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Structure and expression of the *br-c* locus in *Drosophila melanogaster* (Diptera: Drosophilidae)

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Development, *Drosophila*, genes, ecdysterone, puffs

Abstract. We review data on the structure and expression of the *Broad Complex*, a key gene in the response to ecdysterone (20E) in *Drosophila* cells.

The 2B3-7 X chromosome region, containing an early (20E) inducible puff, was saturated with mutations in the genes *dor, hfw* (*swi*) and *BR-C* that cause disturbances in the normal ecdysterone-inducible puffing sequence. By analysis of chromosome rearrangements localized on the genetic, cytological and physical maps, the *BR-C* locus has been mapped exactly within the 2B3-5 puff. Homozygous mutations for *BR-C* result in the multiple distortions of metamorphosis and female fertility due to loss of sensitivity of the genes in this chromosomal region to ecdysteroids.

Using interstrain larval ovary transplantation, the causes of female sterility were studied in females mutant for the *BR-C*. Transplantation of *BR-C* ovaries into females heterozygous for a dominant sterility mutation followed by genetic analysis of the progeny demonstrated that the transplanted ovaries were capable of normal function. The sterility of the *BR-C* mutant females is therefore associated with a somatic extra-ovarial defect rather than a germ line malfunction.

According to cytogenetic and molecular analysis, the *BR-C* occupies more than 115 kb and contains at least three regulatory elements. One of these elements (in position 146.5–161 kb) may respond to 20E and this results in puff activation in the salivary gland cells. The second element (at 99–120 kb) is located upstream in the first exon and is necessary for transcription activation, while the third element (to the left of 99 kb) is necessary for normal fertility.

The strongest *BR-C* mutations result in underdevelopment of almost all ecdysterone-inducible puffs. In animals homozygous for the weaker *BR-C* mutations (*rbp* group) which survive until organ formation in the pupa, the larval salivary glands do not histolyse until at least 72 hour after pupariation (Restifo & White, 1992). Analysis of polytene chromosomes has shown that in the rbp^4 mutant all ecdysterone-inducible puffs are extremely reduced in 0, 3, and 10 h prepupae. Some of the puffs including the 2B3-5 are active until 24 h pupae.

GENERAL CHARACTERISTICS OF THE *BR-C* LOCUS

The changes in puffing activity in the third-instar larva and prepupa are known to be of sequential character and triggered by 20E (for review see Ashburner et al., 1974). Therefore, it has been suggested that mutations induced in the early ecdysterone-induced loci, which propagate the effect of the hormone to other loci, should cause disturbances in the normal puffing sequence.

The 2B1-10 region which contains an early ecdysterone-inducible puff, was saturated with mutations of which those at the genes *ecs* (ecdysterone sensitivity), *swi* (singed wings) and *dor* (deep orange) cause disturbances in the normal puffing sequence (Belyaeva et al., 1981, 1989; Belyaeva & Zhimulev, 1982; Zhimulev et al., 1982; Biyasheva et al., 1985). In independent experiments several lethals were isolated which affected puparium formation and were located near the tip of the X chromosome (Kiss et al., 1976).

Mutations of the *npr* (nonpupariating) series were mapped genetically very close to the *ecs* gene and appeared to be alleles of the *ecs* mutations and previously known *br* visible mutations (Belyaeva et al., 1980). Subsequently, this gene which controls sensitivity of *Drosophila* cells to 20E was renamed *BR-C* (*Broad Complex*) (Lindsley & Zimm, 1992). The *BR-C* gene coincides exactly in position with the cytological limits of the 2B3-5 early ecdysterone-inducible puff (Belyaeva et al., 1987).

Homozygous mutations for the *BR-C* gene result in multiple distortions of larval and imaginal organs and functions related to their insensitivity to the hormone:

1. In gynanders obtained in heterozygotes of *npr-1* (which appeared to be an allele of the *BR-C* and is now named npr^3) with a ring X chromosome, *npr-1/0* tissue can not respond to ecdysterone, and tanned puparial tissue (*npr-1*/ring X) is sharply separated by distinct boundaries from the white larval tissue (*npr-1/0*) (Kiss et al., 1976). Tissues homozygous for *BR-C* mutations do not undergo metamorphosis in vitro (Kiss & Molnar, 1980). The larval salivary gland of the *rbp*-group of mutants do not histolyse and can be found in very late pupae (Restifo & White, 1992).

2. The strongest nonpupariating mutations like *lt324* and *lt435* (npr^5 and npr^6 according to Lindsley & Zimm, 1992) result in loss of development of all ecdysterone-inducible puffs, except 2B3-5. These puffs cannot be induced by exogenous 20E in vitro (Belyaeva et al., 1981).

3. At the molecular level it was shown that the product of the *BR-C* gene controls the activity of genes localized in the intermoult (ecdysterone-repressible) puffs (Crowley et al., 1984; VijayRaghavan et al., 1988; Galceran et al., 1990; Guay & Giuld, 1991) and activation of the genes located in early (Dubrovsky & Zhimulev, 1988) and late (Restifo & Guild, 1986; Galceran et al., 1990; Guay & Guild, 1991) ecdysterone-inducible puffs. Lepesant et al. (1986) demonstrated trans-regulatory action of the *BR-C* gene on the expression of *LSP2* and *FBP1* ecdysterone-inducible genes in the fat body.

All of these data show that *BR-C* gene is a key gene in the induction of gene activity by ecdysteroids.

LETHALITY, br, rbp AND HYPOMORPH PHENOTYPES

In the saturation experiments, numerous mutations (EMS-, X-rays-induced, P-M insertional) have been isolated and they have revealed complex complementation behavior (Belyaeva et al., 1980, 1989; Solovëva & Belyaeva, 1989). The complementation map of some of the mutations [mainly those which were induced by insertions or associated with chromosome rearrangements, and some EMS-induced mutations: npr^5, npr^6, $l(1)2Bc^2$, and $l(1)2Bd^1$] is shown in Fig. 1. Complete complementation maps can be found in Belyaeva et al. (1980, 1989) and Solovëva & Belyaeva (1989). Some of the mutations have been located on the physical map (Galceran et al., 1986, 1990; Belyaeva et al., 1987; Izquierdo et al., 1988; Orr et al., 1989; Protopopov et al., 1991; Solovëva, 1992) and data on the mapping are shown in Fig. 2.

Mutations resulting in *br* phenotype are located in the interval between the breakpoint of $In(1)Hw^{49c}$ and the mutation br^{28} (Fig. 2e). The latter mutation is associated with a P-element insertion into zinc-finger domain-2. Therefore, the *br* function maps between 110 and 200 kb on physical map.

Mutations essential for survival and shown in Fig. 2g [except $In(1)Hw^{49c}$] are lethal when homozygous or heterozygous with each other. $In(1)Hw^{49c}$ breaks DNA to the left of

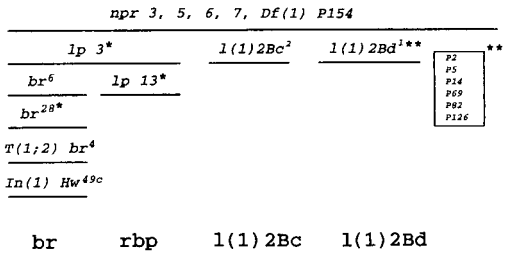

Fig. 1. Complementation map of some mutations and rearrangements affecting viability in the *BR-C* locus. Asterisks denote mutations induced by P-M hybrid disgenesis. The rectangular box and *l(1)2bd* (two asterisks) designates hypomorphic mutations which reduce viability only when heterozygous with deletions or "long" noncomplementing alleles. Therefore their positions on the complementation map are arbitrary (modified from Belyaeva et al., 1989; Mazina et al., 1991; Solovëva, 1992). See Lindsley & Zimm (1992) for explanations of symbols.

the first exon of the gene. All the other rearrangements that disrupt the continuity of DNA between the first exon and last z-3 domain are homozygous lethal. However, their behaviour in heterozygous combination with $In(1)Hw^{49c}$ is peculiar. Heterozygotes between the inversion and rearrangements breaking the locus between the first and second exons (br^{26}-br^{6}) are almost completely viable. Heterozygotes for P-element induced mutations *P3* and *P13* inserted in the exon to upstream of the core, with the inversion are also viable. Heterozygotes of $In(1)Hw^{49c}$ with rearrangements damaging the core and Zn-finger exons (*npr7* and *Df154*) are completely lethal. This may mean that full lethality results only from disruption of core and z-exons in one chromosome and some DNA sequence on 5' end of the gene on the other chromosome. Combination of a defect on 5' end on one chromosome and breaking the continuity of the gene between first and core exons in the other chromosome does not result in lethality. All of these results indicate that the function of viability is distributed along the gene, to about the same extent as the *br* function.

The *rbp* phenotype (Fig. 2f), absence of visible phenotypes in hypomorphs (Fig. 2h) and *br* function of $In(1)Hw^{49c}$ are associated with insertions of mobile elements in exon 4, situated distal by to the core (P element insertions in Fig. 2h), in an intron (rbp^{in}), or upstream of the transcribed portion of the gene [like $In(1)Hw^{49c}$].

In conclusion, no clear correlation was found between location of mutations on the physical map of the DNA, and their presence in different complementation groups.

FERTILITY FUNCTION

Mutations resulting in sterility are located at both ends of the physical map. *Tp(1;3)sta* is sterile as a homozygote. Females heterozygous for the transposition or *Df(1)St490* and any of the other mutations situated to the right of them (Fig. 2d) are sterile as well, except $Tp(1;3)sta/npr^{7}$ and $Df(1)St490/npr^{7}$ which are partially fertile. $Df(1)St490/T(1;2)br^{4}$ females are also partially fertile. Eggs in the ovaries of sterile females appear to have developed normally but are not laid. Females heterozygous for *Df(1)St472*, delimiting the right zone of *BR-C*, and any of the *sta* alleles including *Df(1)sta* and *Df(1)St490* have normal fertility (Mazina et al., 1991). Therefore, on the physical map of the *BR-C* the fertility function is mapped between 95 and 210 kb. Because breaks of DNA in *Tp(1;3)sta* or *Df(1)St490* also affect fertility, some part of the locus must be situated to the left of these rearrangements break points.

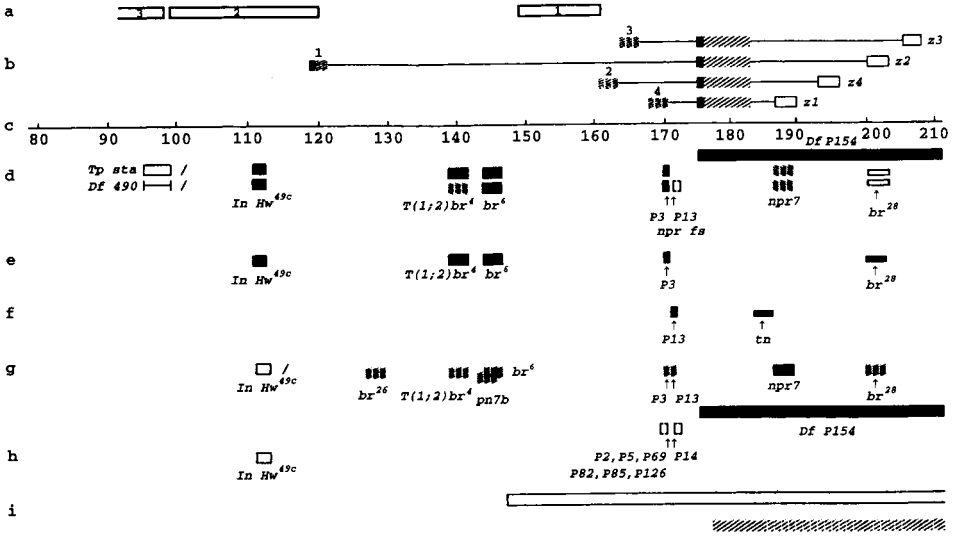

Fig. 2. Molecular map of the *BR-C* gene. a – predicted regulatory elements, 1–3 (see text for explanation); b – intron-exon map of transcripts, z1–z4 – zinc finger domains (according to DiBello et al., 1991; G.M. Guild, pers. comm.); c – physical map, in kb (Chao & Guild, 1986; Belyaeva et al., 1987); d – localization of female sterility function in heterozygotes when *Tp(1;3)sta* or *Df(1)St490* is in one chromosome and any of the mutations listed to the right are in the other chromosome. Black rectangles indicate full sterility, shadowed ones partial sterility, open rectangles the fertile combinations (according to Mazina et al., 1991). Data on gypsy element location (npr^{fs}) are taken from Orr et al. (1989) and Huang & Orr (1992); e, f – *br* and *rbp* phenotypes respectively (according to Belyaeva et al., 1987, 1989; Protopopov et al., 1991; Solovëva, 1992). *tonock* (rbp^{m}) was isolated by C. Moran and recognized as a P-element insert in the intron upstream of the z1 exon and a *rbp* allele (J. Fristrom, pers. comm.); g – localization of breaks resulting in reduced viability. Homozygotes for $In(1)Hw^{49c}$ have normal viability, heterozygotes between the inversion and breaks, shadowed or dark show normal viability or lethality, respectively. Shadowed break points are lethal as homozygotes; h – hypomorphs (according to Solovëva, 1992); i – ecdysterone-induced puff develops normally in the DNA fragment (open rectangular) or is reduced sizes (shadowed) in chromosomes of larvae homozygous for br^{6} inversion or *Df(1)P154* deficiency.

Using gynandromorph fate mapping (Hotta & Benzer, 1972) the foci of the lethal allele *lt76* [$l(1)Bc^{2}$ in Lindsley & Zimm, 1992] were localized to two map sites. One of them was found in the anterior part of blastoderm in the zone giving rise to the anterior region of the nervous system and head. The second covers the blastoderm anlagen which are involved in the development of abdomen and the genital imaginal disc (Balasov & Bgatov, 1992).

To understand the cause of the sterility associated with *BR-C* mutations, ovaries of $y,npr^{3},w/y^{+},In(1)Hw^{49c},w$ female late third instar larvae were transplanted into larvae of the same age heterozygous for the *Fs(1)K1237* dominant sterility mutation. Females with this mutation have underdeveloped ovaries in which oogenesis is blocked at the stage of vitellogenesis and eggs never develop.

47 transplantations were performed and 20 imagos eclosed. Among them 11 females were fertile. They showed normal donor ovaries attached to oviducts of recipients ovaries.

The other 9 flies had normally developed donor ovaries but these were not attached to the oviducts and were consequently sterile.

In the second series, ovaries of sterile *y,npr*^{3}*,w/y,br*^{6}*,w*$^{+}$ females were transplanted into *Fs(1)K1237* recipients. From 42 transplantations 14 imagos eclosed and four flies were fertile. They also had ovaries attached to the oviducts of the *Fs(1)K1237* recipients.

The progeny of all fertile females were tested and shown to carry donor genotypes. Therefore, the donor ovaries are capable of normal function provided they attach to the host oviduct. The analyzed female-sterile mutations do not appear to affect the ovaries themselves.

Genes coding for yolk proteins are expressed in the fat body or the ovarian follicular cells and the proteins are transported to the oocyte (Postlethwait et al., 1980). The synthesis of yolk proteins in fat bodies is controlled by 20E (Bownes et al., 1983), and underproduction of these proteins results in female sterility (Komitopoulou et al., 1983). Sterility of the *BR-C* mutants may therefore be related to underproduction of yolk protein. To check this possibility, hemolymph of *In(1)Hw*^{49c} and *Tp(1;3)sta* sterile homozygotes was collected and the proteins were separated in SDS-PAAG electrophoresis. The results showed normal levels of the yolk proteins in mutant females (Mazina & Korochkina, 1991). An alternative possibility is that the sterility of *BR-C* mutants arises from abnormal morphogenesis of their genital discs due to loss of sensitivity to 20E.

CONTROLLING ELEMENTS OF THE *BR-C* GENE

Complex transcriptional organization of the *BR-C* implies a complex pattern of controlling elements in the gene. It has been shown that 20E induces a normal puff in the 2B3-5 region of the salivary gland chromosomes with *Df(1)pn*^{7b}, *Df(1)br*^{26} and *T(1;2)br*^{4} as well as in females homozygous for inversions *br*^{6} and *Hw*^{49c} (Belyaeva et al., 1980, 1987 and E.S. Belyaeva, unpublished). These break points have been mapped in positions between 113.7 and 148 kb (Fig. 2g).

All DNA sequences necessary for ecdysterone-activated RNA transcription in the salivary gland cell and for puff activation are present between break point of the *br*^{6} inversion (146.5–148 kb) and the start of transcription of the exon 2. Hence, there must be a promoter element (controlling element 1 on Fig. 2a) that responds to hormonal signal in this fragment, as suggested by Belyaeva et al. (1987). This element (between 148 and 160 kb) permits activation of all zinc-finger RNAs and puff formation. Two promoters, in positions 165 and 167 kb have been suggested by DiBello et al. (1991).

Expression of all transcripts is essential for viability and normal external phenotype. Therefore, an additional controlling element (2 on Fig. 2a) located between *Tp(1;3)sta* and *Df(1)St490* at the distal end and first exon (between 99 and 120 kb on Fig. 2a) may also be required for normal *BR-C* gene function (Belyaeva et al., 1987). Galceran et al. (1990) identified a "*cis*-acting long-distance element" and positioned between 102 and 115 kb on the map. According to DiBello et al. (1991) this promoter is situated at 120 kb on the map.

Finally, a third element (3 on Fig. 2a) appears to be required for complete gene function, because chromosome breakage by *Tp(1;3)sta* and *Df(1)St490* results in sterility. Its proximal border coincides with break points of these rearrangements. The distal limit of this element has not been mapped.

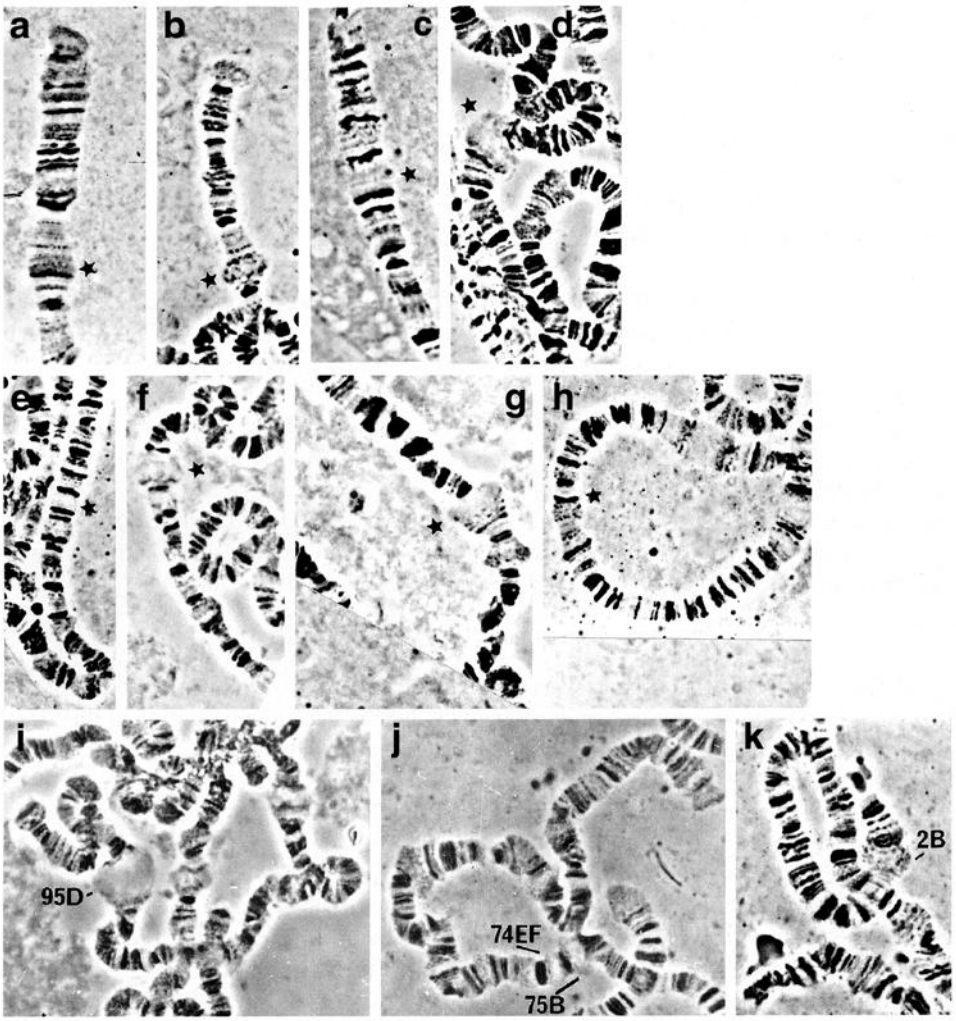

Fig. 3. a–d: Puffing in regions 63E (a, b) and 71F (c, d) of 0h prepupae; a, c – *y,rbp*^{4}; b, d – control, *y*. Regions are labelled by asterisks. e–h: Puffing in regions in 71F (e, f) and 85D1-2 (g, h) of 3h prepupae; e, f – *y,rbp*^{4}; g, h – control, *y*. i–k: Puffs in 24h pupae of *y, rbp*^{4}; i – 95D heat shock puff; j, k – early ecdysterone-inducible puffs, 74EF/75B (j) and 2B3-5 (k).

PUFFING PATTERNS IN *rbp* MUTANTS

As mentioned above, strong mutations of the *BR-C* gene, including *npr*^{5} or *npr*^{6}, result in an almost complete absence of ecdysterone-induced puffs. In larvae homozygous for these mutations only the 2B3-5 puff itself is still active and other early ecdysterone-inducible puffs, 74EF and 75B, are activated to some extent (Belyaeva et al., 1981). Restifo & White (1992) have shown that salivary glands of larvae homozygous for *rbp* mutations do not histolyze and can be found even in late pupae.

No ecdysterone-induced puffs were found in 0h prepupae homozygous for *rbp*^{4} (Fig. 3a,c). In wild-type animals puffs in regions 85D1-2 and 71F1-2 are induced in 0h

prepupae and are active during several hours of prepupal development. However, in *rbp*^{4} animals both of these puffs are missing in 0h prepupae and are inactive in 3h prepupae (Fig. 3e,h). In general, puffing patterns in the *rbp*^{4} mutant are very similar to the patterns seen in the mutation *l(1)ppt10* from *l(1)2Bc* complementation group (Zhimulev et al., 1982). In both of these mutants the late ecdysterone dependent puffs in 0h prepupae do not appear, even though the larvae pupariate and the arrest of development occurrs much latter.

An unexpected pattern of puffing activity is seen in the salivary glands persisting in *rbp*^{4} pupae. In 24h pupae of *rbp*^{4} homozygotes, activity of the 2B3-5 and 74FF/75B puffs is restored. In some salivary glands very large heat-shock puffs can also be found (Fig. 3i–k).

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