Isolated Metaphase Chromosomes Stabilized by DNA-Intercalation or Polyamine Addition: a Comparison.

Interest in the isolation of metaphase chromosomes has increased by the recent application of flow cytometry to chromosome suspensions to detect aberrations or to sort specific chromosomes. A most critical aspect of chromosome isolation procedures is the stabilization of chromosomes upon release from the cells. A recent procedure includes addition of polyamines to the surrounding medium for stabilization. In a very simple procedure, we use DNA-intercalators for the same purpose. Here, we compare the morphological and biochemical characteristics of chromosomes stabilized by these methods, as well as their suitability for flow karyotyping. In this study, the DNA-intercalator was propidium iodide.

The intercalated chromosomes were much longer that those stabilized by polyamines. Length measurements of a specific chromosome after fixation and air-drying resulted in a 43% difference. This less condensed appearance of the intercalated chromosomes did certainly not detract from their suitability for flow karyotyping. Irrespective of the isolation procedure, a very good resolution of peaks in the flow histogram was obtained. DNA extracted from samples of chromosomes isolated by each of both methods and subsequently electrophoresed in agarose appeared to be about 100 kb long. Patterns of chromosomal proteins resulting after SDS-polyacrylamide electrophoresis, fixation, and silver staining were very similar with the exception of a few specific bands. The propidium iodide-chromosomes gave an extra band at about MW 27,000 which was absent in the pattern from the polyamine chromosomes, whereas the latter gave extra bands at Mws 33,000 and 52,000.

In conclusion, both methods are equally suitable for the isolation of metaphase chromosomes to be used for biochemical studies. Although they can also both be applied very successfully for flow karyotyping, the intercalation method should be preferred because—in contrast to the polyamine method—it has been shown to be compatible with banding of the chromosomes allowing their direct identification.

As early as 1897 Sherrington proposed that the tiny knobs (the 'synapses') in the place where neurons contact other neurons were specialized sites of communication. Later it was appreciated that this communication occurred through the release of a chemical substance (the neurotransmitter) via which the membrane potential of the postsynaptic structure was influenced. Therefore, it is not surprising that a great deal of effort has been put into identifying the contents of the myriads of neurons in the CNS. Besides the well-known amines, aminoacids and acetylcholine, a still growing number of peptides is added to the list of putative neurotransmitters. Apart from the site of synthesis (the cell body) no difference exists between peptides and classical neurotransmitters in localization, release and action on the postsynaptic membrane. It is proposed that functions of the hypothalamic peptides as transmitters in the CNS may be related to their function as a hormone in the periphery.