of this hypothesis, they provide molecular evidence that auditory epithelial cells of the female fish express estrogen receptors.

Although by no means contradicting theories of sensory exploitation and drive (*7*, *8*)—how male vocalizations have evolved as a result of detection and choice by females—the present study reminds us that sensory receptivity can be dynamic and context dependent. This should be taken into consideration when exploring cases of apparent mismatches between sensory signals and the receptors that respond to them. Furthermore, the new work extends the observations of hormone-dependent optimization of tuning from the fish electrosensory system to its evolutionary cousin, the auditory system. Indeed, there are scattered but intriguing reports that estrogen or testosterone may be crucial for the development and maintenance of the cochlea of the mammalian inner ear (*9*).

As a good study should, this one raises further questions. Why is the female midshipman's ear not tuned to the components of male vocalizations during the rest of the year? How are the ears of male fish tuned in and out of breeding season? The tuning of the auditory hair cells in nonmammalian vertebrates is largely based on the properties of their ion currents, less so on their mechanical properties. It is likely that steroid hormones act directly on the auditory receptors of the midshipman fish inner ear by modifying the ion currents responsible for electrical tuning and neurotransmitter release. The mammalian cochlea has dispensed with electrical tuning of its hair cells and has, instead, "invested" in a number of remarkable adaptations (both passive and active mechanical processes) for extending the high-frequency range of hearing. It is possible that the influence of steroid hormones on vertebrate auditory organs is an ancestral condition. The co-opting of hormones for regulating development of the mammalian cochlea rather than for hair-cell tuning may be a relatively recent evolutionary innovation. Stay tuned for further revelations!

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BIOCHEMISTRY

Completing the View of Transcriptional Regulation

Peter H. von Hippel

The *lac* repressor of *Escherichia coli* and its interactions with inducer and with specific and nonspecific DNA have long been the central model system for understanding transcriptional control. A molecular view of the system has come from the crystal structures of the *lac* repressor in its free form, bound to its specific operator DNA target site (RO), and bound to inducer (RI) (*1*). However, the picture lacked an important piece, namely *lac* repressor in complex with nonspecific DNA (RD). On page 386 in this issue, Kalodimos *et al*. (*2*) complete the picture by solving the structure of a dimer of the head-group DNA binding domains of *E. coli lac* repressor in complex with nonspecific DNA, a good representation of the RD complex (*3*). Comparing the structure and dynamics of this complex with a structure of the same domains bound to the specific *lac* operator site (representing the RO complex) provides a structural view of how these complexes might interconvert.

The first major effort to understand the control of gene expression grew out of the seminal genetic studies of Jacob, Monod, and co-workers on the *lac* operon. Their work established that (i) regulation of the three enzymes involved in lactose metabolism that are coded within the *lac* operon occurs at the level of gene expression rather than by activating enzyme precursors (*4*); and (ii) the inducer ligand acts on a repressor of transcription, rather than by activating an "inducer protein" (*5*). [A description of these early studies and their interpretations can be found in (*6*).]

The next step was taken by Gilbert and Muller-Hill, who isolated the *lac* repressor (*7*) and showed in vitro that it acts by binding to a specific DNA site located near the promoter of the *lac* operon (*8*). This established the notion of a binding complex of protein and DNA in which specific protein residues "recognize" a particular sequence of base pairs on the same principles as the residues in the active site of an enzyme recognize its substrate. Furthermore, the natural inducer of the *lac* repressor was shown to be a small-molecule intermediate in the lactose reaction pathway that binds specifically to *lac* repressor to give a complex that has lower affinity for the operator DNA target site.

Specific interactions between the hydrogen bond donors and acceptors of the protein binding site and those of the base pairs in the grooves of the DNA double helix provide the molecular basis of binding specificity and target recognition for DNA binding proteins such as *lac* repressor (see the figure). Thus, specific binding occurs in the grooves of double-stranded DNA (dsDNA). However, this binding is supported and stabilized by electrostatic interactions between the negatively charged phosphates of the sugar-phosphate backbones of the dsDNA and the basic amino acid residues that surround the binding site of the protein, and these interactions are largely independent of base pair sequence (see the figure).

This nonspecific binding of regulatory proteins to dsDNA can complicate efforts to establish binding specificity, but it is more than just an experimental nuisance. In fact, in the *lac* repressor regulatory system (and in many others), nonspecific DNA binding plays at least three central mechanistic roles.

First, RD complexes play an important role in the overall thermodynamics of the regulatory process (*9*). Binding of repressor to nonspecific DNA sites decreases the amount of R that is free in solution and thus is directly available to support the RO binding equilibrium. On a per-site basis RD binding is ~10^8-fold weaker than RO binding at physiological salt concentrations. However, the RD binding interaction is functionally nontrivial because the genome of *E. coli* contains ~10^7 base pairs, and thus ~10^7 overlapping nonspecific DNA binding sites that can potentially compete with operator for repressor binding. In addition, unlike RO binding, RD binding is not weakened by RI complex formation (*9*). Consequently, the successful removal of the *lac* repressor from its operator target site by inducer binding, both in vitro and in vivo, is critically dependent on the binding of both R and RI to nonspecific DNA (*9*, *10*).

In subsequent work, Record and his coworkers showed that nonspecific binding of

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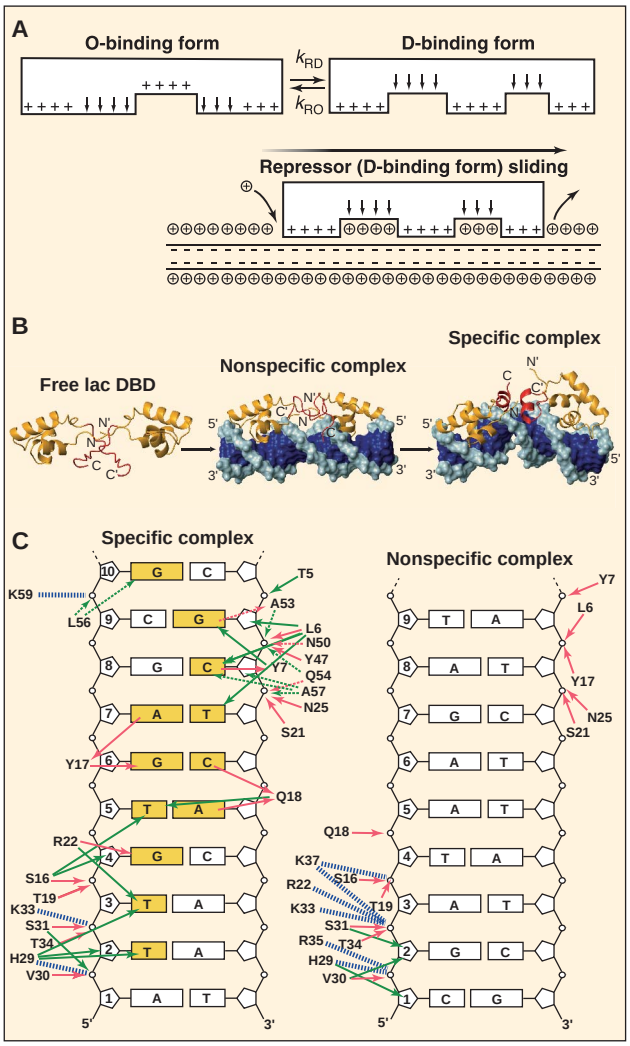

Specific and nonspecific DNA binding complexes of lac repressor. (**A**) Schematic models of the specific (RO) and nonspecific (RD) complexes. Small arrows denote specific hydrogen bond donor and acceptor groups of amino acid residues in the protein binding site. Plus signs (+) denote basic side chains located in and around the same site. In the "down" position these groups are in "interactive contact" with the underlying dsDNA, and in the "up" position these contacts are broken. In the RO complex, there are seven hydrogen bond donor and acceptor "recognition" contacts with the base pairs of the DNA operator site, and only six electrostatic interactions with the charged DNA backbones. In the RD complex, there are 11 charge-charge interactions with the dsDNA backbone, but all the specific interactions with the DNA base pairs have been "withdrawn." As indicated by the double arrows, these conformations are dynamic and interconvert with rate constants k_{RO} and k_{RD}. The lower diagram shows the RD complex engaged in one-dimensional diffusion-driven sliding along dsDNA. Condensed monovalent salt cations are displaced from the backbone "ahead" of the sliding complex, and rebind "behind." Because this "relaxation" of the ion atmosphere is much faster than the sliding rate of the repressor, this sliding represents a true one-dimensional diffusion. [Reprinted from (*17*) with permission] (**B**) Structures of R, RD, and RO forms of *lac* repressor. Note the bending of the complex and the repositioning of the protein helices that occur in the RD to RO interconversion, as well as the dehydration of the protein-DNA interface. [Reprinted from (*2*)] (**C**) Base pair–specific and –nonspecific (backbone) contacts of a single *lac* repressor head-group in RO (left) (bases that are specifically defined by these contacts are colored yellow) and with the DNA of a representative nonspecific DNA binding site (right) (these contacts involve only sugar-phosphate backbone positions). [Reprinted from (*2*)]

proteins such as *lac* repressor to polyelectrolytes such as DNA is purely electrostatic, and can be quantitatively explained in terms of competitive condensed counterion displacement from the DNA by polycationic proteins and related ligands (*11*). Using this model, these workers (*12*, *13*) and others (*14*) estimated that nonspecific DNA binding by a pair of repressor subunit binding domains involves ~11 charge-charge interactions, whereas only six or seven such interactions seemed to be involved in binding R specifically to its operator target.

In addition to its thermodynamic role, nonspecific DNA binding is also central to the kinetics of RO complex formation. In 1970 Riggs *et al*. (*15*) observed that the *lac* repressor seemed to find its operator target at a much faster rate than expected for a diffusion-controlled reaction. This excluded a direct binding process and implied that there must be intermediate states involving RD complexes in the RO binding reaction, and that this nonspecific binding must increase (rather than decrease) the rate of operator target location by the repressor. The kinetics of the RO binding pathway were quantitatively analyzed by Berg and co-workers (*16*, *17*). They proposed three mechanisms for how *lac* repressor locates its operator that all depended on the "facilitated transfer" of R to O by one-dimensional diffusion while in the RD complexed state (*18*); these were called sliding, intersegment transfer, and hopping (*16*).

Subsequent work has shown that all three processes are involved in the kinetics of specific site target location. The sliding process is dominant on naked DNA at low salt concentration, whereas intersegment transfer is likely to be more important within the cell, where the DNA is notably covered with protein and also exists in a compacted state. Based on the Record *et al*. description of nonspecific binding (*11*), and the resulting estimates of the number of charge-charge interactions involved in the RD and the RO complexes, Berg and co-workers (*17*) developed a model of how repressor might slide along DNA and how it might undergo fast conformational changes to permit recognition of the operator when this specific binding site was traversed (see the figure). By interpreting data on heat capacity change, Record and co-workers also predicted that the water of hydration located at the protein-DNA interface should not be appreciably displaced in the RD complex, but should be largely displaced in the RO complex (*19*).

Finally, because most DNA regulatory proteins seem to display a net positive charge around their binding site and a net negative charge elsewhere, the nonspecific binding of repressor and other regulatory proteins to DNA can also play a structural role. Thus, this binding serves to orient the protein properly with respect to the DNA sugar-phosphate backbones so as to facilitate recognition, within the grooves of the dsDNA, of the DNA hydrogen bond donors and acceptors of the specific target sequence. This orientation occurs whether the overall approach of the protein is by diffusion from free solution or by sliding from vicinal nonspecifically bound positions on the same DNA molecule (see the figure).

Pleasingly, most of these predictions based on thermodynamic and kinetic treatments of the RD and RO interactions are borne out by the structures of the RD and RO complexes elucidated by Kalodimos *et al*. (*2*). These include the number of charge-charge interactions seen in the specific and nonspecific DNA complexes; the dehydration observed when the RD complex changes conformation to specifically form an RO complex; and the conformational dynamics of the two binding forms of the repressor (see the figure) (*2*). Direct structural proof of sliding in the RD complexed state comes from the finding that the nuclear magnetic resonance (NMR) structure of the RD complex could only be solved when the length of the dsDNA oligomer used to form the complex was exactly the same length as the DNA binding site of the repressor (*2*). Even one addi-

tional base pair on the DNA oligomer caused the RD complex to "disappear," presumably because the repressor could slide back and forth along the extended DNA and thus prevent the determination of a defined static (on the NMR time scale) RD complex structure.

The *lac* repressor and its complexes with DNA have long served as a central paradigm in the quest for an overarching molecular basis for protein-DNA recognition and for the mechanisms of transcription regulation. The study by Kalodimos *et al*. (*2*) provides a thoroughly satisfying structural conclusion to this saga.

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OCEAN SCIENCE

The Fate of Industrial Carbon Dioxide

Taro Takahashi

The atmospheric CO_2 concentration has increased from about 280 parts per million (ppm) in 1800—the beginning of the industrial age—to 380 ppm today (*1*). During this time, the observed annual rate of increase has been about 50% of that expected from the estimated industrial CO_2 emission rate into the atmosphere. This means that an amount of CO_2 equivalent to about one-half of the industrial CO_2 emitted each year has been missing, and thus Earth's atmosphere is receiving only one-half the full impact of the anthropogenic CO_2 emissions. What process has been taking up the "missing carbon"? An answer to this question is fundamental not only for our understanding of the natural carbon cycle, but also for formulating a sound global CO_2 emission strategy. As reported on page 367 of this issue, Sabine *et al.* (*2*) used an extensive data set obtained for CO_2 concentration and other chemical properties during recent global oceanographic programs, together with a computational method developed by Gruber *et al*. (*3*), and provided a solid estimate for the total amount of CO_2 taken up by the global oceans from 1800 to 1994. Their results show that the oceans store a major proportion of the anthropogenic CO_2 and provide a better understanding of the carbon cycle. In an accompanying report on page 362, Feely *et al*. (*4*) show how the acidification of ocean waters that resulted from the dissolution of anthropogenic CO_2 has changed an important carbon pathway in the oceans—the production, dissolution, and accumulation of biogenic $CaCO_3$.

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Ocean waters are stratified according to their density. In subsurface regimes, waters flow from polar regions toward lower latitudes along constant-density horizons, with little mixing between different densities. A parcel of water that is found at depths today was located at some past time near the sea surface, where it acquired CO_2 and oxygen from the overlying atmosphere (see the figure). During its course of subsurface travel, CO_2 was added from the oxidation of biogenic debris and dissolved organic compounds as well as from the dissolution of skeletal $CaCO_3$ falling through the water column. To compute the amount of CO_2 addition attributable to the atmospheric CO_2 increase, the background and biogenic additions must be subtracted from the measured value. Sabine *et al*. used several assumptions for estimating the biogenic contributions. The P:N:C:O_2 stoichiometry for decomposing organic matter is assumed to be constant. The amount of oxygen used for the oxidation is estimated to be the difference between the observed concentration and the original oxygen concentration, which in turn is obtained by assuming saturation with the atmosphere at the temperature of water. The amount of $CaCO_3$ dissolution is estimated from the difference between the observed alkalinity value and the original at-surface (or preformed) value that is estimated as a function of salinity, phosphate, and oxygen concentration (*3*). The background CO_2 concentration in seawater is estimated assuming that the water contained the preformed alkalinity and was in equilibrium with the preindustrial air for waters at depths below about 2000 m, or with atmospheric CO_2 at the time of the last contact with air for depths above about 2000 m. The age of the water sample is estimated from chlorofluorocarbon or tritium data. The overall uncertainty of the global inventory of anthropogenic CO_2 has been estimated to be about ±20% (*3*).

In table 1 of the Sabine *et al*. report (*2*), the 1800–1994 inventory for anthropogenic CO_2 thus estimated for the global ocean and other carbon pools is summarized. Two well-known quantities in the global carbon cycle are the emissions from fossil fuel combustion plus cement production of 244 ± 20 Pg C (petagrams of carbon; 1 Pg = 10^{15} g) and the amount of excess carbon in the atmosphere of 165 ± 4 Pg C as of 1994. The 1800–1994 ocean inventory of 118 ± 19 Pg C reported by Sabine *et al*. yields the following important information. First, the land biosphere carbon pool has decreased by 39 ± 28 Pg C (244 – 165 – 118 = –39) since 1800. This means that the ocean is the major repository of anthropogenic CO_2 and stores nearly 48% of hitherto emitted CO_2 into the atmosphere. Second, if the change in carbon emission from land use of 100 to 180 Pg C (*5*) is accepted, the terrestrial biosphere should have accordingly in-

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