the vortex array, a magnetic field was required to focus the pulses of electrons leaving the liquid, and a very high quality optical system, using fibre optics to bring the feeble flashes of light up from the cold end to an image intensifier at room temperature, had to be developed. The light from the image intensifier passed into a wide aperture camera loaded with high speed film.

It was found that, with the apparatus in rotation, a series of spots did indeed appear on the film whereas, at rest, a completely blank negative resulted. The density of spots was roughly equal to the expected density of vortex lines in the liquid, and was proportional to the angular velocity.

It turned out, however, that the vortex lines were not in fact arranged in a regular manner, and moreover they appeared to wander around in the liquid in a random manner. The reason for this is not yet clear but, now that a successful technique for photographing individual vortex lines has been developed, the topic will no doubt be the subject of further theoretical and experimental investigations.

Control sites in the lactose operon

from Benjamin Lewin

THE lactose operon has been subjected to such intensive genetic analysis during the past decade that it is perhaps surprising that not all possible classes of mutant were isolated long ago. That the mutants initially taken to identify the promoter in fact correspond to impairment in the action of the cyclic AMP system is, of course, now well known. The wild type lactose operon can be expressed at its full level only in the presence of the cyclic AMP receptor protein (CRP), activated by cyclic AMP. The mutants previously isolated which reduce to 2% of its wild type maximum the expression of the lactose operon all map at the left end of the region between *lacI* and *lacO*, since they fail to recombine with the L1 deletion that covers this region; the reduction to 2% maximum expression is shown also by cells which contain a wild type lactose operon but lack a functional cyclic AMP system. The far left end of the lactose control region therefore seems to provide a DNA site which is necessary for the interaction of CRP and RNA polymerase. Mutants located between this site and the operator have been isolated which allow a high degree of operon expression in the absence of the cyclic AMP system, presumably by creating a site which allows the RNA polymerase alone to initiate transcription.

The model accepted to explain these results supposes that the promoter of the lactose operon is a complex structure, its left end providing for an interaction with the CRP and its right end providing the site where RNA polymerase binds and initiates transcription. How the interactions at these sites are related is not clear. A prediction of this model is that mutants which reduce the level of expression of the operon by lowering the affinity for RNA polymerase of its binding site should map at the right end of the promoter region.

The isolation of these mutants, constituting the first new mutant class to be reported for some time in the lactose operon, has now been accomplished by Hopkins (*J. molec. Biol.*, **87**, 715–724; 1974). Certain repressor mutants share with promoter mutants the phenotype of reduction in maximum operon expression since they prevent induction, but the promoter mutants alone reduce the basal level of lactose enzymes in the uninduced cell. Using this distinction to identify promoter mutants, Hopkins isolated two classes; the first comprise the previously identified kind of mutation at the left end of the promoter and show failure to respond to the cyclic AMP system; the second class, isolated for the first time, are located at the right end of the promoter region as shown by their ability to recombine with the L1 deletion, and have the properties expected of mutants altered in the interaction with RNA ploymerase—they show about 6% of wild type expression in cells with an active cyclic AMP system and this level is reduced to less than 0.5% when the cyclic AMP system is inactive (wild type operons show 2% expression in the absence of the cyclic AMP system). All three mutations of this type lie between the right end of L1 and the operator. The isolation of these mutants opens the possibility of analysis *in vitro* to determine exactly which stage of action of RNA polymerase is inhibited, clearly the next step in identifying the action of this part of the promoter.

The binding of repressor protein to the lactose operon, hitherto thought to implicate only the operator site immediately to the left of the *lacZ* structural gene also may involve more than one site. Using a competitive binding assay for repressor, in which the DNA to be tested is mixed with repressor in the presence of a standard (labelled) operon and the reduction in retention of label on filters is measured, Reznikoff, Winter and Hurley (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2314–2318; 1974) confirm that deletions of the lactose operon have no affinity for repressor and constitutive operator mutants have a reduced affinity depending on the mutation. Promoter mutants show no reduction in repressor binding, including one deletion which appears to remove virtually all the promotor though leaving the operator intact; the independence of these two sites contrasts strongly, of course, with the overlap between them which is seen in phage lambda. A deletion which removes *lacZ* but does not extend into the operator has the same affinity for repressor as wild type lac DNA, confirming that the operator does not extend into the structural gene.

But the strong *lacO*^{c} mutant DNAs have a much greater affinity for repressor *in vitro* than would be expected from the effect of these mutations in the cell; two mutants which reduce the induction of lactose enzymes in the cell by 100 and 300 times show a reduction in affinity for lactose repressor of only 25 or 30 times. And deletions which remove a large part of the operator show the same affinity for repressor as the operator-constitutive point mutations. This suggests that some common residual binding of repressor might take place at a site present in all these mutant DNAs. When these deletions extend into the first part of the *lacZ* gene, the residual binding remains, but when they extend further, the affinity for repressor is much lowered. This suggests that a secondary binding site is present in the *lacZ* gene, some distance from the operator but within the first thousand bases.

Since the operator seems to be at least 16 bases long, it is very unlikely that this second binding site might be the result of a coincidence of sequence; the probability of independent origin of a sequence with an affinity for repressor as great as 3-4% of that of the operator is low—some *lacO*^{c} point mutations might well show this order of binding. No obvious role can yet be seen for this second binding site, although it is possible that it might act as a failsafe signal for blocking progress of polymerases that have passed the operator or that it might interact in some secondary structure with the primary operator; there is, however, some evidence from earlier work to argue against both these possible models. The evolutionary origin of this site is an interesting question, some light on which may be cast by sequence studies of the *lacZ* gene as well as the control region. If the second site does play some part in operon function, then its nucleotide sequence must be under two sets of selective constraints, one concerned with its binding of repressor and one with the amino acid sequence specified in β-galactosidase.