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Perspectives in Biochemistry

Chemotaxis as a Model Second-Messenger System[†]

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Bacterial chemotaxis was studied initially as a model sensory system, and indeed much of that expectation has been fulfilled. The covalent modification of receptors, the integration of signals, the importance of electrical and chemical potentials, adaptation, and other features are now seen to be common to many signaling strategies. Moreover, studies in neural and hormonal cells indicate that there is a common pattern of receptors, signal integration, and output. But one of the main puzzles of second messengers is the complex interactions between pathways. Instead of entirely separate pathways—one for phospholipids, one for cyclic AMP, one for Ca^{2+}, etc.—intricate cross-reactions are observed. The finding that phosphorylation plays a major role in the excitation pathway of chemotaxis, together with the role of methylation in adaptation, presents a similar challenge in interrelationships. The possibly simpler and certainly genetically more accessible bacterial chemotaxis systems thus continue to offer a model for significant aspects of other systems.

Overview of the Chemotaxis System

Bacterial chemotaxis was discovered in 1880 by Pfeffer and Engleman and brought into the modern world by Julius Adler in his pioneering studies (Adler, 1966). Bacteria under the microscope tend to swim randomly in the absence of any stimulus but can respond to a repertoire of attractants and repellents, which generally correlate with molecules that are good for them (oxygen, aspartate, serine, glucose) and those that are bad for them (low and high pH, low and high temperature, phenol, etc.). Flagella are driven by membrane potential.

Bacteria respond to changing conditions by modulation of their tumbling pattern (Brown & Berg, 1972; Macnab & Koshland, 1972). When bacteria go in a favorable direction, they suppress tumbling and continue in the same direction. When going in an unfavorable direction, they generate tumbling, which results in a change in direction. This behavior is controlled by a rudimentary memory, which allows the bacteria to compare past and present, and by the phenomenon of adaptation, which allows them to desensitize constant background stimulation (Macnab & Koshland, 1972). The chemistry of this adaptation was demonstrated to involve methylation of membrane-bound proteins, initially called MCPs and later identified as receptors of aspartate, serine, and dipeptides and as tranducers of ribose, galactose, and maltose. A schematic model of the receptor and a role for methylation in adaptation are shown in Figure 1.

The World of Methylation

One of the important areas involving both controversy and mystery is the role of covalent modification. The early identification of methylation with membrane proteins (the MCPs) (Kort et al., 1975; Ridgway et al., 1977) followed by the identification of these MCPs as receptors (Clarke & Koshland, 1979; Wang & Koshland, 1980) provided a central function for methylation (Springer et al., 1979; Koshland, 1980). Moreover, the early studies on this process clearly identified methylation with the adaptation property (Aswad & Koshland, 1975), rather than with excitation, since excitation can occur in the absence of methylation (Springer & Koshland, 1977) and the timing of adaptation correlates with methylation (Kleene et al., 1979). A large amount of knowledge could be explained by simple models, as shown in Figure 2. Although the concept of "memory" (Macnab & Koshland, 1972) did not require that the slow step be identified with methylation, a series of findings led to this conclusion.

Consistent with this model was the finding that mutants which eliminated the methyl esterase (Es^-) prevented chemotaxis (Stock & Koshland, 1978) and mutants which eliminated the methyl transferase (Tr^-) also prevented chemotaxis (Springer & Koshland, 1977). This applies, of course, to those receptors that were identified with methylation since others are known which can signal without methylation (Niwano & Taylor, 1982). Therefore, it was a considerable surprise that a double mutant (to be referred to as the Tr^-Es^- mutant) in which both the esterase and transferase were removed restored chemotaxis (Stock et al., 1981, 1985b). The chemotactic ability in the Tr^-Es^- mutant appeared to be approximately 10%

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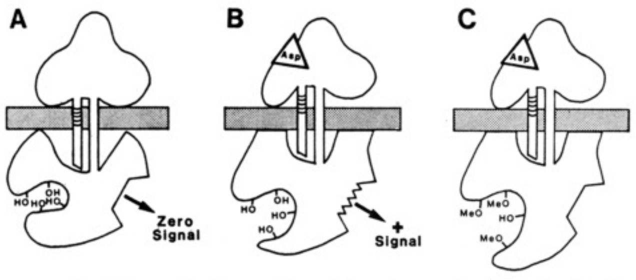

FIGURE 1: Schematic illustration of signaling and adaptation in the transmembrane aspartate receptor. (A) The unstimulated receptor is shown with an external domain that is responsible for ligand binding, two transmembrane segments, and a cytoplasmic domain that can send a signal and be methylated. The glutamic acid groups are symbolized here by OH groups to emphasize the net change of methylation in the following steps. (B) The conformational change induced by the binding of aspartate causes the transmembrane region to be pulled up slightly (shown as three notches) into the external space. This in turn causes an alteration of the cytoplasmic portions (shown here as compression against the membrane), which brings about changes in the signaling region (indicated by the jagged line) and in the methylation region (indicated by the exposure of the OH groups of the relevant glutamic acids). As a result, the aspartate-bound receptor becomes more highly methylated, which somehow releases the stress at the signaling site caused by binding of aspartate. (C) At the new steady state, the methylation sites are sufficiently modified to bring the structure back to the nonsignaling mode, even though aspartate remains bound to the protein.

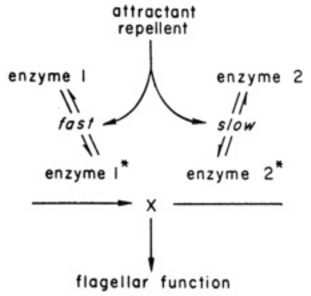

FIGURE 2: Schematic of a memory model. The chemoeffector (attractant and repellent) acts at two places, shown here as involving the formation and decomposition of X, the response regulator. If one enzyme responds rapidly to a stimulus and the other responds slowly, there will be a transient increase (or decrease) in X, depending on the circumstances. If the factor increase in each step is the same, X will eventually return to its identical value (adaptation) with the length of the transient interval depending on the stimulus level. The fast step in effect measures the present concentration of attractant or repellent, whereas the slow step reflects the past concentration and the time; therefore, the level of X reflects the comparison of the past and the present (the memory).

of wild-type chemotaxis as measured on swarm plates (Stock et al., 1985b) and in capillary assay (Stock et al., 1985a).

The analysis of this phenomenon was of considerable interest because it revealed new properties of the sensory system (Stock et al., 1985b). A typical methylatable receptor, the aspartate receptor, contains four potential sites that are reversibly methylated in normal chemotaxis (Kehry et al., 1983; Terwilliger et al., 1986) (cf. Figure 3). Two of these residues initially appear as glutamine residues, which are hydrolyzed to glutamates by the esterase enzyme. Studies of the protein indicate that the properties of the receptor are very similar whether these groups are glutamine or glutamic acid methyl ester. Therefore, the double mutant containing the two amidated glutamates has a zero set point equivalent to that of a receptor which is dimethylated. The behavior of the bacterium in the absence of stimulus is random swimming, and the receptor has about two of the four glutamic acids methylated in that condition. Small perturbations either positive (an increasing attractive gradient) or negative (a decreasing attractive gradient) can perturb the system to alter tumbling frequencies. The double mutant with two amide groups is fortuitously balanced exactly where the normal receptor is balanced, and therefore it could respond to stimuli. The situation is different in the esterase minus (Es⁻) mutant, which tumbles incessantly because it is overmethylated and far removed from the zero point. It is also not available to the transferase minus (Tr⁻) mutant, which is undermethylated and deamidated, and is far removed from the zero point in the other direction. Thus the double mutant anomaly is explained in part by the chemistry that is involved in the "reset to zero" (Figure 3).

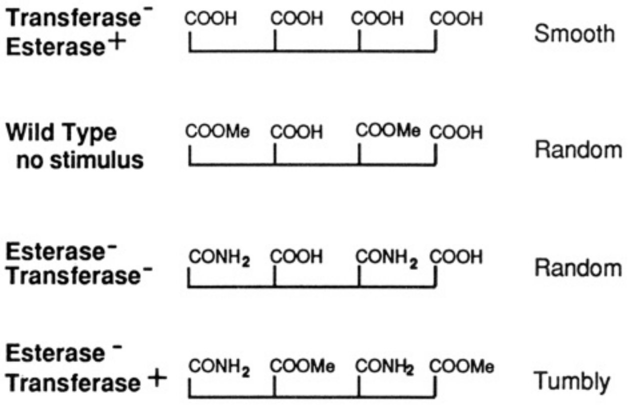

FIGURE 3: Illustration of the way in which the chemistry of the receptor allows a reset to zero mechanism for chemotaxis. The receptor initially has glutamine residues at sites 1 and 3 and glutamic acid residues at sites 2 and 4. In the wild-type cell, the glutamines are deamidated by the esterase and then remethylated by the transferase to produce a protein with two methyl groups on the average in the absence of a stimulus. This produces the appropriate random swimming pattern for the bacterium. In the transferase minus mutant, the esterase removes the two amide groups to form a protein which sends out a constant smooth swimming signal and thus cannot respond to small signals. In the esterase minus mutant, the amide groups are untouched but the remaining carboxyl groups are methylated, thus leading to an overmethylated receptor with a constant tumbling signal and a nonchemotactic phenotype. If, on the other hand, both the esterase and transferase are absent, the initial receptor contains two amide groups that signal a random phenotype similar to the dimethylated species, and thus the bacterium is poised to respond to signals in either direction. The double mutant cannot reset to zero over a wide range of stimuli, but a limited chemotaxis can occur.

This combination of properties led to a number of alternative hypotheses for the role of methylation in the memory process. Hypothesis 1 is that methylation is essential and the mutants are actually leaky, i.e., either small amounts of transferase or esterase activity still remained, or that there were other transferases and esterases not usually involved in chemotaxis which could supply enough covalent modification to enable chemotaxis. Careful tests of the point mutants and deletion mutants (Parkinson & Houts, 1982) that had the same phenotype and a sensitive assay for modification of the central methylated tryptic peptide eliminated this possibility (Weis & Koshland, 1987).

Hypothesis 2 is that the chemotaxis observed on swarm plates was in fact not a chemotaxis directed by the aspartate receptors, which have a requirement of methylation, but actually taxis to oxygen or repellents, which are methylation independent; i.e., the chemotaxis is due to an aspartate-stimulated oxygen or repellent response. Tests in capillary assays and in a defined gradient system (Stock et al., 1985a; Weis & Koshland, 1987) demonstrated that the chemotaxis occurs to aspartate acid in the absence of repellent or oxygen effects.

Hypothesis 3 postulates that methylation is necessary to "reset the system to zero" (Koshland et al., 1982) but is not needed in the sensing of the gradient that occurs by a methylation-independent pathway of the chemotaxis machinery (Stock et al., 1985a; Stock & Stock, 1987). The model in this case is not too different from the adaptation hypothesis postulated for rhodopsin, in which vision is achieved by the short-term excitatory system whereas phosphorylation is only needed for adaptation to the external background. Measurements of the timing of the excitatory system using impulse stimuli showed no adaptation during the period that was theoretically calculated to be required in order to detect the gradients (Berg & Block, 1984). In addition, bacteria swimming in a shallow exponential gradient of aspartate covering the entire range of stimuli from low occupancy to saturation of the receptor showed no appreciable chemotactic response (Weis & Koshland, 1987). Thus, small stimuli both in theory and in experiment failed to give chemotaxis in the Tr^-Es^- mutant. This result would seem to require methylation in sensing as well as "reset to zero". However, if either the transferase or the esterase has a dual function (see below), the mutant could be affecting the timing of the excitation system as well as the adaptation system. thus, hypothesis 3 seems unlikely, but not absolutely excluded.

Hypothesis 4 states that the methylation is involved in resetting the system to zero but that it also plays a role in the optimized memory utilized for the measuring of the gradient. The partial adaptation observed in the double mutant was explained theoretically by Stock et al. (1985b) and can now be identified with phosphorylation (see below). The partial adaptation is sufficient to give the limited chemotaxis observed in the double mutant. The nonmethylating aspartate response system does not operate as efficiently as a wild-type system and is only of importance when the methylation system has been eliminated. A possibility exists that this less efficient chemotaxis was the primordial chemotactic system and was gradually replaced in evolution by the more efficient and fine-tuned methylation system (Weis & Koshland, 1987).

THE RESPONSE REGULATOR

The response regulator has hovered like a grey eminence over the field of bacterial chemotaxis for a number of years. It was proposed at an early stage (Macnab & Koshland, 1972; Koshland, 1977) to explain the excitatory and adaptive properties of the chemotaxis system. So many properties of wild type and mutant, of attractant and repellent, can be correlated under the concept of a response regulator that there is little doubt that some such parameter exists.

In the case of chemotaxis, the response regulator is believed to control behavior and to rise and fall under the influence of attractants and repellents in much the way that temperature controls a furnace through a thermostat. For simplicity, it is assumed that an increase in the response regulator suppresses the probability of turning (or tumbling), and a decrease increases the probability of turning and tumbling. Thus, movement of a bacterium up a gradient of attractant generates an increase in the response regulatory and movement down a gradient causes a decrease. (The "increase" in the response regulator is only an algebraic nomenclature since the system has total logical symmetry and therefore either increase or decrease of a compound would be acceptable just as D and L isomers were arbitrary before an absolute determination of stereochemistry was made.)

An important characteristic of the response regulator is that it controls the probability of tumbling. Thus, the instant the bacterium moves up a gradient of attractant, its probability of tumbling decreases, and the instant it starts down a gradient, its probability increases. The statistical effect, which may seem inefficient to some, is highly efficient for bacteria that reverse quickly on going the wrong way and swim long distances when going the right way. Indeed, visualization in a microscope of bacteria migrating en masse toward a capillary makes it appear as though the bacteria are almost unerringly swimming toward attractant streaming out of the capillary, whereas, in fact, they use a biased random walk.

The early suggestion of response regulator to explain adaptation was bolstered by the finding of additivity between attractants and repellents (Spudich & Koshland, 1975; Berg & Tedesco, 1975), the timing of adaptation (Goy et al., 1977), the behavior of mutants (Slocum & Parkinson, 1985; Matsumura et al., 1977), etc. and has further been supported by the evidence in regard to double mutants (DeFranco & Koshland, 1980) and many other studies.

THE OUTPUT SYSTEM

The response regulator controls the output response by a system that has some similarity to the input via receptor. A membrane protein must at some stage bind the response regulator to change the output of the cell. Some time ago it was postulated that a switch existed in conjunction with the flagella which controlled this process (Rubik & Koshland, 1978; Parkinson et al., 1983). Mutant studies suggested that the CheC gene product was the protein on the flagellar motor which was part of this swtich (Khan et al., 1978). The finding that CheC codes for a membrane protein and probably a peripheral membrane protein added support to this hypothesis (Clegg & Koshland, 1985). Further genetic studies suggested that the CheV gene product performs a parallel function to the CheC gene, and the fact that the CheV protein is also a membrane-bound protein tends to support the assignment (Warrick et al., 1980; Dean et al., 1983). The peripheral membrane location of CheC protein is deduced from the fact that the flagellar apparatus showed no CheC gene product remaining after the washing and isolation procedures. The identification of the CheC gene product as a switch component provided further clues in regard to the signaling system, since intergenic complementation experiments suggested some type of protein interaction, with the gene products of the CheY and CheZ genes (Parkinson et al., 1983; Parkinson & Parker, 1979).

The roles of the CheY and CheZ genes have also been clarified. When the bacterial chromosome was deleted of essentially all the chemotaxis cytoplasmic gene products, i.e., CheA, CheW, CheR, CheB, CheY, and CheZ, the bacterium was found to be continuously smooth swimming (continuous counterclockwise rotation of flagella). Addition of the CheY gene product under the control of a titratable promoter led to increasing clockwise rotation until, with large amounts of CheY protein, essentially all of the bacteria rotated in the clockwise direction (Clegg & Koshland, 1984). These experiments established that the CheY protein could generate tumbling. If the same experiment was repeated in the wild-type cell, the same phenomenon was observed; i.e., increasing amounts of CheY protein produced increasing amounts of clockwise rotation. Moreover, repeating the same experiment in cell envelopes, which were opened, loaded with CheY protein, and then closed, produced the same behavior (Ravid & Eisenbach, 1984). Thus, it is quite apparent that CheY by itself can produce clockwise rotation of the flagella. This does not rule out the possibility that a modified CheY protein may be the actual agent to carry out this function.

These experiments not only identified the role of CheY but also made possible two other types of experiments. In the first place, many other mutations, such as mutations in CheW,

Table I: Signal Transduction Systems in Bacteria

system	sensor gene for kinase	substrate for kinase
chemotaxis	CheA	CheY and CheB
toxicity	CpxA	SfxA
osmolarity	EnvZ	OmpR
nitrogen limitation	NtrB	NtrC
phosphate limitation	PhoR	PhoB
plant exudates	VirA	VirG
dicarboxylic acid metabolism	DctB	DctD

CheR, and CheA, produced constantly smooth swimming bacteria. It is impossible to test such bacteria for changes induced by attractant since the bacteria are already smooth swimming. However, if one transduces the bacterium with the titratable plasmid containing CheY, so that the bacterium shows clockwise rotation, it is possible to test the response of these other components in the sensing system.

Such an applcation added further support to the role of CheA and CheW, for it was found that wild-type bacteria which showed almost exclusive clockwise rotation could be changed to counterclockwise by addition of attractant, but no change was observed in the cells depleted of CheA and CheW (Clegg & Koshland, 1984). Thus the CheA protein and the CheW protein are essential for a signal to be transmitted from the receptor to the flagella.

These experiments were then extended by evaluating the role of CheZ. CheZ was also placed on a plasmid with a titratable promoter and its levels were varied both in wild-type cells and in cells already containing CheY. In both cases, it increased the percentage of smooth swimming (counterclockwise rotation) in proportion to the amount of CheZ in the system. Thus, CheZ plays a parallel but inverse role to CheY in generating smooth swimming in analogy to the tumbling generated by CheY (Kuo & Koshland, 1987).

The question then arises as to the role of CheY and CheZ in relation to each other. By placing the CheY and CheZ genes under the control of different titratable promoters and varying them relative to each other, it was shown that they were competitive. This could be explained by two simple binding constants to the output signal switch or by having CheY and CheZ be catalysts of opposing reactions. The possibility that Y is phosphorylated and that Z dephosphorylates Y (see below) would fit the data.

THE INFORMATION PROCESSING

The principle of a response regulator provides a unifying concept, but identifying the molecular species identified with this parameter has not been easy. One clue arose from the finding that ATP was involved in the signaling process beyond the requirement for synthesizing *S*-adenosylmethionine (Aswad & Koshland, 1975; Kondoh, 1980; Arai, 1981; Shioi et al., 1982). A second clue came from the finding that phosphorylation is utilized in regulation in bacteria (Wang & Koshland, 1981). A third clue came from the sequence homology between the chemotaxis genes and a number of the regulatory systems of other species (Stock et al., 1985b; Ronson et al., 1987). A next step was provided by the work of Ninfa and Magasanik (1986), who showed that the NtrB protein phosphorylates NtrC to activate it. Evidence that the chemotaxis system behaves similarly to the systems shown in Table I was provided by clever experiments of Simon and co-workers (Hess et al., 1987, 1988), who found that purified CheA protein autophosphorylates and also can phosphorylate the Y protein.

The role of protein phosphorylation in chemotaxis is becoming intriguing, as it appears that a diverse array of residues is involved. Wylie et al. (1988) found that phosphorylation of CheA occurs on histidine residues. As is the case with histidine phosphates in the bacterial phosphotransferase (pts) system (Postma & Lengeler, 1985), this CheA phosphate appears to be an intermediate in phosphate transfer and not a regulatory phosphorylation. The instability of the CheY phosphate suggests a carboxyl group phosphorylation, and preliminary information indicates that is so (A. Stock and J. Stock, personal communication). Finally, studies from our own laboratory have identified tyrosine phosphorylation of an *E. coli* protein that also seems to play a role in the signaling pathway (Bollag & Koshland, 1988).

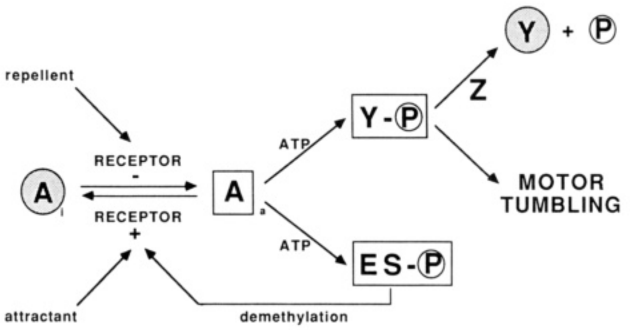

FIGURE 4: Possible mechanism for the activation pathway. The receptor is in an equilibrium between two states, R^-, which is stabilized by repellents, and R^+, which is stabilized by attractants. The R^- state activates the A protein to phosphorylate both the esterase (the B protein) and the tumbling signal (the Y protein). Phosphorylation of Y enhances the tumbling probability, and phosphorylation of B induces demethylation of the receptor, shifting it to the R^+ state. The R^+ state stabilizes the inactive state of the A protein and thus decreases the phosphorylation of B and Y.

The phosphorylation of CheY may affect its binding to the flagellar motor, thus affecting its direction of rotation. The sequence of the CheY protein is very similar to the N terminus of the esterase protein. Thus, the kinase that phosphorylates the CheY protein probably also phosphorylates the N-terminal portion of the CheB protein (Hess et al., 1988). There appears to be an effect of Z on the stability of Y phosphate, which suggests that Z acts as a phosphatase to dephosphorylate the Y protein (Hess et al., 1988). (These ideas have been incorporated in the scheme of Figure 4.)

If, indeed, the phosphorylated Y protein becomes more efficiently bound to the motor to generate tumbling, and the phosphorylated B protein acts as a better esterase to lead to smooth swimming, these two phosphorylations are operating in different directions and at different points in the pathway. Such asymmetry may be desirable to the bacterium. Tumbling is required to reorient the bacterium when it is going in the wrong direction, but prolonged tumbling is unproductive and, hence, should be corrected as soon as possible: ergo, the phosphorylation of the CheB protein. On the other hand, smooth swimming indicates the bacterium is going in the right direction and should be sustained as long as possible: hence, no phosphorylation of transferase protein.

Although many details remain to be uncovered, the determination that phosphorylation controls the response regulator is particularly exciting. It provides roles for the up to recently unidentified chemotaxis genes, it provides the molecular explanation for the response regulator parameter, and it provides another link between the bacterial processing system and those of higher species.

MEMORY

One major property of bacterial sensing that is sometimes overlooked is the ability to have memory without any complex structural apparatus. Bacterial memory derives from the property that there is a fast step and a slow step, both generated by the same chemoeffector (cf. Figure 2). The fast step is a constant reflection of the present concentration of chemoeffector in the current environment, whereas the slow step is a reflection of the past concentration. When the bacterium is moving in a gradient, the two concentrations and the level of X, the response regulator, will be altered. If there is no gradient, then both steps are stimulated equally and the level of the response regulator remains normal. That property means that the relaxation time of the cell can be established purely from the ratio of the rate constants of the two steps producing and degrading X and the properties of the transmembrane receptor. It therefore involves no complicated machinery like our eyes, which require an extremely complicated three-dimensional structure to develop stereopsis. The memory system is ideally designed for bacteria and other species such as leucocytes, which need to detect a gradient without an elaborate internal structure. Thus, the components illustrated in Figure 2 are actually distributed throughout the cell (the receptors are over the total surface, the motors are placed randomly), and yet they can provide a memory by the timing of the steps in information processing.

Although the model for chemotaxis has resulted in optimization for the memory time of the bacterium, the principle would seem to apply very well for neural and hormonal cells in higher speices. Slight modification in the enzymes involved in the phosphatidylinositol system or the cyclic AMP dependent system can greatly change time constants of those cells yet still maintain the basic biochemistry. Thus, the versatility in a complex system is achieved by slight variation of the common components rather than by requiring new chemicals for each cell process.

CONCLUSION

A total understanding of the chemotaxis sensing system has not yet arrived, but it remains one of the most thoroughly understood models for short-term memory and sensing that is available in the biological literature. To those who continually suggest that prokaryotes are different from eukaryotes (true, but vive la difference) the sensing system has turned out to be amazingly similar to other species. The finding of covalent modification of receptors and its relation to sensing and adaptation suggests analogous roles for other receptors. The structure of the chemotactic receptors has been found to be very similar to the predicted topology of transmembrane receptors such as the interleukin receptor, the LDL receptor, the EGF receptor, and the insulin receptor. The system has been capable of integrating both electrical and chemical signals, as do neurons. The ability to compare its past with its present by using the fluctuating levels of a response regulator is a paradigm which seems very likely to be repeated in other examples of short-term memory. And the processing system now appears to involve phosphorylation as in "higher", but not necessarily more interesting, systems.

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