The evolution of bacterial LuxI and LuxR quorum sensing regulators

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Quorum sensing is a widespread form of bacterial communication in which individual cells produce and respond to specific N-acyl homoserine lactone signal metabolites. The different autoinducer synthases that generate these signals and the receptor/activator proteins that mediate the cell’s response to them constitute evolutionarily conserved families of regulatory proteins known as the LuxI and LuxR families, respectively. We have performed a phylogenetic analysis of 76 individual LuxI and LuxR homologues present in diverse members of the Gram-negative Proteobacteria. The results were consistent with an early origin for these regulators during the evolution of the Proteobacteria, with functional pairs of luxI and luxR genes possibly coevolving as regulatory cassettes. In many cases, specific LuxI and LuxR family members appeared to have been inherited horizontally. In particular, those species containing multiple LuxI and/or LuxR homologues usually appeared to have obtained each individual homologue or functional pair of homologues from an independent source. Because multiple homologues interact to form regulatory cascades, this finding suggests that hierarchical signalling pathways can potentially evolve by the sequential integration of pre-existing regulatory circuits acquired from diverse sources.

Keywords: intercellular signalling, cell–cell communication, horizontal gene transfer

INTRODUCTION

Intercellular communication plays a central role in the physiology and development of living organisms. Even unicellular organisms such as bacteria will frequently coordinate their activities so that populations of cells can function as multicellular entities (Shapiro, 1998). Such coordination necessarily requires cell–cell communication, often by means of the production and release of specific signal compounds by individual cells. The extracellular accumulation of these self-produced signals denotes the presence of a relatively dense population of cells and thus the appropriateness of coordinated group behaviour. Bacterial cell–cell communication of this sort is known as quorum sensing.

The best-studied mechanisms of quorum sensing are found among the Gram-negative Proteobacteria, which use N-acyl homoserine lactone ‘autoinducers’ as signal molecules (Greenberg, 2000; Parsek & Greenberg, 2000; Swift et al., 1999). With few exceptions, the proteins that synthesize these autoinducers constitute an evolutionarily conserved family of homologues known as the LuxI family of autoinducer synthases. The proteins that act as the specific response elements for autoinducers form another evolutionarily conserved group known as the LuxR family of transcriptional regulators.

LuxI and LuxR homologues have been identified in diverse species throughout the Gram-negative Proteobacteria, including phototrophic purple non-sulfur bacteria, marine vibrios, rhizosphere bacteria, enteric commensals and opportunistic pathogens of plants and animals. The LuxI and LuxR homologues present in these species regulate such disparate functions as exoenzyme synthesis, conjugation, antibiotic production, luminescence and biofilm formation (Greenberg, 2000; Parsek & Greenberg, 2000; Swift et al., 1999).

The genetic organization of LuxI and LuxR family members is as diverse as their function. Homologues of luxI and luxR genes have been identified both on bacterial chromosomes and on extrachromosomal elements such as the symbiosis (Sym) and tumour-inducing
Table 1. Quorum sensing homologues included in this study

<table>
<thead>
<tr>
<th>Organism</th>
<th>LuxI homologue*</th>
<th>LuxR homologue*</th>
<th>Orientation of adjacent genes</th>
<th>GenBank accession no. or reference</th>
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<td><strong>α-Proteobacteria</strong></td>
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<td>TraR</td>
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<td>TriR†</td>
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<tr>
<td>Agrobacterium tumefaciens (pTiCS8) (nop)†</td>
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<td>TraR</td>
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<tr>
<td>Agrobacterium vitis (pTi2608) (o/c)†</td>
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<td>Non-adjacent</td>
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<tr>
<td><strong>Rhizobium etli</strong>§</td>
<td>Rail</td>
<td>RaiR</td>
<td>→ →</td>
<td></td>
</tr>
<tr>
<td><strong>Rhizobium leguminosarum</strong>§</td>
<td>CinI</td>
<td>CinR</td>
<td>→ →</td>
<td>AF210630</td>
</tr>
<tr>
<td><strong>Rhizobium leguminosarum (pRL1JD)</strong>§</td>
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<td>Rhil</td>
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</tr>
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<td>BisR</td>
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<td>J. A. Downie (unpublished results)</td>
</tr>
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<td>TriR</td>
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<td>Y4qH</td>
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<td>AE000068; AE000069</td>
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<td><strong>Rhodobacter sphaeroides</strong></td>
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<td>CerR</td>
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<td>Cepl</td>
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<td><strong>Chromobacterium violaceum</strong></td>
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<td>CviI</td>
<td>← ←</td>
<td>M. K. Winson (unpublished results)</td>
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<td><strong>Ralstonia solanacearum</strong></td>
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<td>Soll</td>
<td>← ←</td>
<td>AF02184</td>
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<td>Ahyl</td>
<td>← ←</td>
<td>X89469</td>
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<td>Asal</td>
<td>← ←</td>
<td>U65741</td>
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<td><strong>Pantoea (Enterobacter) agglomerans</strong></td>
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<td>EgR</td>
<td>→ ←</td>
<td>X74300</td>
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<td><strong>Erwinia carotovora subsp. carotovora SCC3193</strong></td>
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<td>ExpR</td>
<td>→ ←</td>
<td>X72891; X80475</td>
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<td>HslR (ExpR)</td>
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<tr>
<td><strong>Erwinia carotovora subsp. carotovora GS101</strong></td>
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<td>RexR (ExpR)</td>
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<td>X74299</td>
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<td>CarR</td>
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<td>U17224</td>
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<tr>
<td><strong>Erwinia carotovora subsp. carotovora SCR193</strong></td>
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<td>CarR</td>
<td>Non-adjacent</td>
<td>Holden et al. (1998)</td>
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<td><strong>Erwinia carotovora subsp. betavasculorum</strong></td>
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<td>Ecbl</td>
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<td>EchI (ExpI)</td>
<td>EchR (ExpR)</td>
<td>← ←</td>
<td>U45854</td>
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<td>SdiA</td>
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<td>Esal</td>
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<td>L32184</td>
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<tr>
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<td>ExpR</td>
<td>← ←</td>
<td>X96440</td>
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<tr>
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<td>SdiA</td>
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<td><strong>Serratia liquefaciens</strong></td>
<td>Swrl</td>
<td>SwrR</td>
<td>← ←</td>
<td>U22823; M. Givskov &amp; M. K. Winson (unpublished results)</td>
</tr>
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<td><strong>Serratia marcescens</strong></td>
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<td>Smal</td>
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<td><strong>Serratia marcescens</strong></td>
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<td>CarR</td>
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<td><strong>Yersinia enterocolitica</strong></td>
<td>YelN</td>
<td>YelR</td>
<td>← ←</td>
<td>X76082</td>
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<td><strong>Pseudomonas aeruginosa PA01</strong></td>
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<td>→ ←</td>
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<td>VsmR (RhII)</td>
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<td>U15644</td>
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<td><strong>Pseudomonas aeruginosa PG201</strong></td>
<td>RhII</td>
<td>RhII</td>
<td>→ ←</td>
<td>L08962</td>
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<td><strong>Pseudomonas aureofaciens</strong></td>
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<td>PhzR</td>
<td>→ ←</td>
<td>L33724; L32729</td>
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<td><strong>Pseudomonas fluorescens</strong></td>
<td>PhzI</td>
<td>PhzR</td>
<td>→ ←</td>
<td>L48616</td>
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<td><strong>Pseudomonas syringae pv. tabaci</strong></td>
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<td>PsyR</td>
<td>→ ←</td>
<td>U39802</td>
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<td><strong>Pseudomonas syringae pv. syringae</strong></td>
<td>AhII</td>
<td>AhII</td>
<td>→ ←</td>
<td>AF072537</td>
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</table>
Amino acid sequences

The LuxI and LuxR homologues considered DNA sequences.

METHODS

of different gene functions in response to a single homologues can also permit the independent activation or detection of multiple, distinct signals. Multiple LuxR multiple LuxI and respectively. Many bacterial species even contain multiple Ti plasmids of Rhizobium producing (nop) or octopine/cucumopine-producing (o/c). The homologues listed are present on the Ti plasmids specifically.

† The tumour-inducing (Ti) plasmids of Agrobacterium spp. are grouped according to type as either octopine-producing (oct), nopaline-producing (nop) or octopine/cucumopine-producing (o/c). The homologues listed are present on the Ti plasmids specifically.

‡ The trlR gene contains a frameshift mutation in the 3’ end of the normal reading frame (Oger et al., 1998; Zhu & Winans, 1998). This point mutation was corrected to generate a gene product that more accurately reflects its relationship to other members of the LuxR family.

§ Homologues listed for Rhizobium spp. are present on the chromosome unless a symbiosis (Sym) plasmid is designated (e.g. pRL1JJ), in which case the homologues are present on the Sym plasmid itself.

(Ti) plasmids of Rhizobium and Agrobacterium spp., respectively. Many bacterial species even contain multiple LuxI and/or LuxR homologues for the production or detection of multiple, distinct signals. Multiple LuxR homologues can also permit the independent activation of different gene functions in response to a single autoinducer signal (McGowan et al., 1995; Pirhonen et al., 1993).

The question of where these two families of quorum sensing regulators first arose and how they came to be disseminated across such wide evolutionary distances has yet to be addressed. We have therefore performed a phylogenetic analysis of 76 LuxI and LuxR family members. Our results suggest that these quorum sensing regulators arose early in the evolution of the Proteobacteria and subsequently diverged within each group of organisms, although instances of horizontal gene transfer have also occurred. Surprisingly, most bacteria that contain multiple LuxI and/or LuxR family members appear to have acquired each of their different homologues from separate sources. This result provides new insights into the possible evolution of hierarchical signalling cascades in bacteria.

METHODS

DNA sequences. The LuxI and LuxR homologues considered in this analysis are listed in Table 1. Published sequences were obtained from GenBank, while unpublished sequences were generously provided by individual colleagues. MalT of Escherichia coli (accession no. M13585), EpsR ofRalstonia solanacearum (M61197) and FixJ of Sinorhizobium meliloti (Z21854) were tested as outgroups for the analysis of LuxR family members (Henikoff et al., 1990). Preligned 16S rRNA gene sequences were obtained from the rRNA WWW Server (Van de Peer et al., 1998).

Sequence alignment and analysis. Amino acid sequences were aligned using the default parameters of CLUSTALX (Higgins & Sharp, 1989) and adjusted by eye using GeneDoc (Nicholas & Nicholas, 1997). For purposes of comparison, a second set of alignments was made based on the published alignments of 17 LuxI homologues (Fuqua & Eberhard, 1999) and 15 LuxR homologues (Stevens & Greenberg, 1999). Sites containing gaps were excluded from the analysis to reduce systematic errors. Alignments were analysed using PAUP* version 4.0b4 (Swofford, 2000) to produce maximum-parsimony trees. Confidence in maximum-parsimony trees was determined by analysing 100 bootstrap replicates. Neighbour-joining trees were produced with the MEGA program (Kumar et al., 1994) using the γ model of amino acid substitution with a shape parameter a of 2-0. Confidence in neighbour-joining trees was determined by analysing 500 bootstrap replicates. All trees were drawn with nodes collapsed where bootstrap support was less than 50%.

When tree topologies disagreed with the groupings suggested by rRNA analysis, alternative trees were tested statistically both by a pairwise parsimony method (Templeton, 1983) and by comparison of log-likelihood differences (Kishino & Hasegawa, 1989), each as implemented by PAUP*. Analysis of the LuxI family tree was performed using a strict consensus of the eight most parsimonious trees derived from the modified CLUSTALX analysis. Analysis of the LuxR family tree was performed using a strict consensus of the two most parsimonious trees, while analysis of a combined LuxI/LuxR tree was performed on a strict consensus of the six most parsimonious trees. In all cases, alternative topologies were considered significantly different if P was less than 0.05.

RESULTS AND DISCUSSION

Host bacterial phylogeny as determined by rRNA analysis

The bacteria included in this study can be grouped according to 16S rRNA gene sequences into the α-Proteobacteria (comprising the genera Agrobacterium,
Fig. 1. Neighbour-joining tree of 16S rRNA sequences from bacteria that contain LuxI and/or LuxR family members. Species belonging to the α-, β- and γ-Proteobacteria are grouped within brackets. Different colours within the γ-Proteobacteria identify pseudomonads (blue), aeromonads (grey), vibrios (green) and enterobacteria (red). Scale bar indicates the mean number of substitutions per site. Bootstrap values as the percentage of 500 replicates are shown for each branch.

Rhizobium and Rhodobacter), the β-Proteobacteria (Burkholderia, Chromobacterium and Ralstonia) and the γ-Proteobacteria (Fig. 1; Woese, 1987). The γ-Proteobacteria can be subdivided into families representing the pseudomonads (Pseudomonas), aeromonads (Aeromonas), vibrios (Vibrio) and enterobacteria (Enterobacter, Erwinia, Serratia and Yersinia) (Table 1, Fig. 1). These 16S rRNA groupings were used as the basis for comparison with phylogenetic trees derived from our analysis of LuxI and LuxR family members.

Phylogenetic analysis of the LuxI and LuxR families

Neighbour-joining trees generated from the aligned LuxI and LuxR sequences indicated that the majority of proteins in each family clustered within the same groups defined by 16S rRNA sequences (Figs 2 & 3). Although individual exceptions to this rule were observed, the overall congruity between the quorum sensing and the rRNA trees is consistent with an ancient origin for quorum sensing proteins within the Proteobacteria.

Similar results were obtained with maximum-parsimony trees. Trees generated by either method using alignments based on previously published comparisons of a more limited number of sequences (Fuqua & Eberhard, 1999; Stevens & Greenberg, 1999) were indistinguishable from those produced using our own alignments.

Unfortunately, the amino acid sequences of individual LuxI homologues did not resolve deeper relationships among taxa in this family, as the previously identified groups simply joined with a few unaffiliated homologues.
into a single polytomy (Fig. 2). The LuxR family tree showed a similar pattern: in this case, a basal branch separated most of the α-proteobacterial sequences from the remaining groups, but these were once again united as a large polytomy (Fig. 3). This basal separation of the α-Proteobacteria sequences had relatively weak (71%) bootstrap support in the neighbour-joining trees and was absent from the maximum-parsimony trees, which more closely resembled the single polytomy of the LuxI tree.

In an attempt to resolve these uncertain deeper branches further, we decided to consider the combined sequences of ‘cognate’ pairs of LuxI and LuxR homologues. Cognate pairs are defined as those that are functionally linked, with the LuxI homologue producing the specific acyl homoserine lactone required to activate the corresponding LuxR protein. In most cases, cognate luxI and luxR gene homologues are located adjacently to each other, with ORFs oriented tandemly, divergently or convergently (Table 1). In addition, the TraI and TraR proteins of Agrobacterium tumefaciens and the RhiI and RhiR proteins of Rhizobium leguminosarum are known to function as cognate pairs, despite the fact that their genes are not adjacently positioned. Because functionally related gene pairs have the potential to coevolve, we could repeat our analysis using the combined sequences of these cognate LuxI and LuxR homologues as single taxa.

The resulting phylogenetic tree from the combined LuxI and LuxR sequences was more robust in terms of bootstrap support than those generated from LuxI or LuxR sequences alone, but was otherwise the same as the previous trees (Fig. 4). The fact that this ‘combined’
tree was consistent with the individual LuxI and LuxR
trees supported the hypothesis that cognate luxI/luxR
gene pairs may have become physically and/or func-
tionally linked fairly early in their history and sub-
sequently coevolved as unified regulatory cassettes. The
relative orientation of the ORFs, however, was not a
reliable indicator of phylogenetic relationship. As an
example, the divergently oriented homologues of Vibrio
fischeri grouped most closely with the convergently
oriented homologues of Vibrio anguillarum, while both
sets of Vibrio sequences clustered with the tandemly
oriented Pseudomonas aeruginosa LasI/LasR sequences
(Fig. 4, Table 1).

In a final attempt to resolve deeper relationships among
the different groups of quorum sensing regulators, we
performed an outgroup analysis on the LuxR family
tree. LuxR family members are part of a larger super-
family of regulatory proteins that share similarity only
in their C-terminal DNA binding domains (Henikoff
et al., 1990; Slock et al., 1990). Three members of this
superfamily, Fix) from Sinorhizobium meliloti, EpsR
from Ralstonia solanacearum and MalT from Escheri-
chia coli, were therefore used as representative out-
groups from the α-, β- and γ-Proteobacteria, respectively.
Unfortunately, these outgroup sequences were so dif-
fferent from the quorum sensing family sequences that
the resulting alignments were too weak to provide any
useful information in terms of basal associations with
the LuxR homologues. As a result, we were unable to
establish which of the quorum sensing homologues
appeared to be associated most closely with the out-
group sequences.

The observed groupings of the Chromobacterium vio-
laceum Cvi/CviR sequences with the enterobacterial
cluster and of the Pseudomonas aeruginosa LasI/LasR
sequences with the Vibrio cluster (Fig. 4) represented
notable exceptions to the overall agreement between the
16S rRNA gene tree and the combined LuxI/LuxR tree.
The validity of these anomalous groupings was tested by
placing each pair of sequences into the appropriate host
group as identified by 16S rRNA analysis and subse-
sequently determining the statistical significance of the
resulting differences in the two trees (Templeton,

When the combined Cvi/CviR sequences were moved
to join the base of the β-proteobacterial cluster (Fig. 4),
the resulting tree was significantly longer than the
original (P = 0.0007 and 0.0012 for the Kishino–
Hasegawa and Templeton tests, respectively). The same
result was true of CviR in the LuxR tree alone (P = 0.0002
and 0.0005), but not of CviR in the LuxI tree (P = 0.0513
and 0.0744). These results suggest that the
Chromobacterium CviR is more closely related to the
enterobacterial LuxR homologues than it is to those of
the other β-Proteobacteria. As the association of Cvi
with the enterobacterial LuxI sequences was not sup-
ported statistically, its proper affiliation remains open
to question. By contrast, moving the combined LasI/LasR
sequences of Pseudomonas aeruginosa into the pseudo-
monad cluster also resulted in a significantly longer tree
(P = 0.0137 and 0.0139), but this difference primarily
reflected the contribution of LasI rather than LasR. Moving LasR alone did not produce a significantly
longer tree (P = 0.2971 and 0.2985), while moving LasI
alone did result in a significant difference (P = 0.0024
and 0.0027). These are intriguing results, given our
previous finding concerning the possible coevolution of
functionally linked LuxI/LuxR pairs. Apparently Cvi/CviR
and LasI/LasR are unusual pairs of cognate quorum sensing homologues in that one member of each pair appears to be affiliated with a specific group of
sequences, while the other does not.

Horizontal transfer of individual LuxI and LuxR
homologues

Many other differences between the rRNA and quorum
sensing trees were observed among the unlinked homo-
logues found within the separate LuxI and LuxR trees.
For example, the LuxI homologues of two pathovars of
Pseudomonas syringae grouped strongly (98% boot-
strap support) at the base of the enterobacterial cluster,
well apart from other Pseudomonas LuxI homologues
(Fig. 2). The appearance of the Pseudomonas syringae
sequences within this cluster suggests that this species
may have acquired its quorum sensing regulators by
means of horizontal gene transfer. Moving these
sequences to the base of the pseudomonad cluster resulted in a significantly longer tree (P = 0.005 and 0.006),
indicating that they properly belong with the enter-
obacterial group.

Similarly, the unlinked LuxR homologues, both named
SdiA, of Escherichia coli and Salmonella typhimurium
did not group with other enterobacterial homologues.
Instead, they appeared most closely related to the
Pseudomonas aeruginosa RhlR sequences (Fig. 3), sug-
gesting the horizontal transfer of a pseudomonad
homologue to one or both of these enterobacterial
species. Moving the SdiA proteins to the base of the
enterobacterial cluster created a significantly longer
topology (P = 0.0018 and 0.0019). The SdiA proteins
are physiologically unusual in that no specific autoinducer is
known to be involved in their activation, although it has
been proposed that they might respond to autoinducers
produced by other bacteria (Ahmer et al., 1998). Both
Escherichia coli and Salmonella typhimurium have been
shown to produce extracellular signals (Surette &
Bassler, 1998), but these compounds do not appear to be
acylated homoserine lactones (Surette et al., 1999), nor
is there any evidence of a functional link between them
and the SdiA proteins (Surette & Bassler, 1999).

An alternative interpretation of these results is that
differences between the quorum sensing and the rRNA
trees might reflect gene duplication events followed by
the subsequent loss of certain duplicated genes over
time. The enterobacterial-like LuxI homologues of
Pseudomonas syringae might therefore represent genes
that were lost or remain unidentified in the majority of
pseudomonads. While this argument cannot be ruled out entirely, it does not satisfactorily explain many of the results from this analysis. For example, the complete genome of *Escherichia coli* (Blattner et al., 1997) contains no LuxI homologues and only the single LuxR homologue SdiA, which resembles a pseudomonad rather than an enterobacterial sequence (Fig. 3). To account for this result by gene duplication, *Escherichia coli* would have to have lost the LuxI and LuxR homologues retained by almost all other enterobacteria, while retaining instead the pseudomonad-like SdiA, which has yet to be found in the other enterobacterial taxa. In addition, there are several examples (described below) in which multiple quorum sensing regulators are found within a single bacterial species, yet most of these homologues cluster within the same rRNA group that defines the host. As a result, even in situations where we know that multiple homologues exist, those examples do not support the argument that early gene duplication adequately explains the appearance of a pseudomonad-like homologue within an enterobacterial host. We therefore propose that horizontal gene transfer is the best explanation for these instances of observed non-agreement between the quorum sensing regulator and the rRNA trees.

**Sequence variability within species**

Surprisingly, the variation observed among quorum sensing homologues from different laboratory strains of the same bacterial species was sometimes greater than the variation observed between closely related species. For example, the LuxI and LuxR proteins of *Vibrio fischeri* ES114 are distinct from those of strains MJ1 and ATCC 7744 (Fig. 4), while the LuxI homologue ExpI of *Erwinia carotovora* SCCR3193 differs from the synonymous HsII and Carl sequences of strains 71 and GS101 (Fig. 2). By comparison, the quorum sensing proteins found in *Aeromonas hydrophila* and *Aeromonas salmonicida* are more closely related to each other, as are those of *Pseudomonas fluorescens* and *Pseudomonas aureofaciens* (Fig. 4). This disparity of greater intraspecific than interspecific variability may reflect either recent horizontal transfer events or simply the recent speciation of these closely related taxa. In either case, it is curious that such extreme sequence diversity should exist within regulatory proteins shared by different strains of a single species.

**Evolution of multiple homologues within a species**

Six species of bacteria included in this study, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Rhizobium sp. NGR234*, *Rhizobium leguminosarum* and *Serratia marcescens*, contain more than one LuxI and/or LuxR family member (Table 1). If these multiple homologues arose endogenously by means of recent gene duplication, one would expect them to group most closely with each other in this analysis. Among 13 such possible pairings, however, only two of them – TraR and TrlR of *Agrobacterium tumefaciens*, and CinR and BisR of *Rhizobium leguminosarum* – appeared as closest relatives (Fig. 3). This suggests that in most cases the multiple quorum sensing genes present within a single species were each acquired independently of the other.

This result has important implications for the evolution of hierarchical signalling cascades in general. As an example, *Rhizobium leguminosarum* contains a pair of linked homologues (*cinL* and *cinR*) on its chromosome as well as the *luxI* homologue *rbil* and three additional unlinked *luxR* homologues (*bisR*, *rhlR* and *triR*) on the Sym plasmid pRLJ1 (Table 1). According to our analysis, each of the LuxI and LuxR family members present on this Sym plasmid appears to have been acquired from a separate source (Figs 3 & 4), with only BisR suggesting a possible endogenous origin from the chromosomal CinR (or vice versa). Given this mosaic pattern of inheritance, it will be especially intriguing to understand how the functions of these different homologues are integrated into a unified global regulatory system. The sheer abundance of these diverse regulators within this species may provide continuing opportunities for innovation in the specific role each plays within this global hierarchy.

Similarly, the two LuxR family members CarR and ExpR of *Erwinia carotovora* subsp. carotovora (Table 1) allow the independent activation of different sets of genes in response to a single autoinducer signal (McGowan et al., 1995; Pirhonen et al., 1993). Of these two homologues, CarR groups at the base of the enterobacterial cluster with a homologue of the same name found in *Serratia marcescens*, while ExpR clusters with the other *Erwinia* sequences (Fig. 4). The same pattern is seen with the two *Serratia marcescens* homologues CarR and SmaR, which group separately with the *Erwinia carotovora* CarR and the *Serratia liquefaciens* SwrR, respectively (Fig. 4). These results again suggest that each of the two LuxR homologues found in these species were acquired independently of each other. In the case of the two CarR proteins, these data further imply that horizontal gene transfer may have occurred between *Erwinia* and *Serratia*. Both of the CarR proteins activate carbanem antibiotic synthesis in their respective hosts (Cox et al., 1998; Holden et al., 1998; Thomson et al., 2000). This conservation of function may result from the fact that each homologue is genetically linked to a carbanem biosynthesis gene cluster (Cox et al., 1998; Holden et al., 1998; Thomson et al., 2000), thus allowing the inheritance of biosynthetic and regulatory functions as a single cassette.

Finally, the independent acquisition of multiple homologues is also evident in *Pseudomonas aeruginosa*. As previously noted, the LasI and LasR proteins of *Pseudomonas aeruginosa* showed no affiliation with any of the other pseudomonad homologues, including RhlI and RhlR from the same species (Fig. 4). This suggests that the lasI and lasR genes were acquired independently of *rbil* and *rhlR*. This is an especially significant result given that LasI and LasR serve as dominant regulators of
rhII and rhIR gene expression in *Pseudomonas aeruginosa* (Latifi et al., 1996; Pesci et al., 1997).

Collectively, these results provide additional support for the potential importance of horizontal gene transfer in the evolution of bacteria (Lawrence, 1997; Lawrence & Ochman, 1998). The horizontal transfer of LuxI and LuxR family members is especially noteworthy, however, because these represent regulatory rather than structural genes, and it has been proposed that regulatory functions are not routinely acquired by means of horizontal transfer (Ochman, 1998). The horizontal transfer of LuxI and the potential importance of horizontal gene transfer in Collectively, these results provide additional support for functional capabilities through horizontal gene transfer, therefore suggest that bacteria not only can gain new functional capabilities through horizontal gene transfer, but that they can gain new regulatory circuits and/or signalling abilities as well. As a specific example, *Escherichia coli* and *Salmonella typhimurium* may have the ability to respond to acyl homoserine lactone autoinducers generated by other organisms (Ahmer et al., 1998; Sitnikov et al., 1996), primarily because the ability to process that information through a LuxR homologue was itself acquired from other organisms. The functional organization of the *Pseudomonas aeruginosa* LasI/LasR and RhII/RhIR quorum sensing systems provides a further example of the potential for acquired regulatory functions to be incorporated within existing regulatory pathways. These data therefore suggest that integrated, hierarchical signalling cascades can potentially arise through the sequential addition of independently acquired regulatory genes.

**ACKNOWLEDGEMENTS**

We thank J. A. Downie, M. Givskov, S. Lewenza, P. Sokol, S. Winans, M. K. Winson and J. Zhu for sharing sequence data in advance of publication. We also thank A. K. Chatterjee, C. Fuqua, P. D. Shaw and P. Williams for helpful information about other unpublished sequences. This work was supported by NSF Grant MCB-9600766 to K.M.G. and USDA Grant 9502133 to J.R.G.

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Received 15 February 2001; accepted 1 May 2001.