Evolving the Scientific Article

Keith Wollman
VP of Web Development and Operations, Cell Press
Cell Press Background


- Over next 13 years, Cell Press launches 3 journals:

- Elsevier acquires Cell Press in April 1999
  - acquired *Current Biology, Structure, Chemistry & Biology*
  - launched *Developmental Cell, Cancer Cell, Cell Metabolism, Cell Stem Cell, Cell Host & Microbe*
  - merger with *Trends* journals in 2007
  - Cell Press Society Journals:
Project Goals

- Rethink the online presentation of an article.
- Develop a hierarchical presentation of text and figures so that readers can elect to drill down through the layers of content based on their level of expertise and interest.
- Provide multiple mechanisms for conveying the core content of the article.
- Redefine the unit of publication to address the problem of burgeoning supplemental data.
- Involve the scientific community in developing and refining the ideas.
- Integrate video, sound, and animation into the scientific article.
TAM Receptors Are Pleiotropic Inhibitors of the Innate Immune Response

INTRODUCTION

RESULTS

DISCUSSION
User Feedback Stage 1
User Feedback Stage 2

Abstract, Affiliations & Tabs

- 100% rated the abstract tab as Useful.
  - 57% rated the Article Highlights as Very Useful
  
  "...I think it's a good idea ... I can easily determine what they're getting at."

- About 84% of participants found the highlighting of author affiliations useful, once they tried it out.

  "...the highlighting is kind of handy ... I think would be especially nice on a paper with a lot of authors. Would be very useful to link to personal websites."

- Tabs were considered to be a time saver allowing some people to get to sections of content faster, especially if they were already familiar with the subject area.

Suggestion:

- Move the location of the *graphical overview* to the right side of the page.

- It might be worth exploring an alternative design of displaying author names. When more than two authors are present, consider truncating the list of author names to show,
  - e.g., “First Author Name ... Last Author Name”

- It could be the case that when the author clicks on the Affiliations link that the author list could be displayed. It should be validated with additional user feedback and comparison with the current design.
User Feedback Stage 3

- Survey with > 500 respondents
  - 80% positive
  - 10% neutral
  - 10% negative
- Fantastic -- wish I thought of it!
- It looks absolutely amazing.
- It is simple and practical!
- More transparent - less scrolling!
- I like the graphical overviews.
- Quickly understand concepts.
- Finally a way to quickly distil the essence of the research.
- I like "clickable" summary figures.
- This is a much-needed change in article format.

![Survey Results Table]

*Calculations include blank responses. Total should add up to 100%.*

**Average:** 5.82  
**St Dev:** 1.53
Nuclear Size Is Regulated by Importin α and Ntf2 in *Xenopus*

**Summary**

The size of the nucleus varies among different cell types, species, and disease states, but mechanisms of nuclear size regulation are poorly understood. We investigated nuclear scaling in the pseudotetraploid frog *Xenopus laevis* and its smaller diploid relative *Xenopus tropicalis*, which contain smaller cells and nuclei. Nuclear scaling was recapitulated in vitro using egg extracts, demonstrating that titratable cytoplasmic factors determine nuclear size to a greater extent than DNA content. Nuclear import rates correlated with nuclear size, and varying the concentrations of two transport factors, importin α and Ntf2, was sufficient to account for nuclear scaling between the two species. Both factors modulated lamin B3 import, with importin α increasing overall import rates and Ntf2 reducing import based on cargo size. Importin α also contributes to nuclear size changes during early *X. laevis* development. Thus, nuclear transport mechanisms are physiological regulators of both interspecies and developmental nuclear scaling.

**Highlights**

- Cytoplasmic factors regulate nuclear size and import in *Xenopus*
- Importin α and Ntf2 can account for interspecies nuclear scaling
- Import of nuclear lamins is a crucial determinant of nuclear size
- Importin α scales nuclear size and import during *Xenopus* embryogenesis

**Authors**

Daniel L. Levy, Rebecca Heald

See Affiliations
Nuclear Size Is Regulated by Importin α and Ntf2 in *Xenopus*

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The size of the nucleus varies among different cell types, species, and disease states, but mechanisms of nuclear size regulation are poorly understood. We investigated nuclear scaling in the pseudotetraploid frog *Xenopus laevis* and its smaller diploid relative *Xenopus tropicalis*, which contains smaller cells and nuclei. Nuclear scaling was recapitulated in vitro using egg extracts, demonstrating that titratable cytoplasmic factors determine nuclear size to a greater extent than DNA content. Nuclear import rates correlated with nuclear size, and varying the concentrations of two transport factors, importin α and Ntf2, was sufficient to account for nuclear scaling between the two species. Both factors modulated lamin B3 import, with importin α increasing overall import rates and Ntf2 reducing import based on cargo size. Importin α also contributes to nuclear size changes during early *X. laevis* development. Thus, nuclear transport mechanisms are physiological regulators of both interspecies and developmental nuclear scaling.
Figure 3 Importin α2 and Ntf2 Regulate Nuclear Size and Import

(A) Nuclei were assembled in *X. tropicalis* extract, and at 40 min, importin α-E was added at the indicated concentrations in addition to GFP-NLS. At 80 min, images for at least 50 nuclei per condition were acquired with the same exposure time, and NE surface area was quantified, averaged, and normalized to the buffer control. Error bars represent standard error (SE). Scale bar, 20 μm.

(B) Experiments were performed as in (A) with a fixed concentration (0.8 μM) of added importin α-E or a mutant version lacking the importin β-binding domain (ΔIBB). Average fold change from the buffer control and SD are shown (n = 4 extracts). The ΔIBB mutant did not have a strong dominant-negative effect on import because it was added at a concentration below the endogenous importin α level.

(C) Nuclei were assembled in *X. laevis* extract mock and partially immunodepleted of importin α2 (0.5–1 μM depleted). Kinetics of nuclear assembly were similar in the two extracts. At 40 min, indicated proteins were added at 1 μM as well as GFP-NLS. At 80 min, images for at least 50 nuclei...
Results Section

Nuclear Size and Import Scale between *X. laevis* and *X. tropicalis* In Vitro

Nuclei were assembled in *X. laevis* and *X. tropicalis* egg extracts, using *X. laevis* sperm as the chromatin source. At different time points, nuclei were fixed, visualized by immunofluorescence with an antibody against the NPC (Figure 1A), and quantified for NE surface area (Figure 1B). Nuclei assembled within 30–40 min after chromatin addition and were initially similar in size in both extracts but, over time, grew larger in *X. laevis* extract compared to *X. tropicalis*. Though nuclei in these extracts do not attain a steady-state size, *X. tropicalis* nuclei never reach the size of *X. laevis* nuclei. Extracts prepared from different batches of eggs exhibited some variability, but analysis of five extracts for each species yielded an average NE expansion rate of 70 ± 9 μm²/min in *X. laevis* and 30 ± 9 μm²/min in *X. tropicalis* (mean ± SD, Figure 1B). On average, NE surface area was 2.3-fold greater in *X. laevis* extract compared to *X. tropicalis*. Similar interspecies nuclear growth differences were observed in live samples by time-lapse fluorescence microscopy visualizing nuclear import of green fluorescent protein (GFP) fused to the classical SV40 NLS (Movie S1 and Figure S1A available online). To address whether continual nuclear expansion was a peculiarity of the extract system, we measured nuclear size over time in early cleavage stage *X. laevis* embryos. Nuclei expanded in vivo at a rate comparable to that of egg extracts and failed to reach a steady-state size in arrested embryos (Figure S1B), demonstrating that extracts faithfully recapitulate nuclear dynamics in the early embryo, where cell-cycle timing sets the limit for nuclear growth.

Mixing the two extracts at different ratios produced a graded effect on nuclear size (Figure 1C), suggesting that neither extract possesses dominant activating or inhibitory factors. Addition of extract fractionated by high-speed centrifugation to preassembled nuclei revealed that cytosol had a greater effect on nuclear size than membrane (data not shown). When nuclei were formed with reduced DNA content, using *X. tropicalis* sperm with 55% the DNA of *X. laevis* sperm, only an average 12% reduction in nuclear surface area was observed (Figure 1D). Taken together, these results demonstrate that, in this system, titratable cytoplasmic factors determine nuclear size to a greater extent than the amount of nuclear DNA.
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Experimental Procedures

Nuclear Size Is Regulated by Importin α and Ntf2 in Xenopus

Extended Experimental Procedures

Immunodepletions

For partial immunodepletion of importin α2 from X. laevis extracts, a rabbit polyclonal antibody raised against the entire X. laevis importin α2 protein was used (obtained from Karsten Weis and Petr Kalab). 35 μl Protein A Dynabeads (Invitrogen) were washed with PBS-NP40 and incubated with either 45 μl anti-importin α2 rabbit serum or 45 μl rabbit IgG (0.3 mg/ml in PBS-NP40) for mock depletions at room temperature for 45 min. Unbound antibody was removed and beads were washed in PBS-NP40, then in XB (100 mM KCl, 1 mM MgCl₂, 0.1 mM CaCl₂, 50 mM sucrose, 10 mM HEPES pH7.7). 75 μl CSF-arrested X. laevis extract containing 100 μg/ml cycloheximide was added to the beads and one round of immunodepletion was carried out on ice for 45 min, gently resuspending beads every 10 min. Importin α2 and mock depleted extracts were collected and used for nuclear assembly reactions.

LB3 was immunodepleted from X. tropicalis extracts using a purified rabbit anti-LB3 antibody from Strategic Diagnostic Inc (SDI). 500 μl Protein A Dynabeads (Invitrogen) were incubated with 500 μl anti-LB3 antibody or rabbit IgG (0.2 mg/ml in PBS-NP40). Dynabeads were washed as above and split in two. 200 μl CSF-arrested X. tropicalis extract containing 100 μg/ml cycloheximide was subjected to two rounds of immunodepletion, each at room temperature for 15 min, gently resuspending beads every 5 min. By Western blot, LB3 levels were reduced by 67% ± 6% (mean ± SD, n = 4). LB3 resistant to immunodepletion may be present in an insoluble fraction.

For partial Ntf2 depletion from X. tropicalis, a purified mouse Ntf2 antibody from Abcam was used (IgG2b isotype which binds Protein A). 30 μl Protein A Dynabeads (Invitrogen) were incubated with 30 μl anti-Ntf2 antibody or mouse IgG (0.3 mg/ml in PBS-NP40). 60 μl CSF-arrested X. tropicalis extract supplemented with cycloheximide was subjected to one round of immunodepletion at room temperature for 15 min, gently resuspending beads every 5 min.

Recombinant Proteins

The bacterial expression construct for GST-GFP-NLS was from Mary Dasso. The NLS sequence is the classical nuclear import signal from SV40. A GST-NLS expression construct was made by cloning the SV40 NLS into pGEX-4T-1 (GE Healthcare). These GST recombinant proteins were overexpressed by IPTG induction in BL21(DE3)RIL+E. coli
**Nuclear Size Is Regulated by Importin α and Ntf2 in Xenopus**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
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Review Article

Biological Applications of Protein Splicing

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Summary
Protein splicing is a naturally occurring process in which a protein editor, called an intein, performs a molecular disappearing act by cutting itself out of a host protein in a traceless manner. In the two decades since its discovery, protein splicing has been harnessed for the development of several protein-engineering methods. Collectively, these technologies help bridge the fields of chemistry and biology, allowing hitherto impossible manipulations of protein covalent structure. These tools and their application are the subject of this Primer.
Enhanced SnapShots 1
Contributors

- User Centered Design
- Director of Disruptive Technology
- XML experts
- Cell Press scientific editors
- Cell Press illustrators and designers
- Cell Press readers and authors
- Developers
- Supplier Partners – DJS and TnQ
Timeline

- Beta version launched in July 2009
- Launched for research articles in a production environment on cell.com in January 2010
- Apply format to review articles and introduce Reflect in summer 2010. Respond to user feedback on production version.
- More end user testing
Continuing Initiatives within Elsevier

Prototypes being developed in different domains:

- Parasitology and Tropical Diseases
- Electro-Chemistry
- Materials Science
- Psychology and Cognitive Science