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MINIREVIEW

LexA Cleavage and Other Self-Processing Reactions

JOHN W. LITTLE

Department of Biochemistry and Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721

INTRODUCTION

Beginning with the spectacular discovery of self-splicing RNA over a decade ago (20), molecular biologists and biochemists have uncovered an increasingly wide variety of self-processing reactions involving RNA or protein molecules. For purposes of this minireview, self-processing reactions are defined as those in which a macromolecule carries out a specific covalent modification of itself. For reasons to become clear, this definition may be broadened slightly to include certain modifications of tightly bound ligands. Examples of self-processing reactions are splicing of group I and group II introns (8), cleavage of viral polyproteins (2), phosphorylation and dephosphorylation of proteins in two-component regulatory systems (5, 30, 43), cleavage of LexA and λ repressors (25), and most likely a set of recently described protein splicing reactions (39). A closely related reaction is the GTPase activity of G proteins such as Ras, in which a tightly bound GTP is hydrolyzed, leading to changes in the activity of the protein (3, 47). In some cases, such as Ras GTPase and LexA cleavage, the rate of a self-processing reaction is regulated according to the needs of the cell or organism by interaction with another effector molecule, which acts indirectly to stimulate the self-processing reaction.

Any self-processing reaction involves at least two types of sites: (i) the site to be modified, analogous to the substrate for an intermolecular enzyme reaction, and (ii) the active site, composed of a catalytic center that carries out the chemistry of the reaction and a binding pocket that positions the substrate optimally with respect to the catalytic center. Although many self-processing reactions occur only once in a given molecule and hence are not formally catalytic (since the molecule does not emerge from the reaction unchanged), there is no reason to believe that these highly specific reactions differ in any fundamental way from those catalyzed by enzymes; indeed, many self-processing molecules can be converted into enzymes.

Discovering and documenting that a reaction is self-processing can be difficult experimental tasks, particularly in systems in which the reaction is stimulated by another effector, since the straightforward inference is that such an effector acts directly like most enzymes. It is highly likely that many such reactions have been observed but that their intramolecular natures have not yet been recognized. Accordingly, it is timely to consider known examples for clues as to how this property has been uncovered. In this minireview, I focus first on LexA cleavage and illustrate how various aspects of this system typify the behavior of self-processing reactions. I then describe briefly several other self-processing reactions, most of which have been reviewed recently, and discuss the possible similarities among these reactions. Mechanisms for regulating their rates are then explored, and the means of their discovery is discussed. Because of space limitations, I cite recent reviews in almost all cases; I apologize to many workers for the lack of individual citations.

SPECIFIC LexA CLEAVAGE

The SOS regulatory system of *Escherichia coli* controls the cellular response to conditions that damage DNA or inhibit DNA replication (26, 46). During normal cell growth, LexA repressor is stable and represses a set of about 20 SOS genes. When cells are treated with agents that induce the response, such as UV irradiation or mutagens, an inducing signal is produced, which activates RecA protein to a form that can increase the rate of LexA cleavage. Upon cleavage, LexA is inactivated and the SOS genes are turned on. Accordingly, the rate of RecA-dependent cleavage controls the state of the SOS system (23). If the cell carries a λ prophage, λ repressor is cleaved in a closely similar reaction and prophage induction ensues. Cleavage of λ repressor is far slower than that of LexA, ensuring that prophage induction is efficient only in cells that are likely not to survive the DNA-damaging treatment. In addition, the host UmuD protein is cleaved in the same type of reaction, but in this case cleavage activates the protein for its role in mutagenesis.

Although cleavage of LexA and λ repressors also requires an activated form of RecA in vitro at neutral pH, these repressors cleave themselves at high pH (24). This autodigestion reaction is intramolecular: it displays first-order kinetics, and its rate constant is independent of protein concentration. This behavior is one of the hallmarks of self-processing reactions. Autodigestion cuts the same bond as is cleaved in RecA-mediated cleavage. Several mutant proteins that are resistant to RecA-mediated cleavage also cannot autodigest. These and many other findings suggest that RecA stimulates repressor self-cleavage, rather than acting directly as a protease, and hence we term it a coprotease (25).

In this view of LexA cleavage, the active site that carries out the chemistry of bond breakage lies in LexA, not in RecA. That this site closely resembles that of a typical enzyme is shown by recent evidence that the cleavage product of LexA can act as an enzyme to cleave other molecules of LexA (18). This view of LexA cleavage raises two major questions: What is the mechanism of self-cleavage, and how does RecA protein increase its rate under physiological conditions? Answers to these questions may prove generally applicable to our understanding of self-processing reactions and their regulation.

LexA has several sites involved in cleavage: (i) the cleav-

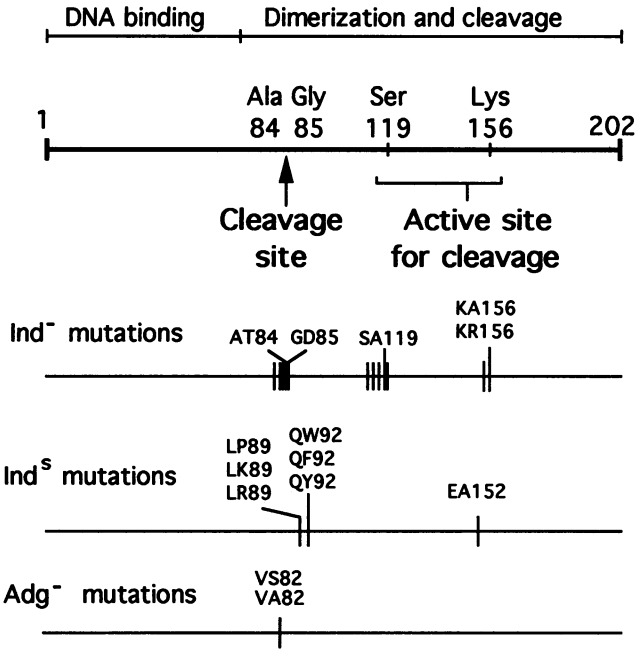

FIG. 1. Schematic depiction of LexA protein. Domain organization of the protein is shown at the top; locations of the cleavage site and active site are indicated. Below are indicated the positions of various types of *lexA* mutations affecting specific cleavage. Ind⁻ (noninducible) mutants show little or no cleavage; Ind^s (superinducible) mutants show increased cleavage rates; Adg⁻ (autodigestion-specific) mutants exhibit specific defects in autodigestion but appear normal for RecA-mediated cleavage. Mutations are named with the wild-type amino acid (one-letter code), followed by the mutant amino acid and the amino acid residue in LexA. See text for details.

age site, the substrate for specific cleavage; (ii) the active site, composed of a catalytic center and a binding site for the substrate; and (iii) a RecA-binding site, where RecA binds to carry out its role in promoting cleavage. The first two types of sites, again, are typical of all self-processing reactions.

The locations of the cleavage site and active site in LexA have been established by a combination of genetic and biochemical analyses (25) and by comparisons with the amino acid sequences of other cleavable proteins (Fig. 1). Mutations that prevent cleavage affect conserved residues lying in three parts of LexA: around the cleavage site and around two residues, Ser-119 and Lys-156, that play direct roles in the chemistry of bond breakage (see below). Similar analysis of λ repressor identified residues important for cleavage in homologous regions of that protein (14, 15).

Identification of the RecA-binding site in LexA and λ repressors has proven more complicated. The analysis of λ repressor mutant proteins identified several that exhibit specific defects in RecA-mediated cleavage but appear normal for autodigestion; these changes probably weaken the interaction with RecA. For reasons that are not clear, similar mutations in *lexA* have not been found. The RecA-specific mutations in λ repressor do not affect residues conserved with other cleavable proteins, suggesting that the RecA-binding site is not conserved among these proteins. In turn, this lack of conservation implies that the interaction between RecA and its substrates is not identical for all substrates, supporting the idea that RecA acts indirectly to stimulate cleavage.

The chemical mechanism of LexA cleavage resembles in some ways that seen for serine proteases such as trypsin. A conserved serine, Ser-119 in LexA, is almost certainly the nucleophile that attacks the peptide bond, since a mutant protein (SA119) changing Ser-119 to Ala has completely lost the ability to cleave (40), and Ser-119 reacts selectively with a serine protease inhibitor (33). Ser-119 is apparently activated as a nucleophile by a mechanism different from that of a classical serine protease, however. The pH rate profile of autodigestion is consistent with the need to titrate a basic group in the protein with a pK_a of about 10. This group is probably a conserved lysine, Lys-156 in LexA; KA156 mutant protein is deficient for cleavage (40), and a change to Arg alters the pH rate profile. Although the role of uncharged Lys-156 is unclear, we suggested (40) that it helps remove a proton from Ser-119 and provides a proton to the α-amino group created when the peptide bond is broken and a covalent ester intermediate is formed. A similar role for deprotonated lysine has recently been proposed on the basis of detailed structural data for β-lactamase (44), which also has an active-site serine that forms a covalent ester intermediate with the substrate. For LexA, we believe it likely that the requirement for titration of a basic group is crucial to the proper control of the rate of cleavage, allowing this rate to be regulated by interaction with RecA, the effector (see below).

How can an effector molecule increase the rate of a self-processing reaction? Several approaches provide at least a tentative answer in the RecA-LexA system. In addition to its effect on self-cleavage, KR156 mutant protein also displays an altered pH rate profile for RecA-mediated cleavage; whereas the wild-type protein undergoes this reaction at about the same rate from pH 6 to 11, KR156 has a rate profile consistent with the need to titrate a group with a pK_a of about 9.5. This finding implies that RecA acts somehow to reduce the pK_a of the Arg residue several pH units and that it does so even more efficiently for the wild-type Lys residue. How might it do so?

LexA is evidently designed to undergo slow cleavage but to be capable of large increases in rate upon interaction with RecA. We reasoned (34, 41) that analysis of mutant LexA proteins with an increased rate of cleavage might help identify the rate-limiting step or steps in cleavage. The rate of any chemical reaction is determined by the free energy difference between the ground state and the transition state. In principle, the rate could be increased either by decreasing the energy of the transition state, as in most enzyme reactions, or by increasing the energy of the ground state, or both. The analysis described below suggests that LexA is designed to allow increases in the energy of the ground state upon interaction with RecA.

We believe that a conformational model (34) for LexA cleavage (Fig. 2) can explain the role of RecA. In this model, LexA can exist in two conformations, a low-energy one termed L and a high-energy one termed L*, that are in equilibrium with an equilibrium constant K_{conf}. The pK_a of Lys-156 in the L form is a normal value of ≈10 but is reduced in the L* form to ≈5 to 6 because it is in a special environment. In this view, the role of RecA is to stabilize greatly the L* form of the protein; effectively, RecA raises the value of K_{conf}, forcing the equilibrium toward L* even at neutral pH. The environment reducing the pK_a is probably created by groups in LexA, not in RecA, since RecA appears to interact differently with different proteins.

This model was suggested by the properties of a new class of *lexA* mutations, termed hypercleavable or Ind^s mutations, that confer increases in the rate of specific cleavage (34, 41).

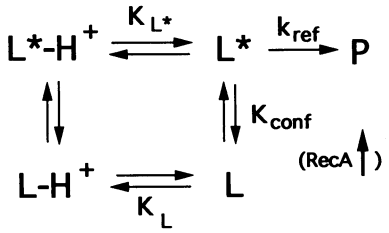

FIG. 2. Model for LexA cleavage reaction. According to this model, only the L* form of LexA can autodigest. K_{conf} is an equilibrium constant between the L and L* forms; activated RecA is thought to stimulate cleavage by greatly raising the value of K_{conf}. K_L and K_{L^*} represent equilibrium constants for titration of Lys-156 in the L and L* forms of LexA, respectively; according to the model, pK_L and pK_{L^*} are 10 and 5 to 6, respectively. k_{ref} is the rate constant for cleavage of L*. See text for details. Modified (with permission) from reference 34.

Mutations changing Gln-92 to Trp, Phe, or Tyr confer large increases in rate; QW92 cleaves about 300-fold faster than wild-type protein at neutral pH. The pH rate profiles for cleavage of these mutant proteins are consistent with the model in Fig. 2, if we suppose that the Ind^s mutations increase the value of K_{conf}, albeit to a lesser extent than RecA does. Other evidence also suggests that the Ind^s mutational changes mimic the role of RecA. This viewpoint suggests that the relationship between RecA-mediated cleavage and autodigestion is even closer than has been imagined in the past. It also emphasizes the indirect role of RecA in promoting the self-processing reaction.

One prediction of this model is that mutants might exist for which the value of K_{conf} would be reduced in such a way that RecA could overcome the effect. Such mutant proteins would appear to exhibit specific defects in autodigestion but would appear normal for RecA-mediated cleavage. Using a screen for suppressors of QW92, we have recently identified such an Adg$^-$ (autodigestion-specific) mutant, VA82; another mutant, VS82, exhibits reductions of about 100-fold for autodigestion but, at most, twofold for RecA-mediated cleavage (37).

Although, as noted above, LexA does not in a formal sense catalyze the self-cleavage reaction, since it is changed during the reaction, the C-terminal cleavage product of LexA can act as a relatively efficient enzyme to cleave other molecules of a truncated LexA protein or an intact protein with a mutation (SA119) in its active site (18). These observations provide support for the idea that the active site in a self-processing molecule does not differ fundamentally from that in an enzyme. Ind^s mutations at the cleavage site improve the substrate but not the enzyme; in contrast, an Ind^s mutation (EA152) near the active-site Lys-156 improves the quality of the enzyme but not of an intact substrate. An intact, noncleavable enzyme (GD85) can act on other substrates. Interpretation of these findings is limited by the fact that the substrate concentration is below the K_m. However, the abilities of intact LexA to work as an enzyme and of an intact substrate to be cleaved suggest that both the active site and the cleavage site are relatively exposed to the solvent in intact LexA. An attractive interpretation is that the L↔L* interconversion may simply represent binding of the cleavage site to the active site. If this is so, it implies that this interaction is not favored in intact LexA, an arrangement that makes sense in terms of the need to control the rate of cleavage.

The interaction between the cleavage site and active site is apparently even weaker in λ repressor. Cleavage of λ repressor is far slower than that of LexA. Strikingly, in the bimolecular reaction the C-terminal fragment of λ repressor catalyzes cleavage of the LexA substrates about as well as the C-terminal fragment of wild-type LexA does and cleaves an Ind^s LexA substrate more rapidly than a wild-type one (18). A truncated λ *CI* substrate is not cleaved by either enzyme. These findings imply that the activity and substrate-binding specificity of the active site in λ repressor are similar to those of LexA and that the rate of cleavage has been modulated during evolution by changes in the interaction between the cleavage site and the active site, rather than changes in the catalytic power of the active site.

In summary, our model (Fig. 2) provides the outlines of an understanding of how LexA self-cleavage is regulated. Two forces operate to restrain the cleavage reaction. First, the reactive conformation is greatly disfavored in the wild-type protein, even at high pH; this means that the ground state of the protein is at a lower energy level. Second, the reactive conformation is further destabilized at neutral pH, because the chemical mechanism of cleavage requires titration of Lys-156, which is disfavored at neutral pH. Both these factors can be overcome by large increases in K_{conf}, an increase which we propose to be carried out by the RecA effector under physiological conditions. Accordingly, the mechanism by which the effector works in the LexA case involves the detailed chemical mechanism of the self-cleavage reaction, and it is unlikely to apply in detail to other systems.

The development of this field (25) illustrates some of the difficulties in identifying a self-processing reaction, particularly in a situation where the rate of this reaction is modulated by another effector. Early experiments were most simply interpreted as reflecting a classical enzyme role for RecA; to be sure, this model was in some ways difficult to accept, given the extreme complexity of RecA for its roles in strand-transfer reactions. A demonstration that self-cleavage could take place required proof that it was not due simply to trace contaminants of a protease. Indeed, the first preparations of LexA underwent slow autodigestion upon storage at 4°C (27), but the significance of this observation was not understood until the reaction was observed with a short incubation period. Finally, it was a matter of luck that intact LexA autodigests at a measurable rate; if LexA contained an Adg$^-$ mutation such as VS82, the SOS system would probably operate normally, but autodigestion might not have been discovered; indeed, λ repressor had been studied intensively as a repressor for about 15 years, and yet its autodigestion had not been observed.

OTHER SELF-PROCESSING REACTIONS

Self-processing reactions may broadly be divided into three classes (Table 1): (i) reactions that apparently proceed spontaneously, (ii) reactions that are facilitated by interaction with other effectors but are not known to be regulated, and (iii) reactions that are facilitated by other effectors in a regulated manner. In this context, we may define regulation as an event that occurs in response to the conditions, such as the environment or cell type of a cell. Although one would expect a teleological rationale for such regulation, it may not be apparent to the investigator, as in any case of regulation (e.g., reference 29). Class 2 reactions may, of course, prove to be regulated in a way that has not yet been experimentally detected. In this section, I consider several of the best-

TABLE 1. Classes of self-processing reactions[a]

Self-processing reaction
Class 1. Spontaneous reactions
rRNA splicing in vitro (under certain conditions)
Histidine decarboxylase cleavage
Viral polyprotein cleavage
Ada protein self-methylation (requires substrate)
Protein splicing?
Autophosphorylation of some sensors in two-component systems
Class 2. Reactions stimulated by other effectors but not known to be regulated
a. Effectors required
Splicing of most group II introns
Dephosphorylation of some response regulators in two-component systems
b. Effectors stimulate reaction, not required
Dephosphorylation of some response regulators
Splicing of some group I introns (may depend on conditions)
Class 3. Reactions whose rates are affected by effectors in a regulated manner
Cleavage of LexA and λ repressors, UmuD protein
Autophosphorylation of some sensors
Dephosphorylation of some response regulators
Receptor tyrosine kinase (usually intermolecular?)
Ras protein GTPase activity

[a] References and details are given in the text. This list is not intended to be complete.

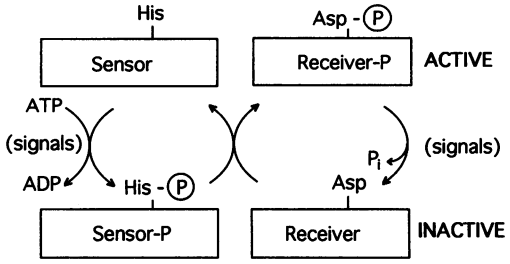

FIG. 3. Organization of a typical two-component regulatory system. Generally these systems involve two proteins, a sensor and a receiver or response regulator. Each reaction shown in this diagram is a self-processing reaction. The inputs and outputs of these systems are exceedingly diverse. In some instances, signals stimulate sensor autophosphorylation; in other cases, signals stimulate receiver dephosphorylation. See text for details.

understood examples of self-processing reactions; their features will be related to each other and to the LexA system in the next section.

Protein splicing. Several examples of reactions in which an internal portion of a polypeptide is removed and the two terminal fragments are joined have recently been described (39). It is not known whether this reaction forms a normal peptide bond, but a simple transpeptidation reaction is biochemically feasible, analogous to the transesterification reactions in RNA splicing. Protein splicing appears to proceed spontaneously, since the known reactions can be observed in heterologous systems where one would not expect specific *trans*-acting catalytic factors to be present. However, these reactions could be regulated in their natural context, for example in a negative manner by interaction with an inhibitor.

Histidine decarboxylase. The enzyme from *Lactobacillus* 30a is made as a single polypeptide chain, which then undergoes an internal cleavage at a Ser-Ser bond. This reaction generates a pyruvoyl terminus, rather than an amino terminus; the pyruvoyl moiety plays a critical role in catalysis (45). The crystal structures of the enzyme and the uncleaved proenzyme have been determined; the only major change upon processing is that the residues at the site of cleavage move apart about 1.7 Å (0.17 nm). Thus, the active site that catalyzes self-processing lies at or very near the active site of the final enzyme, even though the processing reaction is chemically different from the decarboxylation reaction. However, the self-processing event does not need to be highly efficient, since it needs to occur only once in a given molecule; in vitro, self-processing occurs at a rate roughly 10^{-4} to 10^{-5} the rate of the decarboxylation activity (32).

Self-splicing RNA. Despite their distinctive property of being RNA based, two features of these reactions unify them with other self-processing reactions. First, the self-splicing capacity of group I introns has been exploited to develop into an enzyme (or ribozyme) reaction, in which one molecule can catalyze many cleavage or transesterification reactions (8). These findings illustrate once again that self-processing is chemically similar to a catalytic reaction. Second, their rates can be modulated by other effectors. Although self-splicing of group I and group II introns proceeds in vitro, many of these reactions require specialized conditions, such as elevated temperatures, high Mg^{2+} levels, or the presence of polyamines, to take place. In vivo, these reactions require other factors, presumably proteins. A well-characterized example is the splicing of the terminal intron of the *Saccharomyces cerevisiae* mitochondrial *cob* mRNA, which requires the CBP2 protein in vivo and, at low Mg^{2+} concentrations, in vitro (13). Interaction of CBP2 with its RNA target might stabilize a reactive RNA conformation or provide a strong binding site for a required metal ion that otherwise binds very weakly to its binding site in the RNA. It is not known whether the action of CBP2 is regulated.

Two-component regulatory systems. Many signal transduction pathways in prokaryotes rely on proteins that undergo self-processing reactions (5, 30, 43). Although the physiological roles of these proteins are extraordinarily diverse, they are united by a common biochemical mechanism (Fig. 3). These two-component systems each have a sensor protein, whose activity is often regulated by the conditions and which autophosphorylates on a conserved histidine residue, and a receiver or response regulator protein, which is phosphorylated on a conserved aspartate residue in a phosphotransfer reaction with the phosphohistidine of the sensor protein as a donor. In addition, in several systems the dephosphorylation of the receiver protein is catalyzed by interaction with other molecules.

A recent finding sheds light on the molecular nature of receiver phosphorylation. Several receivers can be phosphorylated by low-molecular-weight phosphate donors such as acetyl phosphate or phosphoramidate (28). These findings clearly show that the reaction is an autophosphorylation of the receiver. That is, the active site for phosphorylation lies in the receiver, not in the sensor, and the sensor is a relatively passive partner in the reaction. The sensor does not act in the classical sense as a kinase, but rather as a substrate with (presumably) an especially low K_m for the receiver. In addition, for some receivers, such as CheY and NtrC, phosphorylation appears to be transient, showing that

these proteins have autophosphatase activity as well. In essence, the proteins are acting as specific phosphatases with a phosphoryl-enzyme intermediate (35). However, the purpose of this reaction is to create the phosphoryl-enzyme, which has some distinctive biochemical function, rather than to remove phosphate residues from another molecule. Although small molecules such as acetyl phosphate may serve as donors in vivo, the systems are apparently designed to use the sensors as relatively specific phosphate donors. In some systems, other sensors can serve, although inefficiently, as donors, leading to a phenomenon called cross-talk in which stimulation of one sensor leads to activation of multiple receivers (5, 30). In summary, then, these two-component systems display three different types of self-processing: sensor autophosphorylation, receiver autophosphorylation, and receiver autophosphatase activity.

In addition, each of these types of reaction is regulated in certain cases, either in response to signals (class 3 in Table 1) or in response to the presence of other effectors (class 2). First, many sensors autophosphorylate in response to signals. For example, the chemotaxis protein CheA autophosphorylates when the chemotactic receptors, such as Tar, are activated by ligands. This reaction is also greatly stimulated by another protein, CheW, which apparently holds CheA and the receptors together. Recent evidence with CheA (unpublished data cited in reference 43) and another sensor, EnvZ (48), suggests that sensor autophosphorylation is not truly intramolecular but relies on apposition of two monomers in a complex in which each phosphorylates the other. These systems will require further analysis, since it remains possible that this reaction is naturally intramolecular but that an intermolecular reaction can take place when the intramolecular pathway is blocked; even an inefficient sensor *trans*-phosphorylation reaction could yield high levels of OmpR~P, since the *trans*-acting EnvZ mutant proteins used in these studies cannot stimulate dephosphorylation of OmpR~P (see below).

Second, receiver autophosphorylation is often regulated by the availability of its substrate. For instance, the availability of CheA~P as a substrate for CheY phosphorylation is regulated by chemoreceptor occupancy. There are apparently no examples to date in which the catalytic capacity of the receiver to autophosphorylate is regulated by an interaction with other proteins.

Finally, dephosphorylation of certain receivers is facilitated by interaction with other molecules. When CheY~P interacts with CheZ, the rate of dephosphorylation is greatly increased. There is no evidence that this interaction is regulated, but it seems probable that, again, CheZ is not acting as a specific phosphatase but, instead, facilitates the autophosphatase activity of CheY. The crystal structure of CheY is known, offering the hope that the mechanism of this facilitation will be worked out in detail. Dephosphorylation of NtrC~P is greatly stimulated by interaction with NtrB and another protein called P_{II} that acts as an indicator of nitrogen depletion or excess. Similarly, EnvZ is required for dephosphorylation of OmpR~P, which by itself is as stable as any acyl phosphate. The rate of the EnvZ-mediated reaction is thought to be regulated by the state of EnvZ in response to the osmolarity of the medium, being more rapid at low osmolarity. On the basis of the CheY and NtrC examples, it has been suggested that all instances of phosphatase activity catalyzed by sensors are indirect stimulations of an intrinsic autophosphatase reaction (30). Should this hypothesis be correct, this activity would be directly analogous to the coprotease activity of RecA, and I suggest that the stimulatory activity of the effector be termed a cophosphatase activity.

Ada protein. Ada protein is the central regulator of the bacterial adaptive response to DNA alkylation (22). Methyl groups from two types of DNA lesions, O^6-methylguanine and DNA methyl phosphotriesters, are transferred to Cys residues located in the C- and N-terminal portions of the Ada protein itself, respectively. The latter reaction activates Ada protein to become a transcriptional activator of its own gene and other genes involved in the adaptive response. Both these methyl transfer reactions are irreversible, so that each molecule of Ada can remove only one methyl group from each type of donor. It is unclear why the protein should be designed in this seemingly inefficient way. These reactions are therefore regulated only by the availability of the substrates, an efficient and direct mechanism, since O^6-methylguanine is highly mutagenic and Ada protein removes it.

GTP-binding proteins. A wide variety of GTP-binding proteins play crucial roles in signal transduction. The activities of these proteins are controlled by GTP hydrolysis. Although the GTPase activities of the proteins are not strictly self-processing reactions, they may be considered honorary members of this class if we view the tightly bound GTP as part of the molecule.

The usefulness of this viewpoint is especially evident in the case of Ras, one of the best studied GTP-binding proteins. Ras proteins play crucial roles in signal transduction in eukaryotic cells (3, 47). In its active form, Ras has a tightly bound GTP; hydrolysis of GTP deactivates it, leaving a Ras-GDP complex. Two opposing types of reactions activate and deactivate Ras. Activation is carried out by guanine nucleotide release proteins, which catalyze exchange of GDP for GTP; deactivation is carried out by GAP proteins (GTPase activating proteins) that interact with Ras and greatly increase the rate at which the bound GTP molecule is hydrolyzed. Accordingly, GAP proteins act as effectors to stimulate the self-processing GTPase reaction, in a manner formally analogous to the effect of RecA on LexA.

The structure of Ras is known; however, it is not yet clear whether the GAP proteins contribute functional groups to the chemistry of GTP hydrolysis or whether GAP proteins act indirectly to facilitate the intrinsic activity of Ras (by analogy to the action of RecA on LexA). Stimulation of GTPase activity is also seen with the prokaryotic translation elongation factor EF-Tu, in which interaction with the ribosome stimulates a GTPase reaction involved in kinetic proofreading (3). Similar reactions also take place in heterotrimeric G proteins; recent evidence suggests that the downstream effectors can serve as GAP proteins, increasing the rate at which an activated G protein is deactivated (4).

Mutant forms of Ras exist that have decreased levels of GTPase activity and hence are constitutively activated. These dominant mutant proteins play a role in oncogenesis. Strikingly, these mutant proteins cannot undergo GAP protein-stimulated hydrolysis. Since stimulation by GAP protein is probably the crucial event in vivo, it seems likely that the defect in the ability to be stimulated, rather than the reduction in the intrinsic GTPase activity, is responsible for constitutive activation.

Protein tyrosine kinases. Many membrane-bound receptors have tyrosine kinase activity (6). These proteins autophosphorylate, and the rate of this reaction is increased upon interaction with an extracellular ligand. Autophosphorylation elevates kinase activity toward other substrates. In these cases, as with prokaryotic sensor proteins, it is unsettled whether autophosphorylation can be intramolecular or

must be intermolecular (e.g., references 21 and 38). In some cases, interaction with ligand apparently promotes dimerization, which would favor an intermolecular reaction; however, some receptors, such as insulin receptor, are always dimeric.

REGULATING THE RATES OF SELF-PROCESSING REACTIONS

Any self-processing reaction involves an active site and a substrate, present in the same molecule. In principle, such a reaction can proceed at a rate limited only by the chemistry of the reaction, since the effective local concentration of the active site relative to the substrate can be high (12, 17). Some reactions, such as viral polyprotein self-cleavage (2) and protein splicing reactions, probably do proceed efficiently, if there is no need to restrain their rates. Self-processing of histidine decarboxylase may be slow, by contrast, because the reaction catalyzing it differs from the enzymatic function of the protein.

The issue becomes more complicated when the rate of a reaction is regulated by interaction with other effectors. In such cases, some means must be found to prevent rapid reaction from occurring. Some regulated self-processing reactions appear to be intermolecular in nature, and the effectors promote dimerization or a rearrangement of subunits with respect to each other. Either mechanism might juxtapose an active site and its substrate, greatly increasing the local concentration. For reactions involving a single self-processing molecule, the most plausible mechanism (granted that it is generally invoked when one does not understand a process involving proteins or RNA) is a conformational change promoted by an effector. This could result either in a normally disfavored conformation or in removal of an inhibitory domain, analogous to the effect of cyclic AMP on the two-subunit cyclic AMP-dependent protein kinase. Alternatively, the effector may provide a component that is missing or limiting in the self-catalyzed reaction or, as suggested for CBP2, make that component more available. It is even possible that the mechanism of the reaction may change; the rate-limiting step may be different, or the reaction may follow a different pathway. It seems highly likely that diverse solutions to this problem in different systems will be found.

In several systems, partially constitutive mutants that overcome to some extent the need for an effector have been found. Again, these almost certainly work in different ways in various systems. The Ind^s mutant proteins in LexA probably mimic the effect of RecA on cleavage. Many membrane tyrosine kinases become constitutively active (that is, independent of an inducing ligand) by truncation or loss of inhibitory domains (6). Ras is activated by loss of responsiveness to GAP. In two-component regulatory systems, by contrast, partially constitutive mutants of the response regulator may be ones that no longer need phosphorylation to be active (30). Characterization of constitutive mutants is helpful in sorting out the pathways and mechanisms of signal transduction in all these systems.

DISCOVERY OF SELF-PROCESSING REACTIONS

As exemplified by LexA, the self-processing nature of a reaction can be difficult to discover, especially if the reaction is stimulated by an effector, since this appears to the investigator as an enzyme. The routes to discovery in the various systems reviewed have been diverse. In many cases, such as LexA cleavage, rRNA self-splicing, NtrC or CheY dephosphorylation, and Ras GTPase, the reaction was observed in a system with purified components. In other cases, the reaction was first inferred by analogy with homologous systems, as in the case of λ repressor cleavage or OmpR~P dephosphorylation. In several cases, self-processing was only observed under special reaction conditions, such as high pH for LexA cleavage or high Mg^{2+} concentrations for group I intron splicing. Finally, the self-processing nature of response regulator autophosphorylation was shown conclusively when the reaction was observed with a low-molecular-weight phosphoryl donor. Clearly, the path to discovery in many cases has been a matter of luck in trying the right reaction conditions or in the fortuitous availability of similar reactions in homologous proteins. Certainly, keeping an open mind about interpreting one's findings is also helpful.

Likewise, establishing that a reaction is truly self-processing is difficult. For example, proteolytic reactions might easily result from traces of contaminating proteases attacking at solvent-exposed sites; if a protease were activated by self-cleavage but then could act intermolecularly, the initial events could be obscured. One hallmark of an intramolecular reaction is that its rate constant should be insensitive to dilution, and this criterion can usefully be applied in a test of this property, at least in simple systems involving soluble proteins.

The many precedents discussed above suggest that one should suspect and explore the possibility of self-processing for any reaction that involves a modification of a protein or RNA molecule. One particular type of biological system that seems particularly likely to involve regulated self-processing reactions is one involving cascades of similar biochemical reactions, such as proteolysis (11) or phosphorylation cascades. This mechanism was considered possible, for instance, in the cascade that activates MAP kinase; it was largely ruled out for the activation of MAP kinase because a mutant MAP kinase inactivated in its ATP-binding site could still be phosphorylated normally by MAP kinase kinase (31). However, this and all such studies have two potential caveats, which must be addressed but may be hard to resolve with certainty. First, the rate of the self-processing reaction might be impaired by mutation without destroying the ability of an effector to stimulate the reaction to levels approaching the wild-type level; our ability to isolate autodigestion-defective (Adg$^-$) LexA proteins illustrates this point. Second, the activity of an active site toward an intramolecular substrate might differ from its activity in an intermolecular reaction, so that a mutation might be able to interfere with the latter activity (which is easily assayed) but leave the former activity intact or less impaired.

WHY SELF-PROCESSING?

As with any teleological question, only plausible answers can be given. Evolution does not necessarily arrive at optimal solutions to biological problems but rather modifies and refines existing solutions (16). It seems likely that, early in evolution, relatively simple regulatory systems were the first to develop. Self-processing systems offer the advantage that the specificity is built into a single molecule. In principle, this molecule could be responsive to the conditions, allowing a simple form of regulation. For example, the stability of a proto-LexA protein might have been determined not by interaction with a sensor of DNA damage, but rather in response to the pH, with the interaction with activated RecA evolving later. This, like any interaction

TABLE 2. Enzymes with regulatory properties[a]

Protein	Enzymatic function	Regulatory function	Regulation mechanism
LexA protein	Self-proteolysis	Repression of SOS system	Interaction with activated RecA
RecA protein	DNA strand transfer, specific cleavage	Cleavage of LexA and λ repressors and UmuD protein	Presence of SOS inducing signal such as single-stranded DNA
Biotin holoenzyme synthetase	Synthesis of biotin holoenzyme	Repression of *bio* operon	Excess biotinyl-CoA remains bound to enzyme
Iron regulatory factor	Cytoplasmic aconitase	Stabilizes transferrin receptor mRNA, inhibits translation of ferritin mRNA	Excess iron favors 4Fe-4S cluster, inhibiting RNA binding
Threonyl-tRNA synthetase, thymidylate synthase, T4 DNA polymerase	Self-explanatory	Autoregulation of translation	High levels bind to the mRNA
Ada protein	DNA methylesterase	Transcriptional activator	Presence of DNA methylated on phosphates and O^6 of guanine
Response regulators	Autophosphorylation; autophosphatase	Transcriptional activators, other effector functions	Interaction with sensors (see text)

[a] References and details are given in the text. I would appreciate learning of other examples.

between two macromolecules, must involve a good deal of specificity; however, in the LexA case the specificity is probably limited to the need for a specific protein-protein interaction; the specificity for the cleavage site, as well as the active site that does the chemistry, lies in LexA. Once a self-processing system became established, perhaps it worked well enough that it was difficult to replace by a mechanism that would have to develop from scratch.

Can self-processing reactions evolve into ones that are purely catalyzed by external factors, in the sense that the catalytic center no longer resides in the molecule to be processed? It has been argued (7, 36) that self-splicing, particularly of group II introns, might be the evolutionary precursor to pre-mRNA splicing, based on the close similarity between the fate of the RNA in the two types of reactions. Indeed, many group II introns cannot self-splice in vitro, and require other factors to proceed in vivo. It is possible, however, that these molecules still contain the catalytic machinery but are ineffective because the right conditions have not been tried or the reactive conformation is too disfavored. It does seem plausible that a self-splicing mechanism would relinquish the job to spliceosome proteins and RNAs, which could work with more efficiency, specificity, and flexibility. As with all questions of evolution, an answer to this one will probably be apparent only after we better understand nature in its present form.

POSTSCRIPT—REGULATORS WITH ENZYME ACTIVITIES

As a final note, another type of comparison between LexA and other systems deserves mention. Many gene regulatory proteins which also have enzymatic activities are being discovered (Table 2). Among these proteins are transcription factors belonging to two-component regulatory systems, such as NtrC and OmpR. In several cases, the enzymatic activity is closely related to the regulatory role. The enzyme biotin holoenzyme synthase acts as a repressor of its own synthesis in the presence of excess biotin (10). The mammalian iron regulatory factor (or IRE-BP) binds to specific mRNAs when iron levels are low, stabilizing transferrin receptor RNA and inhibiting translation of ferritin mRNA; in the presence of high iron levels, IRE-BP is activated to a form that has aconitase activity, and when inactivated is an RNA-binding protein (19). The Ada protein catalyzes its own methylation with a DNA methyl phosphotriester as a donor, activating it as a transcriptional activator (22). Several enzymes, including T4 DNA polymerase (1), threonyl-tRNA synthetase (42), and thymidylate synthase (9), bind to their own mRNAs, inhibiting further translation when at high levels. This autoregulation stabilizes cellular levels of these enzymes. The binding site for threonyl-tRNA synthetase in its mRNA resembles the tRNA substrate for the enzyme reaction; a similar mechanism is used in translational control of many ribosomal proteins. Combination of regulatory and enzymatic functions in a single molecule provides a powerful means of integrating regulation and metabolism and can be expected to become an increasingly common theme in biology.

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