T cells. However, there are too few T cells to permit effective immune responses.

The discovery over 40 years ago that some mice lacking a thoracic thymus still exhibit immunocompetence was a major blow to the assumed exclusive role of the thymus in lymphopoiesis (*3*). Since then, other sources of T cells have been contested, and it was recently postulated that in these unusual cases, a neck thymus may be responsible for T cell production (*3*). However, conclusive functional proof of a second thymus was lacking.

Verification of this idea is now presented by Terszowski *et al*., who were investigating the significance of lymphoid structures in the neck of mice. Their analysis reveals that epithelial and lymphoid components of these neck structures correspond to those seen in the thoracic thymus. Importantly, hematological markers displayed by immature, but not mature, T cells are present, indicating ongoing T cell lymphopoiesis. Epithelial cells expressing cytokeratin, a protein present in thoracic thymus but not in lymph nodes, were also apparent, as was the expression of Foxn1, a protein crucial for proper epithelial cell function (*9*). Finally, functional studies with precursor T cells that express a transgenic T cell receptor showed that lymphocyte selection in the neck thymus occurs as in the thoracic thymus. Furthermore, transplants of the neck thymus into nude mice conferred immunocompetence. These results leave no doubt that the neck thymus functions as a primary lymphoid organ. Other observations by Terszowski *et al*. suggest that during development, the neck thymus branches off from the common thymus anlage before the descent of the thoracic thymus into the chest cavity, and that the structure of the neck thymus matures only after birth. This scenario appears consistent with the delayed onset of lymphopoiesis in the neck thymus as compared with the thoracic thymus.

For several decades, the mouse has been the model organism for studying the mammalian immune system, so it is surprising that the functional relevance of a thymus-like organ in the neck has not been fleshed out earlier. The neck thymus is present in strains of mice that are commonly used in experiments (most BALB/c and about half of C57BL/6 mice have it) (*5*). A thymus-like neck structure has also been observed in adult humans, though its presence is considered pathological.

The experiments of Terszowski *et al*. raise concerns about experiments in which the thoracic thymus is removed to study thymus-independent features of the peripheral T cell pool, such as lymphocyte turnover or lymphocyte production in extrathymic tissue. Likewise, the role of the neck thymus in autoimmune disease that occurs after removal of the thoracic thymus shortly after birth needs to be considered. Confirmation of a second mammalian thymus may have settled one debate, but it likely has generated other important questions not previously considered.

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MOLECULAR BIOLOGY

Managing Associations Between Different Chromosomes

Genes present on different chromosomes can be coordinately expressed through a transcription regulatory factor that brings them together in a region of active transcription in the nucleus.

Charalampos G. Spilianakis and Richard A. Flavell

The genetic information of higher organisms is encoded in DNA that is not randomly dispersed within the cell nucleus, but is organized with nucleoproteins into different kinds of chromatin, the building blocks of the chromosomes. Each chromosome resides in a specific region of the nucleus when the cell is not undergoing cell division, and usually genes that are actively being expressed loop out from their condensed chromatin territory and localize to a region of transcriptional activity. These "transcription factory" areas are thus abundant with protein factors that initiate and regulate gene expression (*1*). Although it is well known that expression of a gene is controlled by regulatory elements located in the same region of the same chromosome (in cis), interchromosomal gene regulation has been recently observed in which the transcription of genes located on one chromosome is controlled by regulatory elements located on another chromosome (in trans) (*2*). Now, on page 269 of this issue, Ling *et al.* (*3*) show that a maternal locus on mouse chromosome 7 harboring two adjacent imprinted genes localizes with a paternal locus on chromosome 11 that contains two different genes. This interaction depends on genetic regulatory elements on chromosome 7 and on a protein called the CCCTC-binding factor (CTCF) (see the figure). The result is regulated expression of the two genes on chromosome 11.

Diploid organisms possess two alleles (alternate versions of a gene, maternally and paternally derived) of the same genetic locus. Each allele is thought to function independently of the other, although there are certain phenomena that implicate coordinated or alternate transcriptional regulation of certain loci. Regulatory elements located on one chromosome generally operate in cis on adjacent genes located on the same chromosome, but there are examples of trans-regulation by such regulatory elements on genes located on another chromosome. Such examples include transvection in the fruit fly *Drosophila melanogaster*, a process in which homologous chromosomes come together in a "synapse" and influence gene expression through enhancer elements that act in trans. Another process is X chromosome inactivation, present in many organisms, to ensure that males and females have comparable doses of expressed genes. Interchromosomal pairing of the two homologous X chromosomes allows communication between them, resulting in the mutually exclusive silencing of genes on one X chromosome (*4*, *5*). Similarly, in the plant *Zea mays*, the process of paramutation enables one allele to silence its homolog (*6*).

One allele can sense the presence of the other allele and initiate the above-mentioned processes through epigenetic changes that mark the loci to be regulated. One such epigenetic change involves DNA methylation and demethylation. This modification, known as "genomic imprinting," occurs when both maternal and paternal alleles are present but only one will be expressed. The recently developed chromosome conformation capture technique has revealed (*7*, *8*) that the onset of transcription at an imprinting locus on mouse chromosome 7 depends on the methylation status of an imprinting control region of the locus. It also depends on the looping out of DNA that contains this region, located between the enhancer element of one gene (*H19*) and the promoter element of another gene (*Igf2*). Ling *et al.* applied an alterna-

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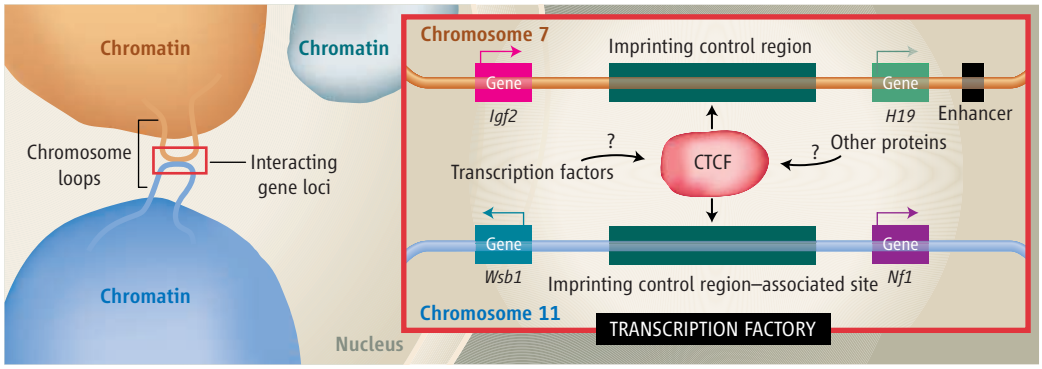

Interchromosomal rendezvous. The interaction between two different gene loci on two different chromosomes is mediated by the transcription regulatory factor CTCF and perhaps other factors. This may occur in regions of the nucleus that are enriched with transcription machinery whereby the genetic elements on one chromosome regulate expression of genes on the partnering chromosome.

tive chromosome conformation capture technique on mouse fibroblast-like cells and identified new chromosomal interacting partners with the imprinting control region of the *H19*/*Igf2* locus. Fluorescence in situ hybridization (FISH) experiments confirmed the overlapping localization in the nucleus of the mouse chromosome 7 *H19*/*Igf2* locus with a locus on chromosome 11 harboring the genes *Wsb1* and *Nf1*. CTCF, a factor that regulates DNA methylation in mammals by binding to the imprinting control region of the maternal *H19*/*Igf2* locus and the paternal loci of *Wsb1*/*Nf1*, is responsible for driving this interchromosomal interaction. Knockdown of the expression of this factor ablated the overlapping localization of the interacting alleles and impaired the transcriptional transactivation of the *Wsb1* and *Nf1* genes by regulatory elements of the *H19/Igf2* locus. The allele specific requirement for CTCF binding on the maternal allele was confirmed by the loss of the interchromosomal interaction when the maternal imprinting control region was deleted. The interaction was preserved when the paternal imprinting control region was deleted. Because there is parental allele specificity for CTCF binding (paternal chromosome 11 and maternal chromosome 7), we could assume that the interchromosomal association is implicated in the imprinting process. There was loss of maternal *Igf2* imprinting in cells lacking CTCF and in cells in which the maternal imprinting control region was deleted. The study suggests that a protein factor is critical to mediating the interaction of the two loci into a common nuclear compartment where transcription may be regulated.

The exact nuclear compartment where these interactions occur remains to be characterized by means of a combination of FISH to visualize DNA loci and immunofluorescence techniques to visualize the cell's transcription machinery. Is the colocalization and physical interaction of loci from different chromosomes a static or a dynamic process in which chromosomal partners transiently associate to be transcriptionally regulated? Interchromosomal interactions are more likely to be a dynamic process in which gene loci enter and leave a specific nuclear environment, possibly changing or exchanging interacting partners. The integration of multiple copies of a specific binding site for a fluorescently labeled protein, within a specific locus, will also permit monitoring the movement of the labeled locus in a living cell, and in real time. RNA FISH experiments, in which newly transcribed RNA on the locus of interest is detected, may also provide answers to such questions. And what about the mechanism by which homologous, associated gene loci exchange epigenetic information? Is it RNA-mediated? Or is it based, as the Ling *et al.* study suggests, on transcription factors (and perhaps homo- or heterotypic interactions between them) that bind each genetic locus?

Gene regulation through interchromosomal interactions may well be a general phenomenon with paradigms in different systems. It has now been implicated in the regulation of alternatively expressed genes in T cells (*2*), for α- and β-globin genes in erythroblasts (*9*), for loci that regulate X chromosome inactivation (*4*, *5*), and in the regulation of imprinting loci (*3*). It is likely that clusters of genes with coordinate or alternate regulation of expression may be controlled by interchromosomal interactions. A genome-wide analysis will reveal interacting chromosome partners with functional consequences in the regulation of gene expression, with possible implications in disease.

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BIOCHEMISTRY

Enzyme Motions Inside and Out

Stephen J. Benkovic and Sharon Hammes-Schiffer

In one enzyme, short-range thermal motions are sufficient to explain the transfer of a hydrogen by tunneling—a transition through a classically forbidden energy state.

A long-standing question in biochemistry is how enzymes catalyze chemical reactions at rates that are, in some cases, millions of times faster than the reaction rate in their absence. The quest for the source of this extraordinary ability has been augmented by recent advances in structural methods [particularly nuclear magnetic resonance (NMR)] (*1*–*3*), in computational power (*4*), and in the sophistication of physical chemical experiments (*5*). The key question is simple: How does the enzyme reduce the free-energy barrier for the chemical transformation? We have reviewed the progression of hypothetical answers to this question (*6*, *7*) and identified a common feature of the various rationales—namely, the requirement for conformational flexibility within the enzyme and substrates—and we noted the diversity of time scales for these movements. On page 237 of this issue, Masgrau *et al.* (*8*) examine the importance of dynamics for catalysis by the enzyme aromatic amine dehydrogenase in the oxidation of tryptamine.

The pathway for oxidation proceeds through a series of intermediates that have been characterized by x-ray crystallography. The step that is the focus of Masgrau and coworkers' study is a proton transfer from the carbon of a Schiff base intermediate to a car-

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