GENETICS

Synthesis of Ribonucleic Acid by Human Chromosomes and a Possible Mechanism of its Repression

IT has recently been suggested that a genetic basis for cellular differentiation in multicellular organisms might be reflected in the distribution pattern of inactivated deoxyribonucleic acid (DNA) in the chromosomes[1]. Inactivated DNA is characterized by three features, namely, heterochromatinization, late replication at the time of doubling, and inactivity of synthesis of ribonucleic acid (RNA). To substantiate this hypothesis, we are now studying the pattern of DNA and RNA synthesis of chromosomes in human cells of various origin by means of autoradiography. In studying the synthesis of RNA by human chromosomes, we encountered a dilemma. The chromosomes are identifiable only in metaphase as are most somatic cell chromosomes in multicellular organisms. During metaphase and anaphase, however, synthesis of RNA completely ceases in all the cells of multicellular organisms so far investigated[2-7]. It is not, therefore, feasible at present to study the pulse labelling pattern of RNA on metazoan chromosomes except polytenic ones. Previous investigators[6,8] have studied RNA synthesis mainly in meiotic chromosomes as these chromosomes remain visible throughout the meiotic cycle. However, they are not chromosomes of differentiated somatic cells in which we are interested. Hsu reported synthesis of RNA in intermitotic nuclei of mammalian somatic cells[9], in which the Barr body was shown to be inactive in the synthesis of RNA. In general, however, the chromosome is not identifiable as a distinct morphological unit in the intermitotic phase of metazoan cells. To overcome this dilemma, we decided to label the cell just before division and examine the distribution of the label at metaphase which immediately follows the labelling. The interval between the labelling and the examination was chosen so as to be sufficiently short to prevent secondary diffusion of the labelled RNA from the site of the synthesis. If the labelled RNA is not released immediately after its synthesis and does persist during mitosis, it would be expected that the label incorporated at late *G2* and prophase would also remain detectable on the metaphase chromosomes.

Leucocytes, from peripheral blood of a normal woman, were collected after addition of phytohaemagglutinin (Difco). They were cultured for 72 h in a medium (tissue culture medium 199, Difco), with penicillin and streptomycin, and squash preparations were made at the termination of the culture. Uridine-^{3}H or thymidine-^{3}H was introduced (0·5 μc./ml.) 3 or 6 h, respectively, before the cells were fixed. All the cultures were subjected to colchicine treatment (10^{-6} M) during the last 3 h. The specimens were stained with acetic acid-orcein, washed for 10–12 h in running water and autoradiographed by dipping them into Kodak '*NTB*-3' emulsion.

In autoradiographs labelled with thymidine-^{3}H, we found metaphase chromosomes with heavy labelling on one of the *X*-chromosomes, identifying this as the late replicating *X*-chromosome. In contrast, however, in metaphase chromosomes labelled with uridine-^{3}H, the *X*-chromosome was free from radioactivity (Fig. 1) while all the other chromosomes were detectably labelled. Structures other than chromosomes showed little labelling. This finding indicates that (1) RNA that is synthesized on the chromosome just before division is not released from the site of synthesis during mitosis, and (2) one of the *X*-chromosomes is inactive in RNA synthesis. The results not only demonstrate the feasibility of this method for investigating the distribution of genetically active and inactive loci in metazoan chromosomes, but also suggest a possible mechanism of genetic repression. In the autoradiographs, it was shown that RNA attaches

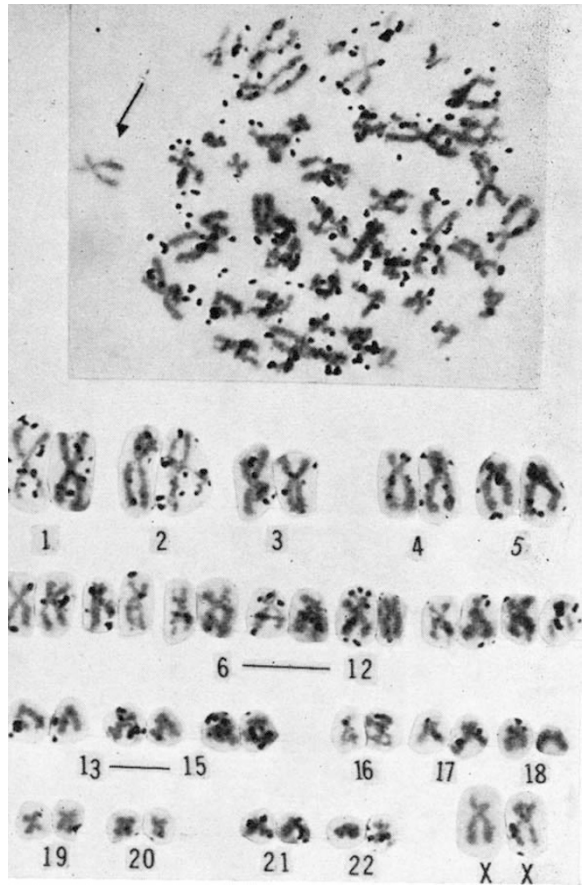

Fig. 1. Autoradiograph of human chromosome, 3 h after introduction of uridine-^{3}H. The cells, derived from the peripheral blood of a woman, were cultured for 72 h. The arrow indicates the genetically inactive *X*-chromosome

firmly to the metaphase chromosomes that are completely repressed in RNA synthesis. Since the turnover rate of RNA synthesis on the chromosome should be regulated both by the rate of RNA synthesis and the rate of removal of the products from the template, failure or suppression of removal of RNA from the template DNA should be followed by repression of RNA synthesis. It is possible to test experimentally the presence of this mechanism in differential inactivation of genes, by introducing a labelled precursor of RNA just before or at the time of gene inactivation and examining at later stages the persistence of the label at the inactivated loci of the chromosomes. If RNA masks the template DNA at the inactivated loci, it should be detectable by means of autoradiography. Experiments are now in progress with the view of substantiating this hypothesis.

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