Review Articles

The Relationship between DNA Replication and Chromosome Structure

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Summary. The results obtained by acridine orange staining of chromosomes, after BrdU treatment, during one or two cell cycles, are described. The alterations of chromosome structure do not depend only on BrdU incorporation into DNA. Some other mechanisms are necessarily involved, and it is postulated that they are disturbances of protein-DNA association, occurring in G1 and in S- or G2-phase. The aspect of metaphase chromosomes then appears as the result of several metabolic steps, all occurring during interphase.

BrdU (5-bromodeoxyuridine) has been widely used in human cytogenetics, since Palmer (1970) and Zakharov et al. (1971) induced chromosomal alterations following the incorporation of this analogue of thymidine.

With the use of fluorochromes, such as acridine orange (Dutrillaux et al., 1973) or dye 33 258 Hoechst (Latt, 1973), acting on mitoses previously treated with BrdU, rapid progress has been made in chromosome analysis.

In this paper, I would like to summarize the main results obtained with BrdU treatment and acridine orange staining, in order to draw some conclusions about the structure of mitotic chromosomes.

Material and Methods

BrdU was added to human lymphocyte cultures, at the final concentration of 1—200 μg/ml. The time of treatment was increased from 2 h to several days. For the discontinuous treatment, the medium containing BrdU was replaced by a fresh one containing thymidine. In some experiments, FrdU (5-fluorodeoxyuridine) was added, at the final concentration of 10 μg/ml. Acridine orange staining was performed according to Couturier et al. (1973).

Results and Discussion

BrdU Treatment during the Last Cell Cycle

Two distinct modifications, often occurring simultaneously, may be induced: a loss or a delay of condensation of some chromosomal segments (segmentation),
Fig. 1. Alterations of chromatids in relation to time of treatment with BrdU during the last cell cycle: \( R \) = locations of the R-bands, \( Q \) = locations of the Q-bands, \( g \) = green fluorescence, \( o \) = orange fluorescence. Y-axis: amount of DNA, X-axis: phases of cell cycle. Horizontal lines indicate time of treatment.

and a modification of the staining with acridine orange which becomes orange instead of green.

The poorly condensed segments frequently emit an orange fluorescence, but they represent only a small part of the segments which may become orange after treatment with BrdU.

The different modifications observed, in relation to the presence of BrdU during a presumed time of the cell cycle, are summarized in Figure 1.

The following different inferences can be made:

1. Treatment in \( G_2 \) phase does not induce any alteration.
2. Treatment at the end of the S-phase alters some G-bands, which are elongated and orange. These G-bands are those whose DNA replicates the latest. These results are in agreement with autoradiography (Ganner and Evans, 1971).
3. Treatment covering the entire late S-phase modifies all the G-bands, which emit an orange fluorescence. Their elongation is not particularly strong. At this moment, the chromosomes have a nearly typical R-banding.