CMLS, Cell. Mol. Life Sci. 55 (1999) 1636–1648
1420-682X/99/121636-13 $ 1.50 + 0.20/0
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CMLS Cellular and Molecular Life Sciences

Review

Minisatellite instability and germline mutation

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Received 15 January 1999; received after revision 11 March 1999; accepted 12 March 1999

Abstract. Tandem-repeat DNA actively turns over in the genome by a variety of poorly understood dynamic mechanisms. Minisatellites, a class of tandem repeats, have been shown to cause disease by influencing gene expression, modifying coding sequences within genes or generating fragile sites. There has been recent rapid progress towards understanding molecular turnover processes at human minisatellites. Instability at GC-rich minisatellites appears to involve distinct mutation processes operating in somatic and germline cells. In the germline, complex conversion-like events occur, probably during meiosis. Repeat turnover appears to be controlled by intense recombinational activity in DNA flanking the repeat array, suggesting that minisatellites might evolve as by-products of localised meiotic recombination in the human genome. In contrast, AT-rich minisatellites appear to evolve by intra-allelic processes such as replication slippage. Curiously, minisatellites in other organisms appear to be more stable than their human counterparts, suggesting species-specific differences in turnover processes. Some yeast models display human-like minisatellite turnover processes at meiosis. However, all attempts to transfer human germline instability to transgenic mice have failed. Finally, tandem repeat instability in various species appears to be extremely sensitive to environmental agents such as radiation via a mechanism which remains enigmatic.

Key words. Minisatellite; mutation; instability; recombination; conversion; model; disease; VNTR.

Introduction

Minisatellites are a class of highly variable tandemly repeated sequences that are generally GC rich, with intermingled variant repeat units ranging from 10 to over 100 base pairs (bp) in length, depending on the locus. Various minisatellite-based DNA fingerprinting and DNA profiling systems have been developed [1, 2] and used for forensic purposes, as genetic markers for linkage analysis and to explore population structure [3–7]. While semi-automated PCR analysis of microsatellites has now largely replaced minisatellite-based systems [8], DNA typing of minisatellites still provides powerful and highly discriminating systems, for example in the recent demonstration of the authenticity of the cloned sheep Dolly by DNA fingerprint analysis (fig. 1) [9]. Minisatellite typing remains an important fingerprinting technique for other species where a large number of microsatellite markers have not yet been developed.

Minisatellites can in some cases display remarkably high levels of germline instability [10] and provide extremely informative systems for analysing complex processes of tandem-repeat turnover in the human genome [11–14]. Minisatellite variant repeat mapping by PCR (MVR-PCR) can be used to chart the interspersion patterns of variant repeats along the repeat array, to provide detailed information on allele structure before and after mutation [15]. Detailed studies in humans have provided essential information for understanding

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repeat turnover processes and allowed various mutation models to be developed.

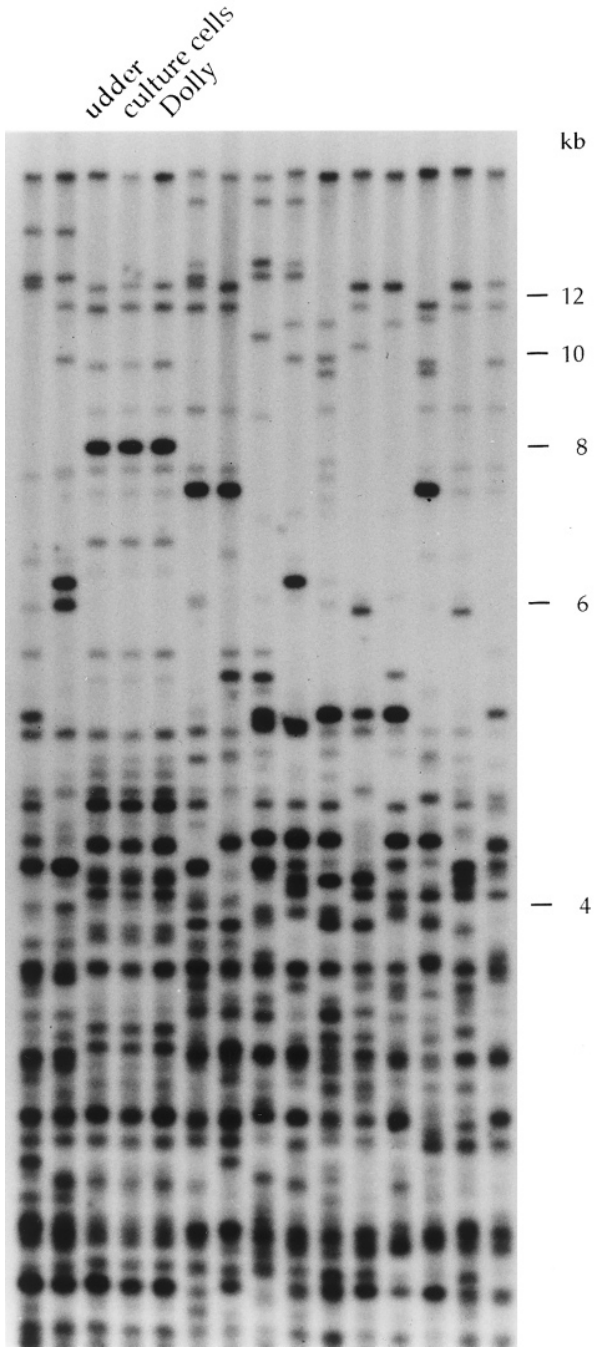

Figure 1. DNA fingerprint analysis of Dolly. DNAs were analysed and compared from the donor udder (udder), the derived cell culture (culture cells) and Dolly's blood (Dolly) and from control sheep (all other samples) (kb, kilobases). Reproduced with permission from Esther Signer.

We now review the influences of minisatellite instability on gene structure and function, plus methods used to detect and analyse de novo mutation in the germline and soma. Turnover mechanisms in other organisms are assessed as well as attempts to model human minisatellite instability processes by transgenic approaches. Finally, we review progress in the use of variable number of tandem repeat (VNTR) loci as a powerful tool for detecting mutation induced by environmental agents such as ionising radiation.

In this review, the term 'minisatellite' is used for loci with GC-rich repeats. Other types of minisatellites (e.g. AT-rich minisatellites) are described as such where appropriate. Unstable mouse 'minisatellites' originally identified by DNA fingerprinting [16–20] are now known to consist of long arrays of short (< 10 bp) repeat units usually sequence homogeneous. These properties distinguish these loci from true minisatellites, and we have therefore renamed them expanded simple tandem-repeat arrays (ESTRs).

Minisatellites and genes

It has long been known that some eukaryote genes contain VNTRs within their coding sequences. It has been suggested that some loci have evolved by concatemerisation of short tandem-repeat sequences to create long open reading frames relatively resistant to point mutations which cause premature chain termination and reading frameshifts [21–26]. Coding minisatellites also have the capacity for rapidly generating new polymorphisms and therefore potential new functions.

Coding minisatellites

A striking example is the human apolipoprotein family. Each member of this family contains a coding minisatellite with a repeat unit of 33 or 66 bp [27]. Apolipoproteins are capable of binding lipids, each being associated with a lipoprotein of specific density [28]. This specificity is due to the variable coding minisatellite region in each gene. Many other genes have been shown to contain a VNTR, such as the human epithelial mucin, involucrin, loricrin and small proline-rich (SPR) genes [29–31] and the D4 dopamine receptor (D4DR) [32, 33]. The coding polymorphisms observed in the latter gene have been shown to affect the ligand-binding affinity of D4DR [33, 34] and are associated with cognitive and emotional disorders, a consequence of the high level of D4DR expression in the limbic areas of the brain [35–39]. Potential functional plasticity is also illustrated by the interferon-inducible gene 6-16 which contains a polymorphic coding minisatellite that

provides multiple splice donor sites [40]. Finally, the prion protein gene contains a 24-bp repeat region coding for an octapeptide. Some inherited forms of prion diseases, including kuru, Creutzfeldt-Jakob disease (CJD), and Gerstmann-Sträussler Scheinker syndrome (GSS), are associated with the amplification of 4–9 extra repeats in the coding repeated region [41]. Deletions of single repeat units have been identified but appear to be polymorphisms and not associated with disease [41]. The biological consequence of these amplifications remains unclear.

Regulating minisatellites

Minisatellite instability can on occasion generate alleles that can disturb the expression of neighbouring genes. The insulin (INS) minisatellite, located 600 bp upstream of the transcriptional start site of the INS gene [42], is likely to be the insulin-dependent diabetes mellitus type 2 (IDDM2) locus [43]. Disease susceptibility appears to arise by different-length VNTR alleles influencing thymic expression, and thus the development of immune tolerance, by transcriptional mechanisms that remain unclear [44]. Similarly, VNTR variants in the vicinity of the H-ras (HRAS1) gene appear to influence the heritable risk of cancers in multiple tissues including colon, breast and bladder [45]. Another VNTR that may influence transcription can be found within the D_H-J_H interval in the human immunoglobulin heavy chain (IGH) locus [46]. The 50-bp consensus sequence of the IGH VNTR contains a sequence similar to the motif binding myc/HLH transcription factor sequence matching a binding site in the adenovirus major late promoter (MLP). Although the IGH VNTR has no enhancer activity, it is able to significantly suppress transcriptional activity of the adenovirus MLP. Another example comes from the correlation between patients affected by progressive myoclonus epilepsy type 1 (EPM1) and expansions at a GC-rich minisatellite in the promoter region of the cystatin B gene (CSTB) implicated in EPM1 [47–49]. A VNTR polymorphism within the second intron of the serotonin transporter gene appears to be associated with susceptibility to major depression [50]. A deletion polymorphism within a second tandem-repeat locus in the promoter region of the same gene [51] is significantly more common in individuals with anxiety-related personality traits such as neuroticism, tension and harm avoidance than in the general population [52]; this deletion appears to be directly responsible for gene dysfunction by reducing levels of transcription.

Closer analysis of all these transcriptional disturbances seems to point to a common process. It appears that for some minisatellites, if not all, their repeat motifs can serve as binding sites for specific transcription factors: Pur-1, rel/NF-κB and myc/HLH for INS, HRAS1 and IGH VNTRs, respectively [44, 46, 53]. The mechanisms responsible for reduced expression of CSTB and the serotonin transporter gene have yet to be elucidated.

Current examples of the association of VNTRs with disease suggest that the isolation of additional human genes associated with minisatellites could provide an important resource for further dissection of complex traits and disease susceptibility. From an evolutionary perspective, it is clear that minisatellite-like sequences played and continue to play an important role in the rapid generation of new and specialised functions [21, 22, 24, 25].

Detecting mutation

Minisatellite mutation generates new-length alleles via the gain or loss of repeat units. Pedigree analysis is the traditional method for detecting germline mutation at human minisatellites [54]. But with mutation rates usually of the order of 1% per gamete, pedigrees are inefficient for detecting mutation and only yield average estimates of mutation rate rather than the detailed information on the mutational behaviour of individual alleles needed for dissecting mutation processes. Most unstable loci show a bias towards mutation in the male germline, though there are exceptions such as MS1 and CEB36 which show similar levels of instability in sperm and oocytes [54, 55]. Studies of somatic instability at minisatellites have proved more problematic due to a very low rate of appearance of new mutant alleles [11, 56, 57]. To circumvent limitations of somatic and germline mutation detection, two different PCR approaches have been developed allowing the detection of new mutant molecules directly in genomic DNA. These methods give access to an unlimited number of somatic and germline (sperm) mutant alleles.

The first approach, small-pool PCR (SP-PCR) [12], involves diluting genomic DNA and amplifying multiple pools each containing typically 100 minisatellite molecules. Mutant molecules can be easily resolved from progenitors by gel electrophoresis and Southern blot hybridisation [58]. Mutation rates can be determined from the number of amplifiable molecules scanned, as estimated by Poisson analysis of single-molecule dilutions of genomic DNA [59, 60]. SP-PCR has been extensively used in sperm mutation analysis [12, 61] and can give reliable quantitative estimates of mutation rates above 10^{-3} per progenitor molecule; measurement of lower rates is impeded by occasional PCR artefacts that interfere with the detection of authentic mutants.

The second approach, size enrichment and SP-PCR (SESP-PCR), involves digesting genomic DNA with a

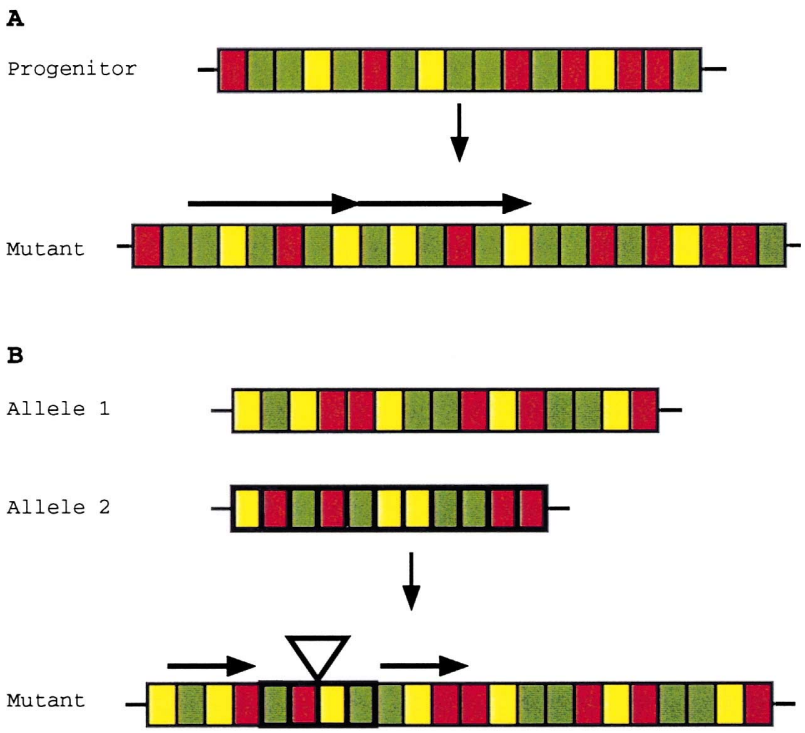

Figure 2. Schematic examples of somatic and germline mutant alleles at human minisatellites. Variant repeats are shown by different colours. Typical examples of mutants are shown (*A*) Somatic mutation with simple duplication in one progenitor allele. (*B*) Complex interallelic conversion in sperm accompanied by a deletion within the transferred DNA segment and duplication around the conversion site in the recipient allele (Allele 2).

restriction enzyme that cuts near the repeat array, followed by gel electrophoresis and recovery of multiple DNA size fractions which are depleted in progenitor allele molecules but which contain abnormal-length mutants that can be detected by SP-PCR [57, 62, 63]. This approach is not only extremely sensitive, allowing detection of mutants as rare as 10^{-6} per cell, but also validates their authenticity by size. However, precise quantitation of mutation rate is not possible due to variation in DNA recovery following gel fractionation [57, 63].

Somatic and germinal mutation processes at GC-rich human minisatellites

Somatic instability

SESP-PCR has enabled rare abnormal-length mutants to be detected, validated and quantitated in somatic (blood) DNA at human minisatellite MS32 [57]. Structural analysis by MVR-PCR revealed simple deletions/duplications of repeat-unit blocks located at random along the tandem-repeat array, with a slight bias towards losses of repeats [57] (fig. 2A). Three different alleles showed somatic (blood) instability occurring at a frequency of $2-7 \times 10^{-5}$ per progenitor molecule. It remains to be seen whether other human tissues show similar frequencies and spectra of somatic mutation. A similar process has been observed at human minisatellite CEB1 (J. Buard, unpublished data), suggesting a common somatic mechanism occurring at human minisatellites. The most plausible mechanisms for somatic instability are unequal sister chromatid exchange (USCE) and intramolecular recombination, though the involvement of replication slippage cannot be excluded [57].

Germinal instability

Minisatellite instability in the germline (sperm) has been extensively characterised at four different human loci (MS205, MS32, B6.7 and CEB1) with average mutation rates of 0.4, 0.8, 4.7 and 9.3% per sperm, respectively [11, 12, 61; K. Tamaki, unpublished data]. Common features are emerging for these loci. First, the level of

instability in sperm is two to three orders of magnitude higher than in blood [57]. This indicates that instability is strongly restricted to the germline. Second, all loci show mutation rate heterogeneity between alleles [11, 12, 61, 62; K. Tamaki, unpublished data]. The most dramatic variation is seen at CEB1 where mutation rates can vary from $< 0.05\%$ up to 25% per sperm per allele [61]. Third, all loci show a bias towards gaining repeats which could explain their recent emergence after the divergence of humans and great apes [64], but raises the issue of what prevents their continuous expansion in the human genome. Preliminary evidence at CEB1 suggests that the longest alleles may become prone to deletions, which could limit their indefinite expansion. For minisatellites CEB1 and B6.7, instability is strongly affected by array size [61; K. Tamaki, unpublished data]; for both loci, instability increases steadily up to 40–50 repeats, above which it appears to reach a plateau [61, 62]. For CEB1, germline stability of short alleles has been shown to be a direct consequence of short array length and not due to internal structural features [61]. Other loci (MS32 and MS205) show no evidence that array length influences mutation rate, suggesting a lack of alleles short enough to lie below the mutation plateau [11, 12].

Structural analysis of germinal mutation

The structural basis of minisatellite instability has been extensively investigated at several minisatellites by MVR-PCR [13–15, 65]. Common features of mutation have emerged. First, all five minisatellites studied (MS31, MS32, MS205, CEB1 and B6.7) show mutation involving gene conversion-like repeat transfers between alleles; mutation is thus recombinational in nature, and almost certainly arises at meiosis. There are, in addition, mutation events apparently restricted to a single allele. The ratio of inter- to intra-allelic events varies between loci and between alleles at a given locus. At minisatellite MS32, 80% of gain mutants in sperm involve interallelic transfers [12]. This rate compares with 75% for MS31 and at least 20% for MS205, respectively [11, 14]. At the hypervariable minisatellite CEB1, the situation is more complex. There is no significant difference in frequencies of interallelic transfer amongst alleles of different length. Instead, it appears that the huge variation in the level of instability between alleles is mainly due to intra-allelic duplication/deletion, which increases in frequency with array length. As a result, the proportion of CEB1 mutants arising from interallelic transfer can vary from 100% for short alleles to 16% or lower for long alleles [61].

Interallelic transfers can be extremely complex, with imperfect duplications of the inserted portion, deletions within the insertion and scrambling of donor repeat segments, as well as duplications or deletions within the recipient allele (fig. 2B) [11, 12, 14, 61]. There is no evidence, however, that new repeat types are created by base substitution. Intra-allelic events usually involve simple duplications and deletions of repeat blocks but can be more complex [11, 12, 14, 61]. Most minisatellites show a polarity of interallelic conversion toward one end of the array. This polarity is strong at minisatellites MS31, MS32 and MS205 but less intense at CEB1 [11, 12, 14, 16]. Polarity suggests that flanking DNA may influence repeat turnover. Further evidence for the role of flanking DNA comes from studies of minisatellite MS32. Alleles associated with a G-to-C transversion 48 bp upstream of the array show reduced variability in human populations [66]. Single-molecule analysis demonstrated a frequently profound reduction in mutation rate at alleles carrying the C variant (O1C polymorphism), with sperm mutation reduced 110-fold compared to G-linked alleles but with no effect on somatic (blood) instability [57]. Sperm mutation suppression acts in *cis*, but does not affect the ability of an allele to act as a sequence donor during gene conversion [66]. This O1C variant provides powerful evidence that flanking DNA regulates meiotic instability, but the mechanism of action of O1C remains obscure. Definitive associations between mutation rate and allele structures or flanking polymorphisms have yet to be found at other human minisatellites [62]. Comparative sequence analysis of three human minisatellites (MS31, MS32 and CEB1) [67] showing meiotic repeat instability showed no significant sequence similarities between different loci, nor any consistent patterns of thermal stability or DNA secondary structure. This suggests that minisatellite instability is not controlled by primary or secondary characteristics of the DNA sequence flanking the repeat array [67].

Meiotic recombination

Various models have been developed to explain the instability processes observed at human minisatellites [12, 68]. The current model (fig. 3) involves mutation initiation by staggered single-strand nicks [68]. The gap created is then repaired via either an intra-allelic pathway or a conversion-like process involving strand invasion of repeats between alleles. This model can account for several features of mutation but not for repeat unit scrambling which instead suggests a more complex multistage process perhaps involving repeated strand invasion [69]. This model predicts that some mutation events could result in meiotic crossover within the repeat array, following isomerisation and resolution of a recombination/conversion initiation complex (fig. 3). Recent work at minisatellite MS32 has shown that array crossovers do occur in sperm, yielding simple

recombinant repeat arrays with exchange of flanking markers, although at low frequency [70]. Meiotic crossover activity is not confined to the repeat array, but extends into DNA flanking the polar end of the array [70, 71], defining an intense and highly localised recombination hotspot centred upstream of the locus and extending into the beginning of the minisatellite. This strongly suggests that the hotspot is responsible for driving repeat turnover at MS32. Analysis of a stable allele with the O1C flanking variant revealed a massive reduction in crossover events, providing further evidence that conversion and crossovers originate by a common process and suggesting that O1C functions by abolishing the hotspot in *cis*, perhaps by disrupting a recombination initiator element in the flanking DNA [70]. The flanking hotspot provides a simple explanation for the polarity of repeat instability arising from recombination (conversion and crossover) events initiated within the repeat array itself. Preliminary analysis of two others unstable minisatellites, MS31 and CEB1 [C. Hollies and J. Buard, unpublished data] suggests that they too are active in meiotic crossover, but that, as with MS32, the vast majority of recombination initiation events are resolved along the conversion pathway without yielding crossovers.

Several issues about minisatellite recombination remain unresolved. The first is whether crossover is reciprocal; preliminary data at MS32 suggest that the flanking hotspot has the same intensity on both alleles of a given man, consistent with reciprocal crossover [A.J.J., unpublished data]. Second, it is still unclear to what extent minisatellites other than MS32 are associated with flanking recombination hotspots. Third, our knowledge of minisatellite instability processes in the female germline is extremely limited, due to the small number of mutants identifiable by pedigree analysis together

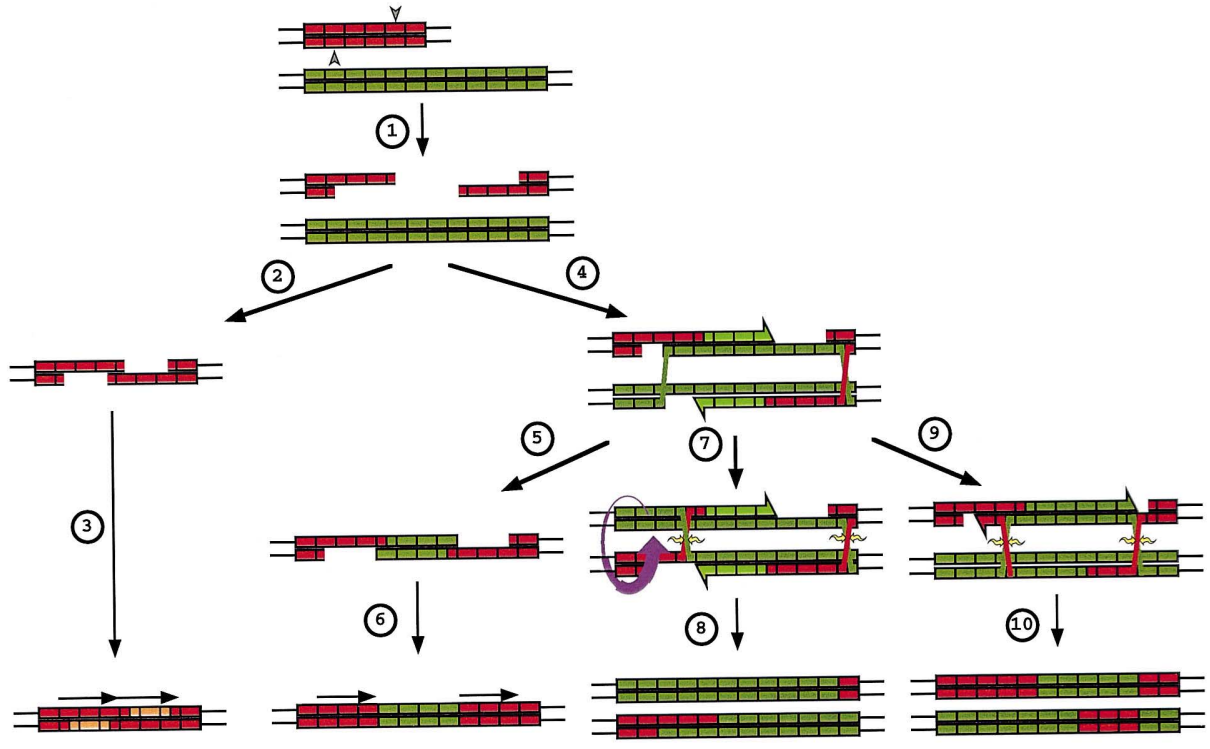

Figure 3. Model for the co-initiation of intra-allelic duplication, conversion and crossover in human minisatellites. The allele destined to become mutated (red allele) is activated for mutation by the introduction of staggered nicks (grey arrowheads) within the array (1). The break expands to a gap and the recipient allele (red) pairs in register with the donor allele (green). For intra-allelic events, the broken strands anneal (2) and the gaps are repaired (light red) creating an intra-allelic duplication (3). The interallelic pathway involves the gap being bridged by strand invasion from the donor allele (4). For conversion, the bridge provides a template for repair synthesis (light green). The donor strand is then extruded (5), leaving the donor allele intact, and the resulting single-strand gap in the recipient allele is repaired to yield a simple conversion product and a duplication of motifs flanking the insertion (6). For recombination, the strand invasion complex is isomerised (purple arrow) and the Holliday junctions resolved (yellow arrows) (7), followed by repair synthesis (8) to yield two recombinant alleles as shown. If isomerisation does not occur, conversion events will arise (10) following resolution of Holliday junctions (yellow arrows) and repair (9).

with the tendency of most minisatellites to mutate preferentially in the male germline, including MS31, MS205 and CEB1 [62]. The few maternal mutants characterised to date suggest a mutation process broadly similar to paternal mutation (e.g. polarity of mutation within the repeat array) but possibly differing in mechanistic detail [12].

Human AT-rich minisatellites

Most minisatellites are GC rich (see above). Five AT-rich loci have, however, been described in humans: the autosomal loci COL2A1 [72], ApoB [73–75], FRA16B [76] and FRA10B [77] and the Y-chromosome-specific minisatellite MSY1 [78]. They all show striking differences from GC-rich minisatellites. Their structure is typically modular, with similar variant repeats clustered into blocks within the repeat array, and not dispersed as in GC-rich minisatellites. AT-rich repeat unit sequences tend to be internally palindromic and thus have the potential to form hairpin structures that may contribute to repeat instability. Mutation processes at AT-rich minisatellites remain uncertain but are likely to be different from GC-rich loci. The Y chromosomal minisatellite MSY1 cannot by definition engage in interallelic mutation events [78], though this locus is highly unstable, with a mutation rate estimated at 5% per sperm [79]. Allele structural comparisons suggest a mutation process dominated by the linear diffusion of variants, probably through slippage within homogeneous blocks of repeats, a process reminiscent of microsatellite mutation [80]. There is additional evidence for a novel sequence homogenisation process at MSY1 whereby new base substitutional variants can spread along an allele by a biased mismatch repair process operating on heteroduplex DNA generated by slippage or USCE [81]; this provides a striking novel example of molecular drive [82]. Similarly, allele diversity studies at the autosomal loci ApoB and COL2A show no evidence of frequent interallelic events [72–74], though ApoB does show polarised variability [74], a characteristic of GC-rich minisatellites. Again, these loci most likely evolve by slippage and constrained USCE [72–74, 78], though in the absence of information on de novo mutants, a possible contribution of interallelic recombination to repeat turnover cannot be ruled out.

Expansions at AT-rich minisatellites can also result in the creation of fragile chromosomal sites such as the common distamycin-A-sensitive fragile site FRA16B [76] or the bromodeoxyuridine-inducible distamycin-A-insensitive fragile site FRA10B [77]. Expansions of up to 2000 copies of what appears to be just one repeat type with palindromic structure have been observed at the FRA16B locus [76], although intermediate-length alleles were not observed. In contrast, the FRA10B fragile site showed shorter expanded alleles [77], revealing that repeat expansions at this locus display the hallmarks of dynamic mutation as described for trinucleotide repeat expansions in neurological disease [80, 83–87]. However, the mechanisms responsible for generating such massive minisatellite expansions in a single generation remain unclear, although they appear to be specific to AT-rich minisatellites.

Minisatellites in non-human organisms

Minisatellites have been detected by DNA fingerprint analysis in most organisms including yeast [88], fungi [89], plants [90] and higher eukaryotes [91–98], suggesting that GC-rich minisatellite-like sequences are present in most genomes. The use of minisatellites detected by DNA fingerprinting in these various species has been largely restricted to studies of population structure and mating patterns, conservation biology, genetic diversity and the development of genetic markers [99–102].

The systematic isolation of rat, mouse and pig minisatellites showed that authentic human-like minisatellites do exist in non-primate species [101, 103, 104], with 10 to 60 bp long GC-rich repeat units and the presence of variant repeat sequences interspersed along the repeat array. In humans, about 90% of minisatellites are localised in subtelomeric regions [103]. In contrast, the pig, rat and mouse genomes show lower levels of subtelomeric clustering (66%, 30% and 15%, respectively) [103, 104]. Closer analysis shows that interstitial sites in pig and rat [103] often correspond to terminal cytogenetic bands in human. This suggests that minisatellites are created near telomeres and that their internalisation arises from secondary events resulting from rearrangements involving chromosome ends. Finally, not a single example of an unstable minisatellite with a high ($>1\%$) mutation rate has yet been reported in the three most thoroughly studied species: pig, rat and mouse [103, 104]. More detailed analysis in mice has shown that mutation rates at endogenous murine minisatellites are well below 10^{-4} per generation [104]. Allele diversity studies by MVR-PCR suggest a mutation process dominated by intra-allelic events [104; P.B., unpublished data], although the occurrence of rare interallelic events of the type seen in humans cannot be excluded. Nevertheless, the low mutation rates and apparently intra-allelic turnover process suggest that the mouse and human genomes process minisatellite sequences differently.

In contrast, ESTRs are common in the mouse but not human genome and often show high levels of germline instability. The ESTR loci Ms6-hm and Hm-2 originate from independent members of the mammalian apparent

LTR retrotransposon superfamily (MT and ORR1, respectively) [16] and show 2.3 and 3.6% germline mutation rates, respectively [17, 18]. More recently, an abundant rodent-specific family of ESTRs (MMS10) has been discovered that has arisen by expansions from within SINE B1 elements [19]. The mouse genome appears to be unique in harbouring expansions of the same sequence in different members of a retroposon family, as seen in mouse MTs (GGGCA and GGCA motifs for Ms6-hm and Hm-2, respectively) and B1 elements (GGCAGA motif) [16, 19, 20]. Unlike human minisatellites, Ms6-hm and Hm-2 also display high postmeiotic instability apparently restricted to the first few zygotic cell divisions following fertilisation [18, 105]. It is not yet known whether MMS10 loci also show postzygotic as well as germline instability [19].

The biological significance of minisatellites remains unclear. The location of human minisatellite MS32 near a recombination hotspot [70, 71], and the telomeric clustering of human minisatellites [103, 106] in chromosomal regions that are proficient in meiotic recombination and involved in initiating meiotic chromosome pairing, both suggest that minisatellites could mark chromosomal sites actively involved in the homology searching between homologous chromosomes required for meiotic chromosome pairing [107]. Minisatellite instability can thus be seen as a by-product of homology searches that involve strand invasion between homologous chromosomes. The apparent stability of minisatellites in other species and the relative lack of subtelomeric clustering do not exclude the involvement of a few subtelomeric VNTRs in meiotic chromosome pairing. However, the relationship, if any, between minisatellite mutation and meiotic chromosome pairing remains obscure.

Modelling minisatellite instability

Detailed analysis of GC-rich minisatellite mutation, including the definition of maternal processes, the developmental timing of germinal instability and the dissection of *cis*-acting regulators of recombination and repeat turnover, requires an experimentally manipulable animal model system. Given the lack of instability of endogenous minisatellites in pig, rat and mouse, modelling has instead been attempted by creating mice transgenic for human minisatellites MS32 and CEB1 [63, 108; J. Buard, unpublished data]. Initial constructs consisting of a short MS32 allele plus a few hundred base pairs of flanking human DNA yielded multicopy and single-copy integrants [108, 109]. The multicopy integrants displayed various modes of instability attributable not to minisatellites but instead to palindromic DNA created during integration [110]. The single-copy integrant could not be established in a homozygous state, preventing analysis of germinal instability involving interallelic interactions [109]. Subsequent constructs contained 29 kb of DNA, including the entire recombination hotspot, surrounding an MS32 allele of known instability in humans [63]. Single-copy lines and a multicopy transgenic line brought to homozygosity showed somatic instability at a frequency comparable to that observed in humans and arising by a similar intra-allelic process. In sharp contrast, sperm DNA analysis showed no trace of the complex recombination-based germline instability seen in humans. This provides further evidence that germline and somatic mutation at human minisatellite MS32 occur via distinct pathways [63]. There also appears to be a major barrier to the transfer of germline instability from humans to mice. Mice transgenic for human minisatellite CEB1 similarly show a lack of germline but not somatic instability [J. Buard, unpublished data]. Finally, for MS32 at least, it appears that the mouse germline is protected from mitotic instability of the type seen in blood [63]. Current data therefore suggest that the mouse is not a good animal model for the analysis of germline instability processes operating at unstable human minisatellites.

Other models of GC-rich minisatellite instability have been attempted. Plasmid constructs containing a consensus hypervariable minisatellite stimulate homologous recombination on transfection into human cells in culture [111]. However, the relevance of this system to processes of meiotic recombination remains highly questionable. Other successful attempts have been made using yeast. Human minisatellite MS1 integrated into *Saccharomyces cerevisiae* showed mitotic instability in haploid cells, establishing that allele interaction is not a prerequisite for the generation of allele length changes in yeast [112–114]. However, MS1 is not a typical human minisatellite but instead consists of a high copy number of a short (9-bp) repeat unit that shows substantial instability by an as yet uncharacterised process in both the male and female germline [54]. It is also the only known human minisatellite that shows somatic destabilisation in tumours deficient in mismatch repair [115]. More recent studies of the human minisatellite MS32 integrated in *S. cerevisiae* in the vicinity of a meiotic recombination hotspot upstream of the LEU2 locus showed evidence for mutation occurring specifically at meiosis [116]. Mutations ranged from simple duplications and deletions to complex interallelic events involving conversion and crossover, thus providing a potentially useful model of a hotspot-associated human minisatellite where the analysis of instability processes will be facilitated by extensive knowledge of pathways of meiotic recombination in yeast [117]. Such analyses could also be extended to endogenous fungal minisatellites. For example, analysis of an AT-rich minisatellite,

MSB1, in *Botrytis cinerea* [89] has revealed a repeat turnover process apparently very similar to that operating at the human MSY1 locus [78].
Finally, a number of hypervariable minisatellite DNA-binding proteins have been detected [118–122]. However, little is known about the function of these proteins and until further mechanistic data are available, the link between these proteins and minisatellite instability must remain speculative.

Minisatellites and the detection of induced mutation

Minisatellites provide excellent systems for the efficient monitoring of germline mutation, and can be used to determine whether mutations can arise following exposure to environmental agents such as ionising radiation. Evidence for radiation-induced mutation first came from studies of mutation in the progeny of irradiated male mice using multilocus DNA fingerprinting [123, 124]. The high frequency of germline mutation at mouse ESTR loci [17–19] allows mutation induction (two- to four fold increase) to be evaluated at low doses of exposure and in very small numbers of families [125]. By varying the interval between irradiation and mating, it was shown that mutation induction occurred only in premeiotic stages of spermatogenesis, with no consistent evidence for induction in postmeiotic cells [124, 125]. A linear dose-response curve for paternal mutation induced at premeiotic stages was found with a doubling dose of 0.33 Gy, a value similar to previous data using classic but far less efficient phenotypic systems for mutation detection [125].
This approach has been extended to humans by using unstable minisatellite loci to study germline mutation rates in children born in areas of Belarus heavily contaminated following the Chernobyl accident [55, 126]. A two fold genome-wide increase in minisatellite mutation rate was found in exposed families compared with unexposed families from the UK. Furthermore, rates in Belarus were correlated with the level of surface contamination by ^{137}Cs, providing evidence that the elevated mutation rate arises directly from exposure to ionising radiation. While this is the first direct evidence for radiation-induced germline mutation in humans, the data should be regarded as suggestive and not definitive and point to the need for extensive additional studies on exposed populations. Similar results among barn swallows, *Hirundo rustica*, breeding close to Chernobyl have been observed. An increased germline mutation rate was obtained from segregation analysis at two hypervariable microsatellite loci, revealing a two- to ten fold excess of mutation events in exposed barn swallows than in birds from control areas in Ukraine and Italy [127].
Chemical pollution has been studied using multilocus DNA fingerprinting to monitor germline mutation rates in herring gulls inhabiting a heavily industrialised urban harbour [128]. The mutation rate was two times higher than in birds living in three different rural sites, suggesting that exposure to complex mixtures of urban and industrial chemicals can play an important role in generating germline mutations. Another study in mice exposed to polychlorinated biphenyls and diesel exhaust emissions demonstrated significantly higher mutation frequencies at two hypervariable mouse minisatellites [129].
Minisatellites provide the most powerful tool currently available for monitoring DNA mutation caused by ionising radiation or genotoxins in human and animal populations. However, the mechanisms of mutation induction are very poorly understood. For radiation, the absolute level of induced mutation is far higher than would be predicted if mutation arises as a direct consequence of radiation-induced damage in minisatellites/ESTRs. This implies an indirect induction mechanism whereby radiation is sensed, inducing an exposure signal that in some way subsequently destabilises tandem repeat loci in the germline [55, 123, 125, 126]. This hypothetical mechanism would represent a completely novel pathway for mutagenesis.

Future issues

Since the first hypervariable minisatellite sequence was fortuitously isolated as a random clone from human chromosome 14 [130], minisatellite instability has been characterised in considerable detail. However, many aspects of the instability process are poorly understood. In the germline, the remarkably complex restructuring of alleles that can accompany mutation still requires mechanistic explanation. Mutation processes in the female germline remain largely obscure. We are completely ignorant about the functional basis of human recombination hotspots, about pathways of repeat turnover and recombination, and about the proteins that are involved in these processes in mammals. It remains to be seen whether detailed genetic and biochemical analysis in model systems such as yeast will give insights relevant to turnover processes in the mammalian germline. The analysis of minisatellites in other species has demonstrated our lack of knowledge in VNTR mutation processes in other genomes, in particular at ESTRs in mice, but does suggest a diversity of mutation processes whose relative importance can vary substantially from species to species. Finally, it appears that minisatellites may be remarkably sensitive to a variety of environmental agents such as ionising radiation and chemical pollutants. The nature of the damage

signal created by these agents, whether in DNA or some other sensor molecule, and the mechanism of subsequent induced mutagenesis, remain major goals for future research.

Acknowledgements. We thank colleagues for helpful discussions, and Esther Signer for the DNA fingerprint shown in figure 1. The work P. B. is supported by the Medical Research Council and of A. J. J. is supported in part by an International Research Scholars Award from the Howard Hughes Medical Institute and in part by grants from the Wellcome Trust, Medical Research Council and Royal Society.

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