

blood) were removed from each sample: the renal cortex, which filters plasma, and the medulla, which alters urine composition to maintain fluid balance. Both deteriorate with age. An extensive clinical history was noted for each sample to account for any potentially confounding medical factors.

Kim and colleagues then isolated RNA transcripts from the samples to determine the activity of every gene, broken down by age and kidney section, through microarray analysis. Looking for differences in gene expression across the genome, they identified genes that showed a statistically significant change in expression as a function of age. Of 33,000 known human genes on the microarray, 985 showed age-related changes, most showing increased activity. These changes are truly age-regulated, the authors conclude, since none of the medical factors impacted the observed changes in gene expression.

Although cortex and medulla have different cell types and perform different functions, their genetic aging profile was very similar, suggesting a common aging mechanism operates in both structures. In fact, these mechanisms may function broadly, as most of the age-regulated kidney genes were also active in a wide range of human tissues. Other organisms appear to lack these changes, however, prompting the authors to argue that understanding aging in humans will require human subjects.

Most importantly, the genetic profile of the tissue samples correlated with the physiological and morphological decline of an aging kidney. An 81-year-old patient with an unusually healthy kidney had a molecular profile typical of someone much younger, while a 78-year-old with a damaged kidney had the profile of a much older person. Using the power of functional genomics, this study has identified a set of genes that can serve as molecular markers for various stages of a deteriorating kidney and predict the relative health of a patient compared to their age group. These gene sets can also serve as probes to shed light on the molecular pathways at work in the aging kidney, and possibly on the process of aging itself.

Rodwell GEJ, Sonu R, Zahn JM, Lund J, Wilhelmy J, et al. (2004) A transcriptional profile of aging in the human kidney. DOI: 10.1371/journal.pbio.0020427

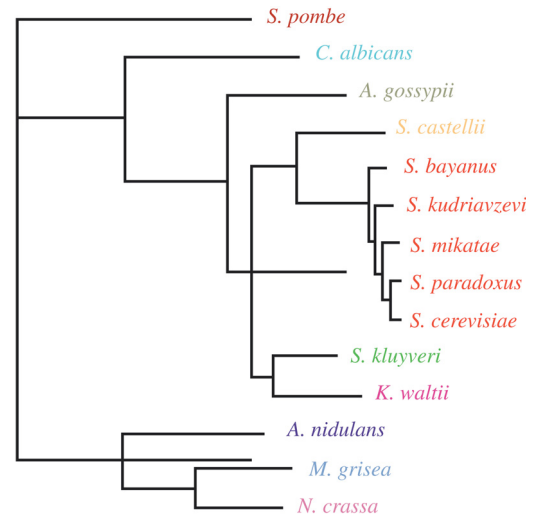
## Genes, Genomes, and the Road to Diversity: How Regulatory Networks Evolve

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Evolutionary biologists have long been interested in understanding the molecular basis for the great diversity in size, shape, and behavior seen in life on earth. Recent attention has focused on the role that gene expression changes play in organismal evolution. Tracing the evolution of gene regulation, however, has proved difficult. This is in large part due to the difficulty in identifying and comparing the regulatory elements that control gene expression in different species.

Gene expression depends on *cis*-regulatory elements, short sequence motifs embedded in the DNA that flank a gene's coding region. Regulatory proteins bind to specific *cis*-regulatory sequences, and command the activation or repression of the corresponding gene. The challenge in studying the evolution of *cis*-regulatory elements lies in identifying those elements in multiple species. Unlike protein sequences, which are typically a few hundred amino acids long and relatively straightforward to identify in related organisms, *cis*-regulatory elements are often short and can have variations in sequence. This makes it very difficult to distinguish the regulatory elements from the nonfunctional DNA that surrounds them. It is even harder to identify corresponding regulatory elements across species. As the evolutionary distance between species increases, so, too, does the difficulty in identifying corresponding *cis*-elements in those species.

In this issue of *PLoS Biology*, Audrey Gasch and her colleagues describe a comparative genomics approach that allows them to identify potential *cis*-regulatory elements in thousands of genes across 14 ascomycete fungi whose diversity represents the effects of several hundred million years of evolution. Ascomycetes are a large class of fungi with extremely diverse morphologies, reproductive strategies, and habitats. A divergence dating back 500 million to 1 billion



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**Phylogeny of fungi used to study evolution of gene regulation**

years ago gave rise to three groups: Archaeascomycetes, Euascomycetes, and Hemiascomycetes. The genome of the brewer's yeast, *Saccharomyces cerevisiae*, a hemiascomycete, was completely sequenced in 1995, and that of fission yeast, *Schizosaccharomyces pombe*, an archaeascomycete, in 2002. Since that time, complete genome sequences have been released for more than nine additional hemiascomycetes and three euascomycetes. This gives the authors an opportunity to compare regulatory systems among progressively more distantly related species, on a genomic scale.

Genome-wide expression studies in the yeast *S. cerevisiae* have revealed groups of genes whose expression levels vary simultaneously under varying experimental conditions. Such co-regulated genes, the authors reasoned, must harbor common regulatory elements that coordinate their response to experimental triggers. Gasch and colleagues looked for such *cis*-elements and found 35 groups of co-regulated *S. cerevisiae* genes with at least one shared *cis*-element. The authors then argued that co-regulation may reflect selection pressures that also apply to other ascomycetes, and so they identified the equivalent of the 35 co-regulated gene groups in each of the 13 other species.

They then looked for shared *cis*-elements within each group and in each species independently, and compared the regulatory systems across the species.

The results of this study show that the majority of *cis*-elements first identified in yeast are retained in the equivalent gene groups in other species, in a manner that reflects the species' evolutionary distance from yeast. One *cis*-element, in a group of co-regulated genes that control the cell cycle, is found all the way from budding yeast to fission yeast, suggesting a selection pressure on the co-regulation of these genes that has withstood greater than 500 million to 1 billion years of evolution.

In contrast, there were other examples in which the same gene groups contained different putative *cis*-elements in each species, suggesting that the regulation of those genes has evolved. In the case of *cis*-elements found in genes controlling protein degradation, a related element was identified in all of the hemiascomycetes, whereas the euascomycetes appear to have adopted a novel *cis*-element for this gene group. Interestingly, the hemiascomycete element displays a sequence variation in *Candida albicans* that is not found in *S. cerevisiae*. The two species diverged 200 million years ago. Gasch and colleagues showed that the protein that binds to the hemiascomycete element has evolved to have slightly different DNA interactions in the two species, allowing the *C. albicans* protein to bind the novel sequence found only in the *C. albicans* genes. This provides evidence for co-evolution between a transcription factor and its target *cis*-element.

Overall, this analysis has uncovered striking cases of conservation and innovation of gene regulatory systems, and therefore provides important insight into the evolutionary forces that have shaped the evolution of gene regulation.

Gasch AP, Moses AM, Chiang DY, Fraser HB, Berardini M, et al. (2004) Conservation and evolution of *cis*-regulatory systems in Ascomycete fungi. DOI: 10.1371/journal.pbio.0020398

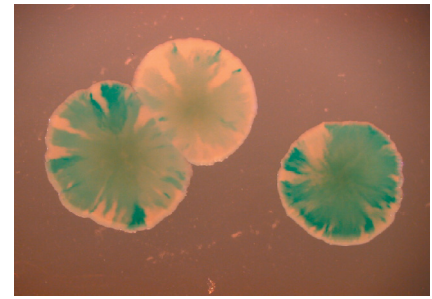
## In Times of Stress, Mutate Early and Often

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For a human, the normal response to stress is to reduce it through some purposeful action, be it indulging in chocolate or calling in sick, at a rate which we can vary to fit the circumstances. For a strain of bacteria faced with stress, the choice is often more stark: it must mutate or die. Among evolutionary theorists, an important question has been whether the rate of mutation is fixed, or instead can adaptively increase in response to stress, thereby increasing the likelihood of a favorable mutation. Something like this latter possibility was envisioned by Darwin, but fell out of favor among some neo-Darwinists, for whom a steady rate of mutation was more in keeping with their overall model of evolutionary gradualism. This debate is taken up in a new study in this issue by P.J. Hastings and colleagues, who examined the mechanism by which *Escherichia coli* lacking the ability to digest lactose, called *lac*<sup>-</sup> mutants, regain that ability when presented with lactose as their only food source.

It has been known for some time that the reversion of *lac*<sup>-</sup> mutants to a *lac*<sup>+</sup> state can be achieved by either of two genetic events: amplification, which creates numerous copies of the nonfunctional *lac* gene, and point mutations, which give rise to functional versions of the gene (many non-useful mutations also occur; thus, there is no directed mutation, in keeping with standard Darwinian evolution). According to the gradualist view, amplification precedes mutation, and the rapid appearance of *lac*<sup>+</sup> cells is explained by a normal mutation rate acting on multiple copies of the gene. In contrast, according to the hypermutation view, amplification and mutation are independent events, and *lac*<sup>+</sup> cells arise quickly because the mutation rate has increased.

While some results from previous studies have supported the gradualist interpretation, the experiments of Hastings et al. show that hypermutation is the most plausible explanation. A variety of procedural improvements allowed them to analyze more individual cells at an earlier stage of colony development. For instance, they analyzed colonies composed of as few as one hundred cells, rather than the ten thousand cells in prior



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### Sected colonies of *lac*<sup>+</sup> and *lac*<sup>-</sup> *E. coli*

experiments, and even nascent colonies at the two-cell stage.

The study produced clear evidence that point mutations arise very early in the development of *lac*<sup>+</sup> colonies, before amplification can account for the number of *lac*<sup>+</sup> revertants observed. Amplification is not only independent from mutation, but occurs relatively late under starvation. The researchers found that amplification, but not point mutation, requires the presence of a particular DNA polymerase, further strengthening the case that amplification need not precede mutation. They also showed that amplification by itself does not induce a so-called SOS response. The SOS system includes a group of genes that cause an increase in mutation in response to stress, and one hypothesis arising from the gradualist model was that amplification turned on the SOS response.

Based on their data, the authors reject the strict gradualist model for the adaptive mutation mechanism in the Lac system. They propose that amplification and hypermutation are independent responses to stress, each of which increases the likelihood of adaptive change. They also suggest that a stress-induced increase in the rate of point mutations may have implications for a variety of mutation-related phenomena, from tumor formation to development of resistance to antibiotics and chemotherapeutic drugs.

Hastings PJ, Slack A, Petrosino JF, Rosenberg SM (2004) Adaptive amplification and point mutation are independent mechanisms: Evidence for various stress-inducible mutation mechanisms. DOI: 10.1371/journal.pbio.0020399