Dispatches

Transcriptional Rewiring: The Proof Is in the Eating

Transcriptional rewiring is an emerging evolutionary principle. Analysis of the galactose genetic pathway in *Candida albicans* and comparison with the classical pathway in *Saccharomyces cerevisiae* have revealed remarkable differences in its regulation in the two yeasts.

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The world around us is a varied and ever-changing place. Understanding how the adaptation of organisms to their natural environments shapes their genotypes is a major challenge in evolutionary biology [1]. Take eating for example — organisms are continuously faced with the task of adapting to the food resources available or risk starvation.

Saccharomyces cerevisiae, the baker's yeast, has evolved the ability to grow rapidly by converting the available sugars in its environment into ethanol. This adaptation allows *S. cerevisiae* to out-compete other microorganisms, because high levels of alcohol are lethal to most of its competitors. In contrast, its distant relative *Candida albicans* has adopted the lifestyle of a human pathogen, whereas *Debaryomyces hansenii* — frequently found in dairy products and brine — has adapted to high salinity levels. A common theme is the knack all these yeasts have to use a variety of sugars as carbon sources. What evolutionary changes equipped yeasts with this diversity of eating habits? A series of comparative studies [2–4] focusing on the catabolism of the sugar galactose has made significant headway toward addressing this question.

The genetic pathway responsible for the breakdown of galactose has been extensively studied in *S. cerevisiae*. Galactose first enters the yeast cell through the action of the galactose permease Gal2p, where it is converted by four enzymes — Gal1p, Gal10p, Gal7p, and Gal5p — into glucose-6-phosphate, which then enters glycolysis [5,6]. In addition to these structural genes, the *GAL* pathway also contains three regulatory genes: Gal4p, a transcriptional activator; Gal3p, a co-inducer; and Gal80p, a co-repressor. In the absence of galactose, Gal80p physically interacts with Gal4p, thereby preventing pathway activation. In the presence of galactose, Gal3p relieves the repression of Gal4p by Gal80p, allowing Gal4p to activate the transcription of the *GAL* structural genes [7]. But how is the *GAL* pathway regulated in other yeasts?

To begin with, several species have lost the ability to utilize galactose as a result of the loss of most or all *GAL* genes [3] (Figure 1A). Polymorphism for galactose utilization is also present within species, as in the case of some *S. cerevisiae* isolates from fermenting grape musts [8]. These studies notwithstanding, the majority of yeasts do utilize galactose even though there may be interesting regulatory variations [2,3]. For example, regulation of the pathway in the dairy-loving yeast *Kluyveromyces lactis* is similar to that in *S. cerevisiae*, but a bifunctional Gal1p is used for co-induction, instead of Gal3p [2,6,9]. But how galactose utilization is regulated in more distant relatives, such as *C. albicans*, has until recently been a mystery. Examination of this pathogen's genome [10] revealed important differences relative to *S. cerevisiae*'s *GAL* regulatory genes (Figure 1A): *C. albicans* lacks a functional homolog of *GAL3*; the *CaGAL80* gene shows limited similarity with its *S. cerevisiae* relative; and the *CaGAL4* gene is only 30% the size of its *S. cerevisiae* homolog.

The lack of a *GAL3* functional homolog is not surprising, because the gene arose as a result of the whole-genome duplication in an ancestor of *S. cerevisiae* after its divergence from *C. albicans* [11,12]. But the differences in the *CaGAL4* and *CaGAL80* genes were suggestive of differences in the regulation of the *GAL* pathway in *C. albicans*. Martchenko *et al.* [4] showed that the structural *GAL* genes are indeed induced in *C. albicans* in the presence of galactose, but, surprisingly, CaGal4p is not involved in their regulation. Next, they identified a palindromic putative regulatory element located upstream of *CaGAL1*, *CaGAL7* and *CaGAL10* genes. Deletion of the element from the *CaGAL10* promoter led to a five-fold reduction of *CaGAL10* expression in the presence of galactose, whereas its insertion upstream of a *lacZ* reporter caused a ten-fold increase in expression [4]. Interestingly, the half-palindrome sequence differs by one nucleotide from the binding site of the *S. cerevisiae* Cph1p ortholog, Ste12p. Experiments with a *CaCPH1* knock-out strain verified that Cph1p is an activator of the *CaGAL* structural genes [4].

Given that the *C. albicans GAL* genes are under the control of Cph1p and not Gal4p, what is the role of Gal4p in this yeast species? Using whole genome expression profiling on *CaGAL4* knock-out strains followed by experimental verification, Martchenko *et al.* [4] demonstrated that Gal4p in *C. albicans* regulates a distinct set of genes, a large fraction of which belong to a telomere-associated gene family of unknown function [13]. Gal4p's involvement in regulating non-*GAL* genes in *C. albicans* may provide a clue as to why *Eremothecium gossypii* (syn. *Ashbya gossypii*) has retained its Gal4p, despite the loss of all other *GAL* genes [3] (Figure 1A).

Martchenko *et al.* [4] propose an evolutionary model to explain the

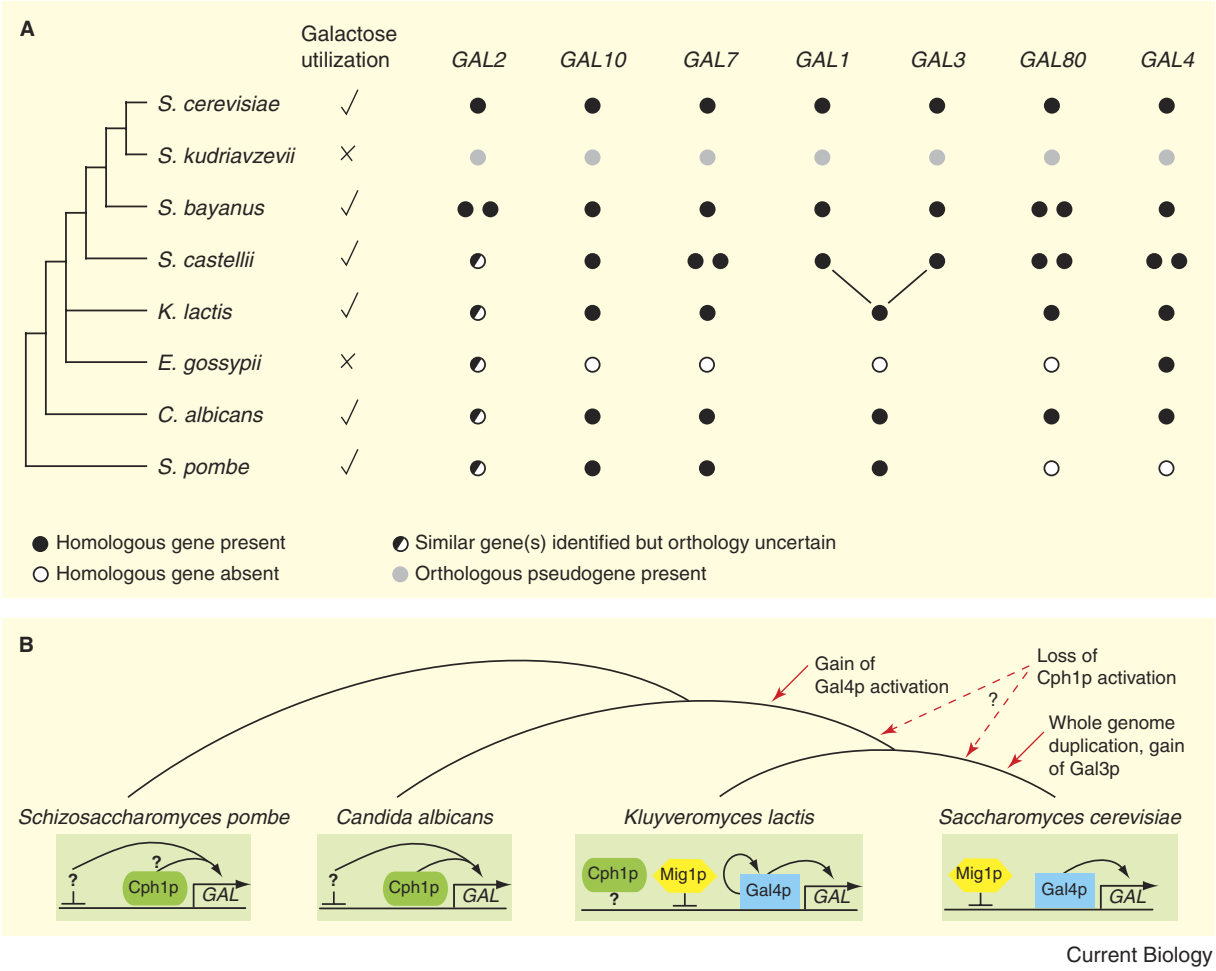

Figure 1. Evolution of galactose pathway regulation across yeasts.

(A) The phylogenetic distribution of the genetic machinery implicated in the catabolism of galactose across exemplar yeast species. (B) A model for the evolution of regulation of the galactose pathway in four yeast lineages. Questionmarks identify putative, as yet untested, regulatory actions.

transition from the inferred ancestral Cph1p regulation — as seen in *C. albicans* — to Gal4p regulation, as in *S. cerevisiae* (Figure 1B). Although some of the proposed steps and interactions remain untested (Figure 1B), this model raises some interesting questions. For example, because CaGal4p regulates only non-*GAL* genes in *C. albicans*, it is presumably not regulated by galactose through CaGal1p or CaGal3p, but is CaGal80p the co-repressor? If so, how is it regulated? If CaGal80p does not inhibit CaGal4p, what, if anything, regulates it?

C. albicans may not be the only yeast that differs from *S. cerevisiae* in the regulation of its *GAL* genes. For example, in the aftermath of the whole-genome duplication some species retained additional copies of certain *GAL* genes. The best-known case is the retention of a subfunctionalized *GAL1/GAL3* duplicate gene pair in *S. cerevisiae* and post-whole-genome duplication relatives, but *S. castellii* also contains two copies of Gal4p and Gal80p in its genome [3,14] (Figure 1A). One is tempted to speculate that these duplicated regulatory genes have also been subfunctionalized in *S. castellii*.

Of course, yeasts regulate both the identity and quantity of the enzymes produced, and the new data [4] suggest interesting differences here as well. *S. cerevisiae* differentially regulates its *GAL* enzymes to an extreme degree, with expression levels increasing >1000-fold in the presence of galactose [5]. Differential regulation in *K. lactis* is more modest (>100-fold) [2], while *C. albicans* apparently displays <10-fold differential regulation [4]. Tight regulation in *S. cerevisiae* may be a by-product of its extreme specialization for using large quantities of glucose [11,15]. In contrast, *K. lactis* likes to eat lactose, a disaccharide composed of galactose and glucose monomers that *S. cerevisiae* cannot hydrolyze. The requirement to induce its *GAL* pathway in conjunction with some glucose consumption probably demands a more modest response. It is intriguing to speculate that the even more limited response of *C. albicans* may be related to its adaptation as a human pathogen.

Several recent studies suggest that transcriptional rewiring similar to that seen in the catabolism of galactose in yeasts is a recurrent evolutionary theme observed in genetic pathways across life's kingdoms [16]. Interestingly, several such rewirings — of the mating circuit [17], of the ribosomal transcriptional module [18], or of the mitochondrial ribosomal genes [19] — have been identified in comparisons between *C. albicans* and *S. cerevisiae*. The most striking differences between the two organisms are the conditions under which they ferment. *C. albicans* — like most yeasts — prefers to respire, whereas *S. cerevisiae* prefers to ferment (even in the presence of oxygen), an adaptation linked to the whole-genome duplication and the emergence of the fruit-bearing angiosperms [11,20]. Could much of this rewiring have been triggered by these extraordinary evolutionary events? Food for thought, at least, and hopefully an appetizer for continued research.

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Language Acquisition: When Does the Learning Begin?

Language acquisition is quite sophisticated by four months of age. Two cues that babies use to discriminate their language from another are the stress patterns of words and visual cues inherent in language production.

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Benjamin Franklin is credited with the invention of bifocal glasses, Franklin said he found them particularly useful at dinner in France, where he could see the food he was eating and watch the facial expressions of those seated at the table with him, which helped interpret the words being said. He wrote: "I understand French better by the help of my Spectacles." Language is a multimodal experience; we obtain linguistic information through hearing, seeing people's lips move, reading and interpreting the context that surrounds the linguistic input. It is an impressive accomplishment that children synthesize all this input into meaningful ideas and that they acquire language in a short amount of time with no formal training. Even more astonishingly, every typically developing child manages to accomplish this feat. The question asked by parents and scientists alike is: how do they do it?

Part of the answer is that there appears to be a language-dedicated system from the outset [1,2]. Evidence in support of this view comes from studies showing that newborns prefer to listen to speech compared to non-speech stimuli [3,4] and that different areas of the brain activate for speech and non-speech stimuli