The nuclear envelope and its involvement in cellular stress responses

Ashraf N. Malhas and David J. Vaux

Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

Abstract
The nuclear envelope is not only important for the structural integrity of the nucleus, but 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 signalling. The nuclear envelope is thus important for development and 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. In the present paper, we review key findings which describe the roles of nuclear envelope components in responses to common types of stress conditions.

Introduction
The NE (nuclear envelope) 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, whereas 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 LAP (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 compartmentalization, the NE is involved in various cellular processes, including chromosome organization, DNA replication, transcription, apoptosis, mechanotransduction and 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 the present brief review, we 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 severe 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 h 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 co-localizes 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 FPLD (Dunnigan-type familial partial lipodystrophy) patients following heat shock suggests that they play an important role [9,10].

Oxidative stress
We have reported previously that lamin B1 is important for cellular response to oxidative stress. Cells lacking a fully functional lamin B1 were found to harbour elevated levels of ROS (reactive oxygen species) 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 (lamin A/C gene) 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 an R439C-encoded mutation in LMNA were found to have similar basal ROS levels to those of healthy control cells, but higher levels following conditions of induced oxidative stress [13].

Intracellular free Ca\textsuperscript{2+} is an important second messenger involved in a range of processes including response to oxidative stress [14]. Elevated ROS levels can alter Ca\textsuperscript{2+} homeostasis through plasma membrane proteins, mitochondria and intracellular Ca\textsuperscript{2+} channels [15]. Elevated ROS levels induced by sublethal levels of tBHP (t-butylhydroperoxide) cause an increase in nucleoplasmic release of Ca\textsuperscript{2+} mediated by IP\textsubscript{3},R2s (inositol triphosphate receptors type 2) located within the NE without having an effect on Ca\textsuperscript{2+} release from the ER (endoplasmic reticulum) into the cytosol [16].

Several antioxidant enzymes are important for the cellular response to oxidative stress and elevated ROS levels. These include catalase, GPX (glutathione peroxidase) and GSTs (glutathione transferases) [17,18]. Although the overall levels of most of these enzymes are not altered under oxidative stress conditions, the local concentrations of GST, catalase 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 disease 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 DEM (diethyl maleate) inhibits classical nuclear import by altering the intracellular distribution of importin-\(\alpha\) and CAS (cellular apoptosis susceptibility protein) [21]. Oxidative stress can also reduce nuclear export through altering the binding of the importin-\(\beta\) family member Crm1 (chromosome region maintenance-1) 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]. CCT\(\alpha\) (CTP:phosphocholine cytidylyltransferase \(\alpha\)) is an enzyme that associates with the NE and promotes the proliferation of the nucleoplasmic reticulum [24]. MDCK (Madin–Darby canine kidney) cells are known to differentiate under hyperosmotic conditions. Under these conditions, CCT\(\alpha\) redistributes from a soluble nucleoplasmic to a focal pattern. Some of these CCT\(\alpha\) foci associate with lamin A/C speckles, whereas some do not. It is thought that the lamin A/C-associating foci may act as store for inactive CCT\(\alpha\), whereas the lamin A/C free foci preserve the enzyme for phospholipid synthesis. These observations suggest a role for CCT\(\alpha\), 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 1), 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 (Hutchinson–Gilford progeria syndrome) 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 (p53-binding protein 1) to γH2AX (phosphorylated histone H2AX) DNA repair foci. These cells also have impaired recruitment of Rad50 and Rad51 to sites of double-strand breaks [29,30]. Cells from Lmna\textsuperscript{−/−} mice also have increased levels of basal DNA damage and defects in repair by NHEJ (non-homologous end-joining) [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. The nuclei of LMNA\textsuperscript{−/−} cells frequently rupture even under low pressure and have decreased cytoskeletal stiffness [32]. Recently, repeated non-lethal nuclear rupture in cells carrying mutations in the LMNA gene was reported [33]. The ERK (extracellular-signal-regulated kinase) and JNK (c-Jun N-terminal kinase) pathways are abnormally activated in cardiac cells from Lmna\textsuperscript{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 mechanotransduction and mechanical stress-related signalling.

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 the control of stress responses, but rarely identify the signalling pathways involved.

Signalling 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 MS and subsequently a phosphopeptide-specific antibody in cell-cycle-selective flow cytometry experiments (A.N. Malhas and D.J. Vaux, unpublished work). 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 signalling platform.

Funding

We gratefully acknowledge the Medical Research Council for funding [grant number G0801917].

References


©The Authors Journal compilation ©2011 Biochemical Society


Received 8 September 2011
doi:10.1042/BST20110719