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A mechanism for memory storage insensitive to molecular turnover: A bistable autophosphorylating kinase

(long-term memory/nervous system/protein phosphorylation)

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ABSTRACT **A mechanism is proposed for a molecular switch that can store information indefinitely, despite the complete turnover of the molecules that make up the switch. The design of the switch is based on known types of biochemical reactions. Central to the mechanism is a kinase that is activated by phosphorylation and capable of intermolecular autophosphorylation. It is shown that such a kinase and an associated phosphatase form a bistable chemical switch that can be turned on by an external stimulus and that is not reset by protein turnover.**

This paper addresses the question of how information can be stably stored by unstable molecules. This problem is discussed with respect to memory storage in the nervous system, but the arguments apply equally to any type of long-term cellular alteration. The difficulty posed by the instability of molecules can be illustrated by considering protein phosphorylation as a mechanism of information storage. Phosphorylation is a common mechanism for switching enzymes on or off (1, 2). Suppose that an elementary bit of information was stored in a neuron by phosphorylating a key enzyme and thereby turning it on. In the absence of a phosphatase capable of dephosphorylating the enzyme, the "on" state could be long-lasting. Nevertheless, since the phosphorylated protein would be gradually destroyed and replaced by newly synthesized unphosphorylated protein, the lifetime of the "on" state would be limited to the lifetime of the protein. The fact that memories can last for human lifetimes poses the question of how more stable molecular switches might be constructed. The conclusion of this paper is that switches capable of storing information indefinitely can be constructed by using known types of enzymatic reactions and that such switches are surprisingly simple.

Fig. 1 illustrates how a switch capable of long-term information storage might be constructed. This model assumes that the switch is composed of two enzymes, a kinase (termed kinase-1) and a phosphatase, with the following properties: Kinase-1 molecules are turned on by phosphorylation and can then act autocatalytically to phosphorylate other kinase-1 molecules, a process termed intermolecular autophosphorylation. Kinase-1 molecules are turned off by dephosphorylation mediated by the phosphatase. It is assumed that the phosphatase reaction becomes saturated when the level of phosphorylated kinase-1 is high. As will be shown below, these reactions constitute a chemical switch with two stable states, one in which the kinase-1 molecules are unphosphorylated and one in which they are almost completely phosphorylated. An elementary bit of memory could be stored in this switch as follows: Initially, kinase-1 would be unphosphorylated and considered "off." Some set of inputs into the cell would activate a second kinase (kinase-2) to

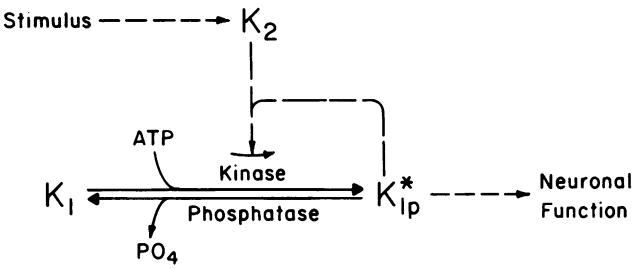

FIG. 1. Reactions in a bistable switch. The switch itself is constructed from two proteins: kinase-1, which can exist in either an inactive state (K_I) or an active state (K^*_{Ip}), and a phosphatase. The transition between inactive and active states is due to a phosphorylation reaction that can be catalyzed by active kinase-1 or by the kinase-2 activated during neuronal stimulation.

an extent that depended on the strength or coincidence of the inputs. Kinase-2 affects the switch by phosphorylating kinase-1. When kinase-2 activation reaches a critical level (to be described below), kinase-1 activity increases regeneratively to a high level. Kinase-1 activity then remains high indefinitely despite the removal of the stimulus and despite the action of the phosphatase. Kinase-1, by its action on other substrates, modifies the morphological, biochemical, and/or electrical properties of the cell in such a way as to alter the behavior of the cell in its neuronal network.

The formal demonstration that the switch shown in Fig. 1 is bistable is given in Fig. 2. The graphs shown in Fig. 2 *B* and *C* are derived from the set of equations in Fig. 2*A*, which are described in the legend. Fig. 2*B* shows how the velocity of the kinase-1 reaction and that of the phosphatase reaction depend on the fraction of the kinase that is active. This fraction is designated K^*_{Ip}/T, where K^*_{Ip} is the concentration of active (i.e., phosphorylated) kinase-1 and T is the total concentration of this protein. The first derivative with respect to time of the active kinase-1 concentration (dK^*_{Ip}/dt) is the difference between the velocities of the kinase and phosphatase reactions and is plotted in Fig. 2*C*. For a state to be stable, dK^*_{Ip}/dt must be zero and $d^2K^*_{Ip}/dt^2$ must be negative. This second condition is required so that a small deviation from a stable state is followed by a spontaneous return to that state. In the example illustrated in Fig. 2, the stability criteria are met by two points: $K^*_{Ip}/T = 0$ and $K^*_{Ip}/T = 0.74$.

Fig. 3 shows the results of calculations that show how the switch could be turned on permanently by an external stimulus. The ability of kinase-2 to activate kinase-1 is assumed to closely follow the strength and kinetics of stimulation. If one starts with kinase-1 "off" and gradually increases the strength of stimulatory pulses, the kinase-1 activity also will increase gradually (Fig. 3). After each pulse of weak stimulation, kinase-1 activity returns to zero because of the phosphatase activity. However, when the kinase-2 stimulation is increased to a critical level, the graded nature of the response disappears; kinase-1 is activated by phosphorylation

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A

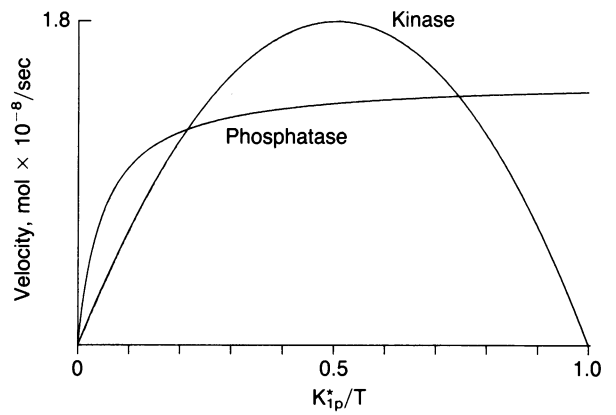
$$dK^*_{1p}/dt = \text{velocity (kinase-1)} - \text{velocity (phosphatase)} \quad [1]$$

$$T = K_1 + K^*_{1p} \quad [2]$$

$$dK^*_{1p}/dt = \frac{K_1 \times K^*_{1p} \times C_1}{(K_{m_1} + K_1)} + \frac{P \times K^*_{1p} \times C_2}{(K_{m_2} + K^*_{1p})} \quad [3]$$

$$dK/dt = \frac{(T - K^*_{1p}) \times K^*_{1p} \times C_1}{(K_{m_1} + T - K^*_{1p})} + \frac{P \times K^*_{1p} \times C_2}{(K_{m_2} + K^*_{1p})} \quad [4]$$

B



C

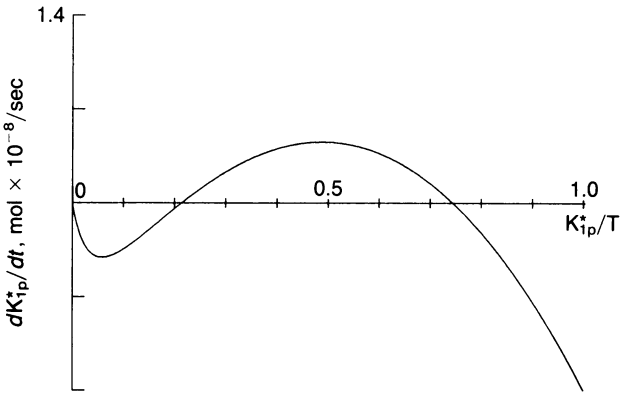


FIG. 2. Bistability of the switch shown in Fig. 1. (*A*) In the absence of kinase-2 activity, the switch is described by four equations. Eq. **1** states that the first derivative of the active kinase-1 concentration, dK^*_{1p}/dt, is the difference between the kinase and phosphatase reaction velocities. Eq. **2** is the conservation condition for kinase-1, where T is the sum of inactive and active kinase-1. Eq. **3** describes the reactions of Eq. **1** in terms of Michaelis–Menten kinetics, where K_{m_1} and K_{m_2} are Michaelis constants for the kinase-1 and phosphatase reactions, respectively; C_1 and C_2 are the turnover numbers of the kinase-1 and phosphatase, respectively; and P is the phosphatase concentration. Eq. **4** is derived by substituting Eq. **2** into Eq. **3**. (*B*) Velocity (moles per second) of the kinase-1 reaction (left term in Eq. **4**) and phosphatase reaction (right term in Eq. **4**) as a function of the fraction of kinase-1 that is active (K^*_{1p}/T). When there is no phosphorylated kinase-1 present, the velocity of the phosphatase reaction is zero. As K^*_{1p} increases, the velocity of the phosphatase reaction increases until saturation occurs. The velocity of the kinase-1 reaction increases as K^*_{1p} is increased, but the velocity falls again at high K^*_{1p} because there is little unphosphorylated protein present to phosphorylate. (*C*) The derivative of K^*_{1p} (Eq. **4**) as a function of K^*_{1p}/T. The points at $K^*_{1p}/T = 0$ and $K^*_{1p}/T = 0.74$ represent the stable states. For instance, if K^*_{1p}/T rises slightly above zero, dK^*_{1p}/dt is negative and K^*_{1p} will return toward zero; if K^*_{1p}/T falls slightly below 0.74, dK^*_{1p}/dt is positive and K^*_{1p}/T will become larger, whereas if K^*_{1p}/T rises above 0.74, dK^*_{1p}/dt is negative and K^*_{1p}/T will return toward 0.74. $C_1 = 30$; $C_2 = 3$; $K_{m_1} = 10^{-6}$; $K_{m_2} = 2.5 \times 10^{-9}$; $P = 5 \times 10^{-9}$; $T = 5 \times 10^{-8}$.

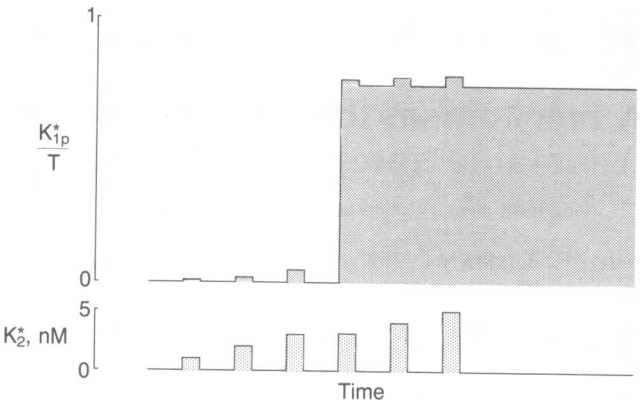


FIG. 3. Effect of stimulation on the switch. Stimulus pulses of different strengths activate kinase-2 in a graded manner. The resulting activation of kinase-1 is also graded until its activation becomes regenerative. Subsequently, kinase-1 activity stays high even after removal of the stimulus. The values of K^*_{1p} shown are the steady-state solutions of Eq. **4** (Fig. 2*A*) with a term added to account for kinase-2 activity. Kinase-1 properties are as in the legend to Fig. 2. Kinase-2 properties: $K_m = 10^{-6}$; turnover number = 30.

faster than the phosphatase can inactivate it. The increasing amount of kinase-1 activity leads to further activation of kinase-1 by autophosphorylation, which in turn increases the rate of kinase-1 activation still further. Thus, a regenerative event occurs that leads to nearly complete activation of kinase-1. Once this event has occurred, removal of the stimulus and the resulting elimination of kinase-2 activity, does not result in a large reduction of kinase-1 activity. Hence, the switch stays on indefinitely.

A switch of this kind is a dynamic switch and will therefore not be reset by reasonable levels of protein turnover. If the switch is on and kinase-1 is active, newly synthesized kinase-1 molecules will become activated, thus replacing active kinase-1 molecules destroyed by protein turnover. Calculations show that for the switch described in Fig. 2, there is only a small reduction in kinase-1 activity, even if a time-constant of only a few seconds is assumed for protein turnover. Thus long-term information can be stored by this switch even though the molecules that make up the switch turn over rapidly and completely. The switch can be reset only by interfering with kinase-1 activity or by increasing phosphatase activity.

Although the system of reactions described above has not yet been observed, the individual reactions themselves are well known. The process of autophosphorylation is widespread (3–5), although it usually occurs by an intramolecular process in contrast to the intermolecular process required here. Similarly, the process by which a kinase is itself activated by phosphorylation is well established (6).

Proper functioning of the switch described in Figs. 1 and 2 has two important requirements. First, the relative concentrations of phosphatase and kinase-1 need to be regulated. For example, when the phosphatase concentration in the model of Fig. 2 is raised by 30%, the switch still works properly, but when the concentration is raised by 50%, the switch fails: it turns on properly when a critical stimulus intensity is reached, but it turns off when the stimulus is removed. In this mode the switch cannot store information [but might serve as a useful detector in a biochemical regulatory system where a high sensitivity to effectors is needed (7)]. Thus, for the switch to operate as a memory-storage device, the concentrations of phosphatase and kinase must be kept within a fairly narrow range. A second requirement is that sufficient energy must be available to maintain the "on" state of the switch. Energy, in the form of ATP, is constantly needed to

rephosphorylate kinase molecules that the phosphatase has dephosphorylated. A way to relax both these requirements is suggested by recent work showing that the same protein can have both phosphatase and kinase activities (8) and that these activities can be reciprocally controlled by phosphorylation of the protein (9). Calculations similar to those described in the legend to Fig. 2 indicate that a bistable switch can be formed from a single type of molecule that is converted by phosphorylation from an autocatalytic phosphatase into an autocatalytic kinase. Moreover, such a switch is very insensitive to concentration changes and requires much less use of ATP. Thus, in principle, a highly efficient and stable switch could be constructed from a single type of molecule† with some advantages over the two-component switch‡ described in Figs. 1 and 2.

In closing, three points should be noted. First, it should be stressed that what is new in the model proposed here is not the concept of bistable chemical switches (see refs. 11–13) but rather the suggestion that they can be simply constructed by using known types of biochemical reactions. Second, such switches could also be involved in other long-term cellular changes, such as growth regulation and transformation. This is an intriguing possibility in view of the observation that many oncogene products and growth-factor receptors are autophosphorylating protein kinases (19–21). Finally, it is of interest to compare the memory mechanism proposed here to the possibility of using DNA for memory storage. Although memory storage in DNA seems plausible if overall properties of a neuron are to be controlled (14), it is hard to see how nuclear DNA could independently control (or be controlled by) events in subcellular compartments, such as the thousands of spines that constitute the input regions of neuronal dendrites. In contrast, it is easy to see how the kinase molecules that are immobilized in dendritic spines (15–17) could preferentially phosphorylate other kinase molecules within the same spine and thereby form local bistable switches that are independent of the switches in other spines. Indeed, an initial test of the ideas proposed here might be to examine dendritic spines for all-or-none differences in the state of kinase phosphorylation.

†Kinase-2 is here considered separate from the switch itself. It would, however, be possible to merge kinase-1 and kinase-2 functions by making kinase-1 autophosphorylation directly activatable by the stimulus-dependent effector, as occurs in the insulin receptor (10).

‡The benefits of reciprocal control of kinase and phosphatase activities in the one-component switch could also be achieved in the two-component switch by allowing the active kinase to inactivate the phosphatase.

Note. After this work was completed, Francis Crick (18) proposed a conceptually related mechanism by which information might be stored in the nervous system.

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