Cell, Vol. 47, 1025–1032, December 26, 1986, Copyright © 1986 by Cell Press 

# A Conserved Family of Nuclear Proteins Containing Structural Elements of the Finger Protein Encoded by *Krüppel*, a Drosophila Segmentation Gene

**Reinhard Schuh,* Wilhelm Aicher,*† Ulrike Gaul,* Serge Côté,* Anette Preiss,*‡ Dieter Maier,*‡ Eveline Seifert,* Ulrich Nauber,* Christian Schröder,* Rolf Kemler,§ and Herbert Jäckle***

* Max-Planck-Institut für Entwicklungsbiologie
Spemannstrasse 35/II
§ Friedrich-Miescher-Laboratorium
der Max-Planck-Gesellschaft
Spemannstrasse 37
D-7400 Tübingen, Federal Republic of Germany

## Summary

***Krüppel* (*Kr*), a segmentation gene of Drosophila, encodes a protein sharing structural features of the DNA-binding "finger motif" of TFIIIA, a Xenopus transcription factor. Low-stringency hybridization of the *Kr* finger coding sequence revealed multiple copies of homologous DNA sequences in the genomes of Drosophila and other eukaryotes. Molecular analysis of one *Kr*-homologous DNA clone identified a developmentally regulated gene. Its product, a finger protein, relates to *Kr* by the invariant positioning of crucial amino acid residues within the finger repeats and by a stretch of seven amino acids connecting the finger loops, the "H/C link." This H/C link is conserved in several nuclear and chromosome-associated proteins of Drosophila and other eukaryotic organisms including mammals. Our results demonstrate a new subfamily of evolutionarily conserved nuclear and possibly DNA-binding proteins that again relate to a Drosophila segmentation gene as in the case of the homeo domain.**

## Introduction

The best-characterized protein structure involved in DNA binding is the helix–turn–helix motif conserved in a variety of prokaryotic regulatory proteins (Pabo and Sauer, 1984), in the yeast *MAT* repressor (Shepherd et al., 1984; Laughon and Scott, 1984), and in the homeo domains of several eukaryotic proteins (McGinnis et al., 1984; Gehring, 1985). A second motif for DNA-binding proteins emerged from sequence analysis of TFIIIA (Miller et al., 1985), a factor involved in the control of transcription of the Xenopus 5S RNA gene. This alternative structure is based on a tetrahedrally coordinated $Zn^{2+}$ ion that allows the folding of tandemly repeated DNA-binding "finger" structures (Miller et al., 1985). The primary structures of several other regulatory proteins are homologous to the TFIIIA protein (Berg, 1986; Vincent, 1986). They include ADR1, which is required for transcriptional activation of the alcohol dehydrogenase gene in yeast (Hartshorne et al., 1986), and Drosophila protein sequences deduced from the DNA sequences of *serendipity* (Vincent et al., 1985) and of *Krüppel* (*Kr*), a segmentation gene (Rosenberg et al., 1986). Aside from having in common the finger structure, these four and other finger proteins (see Berg, 1986) seem not to be directly related with respect to homologous DNA and protein sequences (Vincent, 1986). However, by use of the *Kr* cDNA probe under conditions of low stringency, we have now isolated a second Drosophila gene encoding a *Kr*-like finger protein. Analysis of this gene revealed *Kr*-related molecular properties that are conserved in a small number of genes in Drosophila and in other eukaryotic organisms.

## Results

### Isolation of *Kr*-Homologous Sequences from Drosophila

Southern blots of EcoRI-digested genomic Drosophila DNA were hybridized at low stringency with the *Kr* probe. This probe, the 544 bp BamHI–SalI fragment containing the *Kr* finger domain coding sequence (see Rosenberg et al., 1986), and hybridized to a series of bands (Figure 1a) that were not detected under normal-stringency conditions (see Preiss et al., 1985) or under conditions that revealed the homeo-box-containing genes (data not shown). The multiple bands in the Southern blots suggest the existence of several Drosophila genes that are related to the finger coding region of the *Kr* gene.

To search for such genes, we screened approximately 50,000 recombinant bacteriophages (five genome equivalents) from a genomic Drosophila DNA library under low-stringency conditions. After rescreening the bacteriophages, we picked 22 plaques with positive signals of different intensities. From each clone we purified the DNA, digested it with EcoRI, and prepared Southern blots. These were hybridized, at low stringency, with the *Kr* probe or with DNA from each of the clones isolated. This allowed us to identify eight different fragments of genomic DNA with homology to the *Kr* finger domain. Clones were isolated twice or several times, suggesting that we have identified most of the *Kr*-homologous genes.

### *Kr*-Homologous Transcripts Are Developmentally Regulated

Each of the isolated *Kr*-homologous clones showed one EcoRI fragment bearing the *Kr*-homologous sequence. One of them, a 6 kb Eco RI fragment (designated *Kr h*; Figure 1b), was analyzed in detail. Within the 6 kb fragment two nonadjacent regions showed weak *Kr* homology (Figure 1b). To see whether this 6 kb fragment carries transcribed sequences, we hybridized it to Northern blots with poly(A)$^+$ RNAs from embryos at different stages during early embryogenesis (Figure 1c). The finding of several bands with different intensities at different early stages

† Present address: Max-Planck-Institut für Biologie, Spemannstrasse 34, D-7400 Tübingen, Federal Republic of Germany.
‡ Present address: Department of Biology, Yale University, New Haven, Connecticut.

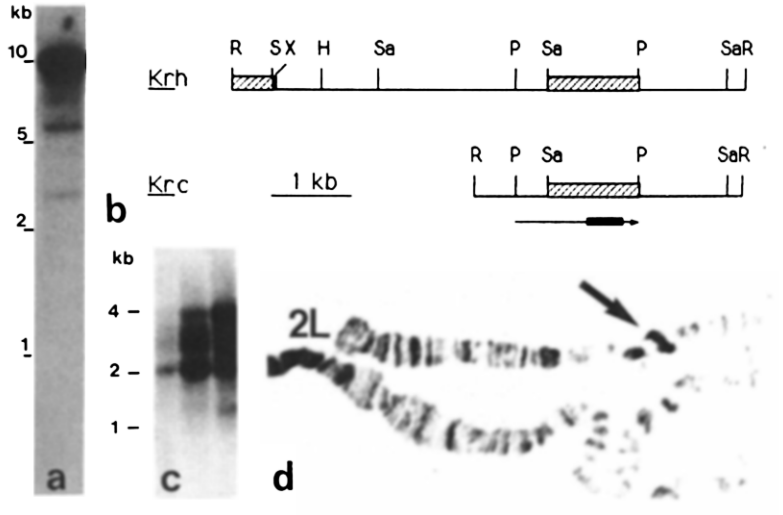


Figure 1. Detection of DNA Sequences Homologous to the *Kr* Finger Coding Region by Southern Blotting, and Characterization of the *Kr h* DNA

(a) Southern blot of genomic Drosophila DNA hybridized with the *Kr* probe at low stringency. Note bands in addition to the 9.5 kb fragment not seen under normal-stringency conditions.

(b) Restriction map of *Kr h*, including the *Kr* probe hybridizing fragments (dashed areas), and localization of the cDNA clone *Kr c*. Sequenced region within *Kr c* (see Figure 3 and Table 1) is indicated by arrows. Restriction sites: S, SalI; P, PstI; R, EcoRI; H, HindIII; Sa, SacI; X, XbaI.

(c) Northern blot loaded with similar amounts of poly(A)$^+$ RNA from 0–2, 2–4, and 8–24 hr old embryos (left to right) hybridized with the *Kr h* fragment.

(d) Localization of the *Kr h* DNA in Drosophila chromosomes using biotinylated probes. A single hybridization signal is observed, on the left arm of the second chromosome (2L) at position 26A/B (arrow).

suggested temporal control of differentially spliced transcripts and/or different transcripts coded by *Kr h* DNA. To analyze these transcripts further and to establish the basis of *Kr* homology, we isolated several *Kr h* cDNA clones of various lengths under normal-stringency conditions from a library prepared from early embryo poly(A)$^+$ RNA (Rosenberg et al., 1986). All of these cDNA clones showed a signal under low-stringency hybridization conditions with the *Kr* probe. For further analysis, we used the *Kr c* clone, which is 3.8 kb (Figure 1b). This clone most likely represents a close-to-full-size copy of the largest *Kr h* transcript (Figure 1c), and detects the same pattern of transcripts as seen with the *Kr h* probe (data not shown).

In situ hybridization of the *Kr c* DNA probe to tissue sections of embryos revealed an unusual pattern of transcript accumulation (Figure 2). Label accumulates over most cells of the blastoderm stage embryo. However, there are clear signs of patchy transcript accumulation along the longitudinal axis of the blastoderm stage embryo (see Figure 2a) and of different levels of transcripts in mesoderm and ectoderm during late gastrulation (Figure 2b). This distribution of the *Kr h* transcripts suggests an underlying

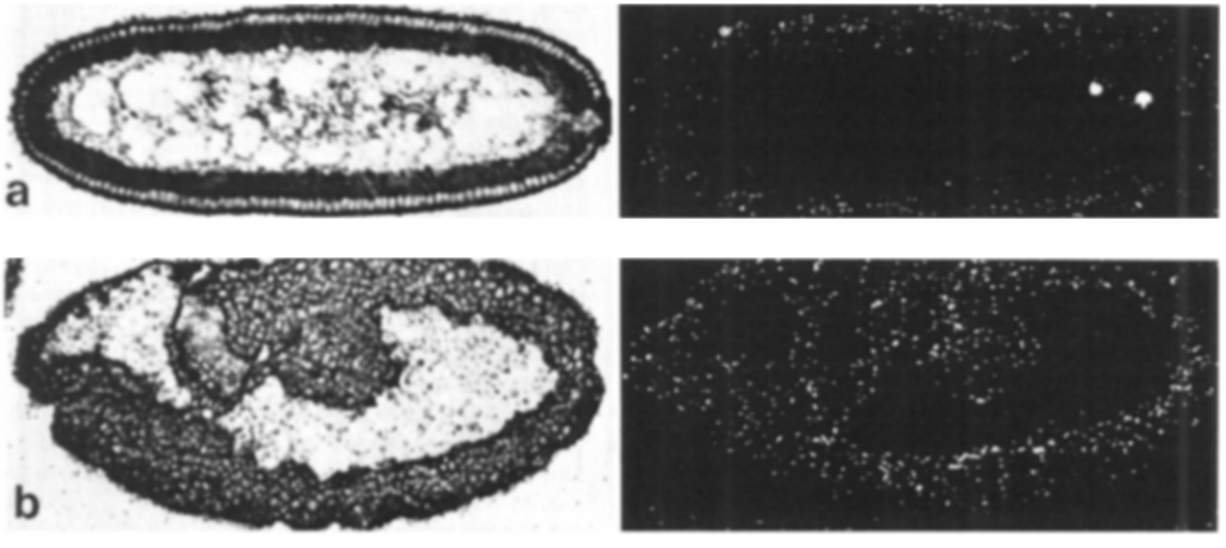


Figure 2. Expression of the *Kr h* Gene in Early Wild-Type Embryos

The bright-field photomicrographs show sections through embryos that were hybridized with the *Kr c* probe. The pattern of *Kr h* transcripts is shown in the accompanying dark-field images. Anterior is to the left. (a and c) Horizontal section through an embryo at blastoderm stage. (b and d) Parasagittal section through a germ-band extended embryo. Note that *Kr h* transcripts accumulate in a patchy pattern along the anteroposterior axis of the blastoderm stage embryo (a), and enrich over the mesoderm (b). Note further that the images possibly represent overlapping patterns of transcripts due to at least four different poly(A)$^+$ RNA species revealed on Northern blots (see Figure 1d).

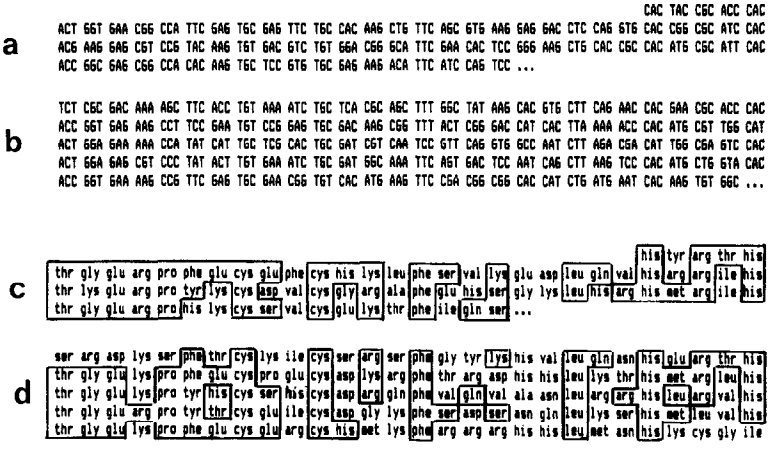


Figure 3. DNA and Amino Acid Sequence of the Finger Region of *Kr c* in Comparison with the Sequence of the *Kr* Finger Domain

(a) Partial *Kr c* DNA sequence. (b) *Kr* finger DNA sequence (for details, see Rosenberg et al., 1985). (c) Amino acid sequence predicted from the single open reading frame of *Kr c* shown in (a). (d) *Kr* finger amino acid sequence. Conserved regions are shown in boxes. For the finger folding schemes and a comparison of *Kr c* and *Kr* finger proteins with other members of the metal-binding Cys-Cys/His-His finger protein family see Table 1, Miller et al. (1985), Berg (1986), and Rosenberg et al. (1986).

spatial control that may be complicated because of several temporally regulated transcripts being recognized by the *Kr c* probe. All of these transcripts contributing to the patchy pattern of expression correspond to a single *Kr h* gene (see below).

### *Kr h* DNA Corresponds to a Single Finger Protein Coding Gene

In situ hybridization of the *Kr h* DNA to polytene salivary gland chromosomes revealed a single site in the 26A/B region on the left arm of the second chromosome. Unfortunately, there is no mutant known for this region that would allow us to analyze the *Kr h* function directly by classical genetics. Thus, we tried first to establish the molecular relation of *Kr* and *Kr h* by means of sequence comparison. For this comparison we used the *Kr c* clone (Figure 1b).

The signal-bearing Sau 3A fragment within the PstI–SacI segment (present in both genomic and cDNA; Figure 1b) was subcloned in M13 phage and was sequenced (Figure 3). A single open reading frame of the 260 bp sequence encodes a predicted 28 amino acid repeat unit as seen in the *Kr* protein (Rosenberg et al., 1986). This repeat unit contains the structural elements to fold at least two adjacent fingers, separated and followed by a seven amino acid link (Figure 3). Both the positions of crucial amino acids within the finger loops (see Miller at al., 1985, for details), involving a stringent positioning within the CysXXCysXXXPheXXXXXLeuXXHisXXXHis structural loop element ("*Kr* motif"), and the seven amino acid links between the fingers (Tyr-Gly-Glu-Arg or Lys-Pro-Phe or Tyr-X; "H/C-link") were identical to those of the *Kr* finger protein (Figure 3 and Table 1). Both these motifs were similar but not identical to the finger motifs of TFIIIA, ADR1, and the *serendipity* protein (see Table 1).

### *Kr*-Homologous Sequences in the Genomes of Various Eukaryotes

Encouraged by the recovery of a *Drosophila* gene that codes for a *Kr*-related finger protein, we searched for *Kr*-homologous sequences in other species. Southern blots loaded with EcoRI-digested DNA from various animals revealed multiple *Kr*-homologous DNA fragments in all eukaryotes tested, but not in bacteria (see Figure 4). This suggests that the *Kr*-related sequences are conserved in several copies in the genomes of eukaryotes ranging from yeast to mammals.

### The H/C Link Is Present in Several Nuclear Proteins and in Chromosome-Associated Proteins of Drosophila

Conservation of the H/C link allowed the *Kr h* gene to be isolated by mismatch hybridization at low stringency (see

Table 1. Comparison of the *Kr h* Finger Domain Sequences with Sequences of Other Proteins Containing the Cis-Cis/His-His Metal-Binding Finger Motif

| | | 1 | $X_2$ | 4 | $X_3$ | 8 | $X_5$ | 14 | $X_2$ | 17 | $X_3$ | 21 | H/C linK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Kr h* | (a) | C | XX | C | XXX | F | XXXXX | L | XX | H | XXX | H | TGEKPYX |
| *Krüppel* | (b) | C | XX | C | XXX | F | XXXXX | L | XX | H | XXX | H | TGEKPYX |
| Mouse *Mkr* | (c) | C | XE | C | GKT | F | XXXSX | L | IX | H | QRI | H | TGEKPYE |
| *serendipity* | (d) | C | XX | C | GKX | F | SXXXX | L | XX | H | MOX | H | XXXXXXXX |
| TFIIIA | (e) | C | XXDG | C | DKR | F | TKKXX | L | KXR | H | XXXX | H | XXXXXXYV |
| ADR1 | (f) | C | XX | C | XRX | F | XRXXX | L | XXR | H | XXXX | H | XXXXXYX |

Note that sequences (d)–(f) represent consensus repeats of the multifinger proteins, and that the highlighted amino acid residues in sequences (a)–(c) are found in all 28 amino acid repeats. Data are from (a) this work, (b) Rosenberg et al. (1986), (c) Chowdhury and Gruss (personal communication), (d) Vincent et al. (1985), (e) Miller et al. (1985), and (f) Hartshorne et al. (1986).

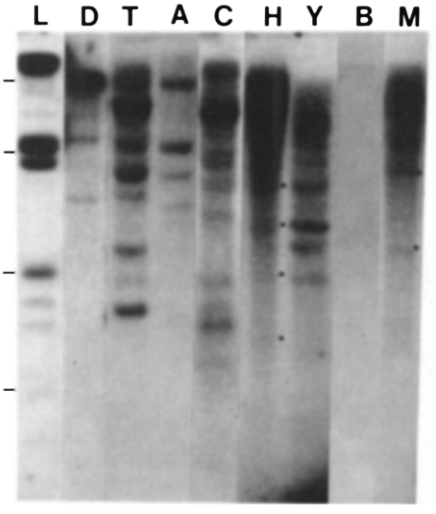


Figure 4. Detection of DNA Sequences Homologous to the *Kr* Finger Coding Region in the Genomes of Various Eukaryotes but Not of Bacteria

Southern blots of EcoRI-digested DNA from each species were hybridized with the *Kr* probe at low stringency (see Experimental Procedures). L, λ size marker DNA; lines to the left refer to 10 kb, 5 kb, 2 kb, and 1 kb fragments (top to bottom). D, Drosophila (see also Figure 1a). T, Tegenaria (spider). A, Artemia (crab). C, Ciona (tunicate). H, Hydra. Y, yeast. B, Escherichia coli. M, mouse. Exposures (1–3 days) were adjusted to optimize the visibility of bands. Dots indicate positions of bands not clearly resolvable in a smeary background, but seen in the original. The hybridization and washing conditions of Southern blots are identical to those described for Figure 1.

Figure 3). This in combination with the conserved *Kr* motif suggested that both *Kr* and the *Kr h* gene encode proteins with a similar biochemical function, which, in view of the structural homology with TFIIIA and ADR1, could involve DNA binding and transcriptional activation. Several attempts using β-galactosidase–protein fusions, which allowed DNA binding studies on homeo domain proteins (Desplan et al., 1985), failed with the finger proteins (U. Gaul and C. Schröder, unpublished observations). Nevertheless, to obtain information on the possible function of the *Kr*-related members of the finger protein family, we raised antibodies against a 12 amino acid peptide (see Experimental Procedures) connecting *Kr* fingers one and two, which includes the H/C link (see Figure 3d, second line). These antibodies were used to study the distribution of H/C-link-containing protein(s) in cells and eventually on chromosomes.

Affinity-purified antibodies against the H/C link recognize nuclear antigens in cleavage stage embryos (Figures 5a and 5b). These nuclei, in the absence of zygotic transcription (Anderson and Lengyel, 1979), possibly accumulate proteins of maternal origin and/or newly synthesized proteins from maternal mRNA. Western blots prepared from nuclear extracts of 0–2 hr old embryos revealed several distinct proteins recognized by the antibodies. In 4–24 hr old embryos, some of these proteins could not be detected anymore (Figures 5g and 5h). This argues for a family of nuclear proteins defined by a common structural element, the H/C link of the *Kr* finger domain, and for developmental control of different members of this family.

The observation that the H/C link is most likely encoded by several maternally active genes (see above), by *Kr*, a blastoderm gastrulation-specific segmentation gene, and by the *Kr h* gene, which extends its action into later embryonic stages, encouraged studies with the anti-H/C link antibodies on polytene salivary gland chromosomes. If the antigen were present on chromosomes, this then would argue for interaction with chromatin, for DNA and/or nuclear RNA binding. The anti-H/C link antibodies recognize a small number of chromosome bands, including transcriptionally active puffs. Examples of antibody reactions with several bands and one puff (85EF) of the 3R chromosome are shown in Figures 5c–5f.

### H/C Link Nuclear Antigen Is Conserved in Vertebrates

The presence of *Kr*-homologous sequences in all eukaryotes analyzed (Figure 4) suggested that the H/C link could again be the basis of the DNA sequence homology. Thus, we should expect the H/C link to be associated with vertebrate proteins. By analogy with Drosophila, the antibodies directed against the H/C link should reveal nuclear antigen, for example, in mammalian cell lines and/or in some nuclei of vertebrate embryos. We studied the anti-H/C link activity on cryostat sections of chicken and mouse embryos, and with several cell lines of mouse, bovine, rat, and human origin. In each material tested, fluorescent staining was concentrated over nuclei, and within the limits of the technique applied, the surrounding cytoplasm was negative. Examples of this study, which is summarized in Table 2, are shown in Figure 6. During mitotic divisions, fluorescence was always found to be associated with chromosomes (see examples in Figures 6e–6h). This indicates that the corresponding proteins containing an H/C link are associated with chromatin, as has been seen more clearly on single Drosophila polytene chromosome bands (Figures 5c–5f).

The observation that all nuclei from all cell lines analyzed contain the H/C link antigen suggested several different proteins, as in the case of Drosophila embryos, rather than a single ubiquitous protein species. To test this assumption, we prepared nuclear extracts from the embryonic mouse carcinoma PCC4 cell line. Western blots containing the nuclear extracts of PCC4 cells revealed several proteins containing the H/C link antigen (Figure 6p).

### Discussion

The primary structures of several transcriptional regulatory proteins, including TFIIIA of frog and ADR1 of yeast, allow the folding of tandemly repeated DNA-binding "finger loops" that are linked by a short and variable stretch of up to eight amino acids (see Table 1; for details, see Miller at al., 1985; Hartshorne et al., 1986). The finger motif for DNA binding, which possibly involves coordinated metal binding, emerged from sequence analysis of TFIIIA. Each finger is thought to specify binding to about six nucleotides, half of a double-helical turn of DNA (Miller et

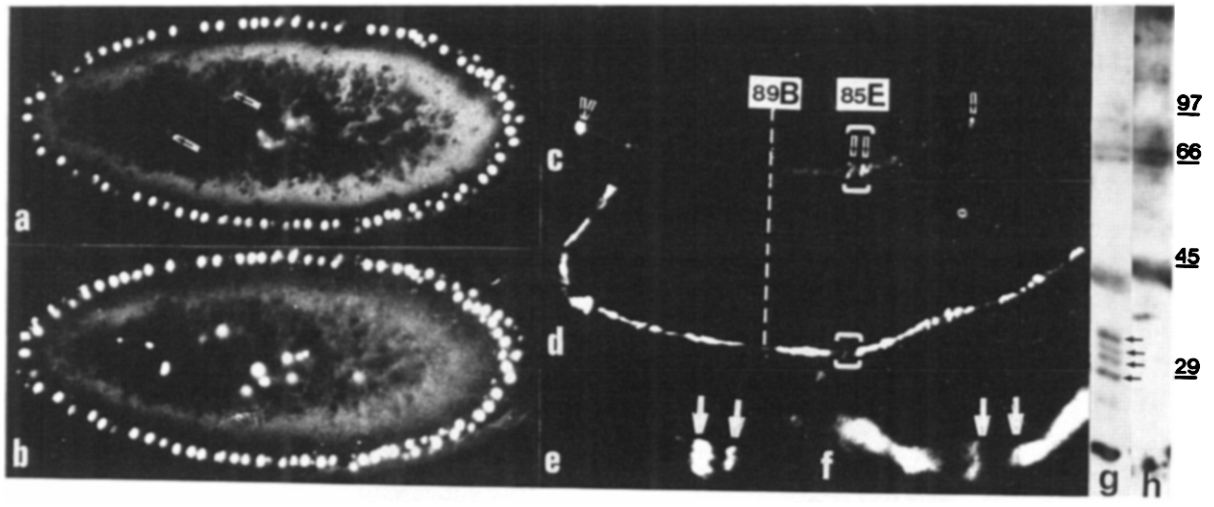


Figure 5. Localization of H/C Link Antigen in Early Embryos and Polytene Chromosomes of Drosophila

Polyclonal antibodies against the H/C link peptide between *Kr* fingers one and two (second line in Figure 3d; see Experimental Procedures) were incubated with Drosophila embryos and salivary gland polytene chromosome squashes; samples were counterstained with DAPI (see Experimental Procedures) to visualize the DNA distribution. (a) Parasagittal section through an embryo after the eighth nuclear division. Note the signal over all peripheral nuclei but not over yolk nuclei (arrows). (b) DAPI image of the same section. (c) Fluorescence signals (arrows) are over the right arm of chromosome 3R. Note that one band is in the puff region 85EF (between brackets; enlargement in [e] and [f]). Dotted line serves as a landmark for chromosome position 89B. (d) DAPI image of the chromosome. (e) Enlarged puff in chromosome region 85EF. Note that the signals are adjacent to and over a DAPI-stained band, respectively (arrows). (f) DAPI image of the same enlarged region . At right are Western blots of nuclear extracts of 0–2 hr old (g) and 4–24 hr old (h) embryos. Note that some bands (arrows) are detected in the earlier but not in later embryos. Apparent molecular weights of marker proteins are given in kd. For details see Experimental Procedures.

al., 1985; Rhodes and Klug, 1986). A similar, invariant finger motif was found in *Kr*, a Drosophila segmentation gene (Rosenberg et al., 1986), and in *Kr h*, a developmentally regulated gene expressing several temporally and spatially controlled transcripts. The function of this gene has not yet been established by genetic analysis. However, the *Kr h* gene product is similar to the *Kr* protein.

The striking similarity of *Kr* and *Kr h* is based on the finger motif per se, an invariant positioning of crucial amino acids within the finger loop, and the H/C link, which is the basis of the nucleotide homology (Figure 3). The latter suggests the existence of a small subfamily of *Kr*-related finger proteins, which is reflected in multiple and conserved *Kr*-homologous DNA sequences and in conservation of several H/C-link-containing nuclear proteins.

The fact that TFIIIA binds to both DNA and RNA in a specific and regulatory manner (Miller et al., 1985) suggests that the *Kr*-related members of the finger protein family may bind also to DNA and/or RNA. However, antibodies produced against an H/C link peptide recognize

Table 2. Recognition and Intracellular Distribution of H/C Link Antigen in Embryonic Tissues and in Cultured Cells

| Organism | Cell Line | Source | Localization | | Remarks |
|---|---|---|---|---|---|
| | | | Nuclei | Cytoplasm | |
| Bovine | BCEC | Capillary endothelium[a] | + | – | |
| | FBHE | Fetal heart endothelium[b] | + | – | |
| Chicken | – | 2.5 day old embryo | + | – | See Figures 6a and 6b |
| | – | 16 day old trachea | +, (+) | – | Different intensities of nuclear signals |
| Human | HEP-2 | Hepatoma[b] | + | – | |
| | MRC-5 | Fibroblast[b] | + | (–) | See Figures 6c and 6d |
| Rat | NRK | Fibroblast[b] | + | – | See Figures 6e–6h, 6l, and 6m |
| Mouse | – | 10 day old embryo | + | – | |
| | PCC4aza1 | Embryonal carcinoma[c] | + | (–) | See Figures 6i and 6k |
| | 3T3 | Fibroblast[b] | + | – | |
| | | Primary fibroblasts from 15 day old embryos | + | – | See Figures 6n and 6o |

[a] Folkman et al. (1979).
[b] American Tissue Type Culture Collection.
[c] Nicolas et al. (1975).

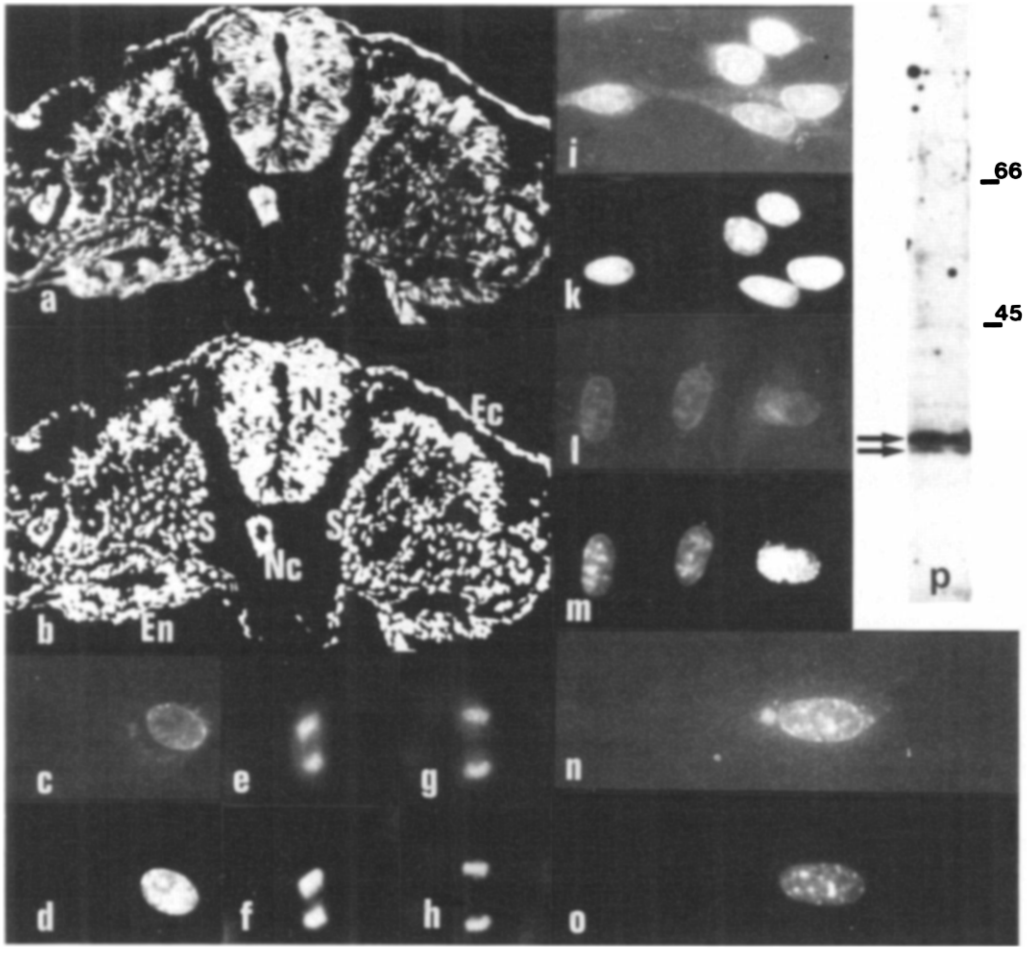


Figure 6. Localization of H/C Link Antigen in the Nuclei of Chicken Embryos and Tissue Culture Cells of Various Origins

(a) Crosssection through a 2.5 day old chicken embryo. Note that all nuclei show fluorescent staining indicative of the H/C link antigen. (b) DAPI image of the same embryo; see Experimental Procedures. N, neural tube; Ec, ectoderm; S, somites; Nc, notocord, En, endoderm. (c), (e), (g), (i), (l) and (n) show tissue culture cells of different origins incubated with antibodies directed against the H/C link antigen; (d), (f), (h), (k), (m), and (o) show the corresponding samples counterstained with DAPI to visualize nuclei. (c and d) Human fibroblast (MRC-5). (e and f) Rat fibroblasts (NRK) in metaphase. (g and h) NRK cells in metaphase; chromosomes are already separated in daughter nuclei. (i and k) Mouse embryonic carcinoma cells (PCC4). (l and m) NRK cells in interphase. (n and o) Primary mouse fibroblast cells from BALB/c embryos. (p) Western blot loaded with nuclear extracts from PCC4 mouse embryonic carcinoma cells. Note two bands reacting with the anti-H/C link antibodies (arrows). Apparent molecular weights of marker proteins are given in kd. For details see Experimental Procedures. See also Table 2 for a summary of additional data.

nuclear antigens present in several proteins, which appear to be localized in bands and puffs of polytene salivary gland chromosomes of *Drosophila*, and are associated with mammalian, chromosomal antigen. These observations argue against binding to cytoplasmic RNA and provide evidence for the H/C link being associated with DNA- and/or nuclear-RNA-binding proteins.

The invariant features that emerged from the *Kr* and *Kr h* comparison are shared by a mouse gene that was recently isolated in P. Gruss's laboratory in Heidelberg under the conditions described here (see Table 1; Chowdhury and Gruss, personal communication). In addition, these features are conserved in different domains of TFIIIA, the prototypical finger protein. In TFIIIA, only finger 7 out of the nine fingers shows the *Kr*-related positioning, and the H/C link is found between fingers 1 and 2 (compare Miller et al., 1985, and Figure 3). This observation may provide the first evidence for the proposal of Miller et al. (1985) that the DNA-binding finger proteins may have emerged from a common ancestral finger motif, and that multifinger proteins may have arisen from gene duplications and/or conversions.

In contrast to the homeo domain, which possibly relates to a conserved helix–turn–helix motif for DNA binding (for review, see Gehring, 1985; Laughon and Scott, 1984), the coding sequences of several identified finger proteins (for review, see Vincent, 1986; Berg, 1986) were thought to lack significant nucleotide sequence conservation and "box" character. This was taken to indicate that the evolution of these proteins is constrained by the requirement to maintain basic features of a protein structure that interacts with DNA, and that the specificity of protein–DNA interaction is provided by a combination of amino acids in the "fingertips," as outlined in the finger model of Miller et

al. (1985). The conservation of the two features described above, especially the finding of the H/C-link motif (which has, in fact, box character), would argue that the specificity of DNA binding may involve a defined positioning of the crucial amino acid residues and the link region between the fingers.

The DNA binding and regulatory functions of TFIIIA and ADR1, and the chromosome binding properties of H/C-link-containing proteins, suggest that the members of the *Kr*-related gene family share DNA binding as a common function. In this view, our simple approach has opened the possibility of isolating and characterizing additional members of a widely spread, evolutionarily conserved family of genes that act at the level of chromatin, possibly on DNA directly. In contrast to the homeo box, which seems to be strongly conserved in the classes of genes required for pattern formation in Drosophila (Gehring, 1985), the finger protein family appears to be involved in more general regulatory functions in several, if not all, eukaryotic organisms.

## Experimental Procedures

### Nucleic Acid Preparations and Hybridizations

DNA of Drosophila and other eukaryotes was prepared according to a standard protocol (Preiss et al., 1985). Poly(A)$^+$ RNA was prepared from staged embryos as described in Rosenberg et al. (1986).

Southern blots of EcoRI-digested DNA from Drosophila were prepared as described previously (Preiss et al., 1985). Hybridization to a nick-translated $^{32}$P-labeled BamHI–SalI fragment of the pcK 2b clone (Rosenberg et al., 1986) was carried out at 60°C in 5× SSPE, 5× Denhardt's solution, 0.2% SDS, and 100 μg/ml of denaturated herring sperm DNA using $10^6$ cpm/ml of labeled probe ($10^8$ cpm/μg) for Southern blots and under previously described conditions (Rosenberg et al., 1986) for Northern blots. (1× SSPE = 180 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA.) The Southern blots were washed at 37°C in 4× SSPE, 0.2% SDS for 2 hr and at room temperature in 2× SSPE, 0.2% SDS for 1 hr. Exposure was overnight on preflashed Fuji X-ray film at −80°C. Northern blots were washed in 2× SSPE, 0.2% SDS for 1 hr at 60°C and were exposed as described for Southern blots.

### DNA Sequencing

DNA was sequenced from M13 subclones by the dideoxy chain termination method of Sanger as described previously (Rosenberg et al., 1986).

### In Situ Hybridizations

In situ hybridizations to polytene chromosomes were as described in Mlodzik et al. (1985). In situ hybridization to tissue sections of embryos was performed as described in Knipple et al. (1985) except that $^{35}$S-labeled nick-translated DNA was used.

### Production and Purification of Antibodies

A twelve amino acid peptide (TGEKPFECPECD; H/C link) produced by Novabiochem (Switzerland) was coupled to bovine serum albumin from Sigma (A 7030) using 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide as described by Shapira et al. (1984). The rabbit was injected (subcutaneously and intramuscularly) with this conjugate emulsified in Freund's adjuvant. After a single booster injection the rabbit was bled on a weekly schedule. The serum was affinity-purified on a peptide–ovalbumin–Affigel 10 column as outlined by Carroll and Scott (1985). Specificity of antibodies was tested on Western blots (see below) of a *Kr*–Lac Z fusion protein (data not shown).

### Western Blotting

Nuclear extracts prepared from staged Drosophila embryos and from PCC4 cells were fractionated on a 10% SDS–polyacrylamide gel and were transferred to nitrocellulose (Frasch, 1985). Western blots were first incubated (30 min) with 0.5% Tween 20 in phosphate-buffered saline (PBS) and then in the same solution containing primary antibody (10 μg/ml, overnight at 0°C). Blots were washed (10 min at room temperature) three times in the Tween 20–PBS solution and then allowed to react (120 min, 20°C) with 5 μCi of $^{125}$I-labeled protein A (Amersham; spec. act. 30 mCi/mg). After repeated washes as above, blots were dried and exposed to Fuji X-ray film.

### Immunofluorescence on Cryostat Sections of Embryos and on Polytene Chromosomes of Drosophila

Embryos were dechorionated, permeabilized, and fixed as described in Carroll and Scott (1985) except that we used paraformaldehyde for fixation. After rehydration, embryos were embedded and sectioned as described by Dequin et al. (1984). Cryostat sections (5–10 μm) were collected on slides, incubated (30 min) with PBT (1% bovine serum albumin and 0.1% Triton X-100 in PBS) and then in the same solution containing 1–10 μg/ml affinity-purified antibodies (1 hr at room temperature). After two washes in PBT (10 min at room temperature), sections were incubated (30 min) with fluorescein isothiocyanate (FITC)–conjugated goat anti-rabbit antibodies (Dianova; 1:100 dilution) and were washed as described above. After being counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Frasch, 1985), sections were mounted in 80% glycerol–PBS. Sections were viewed under a Zeiss epifluorescence microscope and were photographed with a Kodak Plus-X pan film.

Polytene chromosomes of salivary glands were prepared as described by Frasch (1985). Immunofluorescence and DAPI staining were as described above.

### Immunofluorescence on Cryostat Sections of Vertebrate Embryos and on Cultured Cells

Postimplantation mouse embryos (10 days) were removed from the maternal decidua and were immediately frozen in Tissue-Tek II (Lab-Tek Products). Sections were cut on a cryostat (Reichert-Jung), fixed for 10 min in precooled methanol–acetone (40/60 v/v), and washed in PBS (pH 7.2). Cell cultures (see Table 2) were grown in Dulbecco's modified Eagle's medium containing 15% fetal calf serum on cover slips for 2 days, washed in PBS (pH 7.2), and fixed for 10 min in methanol–acetone (−20°C). For indirect immunofluorescence tests, binding of anti-H/C link antibodies (15 μg/ml, 30 min) was revealed with FITC-conjugated goat anti-rabbit antibodies (Dianova; 1:100 dilution, 30 min at room temperature). Sections through 2.5 day old chicken embryos were prepared and treated the same way. After being counterstained with DAPI (see above), the specimens were examined under a Leitz Dialux 20 fluorescence microscope, and photographs were taken using Ilford HP-5 film.

### Acknowledgments

We thank Drs. D. Marcey, S. M. Cohen, D. Tautz, P. Gruss, I. Baxivanelis, and A. Kienlin for their many contributions. S. C. was supported by an NSERC of Canada postdoctoral fellowship. The work was supported by a Deutsche Forschungsgemeinschaft grant (Leibniz program) to H. J. U. G. is a Studienstiftung des Deutschen Volkes fellow.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 8, 1986.

### References

Anderson, K. V., and Lengyel, J. A. (1979). Rates of synthesis of major classes of RNA in Drosophila embryos. Dev. Biol. *70*, 217–231.

Berg, J. M. (1986). Potential metal-binding domains in nucleic acid binding proteins. Science *232*, 485–486.

Carroll, S. B., and Scott, M. P. (1985). Localization of the *fushi tarazu* protein during Drosophila embryogenesis. Cell *43*, 47–57.

Dequin, R., Saumweber, H., and Sedat, J. W. (1984). Proteins shifting from the cytoplasm into the nuclei during early embryogenesis of *Drosophila melanogaster*. Dev. Biol. *104*, 37–48.

Desplan, C., Theis, J., and O'Farrell, P. H. (1985). The *Drosophila* developmental gene, *engrailed*, encodes a sequence-specific DNA binding activity. Nature *318*, 630–635.

Folkman, J., Handenschild, C. C., and Zetter, B. R. (1979). Long-term culture of capillary endothelial cells. Proc. Natl. Acad. Sci. USA *76*, 5217–5221.

Frasch, M. (1985). Charakterisierung chromatinassoziierter Kernproteine von *Drosophila melanogaster* mit Hilfe monoklonaler Antikörper, Ph.D. thesis, Universität Tübingen, Tübingen, Federal Republic of Germany.

Gehring, W. J. (1985). Homeotic genes, the homeo box, and the genetic control of development. Cold Spring Harbor Symp. Quant. Biol. *50*, 243–252.

Hartshorne, T. A., Blumberg, H., and Young, E. T. (1986). Sequence homology of the yeast regulatory protein ADR1 with *Xenopus* transcription factor TFIIIA. Nature *320*, 283–287.

Knipple, D. C., Seifert, E., Rosenberg, U. B., Preiss, A., and Jäckle, H. (1985). Spatial and temporal patterns of *Krüppel* gene expression in early *Drosophila* embryos. Nature *317*, 40–44.

Laughon, A., and Scott, M. P. (1984). Sequence of a *Drosophila* segmentation gene: protein structure homology with DNA-binding proteins. Nature *310*, 25–31.

McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A., and Gehring, W. J. (1984). A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans. Cell *37*, 403–408.

Miller, J., McLachlan, A. D., and Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. EMBO J. *4*, 1609–1614.

Mlodzik, M., Fjose, A., and Gehring, W. J. (1985). Isolation of *caudal*, a *Drosophila* homeo box-containing gene with maternal expression, whose transcripts form a concentration gradient at thepre-blastoderm stage. EMBO J. *4*, 2961–2969.

Nicolas, J. F., Dubois, P., Jacob, H., Gaillard, J., and Jacob, F. (1975). Teratocarcinome de la souris: differentiation en culture d'un linée de cellules primitives à potentialités multiples. Ann. Microbiol. (Inst. Pasteur) *126A*, 3–22.

Pabo, C. O., and Sauer, R. T. (1984). Protein–DNA recognition. Ann. Rev. Biochem. *53*, 293–321.

Preiss, A., Rosenberg, U. B., Kienlin, A., Seifert, E., and Jäckle, H. (1985). Molecular genetics of *Krüppel*, a gene required for segmentation of the *Drosophila* embryo. Nature *313*, 27–32.

Rhodes, D., and Klug, A. (1986). An underlying repeat in some transcriptional control sequences corresponding to half a double helical turn of DNA. Cell *46*, 123–132.

Rosenberg, U. B., Schröder, C., Preiss, A., Kienlin, A., Côté, S., Riede, I., and Jäckle, H. (1986). Structural homology of the product of the Drosophila *Krüppel* gene with *Xenopus* transcription factor IIIA. Nature *319*, 336–339.

Shapira, H., Dibson, M., Muller, G., and Arnon, R. (1984). Immunity and protection against influenza virus by synthetic peptide corresponding to antigenic sites of hemagglutinin. Proc. Natl. Acad. Sci. USA *81*, 2461–2465.

Shepherd, J. C. W., McGinnis, W., Carrasco, A. E., De Robertis, E. M., and Gehring, W. J. (1984). Fly and frog homoeo domains show homologies with yeast mating type regulatory proteins. Nature *310*, 70–71.

Vincent, A. (1986). TFIIIA and homologous genes. The "finger" proteins. Nucl. Acids Res. *14*, 4385–4391.

Vincent, A., Colot, H. V., and Rosbash, M. (1985). Sequence and structure of the *serendipity* locus of *Drosophila melanogaster*: a densely transcribed region including a blastoderm-specific gene. J. Mol. Biol. *186*, 146–166.