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The Nuclear-to-Cytoplasmic Ratio: Coupling DNA Content to Cell Size, Cell Cycle, and Biosynthetic Capacity

Shruthi Balachandra,[1] Sharanya Sarkar,[2] and Amanda A. Amodeo[1]

[1]Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, USA; email: shruthi.balachandra@dartmouth.edu, amanda.amodeo@dartmouth.edu

[2]Department of Microbiology and Immunology, Dartmouth College, Hanover, New Hampshire, USA; email: sharanya.sarkar.gr@dartmouth.edu

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Abstract

Though cell size varies between different cells and across species, the nuclear-to-cytoplasmic (N/C) ratio is largely maintained across species and within cell types. A cell maintains a relatively constant N/C ratio by coupling DNA content, nuclear size, and cell size. We explore how cells couple cell division and growth to DNA content. In some cases, cells use DNA as a molecular yardstick to control the availability of cell cycle regulators. In other cases, DNA sets a limit for biosynthetic capacity. Developmentally programmed variations in the N/C ratio for a given cell type suggest that a specific N/C ratio is required to respond to given physiological demands. Recent observations connecting decreased N/C ratios with cellular senescence indicate that maintaining the proper N/C ratio is essential for proper cellular functioning. Together, these findings suggest a causative, not simply correlative, role for the N/C ratio in regulating cell growth and cell cycle progression.

1. INTRODUCTION

Over a century ago, Oscar Hertwig coined the term karyoplasmic ratio for the nearly constant ratio of nuclear size to cell size that he discovered in protozoans under different growth conditions (66). His student, Theodor Boveri, went on to find that when the DNA content of sea urchin (*Sphaerechinus* and *Echinus*) embryos was altered during fertilization, the nuclear and cell size also changed correspondingly (13). This led to the enduring hypothesis that DNA content is a conserved regulator of cell size. Since then, numerous studies have observed consistent relationships between a cell's DNA content, nuclear size, and cell size across species from single cellular organisms, such as yeasts, to multicellular organisms, including animals and plants (7, 19, 32, 61, 111, 116, 125). In agreement with these observations, cells frequently vary their DNA content and nuclear size as their developmental and cell size needs require (45). One dramatic example is the human pathogenic fungi *Cryptococcus neoformans*, which increases its genomic content in response to host cues to form giant cells and thereby escape being engulfed by the host phagocytic cells (177). Moreover, experimental manipulations of DNA content often result in corresponding changes in cell size (42, 76, 78, 82, 83, 118, 120, 121, 163). Since nuclear volume and DNA content are usually tightly coupled, we will use the term nuclear-to-cytoplasmic (N/C) ratio to represent the ratio of both nuclear volume to cytoplasmic volume and DNA content to cytoplasmic content interchangeably, except in specific instances where they have been shown to be separable.

The observed consistency of the N/C ratio entails that increases in DNA, nuclear volumes, and cytoplasmic growth must be tightly coordinated. Since DNA is the precursor for cellular RNA and protein, it could theoretically set an upper bound for nuclear and cell size by limiting biosynthetic capacity (91, 119). At the same time, the N/C ratio appears to be an important determinant of cell cycle progression and may thereby help to couple growth with division (88, 91, 119). Since cell size, nuclear size, and DNA content are all well correlated, the causality in this relationship is unclear and likely multifaceted.

Whether the robustly reproducible N/C ratio observed in many systems is actively controlling or a passive consequence of some action of DNA on the biosynthetic activity for a given physiological role of a cell remains an open question. In this review, we explore the conserved role of the N/C ratios across species, tissues, and developmental time, including notable exceptions. We highlight the known connection between N/C ratio, biosynthetic capacity, and cell cycle progression, including the many remaining questions about this relationship. Finally, we briefly discuss the role of the N/C ratio in aging and disease.

2. EVIDENCE FOR N/C RATIO SELECTION IN GENOME SIZE VARIATION ACROSS SPECIES

Nuclear DNA content is the genome size multiplied by the number of genomes within a cell (ploidy). Total cytoplasmic volume correlates with total dry mass, although cellular density can also change during the cell cycle (108). Genome size has evolved divergently across species, and ploidy can vary between cell types within the same species, creating a natural experiment for how DNA content corresponds to cell size. Genome size varies from 160 kilobase pairs (kbp) in the bacteria *Candidatus Carsonella ruddii* (117) to 150×10^6 kbp in the understory plant *Paris japonica* (129). Genome size is a major correlate of both nuclear and cell size in a variety of taxonomic groups, including fungi, animals, and plants (65, 111, 113, 114, 126). For example, among salamanders of the genus *Batrachoseps*, genome size varies by billions of base pairs, and as genome size increases, there are corresponding changes in nuclear volume and erythrocyte area (114). A similar relationship is seen within the frogs of the genus *Xenopus*, which have had several recent

whole-genome duplications. The allotetraploid *Xenopus laevis*, which has two diploid genome equivalents, produces egg cells, blood cells, and even has a body size that is approximately twice as large as its diploid cousin, *Xenopus tropicalis* (68, 109). However, this relationship is not perfect, as *Xenopus longipes* is dodecaploid (has 12 copies of the ancestral genome) but has an egg size and body size smaller than *X. laevis* (68, 109). The correlation between genome size and cell size extends beyond an individual genus. A comparative study between 67 species of mammals demonstrated that DNA content and erythrocyte size correlate well over a wide range of body sizes, taxa, and life histories ($R^2 = 0.48$) (61). The correlation between genome size and cell size becomes even more striking as larger ranges of species are included in the comparison. Across vertebrates from fish to birds, erythrocyte cell mass corresponds to DNA content over a range of cell masses from 4.58×10^{-8} µg to 161×10^{-8} µg ($R^2 = 0.95$) (111) (**Supplemental Table 1**). Similar to that in animals, a positive correlation between genome size and cell size is evident in various species of plants. For example, guard cell length and epidermal cell size are positively correlated with genome size in angiosperms (10). A correlation between genome size and cell size does not require a nucleus, as a similar correlation, albeit with a different slope, has been observed across prokaryotes (19).

Supplemental Material >

The dependence of RNA and protein synthesis on DNA could, in principle, explain the correspondence of genome and cell size across species. If transcription were limiting for cell growth, then species with a need for larger cells might tend to evolve larger genomes with more gene copies. However, across divergent species, genome size does not correlate with the number of coding genes due to large differences in the amount of noncoding DNA (this is often called the C-value paradox) (19, 62, 157). Changes in DNA content that do not increase the transcriptional capacity of the cell, such as the accumulation of silenced transposable elements, also result in corresponding changes in cell size (17, 138). For example, the extraordinary variation in genome size found in salamanders is primarily due to the inclusion of long terminal repeat (LTR) retrotransposons in some lineages (153). Nonetheless, the correlation between genome size and cell size remains consistent across this clade, indicating that increased coding capacity is not required for cell size increase in response to changes in total DNA content (126).

Across species, increased cell size is generally correlated with a decrease in metabolic rate due to surface area constraints on nutrient uptake (135). Therefore, since species with larger genomes tend to have larger cell sizes, one would expect a negative correlation between genome size and metabolic rate. Several studies have shown a negative correlation or no correlation between genome size and organismal basal metabolic rate (56, 95, 167, 168). It has been suggested that birds and bats, which have unusually high metabolic demands due to their ability to fly, have experienced evolutionary pressure toward smaller genome sizes to allow for smaller cell sizes and thereby increased metabolic output (61, 151, 168). It is important to note that the relationship between genome size and metabolism is confounded by body size, which is strongly correlated with basal metabolic rate but only weakly correlated with cell size, as cell number can change independently of cell size across species (135).

The relationship between genome size and cell size does not extend directly to body size outside of unicellular organisms and those with tightly controlled cell numbers, such as *Caenorhabditis elegans* (72, 170). Instead, organism size is frequently an independently regulated feature. For example, mouse embryo fusions that have twice the number of normal-sized cells will undergo selective apoptosis to produce normal-sized pups (127). In many multicellular groups, changes in cell size are compensated by changes in cell number. In five genera of the free-floating aquatic duckweed plants, genome size correlates well with the nuclear size and cell size but is negatively correlated with frond (a leaf-like organ) size (67). In the newt *Eurycea bislineata*, wild populations occur as diploids, triploids, and tetraploids with a corresponding increase in cell size. However,

the triploids and tetraploids show no signs of organismal gigantism but are morphologically similar to diploids of the same age with reduced cell numbers (47).

3. THE N/C RATIO IS MAINTAINED WHEN PLOIDY IS MANIPULATED

In addition to the natural experiments provided by related species, experimentalists have created a range of organisms with variant ploidies to study how genome content affects cell size and developmental progression. Along with the sea urchins studied by Boveri (13), several species of yeasts, insects, fish, and amphibians have proved amenable to artificial ploidy manipulation under laboratory conditions (42, 76, 78, 82, 83, 118, 120, 121, 163). Fungi provide a set of excellent systems to study the N/C ratio, as they represent a diverse array of cellular morphologies and many are able to be cultured at multiple ploidies (118). The filamentous fungus *Ashbya gossypii* exists as a syncytium with multiple nuclei spaced throughout a common cytoplasm. Here, nuclear divisions are coupled to cell growth to ensure a consistent N/C ratio as the cell elongates (8, 41) (**Figure 1**). The budding yeast *Saccharomyces cerevisiae* exists naturally as a free-living haploid or diploid, with

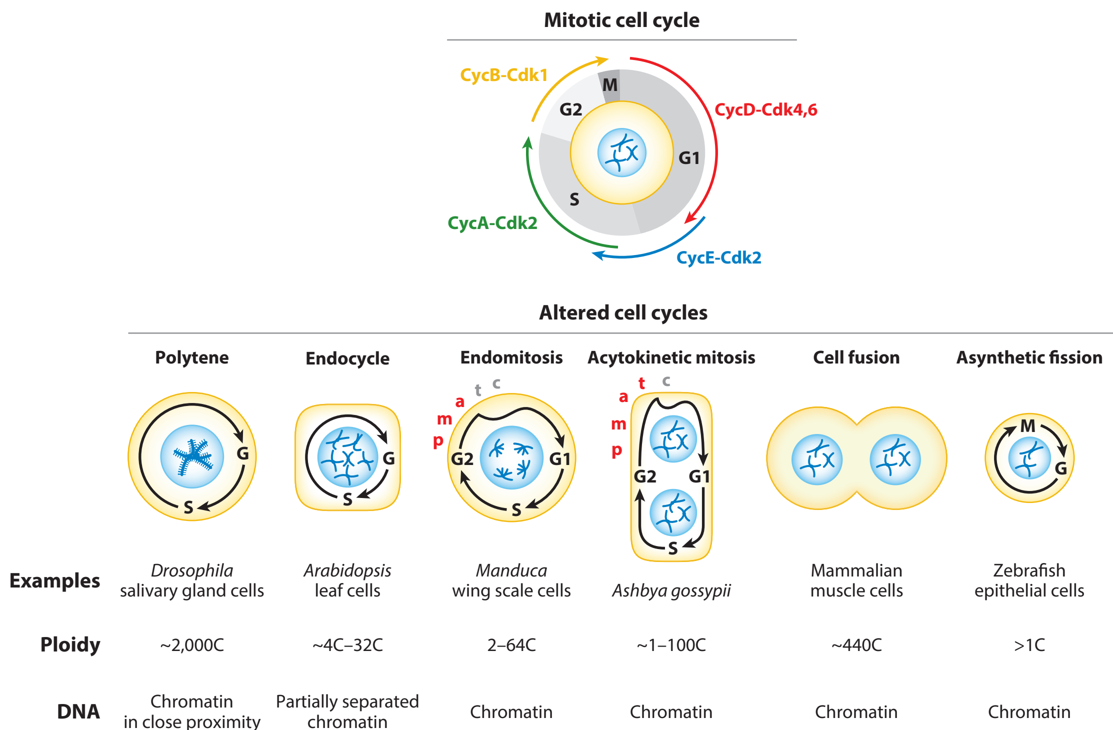

Figure 1

Altered cell cycles can change cellular DNA content. A typical mitotic cell cycle consists of two growth phases (G1 and G2) punctuated by DNA synthesis (S) and mitosis (M). Specific cyclins and cyclin-dependent kinases facilitate transitions between the cell cycle phases. Diverse altered cell cycle modes operate in different tissues. Polytene salivary gland cells of *Drosophila* larva endocycle between S and G phases, resulting in increased ploidy and cell size. The sister chromatids are held in close proximity. Endocycling *Arabidopsis thaliana* leaf cells cycle between the G and S phases, but the chromatin is dispersed. In some cell types, including tobacco hawk moth (*Manduca*) wing scales, the cells show truncated mitosis that includes prophase (p) and metaphase (m) but abort during anaphase (a), thereby omitting telophase (t) and cytokinesis (c). *Ashbya gossypii* undergoes karyokinesis during telophase but omits cytokinesis, resulting in multinucleate cells. Multinucleated cells also occur in mammalian muscle through cell fusions. In asynthetic cell fission observed in zebrafish skin cells, cell division occurs without S phase to rapidly increase the cell number but reduce DNA content.

haploids slightly more than half the size of diploids (140, 160). Furthermore, budding yeast G2 cells are generally larger than those in G1. However, there is no step function in nuclear or cell size during DNA replication; instead, both nuclear and cell volumes increase together gradually during G1 and G2. This results in small fluctuations in the ratio of DNA per nuclear volume over the course of the cell cycle (78). A more extreme version of this phenomenon is seen in the fission yeast *Schizosaccharomyces pombe*, in which nuclear volume scales with cell volume over a 35-fold range in cell size, regardless of DNA content. However, DNA content does determine the size of the cell at division and the cell and nuclear size of the subsequent daughter cells (118). These results highlight the complexity of the relationship between DNA content, nuclear size, and cell size and caution against assuming that the volume occupied by the DNA and associated proteins directly determines the nuclear volume.

Ploidy manipulations in multicellular eukaryotes ranging from insects to vertebrates have substantiated the role of the N/C ratio in regulating cell size. In *Drosophila*, zebrafish, and *Xenopus*, haploid embryos can be produced through several genetic manipulations that disrupt fertilization. In all cases, the resulting animals compensate for the changes in DNA content by increasing the number of reductive cleavage divisions before zygotic genome activation (12, 42, 76, 82, 83, 99, 120, 121, 163). These manipulations are discussed in greater detail in Section 7. Zebrafish triploids survive to adulthood, but their cell cycles are slowed, resulting in adults with fewer, larger cells similar to the naturally existing triploid newts discussed in Section 2 (163). In frogs, hybridization of *X. laevis* (allotetraploid) and *X. tropicalis* (diploid) results in viable adults with an intermediate genome, erythrocyte, and body size. In the early cell cycles immediately after fertilization, nuclear size is similar to that of the *X. laevis* egg donor, consistent with the dominance of maternal control early on. However, by later stages, the nuclear size falls below that of *X. laevis* haploids, which have less total DNA than the hybrid, indicating that genome size alone cannot explain nuclear scaling in this cross (58). These ploidy manipulation experiments demonstrate the importance of DNA content in determining final cell volume.

4. TISSUE-SPECIFIC PLOIDY CHANGES ARE ADAPTATIONS TO CELLULAR PHYSIOLOGICAL DEMANDS

In many multicellular species, a subset of developmentally specified large cell types becomes polyploid. Polyploidy can be achieved through multiple altered cell cycle types or cell fusions. During normal mitosis, cells grow in G1 phase, replicate their DNA in S phase, grow again in G2 phase, and then divide into two new cells in M phase. However, many cell types omit cell division and thereby increase the DNA content within a single cell. A subset of these modified cell cycles in which the increased DNA is found in a single nucleus is known as endoreplication or endoreduplication. Endoreplication can be accomplished in several ways (**Figure 1**).

4.1. Endocycles

Endocycles are a form of endoreplication where cells skip mitosis and progress directly between S and G phases. After endoreplication, the association of the sister chromatid varies. In endocycling *Drosophila* salivary gland cells, the sister chromatids are held together, forming polytene chromosomes. By contrast, in endocycling *Arabidopsis* leaf cells, the sister chromatids separate with increase in ploidy (137). In some cases, the entire genome is not replicated uniformly. Both *Drosophila* nurse cells and mammalian placenta giant cells are highly polyploid and grow to thousands of cubic micrometers to support the growth of other cell types during organismal reproduction (31, 57, 139). In *Drosophila* nurse cells, the genome is fully replicated during the first four endocycles, but later endocycles omit the replication of heterochromatin (34). A group of 15 nurse

cells undergo a variable number of endocycles (between seven and eleven) before dumping their cytoplasmic contents into the egg (34, 133). Nurse cells that are closer to the oocyte end up with higher ploidies and grow larger than those that are more distal. The N/C ratio is tightly maintained across the resulting ploidy gradient within the egg chamber (71). However, it is important to note that not all species with large eggs employ large polyploid nurse cells with a constant N/C ratio to support the biosynthetic activity during oogenesis (27). In many species, including many insects and amphibians, the oocyte nucleus is transcribed at extremely high levels, which often leads to a characteristic lampbrush chromosome (LBC) morphology (54, 55, 112). These oocytes are notable exceptions to standard N/C ratio scaling and provide a dramatic counterexample for the argument that the N/C ratio must be maintained due to biosynthetic constraints (discussed further in Section 8.1).

4.2. Endomitosis

Endomitosis is another altered cell cycle type resulting in increased ploidy. In endomitosis, mitotic events separate the sister chromatids but subsequently abort in anaphase, resulting in a single nucleus with two or more copies of the diploid genome. For example, the wing scales of the tobacco hawk moth, *Manduca sexta*, can undergo endomitosis zero to four times to produce cells between 2 and 64C. Across the wing axis, the nuclear volume scales with ploidy. Moreover, the size of the moth wing scale is directly proportional to the ploidy of the scale-producing cell (26). Another example of a cell size change in endomitosing cells is the platelet-generating megakaryocytes. Human megakaryocytes start out as diploid hematopoietic stem cells that begin endocycling to produce megakaryoblasts with very large nuclei and a relative lack of cytoplasm. Eventually, cytoplasmic content catches up to nuclear volume to produce a mature megakaryocyte with a ploidy up to 128C and cell diameter between 35 and 160 μm. These are the largest cells in the bone marrow (101). Mature megakaryocytes fragment into thousands of tiny anucleate platelets (37, 101, 124). In this way, the megakaryocyte switches from the largest blood cell type with the highest DNA content to the smallest cell type without any nuclear DNA.

4.3. Acytokinetic Mitosis

In acytokinetic mitosis, cells proceed through mitosis and nuclear envelope reformation but without cytokinesis, resulting in cells with multiple nuclei. This arrangement of multiple nuclei in a common cytoplasm is known as a syncytium. This form of multinucleation underlies the N/C ratio–conserving nuclear division cycles of *A. gossypii* (discussed in Section 3). Mammalian hepatocytes also undergo acytokinetic mitosis as well as endocycles to produce cells with one to two nuclei that are either diploid or tetraploid in each nucleus. In general, as the total DNA content doubles, the cell volume also doubles. There is little difference between the cell volumes of hepatocytes with two 2C nuclei and those with one 4C nucleus, indicating that total DNA content is more important in determining cell size than the nuclear number (105).

4.4. Cell Fusions

Cell fusion events are another mechanism of increasing ploidy within a diploid organism. Here, one or more diploid cells fuse to create a large multinucleate cell. Since the initial volume of the cell fusion is the sum of the two starting cells, the resulting N/C ratio is just the average of the two diploid N/C ratios. *Drosophila* epidermal cells form a syncytium through cell fusion in response to injury. The cells surrounding the wound do not divide to replace the injured cells. Rather, the surrounding cells grow larger by either fusing with their neighbors or entering endocycles. In either case, the total cell number is reduced, but the original N/C ratio is maintained (89, 97, 98).

Cell fusions are also common during striated muscle development and regeneration. In general, larger muscle cells have more nuclei. In *Drosophila*, muscle nuclei further endocycle after fusion to maintain a constant N/C ratio during cell growth (171). However, mammalian muscle nuclei have not been observed to endocycle, and the number of nuclei does not increase linearly with cytoplasmic volume, resulting in a modest decrease in N/C ratio in large muscle fibers compared to small (63).

4.5. Mitosis Without DNA Synthesis and Polyploid Mitotic Cells

In contrast to the ploidy-increasing examples discussed above, cells can also divide without replicating their DNA, resulting in reduced ploidy. Polypoid cells are not always postmitotic; for instance, the polyploid cells of *Drosophila* adult rectal papillae and the *Culex mosquito* ileum reenter mitosis to increase the cell number rapidly. The newly formed cells have reduced DNA content and smaller size than their polyploid progenitors (51, 52). Moreover, ploidy reductive divisions are not limited to initially polyploid cell types. It was recently shown that diploid zebrafish superficial epidermal cells can undergo one to two rounds of mitotic divisions without DNA replication, resulting in haploid or lower DNA content. Since these skin cells are short lived, their reduced DNA complement does not affect the tissue integrity (20). In the various examples provided above, cells adopt different strategies to meet physiological demands; however, the N/C ratio remains tightly regulated despite the wide degree of ploidy changes.

5. THE RELATIONSHIP BETWEEN CELL SIZE AND NUCLEAR SIZE DOES NOT ALWAYS DEPEND ON DNA

Although DNA content, nuclear volume, and cytoplasmic volume are all tightly correlated, studies from several species have shown that the nuclear volume is more tightly coupled to cell volume than DNA content (18, 78, 118). In yeasts and mammals, the nuclear size increases as the cell grows within a cell cycle growth phase, which necessarily has a constant DNA content (59, 78, 118, 172). In *S. cerevisiae* and *S. pombe*, nuclear and cytoplasmic volumes also remain tightly correlated when cell size is altered through changes in nutrient availability or mutation of cell cycle regulators that do not affect DNA content (78, 118). In *S. pombe* containing multiple nuclei, the nuclear growth rate depends on the nuclear density in the surrounding cytoplasmic region. Nuclei with larger cytoplasmic domains grow faster than those with a greater local nuclear density, suggesting that nucleocytoplasmic transport, not DNA content, couples nuclear size to cell size (118). The importance of nuclear transport in the coupling of nuclear to cytoplasmic volume is illustrated by the aberrant N/C volume ratios that result from nuclear envelope integrity defects (18). These findings suggest that the balance between the synthesis of macromolecules in the cytoplasm and their transport into the nucleus through the nuclear envelope drives osmotic shifts to regulate the volume of both compartments (90).

Nonetheless, DNA content and nuclear volume cannot be fully uncoupled. At the most extreme, DNA and DNA-bound proteins must set the absolute minimum for the nuclear volume. However, DNA itself makes up only a tiny fraction of total observed nuclear volumes. For instance, the estimated volume of the 1.2×10^4 kbp *S. cerevisiae* genome comprises less than 0.5% of the total nuclear volume (110). Nonetheless, DNA content sets the nuclear growth rate and final nuclear size in *Xenopus* cytoplasmic extracts (65). Moreover, in contrast to multinucleate *S. pombe* (118), in mammalian muscle fibers, nuclear volumes remain constant regardless of the size of their cytoplasmic territories (63). This difference in nuclear scaling may reflect differences between the initial and steady-state outcomes or different modes of regulation between species, and it highlights the many remaining questions in the field.

6. N/C RATIO CONTROL OF CELL CYCLE PROGRESSION IN GROWING CELLS

The remarkable conservation of the N/C ratio across species, experimental manipulations, and cell types suggests a possible role for the N/C ratio in cell size control. Cell size control in turn requires the coupling of cell cycle progression to cell growth (132). Therefore, if the N/C ratio were involved in cell size regulation, it would have to influence cell cycle progression, cell growth, or both. Most cellular contents, including proteins, increase proportionally with volume as cells grow (**Figure 2**), but DNA content, by definition, does not scale with cell size within a given cell cycle phase (24, 93, 104, 128, 178). Therefore, researchers have suggested that DNA could act as a molecular yardstick against which to measure cytoplasmic volume to control cell cycle progression (7, 32, 65, 173) (**Figure 3**). This regulatory framework would potentially explain the conservation of cell size with DNA content and, therefore, conserved N/C ratio. However, careful mechanistic dissection of the relationship between cell size, DNA content, and cell cycle progression has heretofore been limited to a small subset of model organisms and cell types. Whether the insights

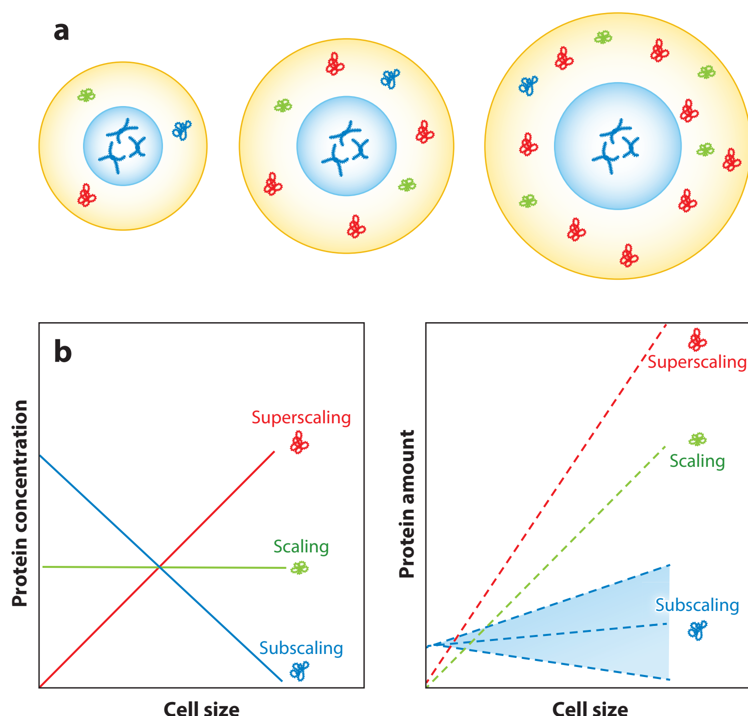

Figure 2

Macromolecule concentrations respond differently to increasing cell size. (*a*) The production of some proteins increases in proportion to growth, resulting in constant concentration over a range of cell sizes (scaling; *green*). Other proteins fail to keep pace with cell growth or are actively degraded in large cells, resulting in reduced concentration with cell growth (subscaling; *blue*). Finally, some proteins increase faster than the average growth rate or are stabilized in large cells and therefore increase in concentration at larger cell sizes (superscaling; *red*). (*b*) Scaling proteins (*green*) double in amount as volume doubles, resulting in a slope of ~1. Subscaling (*blue*) can be the result of production that does not keep pace with total growth or degradation in large cells. One important class of subscaling proteins are those that are held at a constant amount (such as histones within a cell cycle phase) rather than at constant concentrations. Superscaling proteins increase in amount faster than total growth, resulting in a slope >1.

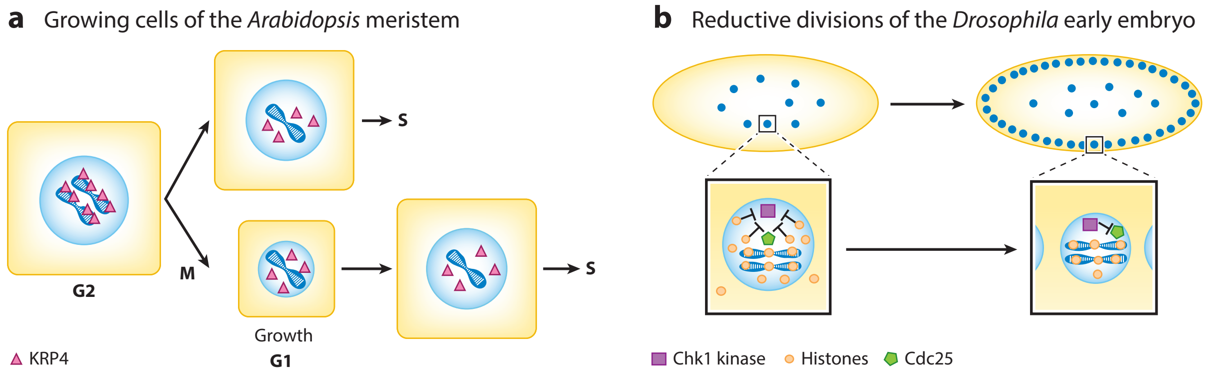

Figure 3

Examples of DNA as a yardstick to measure cell size. (*a*) *Arabidopsis* meristem cells produce the S phase inhibitor KRP4 in G1 phase and partition an equal amount into each daughter cell regardless of cell size. Equal partitioning is ensured through association with DNA, which uncouples the amount of KRP4 that each daughter cell receives from the volume of cytoplasm. In G2 phase, KRP4 disassociates from the chromatin, and its concentration is diluted by cell growth. When KRP4 concentration falls below a set concentration, it triggers cell cycle progression. Therefore, small daughter cells spend more time growing in G1 phase compared to large daughter cells (32). A similar mechanism has been proposed for Rb in mammals (178). (*b*) During pre-midblastula transition embryonic reductive divisions, the cell volume reduces with every cycle. Here, total embryo-wide histone concentration is approximately constant but becomes increasingly incorporated into DNA as the number of nuclei increases. The increasing number of nuclei results in a decrease in the histone concentration in individual nuclei. In *Drosophila*, histone H3 acts as a competitive inhibitor of the cell cycle inhibitor, Chk1. The reduction in H3 nuclear concentrations results in activation of Chk1 to ensure cell cycle slowing at the correct N/C ratio (146).

gained from these examples will be generalizable to the broad range of N/C ratio conservation observed across species remains to be seen.

In many cell types from *S. cerevisiae*, humans, and *Arabidopsis*, cell size is measured in part prior to S phase entry through the dilution of a cell cycle inhibitor that is produced in the prior cell cycle (24, 32, 136, 178). In these systems, it is essential that a constant amount of inhibitor is loaded into each daughter cell so that cell cycle progression will be triggered at the correct cell volume. If the inhibitor were loaded at a constant concentration, as is the case for most cytoplasmic components, then smaller cells would receive less inhibitor than larger cells and therefore would not delay cell cycle progression to allow for sufficient growth to correct initial variations in cell size. Since genomic DNA content does not depend on cell size, binding to chromatin would ensure size-independent, equal partitioning and thereby robust cell size control (173). This exact mechanism was recently identified for the Kip-related, cyclin D repressor KRP4 in *Arabidopsis* (32). KRP4 is produced in G2 phase and then loaded into the daughter cells in equal amounts, regardless of cell size, through its association with chromatin. KRP4 that is not associated with DNA is degraded. In the subsequent G1 phase, KRP4 is released to inhibit cell cycle progression until it is sufficiently diluted by cell growth (32) (**Figure 3*a***). A similar mechanism employing the association of the cell cycle inhibitor Rb with chromatin to ensure equal loading during mitosis has previously been proposed in mammals (178). If this mechanism proves to be generalizable, it could set a lower bound for cell size at a given DNA content.

7. THE N/C RATIO AND THE MIDBLASTULA TRANSITION

The N/C ratio, and specifically DNA content, has long been recognized to control cell cycle progression during the rapid, reductive divisions of early embryos of many species. The newly fertilized eggs of externally developing species must be large enough to produce all the cells within the

resulting offspring, yet at fertilization they only have a single diploid genome. Immediately after fertilization, most species begin a series of reductive cleavage divisions to restore more typical cell sizes and N/C ratios. In some species, these cell cycles are extremely rapid and omit growth phases, cycling directly between S and M phases. They are also transcriptionally inert, depending on maternally provided products for developmental progression. The N/C ratio directly determines the number of initial cleavage divisions, and thereby final cell size, in several model organisms, including *Drosophila*, zebrafish, and *Xenopus.* The transition from rapid, transcriptionally silenced divisions to slower, transcriptionally active cycles is known as the midblastula transition (MBT) (48, 81, 165, 176). Since both cell cycle and transcription at the MBT are directly controlled by the N/C ratio, it is an excellent system to study N/C ratio sensing.

The N/C ratio in the early embryo can be altered in a variety of ways, and all of them result in corresponding changes to the number of divisions before the MBT. In wild-type *Xenopus* embryos, smaller cells with higher N/C ratios slow their cell cycles and initiate transcription sooner than larger cells (23, 82). This is also true when cell sizes or nuclear densities are manipulated experimentally. Haploid embryos undergo one additional division before the MBT (44, 76, 99, 120, 121). Conversely, the addition of extra DNA or removal of cytoplasm results in premature cell cycle slowing even if the DNA is not from the same species (87, 120, 121). In *Drosophila*, where the nuclei reside in a common syncytial cytoplasm prior to the MBT, non-integer changes in the number of diploid genomes result in intermediate cell cycle behaviors, during which only a subset of nuclei undergo extra or fewer divisions (64, 99). The threshold for inducing extra divisions is ~70% of the wild-type diploid genome (99). Strikingly, a recent study found a nearly identical threshold for the genomic content required to induce extra divisions in the neighborhood surrounding a given nucleus in mutant conditions when nuclei are unevenly distributed (64). A similar phenomenon where more densely packed nuclei have slower cell cycles is observed in the cricket *Gryllus bimaculatus*, where nuclei are naturally unevenly distributed in the wild type (39). In *Drosophila*, even small variations in the DNA content of an embryo, such as the difference between male and female karyotypes, produce measurable effects on cell cycle duration (11). These findings indicate that a robust N/C ratio sensor regulates cell cycle progression in the early embryo.

Nuclear size also reduces during the pre-MBT divisions, even though genome size remains constant. This change is likely due to the rapid cell cycle and hyperabundance of nuclear proteins in the early embryo. Nuclei do not reach a steady-state volume before nuclear envelope breakdown. Therefore, nuclear import, not protein production, likely limits nuclear size (58, 92, 93, 115, 122). The composition of the nucleus also changes in the cycles leading up to the MBT. Some proteins become fully nuclear before others, resulting in a different nuclear proteome, depending on the N/C ratio (122, 147). This changing nuclear composition may have profound consequences for timing the MBT, as alteration to nuclear import can both advance and delay cell cycle slowing and transcriptional activation without altering the embryonic DNA content (73, 75, 76).

Exhaustion of a maternally provided cell cycle activator or transcriptional inhibitor by the exponentially increasing number of nuclei is a longstanding hypothesis for how the N/C ratio might time the MBT (120, 121). Several candidates have been identified for such a factor, including histones, deoxynucleotide triphosphates (dNTPs), and replication factors. Histones are loaded in large quantities into the oocyte prior to fertilization (2, 6, 123). Alteration of the maternal histone pool can advance or delay the timing of the MBT in several species, including flies, fish, and frogs (3–6, 22, 79, 148). In pre-MBT cell cycles, histones are rapidly partitioned into the burgeoning number of nuclei and increasing chromatin prior to the MBT and are among the first proteins to become fully nuclear (77, 122). After the cytoplasmic histone pools are exhausted, their nuclear concentrations begin to fall with the loss of free nuclear pools (145). At the same time, there are major changes in chromatin composition and structure (12, 70, 145) (**Figure 3*b***). Recent work

has uncovered a role for histones in cell cycle slowing via their direct interaction with the DNA damage kinase Chk1 (146, 147). Chk1 phosphorylation of the cell cycle activator Cdc25 is essential for cell cycle slowing at the MBT (14, 43, 49, 50, 144, 149, 179). Histone H3 is a substrate of Chk1 and is found in vast excess of Cdc25 in the early embryo (143). A fragment of H3 tail that can interact with Chk1, but not be incorporated into chromatin, is sufficient to suppress Chk1 activity and shorten the early cell cycles. Moreover, the Chk1 phosphorylation site is critical for H3's effect on the cell cycle (146). Therefore, in the early cell cycles the high nuclear concentration of H3 acts as a competitive inhibitor of Chk1 to prevent cell cycle slowing at low nuclear densities. This inhibition is relieved once a critical N/C ratio is met, which allows for cell cycle slowing at the N/C ratio.

A second model for how the N/C ratio can regulate cell cycle slowing at the MBT is based on exhaustion of components required for DNA replication, including dNTPs and replication factors. dNTPs are produced continuously throughout embryogenesis in a feedback-regulated system (152). The limit of maternally provided DNA synthesis capacity has been suggested as a possible cause of cell cycle slowing, as reducing dNTP production results in premature cell cycle slowing (38, 164). The early cell cycles are also sensitive to overexpression or reduction of the factors necessary for DNA replication (29). As embryos approach the MBT, they use fewer origins of replication (29, 141). Overexpression of replication factors without an additional supply of dNTPs leads to increased replication origin firing but also to premature phosphorylation of Chk1 (29). Chk1 in turn feeds back onto S phase entry through the degradation of critical replication factors (30). These two models, which both rely on titratable cell cycle regulators, are not mutually exclusive and, indeed, may reinforce one another to ensure robust cell cycle slowing at the MBT.

The N/C ratio also controls zygotic genome activation (11, 22, 99, 156, 179). There has been wide debate about whether the initial detection of zygotic transcripts at a specific N/C ratio requires direct N/C ratio sensing since the low amount of DNA in early cycles means that there is little template for transcription prior to the MBT. Moreover, the very short pre-MBT cell cycles do not allow much time for transcription before chromatin condensation at mitosis. Indeed, most genes can be prematurely activated simply by stopping the cell cycle, indicating that N/C ratio–dependent cell cycle slowing is the upstream regulator for the majority of zygotic genome activation (46, 49, 86, 142). There is, however, a limit to how early transcription can be initiated, which is set by translation of necessary transcription factors and chromatin remodelers (21). In addition, exogenous templates that are injected into pre-MBT embryos are rapidly silenced (5, 131). Interestingly, a select subset of transcripts appear to respond to the N/C ratio more directly, independently of cell cycle duration or transcriptional template (80, 156). How these transcripts sense the N/C ratio remains unclear but could directly couple the N/C ratio to changes in cellular composition. In addition, early transcription also appears to contribute to Chk1 activation at the MBT, indicating a feedback between transcription and cell cycle (11) and highlighting the many remaining questions concerning the interrelationship between transcription, cell cycle, and the N/C ratio in controlling early development.

8. N/C RATIO AND BIOSYNTHETIC CAPACITY

A constant N/C ratio can be achieved by coupling DNA content to either cell cycle progression (discussed in Section 6) or the cellular growth rate. Since DNA is the template for transcription, DNA content could theoretically set an upper limit for cell size if transcription is the limiting step in cell size maintenance. A rough estimate of the amount of RNA that could be produced from an average gene per minute suggests that transcription is unlikely to be limiting for most transcripts in most diploid cell sizes (1, 9, 53, 85, 166). However, it would only require a single limiting transcript to create an upper bound for cell size based on DNA content. Evidence suggests that

transcription has remarkable plasticity depending on cell state; therefore, DNA content is unlikely to be strictly limiting for cell growth in most unperturbed cells (128). Nonetheless, when cells fall below a minimum developmentally regulated N/C ratio, they experience dramatic changes in their proteomes and proliferative capacity (88, 119). Thus, under certain conditions, DNA may indeed set an upper limit for cell size and growth.

8.1. Transcription Is Not Limiting for Growth in Many Cases

Although many large cell types are polyploid (discussed in Section 4), very large cells can be produced from diploid genomes. In many species—including many insects, amphibians, and birds—the diploid oocyte nucleus (or germinal vesicle) is able to produce the bulk of the necessary transcripts for egg production on its own (54, 55, 100, 112). Chromosomes within the germinal vesicle adopt a characteristic lampbrush morphology due to the extraordinary density of RNA polymerase and nascent transcripts (16, 100). Oocytes take full advantage of these LBCs to produce large amounts of RNA, including transcribing from nearly all copies of multicopy genes as opposed to the small number used in somatic cells of the same species (100, 150). Nonetheless, there is a limit to how quickly a single diploid nucleus can support growth. Oocyte growth in species that rely on LBCs is typically slow (1–6 months or more in many species; in contrast to the ~3 days seen in *Drosophila*, which uses polyploid nurse cells) and requires high RNA stability (55). Moreover, a large fraction of the final volume (more than half in *Xenopus*) is produced by somatic cells and is taken up as yolk granules by the oocyte (40). Notably, the path to large oocytes has evolved increased DNA content multiple times as several frog species in genera outside *Xenopus* employ multinucleated oocytes during growth stages (35). Nonetheless, the existence of large, mononucleate oocytes demonstrates that under the right conditions, very large cells can be produced from limited amounts of DNA.

Even without the extraordinary biosynthetic capacity of LBCs, many diploid cells can scale transcription with cell size without running into a limit from the amount of DNA. For example, when similarly sized cells in G1 and G2 phases are compared, there is no statistical difference in their overall growth rates, indicating that biosynthetic capacity is controlled by cell volume, not limited by DNA content (15, 172). The scaling of most transcripts with cell size rather than DNA content appears to be due to increased recruitment of RNA polymerase to DNA in large cells as well as changes to messenger RNA stability (103, 154, 155). The ability to upregulate transcription in response to cell size is clearly illustrated in cell fusion experiments in which only one of the two starting cells expresses a green fluorescent protein (GFP) reporter construct. After fusion, the transcribing nucleus upregulates transcription of GFP to maintain a constant concentration as the cell suddenly increases in volume without increasing the number of copies of the GFP template DNA (128). These experiments demonstrate that in many instances DNA is not limiting for biosynthesis.

8.2. N/C Ratio Controls Cell Composition and Behavior

Although DNA is not limiting for transcription in many cases, the N/C ratio does impact a cell's proteome and proliferative capacity. In large cells with decreased N/C ratios, global transcription does not scale perfectly with cell size as RNA polymerase becomes limiting (155, 180). Moreover, for some select genes, such as yeast histones, transcription is directly proportional to total ploidy (28). In other species such as *Drosophila*, where histones are transcribed from multicopy genes, when the copy number is reduced, key developmental stages are slowed to allow for sufficient histone RNA accumulation (106). At the protein level, numerous proteins change concentration as cells increase in size, including an increase in senescence-associated proteins and a decrease in cell

cycle regulators (25, 88, 119, 174, 175). Consistent with this observation, *S. cerevisiae* that are forced to grow beyond their normal cell size show decreased proliferative capacity, and excessive growth dilutes their RNA polymerases and factors critical for transcription and ribosome biogenesis (119). Excessive cell growth also leads to senescence in mammals (36, 91, 119). These changes in protein composition are dependent on the N/C ratio in both yeast and mammalian cells, since they do not occur in large cells where DNA content is also increased (88, 119). As cells exceed the maximum cell size that can be supported by their genome size, the production of some components may not keep pace with cell growth (**Figure 2**). In this case, longer-lived constituents might comprise a larger fraction of the resultant proteome in cells with a low N/C ratio (119). This could explain the dilution of cell cycle regulators and DNA-binding proteins. If transcription of these components is limiting, it could put an upper limit on the N/C ratio that a cell can attain and thereby couple DNA content to maximum cell size.

9. N/C RATIO AND DISEASE

N/C ratios that are outside of the optimum range are detrimental to cellular functioning and may contribute to disease. As discussed in Section 8.2, when cells exceed the typical size for their cell type at a given DNA content, the balance of their proteome is disrupted, and their proliferative potential is decreased (88, 91, 119). Moreover, as cells grow larger, their surface-area-to-volume ratio is often reduced, lessening their capacity for extracellular signaling and decreasing their metabolic rate (107). The altered cellular states found in cells with very high and very low N/C ratios may contribute to the misregulation of cell cycle progression that is associated with both aging and cancers.

At the tissue level, aging can be attributed to the reduced regenerative capacity of the constituent cells, especially stem cells. Cellular senescence in turn has long been associated with increased cell size (25, 60, 102, 174). Stem cells are typically small, with higher N/C ratios than their differentiated counterparts, and aged stem cells often have lower N/C ratios due to their increased cell sizes (161, 169). Recent work has shown that increased cell size is causative for the decreased proliferative capacity in aged hematopoietic stem cells (HSCs). Preventing cell size increase in damaged or aged HSCs preserves their reconstitution potential in mouse transplantation experiments (91). At the same time, in tissue culture, large cell size is associated with the increased concentration of senescence markers and a decrease in cell cycle regulators (25, 88). This trend can be reversed when the N/C ratio is maintained by increasing ploidy (88). In budding yeast, large cell size is also associated with reproductive aging. Here, proliferative defects associated with large size can also be rescued by increasing ploidy (119). Together these data suggest that the decreased N/C ratios associated with larger cell size may contribute to senescence in aging tissues. These recent studies represent an exciting new area of future research connecting longstanding observations about the conserved N/C ratio to human disease.

Cancers are in many ways the opposite of senescence since they are marked by uncontrolled proliferation and often the reexpression of earlier embryonic developmental markers (96). Since cancer occurrence and progression are multifactorial, a full treatment of this subject is beyond the scope of this review. Nonetheless, we note that unusually large or small nuclei are common diagnostic features for specific cancer types. For instance, in small cell lung carcinoma, the nucleus makes up a larger fraction of the total cell volume than the nuclei in surrounding healthy tissues (84, 94, 159). Conversely, in B cell lymphoma, the cell volume is significantly increased with a corresponding decrease in the nuclear volume fraction (134). In many cases, the extent of these deviations is predictive of prognosis (33, 69, 162). For example, in prostate cancer cells (PC3s), tumor forming potential inversely correlates with cell size (94). Moreover, recent work has shown

that restoring more typical nuclear volumes reduces the invasive potential of a diverse array of cancer cell types with both increased and decreased nuclear sizes (158). Cancers are also highly variable in their DNA content. It is therefore tempting to imagine that changes in DNA content directly lead to changes in nuclear and cell size in cancer. However, in instances where ploidy, cell size, and nuclear size have been carefully examined, the relationship has not proved to be so simple (74). Nonetheless, polyploidy is associated with drug resistance (96, 130). Understanding the molecular relationships between the N/C ratio and cell proliferation may yield insights into the aberrant behaviors seen in human cancers.

10. CONCLUSION

Cell size varies greatly across species and tissue types. Nonetheless, the N/C ratio is broadly conserved, with large cells generally having more DNA and larger nuclei (45). The basis of this conservation is likely to be multifaceted. However, deviations from cell type–typical N/C ratios are often an indication of malfunction and have been implicated in aging and cancers, highlighting the importance of N/C ratio homeostasis in human health (158). Recent observations coupling DNA content to cell cycle regulation and demonstrating senescence-associated changes in response to reduced N/C ratios have begun to shed light on how the N/C ratio may be sensed to regulate cell growth and proliferation (25, 88, 91, 119, 174). Nonetheless, mechanistic details are sparse. The small number of examples of species-specific molecules that use DNA content to regulate cell cycle progression can hardly explain the widespread conservation of the ratio of DNA to cytoplasm (32, 178). The large-scale changes in cellular composition that have been observed when cells exceed their typical N/C ratios will likely prove to be more generalizable, but their precise cause remains to be elucidated. While some cell types, such as oocytes and the early embryo, can function at extreme N/C ratios, what allows them to escape the apparent constraints imposed on other cell types remains unclear. Careful quantitative studies in diverse organisms and cell types will be required to fully understand how DNA content, nuclear size, and cell size are coupled. A major challenge for the field going forward will be to elucidate general principals from the specific molecular mechanisms that can be probed in detail using available model systems.

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