Minireview

The functional importance of lamins, actin, myosin, spectrin and the LINC complex in DNA repair

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Impact statement

Proteins in the nucleoskeleton, lamins, actin, myosin, and spectrin, have been shown to play critical roles in DNA repair. Deficiencies in these proteins are associated with a number of disorders. This review highlights the role these proteins and their association with the LINC complex play in DNA repair processes, their mechanism of action and the impacts deficiencies in these proteins have on DNA repair and on disorders associated with a deficiency in these proteins. It will clarify how these proteins, which interact with "classic DNA repair proteins" (e.g., RAD51, XPF), represent another level of complexity in the DNA repair process, which must be taken into consideration when carrying out mechanistic studies on proteins involved in DNA repair and in developing models for DNA repair pathways. This knowledge is essential for determining how deficiencies in these proteins relate to disorders resulting from loss of functional activity of these proteins.

Abstract

Three major proteins in the nucleoskeleton, lamins, actin, and spectrin, play essential roles in maintenance of nuclear architecture and the integrity of the nuclear envelope, in mechanotransduction and mechanical coupling between the nucleoskeleton and cytoskeleton, and in nuclear functions such as regulation of gene expression, transcription and DNA replication. Less well known, but critically important, are the role these proteins play in DNA repair. The A-type and B-type lamins, nuclear actin and myosin, spectrin and the LINC (linker of nucleoskeleton and cytoskeleton) complex each function in repair of DNA damage utilizing various repair pathways. The lamins play a role in repair of DNA double-strand breaks (DSBs) by nonhomologous end joining (NHEJ) or homologous recombination (HR). Actin is involved in repair of DNA DSBs and interacts with myosin in facilitating relocalization of these DSBs in heterochromatin for HR repair. Nonerythroid alpha spectrin (αSpII) plays a critical role in repair of DNA interstrand cross-links (ICLs) where it acts as a scaffold in recruitment of repair proteins to sites of damage and is important in the initial damage recognition and incision steps of the repair process. The LINC complex contributes to the repair of DNA DSBs and ICLs. This review will address the important functions of these proteins in the DNA repair process, their mechanism of action, and the profound impact a defect or deficiency in these proteins has on cellular function. The critical roles of these proteins in DNA repair will be further emphasized by discussing the human disorders and the pathophysiological changes that result from or are related to deficiencies in these proteins. The demonstrated function for each of these proteins in the DNA repair process clearly indicates that there is another level of complexity that must be considered when mechanistically examining factors crucial for DNA repair.

Keywords: Lamin A/C, lamin B, actin, myosin, spectrin, nonerythroid alpha spectrin, LINC complex, DNA double-strand break repair, nonhomologous end joining, homologous recombination, DNA interstrand cross-link repair, telomeres, laminopathies, Fanconi anemia, nucleoskeleton

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Introduction

The nucleoskeleton is a major structural and functional network of interacting proteins within the nucleus which is critical for maintenance of nuclear shape, mechanical properties and virtually every essential function in the nucleus.[1] It is comprised of lamins, lamin-binding proteins, nuclear actin, nuclear myosins, spectrins, kinesins, titan, nuclear pore-linked filaments, the nuclear mitotic apparatus and proteins associated with the LINC (linker of cytoskeleton and nucleoskeleton) complex.[1] Three of its major proteins, lamins, actin, and spectrin, are best known for their roles in

maintaining nuclear architecture and the integrity of the nuclear envelope, and in mechanotransduction and mechanical coupling between the nucleoskeleton and cytoskeleton.[1–12] They also take part in essential nuclear functions such as gene expression, regulation of transcription, cell signaling and DNA replication.[1,4–6,8] Less well known and less extensively studied are the critical roles these proteins play in DNA repair.

Maintenance of the integrity of nuclear DNA is essential for genomic stability and preservation of fundamental cellular functions, particularly after exposure to endogenous and exogenous damaging agents such as ultraviolet light, ionizing radiation, genotoxic chemical agents, and byproducts of metabolic activity. Specialized DNA repair systems have evolved to maintain accurate genomic information.[13–15] DNA repair proteins involved in specific repair pathways are available for detecting and repairing different types of DNA lesions.[13–15] Of major importance, and not as widely recognized, is that nucleoskeletal proteins also play important, often critical roles in the DNA damage response in specific DNA repair pathways. These proteins include: lamins, spectrins, nuclear actin, nuclear myosin and the LINC complex.[1,5,16–21] Evidence for the importance of these proteins in various DNA repair pathways is demonstrated in human disorders in which there is a deficiency or defect in the presence or expression of these proteins. This is particularly evident in inherited cancer predisposition disorders, neurological diseases and premature aging syndromes.[21–28] The impact these proteins have on repair processes, therefore, needs to be seriously considered and taken into account when carrying out mechanistic studies on protein interactions critical to repair of damaged DNA. This is another tier in the complexity of the DNA damage response and one that needs serious attention in order to fully understand the critical impact these proteins have on the repair process, particularly in disorders in which a deficiency in these proteins leads to such severe pathological consequences. This review will concentrate on a number of the nucleoskeletal proteins which function in DNA repair, their roles and mechanism of action in this process and the disorders which are associated with a deficiency/defect in each of them.

Lamins and their function in DNA repair

The nuclear lamins are intermediate filaments which are key components of the nuclear lamina, a filamentous protein meshwork underlying the inner membrane of the nuclear envelope. They contribute to the nuclear architecture and maintenance of the shape and mechanical stability of the nucleus.[4,7,12,27–33] They also extend throughout the nucleoplasm and function as a scaffold for tethering chromatin and proteins involved in a number of important nuclear functions including transcription, replication, and DNA repair.[1,4,5,8,31,33] In mammalian cells, there are two major types of lamins, A-type lamins and B-type lamins.[1,4,31–36] Expression of these proteins varies with developmental stage and cell and tissue types, hence deficiencies or defects in the lamins can differentially affect various organ systems at different stages of development.[36,37]

One function of nuclear lamins of significant interest, and in which numerous studies have been carried out, is their role in DNA repair. Mutations in the lamin A gene, *LMNA*, have been linked to a number of degenerative disorders, referred to as laminopathies. These include muscular dystrophies, lipodystrophies, neuropathies, and premature aging diseases, all of which are associated with defects in DNA repair.[26–28,36,38] The role that A-type lamins play in the DNA damage response, particularly the molecular mechanisms they utilize in repair of DNA double-strand breaks (DSB), is being actively investigated since this is the type of repair pathway which is defective in a number of these disorders. The importance of this repair pathway and its link to the pathophysiological changes observed in these disorders is discussed below.

A-type lamins and B-type lamins: Important components of the DNA damage response

Of the myriad of different types of damage that can occur to DNA, one of the most cytotoxic is the double-strand break (DSB) since the integrity of the DNA helix is disrupted when breaks are formed on both DNA strands. Inefficient or inaccurate repair of this type of lesion can result in mutations, chromosomal rearrangements and cell death. In mammalian cells, there are two major pathways involved in repair of DNA DSBs, non-homologous end-joining (NHEJ) and homologous recombination (HR).[39–47] In NHEJ, the two broken ends are fused together with only a little or no homology or processing.[42,47] This is the major pathway for repair of non-replication associated DSBs and can take place throughout the cell cycle, but is more prominent during the G0 and G1 phases (mainly outside of the S and G2 phases).[38,44,45,47–52] It is considered to be relatively error-prone.[40,42,45,47,48] In contrast, HR occurs predominantly in S and G2 phases of the cell cycle and uses the sister chromatids generated during DNA replication to function as templates in the repair process.[39,41,45,46,49] It has a high-fidelity of DNA repair and is considered to be error-free.[39,41,46] NHEJ is a faster and more efficient mechanism of repair of DNA DSBs than HR but has a much lower level of fidelity.[46–48] There are two additional pathways that can be utilized in repair of DNA DSBs: alternative end-joining (A-NHEJ), which involves MRN, DNA end resection and ligation of DNA ends with microhomology; and single-strand annealing (SSA).[53–59] Both of these methods require varying degrees of DNA end resection and usage of sequence microhomologies and are considered to be minor pathways that may act as a backup system when either of the other two systems is compromised and not capable of repairing breaks.[53–59] Deficiencies in these DNA DSB repair pathways can lead to genomic instability, genetic abnormalities, premature aging, cancer and a large number of human disorders.[18,46,47,54,59,60]

Lamins have been shown to play an important role in repair of DNA DSBs.[16–18,38,61] The A-type lamins are encoded by the *LMNA* gene and include lamin A and lamins C1 and C2, which are alternative splicing products of this gene.[35–37] The B-type lamins include lamin B1, which is encoded by the *LMNB1* gene, and lamins B2 and B3,

which result from alternative splicing of the *LMNB2* gene.[18,34,37,62] While B-type lamins are expressed during all stages of development, A-type lamins are expressed only after the onset of cell differentiation.[18,35,37,62] Numerous lines of evidence have shown that both types of lamins are essential for regulation of the NHEJ and/or the HR pathways involved in DNA DSB repair.[16–18,37,38,61] This has been demonstrated by studies in which loss or a deficiency of these lamins has been found to be correlated with defects in repair of DNA DSBs and with genomic instability and by examination of genetic diseases in which there are mutations in the *LMNA* or *LMNB* genes.[28,63,64] A-type lamins (lamins A/C) are also thought to be important in the repair response to DNA ICLs at the time of DNA replication. A deficiency in lamins A/C leads to decreased restart of stalled replication forks occurring after exposure to a DNA interstrand cross-linking (ICL) agent.[28,63,64] Repair of DNA ICLs, which involves homologous recombination, needs to occur before the replication fork can proceed.[28,63,64] These studies point to an important role for lamins in maintenance of genomic stability after DNA damage.

Lamins A/C play a dual role in DNA DSB repair

A particularly interesting question in repair of DNA DSBs is what factors govern the choice of repair pathway which will be used (i.e. NHEJ or HR). Numerous studies have been carried out and are still ongoing to address this. The stage of the cell cycle has been shown to have a major influence on the choice of repair pathways; whereas NHEJ occurs throughout the cell cycle, HR requires that cells be in the S or G2 phase of the cell cycle.[49,58] Thus the phase of the cell cycle in which damage occurs is an important factor in selection of the repair mechanism utilized. Studies suggest that 53BP1 (involved in NHEJ) and BRCA1 (involved in HR) play critical roles in determining which of these two pathways will be utilized by mutually affecting the recruitment of each other to the site of a DSB.[49,57,58] 53BP1 and its protein binding partners inhibit localization of BRCA1 at sites of DNA DSBs in G1 phase.[49,58] BRCA1 is needed in HR for facilitating resection of the 5′ ends of DNA at the site of the break and recruitment of RAD51 to the 3′ ends, where it is involved in homology search and strand invasion during HR repair.[43,44,46,49,58,65–67] Inhibition of BRCA1 localization leads to suppression of HR while promoting NHEJ.[65–68] In contrast, BRCA1 and its associated proteins promote the removal of 53BP1 in S and G2 phases of the cell cycle.[44,49,66,69] 53BP1 is needed for recruitment of proteins involved in NHEJ. In this way, removal of 53BP1 and inhibition of NHEJ allow end resection and HR to proceed.[44,49,66,69] Thus the relative abundance of a particular protein (i.e., 53BP1 or BRCA1) involved in either NHEJ or HR could be important in the choice of repair pathways.[50,57] In addition, studies indicate that the chromatin environment may play a role in regulation of choice of the repair pathway at the site of the DSB, for example whether heterochromatin or transcriptionally active regions of chromatin are present at the DSB.[58]

Lamins A/C have been shown to be important in DNA DSB repair, functioning in both NHEJ and HR and regulating the crosstalk between these repair pathways and the factors involved in them.[5,18,38,61] They have an impact on levels of 53BP1, which is important in NHEJ. They bind to 53BP1 and promote its stability and nuclear retention.[17,61] A model has been proposed in which lamins A/C maintain a nucleoplasmic pool of 53BP1 so as to facilitate its recruitment to sites of DSBs (Figure 1).[17] After damage, 53BP1 dissociates from lamins A/C and can take part in the repair process and in recruitment of proteins involved in NHEJ.[17] The heterodimer, Ku70/Ku80, is recruited and binds to sites of DNA DSBs. DNA-PK forms a complex with Ku70/Ku80 and participates in the end bridging process along with additional proteins involved in NHEJ (Figure 1).[17] In addition, it has been proposed that 53BP1 can also inhibit localization of BRCA1 to sites of DSBs and decrease HR repair in G1 phase.[17,49,61] It may also play a role in inhibition of an alternate method of NHEJ (A-NHEJ, discussed above) involving microhomology end processing (Figure 1).[17,61,70] Depletion of lamin A/C reduces the stability of 53BP1.[17,61] In its absence, 53BP1 can undergo degradation by the ubiquitin-conjugating enzyme H7 (UbcH7) or be cleaved by an endosomal/lysosomal protease, cathepsin L (CTSL).[18,38,61,67] This leads to a deficiency in NHEJ, thus indicating that lamins A/C have an important function in this mode of repair of DNA DSBs.[18,38]

Lamins A/C also play a role in HR after DNA DSB damage (Figure 2).[61] They are important in transcriptional regulation and increased expression of BRCA1 and RAD51, two key proteins essential for HR.[5,61] They inhibit repressor complexes, p130/E2F4, that mediate down regulation of BRCA1 and RAD51.[61] Loss of lamins A/C leads to lack of inhibition of p130/E2F4 and transcriptional repression of the BRCA1 and RAD51 genes leading to decreased expression of BRCA1 and RAD51.[5,61] Recent studies have

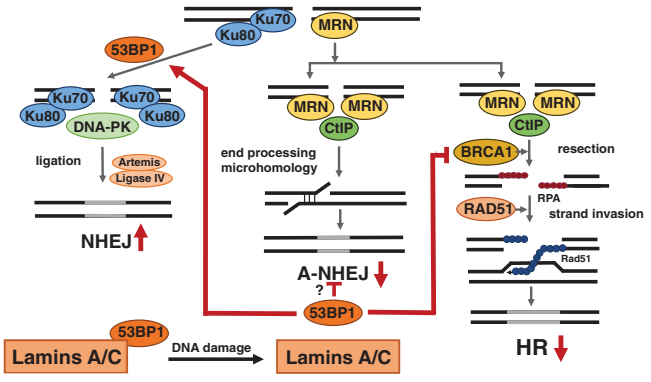

Figure 1. A model for the role of lamins A/C in repair of DNA DSBs by non-homologous end joining (NHEJ). In the cell, lamins A/C bind to 53BP1 to promote its stability. After production of DNA DSBs, 53PB1 dissociates from lamins A/C and takes part in the NHEJ repair process by recruiting proteins involved in this DNA repair pathway. It recruits the heterodimer, Ku70/Ku80, which binds at sites of DSBs. DNA-PK forms a complex with Ku70/Ku80 and participates in the end bridging process along with several other proteins involved in NHEJ. 53BP1 can also inhibit localization of BRCA1 to sites of DSBs and decrease homologous recombination (HR) repair in G1 phase. It may also play a role in inhibition of an alternate method of NHEJ (A-NHEJ) involving microhomology end processing. This model is based on studies and models proposed by a number of investigators.[17,49,61,70] (A color version of this figure is available in the online journal.)

also shown that lamins A/C directly interact with RAD51 and protects it from proteasome-mediated degradation.[71] It has been speculated that RAD51 may undergo a post-translational modification (e.g. phosphorylation) after formation of DNA DSBs which enables it to mobilize to sites of damage (Figure 1).[71] These studies suggest that lamins A/C regulate levels of RAD51 in the cell in the absence of DNA DSBs and provide a reserve of RAD51 for use in HR.[71] Thus lamins A/C can regulate repair of DNA DSBs by HR in two different ways: by transcriptional regulation of BRCA1 and RAD51 and by binding to RAD51 and protecting it from proteasomal degradation.[71] In addition, increased expression of BRCA1 promotes the removal of 53BP1 in S and G2 phases of the cell cycle, leading to decreased NHEJ (Figure 2).[44,49,66,67]

A-type lamins, therefore, play dual roles in the determination of choice of pathways utilized in repair of DNA DSBs. They are important for: (1) NHEJ by regulating levels of 53BP1 in the nucleus, and (2) HR by transcriptionally regulating levels of BRCA1 and RAD51 in the nucleus and by protecting RAD51 from proteasomal degradation.[18,38,61] Determination of exactly how lamins A/C mechanistically interact with proteins involved in these two different pathways for repair of DNA DSBs should significantly aid in the elucidation of the underlying mechanisms involved in choice of repair pathways and their roles in maintenance of genomic stability.

Role of lamin A in mechanosensing protects cells against DNA damage

A relationship has been found between lamin A, cellular stress and DNA damage.[72] Lamin A accumulates early and progressively in tissue such as heart, lung and bone.[73–75] and is highest in adult tissues that are mechanically

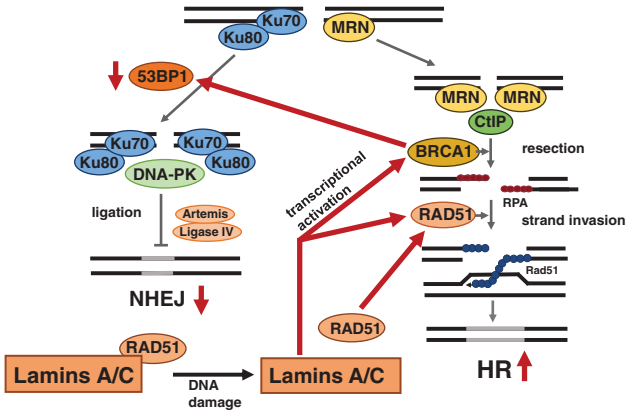

Figure 2. A model for the role lamins A/C play in repair of DNA DSBs by HR. In the cell, lamins A/C bind to RAD51 to promote its stability and protect it from proteasome-mediated degradation. After formation of DNA DSBs, RAD51 is speculated to undergo post-translational modification and be released from lamins A/C which enables it to mobilize to sites of damage and enhance HR.[71] Lamins A/C are involved in HR where they play an important role in transcriptional regulation and increased expression of BRCA1 and RAD51, both key proteins essential in this repair pathway. In addition, increased expression of BRCA1 promotes the removal of 53BP1 in S and G2 phases of the cell cycle leading to decreased NHEJ. This model is based on studies and models proposed by a number of investigators.[44,49,61,66,67,71] (A color version of this figure is available in the online journal.)

stressed, stiff and rich in an extracellular matrix (ECM).[76] As tissues develop, they sustain higher physical stress due to stiffening of the ECM or high actomyosin contractility. Lamin A has been proposed to have mechanosensing ability for detection of these cellular stresses.[72] It has very recently been shown by Cho *et al.* that as embryonic hearts develop they stiffen and strengthen and lamin A accumulates to counter nuclear rupture from the increased stress.[72] Rupture of the nuclear envelope leads to loss of repair factors, which results in increased DNA damage and cell cycle arrest.[72] These studies have demonstrated that decreased levels of lamin A favor nuclear rupture upon physical stress in embryonic cardiac cells and that this leads to mis-localization of DNA repair proteins, such as K80 and 53BP1, to the cytoplasm and an increase in DNA damage, as determined by examining formation of DNA DSBs.[72] It also leads to an increase in loss of telomeres and in cell-cycle arrest[72] It has been proposed that this increase in lamin A after stress is an adaptive response and that lamin A increases during development or after stress to mechano-protect the genome.[72] This mechano-coupled accumulation of lamin A begins in early development and persists into maturation of tissues; this can be an important component in a broad range of diseases in which there are deficiencies in lamin A.[72]

Lamin A is an activator of SIRT6 and stimulates SIRT6-mediated DNA repair

SIRT6 is a member of the sirtuin family of proteins that has a broad range of functions related to aging.[77–79] In mammalian cells, SIRT6 is found in the nucleus; it catalyzes both deacylation and mono-ADP ribosylation and is involved in a spectrum of biological processes which include histone deacetylation, telomere maintenance, DNA repair and genomic stability.[77,80–83] SIRT6 is critical in regulation of the aging process and longevity.[77,80,83,84] It mediates DNA DSB repair via the HR and NHEJ pathways.[82,85,86] Studies on regulation of the activity of SIRT6 have been ongoing, particularly those on the regulation of its activity in repair of DNA damage. It has recently been reported that lamin A can activate SIRT6 and facilitate chromatin localization of SIRT6 upon DNA damage.[79,85,86] Lamin A has been shown to directly interact with SIRT6 and promote SIRT6-mediated deacetylation of histones; it facilitates the localization of SIRT6 to chromatin, and promotes SIRT6 mediated repair of DNA DSBs.[79] After production of DNA DSBs, lamin A aids in SIRT6-dependent recruitment of DNA-PKcs (DNA-PK catalytic subunit) to chromatin, which is involved in NHEJ repair.[79] Lamin A also facilitates SIRT6-mediated deacetylation of CtIP, a protein important for promotion of end resection of DNA DSBs, and its recruitment to sites of DSBs during HR repair.[79] Thus lamin A serves as an endogenous activator of SIRT6 and promotes SIRT6-mediated DSB repair by both HR and NHEJ.

Lamin A/C disorders with DNA repair defects

The role of lamins A/C in DNA DSB repair is further highlighted by disorders in which there are mutations in

the *LMNA* gene that lead to a wide spectrum of disease phenotypes.[5,26–28,61,63] These degenerative disorders, caused by mutations in genes encoding nuclear lamins, are referred to as laminopathies and include muscular dystrophies, lipodystrophies, cardiomyopathies, peripheral neuropathies, and premature aging syndromes such as Hutchinson Gilford Progeria Syndrome (HGPS) and Atypical Werner Syndrome (AWS).[26–28,38,63,87–92] HGPS is among the most severe of these disorders.[26–28,38,89] In most HGPS cases, a mutation in the *LMNA* gene leads to a deletion of 50 amino acids in lamin A resulting in a protein named progerin.[89,91–93] Expression of progerin elicits a number of cellular changes which include nuclear morphological abnormalities, chromatin changes, defective gene expression, chromosomal aberrations, defective repair of DNA DSBs leading to accumulation of elevated basal levels of DNA damage, accelerated telomere shortening and attrition, and premature senescence.[28,61,63,64,89,93–95] Results similar to this have been observed in human and mouse cells with an $LMNA^{-/-}$ genotype, supporting the importance of lamins A/C in maintaining genomic stability.[61,64,71,86]

In HGPS the increase in the basal level of DNA damage is due to deficiencies in both HR and NHEJ.[18,28] Studies carried out on fibroblasts from both HGPS patients and from mouse models of progeria, in which there is loss of expression of lamin A and accumulation of progerin, have enhanced our understanding of the mechanisms that contribute to defects in DNA repair and an increase in genomic instability. These studies have demonstrated that there are defects in recruitment of proteins involved in NHEJ (53BP1) and HR (Rad50, Rad51) to sites of DNA damage (DSBs).[28,63,96] HGPS patient-derived fibroblasts exhibit reduced expression of DNA protein kinase (DNA PK) and associated proteins (Ku70/Ku80) involved in the initial steps of NHEJ.[97] Aberrant accumulation at sites of DNA DSBs of a protein involved in nucleotide excision repair, xeroderma pigmentosum complementation group A (XPA), has also been reported.[96] These studies show that this mislocalization occurs at stalled or collapsed replication forks where there is a concurrent significant loss of proliferating cell nuclear antigen (PCNA), which is needed for DNA synthesis and progression of the replication fork.[98,99] The loss of PCNA could be due to enhanced binding of progerin to PCNA compared to lower levels of binding of lamin A to PCNA.[98] It has been suggested that this sequestration of PCNA by progerin many allow binding of XPA at the replication fork and promote a series of signaling cascades involved in arrest of cell proliferation and promotion of replication fork collapse.[96,98] Both of these factors have been suggested to contribute to premature senescence in these cells.[96,98] Delayed recruitment of proteins in the MRN complex (NBS1 and MRE11), which is involved in the initial steps of HR and aids in the resection of the 5′ ends at the site of a DSB, has also been reported.[100] In addition, recent studies have shown that progerin does not stimulate SIRT6 activity in HGPS cells to the same extent that lamin A does in normal human cells.[79] The functions of SIRT6 in HGPS fibroblasts are significantly impaired following DNA damage, which indicates that this may be a contributing factor in the pathogenesis of HGPS.[79] All of these studies indicate that a number of different mechanisms underly the genomic instability observed in HGPS and that these are the consequence of mutations or alterations in the lamin A gene which hinder the ability of HGPS cells to recruit and retain proteins needed in the DNA damage response for maintenance of genomic integrity.[18,38]

Deficiencies in DNA DSB repair in HGPS occur not only in genomic DNA but also in telomeric DNA. Lamins A/C have been shown to play a key role in maintenance of telomere structure, length and function.[101] In HGPS, dysfunctional telomeres arise due to loss of lamins A/C.[101] Increased shortening of telomeres has been observed in HGPS fibroblasts and fibroblasts from *LMNA*-deficient mice.[101] Studies have shown that lamins A/C interact with TRF2, a core protein of the shelterin complex which binds to telomeric DNA and protects telomeres from loss or shortening at chromosome ends.[102] The mutant form of lamins A/C, progerin, does not interact with TRF2.[102] It has been proposed that in HGPS cells the disrupted interaction between lamins A/C and telomeres, due to the presence of progerin rather than lamins A/C, leads to the telomere instability observed in these cells.[101–103] This telomere instability is in part a result of a deficiency in processing of dysfunctional telomeres through the NHEJ pathway, a process for which lamins A/C are critical.[101–103] Deficiencies in this process contribute to the pathogenesis of HGPS.[101]

A-Type lamins thus play an important role in maintaining genomic stability in cells and in processing damaged DNA in not only genomic DNA but also in telomeric DNA. Deficiencies in A-type lamins can lead to DNA repair defects, which may involve more than one DNA repair pathway, and to disorders such as HGPS where the expression or recruitment of DNA repair components can be disrupted.

Lamin B1 plays a role in the DNA damage response

B-type lamins, like the A-type lamins, in addition to playing an important role in the structural organization of the nucleus, are essential for regulation of DNA metabolism. This includes functions in not only gene activation and silencing but also in DNA replication, transcription and repair.[2–4,16,36,104,105] Lamin B1 has been shown to play a critical role in repair of DNA DSBs by HR (Figure 3).[16] It is involved in maintaining the stability of RAD51 after induction of DSBs by ionizing radiation or from replication fork collapse.[16,106] Lamin B1 binding to RAD51 is thought to occur constitutively.[16] After induction of DSBs, levels of RAD51 increase.[16] Lamin B1 is thought to be involved in this process. It has been proposed that after DNA damage there is increased binding of lamin B1 to RAD51. This enhances the stability of RAD51 by repressing its proteasome-mediated degradation (Figure 3).[16] This leads to increased RAD51 levels which are critical for repair of DSBs by HR. Additional factors may be involved in the regulation of RAD51 stability by lamin B1.[16] Lamin B1 has also been shown to bind directly to the promoters of some genes important in HR including *BRCA1*, *RAD51*, and *MRE11* and can thus be involved in regulation of

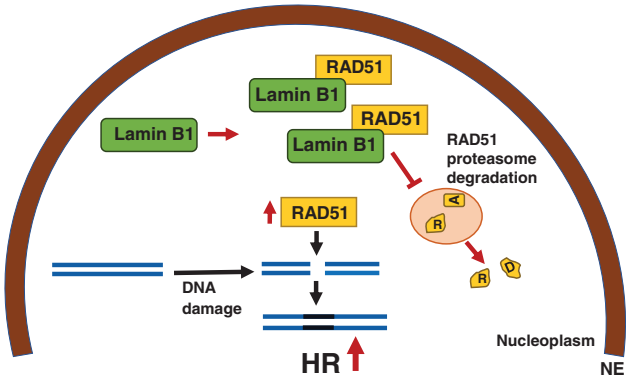

Figure 3. Lamin B1 plays a role in DNA DSB repair by HR. Lamin B1 is involved in maintaining the stability of RAD51 in the cell. It binds to RAD51 and it has been proposed that, after induction of DNA DSBs, there is increased binding of lamin B1 to RAD51. This enhances the stability of RAD51 by repressing its proteasome-mediated degradation. This thus leads to increased RAD51 levels which are critical for repair of DSBs by HR.[16] Not shown, lamin B1 can also bind directly to the promoters of some genes important in HR including *BRCA1*, *RAD51* and *MRE11* and can thus be involved in regulation of expression of these proteins after DSB formation and in increased HR.[106] NE: nuclear envelope. (A color version of this figure is available in the online journal.)

expression of these proteins after DSB formation and in increased HR (Figure 3).[106]

Since the A-type and B-type lamins form interacting networks within the lamina and the nucleoplasm in the nucleus,[16,107] it is possible that interactions between these two types of lamins may play an important role in the regulation of DNA repair processes. In support of this view, lamin B1 has been shown to be important in the mobility and distribution of lamins A/C in the nuclear lamina and nucleoplasm, which in turn may have an influence on DNA repair activity.[16,107] The lamins could act in conjunction with each other. In mature cells, for example, in response to DNA damage, levels of RAD51 may be regulated through repression of its degradation by lamin B1 together with its transcriptional activation by lamins A/C so as to promote HR.[16] The levels of RAD51 could be under the control of both lamins A/C and lamin B1 which differentially function together in a manner dependent upon state of development, cell and tissue type, and physiological circumstances.[16]

Lamin B1 has also been shown to be involved in repair of UV damage by nucleotide excision repair (NER).[106,108] Silencing of lamin B1 expression in tumor (e.g., human osteosarcoma) cells leads to cell cycle arrest and altered expression of several genes involved in NER which leads to defects in the repair of UV-induced DNA damage.[106] In these cells there is downregulation of several proteins required for NER which include DDB1 (DNA damage binding protein 1); CSB (ERCC6) (Cockayne Syndrome B protein), PCNA and Pol η.[106] A strong interaction has been shown between lamin B1 and the promoter of *DDB1* and, when lamin B1 is silenced, levels of DDB1 are greatly reduced.[106] It has been proposed that lamin B1 regulates expression of genes involved in NER at a number of different levels including regulation of transcription and possibly mRNA processing and stability.[108] This regulation could occur either directly or indirectly.[108]

Thus lamin B1, like lamins A/C, plays a pivotal role in regulation of DNA repair processes. Both types of lamins have roles in regulation of expression of genes critical for specific DNA repair processes such as HR, NHEJ and/or NER. These effects may be direct or indirect, just as is their effect on the stability of specific repair proteins. It has been speculated that different lamins may influence distinct patterns of gene expression by interactions with subsets of gene promoters.[108] This could include genes directly or indirectly involved in the repair process. This is an exceedingly interesting area of investigation in which a great deal more work needs to be carried out.

Nuclear actin and its role in DNA repair

Significant advances in the past decade have shown that actin in the nucleus plays a dynamic role in a number of essential nuclear functions in a variety of eukaryotic organisms.[1,109–114] It is actively transported between the cytoplasm and nucleus.[1,110–112,114] In the nucleus, actin exists in monomeric and polymeric forms. Its confirmation differs in different cell types and under different circumstances. There are, however, conformational differences between cytoplasmic and nuclear actin.[112,113,115–117] The majority of actin in the nucleus is present in the monomeric form (80%) with the remaining (20%) in a polymeric (i.e., filamentous) form.[113,115–117] There is evidence that both monomeric and polymeric actin have on impact on the various nuclear processes in which they participate.[109,110,116,118–121] In addition to actin's role in maintaining the structure and integrity of the nuclear envelope, nuclear pore complex, higher-order chromatin structure and structure of sub-nuclear compartments, it participates in many essential steps in gene expression, chromatin remodeling, regulation of transcription, signal transduction and DNA replication.[111–114,122–124] Of particular interest, actin has also been shown to play an important role in DNA repair. A number of recent studies have expanded our knowledge of actin's function in this process, especially its role in DNA DSB repair and its importance in both the HR and NHEJ pathways. These studies will be described below.

Induction of actin after DNA DSB formation

Actin is actively involved in repair of damage to nuclear DNA. Studies have demonstrated that DNA damage as well as environmental stress induce formation of actin filaments in the nucleus of human cells.[19,123,125] Actin filaments induced by various toxic agents display three characteristic morphological patterns: (1) elongated nucleoplasmic filaments; (2) dense nucleoplasmic clusters of filaments; and (3) short, nucleolus-associated filaments.[123] Two actin assembly factors are needed for formation of these structures, Formin-2 and Spire-1/Spire-2.[125] After DNA damage, Formin-2 accumulates in the nucleus.[125] Loss of this protein increases the severity of the damage occurring in the DNA, indicative of the importance of its role in actin filament formation for the repair process.[125]

Significant accumulation of nuclear actin filaments occurs in the nucleus after formation of DNA double-

strand breaks (DSBs) (Figure 4).[19,125,126] A correlation has been found between polymerization of actin into filaments after DNA damage and the number of DNA DSBs formed.[19,125,126] Recent studies have shown that actin and actin-related proteins (Arps) are recruited to chromosomal DSBs and assemble at sites enriched in MRE11, a homologous recombination repair protein (Figure 4).[127] Formation of actin filaments is needed for efficient repair of the induced DSBs.[19,125,127] Actin filaments have also been shown to form after cells are treated with a telomere uncapping agent, which leads to structures resembling DNA DSB formation.[125] These studies are a strong indication that nuclear actin filaments are needed for promotion of efficient DNA DSB repair.

The exact function of these actin filaments and the mechanisms involved in their role in the repair process is still poorly understood. The role of actin polymerization in DNA repair has not been well characterized. It has been hypothesized that actin could potentiate the DNA repair response by several different mechanisms which include: (1) altering the mobility or organization of chromatin; (2) recruiting DNA repair factors to the damage site; and (3) binding to or sequestering DNA repair factors which could inhibit the repair process.[125] Thus there are a number of different avenues that need to be explored in order to fully understand the mechanistic processes governing actin's role in the repair process.

Importance of actin in NHEJ and HR after DNA DSB formation

There is evidence that nuclear actin plays a role in the NHEJ and HR pathways involved in repair of DNA DSBs and that polymerized actin is required for this process. Proteins important in NHEJ (e.g., Ku70-Ku80 heterodimer) and HR (e.g., MRE11 and RAD51) have been found to associate with polymerized actin *in vitro* (Figure 4).[19] It has been suggested that actin aids in the recruitment and stabilization of these repair proteins at the site of damage.[19] Consistent with this viewpoint are studies which show that in human cells disruption of actin polymerization after DNA damage inhibits repair by NHEJ.[19] Similarly, disruption of actin polymerization also reduces the efficiency of HR repair of double-strand breaks.[127,128] The detailed mechanism underlying the role of nuclear actin filaments in DNA repair still has to be elucidated. A number of actin-binding proteins and actin-related proteins (Arps), which aid in actin polymerization after DNA damage and also bind to proteins involved in DNA repair, have been suggested as important factors in this process.[113]

Nuclear actin is also a key component in several chromatin-modifying complexes (i.e., IN080, SWR1, SWI/SNF; BAF and RSC) which play pivotal roles in facilitating DNA repair processes.[113,129] Within these complexes, actin is in a monomeric form and associates with Arps (Figure 4).[113] The presence of Arps in these complexes is critical for assembly of these complexes and maintenance of their structural integrity.[113,129] For one of these chromatin-remodeling complexes, IN080, Arps are thought to maintain the monomeric state of actin within the complex.[113,130–132] The presence of

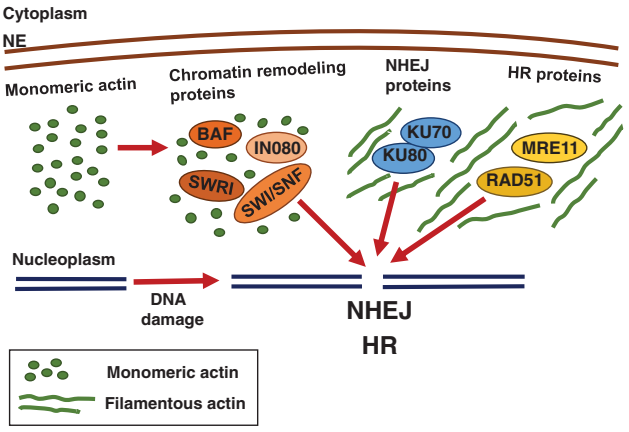

Figure 4. Actin is involved in repair of DNA DSBs by both NHEJ and HR. In the nucleus, after formation of DNA DSBs, polymerization of monomeric actin into a filamentous form is observed.[19,125,126] Actin filaments assemble at sites enriched in proteins important in NHEJ (e.g., Ku70/Ku80) and HR (e.g., MRE11 and RAD51).[19,116–127] They may aid in recruitment and stabilization of these proteins at the site of damage.[19,125–127] Monomeric actin is a key component of several chromatin remodeling complexes (i.e. IN080; BAF, SWRI, and SWI/SNF) which have roles in facilitating DNA repair processes and bind near sites of DNA DSBs.[113] NE: nuclear envelope. (A color version of this figure is available in the online journal.)

actin and Arps in this complex has been shown to promote the response of this complex to chromatin remodeling after DSB formation (Figure 4).[113] This complex binds near sites of DSBs and is required for processing of the DSBs.[133] The mechanism of action of monomeric actin in this process is still unclear.

These studies further demonstrate the importance of actin and its various structural forms in DNA repair processes. Actin polymerization and its formation into filaments is critical for repair of DSBs by NHEJ and HR.[19,126–128] Actin present in a monomeric form is a key component of chromatin-remodeling complexes which play a role in facilitating DNA DSB repair.[113,129] The exact mechanisms of action of actin in these repair processes and the role its structural conformation plays in its activities is an extremely interesting question and one which needs to be studied in greater detail.

Actin and myosin—Partners in DNA repair in the nucleus

Actin's involvement in relocalization of DNA DSBs in heterochromatin

DSBs repaired by HR exhibit extensive movement and relocalization after formation during S and G phases of the cell cycle.[127,128,134,135] When DSB formation occurs in pericentric heterochromatin (referred to as heterochromatin) during S and G2 phases of the cell cycle, these DSBs relocate to the nuclear periphery.[127,128,135,136] Of importance, recent studies from the laboratories of Chiolo and of Schrank have shown that nuclear actin, the Arp2/3 complex, which is an actin nucleator,[137] and the Arp2/3 activator, WASP (a Wiskott–Aldrich syndrome protein family member), are recruited to sites of DSBs.[127,128] WASP brings the ARP2 and ARP3 subunits together, activating

them and enabling actin filament elongation.[127] Actin and proteins in the ARP complex are recruited to MRE11-enriched DSBs in chromatin.[127] Actin filaments, after formation at these sites, extend from the heterochromatin to the nuclear periphery.[128] Chiolo *et al*. have shown that DSBs relocate from the pericentric heterochromatin to repair sites along the actin filaments.[128] The actin filaments then dissemble after relocalization.[128] Actin polymerization is thus required for migration of the DSBs from heterochromatin to localize in discrete sub-nuclear clusters, a process that is essential for homology-directed repair.[127,128] ARP2/3 is not enriched at DSBs repaired by NHEJ and is not involved in regulation of NHEJ.[127] It is postulated that this relocalization and clustering of DSBs may prevent aberrant recombination from occurring, thereby shielding breaks from misrepair, and promote exchanges with the sister chromosome or homologue.[127,128,136,138] This same type of migration of DSBs does not take place during NHEJ repair in heterochromatin.[127,128]

Myosin's role in DNA DSB relocalization in heterochromatin

Of particular interest, Chiolo *et al*. have demonstrated that the actin motor protein myosin is also required for relocalization in heterochromatin of DSBs, which are repaired by HR during S and G2 phase of the cell cycle.[128] Myosin IA, Myosin IB and Myosin V have been shown to have an important role in the movement of heterochromatic DSBs to the nuclear periphery.[128] Recruitment of these nuclear myosins to the site of a DSB depends on targeting by MRE11, a protein involved in HR.[128] A network of actin filaments and interacting proteins coordinates these activities in the heterochromatin. The nuclear myosins direct the DSB repair sites towards the nuclear periphery by "walking along" the Arp2/3 mediated actin filaments allowing relocalization of the damage sites so that they can be repaired.[128] It has been proposed that this type of movement of DSBs may be especially important for allowing repair sites to be released from the more compact heterochromatin and that this type of movement is not as necessary in the less compact euchromatin.[128] These studies thus demonstrate that actin filaments play a critical role in HR repair that takes place in heterochromatin during S and G2 phases of the cell cycle and that their coordinated interaction with both Arp2/3 and myosins is critical for relocalization of sites of DSBs to the nuclear periphery for repair.

Nuclear actin/myosin in DNA DSB repair in transcriptionally active euchromatin

DSBs in transcriptionally active chromatin in human cells are preferentially repaired by HR.[134] Studies have shown that DSBs, induced at defined loci in transcriptionally active genes, cluster and that this clustering occurs mainly during G1 phase of the cell cycle in a period when DNA repair is delayed.[134] This clustering does not occur in genes that are not transcriptionally active.[134] It relies on nucleoskeleton and cytoskeleton networks, as seen by its dependence upon the LINC (linker of nucleoskeleton and cytoskeleton) complex and the nuclear actin organizer protein Formin 2 (FMN2).[134] Data suggest that microtubule- and actin-related networks actively play a role in this process.[134] The MRN complex, which is involved in the initial steps in HR, is also important for sustaining this clustering.[134] It has been suggested that, since HR is suppressed during G1 phase of the cell cycle (i.e., the cells are refractory for repair in G1), clustering may aid in sequestering DSBs until they can be repaired by HR in post-replicative cells (G2 phase).[134] Whether actin filament formation is involved in this process has not as yet been clearly demonstrated, though actin polymerization has been shown to occur in the nucleus shortly after formation of DNA DSBs and is dependent upon FMN2.[134] There is also evidence that, in transcriptionally active chromatin in human cells, after induction of DSBs in G0/G1 phase of the cell cycle, contact between homologous chromosomes can occur and this depends on proteins involved in homology-directed repair (i.e., BRCA1, RAD51, and RAD52), nuclear F-actin polymerization and nuclear myosin.[20] It has been suggested that this is important for the prevention of chromosomal rearrangements after induction of DNA DSBs in transcriptionally active euchromatin.[20]

Actin thus works in concert with myosin in several different aspects of DNA DSB repair. This interaction is important in the relocalization of DNA DSBs in heterochromatin to the nuclear periphery during the HR repair process.[128] Nuclear actin/myosin are also involved in HR in repair of DSBs in transcriptionally active euchromatin where they are also important in mediation of contact between homologous chromosomes at sites of DSBs in the G0/G1 phase of the cell cycle.[128] In addition, the nuclear actin organizer protein, Formin 2, and potentially actin filaments, along with the LINC and MRN complexes are involved in clustering of DNA DSBs in the G1 phase of the cell cycle, possibly sequestering the DSBs until they can be repaired in G2.[128]

The LINC complex in DNA repair

Interactions between the nucleoskeleton and cytoskeleton are mediated by the LINC complex, which spans the inner and outer membranes of the nuclear envelope.[139–142] The key proteins of the complex are the trans-membrane SUN-domain proteins, SUN1 and SUN2, which span the inner nuclear membrane and contain domains that interact with the nuclear lamins, in particular A-type lamins, and with emerin and proteins of the inner nuclear membrane.[139–145] SUN1 and SUN2 interact with the KASH-domain proteins, the nesprins, in the perinuclear space.[139–145] The nesprins span the outer nuclear membrane and interact with microtubules, microfilaments and intermediate filament in the cytoskeleton.[139–145] The LINC complex plays a role in diverse functions which include: nuclear shape and positioning, nuclear migration, mechanotransduction between the cytoplasm and nucleus, maintenance of the centrosome-nucleus connection and movement of chromosomes within the nucleus during meiosis and DNA repair.[141,146–156]

The LINC complex plays a prominent role in the DNA damage response.[150,152,157] Studies indicate that the LINC complex contributes, in human cells and *C. elegans*, to repair of DNA DSBs. The human SUN1 protein and its homolog in *C. elegans* (UNC-84) interact with Ku70/Ku80 and DNA-PK (DNA-dependent protein kinase) driving inhibition of NHEJ and promoting loading of RAD51 to the site of a DSBs which leads to stimulation of HR (Figure 5(a)).[150] SUN1 and UNC-84 have also been shown to play a critical role in the recruitment of FAN-1 nuclease, an endonuclease involved in DNA interstrand cross-link (ICL) repair, to the nucleoplasm after damage with a DNA ICL agent (Figure 5(b)).[150] Though there are other DNA endonucleases involved in production of incisions at the site of DNA ICLs (e.g. XPF), which may depend upon cell type and circumstance, this points to an involvement of the LINC complex in DNA ICL repair.[150] Additional evidence shows that, at sites of DSBs, SUN1 and UNC-84 inhibit NHEJ and promote HR (Figure 5 (b)).[150] Since in the repair of DNA ICLs, DSBs are formed and are repaired by HR, it has been proposed that in ICL repair the LINC complex plays a role not only in the recruitment of FAN1 to the damage site but also in promotion of HR by suppression of NHEJ.[150]

In other studies, SUN1 and SUN2 have been shown in mouse embryonic fibroblasts to interact with DNA-PK.[155] This interaction is not influenced, however, by DNA damaging agents (e.g. hydroxyurea). The role that this interaction may play in the repair process in these cells is not clear and whether it functions in inhibition of NHEJ is unknown but certainly of interest. The LINC complex may also promote mobility of DNA DSBs after damage.[157] Increased mobility of damaged chromatin containing DSBs has been shown to involve 53BP1 and the LINK proteins SUN1 and SUN2.[157] As mentioned above, 53BP1 plays a critical role in limiting the 5′ resection of broken ends of DNA at DSBs. It also has been shown to block resection at dysfunctional telomeres and alter their mobility, which leads to stimulation of NHEJ.[157] It has been proposed that 53BP1-dependent chromatin mobility is mediated by the LINC complex which, through association of nesprin with microtubules, allows transfer of cytoskeletal forces to the nucleus and enhancement of chromatin mobility.[139–141] The 53BP-1 dependent mobility of dysfunctional telomeres thus involves a LINC-microtubule dependent process which promotes NHEJ of DNA DSBs.[157]

A number of different systems, repair proteins and pathways have been investigated in determining the role for the LINC complex in the DNA repair response. This has led to differing viewpoints on its exact mechanistic role in this process. This is a complex but exceedingly important area. The influence the LINC complex has on the DNA repair process can have a major influence on the signaling that occurs between the cytoplasm and the nucleus and can play a significant role in safeguarding genomic stability after DNA damage. This is an intriguing are of investigation which has only been briefly mentioned above. Ongoing explorations in this field should produce some exciting new insights into this process.

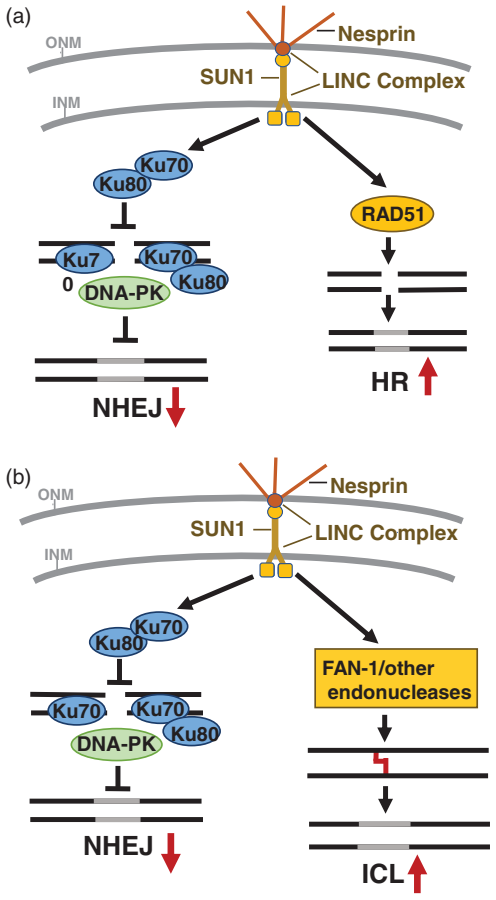

Figure 5. The LINC complex facilitates repair of DNA DSBs and DNA ICLs. (A) The human SUN1 protein and its homolog in *C. elegans* (UNC-84) interact with Ku70/Ku80 and DNA-PK driving inhibition of NHEJ and promoting loading of RAD51 to the site of a DSB, which leads to repair by HR.[150] (B) SUN 1 also plays a role in the recruitment of FAN-1 nuclease to the nucleoplasm after damage with a DNA ICL agent.[150] Other endonucleases may also be involved in this process. NHEJ may be suppressed during this time. This points to an involvement of the LINC complex in DNA ICL repair.[150] ONM: outer nuclear membrane; INM: inner nuclear membrane. (A color version of this figure is available in the online journal.)

Spectrin in the nucleus and its role in DNA repair

Nonerythroid spectrin is present in the nucleus as well as in the cytoplasm.[1,21] Since It is an essential protein in the cell, knowledge of its roles in various cellular processes is critical in order to understand how these processes are disrupted when there is loss or a deficiency in this protein. In the cytoplasm, it is an important component of a cytoskeletal network which provides support to the plasma membrane and helps maintain cell shape, flexibility and elasticity.[158–166] It is involved in cell spreading, lamellipodia extension and cell migration.[167–170] In addition, it has numerous other functions which include trafficking of organelles, vesicles and proteins, signal transduction, cell cycle progression, synaptic transmission in neurons, cell adhesion, immunological synapse formation, and cell growth and differentiation.[158–161,163–166,169–173]

In the nucleus, α-spectrin (αSpII) and two β-spectrins (βSpII and SpβIV) have been identified.[1,13,21,174–180] Structurally α- and β-spectrin are composed of an extended array of triple α-helical repeats and have multiple sites of

interaction with other proteins in the nucleus.[9,10,158–160,180] This helical repeat structure of spectrin is thought to contribute to the elasticity of the nucleoskeleton.[9,158–160,180] In the cytoplasm, spectrin is present as a tetramer composed of heterodimers of α- and β-spectrin.[158–161,163,164,170–173] However, in the nucleus formation of heterodimers or tetramers between αSpII and the β-spectrins has not, as yet, been demonstrated. It is possible that there is some difference in the interaction of αSpII and β-spectrins in the nucleus compared to their interaction in the cytoplasm. αSpII may be present in a monomeric form and not as a heterodimer or tetramer with β-spectrin or, if both forms exist, they may have different nuclear functions. Actin, for example, is present in both a monomeric form and a filamentous form in the nucleus and both forms are involved in different aspects of the DNA repair process.[19,113,126–129] It is possible that a similar situation could hold true for spectrin. Enhanced modes of detection may be required in order to discern formation of heterodimers and tetramers between α- and β-spectrin in the nucleus. Further studies need to be undertaken in order to elucidate the relationship between these proteins.

Like cytoplasmic spectrin, nuclear spectrin also has a structural role; it is a component of a nucleoskeletal network which is important in maintaining the structural integrity and elasticity of the nucleus and recovery of the nucleus from compression.[1,10,158–161,164] It interacts with other nucleoskeletal proteins which include, lamin A and lamin B, actin, emerin, nuclear myosin 1C, protein 4.1, and βSpIV and these interactions are thought to be critical for the nuclear architecture and the biomechanical properties of the nucleoskeleton (Figure 6).[1,8–10,181] Spectrin also plays an important role in the mechanical coupling between the nucleoskeleton and the cytoskeleton. In HeLa cell nuclei, αII-spectrin forms a complex with lamins A and B, emerin, actin, nuclear myosin IC and the LINC complex protein SUN2 and is thought to be an important component in this coupling (Figure 6).[1,9,10] αSpII also interacts with proteins involved in DNA transcription and chromatin remodeling indicating that it has additional functions in the nucleus.[1–7,16,17,181]

Of particular significance, αSpII in human cells has been shown to play an important role in DNA repair.[21,182–185] Lambert *et al*. have clearly demonstrated this in cells in which a deficiency or loss of αSpII leads to defects in DNA repair and genomic instability.[21,22,183–185] There are now multiple lines of evidence that αSpII in the nucleus plays a critical role in DNA repair in human cells in both genomic (non-telomeric) DNA and telomeric DNA.[21,183–187] These studies demonstrate that in repair of DNA ICLs: (1) αSpII localizes at sites of DNA ICLs and is involved in the recruitment of the DNA repair endonuclease, XPF, to these sites; (2) it is needed for incisions produced by XPF at these sites; (3) loss of αSpII from cells results in a deficiency in DNA ICL repair and in chromosomal instability; and (4) correction of loss of αSpII in cells restores chromosome stability and DNA ICL repair capabilities. Evidence for this is presented below.

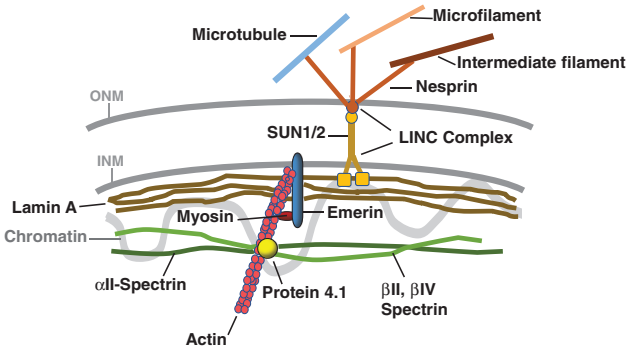

Figure 6. αSpII in the nucleus interacts with the LINC complex and other nucleoskeletal proteins. αSpII is a component of a nucleoskeletal network which is important in maintaining the structural integrity of the nucleus, the biomechanical properties of the nucleoskeleton, and the mechanical coupling between the nucleoskeleton and the cytoskeleton.[1,10,158–161] It interacts with lamin A and lamin B, actin, emerin, nuclear myosin 1c, protein 4.1, βSpIV and the LINC complex protein SUN2, which may be important in this coupling.[1,8–10,181] The nesprins, which are proteins associated with the LINK complex, interact with microtubules, actin filaments and intermediate filaments in the cytoplasm, linking the nucleoskeleton with the cytoskeleton.[1] ONM: outer nuclear membrane; INM: inner nuclear membrane. (A color version of this figure is available in the online journal.)

αSpII binds directly to DNA containing ICLs

Studies by Lambert *et al*. have shown that αIISp from HeLa cell nuclei binds to a DNA substrate containing 4,5′,8-trimethylpsoralen (TMP) plus UVA light induced interstrand cross-links.[182] In addition, purified bovine brain αSpII also binds to this substrate.[182] These studies were the first to demonstrate that αSpII binds directly to DNA containing ICLs.[182] It has been proposed, based on the crystal structure of αSpII, that interaction of αIISp with DNA occurs through hydrogen bonds which form between its protein side chains and the N_3 of purines and O_2 of pyrimidines in the minor groove of DNA.[182] Enhanced binding of αSpII to DNA at sites of ICLs could be due to the increased accessibility of αSpII to DNA when the minor groove of DNA opens after ICL formation.[182] These studies thus demonstrate that αSpII can interact directly with DNA after formation of ICLs, indicating that it plays an important role in the damage-recognition step of DNA ICL repair, as has been proposed.[182]

A deficiency in αSpII leads to reduced chromosomal stability and cell survival after ICL damage

αSpII is a protein which is essential for cell survival; complete loss of this protein leads to cell death.[177,185,188,189] Levels of αSpII can be reduced to 35–40% of normal and cells can survive.[185] However, after knockdown of αIISp by siRNA to these levels in normal human cells, followed by damage with a DNA ICL agent, there is increased chromosomal instability which includes sister chromatid end-to-end fusions, chromatid breaks, chromosome exchanges, and radials.[185,186] In addition, there is decreased cell survival and decreased DNA repair compared to cells transfected with a nontarget siRNA.[185] These results have been observed using two different DNA ICL agents,

8-methoxypsoralen (8-MOP) plus UVA light and mitomycin C (MMC), and two different human cell lines.[185] These studies provide strong evidence for an important role for αSpII in the DNA repair process.

αSpII is required for recruitment of XPF to sites of ICLs

An important indicator of whether a protein is involved in DNA repair is whether the protein localizes to sites of damage in the nucleus. This is usually measured by examining cells for the localization of the protein to sites of damage-induced nuclear foci. After damage of human cells with a DNA ICL agent (8-MOP plus UVA light, or MMC), αSpII localizes to nuclear foci.[22,183,185] Since αSpII has been shown to bind directly to DNA containing a TMP ICL, this indicates that the these foci represent sites of ICLs.[22,183,185] The DNA repair protein, XPF, which creates incisions at sites of DNA ICLs,[187,190,191] co-localizes with αSpII at these damage-induced foci.[22,183,186] The localization of XPF in these foci follows the same time course as that of αSpII, with foci first appearing at 10 h after ICL damage, peaking at 16 h and disappear by 24 h, when the ICLs are presumed to be repaired.[21,183] This indicates that αSpII associates with XPF during the incision events and is involved in the same repair steps as XPF, though their roles in the repair process are different.[21,184]

The importance of αSpII for localization of XPF to sites of damage is seen in studies in which αSpII is knocked down by siRNA. After knockdown of αSpII, XPF fails to localize to damage-induced foci and there is decreased ICL repair.[185,186] These studies indicate that αSpII is critical in recruitment of XPF to sites of ICLs. In support of this, incisions produced by XPF have been examined on a 140 bp substrate containing a site-specific TMP ICL.[182] XPF, present in a complex of chromatin-associated proteins from normal human lymphoblastoid cells, produced incisions on both the 3′ and 5′ sides of the TMP ICL.[182] This was confirmed by studies showing that these incisions could be inhibited by an antibody against XPF.[182] Purified bovine αSpII was shown to enhance the incisions produced by XPF in the normal human cells; αSpII by itself did not create any incisions on the cross-linked DNA.[182] A purified monoclonal antibody, which specifically recognized αSpII from normal human lymphoblastoid cells, inhibited the incisions produced by XPF in this system.[182,192] These combined studies have led to the development of a model in which αSpII acts as a scaffold and aids in the recruitment of repair proteins such as XPF to sites of ICLs; loss of αSpII results in a deficiency in XPF recruitment and a loss in incisions it produces at sites of ICLs leading, to a deficiency in DNA repair.[182]

αIISp acts downstream from FANCD2 in ICL repair

DNA interstrand cross-links are an important block to DNA replication. ICL repair takes place primarily in S phase of the cell cycle at the site of a replication fork stalled at the ICL.[193–195] A large number of proteins are involved in DNA ICL repair, a significant number of which are encoded by genes which are defective in individuals with the inherited bone marrow failure disorder, Fanconi anemia (FA).[25,196,197] FA is characterized by bone marrow failure, chromosome instability, congenital abnormalities, a marked predisposition for development of cancer and a striking cellular sensitivity to DNA interstrand cross-linking agents.[25,196–207] There are 22 complementation groups of FA (FANCA—FANCW), and genes from each complementation group code for a specific protein involved in the DNA ICL repair process.[25,198-200,203] As a result, this pathway has been called the Fanconi anemia repair pathway.[25] Patients from each of the complementation groups are defective in repair of DNA ICLs.[25,196–205] In the initial steps in the repair process, a core complex of FA proteins forms which is critical for the monoubiquitination of the FA proteins, FANCD2 and FANCI.[25,200–203] This usually occurs at the site of a stalled replication fork.[25,200–203] The monoubiquitination of FANCD2 (FANCD2-Ub) and FANCI (FANCI-Ub) initiates a series of events which lead to the repair of the ICL and results in replication fork recovery.[25,196–203]

αSpII, though not a known FA protein, plays a critical role in this repair process during S phase of the cell cycle.[21,186] After ICL damage, FANCD2 is monoubiquitinated and localizes to nuclear foci.[25,196–203] These foci form before those of αSpII and XPF and have a different time course for their formation.[21,208] αSpII does not co-localize in the same foci as FANCD2-Ub; however, it does co-localize with XPF and FANCA foci.[183,208] Formation of αSpII, XPF and FANCA foci all follow the same time course and form after FANCD2-Ub foci.[21,208] In studies using *Xenopus* extracts, XPF is also recruited to damage sites after FANCD2-Ub.[209] Importantly, αSpII is required for the formation of both XPF and FANCA foci. FANCA is a component of the core complex involved in the monoubiquitination of FANCD2 and FANCI.[25,197-200] Its co-localization with αIISp after formation of FANCD2-Ub foci may indicate an additional role for it in the ICL repair pathway. αSpII and XPF are not required for the monoubiquitination of FANCD2 or its localization to foci after ICL damage.[21,208] Collectively, these studies show that αSpII localizes to sites of ICLs after FANCD2-Ub and is not required for monoubiquitination of FANCD2; they also indicate that αSpII acts downstream of FANCD2-Ub, it and recruits XPF and FANCA to sites of ICLs, and it is important in the early incision steps in the repair process.

αSpII functions as a scaffold in recruitment of DNA repair proteins to sites of damage

Since αSpII is proposed to function as a scaffold in the recruitment of repair proteins, such as XPF, to sites of ICLs, an important question is how does it accomplish this. As mentioned above, αSpII consists of 21 triple-helical repeats and a repeat which contains EF hand domains.[158–160] The 10th repeat contains a Src-homology 3 (SH3) domain.[158–160,210] SH3 domains are modular domains important in protein-protein interactions and assembly of protein networks.[211,212] These domains interact with proteins containing proline-rich consensus sequences.[211,212] There are three major classes of protein ligands that bind to SH3 domains, each with a different consensus sequence: class I, class II, and

class 1@ ligands.[212–215] The SH3 domain of αSpII preferentially binds to class 1@ ligands.[215] Of particular interest, a number of the FA proteins contain motifs that bind to SH3 domains and this represents an important class of motifs in FA proteins which can interact with cellular proteins containing SH3 domains, such as proteins involved in signal transduction and intracellular signaling.[216]

Significantly, the FA protein, FANCG, has a class 1@ consensus sequence.[216] Lambert *et al*. have shown, using site-directed mutagenesis and yeast two hybrid analysis, that FANCG binds directly to the SH3 domain of αSpII via this consensus sequence.[216] FANCG also binds directly to XPF-ERCC1.[216] FANCG contains seven tetratricopeptide repeat (TPR) motifs, which are motifs involved in protein-protein interactions.[217–219] TPR motifs 1, 2, 3 and 6 bind to the central domain of ERCC1 (residues 120–220).[220] ERCC1 in turn binds to XPF via its C-terminal domain (residues 220–297).[221–223] XPF binds to ERCC1 via its C-terminal domain, which is different than its nuclease domain, and produces incisions in DNA at sites of ICLs.[223,224] Thus, it has been proposed that αSpII, which binds directly to DNA at sites of ICLs, acts as a scaffold in the recruitment of XPF-ERCC1 to the damage site. It does this via FANCG. αSpII binds directly to FANCG at its SH3 domain.[216] FANCG in turn recruits and binds to XPF-ERCC1,[220] which can then create incision(s) at the site of damage leading to unhooking of the ICL. Thus, once αSpII localizes to the site of damage, FANCG binds to αSpII and essentially acts as an intermediary in recruiting XPF-ERCC1 to the damage site.[21,216,220]

The mechanism of action of αSpII in DNA ICL repair

Several models have been proposed for the repair of ICLs at DNA replication forks stalled at the sites of damage; they are presented in several recent reviews.[24,25,190,193,209,216,226–228] Multiple proteins and steps are involved in this process which include: damage recognition, incision or unhooking of the cross-link, translesion DNA synthesis, homologous recombination, and nucleotide excision repair (NER).[25,190,191,193,197,209] Two of the major models are the dual fork convergence model, which describes ICL repair that is triggered when two replication forks converge at the site of an ICL[24,25,190,193,209,227] and the replication fork reversal model, in which one of the two converging forks at an ICL undergoes reversal.[24,216,227,228] Importantly, it needs to be kept in mind that these models are based on studies using several different organismal systems and the relationship of these studies to human systems and the role of the FA proteins in them is still under investigation.

Lambert *et al*. have proposed a model for the role and importance of αSpII in DNA ICL repair which is supported by studies on the interaction of αSpII with not only damaged DNA but also with proteins involved in DNA ICL repair[21] and which is also based on these previous models for ICL repair (Figure 7).[24,25,184,193–198,226–228] The proposed role of αSpII in ICL repair is shown here for each of the two major models described above. In the dual fork convergence model[24,25,190,193,209,226] (Figure 7(a)): (1) When replication forks converge at an ICL, the CDC45/MCM2-7/GINS (CMG) helicase complex, which translocates along the leading strand template, stalls; (2) CMG is unloaded and FANCM recognizes and localizes to the DNA at the site of an ICL; (3) The FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL) is activated and recruited to the site of DNA damage; (4) The core complex monoubiquitinates FANCD2 and FANCI to form the ID2 complex; (5) The ID2 complex localizes at DNA ICLs; (6) αSpII binds to DNA at the site of the ICL downstream of ID2. Whether αSpII localizes to the site of the ICL directly or is recruited by ID2 is not clear; however, αSpII can localize to sites of damage in the absence of FANCD2-Ub[208]; (7) FANCG binds to the SH3 domain of αSpII and XPF-ERCC1 is recruited to bind to FANCG-αIISp; (8) XPF then takes part in the production of incisions at the site of damage and the unhooking of the ICL. Mechanistically, αSpII thus aids in the recruitment of XPF to sites of ICLs via its association with FANCG. SLX4/FANCP in a number of models functions in assembly of proteins at the damage site.[24,25,191] Additional endonucleases may play a role in this step[25,190]; (9) Translesion DNA synthesis then occurs by a translesion polymerase leading to bypassing the tethered ICL; (10) This is followed by repair of the broken DNA duplex in several steps through NHEJ or HR; (11) The adducted DNA base is removed and the site repaired by nucleotide excision repair.

In the replication fork reversal model for ICL repair (Figure 7(b))[24,225,227,228]: (1) Upon replication fork convergence, the CMG complex is unloaded; (2) One of the two converged forks then undergoes reversal, this promotes re-establishment of a stretch of duplex DNA adjacent to the ICL in the opposing fork; this substrate has been shown to be a much better substrate for unhooking the ICL by XPF[227,228]; (3) Repair proceeds in a manner similar to that in the fork convergence model, FANCM localizes to the ICL, which in this model is on the opposite fork; (4) The FA core complex monoubiquitinates FANCD2 and FANCI to form the ID2 complex which localizes to the damage site; (5) αSpII binds to the DNA at the site of the crosslink; (6) ERCC1-XPF are recruited to the site and bind to αSpII via FANCG; SLX4-SLX1 are recruited and unhooking of the ICL takes place; (7) The monoadducted lesion is then bypassed by the action of translesion DNA polymerases; (8) A double strand break intermediate, which has been generated during the incision process, is repaired by HR and the adduct removed by NER.

These models mainly emphasize the role of αIISp in the ICL repair process. The complexities of protein interactions in these models are still being investigated. For a more extensive review of the additional proteins involved in these processes, the reader is directed to other recent reviews.[24,25,190,191,227,228]

The model outlined above for αSpII's function in ICL repair is supported by numerous lines of evidence, all of which indicate that loss of αSpII leads to decreased localization of XPF to sites of DNA ICLs, to reduced repair of ICLs, and to chromosome instability.[21,184] The important role αSpII plays in this model is that it acts as a scaffold in the recruitment of proteins important in the initial incision steps of the repair process. Whether it has additional

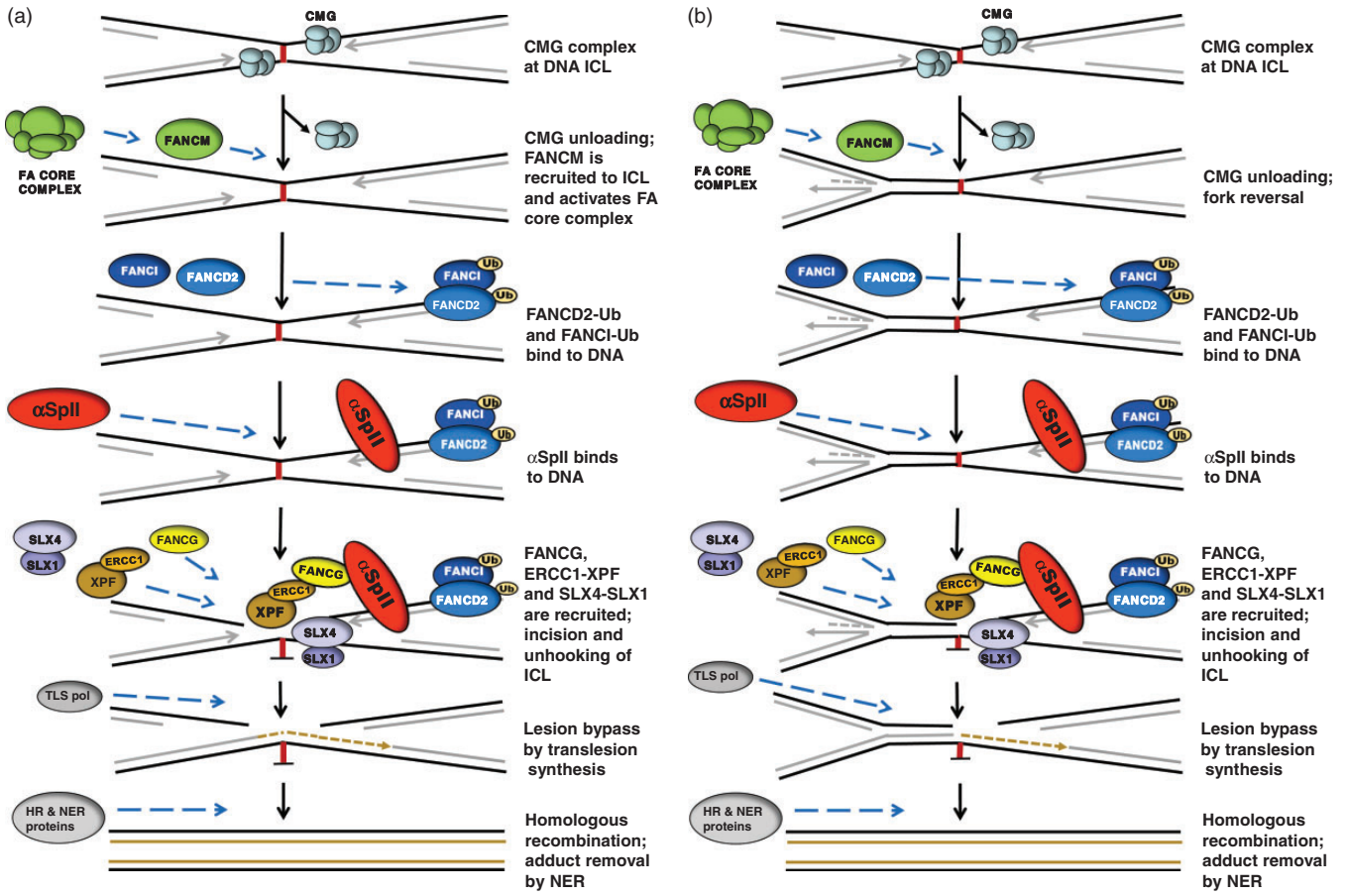

Figure 7. Models for the role of αSpII in repair of DNA ICLs. The role of αSpII in two models of DNA ICL repair are shown. (A) In the dual fork conversion model, when replication forks converge at the ICL, the CMG helicase complex on the leading strand template stalls. CMG is unloaded and FANCM localizes to the DNA at the site of a DNA ICL. The FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL) is activated and recruited to the site of the DNA ICL. The core complex monoubiquitinates FANCD2 and FANCI to form the ID complex which localizes at the ICL. αSpII binds to DNA at the site of the ICL downstream of FANCD2-Ub. FANCG binds to the SH3 domain of αSpII and XPF-ERCC1 is recruited to bind to FANCG-αSpII. XPF then takes part in the production of incisions at the site of damage and the unhooking of the ICL. SLX4/FANCP functions in assembly of proteins at the damage site, additional endonucleases may play a role in this step. Translesion DNA synthesis then occurs by a translesion polymerase leading to bypassing the tethered ICL. This is followed by repair of the broken DNA duplex in several steps through HR or NHEJ. The adducted DNA base is removed and the site repaired by NER. (B) In the replication fork reversal model, when the replication forks converge at the site of a DNA ICL, the CMG complex is unloaded. One of the two converged forks undergoes reversal, this promotes a stretch of duplex DNA adjacent to the ICL in the opposite fork. FANCM localizes to the ICL. The FA core complex monoubiquitinates FANCD2 and FANCI, which localize to the site of damage. αSpII binds to the DNA at the site of the ICL. ERCC1-XPF are recruited to the site and bind to αSpII via FANCG. SLX-SLX1 are recruited and unhooking of the ICL takes place. Translesion DNA synthesis occurs and the monoadducted lesion is bypassed. A double-strand break intermediate, which has been generated during the incision process, is repaired by HR and the adduct removed by NER. These models mainly emphasize the role of αSpII in the ICL repair process.[21] They are based on studies on the interaction of αSpII with damaged DNA and proteins involved in ICL repair[21] and on previous models for DNA ICL repair.[24,25,184,193–198,227] Additional proteins involved in this repair process are not shown and can be found in other recent reviews.[25,190,191,226,227] (A color version of this figure is available in the online journal.)

roles in other steps in this pathway has not yet been explored.

Fanconi anemia and the effects of a deficiency in αSpII on the DNA damage response

Fanconi anemia (FA), as mentioned above, is a bone marrow failure disorder which displays diverse phenotypic characteristic.[23,25,197–203] A distinctive hallmark of this disorder is a defect in ability to repair DNA ICLs.[25,187,191–203,229,230] Of significance, there is a deficiency in αSpII in cells from a number of FA complementation groups.[21,22,174,185] Levels of αSpII in FA are approximately 35–40% of those of normal human cells.[174,185,192] Importantly, the reduced level of αSpII in these cells correlates with a defect in ability to repair DNA ICLs, where levels of repair, as determined by measuring UDS (unscheduled DNA synthesis), are 34–43% of normal.[185,187,192] Reduced levels of αSpII in FA cells also correlate with a defect in production of incisions produced by XPF on a substrate containing a site-directed TMP plus UVA ICL.[229] The deficiency in αSpII additionally correlates with the chromosomal instability in FA cells and an increased number of chromosomal abnormalities after DNA ICL damage such as sister chromatid end-to-end fusions, chromosome exchanges and chromatid breaks.[185,186] These deficiencies in FA can be corrected when levels of αSpII are returned to normal.[22] Collectively these studies emphasize the importance of αSpII in the DNA ICL repair process and the detrimental effect that loss of αSpII can have on cellular functions. Based on these studies, it has been proposed that reduced levels of

αIISp in FA cells play an important role in the DNA repair deficiency and chromosome instability observed in these cells.[21,184]

The critical role of FA proteins in maintaining the stability of αSpII

FA proteins are critical for the ICL repair process.[21,25,196,197] Restoration of levels of individual FA proteins in FA cells from the corresponding complementation group, by transfection with the appropriate FA cDNA, leads to correction of the DNA repair defect and other cellular phenotypic deficiencies observed.[21,25,197,198] It also leads to restoration of αSpII levels to those found in normal cells.[21,22] Thus, FA proteins are important for maintenance of αSpII levels in cells. A major question, however, is what role do the FA proteins play in this process. Studies by Lambert *et al*. have shown that in FA cells the deficiency in αSpII is not due to its decreased expression but rather to its increased cleavage by the protease, μ-calpain.[22,231] The activity of μ-calpain, but not its level, is increased 3–4 fold in FA cells compared to normal cells leading to increased αSpII cleavage.[22] This group has proposed that FA proteins play a critical role in reducing cleavage of αSpII by μ-calpain and have developed a model for the role FA proteins play in this process, which is described below.[21,22]

In normal cells, cleavage of αSpII by μ-calpain is controlled by phosphorylation of Tyr1176. When Tyr1176 is phosphorylated by c-Src, a kinase that binds to the SH3 domain of αSpII, μ-calpain cannot cleave αSpII at its adjacent cleavage site.[232,233] When low-molecular weight phosphotyrosine phosphatase (LMW-PTP) binds to the SH3 domain, it dephosphorylates Tyr1176 and μ-calpain can cleave αSpII.[232,233] According to a model proposed by Lambert *et al*., there are several possible mechanisms by which FA proteins could aid in maintaining stability of αIISp in cells (Figure 8).[21,184]

One proposed mechanism is that in normal human cells FANCG binds to the SH3 domain of αSpII, which prevents binding of LMW-PTP to this domain and inhibits cleavage of αSpII by μ-calpain (Figure 8). In support of this, it has been shown that FANCG binds to the SH3 domain of αIISp via its class 1@ consensus sequence.[216] FANCG could be in equilibrium with LMW-PTP and c-Src kinase for binding to the SH3 domain (Figure 8). When FANCG binds to the SH3 domain, it prevents LMW-PTP from binding to this domain and subsequently prevents dephosphorylation of Try1176 and cleavage of αIISp by μ-calpain. Thus the presence of FANCG, and potentially other FA proteins with consensus sequences that recognize SH3 domains, would prevent the cleavage of αIISp. Of considerable interest, a number of patient derived mutations (at least 18) in the *FANCG* gene result in the FANCG protein having either a defect in the motif that binds to the SH3 domain or this motif is missing.[216] FANCG with these mutations would not be able to bind to the SH3 domain of αIISp which would lead to increased binding of LMW-PTP to this domain and increased cleavage by μ-calpain.[216] Thus, this is one potential mechanism by which an FA protein, in this case

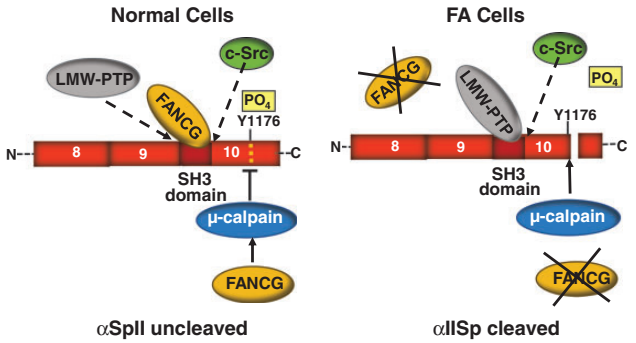

Figure 8. A model for the mechanism by which FA proteins maintain the stability of αSpII. This model proposes several possible mechanisms by which FA proteins could aid in maintaining stability of αIISp in cells. Represented in this figure is a segment of αSpII containing repeats 8–10. FANCG is used to illustrate the role a FA protein may play in maintaining the stability of αIISp. In this model, in normal human cells, an equilibrium exists between low-molecular weight phosphotyrosine phosphatase (LMW-PTP), c-Src and FANCG for binding to the SH3 domain of αSpII in repeat 10. When Tyr1176 is phosphorylated by binding of c-Src to the SH3 domain, μ-calpain cannot cleave αSpII at its adjacent cleavage site.[232,233] When low-molecular weight phosphotyrosine phosphatase (LMW-PTP) binds to the SH3 domain, it dephosphorylates Tyr1176 (Y1176) and μ-calpain can cleave αSpII.[232,233] When FANCG binds to the SH3 domain, it prevents binding of LMW-PTP to this domain and prevents dephosphorylation of Try1176 and cleavage of αIISp by μ-calpain.[21,22] It is also proposed that a FA protein (i.e., FANCG) could bind to μ-calpain and inhibit its ability to cleave αSpII. In this manner, FANCG could play an important role in maintaining the stability of αSpII in cells. In FA cells (for example FA-G cells) in the absence of a functional FANCG, there would be a greater probability that LMW-PTP would bind to the SH3 domain and dephosphorylate Try1176, leading to cleavage of αSpII by μ-calpain.[21,22] FANCG would also not be present to bind to μ-calpain and inhibit it from cleaving αSpII.[21,22] (Modified from Zhang *et al*.,[22] with permission from the American Chemical Society). (A color version of this figure is available in the online journal.)

FANCG, could play an important role in maintaining the stability of αIISp in cells.

Another proposed mechanism is that an FA protein would bind to μ-calpain and inhibit its ability to cleave αIISp (Figure 8). In support of this, it has been shown that FANCA and FANCG bind directly to μ-calpain.[22] This binding could interfere with the ability of μ-calpain to interact with its cleavage site on αIISp. A third proposed mechanism is that FA proteins would modulate binding of calmodulin to αIISp. Binding of calmodulin to a site on αIISp adjacent to the cleavage site of μ-calpain leads to increased cleavage of αIISp by μ-calpain.[234–236] Binding of an FA protein to calmodulin could potentially inhibit calmodulin's binding to αIISp; this in turn would decrease the ability of μ-calpain to cleave αIISp. These studies still need to be carried out. Notably, more than one of these mechanisms may be responsible for the results observed.

Thus, there are a number of mechanisms by which an FA protein could help maintain the stability of αIISp in the cell by reducing its cleavage by μ-calpain. According to the model proposed above, a deficiency in a specific FA protein involved in this process would have deleterious effects on the stability of αIISp and on processes which depend on αIISp such as DNA repair and chromosome stability. This represents another important function for FA proteins—one in which they are involved in maintaining the stability

of a structural protein which plays a critical role in the DNA repair process.

Decreasing μ-calpain activity in FA cells restores DNA repair and chromosome stability after ICL damage

Significantly, the studies of Lambert *et al*. have shown that, when μ-calpain activity is reduced in FA cells, levels of αSpII, DNA repair and chromosome stability are restored to those in normal cells.[22] These studies have demonstrated that in FA-A cells knock-down of μ-calpain activity by μ-calpain siRNA to levels similar to those in normal cells leads to restoration of αSpII to levels found in normal cells.[22] This in turn results in restoration of DNA repair capabilities, localization of XPF in nuclear foci at sites of ICLs and increased chromosomal stability.[22] However, in these FA-A cells there is still a deficiency in the FANCA protein.[22] which raises the question of how the repair defect can be corrected in the absence of this protein. Lambert *et al*. have proposed that when levels of αSpII are restored, by knocking down μ-calpain, the repair process is able to continue since the stability of αSpII has been accomplished by an alternate means, without the need for FANCA. In addition, after restoration of αSpII levels in FA-A cells, non-Ub FANCD2 foci are observed at levels approximately 80% of normal.[208] Formation of these foci follow the same time course as to those of FANCD2-Ub in normal cells after ICL damage.[208] αSpII does not, however, co-localize to any extent with the non-Ub FANCD2 foci.[208] These results thus indicate that αSpII plays a role in localization of non-Ub FANCD2 to sites of damage, though it's role in this process may or may not be direct.[208] Lambert *et al*. have further proposed that in FA-A cells, when levels of αSpII have been restored to normal, non-Ub FANCD2 plays a role in the ICL repair process, (in the absence of FANCA).[208] This proposal is supported by several other studies which provide evidence that non-Ub FANCD2 may be important in the homologous recombination component of ICL repair and in replication fork recovery.[237–241] Thus there may be alternate pathways which can facilitate the DNA damage response and which also involve αSpII.

αIISp is critical for maintenance of telomere function after DNA ICL damage

αIISp functions not only in repair of DNA damage in genomic DNA, it also plays a role in the repair of telomeric DNA where it is critical for maintaining telomere function after ICL damage.[21,186] Telomeres are regions of repetitive nucleotide sequences located at the end of chromosomes which are essential for maintaining genomic integrity.[242–244] They are protected by a multiprotein complex, shelterin, which prevents the ends of telomeres from being recognized as double-strand breaks and thereby prevents end-to-end fusions.[242,244–246] Since the functioning of telomeres is critical for maintenance of chromosome stability, it is important that the integrity of telomeric DNA is maintained after DNA damage.

Lambert *et al*. have shown that αSpII is recruited to telomeres after DNA ICL damage and plays an important role in the repair process.[186] These studies were carried out using telomerase positive normal human lymphoblastoid cells. Telomerase is a ribonucleoprotein enzyme complex which maintains and extends chromosome ends during DNA replication.[247,248] Telomerase positive cells were utilized since highly proliferating cells, peripheral blood cells, bone marrow cells, stem cells such as hematopoietic stem cells and numerous cancer cells express telomerase.[249,250] Use of telomerase positive cells is highly relevant to examination of cellular repair deficiencies in FA. These studies demonstrated, that after ICL damage, αSpII is recruited to telomeres in S phase, indicating that it is important in telomere function during the period when telomeres undergo DNA replication.[242–244,251] They also demonstrated that after ICL damage αSpII co-localizes with two proteins in the shelterin complex, TRF1 and TRF2, and is needed in the recruitment of XPF to the telomeres where it co-localizes with it at sites of damage and is necessary for telomere function after DNA ICL damage. The importance of αSpII is further emphasized by studies in which αSpII was knocked-down by siRNA to levels that are 35% of normal. These studies showed that after ICL damage there was telomere dysfunction, as determined by the formation of telomere dysfunction-induced foci (TIF), which represent sites of DNA double-strand breaks that arise when replication forks are stalled at the site of an ICL.[186] There was also catastrophic loss of telomeres, which is another indication of telomere dysfunction.[186] This group has proposed that reduced levels of αIISp result in defective repair of ICLs in telomeric DNA in S phase and that this leads to stalling of replication forks and formation of DSBs in telomeric DNA, which in turn results in breakage and loss of telomeres.[186]

The studies described above thus demonstrate the importance of αSpII in maintenance of telomere function after DNA ICL damage. The critical role αIISp plays in ICL repair in telomeres is further strengthened by examination of telomeres in FA cells after ICL damage (Figure 9). In FA complementation group A (FA-A) cells, for example, it has been shown that there is a significant increase in telomere dysfunction after DNA ICL damage.[186] Strong evidence supports the proposal that reduced levels of αIISp are an important factor in the production of these dysfunctional telomeres after production of DNA ICLs.[186] In FA-A cells, there is a significant reduction in localization of XPF to sites of damage in telomeres, which is indicative of defective DNA repair and there is a significant increase in the number of TIF positive FA-A cells after ICL damage compared to normal cells as well as a significant loss of telomeres (Figure 9).[186] Importantly, when levels of αIISp are restored to normal by knocking down μ-calpain, the number of TIF positive cells and cells in which there is a loss of telomeres is reduced to normal levels, and levels of XPF foci at telomeres return to normal (Figure 9).[186] These studies further demonstrate that αIISp plays a critical role in telomere maintenance after DNA ICL damage. They also indicate that reduced levels of αIISp in FA cells are a critical factor in the telomere dysfunction observed in FA cells after ICL damage.

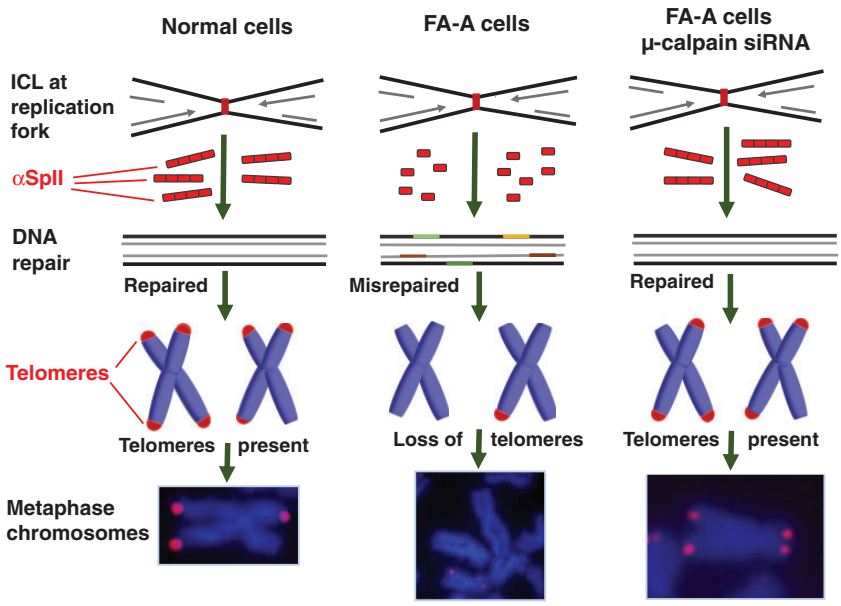

Figure 9. A deficiency in αSpII in FA-A cells results in telomere dysfunction and loss of telomeres after DNA ICL damage. In normal human cells, after DNA ICL damage, αSpII is recruited to telomeres where it plays an important role in the DNA repair process.[186] It is necessary for telomere function after DNA ICL damage. In FA-A cells, reduced levels of αSpII, due to its breakdown by μ-calpain, lead to defective ICL repair in telomeric DNA, telomere dysfunction and catastrophic loss of telomeres.[186] When levels of αIISp are restored to normal by knocking down μ-calpain by μ-calpain siRNA, DNA repair in telomeric DNA takes place, telomere function is restored and levels of telomere positive chromosomes are returned to normal.[186] These studies demonstrate that αIISp plays an important role in telomere maintenance after DNA ICL damage and that reduced levels of αIISp in FA cells are a critical factor in telomere dysfunction in FA cells after ICL damage.[186] Metaphase chromosome inserts showing telomeres are from Zhang *et al*.,[186] and reproduced with permission of Oxford University Press. (A color version of this figure is available in the online journal.)

β-Spectrins and their involvement in DNA repair

Several *β*-spectrins are present in the nucleus. Two isoforms of *β*IV-spectrin have been identified: Sp*β*IVΣ5, a truncated major isoform, and Sp*β*IVΣ1, a full-length minor isoform.[176] Both isoforms have proposed functions in the nucleus; Sp*β*IVΣ5 is thought to contribute to formation of promyelocytic leukemia (PML) bodies.[176] Though PML bodies have been implicated in DNA repair,[252,253] whether Sp*β*IVΣ5 and its association with these bodies plays a role in DNA repair is not known. Sp*β*IVΣ1 is hypothesized to be involved in nuclear skeleton formation; whether it plays a role in DNA repair is also unknown.[176]

Nesprins are members of a family of ubiquitously expressed intracellular proteins containing spectrin repeats.[254,255] Nesprins have a large number of isoforms, and as mentioned above are a component of the LINC complex, extending from the nuclear envelope into the cytoplasm where they associate with microtubules, actin filaments and intermediate filaments. There are also nesprin isoforms that are localized in the nucleus and associate with lamins A/C and emerin.[254] Recent studies indicate that nesprin-2 binds to lamin A and also localizes to PML bodies, which are tethered to the nuclear lamina in vascular smooth muscle cells.[256] This process is thought to be important in the DNA damage response and in localization of signal-regulated kinases to damage-induced repair foci.[256] In addition, studies indicate that nesprin-1 co-localizes with two mismatch repair (MMR) proteins, MSH2 and MSH3, in the nucleus and may have an important role in the functioning of these proteins in mismatch repair (MMR).[257] Knockdown of nesprin-1 also leads to an increase in DNA DSBs.[257] It also leads to failure of recruitment of Ku70, a protein involved repair of DNA DSBs by NHEJ, to sites of DSBs.[257] Since MMR and repair of DNA DSBs by NHEJ are two interlinked processes, this may indicate an involvement of nesprin-1 in both pathways.[257] In various tumor cell lines, nesprin-1 levels are greatly reduced and this correlates with an increase in DNA DSBs and with DNA repair dysfunction.[257] This suggests that nesprin-1 may be important in tumorigenesis and genomic stability.[257] Whether nesprin-1 is associated with PML bodies is not known but is certainly of interest.

Based on the studies described above, it would be interesting to speculate that the association of nesprin-2 with lamin A and PML bodies and their combined involvement in the DNA damage response may be related to the association of Sp*β*IVΣ5[176] and αSpII[21] with PML bodies and that multiple proteins are involved in the repair processes taking place. This is particularly interesting since it has been recently shown that disruption of PML bodies in cells from a mouse model leads to increased sister-chromatid exchanges and chromosome abnormalities and also to impaired HR and NHEJ repair pathways in which there are deficiencies in localization of repair proteins to sites of DNA damage.[258]

Studies on another *β*-spectrin (*β*SpII) (i.e., *β*2Sp) indicate that it may play a role in DNA repair and genomic stability.[177–179,259] *β*SpII is an adapter protein for the Smad3/Smad4 complex which is involved in TGF-*β* signaling and which is translocated from the cytoplasm into the

nucleus.[177–179,259] These studies have shown that, after exposure of βSpII deficient cells to several genotoxic agents (i.e., ionizing radiation or ICL agents), cells exhibit defective restart of stalled replication forks, decreased cell survival, and increased chromosomal aberrations.[177–179,259] This work suggests that βSpII in the nucleus may play a role in the DNA damage response by facilitating repair following replication fork stalling and further suggests that homologous recombination may be involved in this process.[177–179,259] The nuclear functions of βSpII, like those of αSpII, may thus include a role in repair of damaged DNA.

Loss or deficiencies in spectrins and human disorders

Spectrins in the nucleus, in particular αSpII, play a role in a number of important processes which, in addition to DNA repair and chromosome stability, include maintenance of the nuclear architecture, the biomechanical properties of the nucleus, and mechanical coupling between the nucleoskeleton and cytoskeleton.[1,10,158–161,164] αSpII's interactions with proteins, such as those involved in chromatin remodeling, transcription, and RNA processing, suggests that it may be involved in these processes as well.[1,21,181,207] Hence, due to the functional diversity of αSpII, a deficiency or dysfunction in this protein would be expected to lead to a number of different pathological changes or disorders. As mentioned above, one of these is the hematological disorder, FA, which is characterized by bone marrow failure, defective DNA repair and development of cancer (e.g. acute myeloid leukemia (AML), squamous cell carcinoma).[25] Reduced levels of αSpII in FA cells are thought to be an important factor in the DNA repair defects and genomic instability observed.[21] Of particular interest, and as yet unexplored, is the possibility that a deficiency in αSpII could be related to some of the congenital abnormalities observed in FA such as absent radii, radial ray deformities, renal abnormalities and urogenital malformations.[260] Since αSpII has a structural function and is critical in many developmental processes,[173,261–263] it is possible that a deficiency in αSpII in FA could be a factor in a number of these congenital abnormalities.

Evidence also clearly points to a role for αSpII in cancer development. A loss of αSpII in the bone marrow has been reported in patients with AML.[264,265] This indicates that a deficiency in αSpII may play a role in leukemogenesis.[264,265] Since FA patients have a predisposition to develop AML, a deficiency in αSpII may be a contributing factor to the bone marrow failure which occurs.[21,264,265] Altered expression of *SPTANI*, the gene encoding αSpII, has been found in a range of cancers and is reported to play an important role in cancer development and progression.[266,267] It has been proposed that nuclear αSpII acts as a tumor suppressor enabling DNA repair to take place.[267] Loss of αSpII due to either mutations in *SPTAN1* or its breakdown could lead to defects in DNA repair, which could play an important role in tumorgenesis.[266,267] Additionally, in several types of cancer, changes in expression of αSpII are associated with tumor development and progression. In Lynch syndrome and sporadic colorectal cancers, for example, which are deficient in the mismatch repair protein, MLH1, there is a significant reduction in αSpII expression.[266,267] Since αSpII has been shown to directly interact with MLH1, it has been proposed that loss of MLH1 in colorectal cancer leads to destabilization of αSpII which can lead to defective DNA repair and tumor progression.[266,267,269] A reduction in αSpII has also been reported in lung cancer; whether this is due to mutations identified in the *SPTAN1* gene is not clear but it has been suggested that this could lead to DNA repair defects and cancer development.[270]

In the neurological disorders, early infantile epileptic encephalopathy type 5 (EIEE5) and West syndrome, in which there is impaired neurodevelopment and progressive brain atrophy, there are mutations in *SPTAN1*.[271–274] In this disorder there is a defect in binding of αSpII to β-spectrins (II, IV) in the cytoplasm, which is essential for neural development. This is a disorder in which the deficiency in αSpII is due to a mutation in the *SPTAN1* gene leading to a defective protein, not to a breakdown of αSpII after expression. In EIEE5, it is loss of a structural function of αSpII that leads to the neurodevelopmental deficiencies. Whether there is a deficiency in DNA repair is not known nor is it known whether this type of interaction takes place in the nucleus as well as the cytoplasm.

There are also disorders in which there are defects in βSpII. One of these is hepatocellular cancer.[179,275] In human hepatocellular carcinoma (HCC), there are significant reductions in expression of βSpII which are thought to contribute to the tumorgenesis of HCC.[275,276] It has been proposed that loss of βSpII in HCC cells activates Wnt signaling, which leads to development of stem cell-like features and a less differentiated state in these cells, and ultimately contributes to tumor development and invasion.[275] Though mutations in DNA repair genes have been noted in primary liver cancer cells,[277] studies have not been carried out to determine whether loss of βSpII in HHC cells is related to defects in DNA repair in these cells.

These studies emphasize that a deficiency in spectrin can have serious consequences and lead to a number of disorders. It is important to keep in mind that in some disorders a loss or deficiency in αSpII is due to post-transcriptional events. In FA, for example, αSpII is expressed but is broken down after expression. In other disorders, such as EIEE5, the deficiency in αSpII is due to mutations in the *SPTAN1* gene resulting in expression of a defective αSpII. The effects that loss of a functional αSpII protein has on the cell are variable. In FA and MLH1-deficient sporadic colorectal cancers, for example, a deficiency or loss of αSpII is thought to result in defective DNA repair which can lead to the development of cancer. This emphasizes the functional importance of the role αSpII plays in DNA repair. αSpII is also important in cell development, and, as has been proposed, it is possible that a deficiency in αSpII could play a role in the congenital developmental abnormalities observed in FA patients. Spectrins are multifunctional proteins which play critical roles in the nucleus. As their interactions with other nucleoskeletal proteins become more fully recognized and more extensively studied, important insights should be gained as to their role in a number of other disease processes, many of which have not as yet been recognized as being deficient in αSpII.

Conclusions/perspective

Nucleoskeletal proteins play dynamic roles in a number of essential nuclear processes. An important one, and one which has been concentrated on in this review, is the role these proteins play in DNA repair. Their importance in this process is emphasized by the effects that a deficiency or loss in each of these has on specific repair pathways critical for maintenance of important cellular functions and for preservation of genomic stability. The A-type and B-type lamins play critical roles in repair of DNA DSBs and in regulation of the NHEJ and/or HR pathways involved in this process (Figure 10). Loss or a deficiency in these lamins leads to a wide spectrum of disease phenotypes such as the laminopathies which include muscular dystrophies, cardiomyopathies, peripheral neuropathies and premature aging syndromes (e.g. HGPS). Actin is actively involved in repair of DNA DSBs in both its monomeric and filamentous forms. There is significant accumulation of actin filaments after formation of DNA DSBs and these are critical for repair of DSBs by both NHEJ and HR (Figure 10). Monomeric actin is a key component of chromatin remodeling complexes which also facilitate DNA DSB repair. Actin interacts with nuclear myosin in facilitating relocalization of DNA DSBs in heterochromatin to discrete subnuclear clusters. This process is critical for HR repair and is hypothesized to promote exchanges with sister chromosomes or homologues and prevent aberrant recombination. The LINK complex contributes to repair of DNA DSBs and ICLs. The SUN1 protein has been shown to interact with NHEJ proteins to lead to inhibition of NHEJ and stimulation of HR. It is also involved in endonuclease recruitment in ICL repair. αSpII has been shown to play a critical role in repair of DNA ICLs in both genomic and telomeric DNA. It acts as a scaffold in recruitment of repair proteins important in this process, particularly in the initial damage recognition and incision steps of the repair process (Figure 10). It directly binds to DNA at the site of a DNA ICL and to proteins involved in the ICL repair process. Its importance in the repair process is seen in the disorder, FA, where there is a deficiency in αSpII due to its breakdown by μ-calpain. Loss of αSpII in FA is thought to be an important factor in the DNA repair deficiency and genomic instability associated with this disorder. Mutations in the *SPTAN1* gene resulting in expression of a defective αSpII have been found in a number of cancers and this is proposed to lead to defects in DNA repair and play a role in cancer development and progression. There are thus multiple lines of evidence demonstrating the importance of these nucleoskeletal proteins in DNA repair processes.

What has not yet been explored, however, is how a deficiency in one of these nucleoskeletal proteins (i.e., lamin, actin, spectrin) affects the functioning in the DNA repair process of the other nucleoskeletal proteins with which it interacts. For example, does a loss or deficiency in lamin affect actin or αSpII functioning in repair processes in cells? Does a deficiency in αSpII have an effect on lamin or actin repair function? There are some indications that this indeed occurs. It has been shown that lamin A directly interacts with actin *in vitro* leading to actin filament bundling.[278,279]

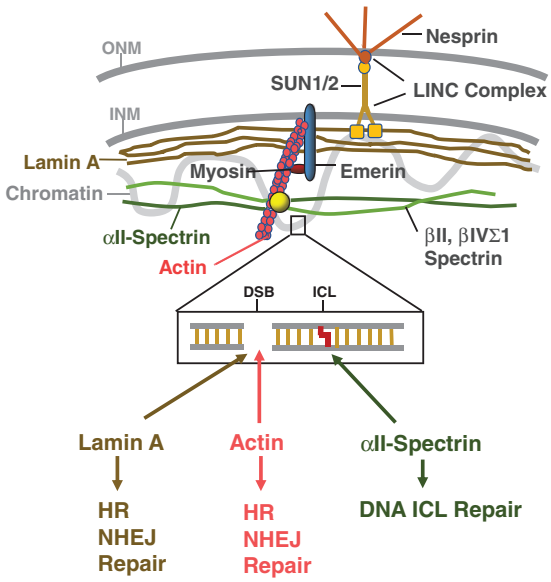

Figure 10. Overview of the major nucleoskeletal proteins which play critical roles in repair of damaged DNA. A-type lamins function in repair of DNA DSBs and in regulation of the NHEJ and HR pathways involved in this process. Actin is involved in repair of DNA DSBs by HR and NHEJ. It also interacts with myosin in facilitating relocalization of DNA DSBs in heterochromatin, a process critical for HR. αII-Spectrin plays a critical role in repair of DNA ICLs. It binds to damaged DNA and acts as a scaffold in the recruitment of repair proteins to the site of damage. The LINC complex and the proteins associated with it contribute to the repair of DNA DSBs and ICLs. The SUN1 protein has been shown to interact with NHEJ proteins leading to inhibition of NHJ and stimulation of HR. It may also be involved in endonuclease recruitment in ICL repair. (A color version of this figure is available in the online journal.)

It has been hypothesized that lamin A may affect the levels of free actin in the nucleus and lead to polymerization of actin.[278] Other studies have shown that emerin, a nuclear membrane protein that caps actin filaments and binds directly to lamins,[280,281] could, together with lamins A/C, modulate actin dynamics and play a role in nuclear actin polymerization.[279] It could be hypothesized that, after exposure to a DNA damaging agent, bundling of actin monomers into filaments, which has been shown to occur, is aided by lamins A/C and emerin and this helps facilitate the repair of DNA double strand breaks. αSpII could also potentially affect actin dynamics in the nucleus, particularly after DNA damage. It has been shown to interact with actin.[1,181] Impaired filamentous actin rearrangements and lack of cellular protrusions have been observed in macrophages from FANCC$^{-/-}$ mice.[282] Of interest, studies have shown that there is a deficiency in αSpII in bone marrow cells from FANCC$^{-/-}$ mice (Lambert *et al.*, unpublished observations). A deficiency in αSpII in human lymphoblastoid cells has been shown to lead to changes in cellular morphology; the cells become rounder and there are fewer pseudopodia.[185] It has been hypothesized that αSpII plays a role in actin filament formation and that reduction of αSpII in cells, such as FANCC$^{-/-}$ cells, leads to defects in formation of actin filaments and to the changes observed in cellular morphology.[21] It is possible that αSpII has a similar role in the organization of actin filaments in the nucleus which would have an impact on repair of DNA damage.[21] These studies provide evidence that an

interaction between some of these nucleoskeletal proteins is an important factor in the DNA repair process.

The role these nucleoskeleton proteins play in the DNA damage response and the effects that interactions between these proteins have on repair pathways represents another level of protein interactions which needs to be considered. These interactions can have an important impact on the outcome of DNA repair processes and the clinical manifestations of disorders in which individual protein components of the nucleoskeleton are defective or deficient. They will have an important impact on development of mechanistic studies on the involvement of these proteins in pathways involved in the DNA repair process and need to be seriously taken into consideration when developing models for these DNA repair pathways.

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REFERENCES

1. Simon DN, Wilson KL. The nucleoskeleton as a genome-associated dynamic network of networks. *Nat Rev Mol Cell Biol* 2011;**12**:695–708
2. Goldman RD, Gruenbaum Y, Moir RD, Shumaker DK, Spann TP. Nuclear lamins: building blocks of nuclear architecture. *Gene Dev* 2002;**16**:533–47
3. Taddei A, Hediger F, Neumann FR, Gasser SM. The function of nuclear architecture: a genetic approach. *Annu Rev Genet* 2004;**38**:305–45
4. Dechat T, Pfleghaar K, Sengupta K, Shimi T, Shumaker DK, Solimando L, Goldman RD. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev* 2008;**22**:832–953
5. Redwood AB, Gonzales-Suarez I, Gonzalo S. Regulating the levels of key factors in cell cycle and DNA repair: new pathways revealed by lamins. *Cell Cycle* 2011;**10**:3652–7
6. Misteli T, Soutoglou E. The emerging role of nuclear architecture in DNA repair and genome maintenance. *Nat Rev Mol Cell Biol* 2009;**10**:243–54
7. Wilson KL, Dawson SC. Functional evolution of nuclear structure. *J Cell Biol* 2011;**195**:171–81
8. Holaska JM, Wilson KL. An emerin 'proteome': purification of distinct emerin-containing complexes from HeLa cells suggests molecular basis for diverse roles including gene regulation, mRNA splicing, signalling, mechano-sensing and nuclear architecture. *Biochemistry* 2007;**46**:8897–908
9. Zohn Z, Wilson KL, Dahl KN. Beyond lamins: other structural components of the nucleoskeleton. *Methods Cell Biol* 2010;**98**:97–119
10. Armiger TJ, Spagnol ST, Dahl KN. Nuclear mechanical resilience but not stiffness is modulated by αII-spectrin. *J Biomech* 2016;**49**:39839
11. Dhal KN, Kalinowski A. Nucleoskeleton mechanics at a glance. *J Cell Sci* 2011;**124**:675–8
12. Lammerding J, Schulze PC, Takahaski T, Kozlov S, Sullivan T, Kamm RD, Stewart CL, Lee RT. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clinical Invest* 2004;**113**:370–8
13. Paul W, Doetsch PW. Mechanisms of DNA repair. In: *Progress in Molecular Biology and Translational Science*. Vol. 110. Cambridge: Academic Press (Elsevier Inc.), 2012, p. 353
14. Nickoloff JA, Hoekstra MF. *DNA damage and repair vol 2: DNA repair in higher eukaryotes*. Totowa: Humana Press, 2013, p. 639
15. Lehmann A, Muzi-Falconi M, Lindahl T, Friedberg E, Elledge S. *DNA repair, mutagenesis, and other responses to DNA damage. Cold spring harbor perspectives in biology.* Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2014, p. 445
16. Liu N-A, Sun J, Kono K, Horikoshi Y, Ikura T, Tong X, Haraguchi T, Tashiro S. Regulation of homologous recombinational repair by lamin B1 in radiation-induced DNA damage. *The FASEB J* 2015;**29**:2514–25
17. Gibbs-Seymour I, Markiewicz EW, Bekker-Jensen S, Mailand N, Hutchison CJ. Lamin A/C-dependent interaction with 53BP1 promotes cellular responses to DNA damage. *Aging Cell* 2015;**14**:162–9
18. Gonzalo S. DNA damage and lamins. *Adv Exp Med Biol* 2014;**773**:377–99
19. Andrin C, McDonald D, Attwood KM, Rodrigue A, Ghosh S, Mirzayans R, Masson J-Y, Dellaire G, Hendzel MJ. A requirement for polymerized actin in DNA double-strand break repair. *Nucleus* 2012;**3**:384–95
20. Evdokimova VN, Gandhi M, Nikitski AV, Bakkenist CJ, Nikiforov YE. Nuclear myosin/actin-motored contact between homologous chromosomes is initiated by ATM kinase and homology-directed repair proteins at double-strand DNA breaks to suppress chromosome rearrangements. *Oncotarget* 2018;**9**:13612–22
21. Lambert MW. Spectrin and its interacting partners in nuclear structure and function. *Exp Biol Med (Maywood)* 2018;**243**:507–24
22. Zhang P, Sridharan D, Lambert MW. Knockdown of μ-calpain in fanconi anemia, FA-A, cells by siRNA restores αII spectrin levels and corrects chromosomal instability and defective DNA interstrand cross-link repair. *Biochemistry* 2010;**49**:5570–81
23. Brosh RM, Jr, Bellani M, Liu Y, Seidman MM. Fanconi anemia: a DNA repair disorder characterized by accelerated decline of the hematopoietic stem cell compartment and other features of aging. *Ageing Res Rev* 2017;**33**:67–75
24. Datta A, Brosh RM, Jr. Holding all the cards – how fanconi anemia proteins deal with replication stress and preserve genomic stability. *Genes* 2019;**10**:170
25. Niraj J, Farkkila A, D'Andrea AD. The fanconi anemia pathway in cancer. *Annu Rev Cancer Biol* 2019;**3**:457–78
26. Worman HJ, Ostlund C, Wang Y. Diseases of the nuclear envelope. *Cold Spring Harb Perspect Biol* 2010;**2**:a000760
27. Worman HJ. Nuclear lamins and laminopathies. *J Pathol* 2012;**226**:316–25
28. Gonzalo S, Kreienkamp R, Askjaer P. Hutchinson-Gilford progeria syndrome: a premature aging disease caused by LMNA gene mutations. *Ageing Res Rev* 2017;**33**:18–29
29. Gerace L, Huber MD. Nuclear lamina at the crossroads of the cytoplasm and nucleus. *J Struct Biol* 2012;**177**:24–31
30. Davidson PNM, Lammerding J. Broken nuclei–lamins, nuclear mechanics and disease. *Trends Cell Biol* 2014;**24**:247–56
31. Gruenbaum Y, Medalia O. Lamins: the structure and protein complexes. *Curr Opin Cell Biol* 2015;**32**:7–12
32. Lammerding J, Fong LG, Jy J, Reue K, Stewart CL, Young SG, Lee RT. Lamins A and C but not lamin B1 regulate nuclear mechanics. *J Biol Chem* 2006;**281**:25768–80
33. Barton LJ, Soshnev AA, Geyer PK. Networking in the nucleus: a spotlight on LEM-domain proteins. *Curr Opin Cell Biol* 2015;**34**:1–8
34. Adam SA, Goldman RD. Insights into the differences between the A-and B-type nuclear lamins. *Adv Biol Regul* 2012;**52**:108–13
35. Lin F, Worman HJ. Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *J Biol Chem* 1993;**268**:16321–6

36. Decchat T, Adam SA, Taimen P, Shimi T, Goldman RD. Nuclear lamins. *Cold Spring Harb Perspect Biol* 2010;**2**:a000547
37. Burke B, Stewart CL. The nuclear lamins: flexibility in function. *Nat Rev Mol Cell Biol* 2013;**14**:13–24
38. Gonzalo S, Kreieinkamp R. DNA repair defects and genome instability in Hutchinson-Guilford progeria syndrome. *Curr Opin Cell Biol* 2015;**34**:75–83
39. Mao Z, Bozzella M, Selaunov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. *DNA Repair (Amst)* 2008;**7**:1765–71
40. Kanaar R, Wyman C, Rothstein R. Quality control of DNA break metabolism: in the 'end'. It's a good thing. *EMBO J* 2008;**27**:581–8
41. Hartlerode AJ, Sully R. Mechanisms of double-strand break repair in somatic mammalian cells. *Biochem J* 2009;**423**:157–68
42. Misteli T, Soutoglou E. The emerging role of nuclear architecture in DNA repair and genome maintenance. *Nat Rev Mol Cell Biol* 2009;**10**:243–54
43. Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. *Annu Rev Genet* 2011;**45**:247–71
44. Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell* 2012;**47**:497–510
45. Goodarzi AA, Jeggo PA. The repair and signalling responses to DNA double-strand breaks. *Adv Genet* 2013;**82**:1–45
46. Wright WD, Shah SS, Heyer W-D. Homologous recombination and the repair of DNA double-strand breaks. *J Biol Chem* 2018;**293**:10524–35
47. Pannunizo NR, Watanabe G, Lieber MR. Nonhomologous DNA end-joining for repair of DNA double-strand breaks. *J Biol Chem* 2018;**293**:10512–23
48. Wayman C, Kanaar R. DNA double-strand break repair: all's well that ends well. *Annu Rev Genet* 2006;**40**:363–83
49. Daley JM, Sung P. 53BP1, BRCA1, and the choice between recombination and end joining at DNA double-strand breaks. *Mol Cell Biol* 2014;**34**:1380–8
50. Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, Kakarougkas A, Meek K, Taucher-Scholz G, Löbrich M, Jeggo PA. Factors determining DNA double-strand break repair pathway choice in G2 phase. *EMBO J* 2011;**30**:1079–92
51. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* 2008;**9**:297–308
52. Kakarougkas A, Jeggo PS. DNA DSB repair pathway choice: an orchestrated handover mechanism. *Br J Radiol* 2014;**87**:20130685
53. Bennardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. *PLoS Genet* 2008;**4**:e1000110
54. Dueva R, Iliakis G. Alternative pathways of non-homologous end joining (NHEJ) in genomic instability and cancer. *Transl Cancer Res* 2013;**2**:162–77
55. Deeriano L, Roth DB. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. *Annu Rev Genet* 2013;**47**:433–55
56. Verma P, Greenberg RA. Noncanonical views of homology-directed DNA repair. *Genes Dev* 2016;**30**:1138–54
57. Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol* 2017;**18**:495–506
58. Her J, Bunting SF. How cells ensure correct repair of DNA double-strand breaks. *J Biol Chem* 2018;**293**:10502–11
59. Sallmyr A, Tomkinson AE. Repair of DNA double-strand breaks by mammalian alternative end-joining pathways. *J Biol Chem* 2018;**293**:10536–46
60. Mendez-Lopez I, Worman HJ. Inner nuclear membrane proteins: impact on human disease. *Chromosoma* 2012;**121**:153–67
61. Redwood AB, Perkins SM, Vanderwaal RP, Feng Z, Biehl KJ, Gonzalez-Suarez I, Mforgado-Palacin L, Shi W, Sage J, Roti-Roti JL, Stewart CL, Zhang J, Gonzalo S. A dual role for A-type lamins in DNA double-strand break repair. *Cell Cycle* 2011;**10**:1–12
62. Vorburger K, Lehner CF, Kitten CT, Eppenberger HM, Nigg EAA. A second higher vertebrate B-type lamin. cDNA sequence determination and *in vitro* processing of chicken lamin B2. *J Mol Biol* 1989;**208**:405–15
63. Liu B, Wang J, Chan KM, Tjia WM, Dneg W, Guan X, Huang J-D, Li KM, Chau PY, Chen DJ, Pei D, Pendas AM, Cadinanos J, Lopez-Otin C, Tse HF, Hutchison C, Chen J, Cao Y, Cheah KSE, Tryggvason K, Zhou Z. Genomic instability in laminopathy-based premature aging. *Nature Med* 2005;**11**:780–5
64. Singh M, Hunt CR, Pandita RK, Kumar R Yang C-R, Horikoshi N, Bachoo R, Serag S, Storoy MD, Shay JW, Powell SN, Gupta A, Jeffery J, Pandita S, Chen BPC, Ceckbar D, Lobrich M, Yang Q, Khanna KK, Worman HJ, Pandita TK. Lamin A/C depletion enhances DNA damage-induced stalled replication fork arrest. *Mol Cell Biol* 2013;**33**:1210–22
65. Bothmer A, Robbiani DF, Feldhahn N, Gazumyn A, Nussenzweig A, Nussenzweig MC. 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. *J Exp Med* 2010;**207**:855–65
66. Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn S, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cai L, Xi X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010;**141**:243–54
67. Pozo FM, Tang J, Bonk KW, Keri RA, Yao X, Zhang Y. Regulatory cross-talk determines the cellular levels of 53BP1 protein, a critical factor in DNA repair. *J Biol Chem* 2017;**292**:5992–6003
68. Zimmermann M, Lottersberger F, Nuonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using rRiF1 to control 5′ end resection. *Science* 2013;**329**:700–4
69. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, Cook MA, Rosebrock AP, Munro M, Canny MD, Xi D, Durocher D. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013;**49**:872–83
70. Brachner A, Foisner R. Lamins reach out to novel functions in DNA damage repair. *Cell Cycle* 2011;**10**:3426–8
71. Li BX, Chen J, Chao B, Zheng Y, Xiao X. A lamin-binding ligand inhibits homologous recombination repair of DNA double-strand breaks. *ACS Cent Sci* 2018;**4**:1201–10
72. Cho S, Vashisth M, Abbas A, Majkut S, Vogel K, Xia Y, Ivanovska IL, Irianto J, Tewari M, Zhu K, Tichy ED, Mourkioti F, Tang H-Y, Greenberg RA, Prosser BL, Discher DE. Mechanosensing by the lamina protects against nuclear rupture, DNA damage and cell-cycle arrest. *Dev Cell* 2019;**49**:920–35
73. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L, Herrmann H, Blum H, Engelkakmp D, Stewart CL, Leonardt H, Joffe B. LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* 2013;**152**:584–98
74. Rober RA, Weber K, Osborn M. Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. *Development* 1989;**105**:365–78
75. Lehner CF, Stick R, Eppenberger HM, Nigg EA. Differential expression of nuclear lamin proteins during chicken development. *J Cell Biol* 1987;**105**:577–87
76. Swift J, Ivanovska Il Busboim A, Harada T, Dingal PC, Pinter J, Pajerowski JD, Spinler KR, Shin JW, Tewari M, Rehfeldt F, Speicher DW, Discher DE. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* 2013;**341**:1240104
77. Khan RI, Nirzhor SSR, Akter R. A review of the recent advances made with SIRT6 and its implications on aging related processes, major human diseases, and possible therapeutic targets. *Biomolecules* 2018;**8**:44
78. Van Meter M, Simon M, Tombline G, May A, Morello TD, Hubbard BP, Bredbenner K, Park R, Sinclair DA, Bohr VA, Gorbunova V, Seluanov A. JNK phosphorylates SIRT6 to stimulate DNA double-strand break repair in response to oxidative stress by recruiting PARP1 to DNA breaks. *Cell Rep* 2017;**16**:2641–50

79. Ghosh S, Liu B, Wang Y, Hao Q, Zho Z. Lamin A is an endogenous SIRT6 activator and promotes SIRT6-mediated NDA repair. *Cell Rep* 2015;**13**:1396–406
80. Gertler AA, Cohen HY. SIRT6, a protein with many faces. *Biogerontology* 2013;**14**:629–39
81. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian healthspan. *Trends Genet* 2014;**30**:271–86
82. Kaidi A, Weinert BT, Choudhary C, Jackson SP. Human SIRT6 promotes DNA and resection through CtIP deacetylation. *Science* 2010;**329**:1348–53
83. Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles of SIRT6. *Trends Biochem Sci* 2014;**39**:72–81
84. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gelson L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu JT, Hong AL, Ford E, Cheng HL, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopoulos GD, Alt FW. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 2006;**124**:315–29
85. Mao Z, Tian X, Van Meter M, Ke Z, Gorbunova V, Seluanov A. Sirtuin 6 (SIRT6) rescues the decline of homologous recombination repair during replicative senescence. *Proc Natl Acad Sci USA* 2012;**109**:11800–5
86. Liu Bm Gosh S, Yang Z, Zheng H, Liu X, Wang Z, Jin G, Zheng B, Kennedy BK, Suh Y, Kaeberlein M, Tryggvason K, Zhou Z. Resveratrol rescues SIRT1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based progeria. *Cell Metab* 2012;**16**:738–50
87. Bonne G, Mercuri E, Muchir A, Urtizberea A, Becane HM, Recan D, Merlini L, Wehnert M, Boor R, Reuner U, Vorgerd M, Eicklein EM, Eymard B, Duboc D, Penisson-Besnier I, Cuisset JM, Ferrer X, Desguerre I, Lacombe D, Bushby K, Pollitt C, Toniolo D, Fardeau M, Schwartz K, Muntoni F. Clinical and molecular genetic spectrum of autosomal dominant Enery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. *Ann Neurol* 2000;**48**:170–80
88. Shackleton S, Lloyd DJ, Jackson SN, Evans R, Niermeijer MF, Singh BM, Schmidt H, Brabant B, Kumar S, Durrington PN, Gregory S, O'Rahilly S, Trembath RC. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat Genet* 2000;**24**:153–6
89. Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci USA* 2004;**101**:8963–8
90. Duer WT, Worman HJ. The nuclear envelope, nuclear lamina, and inherited disease. *Int Rev Cytol* 2005;**246**:231–79
91. Broers JL, Ramackers FC, Bonne G, Yaou RB, Hutchison CJ. Nuclear lamins: laminopathies and their role in premature ageing. *Physiol Rev* 2001;**86**:967–1008
92. Bertrand AT, Chikhaoui K, Yaou RA, Bonne G. Clinical and genetic hererogeneity in laminopathies. *Biochem Soc Trans* 2011;**39**:1687–92
93. De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, Lyonnet S, Stewart CL, Munnich A, Le Merrer N, Levy N. Lamin A truncation in Hutchinson-Gilford progeria. *Science* 2003;**300**:2055
94. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Ribbins CM, Moses TY, Berglund P, Dutra A, Pak eE Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 2003;**423**:293–8
95. Decker ML, Chavez E, Vulto I, Lansdorp PM. Telomere length in Hutchinson-Gilford progeria syndrome. *Mech Ageing Dev* 2009;**130**:377–83
96. Liu Y, Wang Y, Rusinol AE, Sinensky MS, Liu J, Shell SM, Zou Y. Involvement of xeroderma pigmentosum group A (XPA) in progeria arising from defective maturation of prelamin A. *FASEB J* 2008;**22**:603–11
97. Liu GH, Barkho BZ, Ruiz S, Diep D, Qu J, Yang SL, Panopoulos AD, Suzuki K, Kurian L, Walsh C, Thompson J, Boue S, Fung HL, Sancho-Martinez I, Zhang K, Yates J, 3rd, Izpisua Belmonte JC. Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature* 2011;**472**:221–5
98. Hilton BA, Liu J, Cartwright BM, Liu Y Breitman M, Wang Y, Jones R, Tang H, Rusinol A, Musich PR, Zou Y. Progerin sequestration of PCNA promotes replication fork collapse and mislocalization of XPA in laminopathy-related progeroid syndromes. *FASEB J* 2017;**31**:3882–93
99. Moldovan G-L, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. *Cell* 2007;**129**:665–79
100. Constantinescu D, Csoka AB, Navara CS, Schatten GP. Defective DSB repair correlates with abnormal nuclear morphology and is improved with FTI treatment in Hutchinson-Gilford progeria syndrome fibroblasts. *Exp Cell Res* 2010;**316**:2747–59
101. Gonzalez-Suarez I, Redwood AB, Perkins SM, Vermolen B, Lichtensztejin D, Grotsky DA, Morgado-Palacin L, Gapud EJ, Sleckman BP, Sullivan T, Sage J, Stewart CL, Mai S, Gonzalo S. Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. *EMBO J* 2009;**28**:2414–27
102. Wood AM, Rendtle W, Danielsen JM, Lucas CA, Rice EL, Scalzo D, Shimi T, Goldman RD, Smith ED, Le Beau MN, Kosak ST. TRF2 and lamin A/C interact to facilitate the functional organization of chromosome ends. *Nat Commun* 2014;**5**:5467
103. Dimitrova N, Chen YC, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 2008;**456**:524–8
104. Malhas An Lee CF, Vaux DJ. Lamin B1 controls oxidative stress response via oct-1. *J Cell Biol* 2009;**184**:45–55
105. Tang CW, Maya-Mendoza A, Martin C, Zeng K, Chen S, Feret D, Wilson SA, Jackson DA. The integrity of a lamin-B1-dependent nucleoskeleton is a fundamental determinant of RNA synthesis in human cells. *J Cell Sci* 2008;**121**:1014–24
106. Butin-Israeli V, Adam SA, Goldman RD. Regulation of nucleotide excision repair by nuclear lamin B1. *PLoS One* 2013;**8**:e69169
107. Shimi T, Pfleghaar K, Kojima S, Pack C-G, Solovei I, Goldman AE, Adam SA, Shumaker DK, Kinjo M, Cremer T, Goldman RD. The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. *Genes and Dev* 2008;**22**:3409–21
108. Butin-Israeli V, Adam SA, Jain N, Otte GL, Neems D, Wiesmuller L, Berger SL, Goldman RD. Role of lamin B1 in chromatin instability. *Mol Cell Biol* 2015;**35**:884–98
109. Gieni RS, Hendzel MJ. Actin dynamics and functions in the interphase nucleus: moving toward an understand of nuclear polymeric actin. *Biochem Cell Biol* 2009;**87**:283–306
110. Kristo I, Bajusz I, Bajusz C, Borkuti P, Vilmos P. Actin, actin-binding proteins, and acitn-related proteins in the nucleus. *Histochem Cell Biol* 2016;**145**:373–88
111. Moore HM, Vartianinen MK. F-actin organizes the nucleus. *Nature Cell Biol* 2017;**19**:1386–8
112. Virtanen JA, Vartiainen MK. Diverse functions for different forms of nuclear actin. *Curr Opin Cell Biol* 2017;**46**:33–8
113. Klages-Mundt NL, Kumar A, Zhang Y, Kapoor P, Shen X. The nature of actin-family proteins in chromatin-modifying complexes. *Front Genet* 2018;**9**:1–16
114. Kelpsch DJ, Tootle TL. Nuclear actin: from discovery to function. *Anat Rec (Hoboken)* 2018;**301**:1999–2013
115. Grosse R, Vartiainen MK. To be or not to be assembled: progressing into nuclear actin filaments. *Nat Rev Mol Cell Biol* 2017;**46**:33–8
116. McDonald D, Carrero G, Andrin C, de Vries G, Hendzel MJ. Nucleoplasmic beta-actin exists in a dynamic equilibrium between low mobility polymeric species and rapidly diffusing populations. *J Cell Biol* 2006;**172**:541–52
117. Plessner M, Grosse R. Dynamizing nuclear actin filaments. *Curr Opin Cell Biol* 2019;**56**:1–6
118. Pederson T. As functional nuclear actin comes into view, is it globular, filamentous or both? *J Cell Biol* 2008;**180**:1061–4

119. Pederson T, Aebi U. Actin in the nucleus: what form and what for? *J Struct Biol* 2001;**140**:3–9
120. Hofmann WA, de Lanerolle P. Nuclear actin to polymerize or not to polymerize. *J Cell Biol* 2006;**172**:495–6
121. Jockusch BM, Schoenberger CA, Stetefeld J, Aebi U. Tracking down the different forms of nuclear actin. *Trends Cell Biol* 2006;**16**:391–6
122. Simon DN, Zastrow MS, Wilson KL. Direct actin binding to A- and B-type lamin tails and actin filament bundling by the lamin A tail. *Nucleus* 2010;**1**:264–72
123. Belin BJ, Lee T, Mullins RD. DNA damage induces nuclear actin filament assembly by formin-2 and spire-1/2 that promotes efficient DNA repair. *eLIFE* 2015;**4**:07735
124. Rajakyla EK, Vartiainen MK. Rho, nuclear actin, and actin-binding proteins in the regulation of transcription and gene expression. *Small GTPases* 2014;e275394:
125. Pendleton A, Pope B, Weeds A, Koffer A. Latrunculin B or ATP depletion induces cofilin-dependent translocation of actin into nuclei of mast cells. *J Biol Chem* 2003;**278**:14394–400
126. Sun M, Yang M, Xie FY, Wang W, Zhang L, Shen W, Yin S, Ma JY. DNA double-strand breaks induce the nuclear actin filaments formation in cumulus enclosed oocytes but not in denuded oocytes. *PLoS ONE* 2017;**12**:e0170308
127. Schrank BR, Aparicio T, Li Y, Chang W, Chait BT, Gundersen GG, Gottesman ME, Gautier J. Nuclear ARP2/3 DNA break clustering for homology-directed repair. *Nature* 2018;**559**:61–6
128. Caridi CP, D'Agostino CD, Ryu T, Zapotoczny G, Delabaere L, Xiao L, Khodaverdian VY, Amaral N, Lin E, Rau AR, Chiolo I. Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature* 2018;**559**:54–60
129. Shen X, Ranallo R, Choi E, Wu C. Involvement of actin-related proteins in ATP-dependent chromatin remodeling. *Mol Cell* 2003;**12**:147–55
130. Fenn S, Breitsprecher D, Gerhold CB, Witte G, Faix J, Hopfner KP. Structural biochemistry of nuclear actin-related proteins 4 and 8 reveals their interaction with actin. *Embo J* 2011;**30**:2153–66
131. Kapoor P, Chen M, Winkler DD, Luger K, Shen X. Evidence for monomeric actin function in INO80 chromatin remodeling. *Nat Struct Mol Biol* 2013;**20**:426–32
132. Kapoor P, Shen X. Mechanisms of nuclear actin in chromatin-remodeling complexes. *Trends Cell Biol* 2014;**24**:238–46
133. van Attikum H, Fritsch O, Hohn B, Gasser SM. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. *Cell* 2004;**119**:777–88
134. Aymard F, Aquirrebengoa M, Guillou E, Javierre BM, Bugler B, Arnould C, Rocher V, Iacovoni JS, Biernacka A, Skrzypczak M, Ginalski K, Rowicka M, Fraser P, Legube G. Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. *Nat Struct Mol Biol* 2017;**24**:353–61
135. Tsouroula K, Furst A, Rogier M, Heyer V, Maglott-Roth A, Ferrand A, Reina-San-Martin B, Soutoglou E. Temporal and spatial uncoupling of DNA double-strand break repair pathways within mammalian heterochromatin. *Mol Cell* 2016;**63**:293–305
136. Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes SV, Karpen GH. Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* 2011;**144**:732–44
137. Swaney KF, Rong L. Function and regulation of the Arp2/3 complex during cell migration in diverse environments. *Curr Opin Cell Biol 2016* 2016;**42**:63–72
138. Chiolo I, Tang J, Georgescu W, Costes SV. Nuclear dynamics of radiation-induced foci in euchromatin and heterochromatin. *Mutat Res* 2013;**750**:56–66
139. Starr DA, Fridolfsson HN. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. *Annu Rev Cell Dev Biol* 2010;**26**:421–44
140. Wilson KL, Foisner R. Lamin-binding proteins. *Cold Spring Harb Perspect Biol* 2010;**2**:a000554
141. Chang W, Worman HJ, Gundersen GG. Accessorizing and anchoring the LINC complex for multifunctionality. *J Cell Biol* 2015;**208**:11–22
142. Tepley EC, Starr DA. Connecting the nucleus to the cytoskeleton by SUN-KSAH bridges across the nuclear envelope. *Curr Opin Cell Biol* 2013;**25**:57–62
143. Hodzic DM, Yeater DB, Bengtsson L, Otto H, Stahl PD. Sun2 is a novel mammalian inner nuclear membrane protein. *J Biol Chem* 2004;**279**:25805–12
144. Crisp M, Liu Q, Roux K, Rattner JB, Shanshan C, Burke B, Stahl PD, Hodzic D. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol* 2006;**172**:41–53
145. Haque F, Lloyd DJ, Smallwood DT, Dent CL, Shanahan CM, Fry AM, Trembath RC, Shackleton S. SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. *Mol Cell Biol* 2006;**26**:3738–51
146. Hieda M. Implications for diverse functions of the LINC complexes based on the structure. *Cells* 2017;**6**:13
147. Gundersen GG, Worman HJ. Nuclear positioning. *Cell* 2013;**152**:1376–89
148. Zhang X, Lei K, Yuan X, Wu X, Zhuang Y, Xu T, Han M. SUN1/2 and syne/nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron* 2009;**64**:173–87
149. Lei K, Zhu Z, Xu R, Shao C, Xu T, Zhuang Y, Han M. Inner nuclear envelope proteins SUN1 and SUN2 play a prominent role in the DNA damage response. *Curr Biol* 2012;**22**:1609–15.
150. Lawrence KS, Tapley EC, Cruz VE, Li Q, Aung K, Hart KC, Schwartz TU, Starr DA, Engebrecht J. LINC complexes promote homologous recombination in part through inhibition of nonhomologous end joining. *J Cell Biol* 2016;**215**:801–21
151. Luxton GW, Starr DA. KASHing up with the nucleus: novel functional roles of KASH proteins at the cytoplasmic surface of the nucleus. *Curr Opin Cell Biol* 2014;**28**:69–75
152. Swartz RK, Rodriguez EC, King MC. A role for nuclear envelope-bridging complexes in homology-directed repair. *Mol Biol Cell* 2014;**25**:2461–71
153. Chang W, Antoku S, Ostlund C, Worman HJ, Gundersen GG. Linker of nucleoskeleton and cytoskeleton (LINC) complex-mediated actin-dependent nuclear positioning orients centrosomes in migrating myoblasts. *Nucleus* 2015;**6**:77–88
154. Hiraoka Y, Dernburg AF. The SUN rises on meiotic chromosome dynamics. *Dev Cell* 2009;**17**:598–605
155. Luxton GW, Gomes ER, Folker ES, Worman HJ, Gundersen GG. TAN lines; a novel nuclear envelope structure involved in nuclear positioning. *Nucleus* 2011;**2**:173–81
156. Hao H, Starr DA. SUN/KASH interactions facilitate force transmission across the nuclear envelops. *Nucleus* 2019;**10**:73–80
157. Lottersberger F, Karssemeijr RA, Dimitrova N, De Lange T. 53BP1 and the LINC complex promote microtubule-dependent DSB mobility and DNA repair. *Cell* 2015;**163**:880–93
158. Winkelmann JC, Forget BG. Erythroid and nonerythroid spectrins. *Blood* 1993;**81**:3173–85
159. Goodman SR, Zimmer WE, Clark MB, Zagon IS, Barker JE, Bloom ML. Brain spectrin: of mice and men. *Brain Res Bull* 1995;**36**:593–606
160. de Matteis MA, Morrow JS. Spectrin tethers and mesh in the biosynthetic pathway. *Cell Sci* 2000;**113**:2331–43
161. Bennet V, Baines AJ. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. *Physiol Rev* 2001;**81**:353–92
162. Goodman SR, Chapa RP, Zimmer WE. Spectrin's chimeric E2/E3 enzymatic activity. *Exp Biol Med (Maywood)* 2015;**240**:1039–49
163. Goodman SR, Zagon IS, Kulikowski RR. Identification of a spectrin-like protein in nonerythroid cells. *Proc Natl Acad Sci USA* 1981;**78**:7570–4
164. Machnicka B, Grochowalska R, Boguslawska DM, Sikorski AF, Lecomte MC. Spectrin-based skeleton as an actor in cell signaling. *Cell Mol Life Sci* 2012;**69**:19101
165. Sikorski A, Sangerman J, Goodman SR, Critz S. Spectrin (βpII$\sum$1) is an essential component in synaptic transmission. *Brain Res* 2000;**852**:161–6

166. Goodman SR. Discovery of nonerythroid spectrin to the demonstration of its key role in synaptic transmission. *Brain Res Bult* 1999;**50**:346
167. Ponceau A, Albiges-Rizo C, Colin-Aronovicz Y, Destaing O, Lecomte MC. αII-Spectrin regulates invadosome stability and extracellular matrix degradation. *PLoS ONE* 2015;**10**:e0120781
168. Trinh-Trang-Tan M-M, Bigot S, Picot J, Lecomte M-C, Kordeli S. AlphaII-spectrin participates in the surface expression of cell adhesion molecule L1 and neurite outgrowth. *Exp Cell Res* 2014;**322**:365–80
169. Meissner JM, Sikorski AF, Nawara T, Grzesiak J, Marycz K, Boguslawska DM, Michalczyk I, Lecomte MC, Machnicka B. αII-spectrin in T cells is involved in the regulation of cell-cell contact leading to immunological synapse formation?. *PLoS ONE* 2017;**12**:e0189545
170. Metral S, Machnicka B, Bigot S, Colin Y, Dharmy D, Lecomte M-C. αII-spectrin is critical for cell adhesion and cell cycle. *J Biol Chem* 2009;**284**:2409–18
171. Zagon IS, Higbee R, Riederer BM, Goodman SR. Spectrin subtypes in mammalian brain: an immunoelectron microscopic study. *J Neurosci* 1986;**6**:2977–66
172. Gascard P, Mohandas N. New insights into functions of erythroid proteins in nonerythroid cells. *Curr Opin Hematol* 2000;**7**:123–9
173. Stankewich MC, Cianci CD, Stabach PR, Ji L, Nath A, Morrow J. Cell organization, growth, and neural and cardiac development require αII-spectrin. *J Cell Sci* 2011;**124**:3956–66
174. McMahon LW, Walsh CE, Lambert MW. Human α spectrin II and the fanconi anemia proteins FANCA and FANCC interact to form a nuclear complex. *J Biol Chem* 1999;**274**:32904–8
175. Lambert MW. Functional significance of nuclear α spectrin. *J Cell Biochem* 2015;**116**:1816–30
176. Tse WT, Tang J, Jin O, Korsgren C, John KM, Kung AL, Gwynn BB, Peter LL, Lux SE. A new spectrin, beta IV, has a major truncated isoform that associates with promyelocytic leukemia protein nuclear bodies and the nuclear matrix. *J Biol Chem* 2001;**276**:23974–85
177. Thenappan A, Shukkla V, Abdul Khalek FJ, Li Y, Shetty K, Liu P, Li L, Johnson RL, Johnson L, Mishra L. Loss of TGF*β* adaptor protein *β*2SP leads to delayed liver regeneration in mice. *Hepatology* 2011;**53**:1641–50
178. Horikoshi N, Pandita RK, Mujoo K, Hambarde S, Sharma D, Mattoo AR, Chakraborty S, Charaka V, Hunt CR, Pandita TK. *β*2-spectrin depletion impairs DNA damage repair. *Oncotarget* 2016;**7**:33557–70
179. Chen J, Shukla V, Farci P, Andricovich J, Jogunoori W, Kwong LN, Katz LH, Sheety K, Rashid A, Su X, White J, Li L, Wang AY, Blechacz B, Raju GS, Davila M, Nguyen B-N, Stroehlein JR, Chen J, Kim SS, Levin H, Machida K, Tsukamoto H, Michaely P, Tzatsos A, Mishra B, Amber R, Mishra L. Loss of the transforming growth factor-*β* effector *β*2-spectrin promotes genomic instability. *Hepatology* 2017;**65**:678–93
180. Brown JW, Bullitt E, Sriswasdi S, Harper S, Speicher DW, McKnight CJ. The physiological molecular shape of spectrin: a compact supercoil resembling a Chinese finger trap. *PLoS Comput Biol* 2015;**11**:e1004302
181. Sridharan DM, McMahon LW, Lambert MW. αII-Spectrin interacts with five groups of functionally important proteins in the nucleus. *Cell Biol Intl* 2006;**30**:866–78
182. McMahon LW, Sangerman J, Goodman SR, Kumaresan K, Lambert MW. Human alpha spectrin II and the FANCA, FANCC, and FANCG proteins bind to DNA containing psoralen interstrand cross-links. *Biochemistry* 2001;**40**:7025–34
183. Sridharan D, Brown M, Lambert WC, McMahon LW, Lambert MW. Nonerythroid alpha II spectrin is required for recruitment of FANCA and XPF to nuclear foci induced by DNA interstrand cross-links. *J Cell Science* 2003;**116**:823–5
184. Lambert MW. Nuclear alpha spectrin: critical roles in DNA interstrand cross-link repair and genomic stability. *Exp Biol Med (Maywood)* 2016;**241**:1621–38
185. McMahon LW, Zhang P, Sridharan DM, Lefferts JA, Lambert MW. Knockdown of αII spectrin in normal human cells by siRNA leads to chromosomal instability and decreased DNA interstrand cross-link repair. *Biochem Biophys Res Commun* 2009;**381**:288–93
186. Zhang P, Herbig U, Coffman F, Lambert MW. Non-erythroid alpha spectrin prevents telomere dysfunction after DNA interstrand cross-link damage. *Nucleic Acids Res* 2013;**41**:5321–40
187. Kumaresan K, Sridharan D, McMahon L, Lambert MW. Deficiency in incisions produced by XPF at the site of a DNA interstrand cross-link in fanconi anemia cells. *Biochemistry* 2007;**46**:14359–68
188. Lee J, Coyne RS, Dubreuil RR, Goldstein LS, Branton D. Cell shape and interaction defects in alpha-spectrin mutants of Drosophila melanogaster. *J Cell Biol* 1993;**123**:1797–809
189. Norman KR, Moerman DG. Α Spectrin is essential for morphogenesis and body wall muscle formation in Caenorhabditis elegans. *J Cell Biol* 2002;**157**:665–77
190. Faridounnia M, Folkers GE, Boelens R. Function and interactions of ERCC1-XPF in DNA damage response. *Molecules* 2018;**23**:3205
191. Hoogenboom WS, Boonen R, Knipscheer P. The role of SLX4 and its associated nucleases in DNA interstrand crosslink repair. *Nucleic Acids Res* 2019;**47**:2377–88
192. Brois DW, McMahon LW, Ramos NL, Anglin LM, Walsh CE, Lambert MW. A deficiency in a 230 kDa DNA repair protein in fanconi anemia complementation group a cells is corrected by the FANCA cDNA. *Carcinogenesis* 1999;**20**:1845–1853
193. Raschle M, Knipscheer P, Enoiu M, Angelov T, Sun J, Griffith JD, Ellenberger TE, Scharer OD, Walter JC. Mechanism of replication-coupled DNA interstrand crosslink repair. *Cell* 2008;**134**:969–80
194. Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M. DNA replication is required to elicit cellular responses to psoralen-induced DNA interstrand cross-links. *Mol Cell Biol* 2000;**20**:8283–89
195. Williams HA, Gottesman ME, Gautier J. The differences between ICL repair during and outside of S phase. *Trends Biochem Sci* 2013;**38**:386–93
196. Kim H, D'Andrea AD. Regulation of DNA cross-link repair by the fanconi anemia/BRCA pathway. *Genes Dev* 2012;**26**:1393–408
197. Kottemann MC, Smogorzewska A. Fanconi anaemia and the repair of watson and crick DNA crosslinks. *Nature* 2013;**493**:356–63
198. Brosh RM, Jr, Bellani M, Liu Y, Seidman MN. Fanconi anemia: a DNA repair disorder characterized by accelerated decline of the hematopoietic system cell compartment and other features of aging. *Aeging Res Rev* 2017;**33**:67–75
199. Mathew CG. Fanconi anaemia genes and susceptibility to cancer. *Oncogene* 2006;**25**:5875–84
200. de Winter JP, Joenje H. The genetic and molecular basis of fanconi anemia. *Mutat Res* 2009;**668**:11–9
201. Moldovan G-L, D'Andrea A. How the fanconi anemia pathway guards the genome. *Annu Rev Genet* 2009;**43**:223–49
202. Mamrak NE, Shimamura A, Howlell NG. Recent discoveries in the molecular pathogenesis of the inherited bone marrow failure syndrome fanconi anemia. *Blood Rev* 2017;**31**:939
203. Cheung RS, Taniguchi T. Recent insights into the molecular basis of fanconi anemia: genes, modifiers, and drivers. *Intl J Hematol* 2017;**106**:335–44
204. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with fanconi anemia. *Blood* 2002;**101**:822–6
205. Swuec P, Ranault L, Borg A, Shah F, Murphy VJ, van Twest S, Snijders AP, Deans AJ, Costa A. The FA core complex contains a homo-dimeric catalytic module for symmetric Mono-ubiquitination of FANCI-FANCD2. *Cell Rep* 2017;**18**:611–23
206. Nalepa G, Clapp DW. Fanconi anaemia and cancer: an intricate relationship. *Nat Rev Cancer* 2018;**18**:168–85
207. Garaycochea JI, Patel KL. Why does the bone marrow fail in fanconi anemia? *Blood* 2014;**123**:26–34
208. Zhang P, Sridharan D, Lambert MW. Nuclear α spectrin differentially affects monoubiquitinated versus non-ubiquitinated FANCD2 function after DNA interstrand cross-link damage. *J Cell Biochem* 2016;**117**:671–83
209. Klein Douwel D, Boon R, Long DT, Szypowska AA, Raschle M, Walter JC, Knipscheer P. XPF-ERCC1 acts in unhooking DNA interstrand crosslinks in cooperation with FANCD2 and FANCP/SLX4. *Mol Cell* 2014;**54**:460–71
210. Ziemnicka-Kotula D, Xu J, Gui H, Potempska A, Kim KS, Jenkins EC, Trenkner E, Kotula L. Identification of a candidate human spectrin src homology 3 domain-binding protein suggests a general mechanism of

association of tyrosine kinases with the spectrin-based membrane skeleton. *J Biol Chem* 1998;**273**:13681–92
211. McPherson PS. Regulatory role of SH3 domain-mediated protein-protein interactions in synaptic vesicle endocytosis. *Cell Signal* 1999;**11**:229–38
212. Mayer BJ. SH3 domains: complexity in moderation. *J Cell Sci* 2001;**114**:1253–63
213. Ren R, Mayer BJ, Cicchetti P, Baltimore D. Identification of a ten-amino acid proline-rich SH3 binding site. *Science* 1993;**259**:1157–61
214. Kay BK, Williamson MP, Sudol M. The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains. *FASEB J* 2000;**14**:231–41
215. Cesareni G, Panni S, Nardelli G, Castagnoli L. Can we infer peptide recognition specificity mediated by SH3 domains? *FEBS Lett* 2002;**513**:38–44
216. Lefferts JA, Wang C, Sridharan D, Baralt M, Lambert MW. The SH3 domain of αII spectrin is a target for the fanconi anemia protein. *FANCG Biochemistry* 2009;**48**:254–63
217. Blatch GL, Lassle M. The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. *Bioessays* 1999;**21**:9329
218. Blom E, van de Vrugt HJ, de Vries Y, de Winter JP, Arwert F, Joenje H. Multiple TPR motifs characterize the fanconi anaemia FANCG protein. *DNA Repair* 2004;**3**:77–84
219. Hussain S, Wilson JB, Blom W, Thompson LH, Sung P, Gordon SM, Kupfer GM, Joenje H, Mathew CG, Jones NJ. Tetratricopeptide-motif-mediated interaction of FANCG with recombination proteins XRCC3 and BRCA2. *DNA Repair* 2006;**5**:629–40
220. Wang C, Lambert MW. The fanconi anemia protein, FANCG, binds to the ERCC1-XPF endonuclease via its tetratricopeptide repeats and the Central domain of ERCC1. *Biochemistry* 2010;**49**:5560–9
221. de Laat WL, Sijbers AM, Odijk H, Jaspers NG, Hoeijmakers JH. Mapping of interaction domains between human repair proteins ERCC1 and XPF. *Nucleic Acids Res* 1998;**26**:4146–5
222. Tripsianes K, Folkers GE, Ab E, Das D, Odijk H, Jaspers NGJ, Hoeijmakers JHJ, Kapten R, Boelens R. The structure of the human ERCC1/XPF interaction domains reveals a complementary role for the two proteins in nucleotide excision repair. *Structure* 2005;**13**:1849–58
223. Choi YJ, Ryu KS, Ko YM, Chae YK, Pleton JG, Wemmer DE, Choi BS. Biophysical characterization of the interaction domains and mapping of the contact residues in the XPF-ERCC1 complex. *J Biol Chem* 2005;**280**:28644–52
224. Das D, Faridounnia M, Kovacic I, Kaptein R, Boelens R, Folkers GE. Single-stranded DNA binding by the helix-hairpin-helix domain of XPF protein contributes to the substrate specificity of the ERCC1-XPF protein complex. *J Biol Chem* 2017;**292**:2842–53
225. Huang J. Liu S, bellani MA, thazhathveetil AK, ling C, De winter JP, wang Y, wang YW, seidman MM. The DNA translocase FANCM/MHF promotes replication traverse of DNA interstrand crosslinks. *Mol Cell* 2013;**52**:434–46
226. Zhang J, Dewar JM, Budzowska M, Motnenko A, Cohn MA, Walter JC. DNA interstrand cross-link repair requires replication-fork convergence. *Nat Struct Mol Biol* 2015;**22**:242–7
227. Amunugama R, Willcox S, Wu RA, Abdullah UB, El-Sagheer AH, Brown T, McHugh PJ, Griffith JD, Walter JC. Replication fork reversal during DNA interstrand crosslink repair requires CMG unloading. *Cell Rep* 2018;**23**:3419–28
228. Mutreja K, Krietsch J, Hess J, Ursich S, Berti M, Roessler FK, Zellweger R, Patra M, Gasser G, Lopes M. ATR-mediated global fork slowing and reversal assist fork traverse and prevent chromosomal breakage at DNA interstrand cross-links. *Cell Rep* 2018;**24**:2629–42
229. Kumaresan KR, Lambert MW. Fanconi anemia, complementation group A, cells are defective in ability to produce incisions at sites of psoralen interstrand cross-links. *Carcinogenesis* 2000;**21**:741–51
230. Lambert MW, Tsongalis JT, Lambert WC, Parrish DD. Correction of the DNA repair defect in fanconi anemia complementation groups a and D cells. *Biochem Biophys Res Commun* 1997;**230**:687–91
231. Lefferts JA. Lambert MA. Fanconi anemia cell lines deficient in αII spectrin express normal levels of αII spectrin mRNA. *Biochem Biophys Res Commun* 2003;**307**:510–5
232. Nicolas G, Fournier CM, Galand C, Malbert-Colas L, Bournier O, Kroviarski Y, Bourgeois M, Camonis JH, Dhermy D, Grandchamp B, Lecomte MC. Tyrosine phosphorylation regulates alpha II spectrin cleavage by calpain. *Mol Cell Biol* 2002;**22**:3527–36
233. Nedrelow JH, Cianci CD, Morrow JS. C-Src binds αII spectrin's src homology 3 (SH3) domain and blocks calpain susceptibility by phosphorylating Tyr1176. *J Biol Chem* 2003;**278**:7725–41
234. Rotter B, Kroviarski Y, Nicholas G, Dhermy D, Lecomte MC. AlphaII-spectrin is an in vitro target for caspase-2, and its cleavage is regulated by calmodulin binding. *Biochem J* 2004;**378**:161–8
235. Harris AS, Croall DE, Morrow JS. Calmodulin regulates fodrin susceptibility to cleavage by calcium-dependent protease I. *J Biol Chem* 1989;**264**:17401–8
236. Simonovic M, Zhang Z, Cianci CD, Steitz TA, Morrow JS. Structure of the calmodulin αII-spectrin complex provides insight into the regulation of cell plasticity. *J Biol Chem* 2006;**281**:34333–40
237. Wilson JB, Blom E, Cunningham RM, Xiao Y, Kupfer GM, Jones NJ. Several tetratricopeptide repeat (TPR) motifs of FANCG are required for assembly of the BRCA2/D1-D2-G-X3 complex, FANCD2 monoubiquitylation and phleomycin resistance. *Mutat Res* 2010;**689**:12–20
238. Wilson JB, Yamamoto K, Marriott AS, Hussain S, Sung P, Hoatlin ME, Mathew CG, Takata M, Thompson LH, Kupfer GM, Jones NJ. FANCG promotes formation of a newly identified protein complex containing BRCA2, FANCD2 and XR CC3. *Oncogene* 2008;**27**:3641–52
239. Raghunandan M, Chaudhury I, Kellich SL, Hanenberg H, Soeback A. Fancd2, Fancj Brca2 cooperate to promote replication fork recovery independently of the fanconi anemia complex. *Cell Cycle* 2015;**14**: 3:342–53
240. Sobeck A, Stone S, Hoatlin ME. DNA structure-induced recruitment and activation of the fanconi anemia pathway protein FANCD2. *Mol Cell Biol* 2007;**27**:4283–92
241. Yuan F, Hokayem JE, Zhou W, Zhang Y. FANCI protein binds to DNA and interacts with FANCD2 to recognize branched structures. *J Biol Chem* 2009;**284**:24443–52
242. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005;**19**:2100–10
243. Murnane JP. Telomere dysfunction and chromosome instability. *Mutat Res* 2012;**730**:28–36
244. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet* 2008;**42**:301–34
245. O'Sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability. *Nat Rev Mol Cell Biol* 2010;**11**:171–81
246. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. *Science* 2012;**336**:593–7
247. Smogorzewska A, de Lange T. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 2004;**73**:177–208
248. Blackburn EH. Telomeres and telomerase. *FEBS Lett* 2005;**579**:859–62
249. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;**266**:2011–5
250. Broccoli DM, Young JW, de Lange T. Telomerase activity in normal and malignant hematopoietic cells. *Proc Natl Acad Sci USA* 1995;**92**:9082–6
251. Wright WE, Tesmer VM, Liao ML, Shay JW. Normal human telomeres are not late replicating. *Exp Cell Res* 1999;**251**:492–9104
252. Dellaire G, Bazett-Jones DP. PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. *Bioessays* 2004;**26**:963–77
253. Varadaraj A, Dovey CL, Laredj L, Ferfuson B, Alexander CE, Lubben N, Wyllie AH, Rich T. Evidence for the receipt of DNA damage stimuli by PML nuclear domains. *J Pathol* 2007;**211**:471–80
254. Warren DT, Zhang Q, Weissberg PL, Shanahan CM. Nesprins: intracellular scaffolds that maintain cell architecture and coordinate cell function? *Expert Rev Mol Med* 2005;**7**:1–15
255. Liem RK. Cytoskeletal integrators: the spectrin superfamily. *Cold Spring Harb Perspect Biol* 2016;**8**:pii:a018259

256. Warren DT, Tajsic T, Porter LJ, Minaisah RM, Cobb A, Jacob A, Raigor D, Zhang QP, Shananhan CM. Nesprin-2-dependent ERK1/2 compartmentalisation regulates the DNA damage response in vascular smooth muscle cell ageing. *Cell Death Differ* 2015;**22**:1540–50
257. Sur I, Neumann S, Noegel AA. Nesprin-1 role in DNA damage response. *Nucleus* 2014;**5**:173–91
258. Voisset E, Moravcsik E, Stratford EW, Jaye A, Palgrave CJ, Hills RK, Salomoni P, Kogan SC, Solomon E, Grimwade D. Pml nuclear body disruption cooperates in APL pathogenesis and impairs DNA damage repair pathways in mice. *Blood* 2018;**131**:636–48
259. Tang Y, Katuri V, Dillner A, Mishra B, Deng C-X, Mishra L. Disruption of transforming growth factor-β signalling in ELF β2-spectrin-deficient mice. *Science* 2003;**299**:574–7
260. Glanz A, Fraser FC. Spectrum of anomalies in fanconi anaemia. *J Med Genet* 1982;**19**:412–6
261. Riederer BM, Zagon IS, Goodman SR. Brain spectrin(240/235) and brain spectrin(240/235E): differential expression during mouse brain development. *J Neurosci* 1987;**7**:864–74
262. Zagon IS, Riderer BM, Goodman SR. Spectrin expression during mammalian brain ontogeny. *Brain Res Bull* 1987;**18**:799–807
263. Zimmer WE, Ma Y, Zagon IS, Goodman SR. Developmental expression of brain beta-spectrin isoform messenger RNAs. *Brain Res* 1992;**594**:75–83
264. Wolqust LP, Cannizzarro LA, Rameshjk H, Xue X, Wang D, Bhattacharuya PK, Gong JZ, McMahon C, Albanese JM, Sunkara JL, Ratech H. Differential expression in normal hematopoiesis and alterations in neoplastic bone marrow disorders. *Am J Clin Path* 2011;**136**:300–8
265. Gorman EB, Chen L, Albanese J, Ratech H. Pattern of spectrin expression in B-cell lymphomas: loss of spectrin isoforms is associated with nodule-forming and germinal center-related lymphomas. *Mod Pathol* 2007;**20**:1245–52
266. Ackermann A, Brieger A. The role of nonerythroid spectrin αII in cancer. *J Oncol* 2019;**2019**:7079604
267. Ackermann A, Schrecker C, Bon D, Friedrichs N, Bankov K, Wild G, Plotz G, Zeuzem S, Herrmann E, Hansmann M-L, Brieger A. Downregulation of SPTAN1 is related to MLH1 deficiency and metastasis in colorectal cancer. *PLoS ONE* 2019;**14**:e0213411
268. Hinrichsen I, Ernst B, Nuber F, Passmann S, Schafer D, Steinke V, Friedrichs N, Plotz G Zeuzem S, Brieger A. Reduced migration of MLH1 deficient Colon cancer cells depends on SPTAN1. *Mol Cancer* 2014;**13**:11
269. Brieger A, Adryan F, Wolpert S, Passmann S, Zeuzem S, Trojan J. Cytoskeletal scaffolding proteins interact with Lynch-Syndrome associated mismatch repair protein MLH1. *Proteomics* 2010;**10**:3343–55
270. Sun Z, Wang L, Eckloff BW, Deneg B, Wang Y, Wampfler JA, Jang J, Wieben ED, Jen J, You M, Yang P. Conserved recurrent gene mutations correlate with pathway deregulation and clinical outcomes of lung adenocarcinoma in never-smokers. *BMC Med Genomics* 2014;**7**:32
271. Wang Y, Tuo J, Nelson AD, Glanowska K, Murphy GC, Jenkins PM, Parent JM. Critical roles of αII spectrin in brain development and epileptic encephalopathy. *J Clin Invest* 2018;**128**:760–773
272. Saitsu H, Tohyama J, Kumada T, Egawa K, Hamada K, Okada I, Mizuguchi T, Osaka H, Miyata R, Furukawa T, Haginoya K, Hoshino H, Goto T, Hachiya Y, Yamagata T, Saitoh S, Nagai T, Nishiyama K, Nishimura A, Miyake N, Komada M, Hayashi K, Hirai S, Ogata K, Kato M, Fukuda A, Matsumoto N. Dominant-negative mutations in α-II spectrin cause west syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am J Hum Genet* 2010;**86**:881–91
273. Tohyama J, Nakashima M, Nabatame S, Gaik-Siew C, Miyata R, Rener-Primec Z, Kato M, Matsumoto N, Saitsu H. SPTAN1 encephalopathy: distinct phenotypes and genotypes. *J Hum Genet* 2015;**60**:167–73
274. Syrbe S, Harms FL, Parrini E, Montomoli M, Mutze U, Helbig KL, Polster T, Albrecht B, Bernbeck U, van Binsbergen E, Biskup S, Burglen L Denecke J, Heron B, Heyne HO, Hoffmann GF, Hornemann F, Matsushige T, Matsuura R, Kato M, Korenke GC, Kuechler A, Lammer C, Merkenschlager A, Mignot C, Ruf S, Nakashima M, Saitsu H, Stamberger H, Pisano T, Tohyama J, Weckhuysen S, Werckx W, Wickeart J, Mari F, Verbeek NE, Moller RS, Koeleman B, Matsumoto N, Dobyns WB, Battaglia D, Lemke JR, Kutsche K, Guerrini R. Delineating SPTAN1 associated phenotypes: from isolated epilepsy to encephalopathy with progressive brain atrohy. *Brain* 2017;**140**:2322–36
275. Zhi X, Lin L, Yang S, Bhuvaneshwar K, Wang H, Gusev Y, Lee MH, Kallakury B, Shivapurkar N, Cahn K, Tian X, Marshall JL, Byers SW, He AR. βII-Spectrin (SPRBN1) suppresses progression of hepatocellular carcinoma and wnt signalling by regulation of Wnt inhibitor kallistatin. *Hepatology* 2015;**61**:598–612
276. Chen Y, Meng L, Shang H, Dou Q, Lu Z, Liu L, Wang Z, He X, Song Y. β spectrin-mediated differentiation repressed the properties of liver cancer stem cells through β-catenin. *Cell Death and Disease* 2018;**9**:424
277. Lin J, Shi J, Guo H, Yang X, Jiang Y, Long J, Bai Y, Wang D, Yang X, Wan X, Zhang L, Pan J, Hu K, Guan M, Huo L, Sang X, Wang K, Zhao H. Alterations in DNA damage repair genes in primary liver cancer. *Clin Cancer Res* 2019;**25**:4701–11.
278. Simon DN, Zastrow MS, Wilson KL. Direct actin binding to A- and B-type lamin tails and actin filament bundling by the lamin a tail. *Nucleus* 2010;**1**:264–72
279. Ho CY, Jaalouk DE, Vartiainen MK, Lammerding J. Lamin A/C and emerin regulate MKL-SFR activity by modulating actin dynamics. *Nature* 2013;**497**:507–11
280. Holaska JM, Kowalski AK, Wilson KL. Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane. *PLoS Biol* 2004;**2**:e231
281. Clements L, Manilal S, Love DE, Morris GE. Direct interaction between emerin and lamin A. *Biochem Biophys Res Commun* 2000;**267**:709–14
282. Liu Y, Ballman K, Li D, Khan S, Derr-Yellin E, Shou W, Haneline LS. Impaired function of fanconi anemia type C-deficient macrophages. *J Leukoc Biol* 2012;**91**:333–40