PERSPECTIVES: GENE REGULATION

A Paradigm for Precision

Kevin Struhl

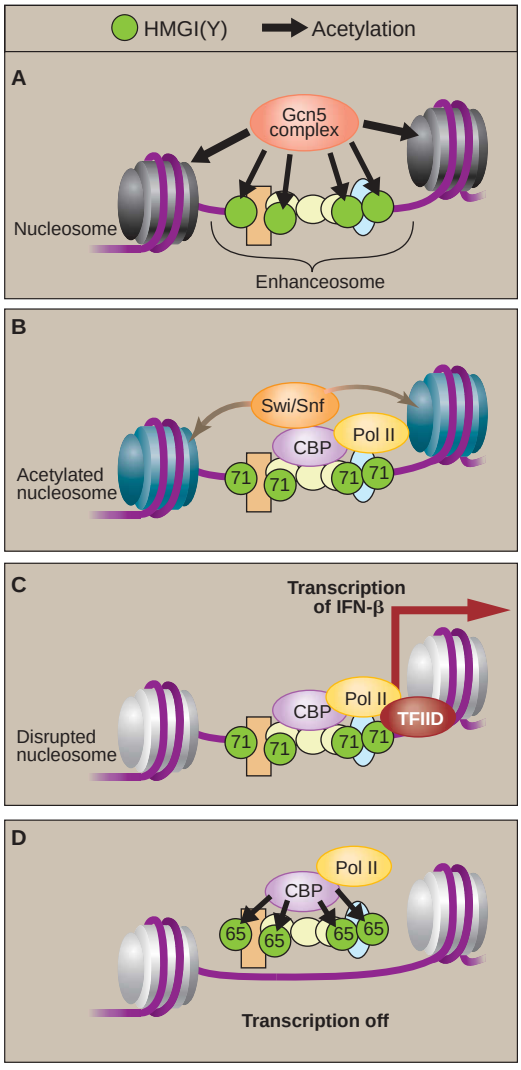
How do eukaryotic organisms express genes in patterns that are extraordinarily diverse and complex, yet precise in their response to environmental and developmental cues? Their secret lies in the enhancer, a relatively short region of DNA that contains multiple binding sites for proteins that activate transcription (activators). Upon binding to an enhancer, activators stimulate transcription by recruiting the RNA polymerase II (Pol II) machinery and chromatin-modifying proteins to the core promoter of the target gene (*1, 2*). The human interferon-β (IFN-β) enhancer, which switches on transcription of the *IFN-β* gene in response to viral infection, is a paradigm for precision in gene regulation. The most recent advances in understanding the molecular dynamics of this on-off transcriptional switch are described by Munshi *et al*. (*3*) on page 1133 of this issue.

Efficient transcription requires the combinatorial and synergistic action of multiple activators bound to the enhancer, and many different combinations of activators are capable of transcriptional synergy. As a consequence, a limited number of activators can be arranged in numerous possible combinations, each of which is biologically distinct. Furthermore, enhancers influence gene expression over long distances, upstream or downstream of the target gene's core promoter. A gene can be regulated by multiple enhancers and hence subjected to the influence of numerous activators, whose target sites are spread out over a large stretch of the chromosome. Individual activators are often restricted to specific cell types, operate only during specific stages of development, and are tightly regulated in response to specific environmental signals. Thus, an enhancer integrates environmental and developmental information to regulate the expression of an individual gene in a biologically appropriate manner.

Enhancers that do not have special combinations or arrangements of activator binding sites are called modular enhancers. They permit extraordinary diversity in gene expression patterns as well as the capacity for evolutionary flexibility (*4*). With modular enhancers, each activator contributes to the overall transcriptional output. However, it is not easy to explain how a modular enhancer can precisely regulate a transcriptional on-off switch in response to an environmental change. For this purpose, the cell needs a different kind of enhancer—one in which each bound activator is essential for transcription. Such nonmodular enhancers would impose the restriction that the target gene would be switched on only when all activators are functionally active.

The human IFN-β enhancer that Munshi *et al*. (*3*) describe is a paradigm for how a nonmodular enhancer can operate as a transcriptional on-off switch. The IFN-β enhancer is a 65–base pair region immediately upstream of the core promoter of the *IFN* gene (*5*). It contains binding sites for activator proteins of the NF-κB, IRF, and ATF/Jun families as well as target sites for the architectural protein HMGI(Y). Each binding site and the precise arrangement of these sites are absolutely required for the activator and architectural proteins to assemble into a structurally discrete nucleoprotein complex termed the enhanceosome (see the figure) (*6–9*). Assembly of the enhanceosome is essential for transcription of the *IFN-β* gene in response to viral infection of cells. Individual activators bound to their sites in the IFN-β enhancer do not by themselves stimulate transcription; enhancers that contain multiple copies of any one activator site are less inducible by virus and respond to other activator-specific inducing conditions. Thus, unlike modular enhancers in which the bound activators operate independently, the enhanceosome is a highly defined structure that is formed only under precise environmental conditions.

In living cells, the process of enhanceosome assembly, recruitment of chromatin-modifying activities, and association of the Pol II machinery is unexpectedly complicated and dynamic (*3, 10*). First, enhanceosome assembly at the nucleosome-free enhancer region occurs in stages. NF-κB and IRF-1 initially bind to the enhancer, followed by the arrival of ATF-2, then IRF-3 and Jun, and finally IRF-7 to yield an intact enhanceosome, 4 hours after viral infection (see the figure). Second, the enhanceosome recruits the Gcn5 histone acetylase, which adds acetyl



The IFN-β enhanceosome switch. (**A**) In response to viral infection, the enhanceosome—consisting of activators (unlabeled colored ovals) and the HMGI(Y) architectural protein (green)—is assembled between nucleosomes (grey). The enhanceosome recruits the Gcn5 complex, which acetylates both HMGI(Y) at lysine 71 and histone proteins in the nucleosome (arrows). (**B**) The stabilized enhanceosome—acetylated at lysine 71 in HMGI(Y) and bound to acetylated nucleosomes (blue)—sequentially recruits the CBP–Pol II enzyme complex and then the Swi/Snf nucleosome remodeling complex. (**C**) Swi/Snf disrupts the positioned nucleosomes flanking the enhanceosome, leading to binding of the TFIID transcription factor and transcription of the gene. Acetylation at lysine 71 inhibits CBP acetylation at lysine 65. (**D**) CBP acetylates HMGI(Y) at lysine 65, leading to disruption of the enhanceosome and the cessation of transcription.

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groups to (acetylates) the histone proteins in adjacent nucleosomes. Gcn5 association and histone acetylation are transient; both disappear 9 hours after viral infection. Third, shortly after recruitment of Gcn5, the enhanceosome recruits the coactivator, CREB binding protein (CBP). Intriguingly, efficient CBP recruitment requires a composite surface that exists only when the enhanceosome is intact; interactions between CBP and individual activators are far less efficient in the absence of the enhanceosome (*11, 12*). Fourth, the Pol II enzyme complex is recruited by the enhanceosome at the same time as CBP is recruited. Fifth, approximately 1 to 2 hours after recruitment of the CBP–Pol II enzyme complex, the Swi-Snf nucleosome remodeling complex is recruited in a manner that depends on CBP and is strongly stimulated by histone acetylation. The recruited Swi-Snf complex disrupts the nucleosome positioned over the core promoter region, thereby permitting the transcription factor TFIID to gain access to the *IFN-β* gene, which is then switched on and transcribed (*10*).

Transcriptional activation of *IFN-β* is transient—the key feature of the regulatory switch is the programmed formation and destruction of the enhanceosome through acetylation of the architectural protein HMGI(Y) by the Gcn5 and CBP histone acetylases (*4, 13*). Acetylation of lysine at position 71 (Lys^{71}) in HMGI(Y) by Gcn5 stabilizes the enhanceosome by increasing the affinity of HMGI(Y) for the activators. In vivo, acetylation of Lys^{71} is transient, and it correlates precisely with the formation of the enhanceosome and recruitment of Gcn5. In striking contrast, CBP acetylates HMGI(Y) at Lys^{65}, which inhibits DNA-binding activity and hence destabilizes the enhanceosome. In vivo, the peak of Lys^{65} acetylation coincides with disruption of the enhanceosome. Importantly, Lys^{65} acetylation does not occur for 1 to 2 hours after CBP has been recruited to the *IFN-β* gene's promoter. Thus, Gcn5-dependent acetylation of Lys^{71} both facilities enhanceosome assembly and protects the enhanceosome by inhibiting CBP-dependent acetylation of Lys^{65}.

These findings demonstrate that the IFN-β enhanceosome uses multiple molecular mechanisms to achieve a precise on-off switch for regulating gene expression. The enhanceosome forms only when all component activators are functional. It has biochemical properties distinct from those of the individual activators, and its existence is regulated by the programmed recruitment of the Gcn5 and CBP acetylases. We still do not have enough information to assess whether cells commonly use enhanceosomes to regulate gene transcription. However, there are indications that enhanceosomes regulate the expression of several other mammalian genes (*14*) and of the Mei3 meiosis inducer gene of the yeast *Schizosaccharomyces pombe* (*15*). Thus, it is highly likely that eukaryotic organisms use modular enhancers to achieve diversity in gene expression patterns and evolutionary flexibility, and enhanceosomes to achieve regulatory precision in gene transcription.

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References and Notes

1. M. Ptashne, A. Gann, *Nature* **386**, 569 (1997).
2. K. Struhl, *Cell* **98**, 1 (1999).
3. N. Munshi *et al.*, *Science* **293**, 1133 (2001).
4. K. Struhl, *Neuron* **7**, 177 (1991).
5. T. Maniatis *et al.*, *In Transcriptional Regulation*, S. L. McKnight, K. R. Yamamoto, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992), pp. 1193–1220.
6. D. Thanos, T. Maniatis, *Cell* **83**, 1091 (1995).
7. T. Maniatis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **63**, 609 (1998).
8. J. Yie *et al.*, *EMBO J.* **18**, 3074 (1999).
9. J. V. Falvo *et al.*, *Mol. Cell. Biol.* **20**, 4814 (2000).
10. T. Agalioti *et al.*, *Cell* **103**, 667 (2000).
11. M. Merika *et al.*, *Mol. Cell* **1**, 277 (1998).
12. J. Yie, K. Senger, D. Thanos, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13108 (1999).
13. N. Munshi *et al.*, *Mol. Cell* **2**, 457 (1998).
14. M. Merika, D. Thanos, *Curr. Opin. Genet. Dev.* **11**, 205 (2001).
15. W. J. van Heeckeren, D. R. Dorris, K. Struhl, *Mol. Cell. Biol.* **18**, 7317 (1998).

PERSPECTIVES: COSMOLOGY

Probing Matter at the Lowest Densities

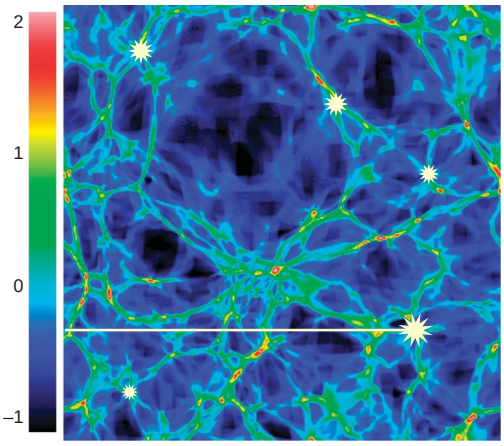
Jordi Miralda-Escudé

The lowest densities of matter in the universe are found in the vast spaces between galaxies. This tenuous intergalactic matter can only be observed through the absorption lines it produces in the spectra of bright, distant sources of light—usually quasars.

Imagine that you are observing one of these distant quasars and that several structures of intergalactic gas lie along the line of sight (see the figure). Because of the expansion of the universe, each gaseous structure recedes from us at a velocity proportional to its distance. Owing to the Doppler effect, the wavelength of a given atomic absorption line is shifted to a different observed wavelength for each of these structures. As a result, the absorption spectrum of the quasar provides us with a splendid map of the density fluctuations in the intergalactic matter along the line of sight to that quasar (*1*, *2*).

For many years, this simple technique has been used to study the intergalactic medium with the Lyman-α line of atomic hydrogen (*3*) at 121.6 nm, which shifts to more easily accessible visual wavelengths for redshifts z greater than 2 (*4*). But important clues about the ionization and evolutionary history of intergalactic matter are also gained from the absorption (*5*) spectra of singly ionized helium, He II, with its Lyman-α line at 30.4 nm. The difficulty of the He II absorption is that, even with the redshift, it must still be observed in the far-ultraviolet, where Earth's atmosphere is opaque, demanding the use of space telescopes.

On page 1112 of this issue, Kriss *et al.* (*6*) report new observations of He II absorption with unprecedented resolution from the Far Ultraviolet Spectroscopic Explorer (FUSE). This new space telescope allows the He II absorption lines to be re-



Simulated structures of intergalactic gas. The projected density in this slice from a numerical simulation shows the characteristic structures formed by gravitational collapse in the expanding universe. Left bar, logarithm of the density in units of mean density. Galaxies can form in the density peaks; supermassive black holes in their nuclei produce quasars (illustrated here as white radiant sources), which are the brightest objects in the universe. When we observe one such quasar from Earth, the gaseous intergalactic structures in the line of sight (thin white line) are detected in the absorption spectrum. The strength of the absorption reflects the density of the structure being intersected but depends also on variations in the intensity of radiation ionizing the gas due to other quasars in the vicinity of the line of sight.

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