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Genetics

The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent *lux* gene activating domain

(luminescence/transcriptional activation/DNA-binding domain/repression)

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ABSTRACT **The *Vibrio fischeri* luminescence genes are activated by an autoinducer and the 250-amino acid residue LuxR protein. To develop a general view of LuxR structure and function, a set of *luxR* 5′-deletion mutations was generated. Ten *luxR* mutant plasmids encoding active LuxR proteins with deletions ranging from residues 2–5 (Δ2–5) to residues 2–182 (Δ2–182) were studied. The degree of transcriptional activation of luminescence genes by the truncated LuxR proteins ranged from 0.01% to >200% of the wild-type level. LuxR proteins with small deletions (up to Δ2–20) were active and remained autoinducer-dependent, LuxR proteins with deletions between residues 2–58 and 2–138 showed low activity and were not affected by autoinducer, and LuxR proteins with large deletions such as the Δ2–162 protein were highly active and autoinducer-independent. However, proteins with deletions equal to or greater than Δ2–20 were unable to autoregulate *luxR*. Our data indicate there is a C-terminal LuxR domain capable of functioning as a transcriptional activator. We suggest that an N-terminal region of LuxR starting between residues 20 and 58 and extending to the region of residues 138–162 masks the activator function of the C-terminal domain. Residues prior to position 20 are needed for autoregulatory function. Experiments showing that wild-type *luxR* is dominant over *luxR* genes coding the Δ2–58 through Δ2–138 proteins indicate the N-terminal arm masks *lux* DNA binding.**

Luminescence of the marine bacterium *Vibrio fischeri*, an essential feature of its light organ symbiosis with certain marine animals, requires autoinduction. *V. fischeri* cells produce a substance termed autoinducer [*N*-(3-oxohexanoyl)-homoserine lactone]. Because cells are freely permeable to it, autoinducer accumulates in the medium during growth. When autoinducer reaches a sufficient concentration, it triggers synthesis of specific luminescence enzymes (1–5). Thus autoinduction provides communication between *V. fischeri* cells allowing them to sense their own population density. At the high population densities attained in the light organ symbiosis, autoinducer can accumulate, and induction of *V. fischeri* luminescence will occur. However, in seawater where *V. fischeri* exists at low densities, autoinduction of luminescence would not be expected (1, 5–8).

A 9-kilobase (kb) fragment of *V. fischeri* DNA that encodes the functions required for luminescence and contains regulatory elements sufficient for their expression in *Escherichia coli* has been isolated (9). Seven *lux* genes are required for autoinducible luminescence in *E. coli*, and these genes are organized as two divergently transcribed units (Fig. 1). One unit contains *luxR*, a gene encoding the LuxR protein, which is required for a response to autoinducer. The other unit, which is activated by the LuxR protein in the presence of autoinducer, contains *luxA* and *luxB*, the genes encoding the α and β subunits of luciferase; *luxC*, *-D*, and *-E*, genes coding for polypeptides involved in synthesis of the aldehyde substrate for luciferase; and *luxI*, the only *V. fischeri* gene required for synthesis of autoinducer by *E. coli* (10). The *luxR* gene is autoregulated at the level of transcription (11–13) and at a posttranscriptional level (14). Induction of luminescence also requires cAMP and the cAMP receptor protein, which activate transcription of *luxR* (15–17).

There are 155 base pairs (bp) between the *luxR* and *luxI* transcriptional start sites. There is a cAMP receptor protein binding sequence centered at position −59 from the *luxR* transcriptional start site (18–20) and, based on a mutational analysis, it appears that a 20-bp inverted repeat centered at position −40 from the *luxI* transcriptional start site is a LuxR-binding sequence required for *lux* regulation (13, 18).

LuxR is a member of a family of transcriptional activators defined by sequence similarities in a C-terminal helix–turn–helix-containing region (21). This so-called LuxR family can be subgrouped based on N-terminal sequence similarities. LasR (22) and the UvrC 28-kDa protein (21) appear to be LuxR homologs. Several members of the family possess similar N-terminal sequences that place them as members of the two-component regulatory system group (21, 23, 24). GerE consists in its entirety of the C-terminal region defining the LuxR family (21), and MalT is so far unique in that it possesses an N-terminal arm that makes it considerably larger than any other members of the LuxR family (at 105 kDa compared to 28 kDa for LuxR). MalT also requires both maltotriose and ATP for activity (21, 25, 26), and it is the only member of the LuxR family that has been demonstrated to bind DNA *in vitro* (27). We cannot explain why it has not yet been possible to develop *in vitro* DNA binding assays for members of the LuxR family other than MalT. Also the conserved C-terminal region of LuxR family members shows significant sequence similarity to the conserved region 4 of bacterial RNA polymerase σ factors (24, 28). Region 4 is a helix–turn–helix-containing region considered to recognize the −35 sequences of promoters (29).

The protein predicted from the *luxR* sequence is 250 amino acid residues (19, 20, 30). Previous studies of randomly generated point mutations that inactivate *luxR* indicate two regions of the LuxR protein are critical for activity; one region spans residues 79–127 and the other spans residues 184–230. Residues 79–127 are presumed to be involved in autoinducer binding, and residues 184–230 are presumed to be involved in *lux* DNA binding (31, 32). In fact residues 184–230 constitute the helix–turn–helix-containing highly conserved region defining the LuxR family (21).

LuxR mutant proteins with a substitution in one of the N-terminal 78 positions were not obtained by random mutagenesis. Thus we hypothesize that the N-terminal arm of LuxR masks the activity of the presumptive C-terminal DNA-binding region in the absence of autoinducer and that

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Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.

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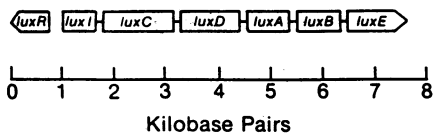

FIG. 1. Organization of the *V. fischeri lux* genes required for autoinducible luminescence in *E. coli*. The *luxICDABE* operon and *luxR* are transcribed divergently.

autoinducer binding to the N-terminal arm allows a C-terminal domain to regulate *lux* transcription. Perhaps many single amino acid substitutions in the N-terminal arm can be tolerated without loss of the proposed masking function. One prediction of our hypothesis is that mutant LuxR proteins that do not contain the N-terminal arm will remain active and in fact exhibit activity in the absence of autoinducer. Thus we describe the construction of *luxR* 5′-deletion mutations that encode polypeptides with N-terminal truncations, and we describe our characterization of those truncated proteins in *E. coli*. Our data are consistent with a model where in the presence of autoinducer an independently folded C-terminal LuxR domain binds DNA but in the absence of autoinducer the N-terminal region inhibits binding of the C-terminal domain to the appropriate *lux* DNA sequences.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Culture Conditions. The *E. coli* strains used were DH5α (33) and JM109 (34). The following plasmids were used: pKK223-3 and pPD723 (Fig. 2), which have been described elsewhere (11); pHK724, which was derived from pPD723 by removal of a 16-bp *Eco*RI–*Kpn* I fragment to position the *luxR* translation initiation codon appropriately with respect to a good Shine–Dalgarno sequence (30); the pSC series plasmids (Fig. 2); pHK555, which contains the complete *lux* gene cluster (Fig. 1) with a Mu dI1681 insertion roughly in the middle of *luxR*; pPD312, which contains the complete *lux* gene cluster with a Mu dI1734 insertion near the 5′ end of the *luxR* coding region (11); pJR551, a pHK555 derivative with a mutation in *luxI* that inactivates this gene (12); and pSH202, which contains the complete *lux* gene cluster without mutation on a *Sal* I fragment subcloned from pJE202 (9). All plasmids except pHK555, pPD312, pJR551, and pSH202 are ColE1 replicons containing ampicillin-resistance markers. pHK555, pPD312, pJR551, and pSH202 are P15A replicons and contain chloramphenicol-resistance markers.

Cultures were grown in L broth or on L agar (35) containing the appropriate antibiotic for plasmid screening or maintenance. Where indicated isopropyl β-D-thiogalactopyranoside (IPTG; final concentration, 1 mM) and autoinducer (final concentration, 0.2 or 2 μM as indicated) were added to cultures (16).

Plasmid Purification and Transformation Procedure. Plasmids were purified by the procedure described by Kraft *et al.* (36). Manipulations of plasmids were performed according to the methods of Sambrook *et al.* (37). The transformation procedure used was that described by Hanahan (33).

DNA Sequencing. The *luxR* 5′-deletion end points in each of the pSC plasmids were identified and the sequence upstream of the *luxR* open reading frame (through the *tac* promoter) was confirmed by DNA sequencing using the chain-termination method (38) as described (32).

Determination of Cellular Luminescence and β-Galactosidase Activity. The light-measuring equipment and standard to calibrate the equipment have been described (39) as have the procedures for measuring luminescence in broth cultures (16, 17). The $CHCl_3$/SDS method described by Miller (40)

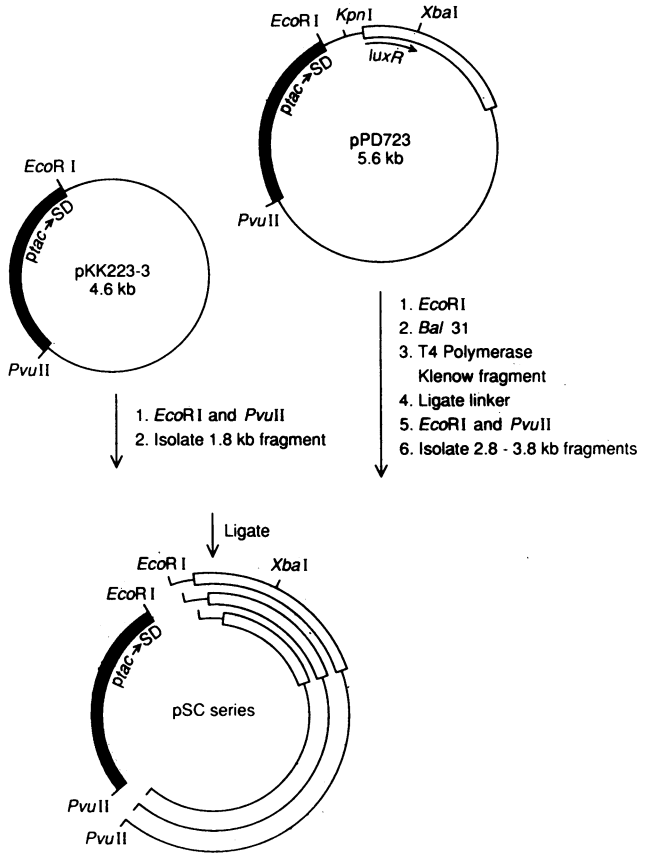

FIG. 2. Construction of *luxR* 5′-deletion plasmids. Plasmids were constructed such that p*tac* and the Shine–Dalgarno sequence from pKK223-3 remained intact. The Shine–Dalgarno sequence was followed by a 6-bp *Eco*RI site, an ATG, and a *luxR* structural gene with a BAL-31-digested 5′ end. This was accomplished using an *Eco*RI linker with an ATG tail (5′-AATTCATG-3′). The *luxR* 5′-deletion plasmids were used to transform *E. coli* DH5α containing pHK555, and the transformants were plated on L agar containing ampicillin, chloramphenicol, IPTG, and autoinducer. Colonies that were luminescent after 2 days at 30°C were picked for further study. Plasmid DNA from each of the luminescent strains was isolated and used to transform *E. coli* DH5α, and ampicillin-resistant chloramphenicol-sensitive transformants, presumed to contain only the *luxR* 5′-deletion mutant plasmids, were obtained. The procedure described above yielded the following plasmids: pSC005, pSC010, and pSC020. Each of these plasmids was used to transform *E. coli* JM109 containing pHK555. Transformants were selected on L agar containing ampicillin, chloramphenicol to maintain pHK555, and IPTG to activate *luxR* transcription from the *tac* promoter. All of the transformants were luminescent. A second series of *luxR* 5′-deletion plasmids was obtained using the procedure described above except that pSC020 was used in place of pPD723. The second series of plasmids included pSC127, pSC130, pSC138, pSC156, pSC162, and pSC182. Finally, pSC058 was constructed by removal of the small *Eco*RI–*Xba* I fragment from pSC020, the ends of the large fragment were filled-in and a blunt-end ligation was performed. The 3.5-kb *Eco*RI–*Pvu* II fragment from the resultant plasmid was isolated and cloned behind the pKK223-3 *tac* promoter. pSC058 should code for a LuxR protein with a 58-amino acid residue N-terminal truncation. The 1.8-kb DNA fragment from pKK223-3, and the 2.8- to 3.8-kb DNA fragments from pPD723 (or pSC020) were isolated after separation by electrophoresis through low-melting-temperature agarose. SD, Shine–Dalgarno sequence; p*tac*, *tac* promoter.

was used to measure β-galactosidase activity. Broth cultures for luminescence and β-galactosidase measurements were grown essentially as described (16). Unless otherwise indicated, luminescence and β-galactosidase activity were measured when cultures reached a density (OD_{660}) of ≈0.5, and growth temperature was 30°C.

RESULTS

Activity of *luxR* 5′-Deletion Products. Because LuxR activity has not yet been demonstrated *in vitro*, we assessed the activity of the truncated *luxR* 5′-deletion products in *E. coli*. Culture luminescence was used as a sensitive index of the ability of a mutant LuxR protein to activate transcription of *luxICDABE* on pHK555. Ten mutant proteins with some degree of activity were studied. The level of activation ranged from 0.01% to 217% of the level of activation by wild-type LuxR, depending on the mutant LuxR protein (Fig. 3). Remarkably, the Δ2–162 protein showed even greater activity than did the wild-type protein. We were unable in several attempts to isolate mutant plasmids encoding active polypeptides smaller than Δ2–182, which showed little activity itself.

The 5′ end of *luxR* including the *luxR* promoter is retained in pHK555 (11, 30). Thus it was possible that the truncated polypeptides encoded by the pSC-series plasmids were forming active multimers with the LuxR N-terminal fragment expected from pHK555; that is the pSC-encoded truncated proteins were not by themselves active. The activities of the pSC-encoded mutant proteins, however, were not appreciably different when pPD312 was used in place of pHK555 (data not shown). In pPD312, Mu dI1734 is inserted within the first 20% of the *luxR* coding region (11). Thus it appears that the N-terminally truncated proteins encoded by the pSC-series plasmids were by themselves active.

Intracellular Levels of Truncated LuxR Proteins. To demonstrate the presence and estimate the level of truncated LuxR proteins in cells containing a *luxR* 5′-deletion plasmid plus pHK555, a Western immunoblot analysis was performed (Fig. 4). When LuxR synthesis was induced with IPTG, the truncated product encoded by each of the pSC-series plasmids except pSC182 could be detected, and the molecular masses of these truncated products were consistent with values calculated from DNA sequence data. As judged from band intensities, levels of the truncated LuxR proteins did not correlate with activity. The Δ2–127 polypeptide and the Δ2–138 polypeptide appeared at roughly the same level as the IPTG-induced wild-type LuxR but showed very little activity. The Δ2–5, Δ2–10, and Δ2–20 polypeptides, which were very active were also detected at roughly the same level as the IPTG-induced pHK724-encoded LuxR. The intensity of the Δ2–58 polypeptide band appeared intermediate between the levels of IPTG-induced and uninduced wild-type LuxR.

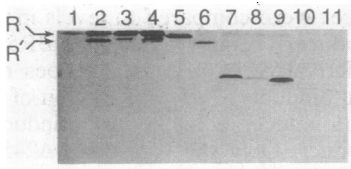

FIG. 4. Western blot analysis of whole-cell extracts of *E. coli* containing pHK555 and pHK724 or pHK555 and a pSC-series plasmid. Cultures were grown as described in Fig. 3. Bands: R, the 28-kDa LuxR polypeptide; R′, a 25-kDa degradation product from LuxR (32) encoded by pHK724. Lanes: 1, pHK555 and pHK724, cells grown without IPTG; 2–11, cells grown in the presence of IPTG; 2, pHK555 and pHK724; 3, pHK555 and pSC005; 4, pHK555 and pSC010; 5, pHK555 and pSC020; 6, pHK555 and pSC058; 7, pHK555 and pSC127; 8, pHK555 and pSC130; 9, pHK555 and pSC138; 10, pHK555 and pSC156; 11, pHK555 and pSC162. SDS/polyacrylamide gel electrophoresis was carried out in a resolving gel that contained 18% polyacrylamide using standard procedures (41, 42). The LuxR antiserum and the Western immunoblot procedures have been described (32, 43); however, blotting was on 0.2-μm (pore size) nitrocellulose filters rather than 0.45-μm (pore size) nitrocellulose filters. Apparent molecular masses of the LuxR mutant proteins were determined using prestained molecular mass protein standards of between 3 and 43 kDa (BRL).

Although the level of LuxR encoded by pHK724 even in uninduced cells is sufficient for full induction of luminescence (30), Δ2–58 showed very little activity. The levels of the Δ2–130, Δ2–156, Δ2–162, and Δ2–182 proteins were less than uninduced levels of the wild-type protein in cells containing pHK724. The Δ2–130 polypeptide showed little activity. However, Δ2–156 and Δ2–162 showed high activity even at low levels. The Δ2–182 polypeptide was not detected on the Western immunoblot. Perhaps this protein showed low activity because it occurred at such low levels in cells (Figs. 3 and 4).

The conclusions that can be drawn from the Western immunoblot analysis are limited for at least two reasons. (*i*) Much of the LuxR polypeptide in IPTG-induced cells containing pHK724 is in the form of inclusion bodies (30), and this may be the case for some or all of the LuxR proteins with N-terminal truncations. (*ii*) During blotting the efficiency of transfer of the various *luxR* deletion products could vary as could their reaction with the LuxR antiserum.

Relationship Between Autoinducer and Induction of Luminescence by *luxR* 5′-Deletion Products. Levels of luminescence in *E. coli* containing pSC plasmids and pJR551 grown

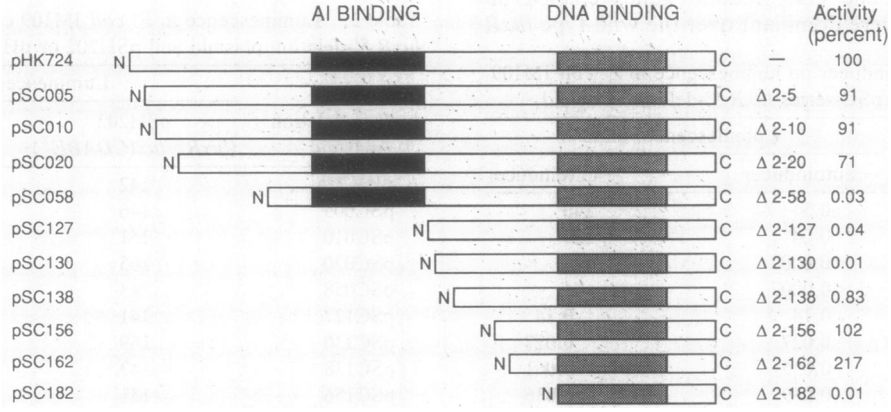

FIG. 3. Schematic representation of the pHK724-encoded LuxR and the pSC-encoded LuxR truncation polypeptides. The pHK724-encoded LuxR is shown on top with the proposed autoinducer-binding region (AI) and the DNA-binding region indicated. The pSC-encoded truncated polypeptides are shown below. The size of the N-terminal truncation follows the representation of each polypeptide. The relative activity of each plasmid-encoded *luxR* product is shown on the right. Activity was assessed by growing *E. coli* containing pHK555 and the indicated *luxR* 5′-deletion plasmid to a culture density of 0.5 (OD_{660}) in the presence of IPTG and autoinducer at 30°C and measuring luminescence. Activity is expressed as the percent of luminescence in a culture of *E. coli* containing pHK555 and pHK724. Luminescence in cells containing pHK555 alone was <0.0001% of the level in cells containing pHK555 and pHK724.

with autoinducer were compared to levels in *E. coli* grown without autoinducer (Table 1). Because pJR551 is a *luxI*$^-$, *luxCDABE*$^+$ derivative of pHK555, it does not direct the synthesis of autoinducer. Thus stimulation of cellular luminescence by autoinducer indicates an autoinducer-dependent *luxR* gene product. Only the wild-type, Δ2–5, Δ2–10, and Δ2–20 polypeptides were autoinducer-dependent, as indicated by a greater activity when autoinducer was added to the growth medium. Apparently, the highly active Δ2–156 and Δ2–162 proteins are autoinducer-independent as are the low-activity deletion proteins.

Influence of *luxR* 5′-Deletion Products on *luxR* Promoter Activity. The *luxR* gene is autoregulated at the level of transcription (11–13) and at a posttranscriptional level (14). It was possible to investigate the role of the LuxR N-terminal arm on transcriptional control of *luxR* by measuring β-galactosidase activity in *E. coli* containing the pSC plasmids and pHK555, which has a *luxR*::*lacZ* transcriptional fusion (Table 2). Our results indicate that the levels of *luxR* promoter activity in *E. coli* containing wild-type LuxR, Δ2–5, or Δ2–10 were about 3-fold lower than the level in the absence of a *luxR* gene. The 3-fold range is about the range of control of *luxR* transcription by autoinducer and LuxR shown elsewhere (11–13). Although some were strong activators of *luxICDABE* transcription, LuxR deletion proteins other than Δ2–5 and Δ2–10 did not significantly reduce *luxR* promoter activity.

Dominance of Wild-Type *luxR* Over *luxR* Deletions Encoding Low-Activity Proteins. LuxR has little activity in the absence of autoinducer and certain of the LuxR deletion proteins have little activity even in the presence of autoinducer (Fig. 3 and Table 1). If LuxR binds DNA and activates transcription, then the low activities could result from a lack of LuxR binding to *lux* DNA or from a lack of transcriptional activation by bound protein. At least for the low activity mutant proteins, we could distinguish between these possibilities. Luminescence was measured in IPTG-grown *E. coli* containing relatively high levels of the truncated LuxR proteins and low levels of wild-type LuxR encoded by pSH202, which contains the complete *V. fischeri lux* gene cluster (Fig. 1). In the presence of saturating levels of autoinducer, *E. coli* containing pSH202 alone or pSH202 plus any of the pSC-series plasmids produced high levels of luminescence (Table 3). Because the Δ2–58 through Δ2–138 and the Δ2–182 LuxR proteins have little activity by themselves, the results indicate that the low-activity mutant proteins do not compete with the wild-type LuxR for the presumptive *luxICDABE* activating sequence. The *luxR* mutant genes carried on pSC156 and pSC182 were dominant over the wild-type *luxR*

Table 1. Effect of autoinducer on luminescence in *E. coli* JM109 containing pJR551 and a pSC-series *luxR* 5′-deletion plasmid

luxR plasmid	Luminescence, units	
	− autoinducer	+ autoinducer
pHK724	0.5	140
pSC005	0.04	150
pSC010	<0.0007	120
pSC020	<0.0007	64
pSC058	0.14	0.14
pSC127	0.027	0.027
pSC130	0.055	0.061
pSC138	0.039	0.036
pSC156	92	97
pSC162	380	320
*	<0.0007	<0.0007

Cultures were grown to a density of 0.5 (OD_{660}) in the presence of IPTG with or without autoinducer (0.2 μM) as indicated. Luminescence units are given as quanta·s^{-1}·ml^{-1}·OD^{-1}·10^{-9}.
E. coli containing only pJR551.

Table 2. β-Galactosidase activity in *E. coli* JM109 containing pHK555 and a pSC-series *luxR* 5′-deletion plasmid

luxR plasmid	β-Galactosidase activity, units
pHK724	8.8 ± 0.7
pSC005	9.2 ± 0.6
pSC010	8.6 ± 0.5
pSC020	25.0 ± 0.8
pSC058	25.7 ± 0.6
pSC127	25.9 ± 0.8
pSC130	26.0 ± 0.3
pSC138	26.2 ± 0.9
pSC156	26.1 ± 0.8
pSC162	26.8 ± 0.6
pSC182	24.9 ± 0.8
*	24.9 ± 0.8

Data are the average of four determinations (± the range of values). Culture conditions were as described in Fig. 3.
E. coli containing only pHK555.

as indicated by the fact that luminescence in *E. coli* containing either of these plasmids plus pSH202 was autoinducer-independent (data not shown).

DISCUSSION

By screening *luxR* 5′-deletion plasmids for the ability to encode mutant LuxR proteins retaining activity in *E. coli*, we have established that the C-terminal 35% of LuxR encoded by pSC162 retains full ability to activate transcription of the *luxICDABE* operon (Fig. 3). This is similar to results with AraC (44) and the LuxR family member FixJ (28). As with AraC (44) and FixJ (28), transcriptional activation by truncated LuxR proteins is inducer independent (Table 1). Based on the results with LuxR and FixJ, we suggest that other members of the LuxR family possess C-terminal domains that can by themselves activate transcription of specific genes in an inducer-independent fashion.

That some mutant LuxR proteins with N-terminal truncations remain active and are autoinducer-independent supports our hypothesis that an N-terminal arm of full-length LuxR serves to mask the activity of the C-terminal transcription factor in the absence of autoinducer and that autoinducer unmasks a functional C-terminal domain within LuxR rather than changing the conformation of the C-terminal region to allow LuxR activation of *luxICDABE* transcription. It is reasonable to suppose that the N-terminal arm is a regulatory

Table 3. Luminescence in *E. coli* JM109 containing a pSC-series *luxR* 5′-deletion plasmid and pSH202 or pHK555

luxR 5′ deletion plasmid	Luminescence, units	
	pSH202 (*luxR*$^+$ *luxICDABE*$^+$)	pHK555 (*luxR*$^-$ *luxICDABE*$^+$)
pHK724	142	116
pSC005	146	125
pSC010	151	112
pSC020	165	140
pSC058	143	0.04
pSC127	141	0.064
pSC130	169	0.021
pSC138	143	0.83
pSC156	151	111
pSC162	284	233
pSC182	163	0.021
*	172	<0.0007

Cultures were grown to a density of 0.5 (OD_{660}) in the presence of IPTG with 2 μM autoinducer. Luminescence units are given as quanta·s^{-1}·ml^{-1}·OD^{-1}·10^{-9}.
E. coli containing only pSH202 or only pHK555 as indicated.

domain that controls the activity of the C-terminal transcription factor in response to autoinducer binding. The extent of the N-terminal masking region or regulatory domain cannot be precisely defined, but we suggest that the low activity exhibited by the Δ2–58, Δ2–127, Δ2–130, and Δ2–138 mutants even in the presence of autoinducer (Fig. 3 and Table 1) results from the existence of a masking region that cannot be appropriately modified by autoinducer. Because the Δ2–156 LuxR is autoinducer-independent but quite active, it appears that the masking region does not extend past residue 156. However, one interpretation of the observation that the Δ2–162 LuxR is more than twice as active as the Δ2–156 protein (Fig. 3) is that residues 157–162 retain some masking activity.

Because the gene encoding full-length LuxR is dominant over those encoding the low-activity Δ2–58, Δ2–127, Δ2–130, and Δ2–138 mutant proteins (Table 3), it appears that with the mutant proteins (and most likely the wild-type protein without autoinducer) the N-terminal regulatory region serves to block binding to the appropriate *lux* DNA sequences. However, the pSC156 and pSC162 *luxR* mutant genes, which encode active autoinducer-independent proteins were dominant over the wild-type gene, conferring an autoinducer-independent phenotype. Because it must be assumed that the highly active LuxR mutant proteins bind DNA, this is the expected result.

Although the Δ2–20, Δ2–156, and Δ2–162 LuxR proteins retained high activity with respect to stimulation of *luxICDABE* transcription, these mutant proteins did not exhibit *luxR* autoregulation (Table 2). Apparently, the N terminus of LuxR has a second function; it is required for autoinducer-dependent autoregulation. Two possible explanations for this (that are not mutually exclusive) are that N-terminal residues between residues 10 and 20 specifically interact with the transcription initiation complex at the *luxR* promoter and that autoregulation involves LuxR–LuxR interactions facilitated by this N-terminal region to allow DNA looping. In any case the data suggest that binding of the C-terminal domain to *lux* DNA is not in itself sufficient for autoregulation of *luxR*, whereas it might be sufficient for activation of *luxICDABE*.

The approach of using *in vitro* random point mutagenesis (32) and *in vitro* DNA resection coupled with *in vivo* LuxR activity screening has allowed us to develop a general view of LuxR structure and function even though specific DNA binding of LuxR *in vitro* has not been demonstrated. There is a C-terminal LuxR domain capable of functioning as a transcriptional activator in an independent fashion. Within this domain the region between residues 184 and 230 appears to be involved in *lux*–DNA binding (32). Autoinducer binding is believed to involve residues in the region between positions 79 and 127 (31, 32). An N-terminal region of LuxR starting between residues 20 and 58 and extending to the region of residues 138–162 contains the autoinducer binding site and serves to mask the activator function of an independently folded C-terminal domain. Residues between positions 10 and 20 are required for autoregulatory function as described above. The dominance experiments with wild-type and mutant *luxR* genes (Table 3) suggest that it is the binding of LuxR to *lux* DNA that is masked by the N-terminal region.

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