Genetics

New aid to human gene mapping

from C. Thomas Caskey

ALTHOUGH the chromosomal localizations of the genes for Duchenne muscular dystrophy[1] and Huntington's disease[2] stand as powerful examples of the use of restriction fragment length polymorphisms (RFLP) to map human genes, considerable technical difficulties still limit the application of the technique. Help is at hand with the report on page 67 of this issue[3] of a new family of DNA sequences, the characteristics of which are highly suited to RFLP linkage analysis.

Typical RFLP analysis has employed cloned functional gene sequences[4], pseudogene sequences[5] or 'anonymous' genomic probes[2], to detect what are presumed to be single base-change polymorphisms that generate or remove recognition sites for restriction endonucleases, the enzymes used to fragment DNA. Alternatively, these probes have been used to detect polymorphisms that are presumed to be due to DNA rearrangements[6]. With the exception of the last, these probes suffer from low heterozygosity because only two alleles are present in the population, one of which often occurs at a low frequency. As a result, no information can be obtained from some critical individuals in a pedigree or even entire pedigrees. Another problem is that genomic recombinants often contain highly repeated sequences that must be removed prior to analysis. Finally, closely linked polymorphic probes may not be available for a chromosomal region of particular interest.

Several of these difficulties should be obviated by the use of the DNA sequence family that is both tandemly repeated and dispersed in the human genome, and is described by Alec Jeffreys and his colleagues in this issue[3]. This family offers the opportunity to recognize multiple unlinked chromosomal loci and, with use of appropriate restriction endonucleases and specific probes, to identify variations in the number of copies of the tandem 'minisatellite' at each locus. The variation in copy number is seen as an RFLP by standard Southern blotting techniques and some loci are shown to be highly polymorphic for copy number of members of this repeat family (multiple alleles).

The minisatellite was first detected during characterization of the human myoglobin gene by Jeffreys and colleagues[7] as an intron sequence that contains four tandem repeats of a 33 base-pair subunit and bears similarities to highly polymorphic sequences that are found near several other genes[8,9]. A probe constructed as a concatamer of this repeat has now been used to identify numerous genomic lambda clones which contain sequences homologous to the repeat. Eight of the clones were selected and further characterized. They differ in their base sequence, number of repeat units and, apparently, in their localization within the human genome (that is, they are not closely linked). All the repeat units, however, share a similar 'core' of 10–15 base pairs. Three of the eight repeats studied are highly polymorphic in copy number when hybridized under high stringency to DNA from various individuals. Pedigree analysis by Southern blotting shows that bands representing different copy numbers of repeats are inherited in a Mendelian fashion. Furthermore, the heterozygosity of these highly polymorphic repeats appears to be close to 100%. Thus, it is highly likely that these probes can yield information for analysing the pedigrees of families affected by a closely linked disease gene.

In order for these probes to be useful the chromosomal localization of the minisatellites will need to be determined. But it is clear that they provide an interesting and potentially significant improvement in the use of RFLP's for prenatal diagnosis, linkage studies, and gene mapping. Other polymorphic satellite DNA probes have been reported[10,11], but they do not seem to have the same widespread potential in human gene mapping. Minisatellite sequence families offer the advantages of multiple chromosomal localization and greater heterozygosity at a given locus. Jeffreys *et al.* have described one, but their report holds out hope that other minisatellite families, which would make useful polymorphic probes available throughout the genome, await discovery.

Because the 'core' sequence of the repeated units resembles the generalized recombination signal of *Escherich coli,* a further use of these minisatellite sequences may be in the study of unequal crossing-over, the presumed mechanism for generating heterogeneity of copy number within a tandem repeat. □

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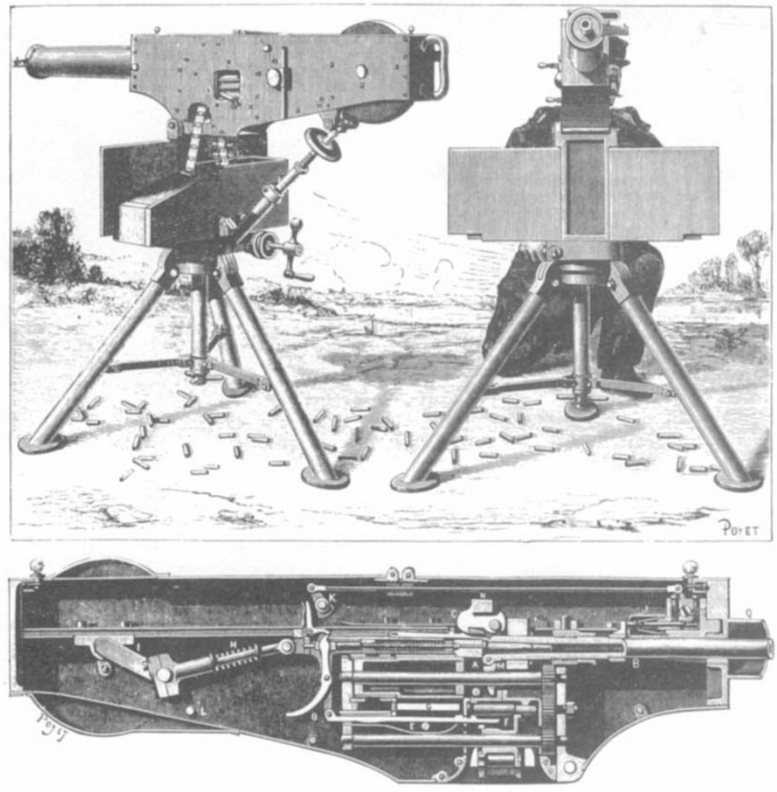
100 Years Ago **The Maxim Gun** **From *Nature* 31 414, 5 March 1885.**



MR. HIRAM STEVENS MAXIM, the well-known American engineer, has lately brought out a new form of a machine-gun, which is attracting a great deal of attention in military and naval circles. This gun is a completely new departure. It takes the cartridges out of the box in which they were originally packed, puts them into the barrel, fires them, and expels the empty cartridges, using, for this purpose, energy derived from the recoil of the barrel.