the mechanisms by which buds arise and differentiate. Recent work has shown that in most species each reiterated sequence — rather than each zooid — arises as a single bud within the growth zone and then gives rise to multiple zooids through subdivision. Siphonophores also have complex symmetry properties that deviate from the simple radial symmetry usually associated with cnidarians. The colonies can even be directionally asymmetric, with some structures consistently displaced to one side or the other just as our own heart is usually displaced towards the left sides of our body.

What is it like to work with siphonophores? Siphonophores are a joy to study. When reading about some aspect of their biology, one is just as likely to reach for a mid-19th century monograph or a paper that came out in the last year. So many questions are wide open — some requiring the same tools and approaches as the naturalists of the Age of Exploration, others necessitating modern high-throughput sequencing technologies. When collecting deep-sea specimens with submersibles, many of the acquired siphonophores are often undescribed species. Rarely do biologists have such an excellent opportunity to pull together such disparate tools in the pursuit of core conceptual questions. In addition, one siphonophore species has been cultivated through its full life cycle in the lab, while others can routinely be collected in the field. Expressed sequence tag (EST) libraries are currently under development that will enable analyses of colony development at the molecular level.

Where can I find out more?

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Essay

Binding reactions: epigenetic switches, signal transduction and cancer

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Simple binding interactions lie at the heart of disparate biological functions. Multiple negative and positive ‘add-ons’, often with small individual effects, make elementary systems that work, work better. Cancer illustrates various of these fundamental processes gone awry.

Molecular biology continues to explode with new facts and details along with the occasional surprise. There is, I believe, an unexpected bonus: a few basic principles underlie many complex processes — signal transduction, gene expression, the maintenance or destruction of gene products, the construction of epigenetic switches, and so on. In some human diseases — cancer, for example — these processes go awry, and a conceptualization of the underlying strategies helps us understand how that can happen. Here I emphasize nature’s reiterated use of the simplest of reactions: *binding*.

By binding, I mean the non-covalent interactions of macromolecules: proteins with other proteins, DNA, RNA, or membranes; of RNA with DNA, and so on. The typical interaction I refer to is reversible under physiological conditions, and its essential function is apposition, bringing one macromolecule in contact with another. In this essay I discuss a few examples of how binding reactions are deployed to different ends. Molecular details differ, but similar general strategies are found at work in these systems. The essentials are illustrated by the workings of an epigenetic switch in bacteria, my starting example.

An epigenetic switch: lessons from lambda

The bacteriophage lambda switch ensures that when one set of genes (those for lysogenic growth) are on, another set (the genes for lytic growth) are off, and *vice versa*. Once the repressor gene (*cI*) is switched on (Figure 1, left) and the lysogenic state established, that pattern of the gene expression is self-perpetuated for many bacterial divisions. The switch can be flipped by an environmental signal — such as UV light — but none of the operations of the switch entails a change in DNA sequence. Rather, the switch comprises a set of binding reactions involving two DNA-binding regulatory proteins (repressor and cro), the enzyme RNA polymerase and DNA. Here are some further salient points describing, or inferred from, the switch. These matters, as well as certain others discussed later in this article and not explicitly referenced, have been discussed previously [1,2].

- *Epigenetics.* The self-perpetuating (and hence epigenetic) character of the switch is not an inherent property of any of its components, but rather is a property of the system conferred by the pattern of binding reactions. There are two ways to make epigenetic switches, and lambda’s switch includes both: a double-negative loop, in which the product of one gene (repressor) turns off expression of the other gene (*cro*) and vice versa; and a positive feedback loop, in which repressor (despite its name) activates transcription of its own gene. The original name my colleagues and I gave to this switch — we called it a ‘genetic’ switch — is misleading because, as just mentioned, there is no change in DNA sequence involved [3]. Epigenetic switches comprising lambda-like components are found in many developmental pathways in eukaryotes.
- *Cooperativity.* The switch requires that proteins bind specifically to sites on DNA. For example, a lysogen repressor must bind to its designated sites in DNA and, more precisely, it must bind predominantly to two of three such sites as shown in Figure 1, on the left. This specificity

is facilitated by cooperativity: two repressor dimers touch (bind) each other as shown, each thereby helping the other bind, and to bind specifically. All binding reactions of the sort discussed in this article face the specificity problem, and cooperativity is widely used to help solve the problem (see appendix one in [1]). I return below to a further role of cooperativity in the lambda switch.

- *Concentration control*. The individual DNA sites in Figure 1 differ only modestly in their affinities for repressor — about tenfold. And so site selectivity tends to be rather readily lost as the concentration of repressor increases. Not to worry: the switch has a 'governor' in the form of another binding reaction — as the repressor concentration increases it tends to turn off transcription of its own gene by binding to the third (lower affinity) site, as shown by the downward arrow in Figure 1, on the left. The binding reactions referred to in this article require that binding domains distinguish between related possible targets. These kinds of interactions risk losing specificity as concentrations increase.
- *Activation of transcription – the imposition of specificity by recruitment.* Lambda repressor works as an activator of transcription in another binding reaction: it simultaneously contacts DNA and RNA polymerase (as shown in Figure 1), thereby recruiting the polymerase to the adjacent promoter. Transcription of the gene is 'activated' — that is, the gene is transcribed at a higher level than it otherwise would be. The gene activated by repressor is the repressor-encoding *cI* gene itself and so, by this positive feedback loop, continuous production of repressor is ensured as these lysogenic cells divide.

We say that polymerase has been given specificity — has been instructed to transcribe a particular gene, the *cI* gene — by this recruiting reaction. The effect is modest (increasing the level of transcription some 10–50-fold) and a potentially significant level of transcription will occur in the absence of the activator. When repressor is destroyed and lysogens induced, cro, the DNA-binding protein produced early upon induction, suppresses this basal transcription as shown on the right in Figure 1.

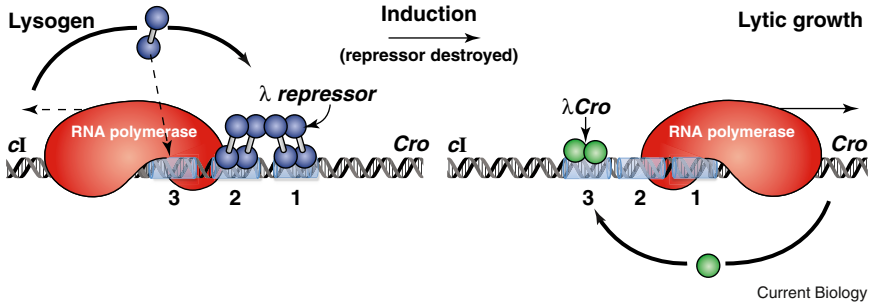

Figure 1. The lambda epigenetic switch.

Two states of the switch are shown: on the left the repressor gene (*cI*) is transcribed but the *Cro* gene is not, and *vice versa* on the right. The scenario on the left is found in lambda lysogens, bacteria that carry an otherwise dormant phage lambda. Inactivation of repressor (induction) results in lytic growth of the phage, an early stage of which is shown on the right. Repressor and cro turn each other's genes off by blocking binding of RNA polymerase to the other's promoter: repressor covers the *Cro* gene promoter when bound at sites 1 and 2 as shown on the left, and cro covers the repressor-gene promoter when bound at site 3, as shown on the right. Repressor bound at sites 1 and 2 activates transcription of its own gene (*cI*), as it represses transcription of *Cro*. Repressor maintains its concentration below a specified level by binding, at higher concentrations, to site 3 (as indicated by the downwards arrow), and turning itself off. All of these effects — auto-activation and repression by repressor, and the opposing effects of repressor and cro — are effected by simple binding reactions with suitably adjusted binding constants. The figure indicates that the switch can be flipped by a dose of UV light which results indirectly in cleavage of repressor. An additional set of interactions involving repressors bound here and at a site some 2000 base pairs away has been omitted.

Many eukaryotic enzymes can, like bacterial RNA polymerase, work on any of a wide array of substrates (different genes in the case of RNA polymerase), and which is chosen, under any given set of conditions, is determined by recruitment, as in the example just discussed. These enzymes include, in addition to polymerases, proteases, ubiquitylators, RNA-splicing enzymes, kinases, phosphatases, transcriptional repressing complexes, nucleosome modifying enzymes, and so on. For example, an E2 ligase can add ubiquitin to many proteins, but the choice is dictated (for one class of E2s) by recruiters called F-box proteins. Each of these recruiters simultaneously binds a specific target protein and the enzymatic machinery, and thus imposes specificity on the enzyme. Ubiquitin is added and, in a further binding reaction, the modified protein interacts with a protease and is destroyed.

Recruiting reactions typically face the problem described for activation of transcription: in the absence of the recruiter there can be an unwanted basal level of activity, and we will see a variety of strategies employed to depress that basal activity.

- *Squelching and self-squelching.* Recruiting reactions are subject to two negative effects as the concentration of the recruiter increases. *Squelching*: an over-expressed transcriptional activator, as it activates its target genes, will tend to depress transcription of other genes. The effect is attributed to competition by activators (the recruiters in this case) for binding common sites on the transcriptional machinery. The effect has been observed in transcription experiments performed with yeast and mammalian cells. *Self-squelching*: At very high expression levels, a transcriptional activator ceases to activate even its designated target genes. The result is explained as follows: successful recruitment requires that a single recruiter (a transcriptional activator in this case) simultaneously contacts the transcriptional machinery and a specific DNA binding site. At very high activator concentrations, the machinery and the DNA site will tend to be occupied by separate copies of the activator, and recruitment will be blocked. The effect has been observed in transcription experiments performed in yeast, and in proteolysis experiments in mammalian cells in which the concentration of an E3 ligase (the recruiter in this case) was varied (Pengbo Zhou, personal communication).

For any given case the extent of squelching and self-squelching will