Developments in Laboratory Diagnosis of Chromosomal Abnormalities

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ABSTRACT

The development of chromosome banding techniques has made possible the detailed analysis of human chromosomes. A vast body of knowledge has accumulated during the last few years as a result of their application in biology and medicine.

Introduction

The demonstration by Caspersson et al.\(^1\) that quinacrine mustard-stained human chromosomes showed a distinctive and reproducible pattern of fluorescence which allowed the identification of each individual chromosome opened a new era in cytogenetics.

Previous techniques were based only on morphologic characteristics of the chromosomes, such as size, position of the centromere and presence of secondary constrictions. Seven groups (A to G) could thus be recognized, but only four of the 22 autosomal pairs (1, 2, 3 and 16) and the Y chromosome could be readily identified.\(^2\) With the aid of autoradiography\(^3\) additional chromosomes could be distinguished: those in group B (4 and 5), D (13, 14 and 15) and E (16, 17 and 18) and the late replicating X. However, its use was restricted almost exclusively to the study of selected chromosomal abnormalities and for investigational purposes because it was too time-consuming and laborious for routine use in chromosomal identification.

It is not surprising, therefore, that the observations of Caspersson and his group were received with great enthusiasm. Their report awakened the interest of cytogeneticists to investigate other techniques for the differential staining of human chromosomes and resulted in subsequent years in the development of a number of different methods for chromosome banding.

To standardize the classification of these methodologies, an ad-hoc committee met in Paris in 1971 and adopted a new nomenclature to designate each of these methods.\(^6\) The technique utilizing quinacrine fluorescence was called Q-banding, while the one producing bands by the denaturation-reassociation technique, and assumed to demonstrate "constitutive heterochromatin", was identified as C-banding. The designation of G-banding was reserved for those methods using Giemsa or related stains following various prestaining treatments of the chromosomes, with the exception of the procedure which utilizes controlled heat treatment. Since this technique produces patterns of bands which are the reverse of the Q- and G-bands, it was called R-banding. Regions and bands within each chromosome were given specific numbers for

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precise identification and a revised system for the notation of structural chromosomal abnormalities by breakage points and band composition was recommended.

**Q-Banding**

Following the observation of distinct banding patterns on plant metaphase chromosomes stained with DNA reacting fluorochromes, Caspersson et al showed that quinacrine mustard (QM), an acridine derivative, enabled the detection of specific banding patterns in human metaphase chromosomes when visualized under fluorescent microscopy. These patterns proved to be different for each one of the 22 pairs of autosomes as well as for the X and Y, thus allowing for easy and unambiguous identification of all chromosomes in the human complement (figure 1).

In addition to the regular pattern of banding, some metaphase chromosomes have been found to exhibit areas of intense fluorescence. The most prominent of these is a highly fluorescent segment located in the distal portion of the long arm of the Y chromosome. Individual variations, including absence, reduction or increase in the length of this segment and the presence of additional bands of fluorescence appear to have no phenotypic effect.

Of great practical application was the observation by Pearson et al and Caspersson et al that this fluorescent area of the Y chromosome could be visualized also in interphase nuclei as a bright and well-defined spot of fluorescence (figure 2). In addition to having been observed in cells from the oral mucosa and cultured fibroblasts, it has been demonstrated in a number of other cell types such as hair root sheath cells, circulating blood lymphocytes and polymorphonuclear lymphocytes. Wharton’s jelly, sperms and amniotic fluid cells. The identification of this fluorescent spot, referred to as the Y body or Y chromatin, has
made possible the positive nuclear sexing of male cells in an analogous fashion to the sexing of female cells by the presence of the Barr body.3

Other small polymorphic regions, the pericentric region of number 3, the juxta-pericentric band in the long arm of number 4 and bright satellites or short arms of the D- and G-group chromosomes, have been described.13,16,17,58,75 Since these variations are heritable they can be useful in tracing the origin of specific chromosomes in trisomies or triploidies,46,53,68 as well as in linkage studies and forensic medicine.47

Initially it was thought that the basis of Q-bandng was an alkylation of deoxyribonucleic acid (DNA) and, more specifically, attachment to the N7 atom of guanine.11 When it was later shown that nonalkylating fluorochromes, such as quinacrine dihydrochloride, could produce identical banding patterns, an intercalation of the acridine nucleus in the double helix of guanine plus cytosine (G+C)-rich DNA regions was postulated. However, Ellison and Barr35 have demonstrated that fluorescent segments on Samoania leonensis, a drosophiloid fly, are characterized by an extremely high adenine plus tyrosine (A+T) content and virtually no G+C. This and in vitro studies59,84 showing that DNA with a high A+T content enhance fluorescence, while G+C-rich DNA has a quenching effect, indicate a strong base specificity in these dyes. It should be mentioned, in addition, that DNAase abolishes Q-bands, whereas removal of RNA or histones has no effect on the banding pattern.22,27

C-Banding

This method, which depicts preferentially the areas of constitutive heterochromatin within the chromosomes (figure 3), is based on the in situ DNA/RNA hybridization procedure developed by Pardue and Gall.60 Most techniques2,34,54,78,79,87 produce darkly stained areas in the juxta-centromeric regions of each chromosome, in the secondary constriction of chromosomes 1, 9 and 16, and the long arm of the Y chromosome. Modifications of these procedures which stain heterochromatic regions in some additional chromosomes6,39 have resulted in the identification of a high degree of polymorphism of these areas.24

Though a useful tool in research20 and in the investigation of specific chromosomal aberrations, the C-banding techniques have very limited application for purposes of practical chromosome identification.

G-Banding

G-bands can be produced by a number of techniques, all of which involve the use of Giemsa or related stains following different types of pretreatments. The banding pattern thus obtained closely corresponds to the Q-bands. Staining of the centromeric regions, secondary constrictions and distal end of the Y chromosome, similar to the C-bands, can be seen in addition to the G-bands when methods derived from the in situ DNA/RNA hybridization procedure60 are employed.31,73,79

The most widely used Giemsa banding techniques37,51,76,83 are based on the pronase digestion method described by Dutrilleaux et al in 1971.33 Exposure of fixed metaphase preparations to proteolytic enzymes prior to
staining produces the characteristic G-banding pattern seen in figure 4.

A great number of modifications can induce a similar pattern of banding. These include: the use of different substances in the pretreatment of chromosomes, such as urea, cesium chloride, and anionic and nonanionic detergents; the alkalinization of the Giemsa stain; and the addition of hydroxyurea, actinomycin D or tetracyclines to the cell cultures prior to harvesting.

It is intriguing to consider how such a distinct and reproducible pattern of banding can be obtained by so many different and varied methods. It has been proposed that the various prestaining treatments remove the chromosomal histones. This would make more phosphate groups in DNA available for dye binding. An opposite point of view has been held by Daniel who postulated that protein in densely-packed heterochromatic regions would be more inaccessible to the action of pretreatment, and that Giemsa would stain the protein remaining at A+T regions after this procedure.

R-Banding

Reverse banding of the chromosomes, that is, the production of bands which are the opposite to the Q- and G-bands, can be obtained by a Giemsa method and by fluorescent techniques. The Giemsa method, which involves controlled heat denaturation of fixed chromosomes in a solution of predetermined pH, was first described by Dutrilleaux and Lejeune. All chromosome pairs can be easily identified and the telomeric regions of the chromatids appear clearly stained. This makes the technique extremely suitable for the detection of minor terminal deletions. The fluorescent techniques usually involve acridine orange
staining preceded by heat denaturation\textsuperscript{5,28} or adjustment of the pH.\textsuperscript{23}

Conclusion

It is important to emphasize that all these techniques rather than being alternatives complement each other in the study of chromosomal abnormalities. Their capability of permitting the identification of each individual chromosome and of regions within them has given impetus to their use in medicine and biology.

A vast body of knowledge has accumulated during the last few years as a result of the application of the banding techniques to the study of patients. The exact chromosomal basis of previously identified syndromes has been confirmed beyond doubt;\textsuperscript{9} new syndromes have been identified;\textsuperscript{18,29,36} the recognition of chromosomal rearrangements has greatly improved;\textsuperscript{8,40} and, in some instances, established and accepted knowledge has given way to newer interpretations. Worthy of mention is the case of the Philadelphia (Ph\textsubscript{1}) chromosome, long believed to be a deletion of the long arm of chromosome 21. Its presence in patients with chronic myelogenous leukemia together with the known association of Down syndrome and leukemia had been the basis for many speculations. These have been shattered by the recognition that the Ph\textsubscript{1} chromosome is not number 21 but chromosome 22.\textsuperscript{57} Further, it has been demonstrated that it results from a 22 to 9 translocation.\textsuperscript{30,70}

Knowledge of the structure and mechanics of chromosomes has been greatly enhanced by the use of the banding techniques, but perhaps their major impact in biology has resulted from their application to chromosome mapping in cell hybridiza-
tion. To date, approximately 50 gene markers have been assigned to chromosomes in man by these methods.\textsuperscript{62}

References


