Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4275–4279, July 1984
Biochemistry

Detecting protein–DNA interactions *in vivo*: Distribution of RNA polymerase on specific bacterial genes

(UV cross-linking/gene regulation/leucine operon/attenuation)

DAVID S. GILMOUR AND JOHN T. LIS

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

Communicated by Norman Davidson, March 23, 1984

ABSTRACT **We present an approach for determining the *in vivo* distribution of a protein on specific segments of chromosomal DNA. First, proteins are joined covalently to DNA by irradiating intact cells with UV light. Second, these cells are disrupted in detergent, and a specific protein is immunoprecipitated from the lysate. Third, the DNA that is covalently attached to the protein in the precipitate is purified and assayed by hybridization. To test this approach, we examine the cross-linking in *Escherichia coli* of RNA polymerase to a constitutively expressed, λ *cI* gene, and to the uninduced and isopropyl β-D-thiogalactoside (IPTG)-induced *lac* operon. As expected, the recovery of the constitutively expressed gene in the immunoprecipitate is dependent on the irradiation of cells and on the addition of RNA polymerase antiserum. The recovery of the *lac* operon DNA also requires transcriptional activation with IPTG prior to the cross-linking step. After these initial tests, we examine the distribution of RNA polymerase on the leucine operon of *Salmonella* in wild-type, attenuator mutant, and promoter mutant strains. Our *in vivo* data are in complete agreement with the predictions of the attenuation model of regulation. From these and other experiments, we discuss the resolution, sensitivity, and generality of these methods.**

The structure and function of chromosomes depend on a constellation of specific interactions between proteins and nucleic acids. In prokaryotes, many specific protein–DNA interactions have been defined *in vitro* and some have been supported by genetic analyses. In eukaryotes such interactions have been defined primarily by *in vitro* analyses using transcription systems and DNA binding assays (1–3). However, the biological implications of *in vitro* studies alone must be interpreted cautiously, because the conditions for detecting specific protein–DNA binding *in vitro* necessarily differ from those within intact cells. For example, the natural state of the DNA substrate (e.g., superhelical density and chemical modification) and the contribution of additional DNA-binding proteins are either poorly defined or unknown.

Here, we describe and test an approach designed to identify specific protein–DNA interactions occurring *in vivo* by using UV light to generate cross-links between protein and DNA. Specific protein–DNA complexes are isolated by immunoprecipitation with antiserum to a specific protein. The cross-linked DNA that is coprecipitated is isolated and characterized by hybridization assays. Although the approach presented should be general, this paper focuses on the association of RNA polymerase with specific bacterial genes. We chose this model system to develop this approach for several reasons. First, UV light has been shown to induce cross-links between RNA polymerase and T7 promoter DNA *in vitro*, and these adducts can be immunoprecipitated with anti-RNA polymerase antibody (4). Second, many RNA polymerase molecules can be associated with an actively transcribed gene, thereby enhancing the probability of generating a cross-link. Third, since regulatory mutations or chemical inducers can modulate the amount of RNA polymerase associated with a gene, the specificity of the interactions detected by our procedure can be rigorously tested. Moreover, the transcription level of some genes will remain unchanged, and these can serve as internal standards.

MATERIALS AND METHODS

Materials. *Escherichia coli* RNA polymerase had been purified as described (5). RNA polymerase antiserum was derived from a rabbit that was immunized as described (6) except 100 μg of purified RNA polymerase was used per injection. This antiserum immunoprecipitates the β and β′ subunits of both *E. coli* and *Salmonella* RNA polymerase. Protein A Sepharose (Pharmacia) was stored at 4°C in 150 mM NaCl/50 mM Tris·HCl, pH 8.0/1 mM EDTA, and was recycled after use by extensively washing with 50 mM $NaHCO_3$/1% $NaDodSO_4$.

All plasmid DNAs were maintained in *E. coli* HB101. Several of the plasmids are described elsewhere: pBGP120 (7), pKK3535 (8), pCV12 (9), and PUC13 (10). Plasmid pLRI was identical to pKB252 (11) except that Ind^s repressor mutant replaced the wild-type repressor. Subclones *leu* 7.1 and *leu* 14.7 were the 2.3-kilobase (kb) *Eco*RI/*Sal* I and the 2.9-kb *Sal* I/*Eco*RI fragments, respectively, from pCV12 and were cloned in pUC13. Plasmid DNAs were prepared using the alkaline procedure as described (12). Restriction fragments from pKK3535, pLRI, and pPBG120 were electroeluted from agarose gels as described (13).

Isolation of Protein–DNA Adducts. Cells were chilled on ice for 5 min prior to irradiation and then transferred to dishes in which the depth of the medium did not exceed 0.5 cm. These dishes were maintained on ice while the samples were irradiated from above with an inverted transilluminator (UV products Chromato-Vue transilluminator Model C-61) whose filter had been removed. The transilluminator was maintained at a distance of 10 cm from the surface, providing a light intensity of 4×10^4 erg/cm^2·sec (measured with a YSI-Kettering Model 65 Radiometer). Cells were collected by centrifugation at 10,000 × *g* for 15 min. The cell pellet was resuspended in 800 μl of 50 mM Tris·HCl, pH 7.4/10 mM EDTA, and transferred to a 1.5-ml microfuge tube; 80 μl of 20% sarkosyl was added, and each sample on ice was sonicated with a microtip sonicator for four 30-sec periods interspersed with periods of cooling. Sonication decreases the average DNA size to 600 base pairs (bp). At this time, samples could be frozen at −70°C for 1 or 2 days with no noticeable effect on subsequent procedures. Each sample was diluted to 0.5% sarkosyl with 50 mM Tris·HCl, pH 7.4/10 mM EDTA, and centrifuged 5 min in an Eppendorf microfuge to remove insoluble material. Antiserum was added to the solu-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IPTG, isopropyl β-D-thiogalactoside; kb, kilobase(s); bp, base pair(s).

ble lysate at 5 μl per 1 ml of bacteria culture and incubated at 0°C for 1 to 3 hr. An aliquot (5 times the volume of antiserum) of suspended 40% protein A Sepharose (vol/vol) was then added, and the lysate was shaken for 1 hr at 4°C with an Eppendorf shaker. The protein A Sepharose was collected with a 30-sec centrifugation in an Eppendorf microfuge. This pellet was washed eight times. Each wash was for 5 min at 4°C with at least 5 vol of 0.1 M Tris·HCl, pH 9.0/0.5 M LiCl/1% Nonidet P-40/1% deoxycholate. Antigen and antibody were eluted from the protein A Sepharose by four successive extractions with 100- to 300-μl aliquots of 50 mM $NaHCO_3$/1% $NaDodSO_4$. Each elution was for 15 min with shaking in an Eppendorf shaker at room temperature. When the DNA was to be analyzed by dot blots, the elution buffer contained 1 μg of calf thymus DNA per ml as carrier.

NaCl to 0.2 M and 2 vol of ethanol were added to the eluate from the protein A Sepharose and to an aliquot of total unfractionated lysate, which served as a standard. The samples were then incubated overnight at −20°C. The precipitate was collected with a 15-min centrifugation in an Eppendorf microfuge at 0°C, then rinsed with 70% ethanol at −20°C, and finally dissolved in 100 μl of 10 mM Tris·HCl, pH 8.0/10 mM EDTA/0.5% $NaDodSO_4$. RNase A was added to 100 μg/ml and incubated 1 hr at 37°C. Proteinase K was added to 50 μg/ml, and the sample was further incubated at 37°C for at least 5 hr. DNA was then precipitated with ethanol. When the DNA was to be purified for nick-translation, the sample was brought to 0.2 M NaCl and extracted with phenol/chloroform/isoamyl alcohol (50:50:1) followed by ether extraction and then ethanol precipitation.

Hybridization Assays. DNAs were radiolabeled with ^{32}P by nick-translation (14) to specific activities of 10^8 to 10^9 dpm/μg. All hybridizations were carried out as described (15), except that the final washes of the hybridized filters were performed at 60°C in 0.2× NaCl/Cit/0.1% $NaDodSO_4$ (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate). Radiolabeled DNA was removed from these filters for rehybridization by rinsing the nitrocellulose in 0.2 mM EDTA (pH 8.0) at 70°C.

RESULTS

Irradiation of *E. coli* Cells with UV Light Cross-Links RNA Polymerase to a Transcribed Gene. The ability to detect cross-links between RNA polymerase and a specific gene should be enhanced by choosing a gene that is transcribed at high levels and is present in multiple copies per cell. The hybrid gene on the plasmid pLRI fits these criteria. This gene consists of the coding region of the *cI* gene of phage λ fused to the *lac* UV5 promoter and is transcribed constitutively (11). *E. coli* cells carrying this plasmid were irradiated with UV light for 0, 1, 2, or 4 min. Total cell lysates were generated by the addition of sarkosyl followed by sonication, which decreased the average DNA size to 600 base pairs. Fig. 1*A* shows that the RNA polymerase antiserum efficiently immunoprecipitates the β and β′ subunits of RNA polymerase from lysates of these cells. These subunits are not precipitated by preimmune serum (data not shown).

DNA covalently bound to the RNA polymerase was recovered from the immunoprecipitate and then assayed for the presence of *cI* gene sequences by dot blot hybridization (18). The results in Fig. 1*B* show that the *cI* gene sequences are precipitated from 10^7 cells irradiated for 1, 2, and 4 min but not from unirradiated cells. The fraction of *cI* gene sequences present in the immunoprecipitate is proportional to the dosage of UV light (Fig. 1*B*). Moreover, the precipitation of *cI* gene is dependent on the addition of the anti-RNA polymerase antiserum; preimmune serum fails to precipitate the *cI* gene in all samples (data not shown). Only a small fraction of RNA polymerase is cross-linked to DNA, because the same amount of the β and β′ subunits are detected in immunoprecipitates from unirradiated cells and cells irradiated for 2 min (the mobility of RNA polymerase is shifted when cross-linked to DNA). Nonetheless, densitometry shows that 0.1% of the *cI* gene sequences are recovered in the immunoprecipitates after a 2-min irradiation (Fig. 1*B*). Although the fraction of DNA immunoprecipitated increases proportionally with the UV dosage, the signal from the total DNA isolated from equivalent numbers of cells decreases with longer irradiation, presumably because of photochemical damage. In addition, UV irradiation for 4 min causes significant protein damage, because the mobility of most of the *E. coli* proteins is changed and the amount of the β and β′ subunit immunoprecipitated is decreased (Fig. 1*A*). For these reasons, we have chosen a 2-min irradiation for all our subsequent work with bacteria.

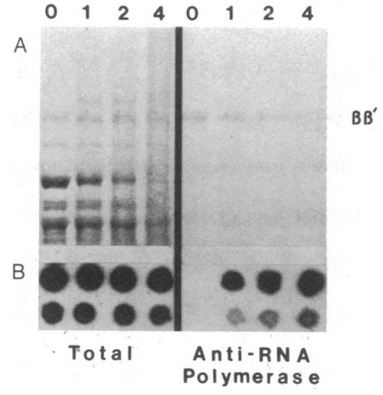

FIG. 1. *In vivo* cross-linking of RNA polymerase to the *cI* gene in the multicopy plasmid pLRI. A 50-ml culture of *E. coli* HB101 containing plasmid pLRI was grown to an OD_{550} of 1.5 in YT medium containing tetracycline at 10 μg/ml (16). Cells were collected by centrifugation at 10,000 × *g* for 10 min and resuspended in 200 ml of 10 mM Tris·HCl, pH 8.0/1 mM EDTA/10 mM $MgCl_2$ at 0°C. Fifty-microliter aliquots were irradiated with UV light for 0, 1, 2, or 4 min as indicated. (*A*) Total proteins and proteins precipitated with anti-RNA polymerase antiserum were fractionated by electrophoresis on $NaDodSO_4$/8% polyacrylamide gel and stained with Coomassie blue (17). Each sample was derived from 5×10^7 cells. Only the region of the gel containing the β and β′ subunits of RNA polymerase are shown. (*B*) Total DNA and DNA precipitated with anti-RNA polymerase antiserum were bound to a nitrocellulose filter as described (18). Total DNA is from 3.3×10^4 cells, and immunoprecipitated DNA is from 1.3×10^7 cells. Each sample is accompanied by a 1:4 dilution. DNA homologous to the *cI* gene was detected by hybridization with a radiolabeled 1.1-kb *Bgl* II/*Pst* I restriction fragment from pLRI.

Several precautions were taken to eliminate RNA from immunoprecipitates, because it too could contribute to the hybridization signal. First, the antiserum contains an endogenous RNase that decreases the RNA present in the cell lysate by at least a factor of 20 (data not shown). This result also indicates that DNA is not immunoprecipitated by virtue of indirect attachment to the RNA polymerase via an RNA bridge. Second, the preparation of immunoprecipitated DNA includes a treatment with RNase A. Third, the samples prepared for the dot blot assay are denatured in 0.15 M NaOH at 85°C for 15 min, which further hydrolyzes RNA.

Efficient Cross-Linking of *lac* Operon DNA to RNA Polymerase *in Vivo* Is Dependent on Gene Transcription. The addition of isopropyl β-D-thiogalactoside (IPTG) causes a dramatic increase in transcription of the *lac* operon (19). To establish that cross-linking of RNA polymerase to DNA is dependent on active transcription, we irradiated uninduced and IPTG-induced cells of strain C600 and examined the cross-linking of RNA polymerase to the single chromosomal copy of the wild-type *lac* operon. Fig. 2 shows that there is

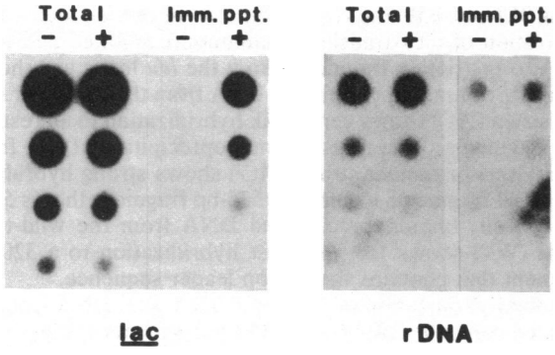

FIG. 2. RNA polymerase cross-links to the *lac* operon in cells induced with IPTG (+) but not in uninduced cells (−). A 120-ml culture of *E. coli* C600 was grown to an OD_{550} of 0.5 at 37°C with shaking in M10 medium supplemented with leucine (50 μg/ml), threonine (50 μg/ml), thiamine HCl (1 μg/ml), and 0.2% fructose (16). One-half of the culture was induced for 10 min in 0.25 mM IPTG. Cells were chilled 5 min on ice and then irradiated for 2 min. Total DNA from 6.35×10^6 cells and anti-RNA polymerase immunoprecipitated (Imm. ppt.) DNA from 6.35×10^8 cells were bound to a nitrocellulose filter supported in a Bethesda Research Laboratories Hybridot Manifold (Schleicher & Schuell sequences application update no. 363). We included a layer of Whatman 3 MM filter paper under the nitrocellulose to prevent the samples from leaking. Dilutions are 1:3. DNA homologous to the *lac* operon was detected with a radiolabeled 2-kb *Hin*dIII/*Hpa* I restriction fragment from pPBG120. After this autoradiographic exposure (left panel, *lac*), the radiolabeled DNA was removed and the rDNA genes (rDNA) were detected by hybridization with a radiolabeled 2.2-kb *Eco*RI/*Eco*RI restriction fragment from pKK3535.

30- to 60-fold more DNA homologous to the radiolabeled *lac* DNA in the anti-RNA polymerase immunoprecipitates from IPTG-induced cells than from uninduced cells. Assays of ribosomal DNA recovered from these immunoprecipitates show that DNA was not lost during the preparation of the uninduced sample (Fig. 2, rDNA). Ribosomal gene transcription should be comparable in these two batches of cells (20).

The 30- to 60-fold increase in the amount of RNA polymerase cross-linked to the *lac* operon in IPTG-induced cells is lower than the 60- to 200-fold increase in the level of transcript (21). This difference may reflect the background in uninduced cells of nonspecific binding of RNA polymerase to the DNA helix (22).

The Cross-Linking of RNA Polymerase to the Leucine Operon Is Consistent with the Attenuation Model. The previous experiments demonstrate that these methods correctly determine the association of RNA polymerase with actively transcribed genes. We now show that these methods have sufficient resolution and sensitivity to test models of gene regulation. In particular, we test the attenuation model of gene regulation with regard to its predictions of the *in vivo* distribution of RNA polymerase on the leader and structural genes of the *leu* operon in wild-type and regulatory mutant strains of *Salmonella*. The attenuation model is supported by many genetic and biochemical studies (23, 24). In the wild-type strain CV468 grown under repressed conditions (leucine present), a leader region of about 165 bp is transcribed at a higher rate than are the structural genes located immediately downstream (25). This is due to premature termination of transcripts 165 bp downstream from the *leu* promoter. Thus, the RNA polymerase density should be higher on this leader region than on the downstream structural genes. In the attenuator mutant strain CV6, a single base change in the leader region causes RNA polymerase to transcribe the entire operon by increasing the frequency of terminator readthrough (25). Thus, the density of RNA polymerase should be high on both the leader and structural genes. In the promoter mutant strain leu500, a single base change in the Pribnow box dramatically decreases transcription initiation (26). Thus, a very low density of RNA polymerase is expected over the entire operon.

Fig. 3 shows that the sequences cross-linked to RNA polymerase in each of the three *Salmonella* strains coincide with the predicted transcription pattern. Very small amounts of RNA polymerase are cross-linked to the *leu* region in the promoter mutant (Pr^-), indicating that very little RNA polymerase is associated with the unexpressed operon. In contrast, significant amounts of RNA polymerase are cross-linked to the leader region of the operon in both the wild-type and attenuator mutant strains (WT and Att^-, respectively). In contrast to the attenuator mutant strain, much less RNA polymerase is cross-linked to the structural

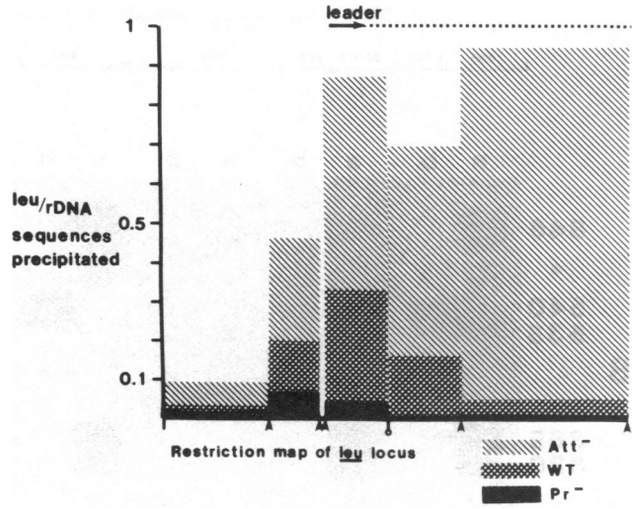

FIG. 3. The density of RNA polymerase on the *leu* operon of wild-type (WT), promoter mutant (Pr^-), and attenuator mutant (Att^-) strains. *Salmonella* strains were grown to an OD_{550} of 0.5 in 46 ml of minimal salt solution (27) supplemented with 0.2% glucose/leucine (50 μg/ml). Cells were chilled 5 min on ice and then irradiated 2 min. Total DNA from 9×10^6 cells and anti-RNA polymerase immunoprecipitated DNA from 9×10^8 cells were bound to each of eight nitrocellulose filters. DNA homologous to particular restriction fragments from the *leu* operon (delineated on the horizontal axis) was detected by hybridization with the corresponding radiolabeled restriction fragment isolated from the plasmid *leu* 7.1. The restriction fragments from *leu* 7.1 were isolated from a 5-cm long 6% polyacrylamide gel cast in a Bethesda Research Laboratories Preparative Gel Electrophoresis System. Manufacturer's instructions were followed, except that 10 μl of *N,N,N',N'*-tetramethylethylenediamine was used per 10 ml of acrylamide gel mix (17), and the running buffer was 90 mM Tris·HCl, pH 8.3/90 mM boric acid/4 mM EDTA. Before nick-translating the restriction fragments from *leu* 7.1, these fragments were treated for 12 hr with 80 units of T4 ligase at 12°C in 10 μl of 50 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/1 mM dithiothreitol/1 mM ATP, and then ethanol-precipitated. After autoradiographic exposure, these radiolabeled DNAs were removed from filters and the rDNA genes were detected by hybridizing all the filters simultaneously with a radiolabeled 2.2-kb *Eco*RI/*Eco*RI restriction fragment from pKK3535. The identity of each restriction site and the molecular weight of each restriction fragment are given in Fig. 4*A*. The hybridization signals from the immunoprecipitates are proportional to the product of fragment length times the average density of RNA polymerase on the fragment. The signal per base pair, which represents the RNA polymerase density, was calculated by dividing the signal from immunoprecipitates by the signal from total DNA. This standardizes for differences in fragment length, in copy number, and in hybridization efficiency. To relate polymerase densities from different strains, these ratios were further divided by the internal standard, which is the density of RNA polymerase on the rDNA genes.

genes in the wild-type strain. This is particularly evident for sequences well-separated from the leader region, such as the 845-bp *Hin*cII/*Hin*cII segment which exhibits 17-fold more RNA polymerase cross-linked in the attenuator mutant strain than in the wild-type.

An alternative method for analyzing the sequence composition of the immunoprecipitated DNA is to nick-translate this DNA and hybridize it to an excess of leucine operon restriction fragments that have been separated by agarose gel electrophoresis and blotted to nitrocellulose (28). The intensity of each band on the resulting autoradiogram reflects the amount of homologous DNA present in the immunoprecipitate. Such an approach eliminates the need to isolate several hybridization probes and can be used to quickly map the distribution of a protein within a particular locus or on several genes simultaneously.

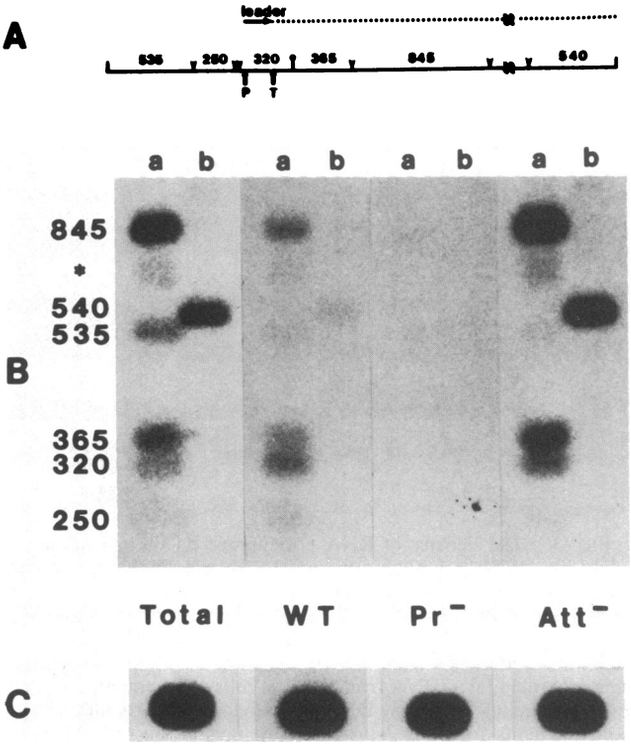

FIG. 4. Hybridization of nick-translated anti-RNA polymerase immunoprecipitated DNA to restriction fragments from the *leu* operon. (*A*) Map identifies the location of restriction fragments, the promoter (P), and the site of premature termination (T) in the *leu* operon. Symbols for restriction enzyme sites are as follows: ▮, *Eco*RI; ⚲, *Pst* I; ▼, *Hin*cII. The 535-, 250-, 320-, 365-, and 845-bp fragments reside on plasmid *leu* 7.1, while the 540-bp *Hin*cII/*Eco*RI fragment at the right end of the map resides in plasmid *leu* 14.7. (*B*) Cells were grown as described in Fig. 3. Total DNA from irradiated wild-type cells and anti-RNA polymerase immunoprecipitated DNA from 3.75×10^9 cells of wild-type (WT), promoter mutant (Pr^-), and attenuator mutant (Att^-) strains were hybridized to restriction fragments from the *leu* operon. Restriction fragments were separated on horizontal agarose gels and blotted to nitrocellulose (28). Lane a contains fragments derived from *leu* 7.1; lane b contains a 540-bp fragment derived from *leu* 14.7. The marked bands (*) are fragments resulting from partial cutting and consist of the 365-bp and 320-bp fragments and the 250-bp and 535-bp fragments. (*C*) The nick-translated DNA in *B* was simultaneously hybridized to restriction fragments from the rDNA clone pKK3535 cut with *Bam*HI, *Eco*RI, and *Hin*dIII. Hybridization to all the restriction fragments homologous to the rDNA was observed; only the signal from the 650-bp fragment containing part of the 16S rDNA gene is shown. The autoradiographic exposure of the immunoprecipitated DNAs is 8 times shorter than those in *B*, while exposure of the total is only 2.5 times shorter.

Fig. 4*A* presents the restriction map of the *leu* locus. Hybridization of nick-translated immunoprecipitated and total DNA to restriction fragments from the *leu* locus are shown in Fig. 4*B*. Immunoprecipitated DNA from the promoter mutant strain (Pr^-) shows very weak hybridization to all restriction fragments. In contrast, immunoprecipitated DNA from the attenuator mutant strain (Att^-) shows strong hybridization to all fragments except the 535-bp fragment that is 5′ to the operon. Immunoprecipitated DNA from the wild-type strain (WT) shows the strongest hybridization to a 320-bp fragment that contains the 165-bp leader sequence.

In the hybridization of nick-translated total DNA from irradiated cells, the 250-bp and 535-bp fragments are less visible than fragments of comparable size. This was unexpected, because the 250-bp and 535-bp fragments are present in molar amounts equal to the remaining fragments. These two fragments possess a high content of adjacent thymine bases, which can form dimers during UV irradiation of cells. Such dimers are known to inhibit nick-translation (29). Nonetheless, measuring the ratio of hybridization signals from immunoprecipitated DNA to total DNA taken from the same irradiated sample permits direct comparison of fragments even if they nick-translate with differing efficiencies.

As a control, the nick-translated DNAs were also hybridized to restriction fragments from a rDNA clone (8). We expect the RNA polymerase densities on these genes in the *Salmonella* strains to be equal, because transcription of ribosomal DNA genes is related to the rate of growth (19), and all three strains grew comparably. Fig. 4*C* shows that similar rDNA signals are obtained from all three immunoprecipitated DNAs, indicating that the hybridization signals to *leu* operon fragments can be directly compared.

The results of Fig. 4 agree well with the results presented in Fig. 3. Both assays show that in repressed wild-type cells most of the RNA polymerase resides on the leader region. Moreover, the density of RNA polymerase on the *leu* structural genes in the attenuator mutant strain is nearly as high as the average density on the rDNA genes under these growth conditions.

The following observations indicate that the hybridization signals are due to DNA and not to contaminating RNA. First, a probe upstream of the *leu* transcript of *Salmonella* hybridizes to DNA in the immunoprecipitates, presumably because of hybridization to randomly sheared DNA molecules that overlap both the transcribed and nontranscribed regions. Such a probe would not hybridize the RNA transcript, which initiates downstream. Second, the DNA in the immunoprecipitates can be nick-translated and hybridized to specific restriction fragments, giving results consistent with those found by dot blot analysis. Finally, treatment of the immunoprecipitated material used for the experiment in Fig. 3 with DNase I before application to the nitrocellulose eliminates the hybridization signal (data not shown).

DISCUSSION

Studies of adducts formed by UV irradiation of a variety of amino acid–nucleic acid mixtures indicate that the formation of covalent bonds between protein and DNA can involve many different amino acids and both purines and pyrimidines (30, 31). Model studies demonstrate that sites of cross-linking are at points of known interaction. For example, RNase A cross-links to pyrimidine dinucleotides via amino acids at the active site (32), while RNA polymerase cross-links to specific nucleotides of the *lac* promoter (33). In this study, we have coupled the use of UV light cross-linking and immunoprecipitation to examine protein–DNA interactions that occur *in vivo*.

Our results indicate that a RNA polymerase can be localized on DNA to within 100–200 bp when the immunoprecipi-

tated DNA is hybridized to restriction fragments of a few hundred base pairs in size. For example, hybridization of nick-translated immunoprecipitated DNA from wild-type *Salmonella* cells to restriction fragments of the leucine operon indicate a higher density of RNA polymerase on the 320-bp fragment that overlaps the leader sequence than to flanking fragments. Also, the proximity of the flanking fragments can be ascertained from the relative hybridization signals. Since the cellular DNA isolated by the immunoprecipitation had been randomly sheared by sonication, sequences flanking sites of protein association are also precipitated. The 365-bp fragment, which is 125 bp downstream from the attenuator, is more enriched in the precipitated fraction than fragments further downstream, but it is still less enriched than the 250-bp fragment, which is only 20 bp upstream from the −35 consensus sequence with which RNA polymerase contacts (34). These *in vivo* data are completely consistent with the attenuation model of regulation of the *leu* operon and are inconsistent with models in which the RNA polymerase transcribes beyond the attenuator under repressed conditions (e.g., retroregulation; see ref. 35).

The methods are sensitive enough to detect a protein bound to a single site on chromosomal DNA even if it cross-links with an efficiency only one-tenth that of RNA polymerase to the *leu* leader. RNA polymerase transcribing at 55 nucleotides per sec will take 3 sec to traverse the entire *leu* leader (36). Given that the strongest *E. coli* promoter initiates transcription once every 5–10 sec (19), we estimate that on average less than one polymerase molecule per cell is associated with this 165-bp region. RNA polymerase is easily detected on this region (Fig. 4, lane WT) after hybridization to the immunoprecipitated DNA from 10^9 cells.

It is possible that UV irradiation could chemically modify protein and DNA and thereby generate abnormal protein–DNA interactions. Two observations indicate that this does not affect these studies. First, our results are consistent with the proposed transcriptional state of the genes analyzed. Second, it has been shown that RNA polymerase cross-links to DNA *in vitro* in a comparable manner for a given UV dosage regardless of whether the UV source is a 100-μsec pulse from a laser or a 20-min irradiation from a germicidal lamp (37).

UV irradiation may also elicit a cellular response that could cause new protein–DNA interactions. We have attempted to minimize this by maintaining the cells at 0°C prior to and throughout the irradiation. The cells are not responding to the UV light in a manner that we can detect, because the amount of DNA cross-linked to RNA polymerase is directly proportional to the irradiation dosage.

The methods should be applicable to other proteins, provided that the proper geometry exists between protein and DNA residues for cross-linking. Studies of UV-induced cross-linking of chromatin and nuclei of chicken erythrocytes reveal that histones cross-link to DNA (38). At present, we are using a minor modification of this approach to map the distribution of RNA polymerase on genes in *Drosophila* cells (unpublished results).

We thank D. Burbee for providing pLRI, H. Noller and A. Dahlberg for providing pKK3535, and J. Calvo for providing pCV12 and *Salmonella* strains. We are indebted to L. Lau for providing purified *E. coli* RNA polymerase. We appreciate A. Bretscher's advice concerning immunological procedures. Finally, we thank M. Wolfner, B. Tye, J. Calvo, J. Roberts, J. Simon, and R. Glaser for critically reading this manuscript. This work was supported by National Institutes of Health Grant GM25232 and March of Dimes Grant 1-868.

1. Bogenhagen, D. F., Wormington, W. M. & Brown, D. D. (1982) *Cell* **28,** 413–421.
2. Dynan, W. S. & Tjian, R. (1983) *Cell* **32,** 669–680.
3. Payvar, F., Firestone, G. L., Ross, S. R., Chandler, V. L., Wrange, O., Carlstedt-Duke, J., Gustafsson, J.-A. & Yamamoto, K. R. (1982) *J. Cell Biochem.* **19,** 241–247.
4. Park, C. S., Hillel, Z. & Wu, C. W. (1982) *J. Biol. Chem.* **257,** 6944–6949.
5. Burgess, R. & Jendrisak, J. (1975) *Biochemistry* **14,** 4634–4638.
6. Bretscher, A. (1983) *J. Cell Biol.* **97,** 425–432.
7. Polisky, B., Bishop, R. J. & Gelfand, D. H. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3900–3904.
8. Brosius, J., Ullrich, A., Raker, M. A., Gray, A., Dull, T. J., Gutell, R. R. & Noller, H. F. (1981) *Plasmid* **6,** 112–118.
9. Gemmill, R. M., Wessler, S. R., Keller, E. B. & Calvo, J. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4941–4945.
10. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
11. Backman, K., Ptashne, M. & Gilbert, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4174–4178.
12. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
13. Yang, R. C. A., Lis, J. & Wu, R. (1979) *Methods Enzymol.* **68,** 176–182.
14. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
15. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
16. Schleif, R. F. & Wensink, P. C. (1981) *Practical Methods in Molecular Biology* (Springer, New York).
17. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
18. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
19. Pace, N. R. (1973) *Bacteriol. Rev.* **37,** 562–603.
20. Kepes, A. (1963) *Biochim. Biophys. Acta* **76,** 293–309.
21. Kumar, S. & Szybalski, W. (1969) *J. Mol. Biol.* **40,** 145–151.
22. Chamberlin, M. J. (1974) *Annu. Rev. Biochem.* **43,** 721–775.
23. Lee, F. & Yanofsky, C. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4365–4369.
24. Keller, E. B. & Calvo, J. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6186–6190.
25. Searles, L. L., Wessler, S. R. & Calvo, J. M. (1983) *J. Mol. Biol.* **163,** 377–394.
26. Gemmill, R. M., Jones, J. W., Haughn, G. W. & Calvo, J. M. (1983) *J. Mol. Biol,* **170,** 39–59.
27. Friedberg, D., Mikulka, T. W., Jones, J. & Calvo, J. M. (1974) *J. Bacteriol.* **118,** 942–951.
28. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
29. Rabkin, S. D., Moore, P. D. & Strauss, B. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1541–1545.
30. Smith, K. C. (1969) *Biochem. Biophys. Res. Commun.* **34,** 354–357.
31. Smith, K. C. & Meun, D. H. C. (1968) *Biochemistry* **7,** 1033–1037.
32. Havron, A. & Sperling, J. (1977) *Biochemistry* **16,** 5631–5635.
33. Simpson, R. B. (1979) *Cell* **18,** 277–285.
34. Siebenlist, U., Simpson, R. B. & Gilbert, W. (1980) *Cell* **20,** 229–281.
35. Gottesman, M., Oppenheim, A. & Court, D. (1982) *Cell* **29,** 727–728.
36. Bremer, H. & Yuan, D. (1968) *J. Mol. Biol.* **38,** 163–180.
37. Harrison, C. A., Turner, D. H. & Hinkle, D. C. (1982) *Nucleic Acids Res.* **10,** 2399–2414.
38. Cao, T. M. & Sung, M. T. (1982) *Biochemistry* **21,** 3419–3427.