The Identification of Human Chromosomes by Fluorescence Techniques

By LORE ZECH. (*Institute for Medical Cell Research and Genetics, Karolinska Institutet, Stockholm, Sweden*)

Various fluorochromes that react with DNA have been shown to bind differentially to metaphase chromosomes from several species of plants and animals (Caspersson *et al.* 1968). Chromosomes that have been treated with such substances show a clear pattern of light and dark cross-striation in the fluorescence microscope. The clearest patterns have been obtained with the bifunctional alkylating agent quinacrine mustard. The mode of binding of this substance to the chromosomes is not yet completely clear. First, it binds specifically to N-7 of guanine. The distribution of quinacrine mustard in the metaphase chromosome is thus influenced by the base distribution along the chromosome. Secondly, the quinacrine group can intercalate in the double helix of DNA and therefore its binding along the chromosome must be influenced by the distribution of DNA in different chromosome parts. Thirdly, the accessibility of the DNA seems to vary along the chromosome, presumably because of differences in the protein linked to the DNA (Caspersson *et al.* 1969). The complex mode of binding is of advantage as the patterns of distribution of the fluorescence along the chromosome have proved to be remarkably reproducible. This is evident from observations that now have been made on chromosomes from quite a large number of plant and animal species. In mammals, especially in man, interest has concentrated on the possibilities afforded by fluorescence techniques for the identification of different chromosome types.

Until recently it has been possible to identify in human metaphases only six autosomes and the Y chromosome on morphological grounds. Some further autosomes and the female X chromosome could be identified by radioautography. The fluorescence method with quinacrine mustard has made it possible to identify all the mitotic chromosomes (Caspersson, Zech, Johansson & Modest, 1970; Caspersson, Lomakka & Zech, 1971).

Visual observation of quinacrine mustard-stained metaphase plates is sufficient for chromosome identification. For a more detailed investigation and for study of structural aberrations, however, it is of value to measure the distribution of fluorescence intensities along the chromosomes photoelectrically. This is preferably done in photographic prints from the fluorescence microscope, as this minimizes the risk of quenching of the fluorescence during the measurement. An instrument for this purpose is described.

For routine work and investigation of a large

amount of material the evaluation of photographic prints is laborious and time-consuming, and therefore a television technique that allows contrast enhancement on the television screen can be of advantage. Those studies can be made either directly on the object in the fluorescence microscope or on a photographic negative. A further advantage of the television technique is the possibility of using a so-called 'line selector', which provides an instantaneous trace on an oscilloscope of a transmittance curve along the length of a chromosome. This tracing corresponds to the fluorescence patterns obtained in the reflexion photometer and can be useful for statistical analysis of the fluorescence patterns (Caspersson, Lindsten, Lomakka, Wallman & Zech, 1970).

Recently the fluorescence technique has been used for the study of chromosomal aberrations. Thus we have investigated a number of different translocations and deletions, and also the chromosomes from patients with Down's syndrome, chronic myelocytic leukaemia, 'cri du chat' syndrome and Turner's syndrome, from XX males and from XY/XO mosaics. In all cases studied so far the chromosomes involved in the aberrations had not changed their characteristic fluorescence patterns (for references see Caspersson & Zech, 1971).

The constancy and reproducibility of the fluorescence patterns of mitotic chromosomes led us to consider the meiotic chromosomes. Until recently only bivalent 9 and the XY bivalent could be identified on morphological grounds. However, staining of meiotic chromosomes with quinacrine mustard made it possible to identify some of the bivalents directly with certainty by their specific patterns (Pearson & Bobrow, 1970; Caspersson, Hultén, Lindsten & Zech, 1971) and others could be referred indirectly to their group. Detailed study proved that in favourable cells all bivalents could be identified if a large enough number of cells was studied. This is of importance for the investigation of the number and the localization of chiasmata on individual bivalents and the estimation of genetic crossing over.

Recently we have used the fluorescence technique for investigation of the chromosomes from hybrid cells between the mouse fibroblast line A9 and human cells from an established Burkitt line. Study of the parental cells showed that the chromosomes from the human cells did not exhibit any exceptions compared with previously investigated normal human chromosomes. The A9-cell chromosomes demonstrated a clear fluorescence pattern that was distinctly different from the pattern of the human chromosomes. Thus it was possible to distinguish between the mouse chromosomes and the human chromosomes in the hybrid cells by means of their

fluorescence patterns (Caspersson, Zech, Harris, Wiener & Klein, 1971).

The work was supported by the Swedish Natural Research Council.

Caspersson, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U. & Zech, L. (1968). *Expl Cell Res.* **49**, 219.

Caspersson, T., Hultén, M., Lindsten, J. & Zech, L. (1971). *Hereditas*, **67**, 147.

Caspersson, T., Lindsten, J., Lomakka, G., Wallman, H. & Zech, L. (1970). *Expl Cell Res.* **63**, 477.

Caspersson, T., Lomakka, G. & Zech, L. (1971). *Hereditas*, **67**, 89.

Caspersson, T. & Zech, L. (1971). *Proc. Conf. Fluorescent Labeling and Identification of Human Chromosomes, Colorado Springs* (in the Press).

Caspersson, T., Zech, L., Harris, H., Wiener, F. & Klein, G. (1971). *Expl Cell Res.* **65**, 475.

Caspersson, T., Zech, L., Johansson, C. & Modest, E. J. (1970). *Chromosoma*, **30**, 215.

Caspersson, T., Zech, L., Modest, E. J., Foley, F. E., Wagh, U. & Zech, L. (1969). *Expl Cell Res.* **58**, 141.

Pearson, P. L. & Bobrow, M. (1970). *Nature, Lond.*, **226**, 959.

Somatic Hybridization as a Tool for the Study of Differentiation: a Review

By M. NABHOLZ. (*Genetics Laboratory, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.*)

Somatic hybridization is rapidly establishing itself as the equivalent of bacterial mating systems in metazoan cell biology. In recent years several groups have successfully used human-mouse hybrid cells to demonstrate linkages between human genes and to allocate such genes to their chromosomes. Another field in which somatic hybridization seems to be destined to become a major tool of enquiry is the investigation of the mechanisms controlling the expression of differentiated functions. In general, the problem has been approached by crossing an 'undifferentiated' cell line with one that, derived from a 'differentiated' tumour, expresses some of the specific characteristics of the

tissue of origin. A number of systems have been studied in this manner, including high activities and inducibility of certain enzymes, the synthesis of certain tissue-specific products and the electrical membrane properties of neurons (Davidson & Yamamoto, 1968; Davidson & Benda, 1970; Schneider & Weiss, 1971; Minna, Nelson, Peacock, Glazer & Nierenberg, 1971; Bloom, Choi & Lamb, 1971). The same approach has been used to investigate virus-mediated transformation (Basilico & Wang, 1971) and malignancy (Harris, Miller, Klein, Worst & Tachibana, 1969). It is concluded that the expression of differentiated functions in hybrids may depend, not only on the genetic make-up of these cells and the mode of regulation studied, but also on the extent to which the latter is intact in the 'differentiated' parent and the tissue of origin of the 'undifferentiated' parent. Recent experiments have demonstrated the possibility of studying the control mechanisms and genetics of such properties that are extinguished on hybridization with an 'undifferentiated' cell line through the use of human-mouse hybrid cells (Klebe, Chen & Ruddle, 1970). Such hybrids may re-express the differentiated function on loss of certain human chromosomes. If it is possible to cross diploid mouse cells isolated directly from their tissue with heteroploid human cell lines and if such cells lose human chromosomes, the latter approach may be greatly expanded.

Basilico, C. & Wang, R. (1971). *Nature, Lond.*, **230**, 105.

Bloom, A. D., Choi, K. W. & Lamb, B. J. (1971). *Science, N.Y.*, **172**, 382.

Davidson, R. & Yamamoto, K. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 894.

Davidson, R. L. & Benda, P. (1970). *Proc. natn. Acad. Sci. U.S.A.* **67**, 1870.

Harris, H., Miller, O. J., Klein, G., Worst, P. & Tachibana, T. (1969). *Nature, Lond.*, **223**, 363.

Klebe, R. J., Chen, T. & Ruddle, F. H. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 1220.

Minna, J., Nelson, P., Peacock, J., Glazer, D. & Nierenberg, M. (1971). *Proc. natn. Acad. Sci. U.S.A.* **68**, 234.

Schneider, J. A. & Weiss, M. C. (1971). *Proc. natn. Acad. Sci. U.S.A.* **68**, 127.

STEROID BIOCHEMISTRY GROUP COLLOQUIUM ON 'STEROIDS AND CHROMATIN'

Chromatin Structure

By B. M. RICHARDS, J. F. PARDON and A. S. POOLEY. (*Searle Research Laboratories, High Wycombe, Bucks., U.K.*)

The complexity of chromosome structure in eukaryotes has been one of the obstacles to extrapolation from prokaryotes of knowledge on mechanisms of transcription and replication. In

recent years new impetus to work on chromatin structure has resulted from a better understanding of histone structure and the molecular structure of the deoxyribonucleohistone complex. This, together with continuing studies on both the structure of chromatin as seen by electron microscopy and the function of chromatin as revealed by studies *in vitro* with cell-free preparations, has produced some