Molecular Analysis of the Fragile X Syndrome

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Summary: The molecular analysis of human X-linked disease has progressed rapidly over the last few years owing to advances in power of mapping techniques. Physical DNA maps covering more than 5 million base pairs have been constructed for several chromosomal regions. Many of these regions have now also been cloned into overlapping cosmid and YAC contigs facilitating the search for disease genes. The recent identification of the mutation in the fragile X syndrome is such an example of the power of YAC technology in the characterization of human genetic disease mutations.

Several genes mutated in X-linked disorders have been mapped genetically to very small regions of the X chromosome. The first genetic disease to be studied using DNA markers which identify restriction fragment length polymorphisms was Duchenne muscular dystrophy (DMD; Davies et al 1983). The DMD gene was subsequently identified by taking advantage of patient deletions and translocations (Hoffmann et al 1987; Koenig et al 1988; see Worton et al this volume). The genetic map of the X chromosome is now based on highly informative markers, which is greatly facilitating the mapping of rarer X-linked disorders (see Davies et al 1991). Notable disease genes that have been identified by cloning sequences based upon their map positions (positional cloning) are the genes responsible in choroideraemia (Cremers et al 1990), chronic granulomatous disease (Royer-Pokora et al 1990), X-linked spinal muscular dystrophy (La Spada et al 1991) and the fragile X syndrome (Oberle et al 1990; Verkerk et al 1991; Yu et al 1991).

This review will concentrate on an approach to the cloning of the region of DNA involved in fragile X syndrome as an example which illustrates the power of genetic disease analysis using current methodologies. In addition, the fragile X syndrome displays a novel genetic mechanism of mutation generation which may occur at several other loci elsewhere in the human genome.

Fragile X Syndrome

The fragile X syndrome is the commonest inherited cause of mental retardation affecting 1 in 1500 males (for review, see Fryns 1989). Fifty-six per cent of female carriers of the mutation are not retarded, while 20% of males are phenotypically

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normal transmitters of the disorder (so-called normal transmitting males) (Sherman et al 1984, 1985). The disorder is associated with the expression of a folate-sensitive fragile site at Xq27.3 (FRAXA), the level of expression of which can vary from a few per cent to as high as 50% (Lubs 1969; Sutherland 1977). Daughters of normal transmitting males are not retarded and either do not express the fragile site or only express the fragile site at very low levels. This can make genetic counselling of individuals at risk particularly difficult. Although closely linked genetic markers have been developed (Richards et al 1991b, Rousseau et al 1991; Suthers et al 1991), their use is sometimes limited because the mode of inheritance (through the male or female line) is unclear.

The unusual inheritance pattern observed in the fragile X syndrome has led to the hypothesis that the development of the full phenotype involves a two-step process (Pembrey et al 1985; Laird et al 1987; Nussbaum et al 1987). The first non-phenotypic, or premutation, event is converted into the full mutation only after passing through oogenesis. Several hypotheses have been proposed as to the nature of this second mutation event, including the genomic imprinting or amplification of DNA sequences at the fragile site.

MICRODISSECTION OF THE FRAGILE X REGION

Approaches to the isolation of sequences at or close to the fragile site have included the mapping of sequences picked at random from chromosome-specific libraries and the isolation of Alu-PCR products from reduced somatic cell hybrids containing only small regions of the X chromosome (Suthers et al 1990; Rousseau et al 1991). One of the most efficient ways of targeting a chromosomal region containing a disease locus of interest is microdissection (Ludecke et al 1989; MacKinnon et al 1990; Senger et al 1990). As little as 20 million base pairs of DNA can be selectively cloned. In this approach the chromosomal region is excised from the chromosome using a micromanipulator and needle. For the Xq27 fragile site library, 20 normal X chromosomes were microdissected, corresponding to approximately 15 fg (10^{-15} gm) of DNA, and the DNA was extracted, digested with a restriction enzyme and ligated into a plasmid vector. Oligonucleotide primers were then used to amplify this small amount of DNA over a million-fold by the polymerase chain reaction (PCR). The resulting DNA was recloned and propagated in Escherichia coli. In this way, a library of more than 20000 clones corresponding to the region around the fragile site, FRAXA, was isolated.

Individual microdissection clones were isolated and localized into intervals within Xq27 by the use of somatic cell hybrid interval mapping (Hirst et al 1991a). Using this technique, 43 new DNA markers were localized in defined positions of Xq27. A typical somatic cell hybrid panel showing the localization of six microclones in the intervals immediately adjacent to the fragile X region is shown in Figure 1.

ISOLATION OF YAC CONTIGS ACROSS THE FRAGILE SITE

Two microclones, M759 and M749, were shown by the hybrid panel to map in the same intervals as markers showing very close genetic linkage to the fragile site (Hirst