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Two "What If" Experiments

Commentary

Mark Ptashne*
Sloane Kettering Institute
New York, New York 10021

Three perspectives are better than one. And it has been over 15 years since the two papers under discussion were published—a long time to remember who was thinking what. So the three of us (Roger, Jun, and I) will have three separate goes at reconstructing the events.

For over 10 years, and through the early 1980s, our lab had concentrated on gene regulation in phage lambda. By 1985 we had clear ideas for how lambda regulatory proteins bind DNA and turn transcription on and off. Would these ideas help us to understand how eukaryotic transcriptional regulatory proteins work? Unlike in the lambda case, eukaryotic genes are wrapped around histones to form nucleosomes; the DNA is sequestered in a nucleus; and, in higher eukaryotes, transcriptional activators can work "at a distance," i.e., when bound to sequences many hundreds, or even thousands, of base pairs from the target gene.

It could have turned out, I guess, that the principles of gene regulation in higher organisms would turn out to be fundamentally different from those found in lambda. Perhaps a new mode of binding to DNA was required, for example; perhaps activation at a distance requires special functions not represented among the lambda regulators; and so on. I found these ideas unpleasant. And in any case, isn't it better to see how far what we know will take us than to assume new complexities?

We picked the simplest eukaryotic example we could find: the Gal (galactose utilization) genes of yeast, the organism introduced a few years earlier to the lab by Lenny Guarente, Sandy Johnson, Bob West, and Roger Yocum. Addition of galactose to growing cells dramatically induces transcription of the Gal genes, an effect mediated by a single transcriptional activator, Gal4. Gal4, we learned, binds to DNA some 250 base pairs away from its target genes (Giniger et al., 1985; West et al., 1984; Guarente et al., 1981). Did Gal4 recognize DNA in some new way, i.e., in some way not illustrated by the lambda regulatory proteins? How did activation at a distance, in this case 250 base pairs, work?

To understand our frame of mind (mine anyway), here is a brief summary of four key concepts extracted from the lambda studies as of 1986 (Ptashne, 1986).

- *DNA binding*. The lambda repressor binds without dramatically changing the structure of DNA. The protein is both an activator and a repressor of transcription, and so neither of these activities requires DNA conformational changes. Where a repressor binding site overlaps a promoter, repressor turns off transcription by excluding RNA polymerase.
- *Transcriptional activation*. Activation requires something else: a protein-protein interaction between repressor and polymerase molecules binding to adjacent sites. This surmise was prompted by the existence and properties of so-called pc (positive control) mutants of repressor. These mutants bind DNA normally but do not activate. The residues changed in these mutants lie on the surface of repressor that, according to model building, contacts polymerase binding to the adjacent promoter. We later called the region of repressor that contacts polymerase its activating region. (Guarente et al., 1982; Hochschild et al.,1983).
- *Cooperativity*. Repressors bind cooperatively to multiple operator sites on DNA. The effect is mediated by a simple binding reaction—a touch as we used to say—between the DNA binding repressors (Johnson et al., 1979). Gene activation could then be seen as an analogous reaction: contact between the activator and polymerase would mediate cooperative binding of the two proteins to adjacent sites on DNA (to an operator and promoter, respectively). In effect, the activator (in this case lambda repressor) would recruit the polymerase to the promoter.
- *Modularity*. The repressor comprises two domains. When separated, each domain retains its functions: the amino domain binds DNA and carries the activating region, and the carboxyl domain contains sites required for interactions between repressors. (Pabo et al., 1979; Sauer et al., 1979).

Roger had been on the scene observing many of these discoveries about lambda. His lambda lab mates included some formidable personalities: Keith Backman, Lenny Guarente, Ann Hochschild, Sandy Johnson, Tom Maniatis, Russ Maurer, Barabara Meyer, Carl Pabo, Bob Sauer, Robin Wharton, Cynthia Wolberger, Rick Bushman, Ann Astromoff, and others. But rather than joining this crowd (members of which came and left during Roger's extended stay), he toyed with another bacterial repressor (LexA, as he explains) until his big moment arrived.

Roger's experiment (Jun's, too) was of the "what if" variety. What if Gal4 worked just like lambda repressor (in its guise as an activator)? What if, that is, Gal4 binds DNA and, using an activating region, contacts the transcriptional machinery and thereby recruits it to the gene? What if Gal4 were modular, carrying different functions on different domains (in this case the DNA binding and activating regions)? And what if there were nothing special about eukaryotic DNA binding domains—perhaps even a simple bacterial DNA binding domain (in this case that of LexA) would work just fine in a eukaryotic cell?

These what ifs came together in the simple experiment: a fusion protein bearing the LexA DNA binding domain attached to a carboxyl fragment of Gal4 is a powerful activator in yeast (and, we now know, in higher eukaryotes as well). Specificity is determined by the DNA binding domain: the fusion protein works only on genes bearing LexA sites introduced nearby. The soon-to-follow paper by Keegan et al. (1986) showed that

*Correspondence: m-ptashne@ski.mskcc.org

Gal4's amino domain bound DNA but did not activate in the absence of the carboxyl portion of the protein.

Roger's experiment left open the possibility that gene activation in yeast was not effected by simple binding interactions as in the lambda case. Perhaps, for example, Gal4's activating region was an enzyme that modified (and thereby literally activated) polymerase prebound to DNA. Indeed, examples of such kinds of activating regions are known in bacteria (see Ptashne and Gann, 2002).

Enter Jun. He was spurred on (I think) by several new ideas in the air, including the finding (Griffith et al., 1986; Hochschild and Ptashne, 1986) that lambda repressor molecules (two dimers in this case) bind cooperatively to operator sites separated by up to some 200 base pairs, with the intervening DNA looping out to accommodate the reaction. What if even activation at a distance might be effected by a simple binding reaction? Sequence gazing, and an experiment by Hope and Struhl (1986), hinted that eukaryotic activating regions might resemble protein signal sequences—many different sequences, all bearing some common (if poorly defined) aspect, might work.

Jun hit the jackpot: he found that many different peptide sequences, each bearing an excess of acidic residues (along with some crucial hydrophobic residues, as was later realized by Regier et al., 1993), work as activating regions when attached to a DNA binding domain. These acid blobs, as they came to be called (though, as Jun explains, that was not his term), presumably could not be little enzymes.

And so, despite the blizzard of proteins involved in gene transcription, gene activation per se (i.e., at which gene transcription ensues) seemed to be mediated by simple binding reactions readily incorporated into the world as revealed by lambda. An array of disparate experimental findings later reinforced this view and produced a description of gene regulation that resonates with that of many other biological regulatory processes (Ptashne et al., 2002; Ptashne and Gann, 2002).

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