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The Drosophila Homolog of the Immunoglobulin Recombination Signal–Binding Protein Regulates Peripheral Nervous System Development

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Summary

The J_κ RBP binds to the immunoglobulin recombination signal sequence flanking the κ-type J segment. We previously isolated the highly conserved homolog of the J_κ RBP gene from D. melanogaster, which is not thought to have immunoglobulin molecules. Using many deficiency mutants and in situ hybridization, we mapped the Drosophila J_κ RBP gene in a region containing two recessive lethal mutations, i.e., *br26* and *br7*, which shows the dominant *Suppressor of Hairless (Su(H))* phenotype in heterozygotes. All six *Su(H)* alleles analyzed at the DNA level contained mutations in the Drosophila J_κ RBP gene. Since the *Su(H)* mutation affects peripheral nervous system development, the Drosophila J_κ RBP gene product is involved in gene regulation of peripheral nervous system development. The results also imply that the immunoglobulin recombination signal sequence and the target sequence of the Drosophila J_κ RBP protein might have a common evolutionary origin.

Introduction

Site-specific recombination in the immunoglobulin and T cell receptor (TCR) gene loci contributes to generation of immune diversity. The variable-region genes of the immunoglobulin and TCR loci are composed of multiple gene segments (V, D, and J) separated by stretches of noncoding DNA (for reviews see Honjo, 1983; Tonegawa, 1983). The recombining DNA segments are flanked by the conserved recombination recognition sequences (RSs) composed of heptamer (CACTGTG) and nonamer (GGTTTTTGT) sequences that are separated by nonconserved spacers. The length of the spacer sequence should be either 12 ± 1 bp or 23 ± 1 bp, but the spacer sequence itself is not conserved. Joining between two DNA segments may occur only if they are flanked by RSs with different spacer sequences (the 12/23 spacer rule) (Early et al., 1980). The heptamer and nonamer sequences are highly conserved during evolution from shark and chicken to human (Litman et al., 1985a, 1985b; Schwager et al., 1988). The presence of RS is essential and sufficient for the correct VDJ recombination, because RS-flanked DNA, which has no sequence homology with either the immunoglobulin or TCR gene, could rearrange in pre-B cell lines (Akira et al., 1987; Hesse et al., 1989) and transgenic mice (Kawaichi et al., 1991). The recombination frequency of RS-flanked DNA in pre-B cell lines was extremely reduced by artificial mutations in the heptamer and nonamer sequences (Akira et al., 1987; Hesse et al., 1989). These facts indicate that the VDJ recombination system should recognize the RS.

We previously isolated a protein that specifically binds to the J_κ RS containing the 23 bp spacer sequence (Hamaguchi et al., 1989). Cloning and characterization of the cDNA encoding this protein (Matsunami et al., 1989) have shown that its amino acid sequence has a 40-residue motif that is a common catalytic domain in a family of site-specific recombinases called integrases, including those of λ and P phages (for review see Craig, 1988). The presence of the integrase motif in the J_κ RS-binding protein (J_κ RBP) suggests that at least a part of the J_κ RBP might have derived from an evolutionarily old ancestor, although the antigen-specific immune system seems to have been created relatively recently. We found that the J_κ RBP gene sequence is conserved not only in mammals but in Xenopus laevis and Drosophila melanogaster (Furukawa et al., 1991). Cloning of the Drosophila J_κ RBP gene homolog showed that the J_κ RBP gene product has 75% homology with the murine J_κ RBP and binds to the J_κ RS with a similar sequence specificity as that of murine J_κ RBP. The results suggest that RS-like sequences might also be conserved in invertebrates including Drosophila and that the J_κ RBP may play essential biological roles in invertebrates as well as vertebrates.

The Drosophila peripheral nervous system (PNS) develops in the form of external sensilla. *Hairless* alleles are characterized by the defect of specific major bristles (macrochaetae) and the shortening of L5 wing veination in heterozygotes, as well as recessive lethality (Plunkett, 1926; Bang et al., 1991). Hypomorphic mutations in the *br7* locus were described as dominant *Suppressors of Hairless (Su(H))* by Nash (1970), who also gave evidence that duplications of the same wild-type chromosomal region act as dominant enhancers of the *Hairless* phenotype (*E(H)*). None of the *Su(H)* lethal alleles show any phenotypes other than recessive lethality in the absence of *Hairless* (Ashburner, 1982). Hypermorphic mutations in the *br7* locus enhance *Hairless*. To clarify a biological function of the Drosophila J_κ RBP, we searched for Drosophila mutants of the J_κ RBP gene. Unexpectedly, we obtained evidence that *Su(H)* and *E(H)* mutants have mutations in the Drosophila J_κ RBP gene, indicating that the Drosophila J_κ RBP gene is located in the *br7* locus.

Results

Deficiency Mapping of the Drosophila J_κ RBP Gene

We previously detected an accumulation of grains in the 35BC region on chromosome 2 by chromosome in situ hybridization using a labeled Drosophila J_κ RBP probe (Furukawa et al., 1991). Fortunately, Ashburner and his colleagues (O'Donnell et al., 1977; Woodruff and Ashburner 1979a, 1979b; Ashburner, 1982; Ashburner et al., 1982a, 1982b, 1983, 1990; Gubb et al., 1984, 1985, 1986)

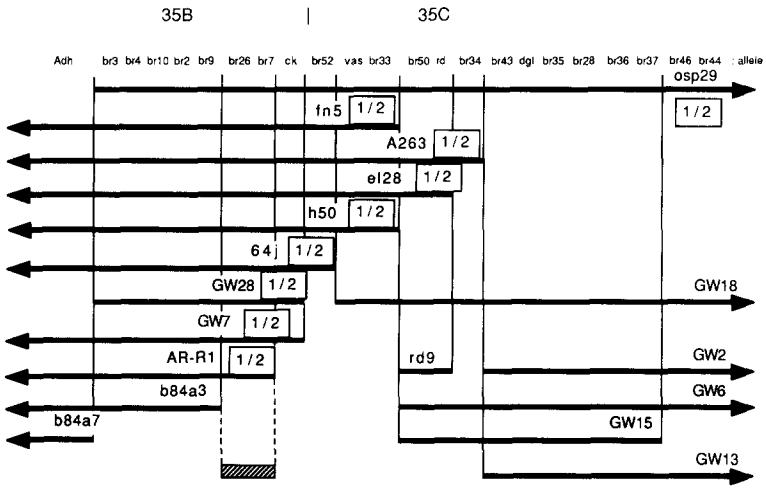

Figure 1. Deficiency Mapping of Mutants Containing Deficiencies over the 35BC Region of Chromosome 2

Names of lethal alleles are given below the 35B and 35C regions. Horizontal arrows show deficiency regions and extensive deficiency in the directions of the arrows. Names of deficiency mutants are given above each bar or arrow. The deficiency mutants that gave a half density band with a Drosophila J_κ RBP probe by Southern hybridization are indicated by 1/2. The hatched bar shows the expected region of the Drosophila J_κ RBP gene locus. The half intensity was evaluated with the internal standard of the Drosophila insulin receptor-like (DIR) gene (Nishida et al., 1986). The heterozygous *Df(3R)e*^{D7} fly deleting the DIR gene showed a half intensity band of the DIR gene compared with the wild-type fly (data not shown). This assures that the DIR gene can be used as an internal standard.

have reported extensive genetic characterization of this region. To map the precise location of the Drosophila J_κ RBP gene, we first studied many mutants containing deficiencies over the 35BC region. These deficiency mutants are maintained heterozygously with Cy balancer chromosomes. Genomic DNAs of the heterozygous deficiency mutants deleting the Drosophila J_κ RBP gene locus are expected to give a band half as intense as that of the wild-type fly by Southern hybridization with the Drosophila J_κ RBP probe. The results of the deficiency mapping are summarized in Figure 1.

GW28, with the region from *br3* to *ck* deleted, and *AR-R1*, with the region left to *ck* deleted, showed half the intensity of the Drosophila J_κ RBP gene, whereas *b84a3*, with the region left to *br26* deleted, contained a normal copy of the Drosophila J_κ RBP gene. The locus of the J_κ RBP gene should be included in the deficiency of all the mutants that gave a half intensity band (indicated by 1/2 in Figure 1) and excluded from the deficiency of all the mutants that showed the wild-type intensity band. Taking these results together, the expected locus of the Drosophila J_κ RBP gene is indicated by a hatched bar, which contains two homozygous lethal loci, i.e., *br26* and *br7*. Mutants of the Drosophila J_κ RBP gene are expected to be lethal, because of its extremely strong conservation in the amino acid sequence (75%) in comparison with its murine counterpart (Furukawa et al., 1991). We therefore considered the possibility that the allele of the J_κ RBP gene might be either *br26* or *br7*.

Rearrangement of the Drosophila J_κ RBP Gene in *br7* Mutants Induced by P-M Mutagenesis

To test the above hypothesis we first analyzed one *br26* mutant allele (*ok5*) and two *br7* mutant alleles (*HG36* and *Pl1*) by genomic Southern hybridization with the Drosophila J_κ RBP probe (data not shown). Although DNAs of *ok5*, *HG36*, and wild-type Conton-S flies contained the 4.0 kb EcoRI and 8.5 kb BamHI fragments that hybridized with the Drosophila J_κ RBP probe, DNA of *Pl1* that was mutagenized by P-M mutagenesis contained a 10.5 kb BamHI fragment in addition to the 8.5 kb BamHI band. Since the appearance of the 10.5 kb fragment was suspected to be due to P element insertion in *Pl1*, we focused our analysis on the *br7* locus.

It has been shown that hypomorphic *br7* alleles dominantly suppress the *Hairless* mutation, which shows characteristic bristle loss, whereas hypermorphic alleles dominantly enhance the *Hairless* mutation (Nash, 1970; Ashburner, 1982). We analyzed eight *Su(H)* mutants and one *E(H)* mutant, as listed in Table 1. Among them, five mutants (*HG36*, *IB115*, *SF8*, *AM1*, and *S5*) have been mutagenized by chemical reagents, three (*Pl1*, *Pl3*, and *MR1*) by P-M mutagenesis, and the other (*AR9*) is a mutant found among a natural population from Greece (Ashburner, 1982). Genomic Southern hybridization of *HG36*, *S5*, and *IB115* DNA using the Drosophila J_κ RBP probe (probe I in Figure 2) detected the 4.0 kb EcoRI and 8.5 kb BamHI fragments that are identical to those of Canton-S DNA (data not shown). The same restriction fragments were detected in *SF8* and *AM1* DNA, but no further studies on these mutants were carried out. The *Pl1*, *Pl3*, and *MR1* DNAs each showed an additional larger BamHI band besides the 8.5 kb BamHI band. *AR9* DNA contained the 8 kb EcoRI and 12 kb BamHI fragments in addition to those of Canton-S DNA (data not shown).

Table 1. Mutant Alleles of *br7*

Name of Allele	Mutation Was Induced by:
Pl1	P-M mutagenesis
Pl3	P-M mutagenesis
MR1	P-M mutagenesis
SF8	Triethylenemelamine
HG36	EMS
S5	EMS
IB115	EMS
AM1	EMS
AR9	Natural population

S5 is an *E(H)* mutation. All the others are *Su(H)* mutations. EMS, ethylmethanesulfonate.

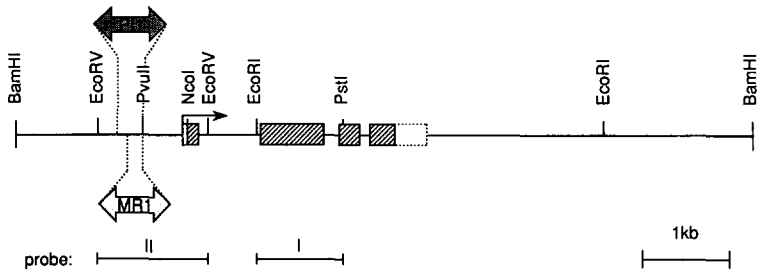

Figure 2. Location of P Elements in the Drosophila J_κ RBP Gene of *br7*^{PI1} and *br7*^{MR1} Mutants

Restriction map of the wild-type 8.5 kb BamHI fragment containing the whole coding region of the Drosophila J_κ RBP gene is shown. A horizontal arrow indicates the starting point of the major transcription initiation site and the direction of transcription (Furukawa et al., 1991). Hatched boxes show the exons deduced from the cDNA sequence. Open and stippled boxes indicate the 5′- and putative 3′-untranslated regions, respectively. The fragments used as probes I and II are shown below. Possible regions of P element insertion in *PI1* and *MR1* are indicated by dotted lines. P elements are represented by double arrows. Estimated sizes of P element insertions in *PI1* and *MR1* are 2.5 kb and 2.0 kb, respectively.

Larger BamHI fragments detected in *PI1*, *PI3*, and *MR1* DNA were likely due to P element insertion. The position of insertion should be inside the 2.8 kb BamHI–EcoRI or 1.7 kb EcoRI–BamHI region (Figure 2), because the internal 4.0 kb EcoRI fragment showed no rearrangement. Since the 2.8 kb BamHI–EcoRI region contains the first exon and the promotor/enhancer region, we further analyzed these three mutants by Southern hybridization using the 1.3 kb EcoRV–EcoRV probe (probe II in Figure 2). This probe detected two sizes of EcoRI–BamHI and EcoRV–NcoI fragments in each mutant, suggesting that the promotor or enhancer of the Drosophila J_κ RBP gene might be disrupted by P element insertion. To confirm that these insertions are due to the P element, both the 8.5 kb and the 11 kb BamHI fragments of *PI1* were cloned into recombinant phages. The 11 kb, but not the 8.5 kb, BamHI fragment was hybridized with P element DNA (data not shown). The P element–inserted fragment of *MR1* was also cloned and analyzed in a similar manner. The location of the P element insertion was estimated by restriction mapping of the cloned BamHI fragments. Possible P element insertion sites of *PI1* and *MR1* are between positions −800 and −500 (the major transcription initiation site is set as the origin of scale) and between positions −700 and −500, respectively (Figure 2). The difference in size of the rearranged bands is probably due to the internal P element deletion, which is generally observed.

AR9 DNA digested with EcoRI or PstI was analyzed by Southern hybridization with three regions of the Drosophila J_κ RBP gene as probes (data not shown). The results did not allow us to determine the nature of the *AR9* mutation, which is either multiple polymorphic restriction sites or very complicated chromosomal aberration. This unclear conclusion is mostly due to the fact that we do not have a control allele of *AR9* derived from a wild Greek population.

Point Mutations in the Drosophila J_κ RBP Gene

To identify mutations of *HG36*, *IB115*, and *S5*, the 8.5 kb BamHI fragments that should include both *br7* alleles of the compound heterozygous flies were purified from each mutant line, cloned into the EMBL3 vector, and screened with probe I (Figure 2). We searched for the *Su(H)* mutation by sequencing the whole coding region and intron–exon boundaries of 5 to 10 independent clones for each mutant. Comparison of the obtained sequences with that from the wild type revealed several deviations from the published sequence of the Drosophila J_κ RBP gene. Most of them were ascribed to polymorphism without any amino acid change, but we identified an error of the published sequence (Furukawa et al., 1991), i.e., transition from cytosine to thymidine at position 2412, leading to amino acid substitution of Val-448 for Ala-448.

In the case of *HG36*, we found an additional mutational transition of guanosine to adenosine at position 1727, which leads to an amino acid substitution of Lys-261 for Glu-261 (Figure 3). As a result, the Drosophila J_κ RBP^{HG36} protein should have an opposite charged amino acid in the integrase motif region. Such a drastic change of Drosophila J_κ RBP^{HG36} would result in a severe, if not complete, loss-of-function mutation of the Drosophila J_κ RBP protein. The *IB115* allele has a mutation of transition from adenine to thymidine at position 1226. This mutation replaces Lys^{94} (AAG) with a stop codon (TAG) (Figure 3). This lesion should result in production of a truncated Drosophila J_κ RBP peptide containing only one-sixth of the Drosophila J_κ RBP protein, which would be very likely to be a loss-of-function mutation. In the *S5* allele, a mutational transition from guanosine to adenosine at position 1611 was identified. This mutation leads to an amino acid substitution of Arg-222 for His-222 (Figure 3). It is interesting to note that the amino acid replacement within the positive charge group causes a dominant enhancer phenotype of the *Hairless* mutation (Ashburner, 1982) in contrast to the *HG36* mutation. Taken together, these results indicate that *Su(H)* and *E(H)* mutations are alleles of the Drosophila J_κ RBP gene.

Developmental Expression of Drosophila J_κ RBP Transcripts

We previously identified Drosophila J_κ RBP transcripts in total embryos and male and female adults (Furukawa et al., 1991). The homozygous loss-of-function mutant of the Drosophila J_κ RBP gene (*Su(H)*) is known to be lethal in the pupal stage (Ashburner, 1982). This suggests that the

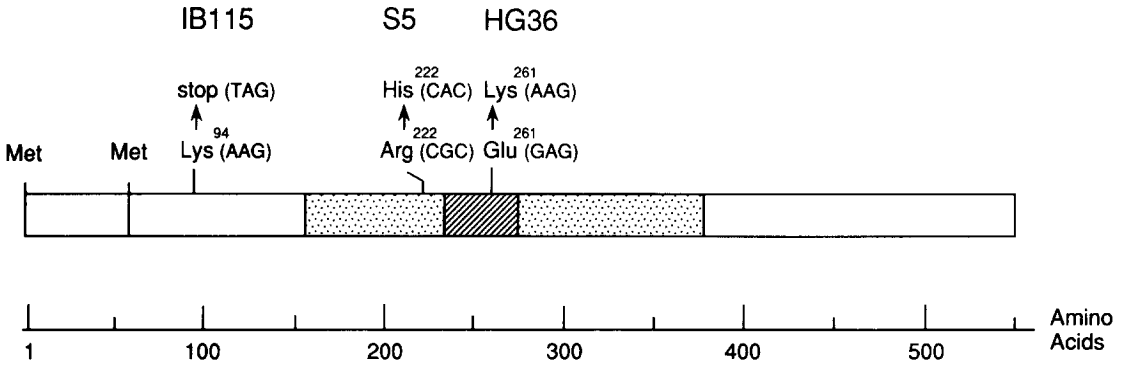

Figure 3. Nucleotide Substitution in the Drosophila J_κ RBP Gene of *br7*^{HG36}, *br7*^{IB115}, and *br7*^{S5} Alleles

Drosophila J_κ RBP DNA was isolated from the *br7* mutants and sequenced as described in Experimental Procedures. Positions of the base and amino acid substitutions observed in the *br7*^{HG36}, *br7*^{IB115}, and *br7*^{S5} mutations are indicated on a diagram of the Drosophila J_κ RBP protein structure. The major transcription unit encodes the protein starting from the methionine at position 66. The hatched and stippled boxes represent the integrase motif domain and highly conserved core region, respectively (Furukawa et al., 1991). The scale in amino acid residues is given below.

Drosophila J_κ RBP protein may be essential at least in the late larval to the pupal stage. To analyze developmental regulation of Drosophila J_κ RBP transcripts, we performed an RNAase protection assay of Drosophila J_κ RBP mRNA (Figure 4). The expected 277-base band was detected in each lane. Although the levels of Drosophila J_β RBP transcripts were decreasing to the second instar larvae, they were up-regulated in the late (third instar) larvae again. The Drosophila J_κ RBP transcript was first detected in 0–3 hr embryos at a relatively high level. Since zygotic transcription begins at approximately 1.5–2 hr after egg laying (Edgar and Schubiger, 1986), the presence of the Drosophila J_κ RBP transcript in 0–3 hr embryos in relative high abundance suggests that at least a part of this transcript may be of maternal origin.

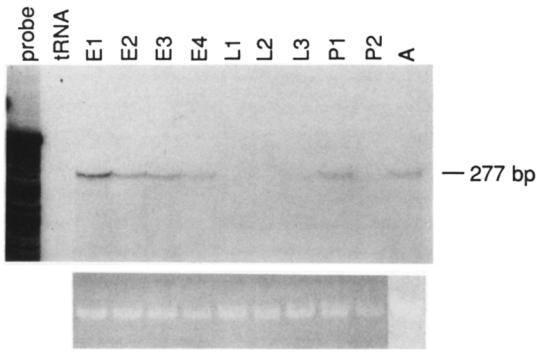

Figure 4. RNAase Protection Assay of Drosophila J_κ RBP Transcripts during Development

The antisense probes were hybridized with 25 μg of tRNA (tRNA) or 8 μg of total RNA isolated from 0–3 hr embryos (E1), 3–6 hr embryos (E2), 6–12 hr embryos (E3), 12–22 hr embryos (E4), first instar larvae (L1), second instar larvae (L2), third instar larvae (L3), early pupae (P1), late pupae (P2), and adults (A) (upper panel). The same amounts of RNAs used for hybridizations were electrophoresed in agarose gel and stained with ethidium bromide as a control for the amount of RNA (lower panel). The 277 bp protected band is indicated. The exposure time was 10 hr.

Discussion

Su(H) and *E(H)* Are Mutant Alleles of the Drosophila J_κ RBP Gene

Molecular analysis of a total of six alleles of *Su(H)* and *E(H)* has identified two classes of mutations in the Drosophila J_κ RBP gene: P element insertion and point mutation. The P element insertion in the upstream region of the Drosophila J_κ RBP gene would alter its promoter or enhancer function, resulting in disregulation of the Drosophila J_κ RBP gene expression temporally and topologically. Although comparison of the expression pattern of Drosophila J_κ RBP transcripts and protein in P-inserted alleles with that of wild type is necessary, the homozygous lethality of these mutants inhibits straightforward comparison.

The *HG36*, *IB115*, and *S5* mutants that were created by chemical reagents contained nucleotide transitions in the J_κ RBP gene, resulting in the amino acid substitution or truncation of the normal Drosophila J_κ RBP protein. Such mutations would distort the J_κ RBP protein activity severely. The fact that a single replacement mutation in the integrase motif results in a loss-of-function mutation (*HG36*) suggests that the integrase motif of the Drosophila J_κ RBP protein may be essential for exerting its function. The truncated mutation of *IB115* also deleted the integrase motif. The amino acid substitution (Arg → His) in the *S5* allele, which seems to enhance the protein activity, is located 12 residues upstream of the integrase motif. Since the integrase motif region is considered to be a catalytic domain in the integrase family (for review see Craig, 1988), modification in the region immediately upstream of the integrase motif in the Drosophila J_κ RBP protein could affect the function of the J_κ RBP protein. Our result with the *S5* allele is consistent with the previous assumption that the *S5* allele is not a simple gene duplication because of its homozygous lethality (Ashburner, 1982).

Hairless and J_κ RBP Are Antagonistic for PNS Development

Hairless mutants show dominant defective phenotypes in

PNS development and recessive lethality (Plunkett, 1926; Bang et al., 1991). Ashburner (1982) reported that hypomorphic alleles of *br7* suppress *Hairless* and that hypermorphic alleles enhance *Hairless*. The bristle number of heterozygous *Hairless* flies is inversely correlated with the copy number of the wild-type *br7* locus. These findings led to an assumption that the *br7* product acts antagonistically with the *Hairless* gene product. A similar type of dosage-sensitive interaction between multiple loci has been characterized in other loci involved in Drosophila PNS development. For example, loss-of-function mutations of *hairy* or *extramacrochaetae* result in the appearance of ectopic sensilla (Moscoso del Prado and Garcia-Bellido, 1984). This phenotype is suppressed by heterozygous deficient alleles of the achaete-scute complex and enhanced by an achaete-scute complex duplication (Moscoso del Prado and Garcia-Bellido, 1984). A total of 7 genes in these groups encode proteins of the helix-loop-helix family (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Caudy et al., 1988; Rushlow et al., 1989; Ellis et al., 1990). The antagonistic dosage-dependent interactions are likely to be due to the formation of heterodimers such as the helix-loop-helix–Id complex (Murre et al., 1989; Benezra et al., 1990; Ellis et al., 1990).

Models for the Function of Drosophila J_κ RBP

One hypothesis for the mechanism of the Drosophila J_κ RBP protein is that it could be a transcription factor and exert its activity by binding to the RS-like sequence in Drosophila. The Drosophila J_κ RBP protein may antagonize the Hairless protein by direct protein–protein interaction and/or competing for binding to the RS-like sequence. Since the Drosophila J_κ RBP protein does not have a known DNA-binding motif, the Drosophila J_κ RBP–Hairless interaction could be a novel type of positive–negative protein interaction, unlike helix-loop-helix–Id and POU-I–POU interactions (Treacy et al., 1991).

Taking the presence of the integrase motif into consideration, another fascinating hypothesis is that the Drosophila J_κ RBP protein might be involved in an unknown site-specific recombination system that regulates gene expression in Drosophila (Furukawa et al., 1991). The Drosophila J_κ RBP protein might also have several functions other than transcription regulator of PNS. The $br7^{LT3}$ allele crossed with $Su(H)^-$ alleles such as *SF8* has an extreme mutant phenotype: their wings, halteres, eyes, and tarsal claws show abnormalities and the flies have fewer than ten macrochaetae per fly (Ashburner, 1982). The pattern of bristle loss is quite different from that of the *Hairless* phenotype. This indicates that the Drosophila J_κ RBP protein could be involved in development of not only PNS but other systems. Homozygous lethality also indicates that the J_κ RBP protein is involved in some essential function of cellular metabolism in addition to PNS development.

Expression of Drosophila J_κ RBP during Embryogenesis

The transcript of the Drosophila J_κ RBP gene is developmentally regulated: it is relatively abundant in the early embryos, but decreases in the early larvae and increases again in the late larvae. The onset of increments of J_κ RBP transcript coincides with differentiation determination of the adult sensilla precursor cells in the late third larval instar and the early pupal stage (Garcia-Bellido and Merriam, 1971; Hartenstein and Posakony, 1989). However, expression of Drosophila J_κ RBP transcripts may not be essential in the embryos because homozygous loss-of-function mutants can survive until the pupal stage. Other genes might also complement the essential biological function of the Drosophila J_κ RBP protein in the embryonic stage. Another possibility is that the loss of the Drosophila J_κ RBP protein may cause nonlethal abnormality in embryos. Since the embryonic PNS precursor cells differentiate at the embryonic stage (for review see Jan and Jan, 1990), the Drosophila J_κ RBP might also be involved in the embryonic PNS precursor cell determination.

Developmental regulation of mRNA expression similar to Drosophila J_κ RBP transcripts was reported in other genes such as the T4 and T5 genes of the achaete-scute complex (Campuzano et al., 1985) and *scabrous* (Mlodzik et al., 1990). All of them have essential roles in the PNS development. Thus, the expression profile of the Drosophila J_κ RBP gene agrees well with the involvement of the Drosophila J_κ RBP gene in PNS development.

Experimental Procedures

Drosophila Strains

The deleted regions of deficiency mutants were as follows: *osp29* (*Df(2L)35B1-3;35E6*), *fn5* (*Df(2L)34F4;35C3*), *A263* (*Df(2L)34 E5-F1; 35C3-5*), *el28* (*Df(2L)35B3;35D4*), *h50* (*Df(2L)34D3;35B10*), *64j* (*Df(2L) 34D1,2;35B8,9-C1*), *GW28* (*Df(2L)35B2;35B7*), *GW7* (*Df(2L)35B3, 35B9,10*), *GW18* (*Df(2L)35C1;36A4,5*), *ARR1* (*Df(2L)35A3,4;35B9-C1*), *rd9* (on Sco), *GW2* (*Df(2L)35D1;35D4*), *b84a3* (*Df(2L)34D3*), *GW6* (*Df(2L)35D1;35D4*), *GW15* (*Df(2L)35C4;35D1-2*), *b84a7* (*D(2L)34C1, 35B1-2*), and *GW13* (*Df(2L)35D2;35F1,2*). These alleles were balanced by balancer chromosomes. All of these mutants were kindly provided by Drs. M. Ashburner and J. Roote at Cambridge University. As an internal control for deficiency mapping, the strain deleting (*Df(3R)e^{D7}/ TM3*) the Drosophila insulin receptor–like gene described by Mohler and Pardue (1984) was provided by Dr. Y. Nishida at Aichi Cancer Center Research Institute.

Southern Hybridization

Genomic DNA was extracted from the adult flies as described previously (Ashburner, 1989). High molecular weight DNA was digested with restriction enzymes under the conditions recommended by the supplier, separated in agarose gels (0.8%), and transferred to nitrocellulose filters. Probes were labeled by oligo labeling (Feinberg and Vogelstein, 1983). Hybridization was performed at 65°C in aqueous solution (Sambrook et al., 1989) using the probes indicated. Washing was performed in 0.1% SDS and 0.01 M sodium citrate–0.01 M citrate at 65°C for 1 hr. Filters were exposed with Imaging plates and analyzed by Image Analyzer (Fuji Film Inc.).

Cloning and Sequencing of *br7* Mutant Alleles

Purified genomic DNA (20 μg) was digested to completion with BamHI (TAKARA Shuzo, Kyoto) and electrophoresed through a 0.8% agarose gel. DNA fractions containing 10–15 kb (*Pl1* and *MR1*) or 7–10 kb (*Pl1*, *MR1*, *HG36*, *IB115*, and *S5*) fragments were isolated by DEAE paper. Following ethanol precipitation, isolated DNA was ligated into BamHI-digested EMBL3 (Stratagene). Recombinant phage ($0.5–1.5 \times 10^5$) were screened by filter hybridization using probe I (Figure 2). Drosophila J_κ RBP–containing DNA was isolated from the phage by digestion with BamHI.

Sequencing of *HG36*, *IB115*, and *S5* alleles was performed with double-stranded plasmids using Sequenase version 2 (US Biochemi-

cals) and [^{32}P]dCTP by the method of Sanger et al. (1977). Sequencing reactions were carried out using specific oligonucleotide primers.

RNAase Mapping Experiments

Total RNA of about 1 g each of embryos, larvae, and pupae or about 500 adult flies was extracted with 4 M guanidine isothiocyanate and purified through a 5.7 M CsCl cushion as described (Chirgwin et al., 1979). The 277 bp ScaI–HincII fragment (from position 1401 to 1677) of the Drosophila J_κ RBP genomic clone was subcloned into the plasmid vector pSP73 (Promega) at the EcoRV site. This clone was linearized by digestion with HindIII. An antisense probe was synthesized from the SP6 promoter by SP6 RNA polymerase and annealed with RNAs specified as described by Melton et al. (1984). Unhybridized RNA was digested by adding 10 μg/ml RNAase A and 2 μg/ml RNAase T1 at 30°C for 30 min. After treatment with proteinase K (100 μg/ml proteinase K, 0.4% SDS at 37°C for 15 min), the surviving fragments were precipitated with ethanol and separated by electrophoresis on a 6% polyacrylamide–urea gel. The dried gel was exposed on Fuji X-ray film.

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