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The power of amphibians to elucidate mechanisms of size control and scaling

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Abstract

Size is a fundamental feature of biology that affects physiology at all levels, from the organism to organs and tissues to cells and subcellular structures. How size is determined at these different levels, and how biological structures scale to fit together and function properly are important open questions. Historically, amphibian systems have been extremely valuable to describe scaling phenomena, as they occupy some of the extremes in biological size and are amenable to manipulations that alter genome and cell size. More recently, the application of biochemical, biophysical, and embryological techniques to amphibians has provided insight into the molecular mechanisms underlying scaling of subcellular structures to cell size, as well as how perturbation of normal size scaling impacts other aspects of cell and organism physiology.

Credit author summary

KEM wrote the first draft of the review, prepared Figures 1 and 2, and did the referencing. CB prepared Figure 3 and Table 1 and helped edit the manuscript. RH edited the review.

1. Introduction

Amphibians are a diverse group of vertebrates divided into 3 separate clades: Anura (tailless frogs and toads), Urodela or Caudata (tailed salamanders and newts) and Gymnophiona (legless caecilians). Anurans are the largest amphibian clade of over 6200 extant species, whereas Urodeles and Gymnophionans comprise ~740 and ~200 species, respectively [1][2]. Amphibians exhibit extreme ranges of size representing a 250-fold difference in body length from the tiny frog *Paedophryne amauensis*, the smallest known vertebrate at 7 mm long [3], to the 33 cm Goliath frog (*Conraua goliath*) [4], to the Chinese giant salamander (*Andrias davidianis*) at 1.8 m [5]. Intriguingly, amphibians also exhibit the largest variability in genome size among vertebrates, with genome sizes occupying both ends of the size spectrum. In contrast to mammalian species that exhibit relatively low variation in DNA

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content (1–4 pg/haploid nucleus) [6], amphibian genome sizes vary from 0.95–120 pg DNA/nucleus [7][8]. Urodele genomes tend to be quite large and vary up to twelve-fold across metamorphic taxa, from 10 pg/nucleus in the plethodontid salamander *Gyrinophilus porphyriticus* to 120 pg/nucleus in neotenic salamanders from the genus *Necturus*, which possess among the largest vertebrate genomes. Anuran genome sizes also vary considerably and broad variation is documented, even among species belonging to the same genus [9]. *Xenopus tropicalis* for instance contains ~1.7 pg/nucleus while *Xenopus longipes* contains ~8 pg/nucleus [10]. Among anurans, the wide variation in genome content may be attributed to interspecific hybridization and whole genome duplication leading to polyploidization, common in frogs and a driver of their evolution and speciation [11]. In contrast, large salamander genomes are frequently diploid, but possess very large chromosomes containing many repetitive DNA elements. For example, the genome of the axolotl *Ambystoma mexicanum* is roughly twenty times the size of the *X. tropicalis* genome but possesses a diploid (2N) karyotype of only 28 chromosomes [12]. Independent of whether the size or number of chromosomes differs across species, somatic cell size correlates linearly with genome size, for example in amphibian neuronal cells [13] and erythrocytes [14]. In contrast to somatic cells, amphibian egg sizes do not necessarily scale with genome size, and scaling relationships emerge following the reductional cleavage divisions that occur during early development. Amphibian eggs are quite large and variable, ranging from ~0.7 mm in the small Pipid frog *Hymenochirus boettgeri* to ~3.5 mm in the caecilian *Ichthyophis glutinosus* [15].

For decades, biologists have pondered the fundamental question: What determines cell size, and how does this impact the size of subcellular structures and the size of the organism? In this review, we discuss the power of amphibian models that have proven instrumental in exploring such questions in vertebrates, starting from basic observations that revealed fundamental features of size scaling to the identification of precise molecular mechanisms that regulate the size of organelles and subcellular structures.

2. Scaling observations made in amphibians lead to molecular questions

It has long been observed that genome size correlates strongly and linearly with cell size [16]–[18]. This principle was first noted in organisms including plants and arthropods, in which increases in genome copy number (ploidy) led to increased cell size, sometimes accompanied by an increase in organism size [19]–[21]. Amphibians present a unique opportunity to study these phenomena in vertebrates, as deviations in ploidy without immediate organism lethality occur spontaneously in nature and can be induced experimentally. Additionally, compared to other model organisms, amphibians lay abundant quantities of large eggs and produce embryos that can easily be manipulated. Some of the earliest known studies in vertebrate size scaling were performed by cell biologist and embryologist Gerhard Fankhauser in the 1930s–1940s using the small newt *Triturus viridescens*, which naturally produces haploid and triploid individuals under certain environmental conditions. By replicating these conditions in the lab, Fankhauser was able to generate embryos of different ploidies and monitor the effects of genome content on cell and organism size. He found that haploid embryos possessed smaller cells and nuclei than diploid embryos, had short and stunted body lengths, and died by metamorphosis [22]. In

contrast, triploid embryos were viable and possessed larger cells and nuclei, with a similar or only slightly increased body size relative to diploids [23]. In the 1950s–60s, similar methods applied to *Xenopus* embryos revealed comparable trends [24], [25]. Remarkably, Fankhauser noted that cell number was altered in triploids, so that embryonic tissues had fewer cells than diploids, but organ size remained constant in terms of total cell mass [26]. A similar compensatory mechanism for maintenance of organ size was also observed in triploid *Xenopus* embryos that contain cells ~1.5x normal size. In this system, tactile sense organs in the lateral line system grew normally and attained normal size through a decrease in cell number [27]. Based on these studies, it was hypothesized that one limitation on body size arises from functional constraints on tissues, which are under homeostatic pressure to maintain their characteristic sizes to preserve proper organ function.

By the 1970s and 1980s, it was noted that the linear relationship between genome size and cell size was conserved among many different species of urodeles [28] and anurans [9], with an inverse correlation between amphibian cell size and cell number [14]. At the cellular level, other studies suggested a direct relationship between amphibian genome size and duration of meiotic and mitotic cell cycles [17], [29], [30] and an inverse relationship between cell size and metabolic rate [31]–[33], thus suggesting possible molecular links between genome size, cell size, and whole organism physiology. Taken together, these observations predicted that amphibians with large genome and cell sizes would exhibit lower metabolic rates, slower growth rates, and possess relatively fewer cells. They also suggested that, if whole-body metabolic rate could be considered as the sum of the individual metabolic rates of its component cells, then an individual composed of smaller cells should have a higher metabolic rate than a similarly-sized individual comprised of larger cells.

These early observations not only established experimental frameworks for testing the effects of ploidy alteration on vertebrate size and physiology, but also outlined the fundamental principle that while genome and cell size are clearly linked, the connection between cell size and cell or whole-body metabolic rates is more complex and likely subject to regulation by other factors in addition to genome size [34]. The issue is confounded by the fact that organism size rarely scales with cell size. For instance, despite their tremendous genome and cell sizes, *Nectarus* salamanders do not grow to be very large [35]. Some of the world's smallest reported salamander species from the genus *Thorius* contain relatively large genomes at 25 pg DNA/haploid nucleus, but are characterized by tiny body lengths of less than 2 cm [36], [37]. Thus, abrupt changes in ploidy induced experimentally usually do not have dramatic effects on organism size. Rather, variation in organism size on evolutionary time scales is thought to be driven by habitat specialization in which larger or smaller animals are better adapted to distinct environmental conditions [38].

What are the molecular mechanisms that operate to alter size and scaling relationships? A number of studies in a variety of other systems including yeast, *Drosophila*, and cultured mammalian cells have identified a plethora of signaling pathways and molecular factors that influence cell size [39]–[42]. However, the size control mechanisms that operate in vivo, for example in response to changes in DNA content across species or within an individual organism, remain very poorly understood, particularly in vertebrate systems. Also unknown is the molecular origin of highly conserved metabolic scaling phenomena, such as Kleiber's

law in which a cell or organism's metabolism scales to ¾ power of its mass [43], [44]. As discussed below, amphibian systems spanning a wide range of size parameters provide a unique opportunity to explore the molecular basis of different scaling behaviors (Figure 1).

3. Using *Xenopus* to study molecular links between genome, cell, and organism size and physiology

Although amphibian body size correlates weakly with genome and cell size in most cases, an important exception exists among *Xenopus* species, which belong to the Pipidae genera of tongueless aquatic frogs. Remarkably, comparing *Xenopus laevis* and *Xenopus tropicalis*, adult body size scales with genome, egg, and somatic cell size: *X. laevis* attains larger adult body length (~10–12 cm) and mass (60–200 g), and is allotetraploid (4N = 36 chromosomes), the product of two diploid progenitor species [11]. *X. tropicalis* is smaller (~4–5 cm and 10–50 g adults) and diploid (2N = 20 chromosomes). Strikingly, whereas fertilization of *X. tropicalis* eggs with *X. laevis* sperm produces inviable hybrid embryos that die prior to gastrulation, adult interspecific hybrids between these two species can be obtained by fertilizing *X. laevis* eggs with *X. tropicalis* sperm [45], [46]. The intermediate-sized genome of these hybrids (28 chromosomes) correlates with their reduced body size relative to *X. laevis* by the tailbud stage and in the adult frog, as well as reduced erythrocyte cell size (Figure 2). What are the molecular mechanisms that drive this scaling effect? One possibility is that changes in bulk genome content influence cell size. However, by stage 21 of development, nuclear to cell size scaling in hybrids was not intermediate between *X. tropicalis* and *X. laevis*, but appeared more similar to that of haploid *X. laevis* embryos (18 chromosomes) at the same stage [47]. Therefore, it appears unlikely that bulk genome content alone is responsible for scaling, at least during embryogenesis. Many alternate hypotheses exist: for example, changes in global transcription or differential gene expression within the hybrid genome may influence scaling. To test whether paternal *X. tropicalis* genes contributed to scaling of the hybrid, a set of 12 differentially expressed *X. tropicalis* transcription factors were microinjected into *X. laevis* zygotes, and effects on embryo and cell size assessed [47]. Although this screen revealed several genes that ultimately modulated tadpole length, none appeared to do so by altering cell size, but rather appeared to affect developmental programs. Thus, the molecular basis of genome size-dependent scaling of cell size remains a mystery.

Variation across egg, genome, and cell sizes can also be used to investigate other interesting questions. For example, the *Xenopus longipes* egg is smaller than that of *Xenopus laevis*, although its genome is three times larger. When does cell size scaling emerge during embryogenesis, and what is the effect of egg size and the allocation of maternal resources on development and metabolism? *Xenopus* and other amphibian systems provide excellent experimental models to explore these questions at the molecular level. Along with the ability to examine a variety of species and generate embryos of differing ploidies, large amphibian embryo size allows for microinjection of mRNAs, Cas9 and sgRNAs, proteins, or morpholinos into the developing zygote, or into specific blastomeres for tissue-specific modification [48], [49]. Whole-embryo transcriptomic [50], proteomic [51], and metabolomic [46] approaches will also be helpful to address the basis of scaling phenomena.

4. Frog egg and embryo extracts reveal size scaling of intracellular structures during early development

To date, the most insight into molecular mechanisms that contribute to size relationships in amphibians has come from studies investigating subcellular scaling (Table 1). One example of scaling occurs during early development, when cleavage divisions without intervening growth phases result in reductional divisions of the large zygote into thousands of smaller cells. As the embryo undergoes no net change in mass, the size of subcellular structures must therefore scale concomitantly with cell size to best adapt their function. Interestingly, subcellular scaling initiates at a specific cell size threshold of approximately 150 μm, above which the size of structures such mitotic spindle size is constant [52], [53]. What initiates subcellular scaling and how is it achieved? Is it by compositional changes in cellular components as development proceeds or via physical mechanisms due to changes in cell volume or shape? Furthermore, how is the organization of subcellular structures altered in response to changes in cell size?

Some answers to these questions have emerged owing largely to experiments using cellular extracts that reconstitute formation of the nucleus as well as the spindle, the dynamic microtubule-based apparatus responsible for faithful segregation of sister chromatids during cell division. Cytoplasmic extracts from metaphase-arrested *Xenopus* eggs have been used to elucidate fundamental principles of cell cycle control and meiotic spindle assembly [54]. This system provides milliliter quantities of undiluted cytoplasm that can be arrested in specific stages of the cell cycle and can drive the spontaneous generation of cytoplasmic domains that likely reflect cytoskeletal self-organization in the egg and early embryo above the observed threshold for cell size-dependent scaling [55]. With the addition of sperm nuclei as a source of chromosomes, cell cycle-specific structures such as spindles [56][57] and interphase nuclei [58] can be formed in vitro, and processes such as DNA replication [59], chromosome condensation [60] and segregation [61]–[63] monitored. In a similar manner, synchronized *Xenopus* embryos can be used to generate cytoplasmic “embryo extracts” at specific developmental stages [64].

Volume-dependent size scaling of organelles in *Xenopus* egg and embryo extracts

Using *Xenopus* extract systems, a number of studies over the past decade have revealed that both physical and biochemical mechanisms operate in concert to adapt the size of subcellular structures to cell size during development, and that size control operates primarily through differences in the amount or composition of the cytoplasm [65]. With respect to cell geometry, cell size has been directly linked to organelle size through cytoplasmic volume, which has been suggested to be a universal regulator of organelle growth [66]–[68]. This theory is supported by experiments encapsulating *Xenopus* egg extracts inside microfluidic droplets to form cell-like compartments of different dimensions [69], [70]. Meiotic spindle size was observed to decrease in droplets of decreasing volume at a specific size threshold, similar to what occurs during early cleavage divisions in embryogenesis. Interestingly, spindle size did not differ in spherical droplets compared to compressed droplets of equal volume but differing droplet diameter, indicating that the scaling trend was dependent on cell volume rather than shape [69]. In similar experiments

examining the effects of confinement on nuclear size, increasing the concentration of sperm per unit volume of *X. laevis* egg extract was sufficient to shrink the resulting interphase nuclei [71]. Additionally, confinement of pre-assembled nuclei in engineered microchannels of decreasing size was sufficient to shrink nuclei as channel size was reduced. As with meiotic spindles, this trend was not due to physical confinement or boundary sensing of nuclei, since altering the volume, but not the aspect ratio of the channel, affected nuclear size. Taken together, these results suggest that cytoplasmic volume plays an important role in regulating the size of subcellular structures independent of a cell boundary.

In addition to volume-dependent mechanisms, specific biochemical mechanisms due to changes in cytoplasmic composition have also been shown to regulate the size of subcellular structures during development. In both cleaving *Xenopus* embryos and egg extracts encapsulated in droplets, spindle length has been observed to scale linearly with cytoplasmic volume at a threshold size, above which spindle size reaches a maximum that is uncoupled from volume [69], [67]. However, spindles and nuclei in extracts prepared from stage 8 (~4000 cell) *X. laevis* embryos were smaller than spindles from stage 3 (4 cell) embryos, even when encapsulated in similar volumes [69], [72]. Similar trends are noted in later-stage embryo extracts. Therefore, cytoplasmic factors also influence intracellular scaling [73]. One mechanism is thought to derive from limiting components as cell or compartment volume decreases, reducing the maternal supplies necessary for organelle assembly, such as the concentration of tubulin required to form a spindle of a specific size [67]. With respect to nuclear size scaling, the histone chaperone nucleoplasmin (Npm2) was recently identified by fractionation of *Xenopus* egg extracts as a key effector. Cytoplasmic levels of Npm2 decrease throughout development and microinjection of Npm2 into stage 10 embryos was sufficient to increase nuclear size [66]. Therefore, factors that become limiting as cell volumes decrease contribute to subcellular scaling[70]–[74],

Sensing the cell surface area to volume ratio coordinately mediates spindle and nuclear scaling in vivo

While limiting amounts of cytoplasmic components provide a simple physical explanation for spindle and nuclear scaling, the underlying molecular mechanisms have proven to be more complex. A major player has emerged as the nuclear transport receptor importin α, which by binding to cargoes and regulating their localization and/or activity can modulate both nuclear and spindle size [64] [72]. Interestingly, as early development proceeds in the cleaving *Xenopus* embryo, an increasing fraction of maternal importin α was found to be associated with the plasma membrane [64]. The resulting decrease in cytoplasmic importin α correlated with decreased import of cargos known to mediate nuclear growth, such as lamin B3 [72], [74]. A similar importin α-based mechanism that modulates spindle size during development was also discovered. Importin α binds and inhibits kif2a, a microtubule depolymerizing motor protein of the Kinesin-13 family, via kif2a's nuclear localization sequence (NLS). These observations led to a model in which progressive titration of importin α to the plasma membrane as cell size decreases reduces inhibition of kif2a in the cytoplasm, allowing it to bind and depolymerize spindle microtubules and decrease spindle size [64], [74]. These findings suggested that in addition to cell volume, cell surface area is a second physical parameter that could function to regulate subcellular scaling.

What is the precise molecular link between cell surface area and volume in scaling? A recent study found that importin α is subject to palmitoylation, a post-translational lipid modification that drives its association with membranes [74]. Experiments using microfluidic droplets fully recapitulated subcellular scaling in the embryo only when importin α could associate with lipids at the periphery of the droplets. Interestingly, pharmacological inhibition or upregulation of importin α palmitoylation in *Xenopus* egg extracts, embryos, and human cells was sufficient to increase or decrease the size of spindles and nuclei, respectively. These results indicate that surface area-to-volume-dependent scaling of subcellular structures mediated by importin α palmitoylation is a conserved, molecular mechanism that operates independently of developmental status in a manner linked to the physical properties of the cell (Figure 3).

5. Interspecies comparison of Pipid frogs provides molecular insight into scaling and architecture of the meiotic spindle

In addition to revealing mechanisms that contribute to the subcellular scaling that occurs during embryogenesis, in vitro systems have also been applied to study scaling across frog species with eggs and genomes of different sizes (Figure 1). Interestingly, the spindles of even closely related species differ not only in size but also in architecture and morphology. ~35 μm long meiotic spindles in *X. laevis* egg extracts possess a high microtubule density in the spindle center, with bundled microtubule arrays extending continuously from pole to pole. In contrast, smaller ~22 μm long spindles in *X. tropicalis* egg extracts have higher microtubule density at the spindle poles and more prominent astral microtubule arrays with a significant lack of microtubule density in the spindle midzone [75], [76]. The size difference is not due to the difference in the DNA content of each species spindle, as using *X. laevis* sperm nuclei as a DNA source in *X. tropicalis* extract or vice versa only exerted a minimal effect on spindle size [65]. Therefore, differences in cytoplasm components such as microtubule-associated proteins were proposed to modulate spindle size. Computational modeling of microtubule dynamics within the spindle predicted that spindle length could be determined by a balance of forces within the spindle that contribute to bipolarity, such as the microtubule-based motor proteins Eg5 and dynein that function to slide microtubules relative to one another, as well as the modulation of spindle microtubule lifetimes controlled by factors that regulate microtubule depolymerization [77], [78]. Interestingly, mixing of *X. laevis* and *X. tropicalis* extracts revealed dose-dependent effects on spindle size and morphology indicating that cytoplasmic factors are sufficient to scale subcellular structures, and that the extract provided a unique approach to identify them [65].

Using this interspecies system, it was determined that the differing sizes and architectures of the *X. laevis and X. tropicalis* spindles were largely due to differences in microtubule stability and forces within the spindle, as the computational models predicted. Microtubule severing rates were higher in *X. tropicalis* egg extracts compared to *X. laevis* due to increased activity of the microtubule severing enzyme katanin, a AAA-ATPase that destabilizes microtubules by severing them along their length, as well as by promoting kinesin-13 driven depolymerization of newly exposed microtubule ends [79], [80]. The increased activity of *X. tropicalis* katanin was found to be due to loss of an inhibitory Aurora

B kinase phosphorylation site in its catalytic p60 subunit, a serine residue at amino acid position 131, which is present in *X. laevis*. Adding a recombinant version of katanin harboring a mutation of this serine to alanine increased severing activity and decreased spindle size in *X. laevis* egg extracts [75]. A second spindle scaling factor was identified as TPX2, a microtubule-associated protein that modulates microtubule nucleation and organization [81]. The concentration of TPX2 is ~3-fold higher in *X. tropicalis* extracts compared to *X. laevis*. By increasing recruitment of the Eg5 motor to spindle poles, TPX2 was shown to locally increase microtubule density and parallel bundling, further reducing spindle size in *X. tropicalis* relative to *X. laevis* [76]. Interestingly, analysis of a third *Xenopus* species, *Xenopus borealis*, revealed meiotic spindles that possess morphological and molecular features of both *X. laevis* and *X. tropicalis*, resulting in a spindle size and architecture intermediate between the other two species [82].

Analysis of spindle scaling in a more divergent frog species provides evolutionary insights

Interspecies studies in *Xenopus* egg extracts have thus allowed for identification of precise molecular scaling mechanisms of subcellular structures. However, these studies have narrowly focused on species within the *Xenopus* genus only. How conserved are these scaling mechanisms among different species? And are there evolutionary constraints on what mechanisms a particular species can utilize? To answer these questions, a recent study used a Pipid frog distantly related to *Xenopus*. The diminutive African Dwarf Frog *Hymenochirus boettgeri* has an average body weight of 2 grams-about $1/15^{th}$ the average weight of *X. tropicalis*, and $1/45^{th}$ that of *X. laevis*. Best known for its role in the pet trade, studies of *H. boettgeri* cover a diverse array of topics including development [83], [84], regeneration [85], feeding behavior [86][87] and medicinal biochemistry [88], [89]. The *Hymenochirus* and *Xenopus* genera diverged over 110 MYA [11], [90]. Differences in morphology and body plan from *Xenopus* are noted, such as a swelling vocal sac, rough, textured skin, and webbing between the digits on the front legs [87], [91]–[93]. *H. boettgeri* eggs are smaller than those of *Xenopus*, ~700 μm in diameter compared to *X. tropicalis* and *X. laevis* at ~800 and ~1200 μm, respectively, corresponding to an overall 5-fold difference in volume. Interestingly, although egg size is smaller, *H. boettgeri* spindle length is similar to that of *X. tropicalis*, at ~23 μm.

Remarkably, despite the small size and number of eggs laid, *H. boettgeri* egg extracts could be prepared that recapitulated meiotic spindle and nuclear assembly. Addition of *H. boettgeri* egg extracts to *X. laevis* egg extracts reduced spindle length in a dose-dependent manner. These observations demonstrated not only that egg extracts from two entirely different frog genera compatible enough to assemble spindles, but also suggested that a common set of cytoplasmic factors control spindle size [94]. Surprisingly, unlike in *X. tropicalis, H. boettgeri* egg extracts were shown to possess lower TPX2 levels and reduced microtubule severing activity. Furthermore, sequence analysis revealed that *H. boettgeri* katanin p60 contains the inhibitory serine 131 residue found in *X. laevis*. Therefore, the mechanisms that scale the *X. tropicalis* spindle smaller are not conserved at the level of the genus in *H. boettgeri*. Instead, it was shown that *H. boettgeri* has evolved a distinct spindle scaling mechanism using the microtubule depolymerizing motor protein kif2a. *H. boettgeri* kif2a contains an activating phosphorylation site at serine 252 that is predicted to be

phosphorylated by Polo-like-kinase 1 (Plk1). *X. laevis* kif2a contains an isoleucine residue at this position which cannot be phosphorylated. These results showed that meiotic spindles scale smaller in *H. boettgeri* compared to *X. laevis* due to increased kif2a-mediated microtubule destabilization [94].

Why have *H. boettgeri and X. tropicalis* evolved different molecular mechanisms for generating similarly sized meiotic spindles? Do these changes provide a fitness advantage by optimizing spindle function? From the standpoint of spindle architecture, it is not surprising that differences in molecular components alter spindle morphology. Examining sequence and expression levels of known spindle size control factors across a diverse range of amphibian species may reveal whether additional mechanisms have evolved together with changes in ploidy to influence spindle architecture and mediate scaling. One particularly exciting opportunity will be to examine spindle architecture in a related species on the extreme end of genome size, such as *Xenopus longipes* [95], [96], which contains an impressive dodecaploid genome (12 n=108 chromosomes, 8 pg DNA/haploid nucleus) to see what kind of adaptations the spindle has acquired to properly segregate such a large genome.

6. Conclusion

Due to wide-ranging sizes differences at the organismal, genome, cell, and subcellular levels, combined with the ease of embryo and ploidy manipulation, amphibians stand out as a unique vertebrate system particularly well-suited to study size relationships. Amphibian embryos have provided a platform for basic observations about the contribution of genome and cell size to organism physiology. These observations coupled with current molecular tools provide a powerful approach to the ongoing study of these topics. Frog egg and embryo extracts, both of *Xenopus* and other genera, recapitulate complex physiological processes in vitro and can be used in multiple capacities to identify precise physical and molecular mechanisms governing the size of organelles and subcellular structures.

While most recent studies have primarily used *Xenopus* systems to investigate size scaling, it will be exciting to apply similar methods to salamanders, as scaling phenomena may relate to their unique physiology. Future studies could shed new light on how extremely large genome sizes in salamanders correlate with large cell sizes, low metabolic rates, and decreased rates of growth and development [16], [97]. These traits may allow them to retain juvenile or larval features in a neotenic state throughout their lifespan, and also enforce ecological constraints such as the need for some species to live in permanent aquatic habitats [98]. Salamanders additionally possess an incredible capacity for regeneration; the Mexican axolotl *Ambystoma mexicanum* regenerates entire lost limbs and large pieces of organs at any age, a feat impossible for most organisms including frogs, which lose the ability to regrow limbs after metamorphosis [99]–[101]. It is tempting to wonder whether and how large genome and cell size may play a role in this unique trait. The recent sequencing of the axolotl genome [102] will aid in the identification of molecular factors that govern size and development in these unique organisms. Thus, amphibian systems promise to continue revealing novel insights into biological size control.

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Species	Avg female body mass (g)/ length (cm)	DNA content (pg/haploid nucleus)	Chromosome #	Avg egg diameter (mm)
H. boettgeri	2/3	2.35	2n=24	0.7
X. tropicalis	30/5	1.8	2n=20	0.8
X. borealis	65/7	3.6	4n=36	1.2
X. laevis	90/10	3.2	4n=36	1.4
X. longipes	12/4	8	12n=108	1.2

Figure 1: Morphometrics and genome content of Pipid frogs.

Pipid frogs display a diverse range of egg, body, and genome sizes, from the large allotetraploid *Xenopus laevis* and *Xenopus borealis* to the tiny diploid *Hymenochirus boettgeri*. Despite the large genome of the dodecaploid *Xenopus longipes*, egg and body size are relatively small.

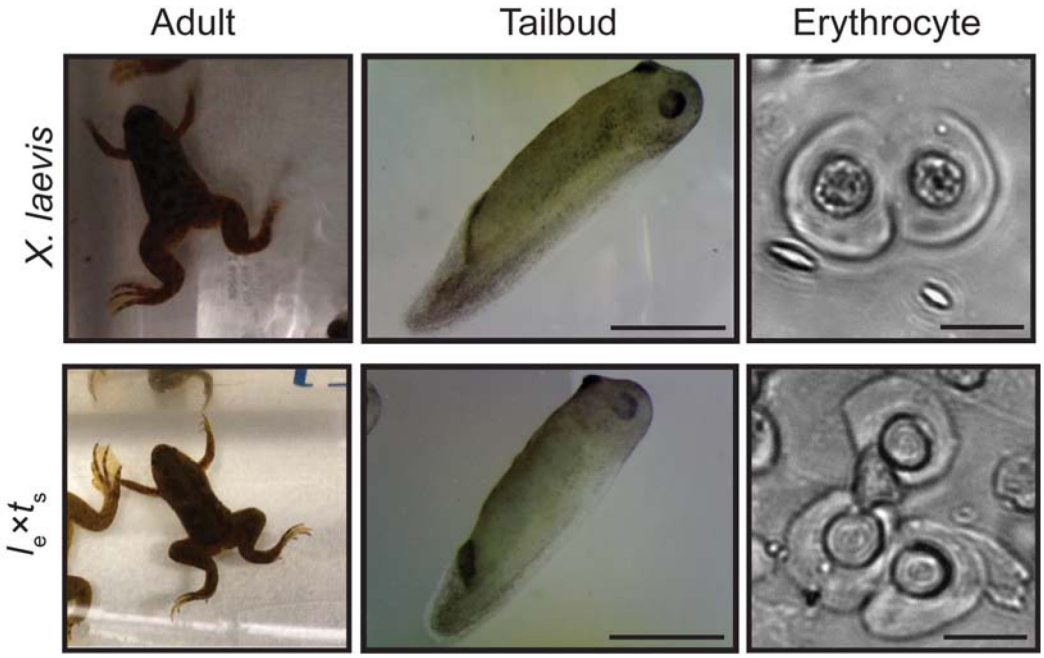

Figure 2: Scaling in *Xenopus* hybrid.

Hybrids generated by fertilizing an *X. laevis* egg with *X. tropicalis* sperm ($l_e \times t_s$) possess an intermediately-sized genome between the parental two species, as well as reduced body size compared to *X. laevis* by the tailbud stage and reduced erythrocyte cell size in adult frogs. Tailbud scale bars = 2 mm, erythrocyte scale bars = 20 μm. Adult frog images are at identical scale. Adapted from [47].

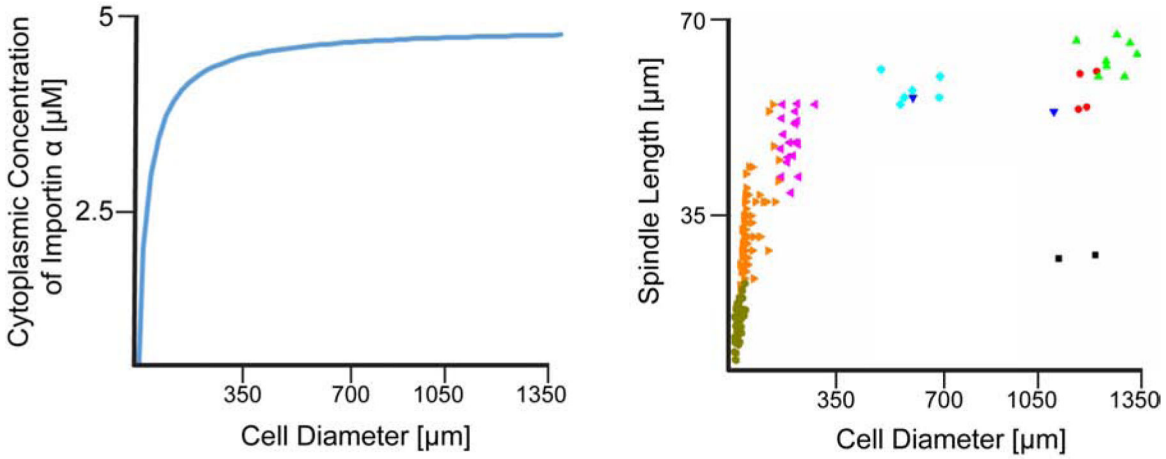

Figure 3: A molecular sensor of the surface area to volume ratio regulates organelle scaling.
The change in the cytoplasmic concentration of importin α as a function of cell size is driven by plasma membrane partitioning and shares the same concomitant logarithmic profile as nuclear and spindle scaling as a function of cell size. Adapted from [52], [74].

Table 1:
Nuclear and spindle scaling factors identified in frogs.

Proteins observed to change spindle and/or nuclear scaling when levels and/or activity are manipulated in frog egg or embryo extracts.

Protein Name	Alteration	Scaling Effect	Frog Species	References
Importin α	Decrease in levels	Smaller spindles Smaller nuclei	*X. laevis, X. tropicalis X. laevis*	[60,70] [68,69,70]
kif2a	Increase in activity Decrease in activity	Smaller spindles Larger spindles	*X. laevis, X. tropicalis* and *H. boettgeri*	[60,70,92],
TPX2	Increase in levels	Smaller spindles	*X. laevis, X. tropicalis*	[73]
katanin	Increase in activity Decrease in activity	Smaller spindles Larger spindles	*X. laevis, X. tropicalis* and *H. boettgeri*	[72,92],
XMAP215	Increase in activity	Larger spindles	*X. laevis*	[100,101]
Ntf2	Increase in levels	Smaller nuclei	*X. laevis, X. tropicalis*	[68]
Lamin B3	Increase in levels Decrease in levels	Larger nuclei Smaller nuclei	*X. laevis, X. tropicalis*	[68,70]
Npm2	Increase in levels Decrease in levels	Larger nuclei Smaller nuclei	*X. laevis*	[69]
cPKC	Increase in levels	Smaller nuclei	*X. laevis*	[102]