Specific Binding of the λ Phage Repressor to λ DNA

by
MARK PTASHNE
Department of Biology, Harvard University

Genetic experiments show that a group of genes may be switched off by the product of a regulator gene, called a repressor. An isolated repressor is shown here to bind specifically and with high affinity to DNA, strongly suggesting that, *in vivo*, repressors block the transcription from DNA to RNA by binding directly to the DNA.

THERE are many examples, in bacteria and their phages, of a group of genes controlled by the product of another gene—a regulator gene. In the classical cases discussed by Jacob and Monod[1], the control is negative and the product of the regulator gene is called a repressor. Repressors act by switching off their target genes; in order to activate these genes the repressor itself must be inactivated. These facts were learned from genetic experiments which do not reveal how repressors work at the molecular level.

The isolation in recent months of two repressors[2,3] makes possible biochemical experiments exploring the mechanism of repression. Many of the models for this mechanism propose different sites for the action of the repressor. According to the simplest model, the repressor binds to a site on the DNA, directly preventing the transcription from DNA to RNA. According to other models the repressor interacts with *m*RNA or *s*RNA to block translation of the genetic message from RNA to protein. A prediction of the first model is that an isolated repressor will bind *in vitro* to DNA containing the receptor site for that particular repressor, but not to DNA lacking this site. The experiments reported here confirm this expectation.

The Genetic System

The protein made by the C_1 gene of phage λ, called the λ phage repressor, is used in these experiments. This repressor blocks the expression of the other phage genes,

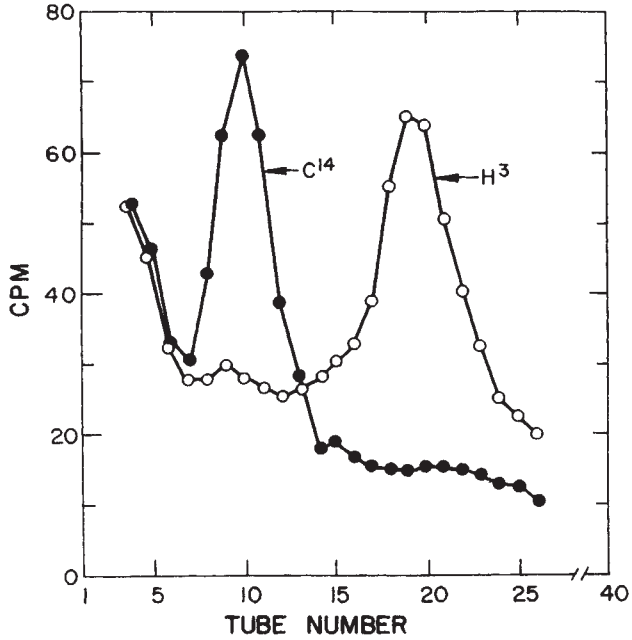

Fig. 1. DEAE-cellulose chromatography of *λind*⁻ and λ wild type repressors. Extracts of *E. coli* phage-infected cells containing ^{14}C-labelled *λind*⁻ repressor in one case and ^{3}H-labelled λ wild type repressor in the other were applied to a DEAE-cellulose column. Fractions from a salt gradient were collected and assayed as described previously[3]. The label used in this and the other experiments reported in this paper was ^{14}C or ^{3}H labelled reconstituted protein hydrolysate.

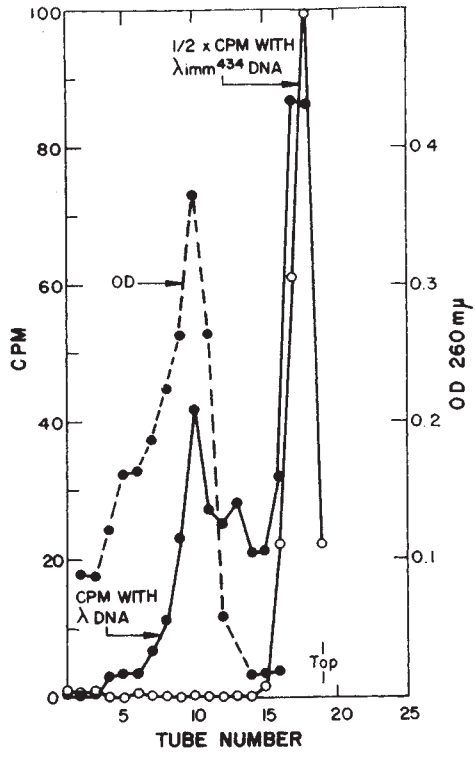

Fig. 2. Specific binding of the *λind* repressor to λ DNA. A portion of ^{14}C-labelled *λind*⁻ repressor, pooled and concentrated from the peak fractions of a DEAE column run, was mixed with 100 μg of λ or *λimm*^{434} DNA. The DNA was preheated at 70° C for 15 min. to minimize aggregation. The solutions were made 0·01 M EDTA, 0·1 M KCl, and 10^{-4} M Cleland's reagent, and 5 μg of commercial *s*RNA was added as an additional inhibitor of possible endonuclease activity. After 5 min. incubation at 37° C, the final volume of 0·7 ml. was layered on a 5–25 per cent sucrose gradient containing 0·05 M KCl, 10^{-4} M Cleland's reagent, and 0·5 mg/ml. BSA as carrier. The gradients were spun at 41,000 r.p.m. for 5 h in an *SB*269 rotor in an IEC centrifuge. Fractions were collected and the DNA peak located by absorbance at 260 mμ. Each fraction was then precipitated on a Millipore filter with TCA and counted in a gas flow counter. The optical density profiles at 260 mμ from the tubes containing λ and *λimm*^{434} DNA are essentially identical. Phages λ and *λimm*^{434} were purified by several bandings in CsCl according to the method of Thomas and Abelson[9], and DNA was then extracted and purified from the two phage preparations using the same phenol and buffer solutions.

keeping the phage chromosome dormant within its host, *E. coli*. Only a very short segment of the phage genome is involved in this control[4,5], a region including the C_1 gene and the sites which determine the sensitivity of the phage to the repressor. Two phages which differ only in this segment are λ and *λimm*^{434} (see ref. 4). Phage *λimm*^{434}, which contains almost all the other known genes of phage λ, makes and is sensitive to the 434 phage repressor only. Therefore, a critical test of specificity is that the isolated λ repressor should bind to λ DNA but not to *λimm*^{434} DNA.

Characterization of the λ Phage Repressor

The isolation of the λ phage repressor was achieved by destroying the host DNA with ultra-violet light, thereby drastically decreasing cellular protein synthesis[3]. These

irradiated cells were infected with many λ phages which, under the conditions of the experiment, synthesized little or no phage protein except repressor. The infected cells were fed radioactive amino-acids, and a single labelled protein was separated from the background label on a DEAE-cellulose column. This protein was identified as the product of the C_1 gene by two criteria: first, it was missing from cells infected with phages bearing amber mutations in the C_1 gene, and second, it was made in modified form by phages which produce temperature sensitive repressors as a result of mutation in the C_1 gene. Electrophoresis and sedimentation of the repressor indicate that it is an acidic protein with a sedimentation coefficient of about 2·8*S*, which corresponds to a molecular weight of approximately 30,000.

The binding experiments to be described were performed mainly with the repressor made by the mutant phage λ*ind*⁻. The *ind*⁻ mutation renders the repressor insensitive *in vivo* to many conditions which inactivate the wild type repressor[6]. For example, a small dose of ultraviolet light delivered to a λ-lysogen will inactivate the wild type but not the *ind*⁻ repressor. The *ind*⁻ repressor can be isolated in the same way as the wild type and has approximately the same sedimentation coefficient, but it chromatographs separately from wild type on DEAE-cellulose columns (Fig. 1). The altered chromatographic behaviour may be due to a charge or a conformational change. However, the fact that this mutation in the C_1 gene, from wild type to *ind*⁻, also changes the behaviour of the protein on DEAE provides further proof that this protein is coded for by the C_1 gene.

Binding of the Repressor to DNA

The labelled λ*ind*⁻ repressor was mixed with λ DNA and sedimented through a sucrose gradient. Fig. 2 shows that some of the label sedimented with the DNA, indicating that the λ*ind*⁻ repressor binds to DNA. Fig. 2 also shows that this binding is specific: when the repressor was mixed with DNA from phage λ*imm*[434], no binding was observed. This experiment has been performed with several different preparations of repressor and phage DNA.

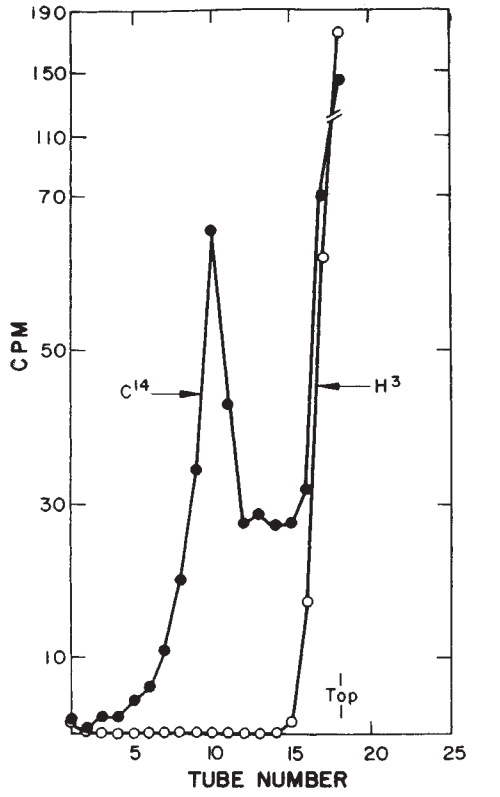

Fig. 3. Selective binding of the λ C_1 product to λ DNA. ^{14}C-labelled λ*ind*⁻ repressor was isolated on a DEAE column from an extract which also contained the ^3H-labelled gene products of the phage λC_1*sus*34. This mixture was then tested for binding to λ DNA as described in Fig. 1. The 260 mμ *OD* profile of the λ DNA is essentially identical to that shown in Fig. 1. The fractions were precipitated with TCA, dissolved in 1/2 ml. 0·1 M NaOH, and counted in 10 ml. of scintillation fluid containing toluene and Triton *X*-100 in the ratio 3 : 1 plus 0·4 per cent PPO and 0·005 per cent POPOP[10].

The repressor used in the binding experiments was not isotopically pure. Depending on the fractions pooled from a DEAE column run, as much as 50 per cent of the label might be present in impurities other than the repressor. In order to guarantee that the label sedimenting with the DNA was in the repressor and not in some contaminant, a double label binding experiment was performed. ^{14}C-labelled λ*ind*⁻ repressor was isolated on a DEAE column from a mixture of extracts which included the ^3H-labelled products of cells infected with the phage λC_1*sus*34. This phage bears in its C_1 gene the amber mutation *sus*34 which blocks production of the repressor[7]. Therefore the ^3H will have labelled all the proteins made except the repressor. Fig. 3 shows that about half the ^{14}C label but none of the ^3H label sedimented with the DNA.

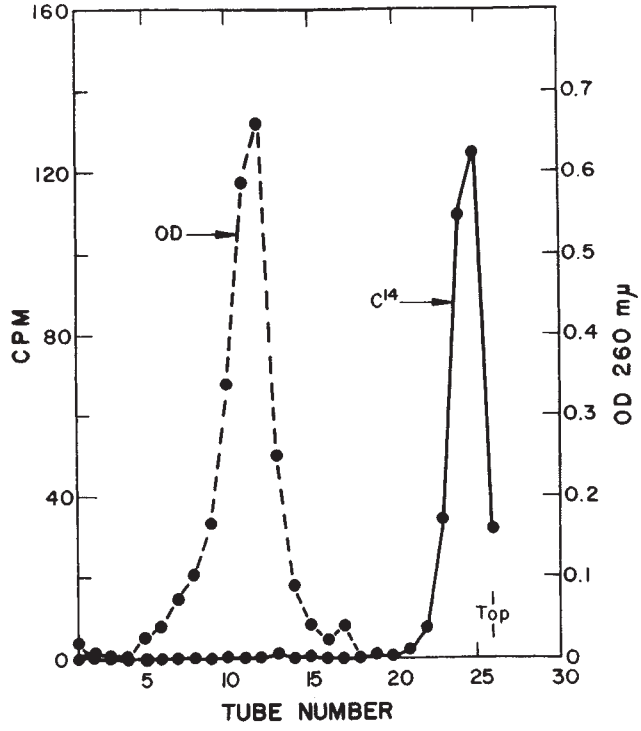

Fig. 4. Binding of λ*ind*⁻ repressor to denatured λ DNA. 100 μg of λ DNA was denatured in 0·1 M NaOH and then neutralized with HCl. The salt concentration was adjusted to 0·1 M KCl and a binding experiment was performed using ^{14}C-labelled λ*ind*⁻ repressor as described in Fig. 1. The gradient was spun for only 2·5 h because of the increased sedimentation coefficient of denatured λ DNA in 0·05 M KCl[11].

The repressor does not bind to denatured DNA. Fig. 4 shows that no counts were displaced from the top of the tube when a mixture of denatured DNA and labelled λ*ind*⁻ repressor was sedimented through a sucrose gradient. The repressor preparation used in this experiment was found to bind efficiently to native DNA, hence its failure to bind here must have been due to the changed configuration of the DNA.

In several experiments, the wild type repressor was tested for binding to DNA. Some binding was detected, but the results were not as striking as with the λ*ind*⁻ repressor. It is possible that the *ind*⁻ form is less susceptible to inactivation during the isolation procedure or that it binds more tightly to DNA.

Nature of the Binding

In order for binding to have been detected under the experimental conditions used, the repressor must bind very tightly to DNA. A close examination of Figs. 2 and 3 shows that some of the bound repressor washes off the DNA as it sediments. This suggests that the dissociation constant is of the same order of magnitude as the concentration of DNA binding sites (called operators) in the peak tubes. Assuming a small number of operators per phage genome (there are probably one or two), this value is roughly 10^{-9}–10^{-10} M. A repressor-operator affinity in this range *in vivo* is suggested by the magnitude of derepression observed with the *lac* operon. Since a 1,000-fold increase in β-galactosidase synthesis occurs on induction, the dissociation constant of the repressor–operator complex should be 1,000-fold less than the concentration of free repressor in the cell[8]. The concentration of free *lac* repressor has been estimated at 10^{-7} M (ref. 2), implying that the dissociation constant is of the order of 10^{-10} M.

The finding of the λ*ind*⁻ repressor to λ DNA was noticeably weaker when the complex was sedimented through a sucrose gradient containing 0·1 M KCl instead of the 0·05 M KCl used in the experiments described here. In a gradient containing 0·15 M KCl, no binding was detected. This observation suggests that the binding is partly electrostatic.

The finding that the λ repressor binds specifically and with high affinity to λ DNA strongly suggests that the simplest model for the mechanism of action of the repressor is correct—namely, that the repressor blocks transcription from DNA to RNA by directly binding to DNA. This conclusion is further supported by the recent observation of Dr. W. Gilbert that the *lac* repressor binds specifically to *lac* DNA and is removed by IPTG (W. Gilbert, to be published).

I thank Nancy Hopkins for technical assistance, and also Drs. W. Gilbert, J. D. Watson and S. E. Luria for help. The work was supported by grants from the U.S. National Science Foundation and the U.S. National Institutes of Health.

Received April 9, 1967.

[1] Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).
[2] Gilbert, W., and Mueller-Hill, B., *Proc. U.S. Nat. Acad. Sci.*, **56**, 1891 (1966).
[3] Ptashne, M., *Proc. U.S. Nat. Acad. Sci.*, **57**, 306 (1967).
[4] Kaiser, A. D., and Jacob, F., *Virology*, **4**, 509 (1957).
[5] Isaacs, L. N., Echols, H., and Sly, W. S., *J. Mol. Biol.*, **13**, 963 (1965).
[6] Jacob, F., and Campbell, A., *C.R. Acad. Sci., Paris*, **248**, 3219 (1959).
[7] Jacob, F., Sussman, R., and Monod, J., *C.R. Acad. Sci., Paris*, **254**, 4214 (1962).
[8] Sadler, J. R., and Novick, A., *J. Mol. Biol.*, **12**, 305 (1965).
[9] Thomas, jun., C. A., and Abelson, J., *Procedures in Nucleic Acid Research*, edited by Cantoni, G. L., and Davies, D. R., 553 (Harper and Row, New York, 1966).
[10] Modified according to E. Kennedy, pers. comm., from Patterson, M. S., and Greene, R. C., *Ann. Chem.*, **37**, 854 (1965).
[11] Studier, F. W., *J. Mol. Biol.*, **11**, 373 (1965).

Tertiary Structure of Ribonuclease

by

GOPINATH KARTHA

Crystallography Center,
Roswell Park Memorial Institute,
Buffalo 3, New York

Two models have recently been proposed for the tertiary molecular structure of ribonuclease (*Nature*, 213, 557 and 862; 1967). The models were based, respectively, on 5·5 Å and 2 Å electron density maps built up over the past decade and a half. An attempt is here made by a proponent of one of the models to reconcile the considerable differences that exist between the two models.

A MODEL has been proposed from this laboratory for the three-dimensional folding of the polypeptide chain in bovine pancreatic ribonuclease[1]. A little earlier, Avey *et al.*[2] had proposed another model of the same protein in the same crystalline form, and in a note appended to that article Dr. C. H. Carlisle had commented on some apparently significant differences between that model and ours.

The purpose of the present article is to point out that in my opinion the disagreement is probably not in the data or the results but in the interpretation of the separate electron density maps computed by the two groups with very different volumes of X-ray diffraction data as input. To be specific: our map was computed at 2 Å resolution with 7,294 reflexions, whereas Avey *et al.* (hereinafter called the London group) used 389 reflexions within 5·5 Å resolution in building their map. To this extent, the two maps which were the starting point for the two separate models are widely different. To indicate the effect of resolution on the quality of the map, three maps are given in Figs. 1–3. Fig. 1 was computed with the 2 Å data of 7,294 reflexions and contours are drawn at intervals of 200 units on an arbitrary scale; the first dotted contour corresponds to 100 units. Figs. 2 and 3 were also computed with our data, but terminated at 5·5 Å and included 362 reflexions to correspond to the data range used by the London group. In Fig. 2 contours are drawn in the same intervals as in Fig. 1; however, in Fig. 3 contours are in intervals of 40 units, starting the dotted with 20. The maps are *xy* sections corresponding to $z = 0{\cdot}357$, this being the co-ordinate of the disulphide bridge we have designated as II–VII. This bridge is denoted in each map by a cross.

In Fig. 1, this point corresponds to a peak density of 1,700 and is one of the five highest peaks in half the unit cell. In Figs. 2 and 3, the peak height at the corresponding point is 120 on the same unit. There are fifteen peaks of height greater than 150 in this map including two regions of peak height of more than 200 units. The first of these corresponds to the region we have identified as in the vicinity of the bulky residues GLN(28), MET(29), and MET(30). The second highest region in this 5·5 Å corresponds to the possibly helical region of the main chain residues 52–58. From a detailed comparison of this 5·5 Å resolution map with 4·0, 3·0, 2·4, and 2·0 Å resolution maps, I conclude that at the 5·5 Å resolution, the most prominent features of the electron density map do not correspond to the disulphide bridges. In the 4 Å maps these bridges do become visible, but one has to go to a resolution of at least, if not better than, 3 Å before the disulphides stand out as the prominent features.

The co-ordinates of the centre of the disulphide bridges obtained from our 2 Å maps are given in Table 1 with corresponding co-ordinates of the London group. We believe our co-ordinates to be accurate to 1 Å. Comparison of these co-ordinates with those of the London group show no direct correspondence even allowing for the possibility of different choice of origins. This is true even for the first of these two bridges, and thus it seems likely that the cystine co-ordinates as listed by the London group differ considerably from our set. Furthermore, I think that a detailed interpretation of the main chain folding in a protein of low helical content from a low resolution map on the assumption that the most prominent features are indeed the disulphide bridges is an extremely

(*Continued on page 330*)