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The alginate regulator AlgR and an associated sensor FimS are required for twitching motility in *Pseudomonas aeruginosa*

(twitching motility/fimbriae/pili/cystic fibrosis/pathogenesis)

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ABSTRACT **Mucoid strains of *Pseudomonas aeruginosa* isolated from the lungs of cystic fibrosis patients produce large amounts of the exopolysaccharide alginate. AlgR has long been considered a key regulator of alginate production, but its cognate sensor has not been identified. Here we show that AlgR is required for twitching motility, which is a form of bacterial surface translocation mediated by type 4 fimbriae. Adjacent to *algR* we have identified a sensor gene (*fimS*), which is also required for twitching motility. However, FimS does not appear to be required for alginate production in mucoid strains. FimS and AlgR are representative of a new subclass of two-component transmitter–receiver regulatory systems. The alternative sigma factor AlgU also affects both alginate production and twitching motility. Therefore, these two virulence determinants appear to be closely associated and coordinately regulated.**

Pseudomonas aeruginosa is an opportunistic pathogen of humans and other species (1). It causes severe chronic infections in patients who are immunocompromised as a result of cancer chemotherapy or AIDS, or who are suffering from burns or cystic fibrosis. It produces an extensive suite of virulence factors including extracellular proteases and toxins, lipases, pyochelins, exopolysaccharides, and type 4 fimbriae. In cystic fibrosis, *P. aeruginosa* produces recurrent and chronic lung infections, which impose substantial morbidity and often mortality as a consequence of accumulated damage to the lung. Isolates from such chronic infections exhibit a "mucoid" colony phenotype due to the production of large amounts of the exopolysaccharide alginate, apparently as a means of evading immune responses (for reviews see refs. 2 and 3).

Transcriptional regulation of the alginate biosynthetic genes involves a variety of components whose interrelationships are not entirely clear. They include the response regulators AlgR and AlgB; the alternative sigma factor AlgU (or AlgT), which is required for the transcription of alginate biosynthetic genes; the anti-sigma factors MucA and AlgN (or MucB), which counteract the function of AlgU; the histone-like protein AlgP; and AlgQ, the exact function of which is unclear (2, 3). AlgR and AlgB have been the subject of considerable study and appear to represent the receiver components of classical sensor-regulator pairs, but their cognate sensors have not been identified.

We have been examining the molecular genetics of the biogenesis and function of type 4 fimbriae in *P. aeruginosa* (4, 5). Type 4 fimbriae are polar filaments produced by a wide range of pathogenic bacteria, including *Neisseria gonorrhoeae*, *Neisseria menigitidis*, *Dichelobacter nodosus*, various *Moraxella* species, and *Vibrio cholerae* (for review, see ref. 6). These filaments mediate adhesion to epithelial cells (7) and surface translocation via a phenomenon termed twitching motility, which is probably their primary function. The mechanical basis of this process is unknown, but is thought to involve fimbrial extension and retraction (8). Loss of fimbriae and/or twitching motility renders the cells avirulent (6, 9). Type 4 fimbriae are assembled by a system closely related to those involved in the formation of cell surface complexes for protein secretion and DNA uptake in a wide variety of bacteria (10, 11), and are composed of a major structural subunit (PilA) whose transcription in *P. aeruginosa* is tightly controlled by a classical RpoN(σ^{54})-dependent two-component sensor-regulator pair, PilS and PilR (4). Here we show that another sensor-regulator pair, AlgR and FimS, is also required for fimbrial function.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *Escherichia coli* strain DH5α (*hsdR recA lacZYA* ϕ*80 lacZ*ΔM15) was used for standard DNA manipulations and preparation of sequencing templates. The *P. aeruginosa* strains used were wild-type PAK (David Bradley, Memorial University of Newfoundland) and *Tn*5-B21 (12) mutants of this strain. Construction of these strains has been described (4). Transformation of *P. aeruginosa* strains was performed as described (13). Antibiotics were used at the following concentrations: *E. coli*, ampicillin (50 μg/ml)/tetracycline (40 μg/ml); *P. aeruginosa*, carbenicillin (500 μg/ml)/tetracycline (200 μg/ml).

DNA Manipulations. Preparation of plasmid DNA, restriction endonuclease digestions, Southern blotting, ligation reactions, and transformation of *E. coli* were carried out using standard procedures (14). *algR* was cloned as an 895-bp *Cla*I–*Sma*I fragment into the broad-host range shuttle vector pUCPKS (15). *fimS* was cloned as a 2.3-kb *Eco*RI fragment into pUCPSK (15). *algU* was cloned by PCR amplification from *P. aeruginosa* PAO1 with amplimers designed against the sequence of *algU* (GenBank accession no. L04794) with flanking 5′ *Bam*HI and 3′ *Xba*I restriction sites and cloned into the corresponding sites of the *P. aeruginosa–E. coli* shuttle vector, pUCPKS. The integrity of the cloned gene was checked by sequencing. DNA sequence analysis was performed using the dideoxy chain termination Applied Biosystems PRISM kit on a 373A automated sequencer. The sequences were completed on both strands.

Assays. ELISA and Western blot analysis were performed as described (16). Twitching motility assays were performed as described using stab inoculation followed by overnight growth and Coomassie stain of the zone of movement at the agar–plastic interface (17). Alginate and total cell protein were assayed as described by May and Chakrabarty (18). Alginate was isolated as described by May and Chakrabarty (18) from a lawn of cells generated by plating 100 μl of an overnight broth culture onto standard 85-mm plates containing 15 ml of agar and grown for 24 hr at 37°C. The media used for alginate assays was *Pseudomonas* isolation agar (PIA, Difco) containing 1%

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Data deposition: The sequence reported in this paper has been deposited in the GenBank data base [accession no. L48729 (*fimS*)].
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glycerol (vol/vol), which is normally used for assaying alginate production by mucoid *P. aeruginosa* strains (18).

RESULTS AND DISCUSSION

The Alginate Regulator AlgR and Its Related Sensor FimS Affect Twitching Motility. We have previously constructed a library of transposon mutants that affect twitching motility in *P. aeruginosa* as determined by altered surface colony morphology (4). These mutants were grouped according to whether they had also acquired resistance to fimbrial-specific bacteriophage and according to the size of the chromosomal restriction fragment into which the transposon had inserted (4). The corresponding loci have since been progressively cloned and sequenced to identify the affected genes. In this way, a large number of genes affecting fimbrial biogenesis or function have been identified and at least 25 genes at several loci are now known to be involved (4, 5).

This study describes the characterization of four phage-sensitive mutants in the library that mapped to a new locus. The mutants S31, S39, S47, and S62 containing the transposon *Tn*5-B21 (12) were grouped to the same *Kpn*I *P. aeruginosa* PAK genomic DNA fragment by Southern blotting using the transposon as a probe. DNA flanking the site of transposon insertion in each mutant was cloned by digestion with *Eco*RI (which cuts the transposon once beyond the tetracycline resistance marker), ligation into pBluescript II (Stratagene), and recovery of tetracycline-resistant *E. coli* colonies. The DNA adjacent to the transposon was then sequenced using the primer "Ollie" (4) and in two cases (S31 and S39) corresponded to the published *algR* sequence (GenBank accession no. M23230) (Fig. 1). Screening a reference PAO1 cosmid library (kindly supplied by B. Holloway, Monash University, Melbourne) with DNA flanking the transposon from S62 enabled isolation of the wild-type locus. Southern blots using the cloned flanking DNA from S39 and S62 showed that these transposons had inserted into the same 2.3-kb *Eco*RI fragment (Fig. 1). This fragment was cloned on the broad host-range plasmid pUCPSK (15) and shown to restore twitching motility to S47 and S62, but not to S31 or S39. This fragment was then sequenced and shown to represent the previously uncharacterized region between *algR* and *argH* (19) (Fig. 1). Sequence analysis of this region identified a gene, which we have designated *fimS* (GenBank accession no. L48729), the product of which shares homology to LytS, a sensor or transmitter that regulates autolysis (murein hydrolase production) in *Staphylococcus aureus* (21) and YehU, a hypothetical protein from *E. coli* (GenBank accession no. U00007) (Fig. 2*a*). Both the *lytS* and *yehU* genes are directly followed by genes (*lytR* and *yehT*, respectively) encoding putative receivers that have overall homology to AlgR (Fig. 2*b*) and that share the same tandem genetic arrangement as *fimS* and *algR*.

AlgR and FimS Are Members of a New Family of Transmitter-Receiver Response Regulators. All response regulators or receivers share a common N-terminal domain showing homology to CheY and include a conserved lysine and three conserved aspartate residues, the third of which is phosphorylated by a cognate sensor or transmitter. (For reviews of the structures of proteins involved in signal transduction by modular phosphorylation in sensor-regulator or receiver-transmitter pairs, see refs. 24–27.) These receivers differ significantly in their C termini and can be subgrouped according to similarities of this region. AlgR clearly has a receiver domain, but shows significant differences from other well-characterized receivers in the remainder of the protein (24, 27). It is however homologous to the recently-described LytR and YehT across their entire sequence, suggesting that these proteins may constitute a new class of response regulators (Fig. 2*b*). Similarly, FimS, LytS, and YehU are also closely related, but differ significantly from other histidine kinases and appear to form a new family of sensors or transmitters (Fig. 2*a*). Together, these protein pairs form a novel subclass of the sensor-regulator or transmitter-receiver superfamily.

There are several differences between FimS, LytS, and YehU and other known types of transmitters such as NtrB and CheA (24–26). First, FimS is similar to LytS and YehU, but differs from known transmitters in the region around the putative histidine phosphorylation site (Fig. 2*a*). It has been proposed that the local structure around the critical histidine in such transmitters can influence the ratio of phosphorylation–dephosphorylation exchange between the histidine and the aspartate in the receiver (24–26). Second, FimS lacks the DXGXG and GXG motifs in region III (Fig. 2*a*), which are thought to comprise nucleotide binding domains (24–26), suggesting that FimS may be incapable of autophosphorylation. YehU lacks the first site, whereas LytS retains both. It is possible that proteins such as LytS may be capable of both autophosphorylation and of blocking or reversing the phosphorylation of its partner(s). The conserved histidine on these proteins may either be able to accept phosphate groups from another transmitter (in which case these proteins may function as an intermediate receiver–transmitter) or act as an acceptor of phosphates from the conserved aspartate in their receiver–partners (24–26). In any case the lack of nucleotide binding domains in FimS suggests that it may operate by an unusual mechanism.

The Role of FimS, AlgR, and AlgU in Alginate Production and Twitching Motility. AlgR and FimS appear to be required for twitching motility in *P. aeruginosa* (Fig. 3). Mutations in *algR* and *fimS* result in either complete loss or severe retardation of twitching motility (Fig. 3 *e* and *i*). Both types of mutants essentially lack surface fimbriae as shown by ELISA (Fig. 4), which is a sensitive quantitative assay, but produce normal amounts of the fimbrial subunit PilA as determined by whole cell Western blots with anti-PilA antiserum (data not shown). Thus AlgR and FimS are not required for *pilA* transcription. These mutants can be restored to essentially wild-type levels of twitching motility by complementation with

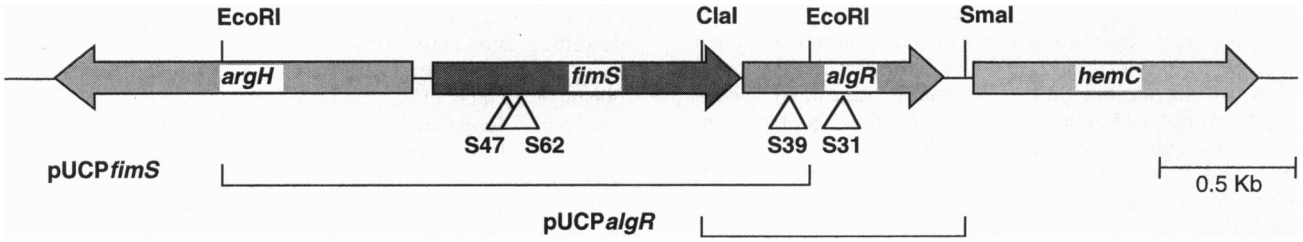

FIG. 1. Map of the *fimS–algR* locus and site of transposon insertions. The sites of transposon *Tn*5-B21 (12) insertion in the mutants S31, S39, S47, and S62 were mapped as described in the text. The 2.3-kb *Eco*RI fragment containing the sites of transposon insertion of S47 and S62 was sequenced and shown to represent the previously uncharacterized region between *algR* and *argH* (19), which encodes arginosuccinate lyase. It has been shown that downstream of *algR* is *hemC*, which encodes porphobilinogen deaminase (20). Triangles indicate the sites of transposon insertion in the different mutants.

FIG. 2. Comparison of the predicted FimS and AlgR sequences with other known transmitters and receivers. Sequence homologies were identified using the BLAST network service at the National Center for Biotechnology Information (22). Proteins were aligned using the CLUSTALV program (23). (*a*) Multiple alignment of the new class of sensor molecules FimS, YehU, and LytS. Identical residues between at least two members are boxed and the conserved His and Asn residues are shaded. The region III motifs of DXGXG and GXG are also shaded. (*b*) Multiple alignment of regulator proteins AlgR, YehT, LytR, NtrC, and CheY [GenBank accession nos. M19277 (NtrC) and K02175 (CheY)]. The identical residues between at least two members are boxed and the critical Lys and Asp residues are shaded. A second putative phosphate-accepting Asp residue is indicated in boldface type. Only the conserved N-terminal domain of NtrC is included, as the remainder of the protein possesses little similarity to this class of regulators.

their respective genes in trans (Fig. 3 *f* and *k*). The level of twitching motility in *algR*-complemented cells is somewhat reduced, but this is also observed in the wild type (Fig. 3*b*), suggesting an inhibitory effect of AlgR overproduction from the multicopy plasmid. This effect has been observed previously in relation to alginate production (28, 29). We have also observed that overproduction of the response regulator PilR, which is required for transcription of the fimbrial subunit gene *pilA*, can substantially overcome the effects of mutations in its cognate sensor PilS (4). Similarly, overexpression of *algR* can overcome *fimS* mutations (Fig. 3*j*), thereby strengthening the conclusion from their related phenotypes and chromosomal juxtaposition that FimS is a sensor or transmitter that interacts with AlgR.

The mucoid colony phenotype associated with high levels of alginate production is observed in fresh clinical isolates of *P. aeruginosa* from cystic fibrosis patients, but this characteristic is unstable in culture (2, 3). As a consequence, studies on the regulation of alginate production have been carried out largely in variants selected for the stable expression of mucoidy (3, 19, 30, 32). Such variants have been subsequently shown to contain mutations in the anti-sigma factors *algN* or *mucA* (which suppress the sigma factor AlgU) and thus result in up-regulation of AlgU activity (2, 3, 33). Mucoidy can also be induced by increasing *algU* gene dosage by transformation with a multicopy plasmid containing this gene (33), presumably due to increased levels of AlgU. We therefore reproduced the mucoid phenotype in our *P. aeruginosa* strains by transformation with the multicopy vector pUCPKS containing *algU* (pUCP*algU*). Under these conditions we confirmed that the *algR* mutant S39 is severely reduced in alginate production (Table 1), in agreement with previous studies in mucoid *P.*

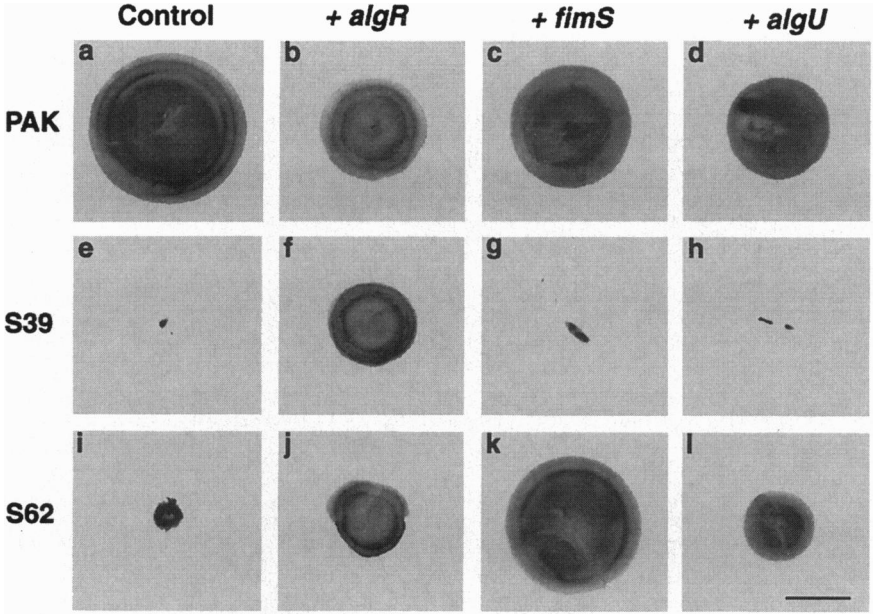

FIG. 3. Subsurface twitching motility assays were performed on *P. aeruginosa* PAK (*a–d*), *P. aeruginosa* S39 (*algR*) (*e–h*), and *P. aeruginosa* S62 (*fimS*) (*i–l*) carrying plasmids pUCPKS (*a*, *e*, and *i*), pUCP*algR* (*b*, *f*, and *j*), *pUCPfimS* (*c*, *g*, and *k*), *or pUCPalgU* (*d*, *h*, and *l*).

aeruginosa strains (19, 32, 34). However, under these conditions, the *fimS* mutant S62 still produces large amounts of alginate similar to the control (Table 1). This does not discount the possibility that FimS may have a role in regulating alginate production under other circumstances.

Overproduction of AlgU from pUCP*algU* restores twitching motility to the *fimS* mutant (Fig. 3*l*) suggesting that the lack of FimS can be overcome by increasing the level of AlgU. This may be due to a direct effect of upregulating transcription from the relevant promoters thereby bypassing the normal environmental signals. Because of this, it is unlikely that the effect of FimS on twitching motility would have been discovered in stable mucoid strains, which have been selected or constructed *in vitro* to overproduce AlgU. On the other hand *algR* mutations cannot be overcome by AlgU overproduction (Fig. 3*h*), indicating that AlgR and AlgU are both required for transcription of the genes involved.

FimS and AlgR appear to have different effects on twitching motility and alginate production, although in other respects they appear to constitute a receiver–transmitter pair. One explanation for this dichotomy is that there are two forms of AlgR, which (in combination with AlgU) recognize different promoters. It is possible that AlgR contains more than one phosphorylation site, one of which is recognized by FimS and the other of which receives independent signals. It is worth noting here that AlgR shares a second aspartate in a conserved domain with LytR and YehT (Fig. 2*b*). AlgR has been reported to be capable of being phosphorylated by both histidine kinases, such as CheA, and by small molecular weight phosphate donors, such as carbamoyl phosphate and acetyl phosphate (35). Alternatively, it is possible that FimS blocks or reverses AlgR phosphorylation. The latter is consistent with the apparent lack of crucial residues required for autophosphorylation activity in FimS.

The mechanism by which AlgR and FimS might regulate twitching motility is unclear at this point. AlgR is thought to be a transcription factor (2, 3), although it has an unusual structure (27). It does not affect the expression of the fimbrial subunit gene, *pilA* (data not shown), but there are more than 30 gene products involved in fimbrial assembly and function (16, 17), one or more of which may require AlgR for expression. Alternatively, AlgR may participate in phosphotransfer interactions with components of the fimbrial system.

An alternative explanation for our observations is that FimS may be a bona fide part of the signal transduction pathway that regulates alginate production in wild-type cells, but that its function is masked by the upregulation of *algU* in mucoid strains, wherein alginate production is normally studied and measured. This occurs with twitching motility (Fig. 3*l*). Because it is difficult to measure alginate production in wild-type

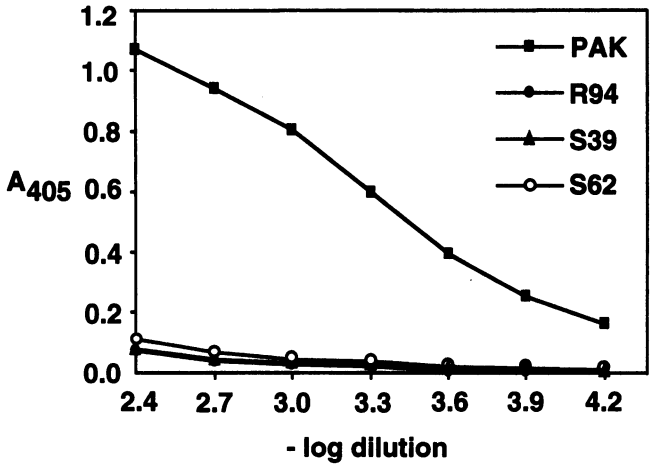

FIG. 4. ELISA against whole cells of the following *Pseudomonas* strains: PAK (■); R94 (●); S39 (▲); S62 (○) using anti-fimbrial antiserum. Bovine serum albumin was used as the antigen control.

Table 1. Alginate production by *P. aeruginosa* PAK, *algR*, and *fimS* mutants containing pUCP*algU* confluently grown for 24 hr on a standard 85-mm *Pseudomonas* isolation agar (Difco) plate containing 1% glycerol (vol/vol)

Strain (+pUCP*algU*)	Mutation	Alginate conc., mg/mg cell protein	% PAK
PAK	—	0.93	100
S39	*algR*	ND	—
S62	*fimS*	0.99	103

Assays were performed in duplicate. ND, not detectable; conc., concentration.

strains under standard media and assay conditions, this issue remains to be resolved. However, if FimS is in fact required for alginate production in the wild type, it is then also possible that the effect of FimS (and AlgR) on twitching motility may be a consequence of a direct link between alginate production and twitching motility. In this respect, it is worth noting that twitching motility appears to be closely related to gliding motility in *Myxococcus xanthus*, which is known to be dependent on the production of extracellular "slime" comprised of complex polysaccharides and proteins (36, 37).

Conclusion. This report shows that the alginate regulator AlgR and an associated sensor FimS are required for twitching motility in *P. aeruginosa*. Although *algR* and *fimS* mutations appear to produce different phenotypes, it seems that they are a genuine transmitter–receiver pair, which are likely to interact with each other, based on the following evidence: (*i*) the *algR* and *fimS* genes are juxtaposed on the chromosome, as is common with other sensor–regulator pairs; (*ii*) AlgR and FimS each show particular sequence characteristics that have only been found in other related sensor–regulator pairs, LytR/LytS and YehU/YehT, the former of which at least is known to function as a cognate pair *in vivo* (21); (*iii*) both AlgR and FimS affect twitching motility; and (*iv*) *algR* overexpression overcomes mutations in *fimS*, indicating that they are on the same pathway, as has been observed for other sensor–regulator pairs (4).

The discovery of a linkage between alginate production and twitching motility is unexpected and has considerable implications. Understanding the details of this linkage will provide further insight into the process of infection by *P. aeruginosa* of patients suffering cystic fibrosis and other diseases.

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1. Fick, R. (1993) *Pseudomonas aeruginosa the Opportunist: Pathogenesis and Disease* (CRC, Boca Raton, FL).
2. Zielinski, N. A., Roychoudhury, S. & Chakrabarty, A. M. (1994) *Methods Enzymol.* **235,** 493–502.
3. Deretic, V., Schurr, M. J., Boucher, J. C. & Martin, D. W. (1994) *J. Bacteriol.* **176,** 2773–2780.
4. Hobbs, M., Collie, E. S. R., Free, P. D., Livingston, S. P. & Mattick, J. S. (1993) *Mol. Microbiol.* **7,** 669–682.
5. Alm, R. A., Bodero, A. J., Free, P. & Mattick, J. S. (1996) *J. Bacteriol.* **178,** 46–53.
6. Tennent, J. M. & Mattick, J. S. (1994) in *Fimbriae: Aspects of Adhesion, Genetics, Biogenesis and Vaccines*, ed. Klemm, P. (CRC, Boca Raton, FL), pp. 127–146.
7. Doig, P., Todd, T., Sastry, P. A., Lee, K. K., Hodges, R. S., Paranchych, W. & Irvin, R. T. (1988) *Infect. Immun.* **56,** 1641–1646.
8. Bradley, D. E. (1980) *Can. J. Microbiol.* **26,** 146–154.
9. Hazlett, L. D., Moon, M. M., Singh, A., Berk, R. S. & Rudner, X. L. (1991) *Curr. Eye Res.* **10,** 351–362.
10. Hobbs, M. & Mattick, J. S. (1993) *Mol. Microbiol.* **10,** 233–243.
11. Mattick, J. S. & Alm, R. A. (1995) *Trends Microbiol.* **3,** 411–414.
12. Simon, R., Quandt, J. & Klipp, W. (1989) *Gene* **80,** 161–169.
13. Mattick, J. S., Bills, M. M., Anderson, B. J., Dalrymple, B., Mott, M. R. & Egerton, J. R. (1987) *J. Bacteriol.* **169,** 33–41.
14. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Watson, A. A., Alm, R. A. & Mattick, J. S. (1996) *Gene* **172,** 163–164.
16. Watson, A. A., Mattick, J. S. & Alm, R. A. (1996) *Gene*, in press.
17. Alm, R. A. & Mattick, J. S. (1995) *Mol. Microbiol.* **16,** 485–496.
18. May, T. B. & Chakrabarty, A. M. (1994) *Methods Enzymol.* **235,** 295–304.
19. Mohr, C. D. & Deretic, V. (1990) *J. Bacteriol.* **172,** 6252–6260.
20. Mohr, C. D., Sonsteby, S. K. & Deretic, V. (1994) *Mol. Gen. Genet.* **242,** 177–184.
21. Brunskill, E. W. & Bayles, K. W. (1996) *J. Bacteriol.* **178,** 611–618.
22. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
23. Higgins, D. G. & Sharp, P. M. (1989) *Comput. Appl. Biol. Sci.* **5,** 151–153.
24. Parkinson, J. S. & Kofoid, E. C. (1992) *Annu. Rev. Genet.* **26,** 71–112.
25. Parkinson, J. S. (1993) *Cell* **73,** 857–871.
26. Swanson, R. V., Alex, L. A. & Simon, M. I. (1994) *Trends Biochem. Sci.* **19,** 485–490.
27. Pao, G. M. & Saier, M. H., Jr. (1995) *J. Mol. Evol.* **40,** 136–154.
28. Kimbara, K. & Chakrabarty, A. M. (1989) *Biochem. Biophys. Res. Commun.* **164,** 601–608.
29. Deretic, V. & Konyecsni, W. M. (1989) *J. Bacteriol.* **171,** 3680–3688.
30. Fyfe, J. A. M. & Govan, J. R. W. (1980) *J. Gen. Microbiol.* **119,** 443–450.
31. Darzins, A. & Chakrabarty, A. M. (1984) *J. Bacteriol.* **159,** 9–18.
32. Deretic, V., Dikshit, R., Konyecsni, W. M., Chakrabarty, A. M. & Misra, T. K. (1989) *J. Bacteriol.* **171,** 1278–1283.
33. Goldberg, J. B., Gorman, W. L., Flynn, J. L. & Ohman, D. E. (1993) *J. Bacteriol.* **175,** 1303–1308.
34. Mohr, C. D., Martin, D. W., Konyecsni, W. M., Govan, J. R. W., Lory, S. & Deretic, V. (1990) *J. Bacteriol.* **172,** 6576–6580.
35. Deretic, V., Leveau, J. H. J., Mohr, C. D. & Hibler, N. S. (1992) *Mol. Microbiol.* **6,** 2761–2767.
36. Wu, S. S. & Kaiser, D. (1995) *Mol. Microbiol.* **18,** 547–558.
37. Shimkets, L. J. (1990) *Microbiol. Rev.* **54,** 473–501.