Hierarchies of Regulatory Genes May Specify Mammalian Development

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An intricate network of regulatory circuitry is likely to underlie the development of mammals. One approach to understanding this complex process is to elucidate the steps that commit a cell to specialize for function in a particular tissue. Experiments involving nuclear transplantation, transdetermination, and cell fusion to form heterokaryons provide evidence that genes are neither lost nor irrevocably repressed in the course of cell differentiation (e.g., Gurdon, J Cell Biol 102, 622–640, 1982; Yamada and McDevitt, Dev. Biol. 38, 104–118, 1974; Blau et al., Cell 32, 1171–1180, 1983). Indeed, the differentiated state appears to be plastic, and changes in cytoarchitecture (Miller et al., Genes Dev. 2, 330–340, 1988) and in the expression of tissue-specific genes can be induced by cell fusion, often in the absence of DNA replication (e.g., Wright, JCB 98, 413–435, 1984; Baron and Maniatis, Cell 46, 591–602, 1986; for review, see Blau et al., Science 230, 758–766, 1985). DNA sequences responsible for tissue-specific expression have been identified by transfection of mutant and chimeric genes into cultured cells. The purification of proteins that bind to these sequences is currently the most widely used strategy for cloning genes with a regulatory role in mammalian cell differentiation. While this biochemical approach provides an efficient means for identifying transcription factors, it is not readily applicable to those regulators that act indirectly to control the expression of tissue-specific genes.

An alternative “genetic approach,” used previously to isolate oncogenes and genes encoding well-characterized products such as membrane receptors, has recently been successfully applied toward the identification of genes that regulate mammalian development. In this approach, DNA from a donor cell is transfected into cultured cells; the recipient cells are then assayed for the heritable expression of novel gene products. This approach differs from the classical genetic approach used to isolate regulatory genes in yeast, nematodes and Drosophila in two important respects. First, in the absence of mutants, a mammalian regulatory gene is identified by its ability to confer an altered phenotype and thus is recognized by a gain of function rather than a loss of function. Second, the analysis of gene function is at the level of the cell, not the organism. This is clearly advantageous for studying genes that regulate the development of organisms with a long life cycle. The “genetic” or transfection approach is particularly attractive in that it permits identification of genes encoding regulators that act indirectly by modulating or interacting with transcription factors. In addition, the results are of nonmutual interest, for, as discussed below, they suggest the possibility that the development of a specialized cell type in higher eukaryotes is dictated by a hierarchy, or ordered progression, of regulatory genes.

Using DNA transfection, Schatz and Baltimore (Cell 53, 107–115, 1988) produced a stable line of fibroblasts capable of rearranging and joining immunoglobulin gene segments—a property characteristic of developing lymphocytes. The recipient cells in their experiments were mouse NIH 3T3 fibroblasts, into which a cleverly designed “substrate” immunoglobulin locus had been stably introduced by retroviral infection. Following transfection of human genomic DNA, the authors identified fibroblasts that had rearranged the immunoglobulin sequences of the substrate, and showed that these cells expressed V(D)J recombinase activity at levels typical of lymphocytes at the pre-B cell stage. Thus, the transfected gene presumably encodes a component of the recombinational machinery or a regulator of B cell development.

Other workers have used a transfection approach to identify genes that regulate muscle cell development (Lassar et al., Cell 47, 649–656, 1986; Davis et al., Cell 51, 987–1000, 1987; Pinney et al., Cell, this issue). The basis for these elegant experiments derives from the seminal finding of Taylor and Jones (Cell 17, 771–779, 1979) that a short exposure to 5-azacytidine stably converts a mouse embryonic “fibroblast” cell line, called 10T1/2 cells, into three different mesenchymal cell types—myoblasts, chondrocytes, and adipocytes. Konieczny and Emerson (Cell 38, 791–800, 1984) speculated that the high frequency of conversion to myoblasts resulted from reduced methylat-
10T1/2 cells. Second, constitutive expression of MyoD1 in nonmesodermal cells does not induce the muscle pheno-
type. Since cells of all three embryonic lineages can be
efficiently induced to express muscle genes upon ex-
posure to muscle regulators in heterokaryons (Blau et al.,
Science, op. cit.), this finding suggests that genes in addi-
tion to MyoD1 are involved. Third, expression of MyoD1 is
antagonistic to cell proliferation, which suggests that this
gene acts at a point after cell determination. Determined
cells are capable of division, whereas differentiation and
division are mutually exclusive choices in muscle, as in
simpler organisms such as Myxococcus and Dictyostelium

DNA transfection should prove useful for identifying
other regulatory genes in the hierarchy, but the choice of
the recipient cell is likely to be critical. Some cells may re-
press the transfected gene, whereas others may lack com-
ponents required for the expression of the novel pheno-
type (Land et al., Nature 304, 596–602, 1983). 10T1/2 cells
were particularly well suited to the identification of Myd
and MyoD1. Another fibroblast cell line that is not my-
genic when stably transfected with MyoD1 may be advan-
tageous for identifying genes that act earlier in the path-
way. To isolate genes acting later in the pathway, DNA
transfection could be used to induce the expression of sta-
bly transfected tissue-specific genes. Recent experiments
suggest that the success of this approach will rely on the
prior identification of individual clones of cells in which the
transfected genes are accessible and responsive to regu-
lators (Hardeman et al., JCB 106, 1027–1034, 1988). Ulti-
mately, however, the developmental significance of the
regulatory genes identified in vitro must be assessed in
the intact animal (Kuehn et al., Nature 326, 295–298, 1987;
Thomas and Capacchi, Cell 51, 503–512, 1987)

Like most techniques, the transfection approach has
some inherent drawbacks. One problem is that the trans-
fected DNA is not always readily identified. In the experi-
ments reported by both Schatz and Baltimore (op. cit.) and
Pinney et al. (op. cit.), there was no evidence that the hu-
man DNA originally introduced and detected in the pri-
mary transfectants was present in the secondary transfec-
tants. This not only poses problems for the isolation of the
genes, but, as acknowledged by Schatz and Baltimore, it
also raises the possibility that the gene transferred to sec-
ondary transfectants was not the same as that originally
transferred. A second drawback is that the regulatory
gene assayed may not always correspond to the trans-
fected DNA. As discussed by both sets of authors, there
remains a formal possibility that it is the endogenous gene
at the site of integration, not the transfected gene, that
constitutes the genetic regulatory locus of interest. Inser-
tion of the donor DNA could, for example, activate a
differentiation-promoting locus or inactivate a locus that is
antagonistic to differentiation. Transposon insertions that
either induce or disrupt gene expression are well docu-
mented in other organisms, such as Drosophila.

How do regulatory genes act to control tissue-specific
gene expression? There are precedents for several types
of mechanisms in simpler systems. A hierarchy of regula-
tory genes is characteristic of lyogenic development in
bacteriophages, certain homeotic genes in Drosophila,
vulval development in nematodes, and sex in yeast,
nematodes and Drosophila. In some cases the product of
one gene is known to regulate the next directly; however,
such cascades of transcriptional regulators are not im-
portant in other temporally ordered progressions of gene ex-
pression. Regulators can control gene expression by act-
ing indirectly to alter the splicing of mRNAs or the activity
of transcription factors by posttranslational modifications
such as phosphorylation or ADP-ribosylation. Specificity
of gene transcription can also be achieved by several
regulators acting together; in such combinatorial schemes,
the stoichiometry and interaction of all components rather
than the nature of any given component may be critical.
Each of these mechanisms allows for complex networks
of control by a limited number of tissue-specific regulatory
components.

A combination of regulatory mechanisms is likely to
operate to specify cell types in mammals. To identify such
different types of regulators, different types of assays are
required. Biochemical purification of regulators based on
their affinity for cis-regulatory sequences in tissue-spe-
cific genes is likely to reveal transcription factors. How-
ever, relatively few of the transcription factors purified by
this approach have proven to be tissue-specific (Bodner
and Karin, Cell 50, 267–275, 1987; Scheidereit et al., Cell
51, 783–793, 1987). Indeed, the tissue-specificity of some
factors that appear ubiquitous by DNA binding assays
only becomes apparent in a functional assay such as in
vitro transcription (Mizushima-Sugano and Roeder, PNAS
This finding not only underscores the necessity for func-
tional assays, but also suggests that in some cases the
regulators that bind DNA directly are not those that deter-
mine tissue specificity. Instead, tissue specificity may be
conferred on ubiquitous factors by posttranslational mod-
ifications as demonstrated for bacteria (Ninfa et al., Cell
50, 1039–1046, 1987), or by cooperative interactions be-
tween proteins (Yoshinaga et al., EMBO J. 5, 343–354,
1986; Ma and Hsia, Cell 60, 139–142, 1990; Issa et al.,
Cell 50, 701–709, 1987). Such factors are unlikely to be
identified by DNA binding assays, but could be revealed
by a genetic approach based on DNA transfection. On
the other hand, when tissue specificity is achieved by a com-
binatorial mechanism, a biochemical approach may be
more effective (Yamamoto, Ann. Rev. Genet. 19, 209–252,
Thus, genetic and biochemical approaches should com-
plement one another in the elucidation of mammalian de-
velopmental pathways.