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# Concentration–affinity equivalence in gene regulation: Convergence of genetic and environmental effects

(phenocopies/parallel evolution/genetic assimilation/expressivity/penetrance)

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ABSTRACT **It is proposed that equivalent phenotypic effects can be obtained by either structural changes in macromolecules involved in gene regulation or changes in activity of the structurally unaltered macromolecules. This equivalence between changes in activity (concentration) and changes in structure can come into play within physiologically plausible limits and seems to represent an important interface between environment and genome—namely, between environmentally determined and genetically determined gene expression. The equivalence principle helps explain the appearance of phenocopies. It also points to a general pathway favorable to the occurrence, during evolution, of frequent episodes corresponding to Waddington's genetic assimilation and is likely to represent one component of the system responsible for the high frequency of recurrence of parallel evolution.**

On the basis of known chemical behavior, it is expected that, at any level of manufacture of gene product, the rate of expression of a given gene may be altered either by a change in the affinity (equilibrium) between interacting regulatory components or by a change in their activity (concentration). It follows that certain changes in affinity and certain changes in concentration must be equivalent in their effects on gene transcription or on the processing of the transcripts (Fig. 1).

The changes in affinity are either those of regulator proteins ("regulators") for polynucleotide receptor sequences ("receptors"), now frequently called response elements or responsive elements (Fig. 1), or of effectors for regulator proteins. Lactose binding to the repressor of the lactose operon is an example of an effector. Proteins may also be effectors, and such protein effectors may in turn be regulated by second-order effectors (1). Regulator proteins either regulate transcription or the processing of the primary transcripts. The set of regulators, effectors, and receptors involved in the control of the transcription of a given gene or of the processing of its transcripts has been called controller node (2–4). The controller node is a genetic unit of regulation. In eukaryotes, a number of controller node components—for instance, cis-acting receptors such as promoters and enhancers—have in recent years been found to be located in the neighborhood of the genes that they control. The known complexity of controller nodes (5) has thus been on the increase. Simultaneously and as a consequence, there is an increase in the theoretical number and range of different mutations that have the potential of leading to equivalent regulatory effects. Such effects are brought about either by mutational changes in controller node components or by changes in their amounts.

As is well known, the affinity of protein for polynucleotide can be altered by a heritable structural modification in the protein resulting from a mutation in its structural gene. This affinity can also be altered by a reversible structural change in the protein, resulting in a modification of specific fit between macromolecules and brought about by a combination with, or by the release of, an effector. An important variant of the latter process is a reversible formation of a covalent compound between a polynucleotide-binding protein and some other organic moiety (e.g., acetylation, ADP-ribosylation; see ref. 6). The potential equivalence is between the effect of a change in component concentration (activity) under a constant structural state and the effect of a structural modification under constant component concentration (activity). Either kind of event could bring about the same decrease or increase in the rate of expression of a gene. This change in rate may be effected at the level of either transcription or processing of the transcript.

Transient covalent modifications of informational macromolecules can, in addition, have effects that fall outside of the applicability range of the concentration–affinity equivalence principle, in that they may not only vary the degree of a given interaction specificity (which is equivalent to the degree of affinity), but may create new interaction specificities between molecules. This might be the case, for instance, of chemical and steric modifications not involving changes in coding that can occur in polynucleotides—e.g., in DNA, through the methylation of cytosines in CpG dimers (e.g., see ref. 7). The methylation of a particular cytosine or, upon DNA replication, the discontinuation of its methylation are likely to be linked to changes in higher-order structure of deoxyribonucleoprotein. These latter changes are expected to establish a new order also in regard to the identity of the interacting components of the system and to their mode of interaction (8). In this changed molecular environment established around the same base sequences, and within the boundaries of such a distinctive structural constellation, the equivalence principle should again apply.

There are various ways that changes in concentrations and affinities can so-to-speak play with each other. For example, as already stated, the mutational change in a protein regulator or in its polynucleotide receptor that alters the affinity of the regulator for the receptor can be expected in many cases to affect the rate of expression of the structural gene that the regulator and receptor control. This structural gene may encode a protein that in turn has regulatory effects, which result in a change in rate of synthesis of other proteins. A change in affinity between controller node components in one part of the genetic system thus can bring about a change in concentration in other parts.

We shall briefly examine the relevance of the principle of concentration–affinity equivalence to the interaction between genomes and environments, using phenocopies as an illustration, as well as comment on the contribution of the equivalence principle to "genetic assimilation" and to the frequent recurrence of parallel evolution. We shall then explore realistic limits of concentrations and affinities within which the concentration–affinity equivalence may come into

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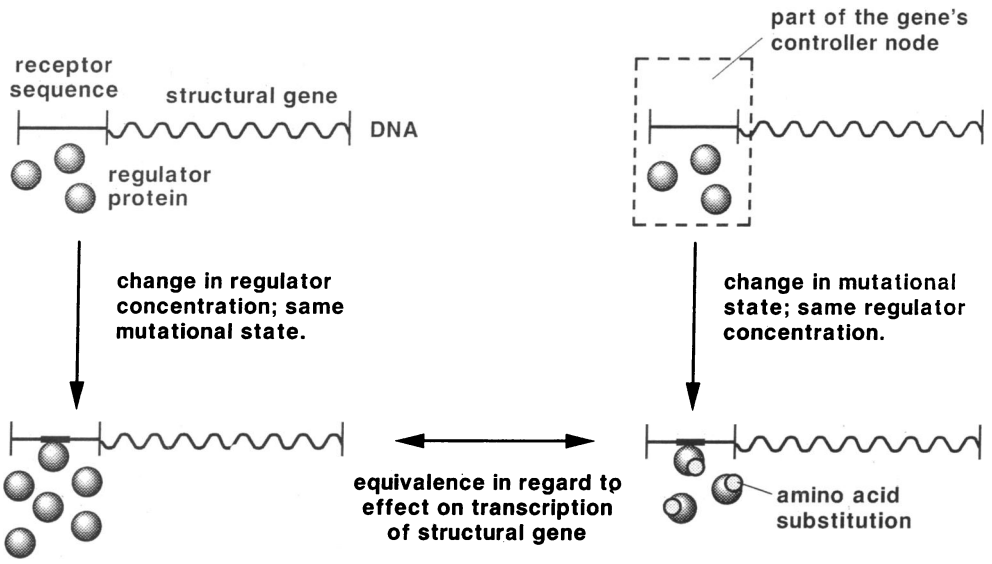



FIG. 1. Equivalence between affinities and concentrations. For simplicity's sake, the figure refers to concentrations rather than to chemical activities. (*Upper Left* and *Right*) The same structural gene in an identical state of transcriptional activity, as determined by a given concentration of regulator molecules. These have a relatively low affinity for their germane receptor sequence, a circumstance that is expressed by the receptor being pictured in the unbound state. (*Lower Left*) The effect of increasing the concentration of the regulator protein: the receptor is now in the bound state and the rate of transcription of the gene is increased by a certain amount. (*Lower Right*) An identical effect obtained through a different pathway: the receptor sequence is in the bound state even though the concentration of the regulator has not been increased, because a mutation has occurred in the structural gene controlling the regulator, the effect of this mutation being to increase the affinity of the regulator for the receptor sequence. Receptor sequence and regulator protein together form part of the structural gene's controller node. The remainder of the controller node, not shown here, includes further receptors and proteins binding to them, plus effectors combining with the proteins.

play. It is understood that in any dependence on concentration, thermodynamic activity is the factor directly responsible for the effect.

### Equivalence Between Genetic and Environmental Effects: The Case of the Phenocopies

Environmental parameters such as temperature, pH, ionic strength, and specific ions can change the activities of the components of a controller node. They have been shown to affect binding equilibria in the *lac* system (9–11). Moreover, the environment can furnish, or withhold, certain types of organic effectors, notably those present among or derived from nutrients. This is attested to, for instance, by the morphogenetic effects of certain vitamin deficiencies (12) that, directly or indirectly, lead to alterations in gene expression.

The functional effects of conformational changes induced by effectors in proteins and polynucleotides can, in principle, be imitated by a large number of different mutations. Given the number of interacting components of a controller node as well as the interaction between controller nodes, the width of the spectrum of different mutational changes that are expected to lead to similar phenotype effects deserves indeed to be emphasized. Here we focus, however, on the expected convergence between the effects of a number of these mutations on the one hand and of changes in environmental conditions on the other in regard to the phenotype produced. The interplay between equilibrium constants and concentrations and, as a result, between mutations and effects of the environment (external and internal) turns out, upon examination, to seem destined to be of general importance in biology.

The convergence and equivalence between mutations and environmental effects have been illustrated many times in nature and in the laboratory by what has been referred to as phenocopies. A phenocopy is an environmentally determined phenotype that appears to be identical with a phenotype brought about by a mutation (13–20). For example, exposing wild-type embryos of *Drosophila* to ether can induce in the adult the characteristics of the bithorax mutant, including an undersized second pair of wings (21). Phenocopies mimicking the action of mutant genes are readily produced by environmental agents such as heat shock and noxious chemicals, when these agents are permitted to act upon well-defined developmental stages of the embryo.

Frequently, a phenocopy and the corresponding mutant phenotype presumably have in common the fraction of time over which a regulator and a receptor are in the bound state. This fraction of time is set by the affinity of the regulators for the receptors and by their concentration and activity. In the case of a mutant, this fraction is determined by a structural change in a regulator or a receptor. In the case of the phenocopy of this mutant, an environmentally determined change in concentration of the active regulator species may cause the receptor to be in the bound state over an identical fraction of time. The half-life of a regulator–receptor complex can be varied to an identical extent, within certain limits, either by modulating an equilibrium constant or by modulating a concentration or activity. For an equivalent half-life of the bound state under both conditions, gene expression integrated over time will be the same.

Not all mutations can be mimicked by an environmental effect, but no doubt all environmental effects can be mimicked by one or several mutations. Indeed, if the genetic system responds to an external agent by certain adjustments in its activities, it thereby indicates that these adjustments are within the range of possible genetic effects, and the equivalence principle implies that all possible effects mediated by the genetic system can also originate in this system through heritable structural changes.

We acknowledge of course that the effect of a mutation can also be mimicked by an environmental interference, during a critical developmental period, not with the expression of a gene, but with the function of a gene product that is not directly involved in gene regulation. For instance, the environment can provide an inhibitor of an enzyme (22, 23), as it probably does in thalidomide embryopathies. Nevertheless, such events are likely in many cases to have effects on the

expression of certain genes. The interference with gene regulation through environmental effects has been brought about artificially by the construction and introduction into cells of antisense genes. The genes, whose lack of expression in the presence of antisense genes imitated mutational dysfunctions, were themselves either directly involved in gene regulation as in the case of the Krüppel gene of *Drosophila* embryos (24), or not so involved as, apparently, in the case of the discoidine gene of *Dictyostelium* (25). Proteins controlled by genes in the second group, however, are expected often to have in turn indirect effects on gene regulation. There can be little doubt, for instance, that the inhibition of discoidin synthesis in *Dictyostelium* (25) eliminates or retards certain changes in gene regulation that the presence of discoidin promotes. Directly or indirectly, a change in the control of gene expression may well be involved in determining the phenotype of most phenocopies, notably of those involving morphological change.

Morphological change indeed is to be considered here. Through regulatory shifts affecting the interactions of different controller nodes, substances of endogenous origin yet under exogenous quantitative control such as hormones, and substances of exogenous origin such as vitamins, or the deprivation of substances, can at critical developmental stages favor one potentially possible morphogenetic pathway over another in certain regions of the body. An example that can tentatively be interpreted as expressing a variation in the underlying quantitative relationships examined here—namely, an interplay between structural and concentration factors—is the production of exencephaly in mice. Certain mutants known in mice, rib fusion and crooked, manifest an increased disposition of the embryo to engage on this teratological developmental course. It seems that the greater the percentage of exencephalic individuals is in different homozygous mutants—namely the penetrance of the mutation in the homozygous state—the smaller is the amount of teratogen, in this case insulin, required in the heterozyote for an equal penetrance of exencephaly to be reached (26). This can be interpreted as indicating that the stronger the teratological effects are of a mutated gene, the smaller is the concentration change in the heterozygote that is required to produce the same effect. The suggestion is that of a mutually complementary relationship between structural change and quantitative change. The quantitative change in question is assumed to occur in a controller node component that is directly or indirectly affected by the teratogen.

In a given body region, no doubt on account of the position of cells with respect to certain gradients of effector substances and of a certain potential of gene activity as defined by cellular determination (27), morphogenesis can in fact be reoriented so as to reproduce processes characteristic of a different body location or of an ancestral evolutionary state. An example of reoriented morphogenesis in an abnormal body location is the induction by administered retinoic acid of feathers instead of scales on the feet of chicken, a phenotype that corresponds to known "ptilopody" mutants (28). An example of a "phenocopy" of an ancestral state is provided by the induction in the limb bones of chicken, through the grafting of additional amounts of normal tissue during development, of anatomical features that are characteristic of *Archaeopterix* (29). Additional portions of tissue imply the presence of additional amounts of factors. A change in amount of these factors generates morphogenetic features very similar to those that the mutational state of its DNA had produced in a long-extinct and distantly related species.

The term mutational state designates the aggregate of all sequence features of DNA or of a designated sector of DNA. Differences in the mutational state between individuals in a population must be largely responsible for the observed degrees of expressivity and penetrance of a phenocopy. Different degrees of expressivity and penetrance probably reflect intrapopulational variations in the stability of certain controller nodes (variations in their "regulatory buffering"; ref. 30). Expressivity and penetrance of phenocopies will be increased through the involvement of controller nodes that are relatively unstable—namely, in which small changes in the activity of regulator or effector molecules are capable of bringing about changes in rate or timing of gene expression large enough to result in a switch to an alternative phenotype. Penetrance is presumably ruled by quantitative differences in either equilibrium constants or thermodynamic activities relative to thresholds, differences that therefore lead to an all-or-none response (the digital mode of regulation of phenotypic expression; ref. 31), whereas expressivity probably depends on such quantitative differences in the absence of threshold effects (the analogue mode of phenotypic response).

## Genetic Assimilation

Thanks to the presumably large number of different mutations that will produce similar regulatory effects within a controller node interaction system, there should be a relatively high likelihood for a mutation promptly to become available that would mimic a given environmental effect on gene expression, on physiology, on morphology, or on behavior. This amounts to a likelihood of genetic assimilation occurring, in Waddington's (32) sense of the phrase. On the basis of the equivalence principle, one can recognize the *a priori* probability that nature will easily be able to proceed with genetic assimilation, which consists of translating, during evolution, environmentally triggered into genetically fixed phenotypic effects. In the past, the concept of genetic assimilation was based on an unqualified appeal to random mutations that implicitly emphasized structural modifications of gene products as a basis for the exquisite morphological, physiological, and behavioral adaptations of organisms. The concentration–affinity equivalence principle does not dispense with random mutations, but, in conjunction with high controller node complexity, it increases, although probably by itself not yet to a conceptually sufficient extent, the plausibility of the prompt occurrence and recurrence of particular heritable changes in gene regulation and, hence, of environmentally directed subtly adaptive evolution. Most adaptations, including behavioral ones, may well turn out to be based on regulatory changes in genes. Altered expression of a gene product can be as adaptive to environmental change as is mutational alteration of the gene product itself.

## Parallel Evolution and Evolutionary Reversibility

Because of the concentration–affinity equivalence, because a variety of different mutational events can produce the same effect on gene expression, and because of the number of macromolecular components entering into a given controller node, to each of which the concentration–affinity equivalence applies, there is a much greater likelihood than would otherwise be the case of multiple mutational recurrences of a same phenotype. At each recurrence, the phenotype is subjected to either natural selection or random genetic drift. We have here one of several plausible internal mechanisms for increasing the rate of parallel evolution, an aspect of directional evolution. The mechanism operates through an increase in the rate at which the opportunity presents itself for a certain mutationally induced phenotype to become evolutionarily effective (fixed).

In addition, the so-called law of Dollo relating to evolutionary irreversibility is thus likely to be inapplicable to the rate of expression of individual genes. Even after multiple regulatory mutations have occurred, leading to changes in

temporal and dynamic modes of gene expression, additional mutations may be expected to be capable of reestablishing an ancestral mode. Although the underlying DNA sequences will be different and will have no chance of reverting collectively to their original state, resulting modes of gene expression can in principle be the same.

**Range of Applicability of the Equivalence Principle**

We now examine ranges of concentrations and affinities within which the equivalence principle can be reasonably supposed to apply.

A simplified approach is taken in modeling the system. Concentrations rather than activities are used, implying an activity coefficient of unity in an idealized situation. The systems being discussed, however, contain molecules and ions in high concentrations. Thus activity coefficients for constituents may be far different from unity. Environmental effects could in fact be reflected in variations of activity coefficients. Such experimental information is not available at present.

A further simplifying assumption is that of thermodynamic equilibrium. It is likely, however, that concentrations (activities) of interacting components within controller nodes would often be determined by rates of reaction rather than equilibrium constants. Thus, a refinement could involve a steady-state kinetic analysis of reacting substances. It seems reasonable to presume that a succession of nonequilibrium states in controller nodes would not compromise the affinity–concentration equivalence principle. When components are in the process of changing in concentration, activity, and conformation without thermodynamic equilibrium being attained, the instantaneous rate of gene expression realized for a given gene at a particular time through a particular constellation of component interactions should be attainable as well via a number of other pathways, although not necessarily with the same kinetics. These other pathways would involve the same controller node components and would be characterized by different combinations of component concentrations and component structures. An example of the kinetic equivalence of gene expression corresponding to structurally distinct states of a given controller node is provided by the induction of β-glucuronidase in mouse kidney by means of different androgens (33). The administration of a "strong" and, alternatively, of a "weak" androgen leads to different rates of β-glucuronidase synthesis, to different β-glucuronidase translational mRNA activities, and to different β-glucuronidase mRNA concentrations. This change in kinetic parameters is mimicked by known mutations. Thus, a structural change in a DNA receptor sequence or in a protein regulator can lead to the same kinetic changes as a structural change in an effector.

For a prokaryotic system such as the *lac* operon, a simple reaction scheme (Scheme I) has been postulated (34) where E, R, and O are effector, repressor, and operator, respectively. Binding of effector alters the affinity of the repressor for operator.

By analogy, consider a simplified controller node system for gene regulation in higher organisms: let

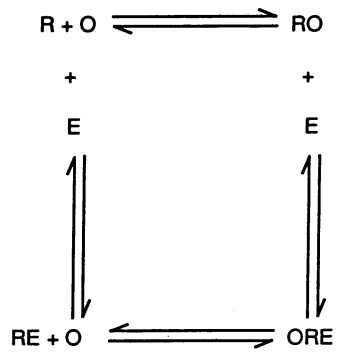
$F_1$ = control element 1 (protein)

$F_2$ = control element 2 (protein)

$D_r$ = polynucleotide receptor segment.

For the system to function, it is assumed that the presence of the ternary complex $F_1F_2D_r$ is required. Moreover, the rate of transcription will be a function of the ternary complex concentration $[F_1F_2D_r]$. Consider binding $F_1$ to $F_2$:

$$K_1[F_1][F_2] = [F_1F_2], \quad [1]$$




Scheme I

where $K_1$ = affinity constant for binding of $F_1$ to $F_2$. Now consider binding of $F_1F_2$ to $D_r$:

$$K_2[F_1F_2][D_r] = [F_1F_2D_r]. \quad [2]$$

Hence, combining Eqs. 1 and 2:

$$K_2K_1[F_1][F_2][D_r] = [F_1F_2D_r]. \quad [3]$$

Thus, the rate of transcription will depend on the expression $K_1K_2[F_1][F_2][D_r]$.

If by mutation $K_1$ and/or $K_2$ are changed, then compensatory changes to offset this could take place in the concentrations of the control elements, $F_1$ and $F_2$. This compensation is at the heart of the equivalence principle.

For three control elements interacting together sequentially and then reacting as a unit, $F_1F_2F_3$, with $D_r$, the relationship for the regulatory complex is

$$[F_1F_2F_3D_r] = K_1K_1K_2K_r[F_1][F_2][F_c][D_r], \quad [4]$$

with

$$K_1 = [F_1F_2]/[F_1][F_2], \text{ as before,}$$

$$K_2 = [F_1F_2F_3]/[F_1F_2][F_3]$$

$$K_r = [F_1F_2F_3D_r]/[F_1F_2F_3][D_r].$$

Eq. 4 can be generalized to $m$ control elements,

$$[F_1F_2 \ldots F_mD_r] = K_1K_2 \ldots K_mK_r[F_1][F_2] \ldots [F_m][D_r]. \quad [5]$$

If only one affinity constant were to be changed, say by 20%, through mutation of the gene encoding $F_1$ or $F_2$ product, then, assuming activity coefficients to remain approximately constant, a change in concentration of $F_1$ or $F_2$ by about 20% would restore the concentration of the complex $F_1F_2F_3 \ldots F_mD_r$ to its value before mutation. Alternatively, $F_1$ and $F_2$ each could change by ≈10%. Several equilibrium constants could be altered. For example, a reduction by 50% of each of four such constants would result in an overall reduction of the complex concentration to $(0.5)^4$, that is, to ≈6% of its former value. Rate of transcription would be diminished accordingly, if transcriptional regulation is unbuffered with respect to activity changes. To compensate for the reduction in binding affinity to 6%, the concentration of one component (neglecting changes in activity coefficients) would have to be increased 16-fold or that of each of four component concentrations would have to be doubled. Such changes in concentration do not seem excessive *a priori* when we deal with

proteins that were present in low amounts to start with, as is likely the case of many regulator proteins. The concentration of receptor sequences can also increase through duplications.

If transcriptional regulation is buffered, then changes in activity in components of the system will not be accompanied by a proportionate change in transcription rate, but will lower the resistance of stabilized gene expression to further activity changes (30), so that now small differences in component concentration or activity are expected to lead in a saltatory mode to qualitatively different results in gene expression.

To evaluate the physiological range of variations, it is of value to examine results of experiments on the *lac* system. A large number of mutants bearing mutations in the *i* gene for *lac* repressor protein have been isolated and classified (35, 36). A group of 12 $i^S$ mutants derived from a *lac* $i^+$ parental strain (32) showed decreased affinity for inducer isopropyl β-D-thiogalactoside (IPTG). When the affinity of the repressor for IPTG was reduced by a factor of 3–9 for $i^S$ mutants, the latter were inducible at high concentrations of IPTG. But when the affinity was reduced by a factor of >60, these mutants were not inducible by large concentrations of IPTG.

In regard to DNA-repressor binding in the *lac* system (37), $K_{DNA}$ (the repressor–DNA association constant) decreased by a factor of ≈20 for a 25% increase in sodium ion concentration. With a pH variation from 7.2 to 7.6, $K_{DNA}$ decreased by a factor of 8. Such changes in $K_{DNA}$, brought about here by nonspecific effectors, could easily be matched by mutations.

It would seem that, based on results for the *lac* system, the notion of a concentration–affinity equivalence is not unreasonable from a physiological point of view. Quantitative differences in regular protein–DNA receptor relationships, differences in their mutual affinities, and effects of these differences on gene expression have begun to be explored in higher organisms (38–40). Clearly, further quantitative work on interaction equilibria and kinetics within and between controller nodes is needed.

The applicability to physiological situations of the principle of concentration–affinity equivalence is explicated by the model and is not contradicted by the data. This permits the inferences of biological interest that have been drawn.

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