slightly greater than that allowed by the uncertainties placed on this column density. It is not the hydrogen, however, that is the dominant soft x-ray absorber but helium and, to a lesser extent, the L shells of heavier elements. If for some reason, such as perhaps the way the gas is ionized, the abundance of neutral helium is different from the expected value (which is attributable mainly to the Big Bang), then agreement can be obtained with no soft excess. Indeed, the best fit to the spectrum (see figure) that I obtained with a single-temperature model is with (i) helium at 70% of the measured value and (ii) the other elements, including the absorbing hydrogen at 100% of the measured value.

It is not possible to measure the column density of neutral helium directly. The neutral-helium absorption model (5) used in most x-ray spectral models has been recently revised and is unlikely to be at fault. If some small absorption correction, attributable say to ionization, were generally applicable to the ISM, then its effect would not have been found with the spectra of active galaxies and quasars because they are often expected to show a soft excess. It is only with clusters that the predicted spectrum in the soft x-ray band should be reliable. An absorption interpretation accounts for the occurrence of the effect in other clusters and for the fact that, as a fraction, the soft excess appears to be constant with radius. This last point would require a rather peculiar emission model.

A further possibility is that the excess is the result of some calibration uncertainty. Such an uncertainty seems unlikely because the result was observed in at least two different detectors, but it would explain why the excess has not been reported before. Some of us who have worked on the ROSAT spectra of clusters have seen a similar effect earlier and dismissed it as either a calibration problem or an error in the galactic column density or have reported it without emphasizing it (6). Lieu *et al.* have done us all a service by highlighting the issue, which represents the discovery of either a new component of the ICM (or at least of clusters), a small correction to the x-ray absorption properties of the ISM, or a problem with the detector calibrations.

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The Whole Lactose Repressor

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When Monod and Jacob and their colleagues began studies of enzymatic adaptation and bacterial genetics in *Escherichia coli* at mid-century, they could not have known that deciphering the intricacies of these processes would open an entirely new field that is only today reaching its zenith. Regulation of genetic expression is central to survival in all living organisms, providing the ability to respond to chemical communication from intra- and extraorganismal sources. Our first glimpse into the elegance of genetic regulatory systems was provided by elucidation of the mechanism by which bacteria regulate expression of the enzymes for lactose metabolism.

Once the *lacI* gene product was identified as the agent that regulates expression of the lactose operon enzymes (*1*), purification of the lactose repressor protein (*2*) and in vitro demonstration of its DNA- and sugar-binding properties followed rapidly (*3*). However, solution of the crystallographic structure of this prototypic genetic regulatory protein, pursued by many research groups, eluded investigators for almost three decades. Attempts to coax diffraction-grade crystals from myriad solutions of this protein, even in the zero gravity of space shuttle missions, were uniformly unsuccessful. Even after yielding to crystallization (*4*), this protein has provided multiple challenges to solving the phase problem. The structures of the dimeric purine repressor and the core domains of *lac* and purine repressors produced by proteolytic removal of the NH_2-terminal DNA-binding domain (*5*) were solved only recently. In this issue, Lewis, Lu, and their colleagues report structures of the intact tetrameric lactose repressor protein and its complexes (6)—our first view of the repressor-operator and the conformational changes in this protein that result in its complex and fascinating behavior.

In the years since its initial purification, the lactose repressor has been a target of intense study. Extensive genetic information, equilibrium and kinetic analyses of ligand binding, as well as chemical and physical characterization of wild-type and mutant proteins have been compiled (*7*, *8*). This tapestry of data defines many of the characteristics of this molecule and enriches the insights provided by these structures. The assignment of binding capacities to core and NH_2-terminal domains, as well as a significant portion of phenotypic data on mutant proteins, can be rationalized effectively by the structures (*7*, *8*). The mobility of the NH_2-terminal DNA-binding domains is reflected in the absence of electron density for this region in the free and inducer-bound forms of the protein (6). Although contacts between the helix-turn-helix binding motif and operator DNA are not well resolved in the structure of the complex, the arrangement nonetheless indicates clearly the binding orientation and the involvement of the hinge helix in high-affinity binding. The rotation of two subdomains within the repressor core monomer in response to inducer binding reflects additional structural flexibility that may account for the difficulties in crystallizing this protein. The results of crystallographic analysis also confirm the utility of homology modeling methods to establish general folding patterns for crystallization-resistant proteins. The essential fold of the core domain monomer was predicted successfully for the lactose repressor on the basis of the crystallographic structures of bacterial periplasmic sugar-binding proteins (9).

Given homomeric assembly of this protein and knowledge of other tetramer structures, the V shape of the repressor tetramer and the consequent absence of 2:2:2 symmetry are surprising (see figure). Only a small buried surface (and hence small free energy) contributes to this quaternary arrangement. The separation of monomer-monomer and dimer-dimer subunit interfaces evident in the tetrameric structure was demonstrated initially by chemical and physical methods (*10*–*13*). Monomers associate to dimer through a surface of the bilobate core domain formed by residues distributed widely in the primary sequence (6); this interface transmits the allosteric communication between monomers (cooperativity) that accompanies inducer binding (*12*). In contrast, dimer-dimer assembly occurs by way of a compact four-helical bundle formed by only 18 COOH-terminal residues from each subunit, an arrangement predicted from genetic studies (*13*) and reminiscent of a similar motif in the eukaryotic regulatory protein p53 (*14*). No allosteric communication appears to occur through this interface. One of the key unanswered questions (not

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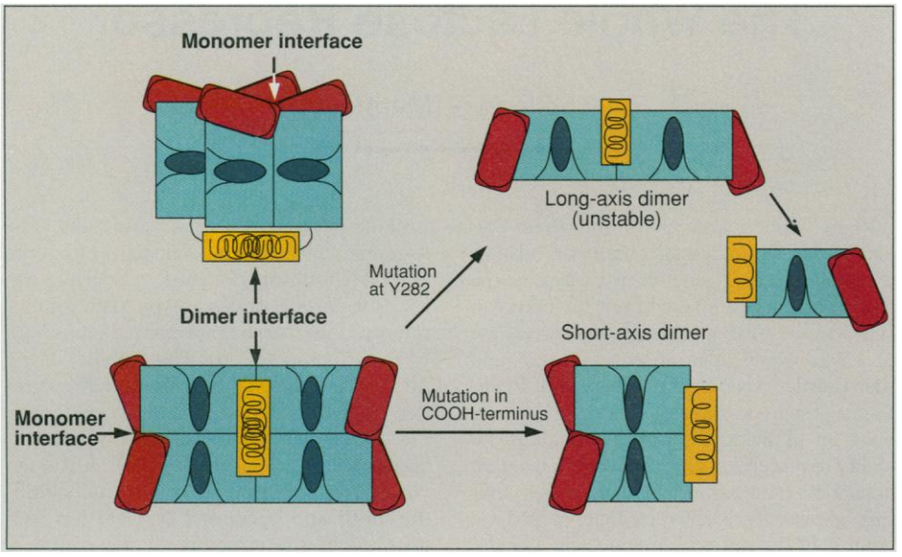

Subunit interfaces in the lactose repressor protein. The large red ellipsoids represent the helix-turn-helix DNA-binding domain, and the smaller dark blue ovals are the binding site for sugar (inducer). The yellow coiled regions represent the leucine heptad repeat sequence. The tetramer structure (upper left) contains two dimers aligned with their NH_2-terminal domains on the same face of the molecule and connected by a four-helical bundle at the base, with an angle of ~28° between the dyad axes of each dimer (*6*). The latter connection abolishes the two potential two-fold axes of symmetry. Opening the structure (lower left) reveals the two subunit interfaces and shows the two potential types of dimeric species. Mutation at Y282 (tyrosine 282) results in disruption of both interfaces to produce the monomer, whereas mutation at the COOH-terminus results in dimeric repressors.

just for this protein) is the mechanism by which cooperativity of ligand binding is mediated. The structures of the repressor and its complexes reveal multiple amino acids that should provide interesting targets with which to explore, by mutagenesis and in vitro characterization, the physical basis of allosteric transitions.

The synthesis of sugar analogs by Monod and his colleagues (*15*) was a key step in deciphering relationships within the lactose operon, and the nonmetabolized but inducing sugars (gratuitous inducers like isopropyl-β-D-1-thiogalactopyranoside) were essential to the insights into genetic regulation generated by Jacob and Monod (*1*). Our understanding of conformational changes in the repressor monomer elicited by binding to inducer sugars will be enhanced as details of the structural shifts that accompany sugar binding come into more precise focus. The influence of sugar binding on protein conformation can be inferred from its contacts in the binding site, and it is apparent that the precise nature of the sugar will determine its effect on protein tertiary arrangement. As seen in the structures, sugar binding alters the orientation of the NH_2-terminal DNA-binding domains, apparently precluding high-affinity contacts of the NH_2-terminal helix-turn-helix motifs and hinge helices with bases in the operator DNA sequence.

This molecular view of the lactose repressor regulatory protein brings us to a new stage in our understanding of the complex process of "enzymatic adaptation." Interestingly, transcriptional regulation by the lactose repressor mirrors many features of eukaryotic systems (*8*). Despite the pessimism expressed by Jacob and Monod in 1969 on prospects for "analyzing down to the ultimate level the programming of the development of a metazoan embryo" (*16*, p. 3), we are discovering in expanding and exquisite detail the specifics of gene regulation, not only in development but throughout the life of an organism. The principles of protein-DNA interaction modulated by binding to signal molecules (whether small ligands or other proteins) that we use today in our quest to understand genetic regulation—be it in development, tumorigenesis, viral infection, or normal cell function—are based in large part on the revolutionary concepts of genetic regulation and protein conformational change that emerged from the bacterial petri dishes of the Institut Pasteur almost 50 years ago.

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