

Chapter 1

Molecular Cytogenetic Applications in Diagnostics and Research: An Overview

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1.1 Introduction

The development of normal (Caspersson et al. 1968, 1971) and “high-resolution” banding of chromosomes (Yunis et al. 1983) made it possible to identify chromosomal anomalies like deletions, duplications, inversions and translocations with a resolution down to about 5×10^6 base pairs, and this enabled the diagnosis of a number of chromosome defects. However, in a number of situations, chromosome aberrations are too small or too complex to be fully diagnosed by banding techniques. Therefore, more sensitive and more refined techniques are sometimes necessary. This need has been met to some extent through the development of in situ hybridization (ISH) techniques. In addition to refining the banding technique, ISH is the only method that can simultaneously give information at both molecular and cellular levels, namely by visualizing DNA sequences on chromosomes and in cells and tissue sections, thereby enabling specific nucleic acid sequences to be visualized in their natural biological microenvironment. As a consequence, ISH has found a number of applications in clinical diagnosis and research.

Gall and Pardue (1969) and John et al. (1969) were the first to (independently) use ISH. Both groups used radioactively labeled single-stranded DNA or complementary RNA as probes and obtained hybridization of denatured cytological preparations. Hybridization was visualized as silver grains after autoradiographic exposure.

In 1981 Langer (Langer et al. 1981) introduced biotinylated nucleotides and the use of labeling with nick translation, where dTTP was replaced with its biotinylated analog. Hybridization was subsequently visualized through the binding of fluorescently labeled avidin or streptavidin. This improvement was of major importance in the subsequent introduction of ISH into routine practice, and this technique is now termed *fluorescence in situ hybridization* (FISH).

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After its initial development, the sensitivity of FISH gradually increased through the development of sandwich techniques based on antibodies and the conjugation of other chemically well-known reporter molecules (e.g., other haptens, fluorochromes, enzymes, colloidal gold particles, etc. (Luke and Shepelsky 1998; Csaki et al. 2007). The introduction of haptens as labels also resulted in the development of multicolored hybridizations (Schröck et al. 1996; Speicher et al. 1996).

At the same time there was a marked improvement in optical systems. For instance, epi-illumination fluorescent, reflection contrast and confocal laser scanning microscopes were developed (Nederlof et al. 1992). Cameras and specific filters for identifying fluorochromes that are not visible to the human eye were also brought into use (for more on filters, see Chap. 8 of this book).

Parallel to the development of FISH (Pinkel et al. 1986a, b), a number of similar techniques were devised, such as primed in situ labeling (PRINS; Koch et al. 1989, 1995), comparative genomic hybridization (CGH; Kallioniemi et al. 1992), reverse chromosome banding (RCP; Carter et al. 1992) and chromosomal band-specific staining (Liehr et al. 2006; see also Chap. 22 of this book).

In the present chapter we will review the set of in situ techniques available—and in-situ-related techniques—in a more general manner from the viewpoint of applications, while the following chapters of this book are then devoted to a more detailed presentation of a number of these FISH techniques and how to perform them in detail. Initially, we will try to identify the different types of problems, especially those encountered in clinical diagnosis, which can be solved through the use of in situ techniques. Subsequently, we will briefly mention a number of examples of applications—within both clinical diagnosis and research—where these techniques can offer extra information.

1.2 FISH Techniques in Clinical Diagnosis

In general, in situ techniques are used within the area of diagnostics in order to demonstrate abnormalities in gross organization or in the localization of endogenous or exogenous DNA or RNA molecules that are causing—or are at least associated with—human disease. In the following sections, endogenous DNA, endogenous RNA and exogenous nucleic acids will be considered separately.

1.2.1 Endogenous DNA

1.2.1.1 The Endogenous DNA Target

When studying man and higher animals, the term “natural DNA targets” refers to nuclear and chromosomal DNA, and to a modest degree mitochondrial DNA. However, since the latter is rarely studied in situ, this section will focus on nuclear

and chromosomal DNA. In both cases, the DNA in the cell has a very high molecular weight and is very tightly packed due to the coiling of the DNA around nucleoproteins, forming chromatin. The difference between nuclear DNA and chromosomal DNA mainly comes down to the macro structure; during mitosis, the genome of the cell is split into a number of chromosomes, which then can be observed individually. However, in terms of detailed DNA structure and accessibility, the differences between chromosomes and nuclei are minor. The dense packing of endogenous DNA is especially problematic when performing FISH analyses on histological sections, where specific DNA targets (chromosome territories and their sub-regions) are studied in the context of functional tissue organization. Here, pretreatment with heat or protease is necessary to unmask the target DNA and achieve efficient penetration of reagents into the nuclei. At the same time, close attention should, however, be paid to the preservation of nuclear morphology (Chin et al. 2003).

Apart from the high degree of condensation of the DNA, this material is rather straightforward to work with as a target. This DNA is extremely stable, which yields a number of practical advantages. Furthermore, the number of copies of DNA in the cell is rather predictable. For autosomal chromosomes there are normally two copies, and while there may be some extra copies or losses for cancer cells, in general there will be a limited number of copies, simplifying FISH analyses.

1.2.1.2 Disease-Associated Aberrations in Endogenous DNA

The genome serves to store the genetic information that encodes all cellular functions. Even though species may appear completely different at a phenotypic level, the basic DNA code and the mechanisms of transcribing the code are extremely well conserved across them (Cromie et al. 2001; Lander et al. 2001). In all organisms, the cellular DNA is constantly exposed to mutational events that can cause alterations in the DNA sequence. At the single-cell level, these mutations sometimes cause disruption of a function, but in many cases mutations are neutral to the cell. This is due to the fact that most (by far) of the human nuclear DNA does not code for proteins. Some of these noncoding DNA sequences evidently have other functions, but there are major parts of the DNA where a minor sequence variation will have no functional effect. In the following section we will mainly concentrate on situations where DNA sequence alterations have deleterious effects on cellular functions.

In general, *mutations* initially occur in only one cell. Therefore, in order to be detected and perhaps cause disease, it is necessary that the mutated cell expands into a larger cell population. At the individual level, this *expansion* can occur either in connection with embryonic development or in connection with tumor formation.

Expansion in connection with embryonic development is what is seen in *inherited diseases*. Here the fertilized egg contains a deleterious aberration that is transmitted to all somatic cells of the individual during its development. This results

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