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A Genetic Analysis of the Drosophila Closely Linked Interacting Genes *bulge, argos* and *soba*

Thomas Wemmer and Christian Klämbt

Institut für Entwicklungsbiologie, Universität zu Köln, D-50923 Köln, Germany

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ABSTRACT

The *Drosophila* gene *argos* encodes a diffusible protein that acts as a negative regulator of cell fate decisions. To define interacting gene products, we performed a genetic analysis of *argos,* which suggests the presence of several partially redundant gene functions in its immediate vicinity at the chromosomal position 73A. Dose titration experiments have identified two of these loci. One of them corresponds to the gene *bulge.* Loss of function *bulge* alleles suppress the rough eye phenotype associated with overexpression of *argos;* conversely, amorphic *argos* mutations suppress the eye phenotype seen in flies bearing a single dominant *bulge* allele. Recombination mapping localized *bulge* 0.15 cM distal to *argos.* A second gene, *suppressor of bulge and argos* (*soba*), corresponds to the recently described lethal complementation group 73Aj. *soba* alleles suppress the eye phenotypes seen in flies expressing either the dominant *bulge* allele or the *hs-argos* construct. *soba* resides 120 kb proximal to *argos.* In addition, we have identified one allele of a new gene, *clown,* which like *soba* suppresses the eye phenotypes associated with *hs-argos* and $bulge^{Dominant}$. *clown* maps on chromosome *3* at the cytological position 68CD.

CELLULAR interactions are essential for the ordered development of all multicellular organisms. Diffusible as well as membrane bound molecules provide a multitude of inductive signals that direct the developmental fate of a given cell. Diffusible inductive signals are exemplified by activin, an important mediator of mesoderm induction during vertebrate embryogenesis (GURDON *et al.* 1994; KESSLER and MELTON 1994). Contact-dependent cell signaling mechanisms involved in vulva induction in *Caenorhabditis elegans* (AROIAN *et al.* 1990; KATZ and STERNBERG 1992) or the induction of R7 photoreceptor cell fate in the Drosophila compound eye (KRÄMER *et al.* 1991; DICKSON and HAFEN 1993) have been well documented.

In many cases individual cells can influence the differentiation of their immediate neighbors by a process called lateral inhibition, which restricts the developmental capacities of the flanking cells (WIGGLESWORTH 1940). In Drosophila, lateral inhibition is in part mediated by members of the neurogenic genes (CAMPOS-ORTEGA 1993). The transmembrane protein serving as a signal is encoded by the *Delta* gene and its transmembrane receptor is encoded by the *Notch* gene (FEHON *et al.* 1990; HEITZLER and SIMPSON 1991; REBAY *et al.* 1991). Both proteins are characterized by a large number of epidermal growth factor (EGF)-like repeats in their extracellular domains.

The Drosophila gene *argos* encodes a secreted diffusible inhibitory signaling molecule. The 444 amino acid Argos protein contains one EGF-like motif and acts noncell autonomously as a negative regulator of cell fate in the developing eye and wing imaginal discs. In each case, *argos* instructs neighboring cells not to adopt the same cell fate as the *argos*-expressing cells (FREEMAN *et al.* 1992; KRETSCHMAR *et al.* 1992; OKANO *et al.* 1992; FREEMAN 1994; SAWAMOTO *et al.* 1994). During eye development *argos* is expressed posterior to the morphogenetic furrow in eye imaginal discs. In the embryonic central nervous system (CNS) *argos* is found specifically in the midline glial cells (FREEMAN *et al.* 1992).

In Drosophila, only a few other diffusible signaling molecules involved in pattern formation have been identified. Examples are the Decapentaplegic (Dpp) protein, which belongs to the transforming growth factor-β (TGF-β) family, and the Spitz protein, which belongs to the TGF-α family of signaling proteins (FERGUSON and ANDERSON 1992; RUTLEDGE *et al.* 1992; WHARTON *et al.* 1993). Much has been learned about their receptors (PRICE *et al.* 1989; SCHEJTER and SHILO 1989; RAZ and SHILO 1992; STURTEVANT *et al.* 1993; BRUMMEL *et al.* 1994; NELLEN *et al.* 1994; PENTON *et al.* 1994), but the receptor(s) for the Argos protein are unknown to date.

In an effort to identify the gene(s) for the *argos* receptor, we conducted a genetic analysis of *argos* using a dose titration strategy (BOTAS *et al.* 1982; BRAND and CAMPOS-ORTEGA 1990). The power of this genetic approach is demonstrated, for example, by the case of R7 photoreceptor cell development; the mutants obtained have been successfully used to unravel the biochemical bases of the signal transduction cascades involved in controlling the R7 fate (SIMON *et al.* 1991, 1993; OLIVIER

Corresponding author: Christian Klämbt, Institut für Entwicklungsbiologie, Universität zu Köln, D-50923 Köln, Germany.
E-mail: cklaemb@cipvax.biolan.uni-koeln.de

et al. 1993). Three gene functions, *bulge, soba* and *clown,* are identified that not only genetically interact with *argos* but also interact with each other. Two of these complementation groups, *bulge* and *soba,* lie close to *argos.* The genes described here are candidates for *argos* receptor genes or may encode proteins that are somehow involved in the elaboration or perception of the *argos* signal.

MATERIALS AND METHODS

Reversion of the bul^D phenotype: Two screens were performed. Three- to 4-day-old bul^D/bul^D males were treated with 25 mM EMS according to ASHBURNER (1989). Mutagenized males were crossed against appropriate balancer flies and scored for suppression of the rough eye phenotype. Isogenized *st, e/st, e* males were mutagenized with 25 mM EMS and crossed against bul^D/bul^D flies. The progeny were scored for mutations that suppress the bul^D eye phenotype.

Excision mutagenesis: Mobilization of *P* elements often results in imprecise excision, leading to small deletions of DNA sequences flanking the insertion site (DANIELS *et al.* 1985). To induce new small deficiencies around the *G105 P*-element insertion, *G105/Ki* p^p $\Delta 2-3$ males were crossed to appropriate balancer flies (ROBERTSON *et al.* 1988). Independent reversion events were selected on the basis of the loss of the *white* eye color marker carried by the *P* element. Homozygous lethal lines were crossed to the flies carrying the alleles $73Aa^{13}$, $73Ab^3$, $argos^{\Delta 7}$ or *scarlet* (LINDSLEY and ZIMM 1992). All crosses were performed on standard fly food at 25°.

Local hopping: To isolate *P*-element induced lethal *bulge* alleles, we performed a series of local hopping experiments (TOWER *et al.* 1993; ZHANG and SPRADLING 1993) starting from the *W11 P*-element insertion in the *argos* gene (FREEMAN *et al.* 1992). *W11/*$\Delta 2-3$ flies were crossed to flies carrying the amorphic $argos^{\Delta 7}$ allele and progeny were scored for viable, red eye (P[*white*] positive) flies. Among 25,000 chromosomes screened, we could identify 26 new third chromosomal insertion events. Twenty-five of these were lethal over the deficiencies of the 73A region and turned out to be insertions in the *73Aa* gene. The flanking genomic DNA sequences of 18 of these *P* elements were cloned by plasmid rescue and all map into a small genomic interval from −5 to −2 between *argos* and *scarlet.* One lethal *P*-element insertion line designated *17.1* was viable over the deficiencies *Df(3L)33f1* and *Df(3L)stf13* but nevertheless mapped within the genomic region uncovered by these deficiencies. We could revert the lethality associated with this *P* element by excision and thus have to conclude that the lethality associated with the *17.1* insertion is *P*-element dependent. The reason for the unexpected viability in *trans* to deficiencies of the region is unknown. Starting from the *17.1* insertion, we generated a second round of local jumps but failed to obtain any lethal *bulge* alleles.

Mitotic recombination: $bulge^{6d7}$, $bulge^{28a1}$, $soba^1$ and $soba^2$ were placed in *trans* to the homozygous viable *P*-element insertion *G105.* First instar larvae were irradiated with 1000 rad at 25 mA and 115 kV. A cell clone homozygous for the *G105* P[*white, lacZ*] enhancer trap insertion revealed by a darker red eye color was observed in a frequency of ~1/200 flies. No twin clone (homozygous mutant *bulge* or *soba* cells), identifiable by a white eye color, was found among the 25–30 clones analyzed for each allele.

DNA methods: All DNA work was carried out according to standard procedures (SAMBROOK *et al.* 1989). To determine the breakpoint of *Df(3L)st7,* genomic DNA was isolated as described (SCHOLZ *et al.* 1993). Southern blots were hybridized with cloned genomic DNA fragments of the 73A region (BUTLER *et al.* 1986; MCKEOWN *et al.* 1987) according to CHURCH and GILBERT (1984). To determine the integration site of the *P* element, genomic DNA sequences flanking the different *P*-insertions were isolated by standard plasmid rescue techniques (BIER *et al.* 1989; WILSON *et al.* 1989) and mapped by restriction site mapping and hybridization analysis.

Antibody staining: CNS axons were visualized with the monoclonal antibody BP102 (kindly provided by N. PATEL and C. S. GOODMAN). Staging of embryos was according to CAMPOS-ORTEGA and HARTENSTEIN (1985). Antibody staining and CNS dissections were performed as described previously (KLÄMBT *et al.* 1991).

Histology: For scanning electron microscopy, heads were dehydrated through an ethanol series and by subsequent critical point drying. Heads were mounted and sputter-coated with a 200 Å thick gold coat and viewed on a Hitachi S520 scanning EM. For semithin eye sections, Drosophila heads of the desired genotype were split with a sharp razor blade and simultaneously fixed with glutardialdehyde and OsO_4 (FRANKE *et al.* 1969). After dehydration the heads were embedded in Araldite and 1-μm sections were viewed under a Zeiss Axiophot. CoS staining of pupal retinas was performed according to MELAMED and TRUJILLO-CENOZ (1975). Dissected pupal retinas were mounted in 70% glycerol and viewed under a Zeiss Axiophot.

RESULTS

***argos*:** The gene *argos* (synonyms are *giant lens* or *strawberry*) was identified on the basis of the eye phenotype caused by several enhancer trap *P*-element insertions (FREEMAN *et al.* 1992; KRETSCHMAR *et al.* 1992; OKANO *et al.* 1992). Hypomorphic *argos* alleles are characterized by a rough eye phenotype (see Figure 3B) and disruptions of the lamina. *P*-element excision mutagenesis resulted in the isolation of the $argos^{\Delta 7}$ mutation, which removes the first exon encoding the putative ATG start codon and the signal sequence. Based on its molecular characterization, this allele has been classified as an amorphic mutation (FREEMAN *et al.* 1992). Homozygous $argos^{\Delta 7}$ embryos are embryonic lethal but show no gross developmental abnormalities. *argos* maps to the genetically well-analyzed region 73A3–4 on the left arm of chromosome *III* in the vicinity of *scarlet* (BELOTE *et al.* 1990). The four *argos* exons extend over ~15 kb of genomic DNA (Figure 1). To define the proximal genomic boundary of *argos,* we performed a deletion analysis. Screening a collection of enhancer trap lines (C. KLÄMBT, unpublished data) for *argos*-like β-galactosidase expression patterns, we have identified the homozygous viable P[w^+, *lacZ*] insertion line, *G105.* The strain *G105* carries a *P*-element insertion located ~500 bp upstream of the first *argos* exon at position −6 (Figure 1) and was used to generate new transposase-induced mutations, which are generally small deletions (DANIELS *et al.* 1985) (see MATERIALS AND METHODS for details). Out of 200 independent excision events, seven were lethal in homozygotes. One of these, the deficiency *Df(3L)G105*Δ*VII* removes the gene *scarlet* as well as the previously described complementation groups *73Aa* and *73Ab.* The

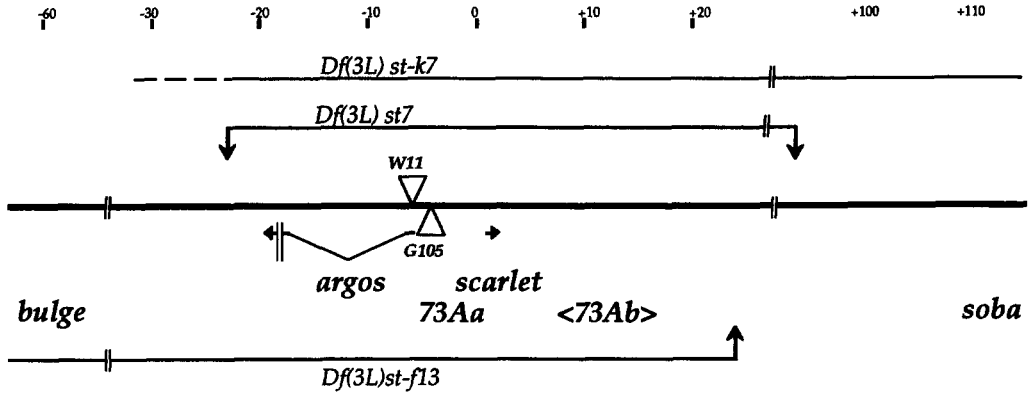

FIGURE 1.—Genomic organization of the argos region. Schematic summary of the genomic region surrounding the genes *argos* and *scarlet* at cytological position 73A. The scale above the map is given in kb from a inversion breakpoint in the *scarlet* transcription unit. The four exons of *argos* span the region from −6 to −19. The orientation of transcription is indicated by arrows. The extent of three deficiencies is indicated by thin lines. The proximal breakpoint of *Df(3L)st-f13* has been mapped to position +25, the proximal position is in 72C/D. *Df(3L)st7* removes DNA sequences in 73A from map position −25 to +90. *Df(3L)st-k7* removes sequences from 73A2 to 74E1–2, it does not affect the gene *bulge*. The positions of two P[*white*$^+$, *lacZ*] enhancer trap insertions, *argos*^{W11} and *G105* are indicated by triangles. The gene *bulge* maps 0.15-cM distal to *argos*, which according to POECK *et al.* (1993) corresponds 30–60 kb of genomic sequences. The complementation group *73Aa* is located between *argos* and *scarlet*, the exact position of the complementation group *73Ab* indicated in brackets is presently unknown.

excision line *G105ΔVI* uncovers only the complementation group *73Aa* (see Table 1). Both *G105ΔVI* and *Df(3L)G105ΔVII* complement the lethality associated with *argos*$^{\Delta 7}$. In addition, we found that the *argos*^{gilΔ4} allele, which carries a small deletion spanning from −9 to −4.5 (KRETSCHMAR *et al.* 1992), fails to complement mutations in *73Aa* as well as *argos* mutations but complements *scarlet*. This places the complementation group *73Aa* between *argos* and *scarlet* and also sets the right boundary of *argos* immediately proximal to the first exon (Figure 1). The chromosomal order of *73Ab* and *scarlet* cannot be deduced from these studies.

Genetic analysis of *argos*: To test whether the *argos*$^{\Delta 7}$ allele leads to a complete loss of *argos* function, we placed *argos*$^{\Delta 7}$ in *trans* to various deficiencies in the 73A region and analyzed the resulting phenotypes (see Figure 2). Embryos carrying *argos*$^{\Delta 7}$ in *trans* to *Df(3L)stg18*, which removes the entire 73A region plus adjacent chromosomal regions (see Table 1 for details), die before hatching and, like homozygous *argos*$^{\Delta 7}$ embryos, do not develop any obviously abnormal CNS phenotype. The embryonic lethal phenotype of homozygous *argos*$^{\Delta 7}$ embryos is very similar to that of *argos*$^{\Delta 7}$/*Df(3L)stg18* embryos, which is in agreement with the notion that *argos*$^{\Delta 7}$ represents an amorphic allele. However, when *argos*$^{\Delta 7}$ is placed in *trans* to smaller deficiencies that remove *argos* together with genomic sequences to the left or to the right of *argos*, rare (1–5% of the expected number) transheterozygous escapers eclosed (*e.g.*, *Df(3L)st-k7*, which removes DNA sequences in 73A2–4 to 74E1–2, or *Df(3L)stf13*, which removes sequences from 72C to 73A map position +25; see Figures 1, 2 and 3C. Thus, despite the fact that both copies of the *argos* gene have been removed, transheterozygous escapers eclose and develop a characteristic eye phenotype that appears stronger than the one associated with hypomorphic *argos* alleles (Figure 3, B and C). We observe a characteristic bulging of the posterior part of the eye that spreads into the anterior part of the eye. It appears unlikely that second site lethal mutations on the *argos*$^{\Delta 7}$ chromosome are the cause for this observations, because the independently isolated amorphic *argos*^{styp2} allele behaved similarly in *trans* to various deficiencies (OKANO *et al.* 1992). Thus, embryonic lethality caused by the loss of *argos* function can be rescued by the concomitant removal of a single copy of genomic DNA sequences lying either to the left or to the right of the *argos* gene. Similar observations have been made for mutations in the *enabled* gene that act as dominant suppressors of the lethality associated with the deletion of the *abl* proto-oncogene (GERTLER *et al.* 1990).

Based on these results, we postulate that at least two interacting genes, named *X* and *Z*, lie on either side of *argos* (see Figure 2). *argos* appears to counteract their function because two doses of *X* and *Z* are lethal in its absence. Reduction of the dosage of *X* or *Z* can rescue the lethality associated with amorphic *argos* alleles, and the surviving adults show a strong *argos*-like eye phenotype. The simultaneous heterozygous removal of *X* and *Z* concomitantly with removal of both copies of *argos* is lethal.

Isolation of genes that interact with *argos*: Hypomorphic *argos* alleles lead to a characteristic eye phenotype and a weak wing venation phenotype. The posterior part of the eye bulges and no regular ommatidial arrays are found. To identify genes that interact with *argos* and thus perhaps components that function in the same genetic pathway, we first focused on existing mutations that produce a similar eye phenotype. Three such mutations listed by LINDSLEY and ZIMM (1992) are *bulge*^{1},

TABLE 1

Alleles and deficiencies used in this study

Original name	New designation	Origin	Genetics	References[a]
bul^{1}	*aos*^{bul}	Spontaneous	*aos*$^{-}$	10
bul^{bp}	*aos*^{bp}	EMS	*aos*$^{-}$	5
bul^{D}		EMS	Viable gain of function	6
argos^{W11}		*P*-element insertion	Viable hypomorph	4
argos$^{\Delta 7}$		*P*-element excision	*aos*$^{-}$	4
argos^{Z}		EMS	*aos*$^{-}$	3
giant lens$^{\Delta 4}$	*Df(3L)gilΔ4*	*P*-element excision	*aos*$^{-}$, *l(3)73Aa*$^{-}$	8
l(3)73Aa^{13}		EMS	*(3)73Aa*$^{-}$	2
l(3)73Aa^{1}		EMS	*(3)73Aa*$^{-}$	2
l(3)73Aa^{6}		EMS	*(3)73Aa*$^{-}$	2
l(3)73Ab^{3}		EMS	*(3)73Ab*$^{-}$	2
Su(bul^{D}*)6d7*	*bul*^{6d7}	EMS	*bul*$^{-}$	11
Su(bul^{D}*) 23a1*	*bul*^{23a1}	EMS	*bul*$^{-}$	11
Su(bul^{D}*) 28al*	*bul*^{28a1}	EMS	*bul*$^{-}$	11
Su(bul^{D}*) 29f*	*bul*^{29f}	EMS	*bul*$^{-}$, viable	11
Su(bul^{D}*) 33f1*	*Df(3L)33f1*	EMS	*bul*$^{-}$*-l(3)73Aj*$^{-}$ *Abl*$^{+}$	11
Su(bul^{D}*) 4a1*	*clown*^{1}	EMS	*clown*$^{-}$	11
Su(bul^{D}*) 5al*	*soba*^{3}	EMS	*l(3)73Aj*$^{-}$	11
l(3)73Aj^{1}	*soba*^{1}	X-ray	*l(3)73Aj*$^{-}$	7
l(3)73Aj^{2}	*soba*^{2}	EMS	*l(3)73Aj*$^{-}$	7
Df(3L)st-f13		X-ray	72C/D-73A4(+25)	1
Df(3L)st7		γ-ray	−25 to +90	9, 11
Df(3L)st-k7		X-ray	*bul*$^{+}$73A2-4-74E1	7
Df(3L)st-g18		X-ray	72E3-74F4	1
G105		*P*-element insertion		11
G105ΔVI	*l(3)73Aa*$^{\Delta VI}$	*P*-element excision	*l(3)73Aa*$^{-}$	11
G105ΔVII	*Df(3L)G105 ΔVII*	*P*-element excision	*l(3)73Aa*$^{-}$*l(3)73Ab*$^{-}$ *st*$^{-}$	11

[a] References are as follows: 1, BELOTE and MCKEOWN (1985); 2, BELOTE *et al.* (1990); 3, E. BRUNNER, unpublished data; 4, FREEMAN *et al.* (1992); 5, GRELL (1955); 6, LINDSLEY and ZIMM (1992); 7, HENKEMEYER *et al.* (1987); 8, KRETZSCHMAR *et al.* (1992); 9, MCKEOWN *et al.* (1987); 10, SPENCER (1937); 11, This study.

bulge^{bp} and *bulge*^{D}. *bulge*^{1} has been placed to the cytological position 72E4–5 in the vicinity of *argos* (VELISSARIOU and ASHBURNER 1981).

In our hands *bul*^{1}/*bul*^{1} flies are viable and show only a weak rough eye phenotype and homozygous *bul*^{bp} flies are lethal. *bul*^{1}/*bul*^{bp} flies are viable and show a more extreme eye phenotype. The amorphic *argos*$^{\Delta 7}$ allele enhances the rough eye phenotype of *bul*^{1}. In addition *bul*^{bp} is semilethal when placed in *trans* to the *argos*$^{\Delta 7}$ allele and the surviving *bul*^{bp}/*argos*$^{\Delta 7}$ adults show an eye phenotype somewhat more severe as seen in homozygous *argos* hypomorphs (see Table 2). Hence, both *bul*^{1} and *bul*^{bp} behave in these complementation tests like alleles of *argos* and thus the published cytological map position of either gene is likely to be incorrect. Taken together, these data suggest that *bul*^{1} and *bul*^{bp} are alleles of *argos* and we refer to them as *aos*^{bul} and *aos*^{bp} in the following.

The third mutation, *bul*^{D} is associated with a dominant rough eye phenotype (E. H. GRELL, cited in LINDSLEY and ZIMM 1992), which becomes more extreme in the homozygous condition (Figure 4, A and B). *bul*^{D}/*bul*^{D} flies are viable and fertile but flightless. The dominant eye phenotype associated with *bul*^{D} is partially suppressed by *argos*$^{\Delta 7}$, *bul*^{1} or *bul*^{bp} and by deficiencies that remove *argos* and sequences proximal to it (*e.g., Df(3L) st-k7* or Df*(3L) st7*; Figure 1). However, larger deficiencies that delete *argos* and sequences distal to it completely suppress the rough eye phenotype of *bul*^{D}.

Screen for modifiers of *bul*^{D}: To clarify the genetic relationship between *bul*^{D} and *argos* alleles, we mutagenized *bul*^{D} homozygotes as well as *st, e/st, e* flies and screened for mutations that revert or otherwise modify the rough eye phenotype. Knowing that mutations in *argos* lead only to a partial suppression of the *bul*^{D} eye phenotype, we scored not only for wild-type revertants but also for intermediate phenotypes. Screening through ~100,000 flies, we isolated 15 mutations, six of which cause complete and five incomplete reversion of the *bul*^{D} eye phenotype.

***bulge*:** Four lethal *bulge*^{D} revertants (*6d7, 23a1, 28a1, 33f1*) define one complementation group. In *trans* to *bul*^{D}, all completely suppress the rough eye phenotype. In addition, we have isolated the homozygous viable

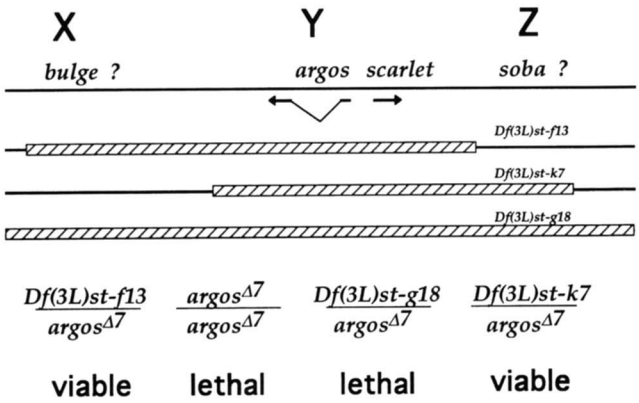

FIGURE 2.—Genes interacting with *argos* reside in the 73A region. Summary of the complementation behaviour of *argos* in *trans* combinations with various deficiencies. The small deletion found in $argos^{\Delta 7}$ that removes the first *argos* exon is lethal in homozygosis and in *trans* to large deficiencies as *Df(3L)st-g18*. However, when placed in *trans* to smaller deficiencies like *Df(3L)st-f13* or *Df(3L)st-k7*, transheterozygous escapers eclose, which show an enhanced *argos*-like eye phenotype (see Figure 3C). Based on these observations, we postulate that interacting genes reside on each side of *argos*. These loci are able to suppress the lethality associated with *argos* amorphs in a dose-dependent manner.

and fertile $bulge^{D}$ reversion allele *29f* that in *trans* to bul^{D} also completely suppresses the rough eye phenotype. Because we were unable to separate *6d7* as well as *29f* from the bul^{D} locus by mitotic recombination (10,000 flies scored for each allele), we suggest these newly isolated mutations are allelic to bul^{D}. Meiotic mapping placed bul^{D} 0.15 cM distant to the gene *scarlet*. Additional deficiency mapping placed the lethal *bulge* revertants distal to *scarlet*. *Df(3L) st7* complements the lethal *bulge* revertants *6d7*, *23a1* and *28a1*. The distal breakpoint of this deficiency has been molecularly mapped to position −25 kb, suggesting that *bulge* has to reside ≥25 kb distal to *scarlet*. Further complementation analysis of the lethal bul^{D} revertants showed that all except *33f1* complement all *argos* alleles, including aos^{bul} and aos^{hp}. Hence, the alleles bul^{D}, *6d7*, *23a1*, *28a1* and *29f* define a new locus residing distal to *aos*, for which we propose to retain the designation *bulge*. The bul^{33f1} chromosome was subsequently shown to carry a deletion that extends from *bulge* to the *73Aj* locus.

***bulge* and *argos* interact in a reciprocal manner:** *argos* and *bulge* are closely linked interacting genes. Amorphic *argos* alleles are able to suppress the dominant eye phenotype of bul^{D}. To date, no dominant *argos* allele is available; however, recent work has described the effect obtained after overexpression of *argos* RNA during development (FREEMAN 1994; SAWAMOTO *et al.* 1994). The transformant line *hs-argos4* made by SAWAMOTO *et al.* (1994) is especially interesting. Flies from this line produce high levels of *argos* mRNA even at 25° and are associated with a fully penetrant rough eye phenotype (SAWAMOTO *et al.* 1994) (Figure 5A). This phenotypic trait is suppressed by amorphic *argos* mutations (SAWAMOTO *et al.* 1994) (Figure 5C). The same suppression is achieved by all lethal *bulge* alleles, whereas bul^{D} enhances the *hs-argos* mediated rough eye phenotype (Figure 5B). The close reciprocal interaction of *bulge* and *argos* and its chromosomal location distal to *argos* make *bulge* a candidate for the gene *X* that we had postulated to explain the results obtained from our initial deficiency analysis (Figure 2).

Phenotypic analysis of *bulge*: The lethal *bulge* alleles *6d7*, *23a1* and *28a1* lead to lethality at the end of embryogenesis, and no abnormalities in the cuticle phenotype could be detected. In the CNS we observed subtle perturbations in the structure of the axonal scaffold. Using specific enhancer trap glia marker lines *AA142* and *rC56* (KLÄMBT and GOODMAN 1991; MENNE and KLÄMBT 1994), we analyzed the organization of the embryonic CNS glial cells in *bulge* embryos. In wild type a regular pattern of individual glial cells can be seen. The segment boundary cells (Figure 6A, arrow heads) mark

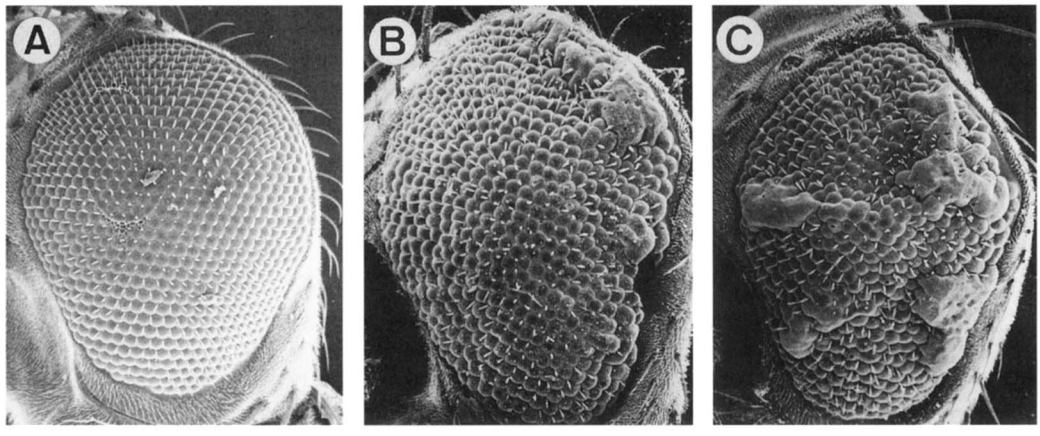

FIGURE 3.—Eye phenotype of *argos*. Scanning electron microscopy of compound eyes from (A) a wild-type fly, (B) a fly homozygous for the hypomorphic allele $argos^{W11}$ and (C) a fly with the genotype $argos^{\Delta 7}/Df(3L)st\text{-}k7$. The characteristic bulging of the posterior portion of the eye can be observed in *argos* flies. In addition to the fusion of ommatidial lenses, frequent bristle duplications can be seen.

TABLE 2

Complementation analysis of *argos* and *bulge* alleles

	aos^{W11}	$aos^{\Delta 7}$	aos^{Z}	bul^{1}	bul^{bp}	bul^{D}	bul^{6d7}	*wt*
aos^{W11}	4	4	4	2	4	1	0	0
$aos^{\Delta 7}$		Lethal	5	2	5	1	0	0
aos^{Z}			5	2	5	1	0	0
bul^{1}				2	2	1	0	0
bul^{bp}					5	1	0	0
bul^{D}						3	0	2–3
bul^{6d7}						Lethal	0	1

Numbers indicate different eye phenotypes: 0, wild-type eye phenotype; 1, weak rough eye phenotype; 2, mild roughening of the adult eye; 3, rough eye phenotype with occasional fusion of lenses; 4, severe rough eye phenotype. Lenses in the posterior region of the eye are fused and the eye appears bulged; 5, semilethal combination. Transheterozygous escapers eclose at reduced frequency (1–5%) and show a dramatic bulging of the eye. Anterior as well as posterior parts of the eye are affected. wt, *Canton S* wild type.

the positions where the intersegmental nerve roots leave the longitudinal connectives. The A and B glia cells labeled by an "x" in Figure 6A are found at stereotypic positions near the segmental commissures. In mutant *bulge* embryos the CNS glial cells appeared to be somewhat less organized when compared to wild type (Figure 6, A and B). Some segments have a reduced number of glial cells, whereas other segments appear to be less affected. A similar phenotype is observed for the exit glial cells; however, no phenotype was observed for the midline glial cells.

The SEM analysis of the eye phenotype of homozygous bul^{D} flies reveals occasional fusion of ommatidial lenses and irregularities in bristle spacing (Figure 4A). Tangential sections through eyes of these flies show a normal number of photoreceptor cells per ommatidial unit but an irregular arrangement of the individual ommatidial clusters (Figure 6, compare C with D). In addition, pigment cells separating the individual ommatidia are missing in ~10% of the ommatidial units. This phenotype is also evident from CoS staining of 60–70-hr-old pupal retinas (Figure 7, A–C). In wild type four lens cells are surrounded by two primary pigment cells and a lattice of secondary and tertiary pigment cells (Figure 7A). In homozygous bul^{D} flies one of the two primary pigment cells is missing in many of the ommatidia (asterisks in Figure 7, B and C). When primary as well as secondary pigment cells are missing, the lens cells of two ommatidial units form a joined structure (Figure 7C). Occasionally, both primary pigment cells are absent (Figure 7C, arrow). In rare cases individual lens cells are also missing (Figure 7C, arrow head). The $bulge^{D}$ eye phenotype is thus reminiscent to the one observed after overexpression of *argos* (FREEMAN 1994; SAWAMOTO *et al.* 1994). To analyze further the function of bulge during development of the compound eye, we generated homozygous mutant cell clones in otherwise heterozygous animals. Because we were unable to recover homozygous mutant cell clones in the compound eye induced by mitotic recombination, *bulge* appears to be cell lethal during eye development.

Second site modifiers of the bul^{D} phenotype: The alleles considered so far are all intragenic revertants of the bul^{D} eye phenotype and define the *bulge* locus. Second site modifiers of the bul^{D} phenotype could possibly reveal other genes acting in a common pathway if the underlying biochemical interactions of the respective gene products are dosage sensitive. Besides *argos*, which is itself a moderate suppressor of the rough eye phenotype associated with bul^{D} (Figure 4C), two other genes were identified in our screen.

***suppressor of bulge and argos* (*soba*):** The recessive lethal mutation *5a1* leads to a similar suppression of the bul^{D} phenotype as do mutations in the *argos* gene (Figure 8C). In addition, *5a1* also suppresses the flightless phenotype associated with homozygous $bulge^{D}$ flies. *5a1* interacts not only with *bulge* but also with *argos* and suppresses the eye phenotype associated with *hs-argos* flies (Figure 8D). Because the eye phenotypes caused by excess *argos* function and those associated with bul^{D} are suppressed equally well, we named the gene identified by the allele *5a1 soba* (*suppressor of bulge and argos*). Interestingly, *soba* is lethal over deficiencies of the 73A region and could be assigned by complementation analysis to the previously described complementation group *73Aj*, which is flanked by the genes *l(3)73Ai* and *abl* (HENKEMEYER *et al.* 1987; MCKEOWN *et al.* 1987; BELOTE *et al.* 1990; SAVILLE and BELOTE 1993). The *73Aj* alleles $73Aj^{1}$ and $73Aj^{2}$ also suppress the bul^{D} eye phenotype (data not shown) and were renamed as $soba^{1}$ and $soba^{2}$. Based on its proximal position the gene *soba* might correspond to the postulated gene *Z* (Figure 2); however, *soba* is not included in *Df(3L)st7*, which also rescues lethality associated with loss of *argos* function (see DISCUSSION). *soba* is a recessive lethal with a lethal period during early larval development. No cuticle abnormalities nor any abnormalities in CNS axon pattern could be detected in homozygous *soba* embryos. We were unable to induce mutant cell clones in the compound eye by mitotic recombination, which is compatible with the notion that *soba* is cell lethal.

***clown* (*cln*):** One additional second site modifier lo-

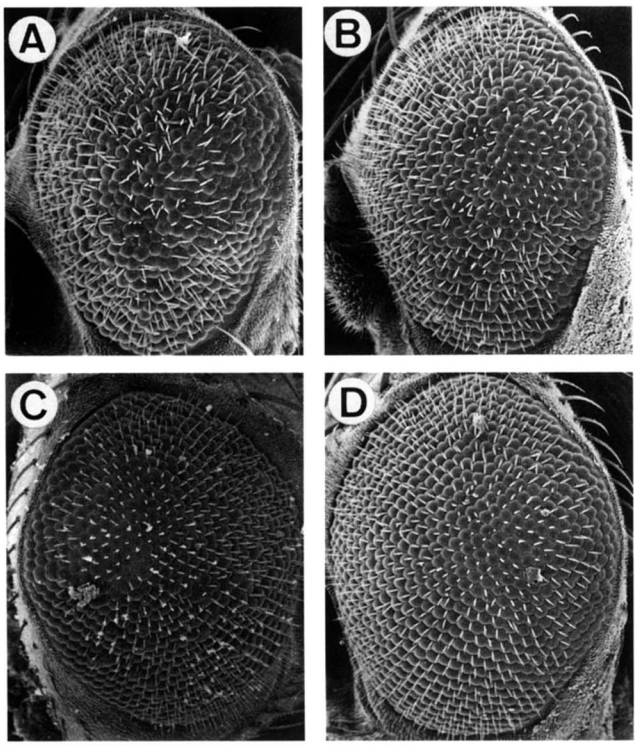

FIGURE 4.—The eye phenotype of $bulge^D$ is suppressed by *argos* and by $bulge^D$ revertants. Scanning electron microscopy of compound eyes from a homozygous (A) and a heterozygous (B) $bulge^D$ fly. The rough eye phenotype is more severe in homozygous bul^D flies. When bul^D is placed in *trans* to amorphic *argos* alleles, the rough eye phenotype is considerably reduced. (C) Eye from an $argos^{\Delta 7}/bul^D$ fly. When bul^D is in *trans* to $bulge^D$ revertants, the rough eye phenotype is completely suppressed. (D) Eye of a bul^{6d7}/bul^D fly.

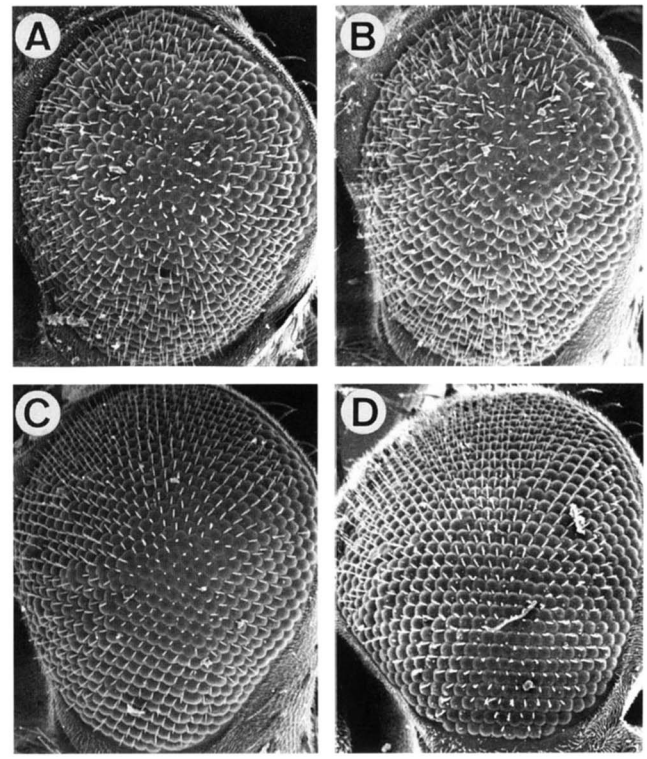

FIGURE 5.—The rough eye phenotype caused by *hs-argos* is suppressed by *argos* and $bulge^D$ revertants. Flies carrying the *hs-argos* construct #4 develop a rough eye phenotype at 25° (A). In *trans* to $argos^{\Delta 7}$ this rough eye phenotype is rescued (C). When *hs-argos* is in *trans* to bul^D revertants, the eye phenotype is rescued as well (D). Flies carrying the *hs-argos* construct together with one copy of the bul^D mutation develop a stronger rough eye phenotype than flies carrying either mutation alone (B).

cus of bul^D identified in our screen is defined by the mutation *4a1*. This mutation leads to complete suppression of both the bul^D eye and the flightless phenotype. Homozygous *4a1* flies are viable and fertile and show a characteristic eye phenotype (Figure 9). The overall morphology of the eye is impaired, with the dorsal compartment being affected most. This appears unpigmented and ommatidial lenses are partly fused. Histological sections show a complete collapse of the ommatidial units, and no pigment cells can be detected in the dorsal part of the eye, whereas some pigment cell are still present in the ventral part (Figure 9B). It is interesting to note that the cone cells are present and secrete the ommatidial lenses. The "white and red" eye phenotype led us to the name *clown* (*cln*). To analyze the development of the *cln* eye phenotype, we stained 60–70-hr-old homozygous *cln* pupal retina with CoS. The regular appearance of the four lens cells in a wild-type ommatidium (Figure 7A) is completely disrupted. We never observed *cln* ommatidia with normal number and shaped lens cells. Some ommatidia contain only two lens cells, whereas others contain up to five lens cells (Figure 7D, asterisks). In many cases the shape of lens cells is unusual and they rather look like pigment cells (Figure 7, D and E, arrow heads). In addition to the lens cell defects, the secondary and tertiary pigment cells are increased in number in mutant *clown* retinas. This aspect of the *cln* phenotype is reminiscent to the phenotype associated with hypomorphic *argos* alleles.

Like mutations in the gene *soba,* mutations in *clown* suppresses both the bul^D eye phenotype and the eye phenotype provoked by overexpression of *argos* (Figure 8). Meiotic mapping placed *clown* at cytological position 3–37.2; further deficiency mapping placed *clown* at 68C-D. Flies carrying the $clown^{4a1}$ allele in *trans* to deficiencies of this region develop a similar eye phenotype as homozygous *clown* flies.

The experiments described above revealed three new genes, *bulge, soba* and *clown,* which are all able to suppress the eye phenotype induced by overexpression of *argos* in a dosage-dependent manner. Furthermore, mutations in *clown, soba* and *argos* are also able to suppress the eye phenotype associated with the dominant *bulge* allele bul^D. *argos, bulge, soba* and *clown* thus represent four interacting genes, whose products might act in a common biochemical pathway.

DISCUSSION

We conducted a genetic analysis of the Drosophila gene *argos* that serves as a negative regulator of cell fate

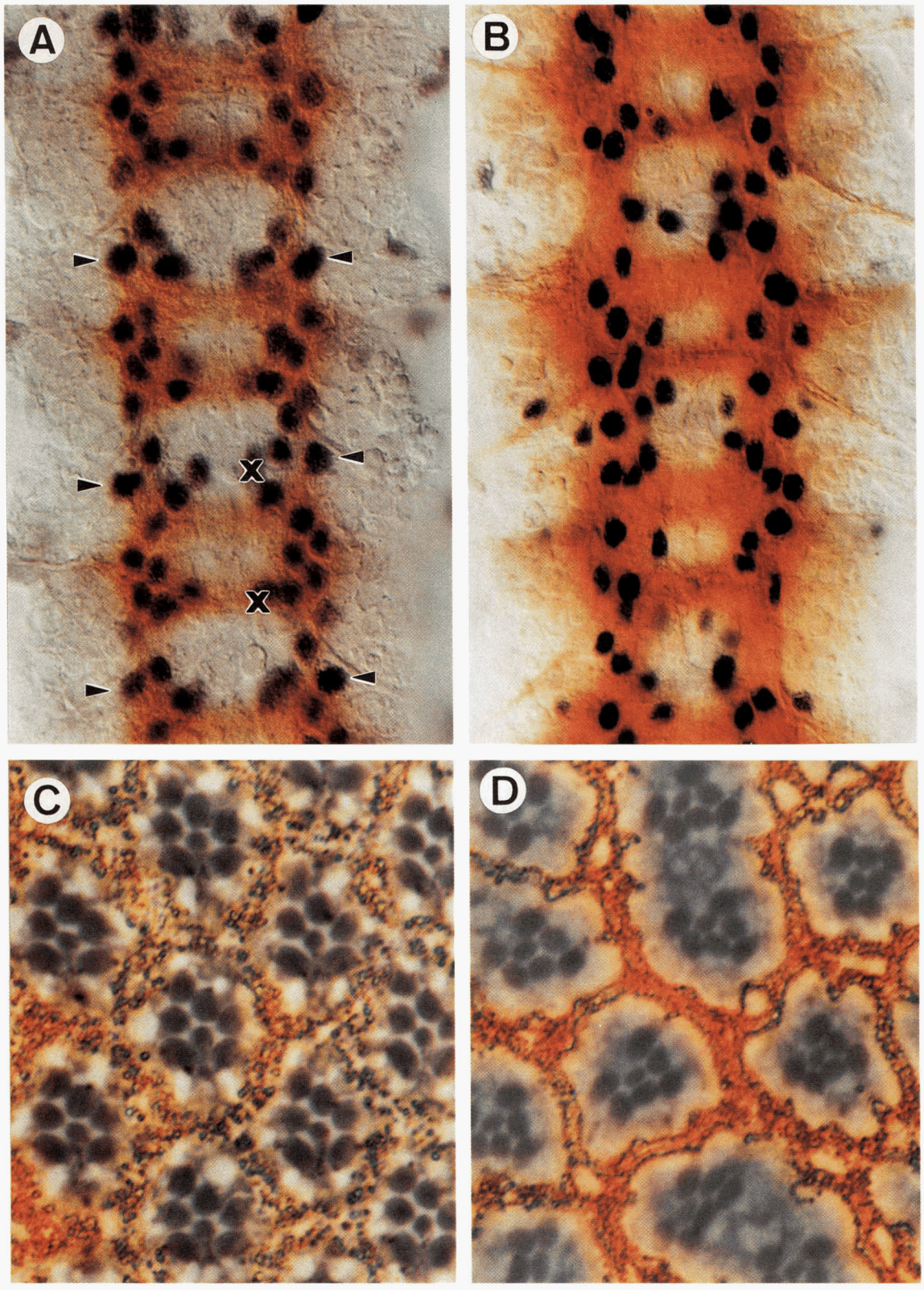

FIGURE 6.—Phenotypes of *bulge* mutants. (A and B) Frontal views of dissected embryonic CNS preparations stained for CNS axons using the monoclonal antibody BP102 (brown) and for β-galactosidase expression driven by the enhancer trap line rC56 (violet). Anterior is up. (A) In the CNS of embryos carrying the *rC56* enhancer trap, eight longitudinal glial cells as well as the two A and B glial cells (x) and the segment boudary cells (arrow head) express β-galactosidase. These glial cells are arranged in a regular and precise pattern. (B) In homozygous bul^{6d7} embryos this regular organization of glial cells is disrupted. (C and D) Tangential sections of adult eyes viewed with phase contrast optics. (C) In wild-type flies the ommatidial units contain six outer photoreceptor cells arranged in a characteristic trapezoidal pattern around the central photoreceptor cells R8 and R7. The orientation of the ommatidial units is highly regular. (D) Flies homozygous for the bul^{D} allele show an irregular orientation of the photoreceptor arrays. In addition, pigment cells are missing between 10 and 20% of the ommatidia.

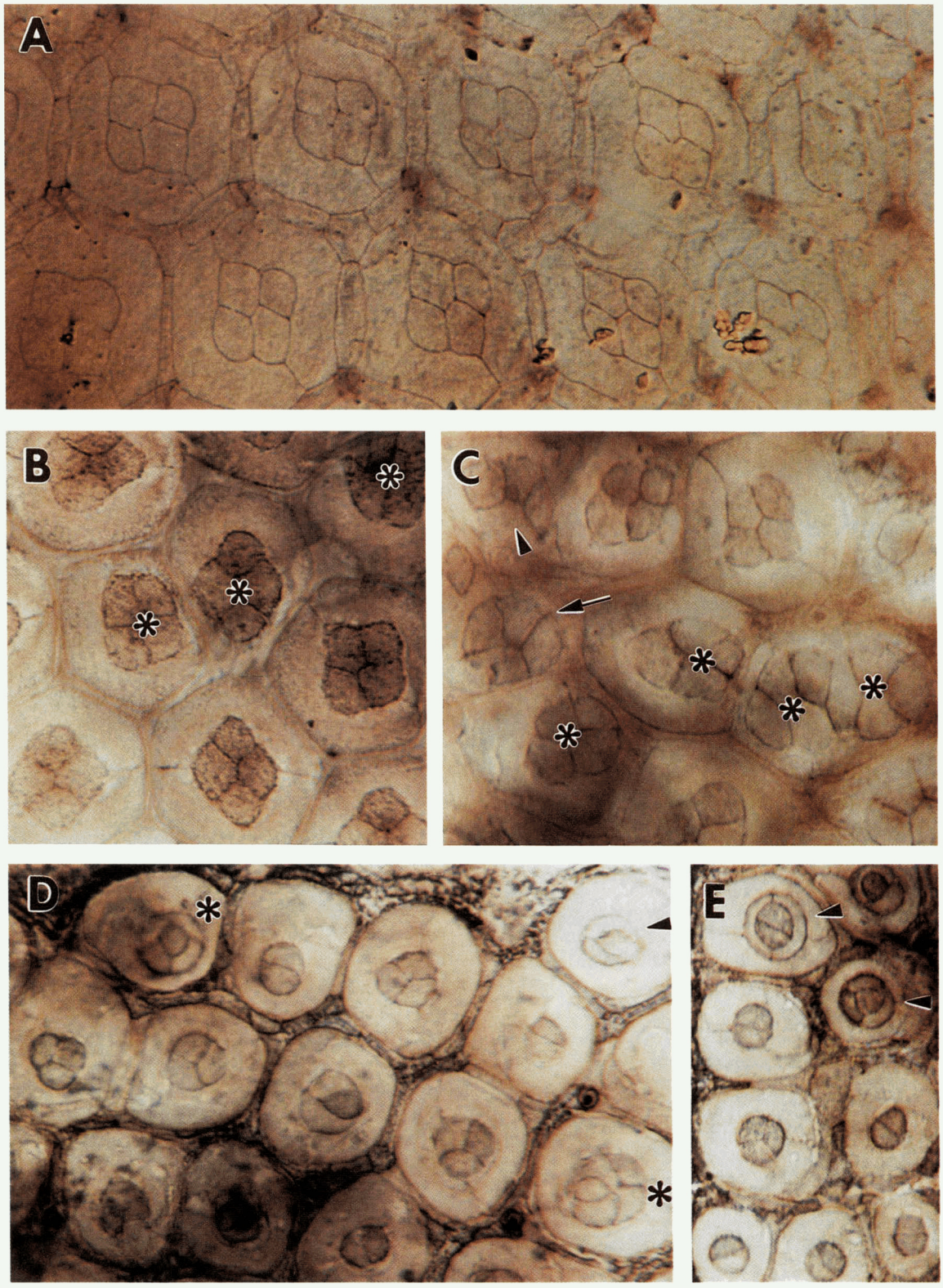

FIGURE 7.—Cobalt sulfide staining of wild-type, homozygous $bulge^{D}$ and homozygous *clown* pupal retinas. Retinas of 60–70-hr-old wild-type (A) homozygous bul^{D} (B and C) and homozygous *cln* (E and F) were dissected and stained with CoS to outline lens and pigment cells. (A) In wild type a regular pattern of four lens cells surrounded by primary and secondary pigment cells is seen. (B and C) In homozygous bul^{D} pupae most ommatidia show the typical organization of four cone cells; however, the orientation of the individual clusters is lost. In many ommatidia one primary pigment cell is missing (asterisk); rarely, a cone cell can be missing (C, arrow head) or a group of four cone cells is not surrounded by any primary pigment cells (C, arrow). (D and E) In homozygous *cln* pupae no ommatidium shows the typical organization of four cone cells. In many cases the number of lens cells appears reduced; however, some ommatidia contain up to five cone cells (D, asterisks). In several cases the cells in position of the lens cells develop a shape typical for primary pigment cells (D and E, arrow heads). The number of secondary and tertiary pigment cells appears increased in number.

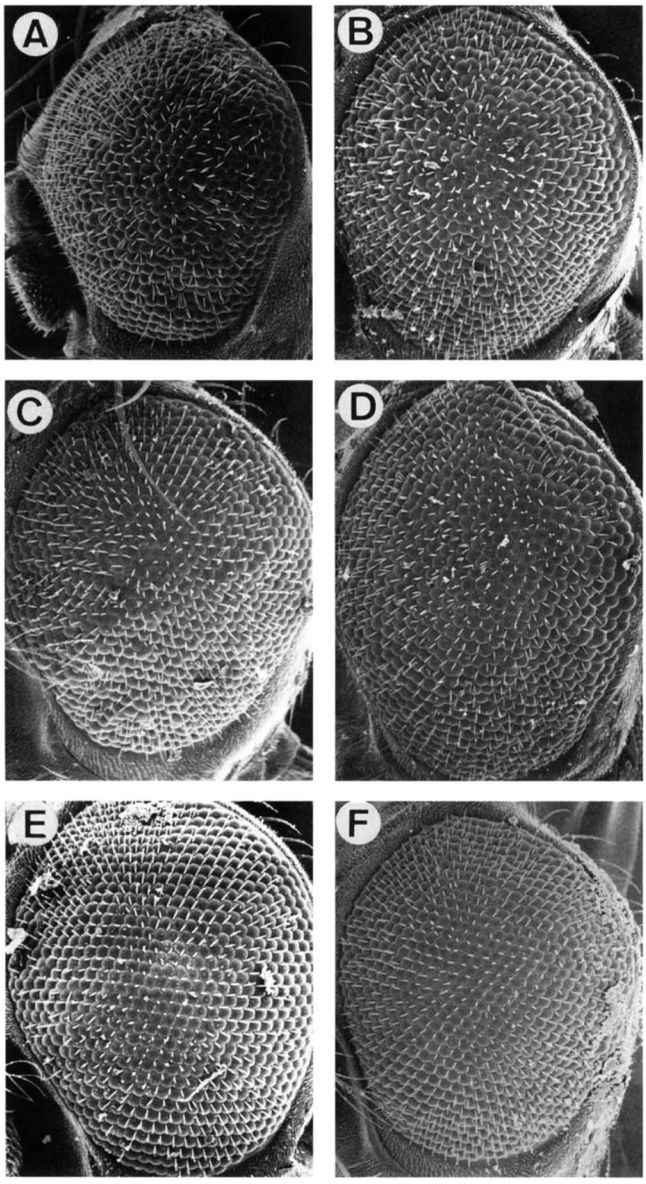

FIGURE 8.—Mutations in *clown* and *soba* suppress the eye phenotypes associated with bul^D and *hs-argos*. (A) Flies carrying one copy of the bul^D chromosome or (B) flies heterozygous for *hs-argos* #4 develop similar rough eye phenotypes. When $soba^{5a1}$ is placed in *trans* to bul^D (C) or *hs-argos* (D), the severity of the rough eye phenotype is reduced. When cln^{4a1} is in *trans* to bul^D (E) or *hs-argos* (F), the rough eye phenotype is completely suppressed.

decisions. Our data lead to a complex picture of the genetic organization of the 73A region, where *argos* resides. Here we describe mutations in three closely linked loci, *bulge, argos* and *soba,* which all show mutual genetic interactions during the development of the compound eye. In addition, we identified the locus *clown,* which interacts with *argos* as well as with *bulge.* Overexpression of *argos* by heat shock and the mutation $bulge^D$ lead to a similar eye phenotype. The possibility that the $bulge^D$ eye phenotype is due to a mutation in a regulatory domain of the *argos* gene leading to increased *argos* expression, however, appears unlikely. First, the suppression of the $bulge^D$ phenotype by the amorphic allele $argos^{\Delta 7}$ is only partial, but it is complete by all bul^D reversion alleles. Second, the viable reversion allele *29f* completely suppresses the eye phenotype associated with $bulge^D$. If this suppression would be due to a decreased *argos* transcription, an *argos*-like eye phenotype should be seen in homozygous *29f* flies, which, however, show no obvious abnormalities. Thus, *bulge* and *argos* appear as two distinct but closely interacting genes. It is interesting to note that the *lin-15* gene of *C. elegans,* which like *argos* acts as a negative regulator of intercellular signaling processes, is also organized in a complex manner. lin-15 activities are encoded by two distinct nonoverlapping transcripts (CLARK *et al.* 1994). The encoded proteins however are unrelated to *argos.*

Localization of the loci *73Aa* and *73Ab*: The genetics of the 73AD region surrounding the eye color locus *scarlet* has recently been studied in some detail (HENKEMEYER *et al.* 1987; BELOTE *et al.* 1990). The complementation groups *73Aa* and *73Ab* have been localized distal to *scarlet.* Alleles of the *73Ab* locus display genetic interaction with mutations in the sex-determining genes (D. ANDREW, personal communication). Furthermore, a transcript localized ~20 kb distal to *argos* has been shown to encode a doublesex cognate protein, making it likely that *73Ab* corresponds to this transcription unit (D. ANDREW, personal communication). This would place *argos* in between *73Ab* and *scarlet,* which, however, is not in agreement with the analysis of deficiencies we have generated starting from a *P*-element insertion just 0.5 kb proximal to the first *argos* exon. The overlap between two deficiencies *Df(3L)gilΔ4* and *Df(3L)G105ΔVII* places *73Aa* within 2 kb upstream of the *argos* gene. Because *Df(3L)G105ΔVII* affects the function of both *73Ab* and *scarlet,* we cannot deduce the absolute order of these two genes, but they must reside proximal to *argos* and *73Aa.* Interestingly, a *scarlet* mutation, st^{sp}, carries a 5.2-kb insert between −3.0 and −2.1 that does not affect either the complementation group *73Aa* or *73Ab* (TEARLE *et al.* 1989) and further restricts the genomic interval within which the complementation group *73Aa* must reside. So far, however, we have identified only middle repetitive DNA sequences in this area (T. WEMMER, unpublished data).

Closely linked interacting genes: We uncovered several mutual interacting genes in the immediate vicinity of *argos.* During the course of our analysis, we noticed that the available *bulge* mutations define two different genes. Whereas bul^1 and bul^{bp} behave genetically as *argos* alleles, bul^D defines a new complementation group. To avoid further confusion, we renamed the first two *bulge* alleles (bul^1 and bul^{bp}) as *argos* alleles and retain the name *bulge* for the newly identified lethal complementation group defined by lethal bul^D revertants. The analysis of the mutations introduced here suggests the presence of partially redundant gene functions in the *argos* region.

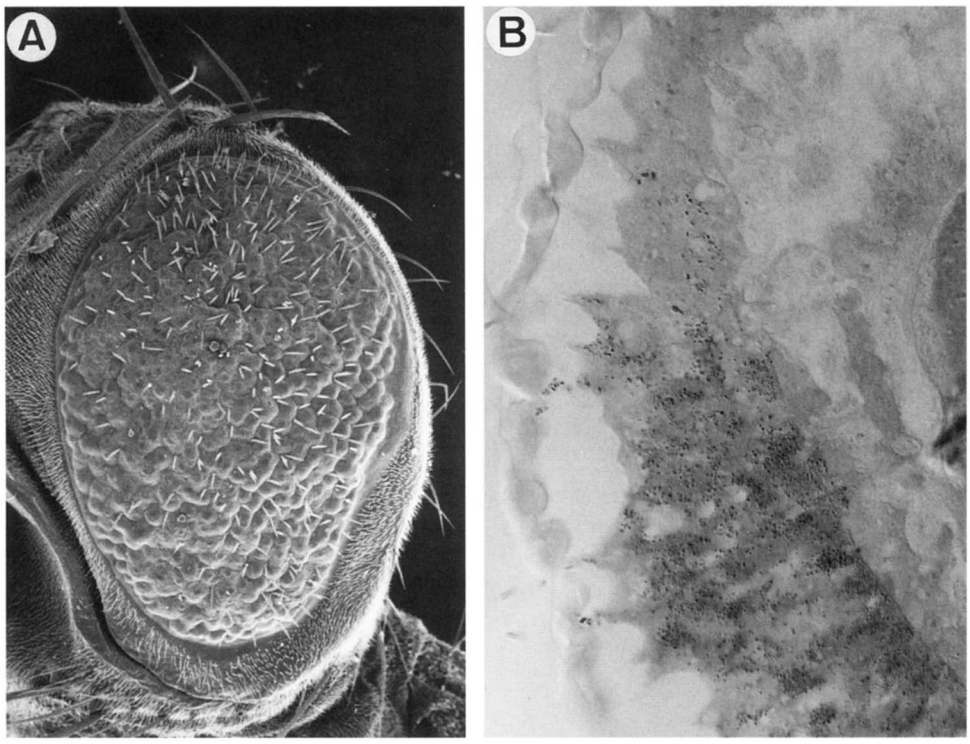

FIGURE 9.—The eye phenotype of *clown*. Homozygous *clown* flies are viable and show a characteristic rough eye phenotype (A). The ommatidial lenses are often fused, as is observed in flies homozygous for hypomorphic *argos* alleles. However, in *clown* homozygotes, fusion of lenses is observed over the entire eye surface. In addition, the arrangement of ommatidial bristles is irregular. These are often missing, but in some cases appear to be duplicated. Although lens structures are present throughout the eye, ommatidial organization is affected more strongly in the dorsal portion of the eye. Here no photoreceptor cells and no pigment cells are observed (B), resulting in the white eye color of the dorsal part of the eye.

In Drosophila redundant gene functions have been described in a number of cases. An example is seen in the *E(spl)-C*. This gene complex encodes seven related bHLH proteins that perform overlapping functions (KLÄMBT *et al.* 1989; DELIDAKIS and ARTAVANIS-TSAKONAS 1992; KNUST *et al.* 1992; SCHRONS *et al.* 1992). Furthermore, flanking the *E(spl)-C* resides the gene *groucho* that closely interacts with the bHLH proteins encoded by the *E(spl)-C* (SCHRONS *et al.* 1992; PAROUSH *et al.* 1994). Redundant gene functions have also been postulated based on the analysis of cell surface molecules like fasciclin I (ELKINS *et al.* 1990). Homozygous *fasciclin I* deficient embryos as well as homozygous *Abelson* tyrosin kinase (*abl*) deficient embryos are fully viable, but a dramatic embryonic CNS phenotype is seen in embryos homozygous for both mutations. Redundancy of gene functions in the *argos* region might also explain the lack of morphological abnormalities seen in the embryonic phenotypes associated with *argos* amorphs (FREEMAN *et al.* 1992). In agreement with this is the finding that deficiencies that remove all three of the above described genes do lead to embryonic CNS abnormalities (T. WEMMER and C. KLÄMBT unpublished).

The apparent complexity of the region is also evident from the discrepancy of our deficiency analysis and gene dose titration experiments. Loss-of-function *argos* mutations are late embryonic lethals. This lethality can be partially rescued by a hemizygous removal of genomic sequences distal or proximal to *argos*. Formally this indicates that *argos* antagonizes *X* or *Z* situated on either side of *argos*. Our genetic analysis has now revealed two closely interacting genes, *bulge* and *soba*, which as shown by deficiency analysis and molecular characterization of mutant RFLPs (T. WEMMER, unpublished results; HENKEMEYER *et al.* 1987), are indeed located to the right and the left of *argos*. These genes thus appear to be excellent candidates for the postulated loci *X* and *Z*. However, based on our deficiency analysis, these interacting genes are formally predicted to counteract *argos* function, whereas *bulge* and *soba* seem to act in the same direction as *argos*.

It is thus conceivable that deletions of control sequences surrounding the *argos* locus could lead to an increased transcription of the remaining gene copy. This is also supported by the fact that the lethality associated with loss of *argos* function is rescued by the deficiency *Df(3L) st7*, which deletes chromosomal DNA sequences from −25 to +90. This interval includes *argos* but not the genes *bulge* or *soba*. Gene regulation involving *trans*-sensing phenomena has been described in several other instances (*e.g.*, KORNHER *et al.* 1986; GEYER *et al.* 1990; SCHOLZ *et al.* 1993). A model using these arguments would predict that homozygous $argos^{\Delta 7}$ flies could be rescued to viability by overexpression of either *bulge* or *soba* cDNAs. An alternative would be that still additional loci interacting with *argos* should be found in the 73A region.

Perspectives: We now have four genes in hand, which based on their close reciprocal interaction, are likely to act in the *argos* pathway and might correspond to the *argos* receptor or be involved in the production or the interpretation of the *argos* signal. The results from the clonal analysis make it unlikely that *bulge* and *soba* encode *argos* like signaling molecules, because both

functions appear to be cell lethal. Preliminary molecular analysis has identified candidate transcripts for *bulge* and *soba* that are both expressed in the developing eye imaginal disc (T. WEMMER, unpublished results). Precise assignment of a function to these genes must await their further molecular characterization.

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