other studies have used higher nicotine concentrations). They show, with the help of careful controls, that this is a direct presynaptic action. Another contribution of this work is the demonstration that the presynaptic receptor is likely to contain the α7 subunit, because the nicotine effect can be blocked by α-bungarotoxin in control conditions, but not after the α7 subunit is eliminated with antisense treatment. In addition, McGehee *et al.* find that nicotine causes an increase in intracellular calcium concentration in presynaptic endings. This observation may explain the effect of nicotine on fast transmission, because homomeric α7 channels have a rather high calcium permeability (*10*). The authors suggest that such presynaptic actions may underlie the behavioral and cognitive effects of nicotine.

Nicotinic enhancement of the spontaneous release of the neurotransmitters γ-amino butyric acid (GABA) and dopamine and of evoked excitatory synaptic transmission in the brain has been reported previously (*11*). But the work of McGehee *et al.* differs in that it provides the first strong evidence for the involvement of α7-containing channels. Other reported cases of presynaptic nicotine effects in the CNS are not sensitive to α-bungarotoxin (*11*, *12*). Most other pre- or postsynaptic nicotine actions also seem to require higher nicotine concentrations (*11*, *13*), although it is puzzling that the potency of nicotine on recombinant α7 receptors is reported not to be very high in absolute terms (*14*). It is also interesting that the "high-affinity nicotine-binding" areas, rather than the α-bungarotoxin–binding areas, are primarily affected in Alzheimer's patients (*9*). One remaining question for all studies is whether the nicotine receptors are ever actually exposed to acetylcholine in real life. This has never been demonstrated, although the presence of neurons containing cholineacetyltransferase makes the possibility plausible.

If most of the important actions of nicotine in the brain are presynaptic effects through α7-containing receptors, what are all the other subunits there for? Nobody knows at present. It is not considered respectable in polite company to suggest that these other subunits might constitute a redundant evolutionary hangover, although one cannot help thinking of the decades that were spent looking for the physiological function of the vast number of histamine receptors in the body. That problem was never really solved—people just got bored with it. We can only hope that the same fate does not await the α4-containing receptors.

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Minisatellites and Human Disease

Theodore G. Krontiris

The appearance of unstable DNA sequences in key regions of the human genome evokes the image of a mischievous Nature casually dropping a box of matches within reach of an adventuresome and unattended child. The predictable consequences emerge in the dramatic example of the unstable trinucleotide repeats: a brushfire of disease-producing mutations in fragile X syndrome, myotonic dystrophy, Huntington's disease, and a growing host of other genetic disease syndromes (*1*). Now evidence is accumulating from studies of the insulin (*INS*) and Ha-ras (*HRAS1*) loci that another class of repetitive sequences, hypervariable minisatellites, contributes a subtler, but potentially more widespread, influence on the heritable risk of disease.

Minisatellites are tandem arrays of a locus-specific consensus sequence that varies between 14 and 100 base pairs (bp) in length (*2*). Such structures are often polymorphic in the number of tandem repeats of the consensus [hence, the alternative designations, variable number of tandem repeats (VNTRs) or variable tandem repetitions (VTRs)]. Dispersed throughout the human genome (and likely those of all vertebrates), minisatellites are often situated just upstream or downstream of genes; many occur within introns. The *INS* VNTR is 600 bp upstream of the transcriptional start site (*3*), and the *HRAS1* minisatellite is 1000 bp downstream of the polyadenylation signal (*4*).

VNTRs are extraordinarily hyperallelic; many loci display dozens of alleles. As a consequence, the heterozygosity rate (het rate), or fraction of individuals in the population with two different alleles, can approach 100%. This means a geneticist may screen an auditorium full of compliant colleagues and never find a homozygote at many VNTR loci. The *INS* minisatellite has a het rate in excess of 90%. Curiously, the *HRAS1* minisatellite displays a het rate of only 65%.

The driving force underlying this genetic plasticity is, of course, a mutation rate that can exceed 10% per gamete (*5*). We are only beginning to understand the mutational processes giving rise to such instability. The intuitively obvious mechanism, single crossovers at the site of slippage and mispairing of tandem repeats, probably occurs infrequently, if at all (*6*). Instead, complex internal rearrangements of the minisatellite appear in new mutations, almost exclusively at one end of the tandem array (*7*). Analysis of the DNA sequence indicates that this process involves interallelic exchange, implicating gene conversion (*7*). The rate of mutation varies from locus to locus and from individual to individual at a given locus (*5*, *8*). The human minisatellite MS32 possesses a cis-acting promoter of mutation on one flank, an observation, if reproduced at other VNTRs, that could explain both the varying rate and the polarity of the mutational mechanism. In addition, some of the

The author is in the Department of Medicine and Program in Genetics, Tufts University School of Medicine and New England Medical Center Hospitals, Boston, MA 02111, USA.

mutations in both humans and mice are somatic, resulting in mosaicism (*9*).

At *HRAS1*, one of the best characterized of the minisatellite loci, the consensus repeat unit is 28 bp long. Four common progenitors, varying in size from roughly 1000 to 2500 bp, comprise 90% of the alleles at the locus. Molecular genetic analysis has revealed that these progenitors have "shed" dozens of larger and smaller mutant alleles (*10*). Alleles of the *INS* VNTR, at which the consensus repeat unit is but 14 bp long, define three size classes distinguished by a modal length of 600 (class I), 1200 (class II), and 2200 bp (class III). Many alleles exist in each class (*3, 11*).

This instability carries with it a high price: cancer (*12*) and insulin-dependent, or type I, diabetes mellitus (IDDM) (*11*). Mutations of the *HRAS1* minisatellite, but not the four progenitor alleles, are associated with multiple forms of cancer: carcinomas of the breast, the colon, and the urinary bladder, and acute leukemia (*13*). Class I alleles of the *INS* VNTR are correlated with a risk of developing IDDM (*12, 14, 15*). Interestingly, pathogenic alleles at both *INS* and *HRAS1* define the same relative risk for their respective disease, slightly more than double that of unassociated alleles. In the case of *HRAS1* minisatellite mutations in cancer, the relatively high prevalence of disease alleles translates this relative risk into a significant attributable risk: *HRAS1* mutations contribute to 1 case of cancer in 11.

The modest relative risk and the possibilities for multiple interactions with other loci leading to disease suggest that minisatellites may actually be modifier genes: Pathogenic VNTR alleles could affect the penetrance or expressivity of cooperating loci. For type I diabetes, the candidates for VNTR interactions are probably in hand. At least six genes, *IDDM1* through *IDDM6*, contribute to risk; the dominant effect is *IDDM1*, the major histocompatibility complex locus (*14*). The *INS* VNTR is now unequivocally established to be *IDDM2* (*16*). So far, we only have a hint of a candidate for gene interactions with the pathogenic *HRAS1* minisatellite: Although *HRAS1* status does not alter penetrance in carriers of a breast cancer gene (*BRCA1*), more ovarian cancers may occur in individuals bearing *HRAS1* mutations (*17*).

What mechanisms are responsible for the association of minisatellites with disease? Linkage disequilibrium with pathogenic alleles of a nearby gene has always been an unsatisfactory explanation at *HRAS1* because high-risk alleles derive from all progenitors and, presumably, from many ancestral chromosomes. Both population- and family-based haplotype analyses have recently excluded all but the *INS* VNTR from the *IDDM2* disease association (*16*). Therefore, the genetic effect resides exclusively within the *INS* VNTR. Additional, albeit indirect, genetic data also implicate the *HRAS1* minisatellite. The *HRAS1* mutation rate in nuclear families and sperm is the same as that of loci with het rates exceeding 90%. The discrepancy between a 65% het rate (in a population of middle-aged and elderly adults) and the much higher level expected from the mutation frequency indicates that certain mutations of the *HRAS1* minisatellite may be dominant lethals.

Both the *INS* and *HRAS1* minisatellites bind specifically to particular transcription factors, in an allele-specific and cell-type–specific fashion. The *HRAS1* minisatellite binds members of the *rel*/NF-κB family (*18*); reporter gene activation occurs in some cell lines, such as the EJ human bladder carcinoma, but not others, such as HeLa (*19*). The amount of activation varies between 2- and 10-fold (*19*).

The *INS* VNTR binds the transcription factor Pur-1, but the relation between high-risk alleles and the resulting transcriptional activation of the insulin promoter is reversed (*20*). The small, high-risk alleles (class I) demonstrate smaller transcriptional effects than larger, low-risk alleles (class III); the effects are noted in vivo, as well. Activation occurs in pancreatic cells but not in HeLa. Most importantly, the sequence composition of the individual repeat units of the tandem array, in addition to overall VNTR length, governs the transcriptional response (*20*).

These results do not yet firmly establish the relation between VNTR effects on transcription and the influence of minisatellites on disease risk, but the hypothesis is worth testing. Although strong disease associations are currently limited to *INS* and *HRAS1*, other minisatellites may also be pathogenic. For example, the VNTR several kilobases upstream of the human immunoglobulin heavy-chain gene (*IGH*) enhancer can bind a protein closely related to the mycHLH factor USF (*21*). The *IGH* VNTR does not activate transcription by binding this factor. Rather, it completely inhibits, in cis and in trans, transcriptional activation through a bona fide USF enhancer element. Because USF and related proteins can activate the *IGH* enhancer, this VNTR may indeed influence immunoglobulin gene expression.

Are the *HRAS1*, *INS*, and *IGH* minisatellites true transcriptional control elements? On evolutionary grounds, the answer must be no. None of these minisatellites is present in homologous, nonprimate genes; therefore, VNTRs would be very recent additions to the constellation of regulatory structures. It is more likely that the mechanisms generating allelic instability depend on the happenstance of transcription factor binding; perhaps the occurrence and variation of minisatellites is, to some extent, an unavoidable feature of certain DNA-protein interactions. However, as long as VNTR effects on transcription are constrained within a narrow range, no selection operates either to fix particular alleles (those with no great effect and, concomitantly, with intrinsically low mutation rates) or to banish a particularly disruptive VNTR altogether. We predict that mutations with effects exceeding those of nonpathogenic progenitor alleles will demonstrate disease effects.

Complex diseases other than cancer and diabetes, especially those with relatively late adult onset, will likely also display contributory effects from minisatellites. The VNTR mutational process may actually be positively selected; by culling those of us in middle age and beyond, evolution brings our species to fighting trim. This is not, alas, too mischievous an image of Nature.

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