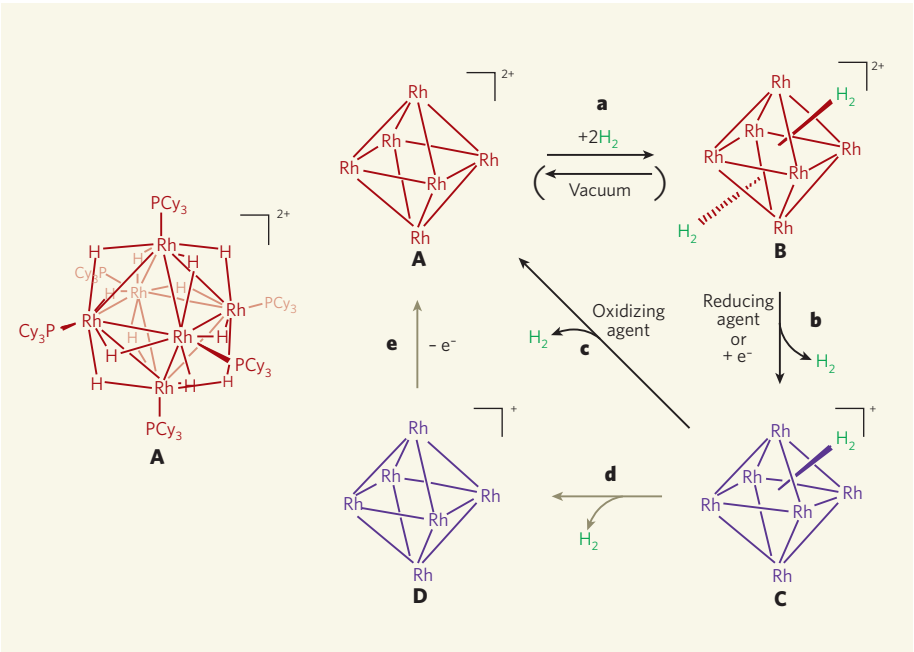


**Figure 1 | Hydrogen uptake–release cycle in molecular clusters.** Structure A is the hydrogen-storage material developed by Weller and colleagues[2]; Rh represents rhodium atoms, and $PCy_3$ are bulky molecules bound to the metals. Clusters in the same colour are in the same oxidation state (also indicated by the number of positive charges on the clusters). The structures are simplified in the depicted hydrogen uptake–release cycle. **a**, The 12-hydrogen-atom cluster (A) takes up two hydrogen molecules to form a 16-hydrogen-atom cluster (B). These hydrogen molecules may be removed under vacuum. **b**, The release of one molecule of hydrogen from B is promoted by a reducing agent or by the transfer of an electron ($e^-$) from an electrode, to give the 14-hydrogen-atom cluster (C). **c**, Chemical oxidation of C promotes the release of one molecule of hydrogen, regenerating the starting material A. **d**, Under electrochemical reduction conditions, the release of one molecule of hydrogen from C occurs spontaneously, yielding a 12-hydrogen-atom cluster (D). This cluster is not at the same oxidation state as A. **e**, Electrochemical oxidation of D regenerates the starting material A.

the lowest-energy molecular orbital that lacks electrons (known as the lowest unoccupied molecular orbital, or LUMO) in the starting material is only slightly higher in energy than that of the highest electron-filled orbital (the highest occupied molecular orbital, or HOMO). This is why the LUMO readily accepts electrons donated from hydrogen molecules. In contrast, the 16-hydrogen cluster has a large HOMO–LUMO gap; the addition of an electron into the LUMO destabilizes the molecule, and induces the release of a hydrogen molecule.

The hydrogen-storage capacity of this rhodium system, expressed as the ratio of the mass of releasable hydrogen to that of the storage system, is only 0.1%. This is clearly not sufficient for practical applications — the US Department of Energy wants hydrogen-storage systems to have a capacity of 6% weight-for-weight by 2010. Improved materials must be developed with a greater number of usable hydrogen molecules, bound to clusters of metals with an overall lower molecular mass. Never-theless, this work[2] provides a well-defined mol-ecular model and a worthwhile strategy for the development of hydrogen-storage materials with high efficiency and convenience. ■

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EVOLUTION

# Different paths to the same end

Antonis Rokas

**Genetic dissection of a yeast gene-regulatory pathway shows that the logical output of such a pathway can remain the same even though the molecular mechanisms underlying the output have diverged remarkably.**

From penguins to mushrooms and baobabs, the world around us harbours a bewildering diversity of life forms. Much of the evolution of this diversity is due to changes in the underlying genetic regulatory architecture[1]. But what happens to such architecture when organisms that diverged long ago retain the same traits (or 'phenotypes')? Can this regulatory architecture diverge while the overlying phenotypes remain similar? On page 415 of this issue, Tsong *et al.*[2] examine the gene-regulatory circuit that governs mating type in several yeast species, and they identify a remarkable example of divergence at the genotypic level (the DNA sequence) despite conservation at the phenotypic level.

The yeast species *Saccharomyces cerevisiae* and *Candida albicans* are seemingly very different: *S. cerevisiae* is the cornerstone of the baking and brewing industries, whereas *C. albicans* is the most commonly encountered fungal pathogen in humans. Both yeasts have one thing in common, however — sex. Although yeasts do not have different sexes *per se*, both species have two molecularly distinct mating types. Mating type in yeasts is controlled by the *MAT* genetic region (locus), which exists in two versions, *MAT**a*** and *MAT*α. A cell's mating type is determined by which version it expresses. Thus, cells expressing the **a** protein become **a** cells by expressing **a**-specific genes (**a**sgs), and are specialized for mating with yeasts of the opposite mating type — α. Likewise, α-expressing cells become α cells, express α-specific genes (αsgs), and can mate only with yeasts of the **a** type.

Underlying this apparent similarity in mating phenotype in the two species (**a**-expressing cells become **a** cells, and α-expressing cells become α cells), the detailed genetic mechanisms by which mating-type identity is achieved seem to diverge considerably (Fig. 1, overleaf). In the **a** cells of *C. albicans* the **a**sgs are only expressed once they have been activated by the **a**2 protein, whereas in *S. cerevisiae* the *MAT**a2*** gene (encoding the **a**2 protein) is

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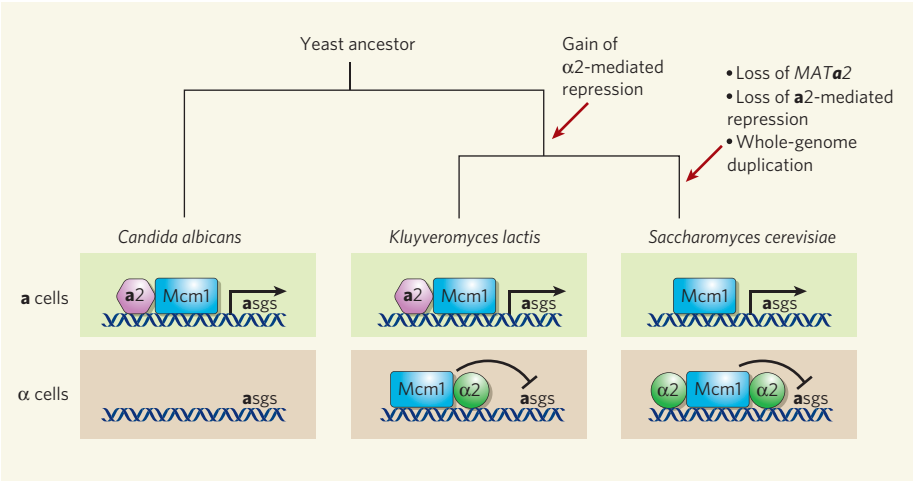


**Figure 1 | The divergent regulatory architectures associated with yeast mating type.** Tsong *et al.*[2] find that in three distantly related yeasts the regulatory architectures are remarkably divergent. This is despite the conservation of the regulatory logic: specifically, in all three yeasts, **a**-specific genes (**a**sgs) are turned on in **a** cells and turned off in α cells. The Mcm1 protein is equally expressed in both cell types and is required for both **a**sg and αsg regulation, sometimes in concert with the **a**2 and α2 proteins. Major transitions in the regulatory architecture are indicated by red arrows.

absent and the **a**sgs are expressed by default in its **a** cells. In the case of α cells, the α1 protein activates αsgs in both yeasts, but in *S. cerevisiae* α2 is also required to repress the **a**sgs (otherwise they are always 'on').

As the regulatory architecture in *C. albicans* also occurs in an evolutionarily diverse set of other yeasts, it is most probably closer to the ancestral one. If so, two key modifications must have occurred in the regulatory architecture of a direct ancestor of *S. cerevisiae*, namely the loss of the *MAT**a**2* gene and the gain of **a**sg repression by the α2 protein.

To piece together this evolutionary path, Tsong *et al.*[2] examined how **a**sgs are regulated in each species. Having identified the set of **a**sgs in *C. albicans*, they then surveyed the regions in front of all **a**sgs to identify putative binding sites for gene-regulatory factors. In *S. cerevisiae*, the expression of **a**sgs is turned on by the binding of the Mcm1 gene-regulatory factor to a characteristic stretch of DNA located upstream of each **a**sg coding region[3] (Fig. 1). But in *C. albicans*, in addition to an Mcm1-binding site, this regulatory motif also turned out to have a binding site for the **a**2 protein. Comparing the upstream regulatory motifs governing **a**sg regulation from several additional yeasts identified two lineages: *C. albicans* and its close relatives possess motifs with binding sites for both Mcm1 and **a**2, whereas *S. cerevisiae* and related species have motifs with only Mcm1-binding sites.

Although *S. cerevisiae* has lost **a**2 and thus the ability to control the activation of its **a**sgs, it has gained the ability to repress **a**sg expression through the α2 and Mcm1 proteins. Specifically, Tsong *et al.*[2] show that the upstream regulatory motif of the *S. cerevisiae* lineage seems to have acquired two α2-binding sites. Furthermore, the domain of α2 that interacts with Mcm1 is similar among all the species with the *S. cerevisiae*-like regulatory architecture, but is not conserved in species with the *C. albicans*-like architecture. It is probable that specific mutations in the Mcm1-interaction domain of α2 enabled the *S. cerevisiae* lineage to acquire the ability to repress **a**sgs.

Intriguingly, a third yeast lineage (*Kluyveromyces lactis* and its relatives) exhibits tell-tale signs of an intermediate architecture between those exemplified by *C. albicans* and *S. cerevisiae*. For example in *K. lactis,* **a**2 participates in **a**sg activation (as in *C. albicans*), but α2 interacts with Mcm1 to suppress **a**sgs in α cells (as in *S. cerevisiae*). *K. lactis* diverged from *S. cerevisiae* after their joint common ancestor split from *C. albicans*, so could it be that it has maintained a 'transitional' regulatory architecture? Although the weight of evidence supports this argument, it has yet to be shown that the *K. lactis* **a**2 and α2 proteins do actually bind to the putative **a**sg binding sites.

How could this transition from positive to negative regulation of **a**sgs occur? From a theoretical standpoint, because natural selection is operating on phenotypes, 'neutral' changes in the underlying genotypes that do not affect the phenotype are not screened by the sieve of selection and can become fixed in natural populations[4,5]. Tsong and colleagues' evidence[2] suggests not only that this rewiring of the mating circuit could have happened in a neutral fashion, but also that it may have required only a few mutational steps. Indeed, increasing the number of A and T bases around the Mcm1-binding site in *S. cerevisiae* can allow Mcm1 to activate **a**sgs in the absence of **a**2 (ref. 6). Moreover, the **a**2- and α2-binding sites in *S. cerevisiae* are remarkably similar, so the transition from positive (by **a**2) to negative (by α2) regulation of **a**sgs might have occurred by only a handful of mutations.

Interestingly, *C. albicans* possesses an extra environment-dependent layer of gene regulation in mating-type control that *S. cerevisiae* does not have and that probably represents an adaptation to its pathogenic lifestyle[7]. This additional control level is unlikely to have been present in the common ancestor of *C. albicans* and *S. cerevisiae*, suggesting that some of the observed differences in regulatory architecture must have been the direct result of natural selection.

As for the bigger picture, several studies indicate that genotypic divergence in the face of phenotypic conservation may be a rather common, but underappreciated, evolutionary theme[4,5]. Examples of such 'developmental system drift' range from ribosomal transcriptional modules in the same yeast lineage[8], to the genetic basis of tooth formation in vertebrates[9], and regulatory evolution in fruitflies[10], although it is still unclear whether all this genotypic divergence was acquired through neutral evolution.

Examination of the mating circuits of other yeast species promises further evolutionary delights. For example, *Pichia angusta* seems to have lost the *MAT**a**2* gene independently[11], and several of the genes involved in *C. albicans* mating are absent from its close relative *Candida parapsilosis*[12]. Finally, considering the technical difficulties associated with the study of mating in species such as *K. lactis* and the tremendous power offered by the *S. cerevisiae* model system, perhaps a fruitful alternative may be to reconstruct the mating circuits of the direct ancestors of *S. cerevisiae* (is this the birth of palaeoregulomics?). For example, engineering a *S. cerevisiae* strain with a *C. albicans*-like or a *K. lactis*-like architecture would allow an analysis of how long-lost ancestors may have regulated mating. Competition experiments of these 'ancestral' strains with modern-day ones would settle the question of whether such a remarkable shift in the architecture of a regulatory circuit could have been pulled off in a neutral fashion. ■

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