, 200], Sgol2 in mouse interacts with PP2A, recruiting it to kinetochores at the first meiotic division. This

recruitment of PP2A to kinetochores is essential for centromeric cohesion protection. Thus, the pathway that is responsible for the step-wise removal of cohesin and ensures two rounds of division in meiosis is conserved from yeast to mammals.

3.3.2 Timing of Anaphase and Sgol2

Previous studies investigating mitotic cells have shown that unattached kinetochores facilitate the formation of the Mad1-Mad2 complex and this complex at kinetochores promotes Mad2 association with APC/C^{Cdc20} [121, 120]. Two of the checkpoint proteins, Mad2 and BubR1 (Mad3), interact directly with APC/C^{Cdc20} and form the Mitotic Checkpoint Complex (MCC) complex. The MCC inhibits the ubiquitylation activity of APC/C^{Cdc20} by interfering with the recognition of certain substrates [112, 113, 114, 115, 116, 117, 118, 126]. Mad2 can exist in two conformational states, either as a closed form (C-Mad2) or in an open conformation (O-Mad2). In its closed conformation Mad2 (C-Mad2), can bind to either Mad1 or Cdc20, and in its open conformation (O-Mad2), Mad2 cannot interact with either of its binding partners [119, 120]. Mad2's interaction with Mad1 and Cdc20 depends on a conserved sequence called Mad2 Interaction Motif in Mad1 and Cdc20. Recently, it has been discovered that Sgol2 has a MIM sequence near its N-terminal coiled-coil domain. Furthermore, in mitotic cells, Sgol2 can associate with the active form of Mad2 (C-Mad2) and with p31comet, a protein implicated in silencing of the checkpoint during the mitotic cell cycle [249]. However, Sgol2-depleted mitotic cells and the knockout mice did not display any defects in mitotic cell cycle progression [222, 249].

In mouse oocytes, APC/C^{Cdc20} activity is restrained by the spindle assembly checkpoint. Knockout or knockdown of checkpoint components, including Mad2, accelerates APC/C^{Cdc20} activation, chiasmata resolution, and polar body extrusion [221, 238, 223]. Similar to mitotic cells, in mouse oocytes, checkpoint proteins can associate with kinetochores [238]. Results presented above show that *SGOL2* knockout oocytes are either arrested or delayed by the SAC. Furthermore, increased expression of Sgol2 accelerates APC/C^{Cdc20} activation suggesting that

in mouse oocytes Sgol2 is involved in the SAC silencing. However, it should be emphasised that *SGOL2* knockout oocytes microinjected with Sgol2 mRNA are not defective in SAC, since these oocytes can still mount a SAC response to nocodazole (data not shown). However, early activation of APC/C^{Cdc20} in oocytes expressing increased amounts of Sgol2 does indicate that Sgol2 can silence the SAC, which is a physiological process, but it does not make it ineffective.

The results presented above show that Sgol2 requires the MIM sequence to silence the SAC. Since, Sgol2 binds to Mad2 through the MIM sequence, it can be suggested that increased expression of Sgol2 in mouse oocytes can sequester C-Mad2, and thereby it can accelerate APC/C^{Cdc20} activation. However, this argument cannot explain why oocytes expressing the Sgol2 mutant defective in PP2A binding with a wild type MIM sequence cannot rescue the SAC induced metaphase I delay/arrest.

Importantly, the interactions of PP2A and Mad2 with Sgol2 are independent. Microinjection of Mad2 binding mutant of Sgol2 into an *SGOL2* knockout oocytes recruits PP2A to kinetochores and protects centromeric cohesion. Likewise, it has previously been reported that an Sgol2 mutant that disrupts its binding to PP2A can interact *in vivo* with Mad2 [249]. Therefore, due to the proximity of the coiled-coil domain and the MIM sequence in Sgol2, and the observation that both the PP2A and Mad2 binding site on Sgol2 are required to silence SAC, it is tempting to speculate that by its phosphatase activity, PP2A can assist in the disassembly of the MCC complex once the checkpoint has been satisfied. However, previous research using Hela cells has shown that the phosphomimetic mutant of Mad2 is checkpoint deficient, suggesting, on the contrary, that the dephosphorylated form of Mad2 might be required to maintain the spindle assembly checkpoint [253].

Intriguingly, microinjection of the T521A T600A mutant into *SGOL2* knockout cell advanced polar body extrusion though these oocytes had high levels Aurora B/C kinase activity at kinetochores. Furthermore, expression of the PP2A mutant in *SGOL2* knockout cells almost completely dephosphorylated Aurora B/C

kinase substrates at kinetochore and accelerated the bi-orientation of homologous chromosomes. However, it was still not able to rescue the delay in APC/C^{Cdc20} activation. In the context of mouse oocytes, these findings could offer an explanation as to how mutants such as *Mlh1*^{-/-} with frequent aberrant attachments and possibly high levels of phosphorylation on Aurora B/C phosphorylation sites can still activate APC/C^{Cdc20} and extrude the first polar body [254]. One explanation could be that in mouse oocytes, the two processes, i.e. error correction machinery and spindle assembly checkpoint that ensure proper segregation, might not be linked as strongly as in the mitotic cell. This research shows that in mouse oocytes, Sgol2 can link the Aurora B/C activity at kinetochores with the SAC silencing. However, the precise details of the molecular network linking Sgol2 to the SAC and the error correction machinery require further investigation.

3.3.3 K Fibre Synthesis and Sgol2

In fully grown *in vitro* maturing mouse oocytes, up to 80 MTOCs originate in the cytoplasm. After GVBD, these scattered MTOCs cluster together and radiate microtubules, forming the apolar microtubule ball. Following DNA condensation, bivalent chromosomes distribute as individual objects on the surface of the microtubule ball. Subsequently, the ball of microtubules forms a bipolar spindle over several hours and at 3-4hrs post-GVBD, it forms a barrel-shaped spindle with poorly focused poles. The dynamical changes in spindle architecture are accompanied by changes in chromosomal orientation and position on the spindle. As oocytes initiate bipolarisation of the spindle, chromosomes start to invade the spindle from their peripheral localisation. Kinetochores correct lateral attachments and the corresponding kinetochores establish stable end-on connections with microtubules emanating from opposite poles. Finally, after several failed attempts, bivalent chromosomes align on the metaphase plate of the barrel-shaped spindle [252, 251].

The assembly of an acentrosomal meiotic spindle depends on the combined activities of RanGTP and CPC-dependent pathways. Coordination between these

pathways is thought to organise the meiotic spindle and arrange homologous chromosomes on the spindle [255]. In mouse oocytes, a fluorescence resonance energy transfer (FRET)-based biosensor showed that RanGTP activity centres on chromosomes throughout the first meiotic division [256]. The inhibition of RanGTP activity during the first meiotic division reduces microtubule density around chromosomes and delays spindle organisation, but it does not prevent the assembly of the acentrosomal spindle [256]. However, Aurora kinase inhibition significantly perturbs assembly of a bipolar spindle [257, 258, 259, 260]. Possibly, three Aurora Kinases are expressed in mouse oocytes, Aurora A, B, and C, with some overlapping functions [261, 260, 258]. However, the precise role and the temporal dynamics of RanGTP and Aurora Kinase-dependent CPC pathways in organising the acentrosomal spindle in mouse oocytes remains unclear.

This study highlights an important role played by Sgol2 at kinetochores in assembling K fibres and in promoting bi-orientation and congression of chromosomes. I show that in wild type cell Sgol2 localises to kinetochores, and is phosphorylated by Aurora B/C kinase. As chromosomes form the pro-metaphase belt Sgol2 concentration at kinetochores increases and it gradually builds up as chromosomes enter the interior of the spindle. Also, I found that Sgol2 promotes K fibres formation and accelerates bi-orientation and congression of homologous chromosomes. Moreover, when chromosomes align on the metaphase I spindle it reduces inter-kinetochore distances. Consistent with this, *SGOL2* knockout oocytes have fewer K fibres, chromosomes are positioned more peripherally on the ball of microtubules and as they enter the ball, bivalents are stretched further resulting in increased inter-kinetochore distances and chiasmata terminalisation. Importantly, I have been able to decouple these functions of Sgol2 from its interaction with PP2A and Mad2 and have shown that these functions depend on Aurora phosphorylation of Sgol2. Also, I have shown that this phosphorylation of Sgol2 by Aurora kinase is required for MCAK recruitment and for Aurora B/C inhibition by Sgol2. Therefore, it possible that Sgol2, by recruiting MCAK to kinetochores and by inhibiting Aurora B/C kinase activity, promotes microtubule-kinetochore interactions.

It is possible that, by interacting with MCAK, Sgol2 can inhibit Aurora Kinase activity at kinetochores, which probably stops the destabilising activity of Aurora B/C Kinase at kinetochores and thereby promotes K fibre stabilisation. Hence, the increased Aurora B/C activity at kinetochores in *SGOL2* could explain the paucity of K fibres in these oocytes. Based on multiple lines of evidence presented in this chapter, it can be concluded that in wild type oocytes after GVBD, during phase 1, Aurora B/C phosphorylates Sgol2, which recruits MCAK to kinetochores. This recruitment, either by directly or indirectly affecting kinetochore microtubule interactions, opposes the kinase activity of Aurora B/C at kinetochores. This results in increased stabilisation of K fibres, which is required for chromosomes to enter the meiotic spindle. Progressively increasing concentrations of Sgol2 and MCAK, possibly by inhibiting Aurora B/C activity at kinetochores, promote bi-orientation and congression of bivalent chromosomes on the barrel-shaped meiotic spindle. MCAK recruitment possibly corrects lateral and side microtubule-kinetochore interactions and maintains inter-kinetochore distances, possibly through its motor activity, as previously reported in mitotic cells [262].

Recent work studying *Xenopus* egg extracts has shown that xSgo2, a newly discovered homolog of shugoshin in *Xenopus*, can promote the activity of Aurora Kinase, which generates a favourable environment for microtubule polymerisation [263]. Although both mouse Sgol2 and xSgo2 can promote microtubule-kinetochore interactions, they might have opposite effects on Aurora Kinase activity.

The bi-orientation and the dephosphorylation of Aurora targets did not require PP2A recruitment to kinetochores in mouse oocytes. However, a recent study on Hela cells using RNAi showed that centromeric PP2A can silence phosphorylation of Aurora B substrates at kinetochore, and interestingly this study also used pSer24 Knl1 as an Aurora B substrate at kinetochores [264]. Discrepancies between the results could simply be due to different cell types used in these studies. However, knocking down PP2A might displace Sgol2 and therefore MCAK from kinetochores [265]. This might also explain why in this study PP2A-depletion in

Hela cells did not produce centromeric cohesion defects [264].

3.4 EXPERIMENTAL PROCEDURES

3.4.1 Generation of Mouse Strains

Generation of the *SGOL2* knockout mice has been described [222]. To detect Rec8, females from a transgenic line expressing Rec8 from a bacterial artificial chromosome (BAC), with nine tandem copies of the human c-myc epitope at its C terminus, were crossed to *sgol2*^{Δ/+} males. Litters with genotypes *sgol2*^{Δ/+}(*Tg*)*Rec8-Myc+* were crossed back to *sgol2*^{Δ/+} mice to generate *sgol2*^{+/+}(*Tg*)*Rec8-Myc+* (controls) and *sgol2*^{Δ/Δ}(*Tg*)*Rec8-Myc+* female.

3.4.2 Isolation and Culture of Oocytes

Prophase-arrested oocytes were harvested from ovaries excised from 8-12 week old female mice. Antral follicles were isolated using sterile insulin needles in M2 medium (Sigma Aldrich) supplemented with 200μM IBMX (Sigma Aldrich). For intracellular microinjections, oocytes were placed in droplets of M2 medium with IBMX. For time-lapse confocal imaging and *in vitro* culture prior to preparation of chromosome spreads, oocytes were transferred into IBMX-free M16 medium and cultured at 37°C and 5% CO₂.

3.4.3 Preparation of mRNAs

Capped mRNA constructs with a poly-A tail were transcribed using T3 or T7 Ultra mMESSAGE kit (Ambion) from plasmid cDNA encoding H2B-mCherry, wild type Sgol2 (Origene), and various mutants of Sgol2, GFP-Sgol2, Securin-eGFP, GFP-MCAK, CENPB-GFP, CENPB-GFP-MCAK, and GFP-EB3.

3.4.4 Microinjection of mRNA

Fully-grown oocytes were injected with 5-10 pL mRNA at a final concentration of 0.1mg/mL in RNase-free water (Ambion) using a Pneumatic PicoPump (World Precision Instruments) [187, 238]. To allow for protein expression, following microinjection of mRNA, GV oocytes were cultured for 1-2hrs in M16 medium supplemented with IBMX. Oocytes were then washed in inhibitor-free M16 medium and thereafter cultured at 37°C and 5%CO₂.

3.4.5 Live Cell Confocal Microscopy

During time-lapse experiments, oocytes were cultured in a PeCon environmental microscope incubator at 37C 5% CO₂. A Zeiss LSM510 META confocal microscope equipped with PC-Apochromat 63x/1.2 NA water immersion and PC-Apochromat 20x/0.8 NA objective lenses was used for image acquisition. For detection of EGFP and mCherry, 488-nm and 561-nm excitation wavelengths and BP 505-550 and LP 575 filters were used. During live-cell imaging, chromosomes labeled with H2B-mCherry were tracked with an EMBL-developed tracking macro [266] adapted to our microscope. Image stacks of 8 to 12 slices of 1.5-2.5 μ m were captured every 5-10min for 12-14hrs.

3.4.6 Chromosome Spreads

Chromosome spreads from mouse oocytes were prepared as previously described [239, 240]. Briefly, zona pellucida was removed by 10mg/ml Pronase (Sigma-Aldrich) at 37°C for 5-10mins. Zona-free oocytes were treated by hypotonic solution (50% FCS in deionized water) for 10 min at 37°. Oocytes were then fixed in paraformaldehyde solution (1% paraformaldehyde, 0.15% Triton-X 100, 3mM dithiothreitol, adjusted to pH 9.2 with NaOH) in 15-well multichamber glass slide (MP Biomedicals) overnight at room temperature in a humidified chamber. After drying, slides were washed twice in 0.4% Photoflo/H₂O (Kodak) and PBS for 5mins, at room temperature. Slides were then processed for immunostaining. In this study, I used CREST (1:500; Davis Lab), rabbit anti-Sgol2 serum

(1:50; gift from J.L. Barbero [241]), anti-phospho T521 Sgol2 (1:500, gift from Yoshi Watanabe [250]), anti-MCAK (1:500, gift from Duane A. Compton [267]), anti-phospho Ser 24 Knl1 (1:1000, gift from Iain Cheeseman [100]), anti-phospho Ser 100 Dsn1 (1:500, gift from Iain Cheeseman [100]) Phosphospecific antibody detecting Thr288, Thr232 or Thr198 on Aurora A/B/C (1:50, Cell Signaling Technology), anti-Aurora C (1:50, Bethly Laboratories), mouse anti-PP2A-C (1:200, BD Biosciences), anti-cMyc (1:500, Millipore).

3.4.7 Immunofluorescence

For analysis of K fibres, oocytes processed as previously described [251]. Briefly, after either 2 or 6hrs post-GVBD, oocytes were treated for 90s with Ca^{+2} buffer 100 mM PIPES pH 7.0, 1 mM MgCl_2 , 0.1 mM CaCl_2 , 0.1% Triton X-100 at 37°C and then fixed in 1% formaldehyde prepared in Ca^{+2} buffer for 30min at room temperature. Following fixation, oocytes were washed and extracted overnight in PBT (PBS supplemented with 0.1% TritonX-100) at 4°C. Cells were then blocked in 3% BSA with PBT (blocking solution) for 1hr at room temperature. For immuno-labeling oocytes were incubated overnight at 4°C with β -tubulin (1:500, abcam) and CREST (1:100, Davis Lab) antibodies, prepared in blocking solution. After four washes in PBT, oocytes were incubated for 1hr at room temp with Alexa 488 and 568 conjugated secondary antibodies (1:500, Invitrogen) prepared in blocking solution. Oocytes were briefly stained with Hoechst 33342 (20mg/ml) before confocal imaging.

3.4.8 Protein Expression and Purification

Recombinant MBP-Sgol2cc-His and MBP-Sgol2cc-Flag were purified from 1L of *Escherichia coli* BL21(DE3) RIPL (Stratagene, La Jolla, CA, USA) carrying plasmids pMAL-c2x (Ampicillin resistance) for the Flag-tagged subunit or pET28b (Kanamycin resistance) for the His-tagged subunit, grown at 37°C in 2xTY media supplemented with 25 $\mu\text{g}/\text{ml}$ chloramphenicol and 100 $\mu\text{g}/\text{ml}$ ampicillin for pMAL-c2x or 50 $\mu\text{g}/\text{ml}$ kanamycin for pET28b. At $\text{OD}_{600} = 0.4$, MBP-Sgol2cc-

HIS or -Flag synthesis was induced by the addition of 0.1mM IPTG, incubated at 30°C. After 4hrs, the cells were harvested by centrifugation, frozen in dry ice, and stored at -80°C. The cell paste was re-suspended in 40ml of lysis buffer (50mM sodium phosphate pH 7.0, 500mM NaCl, 10% glycerol, 0.02% Triton X-100) containing Roche complete EDTA free protease inhibitor, PMSF (1mM) and 5 mM imidazole for the HIS tagged subunit. Each aliquot was sonicated four times for 30s on ice. Insoluble material was removed by centrifugation at 25000rpm for 1hr at 4°C in a Beckman Coulter Avanti-J26XP JLA8.1000 rotor. The supernatant was incubated for 2hrs at 4°C on a 5ml Talon column (BD Biosciences, Palo Alto, CA, USA) or ANTI-FLAG[®] M2 Affinity Gel (Sigma A2220). The M2 Flag beads were washed four times with 50ml of lysis buffer and the Talon beads were washed two times with 50ml of lysis buffer containing 20mM imidazole and two times with 50ml of lysis buffer containing 45 mM imidazole. MBP-Sgol2cc-Flag was eluted with 4 times 1ml of lysis buffer containing 3xFLAG peptide (150ng/ μ l). MBP-Sgol2cc-HIS were eluted with 10ml of lysis buffer containing 1M of imidazole. The concentration of MBP-Sgol2cc-HIS and MBP-Sgol2cc-Flag was determined by nanodrop against the lysis solution.

3.4.9 PP2A-Sgol2 in-Vitro Binding Assay

For all binding assays, highly-purified PP2A subunits (purified as previously described [268]) and MBP-Sgol2cc-Flag were used. To test the binding of PP2A to mutant shugoshin, 20 μ g of GST-tagged PP2A A-subunit was incubated with excess amounts of PP2A B56- and C- subunit and MBP-tagged shugoshin for 1hr on ice. Following incubation, 40 μ g glutathione beads (GE Healthcare) was added and incubated together for 20min. Beads were then washed 4x1mL in 1xPBS, 0.1%Triton X-100, 1mM dithiothreitol, boiled in reduced SDS loading buffer, and subjected to SDS-PAGE analysis using Coomassie blue staining.

3.4.10 Co-Immunoprecipitation of Sgol2 Coiled-Coil Domains

His and Flag-tagged mSgol2cc from wild type and 3A were coexpressed in E.coli BL21(DE3)RIPL for 4hrs at 30° with 0.1mM of IPTG. Cells were spun at 25000rpm for 1hr at 4°C in a Beckman Coulter Avanti-J26XP JLA8.1000 rotor. The pellet was re-suspended into lysis buffer (NaPO₄ 50 mM pH= 7, NaCl 250 mM, Glycerol 10%, Triton 0.02%, protease inhibitor). 10mg of extract was incubated with 100μl of Talon beads for 2hrs at 4°C, the beads were wash 5 times with the lysis buffer containing 23mM of imidazole. His tagged MBP-Sgol2cc was eluted with 10ml of lysis buffer containing 1M of imidazole. IP and input was resolved on a SDS PAGE gel. The abundance of His and Flag-tagged MBP Sgol2 was analyzed by western blot using anti-Flag (1:10,000) and anti-His (1:10,000) antibodies.

Appendix

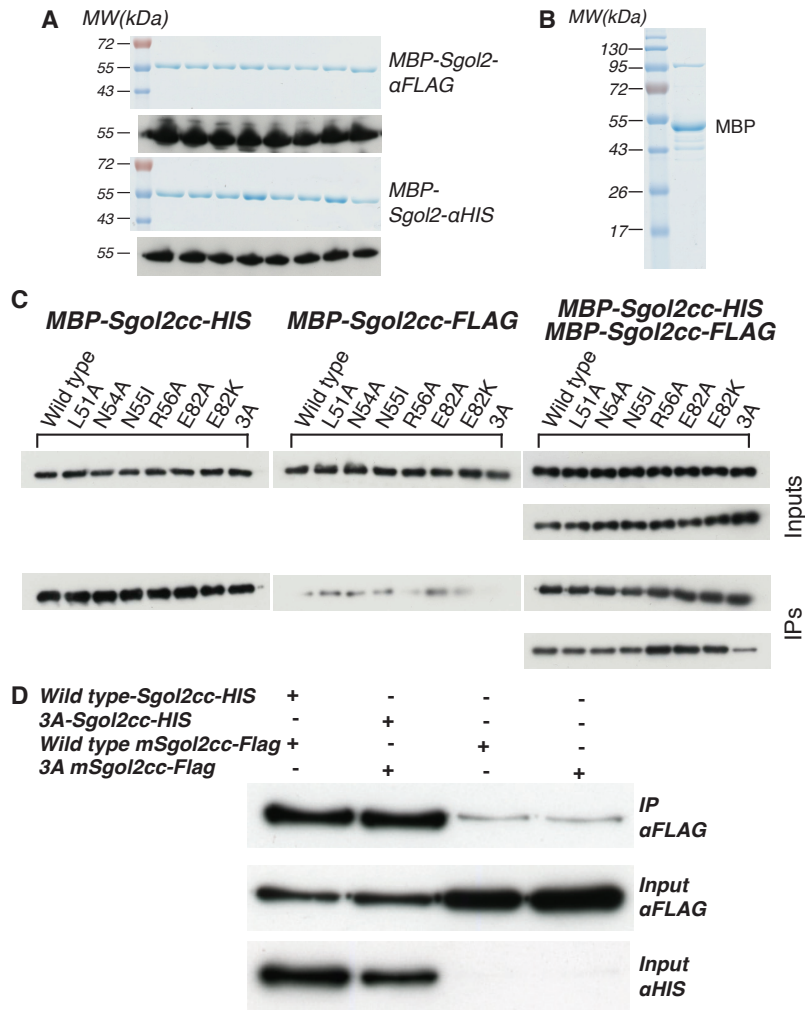


Figure 8: Size and Dimerisation of Sgol2 coiled-coil domains. (A) Coomassie Blue Staining of purified His- and Flag-tagged recombinant MBP-Sgol2cc (37-117) proteins resolved on a 12% SDS-PAGE gel. (B) Dimerisation assay of coiled-coil domains from wild type and mutant Sgol2. The Flag tagged MBP-Sgol2cc (31-117) was pulled down by the His-tagged MBP-Sgol2cc (31-117) with the same substitution. Homodimerisation was assessed by the relative abundance of each form in the IP by western blots. (C) Co-immunoprecipitation assay of MBP-Sgol2cc from wild type and 3A mutant are shown to validate the homodimerisation of coiled-coil domains. MBP-Sgol2cc-Flag and MBP-Sgol2-His were expressed either independently, or co-expressed. Upon anti-His immunoprecipitation, each fraction was separated on a 12% SDS-PAGE gel and analysed by immunoblotting with anti-Flag and anti-His antibodies. Input was resolved on a 12% SDS-PAGE gel. (Performed in collaboration with Dr. Mickael Ploquin.)

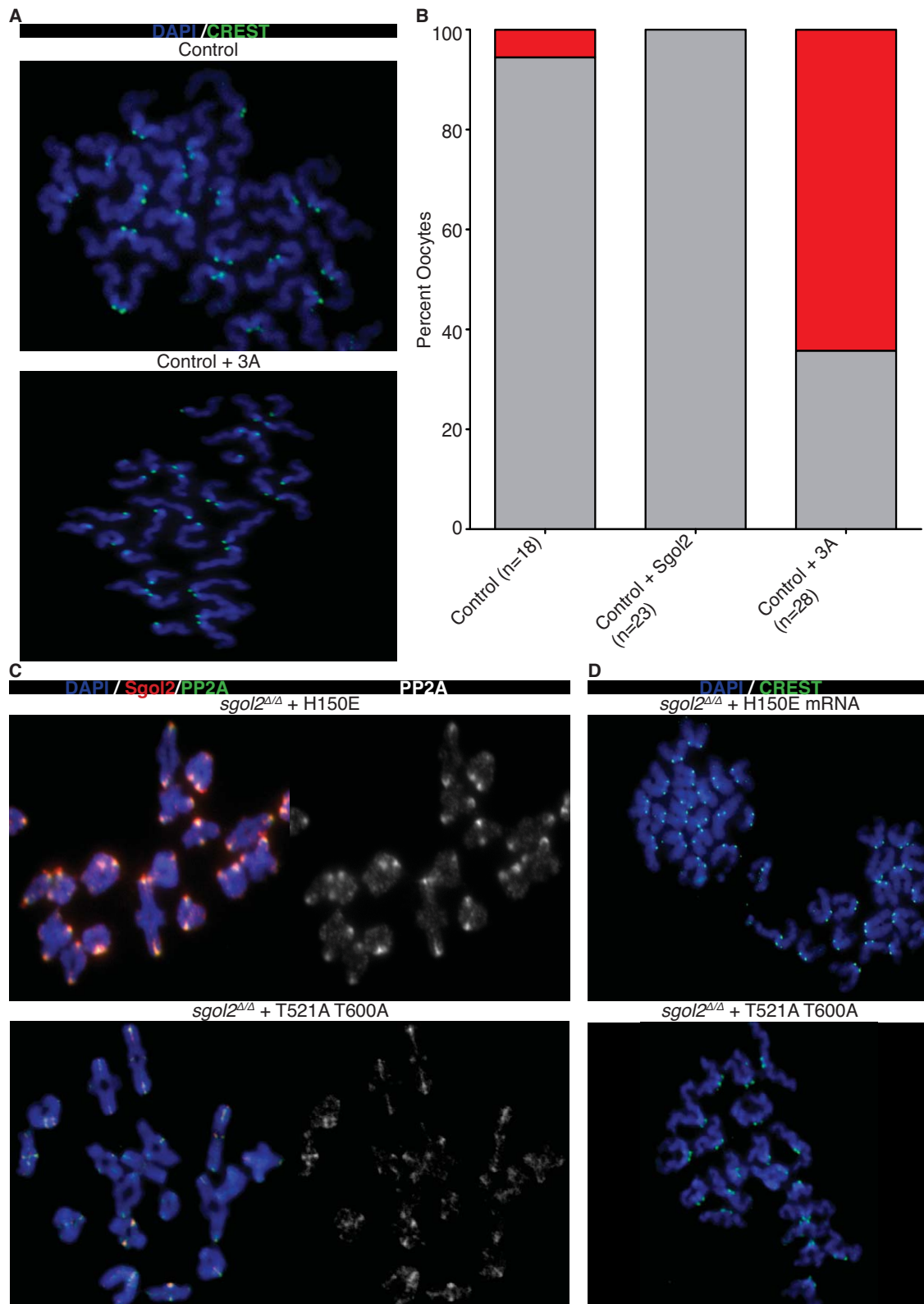


Figure 9: *Sgol2*-*PP2A* interaction is required to protect centromeric cohesion at the first meiotic division. (A) Oocytes harvested at GV stage from wild type (control) mice were microinjected with wild type or 3A mutant *Sgol2* mRNA in M2 medium supplemented with IBMX. After releasing the oocytes in M16 medium without IBMX, chromosome spreads were prepared from metaphase II-arrested oocytes and stained with DAPI and CREST (green). (B) Frequencies of dyads and single chromatids observed on metaphase II chromosome spreads from control oocytes and control oocytes that had been injected with the 3A mutant *Sgol2* mRNAs. Numbers of oocytes examined are indicated (n). (C) Metaphase I localisation of *Sgol2* (red) and *PP2A*-C (green) on chromosomes from *sgol2^{Δ/Δ}* oocytes microinjected with H150E *Sgol2* mRNA and T521A T600A *Sgol2* mRNA. (D) DAPI (blue) and CREST (green) stained chromosome spreads prepared at metaphase II from *sgol2^{Δ/Δ}* oocytes microinjected at GV stage with H150E and T521A T600A *Sgol2* mRNA.

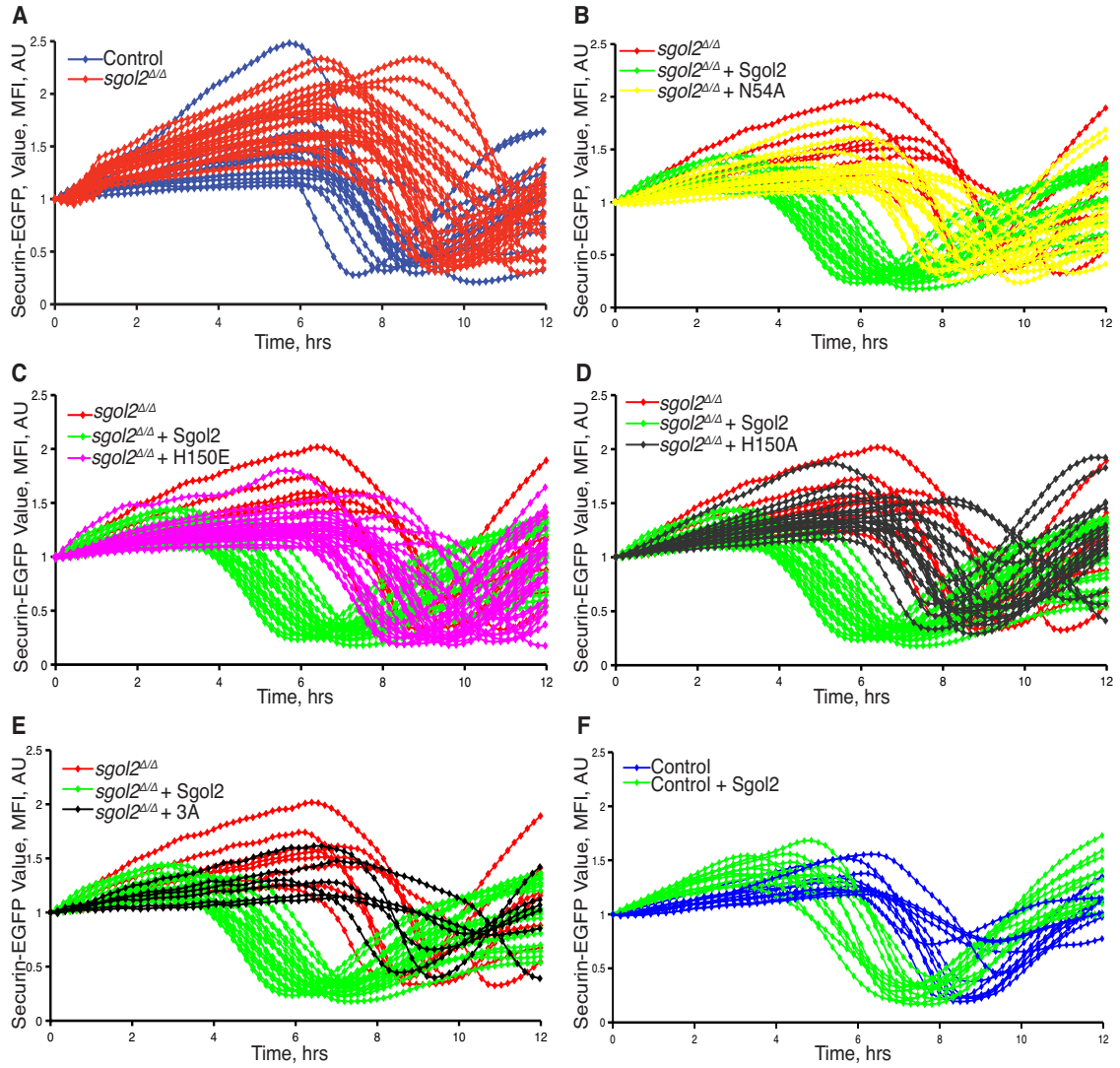


Figure 10: Fluorescence quantifications of Securin-EGFP signals in individual oocytes. (A) Time course measurements of Securin-EGFP mean fluorescence intensity in control and $sgol2^{\Delta/\Delta}$ oocytes. (B-E) Comparison of Securin-EGFP mean fluorescence intensity curves in SGOL2 knockout oocytes and $sgol2^{\Delta/\Delta}$ oocytes expressing the indicated form of Sgol2. (F) Microinjection of Sgol2 mRNA accelerates APC/C^{Cdc20} activation in wild type oocytes. Time course measurements of Securin-EGFP mean fluorescence intensity from individual oocytes.

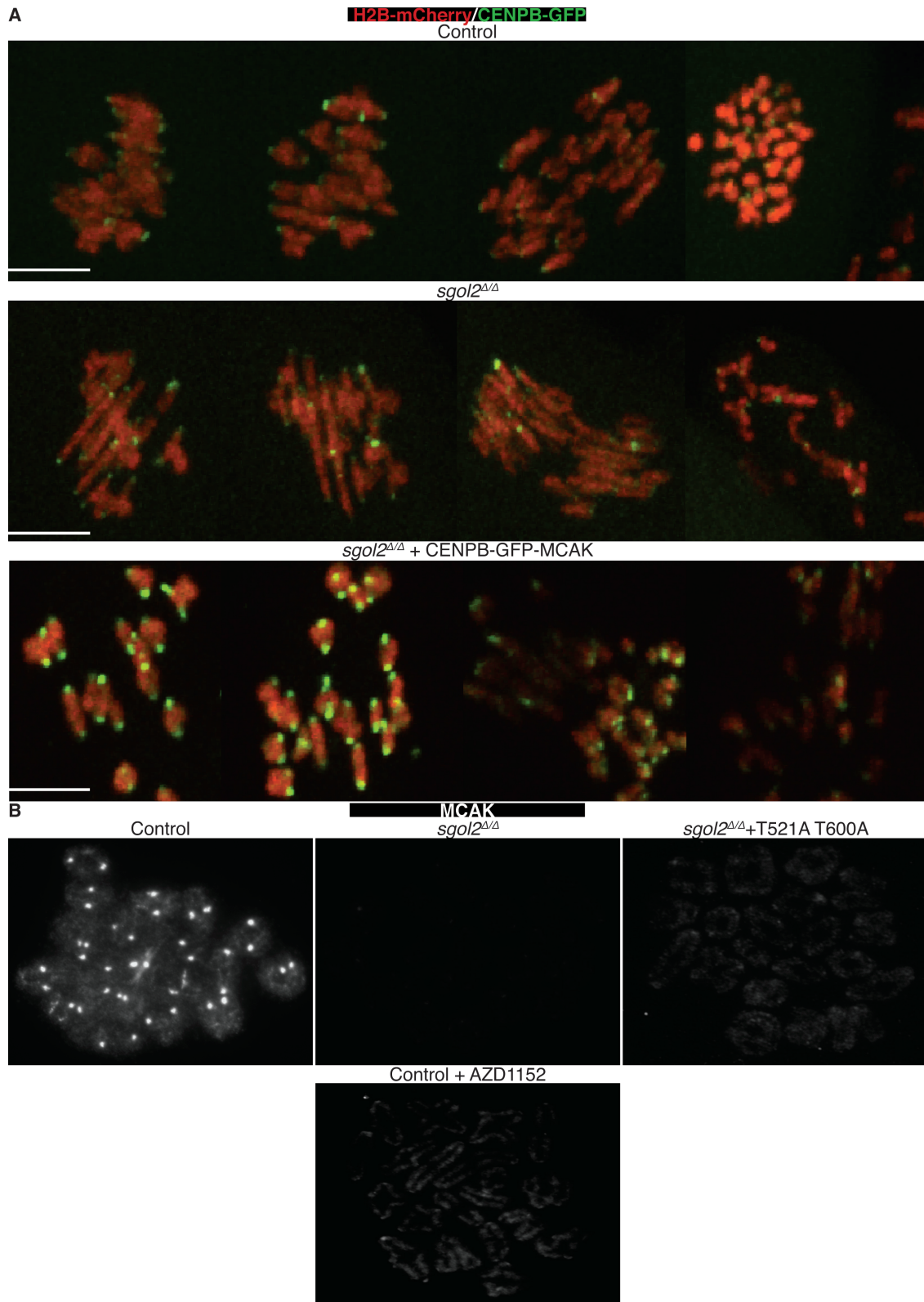


Figure 11: *Sgol2* maintains inter-kinetochores distances by localising MCAK to kinetochores. (A) Oocytes harvested at GV stage were microinjected with either CENPB-GFP or CENPB-GFP fused to MCAK. Time course images from live cell confocal microscopy from metaphase I stage are displayed. Scale bars represent 5 μ m. (B) GV oocytes were harvested in M2 medium supplemented with IBMX. Oocytes were then cultured in M16 medium for about 6-7hrs, corresponding to metaphase I stage. To inhibit Aurora B/C kinase activity oocytes were cultured in M16 medium supplemented with AZD1152 (100nM). Representative chromosome spreads from indicated groups of oocytes at metaphase I stage showing MCAK localisation are displayed.

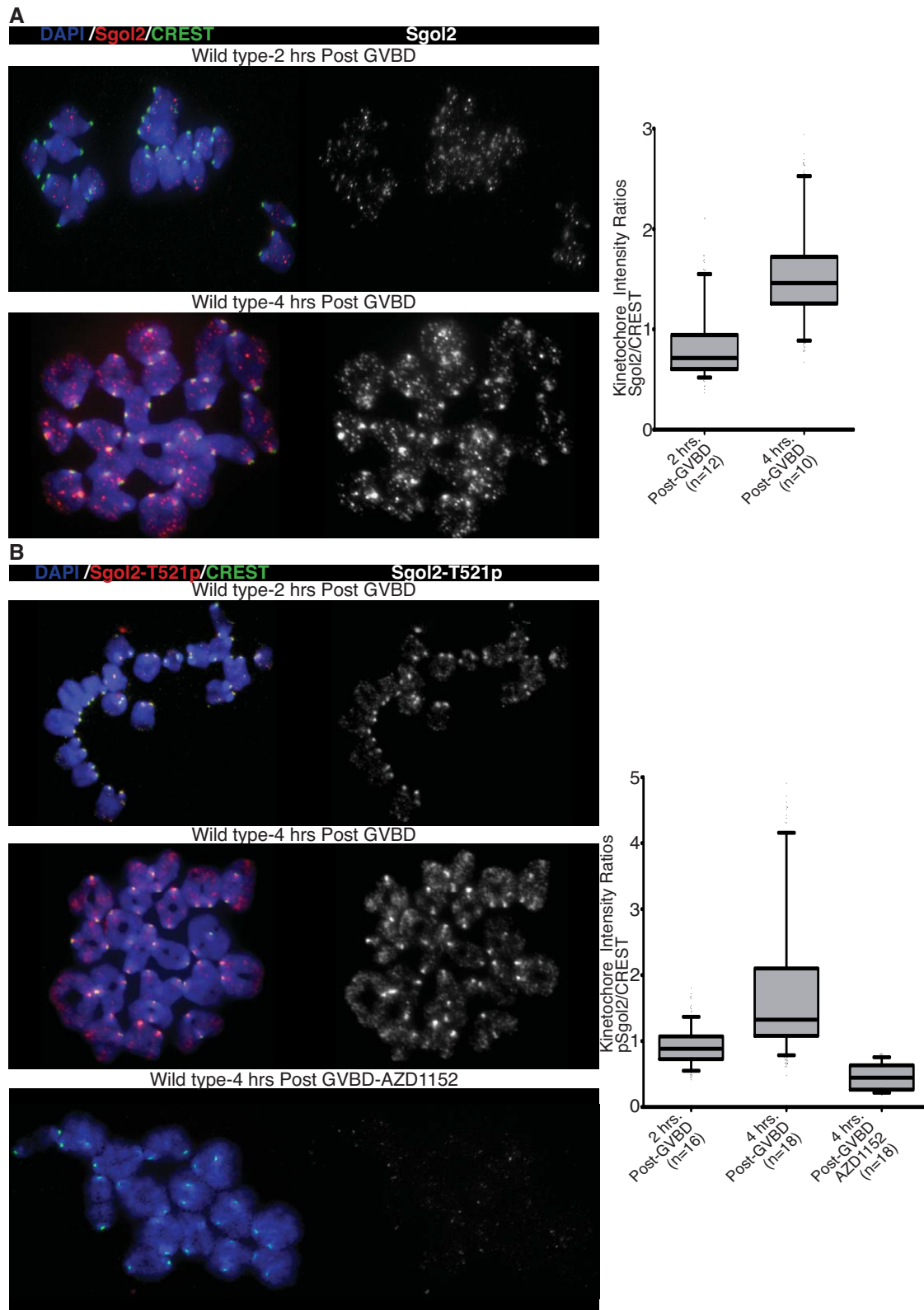


Figure 12: Aurora B/C Kinase phosphorylates Sgol2 on T521. (A) Oocytes were harvested from wild type control females at GV stage. Oocytes were matured in-vitro and chromosome spreads were prepared at 2 and 4hrs post-GVBD. Slides were stained for DNA (DAPI, blue), Sgol2 (red), and CREST (green). Fluorescence intensities of Sgol2 and CREST at kinetochores were quantified. Numbers of oocytes examined are indicated (n). (B) Chromosome spreads were prepared at 2 and 4hrs post-GVBD from wild type control oocytes. Slides were stained for DNA (DAPI, blue), Sgol2-pT521 (red), and CREST (green). Aurora B/C kinase activity was inhibited by culturing oocytes in M16 medium supplemented with the AZD1152 (100nM) inhibitor. Mean fluorescence intensity ratios of Sgol2-pT521 and CREST at kinetochores were quantified.

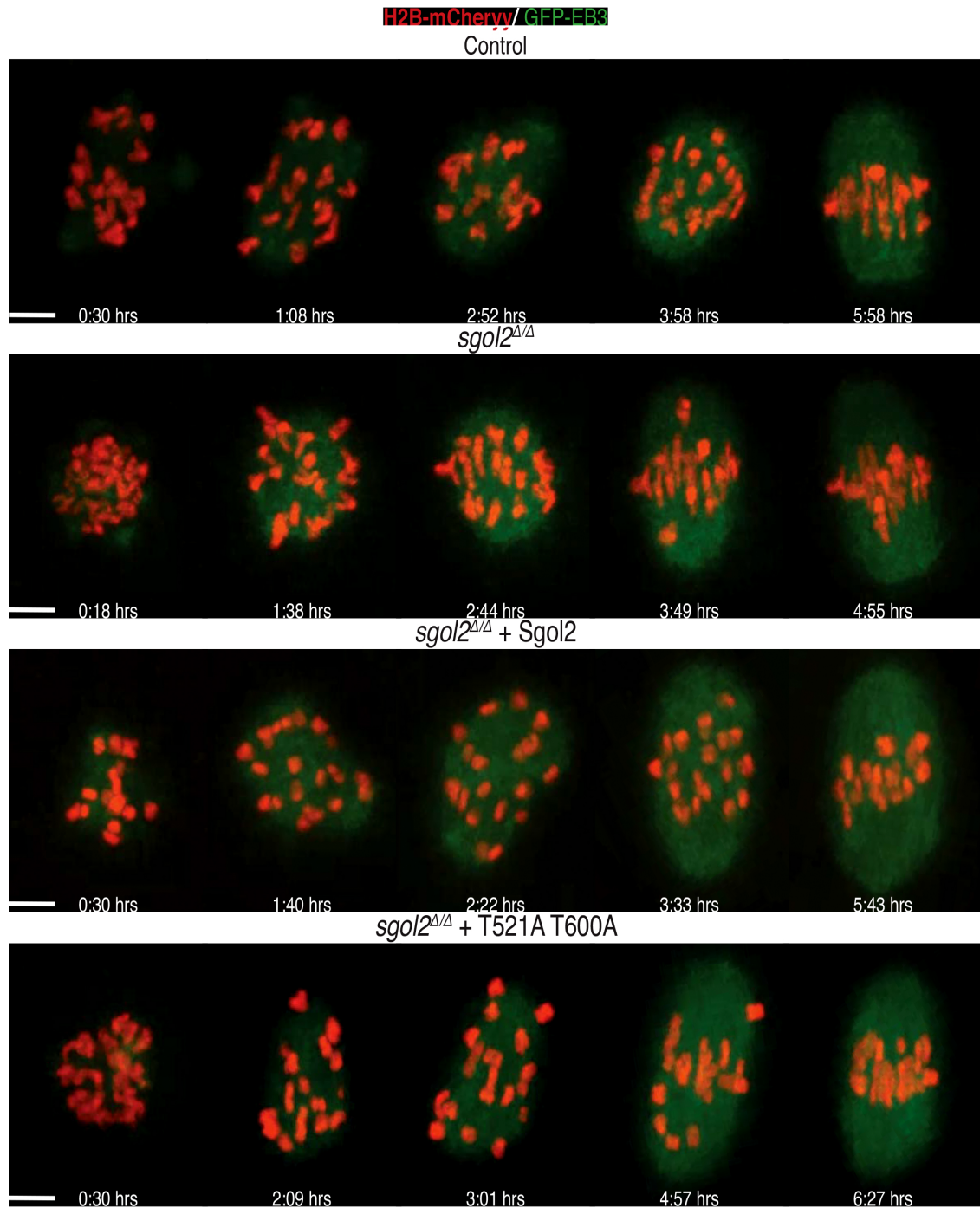


Figure 13: *Sgol2* promotes bi-orientation of homologous chromosomes on the meiotic spindle. Live cell confocal microscopy images from oocytes expressing H2B-mCherry (chromosomes, red) and EB3-GFP (microtubules, green) are shown. Images from littermate control, *sgol2^{Δ/Δ}*, *sgol2^{Δ/Δ}* microinjected with wild type and T521A T600A *Sgol2* mRNA are displayed. Time (in hrs) after GVBD is indicated on each frame. Scale bars represent 5 μ m

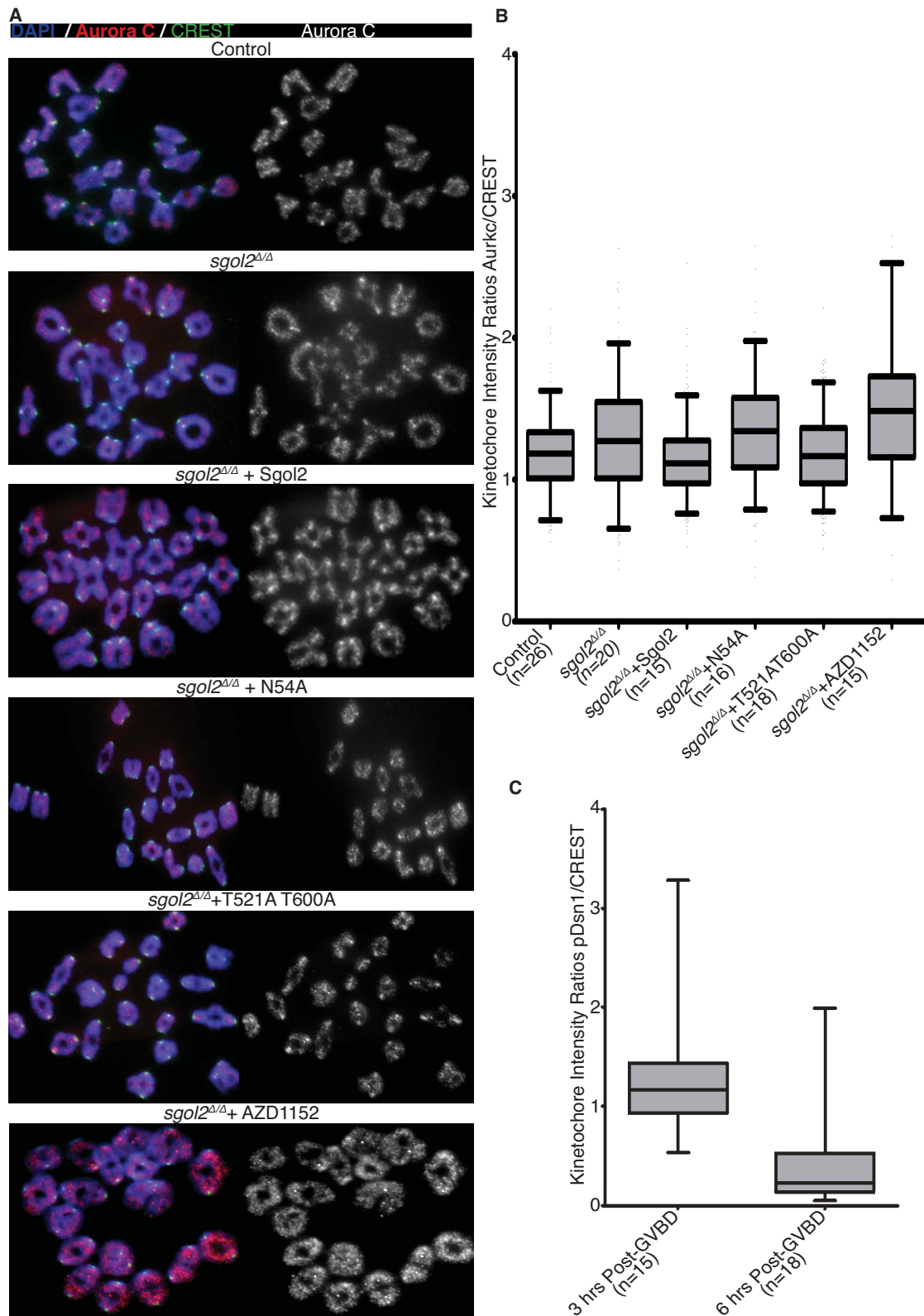


Figure 14: *Sgol2* decreases *Aurora C* activity at the kinetochores without affecting its localisation. (A) Chromosome spreads were prepared from the indicated groups at 4hrs post GVBD. Slides were stained for DNA (DAPI, blue), Aurora C (red), and CREST (green). (B) Mean fluorescence intensity ratios of Aurora-C and CREST at kinetochores. Upper and lower bars indicate 95th and 5th percentiles, respectively. (C) *Dsn1*-Ser100p and CREST kinetochores intensity ratios, quantified from chromosome spreads prepared at 3 and 6hrs post-GVBD from wild type control oocytes.

Contributions

- Experiments presented in Figure 8 were performed in collaboration with Dr. Mickael Ploquin in the laboratory of Prof Kim Nasmyth at the University of Oxford.
- Experiment presented in Figure 3.1C was performed by Seamus Morrone in the laboratory of Prof. Wenqing Xu at the University of Washington.
- Experiment presented in Figure 3.1D was performed by Dr. Bernd Mayer in the laboratory of Prof. Olaf Stemmann at the University of Bayreuth.

References

- [1] R. Hooke. *Micrographia*. Dover Publications, 2003. [1](#)
- [2] M Karamanou, E Poulakou-Rebelakou, M Tzetis, and G Androutsos. Anton van leeuwenhoek (1632-1723): father of micromorphology and discoverer of spermatozoa. *Rev Argent Microbiol*, 42(4):311–4, 2010. [1](#)
- [3] A. van Leeuwenhoek. *The Select Works of Anthony Van Leeuwenhoek: Containing His Microscopical Discoveries in Many of the Works of Nature*, volume 1. translator, 1800. [1](#)
- [4] Robert Brown. On the organs and mode of fecundation of orchidex and asclepiadea. *Miscellaneous Botanical Works, London*, 1(511-514), 1866. [1](#)
- [5] P. Mazzaello. A unifying concept: the history of cell theory. *Nature Cell Biology*, 1(1):E13–E15, 1999. [1](#)
- [6] H. Harris. *The birth of the cell*. Yale University Press, 2000. [1](#), [2](#)
- [7] L.N. Magner. *A History of the Life Sciences, Revised and Expanded*. CRC, 2002. [1](#), [2](#)
- [8] E.B. Wilson. *The cell in development and heredity*, volume 1232. Macmillan New York:, 1925. [2](#), [4](#)
- [9] W. Flemming. *Zellsubstanz, kern und zelltheilung*. Vogel, 1882. [2](#)
- [10] C. Stern and ER Sherwood. A note on the three rediscoverers of mendelism. *Folia Mendeliana*, 13:237–240, 1978. [3](#)

REFERENCES

- [11] Mark Petronczki, Maria F Siomos, and Kim Nasmyth. Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell*, 112(4):423–40, Feb 2003. [3](#), [23](#), [25](#), [26](#), [28](#), [49](#)
- [12] A. Weismann, S.E.B. Poulton, S. Schönland, and S.A.E. Shipley. *Essays upon heredity and kindred biological problems*, volume 1. Clarendon press, 1891. [3](#)
- [13] T. Boveri. *Ein geschlechtlich erzeugter Organismus ohne mütterliche Eigenschaften*. Gesellschaft für Morphologie und Physiologie zu München, 1889. [3](#)
- [14] W.S. Sutton. The chromosomes in heredity. *The Biological Bulletin*, 4(5):231–250, 1903. [4](#)
- [15] K. Nasmyth. A prize for proliferation. *Cell*, 107(6):689–702, 2001. [4](#), [6](#), [7](#)
- [16] D.O. Morgan. *The cell cycle: principles of control*. Sinauer Associates Incorporated, 2007. [4](#), [5](#), [8](#), [17](#), [18](#)
- [17] HP Rusch, W. Sachsenmaier, K. Behrens, and V. Gruter. Synchronization of mitosis by the fusion of the plasmodia of *Physarum polycephalum*. *The Journal of cell biology*, 31(1):204–209, 1966. [6](#)
- [18] P N Rao and R T Johnson. Mammalian cell fusion: studies on the regulation of dna synthesis and mitosis. *Nature*, 225(5228):159–64, Jan 1970. [6](#)
- [19] R T Johnson and P N Rao. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. *Nature*, 226(5247):717–22, May 1970. [6](#)
- [20] R T Johnson, P N Rao, and H D Hughes. Mammalian cell fusion. 3. a hela cell inducer of premature chromosome condensation active in cells from a variety of animal species. *J Cell Physiol*, 76(2):151–7, Oct 1970. [6](#)
- [21] L.H. Hartwell, J. Culotti, and B. Reid. Genetic control of the cell-division cycle in yeast, i. detection of mutants. *Proceedings of the National Academy of Sciences*, 66(2):352–359, 1970. [7](#)

REFERENCES

- [22] L.H. Hartwell et al. Genetic control of the cell division cycle in yeast. iv. genes controlling bud emergence and cytokinesis. *Experimental cell research*, 69(2):265, 1971. [7](#)
- [23] L.H. Hartwell, R.K. Mortimer, J. Culotti, and M. Culotti. Genetic control of the cell division cycle in yeast: V. genetic analysis of cdc mutants. *Genetics*, 74(2):267–286, 1973. [7](#)
- [24] J. Culotti and LH Hartwell. Genetic control of the cell division cycle in yeast. 3. seven genes controlling nuclear division. *Experimental cell research*, 67(2):389, 1971. [7](#)
- [25] P Nurse. Genetic control of cell size at cell division in yeast. *Nature*, 256(5518):547–51, Aug 1975. [7](#)
- [26] P. Nurse, P. Thuriaux, and K. Nasmyth. Genetic control of the cell division cycle in the fission yeast *schizosaccharomyces pombe*. *Molecular and General Genetics MGG*, 146(2):167–178, 1976. [7](#)
- [27] T Evans, E T Rosenthal, J Youngblom, D Distel, and T Hunt. Cyclin: a protein specified by maternal mrna in sea urchin eggs that is destroyed at each cleavage division. *Cell*, 33(2):389–96, Jun 1983. [7](#)
- [28] Damien Coudreuse and Paul Nurse. Driving the cell cycle with a minimal cdk control network. *Nature*, 468(7327):1074–9, Dec 2010. [8](#)
- [29] W Zachariae and K Nasmyth. Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev*, 13(16):2039–58, Aug 1999. [8](#), [9](#), [10](#)
- [30] A Amon, S Irniger, and K Nasmyth. Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of g1 cyclins in the next cycle. *Cell*, 77(7):1037–50, Jul 1994. [8](#)
- [31] Jan-Michael Peters. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol*, 7(9):644–56, Sep 2006. [9](#)

REFERENCES

- [32] Jonathon Pines. Cubism and the cell cycle: the many faces of the apc/c. *Nat Rev Mol Cell Biol*, 12(7):427–38, Jul 2011. [9](#)
- [33] R Visintin, S Prinz, and A Amon. Cdc20 and cdh1: a family of substrate-specific activators of apc-dependent proteolysis. *Science*, 278(5337):460–3, Oct 1997. [9](#)
- [34] J L Burton and M J Solomon. D box and ken box motifs in budding yeast hsl1p are required for apc-mediated degradation and direct binding to cdc20p and cdh1p. *Genes Dev*, 15(18):2381–95, Sep 2001. [9](#)
- [35] Claudine Kraft, Hartmut C Vodermaier, Sebastian Maurer-Stroh, Frank Eisenhaber, and Jan-Michael Peters. The wd40 propeller domain of cdh1 functions as a destruction box receptor for apc/c substrates. *Mol Cell*, 18(5):543–53, May 2005. [9](#)
- [36] M Schwab, M Neutzner, D Möcker, and W Seufert. Yeast hct1 recognizes the mitotic cyclin clb2 and other substrates of the ubiquitin ligase apc. *EMBO J*, 20(18):5165–75, Sep 2001. [9](#)
- [37] Matt Sullivan and David O Morgan. Finishing mitosis, one step at a time. *Nat Rev Mol Cell Biol*, 8(11):894–903, Nov 2007. [10](#)
- [38] R B Nicklas. The forces that move chromosomes in mitosis. *Annu Rev Biophys Biophys Chem*, 17:431–49, 1988. [10](#)
- [39] W Y Miyazaki and T L Orr-Weaver. Sister-chromatid cohesion in mitosis and meiosis. *Annu Rev Genet*, 28:167–87, 1994. [10](#)
- [40] A W Murray and J W Szostak. Chromosome segregation in mitosis and meiosis. *Annu Rev Cell Biol*, 1:289–315, 1985. [10](#)
- [41] C Holm, T Goto, J C Wang, and D Botstein. Dna topoisomerase ii is required at the time of mitosis in yeast. *Cell*, 41(2):553–63, Jun 1985. [10](#)
- [42] C E Shamu and A W Murray. Sister chromatid separation in frog egg extracts requires dna topoisomerase ii activity during anaphase. *J Cell Biol*, 117(5):921–34, Jun 1992. [10](#)

REFERENCES

- [43] C Michaelis, R Ciosk, and K Nasmyth. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*, 91(1):35–45, Oct 1997. [11](#), [15](#)
- [44] V Guacci, D Koshland, and A Strunnikov. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *mcd1* in *s. cerevisiae*. *Cell*, 91(1):47–57, Oct 1997. [11](#)
- [45] A Losada, T Yokochi, R Kobayashi, and T Hirano. Identification and characterization of *sa/scc3p* subunits in the xenopus and human cohesin complexes. *J Cell Biol*, 150(3):405–16, Aug 2000. [11](#)
- [46] I Sumara, E Vorlaufer, C Gieffers, B H Peters, and J M Peters. Characterization of vertebrate cohesin complexes and their regulation in prophase. *J Cell Biol*, 151(4):749–62, Nov 2000. [11](#), [16](#)
- [47] A. Tóth, R. Ciosk, F. Uhlmann, M. Galova, A. Schleiffer, and K. Nasmyth. Yeast cohesin complex requires a conserved protein, *eco1p* (*ctf7*), to establish cohesion between sister chromatids during dna replication. *Genes & development*, 13(3):320–333, 1999. [11](#), [15](#)
- [48] A. Losada and T. Hirano. Dynamic molecular linkers of the genome: the first decade of *smc* proteins. *Genes & development*, 19(11):1269–1287, 2005. [12](#)
- [49] C.H. Haering, J. Lowe, A. Hochwagen, and K. Nasmyth. Molecular architecture of *smc* proteins and the yeast cohesin complex. *Molecular cell*, 9(4):773–788, 2002. [12](#)
- [50] K. Nasmyth and C.H. Haering. The structure and function of *smc* and *kleisin* complexes. *Annu. Rev. Biochem.*, 74:595–648, 2005. [12](#)
- [51] Stephan Gruber, Christian H Haering, and Kim Nasmyth. Chromosomal cohesin forms a ring. *Cell*, 112(6):765–77, Mar 2003. [12](#)
- [52] Christian H Haering, Jan Löwe, Andreas Hochwagen, and Kim Nasmyth. Molecular architecture of *smc* proteins and the yeast cohesin complex. *Mol Cell*, 9(4):773–88, Apr 2002. [12](#)

REFERENCES

- [53] F Uhlmann, D Wernic, M A Poupart, E V Koonin, and K Nasmyth. Cleavage of cohesin by the cd clan protease separin triggers anaphase in yeast. *Cell*, 103(3):375–86, Oct 2000. [12](#)
- [54] R.A. Oliveira, R.S. Hamilton, A. Pauli, I. Davis, and K. Nasmyth. Cohesin cleavage and cdk inhibition trigger formation of daughter nuclei. *Nature cell biology*, 12(2):185–192, 2010. [12](#)
- [55] K. Tachibana-Konwalski, J. Godwin, L. van der Weyden, L. Champion, N.R. Kudo, D.J. Adams, and K. Nasmyth. Rec8-containing cohesin maintains bivalents without turnover during the growing phase of mouse oocytes. *Genes & development*, 24(22):2505–2516, 2010. [12](#), [28](#)
- [56] F Uhlmann, F Lottspeich, and K Nasmyth. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit scc1. *Nature*, 400(6739):37–42, Jul 1999. [12](#), [13](#), [16](#), [48](#)
- [57] R Ciosk, W Zachariae, C Michaelis, A Shevchenko, M Mann, and K Nasmyth. An esp1/pds1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell*, 93(6):1067–76, Jun 1998. [13](#)
- [58] O Cohen-Fix, J M Peters, M W Kirschner, and D Koshland. Anaphase initiation in *saccharomyces cerevisiae* is controlled by the apc-dependent degradation of the anaphase inhibitor pds1p. *Genes Dev*, 10(24):3081–93, Dec 1996. [13](#)
- [59] A Yamamoto, V Guacci, and D Koshland. Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the apc and checkpoint pathway(s). *J Cell Biol*, 133(1):99–110, Apr 1996. [13](#)
- [60] K Furuya, K Takahashi, and M Yanagida. Faithful anaphase is ensured by mis4, a sister chromatid cohesion molecule required in s phase and not destroyed in g1 phase. *Genes Dev*, 12(21):3408–18, Nov 1998. [15](#)
- [61] R Ciosk, M Shirayama, A Shevchenko, T Tanaka, A Toth, A Shevchenko, and K Nasmyth. Cohesin’s binding to chromosomes depends on a separate

REFERENCES

- complex consisting of scc2 and scc4 proteins. *Mol Cell*, 5(2):243–54, Feb 2000. [15](#)
- [62] Prakash Arumugam, Stephan Gruber, Koichi Tanaka, Christian H Haering, Karl Mechtler, and Kim Nasmyth. Atp hydrolysis is required for cohesin’s association with chromosomes. *Curr Biol*, 13(22):1941–53, Nov 2003. [15](#)
- [63] Stefan Weitzer, Chris Lehane, and Frank Uhlmann. A model for atp hydrolysis-dependent binding of cohesin to dna. *Curr Biol*, 13(22):1930–40, Nov 2003. [15](#)
- [64] A Losada, M Hirano, and T Hirano. Identification of xenopus smc protein complexes required for sister chromatid cohesion. *Genes Dev*, 12(13):1986–97, Jul 1998. [15](#)
- [65] N Darwiche, L A Freeman, and A Strunnikov. Characterization of the components of the putative mammalian sister chromatid cohesion complex. *Gene*, 233(1-2):39–47, Jun 1999. [15](#)
- [66] Daniel Gerlich, Birgit Koch, Florine Dupeux, Jan-Michael Peters, and Jan Ellenberg. Live-cell imaging reveals a stable cohesin-chromatin interaction after but not before dna replication. *Curr Biol*, 16(15):1571–8, Aug 2006. [15](#)
- [67] W D Warren, S Steffensen, E Lin, P Coelho, M Loupart, N Cobbe, J Y Lee, M J McKay, T Orr-Weaver, M M Heck, and C E Sunkel. The drosophila rad21 cohesin persists at the centromere region in mitosis. *Curr Biol*, 10(22):1463–6, Nov 2000. [15](#)
- [68] Bin Hu, Takehiko Itoh, Ajay Mishra, Yuki Katoh, Kok-Lung Chan, William Upcher, Camilla Godlee, Maurici B Roig, Katsuhiko Shirahige, and Kim Nasmyth. Atp hydrolysis is required for relocating cohesin from sites occupied by its scc2/4 loading complex. *Curr Biol*, 21(1):12–24, Jan 2011. [15](#)

REFERENCES

- [69] Stewart A Weber, Jennifer L Gerton, Joan E Polancic, Joseph L DeRisi, Douglas Koshland, and Paul C Megee. The kinetochore is an enhancer of pericentric cohesin binding. *PLoS Biol*, 2(9):E260, Sep 2004. [15](#)
- [70] F Uhlmann and K Nasmyth. Cohesion between sister chromatids must be established during dna replication. *Curr Biol*, 8(20):1095–101, Oct 1998. [15](#), [23](#)
- [71] Christian H Haering, Doris Schoffnegger, Tatsuya Nishino, Wolfgang Helmhart, Kim Nasmyth, and Jan Löwe. Structure and stability of cohesin’s smc1-kleisin interaction. *Mol Cell*, 15(6):951–64, Sep 2004. [15](#)
- [72] Jinglan Zhang, Xiaomin Shi, Yehua Li, Beom-Jun Kim, Junling Jia, Zhiwei Huang, Tao Yang, Xiaoyong Fu, Sung Yun Jung, Yi Wang, Pumin Zhang, Seong-Tae Kim, Xuewen Pan, and Jun Qin. Acetylation of smc3 by eco1 is required for s phase sister chromatid cohesion in both human and yeast. *Mol Cell*, 31(1):143–51, Jul 2008. [15](#)
- [73] Elçin Unal, Jill M Heidinger-Pauli, Woong Kim, Vincent Guacci, Itay Onn, Steven P Gygi, and Douglas E Koshland. A molecular determinant for the establishment of sister chromatid cohesion. *Science*, 321(5888):566–9, Jul 2008. [15](#)
- [74] Tom Rolef Ben-Shahar, Sebastian Heeger, Chris Lehane, Philip East, Helen Flynn, Mark Skehel, and Frank Uhlmann. Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. *Science*, 321(5888):563–6, Jul 2008. [15](#)
- [75] Dmitri Ivanov, Alexander Schleiffer, Frank Eisenhaber, Karl Mechtler, Christian H Haering, and Kim Nasmyth. Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. *Curr Biol*, 12(4):323–8, Feb 2002. [15](#)
- [76] R V Skibbens, L B Corson, D Koshland, and P Hieter. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the dna replication machinery. *Genes Dev*, 13(3):307–19, Feb 1999. [15](#)

REFERENCES

- [77] Benjamin D Rowland, Maurici B Roig, Tatsuya Nishino, Alexander Kurze, Pelin Uluocak, Ajay Mishra, Frédéric Beckouët, Philippa Underwood, Jean Metson, Richard Imre, Karl Mechtler, Vittorio L Katis, and Kim Nasmyth. Building sister chromatid cohesion: smc3 acetylation counteracts an anti-establishment activity. *Mol Cell*, 33(6):763–74, Mar 2009. [15](#)
- [78] Kok-Lung Chan, Maurici B Roig, Bin Hu, Frédéric Beckouët, Jean Metson, and Kim Nasmyth. Cohesin’s dna exit gate is distinct from its entrance gate and is regulated by acetylation. *Cell*, 150(5):961–74, Aug 2012. [15](#)
- [79] Izabela Sumara, Elisabeth Vorlaufer, P Todd Stukenberg, Olaf Kelm, Norbert Redemann, Erich A Nigg, and Jan-Michael Peters. The dissociation of cohesin from chromosomes in prophase is regulated by polo-like kinase. *Mol Cell*, 9(3):515–25, Mar 2002. [16](#), [29](#)
- [80] A. Losada, M. Hirano, and T. Hirano. Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. *Genes & development*, 16(23):3004–3016, 2002. [16](#), [29](#)
- [81] I C Waizenegger, S Hauf, A Meinke, and J M Peters. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell*, 103(3):399–410, Oct 2000. [16](#)
- [82] Rita Gandhi, Peter J Gillespie, and Tatsuya Hirano. Human wapl is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase. *Curr Biol*, 16(24):2406–17, Dec 2006. [16](#)
- [83] Stephanie Kueng, Björn Hegemann, Beate H Peters, Jesse J Lipp, Alexander Schleiffer, Karl Mechtler, and Jan-Michael Peters. Wapl controls the dynamic association of cohesin with chromatin. *Cell*, 127(5):955–67, Dec 2006. [16](#)
- [84] Barry E McGuinness, Toru Hirota, Nobuaki R Kudo, Jan-Michael Peters, and Kim Nasmyth. Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. *PLoS Biol*, 3(3):e86, Mar 2005. [16](#), [50](#)

REFERENCES

- [85] Tomoya S Kitajima, Takeshi Sakuno, Kei-ichiro Ishiguro, Shun-ichiro Iemura, Tohru Natsume, Shigehiro A Kawashima, and Yoshinori Watanabe. Shugoshin collaborates with protein phosphatase 2a to protect cohesin. *Nature*, 441(7089):46–52, May 2006. [16](#), [30](#), [35](#), [49](#), [76](#)
- [86] Christian G Riedel, Vittorio L Katis, Yuki Katou, Saori Mori, Takehiko Itoh, Wolfgang Helmhart, Marta Gálová, Mark Petronczki, Juraj Gregan, Bulent Cetin, Ingrid Mudrak, Egon Ogris, Karl Mechtler, Laurence Pelletier, Frank Buchholz, Katsuhiko Shirahige, and Kim Nasmyth. Protein phosphatase 2a protects centromeric sister chromatid cohesion during meiosis i. *Nature*, 441(7089):53–61, May 2006. [16](#), [30](#), [31](#), [35](#), [49](#), [76](#)
- [87] Helga Satzinger. Theodor and marcella boveri: chromosomes and cytoplasm in heredity and development. *Nat Rev Genet*, 9(3):231–8, Mar 2008. [18](#)
- [88] Theodor Boveri. Concerning the origin of malignant tumours by theodor boveri. translated and annotated by henry harris. *J Cell Sci*, 121 Suppl 1:1–84, Jan 2008. [18](#)
- [89] Andrew W Murray. A brief history of error. *Nat Cell Biol*, 13(10):1178–82, Oct 2011. [18](#)
- [90] Iain M Cheeseman and Arshad Desai. Molecular architecture of the kinetochore-microtubule interface. *Nat Rev Mol Cell Biol*, 9(1):33–46, Jan 2008. [19](#)
- [91] R DIETZ. [multiple sex chromosomes in ostracoda cypria, their evolution and division characteristics]. *Chromosoma*, 9(5):359–440, 1958. [19](#)
- [92] R B Nicklas and C A Koch. Chromosome micromanipulation. 3. spindle fiber tension and the reorientation of mal-oriented chromosomes. *J Cell Biol*, 43(1):40–50, Oct 1969. [19](#)
- [93] S Biggins, F F Severin, N Bhalla, I Sassoon, A A Hyman, and A W Murray. The conserved protein kinase ip11 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev*, 13(5):532–44, Mar 1999. [19](#)

REFERENCES

- [94] L Francisco, W Wang, and C S Chan. Type 1 protein phosphatase acts in opposition to ipl1 protein kinase in regulating yeast chromosome segregation. *Mol Cell Biol*, 14(7):4731–40, Jul 1994. [19](#)
- [95] Tomoyuki U Tanaka, Najma Rachidi, Carsten Janke, Gislene Pereira, Marta Galova, Elmar Schiebel, Michael J R Stark, and Kim Nasmyth. Evidence that the ipl1-sli15 (aurora kinase-incenp) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell*, 108(3):317–29, Feb 2002. [19](#)
- [96] Benjamin A Pinsky, Charles Kung, Kevan M Shokat, and Sue Biggins. The ipl1-aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. *Nat Cell Biol*, 8(1):78–83, Jan 2006. [19](#)
- [97] Silke Hauf, Richard W Cole, Sabrina LaTerra, Christine Zimmer, Gisela Schnapp, Rainer Walter, Armin Heckel, Jacques van Meel, Conly L Rieder, and Jan-Michael Peters. The small molecule hesperadin reveals a role for aurora b in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J Cell Biol*, 161(2):281–94, Apr 2003. [20](#)
- [98] Claire Ditchfield, Victoria L Johnson, Anthony Tighe, Rebecca Ellston, Carolyn Haworth, Trevor Johnson, Andrew Mortlock, Nicholas Keen, and Stephen S Taylor. Aurora b couples chromosome alignment with anaphase by targeting bubr1, mad2, and cenp-e to kinetochores. *J Cell Biol*, 161(2):267–80, Apr 2003. [20](#)
- [99] Dan Liu, Gerben Vader, Martijn J M Vromans, Michael A Lampson, and Susanne M A Lens. Sensing chromosome bi-orientation by spatial separation of aurora b kinase from kinetochore substrates. *Science*, 323(5919):1350–3, Mar 2009. [20](#)
- [100] Julie P I Welburn, Mathijs Vleugel, Dan Liu, John R Yates, 3rd, Michael A Lampson, Tatsuo Fukagawa, and Iain M Cheeseman. Aurora b phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. *Mol Cell*, 38(3):383–92, May 2010. [20](#), [73](#), [84](#)

REFERENCES

- [101] Patrick Keating, Najma Rachidi, Tomoyuki U Tanaka, and Michael J R Stark. Ipl1-dependent phosphorylation of dam1 is reduced by tension applied on kinetochores. *J Cell Sci*, 122(Pt 23):4375–82, Dec 2009. [20](#)
- [102] Michael A Lampson and Iain M Cheeseman. Sensing centromere tension: Aurora b and the regulation of kinetochore function. *Trends Cell Biol*, 21(3):133–40, Mar 2011. [20](#), [48](#)
- [103] Fangwei Wang, Jun Dai, John R Daum, Ewa Niedzialkowska, Budhaditya Banerjee, P Todd Stukenberg, Gary J Gorbsky, and Jonathan M G Higgins. Histone h3 thr-3 phosphorylation by haspin positions aurora b at centromeres in mitosis. *Science*, 330(6001):231–5, Oct 2010. [20](#)
- [104] Yuya Yamagishi, Takashi Honda, Yuji Tanno, and Yoshinori Watanabe. Two histone marks establish the inner centromere and chromosome bi-orientation. *Science*, 330(6001):239–43, Oct 2010. [20](#)
- [105] Alexander E Kelly, Cristina Ghenoiiu, John Z Xue, Christian Zierhut, Hiroshi Kimura, and Hironori Funabiki. Survivin reads phosphorylated histone h3 threonine 3 to activate the mitotic kinase aurora b. *Science*, 330(6001):235–9, Oct 2010. [20](#)
- [106] M A Hoyt, L Totis, and B T Roberts. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, 66(3):507–17, Aug 1991. [21](#), [34](#)
- [107] R Li and A W Murray. Feedback control of mitosis in budding yeast. *Cell*, 66(3):519–31, Aug 1991. [21](#), [34](#)
- [108] E Weiss and M Winey. The *saccharomyces cerevisiae* spindle pole body duplication gene *mps1* is part of a mitotic checkpoint. *J Cell Biol*, 132(1-2):111–23, Jan 1996. [21](#)
- [109] K G Hardwick, E Weiss, F C Luca, M Winey, and A W Murray. Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. *Science*, 273(5277):953–6, Aug 1996. [21](#)

REFERENCES

- [110] L S Michel, V Liberal, A Chatterjee, R Kirchwegger, B Pasche, W Gerald, M Dobles, P K Sorger, V V Murty, and R Benezra. Mad2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature*, 409(6818):355–9, Jan 2001. [21](#), [34](#)
- [111] David Perera, Valerie Tilston, Jane A Hopwood, Marco Barchi, Raymond P Boot-Handford, and Stephen S Taylor. Bub1 maintains centromeric cohesion by activation of the spindle checkpoint. *Dev Cell*, 13(4):566–79, Oct 2007. [21](#), [34](#), [45](#)
- [112] Y Li, C Gorbea, D Mahaffey, M Rechsteiner, and R Benezra. Mad2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proc Natl Acad Sci U S A*, 94(23):12431–6, Nov 1997. [21](#), [77](#)
- [113] G Fang, H Yu, and M W Kirschner. The checkpoint protein mad2 and the mitotic regulator cdc20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes Dev*, 12(12):1871–83, Jun 1998. [21](#), [77](#)
- [114] L H Hwang, L F Lau, D L Smith, C A Mistrot, K G Hardwick, E S Hwang, A Amon, and A W Murray. Budding yeast cdc20: a target of the spindle checkpoint. *Science*, 279(5353):1041–4, Feb 1998. [21](#), [77](#)
- [115] S H Kim, D P Lin, S Matsumoto, A Kitazono, and T Matsumoto. Fission yeast slp1: an effector of the mad2-dependent spindle checkpoint. *Science*, 279(5353):1045–7, Feb 1998. [21](#), [77](#)
- [116] V Sudakin, G K Chan, and T J Yen. Checkpoint inhibition of the apc/c in hela cells is mediated by a complex of bubr1, bub3, cdc20, and mad2. *J Cell Biol*, 154(5):925–36, Sep 2001. [21](#), [77](#)
- [117] Z Tang, R Bharadwaj, B Li, and H Yu. Mad2-independent inhibition of apccdc20 by the mitotic checkpoint protein bubr1. *Dev Cell*, 1(2):227–37, Aug 2001. [21](#), [22](#), [77](#)

REFERENCES

- [118] Guowei Fang. Checkpoint protein bubr1 acts synergistically with mad2 to inhibit anaphase-promoting complex. *Mol Biol Cell*, 13(3):755–66, Mar 2002. [21](#), [22](#), [77](#)
- [119] Lucia Sironi, Marina Mapelli, Stefan Knapp, Anna De Antoni, Kuan-Teh Jeang, and Andrea Musacchio. Crystal structure of the tetrameric mad1-mad2 core complex: implications of a 'safety belt' binding mechanism for the spindle checkpoint. *EMBO J*, 21(10):2496–506, May 2002. [21](#), [77](#)
- [120] Andrea Musacchio and Edward D Salmon. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol*, 8(5):379–93, May 2007. [21](#), [22](#), [48](#), [77](#)
- [121] Anna De Antoni, Chad G Pearson, Daniela Cimini, Julie C Canman, Valeria Sala, Luigi Nezi, Marina Mapelli, Lucia Sironi, Mario Faretta, Edward D Salmon, and Andrea Musacchio. The mad1/mad2 complex as a template for mad2 activation in the spindle assembly checkpoint. *Curr Biol*, 15(3):214–25, Feb 2005. [22](#), [77](#)
- [122] Michelle J Hayes, Yuu Kimata, Samantha L Wattam, Catherine Lindon, Guojie Mao, Hiroyuki Yamano, and Andrew M Fry. Early mitotic degradation of nek2a depends on cdc20-independent interaction with the apc/c. *Nat Cell Biol*, 8(6):607–14, Jun 2006. [22](#)
- [123] N den Elzen and J Pines. Cyclin a is destroyed in prometaphase and can delay chromosome alignment and anaphase. *J Cell Biol*, 153(1):121–36, Apr 2001. [22](#)
- [124] S Geley, E Kramer, C Gieffers, J Gannon, J M Peters, and T Hunt. Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin a starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. *J Cell Biol*, 153(1):137–48, Apr 2001. [22](#)
- [125] R S Hames, S L Wattam, H Yamano, R Bacchieri, and A M Fry. Apc/c-mediated destruction of the centrosomal kinase nek2a occurs in early mitosis and depends upon a cyclin a-type d-box. *EMBO J*, 20(24):7117–27, Dec 2001. [22](#)

REFERENCES

- [126] William C H Chao, Kiran Kulkarni, Ziguo Zhang, Eric H Kong, and David Barford. Structure of the mitotic checkpoint complex. *Nature*, 484(7393):208–13, Apr 2012. [22](#), [77](#)
- [127] S K Reddy, M Rape, W A Margansky, and M W Kirschner. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature*, 446(7138):921–5, Apr 2007. [22](#)
- [128] Ilana Braunstein, Shirly Miniowitz, Yakir Moshe, and Avram Hershko. Inhibitory factors associated with anaphase-promoting complex/cylosome in mitotic checkpoint. *Proc Natl Acad Sci U S A*, 104(12):4870–5, Mar 2007. [22](#)
- [129] Mathew J Garnett, Jörg Mansfeld, Colin Godwin, Takahiro Matsusaka, Jiahua Wu, Paul Russell, Jonathon Pines, and Ashok R Venkitaraman. Ube2s elongates ubiquitin chains on apc/c substrates to promote mitotic exit. *Nat Cell Biol*, 11(11):1363–9, Nov 2009. [22](#)
- [130] Luying Jia, Bing Li, Ross T Warrington, Xing Hao, Shixuan Wang, and Hongtao Yu. Defining pathways of spindle checkpoint silencing: functional redundancy between cdc20 ubiquitination and p31(comet). *Mol Biol Cell*, 22(22):4227–35, Nov 2011. [22](#)
- [131] Hoi Tang Ma and Randy Y C Poon. Orderly inactivation of the key checkpoint protein mitotic arrest deficient 2 (mad2) during mitotic progression. *J Biol Chem*, 286(15):13052–9, Apr 2011. [22](#)
- [132] Shirly Miniowitz-Shemtov, Adar Teichner, Danielle Sitry-Shevah, and Avram Hershko. Atp is required for the release of the anaphase-promoting complex/cyclosome from inhibition by the mitotic checkpoint. *Proc Natl Acad Sci U S A*, 107(12):5351–6, Mar 2010. [22](#)
- [133] Gianluca Varetto, Claudia Guida, Stefano Santaguida, Elena Chiroli, and Andrea Musacchio. Homeostatic control of mitotic arrest. *Mol Cell*, 44(5):710–20, Dec 2011. [22](#)

REFERENCES

- [134] Roberta Visconti, Luca Palazzo, and Domenico Grieco. Requirement for proteolysis in spindle assembly checkpoint silencing. *Cell Cycle*, 9(3):564–9, Feb 2010. [22](#)
- [135] Adam Williamson, Katherine E Wickliffe, Barbara G Mellone, Ling Song, Gary H Karpen, and Michael Rape. Identification of a physiological e2 module for the human anaphase-promoting complex. *Proc Natl Acad Sci U S A*, 106(43):18213–8, Oct 2009. [22](#)
- [136] Xing Zeng, Frederic Sigoillot, Shantanu Gaur, Sungwoon Choi, Kathleen L Pfaff, Dong-Chan Oh, Nathaniel Hathaway, Nevena Dimova, Gregory D Cuny, and Randall W King. Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. *Cancer Cell*, 18(4):382–95, Oct 2010. [22](#)
- [137] Jörg Mansfeld, Philippe Collin, Mark O Collins, Jyoti S Choudhary, and Jonathon Pines. Apc15 drives the turnover of mcc-cdc20 to make the spindle assembly checkpoint responsive to kinetochore attachment. *Nat Cell Biol*, 13(10):1234–43, Oct 2011. [22](#)
- [138] Luca L Fava, Manuel Kaulich, Erich A Nigg, and Anna Santamaria. Probing the in vivo function of mad1:c-mad2 in the spindle assembly checkpoint. *EMBO J*, 30(16):3322–36, Aug 2011. [22](#)
- [139] Toshiyuki Habu, Sang Hoon Kim, Jasminder Weinstein, and Tomohiro Matsumoto. Identification of a mad2-binding protein, cmt2, and its role in mitosis. *EMBO J*, 21(23):6419–28, Dec 2002. [22](#)
- [140] Robert S Hagan, Michael S Manak, Håkon Kirkeby Buch, Michelle G Meier, Patrick Meraldi, Jagesh V Shah, and Peter K Sorger. p31(comet) acts to ensure timely spindle checkpoint silencing subsequent to kinetochore attachment. *Mol Biol Cell*, 22(22):4236–46, Nov 2011. [22](#)
- [141] Marina Mapelli, Fabian V Filipp, Giulia Rancati, Lucia Massimiliano, Luigi Nezi, Gunter Stier, Robert S Hagan, Stefano Confalonieri, Simonetta Piatti, Michael Sattler, and Andrea Musacchio. Determinants of conformational

REFERENCES

- dimerization of mad2 and its inhibition by p31comet. *EMBO J*, 25(6):1273–84, Mar 2006. [22](#)
- [142] Adar Teichner, Esther Eytan, Danielle Sitry-Shevah, Shirly Miniowitz-Shemtov, Elena Dumin, Jonathan Gromis, and Avram Hershko. p31comet promotes disassembly of the mitotic checkpoint complex in an atp-dependent process. *Proc Natl Acad Sci U S A*, 108(8):3187–92, Feb 2011. [22](#)
- [143] Frederick G Westhorpe, Anthony Tighe, Pablo Lara-Gonzalez, and Stephen S Taylor. p31comet-mediated extraction of mad2 from the mcc promotes efficient mitotic exit. *J Cell Sci*, 124(Pt 22):3905–16, Nov 2011. [22](#)
- [144] Guohong Xia, Xuelian Luo, Toshiyuki Habu, Josep Rizo, Tomohiro Matsumoto, and Hongtao Yu. Conformation-specific binding of p31(comet) antagonizes the function of mad2 in the spindle checkpoint. *EMBO J*, 23(15):3133–43, Aug 2004. [22](#)
- [145] Maojun Yang, Bing Li, Diana R Tomchick, Mischa Machius, Josep Rizo, Hongtao Yu, and Xuelian Luo. p31comet blocks mad2 activation through structural mimicry. *Cell*, 131(4):744–55, Nov 2007. [22](#)
- [146] S.L. Page and R.S. Hawley. The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol.*, 20:525–558, 2004. [23](#)
- [147] V Borde, A S Goldman, and M Lichten. Direct coupling between meiotic dna replication and recombination initiation. *Science*, 290(5492):806–9, Oct 2000. [23](#)
- [148] M D Cervantes, J A Farah, and G R Smith. Meiotic dna breaks associated with recombination in *s. pombe*. *Mol Cell*, 5(5):883–8, May 2000. [23](#)
- [149] M Molnar, J Bähler, M Sipiczki, and J Kohli. The rec8 gene of *schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. *Genetics*, 141(1):61–73, Sep 1995. [23](#), [28](#)

REFERENCES

- [150] F Klein, P Mahr, M Galova, S B Buonomo, C Michaelis, K Nairz, and K Nasmyth. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell*, 98(1):91–103, Jul 1999. [23](#), [26](#), [28](#)
- [151] Y Watanabe, S Yokobayashi, M Yamamoto, and P Nurse. Pre-meiotic s phase is linked to reductional chromosome segregation and recombination. *Nature*, 409(6818):359–63, Jan 2001. [23](#), [28](#)
- [152] Adèle L Marston and Angelika Amon. Meiosis: cell-cycle controls shuffle and deal. *Nat Rev Mol Cell Biol*, 5(12):983–97, Dec 2004. [23](#), [25](#), [31](#), [49](#)
- [153] Y Watanabe and P Nurse. Cohesin rec8 is required for reductional chromosome segregation at meiosis. *Nature*, 400(6743):461–4, Jul 1999. [25](#), [26](#)
- [154] S B Buonomo, R K Clyne, J Fuchs, J Loidl, F Uhlmann, and K Nasmyth. Disjunction of homologous chromosomes in meiosis i depends on proteolytic cleavage of the meiotic cohesin rec8 by separin. *Cell*, 103(3):387–98, Oct 2000. [25](#), [28](#), [29](#)
- [155] Tomoya S Kitajima, Yousuke Miyazaki, Masayuki Yamamoto, and Yoshinori Watanabe. Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast. *EMBO J*, 22(20):5643–53, Oct 2003. [25](#), [29](#)
- [156] A Tóth, K P Rabitsch, M Gálová, A Schleiffer, S B Buonomo, and K Nasmyth. Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis i. *Cell*, 103(7):1155–68, Dec 2000. [25](#), [27](#)
- [157] Huiling Xu, Matthew D Beasley, William D Warren, Gijsbertus T J van der Horst, and Michael J McKay. Absence of mouse rec8 cohesin promotes synapsis of sister chromatids in meiosis. *Dev Cell*, 8(6):949–61, Jun 2005. [25](#)

REFERENCES

- [158] Takeshi Sakuno, Kenji Tada, and Yoshinori Watanabe. Kinetochore geometry defined by cohesion within the centromere. *Nature*, 458(7240):852–8, Apr 2009. [25](#)
- [159] Liudmila Chelysheva, Stéphanie Diallo, Daniel Vezon, Ghislaine Gendrot, Nathalie Vrielynck, Katia Belcram, Nathalie Rocques, Angustias Márquez-Lema, Anuj M Bhatt, Christine Horlow, Raphaël Mercier, Christine Mézard, and Mathilde Grelon. Atrec8 and atsc3 are essential to the monopolar orientation of the kinetochores during meiosis. *J Cell Sci*, 118(Pt 20):4621–32, Oct 2005. [25](#)
- [160] Laura A Bannister, Laura G Reinholdt, Robert J Munroe, and John C Schimenti. Positional cloning and characterization of mouse mei8, a disrupted allele of the meiotic cohesin rec8. *Genesis*, 40(3):184–94, Nov 2004. [25](#)
- [161] Ekaterina Revenkova, Maureen Eijpe, Christa Heyting, Craig A Hodges, Patricia A Hunt, Bodo Liebe, Harry Scherthan, and Rolf Jessberger. Cohesin smc1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and dna recombination. *Nat Cell Biol*, 6(6):555–62, Jun 2004. [25](#)
- [162] Craig A Hodges, Ekaterina Revenkova, Rolf Jessberger, Terry J Hassold, and Patricia A Hunt. Smc1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. *Nat Genet*, 37(12):1351–5, Dec 2005. [25](#)
- [163] I Prieto, J A Suja, N Pezzi, L Kremer, C Martínez-A, J S Rufas, and J L Barbero. Mammalian stag3 is a cohesin specific to sister chromatid arms in meiosis i. *Nat Cell Biol*, 3(8):761–6, Aug 2001. [25](#)
- [164] Kei-ichiro Ishiguro, Jihye Kim, Sally Fujiyama-Nakamura, Shigeaki Kato, and Yoshinori Watanabe. A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. *EMBO Rep*, 12(3):267–75, Mar 2011. [25](#)
- [165] Cristina Gutiérrez-Caballero, Yurema Herrán, Manuel Sánchez-Martín, José Angel Suja, José Luis Barbero, Elena Llano, and Alberto M Pendás.

REFERENCES

- Identification and molecular characterization of the mammalian -kleisin rad21l. *Cell Cycle*, 10(9):1477–87, May 2011. [25](#)
- [166] Elena Llano, Yurema Herrán, Ignacio García-Tuñón, Cristina Gutiérrez-Caballero, Enrique de Álava, José Luis Barbero, John Schimenti, Dirk G de Rooij, Manuel Sánchez-Martín, and Alberto M Pendás. Meiotic cohesin complexes are essential for the formation of the axial element in mice. *J Cell Biol*, 197(7):877–85, Jun 2012. [25](#)
- [167] Jibak Lee and Tatsuya Hirano. Rad21l, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. *J Cell Biol*, 192(2):263–76, Jan 2011. [25](#)
- [168] Silvia Polakova, Lubos Cipak, and Juraj Gregan. Rad21l is a novel kleisin subunit of the cohesin complex. *Cell Cycle*, 10(12):1892–3, Jun 2011. [25](#)
- [169] Aaron F Severson, Lorraine Ling, Vanessa van Zuylen, and Barbara J Meyer. The axial element protein htp-3 promotes cohesin loading and meiotic axis assembly in *c. elegans* to implement the meiotic program of chromosome segregation. *Genes Dev*, 23(15):1763–78, Aug 2009. [25](#)
- [170] Tomoya S Kitajima, Shihori Yokobayashi, Masayuki Yamamoto, and Yoshinori Watanabe. Distinct cohesin complexes organize meiotic chromosome domains. *Science*, 300(5622):1152–5, May 2003. [26](#)
- [171] L V Paliulis and R B Nicklas. The reduction of chromosome number in meiosis is determined by properties built into the chromosomes. *J Cell Biol*, 150(6):1223–32, Sep 2000. [26](#)
- [172] L S Goldstein. Kinetochore structure and its role in chromosome orientation during the first meiotic division in male *d. melanogaster*. *Cell*, 25(3):591–602, Sep 1981. [26](#)
- [173] JA Suja, J. De La Torre, JF Gimenez-Abian, C.G. de la Vega, and JS Rufas. Meiotic chromosome structure. kinetochores and chromatid cores in standard and b chromosomes of *arcyptera fusca* (orthoptera) revealed by silver staining. *Genome*, 34(1):19–27, 1991. [26](#)

REFERENCES

- [174] A Tóth, K P Rabitsch, M Gálová, A Schleiffer, S B Buonomo, and K Nasmyth. Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis i. *Cell*, 103(7):1155–68, Dec 2000. [26](#), [27](#), [29](#)
- [175] Kirsten P Rabitsch, Mark Petronczki, Jean Paul Javerzat, Sylvie Genier, Barbara Chwalla, Alex Schleiffer, Tomoyuki U Tanaka, and Kim Nasmyth. Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis i. *Dev Cell*, 4(4):535–48, Apr 2003. [26](#), [27](#)
- [176] Mark Petronczki, Joao Matos, Saori Mori, Juraj Gregan, Aliona Bogdanova, Martin Schwickart, Karl Mechtler, Katsuhiko Shirahige, Wolfgang Zachariae, and Kim Nasmyth. Monopolar attachment of sister kinetochores at meiosis i requires casein kinase 1. *Cell*, 126(6):1049–64, Sep 2006. [26](#)
- [177] Kevin D Corbett, Calvin K Yip, Ly-Sha Ee, Thomas Walz, Angelika Amon, and Stephen C Harrison. The monopolin complex crosslinks kinetochore components to regulate chromosome-microtubule attachments. *Cell*, 142(4):556–67, Aug 2010. [27](#)
- [178] Rosemary K Clyne, Vittorio L Katis, Lea Jessop, Kirsten R Benjamin, Ira Herskowitz, Michael Lichten, and Kim Nasmyth. Polo-like kinase cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis i. *Nat Cell Biol*, 5(5):480–5, May 2003. [27](#)
- [179] Brian H Lee and Angelika Amon. Role of polo-like kinase cdc5 in programming meiosis i chromosome segregation. *Science*, 300(5618):482–6, Apr 2003. [27](#)
- [180] Joao Matos, Jesse J Lipp, Aliona Bogdanova, Sylvine Guillot, Elwy Okaz, Magno Junqueira, Andrej Shevchenko, and Wolfgang Zachariae. Dbf4-dependent cdc7 kinase links dna replication to the segregation of homologous chromosomes in meiosis i. *Cell*, 135(4):662–78, Nov 2008. [27](#)
- [181] Juraj Gregan, Christian G Riedel, Alison L Pidoux, Yuki Katou, Cornelia Rumpf, Alexander Schleiffer, Stephen E Kearsey, Katsuhiko Shirahige,

REFERENCES

- Robin C Allshire, and Kim Nasmyth. The kinetochore proteins pcs1 and mde4 and heterochromatin are required to prevent merotelic orientation. *Curr Biol*, 17(14):1190–200, Jul 2007. [27](#)
- [182] Shihori Yokobayashi and Yoshinori Watanabe. The kinetochore protein moa1 enables cohesion-mediated monopolar attachment at meiosis i. *Cell*, 123(5):803–17, Dec 2005. [27](#)
- [183] Shihori Yokobayashi, Masayuki Yamamoto, and Yoshinori Watanabe. Cohesins determine the attachment manner of kinetochores to spindle microtubules at meiosis i in fission yeast. *Mol Cell Biol*, 23(11):3965–73, Jun 2003. [27](#), [29](#)
- [184] K Nasmyth. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu Rev Genet*, 35:673–745, 2001. [28](#), [48](#)
- [185] Jibak Lee, Toshiharu Iwai, Takehiro Yokota, and Masakane Yamashita. Temporally and spatially selective loss of rec8 protein from meiotic chromosomes during mammalian meiosis. *J Cell Sci*, 116(Pt 13):2781–90, Jul 2003. [28](#)
- [186] Maureen Eijpe, Hildo Offenberg, Rolf Jessberger, Ekaterina Revenkova, and Christa Heyting. Meiotic cohesin rec8 marks the axial elements of rat synaptonemal complexes before cohesins smc1beta and smc3. *J Cell Biol*, 160(5):657–70, Mar 2003. [28](#)
- [187] Nobuaki R Kudo, Katja Wassmann, Martin Anger, Melina Schuh, Karin G Wirth, Huiling Xu, Wolfgang Helmhart, Hiromi Kudo, Michael McKay, Bernard Maro, Jan Ellenberg, Peter de Boer, and Kim Nasmyth. Resolution of chiasmata in oocytes requires separase-mediated proteolysis. *Cell*, 126(1):135–46, Jul 2006. [29](#), [45](#), [61](#), [83](#)
- [188] P Bernard, J F Maure, and J P Javerzat. Fission yeast bub1 is essential in setting up the meiotic pattern of chromosome segregation. *Nat Cell Biol*, 3(5):522–6, May 2001. [29](#), [43](#)

REFERENCES

- [189] A W Kerrebrock, W Y Miyazaki, D Birnby, and T L Orr-Weaver. The drosophila mei-s332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. *Genetics*, 130(4):827–41, Apr 1992. [29](#)
- [190] Vittorio L Katis, Marta Galova, Kirsten P Rabitsch, Juraj Gregan, and Kim Nasmyth. Maintenance of cohesin at centromeres after meiosis i in budding yeast requires a kinetochore-associated protein related to mei-s332. *Curr Biol*, 14(7):560–72, Apr 2004. [29](#), [49](#)
- [191] Tomoya S Kitajima, Shigehiro A Kawashima, and Yoshinori Watanabe. The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. *Nature*, 427(6974):510–7, Feb 2004. [29](#), [30](#), [44](#), [49](#)
- [192] Adele L Marston, Wai-Hong Tham, Hiral Shah, and Angelika Amon. A genome-wide screen identifies genes required for centromeric cohesion. *Science*, 303(5662):1367–70, Feb 2004. [29](#), [49](#)
- [193] Kirsten P Rabitsch, Juraj Gregan, Alex Schleiffer, Jean-Paul Javerzat, Frank Eisenhaber, and Kim Nasmyth. Two fission yeast homologs of drosophila mei-s332 are required for chromosome segregation during meiosis i and ii. *Curr Biol*, 14(4):287–301, Feb 2004. [29](#), [30](#), [49](#)
- [194] Cristina Gutiérrez-Caballero, Luis R Cebollero, and Alberto M Pendás. Shugoshins: from protectors of cohesion to versatile adaptors at the centromere. *Trends Genet*, 28(7):351–60, Jul 2012. [29](#), [30](#)
- [195] Yixin Yao and Wei Dai. Shugoshins function as a guardian for chromosomal stability in nuclear division. *Cell Cycle*, 11(14):2631–42, Jul 2012. [29](#), [30](#)
- [196] D Clift and A L Marston. The role of shugoshin in meiotic chromosome segregation. *Cytogenet Genome Res*, 133(2-4):234–42, 2011. [29](#)
- [197] Sabine Vaur, Fabien Cubizolles, Guillaume Plane, Sylvie Genier, Peter K Rabitsch, Juraj Gregan, Kim Nasmyth, Vincent Vanoosthuyse, Kevin G Hardwick, and Jean-Paul Javerzat. Control of shugoshin function during fission-yeast meiosis. *Curr Biol*, 15(24):2263–70, Dec 2005. [30](#), [35](#), [44](#)

REFERENCES

- [198] S.A. Kawashima, Y. Yamagishi, T. Honda, K. Ishiguro, and Y. Watanabe. Phosphorylation of h2a by bub1 prevents chromosomal instability through localizing shugoshin. *Science Signalling*, 327(5962):172, 2010. [30](#), [44](#)
- [199] T T Tang, S E Bickel, L M Young, and T L Orr-Weaver. Maintenance of sister-chromatid cohesion at the centromere by the drosophila mei-s332 protein. *Genes Dev*, 12(24):3843–56, Dec 1998. [30](#)
- [200] Zheng Xu, Bulent Cetin, Martin Anger, Uhn Soo Cho, Wolfgang Helmhart, Kim Nasmyth, and Wenqing Xu. Structure and function of the pp2a-shugoshin interaction. *Mol Cell*, 35(4):426–41, Aug 2009. [30](#), [49](#), [50](#), [51](#), [76](#)
- [201] Vittorio L Katis, Jesse J Lipp, Richard Imre, Aliona Bogdanova, Elwy Okaz, Bianca Habermann, Karl Mechtler, Kim Nasmyth, and Wolfgang Zachariae. Rec8 phosphorylation by casein kinase 1 and cdc7-dbf4 kinase regulates cohesin cleavage by separase during meiosis. *Dev Cell*, 18(3):397–409, Mar 2010. [31](#), [50](#)
- [202] Tadashi Ishiguro, Koichi Tanaka, Takeshi Sakuno, and Yoshinori Watanabe. Shugoshin-pp2a counteracts casein-kinase-1-dependent cleavage of rec8 by separase. *Nat Cell Biol*, 12(5):500–6, May 2010. [31](#), [50](#)
- [203] Daisuke Izawa, Masuo Goto, Akira Yamashita, Hiroyuki Yamano, and Masayuki Yamamoto. Fission yeast mes1p ensures the onset of meiosis ii by blocking degradation of cyclin cdc13p. *Nature*, 434(7032):529–33, Mar 2005. [31](#)
- [204] C Shimoda, A Hirata, M Kishida, T Hashida, and K Tanaka. Characterization of meiosis-deficient mutants by electron microscopy and mapping of four essential genes in the fission yeast schizosaccharomyces pombe. *Mol Gen Genet*, 200(2):252–7, 1985. [31](#)
- [205] N Grandin and S I Reed. Differential function and expression of saccharomyces cerevisiae b-type cyclins in mitosis and meiosis. *Mol Cell Biol*, 13(4):2113–25, Apr 1993. [32](#)

REFERENCES

- [206] C Dahmann, J F Diffley, and K A Nasmyth. S-phase-promoting cyclin-dependent kinases prevent re-replication by inhibiting the transition of replication origins to a pre-replicative state. *Curr Biol*, 5(11):1257–69, Nov 1995. [32](#)
- [207] Y Masui and C L Markert. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool*, 177(2):129–45, Jun 1971. [32](#)
- [208] Brian J Tunquist and James L Maller. Under arrest: cytostatic factor (csf)-mediated metaphase arrest in vertebrate eggs. *Genes Dev*, 17(6):683–710, Mar 2003. [32](#)
- [209] E Vorlauffer and J M Peters. Regulation of the cyclin b degradation system by an inhibitor of mitotic proteolysis. *Mol Biol Cell*, 9(7):1817–31, Jul 1998. [32](#)
- [210] Jeffrey J Tung, David V Hansen, Kenneth H Ban, Alexander V Loktev, Matthew K Summers, John R Adler, 3rd, and Peter K Jackson. A role for the anaphase-promoting complex inhibitor emi2/xerp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of xenopus eggs. *Proc Natl Acad Sci U S A*, 102(12):4318–23, Mar 2005. [32](#)
- [211] Shisako Shoji, Naoko Yoshida, Manami Amanai, Maki Ohgishi, Tomoyuki Fukui, Satoko Fujimoto, Yoshikazu Nakano, Eriko Kajikawa, and Anthony C F Perry. Mammalian emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via cdc20. *EMBO J*, 25(4):834–45, Feb 2006. [32](#)
- [212] Daigo Inoue, Munemichi Ohe, Yoshinori Kanemori, Toshiya Nobui, and Noriyuki Sagata. A direct link of the mos-mapk pathway to erp1/emi2 in meiotic arrest of xenopus laevis eggs. *Nature*, 446(7139):1100–4, Apr 2007. [32](#)
- [213] David V Hansen, Jeffrey J Tung, and Peter K Jackson. Camkii and polo-like kinase 1 sequentially phosphorylate the cytostatic factor emi2/xerp1 to trigger its destruction and meiotic exit. *Proc Natl Acad Sci U S A*, 103(3):608–13, Jan 2006. [32](#)

REFERENCES

- [214] Nadine R Rauh, Andreas Schmidt, Jenny Bormann, Erich A Nigg, and Thomas U Mayer. Calcium triggers exit from meiosis ii by targeting the apc/c inhibitor xerp1 for degradation. *Nature*, 437(7061):1048–52, Oct 2005. [32](#)
- [215] T Hassold and P Hunt. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet*, 2(4):280–91, Apr 2001. [33](#)
- [216] C L Rieder, A Schultz, R Cole, and G Sluder. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol*, 127(5):1301–10, Dec 1994. [34](#)
- [217] P Hunt, R LeMaire, P Embury, L Sheean, and K Mroz. Analysis of chromosome behavior in intact mammalian oocytes: monitoring the segregation of a univalent chromosome during female meiosis. *Hum Mol Genet*, 4(11):2007–12, Nov 1995. [34](#)
- [218] S Brunet, G Pahlavan, S Taylor, and B Maro. Functionality of the spindle checkpoint during the first meiotic division of mammalian oocytes. *Reproduction*, 126(4):443–50, Oct 2003. [34](#)
- [219] Katja Wassmann, Théodora Niault, and Bernard Maro. Metaphase i arrest upon activation of the mad2-dependent spindle checkpoint in mouse oocytes. *Curr Biol*, 13(18):1596–608, Sep 2003. [34](#)
- [220] Hayden A Homer, Alex McDougall, Mark Levasseur, Alison P Murdoch, and Mary Herbert. Mad2 is required for inhibiting securin and cyclin b degradation following spindle depolymerisation in meiosis i mouse oocytes. *Reproduction*, 130(6):829–43, Dec 2005. [34](#), [35](#)
- [221] Hayden A Homer, Alex McDougall, Mark Levasseur, Katie Yallop, Alison P Murdoch, and Mary Herbert. Mad2 prevents aneuploidy and premature proteolysis of cyclin b and securin during meiosis i in mouse oocytes. *Genes Dev*, 19(2):202–7, Jan 2005. [34](#), [35](#), [58](#), [77](#)
- [222] Elena Llano, Rocío Gómez, Cristina Gutiérrez-Caballero, Yurema Herrán, Manuel Sánchez-Martín, Luis Vázquez-Quñones, Teresa Hernández, En-

REFERENCES

- rique de Alava, Ana Cuadrado, José Luis Barbero, José A Suja, and Alberto M Pendás. Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. *Genes Dev*, 22(17):2400–13, Sep 2008. [35](#), [38](#), [49](#), [50](#), [54](#), [76](#), [77](#), [82](#)
- [223] Barry E McGuinness, Martin Anger, Anna Kouznetsova, Ana M Gil-Bernabé, Wolfgang Helmhart, Nobuaki R Kudo, Annelie Wuensche, Stephen Taylor, Christer Hoog, Bela Novak, and Kim Nasmyth. Regulation of apc/c activity in oocytes by a bub1-dependent spindle assembly checkpoint. *Curr Biol*, 19(5):369–80, Mar 2009. [35](#), [36](#), [38](#), [43](#), [45](#), [49](#), [58](#), [77](#)
- [224] B T Roberts, K A Farr, and M A Hoyt. The *saccharomyces cerevisiae* checkpoint gene *bub1* encodes a novel protein kinase. *Mol Cell Biol*, 14(12):8282–91, Dec 1994. [42](#)
- [225] Cheryl D Warren, D Michelle Brady, Raymond C Johnston, Joseph S Hanna, Kevin G Hardwick, and Forrest A Spencer. Distinct chromosome segregation roles for spindle checkpoint proteins. *Mol Biol Cell*, 13(9):3029–41, Sep 2002. [42](#), [43](#)
- [226] Josefin Fernius and Kevin G Hardwick. Bub1 kinase targets *sgo1* to ensure efficient chromosome biorientation in budding yeast mitosis. *PLoS Genet*, 3(11):e213, Nov 2007. [42](#), [44](#)
- [227] Satoko Yamaguchi, Anabelle Decottignies, and Paul Nurse. Function of *cdc2p*-dependent *bub1p* phosphorylation and *bub1p* kinase activity in the mitotic and meiotic spindle checkpoint. *EMBO J*, 22(5):1075–87, Mar 2003. [42](#)
- [228] Vincent Vanoosthuyse, Rebekka Valsdottir, Jean-Paul Javerzat, and Kevin G Hardwick. Kinetochore targeting of fission yeast *mad* and *bub* proteins is essential for spindle checkpoint function but not for all chromosome segregation roles of *bub1p*. *Mol Cell Biol*, 24(22):9786–801, Nov 2004. [42](#)

REFERENCES

- [229] H Sharp-Baker and R H Chen. Spindle checkpoint protein bub1 is required for kinetochore localization of mad1, mad2, bub3, and cenp-e, independently of its kinase activity. *J Cell Biol*, 153(6):1239–50, Jun 2001. [43](#)
- [230] Rey-Huei Chen. Phosphorylation and activation of bub1 on unattached chromosomes facilitate the spindle checkpoint. *EMBO J*, 23(15):3113–21, Aug 2004. [43](#)
- [231] Victoria L Johnson, Maria I F Scott, Sarah V Holt, Deema Hussein, and Stephen S Taylor. Bub1 is required for kinetochore localization of bubr1, cenp-e, cenp-f and mad2, and chromosome congression. *J Cell Sci*, 117(Pt 8):1577–89, Mar 2004. [43](#)
- [232] Patrick Meraldi and Peter K Sorger. A dual role for bub1 in the spindle checkpoint and chromosome congression. *EMBO J*, 24(8):1621–33, Apr 2005. [43](#)
- [233] David Perera and Stephen S Taylor. Sgo1 establishes the centromeric cohesion protection mechanism in g2 before subsequent bub1-dependent recruitment in mitosis. *J Cell Sci*, 123(Pt 5):653–9, Mar 2010. [44](#)
- [234] Tomoya S Kitajima, Silke Hauf, Miho Ohsugi, Tadashi Yamamoto, and Yoshinori Watanabe. Human bub1 defines the persistent cohesion site along the mitotic chromosome by affecting shugoshin localization. *Curr Biol*, 15(4):353–9, Feb 2005. [44](#)
- [235] Zhanyun Tang, Yuxiao Sun, Sara E Harley, Hui Zou, and Hongtao Yu. Human bub1 protects centromeric sister-chromatid cohesion through shugoshin during mitosis. *Proc Natl Acad Sci U S A*, 101(52):18012–7, Dec 2004. [44](#)
- [236] Christiane Klebig, Dirk Korinth, and Patrick Meraldi. Bub1 regulates chromosome segregation in a kinetochore-independent manner. *J Cell Biol*, 185(5):841–58, Jun 2009. [44](#)

REFERENCES

- [237] M Lewandoski, K M Wassarman, and G R Martin. Zp3-cre, a transgenic mouse line for the activation or inactivation of loxp-flanked target genes specifically in the female germ line. *Curr Biol*, 7(2):148–51, Feb 1997. [45](#)
- [238] Théodora Niaux, Khaled Hached, Rocío Sotillo, Peter K Sorger, Bernard Maro, Robert Benezra, and Katja Wassmann. Changing mad2 levels affects chromosome segregation and spindle assembly checkpoint control in female mouse meiosis i. *PLoS One*, 2(11):e1165, 2007. [45](#), [58](#), [77](#), [83](#)
- [239] Craig A Hodges and Patricia A Hunt. Simultaneous analysis of chromosomes and chromosome-associated proteins in mammalian oocytes and embryos. *Chromosoma*, 111(3):165–9, Sep 2002. [45](#), [83](#)
- [240] A H Peters, A W Plug, M J van Vugt, and P de Boer. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res*, 5(1):66–8, Feb 1997. [45](#), [83](#)
- [241] María Teresa Parra, Rocío Gómez, Alberto Viera, Elena Llano, Alberto M Pendás, Julio S Rufas, and José A Suja. Sequential assembly of centromeric proteins in male mouse meiosis. *PLoS Genet*, 5(3):e1000417, Mar 2009. [46](#), [84](#)
- [242] C.H. Haering, A.M. Farcas, P. Arumugam, J. Metson, and K. Nasmyth. The cohesin ring concatenates sister dna molecules. *Nature*, 454(7202):297–301, 2008. [48](#)
- [243] K. Nasmyth and C.H. Haering. Cohesin: its roles and mechanisms. *Annual review of genetics*, 43:525–558, 2009. [48](#)
- [244] J.M. Peters, A. Tedeschi, and J. Schmitz. The cohesin complex and its roles in chromosome biology. *Genes & development*, 22(22):3089–3114, 2008. [48](#)
- [245] O Stemmann, H Zou, S A Gerber, S P Gygi, and M W Kirschner. Dual inhibition of sister chromatid separation at metaphase. *Cell*, 107(6):715–26, Dec 2001. [48](#)

REFERENCES

- [246] A W Kerrebrock, D P Moore, J S Wu, and T L Orr-Weaver. Mei-s332, a drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell*, 83(2):247–56, Oct 1995. [49](#)
- [247] Jibak Lee, Tomoya S Kitajima, Yuji Tanno, Kayo Yoshida, Takashi Morita, Takashi Miyano, Masashi Miyake, and Yoshinori Watanabe. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. *Nat Cell Biol*, 10(1):42–52, Jan 2008. [50](#)
- [248] Haomin Huang, Jie Feng, Jakub Famulski, Jerome B Rattner, Song Tao Liu, Gary D Kao, Ruth Muschel, Gordon K T Chan, and Tim J Yen. Tripin/hsgo2 recruits mcak to the inner centromere to correct defective kinetochore attachments. *J Cell Biol*, 177(3):413–24, May 2007. [50](#)
- [249] Michael Orth, Bernd Mayer, Kinga Rehm, Ulli Rothweiler, Doris Heidmann, Tad A Holak, and Olaf Stemmann. Shugoshin is a mad1/cdc20-like interactor of mad2. *EMBO J*, 30(14):2868–80, Jul 2011. [50](#), [57](#), [77](#), [78](#)
- [250] Yuji Tanno, Tomoya S Kitajima, Takashi Honda, Yasuto Ando, Kei-Ichiro Ishiguro, and Yoshinori Watanabe. Phosphorylation of mammalian sgo2 by aurora b recruits pp2a and mcak to centromeres. *Genes Dev*, 24(19):2169–79, Oct 2010. [62](#), [84](#)
- [251] T.S. Kitajima, M. Ohsugi, and J. Ellenberg. Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes. *Cell*, 146(4):568–581, 2011. [65](#), [70](#), [79](#), [84](#)
- [252] M. Schuh and J. Ellenberg. Self-organization of mtocs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. *Cell*, 130(3):484–498, 2007. [65](#), [79](#)
- [253] K. Wassmann, V. Liberal, and R. Benezra. Mad2 phosphorylation regulates its association with mad1 and the apc/c. *The EMBO journal*, 22(4):797–806, 2003. [78](#)

REFERENCES

- [254] S.I. Nagaoka, C.A. Hodges, D.F. Albertini, and P.A. Hunt. Oocyte-specific differences in cell-cycle control create an innate susceptibility to meiotic errors. *Current Biology*, 21(8):651–657, 2011. [79](#)
- [255] J. Dumont and A. Desai. Acentrosomal spindle assembly and chromosome segregation during oocyte meiosis. *Trends in cell biology*, 2012. [80](#)
- [256] Julien Dumont, Sebastian Petri, Franz Pellegrin, Marie-Emilie Terret, Markus T Bohnsack, Pascale Rassinier, Virginie Georget, Petr Kalab, Oliver J Gruss, and Marie-Hélène Verlhac. A centriole- and rangtp-independent spindle assembly pathway in meiosis i of vertebrate oocytes. *J Cell Biol*, 176(3):295–305, Jan 2007. [80](#)
- [257] Jason E Swain, Jun Ding, Jingwen Wu, and Gary D Smith. Regulation of spindle and chromatin dynamics during early and late stages of oocyte maturation by aurora kinases. *Mol Hum Reprod*, 14(5):291–9, May 2008. [80](#)
- [258] Kristy Shuda, Karen Schindler, Jun Ma, Richard M Schultz, and Peter J Donovan. Aurora kinase b modulates chromosome alignment in mouse oocytes. *Mol Reprod Dev*, 76(11):1094–105, Nov 2009. [80](#)
- [259] Kuo-Tai Yang, Shu-Kuei Li, Chih-Chieh Chang, Chieh-Ju C Tang, Yi-Nan Lin, Sheng-Chung Lee, and Tang K Tang. Aurora-c kinase deficiency causes cytokinesis failure in meiosis i and production of large polyploid oocytes in mice. *Mol Biol Cell*, 21(14):2371–83, Jul 2010. [80](#)
- [260] Bedra Sharif, Jie Na, Karin Lykke-Hartmann, Stephen H McLaughlin, Ernest Laue, David M Glover, and Magdalena Zernicka-Goetz. The chromosome passenger complex is required for fidelity of chromosome transmission and cytokinesis in meiosis of mouse oocytes. *J Cell Sci*, 123(Pt 24):4292–300, Dec 2010. [80](#)
- [261] Gonzalo Fernández-Miranda, Marianna Trakala, Javier Martín, Beatriz Escobar, Alejandra González, Norbert B Ghyselinck, Sagrario Ortega, Marta

REFERENCES

- Cañamero, Ignacio Pérez de Castro, and Marcos Malumbres. Genetic disruption of aurora b uncovers an essential role for aurora c during early mammalian development. *Development*, 138(13):2661–72, Jul 2011. [80](#)
- [262] Linda Wordeman, Michael Wagenbach, and George von Dassow. Mcak facilitates chromosome movement by promoting kinetochore microtubule turnover. *J Cell Biol*, 179(5):869–79, Dec 2007. [81](#)
- [263] Teresa Rivera, Cristina Ghenoiu, Miriam Rodríguez-Corsino, Satoru Mochida, Hironori Funabiki, and Ana Losada. Xenopus shugoshin 2 regulates the spindle assembly pathway mediated by the chromosomal passenger complex. *EMBO J*, 31(6):1467–79, Mar 2012. [81](#)
- [264] E.A. Foley, M. Maldonado, and T.M. Kapoor. Formation of stable attachments between kinetochores and microtubules depends on the b56-pp2a phosphatase. *Nature cell biology*, 13(10):1265–1271, 2011. [81](#), [82](#)
- [265] Z. Tang, H. Shu, W. Qi, N.A. Mahmood, M.C. Mumby, H. Yu, et al. PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. *Developmental cell*, 10(5):575–586, 2006. [81](#)
- [266] G. Rabut, V. Doye, and J. Ellenberg. Mapping the dynamic organization of the nuclear pore complex inside single living cells. *Nature cell biology*, 6(11):1114–1121, 2004. [83](#)
- [267] G.J. Mack and D.A. Compton. Analysis of mitotic microtubule-associated proteins using mass spectrometry identifies astrin, a spindle-associated protein. *Proceedings of the National Academy of Sciences*, 98(25):14434–14439, 2001. [84](#)
- [268] U.S. Cho and W. Xu. Crystal structure of a protein phosphatase 2a heterotrimeric holoenzyme. *Nature*, 445(7123):53–57, 2006. [85](#)