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Genetics

Regulation of differentiated cell-specific functions

(switching of regulatory mechanism/positive vs. negative control/yeast mating type/bacteriophage λ/*Escherichia coli*)

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ABSTRACT **The demand theory of gene regulation predicts that *regulated* cell-specific functions in high demand (i.e., high level of gene expression frequently required) are under the influence of a positive regulatory element whereas those in low demand (i.e., high level of gene expression not frequently required) are under the influence of a negative regulatory element. Furthermore, during differentiation, when the demand regimen for cell-specific functions changes, a switch in the regulatory mechanism itself is predicted. For the case in which a function is regulated in both demand regimens, the mode of regulation will switch from positive (high demand) to negative (low demand) or *vice versa*. These predictions are compared with published experimental evidence and found to be in good agreement.**

In prokaryotes, regulation of gene expression by a negative element, a repressor, has been well established for many systems (1). Mechanisms involving positive elements, activators affecting the initiation of transcription (2) and antiterminators affecting the termination of RNA transcripts before they can be extended into the regulated structural genes (3–7), also have been thoroughly demonstrated. There are a number of established variations on these themes. Although we are currently at a loss to rationalize the entire variety of molecular designs, some success in understanding the role of positive vs. negative elements has been achieved with the demand theory of gene regulation. This theory is based on (*i*) the response of the system to regulatory mutations, (*ii*) the physiology of the system, (*iii*) the natural environment of the cell, and (*iv*) the population dynamics of mutant and wild-type cells. It predicts a correlation between the mode (positive or negative) of the regulator and the normal demand for expression of the regulated structural genes in the system: The regulator element will be positive (e.g., activator, antiterminator) when in the natural environment there is a high demand for expression of the regulated structural genes; it will be negative (e.g., repressor, proterminator) when there is a low demand (8). The terminology used in this paper, and discussed elsewhere (9), is summarized in Table 1.

These predictions of the demand theory were originally tested against experimental data for seven different inducible catabolic operons in enteric bacteria and no exceptions were found (10). Later, these studies were generalized and experimental data for 20 operons representing physiological functions of five different classes in enteric bacteria and their phages were successfully tested (8). There are now more than 40 examples representing physiological functions of 12 different classes that have been tested and reviewed (ref. 9; unpublished data). In a number of cases, I have used the demand theory to anticipate the molecular nature of the regulatory mechanism from existing knowledge of the organism's natural environment. Predictions of this latter type have recently been confirmed by other investigators (11, 12).

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Table 1. Terminology used in this paper

Mode of control	Regulator molecule	Modulator site	Transcript delimitor
Negative	Repressor	Operator	Promoter
Positive	Activator	Initiator	Promoter
Negative	Proterminator	Arrestor	Terminator
Positive	Antiterminator	Liberator	Terminator

All of the previous work has emphasized regulation of physiological functions within cells of a single type experiencing a uniform demand regimen. In this paper, by contrast, I shall use the demand theory to make predictions concerning the regulation of cell-specific functions in organisms with differentiated cell types and, thus, different demand regimens (13). In addition to predicting the mode of regulatory elements for specific functions in each of the cell types (demand regimens), I shall use the theory to predict that, during differentiation, when the demand for specific functions changes, there will be a switch of the regulatory mechanisms that govern these functions so as to conform to the new demand regimen. These predictions will then be tested against experimental data from simple well-studied model systems. The reasonable agreement between predictions and observations in these cases suggests that as a more general rule one might expect the switching of regulatory mechanisms during differentiation when the expression of cell-specific functions is turned on or off.

DIFFERENTIATION AND DEMAND

Let us assume that differentiated cells of each type in the organism contain all the genetic information to be differentiated cells of any of the other types, as seems to be the case for most organisms (14). Then a given set of structural genes (say those coding for functions specific to type A cells) is in high demand in cells of one type (type A) and in low demand in cells of another type (type B). A straightforward application of the demand theory in this situation leads one to predict positive regulation of A-specific functions in type A cells but negative regulation of these functions in type B cells. Hence, a switch of regulatory mechanisms must occur in accordance with the change in the demand regimen during differentiation. I shall have nothing to say about what "causes" differentiation (or the switch) to occur in a given cell, be it hormones, morphogens, chalones, receptors, etc., that might transmit signals from outside the cell or that might be generated internally in response to some program. Instead, the focus will be on the mode of regulation that can be maintained by selection in cells of each differentiated type.

It should be emphasized that the use of the term switching in this context refers specifically to a change in the mode of regulatory mechanisms governing cell-specific functions and not to the turning on or off of specific functions. For example, expression of the gene for β-galactosidase is turned on/off in response

to lactose but the mode of regulation (repressor) does not switch.

Most interesting examples of differentiation occur in higher organisms for which we currently know very little concerning the underlying molecular mechanisms. Therefore, to test the above predictions, we must use simpler model systems for which requisite molecular information is more readily available at present. I shall consider examples from three well-studied systems: (*i*) the bacterium *Escherichia coli*, (*ii*) the temperate phage λ, and (*iii*) the lower eukaryote *Saccharomyces cerevisiae*.

Escherichia coli

It now seems clear that this bacterium has two principal habitats. The classical one, which I shall call the primary habitat, is the lower intestine (colon) of warm-blooded animals. The other, which I shall call the secondary habitat, is water, sediment, and soil. Although these habitats are complex and not well understood, there is sufficient information available to draw at least some conclusions concerning their general nature (unpublished data).

One can estimate that about one-half of the total *E. coli* population at any given time is found in the intestines of warm-blooded animals (unpublished data). On the average, an *E. coli* cell is "born" in an intestine, spends half of its life there, is excreted onto the earth's surface, spends the second half of its life there, and then with a certain probability colonizes an intestine or dies. Since *E. coli* spends comparable times in each of these two habitats, and since these habitats differ in the spectrum and level of available nutrients, it is likely that at least some operons will be primarily off in one habitat and primarily on in the other. In this restricted sense, *E. coli* may be considered to have differeniated cell types.

The substrate of the inducible arabinose catabolic operon and the end product of the repressible tryptophan biosynthetic operon appear to be available frequently in high concentrations in the primary habitat but seldom in high concentrations in the secondary habitat (unpublished data). Thus, these systems appear to provide reasonable analogues of cell-specific functions: The arabinose enzymes represent a function specific to primary type *E. coli* (i.e., *E. coli* in the primary habitat—the colon) and the tryptophan enzymes represent a function specific to secondary type *E. coli* (i.e., *E. coli* in the secondary habitat—water, sediment, and soil). In the primary habitat, the colon, the arabinose operon ought to be under the influence of a positive regulatory element. In the secondary habitat, water, sediment, and soil, this operon ought to be under the influence of a negative regulatory element. These predictions from demand theory and the experimental evidence for both positive and negative regulation of the arabinose operon in *E. coli* (2, 15, 16) appear to be precisely in accord (Fig. 1). Similarly, demand theory predicts that, in the primary habitat, the colon, the tryptophan operon ought to be under the influence of a negative element. In the secondary habitat, water, sediment, and soil, it predicts that this operon ought to be under the influence of a positive regulatory element. Again, these predictions and the experimental evidence for both positive (6) and negative (17–21) regulation of the tryptophan operon in *E. coli* appear to be in accord (Fig. 1).

Thus, there are four states involving two sets of cell-specific functions (*ara* and *trp*) and two *E. coli* cell types (primary and secondary): *ara* primary, *ara* secondary, *trp* primary, *trp* secondary. In each state, the cell-specific function can be modulated in principle by either a positive or a negative regulatory element and, so, *a priori*, the number of possible patterns of regulation involving four independent states is 16 (i.e., 2^4). Only one of these patterns (+, −, −, +) is predicted by demand theory and this is the pattern found experimentally.

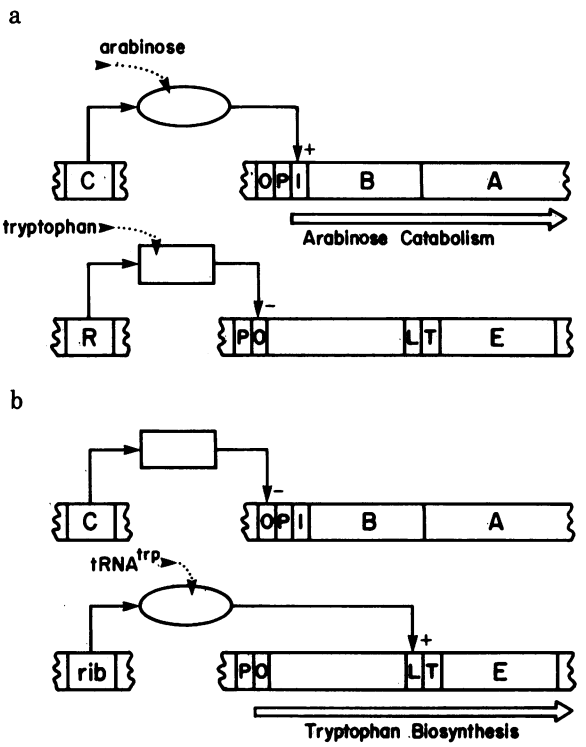

FIG. 1. Regulation of cell-specific functions in *E. coli*. (*a*) Primary type *E. coli* are found in the lower intestine of warm-blooded animals where arabinose and tryptophan are frequently present in high concentrations. (*b*) Secondary type *E. coli* are found in water, sediment, and soil where arabinose and tryptophan are seldom present in high concentrations. Ovals and rectangles denote positive- and negative-acting regulatory elements. C, structural gene for the regulator of the arabinose operon; B and A, regulated structural genes coding for arabinose catabolic enzymes; O, P, and I, the corresponding operator, promoter, and initiator sites on the DNA; R, structural gene for the repressor of the tryptophan operon; E, a regulated structural gene coding for a tryptophan biosynthetic enzyme; rib, coding sequences for a complete ribosome; L, a liberator (see Table 1) sequence (in this case, consisting of two tryptophan codons in tandem). When a translating ribosome becomes stalled at L in the absence of aminoacyl-tRNA^{trp}, the transcribing machinery is freed from the influence of the terminator sequence *T*, and transcription proceeds into the adjacent structural genes.

Temperate phage λ

Temperate bacteriophages such as λ exhibit one of two different life-styles. In lysogenic growth, the virus is stably associated with the host bacterium and is replicated in synchrony with the host's chromosome. In this type of growth, all lytic functions are turned off and only a few lysogen-specific functions are expressed. In the alternative case, lytic growth, the virus grows productively at the expense of the host and eventually a large number of virus particles are released by the host cell. In this case, functions specific for lytic growth are turned on while those specific for lysogenic growth are turned off (for a recent review, see ref. 22). Traditionally, phage particles have been isolated from the environment but it appears that they survive poorly in the intestine (23, 24). Thus, lysogenic and lytic growth are probably associated with primary and secondary type *E. coli*, respectively.

During lysogenic growth, demand theory predicts that lysogen-specific functions, which are in high demand, ought to be under the influence of a positive regulatory element while lytic functions, which are in low demand, ought to be under the influence of a negative regulatory element. During lytic growth, the predictions are the converse: lysogen-specific functions, which are in low demand, ought to be under the influence of a neg-

ative regulatory element while lytic functions, which are now in high demand, ought to be under the influence of a positive regulatory element. [Again, note that the emphasis here is on the two differentiated types of growth and not on the transition (or "decision") between lysogenic and lytic growth.] These predictions and the current experimental evidence for the control of bacteriophage λ are in agreement. The major regulatory themes appear to be the following. During lysogenic growth, the *cI* gene product activates transcription of lysogen-specific functions and represses transcription of lytic functions (25). During lytic growth the *N* gene product acts as an antiterminator necessary for the expression of lytic functions (3–5) and the *CRO* gene product acts as a repressor of lysogen-specific functions (25) (Fig. 2). [The *CRO* gene product also affects expression of *N* and other lytic functions by partial repression midway through infection (22, 25). This and other regulatory mechanisms (22) that manifest themselves at later times during the programmed development of a lytic infection are obviously important, but not critical to the present discussion.]

Again, there are four states involving two sets of growth-specific functions (lysogenic and lytic) and two styles of phage growth (lysogenic and lytic). In each state, the growth-specific functions can be modulated in principle by either a positive or a negative regulatory element and, so, *a priori*, the number of possible patterns of regulation involving the four states is 16. Only one of these patterns of regulation (+, −, −, +) is predicted by demand theory and this is the pattern found experimentally.

Saccharomyces cerevisiae

Conversion of the mating type in yeast cells is currently one of the most attractive model systems for the study of differentiation (26). This simple organism exists in the haploid state as a cell with one of two distinct mating types—*a* and α—and on mating they become the *a*/α diploid cell. Each of these cell types has a number of known functions not expressed in the other two cell types (27). For the remainder of this section, we shall focus only on the haploid cell types to simplify the discussion.

Mating type is determined by the nature of the DNA sequence at a single locus, *MAT*, on chromosome 3 (28). Silent or storage copies of mating type *a* and α sequences are also located on chromosome 3 at loci currently designated *HMRa* and *HMLα* (29–31), respectively (Fig. 3*a*). The type of DNA sequence (*a* or α) resident in the *MAT* locus determines the mating type. Mating-type conversion occurs when the silent copy of the alternative mating-type sequence is duplicated and transposed into the mating-type locus while the resident sequence is ejected (31–33).

Regulation of cell-specific functions in type α cells is summarized in Fig. 3*b*. Two complementation groups have been identified among mutations in the *MATα* sequence (34, 35). The phenotype of mutants altered in the *α1* locus implies that the wild-type allele encodes a positive-acting element that is normally responsible for regulating high-level α-specific functions; by similar criteria, the wild-type *α2* locus encodes a negative-acting element that is normally responsible for regulating low-level *a*-specific functions. The physical organization and divergent transcription of these two genes in the *MATα* sequence has recently been determined (36, 37) and appears to be consistent with ideas based on earlier genetic work. Thus, regulation of cell-specific functions in type α cells according to the current experimental evidence and according to the predictions of demand theory are precisely in accord.

Current views on the regulation of cell-specific functions in type *a* cells are summarized in Fig. 3*c*. In this case, no regulatory mutants analogous to *α1* and *α2* have been isolated in MAT*a* cells (34, 35, 38). As a result, *a*-specific functions are believed to be expressed constitutively—i.e., to exhibit high-level unregulated expression. α-Specific functions are believed to be unexpressed "constitutively"—i.e., to exhibit low-level unregulated expression. In the context of haploid cells, the *MATa* sequence is viewed as a "null" sequence. Recent studies have shown that the physical organization and divergent transcription of two regions within the *MATa* sequence are closely analogous to that in the *MATα* sequence (36, 37), although functions for the two MAT*a* transcripts in type *a* cells are unknown. [The phenotype of mutants altered of the *MATa1* locus in *a*/α-diploid cells, which are not under consideration in this paper, implies that the wild-type allele encodes a positive-acting element that is normally involved in regulating the *a*/α-specific functions of sporulation.

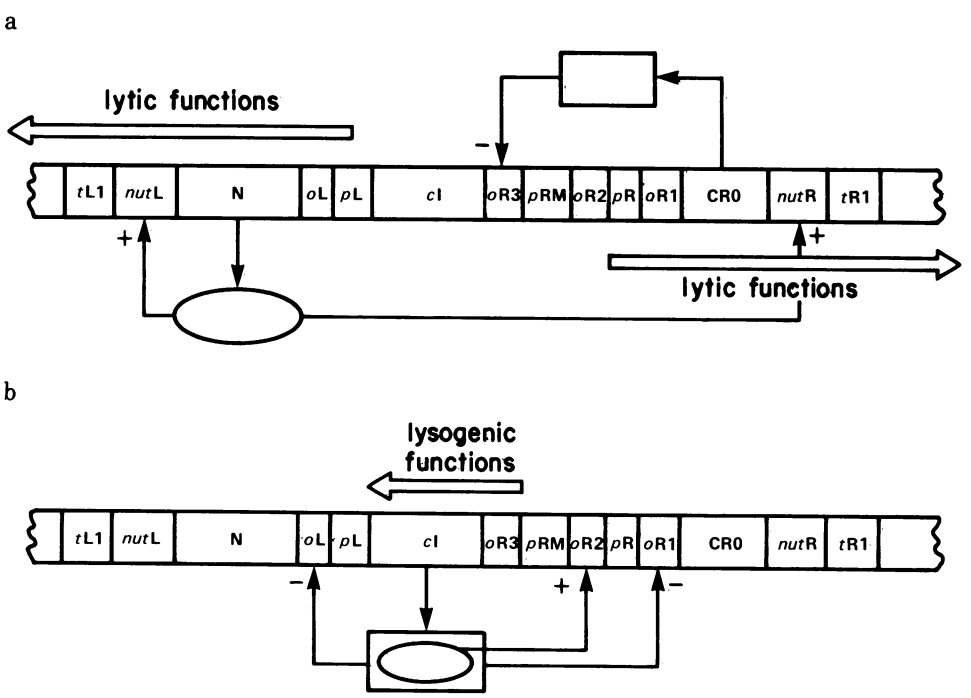

FIG. 2. Regulation of cell-specific functions in λ-infected *E. coli*. (*a*) During lytic growth, expression of the lysogen-specific *cI* repressor is turned off by *CRO* repressor binding to operator *o*R3. Transcripts initiated at promoters *p*L and *p*R extend into lytic functions beyond terminators *t*L1 and *t*R1 because of the antiterminator activity of the *N* gene product, which is recognized by liberator (Table 1) sequences *nut*L and *nut*R. (*b*) During lysogenic growth, lytic functions are turned off by *cI* repressor binding to operators *o*R1 and *o*R2 (and *o*L). This mode of binding the *cI* gene product also activates transcription leftward from promoter *p*RM through the structural gene *cI*. Ovals and rectangles denote positive- and negative-acting regulatory elements. In the case of the *cI* gene product, the same protein has both properties.

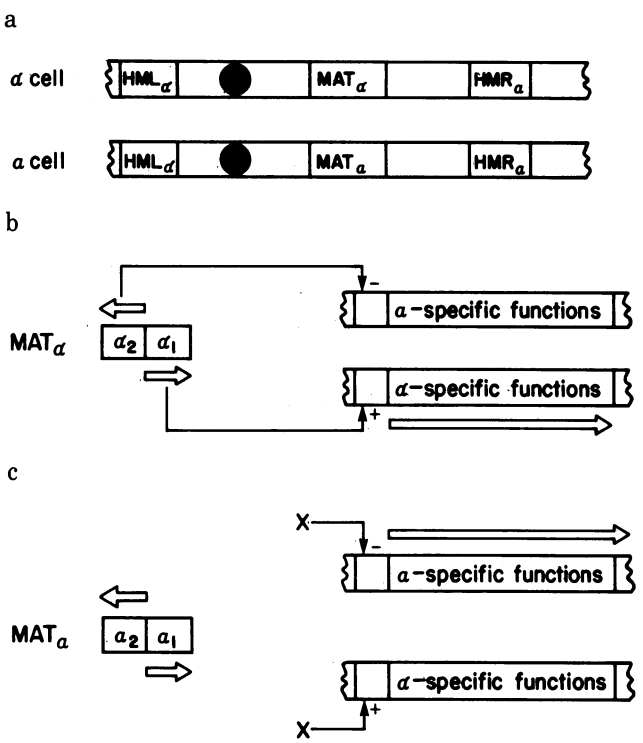

FIG. 3. Regulation of cell-specific functions in haploid yeast cells. (*a*) Location on chromosome 3 of DNA sequences specifying mating-type information. *MAT*, mating-type locus; *HMLα* and *HMRa*, silent copies of mating-type α and *a* sequences, respectively; ●, centromere. (*b*) Regulation of cell-specific functions in type α cells. The *MATα* locus is divergently transcribed to yield two products. One, identified with the *α1* mutation, is a positive-acting element required for expression of α-specific functions. The other, identified with the *α2* mutation, is a negative-acting element required to prevent expression of *a*-specific functions. (*c*) Regulation of cell-specific functions in type *a* cells. The *MATa* locus is divergently transcribed to yield two products. By analogy with type α cells, these cistrons are called *a1* and *a2* even though functions for these genes in type *a* cells have not been established by mutation. *a*-Specific functions are believed to be "turned on" for lack of a negative element and α-specific functions are believed to be "turned off" for lack of a positive element.

Positive regulation of *a*/α-specific functions in *a*/α-diploid cells (*a*/α functions in high demand) is of course expected according to demand theory.]

Again, there are four states involving two sets of cell-specific functions (*a* and α) and two cell types (*a* and α). In each state, the cell-specific functions can be modulated in principle by either a positive or a negative regulatory element and, so, *a priori*, there are 16 possible patterns of regulation involving the four states. Only one of these patterns of regulation (+, −, −, +) is predicted by demand theory.

In *S. cerevisiae*, the experimental evidence follows the predicted pattern with regard to the regulation in type α cells: α-Specific functions are regulated by a positive element and *a*-specific functions are regulated by a negative element. In type *a* cells, the experimental evidence is inconclusive. Cell-specific functions in type *a* cells might still be regulated, in which case we will have to withhold judgement until it is conclusively shown whether or not *a*-specific and α-specific functions are positively and negatively regulated, respectively. On the other hand, at present it appears more likely that cell-specific functions in type *a* cells are unregulated, as is suggested by the current experimental evidence.

This latter situation can be viewed in two different ways. First, as an obvious and rather trivial corollary of demand theory, unregulated structural genes in low demand are predicted to be unexpressed and those in high demand are predicted to be expressed constitutively (10). Second, one can take the position that demand theory, as a theory of gene regulation, has nothing to say about unregulated gene expression. In either case, the evidence is not inconsistent with demand theory, but no new significant information is obtained concerning the situation in type *a* cells.

Thus, the probability of picking at random a pattern of regulation that is consistent with the experimental evidence in yeast would be 1 in 4 rather than 1 in 16, since type *a* cells currently do not provide a significant test for the predictions of demand theory.

CONCLUSIONS

The more obvious predictions of demand theory appear to be well-supported by the results presented above. Of the 12 predictions concerning regulation of cell-specific functions, 10 are supported by the experimental data; 2 are uncertain at this time but, in any case, are not inconsistent with demand theory. In themselves, these data appear to provide additional support for the demand theory of gene regulation.

With regard to the predicted switching of regulatory mechanisms, all three systems exhibit such switching for each of the cell-specific functions examined. Although the prediction of switching in a formal sense is supported by the experimental evidence, it should be pointed out that the molecular details by which the switching is realized are quite different in each case and of course could not be predicted by demand theory. For the arabinose catabolic operon, switching occurs by an allosteric conversion of a single regulator protein; in the case of the tryptophan biosynthetic operon, switching occurs between two separate mechanisms that have different sensitivities to the supply and demand for tryptophan in the cell; switching in bacteriophage λ is accomplished by an interlocking set of genetic regulatory circuits; mating-type conversion in yeast is accomplished by physical transposition of a DNA sequence coding for a battery of regulatory genes.

Thus, demand theory provides a satisfactory explanation for at least certain aspects of the multiple regulatory mechanisms found in several well-studied systems and suggests that switching of regulatory mechanisms may be a general rule for cell differentiation. However, additional examples, particularly from higher organisms, will be required before the full generality of this rule can be ascertained.

Demand theory provides an explanation based on selection for the regulatory mechanisms that are functioning ("active") in a given cell type. It also predicts that the nonfunctioning ("inactive") regulatory mechanisms in these same cells will experience genetic drift and contribute to the genetic load on the population. Two formal strategies for diminishing genetic drift among such nonfunctioning regulatory mechanisms are (*i*) the elements of the regulatory mechanism could be under indirect selective pressure or (*ii*) they could exist in a state in which they experience a reduced net mutation rate. The discussion below suggests that the switching of regulatory mechanisms may be an important factor in the realization of these strategies.

Indirect selection of inactive regulatory mechanisms can be maintained through reciprocally active bifunctional molecules and overlapping recognition sequences. The regulator of the arabinose operon provides an example of a bifunctional protein (2). Both activator and repressor functions reside in the same regulatory molecule but, when the regulator acts as an activator, the repressor function is inactive. Repressor function, however, would be maintained under such conditions largely by the same selective pressure that maintains the activator function. Simi-

larly, ribosomes do not function as antiterminators of the tryptophan operon when tryptophan is in excess (6), yet they are constantly under selective pressure to maintain their other functions in the cell.

Examples of overlapping recognition sequences in DNA are provided by the arabinose *POI* (16), tryptophan *PO* (21), and λ *p*RoR (25) control regions. Since the functional operator and promoter sites overlap within each of these control regions, an unused function can be maintained at least in part by the selective pressure exerted on the overlapping function that is in active use. The tryptophan operator sequence is not used when tryptophan is limited in the cell but, since the tryptophan promoter sequence is in active use, the operator sequence still can be under selective pressure. Conversely, in tryptophan excess, when the operator is used and the promoter is unused, the promoter sequence will still be under selective pressure.

The second formal way of reducing genetic drift is to reduce the local mutation rate for nonfunctioning (inactive) regulatory mechanisms. This might be accomplished by sequestering regulatory genes and sequences in a transcriptionally inactive form that could reduce their mutation rate. For example, the tryptophan liberator and terminator sequences are not transcribed when tryptophan is in excess. The *CRO* and *N* genes of λ are not actively transcribed during lysogenic growth, while the *cI* gene is not actively transcribed during lytic growth. Similarly, *HMLα* and *HMRa*, the silent copies of α and *a* mating-type sequences, normally are not transcribed. Many transcriptionally inactive sequences in higher eukaryotes are methylated more extensively than actively transcribed sequences (39, 40) but whether such methylation reduces mutation rates is not clear. Of course, mechanisms other than methylation could be envisioned; the key point to be demonstrated is whether or not there is a difference in mutation rate for inactive regulatory mechanisms.

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