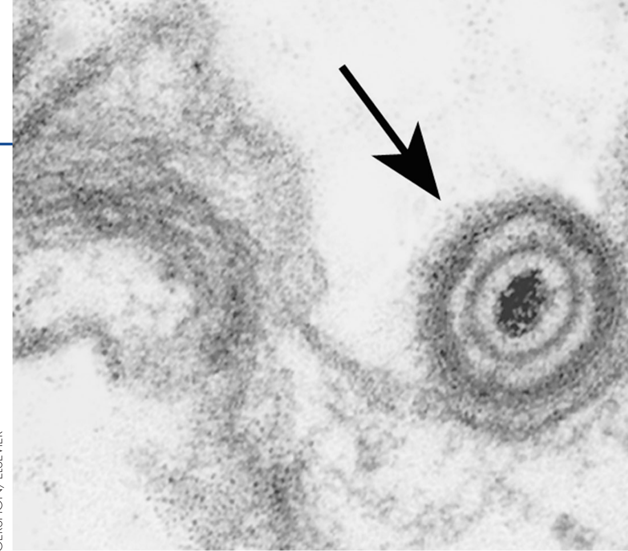
Research Roundup

Saving varicella from suicide

Varicella zoster virus (VZV) causes both varicella (chickenpox) and zoster (shingles). VZV spreads throughout the body, but it does so slowly, and virus only emerges extracellularly in the skin where it can spread to other hosts. That behavior is now explained by Zhenglun Zhu (Harvard Medical School, Boston, MA), Jason Chen, Anne Gershon, and Michael Gershon (Columbia University, New York, NY). They show that in most tissues VZV is diverted to late endosomes for destruction, but in the most superficial epidermal cells that cellular trafficking pathway is turned off, resulting in secretion of VZV.



GERSHON/ELSEVIER

Intact VZV is released when trafficking to late endosomes is shut off in skin.

Trafficking from the Golgi system to the late endosome is mediated by the cation-independent mannose 6-phosphate receptor (MPR^{ci}). MPR^{ci} has been implicated in both VZV entry (mannose 6-phosphate can block infection by free virus) and viral sorting (mature virus ends up in late endosomes). "The idea was around, based on indirect evidence, but it needed to be shown," says Michael Gershon.

The team tested the theory of MPR^{ci} involvement by getting rid of MPR^{ci} with antisense and siRNA. The resulting cells could not be infected with cell-free virus. But if infected by other means they did not send mature virus to late endosomes for destruction but instead secreted intact virus. The latter phenotype was mimicked in the outer skin layer of biopsies from patients, which showed no MPR^{ci} expression and high virus secretion.

Thus VZV uses the MPR^{ci} as a coreceptor for entry, and then uses it to direct intracellular mature virus particles to the degradative late endosome. This slows viral proliferation and prevents host death: the only remaining viral particles are immature nucleocapsids that spread via slow cell fusion events.

The skin is a different proposition. MPR^{ci} and lysosomal sorting are turned off as the outermost skin cells devote their last living hours to the secretion of waterproofing ceramides. The VZV particles follow the remaining trafficking pathway—constitutive secretion to the cell surface—where they are released as infectious virions. The cell-free virus can then either infect and establish latency in exposed sensory nerves or spread to other hosts. JCB

Reference: Chen, J.J., et al. 2004. *Cell.* 119:915–926.

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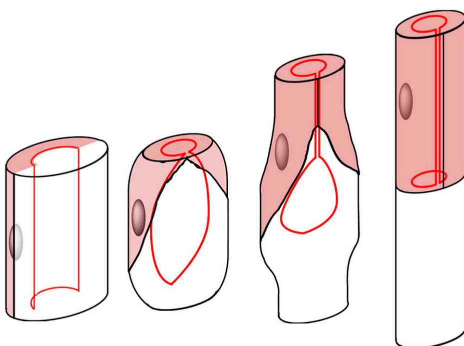
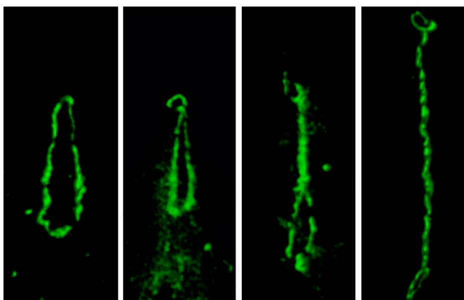
Thinner tubes

A switch that controls cell intercalation determines the diameter of fly embryo tracheal tubes, according to Carlos Ribeiro, Marc Neumann, and Markus Affolter (Biozentrum, Universität Basel, Switzerland). Other tube-like structures such as blood vessels may use similar sculpting techniques.





AFFOLTER/ELSEVIER

Paired cells intercalate (top) as visualized via labeling of adherens junctions (bottom).

The direction of tracheal tube extension is defined by migration of interconnected precursors toward an FGF signal. But tube morphology is also affected by Dpp and Wnt signaling. The Swiss team wanted to know what cellular processes were being controlled by Dpp and Wnt.

They made movies of living fly embryos with labeled adherens junctions (AJs). The label showed cells that were initially paired, side-by-side, in wider tubes. But gradually individual cells reached around at one end until they made contact with themselves, and then started replacing their intercellular AJs with autocellular AJs. This squeezing process continued until the tube was longer but only one cell circumference around.

Expression of the Wnt target Spalt, a transcription factor, was necessary and sufficient to prevent this intercalation process in tubes that remained larger. Dpp's normal function, by contrast, is to repress Spalt expression and thus allow intercalation.

Cells formed autocellular AJs as long as they had a Spalt-free neighbor. So perhaps it is contraction by the neighbor rather than outgrowth by the autocellular AJ-forming cell that is the driving force. Affolter plans to label old and new AJs to help him determine which cell is controlling the process and whether cell sliding, rolling, or detachment are involved in the reassortment of junctional contacts. JCB

Reference: Ribeiro, C., et al. 2004. *Curr. Biol.* 14:2197–2207.

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Pore before seal

A checkpoint coordinates assembly of nuclear pore complexes (NPCs) with formation of the nuclear envelope (NE), according to Wolfram Antonin, Iain Mattaj, and colleagues (EMBL, Heidelberg, Germany).

NPC assembly begins with the binding of the Nup107-160 complex of nucleoporins to chromatin. The EMBL group looked at two other nucleoporins, gp210 and pom121. These nucleoporins span the nuclear membrane and thus might link NPC assembly on the two sides of the NE as it reforms after mitosis.

Although gp210 was not needed for NPC assembly or NE formation in frog egg extracts, pom121 was essential for both processes. After pom121 depletion, vesicles docked onto chromatin but did not fuse with each other to form a complete NE.

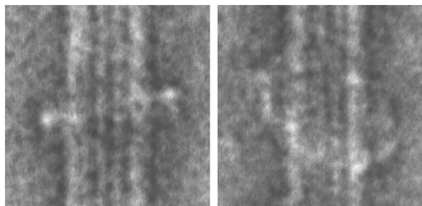
But if Nup107-160 was also depleted from the extracts, NE formation proceeded normally, albeit without insertion of NPCs. Thus pom121 is not absolutely required for NE formation, but pom121's absence can block NE membrane fusion events if Nup107-160 is present.

The assembly of Nup107-160 on chromatin happens early and does not require membranes. But prior NE formation does block the access of Nup107-160 to chromatin and thus its assembly. It may be essential to ensure that this initial assembly and its link to a transmembrane component and the outside world are complete before taking the step of sealing off the nucleus as a separate compartment. **JCB**

Reference: Antonin, W., et al. 2005. *Mol. Cell.* 17:83–92.

Kinetochores hold on with a ring

A complex that links a budding yeast kinetochore to a microtubule (MT) forms a ring around the MT, based on structures from two groups. The ring may help kinetochores to keep hold of an MT, even as the MT shrinks towards the spindle pole during anaphase.



DASH forms a collar around microtubules.

Stephen Harrison (Harvard Medical School, Boston, MA) and Peter Sorger (MIT, Cambridge, MA) are hoping to analyze the structure of the 60 or more yeast kinetochore proteins one subcomplex at a time. The current success with the 10-protein DASH complex, Harrison says, "came about on a dare to see if [first author JJ Miranda] could coexpress the whole thing in *E. coli*." Happily, the bold experiment worked, the purified complex bound MTs, and the electron micrographs clearly showed rings of DASH complex encircling an MT.

The ring structure immediately suggests a mechanistic possibility. "A major way in which evolution has made entities processive is by making rings," says Harrison. In this case, the outward splaying of MT protofilaments as the end of an MT falls apart should keep the ring on the intact section of the MT. This would effectively translocate the ring and thus the attached kinetochore towards the pole-attached end of the shrinking MT. Indeed, Stefan Westermann and Georjana Barnes (University of California, Berkeley, CA) used Miranda's construct to not only come up with a similar structure, but also to gain evidence for mobility of DASH rings along MTs. **JCB**

References: Miranda, J.L., et al. 2005. *Nat. Struc. Mol. Biol.* doi:10.1038/nsmb896.
Westermann, S., et al. 2005. *Mol. Cell.* 17:277–290.

Development is easy

Metazoan cell lineages can be collapsed to a set of rules that is surprisingly simple, according to Ricardo Azevedo (University of Houston, Houston, TX), Armand Leroi (Imperial College, Ascot, UK), and colleagues. Decoding the biochemical basis of the rules should provide a complete recipe book for development.

Azevedo brought three influences to the study: his background in evolutionary biology; his adopted field of worm biology; and computer science from collaborators. When he joined Leroi's worm lab he "was immediately struck by the lineage data," he says. "But there were more data to be extracted."

As in phylogenetic trees, there were repeating patterns. A handful had been noted by others, but no systematic study had been undertaken. This is where a simple computer algorithm helped out.

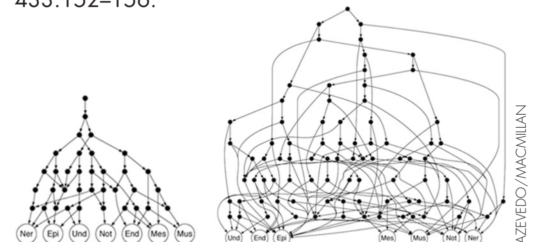
"For any particular cell division we can find another that forms the same pattern," Azevedo explains. "Then we collapse [those into one rule] until we can't do it any more because there is no more redundancy. That gives us the minimal number of states that is required."

In silico evolution of the simplified rule sets did not yield much further simplification, as long as the final distribution of cell types was constrained. Much simpler rule sets could be invented but only by going via intermediate states that had very different cell type distributions.

Azevedo thinks that simplicity arises as evolution strives to minimize the time and genetic information necessary for development. The tendency of evolution to modify what already exists, rather than invent new systems for each new function, may also favor simplicity.

In silico genetic circuits that generate lineages are allowing Azevedo to study the rules behind lineage formation. Eventually he hopes to understand what biochemical combinations of regulators form the basis for each of his rules, but that may have to wait for next-generation expression chips that can analyze individual cells. **JCB**

Reference: Azevedo, R.B.R., et al. 2005. *Nature.* 433:152–156.



An actual lineage (left) is much simpler than a randomly generated lineage (right).

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