having polar functionality, which might result in polymers with new and exciting properties. On page 460, Younkin *et al.* report a major advance that may open the door to such new polymers in the near future (*2*). Their result represents the culmination of two lines of research that diverged from the initial work on Ziegler-Natta catalysts and are reunited in the present work.

One line of research aimed to understand how the Ziegler catalysts work. The nonuniformity of the active sites in these heterogeneous catalysts renders mechanistic study and rational design of modified catalysts extremely difficult. The search for soluble, single-site catalysts was finally rewarded when the groups of D. S. Breslow and Natta discovered that soluble metallocene complexes of early transition metals, when activated by dialkylaluminum halides, were capable of polymerizing ethylene. The introduction of methyl aluminoxane (MAO) as a cocatalyst by H. Sinn and W. Kaminsky boosted the initially low activity of these metallocene catalysts and allowed the polymerization of olefins other than ethylene. This raised the question of how to control the stereoselectivity in the polymerization of α-olefins, which is crucial for influencing the physical properties of the polymers. The development of chiral metallocene catalysts by Brintzinger laid the foundation for the creation of a catalyst "tool-box" that allows the construction of polyolefins with predictable properties, and some of these have just recently been introduced onto the market (*3*).

Despite the great progress made with these metallocene catalysts, one fundamental limitation remains: The highly electrophilic nature of the metal in these early transition metal catalysts generally makes it impossible to use olefins with polar functional groups as monomers or comonomers. Accordingly, a second line of investigation that grew out of the Ziegler and Natta work was aimed toward developing late-transition metal catalysts that might prove more tolerant of polar functionality. Ironically, the first major advances in this area arose from investigation of the "nickel effect" described above. Seminal work by Keim revealed that a nickel complex bearing a ligand that chelates through phosphorus and oxygen atoms (see the figure) catalytically converts ethylene to linear oligomers with chain lengths of between 4 and 20 carbon atoms. This reaction is the foundation of the Shell Higher Olefin Process (SHOP), a commercial process with many applications including the manufacture of detergents (*4*). The SHOP catalyst exhibits high tolerance of polar functional groups such as alcohols or esters, a result of the less oxophilic nature of nickel.

What remained to be done was to combine the best features of the metallocene catalysts with those of the nickel chelate catalysts, and functional group-tolerant polymerization catalysts should result. This, of course, is much more easily said than done, but in effect this is precisely what Younkin *et al.* have accomplished. Through a careful mechanistic analysis of the SHOP systems, they devised a new nickel-based catalyst that incorporates an NO chelate ligand and allows the polymerization of ethylene at ambient temperature and moderate pressure, even without the addition of a cocatalyst (see the figure). The resulting polyethylene is of high molecular weight and exhibits a low degree of branching in the polymer chain. This stands in sharp contrast to the highly branched polymers formed by other known late transition metal cationic catalyst systems (*5*). Most important, perhaps, is the unprecedented functional group tolerance of the new catalytic systems. Polymerizations can be carried out in the presence of polar impurities—severe poisons for Ziegler catalysts—and functionalized olefins can be used as feedstocks.

The discovery of highly active, neutral, single-site late transition metal polymerization catalysts raises several important questions. What sorts of polymers bearing polar functional group can be made and what will be their properties? Will hydrophilic or biodegradable polymers be readily accessible? How can the new catalysts be modified to allow stereoregular polymerization of functionalized α-olefins? One thing is clear, however: The present results will provide the basis for a huge amount of future research, and nickel will be at the middle of it all. This seems only fitting for the metal that inspired the modern era of polyolefin chemistry by serving as the source of Holzkamp's frustrations.

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PERSPECTIVES: TRANSCRIPTION

A Tail of Histone Acetylation and DNA Recombination

Mark S. Schlissel

In the 1960s, molecular biologists first noticed that addition of acetyl groups (acetylation) to histone proteins (constituents of nucleosomes around which the DNA is wrapped) is associated with transcriptionally active regions of the genome (*1*). More recently, many proteins that regulate transcription have been observed to possess (or recruit) histone acetyltransferase (HAT) or histone deacetylase (HDAC) activities that add or remove acetyl groups from proteins, respectively (*2*). The emerging notion is that a sequence-specific DNA binding protein brings histone acetyltransferase activity to an inactive gene in the chromatin. This results in a change in structure of the chromatin and an increase in the accessibility of the gene to other essential components of the transcriptional machinery. The transcription of many groups of genes is regulated by histone acetylation. A report by McMurry and Krangel on page 495 of this issue (*3*) now adds V(D)J recombination in T and B lymphocytes to the list of those reactions potentially regulated by histone acetylation.

Antigen receptors, such as immunoglobulin (Ig) in B cells and the T cell receptor (TCR) in T cells, are encoded by a series of V, D, and J gene segments that are spliced together in different combinations to provide a large repertoire of antigen receptors with different specificities. The assembly of these gene segments into functional Ig and TCR genes during lymphocyte development depends on a site-specific DNA recombination reaction termed V(D)J recombination (*4*). This combinatorial mechanism allows the genome to encode an enormous diversity of antigen receptor molecules with a relatively modest investment of genetic material. All rearranging gene segments are flanked by highly conserved recombination signal sequences. A protein complex containing the lymphoid-specific recombinase

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enzymes, RAG1 and RAG2, recognizes pairs of gene segments and their flanking signal sequences and catalyzes their recombination.

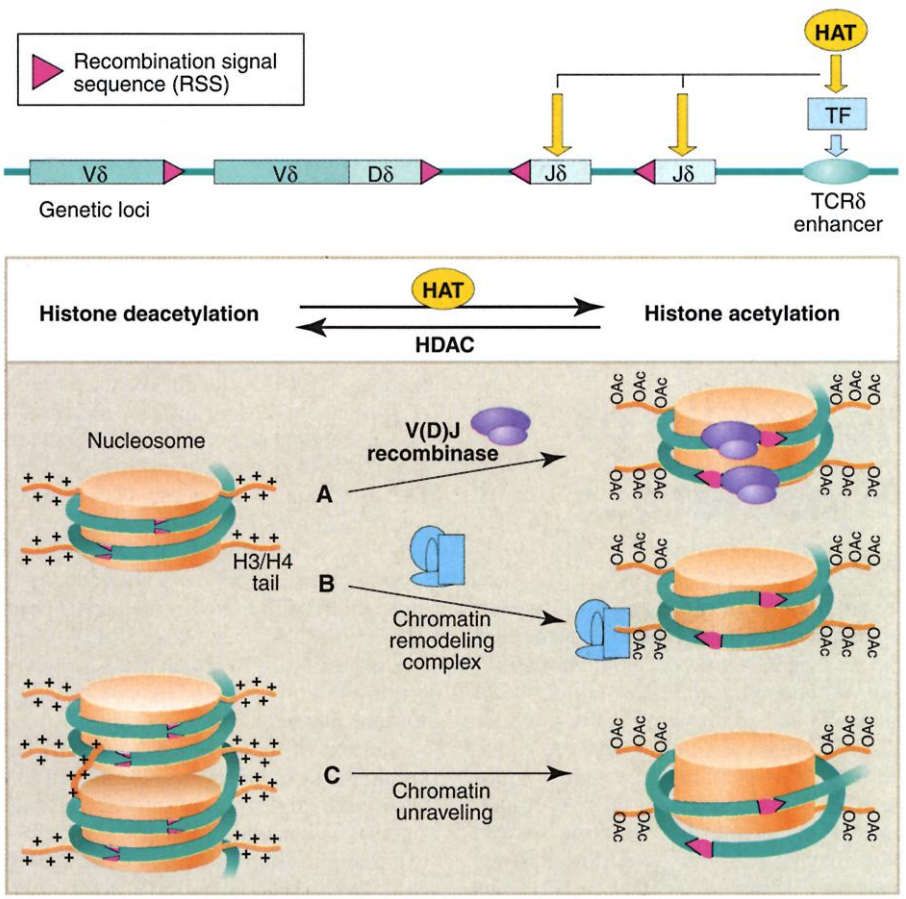
Seven complex genetic loci (Ig μ, κ, and λ, and TCRα, β, γ, and δ) undergo this gene assembly process in a highly regulated fashion. Immunoglobulin genes are fully assembled only in B cells and TCR genes only in T cells (lineage specificity). Within each lineage, Igμ and TCRβ locus rearrangements precede Igκ and TCRα locus rearrangements (ordered assembly). Finally, a given B or T cell generates only one functional Igμ or TCRβ allele because of negative-feedback regulation of rearrangement at the allelic locus (allelic exclusion). How, then, can a common V(D)J recombinase that recognizes highly conserved recombination signal sequences associated with numerous gene segments generate this precisely regulated pattern of gene rearrangements?

An early hint as to the nature of this regulatory mechanism came from the observation that gene segments are transcribed prior to or coincident with their activation for rearrangement (termed germ line transcription). This observation led to the accessibility hypothesis: V(D)J recombination is regulated by differential accessibility of the chromatin structure containing the various gene segment loci (*5*). Germ line transcription may either be a cause or effect of this differential accessibility.

Several groups have shown that targeted disruption of transcriptional enhancers (DNA sequences that bind groups of transcription factors and contribute to gene activation) associated with rearranging loci eliminates germ line transcription and greatly impairs V(D)J recombination (although this is not invariably the case). The accessibility hypothesis has received strong support from experiments demonstrating that recombinant RAG proteins recognize and cleave recombination signal sequences within purified nuclei in vitro, in a pattern defined by the source of the nuclei (*6*). For example, the TCRδ gene locus, but not the Igκ locus, could be cleaved in isolated nuclei from early pro–T cells, whereas the reverse was true in isolated pro–B cell nuclei. Neither locus could be cleaved in fibroblast nuclei, but both loci could be cleaved within purified fibroblast DNA. Thus, accessibility of gene loci to the V(D)J recombinase is a stable and developmentally regulated property of chromatin structure. But, the question remains: What are the properties of chromatin that alter the accessibility of particular DNA sequences to enzymatic processes?

McMurry and Krangel chose to study the regulation of V(D)J recombination in the TCRα/δ locus. During early T cell development, V-to-Dδ rearrangements precede VD-to-Jδ rearrangements. They showed previously that deletion of the TCRδ transcriptional enhancer (Eδ) does not interfere with V-to-Dδ rearrangement, but almost completely prevents VD-to-Jδ rearrangement in a transgenic mouse. In the present study they used an antibody to acetylated histone H3 in a chromatin immunoprecipitation assay to investigate the relationship between histone acetylation and V(D)J recombination. They found a striking correlation between enhancer activity, histone acetylation, and active V(D)J recombination. Vδ and Dδ sequences were packaged in hyperacetylated nucleosomes and underwent Vδ-to-Dδ rearrangement in the presence or absence of an active enhancer, whereas both rearrangement and hyperacetylation of Jδ sequences were strictly enhancer dependent. These investigators then went on to analyze histone acetylation at the TCRα locus. During T cell development, V-to-Jα rearrangement follows productive TCRβ locus rearrangement. They report that nucleosomes associated with the TCRα locus become markedly hyperacetylated as developing cells progress from the pro–T to the pre–T cell stage of development and begin to rearrange the α locus. Furthermore, targeted deletion of the TCRα enhancer eliminates TCRα locus hyperacetylation and completely blocks V-to-Jα rearrangement.

Although these correlations between histone acetylation and accessibility to the V(D)J recombinase are striking, the next challenge in the chromatin structure field will be to understand how histone acetyla-



Getting chromatin to relax. Regulation of V(D)J recombination by histone acetylation. (**Top**) A transcription factor (TF) binds to the enhancer for the TCRδ gene locus and recruits HAT activity to the locus. HAT acetylates local histones, altering chromatin structure and allowing regulatory proteins to gain access to the gene locus. (**Bottom**) (**A**) Acetylation of histone proteins may alter the structure of individual nucleosomes, allowing recombinase enzymes to recognize recombination signal sequences (RSSs) that flank gene segments encoding antigen receptors. (**B**) Acetylated tails of H3/H4 histones may serve as the binding site for chromatin remodeling complexes, resulting in nucleosome sliding or displacement. (**C**) Acetylation may alter interactions between adjacent nucleosomes, leading to an unraveling of the 30-nm chromatin fiber and enhanced accessibility of recombination signal sequences to recombinase. Each of these effects can be reversed by histone deacetylase activity (HDAC).

tion alters the accessibility of specific genetic loci (see the figure). The targets of the acetyltransferase enzyme are lysine residues in the amino-terminal tails of histones, particularly H3 and H4. The crystal structure of the nucleosome core has revealed that these tails extend out from the central disk of the nucleosome around which DNA is twice wrapped (*7*). Acetylation of these histone tails will alter their charge and change their contribution to chromatin structure. These tails are capable of folding back and contacting DNA on the surface of the nucleosome core but can also interact with the DNA or histone proteins of an adjacent core. This adjacent interaction might affect higher orders of chromatin structure such as its assembly into a compact array of supercoiled nucleosomes known as the 30-nm fiber. In addition, the histone tails are available for interaction with other proteins.

First, it is possible that histone acetylation alters the structure of the nucleosome in such a way as to allow binding of the recombinase to the recombination signal sequences. In the absence of acetylation, the RAG recombinases may be unable to recognize a recombination signal sequence on the surface of a nucleosome. Two recent studies have shown that packaging of a DNA substrate containing a recombination signal sequence into a single nucleosome inhibits recognition and cleavage by RAG1 and RAG2 (*8*, *9*). Close contact between the core histones and the wrapped DNA block other proteins from binding to these sequences. But, apparently this inhibition is not relieved when the nucleosome contains hyperacetylated histones (*9*). A second possibility is that histone acetylation causes the localized disassembly of higher order chromatin structure (*10*). This decondensed chromatin might be responsible for the observed deoxyribonuclease sensitivity of accessible loci and might contribute to better access for the recombinase as well. Finally, it is possible that acetylated histones recruit additional chromatin remodeling complexes (Swi/Snf homologs, for example). These remodeling activities might either displace nucleosomes from recombination signal sequences entirely or promote the sliding of nucleosomes, allowing occasional access of the recombinase to the recombination signal sequences (*2*).

The McMurry and Krangel study and the work of other groups suggest that enhancer-binding transcription factors that are developmentally regulated recruit histone acetyltransferase activity to a gene locus destined to undergo rearrangement. However, important questions remain (see the figure). How do these transcription factors gain access to their binding sites in unacetylated chromatin? What determines the boundaries of local histone acetylation? Although histone acetylation clearly correlates with recombinase accessibility, is it necessary? Are additional chromatin structural changes required for the recombinase to gain access to the chromatin? Do the modified histones recruit chromatin remodeling proteins, and if so, what is their role in regulating accessibility? And finally, is overcoming histone-mediated inhibition sufficient to target the recombinase, or are there accessory proteins that specifically recruit the recombinase to accessible loci? The resurgence of interest in how chromatin structure regulates gene expression is sure to lead to a heightened understanding of the regulation of both transcription and V(D)J recombination.

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PERSPECTIVES: PROTEIN SYNTHESIS

Unraveling the Riddle of ProCys tRNA Synthetase

Michael Yarus

Sometimes facts suddenly spin and settle into a more promising arrangement. Partisans of such lovely moments will want to look carefully at a report on page 479 of this issue concerning cysteinyl–tRNA synthetase (CysRS) (*1*). This enzyme is one of 20 aminoacyl–tRNA synthetases—the essential keepers of the genetic code's dictionary. All organisms use these catalysts (which are usually distinct proteins) to covalently connect one of the 20 standard amino acids to its corresponding triplet anticodon within the correct transfer RNA (tRNA) molecule. Thus, these enzymes ensure the subsequent proper alignment of amino acids carried by tRNAs when they pair with complementary triplet codons in mRNA.

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So, it came as something of a surprise to find that the full genome sequences of the archaea *Methanococcus jannaschii* (*2*) and *Methanobacterium thermoautotrophicum* (*3*) lacked an identifiable gene for CysRS. How can such creatures, which have proteins containing cysteines, translate the cysteine codons of mRNA? Apparently, this question has a simple answer: The enzyme was missed during genomic analysis. Extracts of *M. jannaschii* in fact can be shown to have an activity which, given ATP and cysteine, makes Cys-tRNA^{Cys} (*4*). The only unusual quality of this apparent CysRS is that it is "finicky," requiring modified nucleotides in the tRNA^{Cys}; it will not add cysteine to unmodified tRNA^{Cys}.

Stathopoulos *et al.* (*1*) have now followed up on the biochemical identification of the elusive CysRS, purifying the catalyst using a standard biochemical and cloning regimen. CysRS is not elusive for a simple or predictable reason. Instead, it turns out that the missing enzyme is the same protein as prolyl–tRNA synthetase (ProRS), identified in *M. jannaschii* during earlier analyses. Instead of only synthesizing either Pro-tRNA^{Pro} or Cys-tRNA^{Cys}, the purified ProCysRS protein catalyzes both reactions. It does not make the misacylated cross products (Pro-tRNA^{Cys} and Cys-tRNA^{Pro}), and ignores the other 18 amino acids. However, proline inhibits Cys-tRNA synthesis and cysteine inhibits Pro-tRNA synthesis.

Fusions of these two enzymic activities within the same catalyst might conceivably occur with all degrees of intimacy (see the figure). At one end of the scale (panel A), two domains each performing one activity could be simply linked head-to-tail with perhaps a linker peptide in between. Such a dual synthetase, GluProRS, has already been identified in metazoans, such as *Drosophila* (*5*). This 163-kD protein—in which the internal linker is suspected of being necessary for targeting or quaternary structure—has two discrete active sites. It can be cut apart into two separate domains, each fully functional as a GluRS (amino-terminal fragment) or ProRS (carboxyl-terminal fragment).