Q-Banding ☆

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Glossary

Alkylating agent Alkylating agents involve reactions with guanine in DNA. These drugs add methyl or other alkyl groups onto molecules where they do not belong. This in turn inhibits their correct utilization by base-pairing and causes a miscoding of DNA.

Aneuploidy The occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or any chromosome number that is not an exact multiple of the haploid number.

Chromosome An organized package of DNA found in the nucleus of the cell. Different organisms have different numbers of chromosomes. Humans have 23 pairs of chromosomes – 22 pairs of numbered chromosomes, called autosomes, and 1 pair of sex chromosomes, X and Y.

Q-bands Fluorescent (under UV light) bands and chromosomes produced by quinacrine staining. Q-bands are brightest in A–T-rich regions.

Introduction

In 1968, the description of the first heterogeneous banding technique of chromosome based on staining with quinacrine dihydrochloride (Q-banding) was a revolution on cytogenetics. The development of this fluorescent banding technique and its application to human chromosomes have revealed extensive longitudinal differentiation of chromosomes. Q-banding allows the precise identification of the different chromosome pairs and also the identification of structural chromosome rearrangements, such as duplications/deletions, peri- and paracentric inversions, and aneuploidy (chromosome monosmy or trisomy), hard to individuate by means of standard techniques. This unique banding technique made possible the identification of all human chromosomes.

Mechanism of Q-Banding

Q-banding is technically among the simplest banding technique. Fluorescent Q-banding is produced simply as a result of staining, with no other treatment necessary. Q-bandings can be recognized by a yellow fluorescence of differing intensity resulting after treatment of the chromosomes with quinacrine mustard (QM) or quinacrine fluorochromes. Since their introduction as banding agents for chromosomes, there has been a vast mass of literature on the mechanism by which QM and quinacrine fluorochromes produce Q-bands and on the factors affecting the fluorescence. A subsequent interest lies in employing the findings of fluorochrome–DNA interactions to further elucidate the chemical structure of metaphase chromosomes.

QM, an alkylating agent, initially synthesized by E. J. Modest at Boston, gives highly specific banding patterns, particularly of human chromosomes. Various causes have been ascribed to the occurrence of such chromosome bands. First, it has been suggested that it is regarded to bind DNA both through the alkylating group reacting primarily with the guanine content of DNA and by intercalation of the quinacrine group in the double helix of DNA. The pattern of fluorescence exhibited by different segments of a chromosome stained with QM is the result of irregularities in DNA distribution and also by a superimposed pattern due to strong QM binding in particular locations. The amount of QM binding each segment is controlled by steric relations between DNA and chromosomal proteins. In the human karyotype, the pattern of QM binding is ~98% of the length of total metaphase chromosomes, shows very constant and reproducible banding patterns, and is capable of identifying the segments. Some of the smallest bands used in identification, for example, in chromosome 19 of human karyotype, contain the order of 10^{-15} g DNA, corresponding to about 10^6 nucleotide pairs or 100–1000 genes.

However, the discovery that other fluorochomes, like quinacrine dihydrochloride (Q) and ethidium bromide, which lack the alkylating group, can induce similar bands as QM soon led to the abandonment of the hypothesis that the selective binding of QM to the N^7 atom of guanine is responsible for specific banding patterns. Other studies, specifically in vitro studies of quinacrine–polynucleotide complexes, suggest that quinacrine (Q) fluorescence is enhanced by polymers containing adenine (A) and thymine (T), while polymers containing guanine (G) and cytosine (C), or just G, quenched it. Thus strong fluorescence with QM reflects the presence of DNA with high A–T content. Furthermore, the use of photooxidation, which is a treatment believed to preferentially remove guanine (G) residues from chromosomal DNA, allows an increase of Q-staining intensity, supporting the hypothesized primary role of DNA base composition, and specifically that of G interspersion, in determining Q fluorescence response in fixed eukaryotic chromosomes.

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Since the application of Q-banding to chromosomes of species with regions of known base composition, specifically centromeric satellite DNA of mice (*Mus musculus* and *Mus cervicolor*), this explanation is unlikely. In fact, differing patterns of Q-banding in the centromeric regions of these species was explained, first, as a quenching of fluorescence by G in spite of a high proportion of A + T. However, as several studies demonstrated that satellite DNAs contain regions consisting exclusively of several A–T pairs, quinacrine fluorescence of nuclei and chromosomes have been suggested to be greatly influenced by proteins and their interactions with DNA. This postulation was supported when experiments show that, in various species, DNA fractions differing in base composition show similar levels of fluorescence with quinacrine; however, they show differential fluorescence in intact chromosomes or in isolated chromatin fractions. Also it was demonstrated that nuclei show different level of quinacrine fluorescence when they pass from G1 phase to S phase of the cell cycle, although the amount of DNA remains constant. All these experiments indicate that it is not the amount or quality of DNA that determines the level of quinacrine fluorescence, but the state of the chromatin as a whole, that is, the association of DNA with proteins (eg, histone). Moreover, X-ray microanalysis showed that quinacrine binds uniformly throughout the length of chromosomes which confirms that Q-bands result from differential excitation or quenching of fluorescence.

Clearly, the mechanism of Q-banding is far from understood. One may say with confidence, however, that the proteins of chromatin and chromosomes have a clear influence on the quinacrine fluorescence of nuclei and chromosomes, and that at present the evidence that DNA base composition is an important factor in determining Q-banding patterns is tenuous.

Application of Q-Banding

Plant

Although numerous karyotypic studies have been carried out since 1939 in several plant species such as maize, tomato, and rice, the usefulness of standard staining procedures has been limited. Standard staining procedures have facilitated the ascertainment of chromosome numbers and gross morphological features; however, they have not permitted an accurate and unequivocal identification of all the chromosomes of a species because the chromosomes were too small and/or similar in morphology. This led to the introduction of Q-banding in 1968 as the first banding technique. The use of dyes such as quinacrine and QM produced unique patterns of brightly fluorescent regions alternating with nonfluorescent (dark) regions that were produced in each chromosome of *Cricetulus griseus*, *Vicia faba*, and *Trillium erectum*, permitting an identification of all the chromosomes and their homologs. Q-banding has been used successfully to different plant species *Trillium*, *Scilla*, *Allium*, *Crepis*, *Lilium*, *Secale*, and *Vicia*.

Q-banding has been employed to discover the disposition of heterochromatin/repetitive DNA in several species of the genus Crinum and to show that the quantity of heterochromatin is less in cytologically stable species than in hybrid species.

Human

Q-banding was later extended first to human, and even to other animal systems, with modifications. In 1970, the first human banded chromosomes were produced by this technique.

At the Paris conference (1971), Q-bands visualized in human metaphase karyotypes permitted the assignment of a new nomenclature for "arms" of a metaphase chromatid in which letters p and q represent, respectively, the short and long; these arms are then subdivided by numbers. Also new terms have recommended such "variant" in situations where deviations from the norm of chromosome morphology are observed and "heteromorphic" to describe the chromosomes with variable bands. Since then, human chromosome studies become a major field in the biomedical sciences. The development of Q-banding has facilitated the basic research and clinical applications. Several studies have presented their occurrence, distribution, nature, clinical significance, and applications. Clinical applications include the association of chromosome disorders with numerous diseases, including cancers. Furthermore, Q-bands have been shown to be very useful for "population cytogenetics" studies. In fact, Q-banding adds more polymorphic sites to the list of human chromosomal polymorphism with the bright fluorescence in different chromosomes (3, 4,13 14, 21 22, Y). This feature can be found in either the homozygous state or the heterozygous state. Because of difference in frequencies among populations with respect to Q-banding patterns, this may prove to be a valuable taxonomic feature in ethnic anthropology.

Several studies reported the selective value of chromosomal heterochromatin material, of polymorphic Q variants, in the adaptation of human populations to certain extreme environmental factors, particularly to cold and hypoxia.

Q-bands were also compared between sexes. These studies showed an increase of the mean number of Q-bands in females per individual. This highlighted the role of the Y chromosome capable of "compensating" for the large Q-band of the chromosome Y which is present only in the genome of males. This technique was helpful in rapidly determining genotypic sex, in screening for X–Y or Y-autosome translocation and in evaluating a large number of cells when there is concern that a sex chromosome mosaic may have a Y-bearing cell line.

Animal

Advances in Q-banding has been successful in establishing more accurately the degree of genetic homology between closely related species (Primate: *Pan troglodytes*, *Gorilla gorilla gorilla*, *Macaca mulatta*, *Papio sphinx*, *Cercocebus galeritus*, and *Cercocebus torquatus*) and

in determining the structural changes that have taken place at the chromosomal level during evolution leading, in some cases, to the formation of new species through reproductive isolation. In other cases, however, structural chromosome changes are completely independent of the speciation process.

The study of several amphibian, reptile, and fish species showed peculiar results. In fact, it was difficult of obtain multiple euchromatin banding patterns along numerous reptile, fish, and amphibian chromosomes. It has thus, for example, not been possible to induce Q-bands in the chromosomes of the eel *Anguilla anguilla*. For the clawed toad *Xenopus laevis*, chromosomes were uniformly fluorescents following Q-banding. However, all authors agree that Q-banding patterns hitherto demonstrated in a few fishes and amphibians are of the same quality as those obtained under identical experimental conditions in warm-blooded vertebrates. Thus, it was possible to resolve several phylogenetic questions using Q-banding (*Bufo viridis*) or to reveal heteromorphism within species (*Bufo mauritanicus*).

The absence of multiple banding patterns in the euchromatin of amphibians was originally attributed to the strong spiralization of their chromosomes at metaphase. It was concluded from this that the distances between individual bands in amphibian chromosomes are probably too small to be resolved by microscopy. However, later studies, using bromodeoxyuridine (5-bromo-2′-deoxyuridine) (BrdU) replication patterns, suggest that the absence of Q-bands in chromosomes of heterothermal vertebrates might be correlated with the low intermolecular heterogeneity of composition in the long sequences, referred to as "isochores." In fact, different studies demonstrate that the genomes of the homeothermic mammals and birds are strongly compartmentalized by base composition and clearly suggest that the isochores could correspond to the DNA sequences in Q-bands.

Finally the use of modified technique for chromosome preparations coupled to robust modern automatic classification methods proved to be particularly useful to analysis Q-banded metaphase spreads.

Further Reading

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