DIFFERENTIATION REQUIRES CONTINUOUS ACTIVE CONTROL

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INTRODUCTION

The vast number of genes involved in the development of a multicellular organism presents a substantial regulatory problem. Complex controls are required to ensure that the right genes are expressed at the correct level in hundreds of different cell types. Since only a small fraction of the total genes
of a differentiated cell are expressed at any given time, negative regulatory mechanisms for preventing inappropriate genes from being expressed are particularly important. Two types of molecular mechanism that could silence genes in differentiated cells are (a) "passive control," a mechanism for closing down unneeded genes in a given cell lineage so that they do not need active consideration for the life of the organism, and (b) "active control," continual regulation of the expression state of each gene. Under passive control, the commitment to differentiate, like Lyonization of the X chromosome, would result in the permanent inactivation of many unnecessary genes. Under active control, an easily reversible regulatory decision would be made for each gene in each differentiated cell, a decision determined by the protein composition of the cell at any given time. An active mechanism would require that gene expression in the differentiated cells of eukaryotes, like the cells of prokaryotes (1a), be dynamic and subject to continuous regulation (1b).

The control of differentiation by a passive mechanism appears more likely than control by an active mechanism for the following reasons. First, the complexity of chromosome and DNA structure in eukaryotes vastly exceeds that in prokaryotes, making a simple active mechanism seem untenable. Second, plasticity of gene expression in "terminally" differentiated cells appears unnecessary, even risky. Third, active control seems unduly cumbersome. Indeed, the investment in regulators, especially negative regulators, required to maintain most genes at most times in a silent state appears disproportionately large. Fourth, it is unclear how memory and stability could be achieved by an active control mechanism. Although the rationale in favor of passive control appears strong, accumulating experimental evidence in a number of species suggests that the differentiated state is maintained by active continuous regulation, both by positive and by negative regulators.

In this review I first discuss passive control and evidence that this mechanism may be used to regulate gene expression in some settings during development. I then trace historically the key experiments that led to the hypothesis that the development of differentiated cells in tissues and organs is not passively, but actively, controlled. Finally, I discuss the implications of an active control mechanism for differentiation.

**EVIDENCE FOR PASSIVE CONTROL**

As a counterpoint to active control, I have selected two examples that currently provide the strongest cases for passive control. The silencing of genes that results from X-chromosome inactivation or as a consequence of imprinting suggests a passive control mechanism. These two events play a critical role in early development, apparently ensuring the balanced contribution of male and female genomes (2). For X chromosomes, it is clear that only
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one of a pair of genes is active in the same nucleus, an expression state that is established early in embryogenesis and stably transmitted to all progeny cells as methylated heterochromatin. The molecular mechanisms that target genes for this type of repression are not well understood. Once induced, however, this expression state is not easily disrupted and does not appear to be subject to change in the course of normal development; it is altered only in the germline. Imprinting shares several of these properties and, although less well characterized, may share a similar mechanism. X-chromosome inactivation and imprinting both appear to be negative regulatory decisions that persist for the life of the organism.

EVIDENCE FOR ACTIVE CONTROL

It might seem most expedient to silence genes in differentiated cell types by a passive mechanism, such as that used in X-chromosome inactivation. Indeed, why allow muscle genes to be accessible in a liver cell? However, as described below, several lines of experimental evidence suggest that differentiation is actively controlled and requires continuous regulation.

Nuclear Transplantation

Gurdon’s classic nuclear transplantation experiments (3) first began to test whether gene expression in differentiation is controlled by an active or a passive mechanism. Gurdon found that when the nuclei of amphibian intestinal cells were transplanted into enucleated eggs, entire feeding tadpoles developed. Nuclei from keratinocytes and noncycling erythrocytes also displayed this potential for dramatic change in nuclear function (4; for a review, see reference 5). These experiments were interpreted as providing strong evidence that genetic material was generally not lost during vertebrate differentiation. In the present context, these experiments also provide support for an active mechanism of gene regulation, because they show that tissue-specific genes, such as globin genes in intestinal cells, are reversibly inactivated. When passed back through egg cytoplasm, the globin genes are resuscitated and are able to function once red blood cells differentiate among the progeny of the reconstituted egg.

Gurdon’s data, however, do not prove active control, because passage of the differentiated nuclei through the egg might have stripped the DNA of all passive regulatory influences and allowed reprogramming. Indeed, DiBerardino et al (6) showed that the frequency of obtaining feeding tadpoles was increased from approximately 2 to 75% if the nuclei were initially injected into maturing oocytes, conditioned with oocyte cytoplasm, and allowed to progress to the blastula stage before being transplanted into enucleated eggs. The high frequency observed in these experiments ruled out
the possibility that the results reflected the presence of a small subpopulation of residual stem cells. However, it raised the possibility that the incubation step was necessary to alter gene structure and permit access of regulators. Thus, the environment of the maturing oocyte might be a prerequisite for alleviating passive control and allowing resuscitation of genes inactivated during differentiation.

**Transdetermination and Transdifferentiation**

Other lines of evidence show that exposure to ooplasm may not be a requirement for the activation of genes from differentiated nuclei that have no need of those genes. For instance, following serial transplantation of imaginal disc cells into the abdomen of an adult *Drosophila* fly, "transdetermination," or a change in determined state, often occurs so that cells originally destined for genital structures first give rise to leg or head structures and eventually to wings (7, 8). A similar developmental transformation of one body part into another occurs when *Drosophila* homeotic genes are mutated (9).

A change termed "transdifferentiation" has also been observed at sites of tissue regeneration following injury. When the iris of an amphibian, chicken, or human eye is damaged, for instance, some of the cells dedifferentiate, lose their melanin pigment, divide, and give rise to lens cells that synthesize characteristic crystallins (10, 11). Similarly, striated muscle tissue isolated from anthomedusae is capable of generating six diverse cell types including sensory neural cells (12, 13). In some cases, these changes in differentiated state cross lineages, so that mesoderm gives rise to ectodermal cell types.

In transdetermination and transdifferentiation experiments, the fate of the cell is altered without recourse to the regulatory hierarchy characteristic of development from the fertilized egg to differentiated tissue. However, these experiments may not rule out a passive control mechanism. A number of cell divisions typically occur between the two differentiated states, and changes critical to reprogramming and activating silent genes could be associated with DNA replication in the course of those divisions, as suggested by the models of Holtzer et al (14), Brown (15), and Weintraub (16). To address this possibility, experiments that test the need for DNA replication as a prerequisite for activating silent tissue-specific genes are required.

**Heterokaryons**

Strong support for the active-control hypothesis derives from somatic cell hybridization experiments. Upon cell fusion, the influence of one cell type on the function of another can be studied. Experiments with hybrids first showed that gene expression could be altered in transformed cells (17a–c) and in
differentiated mammalian somatic cells (17d–h). In some cases, novel gene expression was induced (18a–c). However, since extensive proliferation was required to select the hybrids, a requirement for DNA replication to alleviate repression by a passive mechanism could not be ruled out. Moreover, in dividing cell hybrids, or synkaryons, nuclei were combined and chromosome loss and rearrangement were the norm. Experiments with nondividing cell hybrids, or heterokaryons (19, 20) in which nuclei remained separate and distinct, demonstrated that this resuscitation of genes could occur without cell division or DNA replication (21–23). Upon fusion of muscle cells with primary cell types representing all three embryonic lineages (endoderm, ectoderm, and mesoderm), genes that encoded a wide range of products including enzymes, membrane components, and contractile proteins were activated. Gene dosage, or the balance of regulators contributed by the two fused cell types, was critical. When the number of muscle cell nuclei exceeded the number of liver cell nuclei, extinction of liver genes and activation of muscle genes were observed; conversely, when the number of liver cell nuclei exceeded the number of muscle cell nuclei, muscle gene expression was repressed. By using a similar heterokaryon approach, these results were corroborated for muscle (24, 25) and extended to a variety of tissue-specific genes including hematopoietic (26), hepatic (27), and pancreatic (28) genes, which were activated in fibroblasts after fusion with cell types derived from blood, liver, and pancreas, respectively.

Several aspects of these cell fusion experiments are consistent with an active control mechanism. First, they suggest that genes are available for expression in cells that normally never express them. Thus, genes typical of mesoderm are readily activated in ectodermal cell types. Second, genes are activated in the absence of DNA replication. The frequency of gene activation did not differ when cells were continuously exposed to an inhibitor of DNA synthesis before and after fusion. Therefore if activation of silent genes requires changes in chromatin structure, these changes are mediated by mechanisms independent of DNA synthesis. Third, cells in which differentiation is well under way are as capable of inducing the expression of previously silent tissue-specific genes as are cells initiating differentiation. From this finding, it appears that the activity of trans-acting regulators is not required transiently at the onset of differentiation, but continuously to maintain it. Fourth, gene dosage, or the balance of regulators contributed by the two fused cell types, is a critical determinant of whether genes are repressed or activated. Fifth, differences among cell types are observed in the kinetics, frequency, and effects of gene dosage on gene activation or gene repression in heterokaryons. This result is likely to reflect differences in the combination of proteins that interact in each type of heterokaryon. Taken together, these observations provide strong support for the active-control hypothesis.
The Helix-Loop-Helix Family of Myogenic Regulators

Compelling evidence that changes in gene expression that accompany differentiation are actively regulated is also provided by the discovery of MyoD. Weintraub and colleagues (29) showed that the constitutive expression of a cDNA encoding a single trans-acting regulator could activate silent muscle genes such as myosin heavy chain and desmin in a range of nonmuscle cell types including fibroblasts, melanocytes, and neuroblastoma cells (30). Indeed, in mesodermal cell types, a complete phenotypic conversion was induced: a muscle-specific distribution of organelles and pattern of gene expression were stably inherited (31). Three additional regulators have now been identified, all members of a helix-loop-helix family of transcription factors (32) that appear to have similar properties (33–37). When constitutively expressed in fibroblasts, each of these myogenic regulators alone is capable of inducing myogenesis, presumably by binding to the consensus E-box sequence found in a large number of muscle-specific genes. These experiments indicate that a single protein, when present at relatively high concentrations, is capable of gaining access to and activating the expression of genes resident in cells that would normally never express them.

Drosophila and C. elegans

Further support for the active-control hypothesis derives from elegant experiments showing that in the course of normal development the continuous activity of positive and negative regulators is required to maintain the differentiated state. Most compelling are the results of experiments in which disruption in expression of a nodal, or key, regulatory gene alters the fate of cells. One might expect that developmental decisions such as segment identity or sex would be made early and locked in place by stable, heritable mechanisms. There would seem to be little advantage to allowing for plasticity in the regulation of these decisions, because the welfare of the organism requires that these decisions remain constant in each of its cells throughout its lifetime. However, experiments with Drosophila and Caenorhabditis elegans indicate that this is often not the case.

The clearest evidence for active control derives from two types of experiments that examine the temporal window during which a particular gene product is required: experiments using temperature-sensitive mutants or somatic mosaics. In the former, gene expression is altered by a shift in temperature. In the latter, mosaic individuals with patches of homozygous mutant cells are generated in heterozygous (normal) individuals by X-ray-induced mitotic recombination or chromosome loss. Studies of this type have shown that the expression of a gene that controls segment identity, such as Ultrabithorax, is required throughout development (38–40). Similarly, both positive and negative regulators of sex determination must be expressed
continuously or else the sexual characteristics of the cells will change, even in adulthood (41-44). Ongoing gene expression is also required to maintain the identity of neurosensory cells (45). Pattern formation, even at late larval stages, is altered if the expression of critical genes, including the large Polycomb family that encodes negative regulators, is disrupted (46). Perhaps the most striking example of plasticity is found in the *Drosophila* central nervous system: an adult female will engage in a complex courtship behavior typical of an adult male if exposed to a shift in temperature that disrupts the expression of the *tra-2* gene (47).

The results of such classic genetic experiments are corroborated by molecular experiments examining the expression pattern of transcripts by in situ hybridization or of proteins by immunofluorescence. They indicate that the products of homeotic selector genes and sex determination genes are present continuously throughout development (48, 49). These and many other experiments show that the uninterrupted expression of certain nodal negative and positive regulators is essential to the expression of the differentiated state in vivo.

**IMPLICATIONS OF ACTIVE CONTROL FOR DIFFERENTIATION**

**Stoichiometry of Positive and Negative Regulators**

The active-control hypothesis suggests that the stoichiometry, or relative concentration, of regulators plays a critical role in the expression of the differentiated state. The effective concentration of a regulator is altered not only when its rate of synthesis or degradation is changed, but also when the concentration of the proteins with which it interacts is altered. Recent evidence indicates that many regulatory proteins form complexes, for example heterodimers via leucine zipper or helix-loop-helix motifs (50, 51). Such interactions can either promote or inhibit the function of a regulator. For instance, the transcription factor MyoD requires the protein E12 to bind DNA efficiently (32), but is prevented from binding DNA when complexed to the protein Id (52). The transcription factor NF-κB is inhibited from entering the nucleus and is therefore inactive when it is complexed to I-κB in the cytoplasm (53, 54). The complexity of these interactions increases as the number of different partners with which a protein can associate increases, as is the case in intact cells (31, 55). Clearly, in addition to abundance, the relative affinity and cooperative interactions of regulators at DNA-binding sites will have a profound impact on gene expression. Recent evidence that synergism among diverse transcriptional regulators occurs even at concentrations at which their DNA-binding sites are saturated suggests that regulators have cooperative effects not just as heterodimers but also as multimeric complexes...
Because proteins act in combinations, small changes in the relative concentrations of a single regulator can have large effects on the expression of the differentiated state of the cell, by shifting a critical balance, reaching a threshold, and setting off a cascade of events. These predictions are borne out in vivo. The dosage of genes encoding the helix-loop-helix proteins daughterless, hairy, and achaete-scute determines sex in Drosophila (58). Gene dosage also determines sex in C. elegans (59) and the phenotype of neurosensory cells in Drosophila (60). Gene dosage is also responsible for several hereditary developmental disorders in humans (61).

**Negative Control of Gene Expression**

As discussed at the outset, a major regulatory dilemma in differentiation is maintaining most genes in an inactive state at any given time. Several lines of evidence suggest that negative trans-acting regulators are continuously present. Following cell fusion, many previously expressed tissue-specific genes are shut off (62–66). In Drosophila, loss-of-function mutants have revealed loci such as hairy and extramacrochaete that encode negative regulators of the achaete-scute complex (60). These gene products are required continuously to repress the differentiation of sensory organs (67). Similarly, the continuous expression of the homeotic Polycomb genes prevents the expression of the Drosophila Ultrabithorax gene. If Polycomb gene expression is disrupted, segment identity is altered (9, 68). Thus, mechanisms that mediate gene repression are of particular importance.

Negative cis-regulatory DNA elements have been revealed in the control regions of many genes, and, like positive cis-regulatory elements, they may eventually prove to be present in all genes. This would lend strong support to the active-control hypothesis. Negative elements are used for spatial control of gene expression, ensuring the repression of tissue-specific genes in a number of inappropriate tissues. Many such sites have been identified, for example in genes encoding growth hormone (69), insulin (70–72), renin (73), interleukin-2 (IL-2) receptor α chain (74), immunoglobulin kappa light chain (75), immunoglobulin heavy chain (76), T-cell receptor α chain (77), urokinase plasminogen activator (78), α-fetoprotein (79), vimentin (80), collagen II (81) and ε-globin (82). Indeed, subsets of differentiated cell types such as T lymphocytes can be distinguished on the basis of the activity of negative cis-regulatory elements: an enhancer controlling the α gene is active in αβ but not γδ cells in which a series of negative regulatory elements is active (77).

Negative regulatory sites are also used for temporal control by preventing gene expression at inappropriate times. These sites are used to silence genes until an extracellular signal induces an intracellular signal transduction pathway, leading to derepression. The beta-interferon gene (87, 88) and the yeast
heat shock genes (89, 90) are cases in point. Extrinsic signals are also likely to mediate the derepression of genes that occurs during differentiation. Negative regulatory sites appear to mediate lysozyme gene repression prior to macrophage differentiation (91), prevent immunoglobulin kappa genes from being expressed until pre-B cells mature to B cells (75, 92), and suppress the expression of major histocompatibility complex class I genes until embryonic stem cells are induced to differentiate by retinoic acid (93).

In many of the cases cited above, negative regulators do not interact with positive regulators directly. Instead, they act at a distance by a mechanism known as "silencing," which provides a means of shutting off gene expression, even in the presence of positive regulators. First described in the yeast Saccharomyces cerevisiae (83, 84), silencers have now been identified in a number of tissue-specific genes in a wide variety of species. Although it has been postulated that they may inhibit transcription (84, 85) or define chromatin loops (86), the mechanism by which silencers act in mammalian cells and the extent to which it parallels that described in yeast remains to be determined.

Negative cis-regulatory DNA elements need not be gene specific. A negative element that appears to play a role in repressing nearby genes has been defined in repetitive alu sequences (92, 94, 95). Other repetitive elements in the vicinity of several chicken genes, including lysozyme, ovalbumin, calmodulin, and UI genes (91), or near rat genes such as the insulin gene that contain the highly reiterated LINES sequence (71), mediate negative regulation. Such repetitive elements constitute putative "global repression-binding sites," that could provide a means of silencing a number of genes with relatively few negative regulators.

Negative regulators need not act directly by binding to repressive sites in DNA. They can affect the expression of specific genes indirectly by preventing the expression of genes encoding positive regulators (96) or by competing with positive regulators for DNA-binding sites (97–99). Alternatively, negative regulators can complex with positive regulators and prevent DNA binding (53, 54, 100) or render activators nonfunctional once bound to DNA (101–103).

In most cases, the derepression of genes, or relief from negative regulation, is accompanied by an induction of expression of positive regulators. The positive regulators may bind at sites distant from the sites occupied by negative regulators and act indirectly (91), or the binding sites of positive and negative regulators may overlap (87, 88, 104). It is generally not known whether positive effects override negative ones by direct or indirect interference with binding, but both mechanisms are likely to operate, and the stoichiometry, or relative concentration of each type of regulator, is likely to play an important role in the outcome.
Establishment and Maintenance of Differentiation

The active-control hypothesis poses problems for differentiation. Do all regulators have to be active at all times to maintain the differentiated state? How can the requisite number of regulators be continuously produced? How are stability and memory ensured? These questions are best addressed by first examining the origin of the differentiated state.

THE FIRST DIFFERENTIATION

The first cell difference, or differentiation, is likely to result from asymmetry. This is readily apparent in *Drosophila* or *Xenopus* development, in which the components of the egg are unequally distributed. The manifestation of asymmetry in mammalian development occurs later and is more subtle. Cells of the early mouse embryo are totipotent: their function is not restricted. This has been most elegantly demonstrated in experiments in which each of the cells at the four-cell stage was isolated and shown to be capable of contributing to both embryonic and extraembryonic tissues in chimeras formed by juxtaposing genetically distinct blastula-derived cells (105). Asymmetry is cytologically apparent within mouse cells as early as the eight-cell stage: organelles, cytoskeletal elements, and cell surface antigens assume a polar distribution that differs among neighbors (106-110). Although chimera experiments show that the cells are still totipotent at this stage (105), the subsequent fate of their progeny can be reproducibly influenced by their position within the reconstituted eight-cell embryo. Cells on the inside are destined for embryonic tissues, whereas cells on the outside contribute to extraembryonic tissues, presumably as a result of exposure to different signals. These signals induce the cells to elaborate novel protein products that, in turn, allow them to respond to novel signals. This signal–response cycle constitutes a hierarchy of regulatory steps that leads to the generation of neighboring cells that are increasingly differentiated from one another, expressing hundreds of different RNA and protein products.

MEMORY AND STABILITY

If differentiation is actively controlled, how is memory of the differentiated state established, maintained, and propagated to progeny? Is the entire hierarchy of regulators that led to the establishment of each distinct differentiated state continuously required to maintain it? If so, this almost infinite regress of regulators would, indeed, seem prohibitive.

The answer to these questions is that cells have developed mechanisms for circumventing the regulatory hierarchy. Thus, past events can be remembered without being continuously repeated and without recourse to a passive control mechanism. Autoregulatory loops, by which a protein product induces transcription of the gene that encodes it, constitute one active control mech-
anism that could maintain protein levels in the absence of early steps. This mechanism appears to be used by the bacteriophage lambda repressor, some of the *Drosophila* homeotic selector gene products, the signal transducer c-jun, and the helix-loop-helix family of myogenic regulators (111–114). Once genes encoding nodal regulators are activated, autoregulation serves to maintain these regulators at a critical threshold concentration, providing both stability and memory. Another cellular memory mechanism involves autocatalytic calcium/calmodulin-dependent protein kinases, which have been proposed as effective mediators of long-term storage by virtue of their multishubunit holoenzyme structure (115). The extracellular matrix components secreted by differentiated cell types help to maintain those differentiated states and can instruct other cells to assume novel fates (116a,b). Additional, as yet unidentified, feedback loops are probably required to maintain the differentiated state by preventing proliferation. By circumventing the regulatory hierarchy, such feedback mechanisms limit the number of regulators required and play a central role in maintaining the differentiated state.

Although actively controlled, the differentiated state is stable. Secreted regulators have limited effects due to differences among cells in membrane components, such as the presence or absence of specific receptors. Cells are also relatively impervious to the effects of a single regulator introduced by injection or transfection. The introduction of tissue-specific transcription factors such as pituitary Pit-I (GHF-1) (117, 118), liver HNF-1 (119), and neural MASH-1 (120) into a variety of cell types causes no phenotypic changes and, in general, does not lead to activation of endogenous target genes. MyoD (see above) is the exception to the rule and is remarkable in its pleiotropic effects. However, even in the case of MyoD, high constitutive expression has diverse effects in different cell types. In some cells MyoD induces a heritable phenotypic conversion; in others it activates the transcription of certain muscle target genes; and in a third category of cells it has no detectable effects at all (30, 31, 121). Experiments examining the ectopic expression or misexpression of single regulators in *Drosophila* corroborate these findings. When *Antennapedia* and *Deformed*, proteins that control the identity of thoracic and head segments, respectively, are expressed at aberrant times and developmental stages under the control of the heat shock promoter, a range of effects is observed (114, 122). A homeotic transformation, such as antenna to leg, is seen only at the highest concentrations of *Antennapedia* protein. The partial response or lack of response may be because regulator concentrations are insufficient, essential endogenous cell proteins are lacking, or proteins that interfere with regulator activity are present. Such proteins may complex with, modify, or compete directly with the foreign regulator, thereby altering its activity. Taken together, these experiments suggest that the effects of a regulator on the differentiated state are buffered by the protein composi-
tion of the cell, which in turn is determined by the cell’s heritage or cumulative responses to cues in the course of development. Thus, the differentiated state is stable and is not easily disrupted.

CONCLUSION

Four reasons were presented at the outset that argued in favor of passive control: DNA complexity, the number of regulators, the need for stability and memory, and the risk of unnecessary plasticity. However, as summarized below, active control can satisfy each of these. In addition, active control is advantageous and possibly essential for differentiation.

It appears that we have come full circle in our views of differentiation. Three decades ago Jacob and Monod (1) showed that a balance of positive and negative regulators controlled the expression of structural genes in bacteria. The possibility that similar mechanisms operated to control gene expression in the differentiated cells of higher organisms was questioned when it became apparent that DNA could exist as tightly coiled fibers coated with histones. The discovery of chromatin structure created the expectation that genes were regulated by a different, possibly passive mechanism. In addition, the correlation of newly induced DNase I-hypersensitive sites and of reduced levels of DNA methylation with cell commitment and tissue-specific gene expression was interpreted as indicating that heritable cis-acting regulatory mechanisms played a central role in controlling differential gene expression. Finally, it seemed improbable that large numbers of trans-acting regulators could efficiently regulate gene expression, because the concentration of DNA in eukaryotic cells was so much greater than in prokaryotes. However, as discussed by Ptashne (111) this is unlikely to present a problem. Therefore, the accumulating evidence suggests that the dynamic mechanism proposed by Jacob and Monod (1) for prokaryotic gene regulation may well be the prevalent mechanism used by eukaryotes to control the expression of differentiation-specific genes.

What is the role of chromatin? Although both active and passive forms of control are associated with changes in "chromatin," the passive forms of gene silencing established early in development are likely to differ at the molecular level from the active forms involved later in the course of differentiation. The repression of genes that accompanies X-chromosome inactivation or imprinting is relatively fixed, or permanent. By contrast, the repression of genes typical of differentiation appears plastic and dynamic. Indeed, contrary to previous models (15, 16), it is now clear that in the absence of DNA replication, inactive genes become hypomethylated, nucleosomes are displaced, and DNase-hypersensitive sites are induced (123, 124). These changes in "chromatin," which alter the expression state of tissue-specific
Differences are actively controlled by 1225 genes, which are readily reversible and can all be accounted for by a change in the stoichiometry of trans-acting factors (125). Therefore, the molecular mechanisms underlying the stable, heritable gene repression by "passive chromatin" are unlikely to be the same as those responsible for readily altered gene repression by "active chromatin." Further distinctions await an elucidation of the underlying molecular mechanisms.

Active control provides both memory and stability. The sequential activity of a series, or hierarchy, of regulators leads to the establishment of each distinct differentiated state. However, this progression from totipotent to differentiated cell does not, in general, restrict the range of possible genes that a cell can express, as concepts such as "committed stem cell," "terminally differentiated cell," and "fate maps" might suggest. Therefore, regulators in the hierarchy that act for short periods to establish a differentiated state, like those that act continuously to maintain it, need not lead to permanent changes. Cells do not have to establish passive control in order to remember the effects of a transiently expressed regulator. Feedback loops can achieve the same end (see above).

A major problem of the active-control hypothesis appears to be the large number of regulators required, in particular negative regulators, since at any given time only a small fraction of the total genes of a cell are expressed. A solution to this problem may be the discovery of negative regulatory elements, identified in repetitive DNA sequences, that silence several different genes (see above). Although to date relatively few such repetitive sequences have been identified, they could, in theory, constitute highly effective "global repression-binding sites," through which a number of related tissue-specific genes could be silenced by relatively few negative regulators. For example, it seems likely that related DNA sequences mediate the effects of the Polycomb class of negative regulators, which bind to at least 60 sites within polytene chromosomes at which diverse homeotic or other Polycomb target genes reside (126).

Plasticity of gene expression in "terminally" differentiated cells appears unnecessary, even risky. Why should the differentiated state be controlled by mechanisms that are dynamic and reversible? Perhaps active control is an evolutionary vestige: a single jellyfish cell can generate numerous different cell types and axolotls can regenerate entire limbs. On the other hand, active control may provide essential plasticity. The same regulatory genes are used at different times in development to specify different processes and therefore must be accessible and activatable. An example is provided by the segment polarity gene, engrailed, in Drosophila: engrailed is expressed at two different developmental stages and the regulatory network governing its expression at these two stages differs (127). In addition, differentiation may not be as rigidly determined as it appears. Upon serial transplantation, Drosophila cells
undergo a transdetermination from leg- to head-type cells and injury to amphibian, chicken, and even mammalian tissues can cause a transdifferentiation, or conversion of melanin-producing iris cells to crystallin-producing lens cells (see above). Moreover, in the course of normal development, cells such as those of the neural crest give rise to a multiplicity of unexpected cell types, including representatives of different embryonic germ layers (128). Diverse postnatal myogenic precursor cells randomly fuse with the entire spectrum of fiber types in their vicinity and, once incorporated, adopt the pattern of gene expression characteristic of the host fiber (129). Finally, neural cells implanted into diverse sites within the brain assume the phenotype of their neighbors (130). Experiments of this type, which use sensitive single-cell markers to monitor the fate of the cells following implantation into novel sites, are revealing unexpected degrees of plasticity of cell function. Possibly all gene expression is actively controlled. An analysis of position effect variegation raises the possibility that even in cases in which gene expression is generally regarded as passively controlled, the underlying mechanisms may be active. Position effect variegation shares properties with X-chromosome inactivation: gene inactivity is associated with heterochromatin, a region of the chromosome that is permanently condensed (131a,b). Translocations lead to the inactivation by heterochromatin of genes not normally subject to this type of regulation in species ranging from Drosophila to humans (131). Recent studies have suggested a link between the spreading of heterochromatin in position effect variegation and the expression of the Polycomb family of genes that encodes trans-acting negative regulators. First, the heterochromatin-associated protein HP1 encoded by a member of the Su(var) family responsible for position effect variegation, shares homology with a protein encoded by a member of the Polycomb family (132). Second, the effects of both types of regulation are dose dependent: the size of the domain encompassed in heterochromatin is altered by Su(var) gene dosage (131), and negative regulation of the bithorax complex by the Polycomb gene products depends on the number of copies of that complex (46). These findings raise the possibility that gene regulation in development is entirely active, and apparent differences reflect a continuum, or spectrum, of more or less perturbatable gene states, controlled by different molecular mechanisms.

SUMMARY

The problems posed by differentiation that appear most soluble by a passive control mechanism can readily be solved by an active mechanism. Given the need for plasticity in gene expression in different cell types at different stages, an active mechanism may be advantageous, even essential. It is striking how
few changes during differentiation are completely irreversible, the gene rearrangements leading to immunoglobulin expression being one clear exception. Indeed, a prediction of the active-control hypothesis is that any nucleus exposed to the appropriate constellation of proteins at the appropriate concentration should be able to perform functions typical of any given differentiated cell type. In the next decade, the elucidation of novel memory mechanisms, or feedback loops, will substantially increase our understanding of how stable differentiated states can be maintained by continuous active control.

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