January 1993
Appearing monthly

Vol. 285, No. 1

ISSN 0027-5107
MUREAV 285 (1) 1–144 (1993)

MUTATION RESEARCH

International journal on mutagenesis, chromosome breakage and related subjects

Fundamental and Molecular Mechanisms of Mutagenesis

Special Issue

In Memory of Max Clark,

US mailing notice — Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis (ISSN 0027-5107) is published monthly by Elsevier Science Publishers (Molenwerf 1, P.O. Box 211, 1000 AE Amsterdam, The Netherlands). Annual subscription price in the U.S.A. US$ 1624.00 (subject to change), including air speed delivery. Second class postage paid at Jamaica, NY 11431.

USA POSTMASTERS: Send address changes to *Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis*, Publications Expediting, Inc., 200 Meacham Avenue, Elmont, NY 11003.

Airfreight and mailing in the U.S.A. by Publications Expediting.

PRINTED IN THE NETHERLANDS

Mutation Research, 285 (1993) 125–144

MUT 00047

Current methods of mutation detection

R.G.H. Cotton

Olive Miller Laboratory, Murdoch Institute, Royal Children's Hospital, Flemington Road, Parkville, Vic. 3052, Australia

(Received 3 July 1992)
(Revision received 17 July 1992)
(Accepted 24 July 1992)

Keywords: Detection of mutations; Mutation detection; Mismatch; Heteroduplex

Summary

Mutation detection is important in all areas of biology. Detection of unknown mutations can involve sequencing of kilobases of DNA, often in many patients. This has lead to the development of methods to screen DNA for mutations as well as methods to detect previously described mutations. This review discusses current methods used for such purposes with special emphasis on genetic diseases of humans. However, savings can be made by similar means in other areas of biology where repetitive or extensive sequencing for comparative purposes needs to be done. This review covers the methods used for detection of unknown mutations, namely the ribonuclease, denaturing gradient-gel electrophoresis, carbodiimide, chemical cleavage, single-strand conformation polymorphism, heteroduplex and sequencing methods. Once mutations have been defined they can be searched for repeatedly by methods referred to as diagnostic methods. Such methods include allele-specific oligonucleotide hybridization, allele-specific amplification, ligation, primer extension and the artificial introduction of restriction sites. We can now choose from a range of excellent methods, but the choice will usually depend on the background of the laboratory and/or the application in hand. Screening methods are evolving to more satisfactory forms, and the diagnostic methods can be automated to screen whole populations inexpensively.

The rate of identification and characterisation of genes which cause specific inherited diseases in humans is rapidly increasing. Also the numbers of mutations identified in a particular gene as causative in disease are increasing rapidly. This is likely to gain further impetus from the Human Genome Project. Thus methods to detect unknown mutations and previously described mutations are assuming increasing prominence and use. The importance of such studies is enhanced as knowledge of the molecular basis of cancer has increased, given that mutations in oncogenes and tumour suppressor genes are now well-documented as causes of cancer. Changes of such magnitude have not occurred in genetics other than the area of human disease, but nevertheless the methods to be discussed are potentially advantageous in these areas.

Correspondence: Dr. R.G.H. Cotton, Olive Miller Laboratory, Murdoch Institute, Royal Children's Hospital, Flemington Road, Parkville, Vic. 3052, Australia.

There are 3 main areas where such methods are used in human disease, (a) the research laboratory attempting to find mutations in a gene which causes a specific disease, (b) the clinical diagnostic laboratory which needs to look for known and unknown mutations causing a specific disease for prenatal or other diagnostic purposes, as well as polymorphic harmless mutations for linkage studies, and (c) the screening of populations for specific mutations (such as has occurred with Tay Sachs disease and as is beginning to occur in cystic fibrosis).

For this review, methods will be divided into screening methods and diagnostic methods. The former are usually used to detect unknown mutations, but there is an increasing tendency to use screening methods to screen for a number of known mutations together with any unknown mutations in the diagnostic setting (see below) (Fig. 1).

Methods of detecting mutations have been reviewed several times in recent years (Caskey, 1987; Grompe et al., 1989; Rossiter and Caskey, 1990; Cotton, 1989, 1991, 1992). One of these was particularly detailed (Cotton, 1991) and reviewed the area up to the end of 1990 and a subsequent review (Cotton, 1992) is essentially an update of this review to near the end of 1991. The field is evolving so rapidly that frequent reviews are necessary to monitor important new methods and modifications of older methods; it is also important to assess the effectiveness of methods after a time in operation.

This review aims to provide a brief description of the principles and practice of methods available at this time together with their variants, and a discussion of their advantages and disadvantages. Key illustrative applications will be given. For more detail (and further examples) the reader is referred to an earlier review (Cotton, 1991). Only those methods used actively at present or those described in the last few years will be covered. Methods to detect the more obvious deletion/insertion mutations have been covered earlier (Rossiter and Caskey, 1990) and will not be covered here, where detection of point mutations will be emphasized. It should be noted that some of the methods mentioned below will be reviewed in more detail in another issue of *Mutation Research*.

Screening methods

The screening methods can be divided into two types: (a) those simple methods which rely on differences in electrophoretic properties being generated between mutant and wild-type nucleic acid by point mutations (these methods cannot, as currently used, detect all mutations, do not localize them within the fragment, and can only be applied to DNA fragments hundreds of bases long), (b) and another group which includes cleavage methods and the carbodiimide method (which can screen kilobase lengths and localise the mutations to within 10 bases in the fragment examined). The subcategory of chemical methods have the potential to detect *all* mutations. Sequencing is more frequently used to detect unknown mutations than it is for diagnostic purposes.

Ribonuclease cleavage (RNAase)

Many ribonucleases cleave single-stranded RNA after pyrimidine residues. This finding was exploited when it was found that single base-pair mismatches in RNA:RNA heteroduplexes were cleaved by ribonuclease (Freeman and Huang, 1981; Winter et al., 1985) as well as in RNA:DNA heteroduplexes (Myers et al., 1985a).

The method was given considerable impetus when uniformly labelled probes could be conveniently produced as described in 1984 using the SP6 system (Melton et al., 1984). Application of the method directly to unamplified genomic DNA has been reported (Myers et al., 1985a; Kaufman et al., 1990). Cleavage of the DNA to which the cleaved RNA was hybridized is possible via S1 nuclease (Atweh et al., 1988). Cleaved RNA has been detected after transfer to a membrane and hybridization with probe (Genovese et al., 1989).

The main advantage of the method is that it is a simple single-step reaction which locates the mutations within the fragment. This is, however, offset by the fact that special RNA probe has to be prepared and that only about 70% of all mutations are detected (Myers et al., 1985a). This is because when purines appear in the probe at the mismatch most mismatches are not cleaved.

Despite the aforementioned disadvantages the method has been used until the present day. For

example, variation in HIV isolates have been studied (Lopez-Galindez et al., 1991), the pattern generated after digestion by RNAase being indicative of geographical distribution and temporal appearance of resistance to AZT. In addition, a number of mutations in the *apc* gene were identified with ribonuclease (Nishisho et al., 1991). The method has also been applied to the intensively studied p53 gene in tumours and cell lines (Kim et al., 1991).

The fact that around 30% of mutations are missed with this method is a considerable shortcoming, if a simple single-step screening method capable of detecting 100% of mutations becomes available the RNAase method is bound to decrease in both use and value.

Denaturing gradient-gel electrophoresis (DGGE) and related techniques

When double-stranded DNA is electrophoresed into a gradient of increasing denaturant a portion of a given strand separates but the strands are anchored together by the portion (higher melting domain) which has not melted at this point. This split in the duplex suddenly arrests the movement of the molecule in the gel. If a single-base change is present in a similar duplex in the split portion, the denaturant concentration for strand separation is usually different, thus the arrest of movement occurs at a different position in the gel and a mutation can be detected by the differential positions of arrest (Myers et al., 1985b). The difference between the positions of arrest is greater if heteroduplex molecules (between mutant and wild-type) are used. The gel is poured with an increasing gradient of denaturant (formamide) and run at 60°C in a special apparatus needed to keep the temperature constant. The length screened is 50–500 bp and it is possible to use unlabelled DNA.

There has been considerable evolution of the method since it was first described, and also there are a number of variants. Changes have been directed either to increasing the percentage of mutations detected or to simplifying the methodology. The most important modification has been the placing of a high melting point 40-base GC rich sequence (the GC clamp) at one end of the fragment to be screened. Most recently this has been achieved using PCR technology, with special primers being synthesized with a clamp attached (Sheffield et al., 1989, 1992a). This means the whole area to be screened is in a low melting point domain and that "almost all" mutations, instead of about 50%, can be detected. In the practical situation it was found that mutant samples had to be mixed with normal DNA to ensure heteroduplexes were formed in order to ensure detection of a maximal number of mutations (Cai and Kan, 1990; Higuchi et al., 1990). Kilobase lengths of genomic DNA can be screened for polymorphisms (60% of any base changes) by digestion with restriction enzymes, separation by DGGE, blotting onto a membrane and then probing with relevant genes (Gray, 1992).

Further modifications have attempted to avoid the use of the special apparatus altogether. Smith et al. (1988) melted the duplexes in solution containing stepwise increases in denaturant and analysed them by standard polyacrylamide gel electrophoresis. Another variation has been to use a temperature rather than a liquid-denaturant gradient (Rosenbaum and Reissuer, 1987). The most recent modification leading to greater simplicity has been the constant denaturant gel electrophoresis (CDGE) method (Hovig et al., 1991). Here separation is undertaken at that concentration of denaturant which corresponds to that of the melting domain of the fragment being analysed. The authors reported detection of 6 of 7 mutations at a particular locus whereas 3 of 7 were found with conventional DGGE. This low detection rate with conventional DGGE was despite the use of a GC clamp which is rather surprising.

One of the special and important advantages of the above method (and other methods separating intact mutant and wild-type molecules during analysis (see below)) is the fact that mutant molecules can be isolated from gels for further analysis such as sequencing. This feature was exploited in the study of errors during PCR amplification (Keohavong and Thilley, 1989). Other advantages are the fact that it can be used in unlabelled mode, it can be used directly on unamplified genomic DNA, and a result can be obtained in 24 h. A particular disadvantage of almost all variants is that either preliminary ex-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.