Satellite Association Frequency and Number of Nucleoli Depend on Cell Cycle Duration and NOR-Activity

Studies on First, Second, and Third Mitoses of Lymphocyte Cultures

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Summary. In human lymphocyte cultures the frequencies of satellite associations in first, second, and third mitoses were investigated using the BUDR-method. A marked decrease of the association frequency with increasing numbers of cell cycles was found. The number of nucleoli seen in interphase is correlated with the satellite association frequency in the respective metaphase. Satellite association is positively correlated to Ag-staining intensity of the NORs. Individual differences in satellite associations are due to differences in NOR activity and in lymphocyte activation. BUDR diminishes somewhat the Ag-staining intensity of the NORs but has no effect on satellite association frequencies. The main reason for the decrease of satellite association frequency in second and third lymphocyte mitoses is presumably a certain dislocation of the original chromosome position during mitosis and a decreased possibility of association during the short interphases. The high association frequency in first mitosis resembles the chromosome position in the long interphase of G₀-lymphocytes.

Introduction

In metaphase figures of human cells the short arms of the acrocentric chromosomes bearing the nucleolus organizer regions (NOR) are often found in association, the so called satellite association (Ferguson-Smith and Handmaker, 1961). Ferguson-Smith (1964) suggested that this is caused by the formation of one
nucleolus by more than one NOR bearing chromosome and that the position of these chromosomes is preserved in the following metaphase. The frequency of satellite associations has been studied in numerous papers (e.g. Cohen and Shaw, 1967; Prokofiewa-Belgovskaya et al., 1968; Zang and Back, 1968; Rosenkranz and Fleck, 1969; Patil and Lubs, 1971; Cooke, 1972; Zankl and Zang, 1974; Mattei et al., 1976) revealing sometimes random and sometimes non-random participation of the different acrocentric chromosomes as well as individual differences. Schmid et al. (1974) showed, that distinct individual differences do exist, which are correlated to individually different sizes of the secondary constrictions and thus individually different NOR-activities. Similar findings were reported by Phillips (1975) who also carried out family studies, and by Hayata et al. (1977). Evans et al. (1974) and Warburton et al. (1976) using the DNA-RNA in situ hybridisation method found also that in general NORs containing larger amounts of rDNA are more frequently involved in satellite associations. Miller et al. (1977) using the Ag-method for staining active NORs (Goodpasture and Bloom, 1976) were able to show that in human lymphocyte cultures the frequency of satellite association is positively correlated to the strength of the Ag-stain which is in turn also dependent on the activity of the NOR (e.g. Miller, 1976a, 1976b). The known individual differences in the number of active NORs (e.g. Bloom and Goodpasture, 1976; Mikelsaar et al., 1977; Varley, 1977) may account for some of the differing results on satellite associations reported in previous papers. There are, however, also reports of differences in satellite association frequencies between cultures of different tissues, such as lymphocytes and fibroblasts (Higurashi and Conen, 1971). In one of our previous studies we could not find, however, any striking differences in the patterns of Ag-stainability of the NORs between cultured lymphocytes and fibroblasts from the same individuals (Mikelsaar and Schwarzacher, 1978). Differences in the frequency of satellite association may therefore also be caused by factors other than NOR activity. Important in this respect are observations on the number of nucleoli in differentiated cells. Nerve cells, for instance, which have ceased to divide show in most instances only one large nucleolus whereas dividing undifferentiated nerve cells contain multiple small nucleoli (see Lavelle and Lavelle, 1970; Breuer, 1978). Another important observation is the decrease in the number of nucleoli with time during the cell cycle (e.g. Schnedl and Schnedl, 1970). These observations suggest that the length of time a cell spends in interphase may influence the association of NOR chromosomes.

In lymphocyte cultures from peripheral blood most of the cells which go into first mitosis after stimulation by phytohaemagglutinin have been in a very long interphase as compared to the short cell cycles which will follow. It had indeed been reported that the satellite association frequency is higher in one day old lymphocyte cultures than in cultures kept for three and four days (Nankin, 1970; Mattevi and Salzano, 1975). In the present paper this problem was investigated in more detail by recording the satellite association frequencies in cultured lymphocytes in first and subsequent mitoses, using the BUDR-method (Latt, 1974). Also the nucleoli and the Ag-staining intensity of the individual acrocentric chromosomes identified by Q-bandimg were studied.