Response: For many years the repressor of the lactose operon has served as a prototypical model for studying gene regulation. The x-ray structures that we reported (1) of the repressor; the repressor bound to an allosteric effector, isopropyl-β-D-1-thio-galactoside; and the repressor complexed to a symmetric 21-bp operator, provide atomic models that describe the conformation of the repressor in the induced and the repressed states. By comparing the three structures, we could describe the transition between states. Interestingly, the allosteric changes are fundamentally analogous to the transition between the T and the R states observed in hemoglobin (2). The structural transformation, however, is localized and requires only a dimeric repressor, even though the observed quaternary structure is tetrameric. From the work of Muller-Hill and his co-workers, it is clear that the intact tetramer is required for maximal repression (3), and high levels of repression are observed only when the tetrameric repressor binds to its primary operator, O1, and an ancillary site, either O2 (93 bp upstream from O1) or O3 (401 bp downstream), and thereby form repression loops (4). We built a model of the tetramer bound simultaneously to O1 and O3 in order to account for the observed tetrameric repressor and to provide a physical representation of these repression loops. Our model was constructed by creating the intervening DNA with a radius of curvature of about 40 Å, thereby linking the O1 and O3 sites. Perros and Steitz raise objections to this model and argue against the proposed looping of the repressor.

When the repressor associates with a symmetric operator, there is a distinct bending of the DNA, and a pair of hinge helices fit into the minor groove analogous to that observed with dimeric purine repressor (5). The binding to the minor groove, by necessity, distorts the DNA. The minor groove widens, which causes the operator to bend away from the repressor. Perros and Steitz suggest that the bending of the DNA is an artifact of using a symmetric sequence and propose that the repressor does not alter the conformation of the wild-type operator DNA. In contrast, the genetic analysis of Miller and his co-workers has shown that mutations in the hinge helix alter the ability of the protein to repress transcription (6). A large number of amino acid substitutions within the hinge helix result in repressor molecules that can no longer bind to the operator. In fact, the hinge helix is nearly as sensitive to mutation as the amino acid substitutions in the recognition helix, suggesting that it plays a crucial role and most likely binds to the DNA, as seen in both the x-ray and the nuclear magnetic resonance structures (1, 7). Moreover, the hinge helix transmits the allosteric signal from the inducer binding site to the DNA binding domain. While the structural studies are admittedly contrary to the results of Crothers and his co-workers (8), they are fully consistent with the position-dependent bending observed by Adhya and his co-workers (9).

Perros and Steitz also argue that the hypersensitivity and protected DNase I cleavage sites as seen by Krämer et al. (10) are inconsistent with the looping model. These experiments demonstrated the importance of correctly phasing the two operators and showed that the position of enhanced and decreased DNase I sensitivity depends on the number of base pairs separating the two operators. Because the spacing of O1 and O2 is much larger than those used in the experiments, and because the intervening sequence was nonnative, it seems quite difficult to extrapolate and predict what will happen with the natural operon.

Using gel retardation assays, Perros and Steitz demonstrate that the repressor and the catabolite gene activator proteins (CAPs) cannot bind simultaneously to a fragment of DNA that encompasses both the O2 and the CAP I sites. The bindings of the two proteins are mutually exclusive, and competition results when the sites are 20.5 bp apart; however, there is cooperativity between CAP and lac when the separation is 72 bp (11). Although the information obtained from these experiments is useful, the DNA fragment is too short for mutual binding; therefore, their design does not address the issue. The correct experiment, which is central to our model, is described in the comment by Hudson and Fried. It is clear from their results that, given an appropriate length of DNA, repressor can bind both O1 and O3 in the presence of CAP. Moreover, CAP increases the affinity of the repressor for its sites. In our opinion, the results of Perros and Steitz support our model by demonstrating that repressor and CAP cannot bind on the same face of the DNA. Repressor and CAP can and do bind simultaneously to O2 and the CAP I sites, as seen in the footprinting gel of Hudson and Fried. The apparent discrepancy is resolved by noting that the repressor contact site is moved upstream by 6 bp, which places the interaction on the opposite face of the DNA from CAP.

When the lac repressor binds to two distinct operators, it is likely that repression loops will form with a structure that depends on the physical properties of DNA, as well as the length of the intervening loop (12). Forming stable looped complexes, particularly for relatively short stretches of DNA, may require additional DNA binding proteins that can dramatically change the physical properties of the nucleic acid. CAP, for example, interacts with a DNA sequence between the primary and an ancillary O3 site. CAP binding induces a bend of approximately 90° over about 30 bp of DNA, causing the DNA to kink (13). In our model, the CAP assists the repressor in forming the repression loop. While it may seem paradoxical that a transcriptional activator (CAP) would stabilize or promote the repressed state, loops between O2 and O3 most likely form when there are low concentrations of glucose (and therefore elevated concentrations of cyclic AMP promoting CAP binding) and lactose. In such instances, the bacterium needs to reduce the basal level of transcription of the lac operon to conserve energy, which is consistent with diauxic growth (14). In this way, the lac repressor and CAP operate as an integrative switch that responds to the relative concentration of these metabolites. Our proposed physical model of the repression loop accounts for the vast amount of experimental data describing the regulation.
of the lactose operon. In our view, the model is fundamentally correct and consistent with experimental results.

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11 November 1996; accepted 13 November 1996

The Loss of Atmosphere from Mars

Luhmann et al. (1) and Jakosky et al. (2) showed that the Martian atmosphere was eroded (sputtered) by energetic O\(^+\) ions that are formed from escaping O and accelerated back into the atmosphere by the solar wind fields. This collisional ejection process appears to explain measured isotope ratios for Ar and N in the martian atmosphere (2, 3) and it may affect the early evolution of this atmosphere (1–3). More recently, D. M. Kass and Y. L. Yung (4) presented a more detailed calculation of the loss of Martian atmosphere. They found that 3 bars of CO\(_2\) are driven off by sputtering, an amount three times greater than the size of Earth’s atmosphere. This is a huge increase in atmospheric loss over the earlier estimate of about 0.1 bars (1, 2). This increase came about because Kass and Young assumed that full dissociation of CO\(_2\) (\(\rightarrow C + 2O\)) occurs readily in collisions of an incident O with CO\(_2\). Therefore, C atoms, which have much lower gravitational escape energies than CO or CO\(_2\), are efficiently formed and energized, which increases the loss of C dramatically.

Because the collisional dissociation cross sections in the energy range of interest (~20 eV to 1 keV) have not been measured, the dissociation cross section used by Kass and Yung essentially maximized the atmospheric loss process. The cross section used for dissociation in O + CO\(_2\) collisions can be compared to a molecular dynamics calculation (Fig. 1). In that calculation, the energetic O interacts with each of the atoms in the molecule that are bound together by pair potentials chosen to reproduce the binding energies and interatomic separations of CO\(_2\) and the dissociation product CO. Although the use of pair potentials in this manner typically leads to an overestimate of the dissociation cross section, the threshold for full dissociation (solid curves) described by Kass and Yung is shifted by about a factor of 5 from that calculated, and the size of their cross section is more than an order of magnitude larger than that calculated. Because the size of their cross section is roughly that of the elastic collision cross section, the net contribution of dissociation to the atmospheric loss process is more than an order of magnitude too large. In addition, the primary collisional dissociation channel is seen to be CO\(_2\) \(\rightarrow O + CO\), so that only a small fraction of the struck CO\(_2\) produces C atoms. Therefore, although it is correct that including CO\(_2\) dissociation in all stages of the cascade of collisions initiated by an incident O\(^+\) increases the C loss rate over that described earlier (1, 2), Kass and

Fig. 1. Dissociation cross sections for O + CO\(_2\) collision plotted as a function of the energy of the O atom. Solid lines: O + CO\(_2\) \(\rightarrow O + C + 2O\); dashed line: O + CO\(_2\) \(\rightarrow O + CO\); dotted line: O + CO\(_2\) \(\rightarrow O + CO\). Line labelled KY, cross section assumed by Kass and Yung (1); curves labelled MD, calculated values using molecular dynamics with the universal interaction potential (6) for the interaction of the energetic O with individual atoms in CO\(_2\). Three pair potentials are used for CO\(_2\), which gives the correct dissociation energy for CO\(_2\) and for the resulting CO.

Yung’s estimate of the effect is an order of magnitude too large. Although over the history of Mars it is certainly possible that more atmosphere may be driven off by sputtering than the amount given by Luhmann et al. (1) and by Jakosky et al. (2), it cannot occur in the manner suggested by Kass and Yung (4), even if their cross sections were correct. That is, as the atmospheric escape rate increases, the region in which the solar wind ionizes and accelerates the escaping atoms occurs at larger distances from the planet (5), reducing the fraction of these ions that impact the atmosphere. In the earliest martian epoch this feedback process is already problematic for the much lower escape rates calculated by Luhmann et al. (1) and by Jakosky et al. (2).

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Response: The results presented in our report (1) indicated that it was necessary to consider dissociation during all collisions in calculating the atmospheric loss from Mars that results from sputtering. With the use of the newly calculated cross sections presented by Johnson and Liu in our model, we find that Mars has lost about 1 bar of CO\(_2\). The revised cross sections reduce our sputtering yields (Table 1), but do not bring our results into agreement with Luhmann et al. (2) and Jakosky et al. (3).

The effective decrease in the collisional cross section pointed out by Johnson and Liu of CO\(_2\) \(\rightarrow CO + O\) (channel I) by a factor of about 5 and of CO\(_2\) \(\rightarrow C + 2O\) (channel II) by a factor of about 50 will not result in decreases of 5 and 50, respectively, in the collision frequency with CO\(_2\). At the important energies for sputtering, collisions with CO\(_2\) result in some form of dissociation (Table 1).

The changes in the cross section (a factor of 5 for channel I and a factor of 10 for channel II) do not have a linear effect on the collision probability, but the ratio of
Response: DNA Looping and Lac Repressor—CAP Interaction
Mitchell Lewis (December 13, 1996)
Science 274 (5294), 1931-1932, [doi: 10.1126/science.274.5294.1931]

Editor's Summary

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