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CONTRACT EE-77-S-02-4328, UNIVERSITY OF COLORADO MEDICAL CENTER

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PROGRESS REPORT

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ERDA Contract #EE-77-S-02-4328

"Genetic Recombination in Mammalian Cells"
in Culture"

Period May 1977 - April 1978

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The objective of this proposal is to show that the process(es) that produces auxotrophic nutritional mutants in Chinese hamster cells includes genetic recombination during mitosis, and which recombination is stimulated by the 3100A light used in the isolation procedure. If these suppositions are true then there are several benefits: genetic recombination during mitosis of mammalian cells in culture will have been unequivocally demonstrated; stimulation of recombination in mammalian cells by a physical agent will have been demonstrated; a method for locating genes on chromosomes and of doing fine structure mapping will be available; the mechanism of mutant formation in diploid cells will be established.

Two general approaches have been made: cytogenetics and the synthesis of a hybrid cell line containing two noncomplementing nutritional mutants, presumably in the same cistron, which can be used to detect crossing over to produce nutritional independence.

The cytogenetic approach has been used in two principal ways: to characterize existing mutants and as a way to study new mutants. In addition it is being used to characterize the parents of the hybrids and the hybrids themselves with a view that prototrophic recombinants selected biochemically will also show recombination cytologically.

1. Mutant characterization cytologically.

The pedigree relationship of the strains under study is as follows:

CHO-K1 pro⁻ser⁺gly⁺

51.11 gly A⁻pro⁻ + Ade A pro⁻ade A⁻

772.56 gly A⁻pro⁻ +

155.25 gly A⁻pro⁻ser⁻ + pro⁺ade A⁻

155.25 pro⁺gly A⁻pro⁺ser⁻

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Cytological study of these hamster cell mutants has been extensive: (1) a standard reference karyotype has been established for the parent CHO-K1; (2) karyotypes for 51.11, 772.56, 155.25, and 155.25 pro^+ have been established. Our model of mutant production by chromosomal recombination yielding homozygosity predicts that these cultures should have different karyotypes with high probability (excepting pro^+ reversions). In fact, these mutants are distinguishable cytologically as predicted. However, as one or two chromosomal rearrangements might be expected at each mutational step (and this may be the case for CHO-K1 $\rightarrow$ 51.11, and 772.56 $\rightarrow$ 155.25) there are an excessive number of differences between CHO-K1 and 772.56. The basis for the increased number of changes between these two strains is not known since the history of the strains is not that complete or clear. It has been indicated that there were some x-ray exposures to the progenitor of 772.56. The question can be handled by considering new mutants whose history is more clear. This is being undertaken. The results thus far, with not the most ideal material, are encouraging.

2. Studies of recombination with hybrid cells.

Hybrids have been selected between 51.11, $\text{ser}^+ \text{gly A}^- \text{pro}^-$ and 155.25, $\text{pro}^+ \text{ser}^- \text{gly A}^-$ using the polyethylene glycol fusion procedure. The gly A mutations are independent and do not complement in the hybrid. Neither parent reverts rapidly to glycine independence (<1 revertant/ 10^6 cells). The gly requiring hybrid which is $\text{pro}^+ \text{ser}^+$ does yield gly^+ spontaneously, which suggests mitotic recombination ($\text{gly A}^- \text{gly A}^{2+} // \text{gly A}^+ \text{gly A}^{2-} \rightarrow ++$) to yield a functional ++ gene. As predicted by the observations on yeast, $\equiv$ where 3100A light has been shown to promote mitotic recombination and gene conversion, irradiation of the glycine requiring hybrid appears to stimulate glycine prototrophic formation. It is now a matter of quantitating the response as a function of light-exposure. Karyotyping of the above hybrid is in progress as well as the glycine independent clones derived from it spontaneously and after light exposure. Hopefully, the $\text{gly}^- \rightarrow \text{gly}^+$ change will be accompanied by a cytological change, which will not only confirm the mitotic recombination basis of the change, but may also indicate the chromosomal location of the gly A gene.