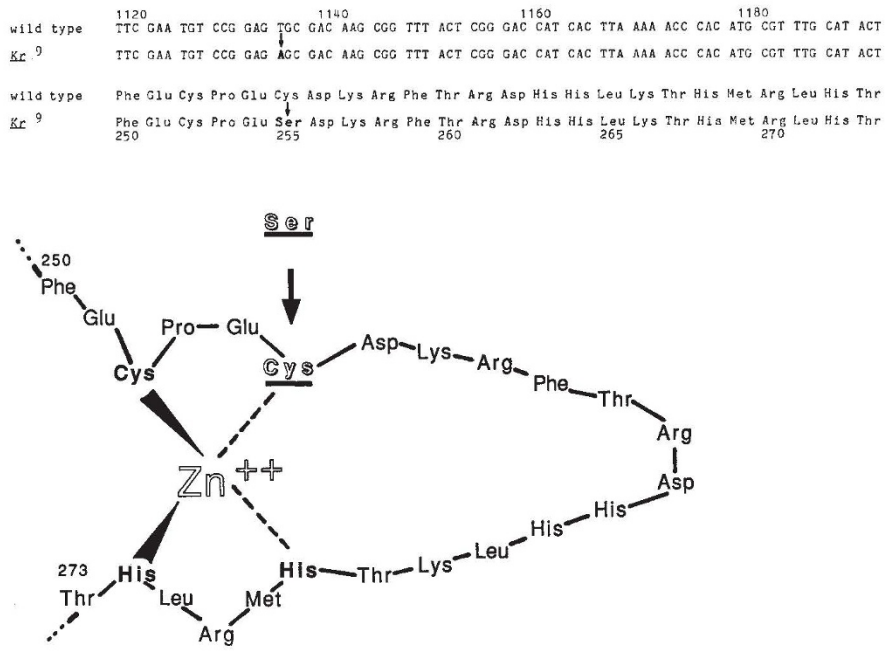

Fig. 3 The Kr^9 mutant is a point mutant leading to an exchange of Cys to Ser. Partial sequence of *Kr* wild-type and Kr^9 DNA. Note the single base-pair difference (transversion T to A; small arrow) between the *Kr* wild-type (first line; for the complete sequence see Fig. 2 in ref. 8) and Kr^9 sequence (second line) which results in a Ser residue in place of a Cys within the second finger region of the putative *Kr* finger protein domain (lines 3 and 4). The position of the exchange of the Cys to the Ser residue within the second putative *Kr* finger structure is schematically shown below (for orientation and the numbering of amino acids see Figs 2, 3 in ref. 8). Note that the exchange occurs in the finger core structure (possibly involving a zinc metal; not experimentally shown for *Kr* protein) that is required for the folding of the DNA-binding finger loop as proposed in the model by Klug and coworkers[4].

Methods. The Kr^9 DNA was isolated as 9 kb *Eco*RI fragment from a genomic DNA library which had been prepared from the DNA obtained from Kr^9/CyO flies[15]; Kr^9 DNA can be distinguished from CyO DNA by a restriction site polymorphism within the *Kr* gene (a *Sal*I site; see ref. 15). Sequencing was carried out[8] with two independent Kr^9 clones covering the sequences between the TATA-box and the first polyadenylation signal (Fig. 2 in ref. 8). To distinguish between trivial polymorphisms between Oregon R DNA (the *Kr* wild-type sequence; ref. 8) and the Kr^9 mutant DNA, we have sequenced the Kr^9 parental DNA in parallel. No difference was observed except that shown in *a*.

significantly in Kr^9 embryos (Fig. 2). Thus, the *cis*-regulatory elements for the spatial and temporal patterns of *Kr* gene expression are left unaffected by the Kr^9 mutation. This suggests that the mutation may cause a rather subtle alteration of the normal *Kr* protein which, however, completely eliminates *Kr* function as indicated by the *Kr* lack of function phenotype observed in Kr^9 embryos (Fig. 1).

Sequence analysis of the Kr^9 DNA revealed a single base-pair difference with respect to the wild-type sequence (Fig. 3; for the complete *Kr* wild-type sequence see ref. 8). This point mutation, a transversion from T to A, replaces a Cys residue by Ser (Fig. 3*a*). This exchange is located within the second of the four putative zinc fingers of the *Kr* protein (Fig. 3*b*). As this conservative amino-acid replacement eliminates the Kr^+ function completely, the SH group of Cys cannot be functionally replaced by the OH group of Ser. This suggests that the Ser–Cys replacement may prevent either the formation of a disulphide bridge or, in the model of finger structure proposed by Klug and coworkers[4], the binding of a metal ion such as zinc. Zinc binding is essential for the tetrahedrally-coordinated core structure (and requires two properly positioned pairs of Cys and His residues; Fig. 3) around which a finger loop can fold. The resulting finger structure is thought to bind to about six nucleotides, half a double-helical turn of DNA[4,18].

Recently, a second motif for metal-binding fingers has been observed in the members of the steroid/thyroid receptor family (see ref. 19). This finger motif is based on a core structure of four Cys residues, so that the pair of His residues (Fig. 3*b*) is replaced by a second pair of Cys[19]. Our results clearly show that a replacement of Cys in the core structure of the TFIIIA-related finger motif (Fig. 3) eliminates the biological activity of the protein, possibly by preventing the formation of the tetrahedrally-coordinated zinc centre. This could result in the lack of *Kr* function directly or it may eliminate the biological activity due to an improper folding of the entire *Kr* finger domain caused by the mutation of the second finger.

Note added in proof: A similar study on ADR1[21], a positive regulator of the ADH2 gene in yeast, revealed that mutations of the Cys or His residues result in non-functional protein. The changes in ADR1, however, were less subtle than the replacement of a SH-group by an OH-group, a relatively minor alteration which completely eliminates *Kr* function.

We thank our colleagues in the laboratory for various contributions, in particular Drs S. M. Cohen and M. P. Pankratz for critically reading the manuscript. U.G. is a Deutsche Studienstiftung fellow. The work was supported by the Deutsche Forschungsgemeinschaft (Leibniz-Programm).

Received 15 December 1987; accepted 19 January 1988.

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Erratum

Parallel gradualistic evolution of Ordovician trilobites

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Nature **330,** 561–563 (1987).

In Fig. 4 legend of this letter, the symbols for the means with 95% confidence interval and number of measurements should read —●—$_n$ and ⊖$_n$.