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Previews

Flagellar Biosynthesis In Silico: Building Quantitative Models of Regulatory Networks

In this issue of *Cell*, Kalir and Alon (2004) describe the construction of an in silico model for the regulatory network responsible for the control of flagellar biosynthesis in *E. coli* based on quantitative gene expression data. They show how the model can be used as a quantitative blueprint to design genetic modifications with predictable influence on the dynamics of the system. The work by Kalir and Alon (2004) provides a general approach for building detailed models of transcriptional regulatory networks.

Recent years have seen an increased interest in developing predictive in silico models of biochemical networks in various organisms. This interest has been fueled primarily by the development of appropriate experimental tools that can produce the data to both build these models and to test and verify them. The major improvements in experimental techniques relate both to the scale of the experiments and to the ability of these methods to produce quantitative data. Quantitative models of biological systems can be used in much the same ways as traditional qualitative connectivity diagrams to interpret large volumes of data, design new experiments, and to understand mechanistic details of biochemical pathways. However, there are also biological questions that can not be fully answered based on connectivity diagrams only, for example the detailed timing of expression programs or the interplay between multiple regulatory pathways. For these types of questions, quantitative in silico models are needed to fully appreciate the particular network designs utilized in nature to achieve the desired network behavior.

One example of such intricate design is the regulatory circuit controlling flagellar biosynthesis in *Escherichia coli*, which is the subject of the study by Kalir and Alon (2004, this issue of *Cell*). For this system, the key transcriptional regulators and their target operons are well characterized (Neidhardt, 1996) giving us a fairly complete qualitative connectivity diagram for this regulatory network. In an earlier study, the target operons in the system were shown to follow a carefully coordinated time-dependent expression program consistent with the order in which the flagellum is assembled (Kalir et al., 2001). How the exact timing was controlled at the molecular level was not obvious from the network diagram and warranted a more detailed study into the dynamics of the system. In the present study, Kalir and Alon (2004) extend this previous work by building a quantitative in silico model of flagellar biosynthesis regulation and show how this model can be used to design variants of the network with altered expression dynamics.

The basic approach utilized by Kalir and Alon is to start with a transcriptional regulatory network with a known connectivity diagram and to find both the functions integrating the regulatory inputs at each promoter (Setty et al., 2003) and the parameters describing quantitatively how all the transcription factors regulate each individual target promoter (Kalir et al., 2001; Ronen et al., 2002). A parallelized GFP reporter gene assay was first used to accurately measure the time courses of promoter activity of flagellar biosynthetic genes in response to induction of this system. Second, the integrated action of the two main regulators in the system, FlhDC and FliA, was determined by constructing a strain where these genes are expressed from independently tunable promoters and measuring the activity of a single target promoter. This approach was used in an earlier study of the classical *lac* regulatory system (Setty et al., 2003), but in the current case the integration function was found to be a linear combination of the two inputs as opposed to the AND-gate like behavior of the *lac* system. Finally, the additive input function was used as a basis to build a parametrized in silico model for the expression response of each of the seven class-2 target promoters (Kalir et al., 2001; Ronen et al., 2002).

With an in silico model in hand, one can ask a number of questions about the regulatory network controlling flagellar biosynthesis. First, how is the timing of the flagellar gene expression program achieved using the two regulators? The answer turns out to be that the primary activator FlhDC is active in the initial phase of induction whereas the secondary activator FliA is itself regulated by FlhDC and is active in the later phase of induction. Also, the influence of FlhDC on individual promoters is variable while the FliA dependent effect is the same for all promoters. These properties of the FlhDC and FliA activators provide both the needed cascaded timing and magnitude of gene expression in the initial induction phase as well as the adaptation to equal level of promoter activity for all seven promoters in the later stage of induction. The in silico model can also be used to design modifications to the molecular components of the flagellar biosynthetic system that would change the expression dynamics in a predictable manner. Kalir and Alon (2004) show that by changing either binding affinities of the transcription factors to particular binding sites on promoters or expression patterns of the transcription factors themselves leads to changes in expression that were correctly predicted by the model a priori.

In addition to the insights provided into the *E. coli* flagellar biosynthetic process the in silico model also has broader implications to other biological systems. The flagellar biosyntheses regulatory network has feed-forward loop architecture with FlhDC as the master controller and FliA as the secondary regulator. Since feed-forward loops appear in significant frequency in currently known transcriptional regulatory network structures (Lee et al., 2002; Shen-Orr et al., 2002) the in silico model of flagellar biosynthesis regulation may prove to be useful for interpreting the dynamic behavior of other transcriptional regulatory networks. It would be particularly interesting to build similar quantitative

models for the regulatory networks controlling flagellar biosynthesis in other bacteria such as *Salmonella*, which have a network architecture similar to the *E. coli* system (Aldridge and Hughes, 2002). Comparative analysis of these models could then elucidate how variability in network behavior is connected to the variability in the network structure and parameters of the model.

The work by Kalir and Alon (2004) builds on efforts to reconstruct transcriptional regulatory networks on a qualitative level, i.e., obtaining the connectivity diagrams through high-throughput technologies such as expression profiling and location analysis (Covert et al., 2004; Lee et al., 2002). The remarkable aspect of this work is that the experimental methods are widely used and can be readily extended to most model organisms. In principle this makes it possible to build detailed models of any transcriptional regulatory network whose connectivity is known. On a more cautious note, while one may be able to build these models for subnetworks, they may not accurately represent the behavior of these networks in the context of the whole cell. For example, the model proposed by Kalir and Alon (2004) does not explicitly include the regulatory processes leading to the activation of the FlhDC regulator (Lehnen et al., 2002) or the negative feedback regulation of FliA by FlgM (Neidhardt, 1996). To complement the small scale in silico models, such as the flagellar biosynthesis network model presented here, there is also need to build models of entire cells or at least subsystems such as metabolism or transcriptional regulation as a whole (Covert et al., 2004).

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Selected Reading

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p53-Mediated Transcriptional Activation: From Test Tube to Cell

Posttranslational modifications of histones have been strongly correlated with transcriptional regulation. In this issue of *Cell*, An et al. (2004) comprehensively examined the nature of arginine methyltransferases and histone modifications in p53-mediated transcription.

The N and C termini, or tails, of histones provide sites for posttranslational modifications, such as phosphorylation, acetylation, methylation, and ubiquitination. Many studies have demonstrated that modifications of these tails correlate with both gene activation and repression (Zhang and Reinberg, 2001). In particular, the histone arginine methyltransferases CARM1 and PRMT1 are under intense study because of their coactivator role in nuclear hormone-mediated transcription (Chen et al., 1999). These enzymes specifically methylate histone H3 at Arg2, Arg17, and Arg26, and histone H4 at Arg3, respectively. Both CARM1 and PRMT1 act in concert with the acetyltransferase CBP/p300, along with the p160 coactivator family to enhance transcription from hormone-responsive promoters (Stallcup, 2001). The tumor suppressor protein, p53, also utilizes CBP/p300 for transcription activation (Gu et al., 1997; Lill et al., 1997), thus, raising the question: do other coactivators, such as arginine methyltransferases, play a key role p53-mediated transcription?

Although many groundbreaking studies correlate histone modifications, such as methylation or acetylation, with transcriptional activation of nuclear hormone and p53-regulated genes, little has been done to test the direct role of these modifications in transcriptional activation. Furthermore, several studies have demonstrated that these coactivators can also modify other proteins (Xu et al., 2001; Chevillard-Briet et al., 2002). Therefore, are histones the relevant target substrates for these coactivators? Do histone modifications play a direct role in transcription? In this issue of *Cell*, An et al. (2004) get to the very nature of these questions.

Many studies have characterized histone-modifying enzymes such as histone acetyltransferases and methyltransferases utilizing either recombinant histones or bulk cellular nucleosomes as substrates. An et al. (2004) utilized the benefits of both substrates by creating recombinant chromatin templates. These templates were unique in that they contained p53 DNA binding sites and were formed with either recombinant wild-type histones or histones that were either lacking tails or mutated at critical lysine or arginine residues that are targets of these methyltransferases. The advantage of this assay system is that it allows monitoring of histone modifications and serves as a template for in vitro transcription. Thus, this system provides a direct way to observe the result of histone modifications on transcription.

Using this assay, An et al. (2004) first determined that CARM1, PRMT1, and p300 are required by p53 to modify the histones in the chromatin template. In addition, p53-dependent transcription was increased significantly by the presence of all three coactivators. Mutations at the