Free calcium transients in chemotactic and non-chemotactic strains of *Escherichia coli* determined by using recombinant aequorin

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Intracellular Ca^{2+} has been previously implicated in the chemotactic response of *Escherichia coli*. However, no correlative measurements of intracellular free Ca^{2+} have been made during bacterial chemotaxis, essential if this is to be established. In order to monitor internal free Ca^{2+} in *E. coli* during challenge with chemotactic agents, the Ca^{2+} -activated photoprotein aequorin was expressed in a chemotactic strain (AB1157) and a non-chemotactic strain [BL21(DE3)] of *E. coli*. Repellents were found to cause an increase (50–150 nM) in intracellular free Ca^{2+} , whereas attractants caused a small but consistent decrease in intracellular free Ca^{2+} . These data are in agreement with the

proposed model that an increase in intracellular free Ca^{2+} causes tumbling. The effect of increasing external Ca^{2+} on the regulation of intracellular free Ca^{2+} in both strains was monitored by using aequorin. The resting level of free Ca^{2+} in *E. coli* (AB1157) was found to be 100 nM, which agrees with previous data [Gangola and Rosen (1987) *J. Biol. Chem.* 262, 12570–12574]. As these results also show differences in the regulation of intracellular free Ca^{2+} between the two strains in the presence of high external Ca^{2+} concentrations, this may have implications for the effect of high- Ca^{2+} environments on *E. coli*.

INTRODUCTION

Ca^{2+} ions have been shown to be a major second messenger in eukaryotic cells [1,2]. This contrasts sharply with prokaryotes, where the role for Ca^{2+} is not well defined [3]. Ca^{2+} -binding proteins have been detected in *Escherichia coli*, and there is evidence both in favour of and against the involvement of Ca^{2+} ions in bacterial chemotaxis (the ability of bacterial cells to be attracted or repelled by chemical agents). Treatment of *Bacillus subtilis* and *E. coli* with ionophores and external Ca^{2+} has been shown to cause tumbling, the swimming response to repellents [4,5]. The critical concentration for external Ca^{2+} , however, seems to vary between 100 nM and 1 mM in these two species. The release of 'caged' Ca^{2+} inside *E. coli* cells has also been shown to induce tumbling, whereas the release of 'caged' Ca^{2+} chelator induces smooth swimming [6]. These data are consistent with an increase in intracellular Ca^{2+} directly mediating tumbling behaviour in bacteria. By contrast, it has been shown that external Ca^{2+} on its own has no effect on chemotaxis, i.e. Ca^{2+} is not a chemotactic agent [7]. This is despite the fact that we have previously shown that increasing external Ca^{2+} elevates intracellular free Ca^{2+} ($[\text{Ca}]_i$) in *E. coli* [8]. Furthermore, growing *E. coli* in the Ca^{2+} chelator EGTA does not decrease its capacity for chemotaxis, suggesting that external Ca^{2+} ions have no role in mediating chemotaxis [9]. Thus the data regarding the role of Ca^{2+} in bacterial chemotaxis are somewhat contradictory. In order to resolve this controversy, and to test whether changes in $[\text{Ca}]_i$ are necessary for chemotaxis, it is essential to measure directly $[\text{Ca}]_i$ in *E. coli* in response to chemotactic agents. However, in an organism as small as *E. coli* difficulties are encountered when using fluorescent dyes for measuring $[\text{Ca}]_i$, due to problems with dye loading, autofluorescence and compartmentalization [10]. Also, manipulating the $[\text{Ca}]_i$ using caged Ca^{2+} compounds could result in only a small sub-

population of cells being investigated, due to problems with electroporation. Here we report the first direct measurements of *E. coli* $[\text{Ca}]_i$ in response to chemotactic conditions, using the recombinant Ca^{2+} -activated photoprotein aequorin from the jellyfish *Aequorea victoria*.

Aequorin has been widely used to study the role of Ca^{2+} in eukaryotic cells [11–13], prokaryotic cells [8] and recently in intracellular organelles [14–17]. The protein is monomeric, with an M_r of 21 400. When it is extracted, it consists of apoequorin and coelenterazine (M_r 423), a prosthetic group linked to apoequorin through a peroxide bond [18,19]. When Ca^{2+} binds to the protein a conformational change takes place, which results in the oxidation of coelenterazine to coelenteramide and the emission of blue light (λ_{max} ~ 470 nm) [20]. By genetically transforming bacterial cells so that they express apoequorin, and then reconstituting the photoprotein with coelenterazine, luminous cells are produced whose luminosity directly reports $[\text{Ca}]_i$.

We have used this system to study changes in $[\text{Ca}]_i$ in both a chemotactic and a non-chemotactic strain of *E. coli*, when challenged with various chemotactic attractants and repellents, and different external Ca^{2+} concentrations.

MATERIALS AND METHODS

Materials

The following derivatives of *E. coli* were used in this study: HB101 as host for DNA manipulations [21], AB1157 [22] and BL21(DE3) [23] for chemotactic measurements. Cos-7 cells [24] were used as a model eukaryotic system. Tryptone, yeast extract and bacterial agar were from Unitpath Ltd. All other analytical-grade reagents were from Sigma Chemical Co. or Fisons.

Abbreviations used: $[\text{Ca}]_i$, intracellular free Ca^{2+} concentration; CM, chemotaxis medium; CL, chemiluminescence.

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Oligonucleotide primers were prepared with an Applied Biosystems 392A DNA synthesizer and purified as 'trityl-off' oligonucleotides. Their primary sequences were as follows:

pET1, 5'CCACACAGATCTATGGTCAAGCTTACATCA3' (5' end sense oligonucleotide to engineer a *Bgl*II site at the 5' end of aequorin cDNA).

pET2, 5'CCCATCAGATCTTTAGGGGACAGCTCC3' (3' end antisense oligonucleotide to engineer a *Bgl*II site at the 3' end of aequorin cDNA).

pET5, 5'ACTGGTTTACAGCAAATGGGTGCG3' (5' end sense similar to region immediately upstream of *Bam*HI site in plasmid pET3a).

Construction of aequorin expression systems for AB1157 and BL21(DE3)

In order to transform *E. coli* strain AB1157, a multi-host-range expression vector, pMMB66EH, had to be used [25]. Aequorin was unidirectionally sub-cloned into this plasmid at the multiple cloning site downstream from the T7 RNA polymerase promoter, by using the *Sal*I and *Pst*I sites to produce pMMB66EH-AEQ.

For expression of aequorin in BL21(DE3) the plasmid pET3a was used, as it was specific for this strain. pET3a plasmid [AMS Biotechnology (Europe)] was prepared from *E. coli* HB101 by the alkaline-lysis method [26]. PCR of the apoaequorin coding region was performed with oligonucleotides pET1 and pET2 as described previously [27], by using Amplitaq DNA polymerase (Promega). The PCR DNA products and pET3a were cut with the restriction enzymes *Bgl*II and *Bam*HI (Boehringer Mannheim) respectively. After purification of the DNA fragments using GENECLAN II (Bio101), the ligation was carried out as described [26] by using T4 DNA ligase and calf intestinal phosphatase (Boehringer Mannheim). The plasmid ligation mixture was used to transform *E. coli* HB101 by using CaCl_2 and heat shock. Colonies growing on carbenicillin plates were screened for aequorin cDNA inserts by PCR, with 1 in 3 colonies containing inserts. Insertion was established by the size of the new plasmid (5500 bases), and determination of correct orientation downstream of the T7 RNA polymerase promoter by PCR using oligonucleotides pET1 and pET5. This plasmid, pET-3AEQ, was used for subsequent expression of aequorin in BL21(DE3).

Expression of aequorin in *E. coli* strains AB1157 and BL21(DE3)

The plasmids pMMB66EH-AEQ and pET-3AEQ were used to transform AB1157 and BL21(DE3) respectively, by using CaCl_2 and heat shock [26]. A single colony was then picked and used to inoculate 5 ml of LB medium containing 50 mg of carbenicillin/ml. This culture was then incubated overnight at 37 °C with shaking. The next morning 1 ml of this culture was used to inoculate 30 ml of LB + 50 mg of carbenicillin/ml. This was then incubated at 37 °C for 2 h with shaking. Isopropyl β -D-thiogalactoside was then added to a final concentration of 0.5 mM, and the incubation was continued at 37 °C with shaking for 2 h.

E. coli motility assays

The chemotactic ability of the two strains of bacteria was determined by using 'swarm plates' [28]. Swarm plates have a low percentage of agar, and this allows chemotactic bacteria to migrate out of the centre of the plate, in response to chemo-attractants. After incubation at 37 °C overnight, the bacteria

spread out and form a concentric ring pattern, which is characteristic of a chemotactic strain [28].

The swarm plates had the following composition: 1% tryptone, 0.5% NaCl, 0.2% agar, 1 mM CaCl_2 in chemotaxis medium (CM; 10 mM potassium phosphate, 1 mM L-methionine, 0.1 mM potassium EDTA, pH 7.0). In order to determine whether the cells were chemotactic, 10 ml cultures of BL21(DE3) and AB1157 were grown overnight. The cultures were then spun down and resuspended in 200 μ l of LB, and a 50 μ l portion was placed at the centre of each plate. The plates were then incubated overnight at 37 °C.

Aequorin reconstitution and luminescence measurements

Reconstitution was carried out in Krebs-Ringer Hepes buffer (KRH: 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 25 mM Hepes, pH 7.5) containing 2.5 μ M coelenterazine, for 1 h in the dark at room temperature. Before Ca^{2+} measurements were made, the cells were washed twice in KRH containing 0.5 mM EGTA, in order to remove the excess coelenterazine. The final concentration of cells containing reconstituted aequorin used in each of the measurements was 50×10^7 /ml.

For the chemotaxis experiments, the bacteria were placed in CM, with 1 mM CaCl_2 added: 10 mM L-serine was used as the attractant, and 5 mM NiSO_4 and 1 mM CoCl_2 were used as repellents. The chemiluminescence (CL) counts were monitored for 6 min after the injection of the repellent or attractant.

For external-calcium experiments the bacteria were placed in KRH + 0.5 mM EGTA, and then KRH containing the appropriate concentration of Ca^{2+} was injected to give final concentrations of 0.1, 1.0 and 10 mM. A second addition of KRH + Ca^{2+} was made after 90 s in order to double the concentrations to 0.2, 2.0 and 20 mM respectively, and CL counts were monitored for another 210 s.

In both the chemotaxis and external- Ca^{2+} experiments, the remaining reconstituted aequorin at the end of the experiment was estimated by discharging with the addition of an equal volume of 50 mM CaCl_2 /2% Nonidet P40. CL measurements were made with a digital chemiluminometer with an EMI photomultiplier 9757 AM as previously described [29]. The output was recorded either graphically or by an 8-digit scaler. Rate constants of luminescence were determined and $[\text{Ca}]_i$ was calculated, by using calcium calibration curves obtained for aequorin extracted from AB1157 and BL21(DE3) as described previously [30]. It is also important to note here that it is essential to convert from CL counts into rate constants, as CL counts alone can give a distorted picture, especially when there is high consumption of aequorin.

RESULTS

Swarm plates

In order to test the mobility of the two strains of *E. coli* used in this study, a swarm-plate assay [28] was established in the presence of external Ca^{2+} (1 mM). The swarm-plate assay showed that the *E. coli* strain AB1157 was chemotactic, forming a concentric ring pattern as the bacteria moved out from the centre of the plates [28]. In contrast, *E. coli* BL21(DE3) did not move from the middle of the plates, showing that this strain was not chemotactic. This result is to be expected, as BL21(DE3) is an *E. coli* B strain, having the genes for flagellation and chemotaxis deleted [23]. As chemotaxis was shown to occur in CM containing 1 mM Ca^{2+} , subsequent measurements of $[\text{Ca}]_i$ were performed in this medium.

Figure 1 Effect of L-serine on $[Ca]_i$ in *E. coli* AB1157

For this, 50×10^7 bacteria were placed in 1 ml of CM/1 mM $CaCl_2$. The $[Ca]_i$ of the bacteria was allowed to return to a low resting level; then 20 μ l of either 0.5 M L-serine in CM ($\blacktriangle$) or CM buffer alone ($\blacksquare$, control) was injected in front of the photomultiplier with a spring-loaded syringe. The CL counts were then monitored for 5 min. Then 1 ml of 50 mM $CaCl_2$ /2% Nonidet P40 was injected to discharge the remaining aequorin CL (results not shown).

Figure 3 Effect of $NiSO_4$ and $CoCl_2$ on $[Ca]_i$ in *E. coli* AB1157

For this, 50×10^7 bacteria were mixed with 1 ml of CM/1 mM $CaCl_2$. The $[Ca]_i$ of the bacteria was allowed to return to resting values; then 20 μ l of 250 mM $NiSO_4$ ($\triangle$) or 50 mM $CoCl_2$ ($\circ$) in CM was injected in front of the photomultiplier ($\blacksquare$, buffer control). The CL counts were then monitored for 5 min, and 1 ml of 50 mM $CaCl_2$ /2% Nonidet P40 was then injected to discharge the remaining aequorin CL (results not shown).

Figure 2 Effect of L-serine on $[Ca]_i$ in *E. coli* BL21(DE3)

For this, 50×10^7 bacteria were placed in 1 ml of CM/1 mM $CaCl_2$. The $[Ca]_i$ of the bacteria was allowed to return to a low resting level; then 20 μ l of either 0.5 M L-serine in CM ($\blacktriangle$) or CM buffer alone ($\blacksquare$, control) was injected in front of the photomultiplier with a spring-loaded syringe. The CL counts were then monitored for 5 min. Then 1 ml of 50 mM $CaCl_2$ /2% Nonidet P40 was injected to discharge the remaining aequorin CL (results not shown).

Figure 4 Effect of $NiSO_4$ and $CoCl_2$ on $[Ca]_i$ in *E. coli* BL21(DE3)

For this, 50×10^7 bacteria were mixed with 1 ml of CM/1 mM $CaCl_2$. The $[Ca]_i$ of the bacteria was allowed to return to resting values; then 20 μ l of 250 mM $NiSO_4$ ($\triangle$) or 50 mM $CoCl_2$ ($\circ$) in CM was injected in front of the photomultiplier ($\blacksquare$, buffer control). The CL counts were then monitored for 5 min, and 1 ml of 50 mM $CaCl_2$ /2% Nonidet P40 was then injected to discharge the remaining aequorin CL (results not shown).

Effects of attractants on $[Ca]_i$ in *E. coli*

To test the effect of chemoattractants on *E. coli* $[Ca]_i$, L-serine was chosen as the model attractant [6]. Figures 1 and 2 show the effects of adding L-serine in the presence of an external Ca^{2+} concentration of 1 mM on AB1157 and BL21(DE3), respectively. Initially, when the bacteria were placed in CM containing 1 mM Ca^{2+} a rapid rise in $[Ca]_i$ was observed. The level of Ca^{2+} then fell rapidly. When it reached a resting level of 180–210 nM for AB1157 and 500–550 nM for BL21(DE3), L-serine was injected to a final concentration of 10 mM. Addition of L-serine induced a slight, but consistent, decrease (10 ± 2 nM, $n = 6$) in

$[Ca]_i$ in AB1157, which returned to the resting level within 5 min. By contrast, in BL21(DE3) the addition of serine caused a rise (200 nM) in $[Ca]_i$. The decrease in $[Ca]_i$ observed in response to serine, even though consistent, was very small. It seems likely that this represents a significant decrease in $[Ca]_i$ in a small number of cells, rather than a very small decrease in all cells. A similar small decrease was seen with another chemoattractant, L-aspartate (results not shown). As suggested previously [6], this decrease was only temporary, and $[Ca]_i$ reached its resting level after 4–5 min.

Figure 5 Effect of external Ca^{2+} concentration on the regulation of *E. coli* $[\text{Ca}]_i$.

E. coli AB1157 (a) and BL21(DE3) (b) were induced and re-activated as described in the Materials and methods section. Then 10 μl portions of the re-activated cultures were placed in 500 μl of KRH/0.5 mM EGTA. CaCl_2 was then added to give external Ca^{2+} concentrations of 0.1, 1 and 10 mM. CL counts were monitored for 90 s; then another 0.1, 1.0 or 10 mM of CaCl_2 was added, and the counts were monitored for 4 min. Then 1 ml of 75 mM Ca^{2+} /2% Nonidet P40 was added to discharge the remaining aequorin CL (results not shown).

Effects of repellents on $[\text{Ca}]_i$ in *E. coli*

To test the effect of chemorepellents on *E. coli* $[\text{Ca}]_i$, NiSO_4 and CoCl_2 (Figures 3 and 4) were chosen as the model repellents [31]. Ni^{2+} and Co^{2+} ions inhibit the energy-dependent transport of Mg^{2+} ions into *E. coli* [32,33]. Initially, when the bacteria were placed in CM containing 1 mM Ca^{2+} a rapid rise in $[\text{Ca}]_i$ was observed, as above. The level of Ca^{2+} then fell rapidly, and, when it reached a resting value of 190–210 nM for AB1157 and 500–550 nM for BL21(DE3), NiSO_4 and CoCl_2 were injected to final concentrations of 5 mM and 1 mM respectively. The addition of repellents to AB1157 caused an immediate large spike in $[\text{Ca}]_i$ of 50–150 nM, which decayed rapidly. This was followed by a sustained increase in $[\text{Ca}]_i$. CoCl_2 caused a larger rise than NiSO_4 , 150 and 50 nM respectively after 4 min. In contrast, the effect of the repellents on BL21(DE3) were of about the same magnitude, but no spike was seen. NiSO_4 caused an increase after 60 s of 100 nM, whereas CoCl_2 caused an increase after 90 s of 30 nM. Similar, but decreased, responses to those

described above were observed when *E. coli* were challenged with attractants and repellents in CM containing no Ca^{2+} (results not shown).

Effect of increasing external Ca^{2+} on $[\text{Ca}]_i$ in *E. coli*

It has been suggested that chemotaxis in *E. coli* is unaffected by external Ca^{2+} concentration [7,9], and the Ca^{2+} ion itself is not a chemotactic agent, but more recent data have shown that increasing external Ca^{2+} increases $[\text{Ca}]_i$ [8]. If changes in $[\text{Ca}]_i$ were responsible for initiating the chemotaxis response in *E. coli*, then changes in external Ca^{2+} would not be expected to alter $[\text{Ca}]_i$ significantly in *E. coli*. In order to test this hypothesis, $[\text{Ca}]_i$ was measured in AB1157 and BL21(DE3) in response to challenge with 0.1, 1 and 10 mM CaCl_2 respectively (Figures 5a and 5b). For AB1157 the resting level of $[\text{Ca}]_i$ in 0.5 mM EGTA (zero Ca^{2+}) was between 90 and 100 nM (Figure 5a). By contrast, the resting $[\text{Ca}]_i$ in BL21(DE3) was around 50 nM in EGTA, indicating that the cells were perhaps unable to regulate their internal Ca^{2+} levels (Figure 5b).

Addition of 0.1 and 1 mM CaCl_2 gave a transient burst of $[\text{Ca}]_i$, followed by a decline, reaching a plateau within approx. 40 s. The cells maintained for several minutes a $[\text{Ca}]_i$ of 175–200 nM in response to 0.1, 1 and 10 mM external Ca^{2+} . A second addition of CaCl_2 was made 90 s after the first, to double the concentration. At an external Ca^{2+} concentration of 0.2 and 2.0 mM, *E. coli* AB1157 cells were still able to maintain a more or less steady $[\text{Ca}]_i$ and begin to bring the level of Ca^{2+} back down to the resting value. However, at external Ca^{2+} concentrations of 20 mM, the $[\text{Ca}]_i$ inside cells stayed around 200 nM. The BL21(DE3) cells, on the other hand, were able to maintain their $[\text{Ca}]_i$ around 200 nM when the external concentrations were 0.2 mM. But in 2 and 20 mM external calcium, $[\text{Ca}]_i$ increased in an apparently uncontrolled manner. These results show that AB1157 is better at regulating its $[\text{Ca}]_i$ than are BL21(DE3) cells, which would be necessary if Ca^{2+} is required for regulating chemotaxis. The results for the AB1157 cells are very similar to those obtained with the mammalian cell line Cos-7 (results not shown). This indicates that some prokaryotic cells are able to regulate their internal Ca^{2+} levels as well as eukaryotic cells can.

DISCUSSION

The results reported here show, for the first time, a direct effect of chemotactic agents on free Ca^{2+} inside live *E. coli*. The most dramatic result is that the two strains respond to changes in external Ca^{2+} and respond to chemotactic agents differently. The chemotactic strain (AB1157) can regulate its $[\text{Ca}]_i$ when the outside concentration is high, whereas the non-chemotactic strain cannot regulate its $[\text{Ca}]_i$ in response to a high external Ca^{2+} gradient. Furthermore, chemorepellents were found to cause a rise in $[\text{Ca}]_i$, whereas chemoattractants caused a decrease. This is consistent with the result where $[\text{Ca}]_i$ was lowered by using the light-activated caged calcium chelator Diazo-2, which resulted in smooth swimming, i.e. the attractant response [6]. The attractant L-serine was found to cause a decrease in $[\text{Ca}]_i$ in *E. coli* (Figure 1), whereas the addition of the repellents NiSO_4 and CoCl_2 caused an increase in $[\text{Ca}]_i$ (Figure 3). For AB1157 the resting level of $[\text{Ca}]_i$ in 0.5 mM EGTA (zero Ca^{2+}) was between 90 and 100 nM (Figure 5a), approximately the same as in previous studies using fura-2 [10].

In contrast, in the non-chemotactic strain BL21(DE3) the addition of L-serine caused an increase in internal free Ca^{2+} (Figure 2). As these bacteria are lacking the genes necessary for chemotaxis, this increase may be due to transport of the amino

acid into the bacteria affecting the $\text{Ca}^{2+}/\text{H}^{+}$ antiport, which regulates internal Ca^{2+} levels [34]. Furthermore, in this strain NiSO_4 caused an increase after a few minutes in internal Ca^{2+} , while CoCl_2 caused a small effect also after a few minutes (Figure 4). This also showed that the Ni^{2+} and Co^{2+} ions were not triggering the aequorin, as no immediate response was seen as in BL21(DE3) cells (Figure 4). It has been demonstrated that external Ca^{2+} in the presence of ionophore causes tumbling in bacteria [4,5]. This would suggest that an increase in $[\text{Ca}]_i$ can induce tumbling. The critical values for external Ca^{2+} in these studies varied from 100 nM external Ca^{2+} in *Bacillus subtilis* [4] to 1 mM in *E. coli* [5]. Our results for the effect of repellents on AB1157 fit in this range and they provide a more accurate estimation of the true $[\text{Ca}]_i$ changes compared with those estimated by using ionophore.

During our experiments it was important to determine how well *E. coli* maintained $[\text{Ca}]_i$ when the external Ca^{2+} concentration varied. In its natural environment, the small intestine, *E. coli* is exposed to an external Ca^{2+} concentration of about 1 mM [1]. Using recombinant aequorin in *E. coli*, we have demonstrated that the wild-type strain (AB1157) was able to regulate its internal Ca^{2+} levels as well as any eukaryotic cell (Figure 5a). This contrasted sharply with the *E. coli* B-strain BL21(DE3), which was unable to maintain a stable internal Ca^{2+} concentration when the external Ca^{2+} concentration was above 1 mM (Figure 5b). The large spiking seen in both strains of bacteria when Ca^{2+} was first injected may have been due to mechanical stimulation of Ca^{2+} channels; this has also been seen with Cos-7 cells (results not shown). The time scale of the spikes was too long for them to be due to the presence of dead or permeabilized cells in the bacterial population, which would have readily released their aequorin. Furthermore, we showed that the removal of the outer wall of *E. coli* did not release any significant quantities of expressed aequorin, and thus the aequorin was truly monitoring $[\text{Ca}]_i$ (results not shown). These results have implications for other strains of bacteria which become dormant in environments with a high Ca^{2+} concentration such as sea water [35]. Ca^{2+} -binding proteins similar to those found in eukaryotic cells have been isolated from *E. coli*, and Ca^{2+} has been implicated as a key control mechanism in the bacterial cell cycle [3]. Furthermore, these results may have implications for bacterial expression systems, as the question arises whether the external Ca^{2+} concentration could be critical for the efficiency of these systems for producing the desired proteins. Removing Ca^{2+} may improve yields of protein from these systems.

The results presented here show that aequorin expressed using the pMMB66EH and pET vectors is a powerful tool for measuring changes in internal free Ca^{2+} in *E. coli*. However, at high internal free Ca^{2+} the total photon emission (10^3 – 10^4 photons/cell), is sufficient to detect light from individual bacteria. Our experiments were carried out on a large population of cells; it will now be possible to monitor Ca^{2+} changes in individual bacteria by using chemiluminescence imaging. This will be important in quantifying the heterogeneity of the response of individual bacteria. Our measurements have so far been made on bacterial populations. We were therefore unable to rule out the possibility that small apparent changes in $[\text{Ca}]_i$ (e.g. in response to L-serine; Figure 1) were actually caused by a large change in a small sub-population of cells. The experiments described here

provide the essential strategy to investigate whether Ca^{2+} plays a major role in signalling in prokaryotes.

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