ELSEVIER

Mechanisms of Development 79 (1998) 161–164

M@D

A transcription unit at the *ken and barbie* gene locus encodes a novel *Drosophila* zinc finger protein

Ronald P. Kühnlein, Chao-Kung Chen, Reinhard Schuh*

Max-Planck-Institut für biophysikalische Chemie, Abt. Molekulare Entwicklungsbiologie, Am Fassberg, D-37077 Göttingen, Germany

Received 5 August 1998; revised version received 21 September 1998; accepted 24 September 1998

Abstract

We describe a novel *Drosophila* transcription unit, located in chromosome region 60A. It encodes a zinc finger protein that is expressed in distinct spatial and temporal patterns during embryogenesis. Its initial expression occurs in a stripe at the anterior and the posterior trunk boundary, respectively. The two stripes are activated and spatially controlled by gap-gene activities. The P-element of the enhancer trap line *l(2)02970* is inserted in the 5′-region of the transcript and causes a *ken and barbie* (*ken*) phenotype, associated with malformation of male genital structures.

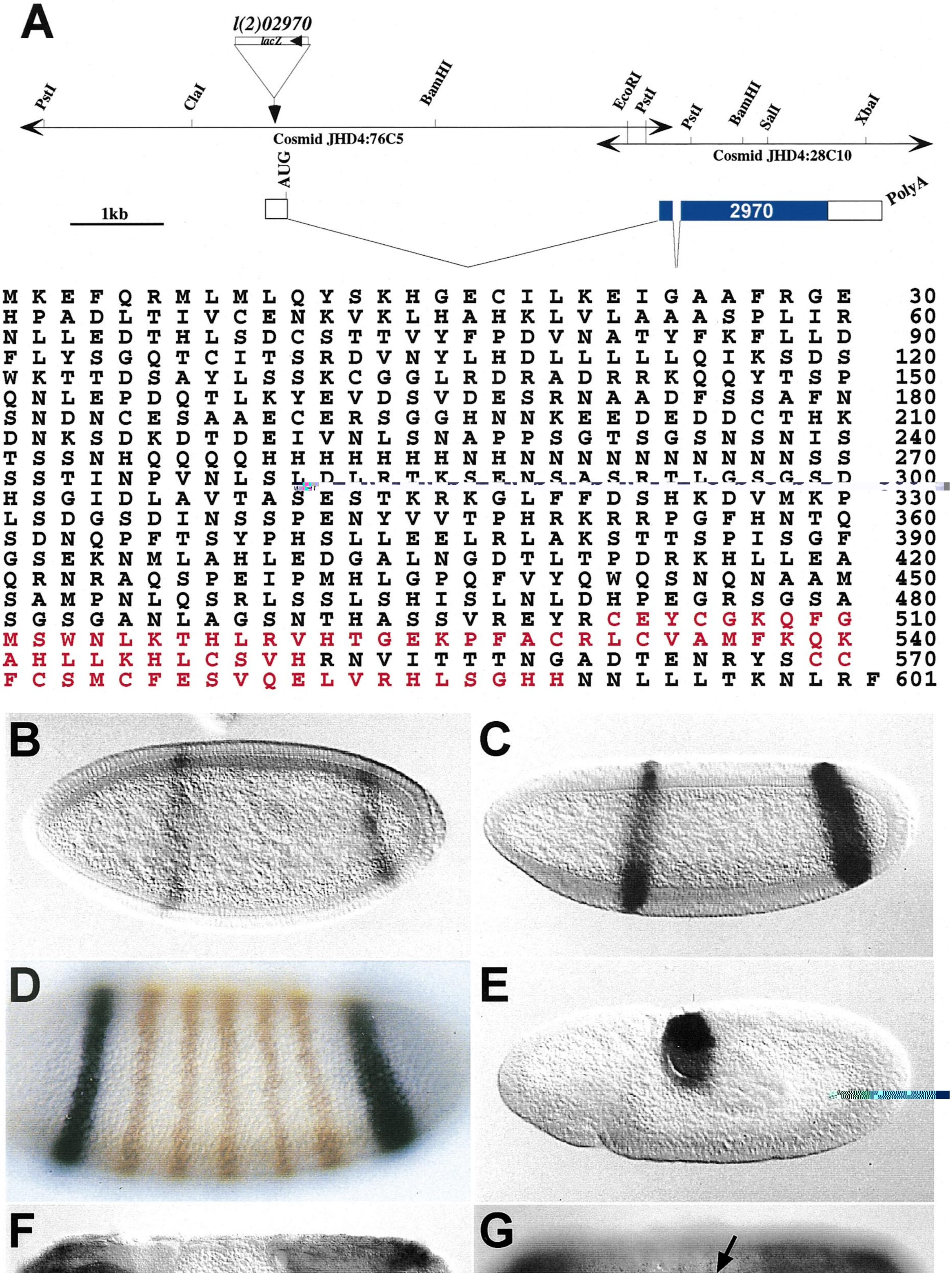
Keywords: l(2)02970; *ms(2)00331*; *l(2)08253*; *ken and barbie*; Zinc finger protein; Gap-genes *Drosophila*

The reporter gene expression of the P-element enhancer trap line *l(2)02970* (Karpen and Spradling, 1992) is defined by two stripes during blastoderm stage (not shown). This suggests that it is regulated by the cis-acting elements of a corresponding endogenous gene. The cloning of this putative gene was initiated by plasmid rescue experiments (Wilson et al., 1989) resulting in genomic DNA fragments flanking the P-element integration. They were used to conduct a chromosomal walk and to characterize the nearby transcription unit by cDNA and Northern blot analysis. The coding capacity of the 2970-transcription unit was determined by sequencing of three overlapping cDNAs and corresponding portions of the genomic DNA. The transcript contains three exons and the combined cDNAs add up to 2.8 kb (Fig. 1A) matching nearly the size of the 3.2 kb long embryonic poly(A)$^+$ RNA detected by Northern blot analysis (not shown). The 2970-transcript contains a single 1803 bp open reading frame that codes for a putative 601 amino acid protein characterized by three diagnostic C2H2 zinc finger motifs (Fig. 1A; Miller et al., 1985). Two zinc finger motifs are connected by the evolutionarily conserved 'H/C-link' motif (Schuh et al., 1986), the third zinc finger is separated by 17 amino acids from the others (Fig. 1A). Zinc finger motifs define a distinct class of nucleic acid binding proteins (El-Baradi and Pieler, 1991) suggesting that the 2970-protein acts as a DNA and/or RNA binding protein.

To visualize 2970-transcript expression during embryogenesis we performed in situ hybridizations on whole-mount embryos using antisense 2970-RNA probes. Transcripts were first detected at the beginning of cellular blastoderm (early stage 5; stages according to Campos-Ortega and Hartenstein, 1985) forming two circumferential rings around the embryo which cover three cell rows at 64% (anterior domain; AD) and 17% (posterior domain; PD) of egg length, respectively (Fig. 1B). At the end of stage 5, the transcripts cover the first and seventh stripe domain of the pair-rule gene *fushi tarazu* (Fig. 1C,D) marking parasegment 2 (posterior maxillary and anterior labial segment) and parasegments 14 and 15 (posterior abdominal segment 8 to anterior abdominal segment 10), respectively (Lawrence and Johnston, 1989). The AD fades away during gastrulation (stages 6–8) whereas the PD persists until the end of embryogenesis in the posterior spiracles (Fig. 1E,F). 2970-transcript expression is also found at stage 16 in the foregut, the proventriculus, the dorsal pouch, the hindgut

* Corresponding author. Tel.: +49-551-201-1758; fax: +49-551-201-1755; e-mail: rshuh@gwdg.de

0925-4773/98/$ - see front matter
PII S0925-4773(98)00183-X


A
l(2)02970
lacZ
PstI
ClaI
BamHI
EcoRI
PstI
Cosmid JHD4:76C5
PstI
BamHI
SalI
XbaI
Cosmid JHD4:28C10
AUG
1kb
2970
PolyA
M K E F Q R M L M L Q Y S K H G E C I L K E I G A A F R G E 30
H P A D L T I V C E N K V K L H A H K L V L A A A S P L I R 60
N L L E D T H L S D C S T T V Y F P D V N A T Y F K F L L D 90
F L Y S G Q T C I T S R D V N Y L H D L L L L L Q I K S D S 120
W K T T D S A Y L S S K C G G L R D R A D R R K Q Q Y T S P 150
Q N L E P D Q T L K Y E V D S V D E S R N A A D F S S A F N 180
S N D N C E S A A E C E R S G G H N N K E E D E D D C T H K 210
D N K S D K D T D E I V N L S N A P P S G T S G S N S N I S 240
T S S N H Q Q Q Q H H H H H H H N H N N N N N N N N N N S S 270
S S T I N P V N L S L D L R T K S E N S A S R T L G S G S D 300
H S G I D L A V T A S E S T K R K G L F F D S H K D V M K P 330
L S D G S D I N S S P E N Y V V T P H R K R R P G F H N T Q 360
S D N Q P F T S Y P H S L L E E L R L A K S T T S P I S G F 390
G S E K N M L A H L E D G A L N G D T L T P D R K H L L E A 420
Q R N R A Q S P E I P M H L G P Q F V Y Q W Q S N Q N A A M 450
S A M P N L Q S R L S S L S H I S L N L D H P E G R S G S A 480
S G S G A N L A G S N T H A S S V R E Y R C E Y C G K Q F G 510
M S W N L K T H L R V H T G E K P F A C R L C V A M F K Q K 540
A H L L K H L C S V H R N V I T T T N G A D T E N R Y S C C 570
F C S M C F E S V Q E L V R H L S G H H N N L L L T K N L R F 601
B
C
D
E
F
G

[redacted]

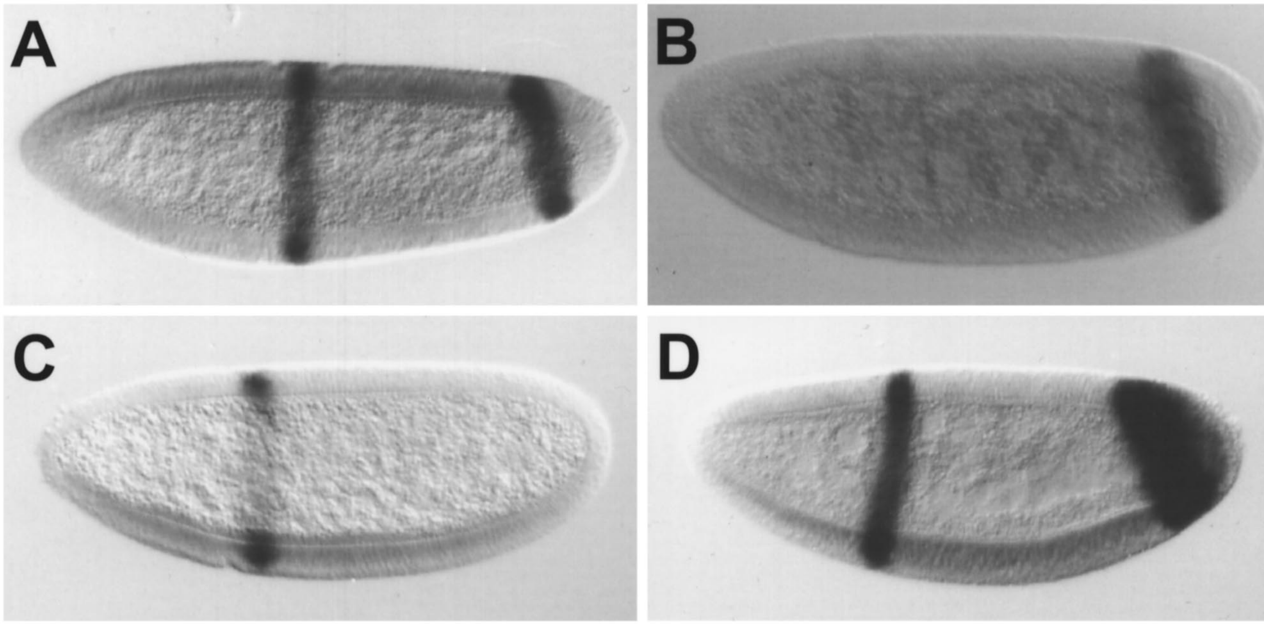


Fig. 2. In situ hybridization of stage 5 *bcd4* + derived (A), hb^{7M48} (B), tll^{g} (C) and hkb^{2} (D) embryos using 2970-cDNA.

and the anal pads (Fig. 1F). Weak 2970-expression is detectable in the tracheal system and segmentally repeated epidermal stripes (Fig. 1G).

The initial expression domains at blastoderm stage suggest that the 2970-transcript is controlled by maternal and segmentation gene activities. The AD depends on the gene *bicoid* (*bcd*), the key component of the anterior organizer system (Frohnhöfer and Nüsslein-Volhard, 1986), as shown by its absence in embryos from homozygous *bcd* females (not shown). In embryos from females that contain multiple *bcd* gene copies (Driever and Nüsslein-Volhard, 1988) the AD is shifted posteriorly to 55% instead of 64% of egg length (compare Fig. 1C with Fig. 2A). The AD is also absent in embryos lacking zygotic gap-gene activity *hunchback* (*hb*; Fig. 2B) indicating that AD expression is mediated by the *bcd* target gene *hb* (Tautz, 1988). The PD expression is controlled by the maternal terminal organizer system, mediated by the terminal gap-gene activities *tailless* (*tll*) and *huckebein* (*hkb*) (Weigel et al., 1990). In *tll* mutant embryos, the PD is absent (Fig. 2C). In *hkb* mutant embryos, the PD extends to the posterior pole (Fig. 2D). Therefore, PD expression is formally activated by *tll* activity and repressed by *hkb* activity as it has been observed for the posterior expression domain of the *spalt* (*sal*) gene (Kühnlein et al., 1997). However, *sal* is not involved in PD regulation since *sal* mutant embryos exhibit a normal PD expression (not shown).

The P-insertion line *l(2)02970* fails to complement the enhancer trap lines *ms(2)00331* and *l(2)08253* (Karpen and Spradling, 1992) which contain P-element insertions in the same chromosomal position. The three alleles are semilethal in transheterozygous conditions and the corresponding flies develop distally unpigmented, less branched aristae as well as malformation of male genital structures (not shown) as has been described by Castrillon et al. (1993) for homozygous *ms(2)00331* flies. The authors refer to the targeted gene locus as *ken and barbie* (*ken*). Thus, the three P-insertion lines represent alleles of the *ken* gene locus. The tight association of the P-insertion *l(2)02970*, which can be reverted to full viability by P-element jump-out experiments (not shown), with the 2970-transcription unit (Fig. 1A) suggests that the zinc finger protein described here represents a *ken* gene product. Consistent with this view is the fact that the genitalia disc anlagen is derived from PS 14 (Ehrensperger, 1983), a region that expresses the 2970-transcript.

1. Materials and methods

Methods of DNA manipulation and whole-mount in situ hybridizations were performed as described (Ashburner, 1989; Sambrook et al., 1989; Tautz and Pfeifle, 1989). The fly stocks bcd^{E1}, hb^{7M48}, hkb^{2}, sal^{16}, tll^{g}, $Df(2R)bw^{S46}$ were used in this study (Lindsley and Zimm, 1992).

Fig. 1. (A) Molecular organization of the *l(2)02970* P-element associated gene. The isolated cosmid clones (Hoheisel et al., 1991), the P-element localization, the composite cDNA with the open reading frame (blue) and the putative protein sequence is shown; the zinc fingers are indicated in red; cDNA and protein sequence information is available under accession no. AJ012576. In situ hybridization of whole-mount early (B) and late (C,D) stage 5, stage 9 (E) and stage 16 (F,G) embryos using 2970-cDNA and in addition *ftz*-cDNA (brown; D). Note: Overstained embryo (G) reveals weak 2970-expression in the tracheal system (arrow) and in epidermal stripes (arrowhead).

References

Ashburner, M., 1989. *Drosophila*: A Laboratory Handbook and Manual. Cold Spring Harbor Laboratory Press, New York.

Campos-Ortega, J. A., Hartenstein, V., 1985. The embryonic development of *Drosophila melanogaster*. Springer, Berlin.

Castrillon, D.H., Gonczy, P., Alexander, S., Rawson, R., Eberhart, C.G., Viswanathan, S., DiNardo, S., Wasserman, S.A., 1993. Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single P element mutagenesis. Genetics 135, 489–505.

Driever, W., Nüsslein-Volhard, C., 1988. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell 54, 95–104.

Ehrensperger, P.C., 1983. Die Entwicklung der bisexuellen Anlage der Genitalien und Analien, untersucht an verschiedenen Geschlechtsmutanten der Taufliege, *Drosophila melanogaster*. Dissertation, Universität Zürich, Switzerland.

El-Baradi, T., Pieler, T., 1991. Zink finger proteins: what we know and what we would like to know. Mech. Dev. 35, 155–169.

Frohnhöfer, H.G., Nüsslein-Volhard, C., 1986. Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. Nature 324, 120–125.

Hoheisel, J.D., Lennon, G.G., Zehetner, G., Lehrach, H., 1991. Use of high coverage libraries of *Drosophila melanogaster* for relational data analysis. J. Mol. Biol. 220, 903–914.

Karpen, G.H., Spradling, A.C., 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single P-element insertional mutagenesis. Genetics 132, 737–753.

Kühnlein, R.P., Brönner, G., Taubert, H., Schuh, R., 1997. Regulation of *Drosophila spalt* gene expression. Mech. Dev. 66, 107–118.

Lawrence, P.A., Johnston, P., 1989. Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even-skipped* and *fushi tarazu*. Development 105, 761–767.

Lindsley, D.L., Zimm, G.G., 1992. The Genome of *Drosophila melanogaster*. Academic Press, San Diego, CA.

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

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Schuh, R., Aicher, W., Gaul, U., Cote, S., Preiss, A., Maier, D., Seifert, E., Nauber, U., Schroder, C., Kemler, R., Jäckle, H., 1986. A conserved family of nuclear proteins containing structural elements of the finger protein encoded by *Krüppel*, a *Drosophila* segmentation gene. Cell 47, 1025–1032.

Tautz, D., 1988. Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. Nature 332, 281–284.

Tautz, D., Pfeifle, C., 1989. A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma 98, 81–85.

Weigel, D., Jürgens, G., Klingler, M., Jäckle, H., 1990. Two gap genes mediate maternal terminal pattern information in *Drosophila*. Science 248, 495–498.

Wilson, C., Pearson, R.K., Bellen, H.J., O'Kane, C.J., Grossniklaus, U., Gehring, W.J., 1989. P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. Genes Dev. 3, 1301–1313.