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Subcellular Scaling: Does Size Matter for Cell Division?

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Abstract

Among different species or cell types, or during early embryonic cell divisions that occur in the absence of cell growth, the size of subcellular structures, including the nucleus, chromosomes, and mitotic spindle, scale with cell size. Maintaining correct subcellular scales is thought to be important for many cellular processes and, in particular, for mitosis. In this review, we provide an update on nuclear and chromosome scaling mechanisms and their significance in metazoans, with a focus on *Caenorhabditis elegans*, *Xenopus* and mammalian systems, for which a common role for the Ran (Ras-related nuclear protein)-dependent nuclear transport system has emerged.

Introduction

Absolute and relative size of biological entities varies widely, both within and among species at all levels of organization above the atomic/molecular: the organism, the cells that make up the organism, and the components of the cells. How does scaling occur so that everything fits and functions properly? Until recently, the control systems that a cell uses to regulate and coordinate the size of its internal structures were virtually unknown. One candidate coordinator is the small GTPase Ran and its downstream transport machinery, which are involved in many cellular processes in both interphase and mitosis, from nucleo-cytoplasmic transport to spindle morphogenesis to nuclear envelope assembly [1,2]. We will start with a brief overview of the Ran pathway and discuss recent work that elucidates mechanisms of subcellular scaling and the potential importance for cell function and division.

The RanGTP pathway and spindle assembly

RanGTP marks the genome in both interphase and mitosis and acts as a molecular switch. In the nucleus, Ran is concentrated in its GTP state due to the chromatin-associated RanGEF (Guanine nucleotide Exchange Factor) RCC1. In the cytoplasm, Ran is found in its GDP form due to the activity of cytoplasmic RanGAP (GTPase Activating Protein). RanGTP binds both importins and exportins, stabilizing the exportin-cargo interaction required for

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nuclear export, while releasing cargoes from importins. As a result, proteins with an NLS (Nuclear Localization Signal) are transported into the nucleus by importins and accumulate in the nucleus, while NES (Nuclear Export Signal)-containing proteins are transported out of the nucleus (Figure 1A).

During mitosis, RCC1 remains associated with the chromosomes following nuclear envelope breakdown, enriching RanGTP in the zone where the spindle will assemble. As RanGTP diffuses away from the chromatin, RanGAP in the cytoplasm converts it to RanGDP, creating a RanGTP gradient. Numerous NLS-containing SAFs (Spindle Assembly Factors) are released within this gradient where they contribute to spindle assembly by nucleating and organizing microtubules [2] (Figure 1B). A recent study in cultured cells has revealed an interesting feedback mechanism that results from the binding of RanGTP-activated SAFs to microtubules [3]**. Microtubule binding serves to concentrate microtubule nucleators on the forming spindle, amplifying microtubule polymerization and rendering the length of the spindle insensitive to the size of the Ran gradient (Figure 1C). This microtubule-dependent amplification mechanism would explain why increasing the amount of chromatin in the spindle, and therefore the amount of RCC1 and RanGTP, does not increase spindle length in *Xenopus* egg extracts [4], while increasing the amount of microtubule polymer by addition of a drug dramatically increases spindle size [5]. Importantly, however, NLS-containing SAFs that regulate microtubule nucleation and dynamics downstream of RanGTP, including TPX2 and kif2a, have been shown to act as scaling factors for centrosomes and/or spindles and whose activities are regulated by importin α [6–8]. Thus, the emerging concept is that while RanGTP acts as a trigger for spindle assembly, the complex interplay between SAFs, microtubules and importins contributes to spindle scaling. Mechanisms of spindle size regulation have been elucidated in a variety of systems, particularly *Xenopus*, and are discussed in detail elsewhere [2,9].

Mechanisms of nuclear scaling

In contrast to the apparent independence of spindle size from the RanGTP gradient, strong evidence has accumulated that nucleocytoplasmic transport via the Ran pathway regulates nuclear scaling through the import of nuclear lamins. The nuclear lamina, which forms an intermediate filament meshwork underlying the inner nuclear membrane, is a major regulator of nuclear morphology in metazoans [10,11]. In *Xenopus*, lamin B3, the major lamin isoform in the egg, was found to be a key cargo regulating nuclear size differences between two different-sized frog species, *X. laevis* and *X. tropicalis*, which scale at organismal, cellular and subcellular levels [12,13]. Slower nuclear import rates and reduced accumulation of lamin B3 in egg extracts of the smaller *X. tropicalis* was shown to be due to differences in the levels of two nuclear transport factors, importin α and Ntf2 [13]. In contrast to importin α that promotes lamin import and nuclear growth, Ntf2 negatively regulates import of large cargoes such as lamin oligomers, and its expression decreases lamin import and nuclear size [14]*. In *C. elegans*, nuclear transport and lamin levels were also demonstrated to regulate nuclear size [15,16]. Thus, it appears that the more import of lamin into the nucleus, the larger it gets (Figure 2A).

But is the situation reversed when nuclei scale smaller? During both early *Xenopus* and *C. elegans* embryonic development, the size of the nucleus decreases with decreasing cell size [13,17,18], which reduces the amount of cytoplasm surrounding the nucleus and the materials (like lamins) necessary for nuclear growth. Interestingly, microfluidic encapsulation of *Xenopus* egg extracts also revealed a role for microtubules in determining the speed of nuclear expansion through dynein-mediated membrane transport [19]. In vivo studies revealed that prior to the mid-blastula transition (MBT) and the onset of zygotic transcription, the reduction in nuclear size in *Xenopus* correlates with reduced import rates and levels of cytoplasmic importin α. Ectopic importin α expression was sufficient to increase nuclear size in pre-MBT embryos [17]. Although lamin isoform expression changes during *Xenopus* development, nuclear scaling was sensitive to the total lamin concentration, and was not altered by specific lamin isotypes [10]. After MBT, but prior to gastrulation, nuclear size reduction was shown to depend on protein kinase C (cPKC) activity, which correlated with the removal of lamins from the nuclear envelope [20]. Moreover, PKC-mediated phosphorylation of lamins in interphase contributed to reductions in nuclear size in both *Xenopus* and mammalian cells [21] (Figure 2B). However, increasing lamin levels in post-MBT embryos or mammalian tissue culture cells also decreased nuclear size [10]. A possible explanation for this paradox is that lamin expression is precisely tuned in transcriptionally active cells, and their incorporation above a certain threshold alters mechanical properties of the lamina and, as a consequence, distorts nuclear size and shape. Consistent with this idea, a recent biophysical study revealed that lamin levels control nuclear stiffness in response to large deformations in mammalian cells [22]*.

What are the functional consequences of nuclear scaling? As well as nuclear mechanics, nuclear size is thought to impact chromatin organization and gene expression, and defects in nuclear size are associated with disease [23]. In *Xenopus*, altering the nucleo-cytoplasmic ratio by modulating either DNA content (ploidy) or nuclear scaling factors affected MBT timing and the onset of zygotic gene expression [17,24]. A recent study in embryonic stem cells demonstrated that changing nuclear size and shape by altering nuclear envelope components impacted gene regulation and lineage differentiation [25]. Interestingly, however, manipulations of nuclear scaling factors in frog or mouse did not negatively affect embryonic development, and the functional significance of nuclear size remains to be elucidated.

Mechanisms of chromosome scaling

A current limit to our understanding of mitotic chromosome scaling is the fact that chromosome architecture itself is poorly understood. Recently, a technical breakthrough that allows much improved visibility of the DNA by electron tomography showed definitively that rather than orderly packaging, chromatin exists as a disordered granular chain with a diameter of 5 to 24 nm. These chains are packed at variable concentration densities inside the nucleus, and at even higher densities in mitotic chromosomes [26]**. This apparent disorder allows flexible bending, enabling high packing densities of DNA. Thus, rather than higher order folding of a nucleosome fiber, mitotic chromosomes contain the same 5 to 24 nm chromatin chains as in interphase, but packed at a higher concentration density.

Thus, a candidate mechanism for how mitotic chromosomes scale with cell size is through changes in chromatin packing density. Consistent with this idea, mitotic chromosome size decreased with nuclear size during both *Xenopus* and *C. elegans* embryogenesis [15,16,27]. Furthermore, intra-nuclear DNA density was shown to correlate with the packing ratio of mitotic chromosomes across a variety of species [28]. Interestingly, when nuclei from small embryonic cells were allowed to expand in an interphase *Xenopus* egg extract prior to inducing chromosome condensation, small mitotic chromosomes were still produced, arguing against a relationship between nuclear size and chromosome size. However, progression through a full cell cycle in egg extract re-established characteristic chromosome lengths [27]. In contrast, artificially reducing nuclear size by blocking nuclear transport or adding an inhibitory lamin antibody to egg extracts, or by reducing RanGTP levels or nuclear import in *C. elegans*, led to the formation of smaller mitotic chromosomes [15,16]. These studies link mitotic chromosome size scaling with the Ran pathway and nuclear import and are consistent with a model in which chromatin has a “memory” of how compact it was in the interphase nucleus. In this model, scaling factors would be imported or exported from the nucleus during interphase and loaded on to chromatin during DNA replication, thereby setting chromosome size (Figure 3).

Even if chromosome scaling were simply due to physical effects of nuclear scaling, factors likely act to maintain higher levels of chromatin compaction in smaller cells to facilitate mitotic chromosome scaling. A screen for proteins essential for embryogenesis in a *C. elegans* strain harboring a long chromosome identified two potential mitotic chromosome scaling factors as topoisomerase II (topo-II) and the centromere-specific histone H3 variant CENP-A [29]**. Since *C. elegans* chromosomes are holocentric, CENP-A is found periodically all along the length of mitotic chromosomes, forming a platform for kinetochore assembly and spindle microtubule attachment [30]. Interestingly, CENP-A levels and chromosome staining decrease during development, and depletion of CENP-A, or reduction of its nuclear import, further reduced chromosome length. These findings implicate CENP-A as one nuclear cargo that could act at the chromosome surface, perhaps by organizing chromatin domains whose abundance correlates with chromosome size. While a CENP-A-driven mechanism of chromosome scaling would be limited to holocentric chromosomes, it suggests where and how a vertebrate scaling factor could operate. However, recent functional studies of proteins at the periphery of human mitotic chromosomes, including Ki-67 that act as a surfactant to disperse mitotic chromosomes [31] and the BAF protein that clusters mitotic chromosomes together [32] did not reveal any role in setting or maintaining their size.

In contrast to CENP-A, partial depletion of topo-II from *C. elegans* embryos was found to increase chromosome length [29]**. Topo-II levels or staining was unchanged during development, however, and the effects of its depletion may reflect a more general role in establishing chromosome architecture. Indeed, depleting or interfering with chromosome structural proteins often results in chromosomes with improper length, shape or compaction; these include condensin and cohesin [33], as well as linker [34] and core histones [35]**. It is thus plausible that chromosome scaling factors include chromatin structural proteins with known functions in compaction and organization. However, elucidating scaling roles for one or more of these factors may prove challenging considering the complex relationships

among them, such as the interplay between condensin, cohesin and topo-II [33,36], and post-translational modifications that alter their activity and distribution [37,38].

Importance of subcellular scaling in mitosis

Spindle and nuclear scaling with cell size is conserved across metazoans [23,39]. Furthermore, scaling factors have evolved to adapt nuclear and spindle size to cell size in different *Xenopus* species [13,40], and scaling mechanisms operate during development [7,20]. One would therefore expect these mechanisms to be important for cell and organism viability. However, since few scaling factors have been identified, functional data are limited. As discussed above, modest changes in nuclear scaling affect developmental timing and gene expression, but not embryo viability [17,24]. Decreasing spindle size during the early cleavage divisions of *Xenopus* embryos by increasing levels of the microtubule depolymerizing spindle scaling factor kif2a caused metaphase spindle alignment defects, but cleavage plane positioning was corrected by interactions of astral microtubules with the cell cortex, resulting in normal development [7]. We can think of two reasons why scaling functions in cell division might be difficult to disrupt. First, in addition to molecular scaling factors, an intrinsic physical mechanism based on cell volume and limiting components plays an important role in subcellular scaling [41,42]. Measurements comparing size variants within and between closely related nematode species indicated that natural selection acts predominantly on cell/embryo size, which then indirectly influences the spindle size [43]. Thus, cell size itself contributes to spindle size and the fidelity of cell division. Second, multiple mechanisms operate across a wide range of cell sizes to facilitate cell division. For example, microtubule amplification and trigger waves function to spatially and temporally coordinate chromosome segregation and cytokinesis in large cells [44,45].

Scaling of chromosome length to anaphase spindle length, which scales to cell length, is obviously crucial for proper chromosome segregation. Landmark studies in plants showed that artificially lengthened chromosomes fail to clear the spindle mid-zone and lead to the formation of micronuclei [46,47]. In animal cells, Aurora B kinase at the spindle mid-zone causes hypercondensation of chromosomes [48,49], which helps to avoid such defects. However, gross inhibition of chromosome condensation by depletion of linker histone H1, for instance, prevents chromosome alignment and segregation [34]. Due to the intimate relationship between chromosome size and architecture, and compensatory mitotic mechanisms, demonstrating a function for mitotic chromosome scaling is a current challenge in the field.

Studies analyzing the effects of variation in size relationships have revealed their relevance to spindle function and accurate chromosome segregation. Manipulating nuclear-cytoplasmic volume ratio by halving or fusing mouse oocytes affected meiotic spindle architecture, assembly kinetics, and chromosome alignment [50]**. Large cytoplasmic volume limited the spindle's capacity to prevent anaphase entry with misaligned chromosomes, consistent with a study showing that cell size determines the strength of the spindle assembly checkpoint during *C. elegans* development [51]*. Modeling also predicts that checkpoint silencing entails proper size scaling of the spindle [52]*. Thus, although

cells possess robust systems to ensure accurate chromosome transmission, cell size and scaling relationships impact the fidelity of cell division.

Conclusions

The role of the Ran pathway in spindle assembly and nuclear scaling has been studied for quite some time, but its implications for mitotic chromosome scaling are only starting to be appreciated. Although the RanGTP gradient itself does not appear to set spindle size, the transport machinery, particularly importin α, regulates known spindle scaling factors. Lamin import, which is also regulated by importin α, scales nuclear size to cell size during development. In turn, nuclear size affects DNA density and correlates with mitotic chromosome length, though the factors that scale chromosome condensation to cell size are still poorly understood. A mechanistic link between importins and coordinated spindle, nuclear, and mitotic chromosome scaling seems likely, but remains to be elucidated. Although proper scaling relationships are central to the fidelity of chromosome segregation, they have proven difficult to manipulate experimentally and compensating mechanisms operate to facilitate the vitally fundamental process of cell division.

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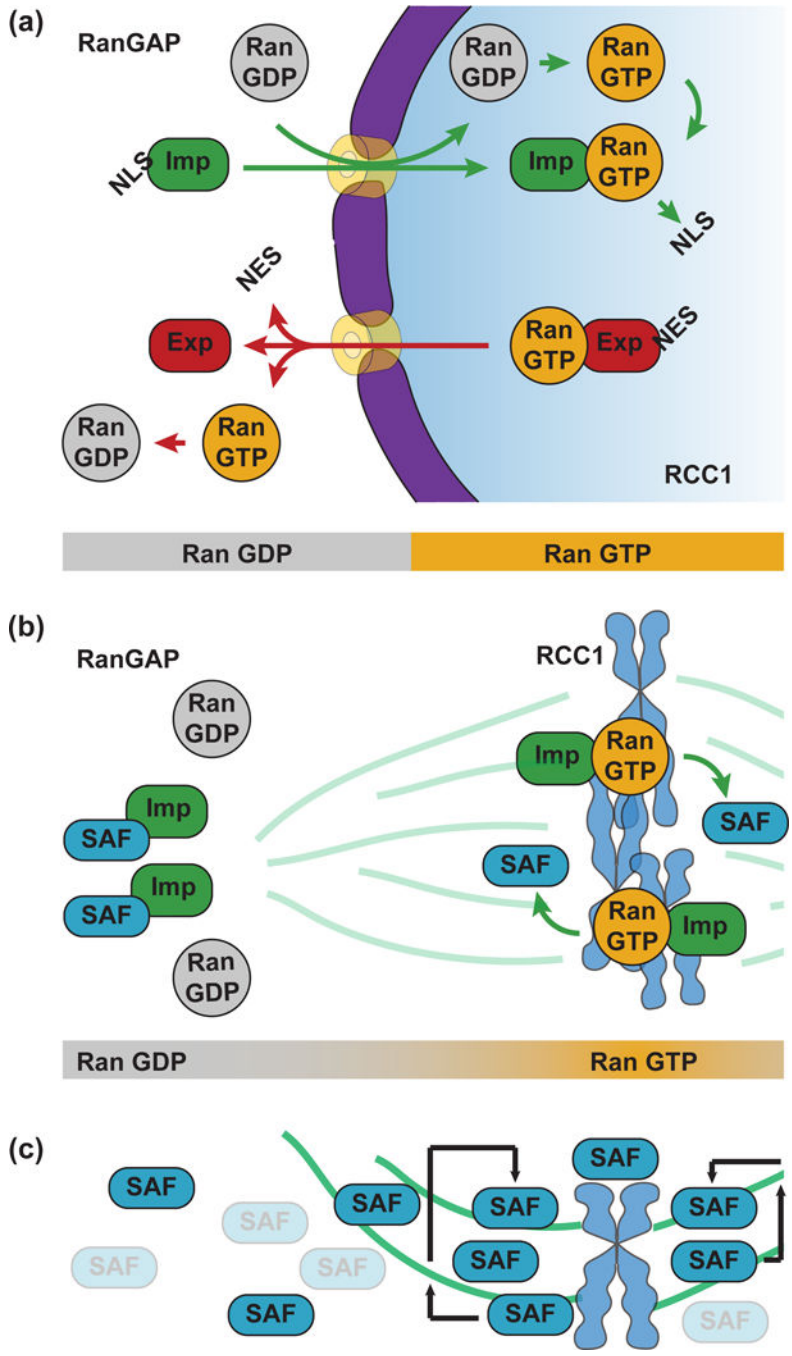

Figure 1.

The RanGTP pathway and spindle assembly. (a) In interphase, Ran is GTP-bound in the nucleus due to the chromatin-associated RanGEF, RCC1, and GDP-bound in the cytoplasm due to cytoplasmic RanGAP. Proteins harboring an NLS are imported into the nucleus by importins and released when importins interact with RanGTP. Proteins containing an NES are exported out of the nucleus by RanGTP-bound exportins and released by GTP hydrolysis. (b) In mitosis, chromosome-bound RCC1 creates a Ran-GTP gradient near the chromosomes where NLS-containing SAFs are released from importins, promoting

microtubule nucleation and stabilization. (c) Following microtubule nucleation by SAFs, the interaction between SAFs and microtubules leads to a feedback that further enriches SAFs on microtubules.

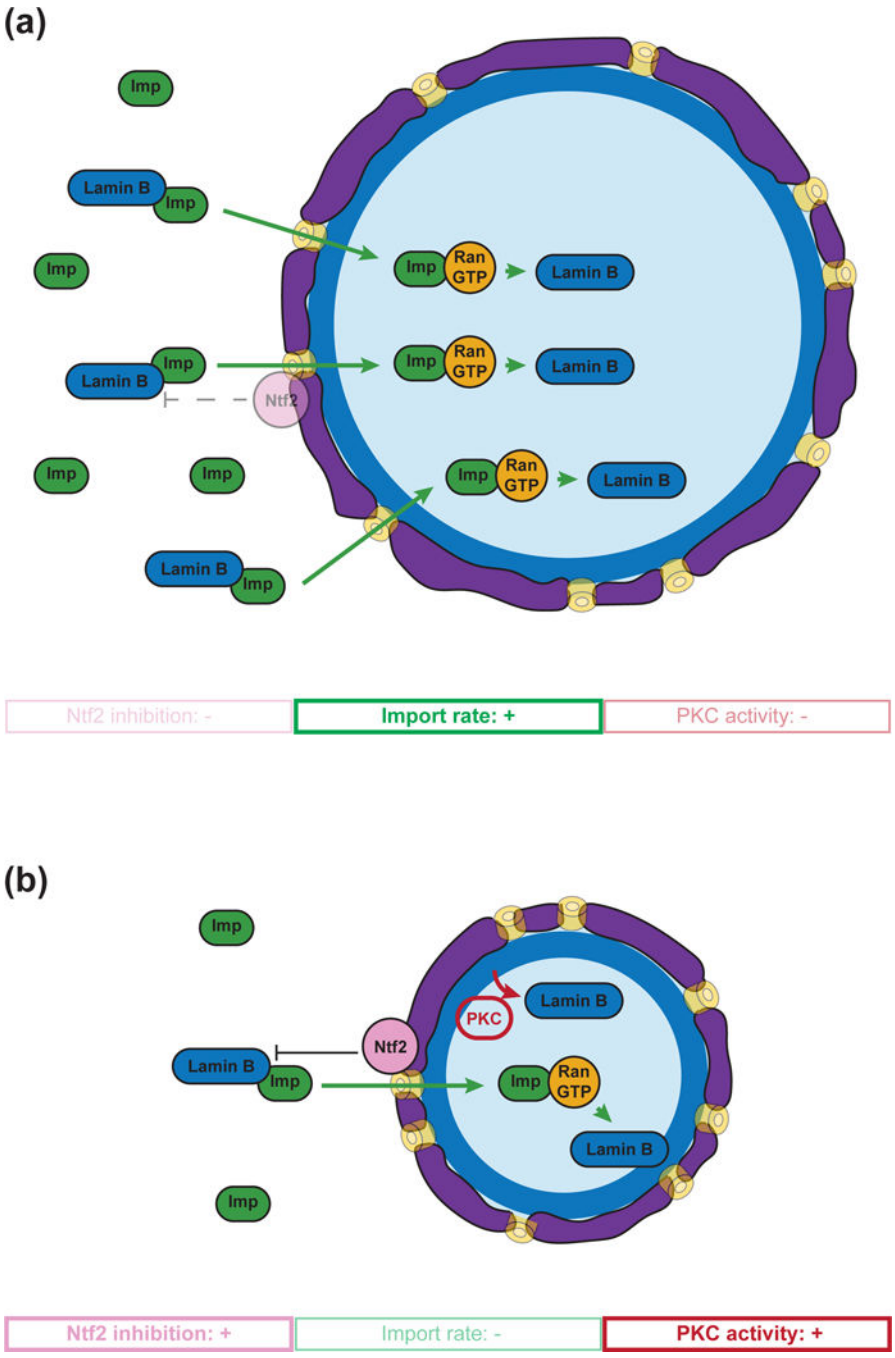

Figure 2.

Mechanisms of nuclear scaling. (a) Nuclear import of lamins promotes nuclear growth. (b) Reduced import rate of lamins and PKC-driven lamin removal scale nuclei smaller. Moreover, Ran-dependent association of Ntf2 to the nuclear pore also affects nuclear size by inhibiting lamin import, perhaps by reducing the diameter of the nuclear pore complex.

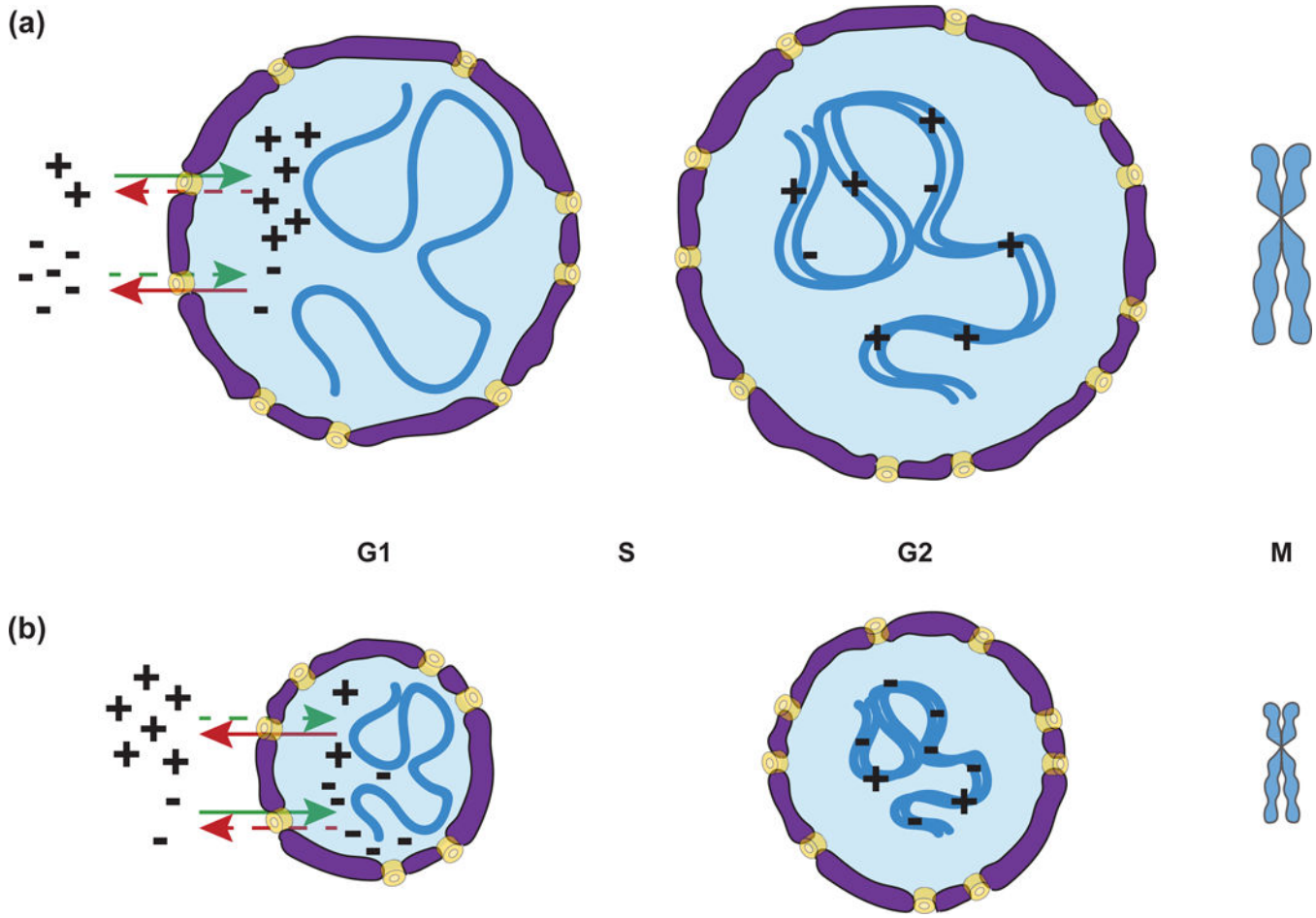

Figure 3.

A possible model for chromosome scaling. Scaling factors that increase (+) or decrease (−) chromosome size are differentially imported/exported in interphase leading to more (+) factors and/or less (−) factors in large nuclei (a) and vice-versa in small nuclei (b). Scaling factors are then loaded during DNA replication and thus set chromosome size for mitosis.