DIFFERENTIATION

Hybrid Responses

from our Cell Biology Correspondent

LAST week I drew attention to the way in which the technique of somatic cell hybridization is being used to establish linkage groups and map human genes. But apart from the genetic analysis of somatic cells, hybridization is proving to be an extremely useful tool for probing the mechanisms of cellular differentiation. It provides the experimenter with a way of putting two genomes—one differentiated, one undifferentiated—into a single nucleus; one can then ask whether a particular differentiated function is maintained in such a situation. Ephrussi and his colleagues were the first to exploit this approach; they found that inter and intraspecific hybrids between pigment-producing melanoma cells and fibroblasts universally fail to make pigment or dopa oxidase and they suggested that the fibroblasts contain something which prevents the continued production of pigment—in other words, that this particular differentiation is "repressed". But how generally valid is this conclusion? Only by collecting catalogues of such examples are we likely to find the answer to that central question.

Schneider and Weiss in Ephrussi's laboratory have apparently added one more case to the list as a result of similar experiments involving a different pair of cells (*Proc. US Nat. Acad. Sci.*, **68**, 127; 1971). A line of rat hepatoma cells exhibits several stable differentiated functions; in particular these cells are characterized by high tyrosine aminotransferase activities which are further increased some four to six-fold when the cells are exposed to the steroid, glucocorticoid hormones. Hybrids obtained by fusing these cells with mouse fibroblasts, however, have only low levels of TAT activity, characteristic of fibroblasts, and the hybrid cells are not susceptible to induction by the glucocorticoids. Both rat and murine tyrosine aminotransferases are present in the hybrid cells, which indicates that the structural genes for the enzyme from both parental cells are maintained. The low activity presumably therefore reflects the absence of expression of other genes which regulate the synthesis of the enzyme and are involved in its induction by hormones. Although they have not rigorously excluded every alternative explanation, that is how Schneider and Weiss interpret their data—another example of the repression of a differentiated function in hybrid cells.

Such "repression" is not, however, an inevitable consequence of hybridization, as Schneider and Weiss note, and in the same issue of the *Proceedings* (*ibid.*, 234) Minna, Nelson, Peacock, Glazer and Nirenberg report an impressive example to add to the opposite page of the catalogue. Clonal lines of murine neuroblastoma cells possess at least ten properties characteristic of differentiated neurones including electrically excitable membranes and acetylcholine receptors. Do any of these survive in a hybrid obtained by fusing neuroblastoma cells with mouse L cells, a fibroblastic cell which responds to electrical stimulation in a way quite distinct from neurones?

The response of the two sorts of parental cells to depolarizing stimuli can be used as markers. The neuroblastoma cells may be passive or give either an A type or a B type response and the L cells give a third, C type, response. (The precise nature of these responses is not essential to this discussion.) Individual cells in six clonal and one uncloned line of hybrids, derived by fusion of neuroblastoma and L cells, were tested by Minna *et al.* In all these hybrid populations cells giving either A, B or C type responses were detected. The C type response was less frequent in hybrid populations than in L cell or L × L hybrid-cell populations, but the A and B type responses were at least as frequent in the hybrid populations as in populations of parental neuroblastoma cells.

Clearly at least some differentiated neuronal functions survive in a cell with a neuronal and fibroblastic genome; indeed it has yet to be excluded that neuronal genomes may induce the neuronal differentiation of fibroblastic genomes. Furthermore, hybridization of normal neuroblasts to L cells may be a way of getting into culture those genes which control different types of neuronal differentiation and by correlating the loss of chromosomes from such hybrids with the loss of neuronal functions it may be possible to determine which chromosomes carry those genes. Nirenberg and his colleagues seem to be on the doorstep of the genetic analysis of nerve cell differentiation.

Controlling Cell Wall Extension

ETHYLENE is unique among the naturally occurring plant growth regulators in having a simple molecular construction and being a gas at normal temperatures and pressures. One of its manifold effects on plant growth is to inhibit cell extension in both roots and shoots allowing the cells to fatten by lateral growth. Drs Irene Ridge and Daphne Osborne at the University of Oxford have looked at the dramatic change in the growth pattern of pea shoots caused by ethylene and in particular have considered whether ethylene controls the growth of plant cells by regulating hydroxylation of certain proteins bound to the cell walls. They report their findings in next Wednesday's *Nature New Biology*.

Ridge and Osborne had previously found that application of ethylene to pea apices caused an increase in the amount of hydroxyproline located in the cell walls. Concomitant with this effect was an increase in the levels of peroxidases found in the cytoplasm as well as covalently and ionically bound to the walls.

The cell walls of higher plants are known to contain proteins rich in hydroxyproline which can cross link with wall polysaccharides, notable arabinose, thereby increasing the strength but limiting the extension of the walls. Hitherto, these proteins were considered to be simply structural and to possess no enzymatic action. By fractionation on DEAE cellulose of a cellulase wall extract containing covalently bound peroxidase as a significant proportion of the hydroxyproline, Ridge and Osborne found that the peroxidase and hydroxyproline components show similar fractionation characteristics. This finding suggests that some of the hydroxyproline-rich wall proteins may be peroxidase.

The peroxidase which is covalently bound to the cell wall differs in two respects from those peroxidases either found in the cytoplasm or just ionically bound to the walls. First, it contains a high concentration of hydroxyproline, and, second, on electrophoresis it separates into isoenzymes which have different properties from the isoenzymes of the other types of peroxidase. From the results of enzyme assays, Ridge and Osborne dismiss the idea that the covalently bound wall peroxidase may act as an additional hydroxylase by converting proline to hydroxyproline in the wall. Ethylene had little effect in the systems tested and they reason that the effect of ethylene on increasing the levels of hydroxyproline in the proteins of pea cell walls is regulated in the cytoplasm, possibly at the level of hydroxylation of specific peptide sequences, the hydroxylated peptides subsequently being transported to the walls.

Ridge and Osborne's article stimulates the question of the possible inter-relationship between ethylene and auxin in the regulation of cell extension. Rayle *et al.* (*Proc. US Nat. Acad. Sci.*, **67**, 1814; 1970) have suggested that the control of rapid cell elongation may be achieved by the degradation of cell wall bonds and it is interesting to speculate that the hydroxyproline–arabinose bond in the cell wall may provide a control site for the action of these hormones. Moreover, another aspect yet to be satisfactorily explained is the control of lateral cell growth in tissues treated with ethylene.