Chromobility: the rapid movement of chromosomes in interphase nuclei

Joanna M. Bridger

Abstract

There are an increasing number of studies reporting the movement of gene loci and whole chromosomes to new compartments within interphase nuclei. Some of the movements can be rapid, with relocation of parts of the genome within less than 15 min over a number of microns. Some of these studies have also revealed that the activity of motor proteins such as actin and myosin are responsible for these long-range movements of chromatin. Within the nuclear biology field, there remains some controversy over the presence of an active nuclear acto–myosin motor in interphase nuclei. However, both actin and myosin isoforms are localized to the nucleus, and there is a requirement for rapid and directed movements of genes and whole chromosomes and evidence for the involvement of motor proteins in this relocation. The presence of nuclear motors for chromatin movement is thus an important and timely debate to have.

Introduction

It is now well accepted that the genomes of eukaryotic cells are highly spatially organized in interphase nuclei as individual chromosome territories. These territories exhibit non-random radial positioning [1]. The non-random nuclear location and positioning of individual chromosome territories can be differential in cells from different origins, before and after differentiation pathway steps, or in disease [2]. This implies that, whatever state cells are in, chromosomes have preferred and regulated locations within nuclei. However, randomness of chromosome territory positioning can be seen as cells are reorganizing or rebuilding their nuclei, for example, at the very beginning of G1-phase [3–5].

With respect to gene movement, there are now numerous examples of where small regions of chromosomes loop away from the main body of a chromosome territory [2]. Further studies have revealed where these genes are headed, revealing an association with a facilitating nuclear structure, such as various nuclear bodies and transcription factories [6–9].

The relocation of individual genes to nuclear structures that may aid their expression is perhaps easier to comprehend than why whole chromosome territories would relocate. After mitosis, a chromosome would have the opportunity to change its nuclear location, presumably by having its chromatin modified and/or being able to interact with different nuclear structures. This situation would require a mechanism presumably within nuclei that elicits this movement, although there could be connections with the cytoplasm. Studying a system such as this will allow us to ask where the chromosomes are being directed, which nuclear compartments and/or structures are functionally important, what are the consequences of repositioning chromosomes with respect to gene expression and silencing and what can happen to a cell/organism when this repositioning is faulty. My group are now trying to answer these questions using assay systems where genes and chromosomes do change position rapidly after stimuli such as removal of growth factors [10], addition of differentiation factors [11] and parasite exposure [12]. One of the keys to understanding what is happening to chromosomes and genes is the rate at which this repositioning can occur. Whole chromosomes and gene loci on loops have been observed changing nuclear location as rapidly as 1 μm/min with neighbouring chromosomal regions and chromosomes remaining stationary. It most certainly involves chromatin modification, but how does this alone allow a change in position, sometimes of over 10 μm?

The nucleus is full of structural proteins that could create a dynamic scaffolding network that chromatin could bind to and be released from through signalling and protein modification. This could mean that, if chromatin were released from one region of the nucleus, it could be random or corralled diffusion that influences where the chromatin ends up being located and/or re-anchoring. The data from some studies do not necessarily support this serendipitous route of relocation, but favour active and directed movement to new non-random locations or specific structures within the nucleus. If a gene needs to be located at a specific nuclear structure such as a nuclear body, a speckle or transcription site to another without rebuilding a new nucleus after mitosis. We have demonstrated this in proliferating human dermal fibroblasts that have been placed in low serum [10].
factory, then it may be that the gene is relocated to the nearest one. However, since co-transcribed regions of the genome are from different chromosome territories and are found co-localized at the same nuclear entity [7,9,13,14], this implies that there is co-ordinated movement of genes from different chromosomes to co-occupy nuclear bodies or factories.

So the hypothesis examined in the present paper is that whole chromosomes and subchromosomal regions can be relocated rapidly and directed to specific nuclear locations. We believe that these regions of chromatin are moved around the nucleus on a network of molecular motors, similar to those found in the cytoplasm. Indeed, the components of such a transport system could include nuclear actin and nuclear myosins working together in concert to move DNA/chromatin cargo around the nucleus.

**Nuclear presence of actin and myosin**

Both actin and myosin have been located in the nucleus from early on in the study of nuclear biology, but the belief in the presence of either molecule was blighted by accusations of cytoplasmic contamination. However, with more sophisticated methodologies and technologies, the acceptance of both of these proteins in the nucleus is gaining credence.

The first papers to discuss the presence of nucleoplasmic actin showed the actin in networks in *Xenopus* oocytes (see [15]). Other reports followed describing actin in the nuclei of cells from a range of different species, such as bovine lymphocytes and slime mould (see [15]). With respect to the different forms of actin, G-actin (globular actin) and F-actin (filamentous actin) have been revealed in the nucleus using anti-actin antibodies [16,17], as well as in FRAP (fluorescence recovery after photobleaching) studies using GFP (green fluorescent protein)-actin [18]. β-Actin was shown to bind the chromatin-modelling protein BAF (barrier to autointegration factor) in *vivo* [19]. However, there may be less conventional forms of actin in the nucleus that are not polymeric in form and are revealed with specially designed antibodies. These antibodies reveal accumulations of actin and a concentration of actin throughout the nucleoplasm [20].

With respect to the movement of chromatin, perhaps the most important study so far revealing nuclear actin employment in chromatin movement is that a β-actin dominant-negative mutant expressed in HeLa cells blocked the movement of gene loci to Cajal bodies [6].

Myosin isoforms were originally found in the nucleus of *Acanthamoeba* cells when an antibody against myosin I revealed myosin in the nucleoplasm [21]. Furthermore, myosin II heavy-chain-like protein was found proximal to nuclear pore complexes in *Drosophila* [22]. More recently, different myosin isoforms have been located in nuclei. These are NM1β (nuclear myosin 1β) [23,24], myosin VI [25], myosin 16b [26], myosin Va [27] and myosin Vb [28]. Myosin VI has been found to have a role in the DNA-damage response [29], and myosin 16b appears to be involved in DNA replication [26]. So far, NM1β has been the myosin candidate most investigated for transposing chromatin around the nucleus [10,15].

NM1β has a single globular head and a single heavy chain structure [15]. NM1β also contains a unique 16-residue amino acid extension at its N-terminus which is required for nuclear import [24]. The nuclear distribution of NM1β is throughout the nucleoplasm with a concentration at nucleoli [10,23,24,30,31]. In addition, NM1β has also been found localized at the nuclear envelope in proliferating HDFs (human dermal fibroblasts) [10,15]. In non-proliferating HDFs, NM1β is reorganized into large aggregates deep within the nucleoplasm ([10,15], and I.S. Mehta, K.J. Meaburn, M. Figgitt, I.R. Kill and J.M. Bridger, unpublished work). Interestingly, these large aggregates are found in proliferating HDFs from Hutchinson–Gilford progeria syndrome patients, but a normal distribution of NM1β is restored after treatment with drugs that ameliorate some of the abnormalities in these cells [32].

**Repositioning of gene loci and whole chromosomes**

Individual chromosomes and gene loci have been shown to change their nuclear location upon stimuli, whether be stimuli to induce differentiation [11,33–36], signals that change growth status [10,37,38], environmental stimuli [12] or compounds to induce exogenous gene expression [39]. The consensus hypothesis in the field is that whole chromosomes are moved to allow some control over genes that are housed upon that chromosome, whether for expression or for repression. Gene loci may be relocated to a new region of the nucleus with the movement of the whole chromosome territory, but genes can also be repositioned by chromatin looping outside the main body of the chromosome territory. Chromatin looped out of territories has been shown to become associated with nuclear structures such as Cajal bodies [6], splicing speckles/SC35 domains [7,14] and transcription factories [9]. Individual genes and genes from different chromosomes that are co-regulated can be found at the same transcription factories [9].

**Evidence for directed chromosome and gene relocation elicited through nuclear motor activity**

The hypothesis that a nuclear motor mechanism is required to relocate a gene/genomic regions or whole chromosome has been tested in a few studies. The involvement of actin and/or myosin in the directed movement of a chromosome or a region of a chromosome can be assayed by employing drugs that interfere with the polymerization and the activity of actin and myosins, by introducing mutant nuclear motor proteins into cells, or using RNA interference technology to knock down specific nuclear motor proteins. Indeed, Belmont and colleagues used both drugs to treat cells and transfected with mutant actin, to test whether an activated exogenous lac operator integrated array, tagged by bound
movement, but it was employed in such a way as to only affect jasplakinolide. However, phalloidin oleate did not block the movement: butane-2,3-dionemonoxime, latrunculin A and activity. Three drugs did prevent the chromosomal nuclear motor proteins were involved. This was achieved by using drugs to inhibit actin and/or myosin polymerization and activity. Three drugs did prevent the chromosomal movement: butane-2,3-dionemonoxime, latrunculin A and jasplakinolide. However, phalloidin oleate did not block the movement, but it was employed in such a way as to only affect cytoplasmic actin, emphasizing further that it is probable that it is nuclear motor proteins that are affected and that chromosome relocation is not a consequence of long-range actions from the cytoplasmic motors [10,15].

The involvement of NM1β in whole chromosome relocation was specifically assessed by using RNA interference to knock down MYO1C expression. Again this intervention blocked the rapid chromosomal movement upon serum removal, putting NM1β in the frame as the nuclear myosin involved. From this study and studies from Belmont’s laboratory, it appears that F-actin and NM1β are active members of the molecular motor responsible for moving chromatin around the nucleus [10,39].

Nuclear structure meets nuclear motor

In the cytoplasm, in order to create force, myosin 1 moves over actin filaments and contains actin-binding domains in its globular head and with sites in its tail domain [15]. But how does a nuclear motor comprising actin and myosin work? Using anti-(myosin 1β) antibodies, NM1β is found throughout the nucleoplasm, but with a definite concentration at nucleoli and the nuclear envelope. In addition, actin has been described as occurring throughout the nucleus [42]. If actin is part of a nucleoskeleton that, although dynamic, can take tension and force, then by NM1β binding to DNA/chromatin, genomic cargo could be envisaged moving over short actin filaments, even if they are localized to compartments within the nucleus. There are some interesting binding capabilities between NM1β, actin and other members of a nuclear structure such as nuclear lamins. Nuclear lamins are at the nuclear envelope in the nuclear lamina which underlies the nuclear membrane and throughout the nucleoplasm [43,44]. Actin will bind to A-type lamins [45]. Emerin, an integral membrane protein embedded in the nuclear membrane, is an interesting protein with respect to its binding, since it binds A-type lamins [46], but also NM1β and F-actin [47]. These proteins at the nuclear envelope could be the link to the LINC (linker of nucleoskeleton and cytoskeleton) complex that would connect a nuclear motor to the cytoskeleton [48].

In conclusion, there a number of examples where whole chromosomes and gene loci move rapidly and apparently directionally through interphase nuclei in response to stimuli. The evidence is starting to accumulate of these movements being elicited by a nuclear motor complex that employs actin as a filamentous base for myosins to work against. Both actin and myosin maybe anchored at various points around the nucleus by being bound to nucleoskeleton proteins such as nuclear lamins and emerin. However, more evidence of nuclear motors in moving chromatin in a directed manner is required.

Acknowledgements

I thank Dr Christopher Eskiw and Dr Ian Kill for helpful discussions and reading this paper before submission.
Funding

Our work on chromosome positioning in Hutchinson–Gilford progeria syndrome cells was supported in part by the Brunel Progena Research Fund.

References


1750 Biochemical Society Transactions (2011) Volume 39, part 6
42 Dingová, H., Fukaťová, J., Mannová, M., Philimonenko, V.V. and Hozák, P.
(2009) Ultrastructural localization of actin and actin-binding proteins in
lamin structures within G1 nuclei of human dermal fibroblasts. J. Cell Sci.
104, 297–306
proteins form an internal nucleoskeleton as well as a peripheral lamina
carboxy-terminal domain of lamin A with actin. FEBS Lett. 425, 485–489
46 Holt, I., Ostlund, C., Stewart, C.L., Man, N., Worman, H.J. and Morris, G.E.
(2003) Effect of pathogenic mis-sense mutations in lamin A on its
interaction with emerin in vivo. J. Cell Sci. 116, 3027–3035
of distinct emerin-containing complexes from HeLa cells suggests
molecular basis for diverse roles including gene regulation, mRNA
splicing, signaling, mechanosensing, and nuclear architecture.
Biochemistry 46, 8897–8908

Received 4 August 2011
doi:10.1042/BST20110696