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Misexpression of the *Drosophila argos* gene, a secreted regulator of cell determination

Matthew Freeman

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

SUMMARY

I have examined the effects on cells in the developing eye of over-expressing the *argos* gene. Transgenic flies carrying *argos* expressed under *hsp70* and *sevenless* control sequences were analysed. All cell types in the developing eye (except bristles) are sensitive to argos concentration: over-expression leads to too few cells forming, the opposite phenotype to that seen in *argos* loss-of-function mutants. This effect was only seen with HS-argos flies: sev-argos flies, which over-express the protein at a lower level are not affected, suggesting that a considerable over-expression is required to disrupt cell fate. However, sev-argos is able to rescue *argos* eye mutations completely, indicating that the normal expression pattern is not critical for wild-type eye development. By transfecting *argos* into tissue culture cells, I show that the protein is secreted in a soluble form.

Key words: *argos*, *Drosophila*, eye development, secreted protein, determination, heat shock

INTRODUCTION

The interaction between cells plays an important part in the determination of cell fate. These interactions range from the communication between abutting cell membranes to long-range signalling mediated by diffusible factors. The *argos* gene (also known as *giant lens* and *strawberry*) encodes a protein that is needed for cell fate decisions in the *Drosophila* eye, and is believed to be diffusible (Freeman et al., 1992b; Kretzschmar et al., 1992; Okano et al., 1992). Although null mutations in *argos* are embryonic lethal, there is a viable class of loss-of-function mutations with an eye phenotype. These have supernumerary photoreceptor neurons, cone cells and pigment cells. Analysis of the loss-of-function phenotype of *argos* eye mutations previously led to the proposal that it acts somehow to regulate the inductive signals responsible for cell determination.

Since the embryonic phenotype of *argos* null mutations has proved difficult to interpret, I have concentrated on its effects in the eye. Eye development starts in the third instar larva, as the morphogenetic furrow crosses the eye imaginal disc: in the wake of the furrow, ommatidial clusters begin to form (Tomlinson, 1988; Ready, 1989; Banerjee and Zipursky, 1990). It is widely accepted that cells in the developing eye acquire their specific fates by a series of inductive interactions in which undetermined cells are sequentially recruited into the ommatidium (Tomlinson and Ready, 1987). Since supernumerary cells are recruited in *argos* loss-of-function mutations, cell fate determination may result from a balance of opposing influences; these influences being the inductive signals and a repressor system, of which *argos* forms a part.

argos is expressed broadly during eye development. The product appears weakly in all cells immediately behind the morphogenetic furrow, where the earliest stages of determination occur. Soon after, expression increases in cells which acquire a specific fate. Thus, it is first strongly expressed in photoreceptors, in the temporal order in which they are determined, and then in cone cells; later, in pupal development it is also expressed in primary pigment cells (Freeman et al., 1992b). Based on this expression pattern and the prediction that argos is secreted, it seems likely that most of the cells in the developing eye are exposed to argos protein early in their development.

If argos is acting as a repressor of inductive signalling, increasing its concentration might disrupt cell determination in the opposite direction from the loss-of-function *argos* mutations; that is, there might be loss of cells instead of the extra recruitment seen in mutants. Alternatively, since the expression level is quite high, and all developing cells in the eye appear to be exposed to the protein, it may already be above a threshold of effect in wild-type discs, and increasing the dose will then have no effect. To examine these possibilities, I have misexpressed the *argos* gene under two different heterologous promoters in transformed flies. All the cell types that are affected by loss-of-function mutations are also sensitive to over-expressed *argos*; in each case, too few cells form in the presence of elevated protein. I also show that the wild-type expression pattern of *argos* is not critical for its function: *argos* driven by the *sevenless* promoter/enhancer can effectively substitute for the normal pattern. Finally I provide direct evidence that argos is secreted from cells and that the secreted form is freely soluble once outside the cell.

MATERIALS AND METHODS

Fly strains and heat-shock conditions

Standard strains were as described by Lindsley and Zimm (1992). All flies were kept at 25°C, unless otherwise noted. Host embryos for transformation were of the genotype *cn;ry*.

HS-argos flies used in the experiments described had four copies of the HS-argos transposon (they were homozygous for inserts on the second and third chromosomes). To examine the phenotype of the misexpressed argos, these flies were heat shocked at 38°C in regimes described in the results. Pupae were staged by collecting white prepupae and ageing them at 25°C.

Ectopic expression constructs

The HS-argos construct was made by cloning a 306 nucleotide fragment of the *hsp70* gene promoter upstream of an *argos* minigene, which has the large first intron removed. The *hsp70* fragment corresponds to coordinates −189 to −495 of Fig. 4 of Ingolia et al. (1980), and includes the TATA box and the heat-shock element. The *argos* minigene was made by fusing an *Eco*RI to *Bam*HI fragment of a cDNA (corresponding to nucleotide positions 1435 to 2402 in Fig. 5 of Freeman et al., 1992b) to a 2.25 kb *Bam*HI to *Hin*dIII fragment from a genomic clone that includes the 3′ end of the gene. The resulting HS-argos fragment was cloned into pDM30, a plasmid containing P-element sequences and the *rosy* gene as a selectable marker (Mismer and Rubin, 1987).

Sev-argos was made by inserting the same *argos* minigene into a plasmid containing the *sevenless* promoter (–967 bp to +89 bp, Bowtell et al., 1988), giving a transcriptional fusion. The *sevenless* promoter/*argos* fragment was then cloned into a plasmid that carries three copies in tandem of a 700 bp *sevenless* enhancer fragment in pDM30 (Fortini et al., 1992). This has been shown to produce a higher level of expression in *sevenless*-expressing cells than previous enhancer fragments (R. Carthew, personal communication).

Both constructs were introduced into *Drosophila* by P-element-mediated transformation (Rubin and Spradling, 1982; Spradling and Rubin, 1982), using standard techniques. Several transformants were isolated for each transposon.

Histology

Scanning electron micrographs were carried out as described by Kimmel et al (1990). Cobalt sulphide and acridine orange staining was carried out as described by Wolff and Ready (1991). *Drosophila* heads were fixed and prepared for sectioning as described by Freeman et al. (1992b).

Anti-argos antibodies

Antisera were raised in mice and rabbits against a fusion protein produced under the T7 promoter in the vector pET3b (Rosenberg et al., 1987). An *Xho*I (at nucleotide 1674, Freeman et al., 1992b) to *Xmn*I (in the 3′ non-coding sequence) fragment of *argos* cDNA was cloned into the *Bam*HI site of pET3b. The resulting protein contains 13 amino acids derived from the vector fused to most of the *argos* open reading frame (lacking only the signal peptide and approximately seven N-terminal amino acids). This construct gave an IPTG inducible protein of approximately 55×10^3 M_r, the predicted size. The protein was purified as inclusion bodies by standard methods. Anti-argos monoclonal antibodies were made by standard techniques (Harlow and Lane, 1988).

argos expression in cell culture

The COS cell expression construct was made by cloning an *argos* cDNA, from the *Eco*RI site at nucleotide 1435 (Freeman et al., 1992b) to a synthetic *Xba*I site just 3′ to the stop codon into a vector containing an efficient COS cell promoter. This promoter is a hybrid of cytomegalovirus and human immunodeficiency virus promoters (Aruffo and Seed, 1987). The expression construct was transfected into COS cells using DEAE dextran in the presence of chloroquine (Luthman and Magnusson, 1983), and the cells were stained with a rabbit anti-argos serum. To look for secreted argos in the medium, the transfected cells were incubated for 24 hours in serum-free medium, and were then harvested and washed twice in PBS prior to solubilising in Laemmli buffer; cell medium was cleared of all insoluble material before adding sample buffer. Western blotting was performed by standard techniques (Harlow and Lane, 1988).

The *Drosophila* SL2 cell expression construct was made by cloning the same *Eco*RI to *Xba*I cDNA fragment into the *Eco*RI site of pUC-hsneoact (Thummel et al., 1988). This plasmid expresses the inserted gene from the *actin 5C* promoter. The construct was introduced into SL2 cells using calcium phosphate precipitation (Nocera and Dawid, 1983), and the cells were stained with rabbit anti-argos serum. I was unable to grow these cells on serum-free medium, and the 15% foetal calf serum in their medium disrupted western blots to look for secreted argos accumulation.

RESULTS

Ectopic *argos* driven by the *hsp70* promoter causes a disruption of eye development

To examine the effects on eye development of over-expressing the *argos* gene, I produced a hybrid gene in which *argos* coding sequences were placed under the control of the heat-shock gene, *hsp70*, promoter. This promoter has been shown to be inducible in most cells (Lis et al., 1983; Bonner et al., 1984), so flies with the HS-argos transposon produce ubiquitous high levels of argos upon heat shock.

These HS-argos flies were reared under a variety of heat-shock regimes. Any significant degree of heat shock during the third instar larval period, when most photoreceptor and cone cell determination occurs, is lethal; the larvae die before pupariation. Because of this lethality, the following analysis of HS-argos is restricted to its effects on eye development that occurs during pupal stages. Heat shocks during pupal stages are not fully lethal and, during the first 30 hours of pupation, they cause severe disruptions in eye development (Fig. 1). The external defects are characterised in the adult eye by a loss of the regular array of ommatidia and frequent ommatidial fusions. The specific effects caused by pupal heat shocks depend on the time of heat shock. In very young pupae (heat shocked before 12 hours post-pupariation [PP]), extensive heat shock causes about 75% of the pupae to die; the survivors have a zone of roughness limited to the anterior part of the eye. Heat shocks after 24 hours cause much less lethality, and lead to considerable roughness throughout the eye; in this later phase, the peak of sensitivity occurs at about 28 hours PP. After 32 hours, the eye is unaffected by over-expressed argos. This pattern of two phases of sensitivity is explained by the way the pupal eye develops. The morphogenetic furrow is still traversing the anterior part of the eye disc until about 10 hours PP so that any affect on photoreceptor and/or cone cell determination, which are both determined in a posterior to anterior temporal gradient, will be manifest early, and affect anterior regions only. Later, secondary and tertiary pigment cells develop more synchronously across the whole retina, and their determination occurs around 20-30 hours PP (See Fig. 2; Cagan and Ready, 1989a; Wolff and Ready, 1991).

Early heat shocks cause loss of photoreceptors and cone cells

Sections through HS-argos adult eyes that were heat shocked prior to 12 hours PP show that there are missing photoreceptors in the anterior region (Fig. 3A). The number of missing cells is variable — more than half the ommatidia have at least one cell fewer, and some are missing up to three. To look for

an effect on cone cells, which are not seen in adult eye sections, I stained pupal retinas with cobalt sulphide (Fig. 3B). Many ommatidia have fewer cone cells than in wild type, and again this effect is limited to the anterior region of the eye, though it spreads further posteriorly than the effect on photoreceptors. Argos protein has a half-life of greater than 4 hours after heat shock (M. F., unpublished observation) so that the elevated levels of argos persist into later development. This includes the period when primary pigment cells start being determined, at approximately 15 hours PP. It is harder to detect lost primary pigment cells unambiguously since they have a less characteristic morphology in disrupted eyes than cone cells, and it is difficult to distinguish a distorted primary from a secondary that has replaced a missing primary. Nevertheless, there are some clear cases of missing primaries, including a few instances where an ommatidium with the normal complement of four cone cells only has one primary pigment cell. This observation implies that the loss of these cells is unlikely to be a secondary consequence of the inability of a diminished number of cone cells to recruit primaries.

Later pupal heat shocks cause loss of secondary and tertiary pigment cells

Heat shocking during the second sensitive period, when the determination of photoreceptors, cone cells and primary pigment cells is complete, causes the loss of secondary and tertiary pigment cells (Fig. 4). The loss of these cells completely disrupts the lattice, leading to a breakdown of the regular array and ommatidial fusions. The pattern of bristles, which form part of the pigment cell lattice, is also disrupted, but their overall number appears about normal; this suggests that their disruption may be secondary to the loss of pigment cells. The loss of secondary and tertiary pigment cells caused by heat shocks in the 22-32 hour PP period corresponds to the time when these cells are being determined (Fig. 2).

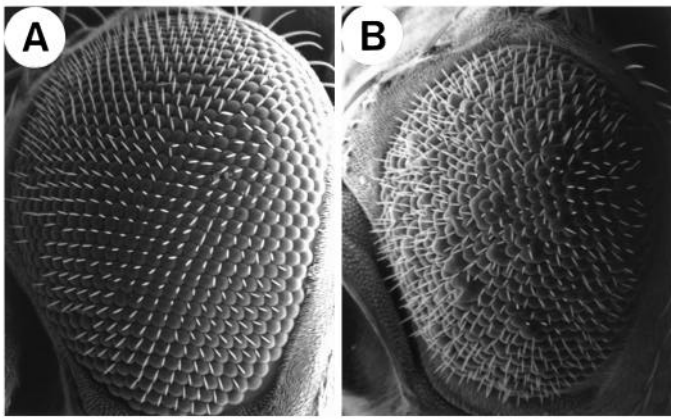

Fig. 1. Pupal heat shocks cause rough eyes in HS-argos flies. Scanning electron micrographs of flies subjected to five 38°C, 30 minute heat shocks at two hour intervals, starting at 24 hours post-pupariation. (A) Wild-type fly and (B) a fly carrying four copies of the HS-argos transposon. The HS-argos fly has a rough eye caused by a disruption of the ommatidial array and frequent ommatidial fusions. The degree of roughness obtained correlates with the amount of ectopic argos expressed: fewer heat shocks, heat shocks at lower temperatures, and heat shocks of flies carrying fewer copies of the transposon all lead to a less rough eye.

Increase in pupal cell death after late heat shocks

What happens to the cells that have their fate denied them in HS-argos eyes? Two obvious possibilities are that they are killed by the excess argos, or that they remain in an uncommitted state. After the late phase heat shocks, there is a clear increase of cell death (Fig. 5), suggesting that the cells which were due to become secondary and tertiary pigment cells instead die. The normal fate of cells that have failed to acquire a specific fate by this late stage is to be removed by a burst of programmed cell death (Cagan and Ready, 1989a; Wolff and Ready, 1991). Therefore the increase of cell death at this stage in heat-shocked pupae does not distinguish between the two possibilities. Unfortunately it is technically extremely difficult to examine younger pupal retinas — a few hours after the earlier heat shocks — and I have been unable to dissect them intact. However, by examining fragments of retina, I have found no evidence of a dramatic increase in cell death after these early heat shocks, suggesting that the excess argos protein does not lead to the immediate death of cells whose fate is altered.

Sevenless-argos causes few defects in photoreceptor determination

I have also expressed *argos* under the *sevenless* gene promoter and enhancer. *sevenless* is eye-specific and is expressed in most of the developing photoreceptors, the mystery cells and the cone cells of third instar larvae (Banerjee et al., 1987; Tomlinson et al., 1987); significant levels of *sevenless* expression have not been detected in the pupal retina. Sev-argos flies have very few defects in eye development, even in the presence of four copies of the transposon. Since it is clear from the HS-argos results that over-expressed *argos* does disrupt photoreceptor and cone cell determination, it appears that the lower level of misexpressed *argos* in sev-argos flies (see Fig. 7) accounts for the lack of effect.

Misexpressed *argos* can rescue *argos* mutations

The availability of flies with ectopically expressed *argos* allowed me to examine whether the normal expression pattern of *argos* is essential for wild-type eye development. I crossed the transgenes into flies with *argos* eye mutations and looked at the ability of the ectopic *argos* to rescue the phenotype. I found that sev-argos effectively suppresses strong or weak eye-specific alleles (Fig. 6). One copy of sev-argos was sufficient to rescue the eye completely. HS-argos was also able to rescue *argos* mutant eyes (data not shown), although I have not found conditions in which the rescue is complete.

Western blots confirm the misexpression results

I have made antibodies against argos protein and have used them to confirm that the ectopic expression under the *hsp70* and *sevenless* promoters did indeed lead to the over-expression predicted. Despite trying a variety of fixation and staining conditions, I have been unable to obtain convincing immuno-localisation of argos in tissue, but the antibody does work well on western blots. Fig. 7 shows that argos protein, which exists in two forms, is present at 2-3 times the wild-type level in eye discs with three copies of the sev-argos transposon, and that four copies of the HS-argos transposon produces about five times as much as wild type, upon heat shock (see legend to

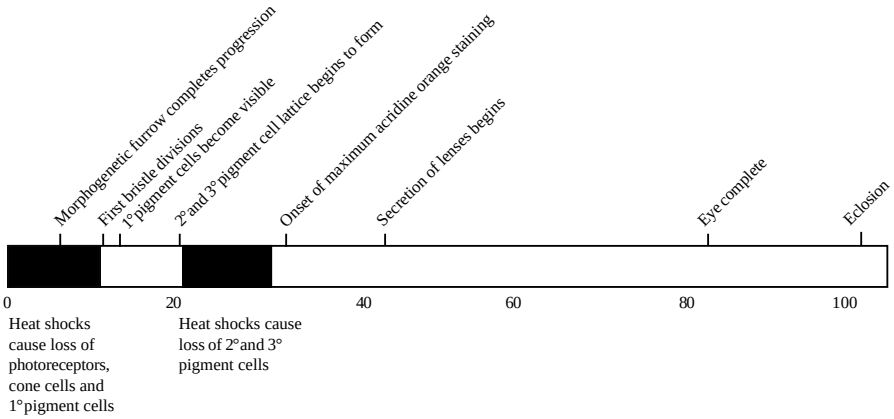

Fig. 2. HS-argos pupae are sensitive to heat shock at two periods. Pupae were collected as white prepupae and aged at 25°C; they were given a single heat shock of one hour at 38°C and allowed to eclose. The eyes were scored for roughness, so minor defects that did not disrupt the surface of the adult eye might not have been detected. Under this regime the only period sensitive to over-expressed argos was between 20 and 32 hours post-pupariation. Using a more intensive heat-shock regime (four 30 minute heat shocks at 2 hour intervals), the period between 2 and 12 hours PP was also found to cause a rough region in the anterior of the eye. It should be noted that the half life of argos protein after heat shock is greater than 3 hours (M. F. unpublished observation). The diagram shows an approximate time line of pupal eye development (adapted from Cagan and Ready, 1989a). Wild-type flies similarly heat shocked were unaffected at all times by the heat shock, except that intense heat shock between 2 and 12 hours PP caused significant lethality.

Fig. 7). These results confirm that the phenotypes observed do arise from over-expression and is consistent with the lack of disruption caused by sev-argos.

Argos protein is secreted by tissue culture cells

I have tested whether argos protein is secreted, as predicted, by transfecting the *argos* gene into tissue culture cells (Fig. 8). In the first experiment, I expressed the argos gene in monkey COS cells. In general the secretory pathway seems to be functionally conserved amongst eukaryotes. Argos protein is expressed in COS cells and is located primarily in the Golgi apparatus (Fig. 8A), which is the route by which secreted proteins leave the cell. Furthermore, western blots show that argos protein accumulates to high levels in the cell medium in a soluble form (Fig. 8B). Note that the secreted form of argos runs on an SDS gel as a larger molecule than the full-length protein predominant in the cell (an over-exposure of the western blot shown in Fig. 8B shows that there is a small amount of the larger form in the cell fraction). I have not investigated this, but assume that it is a late modification that happens in the Golgi just before the protein is secreted. Its significance is not clear, since I have not identified the larger form on western blots of argos from fly tissue (see Fig. 7).

In a second experiment, I transfected *argos* into *Drosophila*

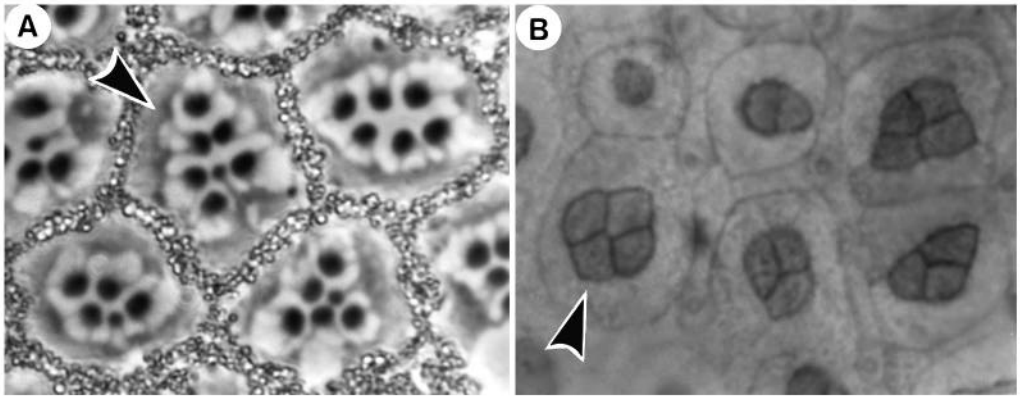

Fig. 3. Early pupal heat shocks cause the loss of photoreceptors and cone cells. (A) 2 μm section through an adult eye that had received four 30 minute heat shocks, at 2 hour intervals starting at 2 hours PP. Under this regime, about 75% of the pupae fail to eclose, but survivors have a rough region in the anterior part of the eye. Sections show that this roughness is primarily caused by the loss of photoreceptors. The arrow indicates a wild-type ommatidium, the rest of the ommatidia in the panel have variable numbers of missing cells. Inner and outer photoreceptors are both affected. (B) A cobalt sulphide stained pupal retina after the same heat-shock regime. Cobalt sulphide highlights cell contacts on the epithelial surface (Melamed and Trujillo-Cenoz, 1975). At this stage, all the retinal cells except the photoreceptors have apical profiles. The retina was dissected and stained at 64 hours PP (the developmental stage of these pupae and heat-shocked wild-type controls implied that the early heat shocks had delayed eye development by approximately 24 hours). The arrow shows an ommatidium with the normal complement of four cone cells; examples of ommatidia with one, two and three missing cone cells are visible (See Fig. 4A for example of a wild-type ommatidium).

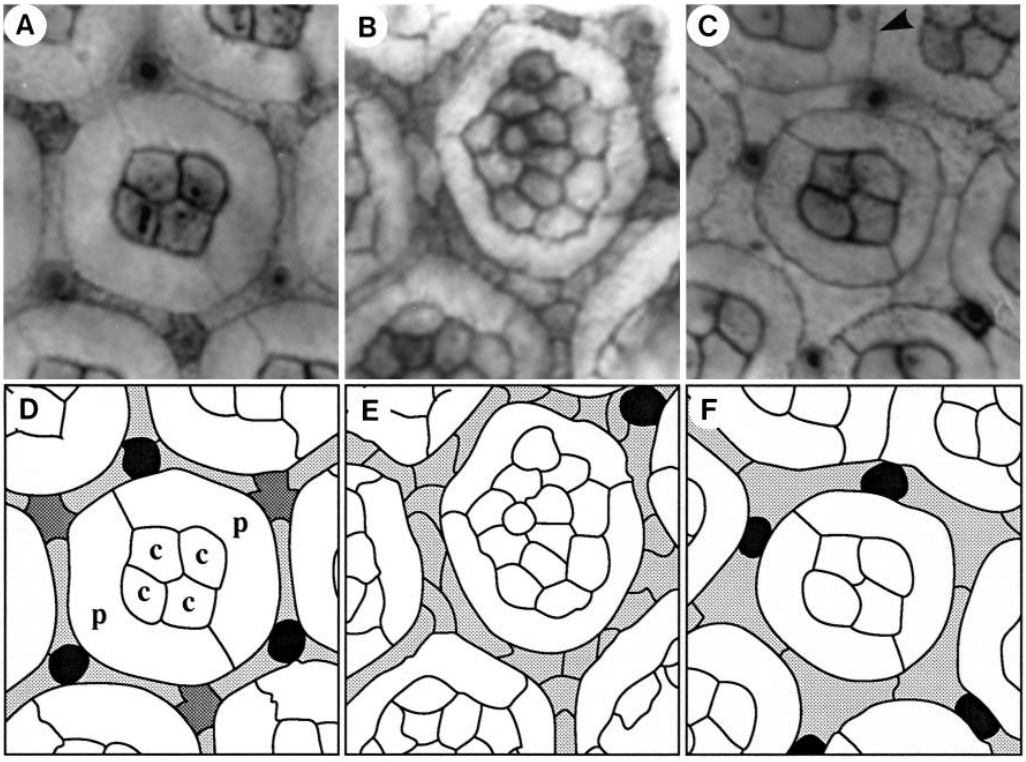

Fig. 4. Late pupal heat shocks cause the loss of 2° and 3° pigment cells. Retinas were dissected from pupae aged 40 hours after they had been heat shocked with the same regime as described in Fig. 1; they were stained with cobalt sulphide. (A-C) Cobalt sulphide-stained retinas, and (D-F) tracings of these photographs. (A,D) Wild type; (B,E) *argos*^{w11}/*argos*^{w11}; (C,F) HS-argos (4 copies). (A,D) Wild-type pupal ommatidia have a regular array of cells: four cone cells (c), two primary pigment cells (p), and they are surrounded by a lattice of secondary and tertiary pigment cells, and bristles. In D, secondary pigment cells are shaded light grey, tertiary pigment cells dark grey and bristles are black. (B,E) *argos*^{w11} ommatidia have extra pigment cells. The example shown has 13 cone cells, 2 primary pigment cells and is surrounded by 16 cells in the lattice; these cannot be identified as secondary or tertiary since the only way of distinguishing these is by their position in the wild-type lattice. (C,F) Over-expression of *argos* causes too few secondary and tertiary pigment cells to form; again it is not possible to identify the remaining cells as secondary or tertiary. The ommatidium shown is surrounded by a total of 7 cells, instead of 12 in wild-type ommatidia. The arrowhead in C indicates an example of abutting primary pigment cells from neighbouring ommatidia, caused by loss of lattice cells between them. The shading in E and F represents secondary or tertiary pigment cells, and bristles are black.

SL2 cells. As can be seen in Fig. 8C, the argos protein is again located in the Golgi apparatus (which does not show the same perinuclear appearance in SL2 cells as in COS cells), indicating that the protein is also secreted from these cells. For technical reasons I have been unable to look for argos accumulation in the medium of the SL2 cells.

These results indicate that argos protein is indeed secreted, which is consistent with previous data showing a long range non-autonomy in mitotic clones (Freeman et al., 1992b), and with the result described above that argos does not need to be expressed in exactly the normal cells to be functional: presumably the extracellular concentration is critical, but the precise pattern of cells that contributes to that concentration is not important.

DISCUSSION

Over-expression of *argos* causes the loss of cells in the developing eye

It is striking that all the different classes of cell in the developing eye (except bristle cells) are affected similarly by *argos*. In all cases loss-of-function mutations cause too many cells to acquire their specific fate (Freeman et al., 1992b; Kretschmar et al., 1992; Okano et al., 1992), while over-expression of the gene causes too few cells to be determined (this work). Although it is clear that the photoreceptors are recruited into the developing ommatidium by a series of inductive signals generated by cells already determined, little is known about the mechanisms by which cone cells and pigment cells form. The common effects of *argos* in all cell types suggests that similar mechanisms may govern the recruitment of these later developing cells. A similar conclusion can be drawn from the work of Cagan and Ready (1989b), who showed that loss of *Notch* function affected all cells in the developing eye similarly.

It is clear from data presented here that photoreceptors are susceptible to argos very early in their determination. Although it is not possible to detect exactly where the furrow is at the time of heat shock, it is about eight rows from the anterior of the eye at pupariation. I applied heat shocks from two hours post-pupariation, and found that photoreceptors were lost from about eight rows of ommatidia at the anterior of the eye. This implies that argos affects photoreceptors at about the same time as the furrow passes – the earliest stage of photoreceptor determination. Similarly, the effects on cone cells and pigment cells coincide with the early stages of their determination.

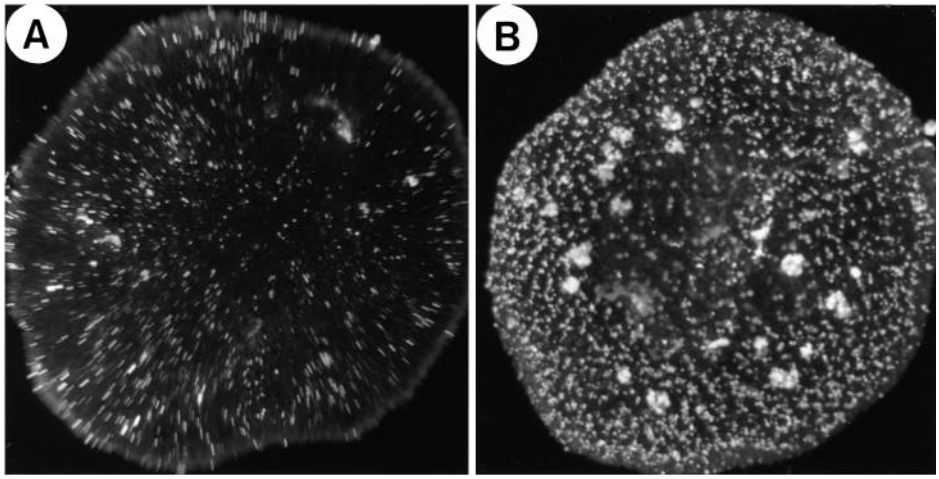

Fig. 5. Extra cell death in HS-argos retinas after late heat shock. Pupal retinas from wild-type (A) or HS-argos (B) flies were heat shocked four times at 2 hour intervals (38°C, 30 minutes) starting at 24 hours post-pupariation; they were dissected at 34-35 hours in acridine orange which stains fragments of dying cells, but is excluded from living cells (Spreij, 1971; Wolff and Ready, 1991). There is a significant increase in the amount of cell death apparent in the HS-argos retinas. Retinas of varying ages were examined to ensure that the extra staining could not be accounted for by staging differences in the different genotypes: in all cases examined, HS-argos retinas showed increased cell death. HS-argos retinas that were not heat shocked were identical to wild-type (not shown).

It appears that cells that develop early do not die immediately when their fate is altered by abnormally high levels of argos protein. They may be free to be recruited as later-developing cell types, and eventually the excess cells are presumably removed by the burst of apoptosis that removes all undetermined cells that remain when the eye is complete (Cagan and Ready, 1989a; Wolff and Ready, 1991). It is important to reiterate, however, that the acridine orange analysis of cell death after early heat shocks is made difficult by the fragile and rather amorphous state of the retina at this period. In practice, whole retinas cannot be dissected out, so the conclusion that there is not a substantial increase in cell death after early heat shocks is based on stained fragments of retina, and should be considered tentative. After late heat shocks, there is a significant and clear increase in cell death, presumably caused by those cells that were destined to become secondary and tertiary pigment cells remaining undetermined and joining the normal burst of apoptosis.

A system to block the response to inductive signals?

Argos appears to block cells from responding to inductive signals: in loss-of-function mutants, extra cells that are in a position to receive inductive signals are recruited; conversely, over-expression of *argos* leads to the same cells being blocked

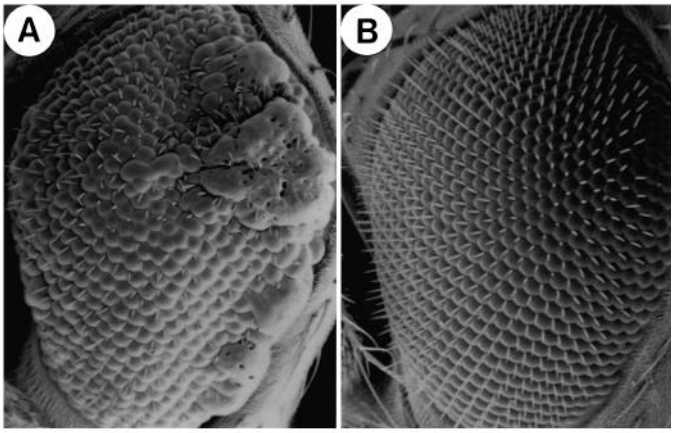

Fig. 6. sev-argos rescues the *argos* mutant phenotype. Scanning electron micrographs of genotypes: (A) $argos^{gil5}/argos^{gil5}$; (B) sev-argos/+; $argos^{gil5}/argos^{gil5}$. One copy of the sev-argos transposon rescues even strong alleles of *argos*.

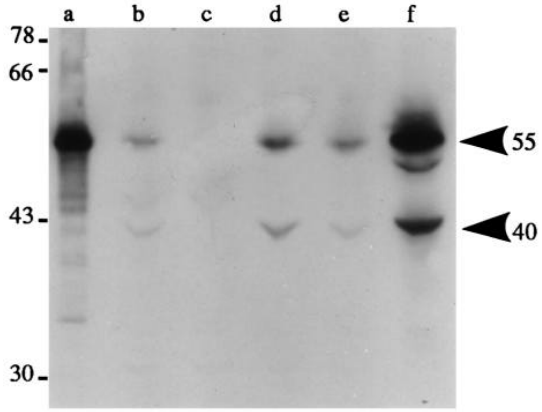

Fig. 7. The ectopic expression constructs do over-express *argos*. A western blot of third instar eye/antennal imaginal discs from larvae of the following genotypes: (a) full-length argos protein purified from bacteria expressing *argos* under the T7 promoter; (b) wild type (Canton-S); (c) $argos^{w11}/argos^{w11}$ (extreme eye mutation); (d) sev-argos (3 copies) in a wild-type background (i.e. these larvae also have their wild-type argos gene); (e) HS-argos (4 copies)in a wild-type background, no heat shock; (f) HS-argos (4 copies), plus heat shock (38°C, 1 hour). Lanes b to f each contain protein from 50 pairs of eye/antennal imaginal discs. A monoclonal antibody raised against the full-length bacterially expressed protein was used to probe the blot. Argos protein occurs in two main forms, a full-length form of approximately M_r 55×10^3, and a smaller form of M_r 40×10^3. The details of the apparent cleavage are not known. $argos^{w11}$ eye discs have no detectable protein. Three copies of sev-argos in an otherwise wild-type fly causes an over-expression of approximately 2- to 3-fold. There is no significant increase in HS-argos (four copy) discs before heat shock, but the overall protein level increases more than ten-fold upon heat shock. This is not a direct measure of the actual increase in concentration of argos around the developing cells, since the expression changes from being restricted to cells behind the morphogenetic furrow to the whole eye/antennal complex. The portion of the disc behind the furrow at this stage represents about a third of the entire disc complex, so that the effective increase in argos concentration around the developing eye cells is about one third of the induced expression seen in lane f – approximately 5-fold.

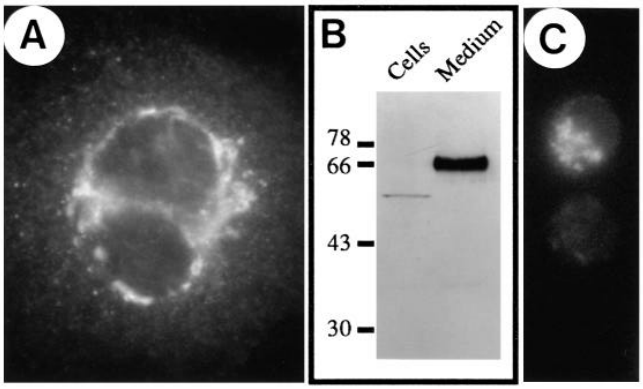

Fig. 8. Argos protein is secreted by tissue culture cells. (A) The *argos* gene was expressed in COS cells (see Materials and Methods) and the cells were immunostained with anti-argos antiserum. Staining is localised to the Golgi apparatus, which forms a characteristic perinuclear structure. (B) The transfected cells and the medium in which they had grown were western blotted; the argos protein is seen to accumulate efficiently in the medium. The extracellular form is larger than that associated with the cells, although with an over-exposure of this blot a small amount (<5%) of the larger form is seen in the cell fraction. This blot was performed using a polyclonal antiserum that only recognises the full-length argos protein in *Drosophila* tissue. Therefore I do not know if there is any of the much shorter, presumably cleaved form of the protein seen in Fig. 7, in COS cell medium. (C) The *argos* gene was also expressed in *Drosophila* SL2 tissue culture cells. Again the protein is located in the Golgi apparatus, which has a different morphology to that seen in COS cells.

from acquiring their normal fate. These results suggest that the normal function of argos may be to block inappropriate cells from being recruited into the ommatidium. This would imply that the intricate patterning in the eye is a consequence of a balance of opposing inductive and repressive influences.

Since *argos* is not expressed in front of the morphogenetic furrow and shows only a limited diffusion range (Freeman et al., 1992b), it cannot be required by all undetermined cells. Indeed, the model suggests that it is only those cells that are in a position to receive inductive signals, but which should not respond to them, that need argos. These include the mystery cells, cone cells and pigment cells. Mystery cells are found in the precluster of the early ommatidium; they undergo some of the very early steps of photoreceptor determination but never proceed as far as expressing neuronal antigens. Their fate is uncertain, although they are expelled from the precluster and probably rejoin the pool of undetermined cells (Tomlinson et al., 1987). The mystery cells have an intimate association with the developing photoreceptors, and so could be in a position to receive inductive cues. It is notable that some mystery cells make contact with R8, and it is possible that it is those cells that are transformed into photoreceptors in *argos* mutants (even in *argos* null mutations only about 70% of ommatidia have a transformed mystery cell). The close relationship between mystery cells and photoreceptors is also indicated by the number of mutants that cause a similar transformation (Mlodzik et al., 1990; Fischer-Vize et al., 1992a,b; Freeman et al., 1992a,b). Although less is known about the determination of the cone cells and pigment cells, it is thought that they are also recruited into the ommatidium from the surrounding naive cells by inductive signals.

This emerging picture of *argos* acting to repress the inappropriate response to inductive signals could also occur in other developing tissues. *argos* functions in the embryo, the wing and the optic lobe, as well as the eye, but it has not been studied sufficiently to understand its function in these tissues (Freeman et al., 1992b; Kretzschmar et al., 1992; Okano et al., 1992). Experiments are currently underway to examine further the role of *argos* in tissues other than the eye. It should be noted that the photoreceptor decay that was seen in *argos*^{w11} flies (Freeman et al., 1992b) has turned out to be caused by a second mutation, in the chaoptic gene (van Vactor et al., 1988), that was later detected to exist on the chromosome (M. Freeman, unpublished observation). Therefore the function of *argos* in the eye does seem to be limited to the period when cells are being determined.

It has recently been proposed that *Notch* encodes a regulator of a cell's competence to respond to inductive cues (Fortini and Artavanis-Tsakonas, 1993; Fortini et al., 1993) and, although there are significant differences between the eye phenotypes, there are also some striking similarities between the apparent function of *Notch* and *argos*. Both are widely expressed and pleiotropic; both have phenotypes in the eye that imply they act to repress inappropriate cells from acquiring a particular fate and, in both cases, they have this effect on photoreceptors, cone cells and pigment cells. However, the phenotypes are not identical. For example, loss of *Notch* function early in ommatidial development leads to all cells acquiring a photoreceptor fate (Cagan and Ready, 1989b), whereas loss of *argos* function has the effect of transforming the mystery cells into photoreceptors (Freeman et al., 1992b). Furthermore, they appear not to have similar phenotypes in other tissues (Simpson, 1990). Therefore, it does not seem likely that *argos* and *Notch* act in the same pathway, and no genetic interaction has been detected between them (Freeman et al., 1992b; M. Freeman, unpublished observations). Nevertheless, both genes point to the possible existence of a general mechanism that controls the ability of cells to respond to inductive cues.

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