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Amplification and Adaptation in Regulatory and Sensory Systems

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Summary. Biological systems respond to sensory inputs and changing metabolic conditions both by amplifying signals and by adapting to them. The mechanisms by which these apparently opposing goals are achieved by the chemistry of the cell are described and evaluated.

Living organisms have devices for sensing the external environment and internal metabolic changes, for adapting to them, and for regulating their internal machinery as a result of these signals. The basic mechanisms of control in most cases are mediated by induced conformational changes of proteins which either "turn on" or "turn off" the processing system. In this way, signals can feed back to inhibit synthesis of a product (*1*) that is in excess or can feed forward to activate a pathway that must be mobilized for a particular function. These changes in activity can be effected either by molecules that induce conformational changes by binding noncovalently (allosteric effectors) (*2*) or by covalent modification of residues on the protein surface (*3*). Such molecular mechanisms seem to operate within the cell in metabolic regulation, between cells in hormonal and neural signaling, and between cells and the environment in sensory receptors. Thus, similar molecular mechanisms operate for both sensing and regulation in biological systems.

Biological sensing and regulatory systems are particularly effective because of two additional properties, namely, the ability to generate amplified responses to low levels of stimuli and the ability to adapt to constant backgrounds of stimuli. Certain signals, such as a photon of light, the odor of a perfume, a faint sound in the distance, do not per se have the energy to generate a behavioral response and must be amplified within the organism by its metabolic and neural machinery. A highly sensitive amplification system would cause problems, however, because living organisms are constantly bombarded by background stimuli such as light, odor, and sound, which could saturate the sensory system. The organism prevents this by adaptation that tends to desensitize the sensing apparatus toward the background stimuli. In most systems the cell is the primary unit that can both amplify and desensitize signals (*4–6*); thus we must look largely to the biochemistry within the cell to explain fundamentals of the phenomena.

Since amplification involves enhancement of a signal and adaptation involves its diminution, the occurrence of both processes within the cell raises questions in regard to their mechanisms and compatibility. Can both occur in the same cell or are they mutually exclusive? Is there is fundamental difference between regulatory and sensory systems? Are there limits to the amplification of a signal and can adaptive systems show amplification? What molecular mechanisms can explain such processes and what are their potentialities? In this article we attempt to address these questions.

Nomenclature of Amplification

Amplification and adaptation have fascinated biologists for a long time and various aspects of these problems have been described and discussed (*4–10*). Inevitably, each investigator has defined terms in his own system and considerable redundancy and ambiguity has de-

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veloped. In that we introduce new concepts and reexamine old ones, it may be worthwhile to define terms precisely in ways that are generally useful.

The amplification of a signal in a biological system generally takes two forms. One, which is referred to here as *magnitude amplification*, occurs whenever the output molecules (which we refer to as ϕ) are produced in far greater numbers than the stimulus molecules (represented as S). Examples are the bleaching of one rhodopsin molecule in the visual system which can cause hydrolysis of 10^5 molecules of cyclic guanosine monophosphate (cyclic GMP) (*11*) or a neurotransmitter which activates an ion gate to let 10^4 molecules of sodium ion enter a cell (*12*). The stimulus, S, can thus represent such diverse species as substrates, activators, inhibitors, neurotransmitters, hormones, and light, and all can show magnitude amplification.

The second form of amplification, which we call *sensitivity amplification*, deals with the percentage change in a response compared to the percentage change in the stimulus. This type of amplification may become particularly important in an adaptive system where there is a significant background or in a "futile cycle" regulatory system in which there are two pathways, both operating, one of synthesis and the other of degradation. In those cases the organism must be able to respond to a signal introduced over background noise. One useful measure of such change, $(d\ln\phi)/(d\ln S)$, has been utilized by Higgins (*9*) and Savageau (*10*) for the analysis of systems; however, we define a sensitivity amplification factor, A_S, in terms of finite intervals because, as already discussed in detail (*13*), it gives the best picture of the limits of amplification in physiological process. The amplification factor, A_S, is therefore defined

$$A_S = \frac{\Delta\phi/\phi_i}{\Delta S/S_i} = \frac{(\phi_f - \phi_i)/\phi_i}{(S_f - S_i)/S_i} \qquad (1)$$

where the subscripts i and f refer to the initial and final values, respectively. If A_S is greater than 1, the percentage change in the response is greater than the percentage change in stimulus.

It is also helpful to have general terms for systems that show greater or less sensitivity than the usual Michaelis-Menten relationship. Cooperativity is one device for achieving adding sensitivity, but it is not the only one. We therefore refer to input-output relations which give the usual hyperbolic relationship, such as Michaelis-Menten, as *hyperbolic sensitivity* (Fig. 1, curve b). Those that are more sensitive to stimulus are called *ultrasensitive* (Fig. 1, curve a) and those that are less are called *subsensitive* (Fig. 1, curve c). Positive cooperativity (*14*) would be an example of ultrasensitivity and negative cooperativity (*15*) an example of subsensitivity. As is discussed below, the sensitivity amplification factor is not automatically larger than 1 if a system is an ultrasensitive system; it will depend on the range over which the stimulus-response ratio is observed. In contrast, a subsensitive system will never have a sensitivity amplification factor greater than 1 but may have very large magnitude amplification factors.

By adaptation we mean the observed decrease in response as a function of time to a change in background level of a stimulus that has elicited an initial transient response. Absolute and partial adaptation is described below.

Limits on Magnitude Amplification

In many discussions of amplification, it is stated or implied that the larger the amplification, the better the performance. If one step of a cascade is shown to produce an amplification of 10^3, a multistep cascade is rationalized on the basis

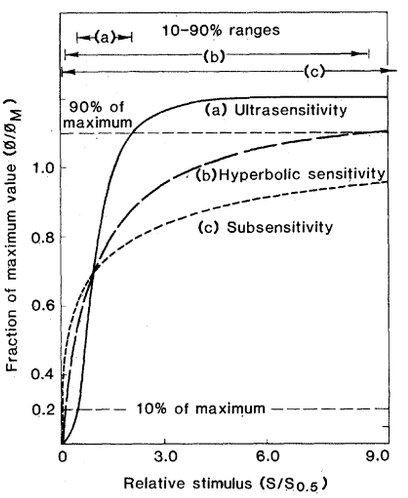

Fig. 1. Sensitivity to stimuli by various response systems. Curve b is the usual type of Michaelis-Menten binding, referred to in this article as hyperbolic sensitivity. Curve a is typical of a system illustrating ultrasensitivity in which the change from 10 percent of maximum response to 90 percent of maximum response occurs over a much narrower range (4.3-fold in S) than does the hyperbolic response (81-fold in S). Curve c illustrates a case in which each system is subsensitive, and the change from 10 to 90 percent occurs over a much wider range than the hyperbolic curve (6561-fold in S). The curves are calculated with the use of the formulas $\phi = (S/S_{0.5})^n/[1 + (S/S_{0.5})^n]$ where $n = 3$ for (a), 1 for (b), and 0.5 for (c).

that three steps can then give a factor of 10^9. The actual situation can be quite different and is illustrated for the best known cascade process in Fig. 2. In this example, the hormone epinephrine (H), released by a neural stimulus at the adrenal medulla, binds to a receptor on a muscle cell converting the inactive receptor (R) to an active form (R*). The conformational change in R to R* induces a conformational change in a neighboring GTP-binding protein (G protein) yielding an activated complex (G*) which converts adenylate cyclase to an active form AC*. The activated adenylate cyclase then catalyzes the conversion of many molecules of adenosine triphosphate (ATP) to produce cyclic adenosine monophosphate (cyclic AMP) which then proceed to activate other elements in the cascade as shown in Fig. 1, where symbols are used to illustrate steps involving large amplifications, large diminutions, moderate amplifications, and moderate diminutions, respectively. In the fifth step, for example, a few molecules of the enzyme adenylate cyclase convert many molecules of ATP to cyclic AMP and in the last step a few molecules of phosphorylase produce many molecules of glucose-1-phosphate from glycogen, both large amplifications. However, of the many molecules produced at the adrenal medulla, only a few actually bind to receptors at the muscle cell, a large diminution step. Thus, the real situation as shown in the figure is large amplification steps followed by large diminution steps and other steps with moderate amplifications and moderate diminutions.

If we examine why this pattern occurs, several facets become apparent. The amplification in the first step is needed to offset the dilution of the hormone in the bloodstream during the second step. The low concentration of hormone in the blood ($10^{-10}M$ in some cases) requires an amplification to generate a significant signal within the cell. Most cascades therefore proceed in alternating magnitude amplification and magnitude diminution.

Within a cell, large magnitude amplifications may even be undesirable. A single molecule in a mammalian cell having a volume of 10^{-9} milliliters has a concentration of $10^{-12}M$. A cascade of three steps of 10^4 each would generate a final concentration of $1M$, a concentration never achieved in physiological situations.

In fact, the biological system is clearly designed to amplify the external signal moderately but not excessively. Shown in Fig. 2 are various "leak" steps de-

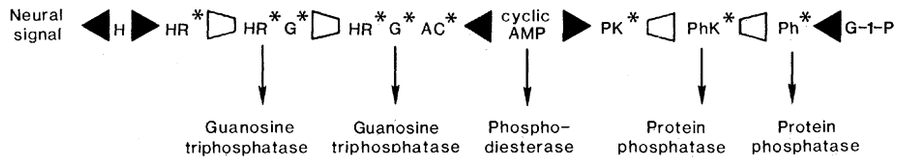

Fig. 2. The sequence of events in a cascaded pathway illustrating amplifications and diminutions. The first step, a neural signal received in the adrenal medulla, causes the release of many molecules of epinephrine (*H*), a large amplification (indicated by ◀) of an initial signal. The hormone is diluted in the bloodstream so that only a few molecules actually bind to the adrenergic receptor on a muscle cell, giving a large diminution in molecules of response per original molecule of stimulus (indicated by ▶). Most of the receptor molecules are effective in activating a G protein to its active state (labeled G*) (moderate diminution ▷). The G protein in its active state can induce a conformational change in the adenylate cyclase molecule to activate it (*AC**). The cyclase can then generate many molecules of cyclic AMP for each initial molecule of activated adenylate cyclase, a very large amplification. Of the many molecules of cyclic AMP, a small fraction binds to the protein cyclic AMP–dependent protein kinase (*PK**), which can then activate the enzyme phosphorylase kinase (*PhK**), which in turn activates by phosphorylation the enzyme phosphorylase (*Ph**). Although each phosphorylating enzyme would be capable of a large amplification, the increase in enzyme concentration from the protein kinase to phosphorylase is approximately 700-fold (*17*)—thus there is only moderate amplification. The final enzyme can convert glycogen to glucose-1-phosphate (*G-1-P*), a large amplification. In a variety of steps there are "leak" reactions that diminish the "gain" of the large amplification steps. The activated G protein contains bound GTP which is hydrolyzed by the G protein itself to guanosine diphosphate to convert G* back to the inactive form (G). Likewise, there is a phosphodiesterase that diminishes the amount of cyclic AMP by hydrolysis to AMP. The protein phosphatases convert the active phosphorylated form of phosphorylase kinase and phosphorylase to the less active dephosphorylated forms.

signed to control the degree of amplification (the gain) of the system. The G protein, which in its active form binds guanosine triphosphate (GTP), also acts as a guanosine triphosphatase that produces an inactive form of the G protein (*16*). A phosphodiesterase is present to drain off cyclic AMP, and phosphatases are present to deactivate the phosphorylated enzymes to inactive forms (*3, 17*). If the rate constants for the leak steps in Fig. 2 were reduced to zero, one hormone receptor would catalyze cyclic AMP formation ad infinitum and lead to an enormous amplification factor. It does not do so because further amplification would be excessive. Leak reactions and substrate limitation provide a safeguard against excess amplification and are necessary components of a reversible system.

The principles outlined here are seen to apply in general in biological systems. An acetylcholine molecule opens a receptor channel allowing 10^4 sodium ions to cross the membrane in a millisecond (*12*). Since the sodium ions which flood into the cell must later be pumped out, too large an amplification is undesirable. Therefore, the channel is designed to stay open for only a brief period before a second conformational change occurs closing the channel as shown in Eq. 2, where S represents acetylcholine and R represents the receptor

$$\underset{\text{(closed channel)}}{S + R} \rightarrow \underset{\text{(open channel)}}{S \cdot R^*} \downarrow \underset{\text{(closed channel)}}{S \cdot R^{\ddagger}} \quad (2)$$

The above examples should not be construed to indicate that successive magnitude amplifications are nonexistent. They seem to occur in the blood clotting and complement fixation processes, but in those cases the protein molecules have low turnover numbers. The cascade may be present in some cases for kinetic reasons since Stadtman and Chock (*18, 19*) have shown that cascades can accelerate reaction velocities.

Thus magnitude amplification of the sizes necessary in biological systems can readily be achieved in single steps. In fact, "leak" or timed shutdowns may be necessary to prevent excessive magnitude amplification. Cascades may produce magnitude amplification, but they are not needed per se to produce large factors.

Sensitivity Amplification

When there is need to change an existing steady state to a new one, a different problem exists. Sensory cells must frequently detect a change in stimulus intensity over a steady background stimulus which is not negligible, such as an object seen in broad daylight. Enzymes in pathways of synthesis and degradation must repond to changes in metabolites, which are never at zero concentration in the cell. The regulators which change the direction of net flow often do not change greatly in concentration, and hence a need to amplify the difference arises. Thus, sensitivity amplification becomes important when there is an existing steady state which must be changed to a new steady state by a finite change in a regulator level.

Three types of sensitivity amplification have been uncovered, and each has advantages and limitations. One is achieved by the positive cooperativity of a protein with a high Hill coefficient (*14, 15, 20*). A second is the effect of multiple inputs along a pathway, for example, the entry of cyclic AMP at several steps along the glycogen cascade (*3, 21*). A third is a newly described (*22*) "zero order ultrasensitivity," arising from the kinetics of covalent regulation, which may provide great regulatory sensitivity but has yet to be demonstrated experimentally. Substrate cycles can also give amplification as suggested by Newsholme and Crabtree (*8*), but this alternative requires cooperative or zero-order effects which are discussed elsewhere (*13*). We next evaluate the sources and limitations of the sensitivity amplification from each of these molecular phenomena.

Limits of Amplification Through Cooperativity

The response relation between a cooperative protein and its stimulus can be expressed roughly in terms of the Hill coefficient (n_H) as shown in Eq. 3 (*14*).

$$\phi/\phi_{max} = \frac{(S/S_{0.5})^{n_H}}{1 + (S/S_{0.5})^{n_H}} \quad (3)$$

In the case of an enzyme, ϕ can be velocity and $S_{0.5}$ the substrate concentration at half-maximal response (*20*). The sensitivity amplification factor, as defined in Eq. 1, is for a cooperative protein,

$$A_S = \frac{(S_f/S_i)^{n_H} - 1}{[(S_f/S_i) - 1][1 + (S_f/S_{0.5})^{n_H}]} \quad (4)$$

Analysis of this equation shows that a noncooperative protein in which $n_H = 1$ will always have an amplification factor less than 1, and it will approach 1 only when S_f is much less than $S_{0.5}$.

The maximum sensitivity amplification available from cooperative systems can be obtained by differentiating Eq. 4 and setting the derivative equal to zero (*13*). The maximum sensitivity amplification factor, obtained by such an analysis, the range over which the maximum is observed, and the relation to the Hill coefficient is given in Table 1. This table illustrates several principles. First, the maximum amplification is dependent on the initial state of the system. In case 1, in which the initial steady-state level is

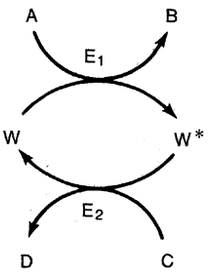

Fig. 3. A cyclic pathway representing biosynthesis and degradation or protein modification and demodification. Thus W and W* could represent unmodified and modified protein, respectively. Because the enzymes have different specificities and different reactants, A versus C, and different products, B versus D, both pathways can be thermodynamically favorable at the same time and thus controlled by the kinetics of the converter enzymes E_1 and E_2. Regulation of the converter enzymes E_1 and E_2 can be by allosteric effectors which may in some cases be sensory stimuli.

only 1 percent of the maximum ($\phi_i = 0.01\ \phi_{max}$), the amplification factors are larger than those observed when it is initially at 10 percent of ϕ_{max}. Second, the sensitivity amplification factor is appreciable but modest in the range of cooperativity values usually observed for biological systems. Very few cooperative systems show a Hill coefficient greater than 4, and the maximum sensitivity amplification for a protein with $n_H = 4$ is 24. For a system with a Hill coefficient of 2, this maximum amplification factor drops to 5.5. Third, if the range over which regulation occurs is different from the optimum, the amplification factor decreases further. Thus the sensitivity amplification factors for the range from 10 to 90 percent of maximum response for proteins with Hill coefficients 2, 4, and 6 are found to be 1, 4, and 9, respectively. These are appreciably less than the optimal values of 5.5, 24, and 47 shown in Table 1. The results of Table 1 therefore indicate that positive cooperativity can give sensitivity amplification factors that are significantly greater than 1, but to do so the Hill coefficients must be appreciably greater than 2 and the interval over which regulation occurs must be near the optimum range.

Multistep Ultrasensitivity

Another mechanism for obtaining ultrasensitivity is to have the same effector enter at several steps in a pathway. In principle, if an effector J entered a multistep pathway at n loci, we might imagine that the reaction would be accelerated by J^n. In fact, this is rarely so. Stadtman and Chock have calculated by computer simulations the response relations when an effector enters more than one step in a cascade (*18*). They find an added sensitivity, but examination of their computer simulation shows less increase in sensitivity that one might expect from a simple J^n relationship. Analytical solutions have been obtained (*18, 19, 22*) for the case of protein modification by two alternative pathways that can be used to illustrate the condition for multistep ultrasensitivity. In Fig. 3 is shown a typical situation in which one pathway, such as a kinase-activated phosphorylation, produces an active enzyme W* and a reverse reaction, such as a phosphatase-catalyzed dephosphorylation, produces the demodified inactive protein. If a compound J activates the forward reaction and inhibits the reverse reaction (both in a Michaelian manner), the equation for the amount of W* is

$$\frac{W^*}{W_t} = \frac{f_{VK} f_J}{1 + f_{VK} f_J} \tag{5}$$

in which

$$f_{VK} = \frac{V_{m_1} K_{m_2}}{V_{m_2} K_{m_1}} \qquad f_J = \frac{J(K_{J_2} + J)}{K_{J_2}(K_{J_1} + J)} \tag{6}$$

and V_m's are maximum velocities, K_m's Michaelis constants, and K_{J_1} and K_{J_2} binding constants of J to the respective enzymes. Equation 5 holds when the modifying enzymes operate in the domain of first order kinetics relative to the target protein.

Examination of Eq. 5 shows that significant ultrasensitivity will not be observed unless the f_J term is higher than first order in J. This will occur only when J is much greater than K_{J_2} and much less than K_{J_1}. For example, if $K_{J_2} = 10^{-8}$ and $K_{J_1} = 10^{-3}$, this condition would be filled for J, ranging from 10^{-6} to $10^{-5} M$. On the other hand, if $K_{J_1} = K_{J_2}$, f_J is first order in J at all levels of J. The limitations on J and its binding constants is not the only condition, however. The f_{VK} term, which comprises the constants of the respective enzymes, must also be appreciably less than 1 in order to have appreciable ultrasensitivity.

This simple example illustrates the principle that multistep ultrasensitivity will occur only in special circumstances and over ranges that are closely circumscribed by the properties of the enzymes involved. Since Eq. 5 has the form of a Hill equation, the factors derived in Table 1 calculated for cooperative proteins will apply, and it is seen that the maximum sensitivity amplification factor for J acting in two steps as shown in Fig. 3 is 5.5, an appreciable but not extraordinarily high figure. The same type of reasoning can be applied to situations in which J activates two sequential steps in a pathway. The mathematics suggest that multistep ultrasensitivity will give substantial sensitivity amplification only if the regulatory molecules enter at more than two steps. It is intriguing in this regard that cyclic AMP exerts an effect at five different loci in the glycogen cascade.

Table 1. Maximum sensitivity amplification factor obtainable from a cooperative protein with a given Hill coefficient. The right-hand column gives the maximum amplification factor obtainable for the ϕ_i value and Hill coefficient (n_H) shown at the left. The other columns give the values of S_i, S_f, and ϕ, which describe the interval.

n_H	Background stimulus ($S_i/S_{0.5}$)	Stimulus range (S_f/S_i)	Final response (ϕ_f/ϕ_M)	$A_S = \frac{\Delta\phi/\phi_i}{\Delta S/S_i}$
Case 1 in which background $\phi_i = 0.01\ \phi_M$				
2	0.1	9	0.45	5.5
4	0.32	3.7	0.66	24
6	0.46	2.5	0.73	47
Case 2 in which background $\phi_i = 0.1\ \phi_M$				
2	0.33	2.2	0.34	2.1
4	0.58	1.8	0.54	5.5
6	0.69	1.6	0.61	9.2

Zero-Order Ultrasensitivity

In view of the difficulties described above in achieving high-sensitivity amplification, it is intriguing that analytic derivations have revealed (*22*) that a third type of ultrasensitivity may be available in biological systems. A typical covalent modification scheme is shown in Fig. 3, in which one converter enzyme, E_1, modifies the protein W and a second, E_2, removes the modification. It was shown (*22*) that an effector, J, binding to only one of the converter enzymes in a Michaelis-Menten manner, will cause ultrasensitive changes in the amount of W*. One example of ultrasensitivity generated by such a system is shown in Fig. 4. If the total amount of enzyme is such that $(K_{m_1}/W_T) = (K_{m_2}/W_T) = 10^{-1}$, the modification system will have an ultrasensitivity equivalent to a cooperative enzyme with a Hill coefficient of 2.9. A system with ratios of 10^{-2} would correspond in sensitivity to a cooperative protein with a Hill coefficient of 13. To obtain such conditions, the enzyme modified has to be in appreciable excess compared to the modifying enzymes, and the binding constants of the proteins must be in the appropriate range. The conditions are stringent, but, since it offers a potential for high-sensitivity amplification, it would be extremely useful when there are two opposing

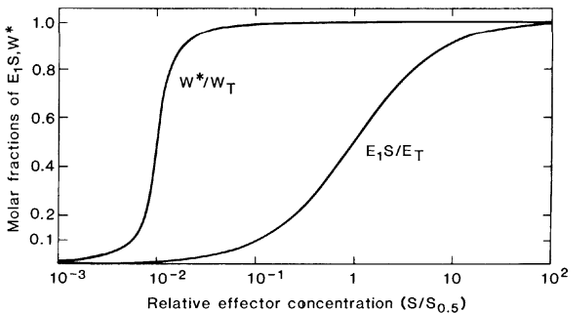

Fig. 4. Generation of ultrasensitivity by the zero-order mechanism. The molar fractions of W* are shown as a function of the effector, S, which activates the converter enzyme E_1 by hyperbolic binding; that is, the fraction of active enzyme, E_1S, is equal to the saturation function, $S/(S_{0.5} + S)$. The ultrasensitivity of the curve for W* is equivalent to a cooperative curve with a Hill coefficient of 3.6 [curves redrawn from (*22*)].

pathways, such as degradation and synthesis, in which ultrasensitive responses would prevent excessive waste due to "futile cycles." Since purification of enzymes in such pathways is now proceeding, evidence for the existence of this type of ultrasensitivity may be available in the near future.

The conclusion from these analyses, therefore, is that sensitivity amplification is possible, but is much more difficult to achieve than magnitude amplification and will occur only when the constants of the system are optimally selected. Thus, the conditions for significant sensitivity amplification are not only that the overall system must exhibit ultrasensitivity, but also that the range over which the effector (or substrate) is changing is close to optimal.

Sensitivity of the Sensory System

In the sensory cell, a small fluctuation in background intensity of a stimulus must be amplified in order to achieve a behavioral response. The mathematics discussed above give a clue as to how this goal might be achieved. If sensitivity amplification, which is difficult to achieve, could be partially changed into a magnitude amplification which is more easily achieved, then a small stimulus might be converted into the easily detectable level. The sensory cell apparently does this by utilizing a combination of temporal change and adaptation.

To illustrate the situation, three types of cells, operating in a stimulus level of S_i, are imagined to receive a sudden increase in stimulus to a new background S_f at time = 0 (Fig. 5). One type responds by a transient alteration of behavior which returns to precisely the same behavioral level as prestimulus, a situation that we call absolute adaptation (Fig. 5, column A). The second is a behavioral response that peaks but does not return to precisely the prestimulus level (Fig. 5, column B), which we call partial adaptation. The last is the change from one steady state to another without any adaptation (Fig. 5, column C). Absolute adaptation occurs in bacterial chemotaxis (*23*) and in the visual system if we define the response as the ability to detect percentage changes in background stimulus (*24*). Partial adaptation occurs with hormones (*5*, *6*). The response without adaptation is common to metabolic control mechanisms. That mechanisms within a category should operate identically seems unlikely, but it is intriguing that the recently identified receptors in bacterial chemotaxis are multiply methylated (*25*), that the light receptor rhodopsin is multiply phosphorylated (*26*), and that the acetylcholine receptor is multiply phosphorylated (*27*). All three systems show adaptation suggesting that the principles may be similar even if the details are not.

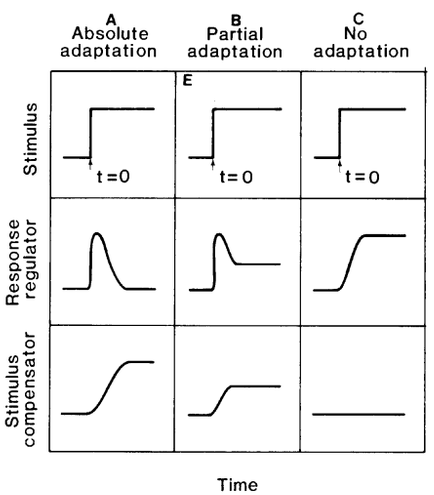

Fig. 5. Adaptive responses to changes in environmental stimuli. Top portion shows identical step changes in stimuli applied to all three systems. In (A) after a transient response the system adapts absolutely; that is, the behavior returns precisely to the prestimulus level even though the stimulus remains at the new higher level. In (B) the transient response adapts only "partially" so that the regulator, X, controlling behavior goes through a maximum but returns to a new value. In (C) there is no adaptation and the system adjusts to a new steady state. In the bottom portion of the figure the compensatory machinery of the cell is seen to be present for the absolute-adaptation situation and unchanged for the no-adaptation model. It may or may not be present for the partial-adaptation model. In the latter case a feedback of the product must be present in one of the proteins of the system.

Absolute Adaptation

Let us examine for a moment an illustrative mechanism by which a transient response with absolute adaptation can be achieved. If a response regulator, X, which controls behavior is formed and removed as shown in Eq. 7, a stimulus J which activates v_f and v_d equally will produce the same level of X at all levels of J. That would produce adaptation but no signal. If J activates v_f rapidly and v_d slowly, a change in J produces a transient signal which adapts absolutely as shown in Fig. 5, column A (*23*). Any size signal can be generated depending on the kinetic characteristics of the system, that is, the relative values of v_f and v_d and the rapidity of their responses to changes in J.

$$\xrightarrow{v_f} X \xrightarrow{v_d} \quad (7)$$

The absolutely adapting system is sensitive to a change in J over time, not to the absolute level of J. When J is unchanging, even though present at a high level, it is a zero stimulus. An externally generated change in J over time is recognized as a stimulus and is governed, to a first approximation, by constraints of magnitude amplification, not sensitivity amplification. The adaptive apparatus thus resets the cell to zero and converts the difficult problem of detecting a diference between 10,000 and 10,001 to the easier problem of detecting a difference between zero and 1.

It may first be asked why the same device does not function in metabolic systems. The answer is that the output of a metabolic system is a net product, not a transient signal.

It is next appropriate to ask the cost of an adaptive system. The answer seems to be that such a system is expensive in energy terms. The systems known so far have a number of enzymes designed for the adaptive apparatus, and there is a steady-state level of covalent modification and demodification that requires energy to maintain. This energy is utilized to bring the system back to the same

behavioral pattern despite the increase in external stimulus. In the case of bacterial chemotaxis, the level of the methylation appears to be proportional to that of the chemoeffector (*28*), and in the visual system the level of phosphorylation is proportional to the light intensity (*29*). This can be interpreted (Fig. 5, column A, in the bottom line) as a compensatory system that provides a counterweight to the stimulus. It is not possible to permanently change a background and return to the same behavior without some compensatory chemistry (*30*). The cell, therefore, synthesizes the enzymes for this compensatory machinery and provides a constant supply of energy, ultimately in the form of ATP hydrolysis, to maintain the adaptive state.

Partial Adaptation and Simple Regulation

In sensory systems that must detect small changes absolute adaptation exquisitely increases the sensitivity of the system. In other systems such sensitivity may not be needed, and the price of absolute adaptation in terms of energy is not worth paying. For example, hormones provide an external signal from one cell to another in the same organism. After a brief period of stimulation, the hormone level is reduced because it is no longer produced in the primary organ and is washed away from receptor cells in the bloodstream. In such a case the partial adaptation (Fig. 5, column B) can provide a large initial signal which is partially dampened to prevent excessive stimulation. The mathematics of partial adaptation show that a single feedback system can give the pattern of Fig. 5, column B, provided that X, which is generated as described in Eq. 7, inhibits v_f by a slow process on a delayed time scale. Partial adaptation may, of course, also utilize a covalent modification machinery for the compensatory process.

For a regulatory process involving change from one steady state to another (Fig. 5, column C) no adaptation is necessary and hence it is unnecessary to expend energy on a compensatory adaptation mechanism.

Conclusion

If we now return to the questions posed earlier, the above analysis provides us with answers. Both amplification and adaptation occur in the same cells, and in fact are part of an integrated system designed to obtain maximum responsiveness to changes in environmental conditions. The molecular mechanisms that amplify signals in both regulatory and sensory systems are similar, and both systems respond to environmental changes. There is a fundamental difference, however, in that the sensory system is more complex than pathway regulation and is designed for a transient signal as well as a permanent change in output.

In the molecular mechanisms available, it is seen that "magnitude amplification" of a small signal is readily achieved and, in fact, can give such large factors that the cell builds in devices to prevent excessive amplification, that is, "leaks" to offset the "gains." "Sensitivity amplification," on the other hand, in which a percentage change in stimulus leads to a larger percentage change in response, is difficult to achieve and requires cleverly designed, ultrasensitive systems. Three distinct molecular mechanisms for achieving ultrasensitivity—cooperative, multistep input of regulators, and zero-order ultrasensitivity—exist, each of which is capable of achieving appreciable ultrasensitivity. Several of these molecular processes may occur in concert, but in each case there are serious limitations with regard to the kinetic constants of the system and the range over which control is exerted.

It is important to emphasize that the larger the sensitivity amplification, the narrower the range over which control is exerted. In some regulatory systems, therefore, it may be advantageous to have hyperbolic sensitivity or even subsensitivity to extend the range of control when high sensitivity is not needed. For sensory systems, adaptation provides this function.

To achieve control, a price must be paid in terms of energy. This is paid either by (i) the synthesis of proteins with appropriate kinetic and feedback properties or by (ii) the continual use of energy to maintain the system. The latter is achieved through covalent modification and demodification in which the ultimate source is ATP.

A sensory system can use all of the devices of a regulatory system; but since it produces a transient signal, it has the potential of utilizing some different devices. One of these allows a sensory system to detect extremely small changes in stimulus over a large background by means of an adaptive response, which in essence resets the system to zero. It does this by making the system responsive to a changing stimulus rather than to the absolute level of stimulus, thus allowing the cell to maintain high sensitivity over a wide range of background intensities.

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