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Correction of genetic defects in vitro – prospects and problems

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Fusion of somatic cells from human individuals with different X-linked enzyme defects has shown that intergenic complementation can take place in hybrid cells (SINISCALCO et al.: Proc. nat. Acad. Sci. [Wash.] *62*, 793 [1969]). However, such hybrid cells possess most or all of the genetic characteristics of both parental cells, including cell surface antigens, and would be rejected immunologically by either of the donors of the parental cells. Thus, if cell fusion techniques are to be used as a means of correcting genetic defects, a system for selectively transferring only the normal gene from a donor cell into a mutant cell would have to be developed. In this direction the system reported by SCHWARTZ et al. (Nature new Biol. *230*, 5 [1971]) seemed to hold promise. These authors believe they have succeeded in incorporating genetic material carrying the gene for the enzyme inosinic-acid-pyrophosphorylase, IMP, from chicken erythrocyte nuclei into heteroploid mouse nuclei of strain A9, which are deficient for this enzyme. Their hybrid cells had chicken-like IMP after growth in selective medium (HAT) but they had no chick cell surface antigens or chick chromosomes. On the basis of these findings these authors suggested that only a very small amount of genetic material had been transferred from the chick cell to the deficient mouse cell. Further investigations by the present author reveals that this system may be more complex. Although some clones of cells were obtained with chick-like enzyme after appropriate fusions are made, other clones had very little enzyme despite their ability to grow in the selective (HAT) medium which contains aminopterin so that only cells which produce IMP should be able to grow in it. In addition, some clones were recovered which have an enzyme whose electrophoretic mobility is much more like that of the mouse than of the chicken. Since no clones able to grow in HAT medium were obtained from control fusions where only mouse A9 cells were fused with each other, or from A9 cells grown only in the presence of the selective medium, it must tentatively be assumed that fusion with a chick nucleus is required. How-

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ever, the ability of the hybrid cell to produce IMP may not in all cases be due to transfer of genetic material from the chicken nucleus to the mouse. Possibly the mouse A9 cell has a mutation affecting the genetic mechanism regulating the IMP gene locus and the cell fusion procedure in some way affects this regulatory mechanism allowing expression of the structural locus. Transduction or transformation phenomena are alternate possibilities.

Although the way in which this "correction" phenomenon occurs still remains obscure, and is under further investigation, the long-range potential of using procedure for correcting genetically defective cells prompted attempts with diploid cells of individuals with the Lesch-Nyhan syndrome. Such cells also lack the ability to produce IMP (also called hypoxanthine-guanine-phosphorybosyl-transferase, HGPRT). Therefore, like A9 cells, they are unable to grow in HAT medium. When they are fused with chick erythrocyte nuclei, some of the hybrid cells can grow in the selective HAT medium and are able to incorporate much more hypoxanthine than they could prior to fusion, but less than normal cells. They possess a low level of IMP which has not yet been adequately characterized electrophoretically. These cells have a normal human chromosome complement and no chick specific cell surface antigens. Thus, the system holds some promise of allowing the correction of a genetic defect *in vitro* but there are many problems which must still be overcome. These include the development of a faster and more reliable means for completing the procedure and the establishment of the genetic stability of the "corrected" cell. In addition, it must still be determined if the cells will be accepted by the body of the mutant individual from whom they were derived, if they will not undergo malignant alteration, and if they will produce enough enzyme to alleviate the metabolic defect. Clearly, much work still remains to be done. If this particular system can be made to work then it may serve as a basis for developing methods for correcting other more frequent genetic defects. Even if this should not prove possible these experiments are providing considerable insight into mechanisms of gene regulation in mammalian cells which may ultimately allow other approaches to the alleviation of hereditary defects.

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