Intranuclear DNA density affects chromosome condensation in metazoans

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ABSTRACT Chromosome condensation is critical for accurate inheritance of genetic information. The degree of condensation, which is reflected in the size of the condensed chromosomes during mitosis, is not constant. It is differentially regulated in embryonic and somatic cells. In addition to the developmentally programmed regulation of chromosome condensation, there may be adaptive regulation based on spatial parameters such as genomic length or cell size. We propose that chromosome condensation is affected by a spatial parameter called the chromosome amount per nuclear space, or “intranuclear DNA density.” Using Caenorhabditis elegans embryos, we show that condensed chromosome sizes vary during early embryogenesis. Of importance, changing DNA content to haploid or polyploid changes the condensed chromosome size, even at the same developmental stage. Condensed chromosome size correlates with interphase nuclear size. Finally, a reduction in nuclear size in a cell-free system from Xenopus laevis eggs resulted in reduced condensed chromosome sizes. These data support the hypothesis that intranuclear DNA density regulates chromosome condensation. This suggests an adaptive mode of chromosome condensation regulation in metazoans.

INTRODUCTION During cell division, DNA becomes highly compacted for segregation of genetic information into future daughter cells. Chromosome condensation is a crucial process because defective chromosome condensation can lead to segregation defects such as aneuploidy and cancer (Jallepalli and Lengauer, 2001; Nasmyth, 2002). The degree of condensation is actively regulated, as different condensed chromosome sizes are observed even in individual organisms possessing identical base-pair lengths of DNA. Condensed metaphase chromosomes become shorter and thicker as development progresses during the late embryonic phase of Xenopus laevis (Micheli et al., 1993). This trend can be reproduced in a cell-free system from X. laevis egg (Kieserman and Heald, 2011). Similar observations have been reported in other species (Conklin, 1912; Frankhauser, 1934; Berardino, 1962; Belmont et al., 1987). Therefore the sizes of condensed chromosomes are controlled by developmental stage-dependent regulation.

Several mechanisms mediate the developmentally programmed regulation of condensed chromosome size. The first mechanism involves condensin, a major regulator of chromosome condensation (Hirano and Mitchison, 1994; Hirano et al., 1997). The ratio of condensin I and II changes between embryonic and somatic cells (Ono et al., 2003), and this change is sufficient to reconstruct relatively long and short condensed chromosomes in a cell-free extract of X. laevis egg (Shintomi and Hirano, 2011). Similarly, two different developmental isoforms of the linker histone H1—embryonic H1M and somatic H1—may also define the size of embryonic and somatic chromosomes in X. laevis (Maresca et al., 2005; Freedman and Heald, 2010). Density at the DNA replication origin may be involved in developmental control of condensed chromosome size. Because the position of the DNA replication origin can define the size of DNA loop structures (Buongiorno-Nardelli et al., 1982; Courbet et al., 2008) and the density of the origin decreases as development proceeds, it has
in metazoans. In multicellular organisms, the size of cell varies among cell stages.

In addition to the developmentally programmed regulation, chromosome condensation is likely controlled by adaptive mechanisms (Neurohr et al., 2011). Here “adaptive” means that the degree of chromosome condensation is adjusted to the physical environment of the chromosome, even in cells of the same type and developmental stage. In Saccharomyces cerevisiae, an introduced extra-long chromosome is condensed to a higher degree to make the physical size of the introduced chromosome comparable to that of native chromosomes (Neurohr et al., 2011). This adaptation is likely important to segregate daughter chromosomes completely over a limited distance of spindle elongation and may be regulated by a “midzone-based ruler” in which insufficient condensation is detected at the midzone during chromosome segregation and triggers more condensation.

Adaptive regulation may be appropriate for intact chromosomes in metazoans. In multicellular organisms, the size of cell varies within the organism, in particular during embryogenesis, and thus the adaptive regulation of chromosome condensation may take place (Kieserman and Heald, 2011; Ladouceur et al., 2011). Caenorhabditis elegans embryogenesis is a good model in which to study the dependence of cellular processes in various spatial contexts, as transparent embryonic cells with various sizes of cell, nucleus, or spindle are observed (Carvalho et al., 2009; Hara and Kimura, 2009, 2011; Greenan et al., 2010; Ladouceur et al., 2011). We identified variety in the extent and speed of spindle elongation in C. elegans embryogenesis (Hara and Kimura, 2009). Considering the possible connection between chromosome condensation and spindle elongation in S. cerevisiae (Neurohr et al., 2011), we expected to see similar variety in condensed chromosome size during C. elegans embryogenesis.

RESULTS

Condensed chromosome length varies during C. elegans embryogenesis

To characterize the variation in condensed chromosome size, we observed separated chromosomes in C. elegans embryos at various cell stages. Squashes from acetic acid–fixed embryos allowed us to visualize 12 individual, rod-shaped mitotic chromosomes in a single cell of a C. elegans diploid hermaphrodite (Figure 1, A–I; Albertson and Thomson, 1982; Yoshida et al., 1984). We measured samples in which the chromosomes were uniformly spread in what we define as prometaphase and metaphase chromosomes. We did not measure samples in which chromosomes segmented into two subgroups, as these represent anaphase or telophase. We measured the physical length and width of the condensed chromosomes, hereafter referred to as chromosome length and width, respectively (Figure 1, J and K, and Supplemental Figures S1 and S2). At different cell stages, chromosome lengths varied, as shown in the box plot (Figure 1J) and histograms (Supplemental Figure S1). The possible causes of this variability include 1) variable genomic lengths (e.g., chromosome III is 12.77 Mbp and chromosome V is 20.82 Mbp long; C. elegans Sequencing Consortium, 1998), 2) variability in cell cycle phase (i.e., prometaphase or metaphase), and 3) experimental variability. Note that the coefficient of variation (i.e., SD/mean) of the chromosome length was similar in all cell stages (Table 1), suggesting that our method was not biased toward a specific chromosome or cell cycle phase. Regardless of the variability, chromosome length clearly appeared to decrease as embryogenesis proceeded (Figure 1J). For example, chromosome length at the 2-cell stage is significantly larger than at the 100-cell stage (p < 0.001; mean value ± SE for 2-cell stage, 3.0 ± 0.1 [n = 50]; for 100-cell stage, 1.9 ± 0.04 [n = 135]). No apparent differences in chromosome width were noted (Figure 1K and Supplemental Figure S2). We do not have a good explanation for why the C. elegans chromosomes shorten but do not widen. In summary, our measurements indicated that the length, but not the width, of individual chromosomes decreases during C. elegans embryogenesis. The data indicate that the degree of chromosome condensation differs among cell stages.

FIGURE 1: Individual condensed chromosome size is not constant during early embryogenesis. (A–I) Chromosomes in squashed embryos at each cell stage; bar = 5 μm. (J) The lengths and (K) widths of chromosomes at each cell stage are shown in the box plot. The middle line of each box is the median. The top and bottom lines are the third and first quartiles, respectively, and the whiskers indicate the 90th and 10th percentiles. The distributions of chromosome length and width in each cell stage are shown in histograms (Supplemental Figures S1 and S2).
| Stage          | Chromosome | Length (micrometers) | Width (micrometers) | CV | N |
|----------------|------------|----------------------|---------------------|----|----|
| Wild type      | Chromosome length | Mean | 3.6 (1.3) | 3.0 (0.7) | 3.2 (0.8) | 2.6 (0.7) | 2.8 (0.8) | 2.4 (0.6) | 2.0 (0.5) | 1.9 (0.5) | 1.9 (0.5) |
|                | CV         | 36.0 | 23.9 | 26.2 | 28.7 | 26.7 | 25.2 | 25.6 | 25.2 | 24.2 |
|                | N          | 47   | 50   | 56   | 84   | 151  | 212  | 193  | 135  | 107 |
|                | Mean       | 0.6 (0.1) | 0.6 (0.1) | 0.6 (0.1) | 0.6 (0.1) | 0.6 (0.1) | 0.6 (0.1) | 0.6 (0.1) |
| mei-1 (RNAi)   | Chromosome length | Mean | 3.0** (0.7) | 3.4** (1.1) | 3.3** (0.9) | 3.0 (0.7) | 3.0* (1.0) | 2.7* (0.8) | 2.4** (0.8) | N.D. |
|                | CV         | 23.7 | 20.9 | 28.8 | 28.5 | 23.3 | 32.2 | 30.2 | 31.0 |
|                | N          | 23   | 20   | 33   | 29   | 66   | 61   | 55   | 74   |
|                | Mean       | 0.7 (0.1) | 0.7* (0.1) | 0.7 (0.2) | 0.6 (0.1) | 0.6* (0.1) | 0.6 (0.1) | 0.6** (0.1) | N.D. |
| klp-18 (RNAi)  | Chromosome length | Mean | 3.4 (0.8) | 3.8* (0.9) | 3.5** (1.0) | 3.3** (0.8) | 3.2* (0.9) | 2.8* (0.7) | 2.7* (0.8) | 2.1** (0.7) |
|                | CV         | 22.2 | 23.6 | 27.7 | 25.8 | 26.5 | 25.0 | 28.1 | 32.3 |
|                | N          | 28   | 39   | 43   | 37   | 73   | 96   | 105  | 74   |
|                | Mean       | 0.7** (0.2) | 0.7** (0.2) | 0.6 (0.1) | 0.6* (0.1) | 0.6 (0.1) | 0.6* (0.1) | 0.5** (0.1) |
| ran-3 (RNAi)   | Chromosome length | Mean | 2.5* (0.9) | 2.3* (0.7) | 2.1* (0.6) | 2.0* (0.6) | 1.7* (0.5) | 1.4* (0.4) | N.D. |
|                | CV         | 35.6 | 30.6 | 30.6 | 28.1 | 27.2 | 24.5 |
|                | N          | 40   | 50   | 68   | 77   | 100  | 64   |
|                | Mean       | 1.0* (0.1) | 0.8* (0.1) | 0.8* (0.1) | 0.9* (0.2) | 0.8* (0.1) | 0.8* (0.1) |
| irma-3 (RNAi)  | Chromosome length | Mean | 3.1** (1.3) | 2.4** (0.9) | 2.5* (0.6) | 2.3** (0.6) | 2.1* (0.6) | 2.2* (0.5) | 1.8** (0.5) | 1.5* (0.3) |
|                | CV         | 43.8 | 36.7 | 25.3 | 24.5 | 30.9 | 24.7 | 29.7 | 19.5 |
|                | N          | 39   | 23   | 88   | 71   | 95   | 63   | 56   | 42   |
|                | Mean       | 0.7 (0.1) | 0.6 (0.1) | 0.7* (0.2) | 0.7 (0.1) | 0.6* (0.1) | 0.6 (0.1) | 0.5* (0.1) |
| C27D9.1 (RNAi) | Chromosome length | Mean | 4.2 (0.8) | 3.8* (1.1) | 2.9 (0.9) | 3.2* (0.8) | 2.8 (0.7) | 2.3 (0.6) | 2.5* (0.8) | 2.0** (0.6) | 2.1 (0.5) |
|                | CV         | 19.5 | 30.1 | 29.0 | 24.9 | 23.9 | 27.8 | 30.6 | 27.3 | 22.9 |
|                | N          | 10   | 67   | 27   | 110  | 163  | 61   | 81   | 96   | 28   |
|                | Mean       | 0.8 (0.1) | 0.7 (0.1) | 0.6* (0.1) | 0.6 (0.1) | 0.6* (0.1) | 0.6* (0.1) | 0.6** (0.1) | N.D. |

Mean length and width of the condensed chromosome (micrometers). SDs are shown in parentheses. CV, coefficient of variation; N, sample size examined; N.D., not determined. Asterisks indicate statistical differences from the wild type (*p < 0.005, ** 0.005 < p < 0.05).
Chromosome condensation dynamics is similar during early embryogenesis

Possible explanations for the difference in condensed chromosome sizes include that 1) chromosome condensation is regulated by varying amounts of condensation regulators inside the cell (Maresca et al., 2005; Maddox et al., 2006; Shintomi and Hirano, 2011) and 2) the duration of chromosome condensation differs. Higher amounts of condensation regulators should lead to swifter condensation, and longer condensation time should lead to greater condensation. To examine whether the speed or the duration of chromosome condensation increases as embryogenesis proceeds, we compared the dynamics of chromosome condensation among various cell stages using an established method (Maddox et al., 2006). The fluorescence intensity distribution of green fluorescent protein (GFP) fused with histone (GFP::histone) allows us to detect changes in chromosome condensation during mitosis. First, we conducted time-lapse imaging of GFP::histone during prophase until the onset of nuclear envelope breakdown (NEBD; Figure 2A). Using the histone distribution data, we quantified and compared chromosome condensation profiles in various cell stages (Figure 2B). In one-cell-stage embryos, the condensation score increases monotonically as chromosome condense, and the difference in condensation kinetics between wild-type and condensin-perturbed embryos (smc-4 (RNAi); RNA-mediated interference [RNAi]) was clear (Maddox et al., 2006; Figure 2B). When we compared the kinetics among cell stages, the overall profile of chromosome condensation was similar (Figure 2B). Thus the speed and duration of chromosome condensation were similar among different cell stages during early embryogenesis. These data suggest that a drastic change in condensation dynamics (such as changes in major chromosome condensation regulators during the stages of interest) is unlikely. Because the condensation kinetics is similar among the different cell stages but the final condensed chromosomes differ in size, we hypothesized that precondensation differences influence the degree of condensation.

Ploidy changes the size of the condensed chromosomes

Because nuclear size changes during embryogenesis, chromosome density in the interphase nucleus varies and may affect the initial...
state of the chromosomes before condensation. The involvement of chromosome density in chromosome condensation is consistent with our observation that condensed chromosomes from one-cell-stage embryos are significantly longer than in two-cell-stage embryos (Figure 1J; p < 0.05). The one-cell-stage embryo has two pronuclei and contains a haploid genome, whereas the nuclei at the two-cell stage contain diploid genomes. Because nuclear size at the two-cell stage is almost comparable to that of pronuclei at the one-cell stage, chromosome density inside the nucleus differs between stages and correlates negatively with chromosome length.

To manipulate chromosome density in the nucleus, we generated haploid embryos by RNAi knockdown of the klp-18/kinesin 12 gene. This RNAi embryo occasionally extrudes all of the oocyte genome into polar bodies due to meiotic division failure (Supplemental Figure S3; Segbert et al., 2003). This causes the klp-18 (RNAi) embryo to possess chromosomes only from the sperm, yielding a haploid genome (Hara and Kimura, 2013). From the klp-18 (RNAi) embryos, we selected haploid embryos by confirming the absence of the female pronucleus or six chromosomes per nucleus (Supplemental Figures S3 and S4). These are called “klp-18 (RNAi) haploid” embryos. The chromosome lengths of klp-18 (RNAi) haploid embryos were significantly larger than those at the corresponding cell stage in the wild type (Figure 3 and Table 1).

klp-18 is unlikely to be directly involved in chromosome condensation. Some klp-18 (RNAi) embryos retained the majority or even maternal compliment of DNA in the embryo, yielding an excess of DNA (polyploid; Segbert et al., 2003; Hara and Kimura, 2013). We observed chromosomes of klp-18 (RNAi) polyplody embryos by selecting embryos with excess DNA or >12 chromosomes per nucleus (Supplemental Figure S4). The chromosomes of klp-18 (RNAi) polyploid embryos were shorter than those of klp-18 (RNAi) haploid and those of wild-type diploid embryos (Figures 3 and Table 1). This observation indicated that the change in chromosome length is negatively correlated with chromosome number in the embryos. In addition, one-cell (pronuclear)-stage klp-18 (RNAi) and wild-type embryos and two-cell-stage klp-18 (RNAi) haploid embryos, all of which possess the same DNA content per (pro)nucleus, had condensed chromosomes of almost the same size (Table 1). Finally, we obtained haploid embryos by mei-1/katanin (RNAi) (Mains et al., 1990; Hara and Kimura, 2013) and observed longer chromosomes (Figure 3 and Table 1). On the basis of these data, we concluded that the change in chromosome number caused a change in the length of the condensed chromosome. Thus the degree of chromosome condensation is not solely defined by developmental stage but might depend on spatial context.

The changes in chromosome length induced by changing the chromosome number should be independent of the midzone-based ruler mechanism proposed in S. cerevisiae (Neurohr et al., 2011). First, we are observing chromosomes of prometaphase and metaphase, and the change should take place before chromosome segregation at anaphase. Second, with this mechanism, the longer chromosome can be explained by faster chromosome separation at anaphase or larger cell size (Ladouceur et al., 2011). This was not the case for klp-18 (RNAi) haploid embryos. The speed of chromosome separation (measured as the speed of spindle elongation) in klp-18 (RNAi) haploid embryos was not faster than the controls at corresponding cell stages (Supplemental Table S1). The cell size in klp-18 (RNAi) haploid embryos was comparable to that in wild-type embryos (cell length in the one-cell stage of wild type, mean ± SE, 43.1 ± 0.3 μm [n = 26]; in the one-cell stage of klp-18 (RNAi) haploid, 42.7 ± 0.9 μm [n = 12]). Third, the midzone-ruler mechanism predicts higher chromosome compaction with shorter spindles. This was not the case in our previous observation, as the short spindle in spd-2/Cep192 (RNAi) or tpxl-1/TPX2 (RNAi) did not affect chromatin condensation (Hara and Kimura, 2013). Instead of the midzone-based ruler mechanism, we propose that reduced DNA density inside the nucleus increased the size of the condensed chromosomes. The nuclear diameters in the klp-18 (RNAi) haploid embryo were similar to those in the wild-type embryo (Supplemental Figure S3 and Table 2). A spatial parameter of intranuclear DNA density may affect the degree of chromosome condensation.

The ratio of DNA amount to nuclear size is correlated with chromosome size

To test our idea that intranuclear DNA density affects the degree of chromosome condensation, we further investigated the relationship between DNA density and chromosome condensation in the C. elegans embryo. First, we evaluated the quantitative relationship between intranuclear DNA density and condensed chromosome size during wild-type embryogenesis. As embryogenesis proceeds, the size of the nucleus decreases (Hara and Kimura, 2009; Figure 4A and Table 2), increasing the intranuclear DNA density. We plotted the relative ratio of the DNA amount to the nuclear size against the mean chromosome length in each cell stage (Figure 4B) for wild-type (including pronuclear stage) and klp-18 or mei-1 (RNAi) haploid nuclei. We did not include data from klp-18 (RNAi) polyplody embryos because their exact ploidy could not be determined (Hara and Kimura, 2013). The relative ratio was calculated by dividing the genome length per (pro)nucleus (Mbp) by nuclear volume. The regression line fitted to the wild-type plot showed that the chromosome length correlated well with the relative DNA ratio; the line also explains the data from the haploid nucleus.
Next we attempted to manipulate nuclear size by knocking down genes. We knocked down ran-3/RCC1, npp-13/Nup93, imr-1/Lamin, emr-1/Emerin; lem-2/MAN1, ima-3/Importin α3, and arf-1/ARF by RNAi because knockdown of each gene reduces nuclear size in one-cell-stage embryos (Poteryaev et al., 2005; Sonnichsen et al., 2005; Meyerzon et al., 2009). RNAi of ran-3 or ima-3 caused a consistent reduction in nuclear size during embryogenesis at the 1- to 16-cell stage (Figure 4C and Table 2). RNAi knockdown of the other candidate genes revealed little effects on nuclear size after the two-cell stage (Supplemental Figure S5 and Supplemental Table S2). In ran-3 or ima-3 (RNAi) embryos, the individual chromosome length at all cell stages significantly decreased in comparison to the wild type (Figure 4D and Table 2). In addition, we examined the larger nuclei in C27D9.1 (RNAi) embryos, which have larger cells (Figure 4C and Table 2; Hara and Kimura, 2009). The chromosomes in C27D9.1 (RNAi) embryos were longer than in the wild type (Figure 4D and Table 1).

When we plot the data from ran-3, ima-3, and C27D9.1 (RNAi) chromosome length versus DNA density, we find an overall trend similar to the data from wild-type and klp-18 or mei-1 (RNAi) haploid embryos and near the regression line (Figure 4B). In summary, changing the DNA ratio by changing nuclear size during embryogenesis or RNAi treatment caused chromosome length to change, supporting the hypothesis that intranuclear DNA density affects the degree of chromosome condensation.

### Reduced nuclear size induces chromosome size reduction in a cell-free system from X. laevis eggs

To obtain completely independent experimental support for the idea that intranuclear DNA density affects the degree of chromosome condensation and to evaluate the generality of the idea, we tested our hypothesis using a cell-free system from *X. laevis* eggs. In this system, functional nuclei can be reconstituted by incubating sperm nuclei in *X. laevis* egg extract at the interphase (Figure 5A). Furthermore, the nuclei can be transformed to condensed chromosomes by inducing the cell-cycle transition of the extract to M phase (Hirano and Mitchison, 1994; Desai et al., 1997). We could reduce the nuclear size in this system by inhibiting nuclear growth with antibody against lamin LIII protein, which is a primary component of the nuclear lamina in *X. laevis* oocytes and early embryonic cells (Lourim et al., 1996), or by adding wheat germ agglutinin (WGA), which blocks nuclear pores by binding to nucleoporins that contain N-acetylglucosamine residues (Finlay et al., 1991; D’Angelo et al., 2006). We preincubated sperm nuclei in the interphase egg extracts to allow nuclear formation and added the antibody or WGA to the extracts to suppress nuclear growth. Although complete inhibition of nuclear import without preincubation impairs DNA replication and nuclear envelope assembly (Cox, 1992; Hachet et al., 2004), our experimental conditions allowed nuclear envelope formation and complete DNA replication (D’Angelo et al., 2006; Supplemental Figure S6). We obtained functional small nuclei by adding antibody against lamin LIII antibodies or WGA to the cytoplasmic extract (Figure 5B and Table 2).

### TABLE 2: Diameter of the prophase nucleus in *C. elegans* embryos

| TABLE 2: Diameter of the prophase nucleus in *C. elegans* embryos |
|---------------------------------------------------------------|
| **Wild type** | **1-cell stage** | **2-cell stage** | **4-cell stage** | **8-cell stage** | **16-cell stage** | **28-cell stage** | **50-cell stage** | **100-cell stage** | **200-cell stage** |
| Mean | N | Mean | N | Mean | N | Mean | N | Mean | N | Mean | N | Mean | N | Mean | N | Mean | N | Mean | N |
| Wild type | 8.8 (0.4) | 24 | 9.1 (0.5) | 42 | 8.0 (0.6) | 47 | 7.1 (0.8) | 49 | 6.2 (0.5) | 60 | 5.4 (0.6) | 45 | 3.8 (0.3) | 28 | 3.3 (0.4) | 42 | 3.1 (0.3) |
| mei-1 (RNAi) haploid | 8.6 (0.5) | 7 | 8.6** (0.7) | 12 | 7.6** (0.6) | 13 | 6.6** (0.7) | 16 | 5.4* (0.5) | 17 | 4.1* (0.5) | N.D. | N.D. | N.D. |
| klp-18 (RNAi) haploid | 9.2** (0.2) | 8 | 9.2 (0.5) | 17 | 7.9 (0.6) | 13 | 6.9 (0.7) | 18 | 6.4** (0.3) | 7 | 5.4 (0.3) | 9 | 4.3** (0.6) | N.D. | N.D. |
| klp-18 (RNAi) polyploid | N.D. | N.D. | 9.7** (0.5) | 8 | 8.7 (0.8) | 9 | 7.6 (0.8) | 10 | 6.0 (0.7) | 17 | 5.2 (0.5) | N.D. | N.D. | N.D. |
| ran-3 (RNAi) | 5.4* (0.4) | 7 | 6.3* (0.3) | 6 | 6.1* (0.6) | 11 | 5.3* (0.8) | 11 | 5.2* (0.5) | 26 | 4.6* (0.7) | N.D. | N.D. | N.D. |
| ima-3 (RNAi) | 8.0** (0.7) | 8 | 7.9* (0.6) | 11 | 6.8* (0.6) | 15 | 5.7* (0.9) | 11 | 4.8* (0.6) | 14 | 3.8* (0.7) | N.D. | N.D. | N.D. |
| C27D9.1 (RNAi) | 9.8* (0.9) | 22 | 9.8* (0.5) | 13 | 8.6* (0.5) | 16 | 7.9* (0.6) | 16 | 7.0* (0.6) | 16 | 6.0 (0.7) | 23 | 4.3* (0.5) | 30 | 3.6** (0.3) | 14 | 3.5 (0.6) |

Mean nuclear diameter (micrometers). Diameter was measured just before nuclear envelope breakdown. Male and female pronuclei were measured in 1-cell wild-type and ran-3, ima-3, C27D9.1 (RNAi) embryos. Only male pronucleus was measured in klp-18 and mei-1 (RNAi) haploid embryos. SDs are shown in parentheses. N, sample size; N.D., not determined. Asterisks indicate statistical differences from the wild type (*p < 0.005, **0.005 < p < 0.05).
The packing ratio is the DNA length (0.34 nm/base pair) divided by the length into which it is packaged. The average DNA length per chromosome in *C. elegans* and *X. laevis* is ∼5700 μm (17 Mbp/6 chromosomes) and 59,000 μm (170 Mbp/18 chromosomes), respectively. Thus we assumed an allometric relationship between the packing ratio (PR), genome size (GS [Mbp]), and nuclear radius (NR [μm]) as PR = GS^α × NR^β × C, where α, β, and C are constants. We calculated these constants by the least-squares method.

### FIGURE 4: Nucleus size affects condensed chromosome size.

A) Relationship between measured nuclear diameter and cell length at various cell stages of wild-type embryos. Different shapes and colors represent each cell stage: blue circle, 1-cell stage; light blue diamond, 2- and 4-cell stages; green triangle, 8- and 16-cell stages; orange cross, 28- and 50-cell stages. (B) Mean chromosome length at each cell stage was plotted as function of the calculated relative DNA density. Data for wild-type (blue diamond), *klp-18* (RNAi) haploid (yellow rectangle), *mei-1* (RNAi) haploid (green rectangle), *ran-3* (gray triangle), *ima-3* (brown circle), and *C27D9.1* (RNAi) embryos (pink cross). We do not include data from *klp-18* (RNAi) polyploid embryo because the exact ploidy of the embryos could not be determined. The regression line for the wild-type data is shown. Error bars are SE. (C) Individual nuclear diameters for wild-type (blue), *ran-3* (gray), *ima-3* (brown), and *C27D9.1* (RNAi) embryos (pink) at each cell stage are shown in the box plot. The nuclear diameters in each RNAi embryo in all cell stages were significantly different from those of wild-type embryos (Student’s t test). *(D) Chromosome length at most cell stages in *ran-3*, *ima-3*, *C27D9.1* (RNAi) embryos significantly differed from those of wild-type embryos (Student’s t test). *p < 0.005; **0.005 < p < 0.05. The middle line of each box is the median. The top and bottom lines are the third and first quartiles, respectively, and the whiskers indicate the 90th and 10th percentiles. Mean chromosome length, nuclear diameter, SD, and sample size are shown in Tables 1 and 2.

and thicker than those in the control extract (Figure 5, D and E and Table 3). Thus intranuclear DNA density affects the degree of chromosome condensation.

**Estimation of the linear packing ratio suggests general trends between packing ratio and intranuclear DNA density**

To compare chromosome condensation between species, we used linear packing ratio to represent chromosome condensation. Linear packing ratio is the DNA length (0.34 nm/base pair) divided by the length into which it is packaged. The average DNA length per chromosome in *C. elegans* and *X. laevis* is ∼5700 μm (17 Mbp/6 chromosomes) and 59,000 μm (170 Mbp/18 chromosomes), respectively. Thus we assumed an allometric relationship between the packing ratio (PR), genome size (GS [Mbp]), and nuclear radius (NR [μm]) as PR = GS^α × NR^β × C, where α, β, and C are constants. We calculated these constants by the least-squares method.
Abut 100 years ago, Conklin (1912) proposed that the size of a chromosome depends on the size of the nucleus, based on cytological observation. The role of nuclear size in chromosome condensation, however, has been largely overlooked. In this study, we propose that intranuclear DNA density is one of the determinants of mitotic chromosome condensation. In this study, we demonstrated a correlation between nuclear size and chromosome condensation in *C. elegans* 1) during normal embryogenesis, 2) when nuclear size was reduced by knocking down *ran-3*/*RCC1* and *ima-3*/*Importin α3*, and 3) when nuclear size was increased by knocking down *C27D9.1*.

In addition, the smaller nucleus reconstituted in *Xenopus* egg cell-free extract resulted in shorter chromosomes. It has been proposed that the size of the nucleus does not directly affect the length of condensed chromosomes in a cell-free system of *Xenopus* egg (Kieserman and Heald, 2011). The proposal is superficially inconsistent with our argument. Our data and the previous report, however, are quantitatively consistent. Kieserman and Heald (2011) method. We used literature data for *X. laevis* (Supplemental Table S3), as well as the *C. elegans* and *X. laevis* data obtained in this study. We calculated α and β as 0.41 and ~0.35, respectively. Because α and β had similar absolute values, we simplified the allometric relationship as PR = (GS/NR)^α × C. We recalculated the value of γ and C and obtained a fitting equation, PR = 440(GS/NR)^0.41 (Figure 6). Of interest, not only the data used for the fitting but also some data measured in *Drosophila*, *Xenopus tropicalis*, mouse, and human cells (Supplemental Table S3) were plotted near the line (Supplemental Figure S7). Note that other data from humans, rats, and mice were plotted far from the fitting curve (Supplemental Figure S7). It will be an interesting future project to compare the sizes of condensed chromosomes from different species under a controlled experimental condition. Although some data from mammalian cells did not fit, our observation that plots from different organisms aligned to a common line implies that the ratio between genome size and nuclear size might be a universal parameter of chromosome condensation.

**DISCUSSION**

About 100 years ago, Conklin (1912) proposed that the size of a chromosome depends on the size of the nucleus, based on cytological observation. The role of nuclear size in chromosome condensation, however, has been largely overlooked. In this study, we propose that intranuclear DNA density is one of the determinants of mitotic chromosome condensation. In this study, we demonstrated a correlation between nuclear size and chromosome condensation in *C. elegans* 1) during normal embryogenesis, 2) when nuclear size was reduced by knocking down *ran-3*/*RCC1* and *ima-3*/*Importin α3*, and 3) when nuclear size was increased by knocking down *C27D9.1*. In addition, the smaller nucleus reconstituted in *Xenopus* egg cell-free extract resulted in shorter chromosomes. It has been proposed that the size of the nucleus does not directly affect the length of condensed chromosomes in a cell-free system of *Xenopus* egg (Kieserman and Heald, 2011). The proposal is superficially inconsistent with our argument. Our data and the previous report, however, are quantitatively consistent. Kieserman and Heald (2011)
demonstrated that the lengths of condensed chromosomes reconstituted from *X. laevis* extract and *X. tropicalis* extract were comparable, although the sizes of the nuclei before induction of chromosome condensation were significantly different. Here the expected size difference of the nuclei is ~1.5-fold in radius, based on a previous report from the same group (Levy and Heald, 2010). In our experiment using *X. laevis* extract, the radius difference between normal and small nuclei was about twofold. Under this condition, the length difference was statistically significant but only ~1.3-fold. Our measurement suggested that, for us to see a significant length change in condensed chromosomes, the nuclear radius should differ dramatically. Our measurement in *C. elegans* supports this notion. In comparison between 2- and 16-cell-stage wild-type embryos, the difference in nuclear radius was significant (*p < 0.01*) and 1.5-fold, whereas the difference in condensed chromosome size was not significant. In contrast, between cell stages in which the difference in nuclear radius is greater than twofold, the condensed chromosome length was always significantly different (*p < 0.01*). Therefore our measurements and the previous measurements (Kieserman and Heald, 2011) are quantitatively consistent.

We also demonstrated a correlation between chromosome amount (ploidy) and chromosome condensation in *C. elegans*. In wild-type embryos, chromosomes from one-cell-stage pronuclei (haploid) were longer than those from two-cell-stage nuclei (diploid). Longer chromosomes were observed in haploid embryos induced by knocking down *klp-18/kinesin 12 or mei-1/katanin*, whereas shorter chromosomes were observed in *klp-18 (RNAi)* polyploid embryos. The correlation is consistent with previous studies in other species. Kieserman and Heald (2011) demonstrated that chromosomes from *X. tropicalis* sperm nuclei are longer than those from *X. laevis* sperm nuclei when incubated in the same extracts. *X. tropicalis* has a smaller genome, and the sizes of the reconstructed nuclei are similar (Levy and Heald, 2010). In the new Triturus palmaeus, the chromosomes of haploid blastulae are larger than those of diploid blastulae (Frankhauser, 1934). (In this study, nuclear size in the haploid *T. palmaeus* blastulae was not measured.) In *Rana pipiens*, although the chromosome size in haploid embryos at stage 25 tail is comparable to that in diploid embryos (Berardino, 1962), the size of the nucleus is reduced in haploid embryos (Briggs, 1949). The aforementioned observations support our hypothesis that intranuclear density affects the length of compacted chromosomes.

How can the ratio of DNA amount to nuclear size affect the size of mitotic chromosomes? One possible scenario involves the formation of DNA loops. DNA loop formation was proposed to mediate the control of chromosome condensation by varying density of the replication origin (Pfumm, 2002). Because the replication origin is believed to be a bundle of DNA loops, the number of DNA loops is expected to decrease as the number of replication origins decreases, producing shorter condensed chromosomes. In fact, DNA loop size increases as development proceeds during Xenopus embryogenesis (Buongiorno-Nardelli et al., 1982; Courbet et al., 2008). The change in transcriptional status during development may also change DNA loop formation (Seydoux and Dunn, 1997; Cremer and Cremer, 2001). We believe that we can apply the concept of DNA loops defining the size of condensed chromosomes to the effect of intranuclear DNA density. For example, if we assume that the anchorage structures for DNA loop formation reside on the nuclear envelope with constant density, a large nucleus or fewer chromosomes should result in many DNA loops per chromosome and thus smaller chromosomes are shorter than those from cells incubated in buffer alone (Hancock, 2012). Because the DNA/chromosome itself is a macromolecule, the effect of intranuclear DNA density on
chromosome condensation may be mediated through general changes in macromolecular crowding.

Intranuclear density-dependent chromosome condensation relies on spatial parameters and is not developmentally programmed; the mechanism can be described as adaptive regulation of chromosome condensation. Adaptive regulation of chromosome condensation has been demonstrated in *S. cerevisiae*, where engineered long chromosome results in higher-order compaction at anaphase (Neurohr et al., 2011). This adaptive regulation ensures that the resultant mitotic chromosome is not too long, so that chromosome segregation proceeds faithfully with a limited distance for spindle elongation (Neurohr et al., 2011). The authors proposed that the midzone of the mitotic spindle at anaphase works as a ruler to measure the length of the mitotic chromosome and induce adaptive hypercondensation (Neurohr et al., 2011). Thus chromosome size may be regulated by other factors, such as the environment inside the cell at other cell cycle stages (Ladouceur et al., 2011). In this study, we observed changes in chromosome size before spindle formation; thus the regulation must be independent of the midzone-ruler mechanism. We were able to demonstrate intranuclear DNA density before nuclear envelope breakdown serves as a spatial constraint for setting mitotic chromosome size.

The biological significance of the intranuclear DNA density-dependent control of chromosome condensation is unclear. Adaptive chromosome condensation can be significant, as it depends on intranuclear density, and optimization requires a trade-off between chromosome compaction in mitotic phase and DNA transactions in interphase. Chromosome compaction is a crucial process, since defective compaction results in abnormal segregation (Hudson et al., 2003; Ono et al., 2003). In contrast, after cell division, the condensed chromosomes become decondensed and reconstitute the nucleus. This decondensation is believed to be important in allowing various transacting factors to bind DNA and activate transcription and replication. Such control may be particularly evident during embryogenesis, since the travel distance of a chromosome in segregation differs dramatically, depending on cell size (Hara and Kimura, 2009). When a chromosome segregates over a short distance in smaller cells, the chromosome must condense extensively to ensure complete condensation; this is less of a requirement in larger cells (Levy and Heald, 2012). Because nuclear size depends on the cell size (Goehring and Hyman, 2012), intranuclear DNA density-dependent adaptive chromosome condensation may be a mechanism to optimize the degree of chromosome condensation in response to cell size.

**MATERIALS AND METHODS**

**C. elegans** strains and RNAi procedure

To visualize individual chromosomes and the cell nucleus, we used wild-type *C. elegans* N2 and RNAi-treated N2 worms, strain CAL0061 expressing GFP–γ-tubulin (tbq-1), GFP–histone H2B (his-11), and GFP–PHPLCdelta-1, and strain TH32 (Hara and Kimura, 2009). For RNAi, double-strand RNA was prepared and injected as described previously (Kimura and Onami, 2005). The templates for smc-4, klp-18, mei-1, ran-3, ima-3, and C27D9.1 RNAi were PCR amplified from genomic DNA. Primer sequences were based on the PhenoBank database (http://worm.mpi-cbg.de/phenobank2; Sonnichsen et al., 2005).

**Separation of individual chromosomes from *C. elegans* embryos**

Condensed chromosomes were separated individually with slight modifications of standard procedures (Albertson and Thomson, 1982; Yoshida et al., 1984). Slides were coated with 1% poly-l-lysine (P9820; Sigma-Aldrich, St. Louis, MO) in distilled water. Distilled water (3 μl) was pipetted onto the slide, and several gravid hermaphrodites (wild-type N2 or treated with RNAi) were placed in the drop and cut open with a scalpel to release the embryos. To prevent artificial contraction of the chromosomes, drugs inducing metaphase arrest (e.g., colchicine and nocodazole) were not used. After removing most of the distilled water, we placed a drop of 50% acetic acid on the underside of an 18 × 18-mm coverslip, and laid this on top of the drop on the slide. The slide was inverted, and gentle pressure was applied to squash the embryos. The slide was placed on dry ice for at least 10 min. The coverslip was removed, and the slide was placed in 100% ethanol for at least 15 min and then in methanol:acetic acid (3:1) for at least 30 min. The fixed slides were air dried and stained with 2 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) solution for 5 min. A coverslip was placed on the sample and sealed. The sample was visualized at room temperature with an LSM5 LIVE confocal laser-scanning microscope equipped with an oil-immersed plan Apochromat 63× objective of numerical aperture (NA) 1.4 (Carl Zeiss, Jena, Germany) or an FV1000 confocal microscope equipped with an oil-immersed plan Apochromat 100× objective of NA 1.4 (Olympus, Tokyo, Japan).

**Nucleus and chromosome assembly in X. laevis egg extracts**

*X. laevis* egg extracts and CSF extracts were prepared as described (Iwabuchi et al., 2000). To reconstruct interphase nuclei, CSF extracts were supplemented with demembranated sperm nuclei (750–1000 nuclei/μl final concentration; Ohsumi et al., 1993), energy solution (1 mM ATP, 10 mM creatine phosphate, and 1 mM MgCl2; Murray, 1991), 5 μM Cy3-dUTP (GE Healthcare, Piscataway, NJ; to visualize DNA replication), and 100 μg/ml cycloheximide (Sigma-Aldrich; to inhibit spontaneous transition to mitosis). All incubations were performed at 22°C unless otherwise stated. After 15 min of incubation, CaCl2 in extraction buffer (EB: 100 mM KCl, 5 mM MgCl2, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–KOH, pH 7.5) was added to a final concentration of 0.6 mM to release the extract into interphase, allowing nuclear formation and DNA replication. To inhibit nuclear growth, 200 μg/ml WGA (Wako Pure Chemical Industries, Osaka, Japan) or 0.5 mg/ml anti-lamin LII antibody (Hasebe et al., 2011) was added after verifying assembly of round nuclei surrounded by the membrane structure. The duration of this preincubation was 30–40 min. In the control experiment, we added 0.5 mg/ml rabbit immunoglobulin G instead of the anti-lamin LII antibody or 50 μg/ml aphidicolin (Sigma-Aldrich) to inhibit DNA replication. After 90-min incubation (total 120 min after adding sperm nuclei) at 22°C, the mixture was supplemented with a nondestructible cyclin B (60 nM; Iwabuchi et al., 2002) to induce mitosis and incubated for another 60 min. Metaphase spindles with aligned replicated chromosomes generally formed after 60 min.

**Observation of reconstructed nuclei and individual chromosomes**

To observe reconstructed nuclei in the egg extract, 2 μl of extract containing the interphase nuclei was fixed and stained with 4% formaldehyde in 30% glycerol in EB containing 5 μg/ml Hoechst 33342 and 20 μg/ml 3,3′-dihexyloxacarbocyanine iodide (DiOC6(3); Kodak, Rochester, NY) on the glass slide and observed under a microscope (Nikon Eclipse 80i). To observe individual chromosomes in the *X. laevis* egg extract, extracts containing mitotic spindles were fixed and stained as described, with slight modifications (Funabiki and Murray, 2000). Briefly, 10 μl of egg extract containing condensed chromosomes was mixed with 40 μl of chromosome dilution buffer.
(10 mM K-HEPES, pH 7.6, 200 mM KCl, 0.5 mM MgCl₂, 0.5 mM ethylene glycol tetraacetic acid [EGTA], and 250 mM sucrose). After 15 min at room temperature, 400 µl of fix solution (4% formaldehyde, 0.1% Triton X-100, 10 mM HEPES-KOH, pH 7.5, 100 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂, and 5 mM EGTA) was added to the diluted chromosomes. After 15 min at room temperature, the fixed chromosome fractions were sedimented onto a poly-l-lysine–coated coverslip through a 1-mL cushion (30% glycerol, 10 mM HEPES-KOH, pH 7.5, 100 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂, and 5 mM EGTA) at 2500 × g for 15 min at 4°C. The coverslips were stained with 1 µg/ml Hoechst 33342 and observed by FV1000 confocal microscopy with a 100x objective.

Quantification of chromosome and nuclear sizes
To quantify chromosome lengths in C. elegans and X. laevis, we manually measured the length of a segmented line traced along DAPI- or Hoechst-positive chromosomes using ImageJ software (National Institutes of Health, Bethesda, MD). For chromosome widths, lines were drawn perpendicular to the path of sister chromatids (avoiding centromeric regions). To measure nuclear size in C. elegans, embryos expressing GFP-histone in M9 solution were visualized, and several z-sections at 2-µm steps were acquired every 30 s (Hara and Kimura, 2009). The maximum diameter of the nuclei in C. elegans embryos and X. laevis egg extract was measured using ImageJ.

Chromosome condensation dynamics in C. elegans embryos
Chromosome condensation dynamics was characterized as described previously (Maddox et al., 2006). We acquired z-sections of GFP–histone-H2B–positive nuclei at 2-µm steps and 10-s intervals from the end of the previous cell division to spindle formation. A maximum intensity projection was selected for each time point. We cut the largest square region that fit within the nucleus for further analysis and set the minimum pixel intensity in each image to 0 and the maximum to 255. After we summed five intensity distributions from 20 s before the target time point to 20 s later, we calculated the percentage of pixels with below-threshold intensity (40%) per total pixels. This threshold (40% = 102) reveals a progressive change in the shape of the fluorescence intensity distribution in one-cell-stage embryos and smc-4 (RNAi) embryos (Portier et al., 2007). For comparison, we examined these data in different developmental stages.

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REFERENCES
Albertson DG, Thomson JN (1982). The kinetochores of Caenorhabditis elegans. Chromosoma 86, 409–428.
Belmont AS, Sedat JW, Agard DA (1987). A three-dimensional approach to mitotic chromosome structure: evidence for a complex hierarchical organization. J Cell Biol 105, 77–92.
Berardino MAD (1962). The karyotype of Rana pipiens and investigation of its stability during embryonic differentiation. Dev Biol 5, 101–126.
Briggs R (1949). The influence of egg volume on the development of haploid and diploid embryos of the frog, Rana pipiens. J Exp Zool 111, 255–294.
Buongiorno-Nardelli M, Micheli G, Carri MT, Marille M (1982). A relationship between replication size and supercoiled loop domains in the eukaryotic genome. Nature 298, 100–102.
C. elegans Sequencing Consortium (1998). Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282, 2012–2018.
Carvalho A, Desai A, Oegerma K (2009). Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. Cell 137, 926–937.
Conklin EG (1912). Cell size and nuclear size. J Exp Zool 12, 1–98.
Courbet S, Gay S, Arnoult N, Wronka G, Anglana M, Brison O, Debatisse M (2008). Replication fork movement sets chromatin loop size and origin choice in mammalian cells. Nature 455, 557–560.
Cox LS (1992). DNA replication in cell-free extracts from Xenopus eggs is prevented by disrupting nuclear envelope function. J Cell Sci 101, 43–53.
Cremer T, Cremer C (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2, 292–301.
D’Angelo MA, Anderson DJ, Richard E, Hetzer MW (2006). Nuclear pores form de novo from both sides of the nuclear envelope. Science 312, 440–443.
Desai A, Deacon HW, Walczak CE, Mitchison TJ (1997). A method that allows the assembly of kinetochore components onto chromosomes condensed in clarified Xenopus egg extracts. Proc Natl Acad Sci USA 94, 12378–12383.
Finlay DR, Meier E, Bradley P, Horecka J, Forbes DJ (1991). A complex of nuclear pore proteins required for pore function. J Cell Biol 114, 169–183.
Frankhouser G (1934). Cytological studies on egg fragments of the salmonerid Trigonostigma hermanni. J Morphol 59, 407–434.
Hachet V, Kocher T, Wilm M, Mattaj IW (2004). Importin alpha associates with membranes and participates in nuclear envelope assembly in vitro. EMBO J 23, 1526–1535.
Hancock R (2012). Structure of metaphase chromosomes: a role for effects of macromolecular crowding. PLoS One 7, e36045.
Hara Y, Kimura A (2009). Cell-size-dependent spindle elongation in the Caenorhabditis elegans early embryo. Curr Biol 19, 1549–1554.
Hara Y, Kimura A (2011). Cell-size-dependent control of organelle sizes during development. Results Probl Cell Differ 53, 93–108.
Hara Y, Kimura A (2013). An allometric relationship between mitotic spindle width, spindle length, and ploidy in Caenorhabditis elegans embryos. Mol Biol Cell 24, 1411–1419.
Hasebe T, Kajita M, Iwabuchi M, Ohsumi K, Ishizuya-Oka A (2011). Thyroid hormone-regulated expression of nuclear lamin correlates with dedifferentiation of intestinal epithelial cells during Xenopus laevis metamorphosis. Dev Genes Evol 221, 926–937.
Hatou M, Hirose S, Tanaka K, Goto Y (2011). Regulation of M phase exit and S phase entry by the Cdc2 activity level during embryonic differentiation in budding yeast: the nuclear pore connection. Cell 109, 551–562.
Hediger M, Earnshaw WC (2003). Condensin Imediates chromosome condensation during pachytene before synapsis. Dev Cell 5, 323–336.
Hirano T, Kobayashi R, Hirano M (1997). Condensins, chromosome condensation protein complexes containing SCP1-SCC1, SCP3 and a Xenopus homolog of the Drosophila Borealin protein. Curr Biol 20, 353–358.
Hochest V, Kocher T, Wilm M, Mattaj IW (2004). Importin alpha associates with membranes and participates in nuclear envelope assembly in vitro. EMBO J 23, 1526–1535.
Hancock R (2012). Structure of metaphase chromosomes: a role for effects of macromolecular crowding. PLoS One 7, e36045.
Hara Y, Kimura A (2009). Cell-size-dependent spindle elongation in the Caenorhabditis elegans early embryo. Curr Biol 19, 1549–1554.
Hara Y, Kimura A (2011). Cell-size-dependent control of organelle sizes during development. Results Probl Cell Differ 53, 93–108.
Hara Y, Kimura A (2013). An allometric relationship between mitotic spindle width, spindle length, and ploidy in Caenorhabditis elegans embryos. Mol Biol Cell 24, 1411–1419.
Hasebe T, Kajita M, Iwabuchi M, Ishizuya-Oka A (2011). Thyroid hormone-regulated expression of nuclear lamin correlates with dedifferentiation of intestinal epithelial cells during Xenopus laevis metamorphosis. Dev Genes Evol 221, 199–208.
Hirano T, Mitchison TJ (1994). A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell 79, 449–458.
Hirano T, Kobayashi R, Hirano M (1997). Condensins, chromosome condensation protein complexes containing CAP1-CAP2, CAP3 and a Xenopus homolog of the Drosophila Borealin protein. Cell 89, 511–521.
Hudson DF, Vagnarelli P, Gassmann R, Earnshaw WC (2003). Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes. Dev Cell 5, 323–336.
Ishii K, Arii G, Lin C, Van Houwe G, Laemmli UK (2002). Chromatin boundaries in budding yeast: the nuclear pore connection. Cell 109, 551–562.
Iwabuchi M, Ohsumi K, Yamanaka TM, Kishimoto T (2002). Coordinated regulation of M phase exit and S phase entry by the Cdc2 activity level in the early embryonic cell cycle. Dev Biol 243, 34–43.
Iwabuchi M, Ohsumi K, Yamamoto TM, Sawada W, Kishimoto T (2000). Residual Cdc2 activity remaining at meiosis I exit is essential for meiotic M-M transition in Xenopus oocyte extracts. EMBO J 19, 4513–4523.

Jallepalli PV, Lengauer C (2001). Chromosome segregation and cancer: cutting through the mystery. Nat Rev Cancer 1, 109–117.

Kieserman EK, Heald R (2011). Mitotic chromosome size scaling in Xenopus. Cell Cycle 10, 3863–3870.

Kimura A, Onami S (2005). Computer simulations and image processing reveal length-dependent pulling force as the primary mechanism for C. elegans male pronuclear migration. Dev Cell 8, 765–775.

Ladouceur AM, Ranjan R, Maddox PS (2011). Cell size: chromosomes get slapped by a midzone ruler. Curr Biol 21, R388–R390.

Levy DL, Heald R (2010). Nuclear size is regulated by importin alpha and Ntf2 in Xenopus. Cell 143, 288–298.

Levy DL, Heald R (2012). Mechanisms of intracellular scaling. Annu Rev Cell Dev Biol 28, 113–135.

Maddox PS, Portier N, Desai A, Oegema K (2006). Molecular analysis of mitotic chromosome condensation using a quantitative time-resolved fluorescence microscopy assay. Proc Natl Acad Sci USA 103, 15097–15102.

Mains PE, Kemphues KJ, Sprunger SA, Sulston IA, Wood WB (1990). Mutations affecting the meiotic and mitotic divisions of the early Caenorhabditis elegans embryo. Genetics 126, 593–605.

Maresca TJ, Freedman BS, Heald R (2005). Histone H1 is essential for mitotic chromosome architecture and segregation in Xenopus laevis egg extracts. J Cell Biol 169, 859–869.

Meyerzon M, Gao Z, Liu J, Wu JC, Malone CJ, Starr DA (2009). Centrosome attachment to the C. elegans male pronucleus is dependent on the surface area of the nuclear envelope. Dev Biol 327, 433–446.

Michel G, Luzzatto AR, Carri MT, de Capoa A, Pelliccia F (1993). Chromosome length and DNA loop size during early embryonic development of Xenopus laevis. Chromosoma 102, 478–483.

Murray AW (1991). Cell cycle extracts. Methods Cell Biol 36, 581–605.

Nasmyth K (2002). Segregating sister genomes: the molecular biology of chromosome separation. Science 297, 559–565.

Neurohr G, Naegeli A, Titos I, Theiler D, Greber B, Diez J, Gabaldon T, Mendoza M, Barral Y (2011). A midzone-based ruler adjusts chromosome compaction to anaphase spindle length. Science 332, 465–468.

Ohsumi K, Katagiri C, Kishimoto T (1993). Chromosome condensation in Xenopus mitotic extracts without histone H1. Science 262, 2033–2035.

Ono T, Losada A, Hirano M, Myers MP, Neuwald AF, Hirano T (2003). Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell 115, 109–121.

Pfliumm MF (2002). The role of DNA replication in chromosome condensation. Bioessays 24, 411–418.

Portier N, Audhya A, Maddox PS, Green RA, Dammermann A, Desai A, Oegema K (2007). A microtubule-independent role for centrosomes and aurora a in nuclear envelope breakdown. Dev Cell 12, 515–529.

Poteryaev D, Squirrel JM, Campbell JM, White JG, Spang A (2005). Involvement of the actin cytoskeleton and homotypic membrane fusion in ER dynamics in Caenorhabditis elegans. Mol Biol Cell 16, 2139–2153.

Segbert C, Barkus R, Powers J, Strome S, Saxton WM, Bossinger O (2003). KLP-18, a Klp2 kinesin, is required for assembly of acentrosomal mitotic spindles in Caenorhabditis elegans. Mol Biol Cell 14, 4458–4469.

Seydoux G, Dunn MA (1997). Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of Caenorhabditis elegans and Drosophila melanogaster. Development 124, 2191–2201.

Shintomi K, Hirano T (2011). The relative ratio of condensin I to II determines chromosome shapes. Genes Dev 25, 1464–1469.

Sonnichsen B et al. (2005). Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. Nature 434, 462–469.

Yoshida TH, Sadaie T, Sadaie Y (1984). Somatic and meiotic chromosomes of the small free-living nematode, Caenorhabditis elegans. Proc Jpn Acad B 60, 54–57.