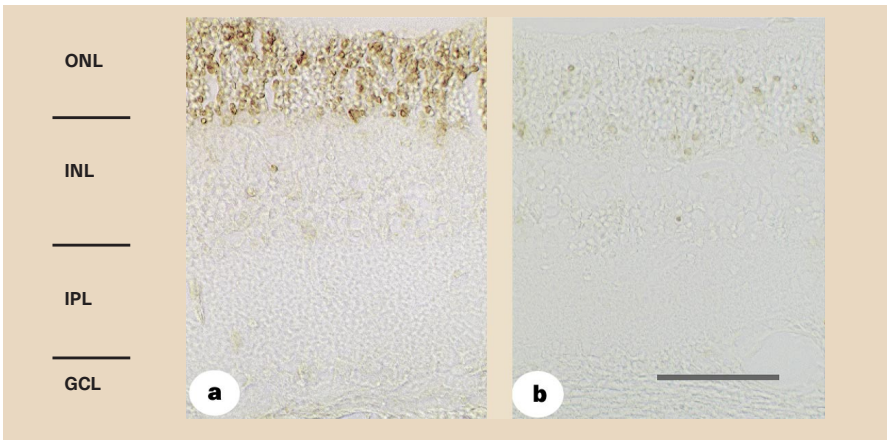

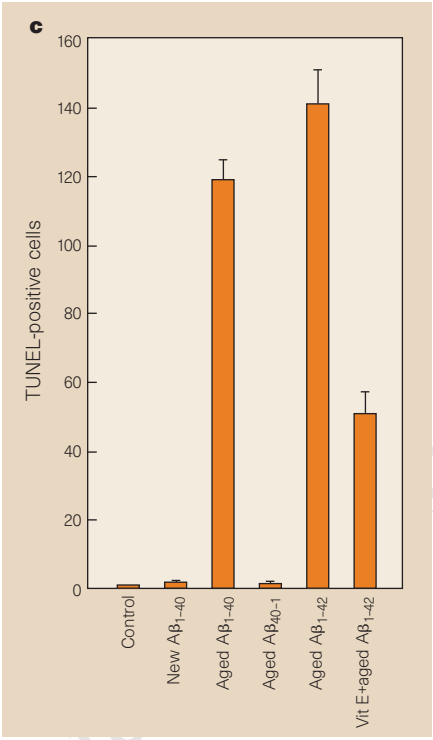

Figure 1 Representative photomicrographs showing TUNEL staining in retinal sections from eyes of rats given unilateral intravitreal injection of **a,** aged $A\beta_{1-42}$ (2 nmol in 3 μl); or **b,** vitamin E (1.3 μg in 3μl) plus aged $A\beta_{1-42}$ (2 nmol in 3 μl) 30 min later. After two days, rats were perfused with fixative and retinas were processed for TUNEL staining. **c,** Number of TUNEL-positive cells (mean of ten 390-μm-long sections) in the outer nuclear layer (ONL) of 2–4 retinas injected with control (3 μl vehicle), new $A\beta_{1-40}$ (2 nmol in 3 μl), aged $A\beta_{1-40}$ (2 nmol in 3 μl), aged $A\beta_{40-1}$ (2 nmol in 3 μl) and the two groups mentioned above. Vitamin E pretreatment produced a significant reduction in the number of TUNEL-positive ONL cells. The purity of the Aβ peptides was confirmed by sequencing and mass spectrometry (Bachem). INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Bar, 50 μm.

the hippocampus *in vitro*[1–3]. Although the mechanisms by which Aβ causes neuronal death are not fully understood, *in vitro* results suggest that an increase in oxidative stress and destabilization of calcium homeostasis[2,3] are involved.

However, in contrast to these *in vitro* observations[2–4], only a slight neurotoxicity results when Aβ is directly administered to the brain[5,6], perhaps because of the rapid efflux of injected compounds from this organ.

In this respect another organ, the retina, offers several advantages. It is an integral part of the central nervous system; its structure is well organized; and, because it is a closed system, injected compounds remain in the vitreous body for a long time[7].

We investigated the cytotoxic effects of Aβ on retinal cells after intravitreal injection and the amelioration of these effects by the antioxidant vitamin E. The technique used to assess apoptosis and therefore cytotoxicity was terminal deoxynucleotidyl transferase dUTP-end-labelling (TUNEL) staining. This technique identifies the 3′ ends of DNA strands — a phenomenon resulting from the fragmentation of DNA that occurs in apoptotic cells.

When the retinas of rats were injected with either aged $A\beta_{1-42}$ or aged $A\beta_{1-40}$ (aggregated; incubated for 4 days at 37 °C), a distinct band of TUNEL-positive photoreceptor cells was evident in the retina's outer nuclear layer. However, in the inner nuclear layer and ganglion cell layer, only a few scattered cells were TUNEL positive (Fig. 1a). This suggests that proximity to the Aβ injection site is not essential for cytotoxicity.

In addition, all retinas injected with the freshly prepared, 'new' $A\beta_{1-40}$ behaved like vehicle-injected and uninjected retinas in showing only occasional TUNEL-positive cells (Fig. 1c). Therefore the cytotoxic effect was specific to treatment with aggregated Aβ. Moreover, unlike active aged $A\beta_{1-40}$, inactive aged reverse $A\beta_{40-1}$ did not induce any changes in photoreceptor cells (Fig. 1c). No DNA fragmentation was seen in the glial cells. But, as with cultured neural cells *in vitro*[8], treatment with $A\beta_{1-42}$ or $A\beta_{1-40}$ severely decreased Bcl-2 immunoreactivity in the endfeet and proximal part of radial processes of Müller glial cells.

In vitro studies also suggested that Aβ causes an accumulation of hydrogen peroxide and lipid peroxides in the cells, and that antioxidants protect cells from Aβ toxicity[2,3]. We found that TUNEL-positive photoreceptor cells in retinas that had been pretreated with vitamin E (1.3 micrograms per 3 microlitres) showed a reduction of about 65% compared with retinas treated with aged $A\beta_{1-42}$ (Fig. 1). This indicates that Aβ cytotoxicity is caused, at least partly, by a free-radical mechanism.

Doubts have been raised in the past about extrapolating *in vitro* findings on Aβ cytotoxicity to the situation *in vivo*. Our results show that some of the proposed mechanisms underlying Aβ neurotoxicity do operate *in vivo*. In addition, the closed 'retina–vitreal' system we describe should serve as an experimental model for further *in vivo* studies and for testing appropriate compounds for their ameliorating effects on Aβ-mediated neurotoxicity.

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Are retrotransposons long-term hitchhikers?

Transposable elements represent a large fraction of the genomes of eukaryotes, and yet we know little of their origins or stability. Striking examples of cross-species transfer have been discovered among *mariner* elements[1] (transposable elements that are widespread in insects and other animals), confirming the impression that horizontal transfers are essential to the long-term success of transposable elements. We show that R1 and R2, two distantly related non-long-

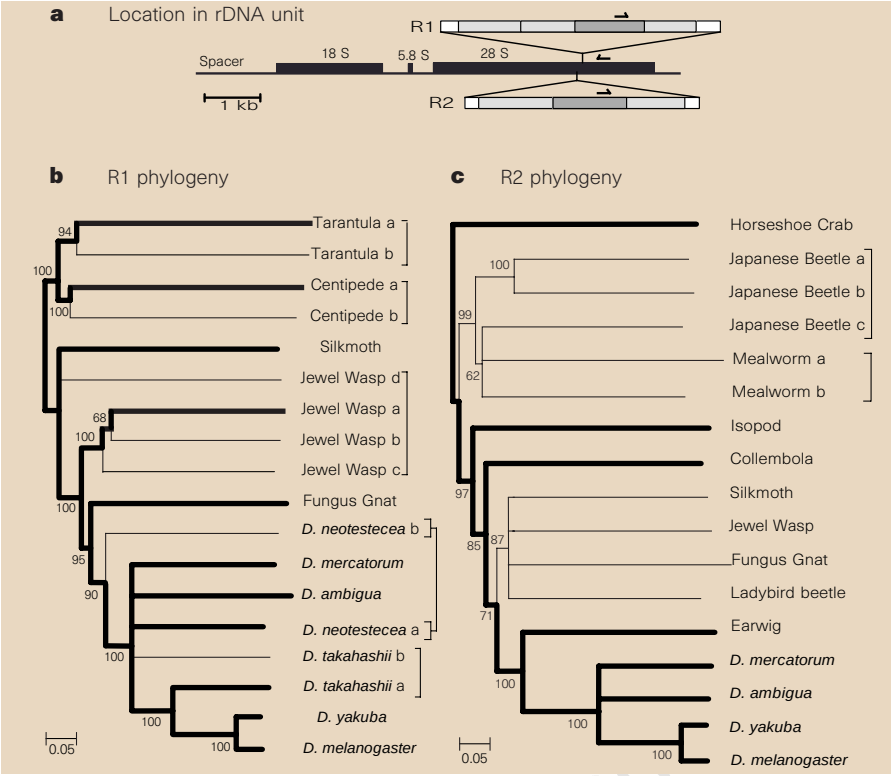

Figure 1 R1 and R2 phylogeny. **a,** Location of R1 and R2 in the rRNA genes. Light shading represents the open reading frames (ORFs); darker shading represents the reverse transcriptase (RT) domain. Short arrows: PCR primer positions[3,5,7] used to amplify the 3′ half of each element. **b, c,** Phylogeny of R1 and R2 elements. Shown are the 60% consensus trees based on 'neighbour-joining' methods with bootstrap values indicated[10]. Trees have been rooted by other non-long-terminal-repeat retrotransposons using their reverse transcriptase domains. Brackets to the right indicate multiple families in the same species, with each family given a letter classification. Bold lines represent a lineage consistent with the species phylogeny. The topology of each tree is also supported by 'maximum parsimony' algorithms and 'maximum likelihood' methods based on both amino-acid and nucleotide sequences, except for minor rearrangement of the multiple R2 lineages in Japanese beetle and mealworm. Accession numbers for previously published elements can be found in refs 3–7; those for the newly determined elements are AF015489 and AF015813–AF015821.

terminal-repeat retrotransposons which insert at specific sites 74 base pairs apart in 28S ribosomal RNA genes[2], have been maintained by vertical transmission since the origin of the phylum Arthropoda, that is, for at least 500 million years.

The insertion specificity of R1 and R2 (Fig. 1a) offers advantages for assessing the extent of these retrotransposons' vertical transmission in an old and diverse lineage, because defective elements are eliminated from the host, and all elements can be cloned irrespective of their relationship to previously characterized elements. In previous studies[3-6] we have shown that R1 and R2 are stable components of the ribosomal DNA loci within the genus *Drosophila*, and are evolving at similar rates to typical nuclear genes.

We cloned the 3′ half of R2 elements from the insects: *Tenebrio molitor* (mealworm), *Hippodamia convergens* (ladybird beetle), *Forficula auricularia* (earwig), *Anurida maritima* (collembola), and the non-insect arthropods: *Porcellio scaber* (isopod) and *Limulus polyphemus* (horseshoe crab). The 3′ half of R1 elements were cloned from *Dugusiella* sp. (tarantula) and *Scolopendra* sp. (centipede). The sequences were obtained by the polymerase chain reaction using one primer to the YADD region of the reverse transcriptase domain, and a second primer to the 28S rRNA gene downstream of the insertion sites[3–5].

These sequences were combined with the sequences of elements previously obtained from the insects *Sciara coprophila* (fungus gnat), *Bombyx mori* (silkmoth), *Nasonia vitripennis* (jewel wasp) and *Popillia japonica* (Japanese beetle)[7]. Multiple lineages of either R1 or R2 elements were found in several of these species. As found previously[5,7], the level of nucleotide divergence between elements is either less than 1% (the same family) or greater than 25% (different families).

To resolve the deep phylogenies of these R1 and R2 elements, conceptual translations of their open reading frames (minimum 360 and 480 amino-acid positions for R1 and R2, respectively) were used in phylogenetic reconstructions (Fig. 1). The phylogenies determined for R1 reveal that in each arthropod, at least one family of elements is congruent with the generally accepted phylogeny of the host species (lineages represented by bold lines), indicating stable vertical inheritance of the elements. Among the species with multiple R1 families, there are several examples where one of the families appears to be more divergent than expected (*D. takahashii*, b; *D. neotestecea*, b; jewel wasp, d). The R2 phylogeny (Fig. 1c) differs from the R1 phylogeny in that multiple families are only found in beetles.

There are two apparent violations of the host species phylogeny on the R2 tree: the families from Japanese beetle and mealworm are too divergent, branching before the other insects; and the *Drosophila* R2 elements are more similar to those of an earwig than to another dipteran (fungus gnat). Although these incongruities could be due to old horizontal transfers, as they represent older than expected divergences, it is more likely that different active lineages of R2 have been propagated in various insects.

This analysis of R1 and R2 sequences suggests these elements have been present during the entire history of the arthropod lineage, a time that is estimated to be at least 500 million years[8,9]. Because their insertion precludes the use of the 28S rRNA gene, R1 and R2 are presumed to be deleterious. How then do we account for their remarkable stability? Is it a case of selfish elements occupying an ideal niche in a conserved multigene family, or do these elements provide useful functions for their hosts which have for some reason maintained them as transposable elements?

Although these phylogenetic data cannot answer these questions, they do establish the potential for retrotransposons to be long-term hitchhikers of eukaryotic genomes. Indeed, if R1 and R2 are used as standards for divergence time, the lineages of many non-long-terminal-repeat retrotransposons appear to date back to the Cambrian era.

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