Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Genes and Development: Molecular and Logical Themes

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"INFLUENTIAL ideas are always simple," said HERSHEY (1970). The operon model of JACOB and MONOD (1961a) introduced the idea of the control unit for gene expression–a regulatory protein and its DNA target. The regulatory protein can respond to one or more effectors; the target controls the initiation step for the transcription of sequences joined to it. And, or course, there can be multiple targets. In bacteria, each target can control one or many separate genes, whereas in eukaryotes each target usually controls a single gene. Thus, the concept of the operon as a mechanism of regulation transcends prokaryotes and provides a simple, universal mechanism for coordinate regulation of gene expression.

Whole organisms contain multiple control units. For example, the bacteriophage λ utilizes an ensemble of control units to mediate the choice between lysogeny and lytic viral growth and to drive the succession of stages in gene expression characteristic of viral multiplication. A chain of control units can be either open (a cascade) or closed (a loop).

Do control units of the sort described for microbial gene regulation play a universal role in development? The proponents of the operon idea noted ways in which control loops could maintain alternative stable patterns of gene expression (JACOB and MONOD 1961b). Indeed, phage λ can display just such a pair of stable regulatory states (EISEN *et al.* 1970; NEUBAUER and CALEF 1970). This gives flesh to the abstract notion of epigenetic determination raised by DELBRÜCK (1949). But does development involve regulatory proteins of the conceptual class revealed by the analysis of prokaryotic operons?

Many different experimental systems are being employed to study aspects of development and may provide answers to the following questions. How do bacteria, such as *Caulobacter crescentus* and *Bacillus subtilis*, coordinate their complex morphogenetic programs? How do unicellular eukaryotes, such as yeast, manage to exhibit three different cell types? And, of course, how do multicellular eukaryotes do all of the above and a lot more?

Genetic analysis played a major role in uncovering the operon mechanism: mutations affecting the regulatory proteins and the target sites had striking and informative effects. Amphibia, marine invertebrates and avian vertebrates have their own strong points for the study of development, so that one asks whether genetic analysis can play a role in answering the questions posed above. To what extent is the *modus operandi* of molecular genetics–identification of the important molecular players by isolation of defective mutants–a sufficient approach?

A number of investigators accustomed to the study of gene regulation in microbes have been involved in studying development directly with metazoons. A group of such biologists joined together, *autour de* FRANÇOIS JACOB, in May, 1990, at the Fondation Les Treilles in Provence, France, to discuss their experiences and prospects. This essay explores four themes on which there was extensive conversation: the strengths and limitations of the operon paradigm; the dialogue between gene action and morphogenesis; the formal logical elements of complex biological systems; and "back to the bench," challenges for developmental genetics in the 1990s.

The operon paradigm and its limitations: MARK PTASHNE presented a scheme, supported by considerable experimental evidence, in which the molecular mechanism of action of regulatory proteins in eukaryotes, including higher organisms, could constitute a

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reenactment of the same molecular principles discovered initially in the regulation of bacteriophage λ. In outline, the following molecular modules could be defined as sufficient and necessary:

- A DNA-binding domain that recognizes a short sequence of DNA, usually about 5–8 nucleotides, which provides the address.
- A dimerizing domain that allows the protein to form homodimers with itself or heterodimers with another recognition protein to enhance both selectivity and affinity.
- A patch for direct or indirect interaction with another protein that itself has a patch interacting with the transcriptional machinery.
- Patches for interaction with other sets of proteins of the same type. This feature allows action at a distance, from several addresses, which in higher organisms are not confined to a few dozen base pairs at the 5′ ends of the genes. There are even effects from paired homologs. The network of interactions permits the assembly of a complex regulatory agglomerate ("regglomerate") with many different components. The specificity rules for interaction may be quite relaxed (LIN *et al.* 1990). All of this can be, and has been, tested by experiments of the kind done most extensively with the GAL4 regulator of yeast, by artificially joining different domains (BRENT and PTASHNE 1985).

A good example of formation of heterodimers to create a new regulatory protein comes from yeast, where the regulator **a**1/α2 is formed by association of **a**1 and α2 polypeptides (GOUTTE and JOHNSON 1988). A spectacular example of this combinatorial association has been described by SCHULZ *et al.* (1990).

The howls of protest were generally of two types:

The molecular mechanism: If the operon paradigm is confined exclusively to the control of transcription initiation, then it is not enough, even for λ, where we also have control by antitermination (*N* and *Q*) and by protein stability (*N* and *cII*). In higher organisms, splicing is regulated; TOM CLINE explicated its role in the mechanism of sex determination in Drosophila (SALZ *et al.* 1989). There are also likely to be mechanisms at other levels, such as messenger lifetime or even translation, as discussed by JONATHAN HODGKIN. One can also imagine that the regulating molecule could be made of RNA rather than protein. If the operon paradigm of genetic regulation merely implies that somewhere, in DNA or RNA, nucleotide sequences in a particular gene provide a specific address for protein to assemble a regulatory apparatus, there would be few objections.

How might such regulation occur in cellular development? This concerns programming and signaling. In prokaryotes and yeast, regulation is mostly coupled to outside environmental variation. Thus, the induction of λ by UV or of β-galactosidase by lactose involves chains of initial events until the λ repressor is proteolyzed or the *lac* repressor dissociates from its operator. These are "ready to go" systems that require a trigger, and triggers often have induction pathways. IRA HERSKOWITZ pointed out that there is, in fact, the equivalent of an outside environment in higher organisms; it is not the world, but other cells. So there may be many "ready to go" systems in development requiring triggers provided by other cells either as molecules or by cell contact. The induction pathways are very complicated and include receptors, G proteins, protein kinases, internal messengers and so on; and their final acts could be phosphorylation or dephosphorylation, or other chemical alterations of the regglomerate. The intercellular signaling system of Myxococcus, discussed below, illustrates the intimate environment.

HERSKOWITZ summarized the yeast situation (see HERSOKWITZ 1989a). Haploid yeast cells (either **a** or α) are partially differentiated: they have receptors on their surface and they produce the mating factors. When prospective mating partners approach each other they signal their presence with these factors, thereby inducing the final differentiation into cells competent for mating. The mating factors are differentiation signals that induce the expression of various genes involved in mating (such as for cell fusion *per se*). There is a good argument that the mating pathway in yeast culminates by regulating the activity of a transcriptional activator protein, STE12, which might well be activated by phosphorylation.

For more solipsistic acts of development, transcription could be timed and located, not by "ready to go" systems but by the stepwise accumulation, substitution or modification of the components of the regglomerate. These might be called "go when ready" systems, so that each control state not only does something to the cell by altering the transcription pattern, but prepares for the next control state, reached by reiteration of the same mechanism. The cascade of *Bacillus subtilis* transcription factors, discussed below, illustrates successive steps of "readiness."

Because both "ready to go" and "go when ready" systems operate in development, perhaps this is the only way to maintain a self-consistent organization in development. It seems that development could not involve dead reckoning in a purely solipsistic system.

The operon paradigm survives, but with numerous twists.

The dialogue between gene action and morphogenesis: Embryogenesis involves processes that are visibly complex in both space and time. Gastrulation reaches levels of complexity that baffle the imagination of those comfortable with the one-dimensional character of chromosomes, genes, and polypeptides.

Are developmental genes qualitatively distinct in their complexity (WAELSCH 1989)?

A reduction of this issue of developmental complexity is to ask how one-dimensional genes and their polypeptide products can give rise to three-dimensional patterns. In *Caulobacter crescentus*, the products of certain flagellar (*fla*) and chemotaxis (*che*) genes are specifically directed to the swarmer cell in the polarized division process. Two mechanisms were discussed by LUCILLE SHAPIRO. The methylated chemotactic protein encoded by the *mcp* gene carries a carboxy-terminal segment that is necessary and sufficient for segregation to the swarmer cell. By contrast, the gene (*flaK*) encoding the flagellar hook protein directs its transcript selectively to the swarmer cell. Fusions of the *flaK* promoter alone to the marker polypeptide neomycin phosphotransferase can direct the marker antigen to the swarmer cell. In this case, the problem of gene product segregation is replaced by a problem in selective transcription. The issue becomes whether the swarmer cell contains a distinct transcription factor for the *flaK* promoter, or a distinct chromosomal template in which the *flaK* gene is active, or both.

The patterns of successive cell divisions can engender three-dimensional arrays of distinct cell types by these and perhaps other mechanisms. In this reduction of the problem of multidimensionality, the issue becomes the determination of cell division pattern. *Saccharomyces cerevisiae* displays two distinct patterns of bud formation: axial in haploid **a** or α cells and polar in **a**/α diploid cells (J. CHANT and I. HERSKOWITZ, unpublished results). HERSKOWITZ summarized a mutational analysis indicating that the axial pattern requires the action of five genes, two of which are unnecessary for the polar pattern. For at least three of these pattern-determining genes, null alleles are fully viable. Thus, the cell division patterns of *S. cerevisiae* seem to be completely dispensable in the laboratory; the prospects for an extensive genetic analysis are correspondingly large!

The nematode *C. elegans* illustrates the importance of cell division patterns in metazoan development (see HORVITZ and SULSTON 1990). BILL WOOD summarized the three early cleavage divisions by which the anterior/posterior, dorsal/ventral and left/right assymetries are established in this species. He has begun to describe mutants affected in left-right asymmetry.

Spatial morphogenesis is but one aspect of embryogenesis. How does the morphology of a particular stage lead to the patterns of gene expression that generate the next stage? PATRICK STRAGIER gave several illustrations, from studies of the sporulation process in *B. subtilis*, of particular morphogenetic steps that activate new patterns of gene expression (STRAGIER and LOSICK 1990). The proteolytic activation of the late-acting transcription factor σ^E requires the formation of the asymmetric septum that divides the forespore from the mother cell. Three layers of morphogenetic control play a role in elaborating active transcription factor σ^K in the mother cell. Firstly, genetic rearrangement deletes an interrupting sequence from the coding region for σ^K. For reasons not yet known, this rearrangement occurs only in the terminal mother cell compartment. Secondly, the transcription of the rearranged gene for σ^K depends on a factor that is present only in the mother cell. Finally, the σ^K product is activated by a protease that depends on the formation of the forespore compartment for activation. The developmental companion to σ^K is the forespore-specific transcription factor σ^G. Its synthesis is coupled to morphogenesis by its dependence upon a set of DNA-binding proteins that fail to act unless an intact forespore membrane is formed. A mutant described by STRAGIER exhibits a perforated forespore membrane and fails to synthesize σ^G even though the DNA-binding regulators of σ^G have been synthesized. Thus, the cascade of transcription factors that drives the sporulation process in *B. subtilis* is thoroughly coupled to morphogenesis in the developing system.

DALE KAISER gave examples of factors that act between cells to promote development of the microbe *Myxococcus xanthus*. Unlike the solitary sporulation habit of *B. subtilis, M. xanthus* sporulates in clusters of approximately 10^5 cells. Mutants with nonautonomous defects in sporulation identify four stages of interaction, A, B, C, and D. The gene product of the *csgC* gene that acts at the final stage, C, is a 17-kD protein tightly associated with the cell membrane. This protein, C factor, can be solubilized and can act to rescue the sporulation of stage-C mutants. Interestingly, nonmotile mutants affected in any of six motility loci of *M. xanthus* mimic the sporulation defect of stage-C mutants and can be rescued by solutions of C factor. But paradoxically, normal amounts of C factor are produced by these nonsporulating motility mutants. KIM and KAISER (1990) have shown that these nonmotile mutants become competent to sporulate when they are aligned within fine grooves on agar surfaces. Thus, the successful transfer of C factor requires intimate cell alignment, which normally depends on the motile behavior of the bacterium. The notion of "microenvironment" is appropriate to the C-factor interaction in *M. xanthus*, just as it is to the action of the *Steel* gene in murine hematopoiesis.

The richness of analysis of these issues in the development of Drosophila was conveyed by SPYROS ARTAVANIS-TSAKONAS, SEYMOUR BENZER, TOM CLINE, ANTONIO GARCÍA-BELLIDO, DAVE HOGNESS, FOTIS KAFATOS and RUTH LEHMANN, but we lack the space to describe their observations. However, certain of

these issues have recently been summarized (HARTLEY and WHITE 1990).

Formal logical circuits: The concept of operon is generative; not only is it sufficient to cover the diverse situations that prompted its discovery, but it helps in imagining new situations that it may also cover. An amplification of the operon paradigm prolongs and extends it rather than replacing it; the paradigm uses the elements of the operon and similar elements as building blocks for ever more elaborate networks. As developed by RENÉ THOMAS, the individual wheels of these nets are feedback loops (THOMAS and D'ARI 1990). According to this view, the elementary principles governing regulatory nets are the following.

- Any regulatory net can be decomposed into a well-defined set of simple feedback loops that usually interact with each other.
- A feedback loop is either positive (each element in the loop exerting a positive influence on its own later development) or negative (each element exerting a negative influence on its own later expression).
- Whether a loop is positive or negative depends only on the even versus odd parity of the number of negative control units.
- Negative feedback loops generate homeostasis with or without periodicity. Whereas a negative control unit reduces expression without influencing gene dosage effects, a negative loop not only attenuates expression but also tends to abolish gene dosage effects.
- Positive feedback loops (that is, direct or indirect autocatalysis) generate multiple alternative steady states; m independent positive loops can generate up to 3^m steady states, 2^m of which can be stable (that is, m binary choices).

A number of illustrations of these principles were heard in conversations at Les Treilles.

A negative feedback loop can abolish gene dosage effects. Thus, SHAPIRO observed that when the *Hook* operon of Caulobacter is cloned in a multicopy plasmid, there is no gene dosage effect. And the negative loop involving the λ *cro* gene presumably results in the levels of products of *cII, O* and *P* being relatively insensitive to the number of gene copies. In the absence of such regulation, the rate of synthesis of these products (which is already sufficient for replication when there is a single copy of the λ chromosome) would be over 100 times higher after 20 min. Such levels are probably unnecessary for O and P and toxic with regard to cII.

A positive feedback loop may associate with a choice. The detailed mechanisms vary from case to case but the principle is the same: positive autocontrol by the λ *cI* gene (PTASHNE); positive autocontrol by various σ factors in *B. subtilis* (STRAGIER); induced synthesis of receptor and mating factor by the yeast mating factors so that, by positive reinforcement, **a** and α cells are indeed making a commitment to differentiation and a commitment to each other (HERSKOWITZ); a positive control loop involving the *Sxl* gene in sex determination in Drosophila (CLINE); and a positive control loop of the C-factor gene in Myxococcus (KAISER). THOMAS suggested that a fruitful avenue for cloning developmental regulatory genes is to screen for activities that have a positive effect on their own expression level, directly or indirectly.

Chains of control units are also an important feature of the networks involved in development. Primary sex determination in Drosophila and Caenorhabditis involves multiple regulators acting in series, as summarized by HODGKIN (see HODGKIN 1990). In contrast, ANNE MCLAREN noted the apparent simplicity of primary male determination in mammals by a positive regulator [see MCLAREN (1990) and references cited therein]. But λ regulation was also simple at first glance.

The genetic analysis of development: Three topics were intensively discussed.

The saturation genetics approach: One of the fundamental genetic strategies for identifying the components of a process, whether it be biosynthesis of histidine or mating ability of yeast, is to isolate mutants defective in the process and then figure out what the wild-type genes do. A variation on this scheme has been used to study sex determination in multicellular organisms (fruit flies, nematodes, mammals), in which mutants are identified that are transformed from one sex into the other. Assuming that the logistics of such an extensive mutant hunt can be worked out, can this "saturation genetics approach" identify all of the important molecular protagonists?

The simple answer is that many important genes and proteins have been identified in this way, including several that fill the bill of being regulatory proteins of the type conceived by JACOB and MONOD. These include the regulatory proteins encoded by the yeast mating-type locus, the cascade of regulatory proteins in early Drosophila development (*bicoid, hunchback, ftz*, etc.) (INGHAM 1988), and the regulators responsible for sex determination in nematodes and fruit flies (*her-1, tra-1*, etc., in nematodes; *Sxl, dsx*, etc., in fruit flies) (HODGKIN 1990). The discovery of these proteins by genetic methods represents a tremendous advance in understanding the programming of development, but is it complete? We are sensitive to the issue of whether the tools that we use to study the process may perturb the process or introduce a bias in our view of the process.

Essentiality and redundancy: Genetic analysis may give an incomplete or distorted view by failing to identify important genes through the isolation of mutants: a gene might be essential or functionally redun-

dant. The essentiality issue was dramatized by HERSKOWITZ with an example from cell-type determination in yeast. It is clear that the mating-type locus determines whether a yeast cell is **a** or α (HERSKOWITZ 1989b). The α1 protein coded by the α mating-type allele activates transcription of α-specific genes, and the α2 protein turns off transcription of the **a**-specific genes in these cells. Genetic analysis, however, failed to reveal that both the α1 and α2 proteins work in conjunction with a general transcription factor, MCM1 (KELEHER, GOUTTE and JOHNSON 1988; BENDER and SPRAGUE 1987). As noted earlier, α1 stimulates transcription by assisting MCM1 protein and α2 inhibits transcription by blocking the function of MCM1. These relationships were discovered not by isolating mutants defective in *MCM1*–we now know that inactivation of this gene leads to cell inviability (PASSMORE *et al.* 1988)–but instead by good old biochemistry: α1 binds to DNA only in the presence of MCM1 (BENDER and SPRAGUE 1987) and α2 binds to DNA more strongly with MCM1 (KELEHER, GOUTTE and JOHNSON 1988).

There are many genes identified in fruit flies, nematodes, mice and other organisms in which the canonical mutation is leaky and in which null alleles cause inviability. In fact, *MCM1* was originally identified genetically in this way (PASSMORE *et al.* 1988). Although genetic analysis can identify important genes such as *MCM1* of yeast and *daughterless* of Drosophila (CLINE 1983), special mutations of these genes that allow viability but confer a mutant phenotype are obviously much rarer than mutations that simply inactivate genes. The flip side to this coin is that some viable mutations turn out to affect essential genes, as exemplified by the recently identified lethal alleles at the murine *quaking* locus discussed by BILL DOVE (SHEDLOVSKY, KING and DOVE 1988). When BENZER discussed some of the more than 100 genes known to affect eye development in Drosophila, GARCÍA-BELLIDO pointed out that, for the vast majority of these genes, null alleles are lethal. BARBARA MEYER developed this theme in depth for sex determination in *C. elegans*. The control gene *xol-1* (*XO lethal*) is vital for dosage compensation (for review see HODGKIN 1990).

Redundancy strikes fear in the hearts of geneticists. KOREN has drawn a cartoon in which a monster is looming over one couple while they are conversing with another couple. They know the monster is there and explain, "We deal with it by talking about it." BOB HORVITZ described at least three different types of functional redundancy, and he and GERRY FINK gave examples of these from nematodes and Arabidopsis. He characterized them as redundancies of genes, of pathways and of cells. MCLAREN noted the analogy to the principle of "double assurance" from experimental embryology.

Many examples are now known in which there are duplicate genes, or else two genes that code for proteins with similar amino acid sequences and similar functions. There are even examples in nematodes and yeast in which genes are triply redundant: three *ACE* genes in nematodes (RAND and RUSSELL 1984) and three *CLN* genes in yeast (RICHARDSON *et al.* 1989). In these cases, certain mutant phenotypes are observed only in triply mutant strains!

Arabidopsis provides a good example of redundant pathways. FINK described recent studies indicating that it has two independent pathways for tryptophan biosynthesis (BERLYN, LAST and FINK 1989). Inactivation of any of three genes in one of the pathways causes a leaky tryptophan requirement, apparently because the other pathway can provide some tryptophan. It appears that the second pathway is located in the chloroplast. FINK suggested that the two pathways may be regulated differently, one, for example, being specialized for a stress response and used to synthesize various protective products.

Nematodes provide an example of redundancy at the cellular level. A particularly valuable experimental approach in nematodes is cell ablation with a laser microbeam followed by analysis of behavior. This technique provides a preview of the phenotype that might be exhibited by mutants defective for the functions of the ablated cells. HORVITZ described studies of the excitation of certain intestinal cells by neighboring neurons called AVL and DVB. Although ablation of either neuron has no phenotype, ablation of both causes a mutant phenotype, defective defecation. Thus, genetic analysis of either the AVL or DVB cell type must be performed in a strain defective in the reciprocal neuron.

Given functional redundancy, is it ever possible to obtain mutants defective in such a function? It is indeed and, as described by HORVITZ, functional redundancy provides one explanation for a puzzling type of mutant that has cropped up in nematodes. These carry mutations that have an observable phenotype (such as altered body shape or movement) but which then exhibit no phenotype when the original mutation is converted to a null. A tidy explanation is that the affected gene is a member of a multigene family and that the original mutation is analogous to a dominant negative mutation, one that creates an abnormal protein that inhibits the proteins encoded by the other, redundant loci (PARK and HORVITZ 1986). In cases where one gene of a multigene family has been cloned, such dominant negative versions can be designed *in vitro* and then introduced into the genome, where overproduction is likely to cause a mutant phenotype (HERSKOWITZ 1987).

HODGKIN described mutants of nematodes that can be used to reveal the inhibitory effects of a mutant

product on a wild-type product. In *smg* mutants (HODGKIN *et al.* 1989), it appears that certain transcripts are more stable than in wild-type strains (R. PULAK and P. ANDERSON, unpublished results). The striking finding is that some truncated polypeptides (resulting from nonsense mutations) become dominant in a *smg*$^-$ background. Even amber mutations, which are expected to be well-behaved losses of function, can become dominant-negatives if the amber fragment is sufficiently long.

JANET ROSSANT described the new era of gene knock-out in mouse genetics: it is now possible to use a mouse gene mutated *in vitro* to inactivate the wild-type gene, by first modifying cells in culture and then deriving a mouse with the mutation in its germ line. That is the good news. Now the bad news: sometimes the mutant has no overt phenotype! One explanation for this disappointing result is functional redundancy. A strategy to contend with this difficulty is to begin another mutant hunt using the silent mutant as the starting strain. This approach has been used successfully in yeast, nematodes, and fruit flies, but of course it has no guarantee of success and can be quite cumbersome.

For the mouse, the tools for genetic analysis are under active development. ROSSANT discussed enhancer-trap protocols and DOVE summarized point mutagenesis by ENU, each of which very efficiently generates mutants on the basis of phenotype (GOSSLER *et al.*, 1989; MCDONALD *et al.* 1990). JACOB raised the conundrum that patterns in the expression of *lacZ* insertions do not match classical embryological lineages. PETER GOODFELLOW noted the difficulties of identifying sex-determining elements by analyzing translocation chromosomes. GYORGY GEORGIEV explored the development of transfection assays for genes controlling metastatic cell behavior. The new genetic tools in mice may soon participate as partners comparable to transplantation and cell culture analysis of the neural crest as summarized by NICOLE LE DOUARIN.

Identifying interacting components: Along with saturation genetics and gene knock-outs using cloned genes, another important genetic strategy for the nineties is the identification of interacting components. At least four different schemes were discussed in the formal sessions and during the unforgettable Provençal meals. The central idea is to use one mutant strain as a springboard to identify another gene, one that is functionally related in some way to the original gene. The standard fantasy is that the two gene products physically interact (the "geneticist's immunoprecipitate"). But this should not be assumed *a priori* to be the case.

The first method, described by HOGNESS, involves identifying interacting components through mutations that are nonallelic noncomplementers. These present themselves as genetic anomalies in which mutations at two separate loci are recessive to their corresponding wild-type alleles but whose double heterozygote exhibits a mutant phenotype. Mutations of this sort have been interpreted to affect components of a multisubunit structure, for example of the mitotic apparatus (HAYS *et al.* 1989).

A classical method to identify interacting components, and one that remains powerful, is to identify modifier mutations. These are mutations distinct from the initial mutation that cause the original phenotype to be relieved (a suppressor mutation) or exacerbated (an enhancer mutation). GARCÍA-BELLIDO is analyzing the morphogenesis of the Drosophila wing. He has classified the 30 genes known to be involved into five synergy groups. Combinations of two mutations from the same synergy group give an enhanced phenotype. DOVE mentioned that the dominant allele *Min*, causing multiple intestinal adenomas in the mouse (MOSER, PITOT and DOVE 1990), responds to dominant suppressor alleles carried by particular mouse strains. ARTAVANIS-TSAKONIS described the use of this approach to identify genes that interact with the Drosophila *Notch* gene. He created a situation in which a certain *Notch* heterozygote was inviable and identified numerous revertants to viability. In so doing, he found mutations in several known genes and in a previously unidentified gene, *deltex* (XU and ARTAVANIS-TSAKONIS 1990).

HERSKOWITZ described the strategy of searching for high-copy-number plasmids carrying one gene that can compensate for a mutation in another gene. This technique has been used profitably in fission and budding yeast to identify genes whose products interact with the cdc2/CDC28 protein kinase (HAYLES *et al.* 1986; HADWIGER *et al.* 1989). In these cases, high-copy-number plasmids were identified that could suppress the growth defect of thermosensitive mutants at semipermissive temperature. One mechanism for this suppression is expected to be by mass action, providing more of one interacting component for association, and this strategy ought to identify proteins that bind to mutated target sites. Other mechanisms for high-copy-number suppression can also be imagined. For example, increased gene dosage might enable the synthesis of sufficient product to bypass a defect in expression of that gene. Although the technique of high-copy-number plasmid suppression is currently available only for microbial systems, it might be worthwhile to develop an analog for nematodes, fruit flies or mice.

The prototype has recently been described for a potentially powerful new method for identifying interacting components. Dubbed by the group the protein interaction trap, this method is based on the two-

domain structure of the yeast transcriptional activator protein GAL4–one (D) the DNA-binding domain and the other (A) necessary for transcriptional activation (BRENT and PTASHNE 1985). FIELDS and SONG (1989) have exploited the fact that these two domains must be physically associated in order to function. They have shown that the two domains can be brought together with two hybrid proteins, D/P_1 and A/P_2, where P_1 and P_2 are protein segments that associate with each other. FIELDS and SONG suggest that it is in principle possible to use this as a screening method. For example, a protein segment of interest (P_3) could be attached to the GAL4 DNA-binding domain to form D/P_3. Then a library of hybrid proteins (A/P_i), formed by joining the GAL4 activation domain to random coding segments, could be examined for one that can associate with the DNA-binding domain. Although not yet implemented, this scheme presents exciting possibilities. The regglomerate may be dissected in this way.

Concluding remarks: SYDNEY BRENNER gave the following overview in the opening conversation.

Biology is concerned just as much with particular implementations as with general principles. GUNTHER STENT long ago put forward the idea that development was a trivial problem because, given the JACOB-MONOD model of gene regulation, all development could be reduced to that paradigm. It was simply a matter of turning on the right genes in the right places at the right times. Of course, while absolutely true this is also absolutely vacuous. The paradigm does not tell us how to make a mouse but only how to make a switch. The real answers must surely be in the detail. It is also a mistake to think that general principles float about in the cosmos waiting for God or Nature or even the NIH to pluck them out and embed them in the real world. But I can hear a complaint: we cannot accept that everything exists separately in unique worlds, and what is true of λ should be true of lambs. However, there is a global constraint, and that is the connectedness by descent of all present living systems and the impossibility in evolution of going back to the drawing board once a certain level of complexity has been reached. Thus, anything successful that appears in evolution will be retained and elaborated as organisms advance in complexity and will be found over and over not as the instantiation of a general principle, but only thorough the continuity of utility.

A skeptic might at this stage ask what is meant by the terms complex and simple. The answer is, it depends. Thus, from the point of view of amino acids, λ repressor is a complex piece of machinery; but from the point of view of *Escherichia coli*, it is part of a simple switch. Biological systems have a hierarchical structure, and a poem at one level is an expletive at another. The same relativity applies to the words primitive and advanced. There is a view that *E. coli* is primitive and we are advanced. That is true from the point of view of function and action. But it is not true from the point of view of genome structure. Here it is *E. coli* that is streamlined and sophisticated, whereas it is our genome that has preserved a far more primitive condition.

A paradigm is a fancy word for an example, but it has come to mean more than that today. It carries the more distinctive flavor of a canonical case, something we all have to follow. But the operon paradigm should have no statutory requirements; it is a classical example and one which we will find, perhaps in more baroque contexts, as we disentangle the regulatory switches of higher organisms.

We thank JONATHAN HODGKIN for helpful comments. We are deeply grateful to ANNETTE GRUNER SCHLUMBERGER for sharing with us the spirit of the Fondation Les Treilles. Additional support came from the U.S.-Western Europe Cooperative Program of the National Science Foundation.

LITERATURE CITED

BENDER, S., and G. F. SPRAGUE, JR., 1987 MATα1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. Cell **60:** 681–691.

BERLYN, M. B., R. L. LAST and G. R. FINK, 1989 A gene encoding the tryptophan synthase β subunit of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **86:** 4604–4608.

BRENT, R., and M. PTASHNE, 1985 A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell **43:** 729–736.

CLINE, T. W., 1983 The interaction between daughterless and sex-lethal in triploids: a lethal sex-transforming maternal effect linking sex determination and dosage compensation in Drosophila melanogaster. Dev. Biol. **95:** 260–274.

DELBRÜCK, M., 1949 Pp. 33–35 in *Symposium sur Unités Biologiques Donées de Continuité Génétique*. Publications Centre National de la Recherche Scientifique, Paris.

EISEN, H., P. BRACHET, L. PEREIRA DA SILVA and F. JACOB, 1970 Regulation of repressor expression in λ. Proc. Natl. Acad. Sci. USA **66:** 855–862.

FIELDS, S., and O.-X. SONG, 1989 A novel genetic system to detect protein-protein interactions. Nature **340:** 245–246.

GOSSLER, A., A. L. JOYNER, J. ROSSANT and W. C. SKARNES, 1989 Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. Science **244:** 463–465.

GOUTTE, C., and A. D. JOHNSON, 1988 a1 protein alters the DNA binding specificity of α2 repressor. Cell **52:** 875–882.

HADWIGER, J. A., C. WITTENBERG, M. E. RICHARDSON, M. DE BARRO LOPES and S. I. REED, 1989 A family of cyclin homologs that control the G_1 phase in yeast. Proc. Natl. Acad. Sci. USA **86:** 6255–6259.

HARTLEY, D., and R. WHITE, 1990 *Drosophila* in Crete: a flying visit. Trends Genet. **6:** 199–201.

HAYLES, J., D. H. BEACH, B. DURKACZ and P. M. NURSE, 1986 The fission yeast cell cycle control gene $cdc2^+$: isolation of a sequence $suc1^+$ that suppresses $cdc2^-$ mutant function. Mol. Gen. Genet. **202:** 291–293.

HAYS, T. S., R. DEURING, B. ROBERTSON, M. PROUT and M. T. FULLER, 1989 Interacting proteins identified by genetic interactions: a missense mutation in α-tubulin fails to complement

alleles of the testis-specific β-tubulin gene of *Drosophila melanogaster*. Mol. Cell. Biol. **9:** 875–884.

HERSHEY, A. D., 1970 Genes and hereditary characteristics. Nature **226:** 697–700.

HERSKOWITZ, I., 1987 Functional inactivation of genes by dominant negative mutations. Nature **329:** 219–222.

HERSKOWITZ, I., 1989a Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52:** 536–553.

HERSKOWITZ, I., 1989b A regulatory heirarchy for cell specialization in yeast. Nature **342:** 749–757.

HODGKIN, J., 1990 Sex determination compared in *Drosophila* and *Caenorhabditis*. Nature **344:** 721–728.

HODGKIN, J., A. PAPP, R. PULAK, V. AMBROS and P. ANDERSON, 1989 A new kind of informational suppression in the nematode *Caenorhabditis elegans*. Genetics **123:** 301–313.

HORVITZ, H. R., and J. E. SULSTON, 1990 Joy of the worm. Genetics **126:** 287–292.

INGHAM, P. W., 1988 The molecular genetics of embryonic pattern formation in *Drosophila*. Nature **335:** 25–34.

JACOB, F., and J. MONOD, 1961a Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3:** 318–356.

JACOB, F., and J. MONOD, 1961b On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26:** 193–211.

KELEHER, C. A., C. GOUTTE and A. D. JOHNSON, 1988 The yeast cell-type-specific repressor α2 acts cooperatively with a non-cell-type-specific protein. Cell **53:** 927–936.

KIM, S. K., and D. KAISER, 1990 Cell alignment required in differentiation of *Myxococcus xanthus*. Science **249:** 926–928.

LIN, Y.-S., M. CAREY, M. PTASHNE and M. R. GREEN, 1990 How different eukaryotic transcriptional activators can cooperate promiscuously. Nature **345:** 359–361.

MCDONALD, J. D., V. C. BODE, W. F. DOVE and A. SHEDLOVSKY, 1990 $Pah^{bph\text{-}5}$: a mouse mutant deficient in phenylalanine hydroxylase. Proc. Natl. Acad. Sci. USA **87:** 1965–1967.

MCLAREN, A., 1990 What makes a man a man? Nature **346:** 216–217.

MOSER, A. R., H. C. PITOT and W. F. DOVE, 1990 A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science **247:** 322–324.

NEUBAUER, Z., and E. CALEF, 1970 Immunity phase-shift in defective lysogens: non-mutational hereditary change of early regulation of λ prophage. J. Mol. Biol. **51:** 1–13.

PARK, E. C., and H. R. HORVITZ, 1986 Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans*. Genetics **113:** 821–852.

PASSMORE, S., G. T. MAINE, R. ELBLE, C. CHRIST and B.-K. TYE, 1988 *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of *MATα* cells. J. Mol. Biol. **204:** 593–606.

RAND, J. B., and R. L. RUSSELL, 1984 Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. Genetics **106:** 227–248.

RICHARDSON, H. E., C. WITTENBERG, F. CROSS and S. I. REED, 1989 An essential G1 function for cyclin-like proteins in yeast. Cell **59:** 1127–1133.

SALZ, H. K., E. M. MAINE, L. N. KEYES, M. E. SAMUELS, T. W. CLINE and P. SCHEDL, 1989 The *Drosophila* female-specific sex-determination gene, *sex-lethal*, has stage-, tissue-, and sex-specific RNAs suggesting multiple modes of regulation. Genes Dev. **3:** 708–719.

SCHULZ, B., F. BANUETT, M. DAHL, R. SCHLESINGER, W. SCHÄFER, T. MARTIN, I. HERSKOWITZ and R. KAHMANN, 1990 The *b* alleles of U. maydis, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell **60:** 295–306.

SHEDLOVSKY, A., T. R. KING and W. F. DOVE, 1988 Saturation germ line mutagenesis of the murine *t* region including a lethal allele at the quaking locus. Proc. Natl. Acad. Sci. USA **85:** 180–184.

STRAGIER, P., and R. LOSICK, 1990 Cascades of sigma factors revisited. Mol. Microbiol. (in press).

THOMAS, R., and R. D'ARI, 1990 *Biological Feedback*. CRC Press, Boca Raton, Fla.

WAELSCH, S. G., 1989 In praise of complexity. Genetics **122:** 721–725.

XU, T., and S. ARTAVANIS-TSAKONAS, 1990 *deltex*, a locus interacting with the neurogenic genes, *Notch, Delta* and *mastermind* in *Drosophila melanogaster*. Genetics **126:** 665–677.