Interchromosomal Huddle Kickstarts Antiviral Defense

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Long-distance chromosomal interactions are emerging as a potential mechanism of gene expression control. In this issue, Apostolou and Thanos (2008) describe how viral infection elicits interchromosomal associations between the interferon-β (*IFN*-β) gene enhancer and DNA binding sites of the transcription factor NF-κB, resulting in the initiation of transcription and an antiviral response.

Until recently, interactions between regulatory DNA elements such as enhancers and promoters in higher eukaryotes were thought to be confined to relatively short-range contacts between elements a few hundred to tens of kilobases apart on the same chromosome. This is based primarily on the assumption that a gene and its regulatory DNA elements must be located in close chromosomal proximity in order to "find" each other in the crowded nucleus. However, several recent studies in mammals have challenged this view by showing that genomic regions separated by tens of megabases on the same chromosome or even located on different chromosomes can undergo long-range interactions at high frequencies in the nucleus (reviewed in Fraser and Bickmore, 2007). In at least two reported cases, the introduction of small mutations in a sequence on one chromosome has altered the expression of an interacting gene on another chromosome, thus implying that interchromosomal interactions can regulate gene expression (Spilianakis et al., 2005; Zhao et al., 2006). Apostolou and Thanos (2008) now reveal a role for interchromosomal interactions in the response of cultured cells to Sendai virus infection.

The human antiviral response is triggered by the transcriptional activation of type I interferon genes such as *IFN*-β that encode a secreted interferon protein (IFN). IFN binds to receptors on the surface of both infected and uninfected cells, resulting in the increased expression of hundreds of antiviral genes. The transcriptional activation of *IFN*-β requires the formation of the *IFN*-β enhanceosome, a complex that is assembled stepwise through the sequential binding of three sets of transcription factors (NF-κB, ATF-2/cJun, and IRFs) at an upstream enhancer sequence (Lomvardas and Thanos, 2002). The master transcription factor NF-κB binds at the enhancer 2 hr after virus infection, followed by ATF-2 (activating transcription factor 2) an hour later. IRF-7 (interferon regulatory factor 7) joins the complex 5 to 6 hr post-infection, just prior to *IFN*-β transcription. *IFN*-β is initially only transcribed in a subset of cells in the population. Furthermore, most cells expressing *IFN*-β have only a single copy of the gene that is active (monoallelic) at any given moment rather than both copies (biallelic). It is not until later in the course of viral infection, after the secreted IFN has increased antiviral gene expression in both infected and uninfected cells, that the two *IFN*-β alleles are simultaneously transcribed. Specifically, the increased expression of the transcription factor IRF-7 boosts *IFN*-β expression throughout the cell population by increasing both the number of cells expressing *IFN*-β and the number of active alleles per cell. Apostolou and Thanos now show that during the first few hours of Sendai virus infection in cultured HeLa cells expressing the transcription factors necessary for *IFN*-β transcription, individual alleles of the *IFN*-β gene engage in specific long-range intrachromosomal and interchromosomal interactions that may potentiate gene expression. Using a circular chromosome conformation capture (4C) assay (Zhao et al., 2006) coupled with an NF-κB chromatin immunoprecipitation (ChIP) step to detect chromosomal interactions, the authors identified three genomic loci that interact with the *IFN*-β enhancer in response to viral infection. One of the identified loci is located on the same chromosome as the *IFN*-β gene, whereas the other two are situated on different chromosomes. Interestingly, all three sequences contained specialized Alu repeats (a common repetitive DNA element) that harbor potential NF-κB binding sites. Apostolou and Thanos detected interchromosomal interactions between the *IFN*-β enhancer and the specialized Alu repeats as early as 2 hr post-infection but found that the loci displayed the highest frequencies of colocalization during enhanceosome assembly (4 hr post-infection). When *IFN*-β transcription is initiated (6 hr post-infection), the transchromosomal associations are markedly reduced, although a percentage of the monoallelically active *IFN*-β genes could still be seen associating with the Alu repeats. At the peak of IRF-7-mediated expression when biallelic *IFN*-β transcription occurs in most HeLa cells (8 to 10 hr post-infection), the association of the enhanceosome with the Alu repeats can no longer be detected.

It has been demonstrated that specific genes or genomic regions can preferentially cluster at specific subnuclear compartments such as transcription factories (Osborne et al., 2004, 2007) or splicing

factor-enriched nuclear speckles (Nunez et al., 2008). As with known examples of such clustering, it is not clear if the interactions the authors observed between the enhanceosome and the Alu repeats are indicative of actual direct interchromosomal regulatory interactions between DNA elements or whether the colocalized genomic loci are simply taking advantage of increased local concentrations of specific factors. Thus, Apostolou and Thanos examined the nature of the interactions by transiently transfecting additional copies of the specialized Alu repeats into HeLa cells before viral infection. Remarkably, the additional Alu repeat copies significantly increased the number of cells expressing *IFN*-β after viral infection, but only if the NF-κB binding sequences in the added repeats were intact. This implication that NF-κB binding is important for the chromosomal interactions was further supported by the observation that specific depletion of nuclear NF-κB resulted in a decrease in virus-induced interchromosomal interactions and *IFN*-β expression. Based on their findings, Apostolou and Thanos propose a two-phase model for stochastic monoallelic activation of *IFN*-β and subsequent biallelic *IFN*-β activation during the course of viral infection (Figure 1). In the first phase, viral infection induces nuclear localization of NF-κB that initially binds to the specialized Alu sequences already interacting with each other at low frequencies. The authors suggest that this may constitute an NF-κB "receptor center" that then stochastically interacts with one allele of *IFN*-β. This contact transfers NF-κB to the *IFN*-β enhancer, allowing it to initiate enhanceosome formation on that *IFN*-β allele to initiate monoallelic activation. During the second phase, secreted IFN protein induces high levels of IRF-7 expression in both infected and noninfected cells, leading to biallelic *IFN*-β expression that is independent of interchromosomal interactions with the specialized Alu repeats. Apostolou and Thanos suggest that interchromosomal associations take place when activating factors such as NF-κB are limiting in the nucleus, such as during early virus infection. Indeed, the authors observed that overexpression of NF-κB in the HeLa cells led to both elevated *IFN*-β transcription much earlier during infection

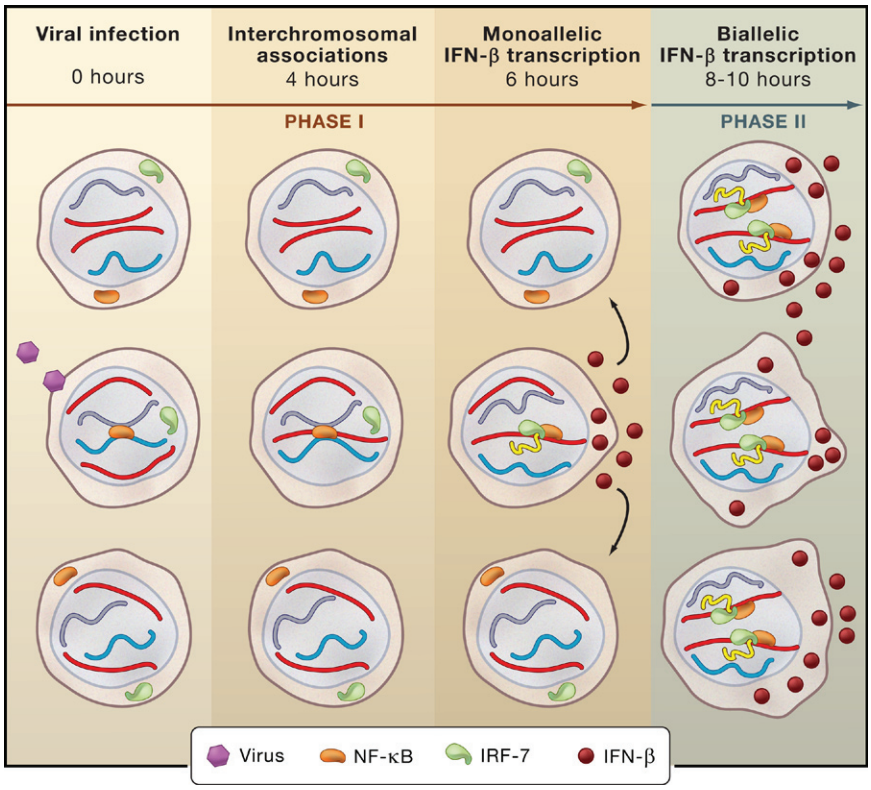

Figure 1. Two Phases of *IFN*-β Expression during Viral Infection

Phase I: Monoallelic *IFN*-β transcription. Virus (purple) infection induces the translocation of NF-κB (orange) and IRF-7 (green) to the nucleus only in the infected cell. NF-κB binds to specialized Alu repeats located on chromosomes 4 (gray) and 18 (blue) to form a proposed NF-κB receptor center. One *IFN*-β allele (located on chromosome 9; red) is recruited to the NF-κB receptor center, resulting in the transfer of NF-κB to the *IFN*-β enhancer and initiation of enhanceosome assembly. Completed enhanceosome assembly results in monoallelic activation of *IFN*-β (*IFN*-β RNA; yellow) and production of secreted *IFN*-β protein.

Phase II: Biallelic *IFN*-β transcription. *IFN*-β signaling induces biallelic transcription of *IFN*-β, leading to signal amplification and *IFN*-β expression in neighboring, noninfected cells.

and a significant decrease in interchromosomal interactions. Though the data presented in this study are consistent with the authors' model, definitive proof is still needed. It will be important for future experiments to determine whether the NF-κB sites in the endogenous Alu repeats are required for chromosomal interactions. Also, ascertaining whether the individual *IFN*-β alleles undergoing interchromosomal interactions during early infection are the same alleles expressing *IFN*-β at later stages of infection will be crucial to further validate the model.

The work of Apostolou and Thanos opens the door to many new questions. For example, given that NF-κB-bound genomic loci show differing associations with Alu sequences, how might this specificity in interactions be achieved and regulated? Also, during later stages of viral infection, could the *IFN*-β locus behave like the interferon-γ locus in which interchromosomal associations are thought to prime the locus for expression but are lost in favor of intrachromosomal contacts upon gene activation (Spilianakis et al., 2005)? Above all, this work, together with other recent advances, highlights the importance of analyzing gene expression in the context of the three-dimensional space of the nucleus. It is tempting to speculate that the activity of most eukaryotic genes is regulated by a complex interplay between transcription factor binding, dynamic relocation to nuclear subcompartments, and specific intra- and interchromosomal associations. Clearly, the discovery of interchromosomal interactions has added a new layer of complexity to our understanding of eukaryotic gene regulation.

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Clearing the Way for Unpaused Polymerases

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Heat shock loci in the polytene chromosomes of the fruit fly *Drosophila* undergo a characteristic change in appearance that coincides with the onset of gene expression. Petesch and Lis (2008) now show that nucleosomes are lost across the entire *Hsp70* locus in an initial wave that precedes transcription by RNA polymerase II.

The nucleosome core, consisting of DNA wrapped around an octamer of histone proteins, provides a significant barrier to transcription by RNA polymerase II. Although this barrier prevents transcription from starting in unsuitable locations, it also poses a problem—when transcription is required, what allows RNA polymerase II to pass through a chromatin template? One solution is provided by ATP-dependent chromatin-remodeling complexes that assist the passage of polymerase through nucleosomes (Carey et al., 2006). However, hints have suggested that in certain situations nucleosomes might be removed altogether, enabling unrestricted access of polymerase to sequences that must be transcribed rapidly (Zhao et al., 2005). In this issue, Petesch and Lis (2008) report that nucleosomes are rapidly lost at heat shock loci in the fruit fly *Drosophila* and that this removal is prior to and independent of transcription by RNA polymerase II.

Heat shock leads to visible changes in the heat shock loci on polytene chromosomes of *Drosophila*, termed "puffing." Unlike neighboring regions of polytene chromosomes that are densely packed, chromosome puffs have a diffuse appearance. Puffing is indicative of changes in chromatin structure, as nuclease hypersensitivity assays showed that extended heat shock results in disruption of nucleosomes along the coding region of *Hsp70* (Wu et al., 1979). However, puffing can be separated from transcription because certain chemical agents can induce puffing independently of transcription (Winegarden et al., 1996). Furthermore, the response of the yeast *HSP82* gene to activation by heat shock involves a rapid loss of histone-DNA contacts that is at least partially independent of the TATA box, indicating transcription by RNA polymerase II might not be required for this nucleosome loss (Zhao et al., 2005).

Despite these findings, it has been unclear just how much the transcription-independent induction of heat shock puffs represents changes in chromatin structure. In their current work, Petesch and Lis show that nucleosomes are lost across the entire *Hsp70* locus within 30 s of heat shock activation. This loss proceeds so rapidly that it occurs before RNA polymerase II even has a chance to reach the end of the *Hsp70* gene. Nucleosome loss extends across adjacent genes, halting only at the scs and scs′ boundary elements (Figure 1). This initial nucleosome loss is followed by a second wave of disruption after polymerase clears the remaining nucleosomes as it moves along the *Hsp70* gene. Strikingly, the initial nucleosome disruption is independent of transcription: Nucleosome loss can be decoupled from transcription with the same chemical agents that induce puffing (but not transcription) of heat shock loci. Moreover, direct inhibition of transcription with a nucleotide analog that prevents elongation but not initiation does not prevent this initial wave of nucleosome loss. Furthermore, although this initial loss is required for optimal transcription of the *Hsp70* gene, it is not sufficient to induce expression of adjacent genes that lie within the boundaries of the heat shock puff region.

What factors are required for this initial, transcription-independent nucleosome disruption? Three proteins appear to have a critical role in this process: heat shock factor (HSF), GAGA factor (GAF; *Trithorax-like*), and poly(ADP)-ribose polymerase (PARP). Notably, all three of these have been shown previously to be critical for puff formation after heat shock. Heat