the ability of *D. pachea* to survive on lathosterol alone, are surprising.

Ultimately, it was the loss of ability to use cholesterol, rather than the use of lathosterol or alkaloid tolerance, that turned *D. pachea* into an obligate specialist. Lang *et al.* [3] have traced this change to at least four amino acid substitutions in the *nvd* gene. Reintroducing the ancestral amino acids into the *D. pachea* Nvd enzyme restores its ability to metabolize both precursors [3]. If the ancestral species could use both substrates, why did *D. pachea* give up cholesterol, cutting it off from every available host plant other than senita? Was this sacrifice driven by positive selection, or does it simply reflect a mutational decay of an unused function?

Transgenic experiments suggest that the same mutations that made Nvd unable to metabolize cholesterol may increase *D. melanogaster* fitness on lathosterol [3]. DNA sequence variation around the *nvd* locus in *D. pachea* shows a signature consistent with past episodes of positive selection, although it is not clear whether the target of selection was *nvd* itself or a nearby gene [3]. It is possible that changes in Nvd occurred after *D. pachea* was already restricted to senita by other, perhaps behavioral, mechanisms. A more intriguing possibility is that these mutations were fixed as a result of a fitness trade-off. Similar to other cactus-feeding species [7], *D. pachea* must have gone through a phase in its evolutionary history where it fed on multiple cactus species. Phylogeographic evidence suggests that this phase may have occurred in Southeastern Mexico, where the range of available host plants was far wider than in the present-day Sonoran Desert [5,7]. Theoretical models indicate that ecological specialization can evolve as long as fitness on different hosts has less than perfect correlation. In other words, selection will favor specialization if alleles that are positively selected on one host are less strongly positively selected, or neutral, on other hosts [20]. Even though ancestral Nvd could metabolize either cholesterol or lathosterol, mutations that conferred fitness benefits on lathosterol-producing cacti could be fixed during a multi-host evolutionary transition even if they were neutral or weakly deleterious on other plants. This change would pre-adapt *D. pachea* to the depauperate ecosystem of the Sonoran desert.

The work of Lang *et al.* [3] provides an excellent example of how molecular insights derived from research in genetic model systems can be brought to bear on long-standing ecological and evolutionary questions. As technological developments increase our ability to interrogate the genomes of non-model taxa, the integration of comparative and functional approaches will help elucidate the deepest mechanisms of evolution.

References

1. Futuyma, D., and Moreno, G. (1988). The evolution of ecological specialization. Annu. Rev. Ecol. Systm *19*, 207–233.
2. Jaenike, J. (1990). Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst *21*, 243–273.
3. Lang, M., Murat, S., Clark, A.G., Gouppil, G., Blais, C., Matzkin, L.M., Guittard, E., Yoshiyama-Yanagawa, T., Kataoka, H., Niwa, R., *et al*. (2012). Mutations in the neverland gene turned *Drosophila pachea* into an obligate specialist species. Science *337*, 1658–1661.
4. Markow, T.A., and O'Grady, P.M. (2005). Evolutionary genetics of reproductive behavior in Drosophila: connecting the dots. Annu. Rev. Genet. *39*, 263–291.
5. Heed, W.B., and Mangan, R.L. (1986). Community ecology of the Sonoran desert Drosophila. In The genetics and Biology of Drosophila, *Volume 3e*, M. Ashburner, H.L. Carson, and J.N. Thompson, eds. (London: Academic Press), pp. 311–345.
6. Fogleman, J.C., and Danielson, P.B. (2001). Chemical interactions in the cactus-microorganism-Drosophila model system of the Sonoran desert. Am. Zool. *41*, 877–889.
7. Oliveira, D.C., Almeida, F.C., O'Grady, P.M., Armella, M.A., DeSalle, R., and Etges, W.J. (2012). Monophyly, divergence times, and evolution of host plant use inferred from a revised phylogeny of the Drosophila repleta species group. Mol. Phylogenet. Evol. *64*, 533–544.
8. Fogleman, J.C., Heed, W.B., and Kircher, H.W. (1982). Drosophila mettleri and senita cactus: fitness measurements and their ecological significance. Comp. Biochem. Physiol. A Mol. Integr. Physiol. *71*, 413–417.
9. Kircher, H.W., Heed, W.B., Russell, J.S., and Grove, J. (1967). Senita cactus alkaloids: their significance to Sonoran desert Drosophila ecology. J. Insect Physiol. *13*, 1869–1874.
10. Niwa, R., and Niwa, Y.S. (2011). The fruit fly Drosophila melanogaster as a model system to study cholesterol metabolism and homeostasis. Cholesterol *2011*, 176802.
11. Campbell, C.E., and Kircher, H.W. (1980). Senita cactus: a plant with interrupted sterol biosynthetic pathways. Phytochemistry *19*, 2777–2779.
12. Etges, W.J. (2007). In memoriam. William B. Heed 1926–2007. Evolution *61*, 2739–2742.
13. Heed, W.B., and Kircher, H.W. (1965). Unique sterol in the ecology and nutrition of Drosophila pachea. Science *149*, 758–761.
14. Yoshiyama, T., Namiki, T., Mita, K., Kataoka, H., and Niwa, R. (2006). Neverland is an evolutionally conserved Rieske-domain protein that is essential for ecdysone synthesis and insect growth. Development *133*, 2565–2574.
15. Legal, L., Chappe, B., and Jallon, J.M. (1994). Molecular-basis of Morinda-Citrifolia (L) - toxicity on Drosophila. J Chem. Ecol. *20*, 1931–1943.
16. Stump, A.D., Jablonski, S.E., Bouton, L., and Wilder, J.A. (2011). Distribution and mechanism of alpha-amanitin tolerance in mycophagous Drosophila (Diptera: Drosophilidae). Environ. Entomol. *40*, 1604–1612.
17. Hall, B.G. (1982). Evolution of a regulated operon in the laboratory. Genetics *101*, 335–344.
18. Nasvall, J., Sun, L., Roth, J.R., and Andersson, D.I. (2012). Real-time evolution of new genes by innovation, amplification, and divergence. Science *338*, 384–387.
19. Copley, S.D. (2009). Evolution of efficient pathways for degradation of anthropogenic chemicals. Nat. Chem. Biol. *5*, 559–566.
20. Fry, J.D. (1996). The evolution of host specialization: are trade-offs overrated? Am. Nat. *148*(*Supplement*), S84–S107.

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Nuclear Transport: Beginning to Gel?

The massive nuclear pore complex mediates nucleocytoplasmic traffic ranging from a single histone to a viral genome. To date, dissecting mechanism has been more an exercise in prediction than biochemical certainty. A recent study combines recombinant proteins with nuclei reconstituted *in vitro* to test predictions in a startlingly productive manner.

Maureen A. Powers[1] and Douglass J. Forbes[2]

Nuclear Pore Complexes (NPCs), approximately 30 times the size of a ribosome, are built at sites of fusion between the inner and outer nuclear membranes. NPCs, together with soluble nuclear transport receptors (NTRs), are responsible for virtually all selective passage of macromolecules between the nucleus and cytoplasm. In a recent publication in *Cell* [1], the Gorlich lab proposes that

Nup98, a nucleoporin that has long captured attention for its involvement in human leukemia [2], plays a critical role in the mechanism of nuclear transport.

NPCs must conduct receptor-mediated transport of cargo as immense as a viral replication complex (~40 nm), while simultaneously posing a barrier to the passage of large non-cargo molecules (>5 nm or ~40 kd) and permitting the diffusion of smaller molecules [1]. The exact nature of this critical permeability barrier and the mechanism by which receptors penetrate the barrier have remained uncertain. Multiple models have been proposed and hotly debated but, to date, biological evidence in favor of any one model has been limited. Hulsmann *et al.* [1] now make a substantial advance in support of their selective phase/hydrogel model, validating its predictions through functional tests of normal and modified pores. This study makes use of the powerful *Xenopus* egg extract system in which chromatin, membrane vesicles and cytosol are combined to reconstitute nuclei capable of functions from nuclear transport to DNA replication (see [3] and references therein).

Gates, Virtual and Otherwise

To appreciate the advance of Hulsmann *et al.* [1], a little knowledge of the players and competing models is needed. From yeast to mammals, the NPC is composed of ~30 nucleoporin proteins or Nups. Most are structural, but about one third are FG-repeat Nups. These contain unstructured repeat domains [4,5], with multiple FG (phenylalanine-glycine) motifs separated by loosely conserved spacers. FG Nups are critical for both the permeability barrier and translocation of receptor/cargo complexes through the pore.

FG domains fall into two classes: those that are *cohesive* and thus can participate in intermolecular interactions among themselves, and those that are *non-cohesive* (Figure 1A) [6]. In general, cohesive domains are enriched in GLFG (glycine-leucine-phenylalanine-glycine) or FxFG repeat motifs, separated by uncharged spacers high in serine and threonine. In contrast, non-cohesive domains consist of FG or FxFG motifs and highly charged spacers. Both classes interact with NTRs (>20 in vertebrates) and NTR/cargo complexes.

For over a decade, controversy has raged over how FG domains are organized within the NPC and how they function in both transport and the permeability barrier. Increasingly, models focus on FG domain cohesiveness. The early 'virtual gate' model did not include distinct roles for cohesive and non-cohesive FG domains. FG domains were proposed to act as flexible filaments that occupy the entrances on either face of the NPC [7]. By repelling large non-cargo macromolecules, these FG filaments create an entropic barrier to entry (Figure 1B). NTRs overcome the entropic energy barrier by binding to the FG domains, enabling the passage of receptor/cargo complexes [7]. The related 'polymer brush' model postulates that *certain* FG domains collapse upon receptor binding, clearing the way for receptor/cargo passage [8]. A 'reduction of dimensionality' model arranges the FG motifs peripherally with the spacers occupying the center of the pore and forming the barrier to large non-cargo molecules. Transport receptors in this model undergo a 2D random walk along the peripheral channel of FG repeats (Figure 1B) [9].

The 'selective phase/hydrogel' model, proposed by the Gorlich group a decade ago [10], posits that cohesive FG domains associate in the center of the NPC to form a sieve-like gel which acts as a barrier to free diffusion of large, but not small, non-cargo molecules (Figure 1B) [10,11]. Transport receptors bind to the FG motifs in the cohesive domain, leading to transient local disruption of the gel. This allows the receptors to move through the gel without disrupting the overall barrier (Figure 1C). In this model, no specific role is proposed for the non-cohesive FG Nups. Finally, the 'forest' model combines several elements of the others in a novel arrangement of FG domains: cohesive FG domains occupy the central NPC channel, as in the selective phase/hydrogel model, while non-cohesive FG domains form an entropic barrier in a concentric outer channel (Figure 1B) [5,12]. Other interesting models derived more from kinetic or microscopic analysis are not covered here.

Nuclear Reconstitution Provides Evidence For the Selective Phase Model

Gorlich and colleagues previously showed that highly concentrated, purified FG domains form gels *in vitro* (see for example [11]). These visually arresting, macroscopic gels, while controversial, showed certain NPC-like properties. Transport receptors were able to penetrate the gels, whereas large, non-receptor bound proteins were excluded.

Hulsmann *et al.* [1], using reconstituted nuclei and modified nuclear pores, now provide functional evidence to support their selective phase model. Depletion of *Xenopus* nuclear reconstitution extracts using the lectin wheat germ agglutinin (WGA) is known to remove a set of glycosylated nucleoporins (Nup62, Nup98, and Nup214) along with their binding partners, and to lead to formation of nuclei incapable of nuclear import, in essence 'biochemically mutant' nuclei [3]. Import is restored by re-addition of the full set of glycosylated nucleoporins [3]. Hulsmann *et al.* find that both reasonable import and the ability to exclude large non-cargo molecules can be restored by the sole addition of one recombinant nucleoporin: Nup98. Although the extent of import was not quantified, presumably Nup98 restored WGA-depleted nuclei only to the very reduced import level typical of nuclei lacking Nup62 [1,13]. Most significantly, the FG/GLFG repeat domain of Nup98 was essential for restoration of function (Figure 1A).

Interestingly, Nup98 is the only vertebrate nucleoporin with numerous GLFG repeat motifs. Additionally, orthologous yeast GLFG domains are the most cohesive when tested *in vitro* [6] and the presence of one or more is essential for yeast viability [14]. The amino-terminal half of Nup98 contains GLFG and FG repeats, while the carboxyl terminus provides targeting to the NPC (Figure 1A) [1]. Together with its position at the center of the NPC [15], Nup98 is thus an ideal candidate for a vertebrate hydrogel permeability barrier.

Two illustrative experiments by Hulsmann *et al.* [1] support the importance of a cohesive domain for import and the permeability barrier. First, when the FG/GLFG domains from the cohesive yeast Nup98 orthologs, ScNup100p and ScNup116p [6], were

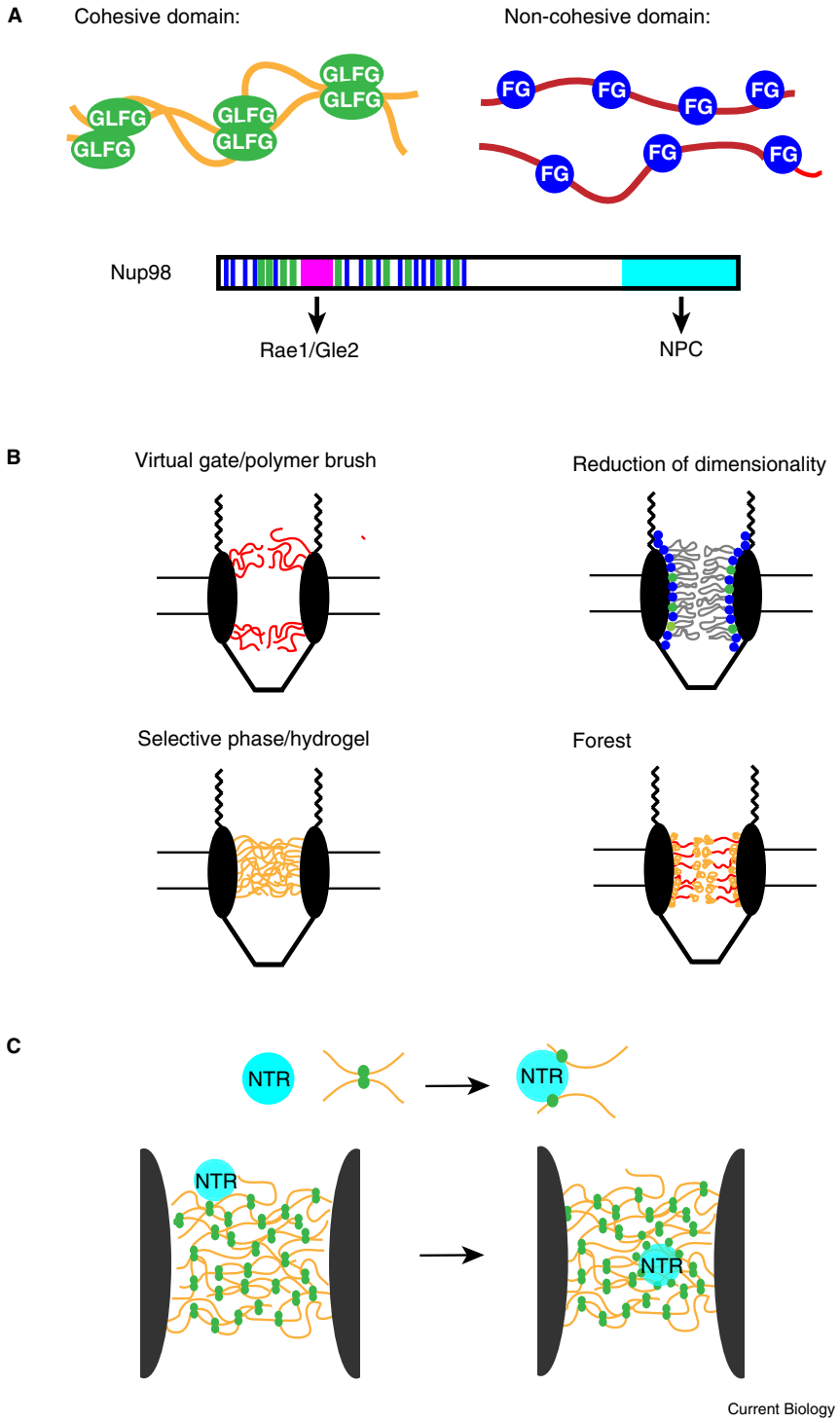

Figure 1. Mechanism of nuclear transport.

(A) Upper: Generalized FG domain types. A cohesive domain with GLFG repeat motifs and uncharged, serine/threonine-rich spacer sequences (gold). A non-cohesive domain with FG repeat motifs and non-cohesive, charged spacer sequences (red). Lower: Nup98 domain arrangement. The cohesive domain contains a mix of FG (blue) and GLFG (green) repeats. Number and placement of each type are representative. Binding site for the nucleoporin Rae1/Gle2 and the NPC targeting domain are indicated. (B) Proposed models of FG domain organization and function in the NPC. In the 'virtual gate/polymer brush' model, FG domains (red) act as filaments that create an entropic barrier to the entry of macromolecules. The energy of binding between transport receptors and FG motifs overcomes this barrier. In the 'reduction of dimensionality' model, FG motifs (blue, green) line the inner face of the NPC scaffold, while spacer regions (gray) fill the central channel forming a barrier to passage of non-receptor-bound macromolecules. NTRs move along the layer of FG repeats. This model does not distinguish between cohesive and non-cohesive FG domains. By contrast, in the selective phase/hydrogel model, cohesive FG domains (gold) interact in the central channel to form a gel-like barrier that selectively allows passage of NTRs and excludes large non-cargo. Finally, in the 'forest' model, FG repeat domains adopt one of two conformations: a collapsed coil (cohesive domains; gold) or an extended coil (non-cohesive domains; red). 'Shrub' Nups have their cohesive FG domain near the NPC scaffold. 'Tree' Nups have an extended coil separating their cohesive FG domain from the NPC scaffold. This arrangement creates a central cohesive channel, and an outer channel where non-cohesive FG domains may form an entropic barrier. (C) Movement of NTRs through the permeability barrier of the selective phase/hydrogel. NTRs locally disrupt the hydrogel by competing for FG motifs. The barrier reforms and seals as NTRs move to each new binding site. FG/GLFG motifs in green. (Panel C is adapted from [1].)

substituted for the Nup98 repeat domain, they restored both transport and the permeability barrier to reconstituted nuclei.

Next, the FG domain of ScNsp1p, a yeast Nup with a bipartite FG domain, was examined. They found that neither its full FG domain nor the large non-cohesive portion of its domain could substitute for the GLFG region of Nup98. However, when four tandem copies of a small cohesive portion of ScNsp1p were fused to the Nup98 carboxyl terminus, the chimera restored transport and the permeability barrier. This is particularly noteworthy since none of the ScNsp1p FG motifs are GLFG, although the spacers share the overall uncharged character of cohesive domains. This experiment is perhaps the strongest support of the selective phase model: a cohesive region from a distant species and a non-orthologous nucleoporin, distinct in sequence but similar in overall properties, provides a functional substitute.

Conclusions

Do the findings of Hulsmann *et al.* [1] sound the death knell for competing models of transport? Not necessarily, as their results are compatible with aspects of other models. For example, loss of the cohesive FG domains, which in the forest model form the central channel barrier [5], could lead to the observed leaky NPCs, even if the outer

entropic barrier of that model remained intact. Alternatively, as in the virtual gate model, the non-cohesive FG Nups could be acting as an initial entropic barrier but require a central hydrogel of Nup98 for effective NPC function. Hulsmann *et al.* [1] themselves did not propose a specific function for non-cohesive FG domains. In sum, it is likely that we are still far from a full understanding of NPC function.

Many questions remain. Firstly, what is the contribution of the non-cohesive FG domains? Secondly, will the exact positioning of the Nup98 repeat domain within the massive NPC prove as important as its cohesiveness? There are hints that this may be the case [1]. Thirdly, if Nup98 is the major component of the permeability barrier, would the amount of Nup98 in an NPC influence its transport properties? Nup98 is a dynamic nucleoporin [16] and its level of association with the NPC can be influenced by phosphorylation [17]. Nucleoporin dynamics may thus provide another level of nuclear transport regulation.

Lastly, Nup98 has an increasing number of additional cellular roles, including but not limited to roles in transcription (reviewed in [18]), intranuclear bodies [16], cell cycle regulation [19], and mitotic spindle assembly [20]. Importantly, the Nup98 gene is also a target of chromosomal translocations that produce fusion proteins containing the FG/GLFG domain of Nup98 and lead to acute myelogenous leukemia [2]. Clearly, determining how the multi-tasking Nup98 serves — and coordinates — so many diverse functions will be of interest for some time to come.

References

1. Hulsmann, B.B., Labokha, A.A., and Gorlich, D. (2012). The permeability of reconstituted nuclear pores provides direct evidence for the selective phase model. Cell *150*, 738–751.
2. Xu, S., and Powers, M.A. (2009). Nuclear pore proteins and cancer. Semin. Cell Dev. Biol. *20*, 620–630.
3. Finlay, D.R., and Forbes, D.J. (1990). Reconstitution of biochemically altered nuclear pores: transport can be eliminated and restored. Cell *60*, 17–29.
4. Denning, D.P., Patel, S.S., Uversky, V., Fink, A.L., and Rexach, M. (2003). Disorder in the nuclear pore complex: the FG repeat regions of nucleoporins are natively unfolded. Proc. Natl. Acad. Sci. USA *100*, 2450–2455.
5. Yamada, J., Phillips, J.L., Patel, S., Goldfien, G., Calestagne-Morelli, A., Huang, H., Reza, R., Acheson, J., Krishnan, V.V., Newsam, S., *et al.* (2010). A bimodal distribution of two distinct categories of intrinsically disordered structures with separate functions in FG nucleoporins. Mol. Cell. Proteomics *9*, 2205–2224.
6. Patel, S.S., Belmont, B.J., Sante, J.M., and Rexach, M.F. (2007). Natively unfolded nucleoporins gate protein diffusion across the nuclear pore complex. Cell *129*, 83–96.
7. Rout, M.P., Aitchison, J.D., Magnasco, M.O., and Chait, B.T. (2003). Virtual gating and nuclear transport: the hole picture. Trends Cell Biol. *13*, 622–628.
8. Lim, R.Y., Fahrenkrog, B., Koser, J., Schwarz-Herion, K., Deng, J., and Aebi, U. (2007). Nanomechanical basis of selective gating by the nuclear pore complex. Science *318*, 640–643.
9. Peters, R. (2005). Translocation through the nuclear pore complex: selectivity and speed by reduction-of-dimensionality. Traffic *6*, 421–427.
10. Ribbeck, K., and Gorlich, D. (2002). The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. EMBO J. *21*, 2664–2671.
11. Frey, S., and Gorlich, D. (2007). A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. Cell *130*, 512–523.
12. Yang, W. (2011). 'Natively unfolded' nucleoporins in nucleocytoplasmic transport: clustered or evenly distributed? Nucleus *2*, 10–16.
13. Finlay, D.R., Meier, E., Bradley, P., Horecka, J., and Forbes, D.J. (1991). A complex of nuclear pore proteins required for pore function. J. Cell Biol. *114*, 169–183.
14. Strawn, L.A., Shen, T., Shulga, N., Goldfarb, D.S., and Wente, S.R. (2004). Minimal nuclear pore complexes define FG repeat domains essential for transport. Nat. Cell Biol. *6*, 197–206.
15. Krull, S., Thyberg, J., Bjorkroth, B., Rackwitz, H.R., and Cordes, V.C. (2004). Nucleoporins as components of the nuclear pore complex core structure and Tpr as the architectural element of the nuclear basket. Mol. Biol. Cell, 4261–4277.
16. Griffis, E.R., Altan, N., Lippincott-Schwartz, J., and Powers, M.A. (2002). Nup98 is a mobile nucleoporin with transcription-dependent dynamics. Mol. Biol. Cell *13*, 1282–1297.
17. Laurell, E., Beck, K., Krupina, K., Theerthagiri, G., Bodenmiller, B., Horvath, P., Aebersold, R., Antonin, W., and Kutay, U. (2011). Phosphorylation of Nup98 by multiple kinases is crucial for NPC disassembly during mitotic entry. Cell *144*, 539–550.
18. Strambio-De-Castillia, C., Niepel, M., and Rout, M.P. (2010). The nuclear pore complex: bridging nuclear transport and gene regulation. Nat. Rev. Mol. Cell. Biol. *11*, 490–501.
19. Jeganathan, K.B., Malureanu, L., and van Deursen, J.M. (2005). The Rae1-Nup98 complex prevents aneuploidy by inhibiting securin degradation. Nature *438*, 1036–1039.
20. Cross, M.K., and Powers, M.A. (2011). Nup98 regulates bipolar spindle assembly through association with microtubules and opposition of MCAK. Mol. Biol. Cell *22*, 661–672.

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Evolution of Gene Regulation: Hybrid Networks Breed Diversity

How do gene regulatory networks evolve? A new study in yeasts shows that *cis*- and *trans*-regulatory changes resulted in a hybrid state of coexisting ancestral and derived regulatory circuits. This hybrid state then diversified into a variety of modern networks.

Andrea I. Ramos and Scott Barolo*

When you come to a fork in the road, take it.

-Yogi Berra

Imagine you're in a car, driving down a highway. How could you convert your power source from an internal combustion engine to an electric motor — traveling at full speed all the while? This is the puzzle facing those who study the evolution of gene regulatory networks. When two related lineages use different strategies to solve the same problem, it can be challenging to retrodict the state of their common ancestor, keeping in mind that all intermediate states must be fully functional (that is, the engine has to keep running during the conversion process). In a recent *Cell* paper, Sandy Johnson and colleagues [1] now report that hybrid states, in which ancestral and derived regulatory mechanisms coexist, can sustain functionality while major transitions in network structure take place.

Evolutionary diversity derives in large part from gradual changes to transcriptional regulatory circuitry [2]. These innovations occur both in *cis* (changes to regulatory DNA sequences