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Genetics

Design of molecular control mechanisms and the demand for gene expression

(positive compared to negative control/evolution/ecological niche)

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ABSTRACT **Regulation by a *repressor* protein is the mechanism selected when, in the organism's natural environment, there is *low* demand for expression of the regulated structural genes. Regulation by an *activator* protein is selected when there is *high* demand for expression of the regulated structural genes. These general conclusions are useful in relating physiological function to underlying molecular determinants in a wide variety of systems that includes repressible biosynthetic pathways, inducible biosynthetic enzymes, inducible drug resistance, and prophage induction, as well as inducible catabolic pathways, for which a special case of this prediction previously was reported [Savageau, M. A. (1974) *Proc. Natl. Acad. Sci. USA* 71, 2453–2455].**

The existence of repressors and activators controlling gene expression has been well documented in recent years. The question naturally arises, are these differences in molecular design significant? Are they simply historical accidents that represent functionally equivalent solutions to the same regulatory problem? Alternatively, have they been selected to meet specific needs and, if so, can we determine the functional implications inherent in each design and the nature of the selective forces that have given rise to them?

Questions of this type are difficult to answer using only the direct experimental approach. For example, one cannot draw conclusions about the differences between repressor and activator mechanisms by comparing directly two representative systems such as the inducible lactose (repressor-controlled) and maltose (activator-controlled) operons because there may be other (unknown) elements involved in their control, and because the systems differ in many ways that are irrelevant to the comparison of the two modes of regulation *per se*.

Ideally, one would like a controlled comparison in which the two systems are identical in every respect except one: the type of control mechanism utilized. Although this is difficult to obtain experimentally, it can be simulated by appropriate mathematical analysis (1). This approach has been applied to inducible catabolic systems in enteric bacteria (2); it led to the prediction of repressor control for pathways whose substrates are seldom present and activator control for pathways whose substrates are often present in the organism's natural environment. This prediction is confirmed by a variety of experimental evidence (2).

Recent analysis has shown that the prediction for inducible catabolic systems can be generalized to a much wider class of systems. In summary, this analysis shows that the only inherent difference in function between systems having activator and repressor mechanisms is their response to regulatory mutations. Repressor-controlled systems tend to become constitutively expressed, while activator-controlled systems tend to become super-repressed, in response to the same types of regulatory mutations. The consequences of these mutations depend upon the physiology and environment of the organism harboring these mechanisms. Super-repressed mutants will be at a selective disadvantage, when compared to the wild-type parent, if they are in an environment requiring significant expression of the regulated structural genes. That is, the functional activator mechanism will be selected when there is high demand for expression of the regulated structural genes; conversely, it will be lost through genetic drift when there is low demand for expression of the regulated structural genes. Constitutive mutants will be at a selective disadvantage, when compared to the wild-type parent, if they are in an environment that does not require significant expression of the regulated structural genes. Under these conditions, constitutive synthesis of extraneous enzymes or proteins is generally wasteful and may actively disrupt the otherwise harmonious operation of the organism. In other words, the functional repressor-controlled system will be selected when there is low demand for expression of the regulated structural genes; conversely, it will be lost through genetic drift when there is high demand for expression of the regulated structural genes. Thus, the more general principle can be stated as follows: *Repressor control is correlated with low demand for expression of the regulated structural genes, whereas activator control is correlated with high demand for their expression.* In this paper evidence supporting the validity of this principle for several different classes of systems is presented. The conditions corresponding to high demand for these systems are summarized in Table 1.

Inducible catabolic systems

The best-studied examples of systems from this class are found in enteric bacteria. In response to an environmentally supplied nutrient, the organism, under appropriate conditions, will induce specific enzymes needed for utilization of that nutrient. In the present context, repressor control, which correlates with low demand for expression, is expected for inducible systems whose substrate is seldom present at high concentrations in the organism's environment, whereas activator control, which correlates with high demand for expression, is expected for inducible systems whose substrate is often available at high concentrations in the organism's environment.

In enteric bacteria there are more than half a dozen inducible catabolic systems for which the nature of the regulator is known. For example, the systems for the utilization of galactose (3), glycerol (4), histidine (5), and lactose (6) are under the control of repressors, whereas those for the utilization of arabinose* (7),

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* Although the regulator of the arabinose system has both activator and repressor properties, in this context it can be treated as an activator (see ref. 1).

Table 1. Conditions corresponding to high demand for gene expression in specific microbial systems

System	High demand for expression
Inducible catabolic pathway	Substrate frequently present in high concentrations
Repressible biosynthetic pathway	End product seldom present in high concentrations
Inducible biosynthetic enzyme (within a repressible biosynthetic pathway)	End product seldom present in high concentrations
Inducible drug resistance	Drug frequently present in high concentrations
Inducible prophage (lytic functions)	Induction frequently occurs

maltose (8), and rhamnose (9) are under the control of activators. From this information one would predict that the first group of substrates is seldom present at high concentrations in the colon, where the enteric bacteria are localized. Conversely, the second group of substrates is expected to be there frequently at high concentrations.

Although the local environment in the animal colon is complex and largely undefined, the relative concentrations of specific nutrients in the colon can be estimated indirectly from their abundance in the diet and their absorption patterns in the small intestine. The results of such studies have been reviewed elsewhere (2) and are in agreement with the predictions in the previous paragraph (see Table 2). The disaccharides lactose and maltose are enzymatically split into their constituent sugars early and late, respectively, during transit through the intestines. Glycerol, the sugar galactose, and the amino acid histidine are all absorbed effectively at the beginning of the small intestine and are unlikely to reach the colon in high concentrations; the sugars arabinose and rhamnose are poorly absorbed and probably reach the colon without extensive attenuation in concentration.

From such absorption data one also can make predictions about molecular control mechanisms that have yet to be fully characterized in enteric bacteria. For example, the sugars mannose and xylose appear to be absorbed slowly by the small intestine (10) and therefore may be present at relatively high concentrations in the colon. In the bacteria one would expect the inducible catabolic systems for the utilization of mannose and xylose to be activator-controlled. Similarly, the relative abundance of tryptophan in the colon (see next section) suggests that the inducible tryptophanase system involves an activator-controlled mechanism. These predictions are summarized in Table 2, although they remain to be tested experimentally.

Repressible biosynthetic systems

The enzymatic machinery required for endogenous biosynthesis of amino acids is considerable (11). It makes economic sense for an organism to repress synthesis of the enzymes in a biosynthetic pathway whose end product is available preformed in the environment; the selective advantage of such repression mechanisms also has been shown experimentally (12). The general principle, restated for this class of systems, is the following: Repressor control, which correlates with low demand for expression, is expected for a repressible system whose end product is often present at high concentrations in the organism's natural environment; activator control, which correlates with high demand for expression, is expected for a repressible system whose end product is seldom present at high concentrations in the natural environment.

Table 2. Nature of regulator correlates with demand for expression of regulated genes

System[a]	Nature of regulator: Observed[f]	Nature of regulator: Predicted	Demand for expression: Predicted	Demand for expression: Observed[f]
Inducible catabolic pathways				
Arabinose	Activator	→	High	High
Galactose	Repressor	→	Low	Low
Glycerol	Repressor	→	Low	Low
Histidine	Repressor	→	Low	Low
Lactose	Repressor	→	Low	Low
Maltose	Activator	→	High	High
Rhamnose	Activator	→	High	High
Mannose	?	Activator ←	High	
Tryptophan	?	Activator ←	High	
Xylose	?	Activator ←	High	
Repressible biosynthetic pathways				
Arginine	Repressor	→	Low	Low
Cysteine	Activator	→	High	High
Isoleucine–valine[b]	Activator	→	High	High
Lysine	Repressor	→	Low	Low
Tryptophan	Repressor	→	Low	Low
Histidine	?	Activator ←	High	
Isoleucine–valine	?	Activator ←	High	
Inducible biosynthetic enzymes (within repressible biosynthetic pathways)				
Isoleucine–valine	Activator	→	High	High
Tryptophan[c]	Repressor	→	Low	?
Inducible drug resistance				
Penicillin[d,e]	Repressor	→	Low	Low
Tetracycline	Repressor	→	Low	Low
Chloramphenicol[d]	?	Repressor ←	Low	
Erythromycin[d]	?	Repressor ←	Low	
Inducible prophages				
λ	Repressor	→	Low	Low
P1	Repressor	→	Low	Low
P2	Repressor	→	Low	Low
P22	Repressor	→	Low	Low

An arrow indicates direction of inference. An entry in an *observed* column adjacent to an entry in a *predicted* column represents the results of independent observations that are used to test the predictions.

[a] Enteric bacteria unless indicated otherwise.
[b] *Saccharomyces cerevisiae.*
[c] *Pseudomonas putida.*
[d] *Staphylococcus aureus.*
[e] *Bacillus licheniformis.*
[f] Evidence of various types was used in constructing this table. The evidence is not equally conclusive in each case.

There is now evidence concerning the nature of the regulator for many repressible biosynthetic systems in microorganisms. Perhaps the clearest examples, in which a repressor element in the control has been demonstrated *in vitro*, are the tryptophan (13–18) and arginine (19, 20) biosynthetic systems in *Escherichia coli*. Less direct but strong genetic evidence suggests control by an activator for the cysteine biosynthetic system in enteric bacteria (21, 22) and the isoleucine–valine biosynthetic system in *Saccharomyces cerevisiae* (23–26). Kelleher and Heggeness (27) have demonstrated "escape synthesis" of diaminopimelate decarboxylase, the last enzyme in the lysine

biosynthetic pathway of *E. coli*, and have suggested repressor control for this system. From this information one would predict that arginine, lysine, and tryptophan are often present and cysteine is seldom present at high concentrations in the colon, where the enteric bacteria are localized. Similarly, one would expect isoleucine and valine to occur infrequently at high concentrations in the natural environment of yeast.

Although the evidence is indirect, in each case it appears to agree with the above predictions (see Table 2). Relative to other amino acids, tryptophan is poorly absorbed by the small intestine (28, 29) and would be likely to reach the colon. Furthermore, the ability to catabolize exogenous tryptophan appears to occur only among those microorganisms that are able to inhabit the gut, and, among the intestinal flora of virtually all the animals examined, there was at least one species of microorganism that was able to catabolize exogenous tryptophan (30). Arginine by itself is slowly absorbed by the intestine (31), but in a mixture with other amino acids its absorption appears to be accelerated (28). The most direct observations show that the concentration of free arginine reaching the distal end of the small intestine is the third highest of all amino acids under a variety of dietary conditions (32). In the study described in ref. 32, the concentration of lysine was the highest of all amino acids at the distal end of the small intestine, whereas that of cysteine was the third lowest.

In the case of *Saccharomyces cerevisiae*, a eukaryotic organism, the expected infrequent occurrence of isoleucine and valine at high concentrations in the natural environment is consistent with the evolution of this organism in sugar-rich nitrogen-poor environments. This is further supported by the observation of Gross (33) that biosynthetic pathways of fungi generally are "set" higher than those of enteric bacteria.

The correlation supported above also can be used to predict the nature of the regulator protein rather than the nature of the organism's environment. Indirect evidence shows that histidine can be absorbed readily by the small intestine (34), although in mixtures of amino acids its rate of absorption is lower (28). By more direct measurements, the concentration of free histidine reaching the distal end of the small intestine is among the lowest of all amino acids under a variety of dietary conditions (32). Thus, one would expect that the system for histidine biosynthesis is under the control of an activator protein, which is consistent with the results of Artz and Broach (35). A similar prediction can be made regarding isoleucine and valine. Each of these amino acids is absorbed rapidly by the small intestine when present alone (36) or in mixtures of amino acids (28, 29). Furthermore, the concentrations of free isoleucine and valine that reach the colon are among the lowest of all amino acids under a variety of dietary conditions (32). Thus, one would expect the system for biosynthesis of isoleucine and valine in *E. coli* to be under the control of an activator protein, which is consistent with the results of Levinthal *et al.* (37). These predictions also are summarized in Table 2, although experimental support cannot be claimed because there is still conflicting evidence concerning the histidine and isoleucine–valine biosynthetic systems in enteric bacteria (see ref. 38).

In addition to repressor/activator mechanisms affecting initiation of transcription, genetic control can be achieved by mechanisms that modulate termination of transcription.† Such mechanisms have been reported for the histidine, tryptophan, and isoleucine–valine biosynthetic systems in enteric bacteria, as well as for bacteriophage λ. This literature is reviewed elsewhere (38).

† In the present context, a mechanism in which the end product (or related metabolite) brings about repression by antagonizing an "anti-terminator" would be formally analogous to activator control; one in which the end product (or related metabolite) brings about repression by combining with an "apoterminator" to form a "terminator complex," which blocks transcription at an attenuator site, would be formally analogous to repressor control.

Inducible biosynthetic systems

There are now several examples of a repressible biosynthetic pathway in which synthesis of one of the enzymes is actually induced by its substrate (33, 39–42). The level of the inducing substrate is in turn decreased by an increase in the level of the pathway's end product, which acts as an allosteric modifier to cause repression of synthesis and/or feedback inhibition of the first enzyme in the pathway.

Repressor control is predicted for those inducible biosynthetic systems whose substrate is seldom present because the end product of the pathway is *frequently* present at high concentrations in the organism's environment; activator control is expected when its substrate is frequently present because the end product of the pathway is *seldom* present at high concentrations in the organism's environment.

Acetohydroxy acid isomeroreductase, one of the enzymes shared for the biosynthesis of isoleucine and valine in *E. coli*, is subject to induction by its substrates, either α-acetolactate or α-acetohydroxybutyrate (42). Genetic studies (43) and recent studies *in vitro* (44) have strongly implicated an activator mechanism in the control of this inducible system. From this information one would predict that isoleucine and valine are seldom present at high concentrations in the natural environment of *E. coli* (see Table 2). Independent, experimental evidence for the relative scarcity of isoleucine and valine in the colon was presented in the previous section.

In another system, tryptophan synthase, the last enzyme in the pathway for biosynthesis of tryptophan, is induced by its substrate indole-3-glycerolphosphate in *Pseudomonas putida* (39, 40) and *Pseudomonas aeruginosa* (41). Proctor and Crawford (45, 46) have presented evidence suggesting that the α chain of tryptophan synthase (or a protein acting with it) is a repressor for this inducible system. On the basis of this evidence one would predict that tryptophan is frequently present at high concentrations in the natural environment of these *Pseudomonas* organisms.

Inducible drug resistance

Natural selection of drug-resistant microorganisms has occurred with expanded clinical and agricultural use of antibiotics during the past two decades (e.g., see refs. 47–49). Simultaneous resistance to several drugs, which is rapidly transmitted to other microorganisms via extra-chromosomal elements (49), is of particular interest because of the obvious implications for clinical practice but also because of the fundamental molecular mechanisms involved. Although resistance is expressed constitutively in most instances (50), there are well-documented examples in which resistance to high levels of a drug occurs in microorganisms following their exposure to low (or subeffective) concentrations of that drug. In some cases this increased resistance is the result of gene amplification among the resistance determinants (51); in others, it is the result of increased gene expression. Examples of the latter type, which are of primary concern in the present context, are inducible resistance to tetracycline (52), penicillin (53, 54), erythromycin (55), and chloramphenicol (56). Repressor control, which correlates with low demand for expression, is expected for such inducible systems when the corresponding antibiotic is seldom present at high concentrations in the organism's natural environment, whereas activator control, which correlates with high demand for expression, is expected for systems of this type when the

corresponding antibiotic is often present at high concentrations in the natural environment.

Recent studies *in vitro* of inducible tetracycline resistance in *E. coli* strongly suggest that this system is under the control of a repressor protein (52). The same conclusion for inducible penicillin resistance in *Bacillus licheniformis* (53) and *Staphylococcus aureus* (54) has been supported by genetic and biochemical studies. From the molecular evidence in these cases one would expect that tetracycline and penicillin are seldom present at relatively high concentrations in the natural environment of these bacteria (see Table 2).

Again there is only indirect evidence to support the prediction. Although use of such antibiotics has increased in the past two decades, the fraction of time that microorganisms are exposed to high doses of these drugs may still be considered relatively small (see ref. 48). This exposure, while certainly significant for selection of drug-resistant organisms, is probably not sufficient for selection of activator mechanisms controlling expression of such resistance.

The above arguments also could be used to predict that induction of chloramphenicol resistance and induction of erythromycin resistance are under the control of repressor proteins (Table 2).

Inducible prophages

Temperate phages or bacterial viruses have two alternative modes of replication. In the lysogenic mode, the virus exists as a prophage or viral genome that replicates along with its host but is otherwise almost totally quiescent (see ref. 57). In the lytic mode, the virus grows vegetatively and eventually progeny are released from the infected cell. The phage can switch from the lysogenic to the lytic mode of replication in response to signals indicating the physiological state of the host cell. This process is called induction.

One can conceive of an inducible prophage under the control of an activator protein. The prophage synthesizes an activator that is normally unable to stimulate transcription of the other viral genes, but in response to the appropriate host signal(s), the activator is converted to a conformation that facilitates expression of viral genes required for lytic growth. Induction also could be under the control of a repressor protein. In this case, the prophage synthesizes a repressor that blocks expression of almost all viral genes. In response to the appropriate host signal(s), the repressor is converted to a form that no longer is able to block transcription of viral genes.

The same physiological function—induction—is realized in each case, but the molecular mechanisms are different. Activator control, which correlates with high demand for expression, is expected for a prophage whose induction is a frequent event, whereas repressor control, which correlates with low demand for expression of the regulated genes, is expected for a prophage whose induction is an infrequent event.

Bacteriophage λ has been studied most thoroughly, and its induction is under the control of a repressor protein (58). Among other temperate phages that have been well studied at the molecular level, repressor control also appears to be the rule [P1 (59); P2 (60); P22 (61)]. From the deductions above and the known molecular nature of the regulator protein for several bacteriophages, one would predict that induction of these prophages is relatively rare in nature (see Table 2).

Although good experimental evidence concerning the frequency of phage induction in nature is not available, there is evidence that tends to support the above prediction. Spontaneous induction is relatively rare under a variety of conditions in which it has been examined (see ref. 62) and most, if not all, natural isolates of coliform organisms are lysogenic for one or more phages (see ref. 63).

Other systems

As stated in the *Introduction*, the correlation between the molecular nature of the regulator protein and demand for expression of the regulated genes may be of quite general importance. This is indicated by the deductions from which this correlation was predicted (1), but it also is evident in the large number of examples, representing systems of four different types, that have been considered in this paper. In almost all cases, the best evidence is available for systems in enteric bacteria and their phages. There are many more examples among prokaryotes and lower eukaryotes, in which there is evidence for either the molecular mechanism or the demand for the physiological function, but not both. Thus, additional cases soon should be available for testing this correlation.

Control of gene expression in differentiated cells of higher eukaryotes has been studied by somatic cell hybridization, and the evidence suggests control by a repressor in some cases and by an activator in others (see refs. 64–66). When these mechanisms are confirmed at the molecular level and more is known about the function of the regulated genes, it will be possible to test the correlation between molecular control mechanisms and demand for expression of the regulated genes in eukaryotic systems.

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