Lambda's Switch: Lessons from a Module Swap

A recent experiment has replaced Cro, a crucial component of lambda's genetic switch, with the lac repressor (plus two *lac* operators). The resulting hybrid phage is viable, but a subtle phenotypic defect explains a puzzle concerning the workings of the switch.

Mark Ptashne

The evolution of complexity, modularity, and systems biology — all of these matters come to mind when reading Atsumi and Little's [1] recent analysis of phage lambda's 'genetic switch'. The switch, which I will describe in a bit more detail below, comprises an integrated set of simple protein–protein and protein–DNA interactions, all of which have been extensively characterized and quantified. A circuit diagram describing these interactions, a part of which is illustrated in Figure 1, has been at hand for some time (reviewed in [2]).

Lambda's switch might strike one as 'irreducibly complex' — perhaps taking away any part of it would be disasterous. But, as shown by Atsumi and Little [1], and by previous work primarily from the same lab [3], various parts of the switch can be removed without totally destroying its function. It seems that evolution can start with a crude version of a switch and, by adding parts seriatum, make the switch work incrementally better, just as Darwin would have liked. The modularity of the switch — which might well have been obscured by later evolutionary modifications — has been so well conserved that, as shown here, one control element can be removed and replaced with a heterologous one without impairing switch function. And — the main point of the new paper under discussion [1] — this hybrid switch can be used to address a thorny question concerning the physiological significance of one of the reactions of the switch. It is sobering to realize that, despite all our knowledge about the system, we evidently cannot calculate its behavior very precisely, and outstanding questions have to be addressed by sophisticated experimentation such as that reviewed here.

To understand these matters we need a brief overview of the switch in action. Consider, to begin with, the lambda phage repressor, the DNA-binding protein that, in a lysogen, turns off transcription of lytic phage genes. The repressor also controls expression of its own gene (*cI*), both positively and negatively. The combination of these effects maintains the concentration of repressor at the right level so as to poise the lysogen to respond to an inducing signal such as UV irradiation. That signal induces lytic phage growth by destroying the repressor, and as lytic genes are turned on the repressor gene is turned off. The switch is amazingly efficient: absent an inducing agent, a lysogen is stable for countless generations, and yet, upon receiving such a signal, in virtually every cell of the population lytic growth of the previously dormant phage ensues [2,4].

These regulatory events are effected by protein–protein and protein–DNA binding reactions shown in part in Figure 1A. In a lysogen, repressor bound to sites O_{R1} and O_{R2} in the right operator (O_R) activates transcription of its own gene by contacting RNA polymerase and thereby recruiting it to the adjacent promoter (P_{RM}) of the repressor gene (*cI*). These DNA-bound repressors simultaneously exclude polymerase from the lytic promoter P_R. When bound to the weaker site O_{R3} repressor turns off transcription of *cI* by excluding RNA polymerase from its promoter. In mutants lacking this auto-negative control, the level of *cI* expression is elevated some two-three-fold. This small increase is sufficient to significantly impair induction, a fact that becomes important later in this story [4,5]. Omitted from Figure 1 is a second operator–promoter sequence (O_L, P_L) positioned some 2400 base pairs away. Interactions between repressors bound simultaneously to sites in O_L and O_R (with concomitant DNA looping) aid the reactions shown here, and are particularly important for the binding of repressor to O_{R3} [6]. Cooperative binding of repressor subunits to DNA is mediated by discrete contacts between repressor molecules [7,8], just as the activation of *cI* transcription by repressor is mediated by a specific contact between repressor and polymerase [9].

One might have guessed that this rather elaborate machinery — built as we have indicated from simple parts — would suffice to make a good switch. But we have long known that there is another key player: the protein called Cro, the gene for which lies adjacent to, and is transcribed in the opposite direction from, *cI* (Figure 1B). The *cro* gene, silent in a lysogen, is one of the first to be transcribed upon induction, and its activity is required for efficient lytic growth. One essential function of Cro is to turn down expression of lytic genes (including itself) that are expressed at a high level immediately following induction. It does this by binding to O_{R1} and O_{R2}, the same sites recognized by repressor [2]. One of the early genes turned off (or down) by Cro encodes a protein, CII, that initiates transcription of *cI*, which then (as mentioned above) becomes self-sustaining, a classic epigenetic event. Thus, Cro indirectly discourages the establishment of repressor synthesis and thereby encourages the phage to enter the lytic cycle after UV irradiation of a lambda lysogen. This picture is extensively supported by genetic and physiological experiments (see, for example, [10]).

But perhaps — and as suggested early on [11] — Cro has another role too: by binding to O_{R3}, Cro would turn off transcription of *cI* directly — just as repressor binding

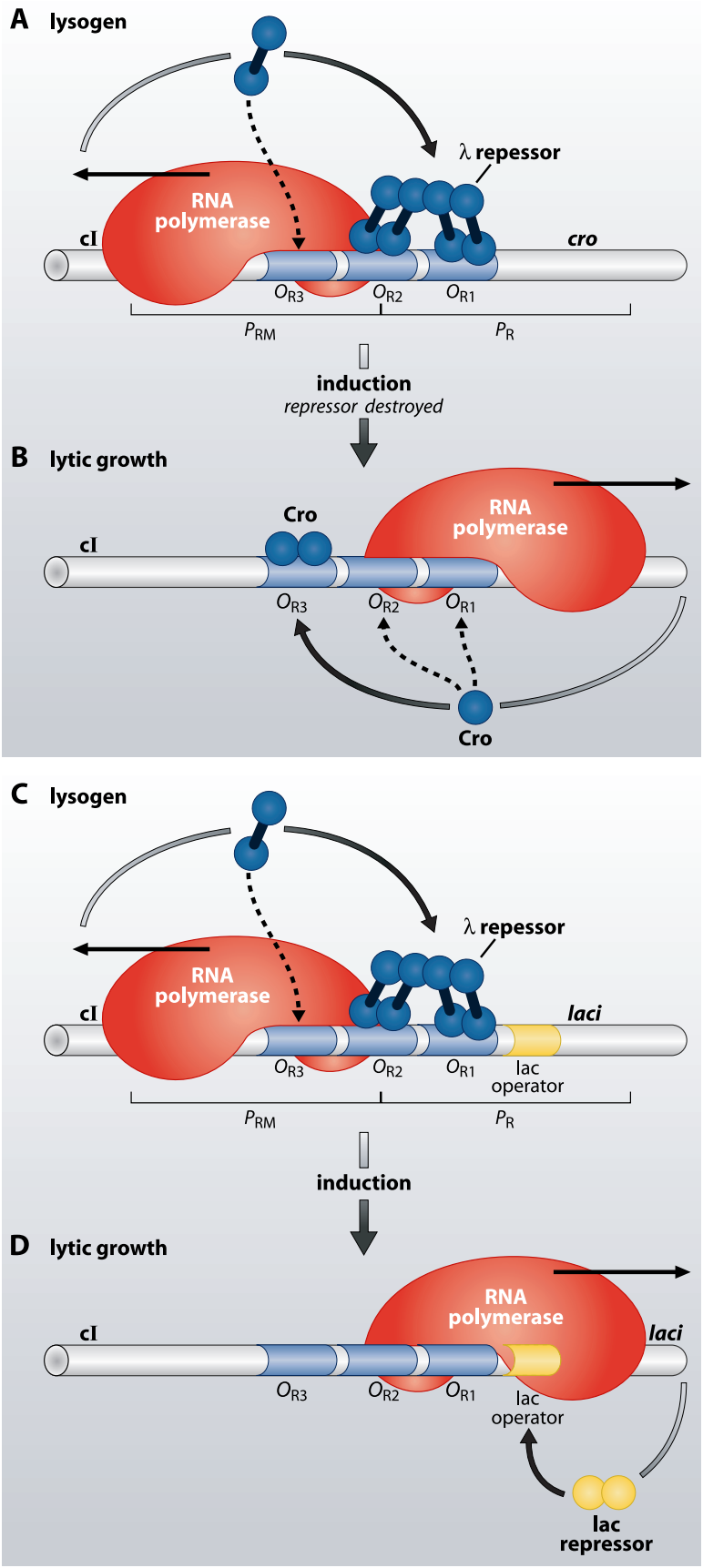

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Figure 1. The state of lambda's right operator (O_R) in a wild-type lysogen.

(A) Two repressor dimers are bound cooperatively to sites O_{R1} and O_{R2} on DNA (solid arrows). The repressor at O_{R2} has — by virtue of a discrete protein–protein contact — recruited RNA polymerase to the promoter (P_{RM}) of the adjacent repressor gene (*cI*). The bound repressors also exclude polymerase from the other flanking promoter (P_R), thereby keeping lytic genes off. With a lower affinity, repressor binds O_{R3} (dashed arrow) and turns off transcription of *cI*. A second promoter of lytic genes (P_L) is positioned some 2400 base pairs away, and interactions between repressors binding to O_L and O_R aid in these reactions (especially the binding of repressor to O_{R3}). (B) The action of Cro upon induction. As repressor is destroyed upon induction, transcription of *cI* falls (because of loss of autostimulation), and rightwards transcription of lytic genes ensues. Cro is expected to bind most avidly to O_{R3} (solid arrow), thereby directly repressing repressor synthesis — the relevance of this reaction is one subject of the paper under discussion. Eventually Cro binds to sites O_{R2} and O_{R1} to turn down transcription of early genes (dashed lines). Amongst the genes turned down in this fashion are Cro itself and *cII*, a transcriptional activator that, to establish lysogeny, initiates transient transcription of *cI* from a promoter to the right of Cro in the figure (not shown). (C) The state of the right operator in a lysogen bearing the hybrid phage of Atsumi and Little [1]. The Cro gene has been replaced with the gene encoding lac repressor (*lacI*), and a *lac* operator has been inserted just to the right of O_R, but within the Pr promoter. Another *lac* operator has been inserted at the distal O_L operator (not shown). The *lacI* gene in this hybrid, like Cro in the wild-type phage, is silent in a lysogen. (D) The action of lac repressor upon induction. Lac repressor binds to the *lac* operator to repress transcription from P_R. The repressor also binds to the second *lac* operator to repress transcription from P_L (not shown). There is no *lac* operator in place of O_{R3}, and so lac repressor cannot repress P_{RM}.

to that site turns off *cI* transcription. As O_{R3} is the site in O_R with the highest affinity for Cro, that effect, plausibly, would be the initial consequence of production of Cro. Is this reaction important in throwing the switch as the phage enters lytic growth? Or, alternatively, might direct inactivation of repressor by the inducing agent, and the consequent loss of cI autoactivation, suffice to keep the level of repressor low enough to allow lytic growth? The straightforward way to distinguish between these scenarios would be to make a lysogen with a phage bearing a mutation in O_{R3} that does not bind Cro at that site. If Cro binding to O_{R3} were important for induction, then such a mutant lysogen would induce only poorly.

The problem facing Atsumi and Little [1] was that every tested mutation in O_{R3} that diminished Cro binding also diminished repressor binding to that site [4,5] (but see below for the latest news on this, which came in while this piece was being finalized for production). And, as mentioned above, if repressor cannot bind to OR3, the consequent overproduction of repressor (a two–three-fold effect) is sufficient to impede induction. And so the question remained: does Cro binding to O_{R3} play any role in the transition from lysogenic to lytic growth upon induction of a lysogen? (For an experiment suggesting it might not, see [12].)

Atsumi and Little [1] addressed this problem by first constructing a hybrid phage bearing the lac repressor in place of Cro (Figure 1C). The hybrid phage was also modified so as to bear *lac* operators just downstream of P_L and P_R, leaving the lambda operators in their usual place. In this configuration, each lytic promoter can be repressed by either the lambda or the lac repressor. Thus, in a lysogen, lambda repressor should act as usual, repressing lytic genes and auto-regulating its own gene. During lytic growth, lac repressor (in place of Cro) should bind the lac operators and turn down transcription of lytic genes.

This conceptually straightforward enterprise was not so easy to realize in practice. Even though the affinities of the two repressors (Cro and lac repressor) for their corresponding wild-type and mutant operator sites have been extensively characterized, there is no way to be confident that any specified level of lac repressor working on any specified lac operator variant will mimic Cro's action *in vivo*. And so Atsumi and Little [1] constructed phage libraries bearing an array of *lac* operator sequences (with varying affinities for lac repressor) at both P_L and P_R; and an array of Shine-Delgarno sequences (which determine the efficiency of translation of the mRNA) in the *lac* repressor gene. They looked among these constructs for plaque formers, testing their constructs at a range of different concentrations of IPTG, a molecule that inactivates lac repressor. And to eliminate needless complications, they used a mutant form of lac repressor that, unlike the wild type, forms dimers but not tetramers. Unlike the wild type, a single molecule of this mutant (a dimer in this case) cannot simultaneously bind to separated DNA sites.

Atsumi and Little [1] found that at least one of the hybrid phages grows lytically and forms lysogens, and those lysogens can be induced to produce progeny, a gratifying result. As shown by various experiments, including noting the lethal effect of inactivating lac repressor with high concentrations of IPTG, lac repressor is performing Cro's critical function of turning down expression of lytic genes by binding to the introduced lac operators. But, because this phage has no *lac* operator in place of O_{R3}, the result strongly suggests that binding of a repressor (Cro or lac) to O_{R3} — the issue raised above — is not absolutely required for lytic growth.

Atsumi and Little [1] took the matter one step further, by showing that higher levels of UV irradiation are required to induce lysogens of the hybrid than are required to induce wild-type lysogens. And so, evidently, binding of Cro to O_{R3}, while not required for that process, does improve the efficiency of induction. The suggested picture is that at a UV dose sufficient to induce say 50% of the lysogens (the 'set point'), the cells are poised to go one way or the other (induce or remain as lysogens) with equal frequency. The higher set point for lysogens of the hybrid phage suggests that Cro binding to O_{R3} helps push the decision to lysis, but this effect can be dispensed with at higher UV doses.

This conclusion has a familiar ring to it: previous work from the Little lab [3,13] has shown that various modifications to the switch, while not inactivating it, do impair its efficiency. For example, eliminating, by mutation, the ability of lambda repressor to activate transcription of its own gene produces a phage that grows lytically very well and even lysogenizes — but the lysogens are less stable (probably because insufficient repressor is made) than are wild-type lysogens. Lambda's switch seems to have evolved from some elemental state by a series of add-ons, each of which improves its function, perhaps as suggested in [2,14].

As this dispatch was going to press, a mutant O_{R3} (bearing three base changes) was described that cannot bind Cro, but binds repressor with nearly normal affinity (K. Shearwin, I. Dodd, R. Schubert and B. Egan, personal communication). Phage bearing the mutant O_{R3} grow lytically and form lysogens, but the efficiency of induction of those lysogens is decreased compared to wild type. The conclusion fits nicely with that of Atsumi and Little [1]: Cro binding to O_{R3} is not essential for induction, but it makes that process more efficient.

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Evolution: The Paradox of Sperm Leviathans

Sexual selection theory predicts that sperm competition will push males to produce more, smaller sperm. Paradoxically, in the fruitfly *Drosophila bifurca* sperm competition is rife but males produce few, giant sperm — the largest known. A recent study reconciles the evolution of giant sperm with theory.

Tommaso Pizzari

The evolution of sexual reproduction typically leads to frequency-dependent disruptive selection on gamete size and numbers, promoting two strategies: large eggs that nurture and protect the embryo and are little mobile; and tiny, mobile, self-propelled, DNA-delivering sperm, which are able to seek out and fertilize eggs [1]. Such sex-specific differential investment in gametes is called anisogamy, and sets the scene for the way sexual selection operates [2–4]. Males, which produce far more sperm than there are eggs available, have a higher potential reproductive rate than females. This means that male reproductive success will be more variable than female reproductive success, leading to more intense sexual selection on males than on females.

In 1948, Bateman [5] demonstrated the implications of anisogamy through an elegant experiment in the fruitfly *Drosophila melanogaster*. Bateman showed that male reproductive success increases with the number of females with whom a male copulates, whereas female reproductive success is largely independent of her re-mating rates. These results indicated that the main reason for why male fitness is more variable than female fitness is that males vary more than females in the number of partners, leading to more intense sexual selection on male re-mating rates.

Anisogamy may be further increased by the fact that, in many species, females mate with multiple males — they are polyandrous — and the ejaculates of different males compete over fertilization [6,7]. Here, sexual selection continues after insemination through sperm competition, and because larger ejaculates tend to have a fertilizing advantage [8,9], and a trade-off exists between sperm number and size [10,11], males are sexually selected to produce numerous, tiny sperm [10].

There is widespread support for this theoretical prediction. In a number of taxa, males of polyandrous species, where sperm competition is intense, invest a larger proportion of their body mass in testes to produce sperm at a faster rate than males of related monandrous species [7,12–14]. With this in mind, it would seem paradoxical that the largest known sperm in the animal kingdom are found in the tiny males of a polyandrous fly where sexual selection and sperm competition appear rife. Males of the fruitfly *Drosophila bifurca* produce very few, giant sperm that are just under six centimetres long [15] (Figure 1). How did this extreme, female-like gametic strategy evolve in a species where sexual selection appears intense?

In a monumental recent study, Adam Bjork and Scott Pitnick [16] set out to unravel the evolutionary paradox of giant sperm using a two-pronged approach. First, they capitalised on the high diversity of male investment in sperm size and numbers across *Drosophila* species, and replicated Bateman's classic experiment on four different *Drosophila* species which vary markedly in sperm size: *D. melanogaster*, *D. virilis*, *D. lummei* and *D. bifurca*. *D. melanogaster* have relatively small sperm (1.87 millimetres) and are anisogamous, whereas at the opposite end of the gradient, *D. bifurca* is as close as *Drosophila* – or any known metazoan species – get to an equal investment per male and female gamete (isogamy). Second, the authors were able to replicate the study in lines of *D. melanogaster* that were experimentally and divergently selected for long and short sperm, which enabled them to study the causal relationship between sperm size and sexual