Synthesis and Turnover of Nuclear Proteins

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The functions of the various nuclear proteins are still largely undeciphered, although the activities in which these proteins must play a central role have been broadly defined as the replication and the selective transcription of the genetic apparatus.

Three classes of nuclear proteins are frequently defined by extraction procedures, i.e. globulins soluble in 0.14 M NaCl, proteins soluble in dilute inorganic acids (histones), and residual or insoluble proteins. Information and hypotheses on the functions of these classes have suggested that such enzymes as nucleoside and nucleotide kinases, DNA polymerase, etc., contribute to the globulins, that histones are involved in control of gene transcription, and that residual proteins have sometimes been equated with structural proteins of the chromosome. The concept of structural proteins is, incidentally, not easily defended. There is no clear evidence that the chromosome contains structural proteins or that the chromosome in its essence is composed of anything except DNA.

Except for a few enzymes, the information on function of strictly nuclear proteins is relatively nonspecific. This applies as well to the recent evidence (1, 2) for a role of histones in regulation of genetic transcription.

From a cytological point of view proteins have sometimes been classified by

their location in nucleoli, chromosomes, or nuclear sap. Although at least some histones and RNA polymerase, for example, are bound to chromosomes, and sRNA methylase is apparently present in nucleoli (3), such data correlating cytological structure with biochemical analysis are still scarce.

The experiments described in this paper were attempts to answer two general questions about nuclear proteins: (a) Are there nuclear proteins which do not show complete turnover (turnover in this case defined as breakdown of the protein or its temporary or permanent exit from the nucleus)?; and (b) is the synthesis of histones restricted to the period of their net accumulation in the nucleus during DNA synthesis, or are histones synthesized during other parts of the cell cycle?

Turnover of Protein in Chinese Hamster Chromosomes

Two years ago one of us (D. P.) and M. A. Bender (4) completed studies on isotope labeling of chromosomal proteins in Chinese hamster cells in culture and the results, with some more recent findings, were as follows. Cells were labeled with a mixture of tritiated amino acids for 8 hr. Colchicine was added to such labeled cultures according to a schedule that would allow collection of metaphase chromosomes in the first, second, and third mitoses after the protein-labeling period. In order to assess by autoradiography the amount of radioactive protein present, metaphase chromosomes were isolated and freed of all cytoplasm
Fig. 1. An autoradiograph of chromosomes isolated at the first metaphase after labeling with tritiated amino acids.

Fig. 2. An autoradiograph of chromosomes isolated at the second metaphase after labeling with tritiated amino acids.

by an acetic acid method. Incorporation of tritiated amino acids into proteins that remained associated with metaphase chromosomes was always light, and long autoradiographic exposure times were required.

At the first metaphase after labeling, all chromosomes had radioactive protein associated with both chromatids (Fig. 1). The result is similar to that obtained following tritiated thymidine labeling of DNA, even to the extent that occasionally the short arm of the X chromosome (in an XY cell line) failed to have radioactive protein. This would suggest that a major part, at least, of the new protein becomes