

The *Drosophila kismet* gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity

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SUMMARY

The *Drosophila kismet* gene was identified in a screen for dominant suppressors of *Polycomb*, a repressor of homeotic genes. Here we show that *kismet* mutations suppress the *Polycomb* mutant phenotype by blocking the ectopic transcription of homeotic genes. Loss of zygotic *kismet* function causes homeotic transformations similar to those associated with loss-of-function mutations in the homeotic genes *Sex combs reduced* and *Abdominal-B*. *kismet* is also required for proper larval body segmentation. Loss of maternal *kismet* function causes segmentation defects similar to those caused by mutations in the pair-rule gene

even-skipped. The *kismet* gene encodes several large nuclear proteins that are ubiquitously expressed along the anterior-posterior axis. The *Kismet* proteins contain a domain conserved in the trithorax group protein *Brahma* and related chromatin-remodeling factors, providing further evidence that alterations in chromatin structure are required to maintain the spatially restricted patterns of homeotic gene transcription.

Key words: Polycomb group, Trithorax group, Transcription, Chromatin, Homeotic, BRM, *Drosophila*, *kismet*

INTRODUCTION

The homeotic genes of the Antennapedia (ANTC) and bithorax (BXC) complexes specify the identities of body segments in *Drosophila* (Duncan, 1987; Kaufman et al., 1990). Homeotic genes encode homeodomain transcription factors that specify the identity of one or more body segments by regulating the transcription of a battery of downstream target genes. Counterparts of the *Drosophila* homeotic genes (the Hox genes) are present in vertebrates and other metazoans where they play highly conserved roles in the control of cell fate (Gellon and McGinnis, 1998; Maconochie et al., 1996; Manak and Scott, 1994). During the past decade, much of the research on Hox genes in *Drosophila* and other organisms has been focused on three questions. How are the spatially restricted patterns of homeotic gene transcription established and maintained during development? How do the homeotic transcription factors regulate the transcription of their target genes? What target genes are regulated by each homeotic transcription factor to specify the identities of the individual body segments?

Of these three issues, perhaps the most is known about the regulation of homeotic gene expression. The initial patterns of homeotic gene transcription are established in the early embryo by transcription factors encoded by segmentation genes.

During subsequent development, these patterns are maintained by two ubiquitously expressed groups of regulatory proteins: the Polycomb group of repressors and the trithorax group of activators (reviewed in Kennison, 1995; Pirrotta, 1997; Simon, 1995). Mutations in Polycomb group genes cause homeotic transformations due to the ectopic transcription of homeotic genes. Conversely, mutations in many trithorax group genes cause homeotic transformations due to the failure to maintain the transcription of homeotic genes. By maintaining states of transcription established earlier in development by the segmentation genes, Polycomb and trithorax group proteins play critical roles in the specification of body segment identities.

Although the mechanism of action of the Polycomb group proteins is not well understood, a growing body of evidence suggests that they act in concert to repress transcription. Polycomb (PC) physically interacts with other Polycomb group proteins, including Polyhomeotic (PH), Posterior sex combs (PSC) and Sex combs on midleg (SCM) (Franke et al., 1992; Kyba and Brock, 1998a,b; Peterson et al., 1997; Strutt and Paro, 1997). Physical interactions between two other Polycomb group proteins, Enhancer of zeste [E(Z)] and Extra sex combs (ESC) have also been detected (Jones et al., 1998; Tie et al., 1998). Many Polycomb group proteins repress transcription of their target genes via *cis*-regulatory elements

known as Polycomb group response elements (PREs). Complexes of Polycomb group proteins may be targeted to PREs via interactions with the Polycomb group member Pleiohomeotic, which directly binds a DNA sequence conserved in several PREs (Brown et al., 1998).

How do PC and other Polycomb group proteins repress transcription once targeted to a PRE in the vicinity of a homeotic gene? One current view is that Polycomb group proteins render homeotic genes inaccessible to activators or components of the general transcription machinery by altering chromatin structure (reviewed in Pirrotta, 1998). The Polycomb protein contains a short domain, the chromodomain, which is conserved in HP1, a component of *Drosophila* heterochromatin involved in heritable gene silencing (Eissenberg et al., 1992; Paro and Hogness, 1991). A direct connection between Polycomb group proteins and chromatin structure has not been proven, however, and it is possible that they repress transcription by blocking enhancer-promoter interactions, interfering with the assembly of the preinitiation complex or altering the subnuclear localization of specific genes (McCall and Bender, 1996; Pirrotta, 1998).

Recent studies of a trithorax group gene, *brahma* (*brm*), have provided a direct connection between the regulation of homeotic gene expression and chromatin. *brm* was identified in a screen for dominant suppressors of *Pc* (Kennison and Tamkun, 1988). The rationale of the screen was that reduction of an activator function could compensate for the reduced PC repressor function. *brm* is highly related to a yeast transcriptional activator, *SWI2/SNF2*, that encodes the ATPase subunit of a 2 MDa chromatin-remodeling complex, the SWI/SNF complex (Tamkun et al., 1992). The SWI/SNF complex facilitates the binding of many transcriptional activators to their binding sites in the context of chromatin by causing ATP-dependent alterations in nucleosome structure (reviewed in Pollard and Peterson, 1998; Kingston et al., 1996; Peterson and Tamkun, 1995; Winston and Carlson, 1992). The BRM protein is also highly related to the yeast STH1 protein, the ATPase subunit of RSC, a chromatin-remodeling complex related to SWI/SNF (Cairns et al., 1996). BRM has been purified from embryos and shown to be the ATPase subunit of a complex related to both SWI/SNF and RSC (Papoulas et al., 1998). Based on these findings, it is likely that BRM uses the energy of ATP hydrolysis to counteract the repressive effects of chromatin on ANTC and BXC homeotic genes.

In addition to *brm*, mutations in eleven other previously unidentified genes were recovered as dominant suppressors of *Pc* (Kennison and Tamkun, 1988). Here we present the genetic and molecular analysis of one of these genes, *kismet* (*kis*). *kis* mutations strongly suppress the homeotic transformations observed in heterozygous *Pc* adults. Conversely, *kis* duplications strongly enhance *Pc* mutant phenotypes (Kennison and Russell, 1987). *kis* plays a dual role in development. Loss of zygotic *kis* function causes homeotic transformations, indicating that *kis* is a member of the trithorax group of homeotic gene activators. Loss of maternal *kis* function causes dramatic segmentation defects similar to those caused by mutations in pair-rule genes. Molecular analysis of the *kis* gene revealed that it encodes multiple, large nuclear proteins that are related to BRM and other chromatin-remodeling factors. These findings suggest that KIS and BRM function by similar mechanisms and

reinforce the importance of chromatin-remodeling factors in developmental processes.

MATERIALS AND METHODS

Drosophila stocks and genetic crosses

Flies were raised on a cornmeal/molasses/yeast/agar medium with either Tegosept or propionic acid at 25°C. Unless otherwise noted, all mutations and chromosome aberrations are described in Lindsley and Zimm (1992). The isolation of the first nine *kis* alleles (*kis*¹⁻⁹) was described previously (Kennison and Tamkun, 1988). Of these, *kis*¹ and *kis*² are the strongest alleles by both the criteria of lethal phase and dominant suppression of *Pc*⁴. All nine appear to be loss-of-function alleles, since deficiencies that include *kis* are also strong dominant suppressors of *Pc*⁴ and males homozygous for *kis*¹ (or heterozygous for *kis*¹ and any of the other eight alleles) are normal if they carry a duplication that includes *kis*⁺ [either *Dp(2;Y)L124* or *Dp(2;1)L124*]. The *kis*^S allele (previously known as *AS760*) was identified in Allan Spradling's laboratory and results from a P-element insertion. The *Df(2L)net* chromosomes are described in Caggese et al. (1988). *Dp(2;1)L124* was generated for the dosage experiments by irradiating *C(1)RM*, *y*² *su(w^a) w^a bb /Dp(2;Y)L124*, *B^S* females with 2000 R of gamma rays, crossing to Oregon R males, and selecting exceptional *y*² *su(w^a) w^a B⁺* sons. One of these sons, which proved to carry a detachment of the attached X chromosome with the duplication for the tip of 2L on the right arm of the X chromosome, was used to balance the *Dp(2;1)L124* chromosome.

Somatic clonal analysis

Clones of homozygous *kis* tissue were induced by mitotic recombination using either X-irradiation (Lawrence et al., 1986) or the FLP-FRT technique (Golic, 1991; Xu and Rubin, 1993). The *Minute* technique was used to increase the size of the clones (Morata and Ripoll, 1975). To generate X-ray-induced clones, *y; kis¹ ck cn bw sp/SM6a* virgin females were mated to *y f^{β6a}; Dp(1;2)sc¹⁹, y⁺ M(2)201/SM6a* males. Blastoderm embryos, first instar larvae and third instar larvae from this cross were irradiated with 500 R, 1000 R and 1260 R, respectively, using a Torrex 120D X-ray generator (Astrophysics Research Corporation). *kis* clones were scored in *kis¹ ck cn bw sp/Dp(1;2)sc¹⁹, y⁺ M(2)201* flies using the markers *yellow* (*y*) and *crinkled* (*ck*). *y* females heterozygous for *kis*² or *kis*^S were similarly crossed to *y f^{β6a}; Dp(1;2)sc¹⁹, y⁺ M(2)201/SM6a* males, irradiated and scored for the presence of clones marked with *y*. As a control, *isol* (*y; cn bw sp*) virgin females (Brizuela et al., 1994) were crossed to *y f^{β6a}; Dp(1;2)sc¹⁹, y⁺ M(2)201/SM6a* males. Progeny were irradiated as above and the frequency of *y* clones was compared to the frequency of *y kis* clones from the experimental crosses. A total of 87 radiation-induced blastoderm clones were observed in 9287 legs (0.94 clones/100 legs). Of these, 7283 legs containing 65 homozygous *kis*¹ clones were mounted and examined (0.89 clones/100 legs). 22 *kis*^S clones were observed in 2004 legs (1.10 clones/100 legs).

To use the FLP-FRT technique, the *kis*¹ and *kis*^S alleles were recombined onto the *P[ry⁺, y⁺J25F P[ry⁺, *hs-neo*, FRT]40A* chromosome (Xu and Rubin, 1993). Virgin *y w P[hsFLP]; P[ry⁺, y⁺J25F P[ry⁺, *hs-neo*, FRT]40A* females were mated to either (1) *y w¹¹¹⁸; kis^S P[ty⁺, *hs-neo*, FRT]40A/SM6a* males, (2) *y w¹¹¹⁸; kis¹ P[ry⁺, *hs-neo*, FRT]40A bw sp/SM6a* males, or (3) *y w¹¹¹⁸; P[ry⁺, *hs-neo*, FRT]40A bw sp/SM6a* males. Mitotic recombination was induced by heat-shocking blastoderm embryos or third instar larvae at 37°C for 60 and 90 minutes, respectively. Adults were dissected, mounted in Gary's magic mountant (Lawrence et al., 1986) and examined under the light microscope.

Germline clonal analysis

Germline mosaics were generated using the dominant female-sterile

technique (Chou and Perrimon, 1992; Chou et al., 1993). Males carrying an insertion of *ovo^{D1}* on the second chromosome (*P/w⁺, ovo^{D1}/CyO*) (Chou et al., 1993) were crossed to females of the genotypes (1) *w; kis¹ cn bw sp/SM6a*, (2) *y; kis^S/SM6a*, or (3) *y Df(1)w67c2*. Germline clones were induced by irradiating late first instar larvae with 1000R. Female offspring of the genotypes (1) *+/w; kis¹ cn bw sp/P[w⁺, ovo^{D1}]*, (2) *+/y; kis^S/P[w⁺, ovo^{D1}]*, or (3) *+/y Df(1)w67c2; +/P[w⁺, ovo^{D1}]* were crossed to males heterozygous for a *kis* deficiency (*w; Df(2L)net-PMC/SM6a*). The cuticle of unhatched larvae were examined as described by Ashburner (1989). As a control for leakiness of the *ovo^{D1}* mutation, crosses were set up as above but were not irradiated.

To generate germline mosaics using the FLP-FRT system, females of the genotypes (1) *y w; kis^S P[ry⁺, hs-neo, FRT]40A/SM6a*, (2) *y w; kis¹ P[ry⁺, hs-neo, FRT]40A bw sp/SM6a*, or (3) *y w; P[ry⁺, y⁺]25F P[ry⁺, hs-neo, FRT]40A/CyO* were crossed to *y w P[ry⁺, hsFLP]¹²; P[w⁺, ovo^{D1}]2L-13X13 P[hs-neo, ry⁺, FRT]40A/CyO* males. First instar larvae from this cross were heat-shocked at 37°C for 90 minutes to induce mitotic recombination. Female progeny of the genotypes (1) *y w/y w P[ry⁺, hsFLP]¹²; kis^S P[ry⁺, hs-neo, FRT]40A/P[w⁺, ovo^{D1}]2L-13X13 P[hs-neo, ry⁺, FRT]40A*, (2) *y w/y w P[ry⁺, hsFLP]¹²/kis¹ P[ry⁺, hs-neo, FRT]40A bw sp/P[w⁺, ovo^{D1}]2L-13X13 P[hs-neo, ry⁺, FRT]40A*, or (3) *y w/y w P[ry⁺, hsFLP]¹²; P[ry⁺, y⁺]25F, P[ry⁺, hs-neo, FRT]40A/P[w⁺, ovo^{D1}]2L-13X13 P[hs-neo, ry⁺, FRT]40A* were crossed to *w; Df(2L)net-PMC/SM6a* males. Embryos produced by females bearing a germline clone were examined as described above.

Isolation of DNA and RNA, and nucleic acid blot analyses

RNA was isolated from developmentally staged embryos, larvae, pupae and adult flies as described in Tamkun et al. (1992). Standard techniques were used for nucleic-acid blot analyses (Sambrook et al., 1989). Filters were probed with DNA fragments labeled by the random-primer method (Feinberg and Vogelstein, 1983) and washed under high stringency (0.1× SSC, 0.1% SDS, 65°C). To control for even loading, the RNA blots were probed with a radiolabeled fragment from the *rp49* gene (O'Connell and Rosbash, 1984). RNA probes for direction of transcription assays were generated and used as described in the 1991 Promega Protocols and Applications Guide.

Isolation and sequencing of cDNA and genomic clones

Genomic and cDNA clones were isolated from phage and cosmid libraries using standard techniques (Elfring et al., 1994; Tamkun et al., 1992). cDNA clones corresponding to the *kis* mRNA were isolated from an iso-1 λgt11 cDNA library (0-24 hour embryos; Tamkun et al., 1992), an OregonR λgt10 cDNA library (3-10 hour embryos; Poole et al., 1985), and a CantonS λZAP cDNA library (0-24 hour embryos; Stratagene). cDNA fragments were subcloned into plasmid vectors and sequenced as described in Elfring et al. (1994). Both strands of DNA were sequenced for all reported sequences. The cosmid isozakB was isolated by screening an iso-1 genomic cosmid library constructed in a *NotBamNot-CoSpeR* vector (Tamkun et al., 1992) with the gt10-3 cDNA. This cosmid was introduced into the *Drosophila* germline using P-element-mediated transformation as described in Tamkun et al. (1991) and tested for the ability to rescue the recessive lethality of *kis* mutations.

In situ hybridization

Digoxigenin-labeled DNA fragments were prepared using the Boehringer Mannheim Genius kit and hybridized to salivary gland polytene chromosomes (Engels et al., 1986), embryos (Tautz and Pfeifle, 1989) or third instar larvae (Kramer and Zipursky, 1992). Anti-digoxigenin antibody (Boehringer Mannheim) was used to detect the bound DNA fragments according to the manufacturer's directions.

Production of antibodies and western blotting

SDS-polyacrylamide gel electrophoresis and western blotting were

performed as described previously (Tsukiyama et al., 1995). Native embryo extracts and purified BRM complex were prepared and analyzed by western blotting as described in Papoulas et al. (1998). To generate a KIS fusion protein, a 962 bp *PstI* fragment from the *kis* cDNA clone Zap1 was cloned into pUR292 (Rüther and Müller-Hill, 1983). A 908 bp *BamHI* fragment from this construct was then cloned into the pGEX 3X glutathione-S-transferase (GST) fusion vector (Pharmacia). This fragment contains 893 bp of Zap1 encoding amino acids 730-1029 of the KIS protein (Figs 4, 6), and 15 bp of the pUR292 polylinker encoding three additional residues. GST-KIS fusion proteins were expressed in *E. coli*, purified on glutathione-agarose columns and used to immunize rabbits as described in Harlow and Lane (1988). Antibodies were affinity purified on columns containing either GST or GST-KIS fusion proteins coupled to Affigel 10 or Affigel 15 resins (BioRad) according to the manufacturer's instructions. Bound antibodies were eluted with 3.5 M MgCl₂ in PBS, dialyzed and assayed by western blotting.

Immunostaining of embryos and larvae

Whole-mount preparations of embryos and third instar larvae were stained with primary antibodies and visualized with secondary antibodies conjugated to horseradish peroxidase (BioRad) as described by Pattatucci and Kaufman (1991). To generate larvae carrying a *Pc* mutation and four wild-type copies of the *kis* gene, *Basc/Dp(2;1)L124, y w* females were crossed to *+*; *Pc⁴ p^p e^s/TM6B, Hu Sb e Tb ca; T(Y;2)L124, y⁺ B^S* males. Larvae of the genotype *Dp(2;1)L124, y w/Dp(2;Y)L124, B^S; +/Pc⁴ p^p e^s* could be distinguished from other progeny by the presence of light-brown mouth parts, colorless Malpighian tubules and the non-*Tb* phenotype. Rabbit polyclonal antibodies against SCR were a generous gift from Thom Kaufman (Indiana University). Charles Girdham and Pat O'Farrell (University of California, San Francisco) generously provided rabbit polyclonal antibodies against EN. Antibodies against EVE were a gift from Michael Levine (University of California, Berkeley).

RESULTS

kis mutations block *Sex combs reduced* derepression in *Pc* heterozygotes

Heterozygous *kis* mutations and deficiencies spanning the *kis* gene strongly suppress the homeotic transformations observed in heterozygous *Pc* adults, including the transformation of second and third leg to first leg [due to *Sex combs reduced* (*Scr*) derepression], wing to haltere [due to *Ultrabithorax* (*Ubx*) derepression] and anterior abdominal segments to more posterior identities [due to *Abdominal-B* (*Abd-B*) derepression]. Conversely, *kis* duplications strongly enhance these transformations (Kennison and Russell, 1987). Since the adult *Pc* phenotypes are due to the ectopic transcription of homeotic genes, it seemed likely that *kis* might act antagonistically to *Pc* to activate the transcription of *Scr* and other homeotic genes.

To test this possibility, we examined the effects of varying the dosage of the *kis* gene on the expression of the homeotic gene *Scr* in the imaginal discs of heterozygous *Pc* larvae (Fig. 1). *Scr* specifies the identities of the labial and first thoracic segments and is normally not expressed in either the second or third leg imaginal discs. As previously observed by Pattatucci and Kaufman (1991), individuals heterozygous for a *Pc* mutation show ectopic expression of SCR proteins in both the second and third leg imaginal discs. The *kis²* mutation strongly suppresses the ectopic expression of SCR proteins in *Pc*

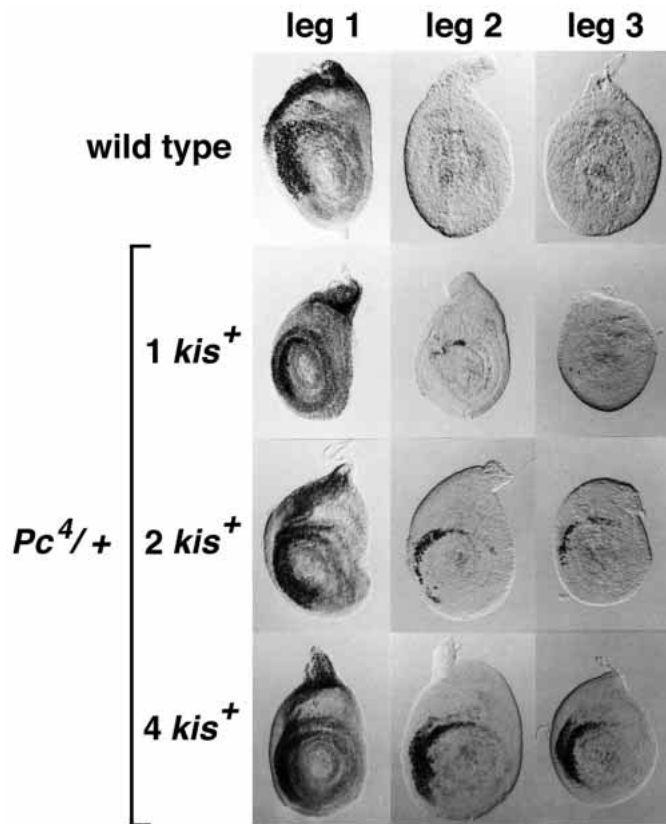


Fig. 1. *kis* is an activator of *Scr* expression in imaginal discs. Leg imaginal discs of wild-type (Oregon R) and heterozygous *Pc⁴* larvae, bearing either one, two or four wild-type copies of the *kis* gene, were stained with antibody against the SCR protein. Note the ectopic expression of SCR proteins in the second and third leg discs of *Pc* heterozygotes bearing two wild-type copies of *kis*. The ectopic expression of SCR in *Pc* heterozygotes is strongly suppressed by a *kis* mutation (1 *kis*⁺), and strongly enhanced in the presence of four wild-type copies of the *kis* gene (4 *kis*⁺). *Pc* mutant larvae bearing four wild-type copies of *kis* were of the genotype *Dp(2;1)L124, y w/Dp (2;Y)L124, B^s; Pc⁴ p^P e^S/+*. The *kis* mutation used was *kis*².

heterozygotes, while four wild-type copies of the *kis* gene strongly enhance the ectopic expression. We observed similar results when we examined the levels of *Scr* RNA by in situ hybridization to imaginal discs (data not shown). These data suggest that *kis* acts antagonistically to *Pc* to activate the transcription of *Scr* and other homeotic genes.

Loss of zygotic *kis* activity causes homeotic transformations

kis is an essential gene; *kis* homozygotes die as first or second instar larvae with no obvious cuticular defects (data not shown). To examine the roles of *kis* during later stages of development, we induced mitotic exchange in first or third instar larvae and examined adult cuticles for changes caused by loss of *kis* function. Loss of *kis* function in the fifth abdominal segment caused a complete transformation toward a more anterior identity, as shown by the loss of the black pigmentation characteristic of this segment (Fig. 2A). This phenotype is identical to that associated with loss-of-function mutations in the *Abd-B* gene of the BXC. Identical results were

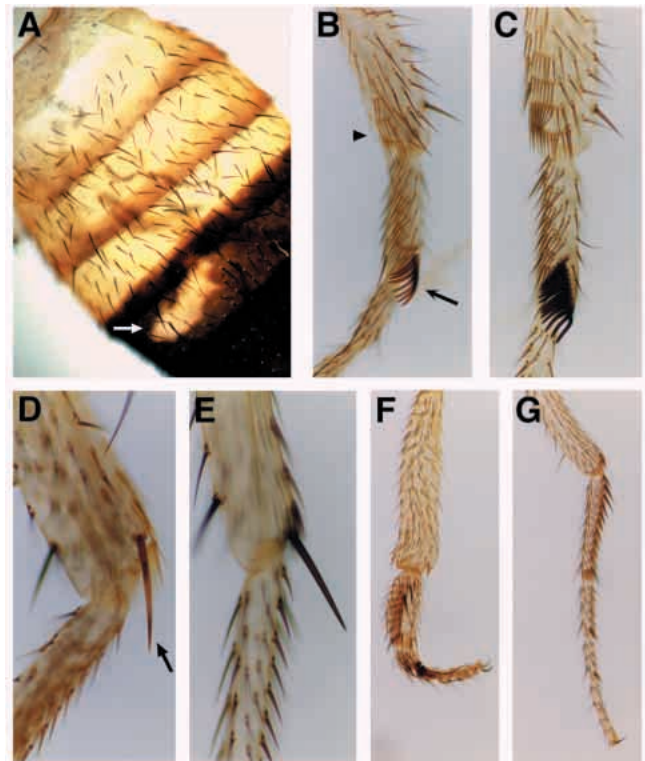
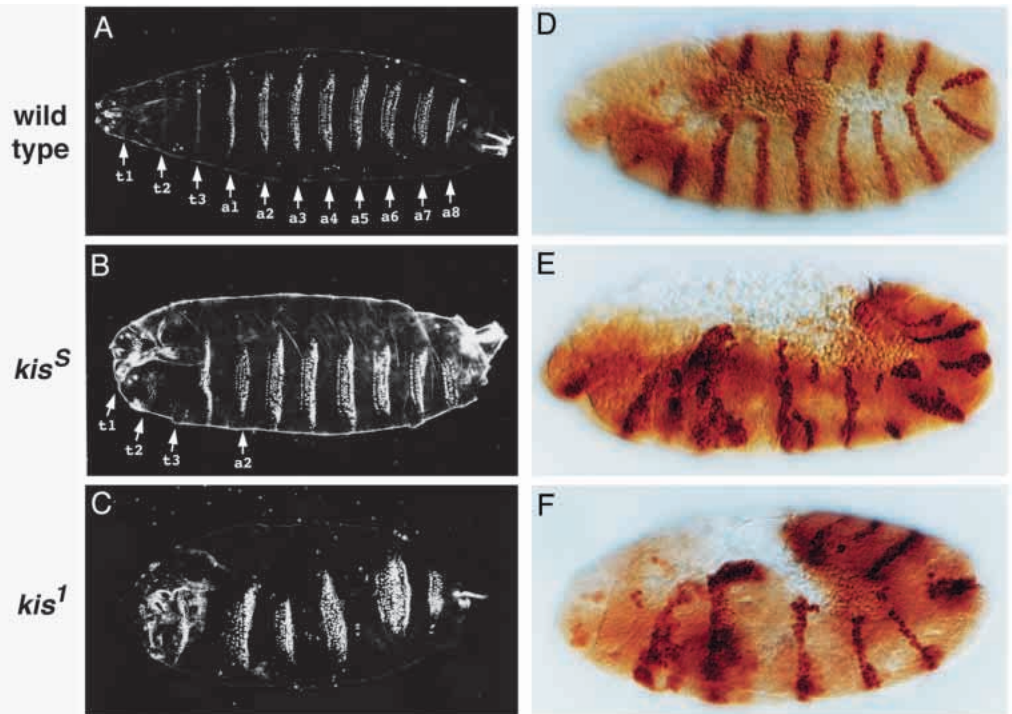


Fig. 2. Loss of zygotic *kis* function causes homeotic transformations. (A) *kis* mutations alter the identity of the fifth abdominal segment. A homozygous clone of *kis*^I tissue (induced at the third instar stage) in the fifth abdominal segment is transformed to a more anterior identity, as evidenced by the loss of pigmentation characteristic of this segment. (B-G) Effects of *kis* mutations on leg development. Clones of homozygous *kis* mutant tissue marked with *yellow* were induced at cellular blastoderm (3±0.5 hours AEL). (B,C) Comparison of the tibia and basitarsus of a first leg bearing a clone of homozygous *kis*^S tissue (B) and a wild-type first leg (C). Note the reduction of first leg character in the *kis*^S clone, including a reduction in both the number of sex comb teeth (arrow) and the number and size of the transverse bristle rows (arrowhead). (D,E) Comparison of a first leg containing a *kis*^S clone (D) and a wild-type second leg (E). Note the appearance of an apical bristle (arrow) within the *kis*^S clone. This marker for second leg identity is evident in the wild-type second leg shown in E. (F,G) Comparison of a third leg bearing a clone of homozygous *kis*^I mutant tissue (F) and a wild-type third leg (G). Development of the tarsal segments is abnormal in the leg bearing the clone.

obtained using three different *kis* alleles (*kis*^I, *kis*² and *kis*^S). The boundaries of homozygous *kis* mutant tissue coincided with the mutant phenotypes, indicating that *kis* is cell autonomous. Clones of homozygous *kis* tissue in other abdominal segments appeared phenotypically normal, although the lack of obvious differences between the second, third and fourth abdominal segments would not allow us to detect subtle transformations between these segments.

Because *kis* mutations suppress the ectopic expression of *Scr* in *Pc* heterozygotes, we anticipated that loss of *kis* function in the developing leg discs would alter the identity of the first leg. Surprisingly, no clear homeotic transformations were observed in homozygous *kis* clones in the thorax when they were induced during larval development. We therefore generated

Fig. 3. Loss of maternal *kis* function causes pair-rule segmentation defects. Cuticle preparations and the complementary pattern of Engrailed protein expression in embryos derived from females bearing germline clones of *kis* mutations. In all panels, anterior is to the left. (A) Wild-type cuticle pattern; (B) embryo derived from a *kis^S* mosaic female; (C) embryo derived from a *kis^L* mosaic female. Note the disruption of head structures and the reduction in the third thoracic and second abdominal segments relative to wild-type. (D-F) Whole-mount embryos stained with a polyclonal antibody against the Engrailed protein. (D) Wild-type embryo; (E) embryo derived from a *kis^S* mosaic female; (F) embryo derived from a *kis^L* mosaic female. Note that the number of body segments is reduced by half. There is a good correlation between the degree of disruption of the Engrailed protein pattern and the severity of the cuticular phenotype.



clones of mutant *kis* tissue at the cellular blastoderm stage of embryogenesis. The average sizes and frequencies of *kis^S* and *kis^L* clones induced at the cellular blastoderm stage were not significantly reduced in any body segments relative to the controls, suggesting that *kis* is not essential for cell viability or division (data not shown). The majority of clones were found in regions of the legs that are similar in each thoracic segment, precluding the detection of homeotic transformations. Several *kis^S* clones did, however, exhibit morphological abnormalities. Homozygous *kis^S* clones in the anterior compartment of the first leg display a reduction in landmark first leg features, such as the transverse row of bristles and the number of sex comb teeth (Fig. 2B). More dramatically, a *kis^S* clone in the first leg also displayed an apical bristle, a landmark feature of the second leg, at the distal end of the tibia (Fig. 2D). This homeotic transformation mimics loss-of-function mutations in *Scr* and is consistent with a role for *kis* as an activator of *Scr* transcription.

Examination of fifteen *kis^L* clones and five *kis^S* clones in the second leg revealed no phenotypic abnormalities, even though they were induced at the cellular blastoderm stage of embryogenesis. In the third leg, twenty *kis^L* and nine *kis^S* clones were examined. Clones of both alleles exhibited abnormal morphologies, particularly in the distal leg structures; the femur and tibia were slightly abnormal while the tarsal segments were severely distorted (Fig. 2F,G). All five tarsi were present but were truncated and exhibited a hooked shape. These abnormalities could be the result of an absence or reduction of portions of one compartment, resulting in the hooked appearance. No homeotic transformations were observed, although these would be difficult to recognize in the distal leg given the degree of pattern disruption. *kis* clones in other body segments, including the head, thorax, wing, haltere and genitalia, did not display any obvious developmental abnormalities.

Loss of maternal *kis* function causes segmentation defects

A possible explanation for the lack of discernible pattern defects in *kis* homozygotes is that sufficient maternal *kis* gene products are present in the embryo to allow normal development in the absence of zygotic *kis* function. To investigate this possibility, we examined the consequences of eliminating both the maternal and zygotic contributions of *kis* gene products using germline clonal analysis. Embryos derived from mothers bearing germline clones of *kis^L* exhibited a deletion of pattern elements approximating alternate segments (Fig. 3C). The most common defect was a reduction in size of one or more alternate segments (T3, A2, A4, A6 and sometimes A8). The second thoracic segment was also often absent or severely reduced and the head region was grossly altered. In more extreme cases, only one or two patches of denticle were observed. The cephalopharyngeal skeleton was internalized and malformed, which may be a secondary result of the major pattern alterations observed. The absence of the T2 and T3 denticle belts was accompanied by the loss of their associated sense organs, both the Keilin's organ and the campaniform sensilla, indicating that the segments were actually missing. The first thoracic segment did not appear to be deleted or transformed, since denticle hairs and the T1-associated hairs (beard) were usually present at the anterior end of the larvae. Similar but less extreme phenotypes were observed in embryos from mothers bearing germline clones of *kis^S* (Fig. 3B). The pattern defects resulting from loss of maternal *kis* function are most similar to those seen in embryos homozygous for mutations in the pair-rule segmentation gene *even-skipped* (*eve*), in which odd-numbered parasegments are deleted (corresponding to the even-numbered abdominal segments). These results indicate that *kis* plays an unanticipated role in embryonic segmentation.

To test whether loss of maternal *kis* function alters the

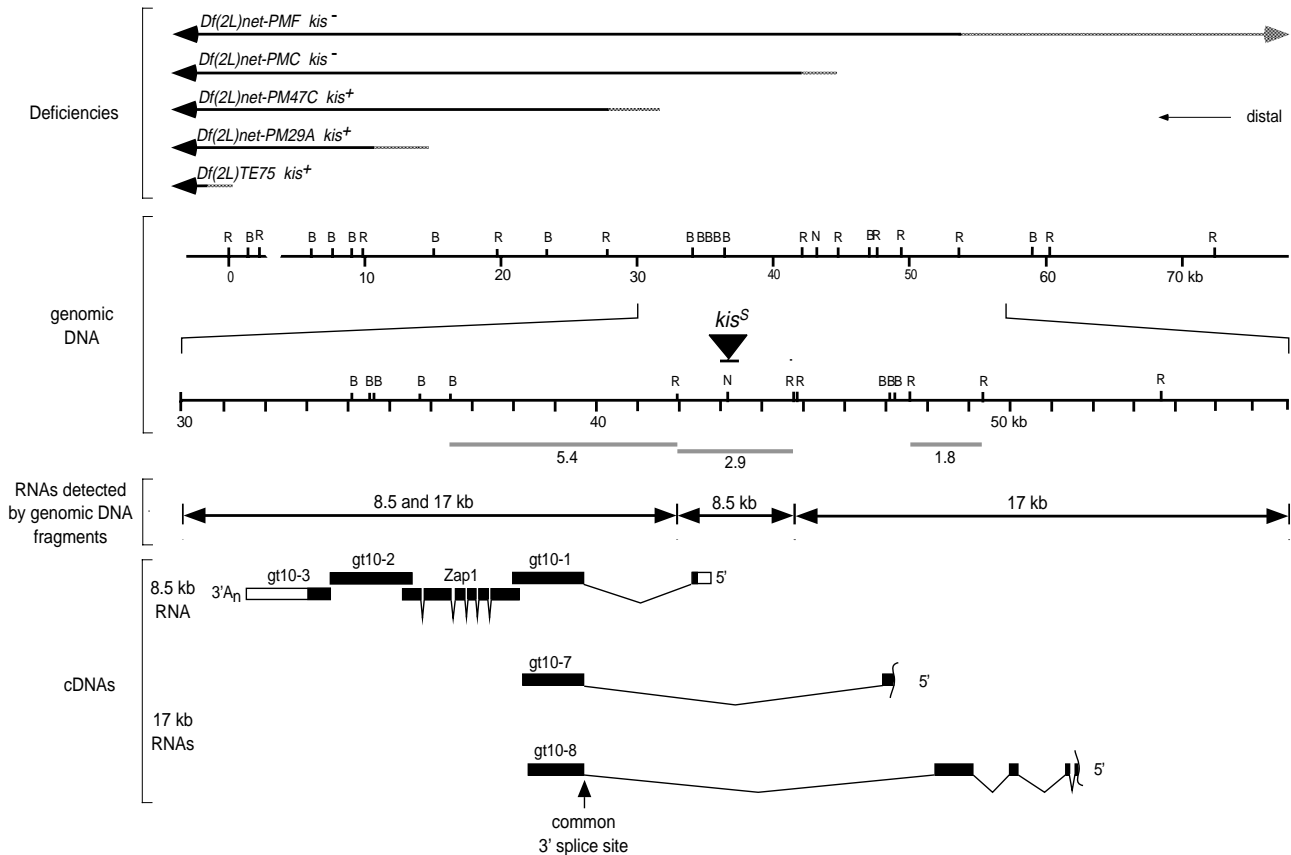


Fig. 4. Molecular map of the *kis* region. The restriction map based on our chromosome walk through the *kis* region is shown (R, *EcoRI*; N, *NotI*; B, *BamHI*). The region shown corresponds to nucleotides 53,093 to 17,401 of GenBank AC005334 fused to nucleotides 64,840 to 28,190 of GenBank AC004274. The deficiencies delimiting the distal end of the *kis* gene are shown above the restriction map (the shaded lines indicate the restriction fragments containing the breakpoints). The position of the *kis*^S P-element insertion is marked by a black triangle. A subset of the genomic DNA fragments used as probes for RNA blots and in situ hybridizations are marked below the restriction map, as are the regions of genomic DNA that recognize the 8.5 kb, 17 kb or both *kis* RNAs. cDNA clones and the exons of the deduced *kis* transcripts are represented by boxes. The four overlapping cDNAs gt10-3, gt10-2, Zap1 and gt10-1 represent the 8.5 kb RNA encoding the 225 kDa KIS protein. cDNAs gt10-7 and gt10-8 are distinct variants of the 17 kb *kis* RNA. The 3' splice site that can be paired with alternative 5' splice sites to generate the different *kis* RNAs is marked by an arrow. The common exon downstream of this 3' splice site begins at nucleotide 453 of the 8.5 kb RNA. Coding and untranslated flanking sequences in the cDNA clones are denoted by black and white boxes, respectively. Coordinates are measured in kb from the most distal *EcoRI* site.

expression of segmentation genes, we examined the distribution of several gap and pair-rule proteins in embryos derived from mosaic mothers. We found that the expression of the gap genes *Krüppel* (*Kr*), *hunchback* (*hb*) and *knirps* (*kni*) was normal, as was the expression of the primary pair-rule gene *eve* (data not shown). However, the expression of the segment-polarity gene *engrailed* (*en*) is dramatically altered by the loss of maternal *kis* function (Fig. 3D-F). *en* is normally expressed in fourteen stripes and this pattern is dependent on the function of pair-rule genes, including *eve*. Embryos from *kis*^S mutant mothers exhibited the fourteen *en* stripes (Fig. 3E), but the stripe borders were not as defined as in wild-type embryos (Fig. 3D). In the *kis*^I mutant embryos, only seven poorly defined *en* stripes were apparent (Fig. 3F). These patterns of *en* expression roughly correlate with the cuticular phenotypes. Taken together, these data indicate that maternal *kis* function is required for the expression or function of one or more segmentation genes, including *en*. The severity of the segmentation defects caused by loss of maternal *kis* function

made it difficult to determine its role in homeotic gene regulation during embryogenesis.

Molecular cloning of *kis*

To further investigate the roles of *kis* in segmentation and segment identity, we cloned and characterized the *kis* gene. Deficiency mapping revealed that the *kis* gene is located in salivary chromosome region 21B6-7 (Kennison and Tamkun, 1988; Lindsley and Zimm, 1992). To isolate DNA from this region, we conducted a chromosome walk in cosmid and phage libraries spanning more than 160 kb of contiguous genomic DNA. To locate the *kis* gene within our chromosome walk, we used in situ hybridization to map the breakpoints of several terminal deficiencies that lie within the 21B region (Fig. 4; data not shown). The breakpoints of five deficiencies allowed us to map an essential portion of the *kis* gene within a 17 kb region of our walk between the proximal breakpoints of *Df(2L)net-PM47C* and *Df(2L)net-PMC* (coordinates +28 to +45, Fig. 4). The position of the *kis* gene was more precisely determined by

the molecular mapping of a P-element allele, *kis^S*. Upon providing P-element transposase, the *kis^S* allele is revertible to wild type, indicating that the P-element insertion disrupts the *kis* gene (data not shown). Consistent with our deficiency mapping, this insertion maps to a 2.9 kb *EcoRI* fragment that lies between the proximal breakpoints of *Df(2L)net-PM47C* and *Df(2L)net-PMC* (Fig. 4; data not shown).

Because of the lack of relevant chromosomal aberrations, we could not determine the proximal boundary of the *kis* locus by deficiency mapping. We therefore used a functional assay to further define the boundaries of the *kis* gene. A cosmid bearing genomic DNA extending from approximately +27 to +61 of our walk was introduced into the *Drosophila* genome by P-element-mediated transformation. Although the distal end of this DNA lies within *Df(2L)net-PM47C* (which complements *kis* mutations), it is unable to rescue the hemizygous or homozygous lethality of several *kis* alleles. This result suggests that the functional limit of the *kis* gene extends beyond the map coordinate +61 of our walk.

kis encodes several large RNAs

To identify potential *kis* transcripts, subcloned genomic fragments from the regions flanking the *kis^S* insertion allele were used to probe northern blots of RNA isolated from *Drosophila* embryos. The 2.9 kb *EcoRI* fragment that spans the site of the *kis^S* insertion hybridized to an 8.5 kb RNA on northern blots (Fig. 5A,B). All fragments between the *kis^S* insertion and the breakpoint of *Df(2L)net-PM47C* detected two major RNAs of 8.5 kb and 17 kb (Fig. 5A). All genomic probes from the region of our walk proximal to *kis^S* recognized only the larger 17 kb RNA (Fig. 5B). Both the 8.5 and 17 kb RNAs are transcribed in the proximal-to-distal direction, as determined by hybridization of single-stranded RNA probes to blots of *Drosophila* RNA (Fig. 5B). The 8.5 and 17 kb RNAs could result from the expression of more than one gene, expression of a single gene with multiple promoters, alternative RNA processing or multiple polyadenylation sites.

To distinguish among these possibilities and identify the *kis* RNA, two embryonic cDNA libraries were screened with genomic probes surrounding the *kis^S* P-element insertion. By probing blots of *Drosophila* RNA with isolated cDNA fragments, we identified six cDNA clones (cDNAs gt10-1, gt10-2, gt10-3, gt10-7, gt10-8 and Zap1) that hybridized to both the 8.5 kb and 17 kb RNA (Fig. 4). These overlapping cDNAs were subcloned and their complete sequences determined. Four of the clones (gt10-1, gt10-2, gt10-3 and Zap1) together define an 8238 nucleotide transcript (GenBank AF113847). The sequence of the predicted *kis* RNA reveals a long 1450 nucleotide 3' untranslated region. The poly(A) tail is preceded by two consensus polyadenylation signals (AATAAA) at nucleotides 8189 and 8204. This 8238 nucleotide transcript appears to

correspond to the 8.5kb RNA and contains a single long ORF (Fig. 6). Using the first AUG as the initiation codon, this 6454 nucleotide ORF extends from nucleotides 316 to 6769. The predicted 2151 residue protein (224.8 kDa and a pI of 5.82) lacks any previously identified sequence motifs indicative of biochemical function (Fig. 6; see below).

The 8.5 and 17 kb *kis* RNAs encode large proteins that contain distinct N-terminal extensions

Comparison of the sequences of three partial cDNA clones (gt10-1, gt10-7 and gt10-8) to each other and to genomic DNA sequences accounted for the origin of the multiple *kis* transcripts (Fig. 4). The three cDNAs hybridize to both the 8.5 kb and 17 kb *kis* RNAs, but correspond to RNAs formed by the pairing of a single 3' splice site to one of three different 5' splice sites. The 5' exon of the gt10-1 cDNA maps to a genomic DNA fragment that hybridizes to only the 8.5 kb RNA. By contrast, the 5' exon of the gt10-7 and gt10-8 cDNAs map to a genomic DNA fragment that hybridizes only to the 17 kb RNA (Fig. 4). The three mRNAs arising from differential processing encode distinct but related proteins. The 8.5 kb RNA encodes the 2151 residue protein described above (Fig. 6), while the 17 kb isoforms encode proteins with extended amino termini.

The pairing of different 5' splice sites with a common 3' splice site generates RNAs encoding proteins bearing a common 2105 amino-acid C terminus. The protein encoded by the 8.5 kb RNA is unique only in its amino-terminal 46 residues (Fig. 6). The amino-terminal extensions encoded by the partial cDNA clones gt10-7 and gt10-8 are 90 and 425 amino acids in length, respectively. Our biochemical studies of the KIS protein (see below) have shown that the 17 kb RNAs encode proteins with molecular masses of greater than 500 kDa, indicating that these N-terminal extensions are quite large. Thus, alternative RNA processing generates *kis* RNAs encoding at least three large proteins.

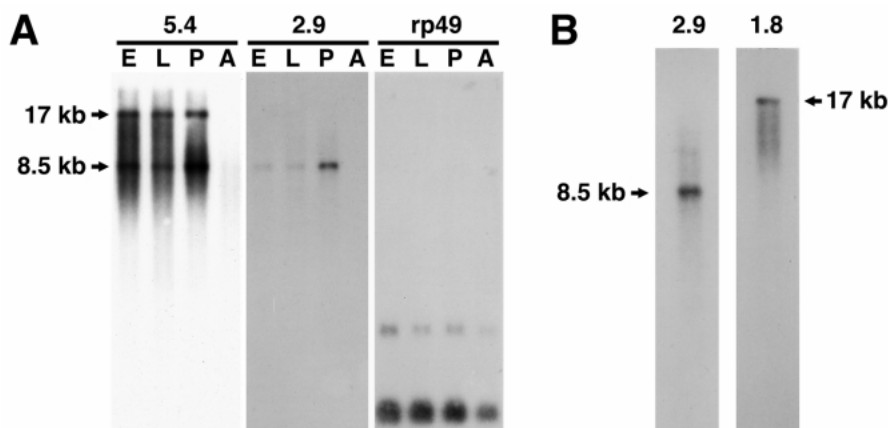


Fig. 5. Developmental expression of the *kis* RNAs. (A) A northern blot containing poly(A)⁺ RNA from 0-24 hour embryos (E), larvae (L), pupae (P) and adult flies (A) was hybridized to the 5.4 or 2.9 kb *kis* genomic DNA fragments noted in Fig. 4. As a control for equivalent loading and integrity of the RNA samples, the blot was probed with rp49 (O'Connell and Rosbash, 1984). Note that the 5.4 kb *kis* genomic DNA fragment hybridizes to both the 8.5 kb and 17 kb transcripts, but the 2.9 kb genomic DNA fragment recognizes only the 8.5 kb RNA. (B) Total RNA from 6-12 hour embryos was hybridized to single-stranded RNA probes. The 2.9 kb probe was derived from the *EcoRI* genomic fragment containing the *kis^S* P-element insertion and only recognizes the 8.5 kb transcript. Probe 1.8 is specific for the 17 kb transcript.

The KIS proteins contain a domain conserved in the trithorax group protein BRM and its human homologs

To identify sequences related to the 225 kDa KIS protein, we searched the current nucleic acid and protein databases as well as the complete sequence of the *Saccharomyces cerevisiae* genome using the TBLASTN and BLASTP programs (Altschul et al., 1997). Although no extensive homologies to other proteins were found, three proteins involved in transcriptional activation showed significant similarity (37–46% identity) to *kis* in a 41 amino-acid segment (Fig. 7), which we call the BRK domain (BRM and KIS). The three other proteins containing this motif are the *Drosophila* BRM protein and its two putative human homologs, BRG1 (hSNF2 α) and hBRM (hSNF2 β) (Chiba et al., 1994; Khavari et al., 1993; Muchardt and Yaniv, 1993). Although this region represents only a small fraction of the KIS protein, its presence in BRM and its human homologs is intriguing given that *kis* and *brm*

were identified in the same genetic screens (Kennison and Tamkun, 1988).

Database searches also identified a partial 6452 bp human cDNA (KIAA0308: GenBank accession AB002306) encoding a large protein that also contains the BRK domain (Fig. 7) as well as an additional 129 amino-acid segment that is highly related (45% identity) to residues 109 to 237 of the KIS protein. The same two domains are present in a predicted *Caenorhabditis elegans* protein (PID 3158516).

The *kis* gene is expressed at uniform levels along the anterior-posterior axis

Alternative splicing of the *kis* RNA thus leads to the production of BRK domain proteins that differ by the presence or absence of long N-terminal extensions. To investigate the significance of this heterogeneity, we compared the developmental expression of the *kis* RNAs by northern blotting and in situ hybridization. Northern blots of staged

<u>MEVIKPMPDFSSFSAFCEHLNKSSQIAATTAATMIPPTINNGKGIYLEKMER</u>	50	MLAEMEQAAMKISSKFSSTNSPHDVKNKWLSDMTSPLGDQLSIDYVGGGGA	1150
QGRMELAAKERESQRLQLIPKKWNRREEYEFRLVLTGYGVDLHVSTPMAS	100	SGSGSGNSRRSNRQQNSQQSSSAAQLKQKQQQQQQQQQQQHQHSMGPQ	1200
SNGSSLSPDWTKFKQMAHLERKSDETLTDYYKVFVAMCKRQAGLKLSESE	150	NLTG <u>EEPVPVINKQTGKRLGGNKAPQLKRLMQWLTEPNYEVDPKWLEQM</u>	1250
RGLBGIIEEIEKEHAKLILDRLEVLAKLREVARNPMLERLKLCTKNADT	200	QNPMTSPSPRASMESAYGSSAVKSHGGRPLSNLSTSSSSHTQQQSSAA	1300
PDWWEPRGRHDKELITAVLKHGLYRSETFIFNDPNFSFGSEKRFIRELEA	250	QSQAGGNSGSSKNSRQQTAAASAALDQAALQFGLAGLNPSSLANLPLGLG	1350
QIQRTIKLEAFNAEKAELAAAEKDAATEKAAATEKAAAEKAAASVKNEVI	300	AFDPKNPLAADFDPKNPLLSMSFGGMPGMGNIPGLGNLNNMNLFASLAGMG	1400
DLDELMTNESVIKESPVTPIKEEIKSEESPEKHDTADNLEDKNSDAEE	350	GLGNLAGMDTQSLAALMAAGPTLGGLTGASGGAGSGKSAQSQSSATSS	1450
STKIKGKEDFNPETTEKEALDESTNLNKDKSSSTETLVPEIEDSKPSEDKM	400	SSSASKKKQQQQQQAQNEAAQLAAAI.SASTGGSGSAGGKNAASASQLA	1500
EVEELPVAENGEKEGSPEKCSDAEDKKFSEKPSPAVPDQKVSMEADE	450	AGFPFLFPNPSLLYPPMGLGGLNPYSLGSSGLGSAYDQLAQYNNLLNGAT	1550
AAPNTEENCSDSESPEKECEDKVVKEVEEEKGENSYEAEQEETEKL	500	SSASNTSSTQSKSHQSQSKSSQRNTTASANSAAASLMNAMASMGGASTV	1600
EKSSIESEPEKSETVLEPEVAVPKKSTGDQDILDLVAGPSDPDDDEVVK	550	TTPSTASGSGRGRQSSSRNQSTTPTAADMAQLSLLMPGADPHLLES	1650
EKEKAVEEECKQAELKARFPDLEVIQPAVTKQKLEKPKLEMCIRWF	600	SRMSNMDLAQATRLMSLQMPPLSGTSSSGGNTSTSKRSSQAANEANV	1700
KDFALERRIAHIVACVESGNVPVDSKYSAFATCKGATDLSIALHESIPHL	650	QAKEQKWLESLARGALPTDLAALQAFSQGKMPSTSGSNTGTSTSSKSS	1750
SSLERRSTTDPVITITTDQGVTKHLQTSHMQQVASSASAASTSSGVPOVT	700	KAAATAAAALPQIPGMSDFPQAFLEAEMAAQMAAAGGSLPLSGPGSLA	1800
QSKPSSNSLPLGLDAKSINAATAAAVAAAA <u>AGGNATSLSSLLPGMSLSAA</u>	750	SLAGLTGGSAGGGSSASGSTSHSSSKRQREQDAFKQQMDYYTKTLGLGS	1850
<u>TGSSAGGVSGLSVPSAGSGVGGKRRRHIAIDVETERAKLHALLNSSTMAP</u>	800	GISLIPTSSAGGSSASSAANAAAAAYAAALDAEQHQHQKALSKRARG	1900
<u>KDWESEIANMEALSGSSGRGRGNSSASSGMOPPAHOHASLSRQSSGQF</u>	850	DLHPTKEELAAAGLPLNLGASMSIEKSLRGGSSSSSSSTPAPMTAEQ	1950
<u>SKPAVPAMKTPPPSMGAMPDLSSSLPKMNMTEMLKSASSGAIDLSEVQD</u>	900	DKVTLTPLNASGGGSSSSSSAAAAGLAANLPSQTTITTIAPPISSGASTS	2000
<u>FSMPKSSKSVHAALSASFSGMGNKSKLDDTLNKLKMKNNCTIEEPVIGKE</u>	950	SERSERSESRLSLTITNAADAACLPPPYEEADELIIQPIILKKPTAANPGS	2050
<u>KKRKLDLIVLGLSAAKEOKTFPPDPSLPSSKKPOIPPSVSVTPANLQSSS</u>	1000	SHGGSDVDDLDTAENTSAANLGSSSSSSSAAAAAEEENRRSSNRLKPR	2100
<u>NOOSNOKPFTITVTTVPKSKSGSSNSGS GTGGSSSASGGGAGGGLSAL</u>	1050	SGNEQGSVVEGQPPEKRRELSTRHTRSSADASTLNLSTGSES GAERN	2150
QNMAMGLSSKDSLNALLAQTMTADPQTFLLKQQQKMMQFLPPAQRKAYEN	1100	E*	2151

Fig. 6. Sequence of the KIS protein. The protein sequence for the 225 kDa isoform of KIS is shown. The sequence was obtained from four overlapping cDNA clones: gt10-3, gt10-2, Zap1 and gt10-1 (Fig. 4). The sequence unique to the 225 kDa protein isoform is marked by a dashed line (residues 1–46). The portion of the protein sequence against which the anti-KIS rabbit polyclonal antibody was raised is underlined (residues 731–1029). The BRK domain (residues 1205–1245) is marked by a double line.

Fig. 7. The 225 kDa KIS protein contains a short domain conserved in BRM and other chromatin-remodeling factors. A 41 amino-acid segment of KIS (residues 1205 to 1245) is aligned to the corresponding regions of *Drosophila* BRM

													<u>% identity</u>																																
kismet	1205	EE	P	P	V	P	V	I	N	K	Q	T	G	K	R	L	G	G	N	K	A	P	Q	L	K	R	L	M	Q	W	L	T	E	N	P	N	Y	E	V	D	P	K	1245	-	
hbrm	588	D	L	P	V	K	V	T	H	T	E	T	G	K	V	L	F	G	P	E	A	P	K	A	S	Q	L	D	A	W	L	E	M	N	P	G	Y	E	V	A	P	R	628	46%	
BRG1	613	D	L	P	V	K	V	I	H	V	E	S	G	K	L	T	G	T	D	A	P	K	A	G	Q	L	E	A	W	L	E	M	N	P	G	Y	E	V	A	P	R	653	46%		
brm	651	D	M	R	V	H	V	V	E	Q	C	T	G	K	L	T	G	D	A	P	M	L	K	H	L	H	R	W	L	N	M	H	P	G	W	Y	W	D	D	691	37%				
KIAA0308	884	E	S	P	V	P	V	I	N	L	K	D	G	T	R	L	A	G	D	A	P	K	R	K	D	L	E	K	W	L	K	E	H	P	G	Y	V	E	D	L	G	924	51%		
consensus		*			V	V							G	L	G				A	P					L	W	L					P	*												

and the human BRG1, hBRM and KIAA0308 proteins. Amino acids that are identical in KIS and one or more of the other proteins are highlighted in black; conserved residues are highlighted in gray. Amino acids that are conserved or identical are marked below the alignment by an asterisk or their single letter code, respectively.

Drosophila RNA were hybridized to a genomic DNA fragment that recognizes all known *kis* transcripts. Both the 8.5 and 17 kb *kis* RNAs are easily detected in embryos, larvae and pupae, but not adults (Fig. 5A). Hybridization of a cDNA probe (Zap1) that recognizes all *kis* RNAs to whole-mount preparations of embryos revealed that *kis* is ubiquitously expressed from the onset of embryogenesis through germ-band extension (Fig. 8). During germ-band retraction, the RNAs gradually become localized to the ventral nerve cord and brain, eventually falling below detectable levels just prior to hatching. Identical patterns were observed using probes that specifically recognize either the 8.5 or 17 kb *kis* RNA (data not shown).

To monitor the expression and subcellular distribution of the KIS proteins, we raised rabbit polyclonal antisera against a 299 amino-acid segment common to all three of the identified protein isoforms (residues 731-1029, Fig. 6). Our molecular analysis predicted the existence of a 225 kDa protein encoded by the 8.5 kb *kis* RNA, and at least two larger proteins encoded by the 17 kb *kis* transcripts (Figs 4, 6). As anticipated, our affinity-purified antibody detected two major polypeptides in *Drosophila* embryo extracts (Fig. 9A). The smaller of the two most abundant polypeptides has an apparent molecular mass of 230 kDa, which is very close to the predicted mass of the protein encoded by the 8.5 kb *kis* RNA. The larger polypeptide has an apparent molecular mass greater than 500 kDa and is likely to be encoded by the 17 kb *kis* RNA. Consistent with this possibility, the independent analysis of cDNA clones corresponding to the 17 kb *kis* RNA has shown that it encodes a protein with a predicted molecular mass of 574 kDa (Allan Wong, Marc Therrien, Debbie Morrison and Gerald Rubin, personal communication; see Discussion). Several much weaker signals were also detected on western blots, which could represent either additional protein isoforms or degradation products.

We next used our affinity-purified antibodies to examine the spatial distribution of KIS proteins in whole-mount preparations of *Drosophila* embryos and third-instar larvae. Consistent with a possible role in transcriptional regulation, the KIS proteins were detected at uniform levels in nuclei from the onset of embryogenesis through germ-band extension (Fig. 9B). The proteins then became localized to the developing central nervous system and were detected in the nuclei of the epidermal cells (data not shown). Levels gradually diminished after dorsal closure, when the intersegmental grooves can first be distinguished. In third-instar larvae, KIS proteins were detected in nuclei of all imaginal discs, with the exception of the labial disc, where little to no protein was detected. KIS

proteins were also detected in the polytene nuclei of the larval salivary gland (data not shown).

KIS is not physically associated with the trithorax group protein BRM

Since the trithorax group genes *brm* and *kis* were identified in the same genetic screens for *Pc* suppressors, it seemed possible that they might physically interact to regulate the transcription of homeotic genes. We recently purified the BRM complex from *Drosophila* embryos and characterized its subunit composition (Papoulas et al., 1998). Although KIS was not identified as a major subunit of the BRM complex in that study, substoichiometric amounts of several large polypeptides reproducibly copurified with BRM. To investigate whether any of these peptides might correspond to KIS, we probed western blots of purified BRM complex and whole embryo extracts with antibodies against KIS. Neither the large nor small forms of the KIS protein were present in purified preparations of BRM complex (Fig. 9A). We have also been unable to coimmunoprecipitate the BRM and KIS proteins from *Drosophila* embryo extracts (data not shown). These findings suggest that the BRM and KIS proteins do not stably interact in the *Drosophila* embryo.

DISCUSSION

kis is a member of the trithorax group of homeotic gene activators

The genetic interactions between *kis* and *Pc* provided the first clue that *kis* plays an important role in the determination of body segment identity. We showed that *kis* mutations suppress the adult *Pc* phenotype by preventing the ectopic transcription of homeotic genes. Thus, *kis* is a member of the trithorax group of homeotic gene activators. Mosaic analyses revealed that loss of *kis* function causes homeotic transformations, including the transformation of first leg to second leg and the fifth abdominal segment to a more anterior identity. These phenotypes are identical to those associated with loss-of-function *Scr* and *Abd-B* mutations, respectively. Taken together, these findings suggest that *kis* acts antagonistically to *Pc* to activate the transcription of both *Scr* and *Abd-B*.

It is intriguing that *kis* mutations alter the fate of only the fifth abdominal segment, since the identities of the fifth through ninth abdominal segments are determined by a single homeotic gene, *Abd-B* (Celniker et al., 1989; Delorenzi and Bienz, 1990). Variations in the levels of ABD-B protein result in the differences between these abdominal segments, with *Abd-B*

expression being lowest in the fifth abdominal segment (Duncan, 1987; Hopmann et al., 1995). Parasegment-specific *cis*-regulatory regions, termed *infra-abdominal* (*iab*) regions (Lewis, 1978) control *Abd-B* expression. Each *iab* region is named for the segment that it affects (*iab-5* through *iab-9*). Mutations in both *iab-5* (Celniker et al., 1990) and *kis* affect the identity of only the fifth abdominal segment, suggesting that the KIS protein may interact specifically with the *iab-5* *cis*-regulatory element of *Abd-B*.

We suspect that *kis* interacts not only with *Scr* and *Abd-B*, but with other homeotic genes as well. For example, the isolation of *kis* mutations as enhancers of loss-of-function *Deformed* (*Dfd*) mutations (Gellon et al., 1997) suggests that *kis* is probably also required to activate transcription of this ANTC homeotic gene. Furthermore, *kis* duplications strongly enhance the transformation of wing to haltere in *Pc* heterozygotes, a phenotype caused by the ectopic transcription of *Ubx* in the wing imaginal disc (J. A. K., unpublished data). However, *kis* mutations do not cause haltere-to-wing transformations due to decreased *Ubx* transcription. A possible explanation for the lack of homeotic transformations in *kis* clones in segments other than the prothoracic and fifth abdominal segment is that the mutations used in these studies are not null alleles. *kis^l* is a strong loss-of-function mutation. It has not been characterized at the molecular level, however, and may not completely eliminate *kis* function. It is also possible that sufficient levels of KIS protein persist in homozygous mutant tissue following mitotic recombination to support normal development. Further genetic studies, including the analysis of conditional *kis* alleles, will be necessary to distinguish between these possibilities.

***kis* mutations cause pair-rule segmentation defects**

Germline clonal analysis revealed an unanticipated role for *kis* in segmentation. Embryos from mosaic *kis^S* females exhibit a deletion or alteration of every other segment, while mutant embryos from mothers bearing germline clones of the stronger *kis^l* allele usually develop only half of the normal number of segments. This variation in phenotypic severity is closely correlated with the extent to which *en* expression is disrupted. The phenotypes associated with loss of maternal *kis* function resemble those caused by mutations in pair-rule segmentation genes that cause the deletion of the odd-numbered parasegments. *kis* thus appears to be necessary for the expression (or function) of one or more pair-rule genes. Recent genetic studies have suggested that *kis* may also be involved in the *Notch* signaling pathway (Go and Artavanis-Tsakonas, 1998; Verheyen et al., 1996). Thus it appears that *kis* plays roles in addition to the regulation of homeotic genes.

What pair-rule genes might require *kis* for their activity? Based on the *kis* mutant phenotype, perhaps the best candidates are *eve* and *hairy* (*h*), both of which are required for the formation of odd-numbered parasegments. Unlike *eve*, *h* and most other segmentation genes, *kis* is uniformly expressed in the early embryo. This raises the possibility that KIS functions as an essential cofactor or modifier of EVE or other pair-rule

proteins. It is also possible that loss of *kis* function might result in pair-rule genes being transcribed outside of their normal expression domains. For example, Cadigan et al. (1994a,b) have shown that ectopic expression of *sloppy-paired* (*slp*) results in phenotypes similar to hypomorphic and amorphic *eve* mutants, and therefore similar to the phenotypes observed in embryos mutant for maternal *kis*. Additional work will be necessary to determine the molecular basis of the segmentation defects resulting from loss of maternal *kis* function.

The KIS proteins contain several domains conserved in BRM and other chromatin-remodeling factors

Our molecular studies of *kis* provide insights into the mechanism of action of the KIS proteins. Alternative RNA processing produces several large nuclear proteins with molecular masses of approximately 225 kDa and ~600 kDa. These proteins share a common 2105 amino-acid C terminus containing a 41 amino-acid segment – the BRK domain – that is conserved in the trithorax group protein BRM and related chromatin-remodeling factors in humans.

A more direct connection between KIS and chromatin-remodeling factors has recently been provided by independent studies in Dr Gerald Rubin's laboratory (Allan Wong, Marc Therrien, Debbie Morrison and Gerald Rubin, personal communication). They recovered *kis* mutations in an unrelated genetic screen and analyzed overlapping cDNA clones corresponding to the 17 kb *kis* RNA. Their analysis of these clones has confirmed and extended our analysis of the *kis* RNAs and proteins. The 17 kb *kis* RNA encodes a protein with a predicted molecular mass of 574 kDa, very similar in size to the largest KIS proteins that we detect by western blotting. This protein contains the same 2105 residue C terminus as the 225 kDa KIS protein.

The N-terminal extension unique to the larger KIS protein is 3217 amino acids in length and contains several interesting

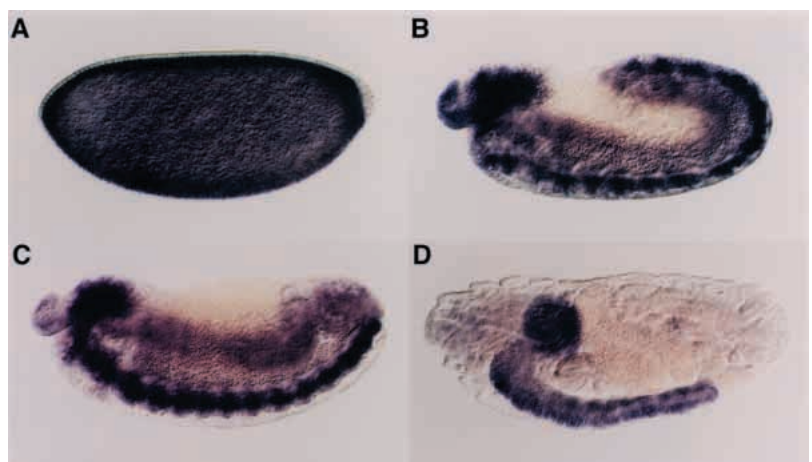


Fig. 8. Distribution of *kis* RNA in *Drosophila* embryos. Whole-mount preparations of *Drosophila* embryos were hybridized to digoxigenin-labeled probes. Embryos were hybridized to a *kis* cDNA fragment that recognizes both the 8.5 kb and 17 kb *kis* transcripts. Transcripts are uniformly distributed in cellular blastoderm stage embryos (A). As gastrulation proceeds (B-D), *kis* transcripts gradually become restricted to the ventral nerve cord and brain (D). Note that the *kis* RNA is uniformly distributed along the anterior-posterior body axis at all stages of development.

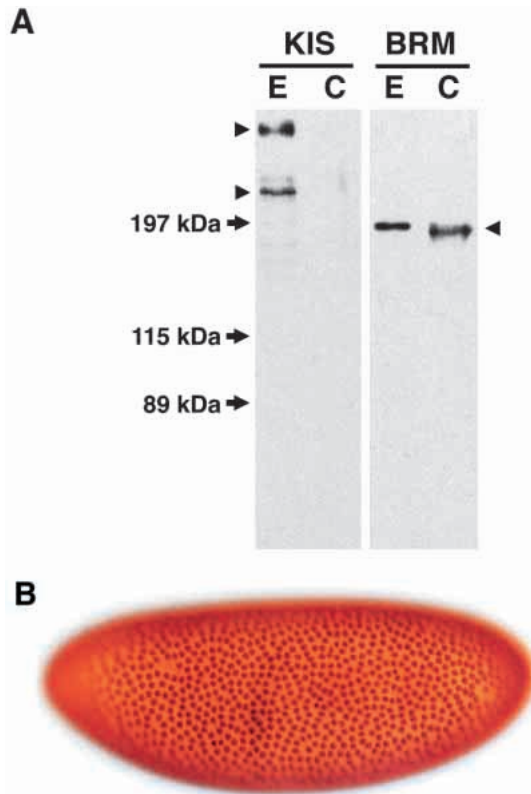


Fig. 9. *kis* encodes several large nuclear proteins. (A) Whole embryo extract (E) and purified BRM complex (C) were resolved on a 6% SDS-polyacrylamide gel and analyzed by western blotting using antibodies against KIS or BRM. The 230 kDa and large (>530 kDa) KIS isoforms are marked by arrowheads. Molecular masses were determined relative to prestained molecular mass markers and cytoplasmic dynein (530 kDa). (B) Affinity-purified anti-KIS polyclonal antibodies were used to detect KIS proteins in whole-mount preparations of *Drosophila* embryos. The KIS protein is nuclear and present at uniform levels along the anterior-posterior axis.

functional domains, including an ATPase domain highly related to those found in BRM (44% identity over 478 amino acids) and other SWI2/SNF2 family members and two chromodomains. The ATPase domain of KIS is most highly related (approximately 50% identity) to that of the CHD (chromodomain-helicase domain) proteins (reviewed in Woodage et al., 1997). Both the KIS and CHD proteins contain a single ATPase domain and two chromodomains. Unlike KIS, however, none of the previously identified CHD proteins contain a BRK domain. Although chromodomains are a distinguishing feature of CHD proteins and many other proteins that interact with chromatin, including the *Drosophila* PC and HP1 proteins (Cavalli and Paro, 1998; Paro and Hogness, 1991; Singh et al., 1991), the function of the chromodomain is currently unknown. It may function as a dimerization or protein-protein interaction domain that targets proteins to the appropriate chromosomal location (Cavalli and Paro, 1998; Messmer et al., 1992; Platero et al., 1995). Both the 225 kDa and 574 kDa forms of the KIS protein contain the BRK domain. Although the BRK domain is conserved in BRM and its human homologs (BRG1 and hBRM), this domain is

not present in yeast chromatin-remodeling factors related to BRM, including SWI2/SNF2 and STH1. This suggests that the BRK domain may interact with a component of chromatin unique to higher eukaryotes.

The discovery that alternative RNA processing produces a 225 kDa protein lacking the ATPase domain and chromodomains was quite surprising, since these regions are likely to be critical for the function of the KIS protein. What is the function of the 225 kDa KIS protein? If this protein, which lacks the ATPase domain, retains the ability to interact with other proteins, it may function as a naturally occurring dominant-negative protein that regulates the activity of the larger form. This restraining influence would be particularly useful for regulating the activity of SWI2/SNF2 family members that are unusually abundant or stable. To investigate the functional significance of the alternative processing of the *kis* RNA, we cloned the *kis* gene from *Drosophila virilis* (G. D., I. Z., W. W. and J. W. T., unpublished data), a species that diverged from *D. melanogaster* more than 60 million years ago. We found that both the 8.5 and 17 kb RNAs are expressed in *D. virilis* embryos, suggesting that both the 225 and 574 kDa KIS proteins are functionally important.

The presence of an ATPase domain, BRK domain and two chromodomains in the 574 kDa KIS protein strongly suggests that it influences chromatin structure. Do *kis* and *brm* play similar roles in chromatin remodeling and development? The KIS and BRM proteins are not highly related outside the ATPase and BRK domain. Furthermore, the KIS protein lacks domain II, an evolutionarily conserved region of the BRM protein that is thought to mediate interactions with the BAP155 subunit of the BRM complex (Elfring et al., 1998; Papoulias et al., 1998). This domain and the C-terminal bromodomain are considered to be distinguishing characteristics of the ATPase subunits of SWI/SNF complexes. Although both BRM and KIS may influence chromatin structure, these differences suggest that the two proteins activate transcription via distinct biochemical mechanisms.

Genetic studies have revealed functional differences between *brm* and *kis*. *brm* is required for oogenesis (Brizuela et al., 1994). Clonal analyses of *brm* revealed that loss of zygotic *brm* function decreased cell viability and caused peripheral nervous system defects, including the twinning of mechanosensory bristles and campaniform sensilla (Elfring et al., 1998). Similar defects were not observed in clones of mutant *kis* tissue, even when we induced clones at the cellular blastoderm stage of embryogenesis. Although *brm* and *kis* are required for the activation of *Scr* and *Abd-B*, these findings suggest that BRM and KIS do not regulate identical sets of target genes. Additional studies will be required to determine the common and distinct functions of these and other trithorax group genes.

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