Second-Site Noncomplementation Identifies Genomic Regions Required for Drosophila Nonmuscle Myosin Function During Morphogenesis

Susan R. Halsell and Daniel P. Kiehart

Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Manuscript received September 19, 1997
Accepted for publication December 10, 1997

ABSTRACT

Drosophila is an ideal metazoan model system for analyzing the role of nonmuscle myosin-II (henceforth, myosin) during development. In Drosophila, myosin function is required for cytokinesis and morphogenesis driven by cell migration and/or cell shape changes during oogenesis, embryogenesis, larval development and pupal metamorphosis. The mechanisms that regulate myosin function and the supramolecular structures into which myosin incorporates have not been systematically characterized. The genetic screens described here identify genomic regions that uncover loci that facilitate myosin function. The nonmuscle myosin heavy chain is encoded by a single locus, *zipper*. Contiguous chromosomal deficiencies that represent approximately 70% of the euchromatic genome were screened for genetic interactions with two recessive lethal alleles of *zipper* in a second-site noncomplementation assay for the malformed phenotype. Malformation in the adult leg reflects aberrations in cell shape changes driven by myosin-based contraction during leg morphogenesis. Of the 158 deficiencies tested, 47 behaved as second-site noncomplementors of *zipper*. Two of the deficiencies are strong interactors, 17 are intermediate and 28 are weak. Finer genetic mapping reveals that mutations in cytoplasmic tropomyosin and *viking* (collagen IV) behave as second-site noncomplementors of *zipper* during leg morphogenesis and that *zipper* function requires a previously uncharacterized locus, *E3.10/J3.8*, for leg morphogenesis and viability.

THE elaboration of complex structures is a hallmark of metazoan development. This elaboration occurs incrementally, initiating with pattern formation and cell fate determination and proceeding through succeeding waves of cellular communications and inductions, cellular differentiation, and ultimately, the generation of mature forms via morphogenetic movements. Morphogenesis requires a link between signaling molecules and the structural molecules that carry out the actual work. Genetic, molecular, biochemical and experimental embryological analyses in many animal systems have expanded our understanding of the signaling events, but we are only beginning to elucidate the connection between signaling and downstream cell biological effects. Further, elucidation of the supramolecular structure of the cytoskeleton required for movement during morphogenesis is in its early stages.

Nonmuscle myosin-II (henceforth referred to as myosin) plays a key role in morphogenesis throughout phylogeny. It drives cell shape changes required for cytokinesis, tissue remodeling and cellular locomotion. Myosin heavy chain has been identified in *Saccharomyces cerevisiae*, and mutations in the MYO1 gene result in aberrations in cytokinesis and cell growth (Watts *et al.* 1987; Sweeney *et al.* 1991). In *Dictyostelium discoideum*, formation of the fruiting body requires myosin function (De Lozanne and Spudich 1987; Knecht and Loomis 1987; Manstein *et al.* 1989). Disruption of the myosin gene does not block the early stages of this morphogenetic process, but formation of the stalk does not proceed past the mound stage. Recent microscopic analyses suggest that myosin-based contraction may be important in generating cell shape changes during Dictyostelium morphogenesis (Knecht and Shelden 1995; Shelden and Knecht 1996). Although a function has not yet been demonstrated, myosin has been identified within the cilium of rat photoreceptor cells, a structure that is the site of new disk membrane morphogenesis (Williams *et al.* 1993). Finally, a recent analysis of the morphogenetic process of ventral enclosure of the hypodermis in *Caenorhabditis elegans* is suggestive of a role for myosin-based contraction (Williams-Masson *et al.* 1997). Laser ablation and cytochalasin treatments suggest that movement of the ventral epidermis occurs via a two step mechanism: first, a quartet of cells migrate ventrally and this is followed by an actin-based purse string contraction at the leading edge of the epidermis, which brings the remainder of the cell sheet to the ventral midline.

Using the available genetic and molecular tools, the role of myosin during Drosophila morphogenesis has been particularly well characterized. Myosin function has been identified at many stages during the life cycle, including oogenesis, embryogenesis and adult metamorphosis. Single-copy genes encoding the myosin heavy

Corresponding author: Daniel P. Kiehart, Duke University Medical Center, Department of Cell Biology, Research Drive, 307 Nanaline Duke Bldg., Durham, NC 27710.
E-mail: d.kiehart@cellbio.duke.edu

chain [*zipper* (*zip*)], regulatory light chain [*spaghetti-squash* (*sqh*)] and essential light chain have been cloned (Kiehart *et al.* 1989; Karess *et al.* 1991; Edwards *et al.* 1995). In embryos, myosin is subcellularly localized in many tissues undergoing cell shape change, including the cleavage furrows of the cellularizing embryo, the apical end of cells that form the ventral furrow during gastrulation and the leading edge of the epidermis during dorsal closure (Young *et al.* 1991; Young *et al.* 1993). Strong alleles of *zip* are embryonic lethal, and mutant embryos have defects in head involution, axon guidance and defects in epithelial sheet movement during dorsal closure (Cote *et al.* 1987; Zhao *et al.* 1988; Young *et al.* 1993; J. Pederson, J. Mahaffey and D. Kiehart, unpublished data). Mutations in *sqh* result in defective cytokinesis and growth of third instar larvae (Karess *et al.* 1991; Edwards and Kiehart 1996). Experimental manipulation of *sqh* expression has revealed additional requirements for myosin function. These experiments include the generation of germline clones in the ovary (Wheatley *et al.* 1995) and stage-dependent depletion of myosin by removing flies carrying a heat shock-*sqh* rescue construct from heat shock (Edwards and Kiehart 1996). These studies reveal that myosin function is required during oogenesis for appropriate somatic follicle cell migrations and ingressions and nurse cell cytoplasmic transport. It is also required for nuclear migrations in cleavage stage embryos (Wheatley *et al.* 1995). During later stages of the life cycle, myosin is required for proper morphogenesis of the adult eye and leg (von Kalm *et al.* 1995; Edwards and Kiehart 1996). Presumably, these examples represent only a subset of morphogenetic processes that rely on myosin-based contraction.

Analysis of the *Broad-Complex* (*Br-C*) suggests that it regulates myosin function during imaginal disc morphogenesis. Molecular genetic analysis shows that zinc-finger transcription factors encoded by *Br-C* are critical for imaginal disc morphogenesis (Kiss *et al.* 1988; DiBello *et al.* 1991). A screen for enhancers of a *Br-C* family member, $broad^{1}$, identified several loci that function during leg imaginal disc morphogenesis (Gotwals and Fristrom 1991). *Ebr* is an EMS-induced enhancer of $broad^{1}$ (Gotwals and Fristrom 1991). Subsequent analysis utilizing a cosmid bearing a *zipper* transgene (Young *et al.* 1993) verified that *Ebr* is a mutation in the myosin heavy chain locus (zip^{Ebr}; Gotwals 1992). Defects in leg morphogenesis give rise to the malformed phenotype (Figure 1; Gotwals and Fristrom 1991). The malformed phenotype reflects aberrations in cell shape changes during morphogenesis in pupal leg imaginal discs (Condic *et al.* 1990; reviewed in von Kalm *et al.* 1995). The malformation ranges in severity from a small deformation in the femur (Figure 1B) to extreme twisting and gnarling of the femur and tibia (Figure 1C). The genetic behavior of myosin, and the observation that myosin is subcellularly localized during leg elongation and during additional morphogenetic events (Young *et al.* 1991; von Kalm *et al.* 1995), strongly support the hypothesis that myosin-based contraction drives these cell shape changes.

Genetic modifier screens have been used to identify a series of gene products required in a number of functional processes. For example, such screens have proven particularly powerful in identifying gene products that function during Drosophila eye development. An example of one such screen is exemplified by a study which utilized a temperature-sensitive mutation in *sevenless*, sev^{B4} (Simon *et al.* 1991). These flies are exquisitely sensitive to changes in function of other gene products required in the *sevenless* pathway. In the screen, EMS-induced mutations were tested for genetic interactions with sev^{B4}, just below its restrictive temperature. Seven *E*(*sev*) loci were identified in this screen and included mutations in *Ras*, *Son of Sevenless*, *Hsp83* and *cdc37* (Simon *et al.* 1991; Cutforth and Rubin 1994). In addition to conducting genetic modifier screens with EMS-induced mutations, a series of contiguous chromosomal deficiencies are available from the Bloomington Stock Center for systematic screening. Chromosomal deficiencies have been successfully utilized to identify genomic regions required for such divergent processes as DNA replication during embryogenesis (Smith *et al.* 1993), embryonic midgut morphogenesis (Bilder and Scott 1995) and germline sex determination and differentiation (Pauli *et al.* 1995). While mapping the location of the *Ebr* locus, Gotwals and Fristrom (1991) identified two chromosomal deficiencies that behave as second-site noncomplementors of *zip* during imaginal disc leg elongation. Their observations indicated that a systematic screen to identify modifiers of *zip* function could be developed utilizing chromosomal deficiencies.

In this study, we have utilized second-site noncomplementation (SSNC) screens utilizing chromosomal deficiencies to identify genomic regions required for *zip* function. These screens assay for the malformed phenotype (mlf). This approach was chosen because: (1) it is an F1 adult viable screen and mlf is readily scorable with a dissecting scope, (2) utilization of the chromosomal deficiencies allow screening of ~70% of the euchromatic genome in a relatively small number of crosses, and (3) redundant usage of gene products throughout development makes it likely that genes identified in this screen will function in additional morphogenetic processes. A total of 158 deficiencies on the *X*, second and third chromosomes have been tested. Forty-seven deficiencies interact genetically with *zip*, resulting in variable penetrance of mlf. Analysis of the overlap between these deficiencies and the behavior of the genetic interactions suggests there are at least 39 SSNC loci uncovered in these screens. Mutations in several genes uncovered by SSNC deficiencies, including cytoplasmic tropomyosin, *viking* [collagen α2(IV)] and a previously identified but uncharacterized locus, *E3.10/J3.8*, also behave as a SSNC of *zip*. This finer genetic

mapping and the identification of *cTm* as a SSNC validates the efficacy of this screening approach. Since myosin function is so well conserved throughout phylogeny, it is likely that gene products identified in these screens will function similarly in other animals.

MATERIALS AND METHODS

Stocks: The Deficiency Kit stocks and *l(2)01209* were obtained from the Bloomington Stock Center (Bloomington, IN). Cytology is based on Lindsley and Zimm (1992) and FlyBase (http://cbbridges.harvard.edu.7081/). The $zip^{6.1}$ allele was from the Kiehart lab (Young *et al.* 1993), and zip^{Ebr} (Gotwals and Fristrom 1991) was obtained from Jim Fristrom (University of California, Berkeley). Bill Saxton (Indiana University) provided khc^{8}, *D2.33*, *D8.13*, *E3.10*, *E3.27* and *E6.17* (Saxton *et al.* 1991), and Ruth Steward (Rutgers University) provided *J3.8*. vkg^{BLK}, vkg^{ICO} and vkg^{SAK} were obtained from Deborah Kimbrell (University of Houston; Rodriguez *et al.* 1996). $E74^{P[neo]}$ and $E74^{DL\text{-}1}$ were obtained from Carl Thummel (University of Utah; Fletcher *et al.* 1995). cTm^{eg9} and cTm^{er4} were obtained from Anne Ephrussi (EMBL, Heidelberg; Erdélyi *et al.* 1995).

Second-site noncomplementation screen: For the autosomal deficiencies, four to five zip^{Ebr}/*SM5* or $zip^{6.1}$/*CyO* virgin females were mated to three to four *Deficiency*/*Balancer* males in 4–5 day broods at either 18° or 25°. At least 20 adult flies of the double heterozygous *zip*; *Deficiency* genotype (*e.g.*, *zip*/+; *Df*/+) were scored for the malformed phenotype (mlf) (Gotwals and Fristrom 1991). A similar mating scheme was used for the *X* chromosome deficiencies, except virgin females bearing the *X* chromosome deficiencies were mated with *zip* males and only adult female progeny were scored. The penetrance of mlf in the double heterozygotes was compared directly to the penetrance in their siblings heterozygous for the deficiency (*e.g.*, *Balancer*/+; *Df*/+). A penetrance of mlf greater than 10% but less than 25% in the double heterozygotes and not attributable to the deficiency alone was classified as weakly interacting. Intermediate second-site noncomplementing deficiencies show a penetrance of mlf between 25–75% and strong interactors have a penetrance in excess of 75%.

Leg mounting: The malformed phenotype was scored under the dissecting scope. For photography, malformed adults were preserved in 70% ethanol. Individual legs were dissected and mounted in CMPC10 (PolySciences Inc., Warrington, PA). The mounted legs were observed by brightfield microscopy using a 5× Neofluar objective (0.15 NA) on a Zeiss Axioplan microscope. Images were captured with a Hamamatsu CCD camera and Metamorph software. Contrast was adjusted using Adobe Photoshop software and the images were then exported to a Canvas file for montaging and labeling (Kiehart *et al.* 1994).

RESULTS

Pilot screens: Pilot screens with previously identified, EMS-induced second-site noncomplementors (SSNCs) of zip^{Ebr} showed that strong, embryonic lethal *zip* alleles, such as zip^{2}, did not give rise to the malformed phenotype in double heterozygous mutant adults (J. Fristrom, S. Halsell and D. Kiehart, unpublished results). Instead, the post-embryonic lethal alleles, $zip^{2.1}$ and $zip^{6.1}$ (Young *et al.* 1993), are sensitive to such genetic interactions (data not shown). In the assay for the malformed phenotype, the $zip^{2.1}$ and $zip^{6.1}$ alleles behave with similar characteristics. In the pilot screens, zip^{Ebr} is the most sensitive allele available for testing for second-site noncomplementation (data not shown), and this is consistent with the observations found in the deficiency screens described here (see below). Based on these observations, zip^{Ebr} and $zip^{6.1}$ were used in the second-site noncomplementation screens described here. The choice of $zip^{6.1}$ instead of $zip^{2.1}$ was arbitrary.

Malformed flies are observed at low levels in the zip^{Ebr} stock. This is not the case with the $zip^{6.1}$ stock. In order to determine the background level of mlf in flies heterozygous for zip^{Ebr}, zip^{Ebr}/*SM5* virgins were crossed to either Oregon-R wild-type males or to males carrying a *CyO* balancer chromosome. In flies outcrossed to Oregon-R wild type (zip^{Ebr}/+), malformed legs are seen in 53/848 adult progeny (6% mlf). In *trans* to a *CyO* balancer chromosome (zip^{Ebr}/*CyO*), 3% of the adults are malformed (2626 flies). Based on these observations, the genetic interactions observed between *zip* and the chromosomal deficiencies were arbitrarily classified as weak (mlf penetrance 10–24%), intermediate (mlf penetrance 25–75%) or strong (mlf penetrance >75%).

Deficiency screens: The Bloomington Stock Center Deficiency Kits were used for F1 adult viable, second-site noncomplementation screens in order to identify genomic regions required for *zipper* (*zip*) function. *zipper*

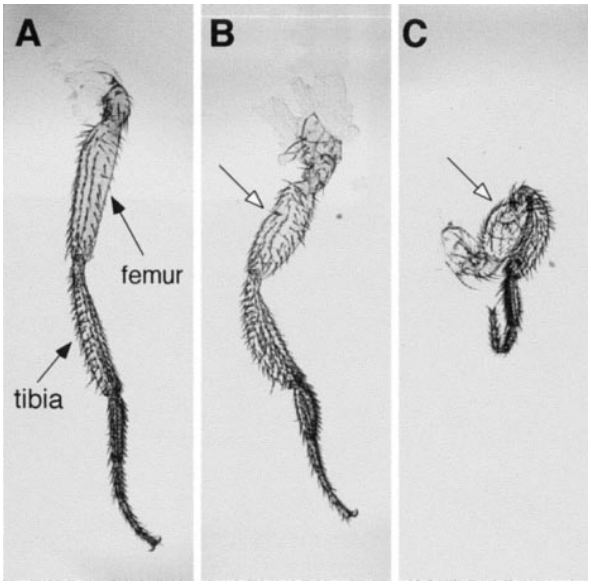

Figure 1.—Malformed phenotype. Defects in cell shape changes driven by myosin-based contraction during pupal leg morphogenesis give rise to the malformed phenotype (mlf) (Condic *et al.* 1990; von Kalm *et al.* 1995). The greatest degree of cell shape change occurs within those cells that will compose the femur and tibia, and the third leg pair is most sensitive to perturbation. (A) In a wild-type adult, the femur and tibia are elongated and slender structures. (B) A weakly mlf leg is evident in a *Df(2R)M41A* +/+ zip^{Ebr} double heterozygous fly. The dent in the femur (open arrow) is characteristic of mlf, and there is a slight thickening of the tibia. (C) In severely mlf legs observed in a *Df(2R)Jp8* +/+ zip^{Ebr} adults, the structure of the leg is grossly affected. The femur is gnarled and twisted upon itself (open arrow) and the tibia is also shortened and twisted. The presence of weakly mlf legs was considered diagnostic in these screens.

encodes the Drosophila nonmuscle myosin-II heavy chain gene (Young *et al.* 1993). A total of 163 contiguous deficiency stocks are available for the *X*, second and third chromosomes, and they uncover approximately 70% of the euchromatic genome (FlyBase). Excluding exceptionally weak stocks and those that uncover *zipper*, 158 of the deficiency stocks were tested for genetic interactions with zip^{Ebr} and $zip^{6.1}$. Second-site noncomplementation of *zip* is indicated by the incidence of the malformed phenotype (mlf) in the adult leg (Figure 1; Gotwals and Fristrom 1991). Initially, at least 20 adult progeny double heterozygous for *zip* and the tested deficiency were scored. In those cases where at least 10% of the flies were mlf, the cross was repeated and at least 50 additional flies were scored. Alternatively, those deficiencies that did not show an interaction with *zip* but overlap deficiencies that did show an interaction, were rescreened to confirm this observation.

Of the 158 tested stocks, 47 deficiencies, when double heterozygous with at least one of the tested *zip* alleles, give rise to mlf adults with a penetrance of at least 10% (Tables 1–3). Expressivity of mlf may be weak or strong (*cf.* Figure 1B to 1C), but it is not necessarily correlated with the degree of penetrance. A weak mlf phenotype is considered diagnostic. Generally, SSNC is observed with greater penetrance with zip^{Ebr} than with $zip^{6.1}$. Of those deficiencies tested at 25° and 18°, 22 show a higher penetrance of mlf at 25°, 8 are more severe at 18° and 13 show no significant temperature sensitivity. Analysis of the relationship between these deficiencies and their genetic behavior suggests that a minimum of 39 loci behave as second-site noncomplementors (SSNC) of *zipper*. The genetic interactions observed between these deficiencies and *zip* are detailed below.

X Chromosome

There are 42 deficiency stocks available in the Deficiency Kit for the *X* chromosome. The data for the SSNC deficiencies are shown in Table 1 and Figure 2, and

TABLE 1

X* chromosome deficiencies that act as second-site noncomplementors of *zipper

Deficiency stock	Cytology	Temperature	Percent malformed(*n*)[a] zip^{Ebr}	Percent malformed(*n*)[a] $zip^{6.1}$	Comments[b]
Df(1)sc-J4	1B; 3A3	18°	7 (30)	2 (42)	Weak
		25°	11 (54)	2 (117)	
Df(1)64c18	2E1-2; 3C2	18°	12 (80)	0 (49)	Weak
		25°	0 (79)	0 (61)	
Df(1)A113	3D6-E1; 4F5	18°	23 (104)	2 (123)	Weak
		25°	6 (93)	3 (124)	
Df(1)JC70	4C15-16; 5A1-2	18°	43 (65)	2 (57)	Intermediate
		25°	37 (49)	0 (44)	
Df(1)G4e[L]h24i[R]	5E3-8; 6B	18°	0 (37)	0 (22)	Weak,
		25°	5 (21)	19 (21)	$zip^{6.1}$ specific
Df(1)ct-J4	7A2-3; 7C1	18°	16 (103)	1 (151)	Weak
		25°	23 (153)	0 (107)	
Df(1)ct4b1	7B2-4; 7C3-4	18°	12 (88)	0 (40)	Weak
		25°	20 (12)	4 (66)	
Df(1)RA2	7D10; 8A4-5	18°	15 (94)	0 (64)	Intermediate
		25°	32 (68)	6 (67)	
Df(1)v-L15	9B1-2; 10A1-2	18°	43 (46)	3 (31)	Intermediate
		25°	40 (65)	10 (63)	
Df(1)v-N48	9F; 10C3-5	18°	13 (117)	1 (121)	Intermediate
		25°	29 (79)	2 (45)	
Df(1)HA85	10C1-2; 11A1-2	18°	ND[c]	5 (19)	Intermediate
		25°	38 (29)	20 (74)	
Df(1)g	12A; 12E or 11F10; 12F1	18°	3 (70)	0 (56)	Weak
		25°	24 (49)	2 (50)	
Df(1)r-D1	14C2-4; 15B2-C1	18°	22 (54)	0 (58)	Intermediate
		25°	29 (92)	0 (53)	
Df(1)HF396	18E1-2; 20	18°	ND	5 (43)	Weak
		25°	22 (50)	17 (24)	

[a] Percent of malformed flies double heterozygous for the deficiency and the indicated *zip* allele (*Df*/+; *zip*/+). *n* is the total number of flies of this genotype that were scored.

[b] Strength of interactions is based on penetrance of the malformed phenotype in the double heterozygous flies and is defined as: weak (10–24% mlf), intermediate (25–75% mlf) and strong (≥75% mlf).

[c] ND, not determined.

those deficiencies which fail to interact in these screens are shown in appendix a. A total of 14 deficiencies on the *X* chromosome behave as SSNCs of *zip*, six with an intermediate penetrance (25–75% penetrance), and the remaining eight as weak interactors (penetrance of 10–24%). There are no strong interacting deficiencies on the *X* chromosome.

Intermediate, second-site noncomplementing *X* chromosome deficiencies: *4C15-16; 4F5:* Overlapping deficiencies *Df(1)A113* and *Df(1)JC70* interact with zip^{Ebr} but not $zip^{6.1}$. At 18°, *Df(1)JC70*; *zip* double heterozygotes exhibit mlf with a penetrance of 43% and a similar penetrance of 37% at 25°. The penetrance of mlf with *Df(1)A113* is lower (23% mlf), but this may be an under-

TABLE 2

Second chromosome deficiencies that act as second-site noncomplementors of *zipper*

Deficiency stock	Cytology	Temperature	Percent malformed(*n*)[a]		Comments[b]
			zip^{Ebr}	$zip^{6.1}$	
Df(2L)JS32	23C3-5; 23D1-2	18°	6 (186)	0 (87)	Weak
		25°	11 (80)	2 (95)	
Df(2L)sc19-5	25A4-5; 25D5-7	18°	ND[c]	ND	Weak
		25°	16 (51)	ND	
Df(2L)c1-h3	25D2-4; 26B2-5	18°	ND	3 (34)	Intermediate
		25°	30 (80)	4 (90)	
Df(2L)TE29Aa-11	28E4-7; 29B2-C1	18°	15 (20)	11 (53)	Weak, zip^{Ebr} and $zip^{6.1}$
		25°	16 (102)	7 (112)	similar
Df(2L)30A-C	29F7-30A1; 30C2-5	18°	7 (124)	2 (99)	Intermediate
		25°	26 (160)	2 (143)	
Df(2L)J39	31A; 32C-E	18°	14 (22)	14 (21)	Weak, zip^{Ebr} and $zip^{6.1}$
		25°	13 (38)	6 (35)	similar
Df(2L)osp29	35B1-3; 35E6	18°	11 (88)	1 (105)	Weak
		25°	7 (94)	1 (101)	
Df(2L)H2O	36A8-9; 36E1-2	18°	7 (73)	3 (34)	Intermediate
		25°	28 (134)	1 (164)	
Df(2L)TW50	36E4-F1; 38A6-7	18°	8 (74)	ND	Intermediate
		25°	28 (162)	5 (73)	
Df(2R)M41A4	41A	18°	7 (29)	13 (60)	Weak
		25°	24 (91)	6 (81)	
In (2R)bw[VDe2L]Cy[R]	41A-B; 42A2-3[d]	18°	29 (49)	ND	Intermediate
		25°	43 (84)	20 (75)	
Df(2R)nap1	41D2-E1; 42B1-3	18°	67 (46)	61 (66)	Intermediate
		25°	82 (93)	75 (227)	(see text)
Df(2R)B5	46A; 46C	18°	5 (55)	0 (55)	Weak
		25°	11 (86)	3 (68)	
Df(2R)vg-C	49A4-13; 49E7-F1	18°	12 (112)	8 (65)	Weak
		25°	1 (75)	1 (89)	
Df(2R)CX1	49C1-4; 50C23-D2	18°	23 (21)	ND	Weak
		25°	19 (106)	0 (84)	
Df(2R)Jp1	51C3; 52F5-9	18°	12 (50)	3 (33)	Intermediate
		25°	40 (143)	2 (119)	
Df(2R)Jp8	52F5-9; 52F10-53A1	18°	84 (19)	67 (64)	Strong, semi-lethal
		25°	98 (86)	76 (55)	
Df(2R)PC4	55A; 55F	18°	24 (110)	5 (76)	Weak
		25°	20 (96)	0 (88)	
Df(2R)or-BR6	59D5-10; 60B3-8	18°	12 (121)	15 (26)	Weak, zip^{Ebr} and $zip^{6.1}$
		25°	5 (130)	6 (160)	similar
Df(2R)bw[VDe2L]Px[Kr]	59D6-E1; 60C-D	18°	36 (137)	ND	Intermediate
		25°	35 (95)	41 (80)	
In (2LR)Px[4]	60B; 60D1-2[d]	18°	56 (25)	24 (90)	Intermediate
		25°	7 (59)	8 (98)	

[a] Percent of malformed flies double heterozygous for the deficiency and the indicated *zip* allele (*Df* + / + *zip*). *n* is the total number of flies of this genotype that were scored.

[b] Strength of interactions is based on penetrance of mlf in the double heterozygous flies and is defined as: weak (10–24% mlf), intermediate (25–75% mlf) and strong (≥75% mlf).

[c] ND, not determined.

[d] Cytology of the deficiency present within the chromosomal rearrangement.

TABLE 3

Third chromosome deficiencies that act as second-site noncomplementors of *zipper*

Deficiency stock	Cytology	Temperature	Percent malformed (n)[a] zip^{Ebr}	Percent malformed (n)[a] $zip^{6.1}$	Comments[b]
Df(3L)M21	62F; 63D	18°	ND[c]	ND	Weak
		25°	22 (55)	8 (90)	
Df(3L)HR370	63A1; 63D1	18°	9 (45)	14 (50)	Weak
		25°	16 (94)	0 (42)	
Df(3L)HR119	63C6; 63E	18°	11 (112)	2 (79)	Weak
		25°	18 (98)	4 (74)	
Df(3L)GN24	63F4-7; 64C13-15	18°	14 (43)	ND	Weak
		25°	4 (71)	3 (71)	
Df(3L)AC1	67A2; 67D7-13	18°	23 (104)	6 (68)	Weak
		25°	13 (120)	1 (136)	
Df(3L)81k19	73A3; 74F	18°	84 (32)	8 (86)	Strong,
		25°	77 (91)	17 (105)	semilethal
Df(3L)W10	75A6-7; 75C1-2	18°	12 (69)	8 (60)	Intermediate
		25°	26 (74)	5 (155)	
Df(3L)Cat	75B8; 75F1	18°	13 (89)	2 (90)	Weak
		25°	20 (140)	1 (142)	
Df(3L)rdgC-co2	77A1; 77D1	18°	11 (106)	3 (180)	Weak
		25°	9 (127)	5 (185)	
Df(3L)Pc-MK	78A2; 78C9	18°	1 (81)	5 (117)	Weak
		25°	17 (109)	1 (111)	
Df(3R)p712	84D4-6; 85B6	18°	9 (33)	23 (30)	Intermediate
		25°	8 (90)	40 (60)	
Df(3R)Ea	88E7-13; 89A1	18°	ND	ND	Weak
		25°	23 (163)	1 (97)	

[a] Percent of malformed flies double heterozygous for the deficiency and the indicated *zip* allele (*zip*/+; *Df*/+). *n* is the total number of flies of this genotype that were scored.

[b] Strength of interactions is based on penetrance of the malformed phenotype in the double heterozygous flies and is defined as: weak (10–24% mlf), intermediate (25–75% mlf) and strong (≥75% mlf).

[c] ND, not determined.

estimation because the stock carries a duplication for region 3C2; 5A1-2 (*Dp(1;2)w[+]*) that cannot be followed phenotypically; thus, the scored class includes flies that carry this duplication. However, this duplication cannot be responsible for the appearance of mlf because 50% of the *zip* heterozygotes (+/+; zip^{Ebr}/+) or deficiency heterozygotes (*Df*/+; +/+) would also be heterozygous for the duplication, but mlf is not seen in these flies. Neither deficiency interacts with $zip^{6.1}$ (2–3% mlf). The smallest overlap between these regions is cytologically 4C15-16; 4F5, although the higher penetrance with *Df(1)JC70* indicates that it might uncover a second locus. Alternatively, these deficiencies may uncover the same locus, but the duplication in the *Df(1)A113* stock masks the full penetrance of mlf in these flies.

7D10; 8A4-5: Df(1)RA2 uncovers the cytogenetic interval 7D10; 8A4-5 and acts as a SSNC of intermediate

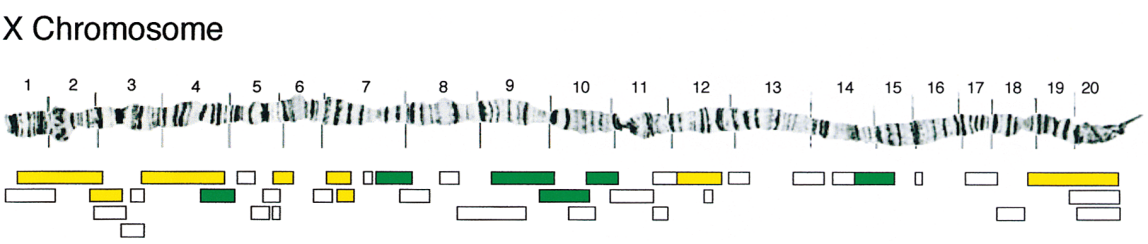

Figure 2.—Second-site noncomplementing deficiencies on the *X* chromosome. Of the tested *X* chromosome linked deficiencies, 14 behave as SSNCs of *zip*. This represents a minimum of 11 loci. Six of the deficiencies act as intermediate SSNCs (green boxes), and eight are weak SSNCs (yellow boxes; Table 1). Tested, noninteracting deficiencies are indicated by the white boxes (appendix a). Of the available deficiencies, no strong SSNC deficiencies were identified on the *X* chromosome. The chromosome map is modified from Lefevre (1976).

penetrance with zip^{Ebr} at 25° (32% mlf) and a weaker penetrance at 18° (15% mlf). There is no observed interaction with $zip^{6.1}$ (6% mlf). An overlapping deficiency, *Df(1)KA14* does not interact genetically with *zip* (7% mlf). Based on the cytology, the interacting locus is confined to 7D10; 7F1-2.

9B1-2; 11A1-2: Cytogenetic interval 9B1-2; 11A1-2 is uncovered by three overlapping deficiencies, *Df(1)v-L15*, *Df(1)v-N48* and *Df(1)HA85*, all of which behave as intermediate SSNCs. Each deficiency displays a slightly different interaction with *zip*. *Df(1)v-L15* shows a penetrance of mlf of approximately 40% with zip^{Ebr} at both temperatures, and a weak interaction with $zip^{6.1}$ at 25° (10% mlf). *Df(1)v-N48* overlaps *Df(1)v-L15* between 9F; 10A1-2, but shows a slightly weaker penetrance with zip^{Ebr} (29% mlf at 25°) and no interaction with $zip^{6.1}$ (2% mlf). This may reflect either genetic background differences in the stocks or it may indicate that *Df(1)v-L15* uncovers an interacting locus not uncovered by *Df(1)v-N48*. Given that *Df(1)C52* does not interact with *zip* (6% mlf), this second possible SSNC locus is confined to 9C-D; 9F. More proximally, *Df(1)HA85* also acts as an intermediate SSNC with zip^{Ebr} (38%) and a weak SSNC with $zip^{6.1}$ (20% mlf). Again, this genetic behavior differs from *Df(1)v-N48*, and may reflect the presence of a SSNC locus in region 10C3-5; 11A1-2. Operationally, the overlap between these three deficiencies defines a minimum number of interacting loci as two, but the differences observed in their interactions with the *zip* alleles indicate that there may be three or more separate loci.

14C2-4; 15B2-C1: In cytogenetic interval 14C2-4; 15B2-C1, a single deficiency, *Df(1)r-D1*, interacts with zip^{Ebr} to give a mlf penetrance of 29% at 25°. This stock is heterozygous for *Dp(1;4)r[+]*, an unmarked duplication covering interval 14A1-2; 16A7-B1, and this duplication may affect the occurrence of mlf flies. However, the presence of the duplication does not result in mlf in the *zip* heterozygous (+/+; zip^{Ebr}/+) or deficiency heterozygous (*Df*/+; +/+) siblings (see above, *4C15-16; 4F5*). Based on these observations, it is likely that the penetrance observed in the *Df(1)r-D1*; zip^{Ebr} double heterozygotes is an underestimate.

Weak, second-site noncomplementing *X* chromosome deficiencies: *1B; 3C2:* Cytological region 1B; 3C2 contains a weakly interacting SSNC. Two deficiencies within this interval, *Df(1)sc-J4* and *Df(1)64c18* interact with zip^{Ebr} with a weak penetrance of mlf (11% and 12% mlf, respectively). Deficiencies *Df(1)BA1* and *Df(1)JC19* do not interact genetically with either *zip* allele (3% mlf); therefore, the interacting locus may be confined to cytological region 2E1-2; 2F6. Alternatively, each deficiency may uncover separate loci, one confined to 2A; 2E1-2 and a second to 3A3; 3C2. The lack of interaction seen with *Df(1)JC19* could result if a suppressor(s) of mlf lies on the chromosome outside the deficiency. It is more likely that two loci in the region act as SSNCs of *zip* because the *Broad-Complex* maps to 2B5, which is uncovered by *Df(1)sc-J4* but not by *Df(1)64c18* . A deficiency specific for the *Broad-Complex*, *npr 1*, is also a weak SSNC of zip^{Ebr} (Gotwals and Fristrom 1991). This genetic behavior is similar to that observed with *Df(1)sc-J4*.

5E3-8; 6B: A single deficiency, *Df(1)G4e[L]h24i[R]* uncovers region 5E3-8; 6B and interacts with $zip^{6.1}$ (19% mlf) but not with zip^{Ebr} (5% mlf). This is only one of two cases in which $zip^{6.1}$ exhibits a higher penetrance of mlf than zip^{Ebr}. The interaction with $zip^{6.1}$ is weak and occurs at 25°. *Df(1)5D* and *Df(1)JF5* do not interact with either *zip* allele, therefore, the smallest interacting interval is 5E8; 6B.

7B2-4; 7C1: Weak SSNC is observed with overlapping deficiencies *Df(1)ct-J4* and *Df(1)ct4b1* and zip^{Ebr} (23% and 20% mlf, respectively). Temperature sensitivity is not observed in either case. The smallest interval uncovered by these deficiencies is 7B2-4; 7C1.

12A; 12E and 18E1-2; 20: Two additional genetic regions are defined by single deficiencies which act as weak SSNCs. *Df(1)g* uncovers 12A; 12E and at 25°, interacts with zip^{Ebr} with a penetrance of 24%. Overlapping deficiency *Df(1)RK2* does not interact (3% mlf), so the smallest defined interval is 12A; 12D2. At 25°, *Df(1)HF396* acts as a weak SSNC with zip^{Ebr} (22% mlf) and $zip^{6.1}$ (17% mlf). Noninteracting deficiencies *Df(1)DCB1-35b* (1% mlf) and *Df(1)A209* (4% mlf) further subdivide this interval to 18E1-2; 19F1-2.

Two genes, regulatory light chain (*spaghetti-squash*; Karess *et al.* 1991) and essential light chain (Edwards *et al.* 1995), are critical for myosin function and map to the *X* chromosome. None of the deficiencies available in the deficiency kit uncover the essential light chain, but two deficiencies, *Df(1)N73* and *Df(1)5D*, should uncover *spaghetti-squash* (*sqh*). Neither deficiency, however, interacts with *zip* (3% mlf). Therefore, a reduction in the gene dosage of *sqh* is not sufficient to elicit the malformed phenotype in combination with *zip*.

Second Chromosome

Fifty-six deficiency stocks on the second chromosome are available for analysis. Twenty-one deficiencies on the second chromosome behave as SSNCs of *zip* (Figure 3; Table 2; appendix b). This represents a greater proportion of SSNCs deficiencies on the second chromosome than those available on the *X* or third chromosomes. One of the deficiencies acts as a strong SSNC nine exhibit an intermediate penetrance of mlf, and the remaining 11 are weak SSNCs.

Strong, second-site noncomplementing second chromosome deficiency: *52F5-9; 52F10-53A1:* On the right arm of the second chromosome, within cytological interval 52F5-9; 52F10-53A1, lies a locus that is a strong SSNC of *zip*. At 25°, 98% of *Df(2R)Jp8* +/+ zip^{Ebr} flies are malformed and the penetrance is 76% in *trans* to $zip^{6.1}$. In addition to displaying mlf with a high penetrance, *Df(2R)Jp8* is semilethal in combination with zip^{Ebr} (Table

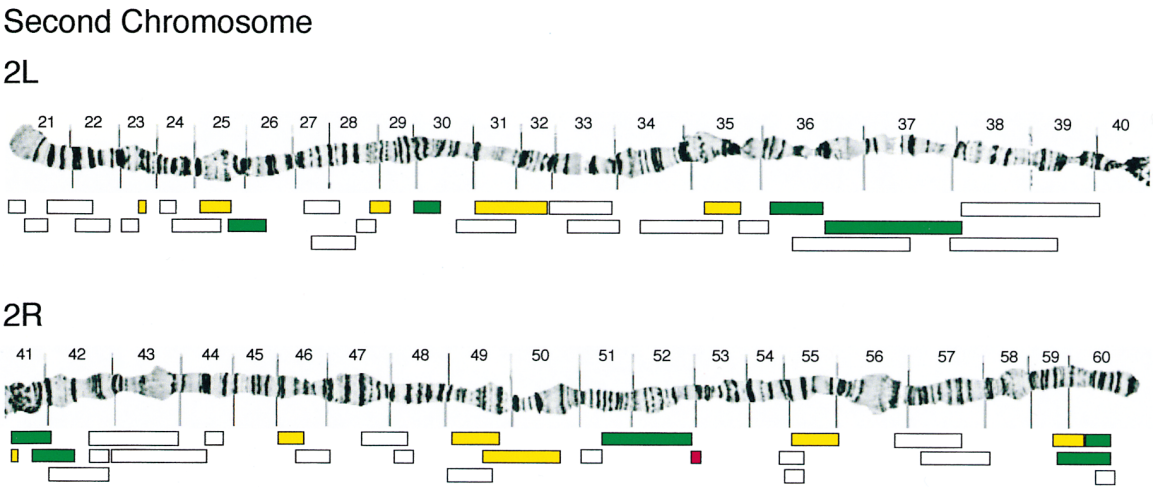

Figure 3.—Second-site noncomplementing deficiencies on the second chromosome. Of the tested second chromosome-linked deficiencies, 21 behave as SSNCs of *zip*. This represents a minimum of 17 loci. One deficiency, *Df(2R)Jp8*, behaves as a strong SSNC deficiency (red box), nine of the deficiencies act as intermediate SSNCs (green boxes), and 11 are weak SSNCs (yellow boxes; Table 2). Tested, noninteracting deficiencies are indicated by the white boxes (appendix b). The chromosome map is modified from Lefevre (1976).

4). *Df(2R)Jp8* + / + zip^{Ebr} flies are recovered at 55% of the expected Mendelian frequency. *Df(2R)Jp8* uncovers the *kinesin heavy chain* (*khc*) locus (Saxton *et al.* 1991). We tested a strong *kinesin heavy chain* mutation, khc^{8}, and six additional EMS mutations uncovered by *Df(2R)Jp8* for genetic interaction with zip^{Ebr} at 25° (Table 4; R. Steward, personal communication; B. Saxton, personal communication; Saxton *et al.* 1991). The khc^{8} allele does not interact with zip^{Ebr} (<1% mlf) nor do four of the six other EMS mutations. Mutations *E3.10* and *J3.8*, however, do interact with zip^{Ebr} with characteristics similar to those observed with *Df(2R)Jp8*. Flies double heterozygous for zip^{Ebr} and *E3.10* are semi-viable (16% of expected Mendelian frequency) and 100% of the escaper adults exhibit mlf. One hundred percent of *J3.8* + / + zip^{Ebr} double heterozygous flies are mlf, and are observed with only 53% of the expected Mendelian frequency. *E3.10* and *J3.8* are recessive lethals that fail to complement each other (data not shown; R. Steward, personal communication) Taken together, it is likely that the genetic interaction seen with *Df(2R)Jp8* is specific to the interval uncovered by the deficiency and that *E3.l0* and *J3.8* are mutations in the locus responsible for this interaction. Further characterization of *E3.10* / *J3.8* is underway.

51C3; 52F5-9: Abutting the interval uncovered by *Df(2R)Jp8* is a second deficiency, *Df(2R)Jp1*, that behaves as an intermediate SSNC of zip^{Ebr}. This deficiency uncovers a locus other than *E3.10* / *J3.8* based on the following: First, the interaction seen with *Df(2R)Jp1* is specific to

TABLE 4

Second-site noncomplementation of *zipper* at 25° in the cytogenetic region uncovered by *Df(2R)Jp8*

Deficiency or mutant stock	Percent malformed (n)[a] + zip^{Ebr}/*Df*(m) +	+ *SM5*/*Df*(m) +	Comments
Df(2R)Jp8/CyO	98 (86)	2 (156)	Semi-lethal in *trans* to zip^{Ebr}
khc^{8}/*CyO*	<1 (163)	<1 (159)	
D2.33/CyO	4 (130)	2 (128)	
D8.13/CyO	2 (163)	<1 (138)	
E3.10/CyO	100 (26)	4 (160)	Semi-lethal in *trans* to zip^{Ebr}; fails to complement *J3.8*
E3.27/CyO	1 (216)	2 (195)	
E6.17/CyO	7 (45)	0 (1)	Lethal in *trans* to *SM5*
J3.8/CyO	100 (61)	0 (116)	Semi-lethal in *trans* to zip^{Ebr}; fails to complement *E3.10*

[a] Percent of malformed flies double heterozygous for the indicated deficiency or mutation and zip^{Ebr} (*Df* or *m* + / + zip^{Ebr}). n is the total number of flies of this genotype that were scored.

TABLE 5

Second-site noncomplementation of *zipper* at 25° in the cytogenetic region uncovered by *Df(2L)sc19-5*

Mutant stock	Percent malformed (n)[a]		Comments
	+ zip^{Ebr}/m +	+ *SM5*/m +	
l(2)01209/CyO	15 (66)	0 (51)	Fails to complement *viking*
vkg^{BLK}/*CyO*	6 (49)	2 (40)	
vkg^{ICO}/*CyO*	10 (49)	0 (66)	
vkg^{SAK}/*CyO*	5 (62)	0 (73)	

[a] Percent of malformed flies double heterozygous for the indicated mutation and zip^{Ebr} (m +/+ zip^{Ebr}). n is the total number of flies of this genotype that were scored.

zip^{Ebr} and penetrance of mlf is lower (40%) and is seen only at 25°. Also, no reduction in viability is seen in these flies (data not shown). Finally, the mutagenesis that generated *E3.10* was designed such that the mutation is uncovered by *Df(2R)Jp8* but not uncovered by *Df(2R)Jp1* (Saxton *et al.* 1991). Therefore, an additional SSNC locus of *zip* falls in interval 51C3; 52F5-9. The myosin light chain kinase maps to this interval (Kojima *et al.* 1996; Tohtong *et al.* 1997; M. Champagne, K. Edwards and D. Kiehart, unpublished results), but mutations in the gene are not currently available for analysis.

Intermediate, second-site noncomplementing second chromosome deficiencies: *25A4-5; 26B2-5:* An intermediate penetrance of 30% of mlf is seen between *Df(2L)c1-h3* and zip^{Ebr}. This deficiency uncovers interval 25D2-4; 26B2-5. *Df(2L)sc19-5* overlaps this interval between 25D2-4; 25D5-7, and it behaves as a weak SSNC. Based on its cytological location, *viking* [*vkg*, encoding collagen α2(IV); Yasothornsrikul *et al.* 1997] should be uncovered by *Df(2L)sc19-5* but not by *Df(2L)c1-h3*. Mutations in the *vkg* locus have been tested for second-site noncomplementation with zip^{Ebr} (Table 5). *l(2)01209* is a *P*-element insertion that fails to complement *vkg* (data not shown). In trans to zip^{Ebr}, 15% of the *l(2)01209* heterozygous flies are mlf. Additionally, vkg^{ICO} acts as a weak SSNC of *zip* (10% mlf), although two additional alleles, vkg^{BLK} and vkg^{SAK} (6% and 5% mlf, respectively) do not interact with zip^{Ebr} by our criteria. The differences in SSNC behavior of these *vkg* alleles with *zip* may reflect differences in their strength, although previous complementation analysis does not address this issue directly (Rodriguez *et al.* 1996).

29F7-30A1; 30C2-5: Cytogenetic interval 29F7-30A1; 30C2-5 is uncovered by *Df(2L)30A-C*. This deficiency is a SSNC of zip^{Ebr}, with a phenotypic penetrance of 26%, but does not interact with $zip^{6.1}$ (2% mlf). The genetic interaction is only seen at 25°. No additional deficiencies within the Deficiency Kits overlap this interval, so the focus of the interaction has not been mapped any more finely.

36A8-9; 36E1-2 and 36E4-F1; 38A6-7: Two adjacent deficiencies, *Df(2L)H2O* and *Df(2L)TW50*, span 36A8-9; 36E1-2 and 36E4-F1; 38A6-7, respectively. Each shows an intermediate penetrance of mlf of 28% with zip^{Ebr} at 25°. This reveals at least two SSNCs loci. *Df(2L)TW137* (6% mlf) is noninteracting and overlaps each of these deficiencies, and noninteracting *Df(2L)TW84* (0%) overlaps the proximal end of *Df(2L)TW50*. Thus, at least one interacting locus is confined to 36A8-9; 36C2-4 and the other is restricted to 37B9-C1; 37F5-38A1.

41A; 42B1-3: Df(2R)nap1 maps cytogenetically to 41D2-E1; 42B1-3. It is an apparent strong SSNC of both zip^{Ebr} and $zip^{6.1}$. At 25°, mlf is seen in 82% of flies double heterozygous for the deficiency and zip^{Ebr} and in 75% of the flies carrying $zip^{6.1}$. A caveat to the interpretation of this data is that the deficiency chromosome shows dominance for mlf since flies that are singly heterozygous for the *Df(2R)nap1* also display mlf (Table 6). The appearance of mlf in the *Df(2R)nap1* heterozygotes is dependent on genetic background. In crosses to zip^{Ebr}/*SM5*, mlf is seen in approximately 45% of the *Df(2R)nap1*/*SM5* flies (Table 6), while the penetrance is 65–82% in *Df(2R)nap1*/*CyO* flies (data not shown). If the penetrance of mlf in the case of the zip^{Ebr} is strictly additive, then the penetrance of mlf in the *Df(2R)nap1* +/+ zip^{Ebr} double heterozygotes would be approximately 40% at 25°. It is unlikely that the genetic interaction seen with zip^{Ebr} and *Df(2R)nap1* is a manifestation of only the genetic background of the deficiency stock, because an overlapping deficiency present in chromosomal rearrangement *In(2R)bw[VDe2L]Cy[R]* (41A-B; 42A2-3) also behaves as an intermediate strength SSNC of zip^{Ebr}, exhibiting mlf in 43% of the flies at 25° (Tables 2 and 6). Flies heterozygous for *In(2R)bw[VDe2L]Cy[R]* also exhibit mlf in the absence of *zip* (10–12%; Table 6) but with considerably lower penetrance than *Df(2R)nap1*. Based on cytology, one of the loci encoding cytoplasmic actin (*Act42A*) maps to 42A (Fyrberg *et al.* 1981), but it is unlikely to be the SSNC because *Df(1)nap9* (42A1-2; 42E6-F1) should also uncover *Act42A* but is not a SSNC (appendix b). Assessing the data observed with these three stocks shows at least one interacting locus falls within 41D2-E1; 42A1-2. Additionally, *Df(2R)M41A4* uncovers 41A and acts as a weak SSNC. Thus, cytogenetic

TABLE 6

Second-site noncomplementation of *zipper* at 25° in the cytogenetic region uncovered by *Df(2R)nap1* and *In(2R)bw[VDe2L]Cy[R]*

Deficiency stock	Percent malformed (n)[a] + zip^{Ebr}/*Df* +	+ *SM5*/*Df* +	Comments
Df(2R)nap1/In(2LR)Gla, Dp(2;2)BG	82 (93)	42 (127)	*Df* heterozygotes display mlf
Df(2R)nap9/In(2LR)Gla, Dp(2;2)BG	4 (73)	9 (90)	
In(2R)bw[VDe2L]Cy[R]/In(2LR)Gla, Dp(2;2)BG	43 (84)	10 (85)	*Df* heterozygotes display mlf

[a] Percent of malformed flies double heterozygous for the indicated deficiency and zip^{Ebr} (*Df* + / + zip^{Ebr}). n is the total number of flies of this genotype that were scored.

interval 41A-F contains at least two loci that interact with *zip*.

59D5-9; 60D: The tip of the right arm of the second chromosome is represented by several SSNC deficiencies that do not uncover *zip*, verified by their ability to complement the two *zip* alleles, but do uncover a number of interacting loci. Flies heterozygous for *Df(2R)bw[VDe2L]Px[Kr]* have blistered wings. In addition to the blistered wings, flies double heterozygous for the deficiency and either zip^{Ebr} or $zip^{6.1}$ display mlf with a penetrance ranging from 35–41% and no temperature sensitivity. *Df(2R)or-BR6* overlaps the proximal region of *Df(2R)bw[VDe2L]Px[Kr]* between 59D5-9; 60B3-8. Unlike *Df(2R)bw[VDe2L]Px[Kr]*, *Df(2R)or-BR6* interacts with zip^{Ebr} and $zip^{6.1}$ only at 18° and results in a weak penetrance of mlf (12–15%). Distally, the deficiency uncovered by *In(2LR)Px[4]* overlaps *Df(2R)bw[VDe2L]Px[Kr]* between 60B; 60D1-2. *In(2LR)Px[4]* is an intermediate SSNC of zip^{Ebr} and $zip^{6.1}$ at 18° (56% and 24% mlf, respectively), consistent with the results seen previously by Gotwals and Fristrom (1991) with zip^{Ebr}. Taken together, these results suggest there are at least two interacting loci uncovered by these deficiencies. One locus interacts specifically at 18° and would fall into 60B1-8, the region overlapped by *Df(2R)or-BR6* and *In(2LR)Px[4]*. At least one additional locus would fall in interval 60B3-8; 60D. Gotwals and Fristrom (1991) identified two loci that interact with zip^{Ebr} in this interval: *bs* and *l(2)B485*. Contrary to their observations, an interaction was not observed between *Df(2R)PX2* and *zip*.

Weak, second-site noncomplementing second chromosome deficiencies: In addition to the three weakly interacting deficiencies on the second chromosome described above, there are eight additional deficiencies, each defining a separate genomic region, that are also weakly interacting. On the left arm of the second chromosome, there are four additional weak SSNC deficiencies in addition to *Df(2L)sc19-5*.

23C3-5; 23D1: Df(2L)JS32 uncovers interval 23C3-5; 23D1 behaves as a weak SSNC (11% mlf).

28E4-7; 29B2-C1: Genomic region 28E4-7; 29B2-C1 is uncovered by *Df(2L)TE29Aa-11* which interacts weakly with zip^{Ebr} at 18° and 25° (15–16% mlf) and also with $zip^{6.1}$ at 18° (11% mlf).

31A; 32C-E: Df(2L)J39 uncovers 31A; 32C-E and also interacts with zip^{Ebr} and $zip^{6.1}$ (13–14% mlf).

35B1-3; 35E6: In cytological location 35, *Df(2L)osp29* uncovers a locus that behaves as a weak SSNC of zip^{Ebr} at 18° (11% mlf). *Df(2L)b87e25* and *Df(2L)r10* do not interact with *zip* (1% and 6% mlf, respectively); therefore this locus is restricted to 35B10-C1; 35E1.

On the right arm of the second chromosome, there are four weak SSNC deficiencies in addition to *Df(2R)or-BR6*.

46A; 46C: Df(2R)B5 uncovers interval 46A; 46C. At 25°, *Df(2R)B5;* zip^{Ebr} double heterozygous flies exhibit mlf at 11% penetrance.

49A4-13; 50C23-D2: In the genomic region 49A4-13; 50C23-D2, two overlapping deficiencies, *Df(2R)vg-C* and *Df(2R)CX1*, act as weak SSNCs of zip^{Ebr}, although they behave with slightly different characteristics. *Df(2R)vg-C* shows the interaction only at 18° (12% mlf) while the interaction shows both a higher penetrance and no temperature sensitivity in the case of *Df(2R)CX1* (19–23% mlf). Either each deficiency uncovers interacting loci within the nonoverlapping regions, or differences in the genetic background of these stocks affect the specificity of the interactions.

55A; 55F: Finally, *Df(2R)PC4* uncovers interval 55A; 55F, and it interacts specifically with zip^{Ebr} but not $zip^{6.1}$ with a penetrance of 20–24%. The more proximal deficiencies *Df(2R)Pc17B* and *Df(2R)Pc111B* (4% and 1% mlf) do not interact, therefore the interacting locus is mapped to 55C1-3; 55F.

Third Chromosome

Of the 65 deficiencies available on the third chromosome, 12 behave as SSNCs of *zip* (Figure 4; Table 3; appendix c). One deficiency behaves as a strong SSNC and in combination with zip^{Ebr} shows reduced viability. Two deficiencies behave as intermediate SSNCs and the remainder interact weakly. A minimum of 11 interacting loci can be inferred.

Strong, second-site noncomplementing third chromo-

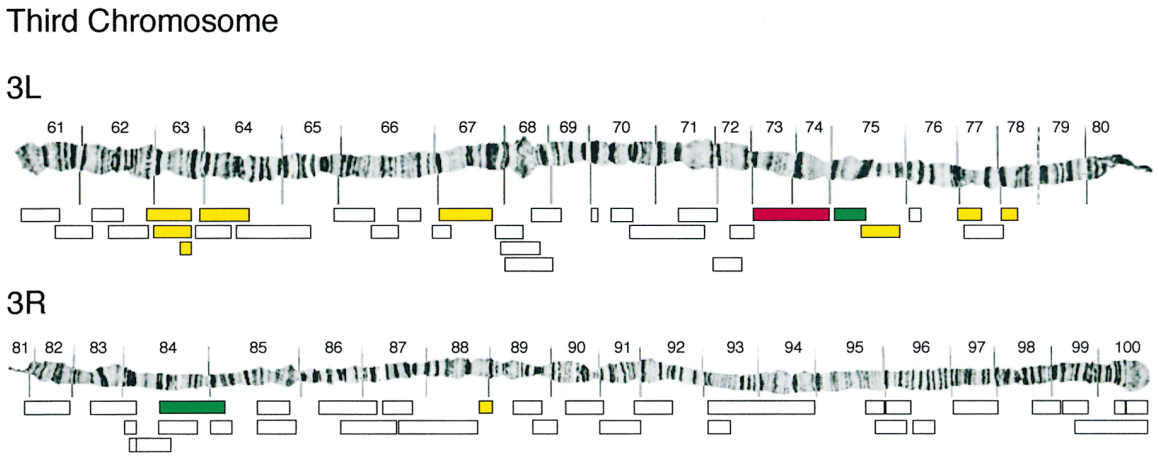

Figure 4.—Second-site noncomplementing deficiencies on the third chromosome. Of the tested third chromosome-linked deficiencies, 12 behave as SSNCs of *zip*. This represents a minimum of 11 loci. One deficiency, *Df(3L)81k19*, behaves as a strong SSNC deficiency (red box), two of the deficiencies act as intermediate SSNCs (green boxes), and nine are weak SSNCs (yellow boxes; Table 3). Tested, noninteracting deficiencies are indicated by the white boxes (appendix c). The chromosome map is modified from Lefevre (1976).

some deficiency: *73A3; 74F:* In the presence of zip^{Ebr}, *Df(3L)81k19* (cytogenetic interval 73A3; 74F) behaves as a strong SSNC at both 18° and 25°, exhibiting a penetrance of mlf in 77–84% of the flies. There is a decrease in viability of double heterozygous mutant flies, as compared to their *SM5*/+; *Df(3L)81k19*/+ siblings (53% of expected Mendelian frequency; Table 7). At 25°, flies with the deficiency in *trans* to $zip^{6.1}$ show a 17% penetrance of mlf. The locus encoding an ecdysone-responsive *ets* domain transcription factor, *E74*, lies within this interval (Burtis *et al.* 1990). Circumstantial evidence that favors *E74* as a SSNC is as follows. First, *E74* is uncovered by *Df(3L)81k19* (data not shown; Fletcher *et al.* 1995). Second, it is expressed at late third larval instar (Thummel *et al.* 1990), coincident with the initiation of the major cell shape changes that occur during the morphogenesis of the leg (Condic *et al.* 1990; Fristrom and Fristrom 1993). Finally, it has been shown to interact genetically with the *Broad-Complex* (Fletcher and Thummel 1995), members of which are also known to interact genetically with *zip* (Gotwals and Fristrom 1991). *E74* is a complex locus, encoding two overlapping transcripts with separate promoters, and two mutations, $E74^{P[neo]}$ and $E74^{DL\text{-}1}$, affect each transcript independently and complement each other genetically (Burtis *et al.* 1990; Fletcher *et al.* 1995). Because *E74* appeared to be a good candidate for acting as the SSNC, we tested it, but found it did not interact genetically with *zip*. Each *E74* mutation was tested with *zip* for second-site noncomplementation (Table 7). For

TABLE 7

Second-site noncomplementation of *zipper* at 25° in the cytogenetic region uncovered by *Df(3L)81k19*

Deficiency or mutant stock	Percent malformed (n)[a]		Comments[b]
	+/zip^{Ebr}; *Df*(m)/+	+/*SM5*; *Df*(m)/+	
Df(3L)81k19/TM6B	77 (91)	10 (170)	Strong, semilethal in *trans* to zip^{Ebr}, 73A3; 74F[c]
Df(3L)st-b11/TM6B	15 (98)	3 (107)	Weak, 72D10-11; 73D1-2[c]
Df(3L)st7/TM3	16 (77)	1 (83)	Weak, 73A3-4; 74A3[c]
Df(3L)st4/TM6	13 (147)	0 (145)	Weak, 72D10; 73C1[c]
Df(3L)st-f13/TM6B	3 (35)	2 (41)	Noninteracting, 72C1-D1; 73A3-4[c]
$E74^{P[neo]}$/*TM6C*	0 (29)	0 (35)	Noninteracting
$E74^{DL\text{-}1}$/*TM6B*	0 (61)	0 (74)	Noninteracting
$E74^{P[neo]}/E74^{DL\text{-}1}$ [d]	0 (59)	0 (53)	Noninteracting

[a] Percent of malformed flies double heterozygous for the indicated deficiency or mutation and zip^{Ebr} (zip^{Ebr}/+; *Df*(m)/+). n is the total number of flies of this genotype that were scored.

[b] Strength of interactions is based on penetrance of the malformed phenotype in the double heterozygous flies and is defined as: noninteracting (<10% mlf), weak (10–24% mlf), intermediate (25–75% mlf) and strong (≥75% mlf).

[c] Cytology uncovered by deficiency.

[d] See text for derivation of *E74* transheterozygotes

each allele independently, no interaction was seen with *zip*^{Ebr} (0% mlf). *Df(3L)81k19*, however, should remove both *E74* transcripts. In order to eliminate both transcripts from the flies, a double heterozygous stock was constructed, *zip*^{Ebr} / *SM5*; *E74P*$^{[neo]}$ / *TM6C*, and males from this stock were crossed to *E74*$^{DL-1}$ / *TM6C* virgin females. The malformed phenotype was then assessed in flies heterozygous for *zip*^{Ebr} and transheterozygous for both *E74* alleles. Again, no malformed flies were observed (0% mlf). Therefore, it is clear that *E74* does not interact genetically with *zip*.

Since *Df(3L)81k19* uncovers a strong SSNC of *zip*, finer genetic mapping was pursued (Figure 5; Table 7). Reported deficiencies that overlap *Df(3L)81k19* are confined to the proximal side of the deficiency. A weakly interacting genetic region has been identified between 73A3-4; 73C1. *Df(3L)st-bll*, *Df(3L)st7* and *Df(3L)st4* interact with *zip*^{Ebr}, giving rise to mlf flies in 13–16% of the cases. *Df(3L)st-f13* defines the proximal limit of this region because it does not interact (3% mlf). The low penetrance of mlf observed with these deficiencies suggests that at least one other SSNC locus of *zip*, falls within the interval 73C1; 74F, and perhaps, based on the distal breakpoint in *Df(3L)st7*, the locus is actually confined to 74A3-74F. Unfortunately, no published deficiencies subdivide this region. Eighteen lethal *P*-element insertions collected by the Berkeley Drosophila Genome Project (University of California, Berkeley, CA) fall within 73A-74F have been tested for SSNC of *zip*, but none of them interact (data not shown). In sum, we have yet to identify the locus or loci responsible for the strong SSNC observed when the *Df(3L)81k19* chromosome is put in *trans* to *zip*.

Intermediate, second-site noncomplementing third chromosome deficiencies: Two regions on the third chromosome contain loci that interact intermediately with *zip*.

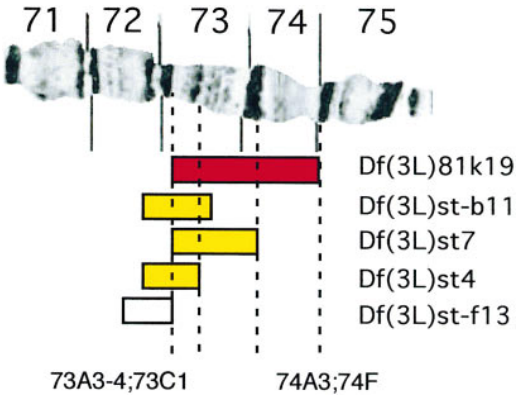

Figure 5.—Finer genetic mapping of the *zipper* SSNC region 73A3; 74F. *Df(3L)81k19* is a strong SSNC of *zip* (red box). To more finely map the regions responsible for this genetic interaction, additional deficiencies were tested for SSNC of *zip*. *Df(3L)st-b11*, *Df(3L)st7* and *Df(3L)st4* behave as weak SSNCs (yellow box), but *Df(3L)st-f13* does not interact genetically with *zip* (white box; Table 7). This maps a weakly interacting region to interval 73A3-4; 73C1. Based on these observations, region 74A3; 74F may also include a locus or loci that behave as SSNC of *zip*.

75A6-7; 75F1: Two deficiencies, *Df(3L)W10* and *Df(3L)Cat*, define one of these regions. They overlap between 75B8; 75C1-2. Each deficiency interacts with *zip*^{Ebr} but not with *zip*$^{6.1}$, and a slight increase in the penetrance of mlf is seen at 25°. The highest observed penetrance of mlf is 26% with *Df(3L)W10* and 20% with *Df(3L)Cat*.

84D4-6; 85B6: Df(3R)p712 defines a unique interval, 84D4-6; 85B6. In all but one other case, *zip*^{Ebr} proves to be the more sensitive allele in these screens. *Df(3R)p712*, however, interacts with *zip*$^{6.1}$ but not with *zip*^{Ebr}. There is a slight temperature sensitivity observed, so at 25°, 40% of the double heterozygous flies are mlf as compared to 23% at 18°. *Df(3R)Hu*, *Df(3R)Ant17* and *Df(3R)p-Xt103* (8% mlf) are noninteracting deficiencies that overlap the proximal and distal breakpoints of *Df(3R)p712*. Thus the SSNC locus is confined to 84F1; 85A2.

Weak, second-site noncomplementing third chromosome deficiencies: Six regions on the third chromosome possess weak SSNC loci of *zip*.

62F; 63D: One region is defined at 63D1. Three overlapping deficiencies, *Df(3L)M21*, *Df(3L)HR370* and *Df(3L)HR119* overlap at 63D1 and each interacts with *zip*^{Ebr} with a penetrance between 11–22%.

63F4-7; 64C13-15: Neighboring this interval, *Df(3L)GN24* also shows mlf with *zip*^{Ebr} with a penetrance of 14% at 18°. Two noninteracting deficiencies, *Df(3L)GN50* and *Df(3L)ZN47* (5% mlf), overlap this region, so the interacting locus is likely to be confined to 64C.

67A2; 67D7-13: Df(3L)AC1 uncovers 67A2; 67D7-13 and is a weak SSNC of *zip*^{Ebr} at 18° and 25° (23% and 13% mlf, respectively). Comparing the cytology of this deficiency with the noninteracting deficiency *Df(3L)29A6* (7%), the locus lies within 67B1; 67D7-13.

77A1; 77D1 and 78A2; 78C9: Distally on the chromosome arm 3L, nonoverlapping deficiencies, *Df(3L)rdgC-co2* (11% mlf) and *Df(3L)Pc-Mk* (17% mlf), interact weakly with *zip*^{Ebr}. *Df(3L)ri-79C* is noninteracting and overlaps each of these deficiencies. Taken together, *Df(3L)rdgC-co2* uncovers a locus in interval 77A1; 77B-C that interacts with *zip*^{Ebr} at 18° with a penetrance of mlf of 11%. Screening with *Df(3L)Pc-Mk* reveals a weakly interacting locus mapping to 78A; 78C9.

88E7-13; 89A1: Df(3R)Ea behaves as a weak SSNC of *zip*^{Ebr}. Testing at 25° shows that *Df(3R)Ea* interacts specifically with *zip*^{Ebr}. The penetrance of mlf in these flies is 23%. The genetic interactions observed with this deficiency are consistent with results obtained when mutations in the *cytoplasmic tropomyosin* (*cTm*) locus are tested (Table 8). *Df(3R)Ea* uncovers *cTm*, and maternal effect and lethal mutations specific for *cTm* have been identified (Erdélyi *et al.* 1995; Tetzlaff *et al.* 1996). A maternal effect allele of *cTm*, *cTm*^{eg9}, was generated by *P*-element insertion and a lethal allele, *cTm*^{er4}, was generated

TABLE 8

Second-site noncomplementation of *zipper* at 25° in the cytogenetic region uncovered by *Df(3R)Ea*

Deficiency or mutant stock	Percent malformed (n)[a]		Comments
	$+/zip^{Ebr}$; $Df(m)/+$	$+/SM5$; $Df(m)/+$	
Df(3R)Ea	23 (163)	2 (197)	
$cTm^{eg9}/TM3$	34 (41)	2 (40)	Maternal effect allele
$cTm^{er4}/TM3$	26 (76)	0 (64)	Lethal allele

[a] Percent of malformed flies double heterozygous for the indicated deficiency or mutation and zip^{Ebr} ($zip^{Ebr}/+$; $Df(m)/+$). n is the total number of flies of this genotype that were scored.

by imprecise excision of the *P*-element (Erdélyi *et al.* 1995). Both *cTm* alleles behave as SSNCs with zip^{Ebr} but not with $zip^{6.1}$ (Table 8; data not shown). The penetrance of mlf with each allele and zip^{Ebr} is similar to each other and to *Df(3R)Ea* (26–34% mlf). Thus, *Df(3R)Ea* defines at least one locus that acts as a SSNC of zip^{Ebr}, and *cTm* appears responsible for the interaction.

DISCUSSION

Second-site noncomplementation of *zipper*: Our screens for second-site noncomplementation of *zipper* with deficiencies from the Bloomington Stock Center Deficiency Kits assay for the malformed phenotype. These screens reveal 47 deficiencies on the *X*, second and third chromosomes that act as SSNCs of *zip*, and 111 that do not. The relative strength of the genetic interactions has been assigned based on the penetrance of mlf in *zip*; *Df* double heterozygous flies. Two of the 47 interacting deficiencies, *Df(2R)Jp8* and *Df(3L)81k19*, are classified as strong SSNCs, with an observed penetrance of mlf in excess of 75%. Intermediate strength penetrance (25–75% mlf) is seen with 17 of the deficiency stocks, and weak interactions (penetrance of 10–24%) are seen with 28 of the stocks. The two strong interacting deficiencies also affect the viability of flies that are double heterozygous with zip^{Ebr}. Expressivity of mlf is not always correlated with the degree of penetrance. Temperature sensitivity was assessed for 43 of the SSNC deficiency stocks. Thirteen of the stocks showed no significant temperature sensitivity while eight deficiencies exhibited cold sensitivity at 18°, and 22 exhibited temperature sensitivity at 25°. Generally, the zip^{Ebr} allele is more sensitive in these screens, such that SSNC is seen only with zip^{Ebr}, or SSNC is observed with both *zip* alleles, but with a lower penetrance when in *trans* to $zip^{6.1}$. The only exceptions to this are seen in tests with *Df(1)G4e[L]h24i[R]* and *Df(3R)p712*. In each of these cases, SSNC is observed only when double heterozygous with $zip^{6.1}$. Thirteen of the deficiencies interact with both zip^{Ebr} and $zip^{6.1}$, and they include the two strong SSNCs, five of the intermediate SSNCs and six weak SSNCs. Among this collection of 13 stocks, five overlap and define two regions. *Df(2R)M41A4* and *In(2R)bw[VDe2L]Cy[R]* overlap in region 41A, and *Df(2R)orBR6*, *Df(2R)bw[VDe2L]Px[Kr]* and *In(2LR)Px[4]* overlap in cytogenetic interval 60B-D. Thus, the SSNC deficiencies that interact with both zip^{Ebr} and $zip^{6.1}$ define 10 regions of the genome that are required for *zip* function and represent strong candidates for pursuit in the future. In addition, the 12 deficiencies that act as intermediate SSNCs, 11 of which interact with zip^{Ebr} and one that interacts with $zip^{6.1}$, are also strong candidates for future analysis.

Second-site noncomplementing loci generally map to the deficiencies: Operationally, it is possible that the SSNC seen between the deficiency stocks and *zip* could map to a region of the chromosome other than that area that is uncovered by the deficiency. In all cases for which appropriate data are currently available, this is not the case. In these instances multiple stocks are available for screening. In the strongest case arguing against the interacting loci mapping outside the deficiency, overlapping deficiency stocks derived from different parental lines show a genetic interaction with *zip*, behaving with similar characteristics. Eight chromosomal regions are defined by overlapping SSNC deficiencies derived from different parental chromosomes (Table 9). In cases where overlapping deficiencies have not been tested, the identification of individual SSNC mutations that are uncovered by the deficiency provide alternate evidence that the SSNC is due to loci uncovered by the deficiency. In this study, we identified three mutations, *E3.10*/*J3.8*, *vkg*, and *cTm*, that are uncovered by SSNC deficiencies and are themselves, SSNCs of *zip*.

Genetic background affects the penetrance of mlf. When $zip^{Ebr}/SM5$ is outcrossed to second chromosome deficiency stocks, mlf is observed in an average of 3% of the zip^{Ebr}/CyO progeny. When outcrossed to Oregon-R wild-type flies, mlf is seen in 6% of the $zip^{Ebr}/+$ progeny. We expect the observed variation in the penetrance arises from genetic differences that could be present on any of the chromosomes within the stocks. The influence of genetic background is of greatest concern for those deficiencies that interact with *zip* with weak penetrance (10–24% mlf), particularly those with an observed penetrance of 10–15%. Indeed, our selection of 10% penetrance as the minimum point for de-

TABLE 9

Origins of overlapping deficiencies that act as second-site noncomplementors of *zipper*

Genetic behavior[a]	Deficiencies	Cytogenetic interval[b]	References
Weak	*Df(1)ct-J4*	7B2-4; 7C1	Lefevre and Johnson 1973
Weak	*Df(1)ct4b1*		
Weak	*Df(2R)M41A4*	41A and 41D2-E1;41F11	FlyBase; Kernan *et al.* 1991
Intermediate	*Df(2R)nap1*		
Intermediate	*In(2R)bw[VDe2L]Cy[R]*		
Weak	*Df(2R)vg-C*	49A4-13;50C23-D2	FlyBase
Weak	*Df(2R)CX1*		
Intermediate	*Df(2R)bw[VDe2L]Px[Kr]*	60B1;60B8 and 60B3-8;60D	FlyBase
Weak	*Df(2R)or-BR6*		
Intermediate	*In(2LR)Px[4]*		
Weak	*Df(3L)M21*	63D1	Wohlwill and Bonner 1991
Weak	*Df(3L)HR119*		
Weak	*Df(3L)HR370*		
Intermediate	*Df(3L)W10*	75B8;75C1-2	Bewley *et al.* 1986; Segraves and Hogness 1984
Weak	*Df(3L)Cat*		

[a] Strength of interactions is based on penetrance of the malformed phenotype when double heterozygous with *zip* and is defined as: weak (10–24% mlf), intermediate (25–75% mlf) and strong (≥75% mlf).
[b] SSNC genomic region that is uncovered by the indicated deficiencies.

fining interaction is somewhat arbitrary, but we believe it is valid. Genetic background is unlikely to be responsible for the observed interaction with all of the weakly penetrant deficiencies. First, of the 28 identified weak class deficiencies, only 11 interact with a penetrance of 10–15%. The influence of genetic background on the interaction has been tested, albeit indirectly, for five of these 11 deficiencies. For example, deficiencies *Df(2L)TE29Aa-11*, *Df(2L)J39* and *Df(2R)or-BR6* interact with both zip^{Ebr} and $zip^{6.1}$ (Table 2). Because each of these *zip* alleles was derived independently from different parental stocks (Gotwals and Fristrom 1991; Young *et al.* 1993) the genetic background for these interacting deficiencies was varied in the double heterozygotes, and yet each deficiency is interacting. Further, *Df(2R)vg-C* and *Df(2R)CX1* are weakly interacting, overlapping SSNC deficiencies derived from different parental stocks that behave similarly in this assay (Table 9). Thus, genetic background is unlikely to solely account for the observed interactions between these deficiencies and *zip*.

Overall, the general applicability of this genetic screen holds strong. To date, based on the criteria listed above, of the 47 SSNC deficiencies, 11 SSNC genomic regions have been definitively mapped, and one additional interval is likely to be specific for SSNC with *zip*. Clearly, verification that SSNC maps to the deficiency and not elsewhere in the genome is a first step in pursuing the identification of individual SSNC loci.

Molecular basis of second-site noncomplementing loci: The expectation of this screen is that loci that are required for myosin function during morphogenesis will be identified. Morphogenetic events that are driven by myosin-based contraction require the appropriate temporal and spatial function of myosin. Players in this process may include the following: cell surface receptors and their ligands that respond to developmental cues; intercellular signaling molecules; transcription factors that direct the synthesis of factors required upstream of myosin function; structural factors, such as other cytoskeletal proteins and extracellular matrix proteins, involved in the appropriate targeting and maintenance of myosin within the cell; and direct, myosin regulatory proteins, such as myosin light chain kinase, that regulate the activity of myosin-based contractions. In this study, the mlf leg phenotype provides the assay for this screen. Therefore, the genomic regions identified in these screens must include loci that are required to collaborate with myosin during imaginal leg disc elongation. It is expected that some of the identified loci will be specific for adult leg morphogenesis, but other loci will encode gene products that are used throughout all stages of development for cell shape change driven morphogenesis. To date, our analysis of loci uncovered by SSNC deficiencies demonstrates that a gene encoding a cytoskeletal protein and one encoding an extracellular matrix protein are each required in concert with myosin for proper imaginal leg disc morphogenesis. In addition, analysis of interactions between *zip* and *E74* reveals that the function of myosin during leg morphogenesis may not be under general transcriptional control of ecdysone-induced transcription factors. Finally, a currently uncharacterized EMS-induced mutation that behaves as a strong SSNC of *zip* has been identified.

Cytoskeletal and extracellular matrix loci: This screen implicates a function for cytoplasmic tropomyosin during morphogenesis. Drosophila cytoplasmic tropomyosin (cTm) is one product of the complex *tropomyosin*

locus. Differential splicing and differential poly-adenylation gives rise to muscle and nonmuscle(cytoplasmic) specific transcripts and protein products (Hanke and Storti 1988). *P*-element insertional mutations in the *cTm* gene were identified either as female-sterile mutations that disrupt *oskar* RNA localization (Erdélyi *et al.* 1995) or as zygotic lethal mutations that affect embryonic head morphogenesis (Tetzlaff *et al.* 1996). Germline-specific mutant alleles reduce the prevalence of some of the *cTm*-specific transcripts in the ovary (Erdélyi *et al.* 1995). Presumably, the lethal alleles would have a more severe effect on the expression of *cTm*, although this was not shown. A germline-specific mutation, cTm^{eg9}, and a lethal allele generated by *P*-element excision, cTm^{er4}, behave as SSNCs of *zip* during metamorphosis. This reflects a postembryonic requirement for the *cTm*, and that, in our assay, the "germline specific" allele has postoogenic defects. The nature of the requirement for *cTm* in myosin function during this morphogenetic event is presently unclear. Cytoplasmic tropomyosin is thought to stabilize actin filaments (reviewed in Pittenger *et al.* 1994). Genetic analysis in yeast reveals this tropomyosin function. Mutations in the *Saccharomyces cerevisiae* tropomyosin gene lead to the loss of actin cables and a reduced growth rate (Liu and Bretscher 1989). In *Schizosaccharomyces pombe*, tropomyosin is localized to the contractile ring during cytokinesis, and mutations in the gene disrupt cytokinesis (Balasubramanian *et al.* 1992). Based on these observations, *cTm* mutations are likely to disrupt myosin function during leg elongation by disrupting the actin cytoskeleton.

Collagen IV is a basement membrane collagen, and its function has been demonstrated during morphogenesis in *C. elegans* and Drosophila. Mutations in the *emb-9* locus [Collagen α1(IV)] and the *let-2* locus [Collagen α2(IV)] cause defects during the late morphogenetic stage that result in embryonic lethality (Guo *et al.* 1991; Sibley *et al.* 1993). In Drosophila, two Collagen IV genes, *DCg1* [α1(IV); Natzle *et al.* 1983] and *viking* [α2(IV); Yasothornsrikul *et al.* 1997], localize to the SSNC interval uncovered by *Df(2L)sc19-5*. Mutations in *viking* act as SSNCs of *zip* during leg morphogenesis. Levels of Collagen IV are detectable throughout the life cycle of the fly, with high levels detected until the onset of pupation (Fessler *et al.* 1993). During leg morphogenesis, the basal lamina detaches from the disc epithelium, and proteolysis of Collagen IV is thought to make the basement membrane more extensible, thus facilitating leg elongation (Fessler *et al.* 1993). This site-directed cleavage of Collagen IV occurs in response to ecdysone (Birr *et al.* 1990; Fessler *et al.* 1993). Identification of *Stubble* as a necessary gene product during leg imaginal disc morphogenesis also demonstrates a role for proteolysis during this process. The *Stubble* locus encodes a type II transmembrane serine protease (Appel *et al.* 1993), and it interacts genetically with $broad^{1}$ and zip^{Ebr} (Gotwals and Fristrom 1991). However, since the existing *viking* mutations are *P*-element insertions, it is more likely that these mutations affect the expression of Collagen IV, rather than its proteolysis, during metamorphosis. Basal cytoplasmic extensions have been observed in the imaginal discs of several insects (reviewed in Fristrom and Fristrom 1993) and are thought to be important for morphogenetic movements. Perhaps mutations in *viking* interfere with such processes in the Drosophila leg imaginal disc, and this effect would occur prior to the observed proteolysis. Mutations are available in the *DCg1* α1(IV) gene (Rodriguez *et al.* 1996), and it is of interest to know if these mutations also act as SSNCs of *zip*.

Transcription factors: The requirement for myosin function during leg morphogenesis is demonstrated by several lines of evidence. zip^{Ebr} was first identified as *E(br)* in screens that identified genetic interactors with the ecdysone-inducible *Broad-Complex* (*BR-C*) family of transcription factors (Gotwals and Fristrom 1991). Later, rescue with a cosmid bearing a *zip* transgene (Young *et al.* 1993) showed that *E*(*br*) is an allele of *zip* (Gotwals 1992). Further, myosin is subcellularly localized within leg imaginal discs undergoing cell shape change (von Kalm *et al.* 1995). Finally, experimental depletion of myosin during larval development gives rise to mlf in adults (Edwards and Kiehart 1996). Transcription of *zip* is not under ecdysone control in the imaginal discs (von Kalm *et al.* 1995); therefore, the gene expression directed by *BR-C* must affect other aspects of leg disc morphogenesis, rather than merely inducing *zip* expression. *E74* also encodes ecdysone-inducible transcription factors whose expression is induced in late third instar larvae (Burtis *et al.* 1990). *E74* is a complex locus, encoding two transcripts, each of which has been mutated separately (Burtis *et al.* 1990; Fletcher *et al.* 1995). Genetically, *BR-C* and *E74* mutations interact, suggesting they function in overlapping pathways (Fletcher and Thummel 1995). Pupae hemizygous for the $E74^{DL\text{-}1}$ mutation exhibit, among other phenotypes, a shortened leg phenotype (Fletcher *et al.* 1995). *Df(3L)81k19* uncovers the *E74* locus, and it is a strong SSNC of *zip*. However, mutations in each of the E74 transcripts, either singly or in *trans* to each other, do not interact with *zip*. Clearly a locus other than *E74* on the *Df(3L)81k19* chromosome is responsible for the SSNC. Finer genetic mapping reveals a more weakly interacting locus may reside in cytogenetic region 73A3-4; 73C1. Identification of the other SSNC locus (loci) uncovered by the *Df(3L)81k19* chromosome awaits finer genetic analysis.

Conclusion: The goal of these screens is to identify factors, both known and novel, that affect myosin function during morphogenesis. In fact, both known and novel loci have been identified. Characterization of SSNC deficiencies identified in this study indicates that they uncover an expected array of loci, including those

that encode cytoskeletal and extracellular matrix proteins and a transcription factor, that are required for myosin function during leg disc morphogenesis. While the role of the *Broad-Complex* and perhaps *viking* may be confined to imaginal disc morphogenesis, it is clear that the functional interactions between myosin and cytoplasmic tropomyosin are likely to occur in morphogenetic and cell migration events during oogenesis, embryogenesis and metamorphosis. In addition, these screens reveal that a previously uncharacterized mutation, *E3.10/J3.8* is a strong interactor with *zip*, and that it is required for both morphogenesis and viability in the fly. Phenotypic and molecular characterization of the *E3.10/J3.8* mutation will proceed along with further characterization of the interacting deficiencies identified in these screens. These studies promise to extend our understanding of the spatial and temporal control of myosin-based contractility in the cell shape changes required for metazoan development.

We acknowledge the attentive help of K. Matthews and the Bloomington Stock Center in providing the deficiencies used in this study. We also thank A. Ephrussi, J. Fristrom, D. Kimbrell, B. Saxton, R. Steward and C. Thummel for providing additional mutant stocks. R. Montague's technical expertise was instrumental in the microscopy and generation of Figure 1. We appreciate the stimulating discussions with the members of the Kiehart, Lin and Titus labs and the critical reading of the manuscript by J. Bloor. This work was supported by National Institutes of Health grants to D.P.K. (GM33830) and S.R.H. (GM17383).

LITERATURE CITED

Appel, L. F., M. Prout, R. Abu-Shumays, A. Hammonds, J. C. Garbe *et al.*, 1993 The Drosophila *Stubble-stubbloid* gene encodes an apparent transmembrane serine protease required for epithelial morphogenesis. Proc. Natl. Acad. Sci. USA **90:** 4937–4941.

Balasubramanian, M. K., D. M. Helfman and S. M. Hemmingsen, 1992 A new tropomyosin essential for cytokinesis in the fission yeast *S. pombe*. Nature **360:** 84–87.

Bewley, G. C., W. J. Mackay and J. L. Cook, 1986 Temporal variation for the expression of catalase in *Drosophila melanogaster*: correlations between rates of enzyme synthesis and levels of translatable catalase-messenger RNA. Genetics **113:** 919–938.

Bilder, D., and M. P. Scott, 1995 Genomic regions required for morphogenesis of the Drosophila embryonic midgut. Genetics **141:** 1087–1100.

Birr, C. A., D. Fristrom, D. S. King and J. W. Fristrom, 1990 Ecdysone-dependent proteolysis of an apical surface glycoprotein may play a role in imaginal disc morphogenesis in Drosophila. Development **110:** 239–248.

Burtis, K. C., C. S. Thummel, C. W. Jones, F. D. Karim and D. S. Hogness, 1990 The Drosophila 74EF early puff contains *E74*, a complex ecdysone-inducible gene that encodes two ets-related proteins. Cell **61:** 85–99.

Condic, M. L., D. K. Fristrom and J. W. Fristrom, 1990 Apical cell shape changes during Drosophila imaginal leg disc elongation: a novel morphogenetic mechanism. Development **111:** 23–33.

Cote, S., A. Preiss, J. Haller, R. Schuh, A. Kienlin *et al.*, 1987 The *gooseberry-zipper* region of Drosophila: five genes encode different spatially restricted transcripts in the embryo. EMBO J. **6:** 2793–2801.

Cutforth, T., and G. R. Rubin, 1994 Mutations in *Hsp83* and *cdc37* impair signaling by the Sevenless receptor tyrosine kinase in Drosophila. Cell **77:** 1027–1036.

De Lozanne, A., and J. A. Spudich, 1987 Disruption of the Dictyostelium myosin heavy chain by homologous recombination. Science **236:** 1086–1091.

DiBello, P. R., D. A. Withers, C. B. Bayer, J. W. Fristrom and G. M. Guild, 1991 The Drosophila *Broad-complex* encodes a family of related proteins containing Zinc fingers. Genetics **129:** 385–397.

Edwards, K. A., and D. P. Kiehart, 1996 Drosophila nonmuscle myosin II has multiple essential roles in imaginal disc and egg chamber morphogenesis. Development **122:** 1499–1511.

Edwards, K. A., X.-J. Chang and D. P. Kiehart, 1995 Essential light chain of Drosophila nonmuscle myosin II. J. Muscle Res. Cell Motil. **16:** 491–498.

Erdélyi, M., A. M. Michon, A. Guichet, J. B. Glotzer and A. Ephrussi, 1995 Requirement for Drosophila *cytoplasmic tropomyosin* in *oskar* mRNA localization. Nature **377:** 524–527.

Fessler, L. I., M. L. Condic, R. E. Nelson, J. H. Fessler and J. W. Fristrom, 1993 Site-specific cleavage of basement membrane collagen IV during Drosophila metamorphosis. Development **117:** 1061–1069.

Fletcher, J. C., and C. S. Thummel, 1995 The ecdysone-inducible *Broad-Complex* and *E74* early genes interact to regulate target gene transcription and Drosophila metamorphosis. Genetics **141:** 1025–1035.

Fletcher, J. C., K. C. Burtis, D. S. Hogness and C. S. Thummel, 1995 The Drosophila *E74* gene is required for metamorphosis and plays a role in the polytene chromosome puffing response to ecdysone. Development **121:** 1455–1465.

Fristrom, D., and J. W. Fristrom, 1993 The metamorphic development of the adult epidermis, pp. 843–897 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fyrberg, E. A., B. J. Bond, N. D. Hershey, K. S. Mixter and N. Davidson, 1981 The actin genes of Drosophila: protein coding regions are highly conserved but intron positions are not. Cell **24:** 107–116.

Gotwals, P. J., 1992 Genetic interactions identify genes involved in Drosophila imaginal disc morphogenesis. Ph.D. Thesis, University of California, Berkeley.

Gotwals, P. J., and J. W. Fristrom, 1991 Three neighboring genes interact with the *Broad-complex* and *Stubble-stubbloid* locus to affect imaginal disc morphogenesis in Drosophila. Genetics **127:** 747–759.

Guo, X., J. J. Johnson and J. M. Kramer, 1991 Embryonic lethality caused by mutations in basement membrane collagen of *C. elegans*. Nature **349:** 707–709.

Hanke, P. D., and R. V. Storti, 1988 The *Drosophila melanogaster tropomyosin II* gene produces multiple proteins by use of alternative tissue-specific promoters and alternative splicing. Mol. Cell. Biol. **8:** 3591–3602.

Karess, R. E., X. Chang, K. A. Edwards, S. Kulkarni, I. Aguilera *et al.*, 1991 The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in Drosophila. Cell **65:** 1177–1189.

Kernan, M. J., M. I. Kuroda, R. Kreber, B. S. Baker and B. Ganetzky, 1991 nap^{ts}, a mutation affecting sodium channel activity in Drosophila, is an allele of *mle*, a regulator of *X* chromosome transcription. Cell **66:** 949–959.

Kiehart, D. P., M. S. Lutz, D. Chan, A. S. Ketchum, R. A. Laymon *et al.*, 1989 Identification of the gene for fly non-muscle myosin heavy chain: Drosophila myosin heavy chains are encoded by a gene family. EMBO J. **8:** 913–922.

Kiehart, D. P., R. A. Montague, W. L. Rickoll, D. Foard and G. H. Thomas, 1994 High-resolution microscopic methods for the analysis of cellular movements in Drosophila embryos, pp. 507–532 in *Drosophila melanogaster: Practical Uses in Cell and Molecular Biology*, edited by L. S. B. Goldstein and E. A. Fyrberg. Academic Press, San Diego.

Kiss, I., A. H. Beaton, J. Tardiff, D. Fristrom and J. W. Fristrom, 1988 Interactions and developmental effects of mutations in the *Broad-complex* of *Drosophila melanogaster*. Genetics **118:** 247–259.

Knecht, D. A., and W. F. Loomis, 1987 Antisense RNA inactivation of myosin heavy chain in *Dictyostelium discoideum*. Science **236:** 1081–1086.

Knecht, D. A., and E. Shelden, 1995 Three-dimensional localization of wild-type and myosin II mutant cells during morphogenesis of Dictyostelium. Dev. Biol. **170:** 434–444.

Kojima, S. I., M. Mishima, I. Mabuchi and Y. Hotta, 1996 A single *Drosophila melanogaster* myosin light chain kinase gene produces multiple isoforms whose activities are differentially regulated. Genes Cells **1:** 855–871.

Lefevre, G., 1976 A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands, pp. 31–66 in *The Genetics and Biology of* Drosophila, edited by M. Ashburner and E. Novitski. Academic Press, New York.

Lefevre, G., and T. K. Johnson, 1973 Evidence for a sex-linked haplo-inviable locus in the *cut-singed* region of *Drosophila melanogaster*. Genetics **74:** 633–645.

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

Liu, H., and A. Bretscher, 1989 Disruption of the single *tropomyosin* gene in yeast results in the disappearance of actin cables from the cytoskeleton. Cell **57:** 233–242.

Manstein, D., M. Titus, A. De Lozanne and J. Spudich, 1989 Gene replacement in Dictyostelium-generation of myosin null mutants. EMBO J. **8:** 923–932.

Natzle, J. E., J. M. Monson and B. J. McCarthy, 1983 Cytogenetic location and expression of collagen-like genes in Drosophila. Nature **296:** 368–371.

Pauli, D., B. Oliver and A. P. Mahowald, 1995 Identification of regions interacting with *ovoD* mutations: potential new genes involved in germline sex determination or differentiation in *Drosophila melanogaster*. Genetics **139:** 713–732.

Pittenger, M. F., J. A. Kazzaz and D. M. Helfman, 1994 Functional properties of non-muscle tropomyosin isoforms. Curr. Opin. Cell Biol. **6:** 96–104.

Rodriguez, A., Z. Zhou, M. L. Tang, S. Meller, J. Chen *et al.* 1996 Identification of immune system and responses genes, and novel mutations causing melanotic tumor formation in *Drosophila melanogaster*. Genetics **143:** 929–940.

Saxton, W. M., J. Hicks, L. S. B. Goldstein and E. C. Raff, 1991 Kinesin heavy chain is essential for viability and neuromuscular functions in Drosophila, but mutants show no defects in mitosis. Cell **64:** 1093–1102.

Segraves, W. A., and D. S. Hogness, 1984 Molecular and genetic analysis of the 75B ecdysone-inducible puff of *Drosophila melanogaster*. Genetics **107:** s96–s97.

Shelden, E., and D. A. Knecht, 1996 Dictyostelium cell shape generation requires myosin II. Cell Motil. Cytoskeleton **35:** 59–67.

Sibley, M. H., J. J. Johnson, C. C. Mello and J. M. Kramer, 1993 Genetic identification, sequence, and alternative splicing of the *Caenorhabditis elegans* alpha 2 (IV) collagen gene. J. Cell Biol. **123:** 255–264.

Simon, M. A., D. D. L. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the *sevenless* protein tyrosine kinase. Cell **67:** 701–716.

Smith, A. V., J. A. King and T. L. Orr-Weaver, 1993 Identification of genomic regions required for DNA replication during Drosophila embryogenesis. Genetics **135:** 817–829.

Sweeney, F. P., M. J. Pocklington and E. Orr, 1991 The yeast type II myosin heavy chain: analysis of its predicted polypeptide sequence. J. Muscle Res. Cell Motil. **12:** 61–68.

Tetzlaff, M. T., H. Jackle and M. J. Pankratz, 1996 Lack of Drosophila cytoskeletal tropomyosin effects head morphogenesis and the accumulation of oskar mRNA required for germ cell formation. EMBO J. **15:** 1247–1254.

Thummel, C. S., K. C. Burtis and D. S. Hogness, 1990 Spatial and temporal patterns of *E74* transcription during Drosophila development. Cell **61:** 101–111.

Tohtong, R., D. Rodriquez, D. Maughan and A. Simcox, 1997 Analysis of cDNAs encoding *Drosophila melanogaster* myosin light chain kinase. J. Muscle Res. Cell Motil. **18:** 1–14.

von Kalm, L., D. K. Fristrom and J. Fristrom, 1995 The making of a fly leg: a model for epithelial morphogenesis. Bioessays **17:** 693–702.

Watts, F. Z., B. Sheils and E. Orr, 1987 The yeast MYO1 gene encoding a myosin-like protein required for cell division. EMBO J. **6:** 3499–3505.

Wheatley, S., S. Kulkarni and R. Karess, 1995 Drosophila nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. Development **121:** 1937–1946.

Williams, D. S., M. A. Hallett and K. Arikawa, 1993 Association of myosin with the connecting cilium of rod photoreceptors. J. Cell Sci. **103:** 183–190.

Williams-Masson, E. M., A. N. Malik and J. Hardin, 1997 An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. Development **124:** 2889–2901.

Wohlwill, A. D., and J. J. Bonner, 1991 Genetic analysis of chromosome region 63 of *Drosophila melanogaster*. Genetics **128:** 763–775.

Yasothornsrikul, S., W. J. Davis, G. Cramer, D. A. Kimbrell and C. R. Dearolf, 1997 *viking*: identification and characterization of a second type IV collagen in Drosophila. Gene **198:** 17–25.

Young, P. E., T. C. Pesacreta and D. P. Kiehart, 1991 Dynamic changes in the distribution of cytoplasmic myosin during Drosophila embryogenesis. Development **111:** 1–14.

Young, P. E., A. M. Richman, A. S. Ketchum and D. P. Kiehart, 1993 Morphogenesis in Drosophila requires nonmuscle myosin heavy chain function. Genes Dev. **7:** 29–41.

Zhao, D. B., S. Cote, F. Jahnig, J. Haller and H. Jackle, 1988 *zipper* encodes a putative integral membrane protein required for normal axon patterning during Drosophila neurogenesis. EMBO J. **7:** 1115–1119.

Communicating editor: R. S. Hawley

APPENDIX A

X chromosome noninteracting deficiencies

Deficiency stock	Cytology	Deficiency stock	Cytology
Df(1)BA1	1A1; 2A	*Df(1)N71*	10B2-8; 10D3-8
Df(1)JC19	2F6; 3C5	*Df(1)N105*	10F7; 11D1
Df(1)N-8	3C2-3; 3E3-4	*Df(1)N12*	11D1-2; 11F1-2
Df(1)dm75e19	3C11; 3E4	*Df(1)C246*	11D-E; 12A1-2
Df(1)C149	5A8-9; 5C5-6	*Df(1)RK2*	12D2-E1; 13A2-5
Df(1)N73	5C2; 5D5-6	*Df(1)RK4*	12F5-6; 13A9-B1
Df(1)5D	5D1-2; 5E	*Df(1)sd72b*	13F1; 14B1
Df(1)JF5	5E3-5; 5E8	*Df(1)4b18*	14B8; 14C1
Df(1)Sxl-bt	6E2; 7A6	*Df(1)B*	16A2; 16A6
Df(1)C128	7D1; 7D5-6	*Df(1)N19*	17A1; 18A2
Df(1)KA14	7F1-2; 8C6	*Df(1)JA27*	18A5; 18D
Df(1)lz6	8D1-2; 8E1-2	*Df(1)DCB1-35b*	19F1-2; 20E-F
Df(1)C52	8E; 9C-D	*Df(1)A209*	20A; 20F

Penetrance of malformed <10% in deficiency; *zip* double heterozygotes (*Df*/+; +/*zip*).

APPENDIX B

Second chromosome noninteracting deficiencies

Deficiency stock	Cytology	Deficiency stock	Cytology
Df(2L)net-PMF	21A1; 21B7-8	*Df(2R)nap9*	42A1-2; 42E6-F1
Df(2L)al	21B8-C1; 21C8-D1	*Df(2R)pk78s*	42C1-7; 43F5-8
Df(2L)ast2	21D1-2; 22B2-3	*Df(2R)cn88b*	42C; 42E
Df(2L)dp-79b	22A2-3; 22D5-E1	*Df(2R)cn9*	42E; 44C
Df(2L)C144	23A1-2; 23C3-5	*Df(2R)44CE*	44C4-5; 44E2-4
Df(2L)ed1	24A3-4; 24D3-4	*Df(2R)X1*	46C; 47A1
Df(2L)sc19-8	24C2-8; 25C8-9	*Df(2R)en-A*	47D3; 48B2-5
Df(2L)J136-H52	27C2-9; 28B3-4	*Df(2R)en30*	48A3-4; 48C6-8
Df(2L)spd	27D-E; 28C	*Df(2R)vg135*	49A-B; 49D-E
Df(2L)Trf-C6R31	28DE	*Df(2R)trix*	51A1-2; 51B6
Df(2L)Mdh	30D-F; 31F	*Df(2R)Pc17B*	54E8-F1; 55B9-C1
Df(2L)Prl	32F1-3; 33F1-2	*Df(2R)Pc111B*	54F6-55A1; 55C1-3
Df(2L)prd1.7	33B2-3; 34A1-2	*Df(2R)AA21*	56F9-17; 57D11-12
Df(2L)b87e25	34B12-C1; 35B10-C1	*Df(2R)Pu-D17*	57B4; 8B
Df(2L)r10	35E1-2; 36A6-7	*Df(2R)Px2*	60C5-6; D9-10
Df(2L)TW137	36C2-4; 37B9-C1		
Df(2L)TW84	37F5-38A1; 39D3-E1		
Df(2L)TW161	38A6-B1; 40A4-B1		

Penetrance of malformed <10% in deficiency; *zip* double heterozygotes (*Df* +/+ *zip*).

APPENDIX C

Third chromosome noninteracting deficiencies

Deficiency stock	Cytology	Deficiency stock	Cytology
Df(3L)emc-E12	61A; 61D3	*Df(3R)Hu*	84A6-B1; 84B3-6 and 84D4-5; 84F1-2
Df(3L)Ar14-8	61C5-8; 62A8	*Df(3R)Antp17*	84B1-2; 84D11-12
Df(3L)R-G5	62A10-B1; 62C4-D1	*Df(3R)p-XT103*	85A2; 85C1-2
Df(3L)R-G7	62B8-9; 62F2-5	*Df(3R)by10*	85D8-12; 85E7-F1
Df(3L)GN50	63E1-2; 64B17	*Df(3R)by62*	85D11-14; 85F6
Df(3L)ZN47	64C; 65C	*Df(3R)M-Kx1*	86C1; 87B1-5
Df(3L)pbl-X1	65F3; 66B10	*Df(3R)T-32*	86E2-4; 87C6-7
Df(3L)66C-G28	66B8-9; 66C9-10	*Df(3R)ry615*	87B11-13; 87E8-11
Df(3L)h-i22	66D10-11; 66E1-2	*Tp(3; Y)ry506-85C*	87D1-2; 88E5-6
Df(3L)Rdl-2	66F5; 66F5	*Df(3R)P115*	89B7-8; 89E7-8
Df(3L)29A6	66F5; 67B1	*Df(3R)C4*	89E; 90A
Df(3L)lxd6	67E1-2; 68C1-2	*Df(3R)P14*	90C2-D1; 91A1-2
Df(3L)vin2	67F2-3; 68D6	*Df(3R)Cha7*	91A; 91F5
Df(3L)vin5	68A2-3; 69A1-3	*Df(3R)D1-BX12*	91F1-2; 92D3-6
Df(3L)vin7	68C8-11; 69B4-5	*Df(3R)e-N19*	93B; 94
Df(3L)Ly	70A2-3; 70A5-6	*Df(3R)e-R1*	93B3-5; 93D2-4
Df(3L)fz-GF3b	70C1-2; 70D4-5	*Df(3R)crb87-4*	95E8-F1; 95F15
Df(3L)fz-M21	70D2-3; 71E4-5	*Df(3R)crb87-5*	95F7; 96A17-18
Df(3L)BK10	71C; 71F	*Df(3R)XS*	96A1-7; 96A21-25
Df(3L)brm11	71F1-4; 72D1-10	*Df(3R)XTA1*	96B; 96D
Df(3L)stf-13	72C1-D1; 73A3-4	*Df(3R)T1-P*	97A; 98A1-2
Df(3L)VW3	76A3; 76B2	*Df(3R)3450*	98E3; 99A6-8
Df(3L)ri-79C	77B-C; 77F-78A	*Df(3R)L127*	99B; 99E
Df(3R)2-2	81F; 82F10-11	*Df(3R)B81*	99C8; 100F5
Df(3R)Tp110	83C1-2; 84B1-2	*Df(3R)awd-KRB*	100C; 100D
Df(3R)Scr	84A1-2; 84B1-2	*Df(3R)faf-BP*	100D; 100F5

Penetrance of malformed $<10\%$ in deficiency; *zip* double heterozygotes (*zip*/+; *Df*/+).