Minireview

Rewiring the transcriptional regulatory circuits of cells
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Abstract

The molecular mechanisms that regulate gene expression can evolve either by changing the cis-acting DNA elements in promoters, or by replacing the trans-acting regulatory proteins. New data from yeast species show that both processes can happen.

Whether you’re driving a Cadillac or an electric golf buggy, you put your foot on the accelerator and it goes. Most of us don’t care what’s happening in the engine so long as it does what we want it to do, and which vehicle we choose depends on whether we want to drive across the country or across the fairway. Natural selection is a bit like us uninformed motorists: it favors organisms that work well in a particular context, without prejudice as to what is the best mechanism to achieve this. But the catch about evolution is that every organism has been produced by descent with modification from ancestor organisms that worked, so an engineer cannot just design your ‘golf buggy’ from scratch. To push the analogy, it has to be made from a Model T Ford by continually adding, subtracting or modifying parts, keeping the car running all the time.

If you find it hard to see how an engineer could do this, you will appreciate why we have a limited understanding of how evolution can alter the regulatory circuitry of cells. Despite the facts that a study of gene regulation in the lac operon was one of the first great successes of molecular biology [1], and that it has long been argued that the molecular differences between species are more likely to hinge on gene regulatory differences than on gene content differences [2], few comprehensive studies into how gene regulation evolves have been attempted. The reason for this is no secret. Whereas the coding region of the lac operon consists of three genes totaling 4,941 nucleotides, the operator and promoter sequences comprise only 160 bases [3]. In addition, cis-regulatory elements do not evolve within a clearly defined structural framework such as that imposed by the triplet code, nor do they exhibit the close relationship between sequence and function that exists in coding regions. So, although incredible progress has been made in the last decade in molecular evolutionary studies of genes and genomes (see [4] for a review), how gene regulation evolves is still a topic about which there is more speculation than hard data.

On the other hand, there is more to regulatory evolution than just cis elements. In this regard, a recent paper by Tsong et al. [5] on the evolution of the yeast mating-type circuitry provides a valuable lesson. By showing that one yeast species uses a regulatory protein that is simply not present in another species, Tsong et al. [5] bring home the message that gene regulation is achieved by cis elements working in conjunction with their cognate transcription factors, with both sets of factors operating in the specific context of a genetic circuit, the final output of which is the object of natural selection. The system studied by Tsong et al. is the MTL (mating-type-like) locus of the pathogenic yeast Candida albicans, which corresponds to the MAT (mating-type) locus of Saccharomyces cerevisiae (Figure 1). The S. cerevisiae system has been studied intensively and comprises two genes, MAT\(\alpha\) and MAT\(\alpha\), encoded by the \(\alpha\) version of the locus, and one gene, MAT\(\alpha\), encoded by the \(\alpha\) version; each haploid cell is either \(\alpha\) or \(\alpha\) type. The combination of genes for one activator protein (\(\alpha\)1) and two repressors (\(\alpha\)2 and the \(\alpha\)1/\(\alpha\)2 dimer) in diploid cells allows the
Figure 1
Genotypes, interactions and outputs of the mating-type loci of Candida albicans and Saccharomyces cerevisiae [5,15]. (a) The C. albicans MTL locus. The α version of the locus encodes the \( \alpha_1 \) protein, which activates the expression of α-specific genes; the a version of the locus encodes the \( \alpha_2 \) protein, which activates the expression of a-specific genes. In a/α cells, where both versions of the locus are present, the \( \alpha_2 \) and a1 proteins act together as a repressor of the phenotypic switch from white to opaque, and of 'haploid-specific' genes. (b) The S. cerevisiae MAT locus. As in C. albicans, the \( \alpha_1 \) protein activates the expression of α-specific genes, and \( \alpha_2 \) and a1 act together in diploid cells as a repressor of MATα1 and haploid-specific genes. The a-specific genes are, however, constitutively expressed in the absence of the \( \alpha_2 \) repressor. The dotted lines highlight the replacement of a positive branch in C. albicans with a negative one in S. cerevisiae.
S. cerevisiae MAT locus to produce three types of output, corresponding to α maters, a maters, and non-mating a/α diploid cell types. In C. albicans, however, the a idiomorph contains a second gene, MTLa2, that is completely absent from S. cerevisiae.

At the start of the recent analysis, Tsong et al. [5] knew only two things about MTLa2. The first is that it encodes a DNA-binding protein with an HMG domain (named for the 'high mobility group' proteins in which it was first found), unrelated in sequence to any of the S. cerevisiae mating-type genes. The second is that it is not responsible for the most obvious difference in mating between the two yeasts, namely that in order to mate a C. albicans cell must undergo a morphological change from the white phase, which is thought to aid its avoidance of the host's immune system, to the mating-competent opaque phase. MTLa1 and MTLa2 together had previously been demonstrated by Miller and Johnson [6] to be necessary and sufficient to regulate (repress) phenotypic switching between white and opaque morphologies. With the exception of this initial requirement, however, mating in the two species was thought to proceed by similar mechanisms.

In order to fully understand the genetic circuit regulating mating, and in particular the role of the novel transcriptional regulator MTLa2, all sixteen possible knockout combinations of the MTLa1, MTLa2, MTLa1 and MTLa2 genes were generated in C. albicans and their mating ability assayed [5]. These experiments showed that, like in S. cerevisiae, α1 is a positive regulator of the ability of a-cells to mate, and α1 and α2 together repress mating ability. Additionally, they revealed that, unlike in S. cerevisiae, in which a-specific genes are constitutively expressed unless the α2 protein is present to repress them, in C. albicans a-specific genes require the novel a2 protein as a positive regulator and are insensitive to a2 as a negative regulator. Interestingly, although these last two observations represent regulatory differences between S. cerevisiae and C. albicans, because the two changes effectively cancel each other out the circuit performs a logically identical operation in the two species and the output remains three cell types (Figure 1).

When genomic data from other yeasts is used to put these observations in a phylogenetic context [5,7], it is clear that the C. albicans circuit is the ancestral one and that in S. cerevisiae α2 has acquired a new, negative regulatory function to compensate for the loss of α2. This rewiring of MAT-locus function occurred in the Saccharomyces lineage of yeasts soon after they had acquired the Ho endonuclease that allows haploid cells to switch mating types efficiently [7]. In fact, comparisons between yeasts and filamentous ascomycetes such as Neurospora suggest that the MAT locus remained in the same chromosomal location (beside the SLA2 gene) for eons while its gene content underwent several changes [7].

In addition to the evolutionary changes in the repertoire and function of regulatory proteins, we can safely infer that there have been multiple instances of change in the cis elements that these proteins recognize. In the a-specific genes of S. cerevisiae many cis elements that were previously acted on by the a2 protein must have been lost, and multiple sites for repression by a2 must have been gained. For example, the RAM2 and STE6 genes, which encode proteins involved in farnesylation and secretion of a-factor pheromone, respectively, have shifted from being activated by a2 (as in C. albicans) to being repressed by a2 (as in S. cerevisiae) [5]. At present we do not know whether this shift occurred by co-opting new cis elements, or by retaining the same cis elements but changing the proteins that bind them. It will therefore be interesting to determine the DNA-binding specificity of C. albicans a2.

Tsong et al. [5] also used microarrays to dissect the transcriptional consequences of the phenotypic switch from white to opaque. In S. cerevisiae, the a1/a2 dimer, which is only present in heterozygous diploids, represses 20-30 genes associated with mating. By contrast, C. albicans this dimer represses only seven genes directly but approximately 400 others indirectly through control of the white to opaque morphological switch. The functions of these 400 genes are not restricted to involvement in mating, but also include aspects of the pathogenic lifestyle of C. albicans. Again, the phylogenetic context suggests an interpretation: during the evolution of virulence in C. albicans (a derived characteristic of this lineage), these 400 genes were brought under the control of a much older genetic circuit, MTL. The question of how hundreds of genes could be marshaled by a new regulator is an interesting one. The evidence suggests that it happened as an indirect consequence of changing a small number of direct targets of the a1/a2 heterodimer. This kind of parsimony in rewiring genetic circuits has also been observed in artificial evolution experiments in S. cerevisiae. Following 500 generations of glucose-limited growth, Ferea et al. [8] observed that many genes involved in energy pathways and carbohydrate metabolism were coordinately up- or down-regulated. Correlated changes in the expression profiles of known regulators of these genes support the idea that, rather than selection for multiple independent downstream changes, a few changes further upstream in the regulatory hierarchy are responsible.

The logic of the MAT locus circuit discussed above shares many similarities with the genetic circuit underlying wing polyphenism in ants. Polyphenism is the ability of a genome to produce different phenotypes in response to different external signals, as dramatically illustrated by winged queen ants and their wingless worker sisters. In ants, polyphenism is achieved by interrupting, in workers, the genetic circuit that would otherwise execute a program of wing patterning [9]. The circuit is believed to have evolved only once, and is conserved among insect species (including Drosophila) that
last shared a common ancestor over 300 million years ago. In spite of this, Abouheif and Wray [9] found that the point at which the program is interrupted is different in different ant species, and is labile over periods as short as 20 million years. As in the MAT locus, the output phenotype (two states of 'wingedness'; three states of 'yeastness') has been conserved across species, but the details of how the regulatory circuits in each species achieve the conserved outputs are different.

Where does this leave us? It is becoming clear that in addition to studies aimed at dissecting the evolutionary conservation of cis elements [10-12], we need many more studies in the style of Tsong et al. [5] - detailed analyses of real genetic circuits over appropriate evolutionary distances, where both cis- and trans-acting factors can change (see also Hinman et al. [13]). The level of detail is also going to be very important. For instance, a recent analysis of the lac operon shows that, contrary to what has been believed for the last 50 years, it has not evolved to permit a smooth transition from fully repressed to fully expressed; instead, the circuit encodes four distinct plateaus and thresholds [14]. It will be vital to have such accurate information in order to be able to determine whether or not a genetic circuit is truly conserved, and to peer a little more closely into the transcriptional engine of the cell.

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