proteins from the strains B and K12 of *E. coli*. In confirmation of previous results, no differences are detected between the proteins from the 50S subunits, but two changes are evident between the 30S subunit proteins.

LACTOSE OPERON

Release of Repression

from a Correspondent

PEOPLE who thought that they knew everything about the lactose operon of *E. coli* must stand confounded by reports that its transcription is controlled in two ways. According to traditional wisdom, a repressor protein binds to the operator, preventing RNA polymerase (which binds at the preceding promotor site) from proceeding into the operon to synthesize messenger RNA. The operon is switched on when this repressor is released from the DNA by β-galactoside inducer (the substrate for the enzymes coded by the operon).

But releasing the repression, although necessary, is not sufficient for full expression of the operon. This requires another control system, which was discovered by studying the effect of glucose in depressing the synthesis of many inducible enzymes in bacteria. Glucose has this effect because it reduces the amount of cyclic AMP in the cell. Reasoning that this nucleotide is unlikely to interact directly with the components of the operon, Zubay, Schwartz and Beckwith sought a protein factor which might mediate its effect.

They report (*Proc. US Nat. Acad. Sci.*, **66**, 104; 1970) the isolation of a mutant unable to grow on lactose or arabinose—a characteristic of a mutant lacking the protein factor, because both the lactose and arabinose operons are susceptible to the glucose effect. Using a DNA-dependent β-galactosidase synthesizing system, they could not synthesize this product of the lactose operon *in vitro* when the supernatant components were derived from the mutants, but they could do so with the corresponding extracts from wild type cells. They have purified an activity that seems to reside in a protein of molecular weight 45,000.

Starting from a biochemical instead of a genetic point of view, Pastan and his colleagues sought a protein that could bind cyclic AMP. They, too, found a protein of molecular weight about 40,000 (*Proc. US Nat. Acad. Sci.*, **66**, 480; 1970). Mutants unable to respond to cyclic AMP had a protein with an altered affinity for the nucleotide. The only significant difference between the two groups apart from the name ascribed to the protein (CAP to Zubay *et al.*, CR to Pastan *et al.*) is a two order magnitude difference in the binding constant for cyclic AMP. Although cyclic GMP competes with cyclic AMP for the protein, no other nucleotide binds.

The cyclic AMP binding protein probably acts at the promotor, for mutations at this site affect the response of the lactose to cyclic AMP (*Proc. US Nat. Acad. Sci.*, **66**, 773; 1970), and this genetic analysis is nicely complemented by the finding that the amount of messenger RNA synthesized by the lactose operon correlates with the level of β-galactosidase produced in response to cyclic AMP (*J. Biol. Chem.*, **245**, 2259; 1970). This implies that, as a result of its interaction with cyclic AMP, the binding protein helps the initiation of transcription at the promotor of the lactose and other operons.

Surprises are also in store so far as the interaction of the repressor and operator itself is concerned. Riggs *et al.* (*J. Mol. Biol.*, **48**, 67; and **51**, 303; 1970) have shown that nitrocellulose in the form of membrane filters may be as powerful a tool as nitrocellulose in the form of centrifuge tubes. They have taken advantage of the way in which repressor protein sticks to these filters but double stranded DNA does not. This means that only the DNA that interacts with the protein is trapped on the filter.

DNA trapped on the filter can be released by addition of IPTG (an inducer of the lactose operon). The rate of dissociation of the complex can be measured by following the disappearance of radioactive DNA counts from the filter when unlabelled DNA is added after a radioactive complex has been formed. An unexpected result was obtained by changing the concentration of IPTG; the inducer increased greatly the rate of dissociation of the complex. Another small molecule, nitrophenyl-fucoside, which opposes the effect of IPTG *in vivo*, decreased the rate of dissociation of the complex.

One of the popular models for the action of the repressor is ruled out by the finding that these small molecules directly affect the release of repressor from the operator. This is the conventional allosteric model which suggests that there is a rapid equilibrium between free and bound repressor, and inducers (or anti-inducers) bind only to the free repressor, thus blocking its DNA binding site. The scheme would predict that the rate of dissociation of repressor from DNA would not be affected by inducer concentration. But the inducer seems to pry the repressor off the DNA, probably by binding to an independent site on the repressor. Another surprise revealed by this technique is that glucose, hitherto thought to exert its effect on repression only by altering the amount of cyclic AMP, seems to interact directly with the repressor as an anti-inducer. But the significance of this effect *in vivo* may be limited, for it takes place *in vitro* only at unphysiologically high levels of glucose.

TRANSCRIPTION

Approaching the *in Vivo* System

from our Nucleic Acids Correspondent

RECENTLY, molecular biologists have come to realize that the instant transcription or replication of DNA offered by the use of purified enzymes *in vitro* may differ significantly from the way in which these processes take place within the cell. The present fashion is to regard these activities as catalysed by cell organelles rather than individual enzymes, and accordingly, two articles in the current issue of the *Journal of Molecular Biology* report the isolation of structural complexes from the cell which contain all the components necessary for transcription or replication.

Pettijohn *et al.* (*J. Mol. Biol.*, **52**, 281; 1970) have established an experimental system for studying the transcription of ribosomal RNA cistrons *in vitro* from *E. coli* or *Bacillus megaterium*, which must at least approximate to the real situation. *In vivo*, the rate of transcription of the ribosomal RNA cistrons is as much as 100 times greater than mRNA copying in bacteria. Pettijohn *et al.* have been able to purify a DNA-protein