Identification of Human Chromosomes by DNA-Binding Fluorescent Agents

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Abstract. The distribution of DNA along metaphase chromosomes that are not excessively contracted can be visualized in the fluorescence microscope with the aid of fluorescent DNA-binding agents. Additional, characteristic details in the fluorescence patterns are obtained with fluorochromes that bind preferentially to certain chromosomal regions. The highly fluorescent alkylating agent quinacrine mustard (QM) effects discrete, fluorescent labeling of both plant and mammalian metaphase chromosomes, presumably by selective binding to guanine residues in DNA, and is also capable of intercalation in the DNA double helix. Chromosome regions fluorescing particularly strongly with QM have been demonstrated in human metaphase chromosomes 3, 13—15 and Y.

A convenient measuring technique has been developed for the rapid and accurate recording of fluorescence patterns in human metaphase chromosomes. These photoelectric recordings of the fluorescence patterns contain far greater detail than can be seen by the human eye.

The fluorescence patterns described are based on measurements of about 1,000 human metaphase chromosomes. This new technique of determining fluorescence patterns in human chromosomes should be particularly valuable for the identification of chromosomes 4—5 and the individual types in the 6—12 group. Individual, typical patterns also occur within the groups 13—15, 17—18, and 21—22.

Introduction

Characteristic fluorescence patterns are produced by quinaeine mustard (QM) in metaphase chromosomes from Vicia faba, Trillium erectum, Scilla sibirica, and certain other plants because QM binds with different intensities to heterochromatic and euchromatic regions (Caspersson, Farber, Foley, Kudynowski, Modest, Simonsson, Wagh and Zech, 1968; Caspersson, Zech, Modest, Foley, Wagh and Simonsson, 1969a and b). Comparisons of the DNA distribution patterns obtained with a specially designed high-resolution UV-microrocptidesgraphic technique (Carlson, Caspersson, Foley, Kudynowski, Lomakka, Simonsson and Sören, 1963) and the fluorescence intensities in different chromosome parts measured with a special high-resolution fluorometer (Caspersson, Zech, Modest, Foley, Wagh and Simonsson, 1969a) show that the ratio of fluorescence intensity per unit of DNA between heterochromatic and euchromatic chromosome regions varies from 1.5 to 2.5.
Chromosome regions fluorescing strongly with QM have been demonstrated in human metaphase chromosomes 3, 13—15 and Y (Caspersson, Zech and Johansson, 1970), while some of the other human chromosomes also appear to display small regions with a preferential binding of the fluorescent substance.

Similar observations have been made in plant chromosomes with other DNA-binding fluorescent substances, particularly those which intercalate in the double helix (Caspersson, Zech, Modest, Foley, Wagh and Simonsson, 1969:a, b). However, the patterns obtained with QM are much clearer than those obtained with the other fluorochromes studied.

**Determinants of QM Distribution in Metaphase Chromosomes**

Quinacrine mustard binds to DNA in two ways: (a) through the alkylating group, which reacts primarily with DNA-guanine (Caspersson.