FIG. 3 a, An autoradiogram of the initial products of digestion showing that T5 5'-exonuclease has endonuclease activity. Lane 1, 5'-end 32P-labelled oligonucleotide markers of 34 and 16 nucleotides. Unlabelled template and adjacent strands were present at slight molar excess over the 34-mer flap strand (0.2 pmol). The sequence for the flap structure used was as in ref. 4. The substrates were incubated for 30 min at 37°C in 12 μl of 25 mM glycine/NaOH, pH 9.3, 5 mM MgCl₂ and 1 mM DTT with different concentrations of T5 exonuclease. Lane 2, 0.003 pmol enzyme; lane 3, 0.03 pmol enzyme; lane 4, 3.0 pmol enzyme; lane 5, 0.3 pmol enzyme. Reaction products were electrophoresed on a 15% polyacrylamide gel. The initial major products of digestion are 19 and 21 nucleotides in length, consistent with cleavage at the bifurcation. b, A conceptual model of how a flap structure could bind to the T5 5'-exonuclease (produced with RIBBONS®). Based upon previous experimental results (refs 3,7 and our own unpublished observations) we have made a model of the single-stranded flap DNA (ssDNA, blue) threaded through the helical arch. The placement of the DNA was chosen with the electrostatic potential surface contour as a guide. The flap DNA structure was docked on the protein, minimizing clashes between the two molecules. The model was not energy minimized.

site occupied by zinc was observed. Thus the distance between the two metal-binding sites we observe is much greater than the usual 4 A observed in nucleases with a putative two-metal-ion mechanism, and it is likely that the details of the 5'-exonuclease mechanism will be different.

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The two metal ions are shown as silver spheres. The precise position of the double-stranded DNA docked to the enzyme needs to be determined experimentally.

A more complete understanding of the mechanism of the enzyme will clearly require co-crystallization with DNA. Our structure will assist in the devising of the site-directed mutagenesis experiments required to elucidate the mode of action for this member of a biochemically important class of enzymes.

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CORRECTION

Two distinct mechanisms for long-range patterning in the Drosophila wing

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The correct address of M.C. is Centro de Biologia Molecular, Universidad Autonoma de Madrid, Canto Blanco, 28049 Madrid, Spain.

ERRATUM

A late Neanderthal associated with Upper Palaeolithic artefacts

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A TYPOGRAPHICAL error was introduced into the last line of Table 1(c), which should read UP – NE > NE – HS.

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