Others, noting the hundreds of sites with Clovis-type artefacts scattered all over the continental United States and dating at the end of the ice age, conclude that Clovis sites really do mark the first settlement of the Americas south of Canada. If (say this second group of authors) there really had been humans in the Americas long before Clovis times, we would by now have discovered hundreds of unequivocal pre-Clovis sites, as we have in Australia and Europe. Palaeontologists and archaeologists who work on the prehistory of the Americas will no doubt continue to argue about these matters after reading the paper by Roberts *et al.*[2]. ■

Jared M. Diamond is in the Department of Physiology, University of California Medical School, Los Angeles, California 90095-1751, USA.
e-mail: jdiamond@mednet.ucla.edu

1. Martin, P. S. & Klein, R. G. (eds) *Quaternary Extinctions: A Prehistoric Revolution* (Univ. Arizona Press, Tucson, 1984).
2. Roberts, R. G. *et al. Science* **292**, 1888–1892 (2001).
3. Baynes, A. *Rec. W. Aust. Mus.* (suppl.) **57**, 391 (1999).
4. Roberts, R. G. *et al. Quat. Sci. Rev.* **13**, 575–583 (1994).
5. Miller, G. H. *et al. Science* **283**, 205–208 (1999).

Neurodegenerative diseases

Origins of instability

Richard R. Sinden

Errors in DNA replication are thought to underlie the lengthening of tracts of repeated DNA that occurs in some neurodegenerative diseases. But mechanisms for repairing damaged DNA may also be responsible.

Many neurological and neurodegenerative diseases, such as Huntington's disease and fragile X syndrome, share a similar genetic basis — the lengthening of tracts of repeated DNA sequence. The molecular mechanisms that bring about this 'repeat instability' have attracted much attention, but have remained somewhat mysterious. At a recent meeting*, however, our understanding of these mechanisms blossomed with the revelation that proteins that repair damaged DNA may be responsible for generating repeat instability in cells that are not dividing.

At least 14 human diseases have been associated with the lengthening (expansion) of tracts of nucleotide triplets, such as CTG (CAG on the complementary DNA strand), CGG (CCG) and GAA (TTC), in various human genes[1]. As well as Huntington's disease and fragile X syndrome, these diseases include myotonic dystrophy type 1, several spinocerebellar ataxias and Friedreich's ataxia. Breaking the 'triplet-repeat' mould, spinocerebellar ataxia type 10 is linked to the expansion of pentanucleotide ATTCT (AGAAT) repeats, from about 14 to 4,500 repeats[2]. The pathological bases of these diseases vary, but repeat expansion is the underlying mutation in all.

Two principal classes of repeat instability are associated with these diseases. First, when the tracts of repeats occur within non-protein-coding regions of an affected gene, the tracts can expand by a factor of 10–20, or even more, between generations. This large-scale expansion occurs in fragile X syndrome, myotonic dystrophy, spinocerebellar ataxias 8 and 10, and Friedreich's ataxia. Massive expansion of these particular repeats has

been seen only in humans (although other repeats expand in mice[3]). The second class involves small expansions. Small-scale expansion of CAG repeats can cause disease when the repeats encode a tract of polyglutamine amino acids within an affected protein; for example, the presence of 30 CAG repeats in the gene encoding the huntingtin protein is in the normal range, whereas more than 36 CAG repeats cause Huntington's disease. In some disorders, such as myotonic dystrophy type 1 and Huntington's disease, short expansions accumulate differently in different cells and tissues throughout life. Small expansions may also occur between generations.

Conventional wisdom says that small expansions or deletions within tracts of repeats are generated during genome replication — that is, as cells divide. During DNA replication, a double-stranded DNA helix must first be unwound and unzipped; the two separated strands are then used as templates for the production of two new strands. When the DNA includes long tracts of simple repeats, it is easy for the growing DNA chain and the original template to become misaligned if DNA-replicating enzymes pause and separate from the template. This can lead to deletions or expansions of sequence when replication recommences. In fact, in a primate replication system, there is a marked bias for expansion from 79 to 107 repeats of the CTG repeat that is found in myotonic dystrophy (C. Pearson, Hospital for Sick Children, Toronto). Moreover, disease-associated triplet repeats can form 'alternative' DNA conformations, including hairpins and slipped-strand structures, as well as intramolecular triplex and quadruplex DNA structures. These structures might promote fractious replication.

It seems, however, that theories involving genome replication cannot account for all

small-scale repeat instability. In mouse models of neurodegenerative disease, instability generally occurs at different rates in different tissues, with continued expansion occurring as the mice age. Instabilities can also show a dependence on their chromosomal context. Most remarkably, it seems that repeat variations can occur in the absence of the DNA replication that is associated with genome duplication. In mice engineered to provide models of myotonic dystrophy or Huntington's disease, instability often occurs to a greater degree in cells that do not divide (such as brain and kidney cells) compared with cells that divide frequently[4–6]. Variations in instability are also observed in cultured cells[7]. Moreover, repeat variability has also been seen in eggs[8] and developing sperm[9], which do not replicate and do not undergo a process called DNA recombination. Both replication
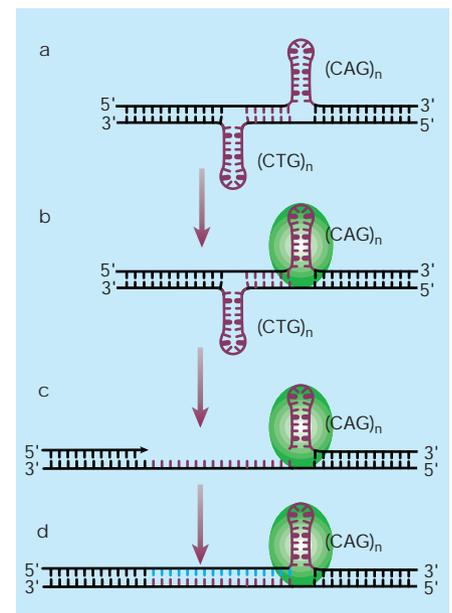

Figure 1 **In one model for the production of unstable tracts of nucleotide repeats in cells that are not dividing, 'alternative' DNA structures are crucial.** a, **During DNA replication, the individual DNA strands can become separated and then paired up again. During this process, slipped-strand DNA structures, as shown, or other 'alternative' structures may form; this is particularly likely when tracts of nucleotide repeats (such as CTG, or CAG on the complementary strand; purple) are present. These structures may also form spontaneously.** b, **The DNA-repair proteins MSH2 or MSH3 (green oval) bind to and stabilize the hairpin in one strand (here the upper strand).** c, **The repair proteins introduce an incision next to the upper hairpin, allowing the strands to separate and the lower hairpin to unfold.** d, **The resulting gap in the upper strand is filled in (turquoise) by using the lower strand as a template. The tract of repeats is thereby lengthened relative to the original DNA. (The thicker tick marks denote T–T and A–A mismatches that occur in the hairpin stems.)**
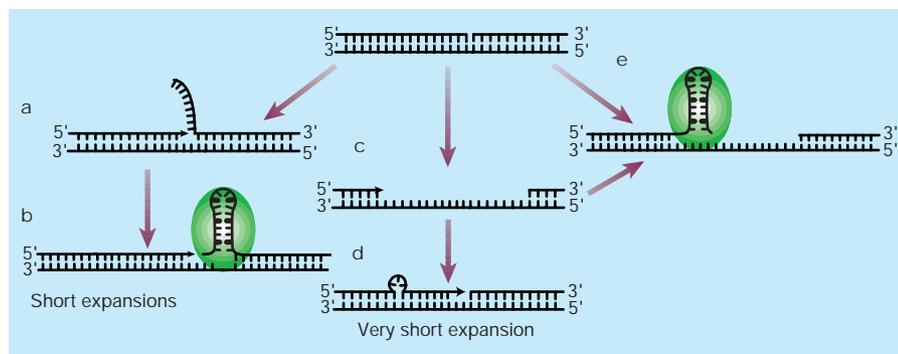
Figure 2 A nick in DNA could promote the instability of tracts of nucleotide repeats. a, During the repair of the gap, too much of the lower strand might be copied, producing a flap. This is 'strand-displacement synthesis'. b, Continued copying will lead to the formation of a hairpin within the flap. The repair proteins MSH2 or MSH3 might bind to and stabilize the hairpin; sealing the nick will then lead to an expansion in the upper strand. The next time the cell duplicates its DNA, the expansion will be copied if unrepaired, and both strands will contain the expansion. c, A large gap might form as a result of the initial nick. d, The error-prone replication enzyme DNA polymerase-β (or another polymerase) fills in the gap; if it slips, it could cause addition or deletion of one or more repeats. e, DNA 'melting' at the nick could lead to hairpin formation, perhaps stabilized by MSH2/MSH3, creating a gap. DNA melting and hairpin formation could also occur at a gap, as well as a nick. The hairpin may form at either end of the gap. If the gap is filled without removing the hairpin, repeat expansion will occur.

and recombination are thereby excluded as obligatory pathways to repeat variability.

What mechanism, then, can explain the age-dependent accumulation of mutations in non-dividing cells? A key revelation was that mice with Huntington's disease that lack the DNA-repair protein MSH2 do not show age-dependent repeat variability in different tissues[10]. Studies of animal models of myotonic dystrophy that lack the DNA-repair proteins MSH2 (G. Gourdon, Hôpital Necker-Enfants Malades, Paris), PMS2 (D. Monckton, Univ. Glasgow) or MSH3 (B. Wieringa, Univ. Nijmegen), as well as further studies of mice with Huntington's disease[9], have now confirmed that age-dependent repeat instability requires repair proteins. Moreover, MSH2 is required for repeat instability in developing sperm in mice with Huntington's disease[9]. However, instability still occurs in animals that lack the repair protein MSH6 (B. Wieringa) or the recombination proteins RAD52 and RAD54 (G. Gourdon).

There are several ways in which DNA can be altered that might lead eventually to DNA-repair-dependent repeat instability. First, alternative DNA structures, such as slipped-strand DNA, might form within repeat tracts after DNA replication. These structures could then bind to MSH2 or MSH3, which would try to mend the unusual structures, perhaps (in fairly complicated ways) resulting in errors such as repeat expansion (Fig. 1; C. Pearson). Second, DNA sustains continuous spontaneous damage, for example as a result of oxidation[8]. Repeat tracts or alternative structures might be particularly sensitive to damage — and so particularly dependent on effective DNA repair.

Third, single- or double-stranded breaks can occur in DNA, either *de novo* or as a result of incomplete DNA replication. Moreover,

larger gaps could accumulate, with flaps, hairpins or other complex structures forming at their ends (C. McMurray, Mayo Foundation, Rochester, Minnesota). DNA-repair proteins might bind to and stabilize these unusual repeat structures, indirectly promoting expansion (Fig. 2). In the case of mice with Huntington's disease, the repeat tracts expand by just one to three repeats[9]; this may be too few to allow a stable repeat secondary structure to form. Here, instability might instead reflect the slippage of replicating enzymes during an error-prone repair process.

With this new knowledge in hand, it may one day prove possible to develop approaches that minimize the lengthening of repeat tracts that can occur throughout life. But another important part of the story — the molecular basis of the large-scale tract expansion that can occur between generations — remains a mystery. ∎

Richard R. Sinden is at the Center for Genome Research, Institute of Biosciences and Technology, Texas A&M University System Health Sciences Center, 2121 West Holcombe Boulevard, Houston, Texas 77030-3303, USA.
e-mail: rsinden@ibt.tamu.edu

1. Cummings, C. J. & Zoghbi, H. Y. *Hum. Mol. Genet.* **9**, 909–916 (2000).
2. Matsuura, T. *et al. Nature Genet.* **26**, 191–194 (2000).
3. Bois, P. R., Southgate, L. & Jeffreys, A. J. *Mamm. Genome* **12**, 104–111 (2001).
4. Kennedy, L. & Shelbourne, P. F. *Hum. Mol. Genet.* **9**, 2539–2544 (2000).
5. Seznec, H. *et al. Hum. Mol. Genet.* **9**, 1185–1194 (2000).
6. Fortune, M. T., Vassilopoulos, C., Coolbaugh, M. I., Siciliano, M. J. & Monckton, D. G. *Hum. Mol. Genet.* **9**, 439–445 (2000).
7. Gomes-Pereira, M., Fortune, M. T. & Monckton, D. G. *Hum. Mol. Genet.* **10**, 845–854 (2001).
8. Kaytor, M. D., Burright, E. N., Duvick, L. A., Zoghbi, H. Y. & Orr, H. T. *Hum. Mol. Genet.* **6**, 2135–2139 (1997).
9. Kovtun, I. V. & McMurray, C. T. *Nature Genet.* **27**, 407–411 (2001).
10. Manley, K., Shirley, T. L., Flaherty, L. & Messer, A. *Nature Genet.* **23**, 471–473 (1999).

Daedalus

The tense road

A road, says Daedalus, is quite a clever mechanical invention. It takes the force applied by a driven wheel, and reacts to it by momentary deformation. The resulting reaction of the road on the vehicle drives it along. Fast or heavy vehicles, by the powerful reaction they impose on the road, soon damage it. Their powered wheels do far more damage than the merely passive load of the coasting wheels. Daedalus sees the force of the engine being applied first to the tyre, and only then to the road. The tyre is canted, at least at the bottom, and the angle of cant applies a force to the vehicle.

So Daedalus wonders what would happen if the tyre were made from his 'slow rubber' of last week. At a slow enough speed, nothing would happen, and the vehicle would proceed with normal engine efficiency along the road. But at some well-defined speed, the slow restitution of the rubber would hold the canting effect well beyond the time of contact. The tyre would become very lossy, and most of the car's energy would appear not in acceleration, but as heat in the tyres. (Carbon black is put into tyres specifically to dissipate heat.)

Thus a tyre of a properly compounded slow rubber would act as an automatic speed limit. On the principle 'never do by law what you can do by engineering', Daedalus advocates the sale of 'slow tyres' to prove to the police that a specific car could never have exceeded the speed limit.

But clearly the law would be better served by a 'slow road' with the equivalent property. Any car or lorry, no matter what its tyres, would then feel a dramatic slowing on passing onto such a road surface. The road would be deformed by the driven tyres, and this deformation would be restored too late to power them along. Instead, the road would get hot.

The slow road might need to be replaced at frequent intervals, as it absorbed the power of many roadhogs, but it could still be a sound investment. On roundabouts, junctions and sharp bends, and on motorways (with the exception of overtaking lanes), it would restrict motorists to the speed limit, without requiring any legal action. A driver would notice nothing, except that no amount of throttle could accelerate him above the legal limit on such stretches of road. Daedalus hopes that such stretches will hardly get warm from the few roadhogs who challenge them, and that their deterioration (thermal and mechanical) will be very slight.

David Jones

758

NATURE | VOL 411 | 14 JUNE 2001 | www.nature.com