Biochemistry. In the article "Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the *lux* promoter region" by Ann M. Stevens, Katherine M. Dolan, and E. P. Greenberg, which appeared in number 26, December 20, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 12619–12623), Fig. 4 was printed incorrectly. Specifically, the lower parts of lanes 3 and 10 in *A* were partially obscured, and the entire figure appeared too light, so that some bands were not clearly visible. The corrected figure and its legend are shown here.

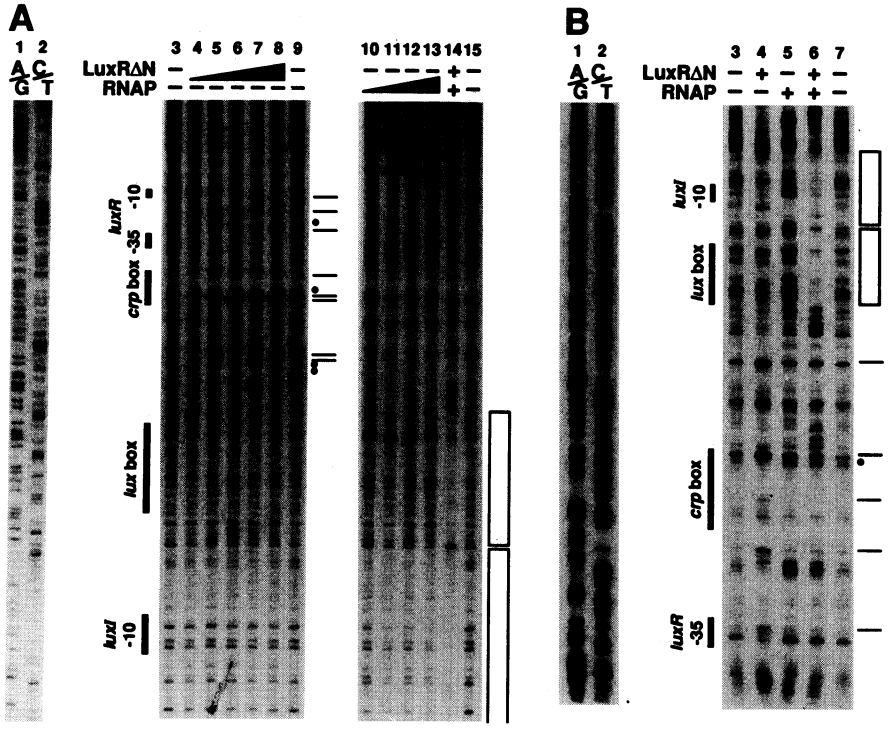

FIG. 4. DNase I protection analysis of LuxRΔN binding and RNAP binding to wild-type *lux* regulatory DNA from pAMS103. (*A*) *luxR* coding DNA strand. Lanes: 1 and 2, A/G and C/T sequencing ladders; 3, 9, and 15, no protein added; 4–8, LuxRΔN at 1.7, 3.5, 6.9, 10, and 14 μM; 10–13, RNAP holoenzyme at 11.5, 23, 46, and 92 nM; 14, LuxRΔN (10 μM) and RNAP (23 nM) together. (*B*) *luxI* coding strand. Lanes: 1 and 2, A/G and C/T sequencing ladders; 3 and 7, no protein added; 4, LuxRΔN (10 μM); 5, RNAP holoenzyme (23 nM); 6, LuxRΔN (10 μM) and RNAP (23 nM) together. The locations of the *luxI* −10 region, *lux* box, *crp* box, and *luxR* −10 and −35 regions are indicated by the solid lines. Hypersensitivity in the presence of LuxRΔN is indicated by the lines, and protection by LuxRΔN is indicated by the dots (not all hypersensitive or protected bands are indicated). The regions protected by LuxRΔN and RNAP together are indicated by the open boxes.

Medical Sciences. In the article "Infectious amyloid precursor gene sequences in primates used for experimental transmission of human spongiform encephalopathy" by L. Cervenáková, P. Brown, L. G. Goldfarb, J. Nagle, K. Pettrone, R. Rubenstein, M. Dubnick, C. J. Gibbs, Jr., and D. C. Gajdusek, which appeared in number 25, December 6, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 12159–12162), the authors request that the following correction be noted. The last entry in the middle column of Table 1 (page 12162) should have been 95.3%, rather than 96.3%. The corrected table reads as follows.

Table 1. Comparison of infectious amyloid precursor nucleotide and predicted amino acid homology to transmission rates of human spongiform encephalopathy in experimental primates

Species	Nucleotide homology, %	Amino acid homology, %	Transmission rate (positive/total animals)
Gorilla (ape)	99.6	99.2	Not inoculated
Chimpanzee (ape)	99.3	99.2	97 (28/29)
Rhesus (Old World) monkey	95.8	96.8	73 (19/26)
Spider (New World) monkey	95.3	96.1	97 (30/31)
Squirrel (New World) monkey	94.3	95.3	93 (196/211)

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Biochemistry

Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the *lux* promoter region

(autoinduction/DNA binding/luminescence/quorum sensing)

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ABSTRACT **LuxR, the *Vibrio fischeri* luminescence gene (*lux*) activator, is the best-studied member of a family of bacterial transcription factors required for cell density-dependent expression of specific genes involved in associations with eukaryotic hosts. Neither LuxR nor any other LuxR homolog has been shown to bind DNA directly. We have purified the LuxR C-terminal transcriptional activator domain from extracts of recombinant *Escherichia coli* in which this polypeptide was expressed. The purified polypeptide by itself binds to *lux* regulatory DNA upstream of the *lux* box, a 20-bp palindrome that is required for LuxR activity *in vivo*, but it does not bind to the *lux* box. However, the LuxR C-terminal domain together with RNA polymerase protects a region including the *lux* box and the *lux* operon promoter from DNase I cleavage. There is very little protection of the *lux* operon promoter region from DNase I digestion in the presence of RNA polymerase alone. Apparently, there is a synergistic binding of the LuxR C-terminal domain and RNA polymerase to the promoter region. The upstream binding region for the purified polypeptide encompasses a binding site for cAMP receptor protein (CRP). Under some conditions, CRP binding can block the binding of the LuxR C-terminal domain to the upstream binding region, and it can also block the synergistic binding of the LuxR C-terminal domain and RNA polymerase to the *lux* box and luminescence gene promoter region. This description of DNA binding by the LuxR C-terminal domain should lead to an understanding of the molecular interactions of the LuxR family of transcriptional activators with regulatory DNA.**

LuxR-facilitated autoinduction controls transcription of luminescence genes in *Vibrio fischeri*. LuxR homologs occur in a number of different Gram-negative bacteria, and these transcription factors are involved in a phenomenon termed quorum sensing and response (for recent reviews, see refs. 1–3). In quorum sensing, the cells produce an *N*-acylhomoserine lactone, the autoinducer. The *V. fischeri* autoinducer is *N*-(3-oxohexanoyl)homoserine lactone (4). Cells of *V. fischeri* are freely permeable to the autoinducer, which therefore accumulates in the medium during growth (5). When autoinducer reaches a sufficient concentration it binds to LuxR (6, 7), which can then activate transcription of the luminescence (*lux*) genes. Thus autoinducer is a signal that allows communication between *V. fischeri* cells, enabling them to monitor their own population density. At low cell densities, the autoinducer will diffuse away from cells. At high cell densities, the autoinducer will reach a sufficient concentration, the cells will sense that a quorum has been attained, and transcription of the *lux* genes will be activated.

There are no reports of *in vitro* activity for LuxR or any LuxR homolog. A general view of the mechanism of autoinduction in *V. fischeri* has been developed from molecular genetic analyses. These analyses were made possible by the cloning of a fragment of *V. fischeri* DNA that encodes all of the functions necessary for autoinducible luminescence in *Escherichia coli* (8). This *V. fischeri* DNA contains two divergent transcriptional units. One unit contains *luxR*, and the other unit, which is activated by the LuxR protein together with autoinducer, contains *luxI*, the gene required for autoinducer synthesis, and genes required for light emission (8–10) (Fig. 1).

The *lux* box, a 20-bp inverted repeat centered at −40 bp from the start of *luxI* transcription (Fig. 1), is required for autoinduction of luminescence (11) and is thus a putative binding site for LuxR. The LuxR polypeptide contains 250 aa (10, 12) and consists of two domains (13, 14). The C-terminal domain, which extends from around residue 160 to the C terminus, is thought to bind *lux* regulatory DNA and activate transcription of the luminescence genes (15). The other domain, which binds autoinducer (7, 14–17), consists of the N-terminal 60–70% of LuxR. In the absence of autoinducer the N-terminal domain inhibits transcriptional activation by the C-terminal domain. This inhibitory role is neutralized by autoinducer binding. In *E. coli*, truncated LuxR polypeptides consisting solely of the C-terminal domain can activate the *lux* genes in the absence of autoinducer (13). LuxR is thought to function as an oligomer and residues in the region of 116–161 in the N-terminal domain appear to be critical for oligomerization (14).

A barrier to developing an understanding of the mechanisms by which LuxR or LuxR homologs activate transcription has been an inability to demonstrate binding of any of these proteins to regulatory regions of target genes *in vitro*. Several obstacles have hindered development of an *in vitro* LuxR activity assay. When overexpressed in *E. coli*, LuxR forms insoluble inclusion bodies (18). Furthermore, LuxR requires the assistance of Hsp60 to fold into an active form (19, 20). Additionally, a number of other DNA-binding proteins, including CRP (21), LexA (21), and Fnr (40) recognize sequences in the *lux* regulatory DNA. This has confounded attempts to use LuxR-containing cell extracts to study binding of LuxR to *lux* regulatory DNA. Finally, full-length LuxR is associated with the membrane fraction of crude *V. fischeri* cell extracts (22). We have overcome these obstacles by purifying the C-terminal domain of LuxR and studying the DNA-binding activity of this polypeptide *in vitro*. We show that by itself this polypeptide binds *lux* regulatory DNA specifically but does not bind to the *lux* box. Together, the purified LuxR polypeptide and RNA polymerase (RNAP) bind synergistically to the *lux* box and the *luxI* promoter region.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Culture Conditions. We used *E. coli* XL1-Blue (23) containing pSC156 (13) to produce the 95-aa C-terminal fragment of LuxR, referred to as

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Abbreviations: CRP, cAMP receptor protein; RNAP, RNA polymerase.

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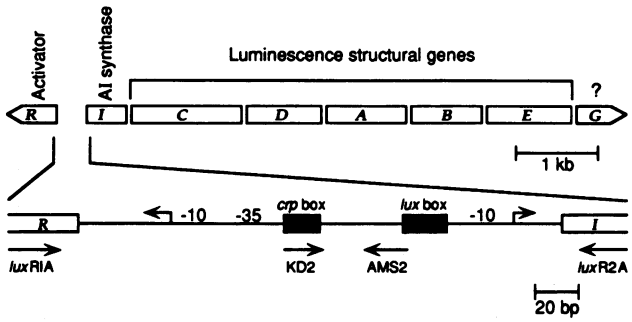

FIG. 1. Organization of the *V. fischeri lux* gene cluster and map of the intergenic *lux* regulatory DNA. (*Upper*) The *lux* gene cluster. Arrowheads indicate the direction of transcription of *luxR* and of the *luxICDABEG* operon. The *luxR* gene codes for the transcriptional activator; *luxI* codes for autoinducer synthase; *luxC*, *-D*, and *-E* code for the fatty acid reductase required for synthesis of the aldehyde substrate for luciferase; *luxA* and *-B* code for the subunits of luciferase; and the function of *luxG* is unknown. (*Lower*) The *lux* regulatory region amplified from pJE202 by PCR with the primers *lux*R1A and *lux*R2A. The open boxes indicate the starting regions of the *luxR* and *luxI* open reading frames. The filled boxes denote the locations of the cAMP receptor protein (CRP)-binding site (*crp* box) and the *lux* box. The transcriptional start regions and the location of the promoter elements of *luxR* and *luxI* are shown. The arrows below the map indicate the lengths, locations, and names of the primers used for PCR generation of *lux* regulatory fragments.

LuxRΔN. For purification of LuxRΔN, cells were cultured at 30°C in 5 liters of Luria broth (24) containing ampicillin (100 μg/ml). Isopropyl β-D-thiogalactopyranoside (IPTG, 1 mM) was added to the culture during the midexponential phase of growth. Cells were harvested 2 hr after addition of IPTG.

The following plasmids were used as templates for PCR synthesis of the target DNAs used in DNA mobility-shift experiments: pJE202, which contains the *V. fischeri luxR*, *luxICDABEG* gene cluster (8); pJHD506, which contains *luxR*, part of *luxI*, and the regulatory DNA between *luxR* and *luxI* except that there is a deletion of the central 12 bp of the 20-bp *lux* box (11); and pUC18 (25).

Two plasmids were constructed for use as sources of DNA for DNase I protection experiments, pAMS103 and pAMS104. Both of these plasmids contained the *lux* regulatory DNA between *luxR* and *luxI* (Fig. 1) cloned into the *Hin*cII site of pUC19 (25). The *lux* regulatory DNA was prepared by PCR amplification from pJE202 for pAMS103, and pJHD506 for pAMS104. The primers for PCR amplification corresponded to nt 32–10 of the *luxR* open reading frame (*lux*R1A), and the *luxI* open reading frame (*lux*R2A) (Fig. 1). Standard procedures were used for PCR amplification and cloning (24). To confirm that pAMS103 contained the intergenic *lux* regulatory DNA and that pAMS104 contained a similar *lux* fragment with a 12-bp deletion in the *lux* box, the nucleotide sequence of the *V. fischeri* DNA in these plasmids was determined by the dideoxy chain-termination method (26) using the pUC forward and reverse primers.

Purification of LuxRΔN. After induction of LuxRΔN synthesis, cells of *E. coli*(pSC156) were harvested by centrifugation and washed once in 0.15 M NaCl. The resulting cell paste was stored frozen at −70°C prior to purification of LuxRΔN. Approximately 15 g of the cell paste was thawed and suspended in 50 ml of cold buffer A [1 mM EDTA/5 mM dithiothreitol/10% (vol/vol) glycerol/20 mM sodium phosphate, pH 6.8]. Prior to disruption of bacteria in a French pressure cell at 15,000 psi (1 psi = 6.89 kPa), the protease inhibitors phenylmethylsulfonyl fluoride (100 μg/ml), leupeptin (0.5 μg/ml), and pepstatin A (0.7 μg/ml) were added. Remaining whole cells and cell debris were removed by centrifugation at 11,000 × *g* for 30 min at 4°C. The soluble cell extract was further clarified by ultracentrifugation at 100,000 × *g* for 1 hr. The clarified cell extract remaining in the supernatant fraction after ultracentrifugation was applied to an SP-Sepharose cation-exchange column (automated FPLC system; Pharmacia LKB). The column was equilibrated and washed with buffer A, and LuxRΔN was then eluted at 600–750 mM NaCl in a linear gradient of 0–1 M NaCl in buffer A. Column fractions were examined for LuxRΔN by Western immunoblotting with LuxR antiserum (17). The LuxRΔN fractions were pooled, concentrated by ultrafiltration (Amicon), and applied to a Sephadex G-75 gel filtration column (2.6 cm × 65 cm, Pharmacia LKB). LuxRΔN was eluted in buffer A plus 0.1 M NaCl. Fractions containing LuxRΔN, identified by Western immunoblotting with anti-LuxR, were pooled and concentrated by ultrafiltration. The gel filtration column was calibrated with protein standards (low molecular weight gel filtration calibration kit; Pharmacia LKB). Protein concentrations were estimated by the Bradford dye-binding procedure with reagents from BioRad. SDS/PAGE has been described (27, 28).

DNA Mobility-Shift Assays. Gel shift assays were based on published procedures (29). Reaction mixtures (60 μl) contained 3 nM radiolabeled DNA, 50 mM NaCl, 1 mM $MgCl_2$, 0.002 μg/μl poly(dI-dC) (Boehringer Mannheim), acetylated bovine serum albumin (2 mg/ml), 1 mM dithiothreitol, 0.1 mM EDTA, and 10% glycerol in 40 mM Hepes (pH 7.4). Reactions were initiated by addition of LuxRΔN, and proceeded at 30°C for 15 min. Reaction mixtures were separated by electrophoresis at 10 V/cm at 25°C in 4% polyacrylamide gels with recirculation of the buffer (20 mM Hepes/3 mM NaCl/1 mM EDTA, pH 8.0). Radioactive bands were visualized by autoradiography as described (24).

Radiolabeled DNAs for the mobility-shift experiments were generated by PCR. Fragments of *lux* regulatory DNA were generated from either pJE202 or pJHD506 using *lux*R1A and *lux*R2A (Fig. 1) as primers. With pJE202 as the template, a 282-bp DNA fragment containing the entire wild-type *lux* regulatory region was generated. A 270-bp fragment with a 12-bp deletion in the *lux* box was generated from pJHD506. A 157-bp DNA fragment extending from the *crp* box through the *lux* box and into the *luxI* open reading frame was generated from pJE202 with KD2 and *lux*R2A serving as primers (Fig. 1). A 182-bp DNA fragment extending from the *luxR* open reading frame through the first 3 bp of the *lux* box was generated with pJE202 as a template and *lux*R1A and AMS2 as primers (Fig. 1). The 104-bp pUC multiple cloning site was amplified from pUC18 by using the pUC forward and reverse primers (25). The PCR products were radiolabeled by inclusion of [α-^{32}P]dCTP in the PCR mixtures (24).

DNase I Protection Experiments. DNase I protection assays were based on published procedures (30). Reaction mixtures (60 μl) contained ^{32}P-end-labeled *lux* regulatory DNA (10,000–15,000 cpm), 50 mM NaCl, 1 mM $MgCl_2$, acetylated bovine serum albumin (2 mg/ml), 2 mM dithiothreitol, 0.3 mM EDTA, 4 mM sodium phosphate, and 10% glycerol in 40 mM Hepes (pH 7.4). Reactions were initiated by the addition of proteins at the concentrations indicated. Purified RNAP holoenzyme was purchased from Promega, and core RNAP was purchased from Epicentre Technologies (Madison, WI). Purified CRP was a gift from T. Steitz. In cases where CRP was added to the reaction mixture, the buffer contained 2 mM cAMP. After 15 min at 30°C, 0.1 μg of DNase I (Promega) in 2 μl of 2.5 mM Tris, pH 8/150 mM $MgCl_2$/300 mM $CaCl_2$ was added to each reaction mixture. DNase I digestion was allowed to proceed for 1 min and was stopped by addition of 15 μl of 3 M ammonium acetate/0.25 M EDTA, containing sonicated calf thymus DNA at 15 μg/ml. Nucleic acids were precipitated in ethanol and dissolved in loading buffer (sequencing stop solution, United States Biochemical). Samples were heated at 80°C for 5 min and analyzed by electrophoresis in a 6% polyacrylamide/urea sequencing gel. The

radiolabeled DNA fragments were also used in Maxam–Gilbert A+G and C+T sequencing reactions (31) to generate a reference sequencing ladder. Radioactive bands were visualized by autoradiography.

Three different *lux* regulatory DNA fragments were end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (24). For labeling the *luxR* sense strand, we used a *Hin*dIII–*Eco*RI *lux* DNA fragment from pAMS103 or an *Eco*RI–*Pst* I fragment from pAMS104. For labeling the *luxI* sense strand, an *Eco*RI–*Pst* I *lux* regulatory DNA fragment from pAMS103 was used.

RESULTS

Purification of LuxRΔN from *E. coli*(pSC156). The *tac* promoter-controlled 5′ *luxR* deletion on pSC156 directs the synthesis of a 95-aa polypeptide that serves as an autoinducer-independent activator of the *V. fischeri* luminescence genes in *E. coli* (13). This pSC156-encoded polypeptide, LuxRΔN, was found predominantly in the soluble supernatant fraction after ultracentrifugation of *E. coli*(pSC156) extracts (<10% in the pellet). LuxRΔN was purified by ion-exchange and gel filtration column chromatography (Fig. 2). About 1 mg of purified LuxRΔN was obtained from a 5-liter culture of *E. coli*(pSC156). As determined by SDS/PAGE LuxRΔN had an apparent molecular weight of about 10,000, consistent with its predicted molecular weight of 10,695. Based on its elution from the gel filtration column, we believe that LuxRΔN exists as a monomer in solution (data not shown). This finding is consistent with the conclusion based on molecular genetic analyses of LuxR that the region required for oligomerization resides in the N-terminal domain of the full-length polypeptide (14). Only a small part of this oligomerization region exists on LuxRΔN. We have not investigated whether LuxRΔN forms oligomers at concentrations higher than that at which it was eluted from the gel filtration column.

DNA Mobility-Shift Studies: Purified LuxRΔN Specifically Binds to *lux* Regulatory DNA. LuxRΔN bound to the 282-bp wild-type *lux* regulatory DNA-containing fragment, and apparently, two LuxRΔN–DNA complexes formed. As the concentration of LuxRΔN was increased, the extent of the DNA mobility shift increased and smearing of the bands was evident (Fig. 3*A*). This type of result would be expected if the LuxRΔN–DNA complexes were dissociating during electrophoresis or if a nucleoprotein complex were forming with DNA binding and additional LuxRΔN binding to the DNA-associated LuxRΔN as the concentration of the protein was increased (32, 33). A shifted complex was not observed when pUC DNA was used in place of *lux* regulatory DNA (Fig. 3*B*). This indicates a specificity of LuxRΔN binding with *lux* regulatory DNA. Furthermore, competition experiments with unlabeled *lux* regulatory DNA and unlabeled pUC DNA indicated that LuxRΔN specifically bound to *lux* regulatory DNA (data not shown).

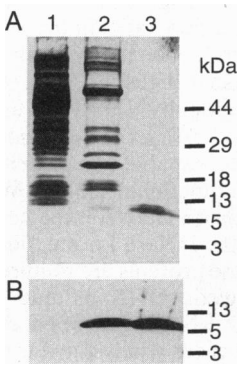

FIG. 2. Purification of LuxRΔN from clarified extracts of *E. coli* containing pSC156. (*A*) SDS/polyacrylamide gel stained with Coomassie brilliant blue. (*B*) Western immunoblot with anti-LuxR serum. Lanes: 1, clarified cell extract (20 μg of protein); 2, LuxRΔN peak from SP-Sepharose column chromatography (20 μg of protein); 3, LuxRΔN peak from Sephadex G-75 column chromatography (5 μg of protein). Locations of molecular mass (kDa) standards are shown on the right.

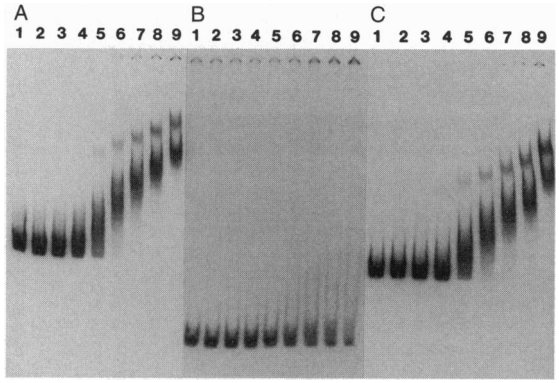

FIG. 3. DNA mobility-shift assays with purified LuxRΔN. (*A*) The 282-bp *lux* intergenic region from pJE202. (*B*) The 104-bp pUC multiple cloning site. (*C*) The 270-bp *lux* intergenic region from pJHD506, containing a deletion of 12 bp from the *lux* box. Lanes: 1, no LuxRΔN; 2–9, LuxRΔN at 0.26, 0.52, 1.0, 2.1, 4.2, 6.3, 8.4, and 13 μM, respectively.

To begin to define the location of LuxRΔN binding on the *lux* regulatory DNA, several different *lux* regulatory DNA deletion fragments were used in DNA mobility-shift experiments. The mobility-shift pattern observed with a 270-bp fragment of *lux* regulatory DNA with a deletion of the central 12-bp of the *lux* box was remarkably similar to the pattern observed when the 282-bp wild-type *lux* regulatory DNA fragment served as the target DNA (compare Fig. 3 *C* and *A*). Because this 12-bp deletion abolishes autoinduction of luminescence *in vivo* (11) the observed DNA mobility-shift result was unexpected. It indicates that LuxRΔN was not binding to the *lux* box but was binding elsewhere on the *lux* regulatory DNA. To confirm this, a 182-bp DNA fragment extending from the *luxR* open reading frame up to, but not including, the *lux* box and a 157-bp *lux* regulatory DNA fragment extending from the CRP-binding site (*crp* box) into the *luxI* open reading frame were used as target DNA. With either DNA fragment, complexes formed with LuxRΔN (data not shown). Apparently, a LuxRΔN binding site in the *lux* regulatory DNA resides in a region including the *crp* box and extending toward but not including the *lux* box.

DNase I Protection Studies: The Influence of RNA Polymerase and CRP on LuxRΔN Binding to *lux* Regulatory DNA. To further investigate the binding of LuxRΔN to *lux* regulatory DNA, DNase I protection studies were done with LuxRΔN by itself or together with RNAP, CRP, or both. CRP binds in the region defined as the LuxRΔN binding site by our DNA mobility-shift experiments (21). When LuxRΔN alone was added to *lux* regulatory DNA no obvious footprint was observed, regardless of the concentration of LuxRΔN used. Rather, a pattern of hypersensitive bands and protected bands was observed in the region identified in the DNA mobility-shift experiments as the LuxRΔN binding region (Fig. 4). Also consistent with the DNA mobility-shift experiments, LuxRΔN did not affect the DNase I banding pattern in the *lux* box region (Fig. 4).

When RNAP by itself was added to the *lux* regulatory DNA, little or no DNase I protection was observed in the region of the *luxI* promoter (Fig. 4). However, when both LuxRΔN and RNAP were present, a clear footprint over the *lux* box and the *luxI* promoter region was observed, and the pattern of upstream hypersensitive and protected bands observed with LuxRΔN alone was lost (Fig. 4). The synergistic binding of RNAP and LuxRΔN required the σ subunit of RNAP. When core RNAP was used in place of the holoenzyme the footprint was not present (data not shown). When the DNA contained a mutation in the *lux* box there was no DNase I protection of the *lux* box region and there was

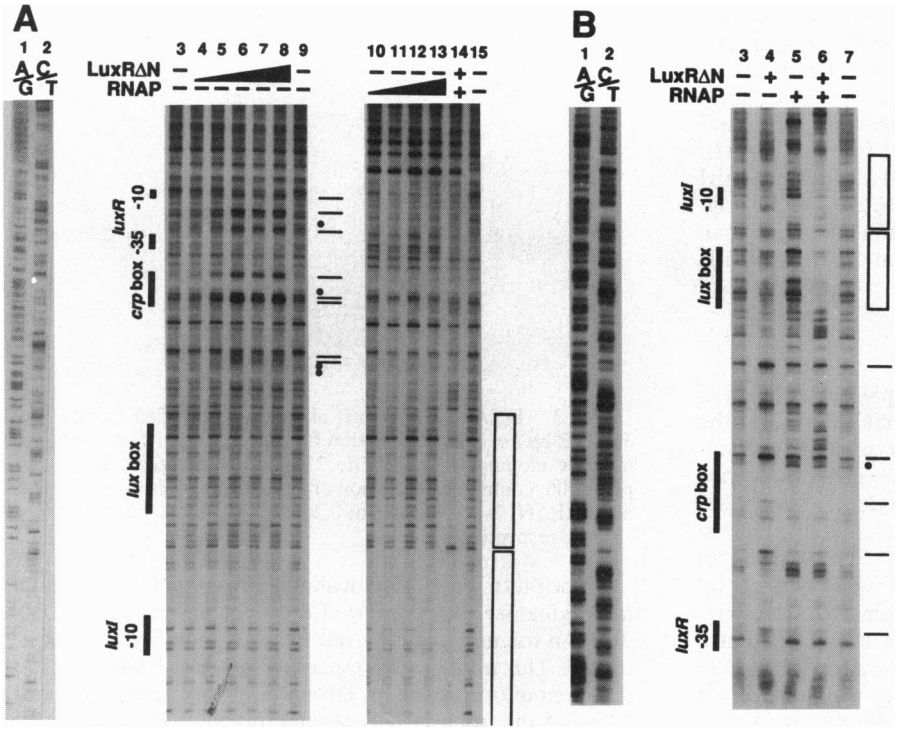

FIG. 4. DNase I protection analysis of LuxRΔN binding and RNAP binding to wild-type *lux* regulatory DNA from pAMS103. (*A*) *luxR* coding DNA strand. Lanes: 1 and 2, A/G and C/T sequencing ladders; 3, 9, and 15, no protein added; 4–8, LuxRΔN at 1.7, 3.5, 6.9, 10, and 14 μM; 10–13, RNAP holoenzyme at 11.5, 23, 46, and 92 nM; 14, LuxRΔN (10 μM) and RNAP (23 nM) together. (*B*) *luxI* coding strand. Lanes: 1 and 2, A/G and C/T sequencing ladders; 3 and 7, no protein added; 4, LuxRΔN (10 μM); 5, RNAP holoenzyme (23 nM); 6, LuxRΔN (10 μM) and RNAP (23 nM) together. The locations of the *luxI* −10 region, *lux* box, *crp* box, and *luxR* −10 and −35 regions are indicated by the solid lines. Hypersensitivity in the presence of LuxRΔN is indicated by the lines, and protection by LuxRΔN is indicated by the dots (not all hypersensitive or protected bands are indicated). The regions protected by LuxRΔN and RNAP together are indicated by the open boxes.

little or no protection of the *luxI* promoter region by LuxRΔN together with RNAP. Addition of either LuxRΔN alone or LuxRΔN and RNAP to the DNA with the *lux* box mutation, however, did result in the same DNase I cleavage pattern observed in the upstream region of wild-type DNA with LuxRΔN alone (data not shown).

For two reasons, we studied the influence of CRP on the DNA binding of LuxRΔN alone or together with RNAP: (*i*) LuxRΔN by itself was found to interact with the *lux* regulatory DNA in a region that encompassed the CRP-binding site and (*ii*) previous studies of *lux* gene transcription in *E. coli* have led to the suggestion that under some conditions LuxR and CRP are transcriptional antagonists (34). Addition of CRP alone to the *lux* regulatory DNA resulted in a footprint over the region previously shown to be protected by this protein (21). When LuxRΔN or LuxRΔN and RNAP were included in the reaction mixtures with CRP, the DNase I protection pattern was similar to that obtained with CRP alone (Fig. 5). At the concentration of CRP used, this protein

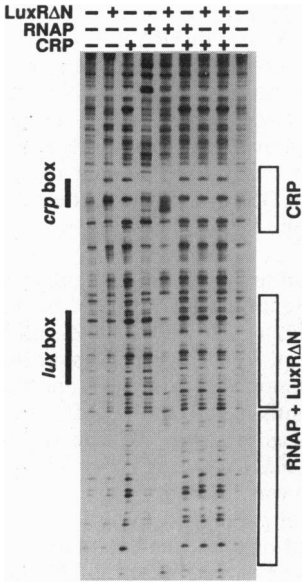

FIG. 5. DNase I protection analysis of the influence of CRP on the binding of LuxRΔN and RNAP to wild-type *lux* regulatory DNA from pAMS103 (*luxR* coding strand). LuxRΔN (14 μM), RNAP (46 nM), and CRP (780 nM) were added as indicated. The locations of the *lux* and *crp* boxes are shown on the left for reference. The regions protected by RNAP plus LuxRΔN alone and by CRP alone are shown on the right.

appeared to block the binding of LuxRΔN to the *lux* regulatory DNA even in the presence of RNAP. It could be that LuxRΔN must bind to the CRP-binding region before it can synergistically bind with RNAP in the *lux* box region, or CRP binding to the *crp* box may distort the regulatory DNA in the region of the *lux* box so as to occlude the region of LuxRΔN–RNAP binding. Further experiments will be required to determine how CRP binding to the *crp* box interferes with the synergistic binding of LuxRΔN and RNAP in the region of the *lux* box and *luxI* promoter and whether concurrent binding of CRP and LuxRΔN is possible under other conditions—for example, at lower CRP concentrations.

DISCUSSION

We have obtained direct evidence that the *V. fischeri* LuxR protein interacts with the *lux* transcription initiation complex. Our previous knowledge of LuxR and its homologs came entirely from *in vivo* experiments. To overcome difficulties encountered in studying full-length LuxR *in vitro*, we purified a region of this protein consisting of the C-terminal DNA-binding and transcriptional activator domain from recombinant *E. coli* (Fig. 2). We have termed the recombinant protein LuxRΔN.

As indicated by the results of DNase I footprint experiments, LuxRΔN and RNAP cooperate to protect the *lux* regulatory DNA in the region of the *lux* box (starting around *luxI* −54 through the *luxI* transcription start site to +6). Alone, RNAP only weakly protected a small area in the −10 region of *luxI*, and LuxRΔN did not influence the sensitivity of the *luxI* promoter region to DNase I digestion (Fig. 4). Protection of the *luxI* promoter region by LuxRΔN and RNAP together required an intact −35–*lux* box region. However, LuxRΔN and RNAP may be capable of a protein–protein interaction in the absence of a functional *lux* box. We cannot discriminate from our experiments between the contributions of LuxRΔN and RNAP to the DNase I protection. We can conclude only that both of these proteins are required for the protection. Although synergistic DNA binding of other transcriptional regulators and RNAP has been described (35–37), we know of no other cases where there is an

absolute requirement for RNAP to achieve DNase I protection as described here for LuxRΔN. Our evidence indicates that when eluted from the gel filtration column the purified LuxRΔN existed as a monomer. Full-length LuxR is thought to function as a multimer, presumably a dimer (14). It is possible that full-length multimeric LuxR would not exhibit an absolute dependence on RNAP for DNase I protection of the *lux* box. It is also possible that under the conditions used in the DNase I protection experiments, LuxRΔN existed in an oligomeric state.

Both gel mobility studies and DNase I protection studies indicate that purified LuxRΔN by itself interacts with *lux* regulatory DNA in a region upstream of the *lux* box (Figs. 3 and 4). This upstream region encompasses the CRP-binding site (Fig. 1). In the gel shift experiments the mobility of the complexes decreased with increasing concentration of LuxRΔN. One interpretation of this result is that two discrete complexes formed. Perhaps one complex consisted of oligomers more tightly bound to the DNA, and the other consisted of monomers more loosely bound to the DNA. The smearing could have resulted from a dissociation of loosely bound complexes during gel electrophoresis. Another interpretation of this sort of pattern is that a nucleoprotein complex formed, with secondary binding of LuxRΔN molecules to DNA-bound LuxRΔN (32). The DNase I protection experiments also showed a peculiar pattern in which a number of hypersensitive bands were the most obvious result of the addition of purified LuxRΔN. Like the DNA mobility-shift results, this is consistent with the formation of a nucleoprotein complex in which the DNA bends around a protein core (32, 33).

By using DNase I protection assays we showed that under some conditions CRP not only interfered with LuxRΔN binding in the absence of RNAP but also interfered with the synergistic binding of LuxRΔN and RNAP to the *lux* box and the *luxI* promoter (Fig. 5). Apparently, CRP binding can occlude binding of LuxRΔN–RNAP even though the two binding regions are separated by about 40 bp. Either CRP can bend the DNA (38) so that it can physically interfere with protein binding at the *lux* box or binding of LuxRΔN to the CRP-binding region is a prerequisite for the synergistic interaction of LuxRΔN and RNAP in the *luxI* promoter region. With the ability to study LuxRΔN binding to *lux* DNA *in vitro* it will be possible to examine this more thoroughly.

In summary, we have found that the C-terminal domain of LuxR binds to *V. fischeri lux* regulatory DNA specifically. By itself, it binds to a region distant from the *lux* box and LuxR-activated *luxI* promoter. Together, RNAP and the C-terminal domain of LuxR bind to a region spanning the *lux* box, the *luxI* −35 and −10 regions, and up through the first several base pairs of the *luxI* open reading frame. There is evidence from *in vivo* studies that the mechanisms of DNA recognition and transcriptional activation are conserved among LuxR homologs (39). Thus, this report of *in vitro* DNA binding by the transcriptional activator domain of LuxR and the characterization of the binding of this protein to *lux* DNA should open the way to further detailed studies of the interactions of members of the LuxR family of transcription factors with their target DNA.

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