suppressor genes are present in natural populations but at low to moderate frequencies[26]. This observation may be related to laboratory studies, indicating that many suppressor alleles seem to exert detrimental effects on viability and/or fertility in the homozygous state. Periods of severe inbreeding, as associated with the founding of genetically isolated populations, could result in the rapid loss of suppressor alleles and a consequent sudden release of new TE-mediated regulatory phenotypes. Such a scenario may help explain how novel regulatory variants, which in a large randomly mating population might be selected against, could become established in small isolated populations and perhaps lead to the emergence of phenotypically distinct species (Fig. 3).

Conclusion

As data continue to accumulate over the next several years, we should be in a better position to evaluate definitively the role played by TEs in regulatory evolution. Nevertheless, based on presently available evidence, it seems clear that the once popular notion that TEs are merely junk DNA and of no evolutionary consequence is no longer tenable. On the contrary, it may turn out that these small viral-like DNA sequences are critically important to the sudden emergence of phenotypic novelties over evolutionary time.

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Pheromones, social behaviour and the functions of secondary metabolism in bacteria

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The functions of secondary metabolites in bacteria are generally not known, although it is to be assumed that their production in nature must be of some benefit to the producer organism. Most microbial secondary metabolites may perhaps best be viewed as pheromones. Their production may thus represent a form of microbial social behaviour. Because cells that are close to each other spatially are normally closely related genetically, a simple application of Hamilton's rule may be used to account for the benefits that such secondary metabolite production afford the producer.

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The term 'secondary metabolite' was first explicitly applied to microbiology by Bu'lock in 1961 (Ref. 1). While there is a certain arbitrariness, and indeed imperfectness[2], in the exact definition of secondary metabolism and a secondary metabolite, Bu'lock's definition distinguished secondary metabolites from the 'general' (i.e. primary) metabolites, which are produced by most organisms, 'as having, by contrast, a restricted distribution (which is almost species specific) and no obvious function in general metabolism'. In submerged batch cultures of microorganisms, the onset of secondary metabolism often correlates with the ending of the most rapid growth phase (the trophophase) and the beginning of the stationary phase or 'an *idiophase* (Greek *peculiar*) in which are displayed metabolic idiosyncracies.... The concept of primary and secondary metabolic processes, as now generally understood, is clearly related to this distinction'[3].

While secondary metabolism is perhaps easier to recognize than to define, we shall adopt the view that its crucial feature is indeed a restricted distribution among a very small number of organisms[4], a fact that alone might lead one to consider that secondary metabolites could be expected to confer specific benefits on their producers. However, in the three decades since Bu'lock's papers, the functions of these molecules generally remain far from obvious. Here, we raise arguments in support of the view that many, if not most, bacterial secondary metabolites are best viewed as pheromones. Although similar arguments may undoubtedly be applied to other unicellular organisms, such as ciliates, algae and fungi, our focus will be on the relevant phenomena in bacteria,

and it is worth pointing out that a number of secondary metabolites, such as tetrodotoxin, that were once ascribed to higher organisms are now known to be the products of symbiotic or commensal bacteria[5].

Bacterial pheromones

> A pheromone is a chemical excreted by an organism into the environment that acts to elicit a specific response from other organisms of the same species. The importance of pheromones in the life cycle of various species of mammals, insects and fungi is well known. In the past decade, it has become apparent that pheromones influence the behavior and development of prokaryotes. Pheromones excreted by myxobacteria, actinomycetes and cyanobacteria elicit specific developmental responses from these organisms. In addition, pheromones excreted by *Enterococcus faecalis* function in conjugation, and pheromones of luminous bacteria regulate bioluminescence of these organisms.

This quotation represents the abstract of the 1986 review by Stephens[6], in which she sought, successfully and from a very dispersed literature, to 'collect and describe cases of pheromone production by prokaryotic organisms and the responses elicited by these signalling substances'. In the cases considered, the organisms exhibited obvious visual or morphological changes on exposure to the relevant pheromone. Thus, myxobacteria aggregated to form fruiting bodies, *Enterococcus* (previously *Streptococcus*) *faecalis* mated (underwent conjugation), *Vibrio* bioluminesced, *Streptomyces* species sporulated (and synthesized antibiotics, *inter alia*), and the filamentous cyanobacterium *Cylindrospermum licheniforme* formed akinetes (spore-like structures). Subsequent events have served amply to flesh out the details of these and other responses, such that it is being increasingly widely recognized that a variety of prokaryotic microorganisms communicate with members of their own species by means of chemical signals that can elicit profound physiological changes.

The types of responses to these pheromones nicely parallel those in the much better-understood social insects[7] and include communicating changes in nutritional availability, and the desire to mate and to aggregate. A number of recent reviews are available[6,8–14], and a summary of a variety of prokaryotic systems appears in Table 1. In many cases, these pheromones are small, diffusible molecules of known structure. For present purposes, however, the conclusion is clear: prokaryotic pheromone production is widespread (and plausibly universal), and pheromones are involved in a number of known microbial activities. By definition, such pheromones are excreted by producer cells (and they sometimes elicit their own production), and in the absence of exogenous molecules their actions necessarily exhibit a cell-density dependence.

Secondary metabolism: in search of function

Although some authors have argued that secondary metabolites may be 'waste' or 'overflow' products[15,16], or evolutionary leftovers with a previous autophysiological function but no *modern* function[17], many commentators do assume that (the chemical activities of) secondary metabolites must be of some benefit to the producer under at least some of the conditions that it encounters in nature. It is therefore reasonable, as for instance Campbell[4], Luckner[18] and Vining[19] do, to distinguish explanations for secondary metabolism that are based on activities that affect the producer species itself (e.g. Refs 4,19–24) ('intrinsic functions') from those (e.g. Ref. 25) that assume that their major role in improving survival is by affecting competing species, for example by antibiosis ('extrinsic functions'). In view of the definition of pheromones above, however, we need a slightly subtler distinction: if the producer organism is a single microbial cell, and the 'target' organism is a different member of its own species, pheromones function neither purely intrinsically nor purely extrinsically. It therefore seems best simply to refer to such a functionality as pheromonal.

Thus, instead of seeking to define the functions of secondary metabolites more specifically, for instance, as antibiotics, mineral scavengers (siderophores), differentiation signals, morphogenetic agents, sporulation inducers, and so on[4,19], a more useful view at this level is that most microbial secondary metabolites may best be construed as pheromones, since they are certainly excreted (and, as Stephens[6] points out, often tend to be moderately lipophilic), and in some cases do have demonstrable activity on the producing cell and its relatives. That no functions have been found in all cases merely reflects the poverty of methods at our disposal for analyzing physiology at the single-cell level, and for dealing with the extraordinary heterogeneity observable within even the simplest laboratory cultures of non-filamentous, planktonic bacteria[26,27].

It pays to advertise: the evolution of social behaviour

If one accepts that at least some microbial secondary metabolites are pheromonal, and thus involved in the social behaviour of members of a given species, how then should we seek to quantify the benefits that their excretion brings to the producer cell? Although neither microbial ecologists nor those natural-products chemists, who seek function in secondary metabolism, seem to have taken up these ideas in any detail at all, the appropriate framework here is the body of ideas of genetical kinship theory encapsulated in Hamilton's rule. Hamilton[28,29] extended the scope of fitness theory to include the effects of the actions of individuals on their genetic relatives, to show how traits that do not increase the likelihood of an individual's successful reproduction can be selected for and spread if they confer a sufficiently great benefit on kin. Specifically (and see Refs 30,31), if we consider an action by an individual organism (such as the production of a pheromone), it has a cost in terms of a decrease in the number of its own offspring, denoted by c, and a benefit in the increase of the recipient's offspring, denoted by b. The donor and the recipient are related to each other by a degree of relatedness, r. (Of course, in the case of microbes in nature that reproduce asexually, the degree of relatedness between parents, children, grandchildren, and so on, is very close to 1.) Hamilton's rule then states that the social action is favoured by selection if $rb - c > 0$. Given that cells in nature will normally be located adjacent to their parents and siblings, and that those on the edge of a colony are aware of the surrounding nutritional status, the potential benefits, in terms of preparing one's kin for nutritional hardships or bonanzas to come, is very great. While this is not the place to develop this in quantitative detail, it should be clear that the costs of producing many of these signals are likely to be very small relative to the benefits, particularly if the molecules are autoinducers in the sense that the secretion of a pheromonal signal molecule actually stimulates its own synthesis by the target cells.

An example of bacterial cooperation: the breaking of dormancy in *Micrococcus luteus*

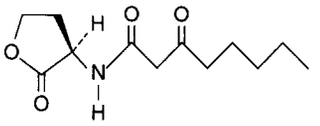
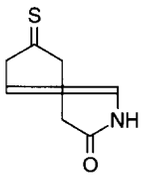
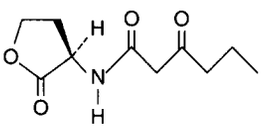
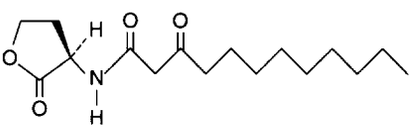
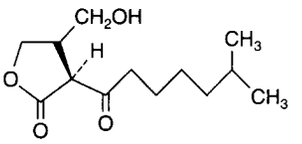
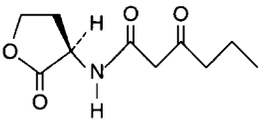
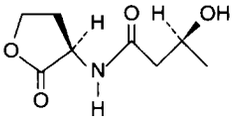
To give an example, (bacterial) dormancy may be defined as *a reversible physiological state of low metabolic activity, in which cells can persist for extended periods without division*. In bacteria, this often corresponds to a state in which cells are not 'alive' in the sense of being able to form a colony when plated on a suitable solid medium, but to a state in which they are not 'dead' in that when conditions are more favourable they may be resuscitated so as to revert to a state of 'aliveness' as

so defined[32]. We have recently found[33–35] that viable cells of *M. luteus* can secrete a pheromone-like substance, which is apparently necessary (though not sufficient) for the resuscitation of starved, dormant cells of the same organism. Why should such cells wish to resuscitate their kin? Clearly because for microbes in balanced growth, growth and reproduction go hand in hand, and in the competition for the new nutrients, it is much better for you and your siblings/clones to be quickest off the mark (without false starts, which would be costly in terms of protein turnover), since while the competition is waking up, you have depleted the new nutrient source and turned it into progeny and reserve material (see also Ref. 36). As well as the more conventional induction of gene expression, then, cooperation and pheromonal activation of metabolic pathways are thus a key to rapid response, and cognate arguments can self-evidently be applied to each of those systems (Table 1) in which the essential exogenous stimulus is a substantial change in nutrient status.

Some corollaries of the view of microbial secondary metabolites as pheromones

A number of authors have espoused the view that it can make sense even for typical laboratory strains that do not exhibit marked morphological differentiation to indulge in physiological differentiation, even in isotropic media, since this maximizes the chance of surviving unfavourable conditions that may arise[26]; Koch[37] refers to such strategies as an insurance policy, and carrying it out almost certainly requires signals. It is also clear from studies of colony dynamics (e.g. Refs 38–42) that cell–cell communication is a major, if unresolved, feature of bacterial growth on solid surfaces. One should also expect that, when viewed in quantitative terms (cf. Refs 13,43–46), these pheromonal signals should be honest in the sense used by Grafen[47] (since r is close to 1) and that there should be a fair degree of specificity within a genus or species; where the same or very similar pheromone molecules are used by different microorganisms (and for different purposes), as with *Erwinia carotovora* and *Vibrio fischeri* (see Refs 12, 48,49) it may be noted that in nature these organisms would not come into contact, the former being a pathogen of terrestrial plants, the latter a marine microbe that can participate in symbioses with the light organs of certain fishes. Correspondingly, where the signal is a molecule with a widespread species distribution (i.e. not a true secondary metabolite in our definition), as with glutamine for swarming in *Proteus mirabilis*[50], and perhaps in colonies

Table 1. Some examples of pheromone-like activity in prokaryotes

Organism	Role	Chemical nature
Agrobacterium tumefaciens[56–58]	Conjugation	N-(3-oxooctanoyl)-homoserine lactone 
Bacillus subtilis[59,60]	Genetic competence	Modified decapeptide
B. subtilis[61]	Sporulation	Not yet known
Cylindrospermum licheniforme[62]	Akinete (spore) formation	 (tentative)
Enterococcus faecalis[46,63,64]	Conjugation, leading to genetic exchange	Hydrophobic, linear hepta- and octa-peptides, e.g. PheLeuValMetPheLeuSerGly
Erwinia carotovora[11,14,65–67]	Autoinducer of virulence, carbapenem biosynthesis	N-(3-oxohexanoyl)-homoserine lactone 
Lactococcus lactis[68]	Aggregation/sex factor	Not yet known (protein has homology with lux)
Micrococccus luteus[32–35]	Resuscitation from dormancy	Not yet known
Myxococcus spp.[69–72]	Fruiting body formation and sporulation	Amino acids, and/or peptides and proteases; fatty acids
Pneumococcus sp.[73]	Genetic competence	Protein, mol. wt c. 10 000 Da
Proteus mirabilis[50,74]	Swarming and virulence	Glutamine
Pseudomonas aeruginosa[49,66]	Autoinducer of virulence genes	N-(3-oxododecanoyl)-homoserine lactone 
P. aureofaciens[75]	Autoinducer of phenazine antibiotic biosynthesis	Not yet known
Streptomyces griseus[9,76–78]	Autoinducer of sporulation and streptomycin biosynthesis	2S-isocaproyl-3R-hydroxymethyl-γ-butyrolactone 
S. virginiae[44,45,76,79]	Autoinducer of virginiamycin biosynthesis	Various butyrolactones/butanolides
Vibrio fischeri, *V. harveyi*[11,12,14]	Bioluminescence	N-(3-oxohexanoyl)-homoserine lactone (*V. fischeri*)  N-(3-hydroxybutanoyl)-homoserine lactone (*V. harveyi*) 

generally, it is reasonable that the high cell density of producer and recipient organisms means that over the relevant spatial location they are virtually axenic.

Finally, it is worth stressing that the existence of pheromones implies the existence of receptors, and that there is, in fact, increasing evidence that prokaryotic microorganisms may possess receptors even for higher eukaryotic hormones such as insulin[51,52]. This would imply that the evolutionary roots of the vertebrate endocrine system may be far more ancient than is generally believed[52–54], and adds weight to the view that intercellular signalling by pheromones may be a property of all bacteria.

In conclusion: (1) the phenomenon of cell–cell communication between prokaryotic organisms of the same species is widespread, and probably not at all confined to differentiating organisms in the usual sense; (2) any major change in physiological state induced by environmental factors is in one sense 'developmental', and may be communicated by chemical signalling to other organisms of the same type; (3) the 'function' of so-called secondary metabolites is actually connected with regulation of their own physiology (and not, say, as an antibiotic weapon against other species – such biological activity usually occurring at higher concentrations; see also Ref. 55); (4) therefore, such secreted bioactive molecules should in many cases be considered as semiochemicals or pheromones; and (5) there are straightforward ecological and evolutionary reasons, based on genetical kinship theory, why bacteria and other unicellular microorganisms should indeed behave in this way. As well as opening up a major area of bacterial physiology, we believe that this realization has substantial implications for microbial drug discovery and production.

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