Dispatches

Transcription: A Mechanism for Short-Term Memory

Yeast growing for a considerable time in glucose 'remember' a previous exposure to galactose, the inducer of its galactose-utilization (GAL) genes. This memory is conveyed by a cytoplasmically transmitted galactokinase working as a signal transducer.

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There is a Lamarkian overtone to epigenetics: a cell, perhaps as instructed by an environmental signal, undergoes a change of state, and a 'memory' of this event is passed on to its descendents. Crucially, no mutation is involved in the initial change in state or its transmission. It is obvious (is it not?) that cells of a complex developing organism undergo many such events [1]. And so the question arises: is there a unifying mechanism involved? Classical examples immediately indicate that the answer is no.

For example, inheritance of lambda lysogeny in bacteria involves transmission in the cytoplasm of a protein that binds DNA and perpetuates its own synthesis by activating transcription of its own gene. In contrast, prion transmission, although self-perpetuating, does not involve gene regulation at all. Even were we to restrict the discussion to gene regulation we would see crucial differences. X chromosome inactivation, in mammals, for example, affects only one of the two female chromosomes, and the identity of the inactivated chromosome is perpetuated (by an unknown mechanism) through cell division (a so called '*cis*' effect). In contrast, in the lambda example, should there be multiple copies of the target gene, all will be activated as they are recognized by the cytoplasmically transmitted self-activator. The latter kind of '*trans*-acting' feedback loop is mirrored by many examples in higher eukaryotes, including heart and muscle development, as well as in yeast (see for example [2]).

As reported recently in *Current Biology*, Zacharioudakis *et al.* [3] have now shown how, in the yeast *Saccharomyces cerevisiae*, the effect of a transient biological signal is 'remembered' for some six or seven generations. Here, as in the lambda and prion examples, a cytoplasmically transmitted protein is involved. But, as we shall see, when working as a memory determinant, the transmitted protein engages in a non-enzymatic signal transduction reaction. And unlike in the lambda and prion cases, here the protein does not self perpetuate, and the cells lose the memory as the protein is diluted away.

The phenomenon under study is realized when the usual procedure for inducing transcription of yeast's GAL genes is modified. Thus, most simply, transcription of the GAL genes is strongly induced — some 1000-fold for the galactokinase gene *Gal1* — when cells are transferred to galactose-containing medium. But, if cells are grown first in galactose, then transferred to medium lacking galactose but containing glucose, and then transferred back to galactose, their GAL genes are induced much more quickly than if they had not had that recent experience of growing in galactose. In other words, the cells 'remember' — for some six or seven generations of growth in glucose — having previously seen galactose [3–5]. The explanation for how this works builds on many years of previous work on the GAL regulatory system.

Galactose induces the GAL genes in the following way. The sugar enters the cell and binds, along with ATP, to the protein Gal3 (typically expressed at low levels even in the absence of galactose). That complex, in turn, binds to and inactivates, perhaps by sequestration to the cytoplasm, the protein Gal80 [6]. Gal80 is an inhibitor of the activator Gal4; it covers Gal4's activating region and thus, while not interfering with Gal4's ability to bind DNA, prevents Gal4 from working. Once freed from Gal80 by the Gal3–galactose complex, Gal4 activates transcription of various GAL genes (including *Gal3*, thereby initiating an auto-catalytic feedback loop). As this description implies, cells deleted for *Gal3* do not, over short time periods, induce the GAL genes when exposed to galactose; however, such cells do induce the GAL genes very slowly, finally expressing these genes to the same level as would wild-type cells. As explained in the next paragraph, analysis of this 'slow induction' effect, taken with other results, reveals that the galactokinase Gal1 can also perform, albeit weakly, the anti-Gal80 function of Gal3. And as we shall see later, this fact is crucial to understanding the memory effect analyzed by Zacharioudakis *et al.* [3].

Cells deleted for *Gal3* do not undergo even slow induction of the remaining GAL genes if the *Gal1* gene is also deleted. *Vice versa*, overexpression of *Gal1* from a heterologous promoter renders cells quickly inducible in the absence of Gal3. Gal1 and Gal3, despite their apparently disparate functions — one an enzyme that helps metabolize galactose, the other a transducer of the galactose signal — are close relatives: 70% of their amino acids are identical, and addition of just two amino acids (a serine and an alanine immediately following amino acid 164) to Gal3 confers upon that protein the enzymatic function of Gal1. These findings, taken together, suggested that Gal1 can perform, at least weakly, the

anti-Gal80 function ordinarily performed by Gal3. Indeed, *in vitro*, just as Gal3 can form a complex with Gal80 (plus ATP and galactose), so can Gal1, albeit with a lower efficiency [7–9]. Now back to Zacharioudakis *et al.* [3].

The crucial experiment was as follows. Cells growing in galactose were transferred to glucose, allowed to undergo about six or seven divisions, and then mated with cells that bore a *Gal1–GFP* fusion gene, and that had not recently been exposed to galactose. Galactose was added and GFP production detected by flourescent microscopy of mating pairs. The first striking result was that induction of the *Gal1–GFP* fusion gene occurred much more quickly than in a parallel experiment where the cells lacking that gene had not been pre-gown in galactose. In other words, the memory was transmitted through the cytoplasm where it worked in '*trans*' on the *Gal1–GFP* fusion gene. The 'remembered' activating effect did not even require fusion of the nuclei of the mating pairs: Zacharioudakis *et al.* [3] used a mutant yeast in which such fusion does not occur (as it otherwise would), and heterokaryons, easily recognized in the microscope, were formed instead.

What factor is transmitted in the cytoplasm that confers the memory effect? Zacharioudakis *et al.* [3] first showed that it is not Gal3: cells growing in galactose, but lacking Gal3, still 'remember' that experience after subsequent growth in glucose as evidenced by their quick induction of the GAL genes upon exposure to galactose. But, using suitable mutants, they confirmed previous suggestions that Gal1, a protein highly expressed in galactose, is required for the effect. Moreover, constitutive high level expression of *Gal1* (from a heterologous promoter) imposed the 'quick induction' phenotype on cells transferred directly from growth on glucose to galactose (and thus with no 'instructional' phase of growth on galactose). These experiments were performed not by flourescent microscopy of colonies on a plate, but rather by FACS analysis of cells in solution, a set of experiments that demonstrate by an independent method that the memory effect can be visualized in individual cells.

The picture that emerges, then, is that growth in galactose causes expression to a high level of Gal1. Enough of this protein is then distributed to some six or seven generations' worth of progeny cells produced in glucose so that, thanks to the Gal3-like activity of Gal1, those progeny cells induce quickly in response to galactose. Gal3 cannot confer memory simply because it is produced at a much lower level than Gal1, and so is quickly diluted out as the cells begin to divide. The information in the following paragraph, while not crucial for understanding the basic points reviewed here, extends our picture of induction of the GAL genes.

The close relationship, in sequence and function, between Gal1 and Gal3 suggests they have evolved from a common precursor. And, indeed, in the yeast *Kluyveromyces lactis*, which lacks the genome duplication that occurred in an ancestor of *S. cerevisiae*, a single gene performs both the galactokinase and inducer-transmitting functions that are performed separately by the products of *Gal1* and *Gal3* in *S. cerevisiae*. The promoters of the *S. cerevisiae Gal1* and *Gal3* genes have diverged — particularly in the dispositions of the Gal4-binding sites in their promoters — so that when grown in the 'neutral' sugar raffinose, the *Gal3* gene is expressed to a low but significant level (as described earlier) and *Gal1* is more efficiently repressed. Upon induction by galactose, expression of *Gal3* rises moderately and that of *Gal1* dramatically. Thus, evolution took advantage of the whole genome duplication to adjust the uninduced and induced levels of these two proteins in accordance with their functions [10]. The careful reader will note, however, that all of the experiments of Zacharioudakis *et al.* [3] used, as the uninduced condition, growth in glucose, not raffinose. When grown in glucose, the preferred carbon source, the *Gal3* gene is also strongly repressed (part of the 'glucose repression' effect). Induction is slower in this case (hours instead of minutes) and the cells induce heterogeneously instead of uniformly. Ectopic expression of *Gal3* (or of *Gal1* to a higher level) speeds up induction and renders it uniform.

As implied by our opening paragraphs, the epigenetic examples we best understand involve cytoplasmically transmitted proteins that, either by activating transcription of their own genes (lambda, for example), or by converting wild-type proteins into mutant forms (prions), perpetuate their own production. In the example of Zacharioudakis *et al.* [3], we see an example of a cytoplasmically transmitted protein that does not self-perpetuate itself, and so the life span of the memory, though considerable, is necessarily limited. The authors point out that the effect they analyze had previously been ascribed to 'chromatin effects'. Perhaps this is not surprising: epigenetic changes are often *defined* as changes in chromatin modifications. Here are two recent examples: "Epigenetics refers to DNA and chromatin modifications that influence chromatin structure and change the state of gene expression …" [11]. "The following could be a unifying definition of epigenetic events: the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" [12]. Such definitions would exclude all cytoplasmically transmitted epigenetic changes, including the lambda and prion cases, as well as the yeast example analyzed here. And there is no compelling evidence that nucleosome modifications are, per se, heritable [13]. It would seem that each clearly defined epigenetic event will have to be understood in its own right. And it would not be surprising were each such example to require detailed background information and incisive experimentation such as are found in the paper of Zacharioudakis *et al.* [3].

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Evolution: Convergent Eye Losses in Fishy Circumstances

Eye loss has occurred independently several times in Mexican cavefish. A new study shows that some aspects of vision can be restored by crossing cavefish from different populations, suggesting that changes at multiple loci contribute to eye loss.

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The animals that inhabit caves have fascinated biologists for over 150 years [1]. Cave ecosystems are often isolated from surface ecosystems and strongly energy limited [2] — a combination of factors that produces depauperate ecosystems, which have much in common with island communities. One of the most striking features of caves is surely their darkness — from twilight at the cave mouth to a profound darkness deeper within. The total absence of light makes the eyes of animals living deep within caves redundant and as a consequence many of these animals have lost eye pigmentation, often accompanied by a marked reduction in eye size or even total eye loss [2,3]. Although eye reduction or loss occurs in numerous cave-dwelling animals, including insects and crustaceans [2–4], its evolution and development has been studied most extensively in the Mexican blind cavefish, *Astyanax mexicanus* (Figure 1) [5–9]. These fish are particularly attractive for studying evolution because there are several independent cave populations, which entered caves independently over the past 1,000,000 years. Moreover, descendants of the surface populations from which they arose are still living today and can form fertile hybrids with the cavefish [5–7].

Several recent studies, for instance [5,7], have taken advantage of this ability to produce fertile hybrids between different populations of *A. mexicanus* (= *A. fasciatus*). Hybrids from crosses between cave and surface fish have enabled the identification of twelve quantitative trait loci (QTL) for eye or lens production in populations of *A. mexicanus* [5]. The latest study, published in this issue of *Current Biology* [9], shows that complementation between these loci in different cave populations is sufficient to restore vision in *A. mexicanus*.

Crosses between individuals from surface and cave populations produced progeny capable of responding to a simple behavioural assay of visual function — the optokinetic response — in which the fish's eyes follow dark stripes

Figure 1. Out of sight.

A Mexican blind cavefish (*Astyanax mexicanus*) from the Pachón cave, accompanied by two surface morphs. Image courtesy of Richard Borowsky.