Nuclear motors and nuclear structures containing A-type lamins and emerin: is there a functional link?

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Abstract

Rapid interphase chromosome territory repositioning appears to function through the action of nuclear myosin and actin, in a nuclear motor complex. We have found that chromosome repositioning when cells leave the cell cycle is not apparent in cells that have mutant lamin A or that are lacking emerin. We discuss the possibility that there is a functional intranuclear complex comprising four proteins: nuclear actin, lamin A, emerin and nuclear myosin. If any of the components are lacking or aberrant, then the nuclear motor complex involved in moving chromosomes or genes will be dysfunctional, leading to an inability to move chromosomes in response to signalling events.

Interphase chromosome positioning and repositioning

Chromosomes are positioned non-randomly in interphase nuclei. In primary proliferating fibroblasts, chromosomes are positioned according to their gene density, with gene-poor chromosomes at the nuclear periphery and gene-rich chromosomes towards the nuclear interior [1,2]. The gene-density-correlated positioning of chromosomes found in different cell types gave credence to the idea that positioning of genes and regulatory elements at specific regions of the nucleus would have an impact on the regulation of gene expression or silencing. Some interphase chromosome positions alter with differentiation, whereas others remain in the same nuclear location (reviewed in [3,4]), adding further weight to the argument that interphase positioning is relevant in genome function. On the other hand, we have found that, when cells leave the proliferative cell cycle, to become either quiescent or senescent, chromosomes are still positioned non-randomly, but now according to size, with large chromosomes at the nuclear periphery and small chromosomes in the nuclear interior (I.S. Mehta, M. Figgitt, L.S. Elcock, K.J. Meaburn, G. Bonne, I.R. Kill and J.M. Bridger, unpublished work). Whether this change in location influences changes in transcription profiles has yet to be determined.

We found that, as fibroblasts became quiescent or senescent, a number of chromosomes would change position and some would remain at the same nuclear location. Quite a few chromosomes, such as chromosomes 13 and 18, change nuclear position as fibroblasts become non-proliferating and relocate to same nuclear compartment regardless of whether cells are quiescent or senescent (Figure 1). Others relocate, but are found in different nuclear compartments in both quiescent and senescent fibroblasts (I.S. Mehta, M. Figgitt, L.S. Elcock, K.J. Meaburn, G. Bonne, I.R. Kill and J.M. Bridger, unpublished work). Chromosome 10 is the most interesting chromosome, since it is found in an intermediate nuclear position in proliferating fibroblast nuclei (Figure 1A), at the nuclear periphery in quiescent fibroblasts (Figure 1B) and within the nuclear interior in senescent fibroblasts (Figure 1C). Indeed, it is one of two chromosomes that are located at three different nuclear locations in these cell states. From these analyses, we have to conclude that chromosome territories move, changing their nuclear location as cells become senescent or quiescent (Figure 1). But whether this was immediate, over a number of hours, or whether cells are required to traverse mitosis for chromosomes to become repositioned was not clear, and not trivial to study in senescent cells. However, using serum removal, we were able to show that chromosome territories are relocated very rapidly, after 15 min. This repositioning is an active process requiring energy, since inhibitors of ATPase and GTPase block the relocation of chromosomes (I.S. Mehta, L.S. Elcock, G. Bonne, I.R. Kill and J.M. Bridger, unpublished work). Rapid repositioning has been observed for individual genetic loci [5,6]. In order to understand how this rapid movement is elicited, we are looking at nuclear motors.

Nuclear motors

Molecular motors of the cell cytoplasm have been studied for many years. Over the years, there have been on/off discussions as to whether there are molecular motors within the nuclei of cells. The discussions started in the 1970s [7,8], with a role for nuclear actin and myosin being suggested in heterochromatin formation [9]. In the 1980s, evidence was...
Figure 1 | Chromosomes occupy differential locations in normal proliferating, quiescent and senescent primary fibroblasts

(A) In a young proliferating fibroblast nucleus, chromosome 10 occupies an intermediate location, whereas chromosomes 13, 18 and X localize at the nuclear periphery. (B) In a young quiescent fibroblast nucleus, this position of chromosomes is altered and chromosome 10 localizes at the nuclear periphery, whereas territories for chromosomes 13 and 18 are positioned in the nuclear interior. Chromosome X remains at the nuclear periphery. (C) In senescent cells, whereas chromosomes 13 and 18 are still localized in the nuclear interior, chromosome 10 is also observed to be positioned in the nuclear interior. Chromosome X remains at the nuclear periphery.

provided that nuclear actin and myosin were involved in nucleocytoplasmic transport [10], and, in the 1990s, more ultrastructural studies were performed, localizing actin and myosin in the nucleus [11,12]. Within the last 10 years, scientists have uncovered several isoforms of myosin that are found in nuclei, including NMI (nuclear myosin I) (reviewed in [13]), myosin VI [14], myosin 16b [15] and myosin Va [16]. In the 1970s, actin was also shown to be a nuclear protein [17,18]. However, it is still an unresolved question as to whether this actin in the nucleus is a polymerized or an unpolymerized form [19].

Using microinjection of antibodies, actin [20,21], and actin and myosin together [22], were shown to be involved in RNA polymerase II transcription, which has been confirmed further using drugs and mutant cell lines not only for nuclear actin and NMI [13,23], but also for myosin VI [14]. These acto–myosin motors also appear to be involved in RNA polymerase I transcription [23–25].

Both nuclear actin and NMI have also been found to associate with chromatin-remodelling complexes. Actin binds to SWI/SWF-like BAF (barrier to autointegration factor) remodelling complexes, whereas NMI associates with components of the WICH (WSTF–SNF2h complex) chromatin-remodelling complex [13].

However, the involvement of nuclear motors in transcription and chromatin remodelling appears not to be their only nuclear function. There is an increasing body of evidence that they are also involved in movement of chromatin around nuclei [5,26].

We have found that the rapid relocation of chromosomes in interphase is blocked by drugs preventing polymerization of actin and myosin (I.S. Mehta, L.S. Elcock, G. Bonne, I.R. Kill and J.M. Bridger, unpublished work).

It is interesting to note that Hozak and colleagues found that both nuclear actin and NMI are distributed quite differently in resting non-stimulated lymphocytes compared with PHA (phytohaemagglutinin)-stimulated lymphocytes [27] and Riddle et al. [28] demonstrated that nuclear actin was decreased in quiescent fibroblasts. We have stained proliferating and senescent fibroblasts with a commercial anti-NMI antibody and have seen quite different distributions of NMI in senescent cells compared with proliferating fibroblasts; it could be described as disorganized (I.S. Mehta, L.S. Elcock, M. Amira, I.R. Kill and J.M. Bridger, unpublished work).

From our studies, we know that, when quiescent cells are re-stimulated with serum, chromosomes do not relocate to a proliferating gene-density distribution until after cells have traversed mitosis with a breakdown and rebuilding of the nucleus [29]. Thus we suggest that not only do quiescent cells have a compromised nuclear motor for moving chromatin, but also the same is true for senescent cells, since we have not visualized any chromosome repositioning in senescent cells after removal of serum. This may indicate that nuclear motors containing actin and myosin do not function in the same manner in non-proliferating cells with respect to dynamic chromatin/chromosome movement.
A-type lamins and emerin

The nuclear lamina is described as a meshwork [30] or a uniparallel array [31] of interconnecting filaments underlying the inner nuclear envelope. This structure, together with the nuclear envelope and nuclear envelope proteins, is important for organizing the structure of the entire nucleus and the chromatin within it [32]. In most vertebrate cells, two major types of nuclear lamin are found: A and B. A-type lamins are encoded by LMNA which undergoes alternative splicing to form lamins A, delta A, C and C2, whereas B-type lamins are encoded by two separate genes LMNB1 and LMNB2 to form lamins B1, B2 and B3 (reviewed in [33]).

A-type lamin proteins not only are located at the nuclear periphery, but also are probably part of the nuclear matrix [34,35]. Further lamin proteins have been observed within the nuclear interior [36,37] with sites of replication [38], transcription factories [39] and splicing speckles [40]. Nuclear A-type lamins seem to be involved in many nuclear functions such as nuclear structural integrity, DNA replication, transcription, splicing, cell signalling, DNA repair, cellular proliferation, transit of mitosis, organization and stability of the genome, differentiation, normal aging, apoptosis and epigenetic control of chromatin (reviewed in [41]).

A-type lamins have a number of binding partners, and this might well explain their multifaceted roles and importance within the nucleus, but this also adds another level of complexity in assigning roles to particular complexes of proteins containing A-type lamins. One of the binding partners of nuclear A-type lamins is emerin (reviewed in [32]). The lamin–emerin interaction has been implicated to be important in maintaining the structural integrity of the nucleus, in correct localization of the complex and in efficient progression of the cell through the cell cycle [42]. The importance of this complex is emphasized by both proteins causing similar diseases when the respective genes are mutated, i.e. EDMD (Emery–Dreifuss muscular dystrophy), HGPS (Hutchinson–Gilford progeria syndrome) is a premature aging syndrome caused by a base substitution in the LMNA gene encoding a G608G mutation [43] resulting in the formation of a truncated lamin A protein termed progerin, which acts in a dominant-negative way [44]. Studies on patients suffering from HGPS have revealed that lamins are important in the maintenance of nuclear structure and shape as well as in heterochromatin formation, chromatin modification and gene expression [45]. Studies using emerin-null cells derived from patients suffering from X-linked EDMD have revealed that emerin may also influence gene expression, heterochromatin organization and be involved in maintaining the structural integrity of the nuclear envelope (reviewed in [32]).

A-type lamins and emerin: are they integral to the function of a nuclear motor?

If lamins A and C and emerin are involved in the function of a nuclear motor comprising nuclear actin and nuclear myosins, then there should be evidence that they bind one another in such a way as to form a complex. Indeed, in vitro studies have demonstrated the binding of actin to the C-terminus of lamin A [46]. In addition, the tail region of lamin A has an additional site with affinity for actin [47]. Emerin binds to both actin [F- (filamentous) and G- (globular) actin] and NMI, revealed by proteomic studies [32]. Thus we know that emerin binds lamin A that can bind to actin, which can bind to both emerin and NMI which can bind emerin. This makes an interesting hypothetical complex (Figure 2) and, although it has been discussed as being present at the nuclear envelope [48], could it also be present and functional throughout the nucleus?

If this complex exists, does it function to move chromatin/chromosomes around the nucleus? We have started to address this question by performing chromosome positioning assays (two- and three-dimensional FISH (fluorescence in situ hybridization)) in proliferating fibroblasts of patients that have mutations in either LMNA or EMD genes (Figure 3B and 3D) [49] and comparing chromosome positioning when these cells have become senescent. Intriguingly, in HGPS cells that have become senescent, the chromosomes do not alter their nuclear location as they would do in normal control cells (Figure 3C). This implies that the nuclear motor function, which would have been involved in chromosome movement during transition from a proliferating to a senescent state, is not functional. Indeed, anti-NMI antibody staining reveals

Figure 2 | Model showing the interactions between the nuclear actin–myosin and the nuclear lamin–emerin complexes for efficient functioning of the nuclear motor

Nuclear lamins are known to bind to nuclear actin and nuclear emerin. Nuclear emerin binds nuclear actin, nuclear myosin and nuclear actin. Nuclear myosins are known to bind to DNA, whereas nuclear actin, emerin and lamins are known to interact with the nuclear matrix. Thus chromosome territories are bound by nuclear myosins which bind emerin which bind both nuclear actin and lamins. Thus this four-protein complex, with the support of the nuclear matrix, may function to move the chromosome territories within the nuclear space.
Nuclear Envelope Diseases and Chromatin Organization 1387

Figure 3 | Chromosome 10 localizes at the nuclear periphery in proliferating HGPS and EDMD fibroblasts, and the position of chromosome 10 does not change once the cell is senescent

In senescent cells that are quiescent, the territories of chromosome 10 are in the nuclear interior (A). In primary proliferating fibroblasts derived from patients suffering from HGPS or EDMD, chromosome 10 territories are at the nuclear periphery (B and D respectively), and this localization of chromosome 10 does not change when these HGPS cells become senescent (C).

(A) Senescent quiescent nucleus
(B) Proliferating HGPS nucleus
(C) Senescent HGPS nucleus
(D) Proliferating EDMD nucleus

Chromosome 10 territories
Chromosome 18 territories
Chromosome 13 territories
Chromosome X territories

the same disorganized distribution of NMI as in senescent cells (I.S. Mehta, L.S. Elcock, M. Amira, I.R. Kill and J.M. Bridger, unpublished work).

If emerin is functionally involved in this complex, then chromosomes would also not be relocated in EDMD cells that became senescent. Unfortunately, we have only looked at chromosomes 13 and 18 (Figure 3D) so far, and they are found in the nuclear interior in both proliferating and senescent EDMD cells [49].

Thus, for efficient movement of whole chromosome territories, the formation and functionality of this protein complex including nuclear actin, myosin, lamin and emerin (Figure 2) may be vital. It has also been shown that, in normal senescent cells, there is an accumulation of the mutant form of lamin A [50,51]. This may well explain why, in senescent cells, chromosome 10 is not repositioned when senescent cells are placed in low serum (Figure 3A), indicating that the nuclear motor controlling chromosome movement in senescent cells is dysfunctional.

In order to test these hypotheses and the functionality of nuclear motors in non-proliferating and diseased cells, we need to perform a number of experiments. These would include interfering with the complex in normal cells as well as rebuilding it in cells where this complex is dysfunctional or missing; these studies are on-going in our laboratory.

References

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