


## MEETING REPORT

# Non-lethal message from the Holy Land: The first international conference on nonapoptotic roles of apoptotic proteins

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Apoptosis is a major form of programmed cell death (PCD) that eliminates unnecessary and potentially dangerous cells in all metazoan organisms, thus ensuring tissue homeostasis and many developmental processes. Accordingly, defects in the activation of the apoptotic pathway often pave the way to disease. After several decades of intensive research, the molecular details controlling the apoptosis program have largely been unraveled, as well as the regulatory mechanisms of caspase activation during apoptosis. Nevertheless, an ever-growing list of studies is suggesting the essential role of caspases and other apoptotic proteins in ensuring nonlethal cellular functions during normal development, tissue repair, and regeneration. Moreover, if deregulated, these novel nonapoptotic functions can also instigate diseases. The difficulty of identifying and manipulating the caspase-dependent nonlethal cellular processes (CDPs), as well as the nonlethal functions of other cell death proteins (NLF-CDPs), meant that CDPs and NLF-CDPs have been only curiosities within the apoptotic field; however, the recent technical advancements and the latest biological findings are assigning an unanticipated biological significance to these nonapoptotic functions. Here, we summarize the various talks presented in the first international conference fully dedicated to discuss CDPs and NLF-CDPs and named 'The Batsheva de Rothschild Seminar on Non-Apoptotic Roles of Apoptotic Proteins'. The conference was organized between September 22, 2019, and 25, 2019, by Eli Arama (Weizmann Institute of Science), Luis Alberto Baena-Lopez (University of Oxford), and Howard O. Fearnhead (NUI Galway) at the Weizmann Institute of Science in Israel, and hosted a large international group of researchers.

The meeting was opened by **Eli Arama** with a concise historical overview of the seminal findings around caspases that started to establish the current notion that these evolutionarily conserved proteins can play essential biological roles beyond apoptosis (Fig. 1).

## 1st Session: Nonlethal roles of caspases in the nervous system

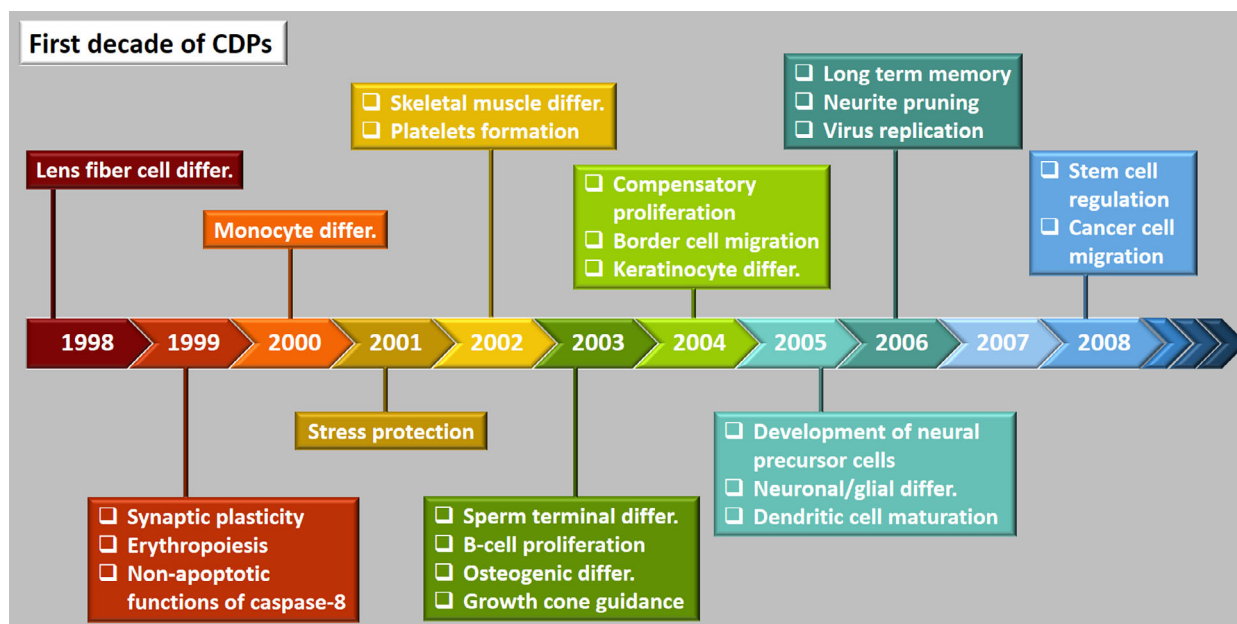
The first session was chaired by **Sarit Larisch** (University of Haifa) and kicked off with two sequential lectures on axon pruning. **Mohanish Deshmukh** (UNC-

Chapel Hill) provided molecular details that allow the activation of the mitochondrial apoptotic pathway in neuronal axons during the process of pruning without causing cell death. Intriguingly, axon pruning following NGF deprivation in *ex vivo* neuronal cultures (using microfluidic chambers that separate between the cell body and the axonal compartments; Fig. 2C,C') requires the activation of JNK signaling and caspases (caspase-9, caspase-3, and caspase-6) without Apaf-1, suggesting a different caspase-9 activation mechanism during axon pruning. Furthermore, axon pruning induces the transcription of multiple BH3 family genes and their spatial localization in the axons that are targeted for pruning. Interestingly, mature neurons undergoing pruning do not express Apaf-1 and specifically upregulate the pan-inhibitor of the BH3 family miR29 in their soma, thus subcellularly restricting the activation of the mitochondrial pathway to the axons. **Avraham Yaron** (Weizmann Institute of Science) reported that inhibition of transcription and translation up until 6 h post-NGF deprivation inhibits axon pruning in DRG neurons. They identified Puma, another BH3-only family gene, in RNA-seq analysis of

the protected axons, and showed, both *ex vivo* (using microfluidic chambers) and *in vivo*, that Puma is required for axon pruning by modulating axonal caspase-3 activity. Additionally, he showed that the restricted exposure of PS in the axons is required for clearance of axonal debris upon fragmentation but not for the degeneration process itself. **Douglas Campbell** (Kyoto University) presented his recent work on axonal arborization and synaptogenesis of retinal ganglion cells in zebrafish. He reported that caspase-8 has a protease-independent role, together with the early endosome protein Rab5c, in restricting axonal arbor growth in the visual system. This study complements earlier findings from his laboratory about localized activities of caspase-9 and caspase-3 in restricting axonal arborization through interaction with Slit-Robo signaling. **Thomas Hummel** (University of Vienna) presented data from *Drosophila* experiments showing that proper innervation of the olfactory glomeruli is controlled by the *Drosophila* orthologs of Apaf-1 (Ark), and caspase-2/caspase-9 (Dronc), as well as the unconventional initiator-like caspase Strica, without the involvement of the so-called effector caspases. Genetic

## Abbreviations

5-FU, 5-fluorouracil; AiP, apoptosis-induced proliferation; ALD, apoptosis-like death; ALS, amyotrophic lateral sclerosis; Apaf-1, apoptotic protease-activating factor 1; Ark, apaf-1-related killer; ARTS, apoptosis-related protein in TGF-beta signaling pathway; ATM, ataxia telangiectasia-mutated; ATP, adenosine triphosphate; ATR, atm- and rad3-related; Bax, bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Bcl2l1, bcl-2-like protein 1; Bcl-xL, B-cell lymphoma-extra large; BCM, border cell migration; BH3, bcl-2 homology domain 3; BID, bcl-2-interacting domain; Bim, bcl-2-interacting mediator of cell death; BOK, bcl-2-related ovarian killer; CAD, caspase-activated DNase; Caspase, cysteine aspartase; CDP, caspase-dependent nonlethal cellular process; CED, cell death protein; cIAP, cellular inhibitor of apoptosis; CIN, chromosomal instability; DAMPs, damage-associated molecular patterns; DAP5, death-associated protein 5; Dcp-1, death caspase-1; DDR, DNA damage response; Diap1, death-associated inhibitor of apoptosis 1; DNase II, deoxyribonuclease II; DRG, dorsal root ganglion; Drice, death-related ice-like caspase; Dronc, death regulator nedd2-like caspase; Drp1, dynamin-related protein 1; Duox, dual oxidase; DUSP, dual-specificity phosphatase; EcR, ecdysone receptor; Egl-1, egg-laying defective 1; eIF4G, eukaryotic translation initiation factor 4 G; EMT, epithelial-mesenchymal transition; EndoG, endonuclease G; ER, endoplasmic reticulum; ESCs, embryonic stem cells; FADD, Fas-associated death domain protein; FAK, focal adhesion kinase; Fbxo7, F-box protein 7; H3K27, methylation of histone H3 on lysine 27; HFSCs, hair follicle stem cells; Hh, hedgehog; Hid, head involution defective; HtrA2, high-temperature requirement A 2; ICAD, inhibitor of CAD; ICM, irradiation-induced cell migration; IP3, inositol trisphosphate; I $\kappa$ B, inhibitor of NF- $\kappa$ B; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KO, knockout; LCD, linker cell death; LINC, linker of nucleoskeleton and cytoskeleton; LPS, lipopolysaccharides; LTA, lymphotoxin alpha; MAC, membrane attack complex; MAP, mitogen-activated protein; Mdm2, mouse double minute 2 homolog; MEFs, mouse embryonic fibroblasts; MFN1, mitofusin-1; MiD, mitochondrial dynamics protein; miR, microRNA; MLKL, mixed lineage kinase domain-like; MMP1, matrix metalloproteinase 1; MOMP, mitochondrial outer membrane permeabilization; mPTP, mitochondrial permeability transition pore; MTCH2, mitochondrial carrier homolog 2; MTs, malpighian tubules; MTS, mitochondrial targeting sequence; Myo1D, unconventional myosin-Id; NE, nuclear envelope; NF- $\kappa$ B, nuclear factor kappa B; NGF, nerve growth factor; NLF-CDPs, nonlethal functions of other cell death proteins; NLRP3, NLR family pyrin domain containing 3; PAMPs, pathogen-associated molecular patterns; PARK15, Parkinson's disease gene 15; PARP, poly (ADP-ribose) polymerase; PCD, programmed cell death; PGCs, primordial germ cells; PI31, proteasome inhibitor 31; PI3K, phosphoinositide 3-kinases; PNS, peripheral nerve system; PS, phosphatidylserine; PtMC5, *P. nonbreakingpacetricornutum* metacaspase 5; Puma, p53 upregulated modulator of apoptosis; Rab, ras-associated binding; RasGAP, Ras GTPase-activating proteins; RIPK, receptor-interacting serine/threonine kinase; Robo, roundabout; ROS, reactive oxygen species; SAMPs, stress-associated molecular patterns; SFK, src family kinase; SIMU, six-microns-under; STAT, signal transducer and activator of transcription; Strica, ser/thr-rich caspase; TDP-43, TAR DNA-binding protein 43; TNF, tumor necrosis factor; TNFR, TNF receptor; TRAIL, TNF-related apoptosis-inducing ligand; TSN, tudor staphylococcal nuclease; UMPS, uridine monophosphate synthetase; VDAC1, voltage-dependent anion-selective channel 1; XIAP, X-linked inhibitor of apoptosis; YAP, Yes-associated protein; Yca1, yeast caspase-1; Zfh-2, zinc finger homeodomain 2; zVAD-fmk, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl] fluoromethyl ketone;  $\Delta\Psi_m$ , mitochondrial membrane potential.



**Fig. 1.** Timeline of the first 10 years of CDP research. This scheme depicts cornerstone discoveries about nonlethal functions of the apoptotic caspases organized by the year of publication of the earliest paper on each topic. These papers, as well as additional prominent papers on the same topics, published within the first decade, are cited herein (if not mentioned otherwise, the CDPs were characterized in mammalian cells). Lens fiber cell differentiation [1,2]; synaptic plasticity [3–5]; erythropoiesis [6–9]; nonapoptotic functions of caspase-8 [10–16]; monocyte differentiation [17–19]; stress protection [20,21]; skeletal muscle differentiation [22,23]; platelet formation [24]; sperm terminal differentiation (*Drosophila*) [25–30]; B-cell proliferation [31]; osteogenic differentiation [32–34]; growth cone guidance (*Xenopus* retina) [35]; compensatory proliferation (*Drosophila*) [36–39]; border cell migration (*Drosophila*) [40]; keratinocyte terminal differentiation [41]; development of neural precursor cells (*Drosophila*) [42,43]; neuronal and glial differentiation [44–46]; dendritic cell maturation [47]; long-term memory (zebra finch) [48]; neurite pruning (*Drosophila*) [49,50]; virus replication (*Caenorhabditis elegans*) [51]; stem cell regulation [52–54]; cancer cell migration [55].

mutant analyses revealed ectopic innervation of unrelated glomeruli due to abnormal axonal pathfinding and synaptic connectivity (pruning and neuronal identity were not affected), hence dramatically rewiring the nervous system and presumably disrupting the proper sensing of environmental cues. **Bertrand Joseph** (Karolinska Institutet) discussed how caspase activity levels regulate different activation modes of microglia in the brain. Low/basal levels of caspase-3 activity, achieved by partial cleavage of this protease, promote pro-inflammatory microglia and consequent neurotoxicity with implications for Parkinson's and Alzheimer's diseases. This is achieved in part through cleavage and activation of protein kinase C- $\delta$ , which leads to I $\kappa$ B kinase complex and subsequent NF- $\kappa$ B transcriptional activity of different pro-inflammatory genes. Full activation of caspase-3 activity, to which he refers as 'the drama', leads to microglial cell death, whereas the S-nitrosylation of the caspase-3 active cysteine residue abrogates its activity and exacerbates pathological

features such as microglia-supported glioma cell migration and invasion. **Kristin White** (MGH/Harvard Medical School) closed this session presenting her recent studies on the gene regulatory mechanisms that determine the precise spatiotemporal pattern of neural stem cell (neuroblast) apoptosis in *Drosophila*. They have previously generated an enhancer (*enh1*)-based reporter of *reaper* family gene expression, which is active specifically in the doomed neuroblasts. Using this reporter, they identified several genetic factors that provide both spatial and temporal cues for neuroblast death. In particular, the activation of Abdominal-A, Grainyhead, and the DNA-binding protein Cut downstream of Notch signaling controls the spatiotemporal activation of key enhancers that regulate the expression of pro-apoptotic factors within the *reaper* locus. At the molecular level, she also reported the repressive/active chromatin state of the *reaper* locus is regulated by Cut through the modulation of cohesins and trimethylated H3K27.

## 2nd Session: Nonlethal roles of caspases in development

This session, chaired by **Yun Fan** (University of Birmingham), included eight talks mostly using *Drosophila*, which illustrated the strong impact that research in this model organism has made on this field. **Ginés Morata Pérez** (Autonomous University of Madrid) presented results illustrating the intriguing links between apoptosis, tumorigenesis, and regeneration in *Drosophila* imaginal discs. His analysis indicated that JNK signaling and the initiator caspase Dronc are crucial to induce cell death and initiate signaling events in healthy cells that stimulate their proliferation through a phenomenon termed apoptosis-induced proliferation (AiP). He also showed the critical involvement of this phenomenon during tissue regeneration. Furthermore, he provided evidence that JNK activation in cells unable to die due to the artificial expression of apoptotic inhibitors promotes tumorigenesis and avoids the elimination of transformed cells through the phenomenon dubbed cell competition. **Hermann Steller** presented an ongoing story about a proteasome-binding protein PI31 that started with seminal experiments performed in *Drosophila* and currently has been proven true in neurodegenerative models of disease in mice. His group identified PI31 as a binding partner of an F-box protein called Nutcracker (the homolog of mammalian Fbxo7/PARK15), which is required for the nonlethal activation of caspases during the terminal differentiation of *Drosophila* spermatids. Subsequent studies in *Drosophila* and mammalian cells revealed that PI31 promotes proteasome activity, while acting as an adapter for neuronal proteasome transport. Accordingly, PI31 knockout (KO) mouse embryos died at mid- to late gestation, and conditional loss of PI31 function disrupted the architecture of synapses, thus leading to compromised motor function. Intriguingly, these phenotypes come together with neuronal loss and become more severe with age, therefore resembling the pathophysiology of several mouse and human neurodegenerative diseases. **Andreas Bergmann** (University of Massachusetts Medical School) expanded on the process of AiP, highlighting remarkably similar mechanisms operating during normal replacement of dying enterocytes in the adult posterior midgut. Similar to their previous findings about AiP in the eye imaginal disc, he showed that dying enterocytes utilize the unconventional myosin Myo1D to subcellularly restrict the activation of Dronc to the basal side of the cells (Fig. 2A). These events then trigger the activation of Duox and subsequent release of reactive oxygen species (ROS). Interestingly, the

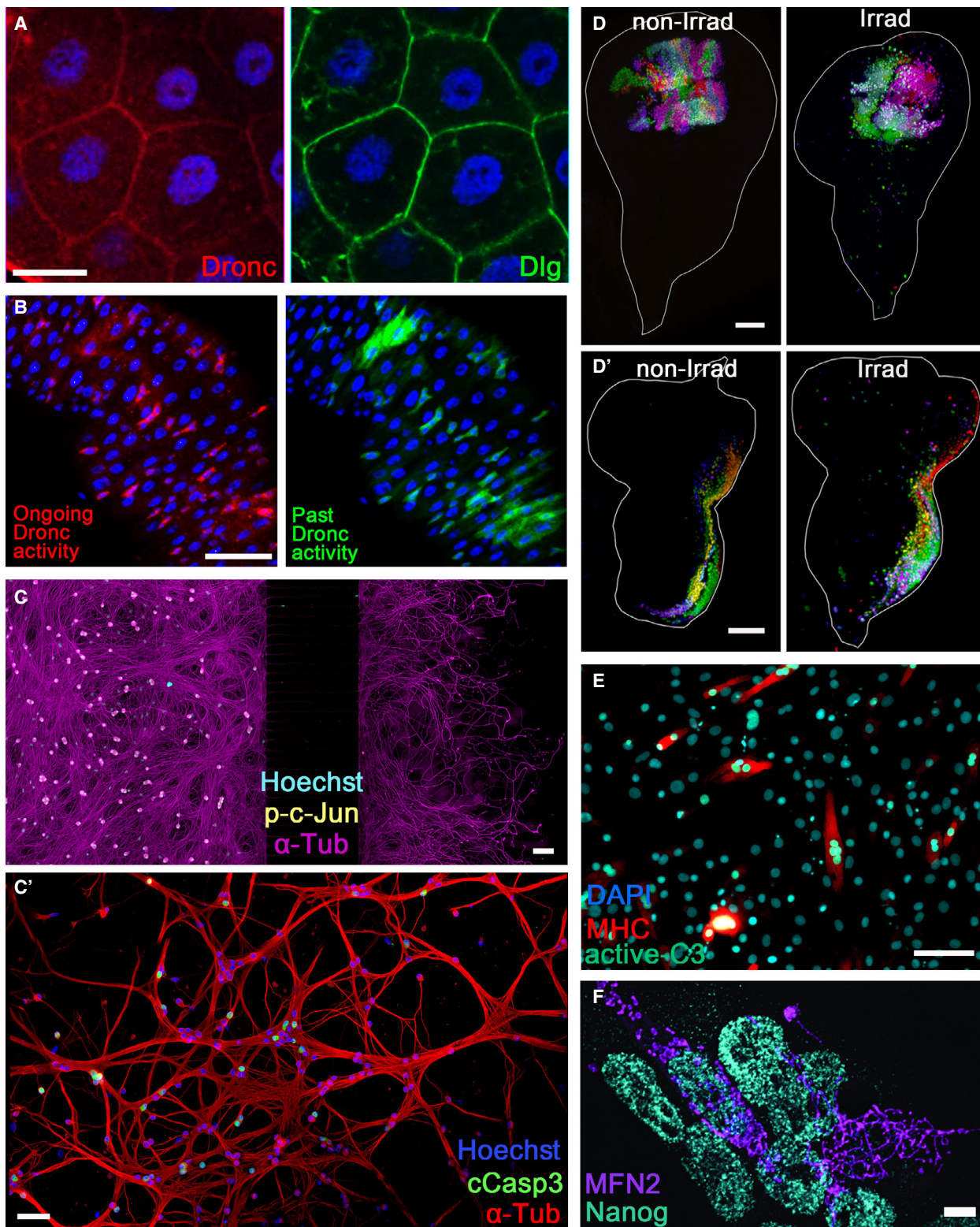
extracellular release of ROS acts as a potent attractant to the damaged intestinal areas of the nearby macrophages. Finally, macrophages can secrete the *Drosophila* TNF superfamily ligand Eiger, which ultimately appears to stimulate the cell proliferation of intestinal precursors for regenerative purposes. **Alessia Galasso** (from the Baena-Lopez laboratory, University of Oxford) presented another physiological paradigm where caspase activation plays a prosurvival instead of apoptotic role. She provided evidence that the *Drosophila* ovarian follicular stem cells under moderate stress activate the caspase pathway in a nonapoptotic manner. This activation is crucial for the fine-tuning of Hh signaling and autophagy through the regulation of the Hh receptor, Patched. Furthermore, caspase activation sustains the proliferation and differentiation of ovarian somatic cells under moderate stress conditions. Importantly, she also showed preliminary data indicating some degree of conservation of these findings in ovarian somatic cells from humans. **Eli Arama** (Weizmann Institute of Science) presented evidence that sublethal (basal) activation of the effector caspases Drice and Dcp-1 inhibits cell migration of *Drosophila* imaginal disc epithelial cells both during normal tissue homeostasis and upon ionizing irradiation (Fig. 2D, D'). Interestingly, the regulation of cell migration upon irradiation relies on the ATR branch of the DNA damage response (DDR) pathway. Furthermore, he provided evidence that tuning down the function of RhoGTPases (Rho1, Rac, and cdc42), which control actin cytoskeleton dynamics and can all be cleaved by caspases, inhibits this process of irradiation-induced cell migration (ICM). He then showed results indicating that deliberate sublethal activation of the effector caspases inhibits normal cell migration of developing primordial germ cells (PGCs) during *Drosophila* embryogenesis. Interestingly, he discovered that a portion of PGCs, which are normally eliminated during this process, activate a DNase II-dependent, nonapoptotic (caspase-independent) form of cell death reminiscent of parthanatos. Following these results, they put forward a yet to be tested, attractive hypothesis, suggesting that nonapoptotic cell death pathways might be the pathways of choice for cells that normally activate CDPs, as these cells require tight control over the levels of caspase activity without compromising the cell sensitivity to undergo PCD during development. **Masayuki Miura** (The University of Tokyo) presented additional information regarding the ability of sublethal levels of caspase activity to inhibit epithelial cell movement. He observed that nonapoptotic caspase activation normally occurs at leading-edge cells during thorax closure in *Drosophila* pupal development.

**Fig. 2.** Representative paradigms of CDPs and NLF-CDPs discussed in the conference. (A) The *Drosophila* initiator caspase Dronc (caspase-2/caspase-9 ortholog) can be localized at the plasma membrane for nonapoptotic functions. For example, shown is the membrane localization of Dronc (red, left), colocalizing with the membrane marker disk large (Dlg; green; right) in salivary gland cells where Dronc has nonapoptotic functions. Nuclei were costained with DAPI (blue). For details, see [56,57]. (B) A *Drosophila* genetic sensor of initiator caspase activity reveals caspase activation signatures without cell death. Ongoing (red; left) and past (green; right) caspase activation in the gut revealed by a sensor of Dronc activity. Note the presence of large green cells (enterocytes) exhibiting caspase activation signature from the past without signs of apoptosis. Nuclei were labeled with DAPI (blue). For details, see [58]. (C) Mouse sympathetic neurons induced to undergo axon pruning in a microfluidic chamber device. Neuronal cell bodies plated in the soma compartment (left) extend their processes through microgrooves into the axon compartment (right). The fluidic separation of the soma and axon compartments allows one to selectively expose only the axons to NGF deprivation to induce axon pruning. Neurons were stained for  $\alpha$ -tubulin (magenta; axons), phospho-c-Jun (yellow; an early signaling event after axonal deprivation of NGF), and Hoechst (cyan; nuclei). (C') Sympathetic neurons undergoing apoptosis. Sympathetic neurons in culture were deprived of NGF to induce apoptosis. Neurons were stained for  $\alpha$ -tubulin (red; axons), cleaved caspase-3 (green), and Hoechst (blue). The images in (C) and (C') were taken by Selena Romero in the Deshmukh laboratory. (D, D') Effector caspase activity potentially inhibits cell migration and invasion of epithelial cells. Shown are 3D projections of *Drosophila* (D) wing and (D') eye-antenna imaginal discs containing multiple fluorescent color clones generated by the *Raeppli* construct and expressed under the *sal-Gal4* driver in the background of the *drice*<sup>-/-</sup> mutant (caspase-3 ortholog). Note the presence of multiple cells which migrated away from the confined *sal* expression domains at 48 h postirradiation (40 Gy X-rays; right panels). Migrating cells are rarely detected in imaginal discs from nonirradiated *drice*<sup>-/-</sup> mutants (left panels) or in imaginal discs from irradiated wild-type larvae (as these cells usually undergo apoptosis 2–4 h postirradiation; not shown). Low levels of effector caspase activity, which are far below the threshold required to induce apoptosis, can potentially inhibit this ICM process. For details, see [59]. (E) Apoptosome-dependent myotube formation involves activation of caspase-3 in differentiating myoblasts. The picture shows caspase activation in myoblasts that have differentiated into myotubes detected with a cell permeable caspase-3 substrate (green; this substrate appears in the nuclei after its cleavage). DAPI labels the nuclei (blue), while cells that express myosin heavy chain (MHC) are red. Note the presence of green nuclei (indicative of caspase activity) in multinucleated myotubes. For details, see [60]. (F) Enforced mitochondrial fusion/elongation is sufficient to drive naïve mouse ESCs to exit naïve pluripotency in both wild-type and *MTCH2*<sup>-/-</sup> mutants. Expression of the promitochondrial fusion protein mitofusin 2 (MFN2; tagged with Myc; magenta) in naïve ESCs induces mitochondria elongation and represses nuclear Nanog expression levels (cyan; anti-Nanog Ab). Note that *MTCH2*<sup>-/-</sup> ESCs fail to elongate their mitochondria, thus leading to expression of naïve pluripotency markers such as Nanog. For details, see [61]. Scale bars in A, B, D, D', 50  $\mu$ m; C, C', E, 100  $\mu$ m; F, 5  $\mu$ m.

Furthermore, consistent with the role of caspases in the inhibition of cell migration during ICM, inhibition of Dronc and the effector caspases led to an increase in thorax closing speed, indicating negative control of basal caspase activity over cell motility. When they wounded the thorax near the closure site, they also observed basal caspase activation in the neighboring cells, although only the inhibition of Dronc but not of the effector caspases increased the wound closing speed. Interestingly, a similar negative control of Dronc over cell migration was previously demonstrated by the Montell group for border cell migration (BCM) during *Drosophila* oogenesis. Together, these findings suggest that there are at least two modes of nonapoptotic negative control of caspases over cell motility: one that requires effector caspase activity (i.e., ICM and thorax closure) and another that depends on the initiator caspase activity alone (i.e., BCM and thorax wound closure). At the molecular level, Dronc negative effect on wound healing mainly relies on its ability to induce the production of ROS, while controlling the intracellular levels of myosin II. **Tin Tin Su** (University of Colorado, Boulder) reported a role of the effector caspases in regeneration after ionizing irradiation. She uncovered the presence of

irradiation-induced cell death-resistant cells in the proximal region of the *Drosophila* wing imaginal disc (wing hinge), which following irradiation, acquire stem cell-like properties and replenish the compromised regions of the wing disc. Interestingly, this property was dependent on the pro-apoptotic protein Hid (but not Reaper)-induced cell-autonomous nonlethal Dronc activation and consequent effector caspase activation. Finally, they identified the downstream effector of the JAK/STAT signaling, the transcription factor Zfh2, as a major component restricting the levels of caspase activation in the hinge, hence preventing apoptosis of the hinge cells while promoting their regenerative behavior following irradiation. The day was concluded by **Sharad Kumar** (University of South Australia), who reported unconventional functions of caspase-2. He and others showed that although not essential for animal development, caspase-2-deficient mice show several subtle phenotypes, including altered basal energy metabolism and premature aging. On the other hand, caspase-2 deficiency protected mice from high fat diet-induced obesity, fatty liver disease, and hyperinsulinemia. These were later attributed to a nonapoptotic function of caspase-2, likely influencing basal metabolism by regulating adipocyte biology and fat





expansion. He then provided compelling data that caspase-2 could have tumor suppressor activity in several murine tumor models. Caspase-2 deficiency increased chromosomal instability (CIN) and aneuploidy in both mouse models and MEFs in culture, and this was independent of the protease catalytic activity. The ability of caspase-2 to limit CIN was explained by two distinct models which might be context-dependent; The Kumar laboratory found that caspase-2 promotes cell death of cells with mitotic defects, whereas others suggested that cytokinesis failure could initiate caspase-2-dependent cleavage of Mdm2, leading to p53 stabilization and cell cycle arrest to prevent polyploidy.

### 3rd Session: Nonlethal roles of apoptotic mitochondrial proteins

This session, which mainly but not exclusively discussed alternative roles of members of the Bcl-2 family proteins, opened the second day and was chaired by **J. Marie Hardwick** (Johns Hopkins University). Marie discussed nonapoptotic functions of the multidomain anti-apoptotic Bcl-2 family member Bcl-xL in healthy cells. Whereas Bcl-xL promotes cell survival by inhibiting outer mitochondrial membrane pores induced by the multidomain pro-apoptotic Bcl-2 family member Bax, the Hardwick group found that in cortical neurons, Bcl-xL and the pro-apoptotic BH3-only protein, BID, can localize to the inner as well as to the outer mitochondrial membranes, and to the endoplasmic reticulum (ER). Consistently, they showed that neurons from Bcl-xL conditional KO mouse brains have increased total ion flux across the inner mitochondrial membrane, which reduces the efficiency of mitochondrial respiration. On the molecular level, they identified in a screen that Bcl-xL directly binds to the  $\beta$  subunit of the  $F_1 F_0$  ATP synthase complex and that this interaction regulates inner-membrane potential by decreasing ion leak within this complex, consequently increasing complex activity, and contributing to enhanced synaptic efficacy. Finally, Marie reported a cooperative function of Bcl-xL and Bax in altering membrane curvature and calcium flux in cells independently of cell death, implying that these proteins can also indirectly regulate the function and topology of other membrane proteins, such as the conductance of channels and transporters. **Sarit Larisch** (University of Haifa) presented her recent work on ARTS, a pro-apoptotic mitochondrial protein that can promote apoptosis by binding and mediating proteasomal degradation of the apoptosis inhibitory proteins, XIAP and Bcl-2. Using MCF10 cell lines and 3D organoid models for normal human morphogenesis of breast

cancer progression, they found that loss of ARTS function could mediate the conversion of normal breast cells to premalignant phenotype. Conversely, restoring the function of ARTS by using an ARTS mimetic small compound (that binds and degrades XIAP but not cIAPs) could promote the reversion of the premalignant cells into normal phenotype. Unexpectedly, however, this tumor-suppressive activity of ARTS did not involve apoptosis although it was associated with an increase in caspase-3 activation, suggesting a nonapoptotic function of caspase-3 in this context. **Germain Gillet** (Université de Lyon) provided molecular details about the regulation of cell migration by the anti-apoptotic Bcl-2 family protein, Bcl-xL. He showed that Bcl-xL silencing was not sufficient to trigger apoptosis, but rather led to impaired migration of mammary cancer cell lines, both *in vitro* and in a zebrafish xenograft model. Interestingly, these migration defects were not inhibited by the addition of zVAD-fmk or BH3 mimetics (which specifically inhibit its anti-apoptotic activity), suggesting that the control of cell migration by Bcl-xL is independent of caspase activity and its interactions with pro-apoptotic Bcl-2 family proteins. He further showed that these promigratory effects depend on Bcl-xL localization in the mitochondria (and not in the ER) and its interaction with the VDAC1 channel. Furthermore, the impaired migration following Bcl-xL silencing was correlated with compromised oxygen consumption and a consequent decrease in mitochondrial ROS production, which were in part attributed to changes in VDAC1 permeability. **Hamsa Puthalakath** (La Trobe University) revealed an unexpected finding that the multidomain pro-apoptotic Bcl-2 family protein, BOK, controls uridine metabolism through its regulation of uridine monophosphate synthetase (UMPS), a key enzyme involved in uridine biosynthesis. As a result, loss of *Bok* confers resistance to 5-fluorouracil (5-FU), an important cancer chemotherapeutic agent. In accordance, cancer cells and primary tissues that acquire resistance to 5-FU downregulate BOK expression. He also showed that loss of *Bok* inhibits 5-FU-mediated p53 induction, although the basal p53 level was elevated. Importantly, loss of *Bok* also slows cell proliferation, which is likely to confer resistance to other cytotoxic chemotherapy whose selectivity relies on targeting proliferating cells. **Liat Hammer** (from the Kimchi laboratory, Weizmann Institute of Science) found that following irradiation, cultured human lung cancer cells (NCI-H460) induce prominent cellular senescence associated with p53 and p21 upregulation, and no caspase activation. Screening for genes that mediate this phenomenon among 97 known components of the

autophagy, apoptosis, and programmed necrosis pathways identified the mitochondrial serine protease HtrA2/Omi (known to mediate both apoptotic and nonapoptotic forms of cell death) as an important mediator of irradiation-induced senescence downstream of p53 induction. Interestingly, senescence induction in these cells was at least in part attributed to the regulation of cytoskeleton dynamics through the proteolytic processing of the intermediate-filament, vimentin, by HtrA2/Omi. **Atan Gross** (Weizmann Institute of Science) described a new role of the mitochondrial carrier homolog 2 (MTCH2) in mediating mitochondrial dynamics. MTCH2 has been originally described by the Gross group as a receptor-like protein for BID. Further studies from the laboratory also identified nonapoptotic functions of MTCH2 in mitochondrial metabolism regulation, such as controlling hematopoietic stem cell quiescence and survival downstream of the ATM kinase/BID pathway. More recently, they found that MTCH2 also plays a role in regulating mitochondrial fusion/elongation, which consequently controls cell response to glucose levels, as well as the exit of mouse embryonic stem cells (ESCs) from naïve pluripotency (Fig. 2F). Molecularly, MTCH2 was shown to colocalize with components of the mitochondrial fission machinery complex MiD49/MiD51/Drp1 and to promote inhibitory phosphorylation of Drp1. MTCH2 was also found to promote mitochondrial fusion by binding to MFN1. Interestingly, immunofluorescence data revealed some overlap between Drp1 and MFN1, raising the attractive hypothesis that MTCH2 might function to coordinate the fusion/fission state of the mitochondria. **György Hajnóczky** (Thomas Jefferson University, PA) described fluorescence-based strategies to resolve local ROS, pH, and  $\text{Ca}^{2+}$  fluctuations at the interface between the ER and the mitochondria. Using this genetically targeted toolkit, they monitored a phenomenon known as ‘flickers’, which refers to transient depolarization of mitochondrial membrane potential ( $\Delta\Psi_m$ ), reflecting transient mitochondrial permeability transition pore (mPTP) opening that occurs both spontaneously and more frequently upon stress. Interestingly, they found that flickers induce oxidative bursts at the adjacent ER–mitochondrial interface, which locally sensitize the intracellular  $\text{Ca}^{2+}$  channels, IP3 receptors (IP3Rs), to release  $\text{Ca}^{2+}$  from the ER. This in turn likely enhances mitochondrial  $\text{Ca}^{2+}$  to stimulate ATP production or further promote mPTP opening, which can contribute to cell signaling mechanisms. This is in contrast to more sustained oxidation of the IP3Rs, which can induce robust  $\text{Ca}^{2+}$  release from the ER, sustained mPTP opening, and the release of pro-

apoptotic factors from the mitochondria. Continuing with noncanonical functions at the interface between neighboring organelles, **Reuven Stein** (Tel Aviv University) reported on the ability of Bax to regulate nuclear envelope (NE) permeability independently of caspases during stress-induced apoptosis. Although permeabilization of the NE during apoptosis is known to depend on caspase proteolytic processing of some of the NE-resident components, such as the nuclear pore complex and the nuclear lamina, the Stein group discovered that Bax can also promote localized NE rupture in the form of transient and repetitive release of nuclear bubbles during early stages of apoptosis. Specifically, he showed data suggesting that Bax promotes subcellular redistribution of nesprin-2 and nesprin-1, components of the linker of nucleoskeleton and cytoskeleton (LINC) complex, and that this effect depends on the mitochondrial targeting sequence (MTS) and the BH3 domains of Bax. He also showed that Bax binds to nesprin-2 and that the interaction occurs in close proximity to perinuclear mitochondria and requires Bax N terminus but not the Bax BH3 domain. **Nikolay Popgeorgiev** (from the Gillet laboratory, Université de Lyon) concluded this morning session with a talk about the origin and evolution of the pleiotropic functions of the Bcl-2 family proteins. They identified four Bcl-2 family members in *Trichoplax adhaerens*, a basal metazoan organism from the phylum Placozoa (Precambrian, 550 mya). Primary structure analysis revealed that all four proteins belong to the multidomain Bcl-2 group, two of which resemble the anti-apoptotic members, while the other two are the Bax and Bak orthologs. Consistently, overexpression of trBax and trBak in HeLa cells and RNA injections to zebrafish embryos could trigger apoptosis, although only trBax could rescue apoptosis induction in *Bak/Bax* KO murine cells. This intriguing functional conservation was not limited to apoptosis, as the anti-apoptotic ortholog trBcl-2l1 was shown to localize to both the ER and the mitochondria, controlling IP3-dependent calcium fluxes and repressing trBax-dependent mitochondrial leakage (MOMP), respectively.

#### 4th and 5th Sessions: Caspase evolution and metacaspases; nonlethal roles of other cell death factors

These sessions were chaired by **Howard Fearnhead** (NUI Galway). The first afternoon session focused on the similarities and differences between caspases (found in metazoa) and metacaspases (found in all



other forms of life except metazoa), both of which belong to the clan C/D family of proteases, although their biochemical properties are highly distinct. **Lynn Megeney** (University of Ottawa) reported on an intriguing functional link between caspases and metacaspases in controlling intracellular proteostasis. Previous studies by the Megeney group and others showed that loss of Yca1, the only type I metacaspase in the yeast *Saccharomyces cerevisiae*, results in an increased retention of insoluble aggregated material and decreased cell fitness, suggesting a role for Yca1 in suppressing protein aggregation. Lynn further showed that this activity of Yca1 requires its monoubiquitination (K355) by the ubiquitin ligase Rsp5. Building on a yeast model of aggregation of TDP-43, a common cytoplasmic inclusion protein in neurons of all ALS-affected individuals, they demonstrated that Yca1 and a yeast-expressed human caspase-3 could both target and disperse the hTDP-43 aggregates. Furthermore, quantitative proteomic analysis of the hTDP-43 yeast model revealed a number of mitochondrial proteins that interact with TDP-43, and these interactions were reversed when caspase-3 was activated. Therefore, both the metacaspases and caspases appear to mediate dispersion of protein aggregates, raising the attractive hypothesis that the robust effector caspase activity in affected and dying neurons of patients with neurodegenerative diseases might be attributed to their primary function in the clearance of toxic protein aggregates. **Assaf Vardi** (Weizmann Institute of Science) discussed the redox control of cell adaption and cell death in algal blooms. Microbial eukaryotes lack the canonical apoptotic genes found in metazoan organisms, including caspases and Bcl-2 family proteins, but do possess metacaspases, and these play a role in adaptive stress responses. Diatoms are a major group of single-celled ocean algae, responsible for about half of marine photosynthesis, through the formation of cycles of massive blooms. Formation and demise of diatom blooms are controlled by the availability of inorganic nutrients and light, and by biotic interactions with grazers, bacteria, and viruses. Under nutrient and biotic stress conditions, diatoms produce bioactive compounds, such as oxylipins, which can act as chemical defense mechanisms, but are also lethal in high doses, through the induction of a signaling pathway, involving  $\text{Ca}^{2+}$  transients, NO production, and mitochondrial glutathione pool oxidation. Interestingly, the Vardi group identified an unconventional,  $\text{Ca}^{2+}$ -dependent, metacaspase in the diatom model *Phaeodactylum tricornutum*, PtMC5, which mediates cell death in response to elevated oxylipin levels, and is regulated by oxidation of a unique cysteine pair.

They thus suggest that PtMC5 may be a major key in understanding the significance of PCD in bloom dynamics. **Peter Bozhkov** (The Swedish University of Agricultural Sciences) highlighted the fact that developmental PCD in plants differs drastically from PCD in animals, due to the anatomical and molecular distinctions between these organisms. For example, plant cells are surrounded by a rigid cell wall that is often not degraded upon execution of developmental PCD, but is left as a structural component of a tissue, while the dying cells are cleared by endogenously forming lytic vacuoles. On the molecular level, unlike caspases, metacaspases function as monomers, usually require  $\text{Ca}^{2+}$ , and cleave their substrates after arginine and/or lysine, thus are likely to mediate a different form of PCD. Consistent with this idea, although a few proteins were found to be cleaved by both caspases and metacaspases, such as the tudor staphylococcal nuclease (TSN), metacaspases were found to mediate vacuolar PCD in several plant species, including in the embryo suspensor of the Norway spruce tree, acting upstream to autophagy to prevent dying cells from early collapse and necrosis.

The second afternoon session was opened with a presentation by **J. Marie Hardwick** (discussed above) followed with a talk by **Adi Kimchi** (Weizmann Institute of Science), discussing a nonapoptotic role of the death-associated protein 5 (DAP5) in human embryonic stem cells (hESCs). DAP5 was previously identified in the Kimchi laboratory in a screen for death-promoting genes. It is an eIF4G translation initiation factor member and was shown to drive cap-independent translation of apoptotic proteins. Adi showed that DAP5 silencing prevents differentiation induction in hESCs, as reflected among other parameters by sustained expression of Nanog and Oct4. Polysomal profiling of DAP5-deficient hESCs, as well as DAP5 immunoprecipitation followed by deep RNA sequencing, revealed dual (opposite) effect of DAP5 on translation of selective mRNAs during the transition from pluripotency to differentiation; DAP5 promotes cap-independent translation of mRNAs indirectly through binding to additional translation factors, while it suppresses translation of other mRNAs through direct binding. Interestingly, DAP5 and many of its directly bound mRNAs were found to localize to stress granules, suggesting a role of DAP5 in retaining untranslatable mRNAs, such as those coding for pluripotency proteins, in stress granules. **Hanna Engelberg-Kulka** (Hadassah-Hebrew University Medical Center) explained the PCD mechanisms of bacteria. Her group previously identified a toxin-antitoxin system module dubbed *mazEF* in *Escherichia coli*, in which the toxin

MazF is an endoribonuclease induced under stress conditions. MazF activity promotes two opposite effects; it leads to cell death of most of the population by inhibiting bulk protein synthesis, but it also promotes survival of a small subpopulation by promoting stress-induced translation of selected mRNAs. She then discussed another cell death pathway, called ALD, mediated by the DNA damage response coordinator LexA-RecA in *E. coli*. Interestingly, as opposed to the *mazEF* system, which is induced by a variety of stresses and is dependent on the density of the population, the ALD-mediated cell death pathway is DNA damage-dependent only and is cell density-independent. The *mazEF* system was found to generally inhibit the ALD pathway, demonstrating how different PCD pathways interact to regulate cell survival and death at the levels of the population and the individual cell, with implications for cell fate choices and the evolution of altruism. **Lazaros Vasilikos** (from the Wei-Lynn Wong laboratory, University of Zurich) concluded this session by revealing an unexpected function of cIAP1 in cancer cell metastasis. He showed that mice injected with a Smac mimetic (which inhibits IAPs), as well as mice mutant for *ciap1* but not *ciap2*, displayed decreased lung tumor load following intravenous injection of cancer melanoma cells. This effect does not occur following subcutaneous injection of the melanoma cells, suggesting a role of cIAP1 in promoting tumor cell metastasis rather than growth *per se*. Interestingly, cIAP1 loss in the endothelial tissue, and not in the hematopoietic system, decreased lung tumor load by making the endothelium less permeable to the extravasation of the melanoma cells. This nonapoptotic function of cIAP1 is negatively regulated by TNFR2 (and not TNFR1) and its ligand lymphotoxin alpha (LTA; and not TNF), which is secreted by the melanoma cells. These findings may have important implications for the use of Smac mimetics against tumor extravasation and metastasis to the lung through the TNFR2/cIAP1 endothelial axis.

## 6th Session: Noncanonical immune cell mechanisms

The third day of the meeting was opened with a session focusing on the connections between the immune system and cell death proteins during apoptosis and nonapoptotic processes, and which was chaired by **Luis Alberto Baena-Lopez** (University of Oxford). The first presenter of the day was **Estee Kurant** (University of Haifa) who used the *Drosophila* adult brain as a model for testing the idea that increased glial phagocytosis may prevent neurodegeneration. This hypothesis

is based on the observed correlation between aging-dependent decline of glial phagocytosis ability and neuronal dysfunction. Unexpectedly, however, overexpression of the phagocytic receptors six-mircons-under (SIMU) and Draper (Drpr) in adult *Drosophila* glia caused neuronal loss and consequent motor dysfunction and a shortened life span, implying that excessive phagocytosis (as opposed to deficient phagocytosis) might also promote neurodegeneration. Interestingly, these hyperphagocytic glia did not trigger neuronal apoptosis but rather engulfed live neurons in a mechanism mediated by PS. These findings highlight an important aspect in neurodegeneration, namely that tight regulation of the glial cells is essential for neuronal survival and proper brain function. **Seamus Martin** (Trinity College, Dublin) discussed the mechanisms that trigger inflammation following cell stress. Whereas inflammation is known to initiate through detection of microbial components (pathogen-associated molecular patterns or PAMPs) or through necrosis-mediated release of intracellular contents (damage-associated molecular patterns or DAMPs), the mechanisms that lead to chronic inflammation responses in pathological conditions, such as cancer, obesity, and neurodegeneration, have been more obscure. The Martin group hypothesized that these pathological conditions might be associated with tissue stress, which is known to provoke cell stress responses, such as the unfolded protein response following ER stress, and consequent inflammation. Interestingly, they showed that ER stress could initiate inflammation through transcriptional upregulation and ligand-independent activation of TRAIL receptors. This in turn leads to caspase-8/FADD/RIPK1-dependent NF- $\kappa$ B activation and inflammatory cytokine production. These findings revealed nonapoptotic roles of the death receptors as 'stress-associated molecular patterns (SAMPs)' driving NF- $\kappa$ B-dependent inflammation in response to cell stress and could be key to interpret and manage the inflammatory response linked to chronic diseases. **Yun Fan** (University of Birmingham) presentation solved an old puzzle regarding TNF-induced cell death in *Drosophila*. Previous studies showed that eye disc overexpression of the sole homolog of TNF in *Drosophila*, called Eiger (Egr), results in severe eye ablation and that this phenotype was not suppressed by coexpression of the effector caspase inhibitor p35, albeit it was readily suppressed by Dronc inhibition. Moreover, the Fan group showed that although Egr triggers apoptosis in the eye discs (revealed by cleaved effector caspase staining, and through suppression of the eye ablation in *hid* mutants and upon Diap1 overexpression), no suppression was detected in effector caspase

mutant flies where active caspase staining was abrogated. The riddle began to unravel when they found that the Egr-expressing cells continue dying upon effector caspase inhibition, as they switch to a non-apoptotic necrotic-like cell death pathway, which is similar to the apoptotic pathway, and is also dependent on JNK and the catalytic activity of Dronc. Interestingly, imaginal disc cells that are mutant for *scribble*, a tumor suppressor gene regulating cell polarity, also triggered a similar necrotic cell death pathway when effector caspase activity was compromised, suggesting that Egr can trigger a two-layered defense system for oncogenic growth inhibition. **Dror Mevorach** (Hadassah-Hebrew University Medical Center) discussed the involvement of the complement membrane attack complex (MAC) in pathological axonal degradation of peripheral nerve system (PNS) neurons. The MAC plays a role in host defense processes by

forming transplasma membrane channels/pores on the surface of pathogenic bacteria, where ions and small molecules can pass, bringing about osmotic lysis of the foreign cells. Interestingly, spontaneous MAC activation is known to occur in different human cells, but self-cell lysis is usually avoided in part through the expression of several MAC inhibitors, such as the membrane protein CD59. Previous work from the Mevorach group and others revealed several mutations in the human *CD59* gene, which are associated with chronic hemolysis and recurrent episodes of Guillain-Barré Syndrome-like disease, where the body's immune system mistakenly attacks part of its PNS. The molecular mechanisms behind this were proposed to involve MAC assembly in myelin sheath gaps of myelinated axons (also known as the nodes of Ranvier) following any viral infection, leading to inflammation-mediated acute motor axonal neuropathy. The session was



**Fig. 3.** Representative photographs taken during the conference scientific program and associated events. (A) The David Lopatie Conference Centre at the Weizmann Institute of Science. (B) Dinner for the speakers who arrived in the evening before the conference started. (C) Left to right: Eli Arama, Howard Fearnhead, Alberto Baena, and Lynn Megeney. (D) Left to right: Sharad Kumar, Masayuki Miura, Shai Shaham, and Kristin White. (E) During the poster session. (F) Left to right: Hermann Steller, J. Marie Hardwick, and Adi Kimchi. (G) A cup of coffee with foam drawing featuring The FEBS Journal. (H) Andreas Bergmann. (I) One of the groups during the tour in the old city of Jerusalem. (J) Left to right: Eva Svandova, Abigail Tucker, and Douglas Campbell (walking behind) wondering in the alleys of the old city. Photographs (A), (B), (I), and (J) were taken by Eli Arama; (C), (D), (E), and (F) by Ohad Herches; (G) by Seamus Martin; and (H) by Itai Belson.

concluded by **Shai Shaham** (Rockefeller University, New York), who discussed the mechanisms governing linker cell death (LCD) in *Caenorhabditis elegans*. LCD occurs in the absence of all *C. elegans* caspases or other apoptosis genes, and is mediated by a signaling pathway consisting of MAPKK, the heat-shock transcription factor, and components of the ubiquitin/proteasome pathway. Accordingly, LCD morphology is distinct from classical apoptosis, characterized by lack of chromatin condensation, a crenellated nucleus, and swelling of organelles. The dying linker cell is engulfed by two neighboring cells which split the cell into two parts. Both the engulfment and degradation of the dying linker cell are also distinct from that of apoptotic cells, involving RAB-35 and ARF-6 GTPases. Linker cell degradation, but not killing, requires the apoptotic caspase, CED-3, and the Apaf-1 homolog, CED-4, but not the BH3-only protein, Egl-1, within the dying cell, which could explain the frequent detection of non-cell death essential caspase activity during developmental cell death in mammals. Shai concluded his talk with the provoking hypothesis that a related form of cell death could be at least as prevalent as apoptosis during mammalian development.

After this session, the scientific program ceased for the rest of the day, during which the conference attendees went on a guided tour in the old city of Jerusalem, followed by a gala dinner in a local traditional restaurant. Selected informal photographs taken at the conference venue and during the associated events are shown in Fig. 3.

## 7th Session: New roles of caspases and cell death

The morning session of the last day of the scientific program was chaired by **Benjamin Weaver** (UT Southwestern Medical Center, TX) and dedicated to lethal and nonlethal roles of caspases. **Magali Suzanne** (Université Toulouse III—Paul Sabatier) opened the session describing an active morphogenetic role of apoptosis during development. Previous studies from her laboratory showed that fold progression in the *Drosophila* leg imaginal disc follows stereotypical spreading of apoptosis. The dying epithelial cells generate apico-basal forces that deform neighboring cells, resulting in local increase of tissue tension and consequent folding. The force-producing machinery consists of a dynamic actomyosin contractile cable, connecting the cell apical surface to the nucleus, which itself is relocalized basally through anchoring to basal adhesions. When looking at non-cell death processes which

involve epithelial–mesenchymal transition (EMT; i.e., induction of cell delamination in the leg disc or the embryonic invagination into the ventral furrow), the Suzanne group found that the initiation of EMT involves a similar apico-basal force generated by myosin II cables. Therefore, key developmental morphogenetic events appear to involve similar generators of mechanical forces. **Christian Widmann** (University of Lausanne) explained his discovery that cleavage of the p120 RasGAP by caspase-3 can generate both prosurvival and prodeath signals, depending on the degree of stress experienced by the cell. RasGAP bears two cleavage sites with very different susceptibilities toward caspase-3-mediated proteolytic attack. Under a mild stress, low levels of active caspase-3, insufficient to cleave classical apoptotic substrates (e.g., PARP and ICAD), efficiently cleave RasGAP, releasing an N-terminal fragment, which stimulates Akt-dependent survival pathways. Mice with point mutation in that cleavage site showed decreased ability to cope with various stresses, displaying increased cell death and reduced Akt activity. A second, less sensitive, caspase-3 cleavage site in the N fragment is cleaved only following intense stress, thus terminally abrogating its capacity to activate Akt. Interestingly, mass spectrometry revealed 37 additional protein substrates which were similarly cleaved under a low stress, although this could be an underestimate, as RasGAP was not detected in this analysis, suggesting that the method is not sensitive enough. **Abigail Tucker** (King's College London) took us into the world of hard tissues and described the roles of apoptotic proteins in the development of bone and cartilage. Comparing PCD patterns with that of caspase expression during the skeletal system development indicated that multiple pro-apoptotic caspases are dynamically expressed in the forming bone and cartilage, in areas not associated with apoptosis. Inhibition of individual caspases in different relevant cell cultures led to specific molecular changes, including downregulation of CD36 (involved in bone resorption and mineralization), supporting roles of caspases in cell differentiation. Indeed, caspase-7 KO mice phenocopy long bone defects observed in the CD36 KO mice. Furthermore, inhibition of caspase-2 and caspase-8 in explant cultures of the Meckelian cartilage (an intermediate structure in the development of the embryonic mammalian mandible) led to persistence of structure, presumably by affecting differentiation of macrophages to chondroclasts that are involved in the resorption of the calcified cartilage. **Luis Alberto Baena-Lopez** (University of Oxford) showed new findings illustrating the diverse and tissue-dependent activity of caspases as regulators of stem



cell function in *Drosophila* organs. His data indicated that transient, nonapoptotic caspase activation in the *Drosophila* gut is critical to prevent premature differentiation of gut enteroblasts, whereas in the ovary it facilitates the proliferation and differentiation of somatic precursors. He linked this dual role of caspases with their ability to intersect the activity of specific signaling pathways in each cellular context. Whereas caspase activation in the gut appears to limit activation levels of Notch signaling, caspase function in the ovary enhances Hh signaling through fine-tuning of its receptor Patched. Reinforcing the nonapoptotic nature of such caspase activities, he then showed that although the Dronc sustained caspase functions in both of the cellular scenarios, the effector caspases were dispensable in the gut (initiator caspase activity is detected with a new genetic tool; an example is shown in Fig. 2B). Continuing with studies in *Drosophila*, **Madhu Tapadia** (Banaras Hindu University) discussed nonlethal functions of apoptotic proteins in the Malpighian tubules (MTs), the functional analogs of the mammalian kidneys. During metamorphosis, the steroid hormone ecdysone instructs the histolysis of the larval structures by PCD, but intriguingly despite expressing the entire repertoire of apoptotic genes and the ecdysone receptor (EcR), the MTs avoid this fatal fate, displaying no dying cells while maintaining fixed numbers of cells throughout life. Survival of these cells is attributed in part to nuclear rather than cytoplasmic localization of the *reaper* family genes and the caspase zymogens, and to specific modulators of the ecdysone pathway. Interestingly, mild enhancement of the *reaper* family genes or caspase expression, as well as inactivation of the effector caspases, both cause deformed MT morphology, altering arrangement, shape, and function of the MT cells. However, how exactly caspase activity promotes MT integrity and development is unclear, although loss of caspase activity in these cells was associated with irregular microtubule arrangement, increased MMP1, and decreased Na<sup>+</sup>/K<sup>+</sup> pump expressions, which could explain the observed phenotypes. The session was concluded by **Howard Fearnhead** (NUI Galway), who provided new molecular insights on the involvement of the mitochondrial death pathway in the regulation of muscle differentiation. Studies from the Fearnhead group and others demonstrated the involvement of caspase-2, caspase-9, and caspase-3 activation in myoblast fusion and differentiation *in vitro*. However, whether this requirement for caspase activity is cell-autonomous or that apoptotic myoblasts trigger differentiation in neighboring healthy myoblasts remained controversial. Howard presented conclusive data showing that the differentiating

myoblasts themselves produce caspase-3 activity (Fig. 2E). Compromising apoptosome formation by targeting Apaf-1 or cytochrome *c* markedly decreased myoblast fusion and differentiation. Furthermore, inhibiting the intrinsic pathway upstream of mitochondrial permeabilization, that is, by targeting caspase-2 and Bid, resulted in a more profound effect on differentiation, although the reduction in active caspase-3 was similarly affected. Since caspase-2 was found to cleave and activate Bid in this cellular scenario, these findings suggest a hierarchical model in which caspase-2 and Bid play additional roles controlling myogenesis besides activation of the mitochondrial pathway.

## 8th Session: Caspase roles in cell signaling

The last session of the meeting was chaired by **Lynn Megeney** (University of Ottawa) and highlighted the roles of caspases in cell signaling. **Dwayne Stupack** (UC San Diego, CA) discussed the crosstalk between caspase-8 and the cytoskeleton, and its regulation by oncogenic Src family kinase (SFK) signaling. Caspase-8, the initiator caspase of the extrinsic apoptotic pathway, has been also implicated in several nonlethal cellular functions, such as cell proliferation, fate, differentiation, and migration. Interestingly, caspase-8 was shown to promote cell migration and metastasis of neuroblastoma cells compromised for caspase-3 (to suppress apoptosis). Molecularly, caspase-8 is frequently associated with the leading edge of lamellae and with activated SFKs. Within focal adhesion complexes, procaspase-8 is associated with calpain-2 and FAK, promoting calpain activation, which in turn cleaves many cytoskeletal- and adhesion-related proteins, including Talin, leading to increased active integrins on the cell surface. Procaspase-8 can be also phosphorylated on several tyrosine residues, of which Y380 is particularly interesting, as it decreases susceptibility to apoptosis and promotes cell migration, either via activation of PI3K signaling and/or the small GTPase Rac. Given the multiplicity of cellular effects that are elicited by caspase-8, these findings begin to clarify why tumor cells that exhibit elevated caspase-8 are associated with worse prognosis. **David Wallach** (Weizmann Institute of Science) highlighted the fact that although ample *in vitro* data indicate a central role for caspase-8 in the extrinsic apoptotic pathway and as a potent inhibitor of the signaling for necroptosis, the exact *in vivo* roles and possible significance of the extrinsic pathway and necroptosis remained controversial. This confusion is derived from *in vivo* studies of caspase-8 KO mice, displaying

pleiotropic effects, which could not firmly be attributed to the known *in vitro* functions. Furthermore, the effector of the necroptotic death, mixed lineage kinase domain-like (MLKL), also serves several nonlethal functions. For example, the RIPK1/RIPK3/MLKL necroptotic pathway can mediate LPS-induced assembly and function of the NLRP3 inflammasome in caspase-8-deficient dendritic cells. MLKL also associates with endosomes, indirectly controlling signaling by facilitating trafficking/degradation of receptors and ligands. Significantly, this constitutive function occurs independently of RIPK3 and necroptosis, although RIPK3 can enhance MLKL association with endosomes. **Chuan-Yuan Li** (Duke University, NC) unraveled the molecular mechanisms underlying the tumorigenesis-promoting effect of sublethal levels of caspase-3, caspase-6, and caspase-7 in human and mouse cells. Caspases are usually considered as tumor-suppressive because of their central roles in apoptosis. Counterintuitively, however, increasing evidence suggests that caspases can also support and promote cancer progression in a variety of tumor cells *in vitro* and *in vivo*. Chuan-Yuan showed that this nonlethal activity of caspases is the consequence of low level of cytochrome c leakage from the mitochondria that occurs in many tumor cells without any stress. Sublethal caspase activity then induces nonlethal activation of apoptotic endonucleases, such as CAD and EndoG, which in turn cause DNA damage and activation of the DDR pathway. ATM, a central DDR component, was shown to activate NF- $\kappa$ B and Stat3, which are important in maintenance of cancer cell tumorigenicity and stemness. Interestingly, these studies, which reveal the dark side of the DDR, correspond with Eli Arama's presentation, showing that irradiation triggers the DDR and consequent epithelial cell migration and invasion in *Drosophila* with reduced caspase activity. **Yaron Fuchs** (Technion—Israel Institute of Technology) discussed the nonlethal functions of caspases in the skin. The group previously reported that caspase-3 is expressed in proliferating cells of the mouse sebaceous gland without inducing apoptosis. Their findings indicate that caspase-3 can cleave  $\alpha$ -catenin, which is known to sequester YAP, resulting in the release and nuclear translocation of YAP, and consequent cell proliferation and organ size increase. Seeking for additional nonapoptotic roles of caspases in this system, the group now found that caspase activation can have a strong nonautonomous effect by governing the behavior of neighboring cells. They showed that apoptotic cells can release mitogenic factors, which drive proliferation and expansion of neighboring cells. These mechanisms are highly reminiscent of the AiP

phenomenon in *Drosophila* (see also above the lectures by Morata and Bergmann) and could potentially contribute to tissue repair and tumorigenesis. **Benjamin P. Weaver** (UT Southwestern Medical Center, TX) expanded on the interplay between caspase (CED-3) activity and p38 MAP kinase (PMK-1) signaling in *C. elegans*. Screening for genes that become essential when CED-3 is inactivated, identified *vhp-1*, the homolog of DUSP, which similar to its mammalian counterpart functions to inhibit PMK-1. Loss of both *vhp-1* and *ced-3* led to a much more substantial delay in larval development than loss of each gene alone. This effect was attributed to increased PMK-1-dependent epidermal expression of multiple stress-response factors, such as antimicrobial peptides and genes regulating stress adaptation. Accordingly, CED-3-positive effect on development was shown to be exerted by direct cleavage and inactivation of PMK-1, suggesting that CED-3 and PMK-1 oppose each other to balance developmental and stress-responsive gene expression programs. **Olivier Julien** (University of Alberta) concluded this last session discussing approaches to address what emerged as another major theme of the meeting: the challenge of identifying caspase substrates and understanding how proteolysis of particular proteins could be mapped to specific fate choices. The strengths and limitations of several different methodologies were presented with particular emphasis on an N-terminomics approaches. He mentioned that the number of substrate targets identified for individual caspases can vary widely, ranging from a few targets for caspase-4, caspase-5, caspase-9, and caspase-14, to hundreds of targets for caspase-1, caspase-2, caspase-3, caspase-6, caspase-7, and caspase-8. Furthermore, different substrates display different cleavage kinetics. It is clear that integrating the proteomic data describing caspase-dependent proteolysis with experiments investigating how caspase induces different cellular fates is extraordinarily challenging.

## Concluding remarks

The meeting stressed the intriguing versatility of many, and perhaps all apoptotic proteins, shown by their central roles in both cell death and disassembly as well as a remarkably diverse range of nondeath processes. This versatility is not limited to a particular component of a cell death pathway playing a nondeath role; large parts of molecular pathways typically described as 'death pathways' in fact also induce noncell death outcomes. Paradoxically, many of these functions facilitate cell survival, cell proliferation and differentiation, cell migration, intracellular signaling, and the release

of signaling molecules, which ultimately control a diverse repertoire of cellular and physiological functions essential for ensuring the development and tissue homeostasis. Furthermore, it is becoming clear that the deregulation of these CDPs and NLF-CDPs could contribute decisively to the initiation and progression of multiple diseases. At the meeting, there were indications (including compartmentalizing and limiting protein activity) of how cells can utilize the same molecular machinery to elicit a range of responses, but there is still much to discover before we can use these novel functions for therapeutic gain.

Together, these findings suggest the intriguing possibility that perhaps some of the nonlethal functions of apoptotic proteins and pathways, initially thought unconventional and odd, could constitute their primary role and that their destructive functions evolved only later with a need to efficiently eliminate either unnecessary or faulty cells. Further experimental work is needed to confirm this hypothesis. Whether true or not, at this stage it is clear that the promiscuity of the 'death' proteins means that there may be no dedicated 'cell death machinery' with a single function. Which raises intriguing questions: Can a quantitative description of the activity of these proteins and pathways reveal how and when different cell fates are elicited? What are the regulatory mechanisms which maintain such proteins under sublethal thresholds of activation?

The success and need for such meetings were shared by all of the participants during the closure events, and a new meeting is expected as a continuation of the initiative in the years coming, which will continue illuminating the bright side of what has been previously characterized as deadly proteins.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work.

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