

COMPARISON OF ASTRONOMICAL AND DEVILS HOLE ICE AGES			
Bathythermal	Chronology (years before present)		Cycle duration (years)
	Devils Hole	Astronomical	
2	—	25,000	45,000
4	70,000	70,000	80,000
6	160,000	150,000	120,000
8	270,000	270,000	85,000
10	358,000	355,000	80,000
12	450,000	435,000	120,000
14	550,000	555,000	

period) to an average of about 90,000 years, and the amplitude increased, with the bathythermals deepening to produce intense glaciations<sup>7</sup>.

The last column in the table shows that the 'periodicity' of 90,000 years actually derives from the isotope cycles ranging

in duration one, two or three obliquity cycles, an apparent result of beating between the obliquity and eccentricity cycles<sup>8</sup>. Before about one million years ago, such beating was much more sporadic and obliquity was the dominant factor timing the ice ages<sup>7</sup>. Although it has long been recognized that eccentricity has a secondary effect on climate compared to obliquity, eccentricity may become important at

times when other conditions (the Tibetan uplift, for instance<sup>9</sup>) have driven climate to criticality.

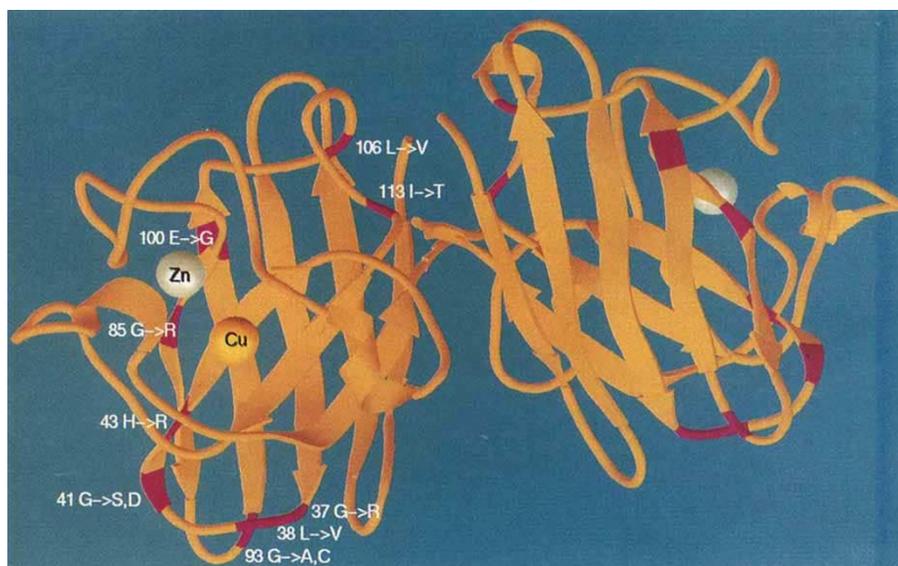
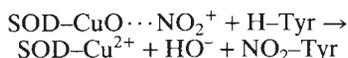
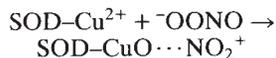
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## ALS, SOD and peroxynitrite

SIR — Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of large motor neuron nuclei in the spinal cord. Because muscle fibres are innervated by only a single motor neuron, each loss is particularly destructive. Interneurons surrounding motor nuclei produce nitric oxide that helps modulate synaptic plasticity<sup>1</sup> and may indirectly contribute to motor neuron destruction in ALS by Cu,Zn superoxide dismutase (SOD). Eleven unique mutations at nine distinct sites in SOD are associated with the autosomal dominant inheritance of familial ALS (see figure)<sup>2</sup>. Because afflicted ALS patients have one normal

SOD allele, SOD activity should decrease by at most 50 per cent, which would double the steady-state concentration of superoxide. Superoxide reacts with nitric oxide at a rate of  $6.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , three times faster than with native SOD, to form the powerful oxidant peroxynitrite (ONOO<sup>-</sup>)<sup>3</sup>. Peroxynitrite in turn reacts with SOD at  $\sim 10^5 \text{ M}^{-1} \text{ s}^{-1}$  to form a nitronium-like intermediate, which nitrates tyrosine residues<sup>4</sup>.



Protein backbone of human Cu,Zn SOD is shown looking down the active site to the copper, based on the human X-ray structure<sup>5</sup>. Mutant amino-acid substitutions are shown in red and are primarily localized on the ends of the  $\beta$ -barrel. Mutations at these diverse sites may push open the loops that form the superoxide-binding pocket and expose more of the copper for reaction with peroxynitrite.

The mutations identified in ALS patients do not directly affect conserved amino acids forming the active site, but could slightly disrupt the active-site pocket to allow greater access of peroxynitrite to the copper (see figure). Chemical modification of several amino acids in the active site allows SOD to react with peroxynitrite but greatly diminishes superoxide scavenging<sup>4</sup>. Thus, the SOD mutations may increase both peroxynitrite formation owing to reduced scavenging of superoxide and nitration by peroxynitrite of critical cellular targets.

Nitration of proteins will slowly injure motor neurons as well as other cells. However, motor neurons cannot regenerate. Motor neuron injury will accelerate as the nervous system adapts by rearranging synapses to other motor neurons, subjecting them to additional fluxes of nitric oxide. Peripheral nerve injury and chronic inflammation can induce nitric oxide synthase in spinal cord<sup>5,6</sup>. Motor neurons may also possess a target that is unusually susceptible to nitration, such as tyrosine kinases. For example, brain-derived neurotrophic factor rescues spinal motor neurons from cell death *in vivo* and binds to the receptor tyrosine kinase *trkB* (ref. 7). Altered phosphorylation also affects the abnormal assembly and distribution of neurofilaments observed in ALS.

Because native SOD also catalyses nitration by peroxynitrite, ALS in some patients with normal SOD genes may result from peroxynitrite formation due to mild trauma or inflammation near motor neurons. Therefore, caution should be exercised before attempting to treat ALS patients with SOD until the mechanisms accounting for the dominant action of the SOD mutants are better understood.

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