The nuclear envelope and its involvement in cellular stress responses

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Abstract

The nuclear envelope is not only important for the structural integrity of the nucleus, but is also involved in a number of cellular functions. It has been shown to be important for maintaining and controlling chromatin organization, sequestering transcription factors, replication, transcription and signaling. The nuclear envelope thus is important for development, differentiation and some of its components are essential for cell viability. Among the many functions which are emerging for the nuclear envelope is its involvement in protecting the cell against different types of cellular stress. Here we review key findings which describe the roles of nuclear envelope components in responses to common types of stress conditions.

Introduction

The nuclear envelope (NE) is a structure composed of the outer and inner nuclear membranes, an inter-membrane space bridged by protein complexes, and an underlying meshwork of nucleoplasmic proteins, the nuclear lamins. Nuclear lamins contribute to the structural integrity of the nucleus and are classified into A and B-type lamins. A-type lamins are developmentally regulated and are expressed in differentiated cells, while at least one B-type lamin is expressed in all vertebrate cells. Nuclear lamins are associated with other components of the NE such as MAN1, lamin B receptor, emerin, nesprins and lamina-associated polypeptide-1. The NE is also pierced by nuclear pore complexes, which are involved in the nuclear import and export [1]. In addition to its role in cellular compartmentalisation, the NE is involved in various cellular processes including chromosome organisation, DNA replication, transcription, apoptosis, mechanotransduction, mitosis and is a platform for various signalling events [2-5]. Through these mechanisms and others, the NE is also involved in cellular responses to stress. The importance of the NE and its roles in cellular stress responses are highlighted by the laminopathies, a group of diseases that result from mutations in genes coding for its components. In this brief review we will discuss the involvement of the NE components in cellular responses to common stress conditions.

Heat shock

Exposure of cells to elevated temperatures induces a stress response that involves synthesis of proteins, some of which are nuclear, that are involved in protecting the cell. Although the distribution of the nuclear envelope components LAP2β, emerin, lamin A/C and lamin B are not altered as a result of mild or sever hyperthermia some differences in expression levels and distribution are observed during recovery from severe heat shock [6]. Emerin levels are reduced following long term recovery from mild heat shock while lamin B levels increased within 40 min of severe heat shock [7] and following 20 hours of recovery [6]. Despite these observations, no correlation between lamin B levels and short term survival following heat shock were reported and it is therefore thought that lamin B might be important for long term recovery

[7]. The latter may be important to mediate gene expression changes and chromatin rearrangements necessary for recovery from stress.

Expression of αB-crystallin has been reported to increase following various stress conditions including heat stress where the protein also translocates from the cytoplasm to the nucleus [8]. In the nucleus, αB-crystallin colocalises with nucleoplasmic lamin A/C speckles. Since peripheral lamin A/C is disrupted upon heat shock, αB-crystallin might play a role in the formation and stabilization of lamin A/C nuclear speckles. Although the exact mechanisms by which the NE and its components contribute to heat shock responses are still unknown the presence of fragmented nuclei in fibroblasts from Dunnigan-type familial partial lipodystrophy patients following heat shock suggests that they play an important role [9, 10].

Oxidative stress

We have previously reported that lamin B1 is important for cellular response to oxidative stress. Cells lacking a fully functional lamin B1 were found to harbor elevated levels of reactive oxygen species (ROS) and to be more susceptible to oxidative stress [11]. We found that this was mediated by the transcription factor Oct-1, which is normally sequestered at the nuclear envelope by lamin B1, and that loss of this sequestration leads to changes in Oct-1 target genes, some of which are involved in cellular response to oxidative stress. Elevated levels of ROS have also been reported in fibroblasts from lipodystrophy patients with *LMNA* mutations and cells in which prelamin A accumulation is induced using HIV protease inhibitors [12]. The exact mechanism by which lamin A/C contributes to oxidative stress responses is probably a complicated one since cells from a FPLD patient with R439C mutation in *LMNA* were found to have similar basal ROS levels to healthy control cells, but higher levels following conditions of induced oxidative stress [13].

Intracellular free calcium (Ca^{++}) is an important second messenger involved in a range of processes including response to oxidative stress [14]. Elevated ROS levels can alter Ca^{++} homeostasis through plasma membrane proteins, mitochondria and intracellular Ca^{++} channels [15]. Elevated ROS levels

induced by sub-lethal levels of tert-butyl hydroperoxide (tBHP), cause an increase in nucleoplasmic release of Ca^{++} mediated by IP3R2s located within the NE without having an effect on Ca^{++} release from the ER into the cytosol [16].

Several antioxidant enzymes are important for the cellular response to oxidative stress and elevated ROS levels. These include catalase (CAT), glutathione peroxidase (GPX) and glutathione transferases (GSTs) [17, 18]. Although the overall levels of most of these enzymes are not altered under oxidative stress conditions, the local concentrations of GST, CAT and GPX are increased up to seven times by electrostatically associating with the outer nuclear membrane to form a so-called nuclear shield [19]. This results in increased DNA protection against ROS. It is therefore possible that disruption of the perinuclear regions in diseases such as Alzheimer's, Huntington's and Parkinson's may interfere with the nuclear shield arrangement and contribute to the pathologies of these diseases [20].

In addition to the above, oxidative stress triggers a response that affects transport into and out of the nucleus. Mild oxidative stress using diethyl maleate (DEM) inhibits classical nuclear import by altering the intracellular distribution of importin-α and CAS [21]. Oxidative stress can also reduce nuclear export through altering the binding of the importin-β family member chromosome region maintenance-1 (Crm1) to several nucleoporins and Ran [22].

Osmotic stress

The NE can form invaginations and a network of intranuclear structures collectively known as the nucleoplasmic reticulum which has been recently reviewed elsewhere [23]. CCTα is an enzyme that associates with the NE and promotes the proliferation of the nucleoplasmic reticulum [24]. MDCK cells are known to differentiate under hypertonic conditions. Under these conditions CCTα redistributes from a soluble nucleoplasmic to a focal pattern. Some of these CCTα foci associate with lamin A/C speckles while some don't. It is thought that the lamin A/C associating foci may act as store for inactive CCTα while the lamin A/C free foci preserve the enzyme for phospholipid

synthesis. These observations suggest a role for CCTα, the nucleoplasmic reticulum and lamin A/C in differentiation of MDCK cells under hypertonic conditions [25] and recovery from osmotic stress.

A novel stress protein involved in stress responses has been identified in fission yeast. It is called Ish1 (induced in stationary phase), and localizes to the NE and plasma membrane and is elevated under glucose starvation and osmotic stress [26]. Ish1 was also found to interact with another novel nuclear protein, Bis1, which is a homologue of the ES2 proteins found in mammalian cells. Both Ish1 and Bis1 are important for cell viability during stress but their functions are still unknown.

DNA damage response

DNA damage is a common event that takes place under several stress conditions including exposure to genotoxic agents, chemotherapeutic agents and irradiation [27]. Cells from patients with mutations in NE components are often characterized by genomic instability. Cells from HGPS patients for example have defective DNA repair, have high levels of basal DNA damage and are more susceptible to DNA damaging agents [28, 29]. They show a delayed recruitment of 53BP1 to γH2AX DNA repair foci. These cells also have impaired recruitment of Rad50 and Rad51 to sites of double strand breaks [29, 30]. Cells from *Lmna*$^{-/-}$ mice also have increased levels of basal DNA damage and defects in repair by non-homologous end joining (NHEJ) [31]. Therefore lamin A and its processing are both required for genomic stability and the ability to cope with genotoxic stress.

Mechanical stress

The NE provides structural support and is therefore important for cellular responses to mechanical stress. One of the proposed pathological mechanism of NE-associated diseases is that mutations leading to disruption of the nuclear lamina result in increased nuclear fragility in mechanically stressed tissues, e.g. muscle. Nuclei of cells lacking lamin A (*Lmna*$^{-/-}$) frequently rupture even under low pressure and have decreased cytoskeletal stiffness [32]. Recently, repeated non-lethal nuclear rupture in cells carrying

mutations in the lamin A/C gene was reported [33]. The ERK and JNK pathways are abnormally activated in cardiac cells from *Lmna*$^{H222P/H222P}$ mice. This super-activation affects normal cardiac function and is thought to contribute to the onset of cardiomyopathy [34]. Therefore, NE abnormalities can lead to physical weakness and fragility but may also lead to impaired mechano-transduction and mechanical stress related signaling.

Mechanisms in NE stress responses

Although the *in vitro* and *in vivo* evidence reveals clear links between the NE and the ability of the cell, and ultimately the entire organism, to respond to stress, the mechanisms by which these responses occur remain almost entirely unknown. Biochemical observations in cells from patients with laminopathies and after therapeutic drug treatment implicate specific proteins in control of stress responses, but rarely identify the signaling pathways involved.

Signaling via reversible post-translational modifications of the proteins of the NE represents one obvious possible mechanism, with phosphorylation as perhaps the leading contender. The issue has been clouded by high levels of mitotic phosphorylation occurring at multiple sites in both A-type and B-type lamins [2]. Recently we have obtained evidence for stress-induced interphase phosphorylation of lamin B1, initially using mass spectrometry and subsequently a phosphopeptide specific antibody in cell cycle selective flow cytometry experiments (Malhas and Vaux, submitted). Whether this will emerge as a general mechanism remains to be seen, but it raises the possibility that the NE plays yet another role in the cell, that of a stress-response signaling platform.

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