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Bacterial observations: a rudimentary form of intelligence?

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Genome sequencing has revealed that signal transduction in bacteria makes use of a limited number of different devices, such as two-component systems, LuxI–LuxR quorum-sensing systems, phosphodiesterases, Ser-Thr (serine-threonine) kinases, OmpR-type regulators, and sigma factor–anti-sigma factor pathways. These systems use modular proteins with a large variety of input and output domains, yet strikingly conserved transmission domains. This conservation might lead to redundancy of output function, for example, via crosstalk (i.e. phosphoryl transfer from a non-cognate sensory kinase). The number of similar devices in a single cell, particularly of the two-component type, might amount to several dozen, and most of these operate in parallel. This could bestow bacteria with cellular intelligence if the network of two-component systems in a single cell fulfils the requirements of a neural network. Testing these ideas poses a great challenge for prokaryotic systems biology.

Introduction

Microorganisms were discovered at the end of the nineteenth century, predominantly as a result of their ability to cause disease (in animals and in plants), and because of their specific role in the biogeochemical cycles on the Earth's surface. This gave rise to the idea that each microorganism would carry out only one particular task in nature. Partly because of this, until relatively recently bacteria were generally considered to be little more than 'bags of enzymes', that is, too small to use the complex processes of signal transduction to regulate cellular processes, such as gene expression, not to mention intra- and intercellular communication [1].

However, this view has changed radically in the past three decades as molecular genetics and subsequently genome sequencing became generally applicable to problems in microbiology. These techniques led to the discovery of a kaleidoscope of regulatory mechanisms that enable even the simplest bacterium to express the required components for a particular process at the appropriate place and time. Although in this context it is difficult to give an exact definition of signal transduction, this process is characterized by the conversion of an (extra)cellular signal of some chemical or physical form into an entirely different form (e.g. from a photon into the phosphorylated form of a protein) that affects gene expression or enzyme activity (e.g. the cell's swimming behaviour through modulation of flagellar rotation).

The diversity among prokaryotes, both at the genus and species levels, is bewildering, particularly when one realizes that the majority of prokaryotes remain to be described [2]. However, even their description will remain problematic as long as the percent identity of the 16S rRNA gene(s) and the percentage DNA–DNA hybridisation are taken as the key assignment criteria for genera and species, respectively. It might be anticipated that in the future these problems will dissolve, as genome sequence comparisons will become the norm.

Considering this, it comes as a surprise that signal transduction in microorganisms is dominated by a restricted number of molecular mechanisms. For the prokaryotes (i.e. the Bacteria and Archaea) the most important of these are: (i) two-component systems [3], (ii) LuxI–LuxR-based quorum-sensing systems [4], (iii) phosphodiesterases [5], (iv) Ser-Thr (serine-threonine) kinases [6] and (v) OmpR-type regulators [7]. For representatives of all of these types of systems, detailed molecular, structural and functional information are available for signal input, signal transmission and signal output processes. Because the most detailed information is available for two-component systems, their properties form the focal point of this review.

For several sequenced model organisms, inventories have been made of the number of different two-component systems in individual cells of a particular species [7]. This analysis has revealed that several of these types of system are present in large numbers in individual cells. Because most of these systems operate in parallel [8], this also makes it relevant to discuss the network properties of the signal transduction processes that they catalyse.

Two-component regulatory systems

Sequence comparisons of widely divergent regulatory systems in Bacteria, each making use of phosphoryl transfer, revealed that signal transduction is frequently mediated by pairs of proteins consisting of a sensor and a response regulator. The sensor displays signalling-modulated histidine protein kinase or phosphatase activity, and the response regulator carries out phosphoryl transfer to one of its aspartyl side chains, coupled to the modulation of output (mostly transcriptional) activity (this is the classical type of two-component system; for a review see Ref. [9]). The word protein must be interpreted with some creativity here because both these activities can be catalysed not only by a single large multi-domain protein, but also by

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several separate proteins that form a cognate set. However, a subset of two-component systems has been described in which three subsequent steps of phosphoryl transfer take place: from the His in the sensor, to an Asp in the first response regulator, to the His in the phospho-transfer domain, to the Asp in the second response regulator. These are the so-called phospho-relay systems [10], which provide (compared with the 'classical' type; see above) additional targets for regulation, for example, through dedicated phosphatases. The sensor is often an integral membrane protein, with its signal-sensitive domain on the extracellular side of the cytoplasmic membrane, but sensing domains can also be present in the cytoplasm. Although the signals received can physically differ widely in nature, and include photons, metabolites, metal ions and turgor pressure [11] (Figure 1), many of these systems function to modulate bacterial virulence. In the presence of a signal, the balance between kinase and phosphatase activity in many sensors shifts towards kinase activity, although both activities can use the same active site [12].

The number of two-component systems within a single bacterial species varies considerably, with the number of systems ranging from zero to several dozen (Box 1). Prototypic bacteria, such as *Escherichia coli* and *Bacillus subtilis*, contain more than 20, whereas free-living organisms tend to contain more of these systems than parasitic bacteria [7,13]. Most systems only provide the cell with a competitive advantage, but some catalyse a vital cellular function [14]. This latter observation has boosted initiatives to search for inhibitors of two-component systems that could be developed into a new generation of antibiotics, the more so because two-component systems are only occasionally identified in lower Eukarya and plants, and have not, to date, been identified in any mammal. Because the sensory kinases form a separate domain within the large GHKL ATPase family [15] it can be anticipated that selective inhibitors might be designable.

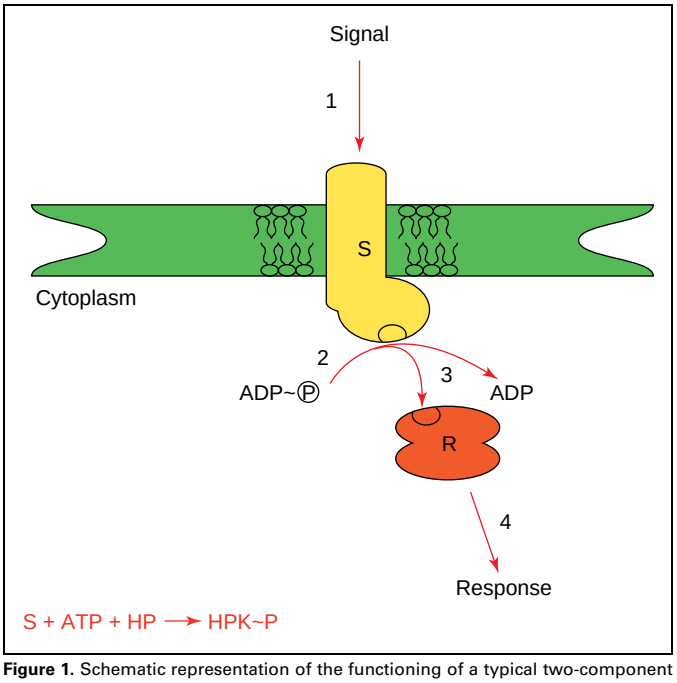

Figure 1. Schematic representation of the functioning of a typical two-component system. (1) The input domain of a transmembrane sensory kinase and/or phosphatase (S) is activated by binding a signal molecule; (2) because of activated kinase activity its transmitter domain phosphorylates itself with ATP (ADP~P); (3) the receiver domain of the cognate response regulator transfers the phosphoryl group to one of its aspartyl side chains; and (4) the output domain becomes activated to, for example, bind to a promoter region of the DNA. The overall balance equation of this reaction is given at the bottom of the figure. In the absence of signal, phosphatase activity of the sensor often dominates over kinase activity. Adapted, with kind permission of Springer Science and Business Media, from Ref. [11].

Box 1. The 'phospho-proteome' of bacteria

In the 'omics' context it is often emphasized that it is very important to characterize, for a specific species, the 'phospho-proteome' (i.e. an inventory of all proteins that can become phosphorylated *in vivo*). Important as this might be, there are significant technical hurdles that must be overcome before this can be achieved. For proteins phosphorylated stably on serine, threonine and/or tyrosine residues (e.g. through the activity of the corresponding Ser-Thr and Tyr kinases) this can be achieved through the application of newly developed proteomics methodology based on mass spectrometry, radiolabelling and/or the use of specific antibodies.

Technically more challenging, however, is the task of characterizing the subset of the phospho-proteome that is phosphorylated on aspartyl and histidyl residues. The inherent chemical instability of their linkages makes it necessary to carry out a stabilization reaction before the phospho-proteome characterization can be reliably carried out with mass spectrometry. Methodology designed to achieve this has been described that involves conversion to homoserine, using a reaction with borohydride in DMSO [54]. It should also be kept in mind that the proteome of lower eukaryotic cells might contain a subset of these non-stably phosphorylated proteins, considering the phylogenetic distribution of the two-component systems.

The spatial structures of some histidine kinase domains and several response regulators have been resolved using X-ray crystallography and NMR (nuclear magnetic resonance) spectroscopy (for a review see Ref. [16]). The kinase domains are composed of an ATP-binding domain plus an H-box in a two- or four-helix bundle domain that also functions as a dimerisation domain. They crystallize as dimers with a similar fold to that of the ROP (replication of plasmids) protein from *E. coli*. The phylogenetic relationship between the sensors and the other kinase groups has not yet been resolved, but a case can be made for the descent of some of the bacterial Ser-Thr kinases from phospho-relay-type histidine protein kinase domains. After all, several response regulators can become phosphorylated on a serine rather than on an aspartyl side chain [17].

Dimer formation is crucial to the kinase activity of the sensors because ATP bound to one-half of a dimer is the source of phosphate that is transferred to an exposed histidine in the helix bundle of the other [18]. The structure of the lid over the ATP-binding site of classical-type and phospho-relay-type sensors correlates with the phosphatase activity of that specific sensor (i.e. particularly when the cognate signal is absent). This provides the phospho-relay systems with very high (zero-order) sensitivity towards variations in the strength (concentration) of the signal to which they respond and makes such sensors more suitable to function in crosstalk [19]. The catalytic function of phosphoryl transfer resides in residues around the aspartyl side chain of the regulators [9]; its specificity in a limited number of residues of both kinase and regulator [20].

For thermodynamic reasons the phosphorylation of proteins on an aspartyl side chain is optimally suited to

elicit conformational change [9]. However, the *in vivo* stability of this linkage is often limited to seconds or minutes. This causes considerable technical constraints in the analysis of the level of phosphorylation of response regulators *in vivo*. Nevertheless, the protein environment can modulate this stability considerably. Examples have been described where a particular response regulator can be isolated in a stable phosphorylated form following heterologous overproduction in *E. coli* [21]. The instability of phospho-aspartyl linkages also provides advantages. Activation of a particular regulator requires a continuous flux of phosphoryl groups, emanating from the cognate sensor. Exhaustion of the signal then leads automatically to a switch-off. Therefore, the rate-limiting factor in this deactivation could, particularly for very-high-affinity sensors, reside in the dissociation rate of the signalling molecule from the sensor. A simple calculation, based on the fact that the affinity of the sensor for its ligand equals the ratio of the rate constant for binding over the rate constant for dissociation of the ligand, illustrates that to achieve nM affinity, even with diffusion-limited ligand binding for a ligand that is present in μM concentration, requires off-rates that are far slower than $1\ s^{-1}$.

Considering this, it is significant that several systems have recently been described in which the sensor not only binds, but subsequently also metabolises, the signalling molecule. Examples are the hydrogen sensor HoxBC from *Ralstonia eutropha*, which functions as a hydrogenase [22]; a sensor from purple bacteria that senses a rate of electron transfer (i.e. oxidises a reduced – unknown – signalling molecule [23]); and the glucose-6-phosphate sensor UhpBC from enterobacteria, such as *E. coli*, which not only binds, but also transports this substrate [24]. Furthermore, the photoactive sensory proteins that show a dark-reversible photocycle, such as the archaeal sensory rhodopsins, also fall in this category [25]. As the turnover rate of these 'enzyme-sensors' is probably faster than $1\ s^{-1}$, this could be one way to limit sluggishness of the response of some systems to deactivation. Nevertheless, the collection of two-component systems in a single cell could have different deactivation time constants.

Environmental sensing, neural networks and intelligence

Although most molecular characterizations of signal transduction in bacteria have been carried out on isolated systems, in their natural habitat bacteria have to respond, with an 'informed decision' [26], to a wide range of simultaneous and fluctuating signals. It is therefore important to analyse how a collection of such systems interacts within an individual cell (i.e. to analyse their network properties). The chemical basis of this network is the transfer of phosphoryl groups from ATP, via histidine and aspartyl side chains, eventually to water.

An important characteristic of transcriptional regulation in general, and signal transduction in bacteria in particular, is that the majority of its components are simultaneously expressed [8,27] (i.e. they mostly function in parallel) and cascade-wise organization [28] is relatively rare. Beyond this parallel functioning, many two-component systems show the phenomenon of auto-amplification [29], which means that when a signal activates such a system, its expression is boosted. Furthermore, because of a branched organization (e.g. the Kin–Spo system from *B. subtilis*) or because of crosstalk, one particular regulator can be subject to phosphorylation by more than one sensor. In this case, the regulator carries out a 'logical operation' by summing the phosphoryl flux through the separate sensors that phosphorylate it. Regulators are often considered to be very simple two-state (i.e. digital) signal transducers [30]. The first-order approximation therefore holds that the steady-state concentration of their signalling state is proportional to the sum of the activity of all sensors acting upon it.

The molecular properties of the sum of the two-component systems in a typical bacterium, such as *E. coli*, can therefore be summarized as follows: (i) multiple (branched) systems operate in parallel; (ii) key components carry out logical operations; (iii) the basic elements of this network are subject to auto-amplification; and (iv) crosstalk does occur between the pathways. The extent, to which this latter process occurs, however, remains to be characterized in more detail (Figure 2). Strikingly, the characteristics of such a network are identical to the properties that have been assigned as the prerequisites to make any network perform as a 'neural' network [31]. This leads to the

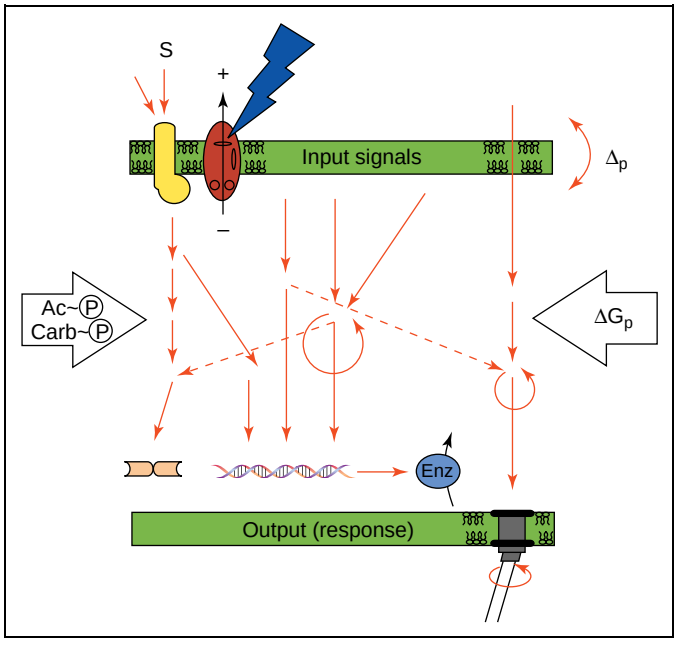

Figure 2. Salient biochemical aspects of the mutual interactions between the various two-component systems in a single bacterial cell. The various two-component systems in a single bacterial cell are represented by sets of two to four sequential arrows. The arrowed circles represent the process of auto-amplification. The two-component systems might modulate using their output gene expression, enzyme activity, cell division and/or cellular (swimming) behavior. Specific input signals, for example, s (metabolic substrate) or hν (photons), activate two-component systems selectively. Response regulators as such can be activated in a non-selective way by low-molecular-weight phosphoryl donors, such as acetyl phosphate and carbamoyl phosphate. Signal transfer across the membrane might be modulated by the (size of the) proton motive force, and the energy state of the cells might modulate signal transfer very generally via the intracellular phosphorylation potential. A crucial (and as yet incompletely resolved) aspect of the functioning of the collection of two-component systems in a single cell is the extent to which the various systems 'crosstalk' (i.e. carry out mutual *trans*-phosphorylation). This process is represented by the broken lines. Adapted, with permission, from Ref. [32].

Box 2. Bacterial intelligence and associative memory

When analysing the characteristics of bacterial signal transfer processes in terms of the functional characteristics of higher organisms, such as memory, learning and intelligence, it is obvious that simplifications have to be made. In this simplification process, however, it is of utmost importance to define the (chemical basis of the) processes under consideration stringently. One example is the difference between adaptation and learning. When the former is defined as an adjustment of the metabolic machinery of the cell in direct response to altered physiological conditions, the latter can be defined as an adaptation that is mediated through altered levels of signal transduction components in the cell [29].

The most crucial feature of human intelligence is the ability to associate (i.e. to identify non-identical systems as being related). The mechanistic basis for this is the so-called associative memory: the ability to generate a certain response, elicited by a complex sum of stimuli, even when the set of stimuli shows small alterations. This is a typical neural network feature that can be identified after training the network with a combination of input signals, followed by a test in which one or more elements of the complex input signal are added or deleted.

idea, as formulated earlier [32], that the combined activity of all two-component systems in a single bacterium, because of their biochemical properties, could bestow bacteria with properties associated with intelligent cellular behaviour, such as associative memory and learning, and thus with a minimal form of intelligence (Box 2). Depending on the exact 'wiring' of the two-component systems in a particular cell even more complexity can be predicted, for example, the occurrence of partial networks that function independently and in parallel. Furthermore, with reference to its molecular basis, it would be appropriate to refer to such a network of two-component systems as a phospho-neural network, in which phosphoryl flow (based on kinase versus phosphatase activity) takes over the role of the action potentials of the neural network in the human brain and the electrical current in computer-based artificial neural networks.

Testing the applicability of the phospho-neural network concept

Of the four conditions listed that have to be fulfilled before the network of two-component systems can be classified as a neural network, three appear rather straightforward. (i) Because of the general structure of transcriptional networks in microorganisms [8] it is reasonable to assume that many two-component systems in a single cell will be operating in parallel. It remains to be seen whether and to what extent a cascade-wise organization can be described as a multi-layered network. (ii) Logical operations are involved via reversible phosphoryl transfer. The most straightforward assumption is that, for example, in the case of convergent crosstalk (i.e in the network that initiates sporulation in *B. subtilis*) the phosphoryl flux through the response regulator (i.e. Spo0A) will be the arithmetic sum of the fluxes through the sensors involved (KinA to KinE), particularly when the average phosphorylation level of the response regulator *in vivo* is less than 30–40%. This idea is supported by observations of CheY, where the *in vivo* level of phosphorylation in the presence of an activated kinase CheA is below 30% [33]. The competing regulator in the Che system (i.e. CheB) and the CheY phosphatase CheZ contribute to this relatively low phosphorylation level [34]. (iii) The degree of auto-induction does vary significantly between different two-component systems in a particular species. Some have been shown to be strongly inducible by their cognate signal, with respect to kinase and/or regulator concentration, whereas others are not. But then, for a network to be classified as a neural network, it is not required that all nodes show this characteristic of auto-amplification.

The fourth prerequisite requires more discussion. Two-component systems were initially defined on the basis of their mutual sequence similarity. This rapidly led to tests of non-cognate phosphoryl transfer between different two-component systems. Such *trans*-phosphorylation (i.e. crosstalk) was readily demonstrated *in vitro* for many non-cognate combinations of transmitter and receiver domains of two-component systems [35]. It is relevant to note, however, that the rate of phosphoryl flow via crosstalk is generally some orders of magnitude (two to four) slower than the corresponding cognate rate(s). However, to characterize crosstalk between two-component systems *in vivo* is much more complicated; this requires a proper definition of 'cognate', which is hard to give. Expression of a sensor and a response regulator from separate operons [36] does not automatically qualify such pairs as non-cognate and thus not all claims for crosstalk are equally relevant [37]. Several studies have reported the existence of crosstalk between unrelated systems in wild-type organisms *in vivo* [38–41], and many more reports are available detailing crosstalk in mutant strains, with a relevant sensor and/or response regulator over-expressed or after heterologous expression of the signal transduction proteins [35]. Two-component systems might also interact more indirectly, through (the intracellular level of) low-molecular-weight phosphoryl donors, such as acetyl phosphate [42]. By contrast, it is also clear that many two-component systems are 'wired' to primarily elicit a cognate response [3,9,13]. The balance between kinase and phosphatase activities of a particular two-component sensor will play a dominant role in determining the extent to which crosstalk occurs between specific two-component systems in a single cell.

Clear demonstrations of associative memory have not yet been detected in any single bacterial cell. When characterizing four randomly chosen major two-component systems in *E. coli* (i.e. Ntr, Pho, Uhp and Arc), some basic predictions of the phospho-neural network model were tested [43]. The results showed that, for the systems selected and analysed, the level of crosstalk precluded a clear observation of neural network behaviour (note that crosstalk is detectable only when it occurs at a rate that is more than a few percent of the cognate rate). For example, crosstalk towards the NtrC response regulator was only measurable when its cognate kinase was eliminated. It therefore appears that the cell 'values' the independent functioning of these systems more than their collective behaviour. More extensive network tests will require stringent physiological control and/or more detailed knowledge about the nature of sensor-activating signals.

It will be of interest to carry out a similar characterization with several two-component systems that all belong to the same subclass of sensors and/or response regulators [44]. Such a choice is relevant particularly in some phototrophic prokaryotes, where the extent of 'redundancy' of phytochrome-like two-component systems is very high (e.g. in *Nostoc punctiformis*).

The concept of the phospho-neural network has, however, been useful in sharpening ideas and definitions. On several occasions in the literature the question of whether or not bacteria have a 'memory' or can 'learn' has been raised. The memory effect in chemotaxis is well-established [45], and in bacterial physiology, memory effects have been reported that are referred to as 'learning' [46]. However, the learning process is best defined as a signal-induced increase in the concentration of signal transduction components in the cell. Even with this more stringent definition it can be concluded that bacteria can learn [29] and have a memory [47].

The most stringent tests of predictions from the phospho-neural network model require measurement of the level of phosphorylation of response regulators in intact cells. Considerable technical barriers must be overcome before such tests can be performed routinely. Recently, the separation of phosphorylated and non-phosphorylated ArcA on an iso-electric focussing gel was reported [48]. Applying this methodology to extracts of cells obtained by rapid sampling [49], in combination with Western blot analyses, should enable the relevant parameters to be assayed. In addition, the recently reported FRET (fluorescence resonance energy transfer) assay of the relative *in vivo* phosphorylation level of CheY [50] might become beneficial in such studies, particularly when information about relative changes suffices.

A further complication in modelling signal transduction is the distribution of the components involved throughout the bacterium. In most models they are assumed to be homogenously distributed throughout the compartment in which they are active (in particular, the two-dimensional volume of the cytoplasmic membrane and the non-nucleoplasmic volume of the cytoplasm are relevant in this respect). Nevertheless, several examples are available in which this assumption is clearly in disagreement with observations. The best known of these is the accumulation of chemotaxis proteins in one pole of *E. coli* cells. However, additional examples are also available, such as the aggregation of taxis proteins in 'organelles' in the cytoplasm of *Rhodobacter sphaeroides*, and the accumulation of two-component sensors involved in cell division in one of the poles of *Caulobacter*. However, evidence is not available for the majority of two-component sensors and response regulators, suggesting a non-random distribution. Furthermore, at this stage it is not at all clear how much models would gain in predictive power from a detailed account of this non-random distribution.

Extension of the application of the neural network concept

One of the most attractive features of the phospho-neural network concept is that it leads to testable predictions for the performance of a complex system, particularly when intuition fails. The occurrence of associative memory in the response to multiple and repetitive, but variable, stimuli is a case in point. Several other types of signal transfer could be analysed in a similar manner to their network characteristics. Obvious candidates are the quorum-sensing systems (Box 3) and the mitogen-activated protein (MAP) kinase pathways in lower eukaryotes (Box 4). However, more systems, such as the cyclic guanylate synthases, in combination with phosphodiester synthases [7], and the Ser-Thr kinases and phosphatases [51] do qualify.

Box 3. Quorum (or diffusion) sensing

Ongoing research has shown that another type of signal transduction mechanism, the process of quorum or diffusion sensing, is widely distributed among bacteria [4,55]. In such a process, an autoinducer (e.g. an N-acyl homoserine lactone in Gram-negative bacteria or a (cyclic) peptide in Gram-positive bacteria) accumulates at equal concentrations intra- and extracellularly, predominantly as a function of cell population density. Subsequently, when a threshold concentration is reached, a transcriptional regulator, which is part of a feedback loop that includes the gene encoding the autoinducer synthetase, is activated. This results in rapid accumulation of the autoinducer up to saturation levels.

These systems, particularly those based on the action of N-acyl homoserine lactones, could also fulfil all the requirements listed for making a network of such systems perform like a neural network. Multiple systems are present in individual species (e.g. five in *Burkholderia thailandensis*) [56]. Crosstalk in these systems can be mediated by sensitivity of one particular transcriptional regulator towards a series of N-acyl homoserine lactones (e.g. with increasing tail length), and also by broad-specificity lactone synthetases [57]. Auto-amplification is widely observed in quorum sensing, via regulator control of the gene encoding the regulator itself and/or the autoinducer synthase.

The future: molecular systems biology in bacteria

If one is not so stringent about the condition that the molecular basis of signal transfer between the interacting systems must be the same, many more systems qualify to be analysed with respect to their network properties. An important example of this is the interaction of the most important global regulatory systems in a single bacterium (i.e *E. coli*), for example, catabolite repression, cold and heat shock, nitrogen regulation, and the stringent and SOS responses. The problem with this approach, however, is the fact that it will be extremely challenging to quantitate the relative strength of the connections between the various regulatory pathways involved.

Nevertheless, the continuing insight into the physiological and regulatory processes in bacteria enables mathematical modelling to become more detailed. In addition, the complex set of chemical reactions that sustain prokaryotic life can be split into conceptually independently functioning modules [52]. This fact can be exploited in the simplifications required for mathematical modelling of a living cell. By founding such models on the molecular basis of these processes, a common ground can be found to link them and eventually extend them to the description of an entire prokaryotic cell. Such models are challenging, particularly because of the need to base them on an explicit molecular basis that takes account of measured numerical values of relevant binding constants, association and

Box 4. A neural network in a single eukaryotic microorganism?

The molecular basis of signal transduction in Eukarya is significantly different from that in Bacteria. There are no examples of eukaryotic organisms in which a multitude of two-component pathways operate in parallel. In Eukarya there is, however, an alternative system that might function similarly as the collection of two-component systems in Bacteria: the MAP kinase pathways. In these systems three activatable Ser-Thr kinases (i.e. a MAP-, a MAPK- and a MAPKK-kinase) [58] function sequentially, to finally activate a eukaryotic transcription factor. The yeast *Saccharomyces cerevisiae,* for example, uses six of these pathways in parallel, and the constituent kinases show significant non-cognate activity (i.e. 'crosstalk'), as has been demonstrated for various combinations (see references within Ref. [58]). In addition, there can be extensive crosstalk with other types of protein kinases [59]. When crosstalk occurs, these systems carry out logical operations in terms of a summation (or subtraction when phosphatases are involved) of the phosphorylation level of the regulatory components. Moreover, auto-amplification of expression of the components of MAP kinase pathways has been demonstrated [60]. This indicates that a network of MAP kinase pathways also displays all the properties required to show neural network-like output characteristics. Whether or not it does, remains to be proven.

One specific factor – absent from two-component systems – complicates the analysis and interpretation of experiments on these MAP kinase pathways. This is the involvement of so-called scaffold proteins [61]. These proteins organize single copies of each of the three kinases of a MAP kinase pathway into one large supercomplex, and thereby considerably increase intra-pathway specificity. Such organization appears to isolate the pathway from external effectors. The molar ratio of scaffold over protein kinase components will therefore be an important determinant of the cell's signalling characteristics. Furthermore, evolutionary mechanisms might be operative in yeasts that minimize interactions through crosstalk between homologous systems [62]. On the positive side is the fact that mathematical modelling of the activity of these MAP kinase modules has advanced significantly and that MAP kinase signalling can be engineered – and thus modulated – with high precision [63].

dissociation rates, measured under *in vivo* conditions. Although key aspects in this approach are lagging behind, such as the proper description of morphogenetic processes (i.e. processes in which regulated gene expression leads to the generation of structures with a specific morphology), significant progress has been achieved. Work along these lines will ultimately lead to a molecular 'systems biology' description of organisms such as *E. coli* [53]. This approach might turn out to be indispensable to sufficiently understand both the profitable and the virulent activities of *E. coli* (and other microorganisms), with respect to the needs of mankind.

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