Cro Regulatory Protein Specified by Bacteriophage λ

STRUCTURE, DNA-BINDING, AND REPRESSION OF RNA SYNTHESIS*

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The Cro protein specified by bacteriophage λ is a repressor of the genes expressed early in phage development and is required for a normal late stage of lytic growth. We have purified Cro protein to virtual homogeneity and analyzed its structure and properties as a DNA-binding protein and repressor of RNA synthesis. To confirm that the protein is the product of the cro gene, we have also shown that a missense mutation in the cro gene leads to a product that is more temperature- and salt-sensitive in its DNA-binding property. As purified, Cro protein is a dimer of identical subunits of molecular weight 8600. The purified protein binds to λ-DNA carrying the specific binding sites (operators o1 and o2) with an estimated dissociation constant of $10^{-10}$ to $10^{-9}$ M; there is also weaker binding to other sites on DNA, as found for other DNA-binding regulatory proteins. In a purified transcription system, the Cro protein is an effective and specific repressor of RNA synthesis from the N and cro genes; thus Cro is an autorepressor which regulates its own synthesis. A comparison of the properties of the two λ repressor proteins, cl and Cro, indicates that cl is a "strong repressor" specialized for complete turnoff of lytic functions needed for the maintenance of lysogeny, whereas Cro is a "weak repressor" specialized for a gradual turnoff of early viral genes that potentiates the late stage of lytic development.

The temperate bacteriophage λ specifies two repressor proteins, cl and Cro, which carry out regulatory functions essential for different aspects of the viral life cycle. The cl protein acts under conditions of stable lysogeny to maintain repression of the integrated viral DNA, Cro protein acts during lytic development to turn off the expression of the phage genes active early after infection (1-6) and thus potentiates the early-late switch in expression of viral genes.

The cl protein has been purified and extensively characterized in vitro for binding to specific operator sites on λ-DNA and ability to repress RNA synthesis initiated at the λ promoter sites active early during viral development (7-11) (Fig. 1). In a previous paper, we presented data indicating that Cro protein is a DNA-binding protein which binds to the same operator region of λ-DNA as does cl (12). This report describes the purification of Cro protein to apparent homogeneity, presents a more detailed characterization of its structure and DNA-binding activity, and establishes the capacity of Cro to function as a specific repressor of RNA synthesis initiated at the early promoter sites of λ-DNA. Our biochemical results indicate that the physiological differences between cl and Cro may be attributable to the different binding capacities of the two proteins.

EXPERIMENTAL PROCEDURES

Materials

Nucleic Acids—Bacteriophage DNA was prepared from purified phage by phenol extraction as described previously (10, 12). "Chicken blood" DNA and Riehchichia coli (rRNA were obtained from Calbiochem.

Proteins—E. coli RNA polymerase was prepared as described by Burgess and Jendrisak (13). Termination factor p, purified according to Roberts (14), was the gift of J. Galluppi and J. Richardson, University of Indiana. PurGase D Nase was obtained from Worthington and ovalbumin, chymotrypsin, myoglobin, and bovine serum albumin from Schwarz/Mann.

Bacterial and Phage Strains—The bacterial host used to prepare Cro protein was O60084. The infecting phage for large scale preparations of wild type Cro protein was A Nam53ulu3cIaml4Sam7; to characterize the temperature-sensitive mutant protein, parallel infections of A Nam53ulu3cIaml4Sam7 and A Nam53ulu3cIaml4Sam7 were used, in which the cro" mutation was tof 2 (15). The λ mutants and the rationale for using them have been described in more detail previously (12). In brief, N" mutation eliminates production of most λ proteins besides Cro, while may increase Cro production, cl" mutation eliminates the DNA-binding activity of cl protein, and S" mutation prevents cell lysis.

Other Materials—Cellulose (CF11) and phosphocellulose (P11) were obtained from Whatman and Sephadex G-75 (140 to 120 μ particle size) from Pharmacia. λ-DNA-cellulose was prepared as described by Alberts and Herrick (16). Ultrapure ammonium sulfate was obtained from Schwarz/Mann, acrylamide and sodium dodecyl sulfate from Bio-Rad, unlabeled nucleoside triphosphates from New England Nuclear, and rifampicin from Leptin.

Methods

DNA-binding Assay for Cro Protein—The standard assay was done essentially as described previously (10, 12). The assay measures the retention of α-32P]DNA on a nitrocellulose filter by virtue of its tight binding to Cro protein; for purification an excess (100-fold) of unlabeled "chicken blood" DNA was added to compete for the binding of proteins that associate with DNA but lack specificity for λ-DNA. To further differentiate the operator-specific Cro protein from other λ-DNA-binding proteins, we did parallel assays with λ-DNA and λimm434-DNA (which is the same as λ-DNA except for the operator-containing immunity region). The standard assay mixture contained 0.2 μg of λ-32P]DNA and 20 μg of chicken blood DNA in 0.1 ml of "binding buffer": 10 mm Tris/Cl (pH 7.3), 20 mm KCl, 10 mm MgCl2, 0.2 mm dithiothreitol, and 0.2 mm EDTA. After incubation for 10 min at 0°, the mixture was filtered with a nitrocellulose filter and washed twice with binding buffer. The amount of λ-32P]DNA retained by the filter is proportional to the amount of Cro protein present in the sample.

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**RESULTS**

**Purification of Cro Protein**

A summary of the purification of Cro protein is presented in Table I. Unless otherwise noted, all operations were performed at 0.4°C.

### Growth of Infected Cells—A culture (100 liters) of Escherichia coli C600S1u was grown in a New Brunswick Fermenter at 30°C in a broth containing 2% Difco Bacto-tryptone, 1% yeast extract, 0.5% NaCl, and 0.2% maltose. When the A660 of the culture reached 1.0, MgCl2 was added to 10 mM and λ phage (strain Nambojlan 14:0; Salmi) was added at a multiplicity of 10 phages/cell. After 60 min at 30°C, the cells were harvested by centrifugation in a Sharples continuous flow centrifuge, resuspended in 50 ml of 10% sucrose, 50 mM Tris/HCl (pH 7.5), quick-frozen, and stored at −20°C. The procedure yielded 150 g of cells.

### Preparation of Extract—The frozen cells were thawed and mixed with 80 ml of 2 mg/ml of lysozyme in 250 mM Tris/HCl (pH 7.5), 1 mM EDTA, and 19 ml of 4 M NaCl, 10 mM 2-mercaptoethanol. After 30 min at 0°C, lysis was completed by raising the temperature gradually to 32°C over a period of 10 to 15 min. Magnesium acetate was added to 10 mM, and pancreatic DNase to 2 μg/ml. After the viscosity was substantially reduced (5 to 10 min), 50 ml of 4 mM NaCl were added, and the lysate was centrifuged for 4 h at 30,000 rpm in a Spinco 30 rotor. The supernatant fraction (550 ml) was dialyzed for 3 h (two changes) against 5 liters of 10 mM KPO4 (pH 6.4), 0.2 mM EDTA, 0.2 mM dithiothreitol, 5% glycerol (Buffer A) containing 0.1 M KCl (Fraction I).

### Phosphocellulose Chromatography—Fraction I was applied at a flow rate of 3 ml/min to a phosphocellulose column (90-ml bed volume) equilibrated with Buffer A containing 0.1 M KCl. The column was washed with 150 ml of Buffer A containing 0.1 M KCl, and then eluted at a flow rate of 0.8 ml/min with a linear gradient (500 ml total volume) from 0.1 to 1.0 M KCl in Buffer A. Cro protein, showing the DNA-binding activity specific for λ-DNA, eluted at a KCl concentration of approximately 0.45 M (Fraction II). Binding specificity for λ-DNA was checked by parallel assays with both λ-DNA and λimm 484-

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**Table I**

| Fraction | Volume | Total protein | Specific activity | Yield |
|----------|--------|---------------|------------------|-------|
| I. Crude extract | 550 | 4455 | a | a |
| II. Phosphocellulose | 50 | 39.5 | 157 | 100 |
| III. Sephadex G-75 | 40 | 5.2 | 415 | 35 |
| IV. DNA-A-cellulose 1 | 6 | 0.68 | 3355 | 48 |
| V. DNA-cellulose 2 | 4 | 0.60 | 4000 | 39 |

*a The binding is not specific for λ-DNA in the crude extract, and so no value for activity can be given at this stage.
DNA, which lacks the specific binding sites for Cro protein.

Sephadex G-75 Gel Filtration—Fraction II was concentrated by (NH₄)₂SO₄ precipitation (70% saturation at pH 6.0 for 20 min), centrifugation for 20 min at 13,000 rpm, and resuspension in 3 ml of 10 mM Tri/HCl (pH 7.3), 0.2 mM EDTA, 0.2 M dithiothreitol, 5% glycerol (Buffer B) containing 0.2 M NaCl. The concentrated protein solution was applied to a column of Sephadex G-75 (90 x 2.5 cm) equilibrated with Buffer B containing 0.2 M NaCl, and eluted with the same buffer at a flow rate of 15 ml/h. The DNA-binding activity specific for λ-DNA eluted between 260 and 300 ml (approximately the position where a marker myoglobin protein eluted) (Fraction III).

DNA-cellulose Chromatography—Fraction III was diluted to 0.1 M NaCl with an equal volume of Buffer B and applied to a λ-DNA-cellulose column (10-ml bed volume; 5 mg of λ-DNA) equilibrated with Buffer B containing 0.1 M NaCl. The column was washed with 20 ml of Buffer B containing 0.1 M NaCl and eluted with a linear gradient (50 ml total volume) from 0.1 M to 1.0 M NaCl in Buffer B. The DNA-binding activity specific for λ-DNA eluted at a NaCl concentration of approximately 0.3 M (Fig. 2a) (Fraction IV). Fraction IV was diluted to 0.1 M NaCl with Buffer B and rechromatographed on λ-DNA-cellulose (5-ml bed volume) with a linear gradient (30 ml total volume) from 0.1 to 1.0 M NaCl in Buffer B (Fig. 2b). The pooled fractions, 18 to 21 (Fraction V), were used for the further studies described in this paper. Fraction V was free of DNAase activity, as judged by sedimentation of 0.6 μg of λ-DNA in an alkaline sucrose gradient after prior incubation with 0.6 μg of Cro protein for 30 min at 30°C. Fraction V was also free of RNAase activity, as judged by no detectable release of acid-soluble radioactivity when 0.01 μg of λ-HIRNA was incubated for 15 min at 30°C with 0.5 μg of Cro protein or by no change in the size of 4 S and 6 S RNA analyzed by polyacrylamide gel electrophoresis. Cro protein was stored at -20°C in Buffer B with 0.4 M NaCl and 50% glycerol without loss of activity over a 1-year period.

Properties of Altered Cro Protein Produced by cro⁻ Mutation—Although the binding specificity and the conditions for synthesis provided a strong indication that the DNA-binding protein purified above is the product of the cro gene, we have also characterized an altered protein produced by a phage with a missense mutation in the cro gene in order to complete the identification of the Cro protein. For this purpose 2-liter cultures of E. coli C600Su⁺ were infected with cro⁺ or cro⁻ (tof2 mutation) phage at 30°C for 60 min, and a smaller scale purification was carried out by phosphocellulose chromatography, followed by concentration with dry Sephadex G-75 and sedimentation in a 10 to 30% glycerol gradient in Buffer A containing 0.4 M KCl. Even when assayed at 0°C, the specific activity of the mutant Cro protein thus isolated was about 15 to 20% that of wild type Cro protein, as judged by the amount of Cro protein determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (both preparations contained about 60 to 70% Cro protein).

The normal and mutant Cro proteins were characterized for sensitivity of the DNA-binding reaction to elevated temperature (Fig. 3) and ionic strength (Fig. 4). There is a more severe inhibitory effect of both temperature and ionic strength on the

1 tof2 is a temperature-sensitive cro mutation (15) and shows temperature-dependent overproduction of λ-exonuclease (Y. Takeda, unpublished results).
Physical and Chemical Properties of Cro Protein

Physical Structure — To estimate the molecular weight of native Cro protein, we carried out velocity sedimentation in a 10 to 30% glycerol gradient (Fig. 5). Two salt concentrations were used in an effort to determine the stability of the subunit structure. In both 0.05 M KCl and 0.5 M KCl, Cro protein has an estimated sedimentation coefficient of 1.9 to 2.0 S, as judged by the sedimentation of marker proteins of known molecular weight. This indicates a molecular weight of 15,000 to 20,000, assuming that the axial ratio is in a typical range for a globular protein (22).

To determine the monomer molecular weight and estimate the purity of the final preparation, we used polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) polyacrylamide gel containing sodium dodecyl sulfate. Cro protein (7 µg) was treated with 1% SDS and 2% 2-mercaptoethanol for 16 h at room temperature, and electrophoresis was carried out for 1.5 h at 8 mA/tube as described by Weber and Osborn (19).

Amino Acid Composition — The amino acid composition of Cro protein is presented in Table II. The composition is high in lysine and alanine and lacks cysteic acid and tryptophan, showing an interesting similarity to the prokaryotic DNA-binding protein HU (23, 24) and to the eukaryotic histone H2B (25). From the amino acid composition, the monomer molecular weight is determined to be 8600. From the combined physical and chemical studies, we conclude that native Cro protein is probably a dimer of identical subunits.

Binding Properties of Cro Protein

Equilibrium Binding — Previous experiments have shown that Cro protein binds to the same operator region used by the λ cl protein, the "λ repressor" that maintains lysogeny (12).

We wanted to determine the dissociation constant for Cro and compare it to the very low value of 10^-13 M estimated for cl protein.

In order to analyze the binding data, we needed to know that Cro binding is sufficiently specific for the operator sites on λ-DNA for this interaction to dominate the binding curve and that one active Cro protein is sufficient to retain the radioactive λ-DNA on the nitrocellulose filter in the standard binding assay. This information is provided by the binding curve of Fig. 7, in which Cro concentration is varied for two DNA substrates, λ and λimm 434; λimm 434 is mainly identical with λ but lacks the region of λ-DNA containing the specific binding sites for cl and Cro proteins (7, 10, 12). The binding to λ-DNA is linear at low concentrations of Cro protein, indicating that one active Cro molecule can retain 1 λ-DNA molecule. The binding is also largely specific for λ-DNA. The "nonspecific" binding that we have observed for λimm 434-DNA has also been found for other DNA-binding regulatory proteins (26, 27), and presumably represents relatively weak binding interactions that can occur anywhere on a DNA molecule (a similar binding curve to that of λimm 434 has been found also for φ60 DNA). From the data of Fig. 7, we conclude that the standard

![Fig. 4](image-url) Salt sensitivity of Cro protein specified by mutant cro gene. DNA binding assays were carried out as described under "Methods," except that the binding mixtures included the KCl concentration indicated on the figure. The nonspecific binding to λimm 434-DNA has been subtracted from the binding to λ-DNA to give the data presented in the figure. The binding to λimm 434-DNA at 0.03 M KCl was 3% with the Cro+ preparation and 11% with the Cro- preparation; this decreased linearly with increased salt concentration and was 9% for Cro+ and 7% for Cro-, respectively, at 0.2 M KCl. O-O, DNA binding for normal (cro+) protein; ●●, DNA binding for cro- mutant protein.

![Fig. 5](image-url) Left. Velocity sedimentation of native Cro protein in a glycerol gradient. Cro protein (0.2 ml of Fraction V) was layered on a 10 to 30% glycerol gradient in Buffer B containing either 0.05 M KCl (a) or 0.5 M KCl (b) and sedimented for 24 h at 49,000 rpm. The vertical arrows denote the sedimentation position of marker proteins: B is bovine serum albumin (M, = 66,000); O, ovalbumin (45,000); C, chymotrypsinogen (25,000); M, myoglobin (17,000). ●●, DNA-binding activity for λ-DNA; ○○, DNA-binding activity for λimm 434-DNA.

Right. Electrophoresis of denatured Cro protein in a polyacrylamide gel containing sodium dodecyl sulfate. Cro protein (7 µg of Fraction V) was treated with 1% sodium dodecyl sulfate and 2% 2-mercaptoethanol for 16 h at room temperature, and electrophoresis was carried out for 3.5 h at 8 mA/tube as described by Weber and Osborn (19). Protein was stained with Coomasie brilliant blue. The molecular weight was estimated from marker proteins (bovine serum albumin, ovalbumin, chymotrypsinogen, myoglobin, and cytochrome c) run in a parallel gel.
TABLE II

Amino acid composition of Cro protein

| Amino acid                  | mol/unit* | mol % |
|-----------------------------|-----------|-------|
| Alanine                     | 9         | 11.5  |
| Arginine                    | 4         | 5.1   |
| Aspartic acid + asparagine  | 7         | 10.0  |
| Cysteic acid                | 0         | 0.0   |
| Glutamic acid + glutamine   | 6         | 7.7   |
| Glycine                     | 5         | 6.4   |
| Histidine                   | 1         | 1.3   |
| Isoleucine                  | 6         | 7.7   |
| Leucine                     | 4         | 5.1   |
| Lysine                      | 10        | 12.8  |
| Methionine                  | 2         | 2.6   |
| Phenylalanine               | 3         | 3.8   |
| Proline                     | 3         | 3.8   |
| Serine                      | 4         | 5.1   |
| Threonine                   | 7         | 10.0  |
| Tryptophan                  | 3         | 3.8   |
| Tyrosine                    | 3         | 3.8   |
| Valine                      | 4         | 5.1   |
| Total                       | 78        |       |

* Calculations were made using the assumption that the protein has 1 histidine residue per monomer unit.

FIG. 7. DNA binding of Cro protein as a function of Cro concentration. Binding assays were carried out using the standard binding conditions (DNA concentration, 2 μg/ml), but the filter was washed with 0.5 ml of 45% ethanol as described under "Methods." O-O, DNA-binding activity for λ-DNA; O---O, DNA-binding activity for λimm434-DNA.

DNA-binding assay will allow us to estimate specific binding constants.

A binding curve in which DNA concentration is varied is most appropriate for the measurement of an equilibrium dissociation constant (28, 29); the results of this experiment for Cro protein are shown in Fig. 8. The detailed interpretation of the binding curve is complicated by the fact that we do not know precisely how many specific binding sites for Cro are present on a λ-DNA molecule. For λ cl protein, there are three binding ("operator") sites on either side of the cl gene, termed oL1, oL2, and oL3, and oH1, oH2, and oH3, and oH6 of these oH1, and oH1 have substantially higher affinities for cl than the others (30). For Cro protein, we only know so far that Cro binds at least two sites in the oL region (see Ref. 12 and below). If we assume two binding sites per 3 × 10⁷ daltons of λ-DNA (31), we calculate a dissociation constant of 8 to 9 × 10⁻¹⁵ M. Comparisons with dissociation constants of other proteins are difficult to interpret because the measurements are subject to substantial variation with ionic strength, temperature, and pH. However, Cro does appear to be a substantially weaker DNA-binding protein than other specific regulatory proteins studied so far; approximate values for λ cl protein, lac repressor, and araC protein are 10⁻¹³ M (7), 10⁻¹³ M (28), and 10⁻¹⁵ M (29), respectively.

Dissociation Rate Constant—To estimate the dissociation rate constant, we formed a Cro-λ-[³²P]DNA complex and measured the loss of λ-[³²P]DNA from this complex with time in the presence of a 30-fold excess of unlabeled λ-DNA (Fig. 9). Since a Cro molecule should not reassociate to a significant extent with the [³²P]DNA once released, the initial decrease in [³²P]DNA bound should follow an exponential first order decay

FIG. 8. DNA binding of Cro protein as a function of DNA concentration. Binding assays were carried out as for Fig. 7, except that λ-DNA concentration was varied as indicated on the figure and Cro concentration was held constant at 15 ng/ml (O---O) or 10 ng/ml (O-O).

FIG. 9. Stability of Cro-λ-DNA complex. λ-[³²P]DNA (0.5 μg/ml) was incubated with Cro protein for 10 min at 0 °C by binding level of 20% of the input DNA (zero time), unlabeled λ-DNA was added to 15 μg/ml, and 0.1-ml aliquots were taken out at intervals and filtered to determine the remaining λ-[³²P]DNA-Cro complex. O-O, at pH 7.5; O---O, at pH 6.6.
Curve. Because of the rapid decay found under our standard binding condition of pH 7.3, we also carried out binding assays at pH 6.6. The calculated dissociation rate constant is about $2 \times 10^{-2} \text{s}^{-1}$ at pH 7.3 and $5 \times 10^{-3} \text{s}^{-1}$ at pH 6.6. As expected from the equilibrium binding data, the half-life for Cro dissociation at pH 7.3 is much less than that found for c1 protein and lac repressor, and substantially less than the 3-min value found for araC protein. In an experiment carried out under identical conditions, we found the half-life for cro dissociation to be greater than 100 min. We have also attempted to measure the dissociation rate for "nonspecific" binding, using DNA lacking the specific operator sites (λimm434- or λ80-DNA). The dissociation was very rapid with a half-life of $\approx 10$ s, which is consistent with the concept that the nonspecific binding is a substantially weaker interaction than the specific binding to the regulatory sites.

We have attempted to determine the association rate constant, but have not been able to do so because Cro protein is unstable at the very high dilution required for this measurement. If the rate constant for Cro is comparable to that estimated for araC protein ($2 \times 10^6 \text{M}^{-1} \text{s}^{-1}$), the value calculated for the equilibrium dissociation constant is $10^{-11} \text{M}$ at pH 7.3.

Effect of Cro Protein on RNA Synthesis

From experiments carried out in vivo, we expect Cro protein to act as a specific repressor of RNA synthesis initiated at the early promoter sites of λ-DNA, p5 and p6 (see Refs. 1 to 6 and Fig. 1). To test this expectation, we used purified λ-DNA as a template for RNA polymerase and studied the effect of Cro protein on RNA synthesis. To ensure that Cro effects derive from the specific binding reaction, we used λimm434-DNA as a template in parallel experiments.

The capability of Cro protein to function as a specific repressor in vitro is shown in Fig. 10, in which strong repression of total RNA synthesis from λ-DNA occurs in the absence of any repression effect on RNA synthesis from λimm434-DNA. The RNA products were analyzed further by separation through polyacrylamide gel electrophoresis and visualization by autoradiography (Fig. 11). The RNA produced in vitro from λ-DNA in the presence of ρ factor is predominantly of four size classes, designated 4 S, 6 S, 8 to 9 S, and 12 S (18, 32, 33). The 8 to 9 S and 12 S transcripts represent RNA chains initiated at the cI and Q genes, respectively (Fig. 1). The results of Fig. 11 show that Cro protein represses 8 to 9 S and 12 S RNA synthesis from λ-DNA but not from λimm434-DNA; 6 S RNA synthesis was not repressed by Cro with λ-DNA as a template (although not shown at this gel exposure, 4 S RNA was also not repressed by Cro). As expected from the failure of Cro to repress total RNA synthesis from λimm434-DNA (Fig. 10), there was no difference in the gel pattern of RNA synthesis from λimm434-DNA in the presence or absence of Cro (data not shown).

If the repression of transcription noted in Fig. 10 and Fig. 11 occurs at the initiation step of RNA synthesis, the binding of
RNA polymerase to DNA before Cro might be expected to abolish repression. Table III shows this is the case (compare Lines 2 and 4). Cro added at the same time as RNA polymerase is an effective repressor (Table III, Line 3), presumably because of the relatively slow formation of a tight binding initiation complex by RNA polymerase (see Ref. 34). Thus, Cro probably represses RNA synthesis by blocking the capacity of RNA polymerase to bind at the promoter site; a similar mechanism has been inferred for cl protein (7-11).

From these results, we conclude that Cro is an effective and specific repressor which inhibits the initiation of RNA synthesis from the early promoters, pL and pR. Since 8 to 9 S RNA is a specific repressor which inhibits the initiation of RNA synthesis, it is probable that RNA polymerase to bind at the promoter site; a similar mechanism might be expected to these proteins will be an interesting study in protein evolution.

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DISCUSSION

Cro protein has a special role among the specific regulatory proteins analyzed so far because it functions during a temporal "switch" in viral development from a replication-oriented "early" stage to a maturation-oriented "late" stage. The repression activity of Cro serves to turn down the transcription of early genes concerned with production of replication and recombination proteins during the period of head and tail production, virus assembly, and cell lysis. The mechanism for this essential time delay in the action of Cro has been a puzzle because the cro gene is transcribed during the earliest ("immediate early") stage of RNA synthesis (1-5). Our biochemical experiments suggest that the delayed action of Cro might result from the relatively low affinity of Cro protein for its specific regulatory sites; thus Cro will begin to function as a repressor only after the time required for the synthesis of a high level of the protein.

In contrast to Cro, the cl protein of phage λ functions as a steady state repressor to maintain lysogeny through a complete turn-off of transcription of early genes. We suggest that the different biological requirements for the repression of early genes during lytic growth or lysogeny has led to the evolution of biochemically different repressors, a high affinity maintenance repressor (cl) and a low affinity lytic repressor (Cro), although both use the same operator region of λ DNA. Cro and cl also appear to differ in their effect on transcription of the cro and cl genes. Because they repress transcription initiated at pL, both cl and Cro are repressors of the cro gene (thus Cro is an "autorepressor"); however, cl can function either as a repressor or an activator of the cl gene transcript initiated at pN, whereas Cro probably functions only as a repressor of this RNA (Fig. 1) (1-12, 30). This additional functional difference between the two repressors should be clarified by a more detailed analysis of binding and transcription.

Cro protein is the smallest DNA-binding regulatory protein purified so far that exhibits specificity for a DNA site. Remarkably the amino acid composition of Cro protein is very similar to that of the transcription factor TF1 of phage SP01 (35), the prokaryotic DNA-binding protein HU (23, 24), and the eukaryotic histone H2B (25); the similarity to HU protein is most striking. HU is also a small protein (molecular weight 7000) but lacks any known specificity for DNA sites (29). An interesting possibility is that HU protein might have a minimal structure for recognition of double-stranded DNA, to which Cro protein has added a minimal structure for specific sequence recognition. A comparable structural analysis of these proteins will be an interesting study in protein evolution.
Cro regulatory protein specified by bacteriophage lambda. Structure, DNA-binding, and repression of RNA synthesis.
Y Takeda, A Folkmanis and H Echols

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