Intracellular Ca\textsuperscript{2+} Mobilization and Kinase Activity during Acylated Homoserine Lactone-dependent Quorum Sensing in Serratia liquefaciens

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Quorum sensing in Gram-negative bacteria involves acylated homoserine lactones (AHLs) and a transcription factor, activated by the AHLs. In this study, a possible involvement of intracellular Ca\textsuperscript{2+} as second messenger and/or protein kinase activity during signal transduction is analyzed. When N-hexanoyl-L-homoserine lactone was added to a suspension of Fura-2-loaded Serratia liquefaciens, there was a decline in [Ca\textsuperscript{2+}], measured as a decrease in the Fura-2 fluorescence ratio. As controls, the addition of the signal molecule N-3-oxohexanoyl-L-homoserine lactone, which is not produced by S. liquefaciens, did not induce changes in [Ca\textsuperscript{2+}]. Using a protein kinase activity assay on AHL-stimulated cells, an increase in kinase activity after N-butanoyl-L-homoserine lactone stimulation of S. liquefaciens cells was detected, whereas the kinase activity induced by N-3-oxohexanoyl-L-homoserine lactone was not statistically significant. The conclusion from this study is that changes in [Ca\textsuperscript{2+}], are involved in quorum sensing signal transduction in the Gram-negative bacteria S. liquefaciens. We also conclude that kinase activity is induced in S. liquefaciens upon AHL stimulation. We suggest that the transient intracellular [Ca\textsuperscript{2+}] changes and kinase activity, activated by the AHL signal, are critical for the quorum-sensing signal transduction.

Many species of Gram-negative bacteria use quorum sensing to regulate important physiological functions, e.g. expression of virulence factors and biofilm formation. Transcription of specific sets of genes is activated at a certain cell density, which the bacteria sense as concentrations of small diffusible signal molecules, produced by the bacteria. Quorum sensing in Gram-negative bacteria involves acylated homoserine lactones (AHLs) as signal molecules, AHL-synthesizing protein, (LuxI homologue), and the transcriptional activator protein (LuxR homologue) (1, 2). It is proposed that the AHL molecule binds to the LuxR, allowing it to function as a transcription activator, and most data are consistent with this hypothesis, although most data may also be explained by a signal transduction mechanism, an indirect effect of AHLs, involving other factors beside the LuxR homologues and the AHL signal molecules. LuxR, the Vibrio fischeri luminescence (lux) gene activator, is the best studied member of the LuxR family of bacterial transcription regulators required for cell density-dependent gene expression. LuxR homologues occur in a number of different Gram-negative bacteria (e.g. TraR in Agrobacterium tumefaciens and LasR and RhlR in Pseudomonas aeruginosa) regulating Ti-plasmid transfer and production of virulence determinants, respectively. Direct interaction between LuxR and its most studied AHL signal, N-3-oxohexanoyl-L-homoserine lactone (OHHL), has not been clearly demonstrated in vitro (1–5). However, recently Zhu and Winans (6) purified TraR from A. tumefaciens, after overexpression in Escherichia coli, as a complex with OHHL, confirming a direct interaction between a LuxR homologue and its AHL signal molecule. Interestingly, Bassler et al. (7, 8) have found, in the Vibrio harveyi luminescence quorum sensing system, a potential AHL binding receptor LuxN, necessary for luminescence, with a membrane-spanning domain. They suggest a two-component signal transduction; i.e. the signal binds to a receptor, which then transfers information (e.g. by a phosphorylation step), leading to transcription. Also, Sitnikov et al. (3) hypothesized involvement of another factor beside the LuxR homologue, which may be species-specific in its AHL response in one strain of bacteria and more unspecific in another strain.

Protein kinases play a key role in signal transduction pathways in both eukaryotic and prokaryotic cells (9, 10). Protein kinase C (PKC) is a heterogeneous group of serine/threonine protein kinases often involved in cellular responses to hormones and neurotransmitters in eukaryotes. Recently, several studies have reported on discoveries of serine/threonine protein kinases also in prokaryotes (10, 11). Protein kinase C requires membrane binding for maximal activity, and this binding requires Ca\textsuperscript{2+}. Ca\textsuperscript{2+} decreases the amount of negatively charged phospholipids, thereby increasing PKC membrane binding (12, 13), activity (14, 15), and regulation (16).

In eukaryotic cells, intracellular Ca\textsuperscript{2+} is thoroughly studied. The level of [Ca\textsuperscript{2+}] is tightly controlled, and changes in [Ca\textsuperscript{2+}] are known to regulate a variety of processes such as secretion, cell cycle transition, fertilization, and chemotaxis. In prokaryotes, however, the role for [Ca\textsuperscript{2+}], is not well studied. As more data continuously are being revealed, it has become evident that many Ca\textsuperscript{2+}-controlled processes in eukaryotic cells have parallels in prokaryotes. As for eukaryotic cells, the [Ca\textsuperscript{2+}], is in bacteria lies in the submicromolar range and is regulated by cell membrane- and organelle-associated Ca\textsuperscript{2+} channels and pumps (9, 17, 18). In bacteria, the expression of certain genes...
has been demonstrated to be, at least in part, both extra- and intracellular Ca²⁺-dependent. Extracellular Ca²⁺ can act as an environmental signal, sensed by outer membrane receptors possibly influencing gene transcription (17). Sensitivity to low Ca²⁺ concentrations is suggested to involve a high affinity sensor protein, having either an extra- or intracellular localization (17). Intracellular Ca²⁺ as a second messenger has been studied in bacterial chemotaxis, and it has been shown for E. coli that stimulation with chemotactic attractants and repellants induce changes in [Ca²⁺] (19, 20).

In the present study, involvement of factors, such as changes in intracellular calcium concentration and protein phosphorylation, during AHL activation of LuxR homologues in quorum sensing was investigated. As models for AHL-induced quorum sensing, the Gram-negative bacteria Serratia liquefaciens and V. fischeri were used.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The potassium salt of Fura-2 was obtained from Molecular Probes, Inc. (Leiden, The Netherlands). Stock solutions of Fura-2 (5 mM) in water were prepared, and light exposure during cell loading and fluorescence measurements with a Radioactivity counter was kept to a minimum. OHHL was purchased from Sigma. N-Hexanoyl homoserine lactone (HHL) was purchased from Quorum Science Inc. (Corvalle, IA). N-Butanoyl homoserine lactone (BHL) was a generous gift from Prof. P. Williams (School of Pharmaceutical Sciences/Institute of Infections and Immunity, University of Nottingham, United Kingdom). Stock solutions, 2.0 mM in phosphate-buffered saline (PBS), of the AHLs were prepared 30 min prior to the experiments. Biotrak™ PKC assay system (RPN 77) and [γ-32P]ATP were obtained from Amersham Pharmacia Biotech.

**Bacterial Strains and Culture Procedures**—As a model for quorum sensing, a mutant S. liquefaciens strain, SwrI, from wild type strain MG44 was used. This mutant strain does not synthesize BHL and HHL, two AHLs known to be produced by wild type S. liquefaciens (21). In addition, a S. liquefaciens strain, MJ-215, was used. This strain is unable to produce OHHL and HHL, two of the known AHLs produced by V. fischeri (22). As a non-AHL-producing or -responding Gram-negative control bacteria, we used the E. coli strain MG1655. Cultures for experiments were grown in LB media, pH 7.5, at room temperature (18). Cultures were grown in a double beam luminescence spectrometer at 340- and 380-nm excitation and 510-nm emission wavelengths. For every measurement, 200 µl of the Fura-2-loaded cell suspension was added to a quartz cuvette filled with 3 ml of Ca²⁺ buffer. The cell suspension was allowed to equilibrate for 10 min before measurement. All additions to the cuvette had a volume of 30 µl, corresponding to 1% of the total cuvette volume. Typically, fluorescence intensity data were collected every 1.9 s for 4 min, and the ratio of light emission at the two excitation wavelengths (340 and 380 nm) were recorded. The addition of PBS as control and the addition of AHL in PBS were performed after 120 and 180 s, respectively. In the Fura-2 ratio fluorescence images (see below), fluctuations in fluorescence intensity sometimes were seen for 30 s after the addition of AHL. Therefore, the average fluorescence ratio value during 60 s following the addition of AHL or PBS was calculated in each sample. AHLs were also added to Fura-2 loaded E. coli cells (strain MG1655) to study possible activation of a Gram-negative bacteria without this BHL- and HHL-dependent quorum sensing system. Results are presented as the mean value ± S.E. (n = 8) for each AHL.

**Fura-2 Ratio Fluorescence Imaging**—Images of intracellular Ca²⁺ transients were monitored using Fura-2-loaded cells. The recordings were performed as described previously (17). In brief, 100 µl of cell suspension—preconditioned at room temperature for 15 min. After three washes in the same medium, the cell pellet was resuspended in 2 ml of Ca²⁺ buffer (120 mM NaCl, 20 mM Hepes-Tris (pH 7.2), 10 mM glucose, 10 mM CaCl₂, 4.7 mM KCl, 1.2 mM KH₂PO₄, and 1.2 mM MgSO₄) and kept at room temperature until use, within 2 h.

**Fura-2 Ratio Fluorescence Spectrophotometry**—Intracellular calcium measurements were performed in a double beam luminescence spectrometer at 340- and 380-nm excitation and 510-nm emission wavelengths. Intracellular calcium was measured at 20 °C with slow magnetic stirring. For every measurement, 200 µl of the Fura-2-loaded cell suspension was added to a quartz cuvette filled with 3 ml of Ca²⁺ buffer. The cell suspension was allowed to equilibrate for 10 min before measurement. All additions to the cuvette had a volume of 30 µl, corresponding to 1% of the total cuvette volume. Typically, fluorescence intensity data were collected every 1.9 s for 4 min, and the ratio of light emission at the two excitation wavelengths (340 and 380 nm) were recorded. The addition of PBS as control and the addition of AHL in PBS were performed after 120 and 180 s, respectively. In the Fura-2 ratio fluorescence images (see below), fluctuations in fluorescence intensity sometimes were seen for 30 s after the addition of AHL. Therefore, the average fluorescence ratio value during 60 s following the addition of AHL or PBS was calculated in each sample. AHLs were also added to Fura-2 loaded E. coli cells (strain MG1655) to study possible activation of a Gram-negative bacteria without this BHL- and HHL-dependent quorum sensing system. Results are presented as the mean value ± S.E. (n = 8) for each AHL.

**RESULTS**

**Measurements of [Ca²⁺]i, in Fura-2-loaded Bacteria in Suspension**—Upon the addition of PBS to a suspension of Fura-2-loaded S. liquefaciens, no change in fluorescence ratio was observed; i.e. there was no change in [Ca²⁺]i. When 20 µM of HHL was added, however, there was a decline in [Ca²⁺]i, here demonstrated as the mean fluorescence ratio value during 60 s after the addition of HHL (Fig. 1A). The change in [Ca²⁺]i differed significantly from the resting level (p < 0.05, n = 8). The addition of 20 µM OHHL (Fig. 1B) did not induce changes in [Ca²⁺]i, that differed significantly from the resting level (n = 8). Fura-2-loaded E. coli, treated with OHHL or HHL as described, did not demonstrate any change in fluorescence ratio (data not shown).

**Imaging of [Ca²⁺]i, in Fura-2-loaded Single Bacteria**—The addition of 200 µM HHL to S. liquefaciens settled to silanized glass induced a transient decline in [Ca²⁺]i, observed as a decrease in light intensity in the ratio fluorescence images (Fig. 2A). Similarly, 200 µM OHHL added to V. fischeri induced a...
transient decrease in observed $[\text{Ca}^{2+}]_i$ (Fig. 2B). Typically, the cells recovered after 5 s but most often they exhibited short bursts of $[\text{Ca}^{2+}]_i$, fluctuations after recovery.

**Detection of Kinase Activity in AHL-stimulated Bacteria**—When *S. liquefaciens* had been stimulated by BHL for 90 s prior to homogenization, the $\gamma$-phosphate group transfer from ATP to a peptide or protein substrate in the cell homogenate was significantly higher compared with unstimulated cell samples ($p < 0.05$, $n = 5$). Stimulation with OHHL, prior to sample preparation, also seemed to increase the amount of transferred phosphate groups; however, this increase was not statistically significant. We assume that the measured phosphate transfer is due to an induction of kinase activity (Fig. 3A). When a PKC-specific substrate, a peptide with a serine/threonine residue of the $\alpha$-$\beta$-$\gamma$ type, was added during sample analysis, the kinase activity of the BHL-stimulated samples were decreased about 20%. Interestingly, when no AHL signal had been added to the bacteria, the samples exhibited PKC activity, although not significantly different from stimulated cells (Fig. 3B).

**DISCUSSION**

This is the first study of the involvement of intracellular $[\text{Ca}^{2+}]_i$ and protein phosphorylation in bacterial quorum-sensing signal transduction. We demonstrate that both intracellular $[\text{Ca}^{2+}]_i$ transients and kinase activity are induced by stimulation of the quorum-sensing bacteria with either of its specific AHL signal molecules, BHL or OHHL. These substances are produced by *S. liquefaciens*, where HHL has the same effect as BHL if used at a higher concentration (21).

The *V. fischeri* LuxR protein is a 250-amino acid polypeptide (23, 24). Based on mutational analysis, it has been shown that LuxR consists of two functional domains where the C-terminal together with RNA polymerase, can interact with the lux-regulatory DNA, and the N-terminal contains an AHL binding domain that inhibits the activity of the C-terminal domain in the absence of the AHL signal molecule (5, 25). A LuxR mutant with the N terminus deleted is independent of cell density and shows the same or higher activity as LuxR in the presence of BHL or OHHL (26). Zhu and Winans (6) observed for the *A. tumefaciens* TraR protein that the AHL binding increases the affinity of TraR for the DNA binding site and that the binding might stabilize the TraR protein against cytoplasmic proteases. This stabilization is hypothesized to be a conversion from unbound TraR monomers to AHL-bound oligomers of the TraR protein (6). LuxR has been found to be associated with the cytoplasmic membrane, but it has no transmembrane structures (27). When trying to purify LuxR from a cell membrane fraction of *V. fischeri*, it has been shown that LuxR remains associated to membranes in the presence of a number of detergents as well as KCl, but LuxR can be solubilized from membranes by EDTA treatment (27). Solubilization of LuxR from membranes by EDTA might indicate the involvement of Ca$^{2+}$ as a factor for membrane attachment. It is known that Ca$^{2+}$ may have a general role in the lateral distribution of membrane...
lipids (9). Accordingly, Ca" might have a role in an eventual multimerization of the membrane-attached LuxR monomers.

We assume that the transcription factor “SwrR” regulated by BHL and HHL in S. liquefaciens is a homologue to the LuxR protein in V. fischeri. In S. liquefaciens, DNA sequencing has revealed the presence of an open reading frame next to the swrI gene (coding for the SwrI protein, which synthesizes BHL and HHL signal molecules), denoted swrR, with homology to the LuxR family of AHL-binding transcriptional regulators (unpublished results referred to by Givskov et al. (28)).

In trying to elucidate the role of intracellular Ca" transients in quorum-sensing signaling, the total amino acid sequence of LuxR protein, as determined by Devine et al. (23, 24), was analyzed for calcium binding motifs, active sites of enzymes, and other functional regions, in the Prosite data base (29). In the LuxR sequence, the data base search revealed identity with an ATP/GTP-binding site motif (residues 194–201) and predicted several sites for phosphorylation by protein kinase C and casein kinase II. The data base analysis also predicted a site for myristoylation (residues 119–124). No identity for EF-hand calcium binding motifs was found in the LuxR sequence. However, when the requirement for similarity was set to 72%, an EF-hand motif was suggested (residues 74–86). The Prosite analysis thus provides support for more than one alternative hypothesis for how Ca" transients and/or protein phosphorylation might be involved in quorum sensing signaling.

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2 The Prousite data base is available on the World Wide Web.

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**Fig. 4.** A model demonstrating alternative mechanisms for transcription activation of LuxR. Both direct AHL interaction with LuxR and AHL binding to a not yet identified non-LuxR AHL-receptor may be essential for transcription activation by LuxR. Direct binding of AHL to LuxR (A), as a one-step event, requires free diffusion of AHL through the periplasmic region. Assuming the involvement of a non-LuxR AHL-receptor (B), an additional, possibly synergistic, pathway is suggested. Binding of AHL to a non-LuxR AHL receptor triggers a change of [Ca"], in addition to activation of an AHR- or Ca"-activated kinase, leading to exposure of an AHR binding site on LuxR or exposure of a membrane attachment site affecting the AHR binding site. Many bacterial regulators are activated by a kinase-dependent phosphorylation. Observed AHR-induced [Ca"], responses might indicate a possible activation of LuxR by a Ca"-dependent phosphorylation, upon AHR stimulation. Activation of the LuxR initiates transcription. A LuxR-associated myristoyl site, i.e. an acyl-binding domain, possibly being the site for membrane association or the AHR-binding site, might initiate the transcription factor as it anchors to the membrane or exposes the AHR binding site.

In both detection approaches used to study [Ca"], mobilization, ratio fluorescence spectrometry of cell populations and ratio fluorescence imaging for single bacteria analysis, we observed a decrease in intracellular [Ca"], upon stimulation with a specific AHL molecule. When HHL was added to a suspension of S. liquefaciens, there was a decline in [Ca"], observed as a decrease in the Fura-2 fluorescence ratio (Fig. 1A). On the other hand, the addition of a nonspecific AHR signal, OHHL (Fig. 1B) or the addition of PBS buffer only did not induce significant changes in [Ca"]. The cells thus respond with a mobilization of [Ca"], to HHL but not to OHHL, an AHR molecule that is not produced by S. liquefaciens. As a comparison, E. coli was not activated by either HHL or OHHL. During fluorescence experiment treatment with salt-free electroporation buffer, V. fischeri rapidly and extensively formed mucus affecting the homogeneity and translucency of the cell suspension, thereby invalidating the results.

In Fura-2 ratio fluorescence imaging experiments, the addition of HHL and OHHL to settled S. liquefaciens and V. fischeri, respectively, induced rapid declines in observed [Ca"]. The cells recovered after a short series of repeated [Ca"], fluctuations (Fig. 2). The ratio imaging of settled single cells, where, roughly, a pixel corresponds to the size of a single bacterial cell, showed [Ca"], transients already after a few seconds, followed by [Ca"], fluctuations. Upon initial recovery, however, the cell [Ca"], continued to fluctuate for ~15 s, sometimes even longer. To be sure to detect all of these fluctuations in the conventional Fura-2 fluorescence measurements, the average fluorescence over a longer time period, 60 s, was measured. Within these 60 s, the rapid [Ca"], responses demonstrated in the single cell measurements are well included. These [Ca"], fluctuations might explain the “noisy” appearance of fluorescence data from cell suspensions (data not

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**Fig. 3.** Kinase activity, measured as "P incorporation upon AHL stimulation in homogenates of S. liquefaciens.** A**, kinase activity without added PKC-substrate; **B**, kinase activity in the presence of a PKC-selective peptide. An overnight culture of the Gram-negative S. liquefaciens SwrI mutant strain, unable to produce AHR signal molecules, was stimulated by BHL or OHHL (20 μM) for 90 s prior to cell disintegration. Kinase activity was assayed by measuring the rate of "P incorporation from [γ-"P]ATP added to the cell homogenates, in the presence or absence of a PKC-selective peptide substrate with serine/threonine residues specific for the α-β-γ isoforms of PKC. When no substrate was added, bacterial samples stimulated by BHL (A), demonstrated γ-phosphate group transfer from ATP to a substrate in the cell homogenate, being significantly higher compared with unstimulated cell samples (p < 0.05, n = 5). Stimulation with OHHL also seemed to increase the amount of transferred phosphate groups. In the presence of a PKC-specific substrate, the kinase activity of BHL-stimulated samples was decreased by 20% (B).
shown). In eukaryotic cells, most often transient increases in calcium concentration have been coupled to signal transduction. The data presented here, showing a transient decrease in \([Ca^{2+}]_i\), are consistent with data from a bacterial chemotaxis study presented by Tisa and Adler (19), where stimulation of Fura-2-loaded *E. coli* cells with a chemotactic attractant (t-serine) also led to a transient decrease in \([Ca^{2+}]_i\). Tisa and Adler present a theory for the coupling between an observed decrease in \([Ca^{2+}]_i\), and signal transduction. They suggest that Ca\(^{2+}\) might help stabilize and maintain a chemotactic protein in its phosphorylated state, stimulating tumbling. A decrease in \([Ca^{2+}]_i\) would result in an unphosphorylated protein, stimulating running.

Possible roles for the demonstrated Ca\(^{2+}\) transients in AHL-dependent quorum sensing may be involvement in the attachment of LuxR (or LuxR homologue) to the membrane or exposure of its AHL-binding site. A myristoylation site in LuxR, suggested by the Prosite data matching (i.e. an acyl-binding domain) is a possible site for membrane association or may be an AHL-binding site. Both of these alternatives might in turn be dependent on changes in intracellular \([Ca^{2+}]_i\), by a "calcium-myristoylation switch," in which an acyl-binding site is exposed upon Ca\(^{2+}\) stimulation (30).

Many bacterial regulators employ a signal transduction system, where a protein is activated by kinase-dependent phosphorylation. The AHL-induced \([Ca^{2+}]_i\), response observed in the fluorescence experiments might indicate a possible activation of LuxR homologues by a Ca\(^{2+}\)-dependent phosphorylation upon AHL stimulation. Kinase stimulation by Ca\(^{2+}\) activated calmodulin-like proteins have been described in bacteria (31). The data base search predicted an ATP/GTP-binding site motif (residues 194–201) in the LuxR sequence, and several sites for phosphorylation by protein kinase C and casein kinase II. We therefore investigated possible protein kinase activity upon AHL stimulation of *S. liquefaciens* cell suspensions. We found an increase in kinase activity in cell homogenates from bacteria that had been stimulated with BHL. However, when a \(\alpha{\beta}\gamma\) PKC isoenzyme substrate was present during measurements the kinase activity in BHL-treated samples was dampened about 20%. The finding may indicate a competitive inhibition of the BHL-induced kinase activity by the PKC-specific \(\alpha{\beta}\gamma\) substrate. If so, a possible "non-\(\alpha{\beta}\gamma\) substrate" is most probably synthesized by the cells and harbored in the homogenate. In contrast, when cell samples were not stimulated with AHLs prior to homogenization, kinase activity was measurable in the presence of the \(\alpha{\beta}\gamma\)-PKC substrate type only, indicating a specific PKC activity.

In Fig. 4 alternative roles for intracellular \([Ca^{2+}]_i\) and kinase activity in LuxR homologue activation is considered. We suggest a model where AHL interaction with the LuxR homologue is dependent on or synergistic with AHL interaction with a "non-LuxR AHL receptor" situated, presumably, in the periplasmic region. A direct binding of OHHL to a LuxR homologue, TraR, has been demonstrated (6). Assuming the involvement of \([Ca^{2+}]_i\), in this interaction, possible mechanisms for this may be exposure of the AHL binding site on the LuxR homologue upon \([Ca^{2+}]_i\), changes or exposure of the site for membrane attachment, which in turn may affect the AHL binding site. In parallel to the suggested Ca\(^{2+}\) effects, LuxR may be activated for transcription through phosphorylation by an AHL-activated, or possibly Ca\(^{2+}\)-activated, kinase. Both direct AHL interaction with LuxR and AHL binding to a not yet identified "non-LuxR AHL receptor" may be essential for transcription activation by the LuxR homologue. However, one AHL interaction in this pathway may be more species-specific than the other.

The conclusion of this study is that changes in \([Ca^{2+}]_i\), are induced by quorum-sensing signals in the Gram-negative bacteria *S. liquefaciens* and *V. fischeri*. We also conclude that kinase activity is induced in *S. liquefaciens* upon AHL stimulation. We therefore suggest that transient intracellular \([Ca^{2+}]_i\) changes and kinase activity following AHL stimulation are critical for the quorum-sensing signal transduction in *S. liquefaciens*.

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REFERENCES

1. Fuqua, W. C., Winans, S. C. & Greenberg, E. P. (1994) *J. Bacteriol.* 176, 7569–7575
2. Salmond, G. P. C., Bycroft, B. W., Stewart, G. S. A. B. & Williams, P. (1995) *Mol. Microbiol.* 16, 615–624
3. Sittikov, D. M., Schineller, J. B. & Baldwin, T. O. (1995) *Mol. Microbiol.* 17, 801–812
4. Kaplan, H. B. & Greenberg, E. P. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 84, 6639–6643
5. Hanseela, B. L. & Greenberg, E. P. (1995) *J. Bacteriol.* 177, 815–817
6. Zhu, J. & Winans, S. C. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 4832–4837
7. Basler, B. L., Wright, M., Showalter, R. E. & Silverman, M. R. (1993) *Mol. Microbiol.* 9, 775–786
8. Basler, B. L., Wright, M. & Silverman, M. R. (1994) *Mol. Microbiol.* 12, 403–412
9. Norris, V., Grant, S., Freestone, P., Canvin, J., Sheil, F. N., Tolh, I., Trinei, M., Molina, K. & Normam, R. I. (1996) *J. Bacteriol.* 178, 3677–3682
10. Metley, S. T. & Lary, S. (1999) *Infect. Immun.* 67, 5386–5394
11. Kenelly, P. J. & Potts, M. (1996) *J. Bacteriol.* 178, 4759–4764
12. Bazi, M. D. & Nebesstuen, G. (1996) *Biochemistry* 35, 7624–7630
13. Newton, A. C. & Keranen, L. M. (1994) *Biochemistry* 33, 6651–6658
14. Hannan, Y. A., Loomis, C. R. & Bell, R. M. (1986) *J. Biol. Chem.* 261, 7184–7190
15. Orr, J. W. & Newton, A. C. (1992) *Biochemistry* 31, 4667–4673
16. Keranen, L. M. & Newton, A. C. (1997) *J. Biol. Chem.* 272, 25959–25967
17. Smith, R. J. (1995) *Ado. Microb. Physiol.* 37, 83–103
18. Jones, H. E., Holland, I. B., Baker, H. L., and Campbell, A. K. (1986) *J. Biol. Chem.* 261, 7184–7190
19. Tisa, L. S. & Adler, J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 10777–10781
20. Watkins, N. J., Knight, M. R., Trewavas, J. & Campbell, A. K. (1995) *Biochem. J.* 306, 865–869
21. Eberl, L., Winans, M. K., Sternberg, C., Stewart, G. S. A. B., Christiansen, G., Chhabra, S. R., Bycroft, B., Williams, P., Molin, S. & Givskov, M. (1996) *Mol. Microbiol.* 20, 127–136
22. Kuo, A., Callahan, S. M. & Dunlap, P. V. (1996) *J. Bacteriol.* 178, 971–976
23. Devine, J. H., Countryman, C. & Baldwin, T. O. (1988) *Biochemistry* 27, 837–842
24. Devine, J. H., Shadel, G. S. & Baldwin, T. O. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 5688–5692
25. Stevens, A. M., Dolan, K. M. & Greenberg, E. P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12619–12623
26. Choi, S. H. & Greenberg, E. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 11115–11119
27. Kolbchak, D. & Greenberg, E. P. (1993) *J. Bacteriol.* 175, 7307–7312
28. Givskov, M., Eberl, L. & Molin, S. (1997) *FEMS Microbiol. Lett.* 148, 115–122
29. Bubrich, E., Bucher, P. & Hofmann, K. (1997) *Nucleic Acids Res.* 26, 217–221
30. Ames, J. B., Ishima, R., Tanaka, T., Gordon, J. I., Stryer, L., and Ikura, M. (1997) *Nature* 389, 198–202
31. Onek, L. A. & Smith, R. J. (1992) *J. Gen. Microbiol.* 138, 1039–1040
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