DNA-Protein Interactions in Transcription

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Preface

This volume is the result of a symposium titled **DNA–Protein Complexes in Transcription,** held at Keystone, Colorado, April 4–10, 1988. When planning first began in 1986, it was anticipated that a meeting of modest size would be held, presenting a broad view of a specialized topic. At that time, very few eukaryotic transcription factors had been isolated, and little was known about them. The phenomenon of transcriptional control at a distance seemed to be restricted to eukaryotic genes, and there was little experimental support for proposed mechanisms of action at a distance. Hints were just beginning to emerge that DNA was more flexible than had been thought and that this might have implications for its recognition by control proteins. Gene activation mechanisms in general were obscure, and critical details were missing even from the well-characterized *Escherichia coli* systems. It seemed like an excellent time to bring together experts in eukaryotic and bacterial systems, ranging from biologists to physicists, to exchange ideas and results.

By the time of the meeting in 1988, the field of study had changed almost beyond recognition. This is reflected in the articles in this volume which discuss aspects of the mechanism of action of dozens of transcription factors. We now know such enormously important details as which factors direct tissue-specific transcription, how some factors cooperate during transcriptional activation, how they can act over long distances via DNA looping, and how some bind DNA at the atomic level. That all of this could have happened over a two-year period is remarkable and a testament to the health and vigor of the international research community.

This explosion of new knowledge was accompanied by an explosion in the number of people who wanted to attend the meeting. As a result, the symposium was much larger than anticipated. I am very grateful to all participants and to the Keystone and UCLA Symposia staff, and especially Robin Yeaton-Woo, for providing the forum for the exchange of exciting new results and ideas. It was a good time to be working on DNA-protein interactions, and the articles in this volume reflect this feeling.

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Jay D. Gralla

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I. PROKARYOTIC TRANSCRIPTIONAL CONTROL

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SPECIFIC REPRESSION IN THE *LAC* OPERON - THE 1988 VERSION[1]

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ABSTRACT. An updated version of the mechanism of repression in the *lac* operon is presented. This is shown to involve two operators separated by 402 base pairs of transcribed DNA. The two operators cooperate in repressor binding *in vivo*. A dual mechanism of repression results: both initiation and elongation of expression is blocked.

Studies on specific repression of the *lac* operon have had an enormous impact on the development of modern Molecular Biology. Among the landmarks were: the operon model for negative control of gene expression (1,2), the isolation of the first control protein (3), and operator control DNA (4). In addition to the intellectual achievements that resulted from these studies, many central technical advances came directly from *lac* repression studies. These include: classical genetic selections, chemical probing of DNA-protein complexes which in turn led to Maxam-Gilbert DNA sequencing, gel-shift and filter-binding methods for detection of DNA-binding proteins, DNaseI footprinting, and gene cloning methods based on the *lac* system. Since a great deal of Molecular Biology derives from these advances in the *lac* repression system, it is worthwhile to review and update the mechanism of specific repression of the *lac* operon.

[1]This work was supported by USPHS grant GM35754.

EARLY GENETICS AND THE FIRST TWO DECADES OF BIOCHEMISTRY

Several decades of pioneering genetic research by Jacob and Monod and co-workers at the Pasteur Institute led, in 1961, to the operon model for *lac* control (1). It was proposed that a transacting repressor interacted with a cis acting operator DNA to regulate *lac* gene expression. This of course proved to be precisely correct. Early models for the nature of the repressor emphasized the likelihood that it would be an RNA molecule (see 5). The evidence for this was very indirect, however, and in the early 1960's the isolation of *lac* repressor was begun

Repressor isolation was first achieved by Gilbert and Müller-Hill (3) and shortly afterwards by Riggs, Bourgeois and co-workers (6). The two groups took quite different approaches to this problem; Gilbert took advantage of the requirement that the repressor must bind IPTG, an inducer of the *lac* operon, while the other isolation assumed, correctly, that repressor would bind selectively to operator DNA. The repressor turned out to be protein. Thus in 1966 the first component of the system was purified, which set off a decade of biochemical studies of its properties and interaction with *lac* operator.

Studies by both groups during this time confirmed that repressor bound operator tightly and specifically and that the interaction was weakened by inducer (7,8). The isolation of the operator, however, proved to be extremely difficult. Finally, in 1973, Gilbert and Maxam isolated and sequenced the *lac* operator (4). This was done by isolating DNA protected by repressor from nuclease digestion and sequencing the region by the laborious methods then available. The operator turned out to have a two-fold symmetric sequence which fit nicely with the multimeric nature of the repressor protein.

During this same decade, Sadler and colleagues began isolating numerous *lac* constitutive mutations, in order to define the operator genetically (9). These mapped to a very small locus just upstream from the *lacZ* gene. In 1975, Gilbert and colleagues determined the DNA sequence of many of these O^c mutations (10). In every case, the mutants were changes in DNA sequence underneath the previously determined binding site for *lac* repressor. Bourgeois and colleagues showed that these same mutant DNA's bound repressor less well *in vitro* (11). Thus, biochemistry and genetics merged, without apparent contradiction, to support a unified model for interactions of *lac* repressor with *lac* operator.

About this same time, work in Gilbert's laboratory led to a model for how this repressor-operator interaction accomplished repression. The operator sequence was shown to include the DNA region encoding the initial bases of the *lac* transcript (12,13). Majors then showed that pre-incubating repressor with operator prevented *in vitro lac* transcription by RNA polymerase (13). Thus was the 1975 paradigm for *lac* repression established (10). Binding of repressor was said to exclude interactions with RNA polymerase due to the physical overlap of the two binding sites. Transcription could proceed when inducer weakened repressor's interaction, or in O^c strains when operator mutations weaken this same interaction.

The 1975 model was presented in texts and reviews as a paradigm for gene regulation. Nevertheless, there were residual doubts about the completeness of the model, although they were rarely discussed. One problem was that the model did not explain fully the quantitative effects of the *lac* O^c mutations. None of the *lac* O^c mutants led to very high constitutive levels of *lac* expression, implying that significant repressibility remained in all these mutant strains. Moreover, some of the genetically defined O^c mutations turned out to be associated with the same nucleotide sequence change within the *lac* operator; these were genetically classified as distinct mutants based on slightly different effects on *lac* operon repression. Also about this time, other sequences that could bind *lac* repressor *in vitro* were found to be associated with the *lac* operon (10,14). One of these was a very weak binder located 93 base pairs upstream of the operator and the other was somewhat stronger and was located 402 base pairs downstream within the *lacZ* gene. Nevertheless, the strong evidence from *in vitro* transcription and binding studies was so unifying and attractive that these considerations did not prevent the 1975 model from gaining widespread acceptance.

After 1975, this model for *lac* repression was fully accepted and research in the next decade centered principally on learning the details of repressor-operator recognition. Although this research was exceptionally fruitful and important, none of it seriously challenged the 1975 repression model. The discovery and characterization of remote control elements in other systems set the stage for the re-evaluation of this model in the mid-1980's.

RE-EVALUATION OF THE MECHANISM - 1986 TO 1988

At the beginning of this period, constructs were studied in which synthetic *lac* operator-like sequences were inserted upstream or downstream from *lac* operator-promoter regions (15-17). These studies demonstrated convincingly that the potential existed for repression of the *lac* operon by distant sites. In some cases, the artificial operator was downstream in the transcribed region and was implicated in blocking elongation of transcription. However, upstream constructs were also effective and this was suggested to involve DNA looping (15), as suggested by previous work in the ara operon. This was shown to be true *in vitro* using artificial constructs containing either two *lac* operators or the natural operator joined to the 93 base pair upstream operator-like sequence (18,19). Binding studies suggested that remote operators strengthened binding of repressor to the primary operator (20,21). In a short time it became obvious that suitably spaced *lac* operators could be brought together to form a tight, specific repression loop. Attention then focused on the naturally-occurring operator-like sequences within the *lac* operon.

That the downstream operator-like sequence, termed O_2, is a part of the natural repression apparatus was demonstrated by inactivating it and observing the consequences for repression (22,23). Full repression could not be achieved with a mutant O_2; β-galactosidase levels were six-fold higher under severe repression conditions. Although this effect is very significant, it is quantitatively modest in the context of the 1000-fold repressibility of the intact *lac* operon containing O_2 and the natural O_1 operator. Therefore, O_2 is an authentic, secondary component of the *lac* repression apparatus. In view of the above studies, O_2 could work either by blocking elongation, by strengthening repressor binding at the initiation site within O_1, or by a combination of both mechanisms.

In vivo footprinting and expression studies have now shown that both mechanisms can contribute to *lac* repression *in vivo* (23,24). Relative *in vivo* binding constants (23,24) were obtained for the repressor operator interactions in various plasmid constructs (Table 1). These showed that, *in vivo*, O_1 and O_2 cooperate to form a repression complex that is tighter than that formed with either operator alone, leading to stronger repression of expression. The effect of O_2 was not as strong in a strain that allows