The Function of the Drosophila argos Gene Product in the Development of Embryonic Chordotonal Organs

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We characterized the embryonic expression pattern and mutant phenotypes of the Drosophila gene argos, which encodes a secreted protein with an epidermal growth factor motif. The argos null mutation caused an increase in chordotonal (Ch) organs in both the thoracic and the abdominal segments, whereas overexpression of the argos gene resulted in a decrease in these organs. We showed that the argos transcripts are expressed transiently in the cells surrounding the Ch organ precursor and that the gene rhomboid (rho), which is involved in the regulation of the number of Ch organs, acts epistatically to argos in this event. Our findings suggest that argos plays a role in Ch organ precursor formation and regulates the final number of Ch organs.

INTRODUCTION

The development of multicellular organisms is a consequence of cell differentiation and assignment of differentiated cells to appropriate spatial locations. In this process, cell–cell interactions play a key role in the generation of cell-type diversity and the regulation of the number of specific cell types. To address the molecular mechanism of cell–cell interaction during morphogenesis, we investigated the function of the Drosophila argos gene, which encodes a diffusible factor with an EGF-like motif (Freeman et al., 1992a; Kretzschmar et al., 1992; Okano et al., 1992). In the adult compound eye, the argos loss of function mutation results in an increase in photoreceptor cells, cone cells, and pigment cells, whereas overexpression of argos causes a decrease in all these cell types (Brunner et al., 1994; Freeman, 1994a; Sawamoto et al., 1994). Based on these observations, it has been proposed that argos gene products secreted by differentiating cells prevent neighboring cells from adopting the same cell fate. The argos mutant also shows embryonic lethality characterized by the failure of head involution, broadening of the ventral epidermis, and an increase in the number of sensilla in the antennal-maxillary complex (Freeman et al., 1992a; Okano et al., 1992). These findings indicate that argos is necessary not only for adult appendage development but for proper development of other embryonic tissues, including the peripheral nervous system (PNS).

The PNS of Drosophila is an excellent model system to address basic developmental mechanisms, such as cell–cell interaction. The embryonic PNS consists of approximately 650 neurons and is made up of three major groups of neurons and support cells located at ventral, lateral, and dorsal levels in each segment (Campos-Ortega and Hartenstein, 1985; Ghysen et al., 1986; Hartenstein, 1988; Ghysen and O’Kane, 1989). Two major types of sensory neurons can be distinguished by their morphology in the PNS of each body segment. One type is the chordotonal (Ch) neurons that innervate the Ch organ. They are subepidermally located internal stretch receptors. The other type is the external sensory (Es) neurons that innervate Es organs. These neurons are mechanoreceptors and chemoreceptors present in the cuticle. The neurons and associated support cells that constitute a sensory organ are derived from a single precursor cell, the sensory organ precursor (SOP), through two or three asymmetric
cell divisions (Bodmer et al., 1989). Each of the SOPs within the domains of equivalent cells in the ectoderm, termed proneural clusters, is endowed with the potential to become a SOP through the expression of the proneural genes (Campuzano et al., 1985; Dambly-Caudière and Ghysen, 1987; Jarman et al., 1993). Removal of the function of the proneural genes of the achaete-scute complex (AS-C) causes loss of ES organs and their SOPs (Garcia-Bellido and Santamaria, 1978; Garcia-Bellido, 1979; Dambly-Caudière and Ghysen, 1987; Ghysen and O’Kane, 1989; Skeath and Carroll, 1991), while one of the other proneural genes, atonal (ato), is indispensable for the formation of the SOPs of Ch organs (Jarman et al., 1993). During mid-embryogenesis, ato-expressing SOPs delaminate to the subepidermal layer in the posterior compartment of each abdominal segment. Although each abdominal hemisegment has eight Ch organs in the late embryonic stage, only five Ch organ precursors expressing ato mRNA in each abdominal segment are observed (Jarman et al., 1993). The cell lineage explaining how these five ato-expressing Ch organ precursors ultimately supply eight precursors is unknown. On the other hand, it is known that mutations in loss of function mutants of spitz group genes, which are classified as modifiers of the Drosophila EGF-receptor homolog (DER) (Price et al., 1989; Schetinger and Shilo, 1989), cause the absence of three Ch organs in each abdominal segment (Bier et al., 1990; Rutledge et al., 1992; Jan and Jan, 1993). As each remaining Ch organ in these mutants contains a neuron and three other support cells, the spitz group genes may play a role in part of the process which regulates the exact number of Ch organ precursors rather than cell fate specifications. Interestingly, loss of function of the spitz group genes causes phenotypes opposite to those of the argos mutant during development of many other tissues, such as embryonic ventral epidermis, adult compound eyes, and wing veins (Mayer and Nüsslein-Volhard, 1988; Heberlein et al., 1993; Raz and Shilo, 1993; Sturtevant et al., 1993, 1995; Freeman, 1994b; Kolodkin et al., 1994; Tio et al., 1994).

In this report, we describe one role of argos during embryogenesis. We studied the pattern of expression of argos during embryogenesis and characterized the effect of loss and gain of argos function in the embryonic nervous system. Our results indicate that argos gene products act negatively to restrict the number of Ch organs but not ES organs and that rhomboid (rho), one of the spitz group genes, acts epi-statically to argos to regulate the correct number of Ch organs in the embryonic PNS.

MATERIALS AND METHODS

Fly strains. The enhancer trap line argos152 was previously described as sty2 (Okano et al., 1992). The A37 enhancer trap line was a gift from Dr. R. Ueda, and rho241 was a gift from Dr. E. Bier; w;Dr(TM3.Sb P[1Δ2-3.3y]+) were obtained from Bloomington Stock Center. The hs-argos 4 transformant line has been previously described (Sawamoto et al., 1994). The rho241 argos257 chromosome was generated by a standard protocol. To discriminate between heterozygous and homozygous mutant embryos, balancer chromosomes carrying P elements expressing lacZ (TM3.Sb P[Ubx-lacZ] or TM6B, Tb Hu P[Abx-lacZ]) were used in all experiments described in this paper. wild-type and Canton special were used as wild-type stock.

In situ hybridization to whole mount embryos. Whole mount in situ hybridization was done by using digoxigenin-labeled RNA probes as described by Tautz and Pfeifle (1989). The argos RNA probe and the β-galactosidase RNA probe were made from the cSty2 cDNA clone (Okano et al., 1992) and pBluescript II Phagemid Vectors (Stratagene), respectively, by in vitro transcription using a standard procedure for a digoxigenin RNA-labeling (Boehringer Mannheim).

Isolation of argos257. A new null allele argos257 was generated by the imprecise excision of original P-lacW insertion in the argos257 enhancer trap line. The P-lacW element in the argos257 was remobilized in the presence of the P transposase source Δ2-3. One hundred excision lines with rough eye phenotype were generated, 10 of which showed severe roughening of the eye with reduced viability and failed to complement argos257 (Okano et al., 1992). To identify imprecise excisions that harbor deletions of argos, these excision lines were analyzed by Southern blotting. The first exon which included the ATG codon and the signal peptide was deleted in argos257.

Antibody staining. Immunohistochimistry was performed as described (Patel, 1994) using the following primary antibodies: anti-Crumbs (Crb) mouse IgG monoclonal antibody (MAb) (Tepa et al., 1993) used at a 1:5 dilution, mouse IgG MAb 22C10 antibodies (Jackson Immunoresearch) used at a 1:500 dilution, rabbit anti-Couch potato polyclonal antibody (Bellen et al., 1992) used at a 1:500 dilution, and rabbit anti-β-galactosidase polyclonal antibody (Cappel) used at a 1:500 dilution. The secondary antibodies used were HRP conjugated goat anti-mouse or rabbit IgG (Jackson Immunoresearch) used at a 1:500 dilution.

Heat shock experiment. Wild-type and hs-argos 4 embryos were collected on apple agar plates for 2 hr in a 25°C moist chamber. The embryos were incubated for 2 hr at 25°C, and then placed in a humidified 37°C incubator for 30 min. Then, the treated embryos were aged to stage 16 at 25°C, collected, fixed, and immunostained to assess the number of Ch organs.

RESULTS

Expression of the argos Transcripts during Embryogenesis

As the first step in elucidating the function of argos during embryogenesis, the expression pattern of argos RNA was characterized by whole-mount in situ hybridization. Embryos were staged according to Campos-Ortega and Hartenstein (1985). Expression of argos was first detected in the cellular blastoderm stage (stage 5) (Fig. 1A). During the fast phase of germband extension (stage 8), argos expression started to be localized in the mesoderm in a pair-rule like pattern (Figs. 1B and 1C). At stage 9, argos expression became equally expressed in the mesoderm of every segment (Fig. 1D). In germband extended embryos, transient expression of argos was observed in the visceral mesoderm at stage 10 (Fig. 2A), and at stage 11, it was observed in
Expression of argos during embryogenesis. The embryonic expression pattern of argos has been analyzed by whole-mount in situ hybridization with a digoxigenin-labeled argos RNA probe. Embryos are staged according to Campos-Ortega and Hartenstein (1985). Anterior is to the left and dorsal at the top. (A) argos expression is first detected in the cellular blastoderm stage (stage 5) with a unique pattern in three dorsal regions. Two distinct populations of argos-expressing cells are observed in the anterior part of the embryo. The posterior population is composed of two fine stripes, and the posterior stripe closely matches the cephalic furrow formed in the next stage (arrow). (B) A surface view of an embryo at stage 7. (C) A sagittal optical section at stage 8; argos expression starts to be strongly localized in the mesoderm of every other segment in a pair-rule-like pattern, indicated by the arrowheads. (D) A stage 9 embryo showing that argos expression gradually increases in the mesoderm of the segments (arrowheads). (E, F) Stage 10 (E) and stage 11 (F) embryos. Strong, but transient, argos expression in tracheal pits (arrowheads) is observed (E) and decays rapidly (F). (G) At stage 12, argos expression begins in the epidermal cells forming the segment boundary in each abdominal segment and the optic lobe placode, indicated by the arrowhead. (H) After germband retraction is complete (stage 13), argos expression starts in the visceral mesoderm located on both lateral sides of the yolk (arrowheads) and in the outer support cells of several sensory organs, including the spiracular sensory organ (arrow). (I) At stage 16, argos is strongly expressed in the midline glia of the ventral nerve cord (arrowhead). The optic lobe primordia located in the ventral region of the brain hemisphere are also labeled (arrow).
FIG. 2. argos mRNA distribution at a higher magnification. Anterior is to the left in all photographs. (A, B) argos expression in mesodermal cells. Shown are ventral views of stage 10 (A) and stage 11 (B) embryos, respectively. (A) argos is transiently expressed in unidentified subset cells of segmentally repeated mesoderm (arrows) and arched arrangements of the visceral mesoderm progenitors (arrowheads). (B) argos-expressing mesodermal cells spread over the surface of the yolk. The labeled cells and yolk granules (arrowhead) are visible on the same focus plane. (C, D) The ventral surface of the embryo at stage 8 (C) and stage 11 (D), respectively. Bilateral two-cell-wide stripes of ectodermal cells (arrowheads) bordering the mesectoderm (arrow) express the argos transcripts (C). Note that these bilateral stripes come to form a pair of parallel one-cell-wide stripes (arrowhead) along the ventral midline (D). The parallel stripe expression persists until germband retraction. (E, F) Ventral view of the CNS showing argos expression at stage 12 (E) and stage 16 (F), respectively. argos expression is visible in midline glia precursors on the ventral midline in each segment (E). It continues until midline glia precursors divide and migrate to the final position in the ventral nerve cord (F). (G, H) Lateral view of stage 13 (G) and stage 15 (H) embryos, showing argos expression in the tracheal system. The tip cells of transversal tubular processes of tracheal trees are highly labeled (G). The argos transcripts come to be observed in the dorsal trunk of the tracheal system after each transversal tubular process comes into contact with its anterior and posterior neighbors (H). Note the apical accumulation of argos mRNA. (I, J) Dorsolateral view of a stage 13 embryo (I) and lateral view of a stage 16 embryo (J). argos transcripts are observed in the developing optic lobe (arrow) and antenno-maxillary complex (arrowheads), the prominent sensory organs in the head.

bryogenesis. In stage 13 (Fig. 1H), argos expression resumed in the tracheal system. Apical accumulation of the argos transcripts was observed in the tip cells of the transversal tubular processes of the tracheal trees (Fig. 2G). After each transversal tubular process came into contact with its anterior and posterior neighbors, the argos transcripts were present in the dorsal trunk of the tracheal system, a structure that ran longitudinally through the embryo (Fig. 2H). This argos expression persisted until stage 15. The argos transcripts were also expressed in the developing optic lobe and in several terminal sensory organs throughout embryogenesis. The optic lobe placode began to be weakly labeled at stage 12, when it invaginated medially to form a deep pouch (Fig. 2I). When the optic lobe primordia arrived at the ventral surface of the brain hemisphere, argos expression became more intense (Figs. 1I and 2J). The argos expression in sensory organs such as the antenno-maxillary complex (indicated by arrows in Fig. 2I) and the spiracular sensory organ, continued throughout the latter half of embryogenesis (Figs. 2I and 2J). Based on the observation of a neuron-specific marker, Mab22C10 (Fujita et al., 1982), and argos^{sty2} enhancer trap (Okano et al., 1992) double staining, the argos-expressing cells in these sensory organs are a subset of outer support cells, as these cells were wrapped around the dendrites of sensory neurons and were located within the epidermis (Fig. 4A–4C). Expression of lacZ in argos^{sty2}, which has a P-lacW insertion in the putative signal peptide, more closely resembled the localization of the argos transcripts than that of another enhancer trap line, argos^{sty1} (Okano et al., 1992).
Consequently, the brain hemispheres remained exposed to various degrees (Figs. 5D and 5F), atria were not formed, and salivary ducts opened outside the body at late stage 16. The brain hemispheres of both sides in one-half of the homozygous embryos had fused (Fig. 5C) or were divided into lobes (Fig. 5E). The developing optic lobe often failed to migrate to the proper position and failed to retain its tubular structure.

**argos Mutation Causes a Gain of Chordotonal Organs**

There are two kinds of Ch organ clusters and three isolated Ch organs in the embryonic peripheral nervous system.

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**FIG. 3.** argos expression in cells surrounding the Ch organ precursor. Lateral view of stage 11 embryos showing argos mRNA distribution (A), anti-β-galactosidase immunostaining of the A37 enhancer trap line (B), and argos mRNA and β-galactosidase mRNA in the A37 enhancer trap line (C). The external sense (Es) organ precursor (indicated by “A”) and the chordotonal (Ch) organ precursor (indicated by “P”) expressing β-galactosidase are observed (B). argos expression is observed in cells surrounding the Ch organ precursor (indicated by “P”) in the posterior compartment and never observed in the anterior compartment in which the Es organ precursor delaminates. Tracheal pits are indicated by asterisks.

**argos Mutant Phenotypes in the Embryonic Brain**

We previously reported that the argos loss of function mutation caused failure of head involution (Freeman et al., 1992a; Okano et al., 1992). To analyze the mutant phenotype in detail, a new null allele, argos257, was generated by imprecise excision of the P-element in the argos2⁰⁰ enhancer trap line. As this null allele showed the arrest of head involution as well, we examined the embryonic cephalic region. The degree of the phenotype in the cephalic region of the argos mutant embryos ranged from slightly warped brain hemispheres to massive disorganization. In approximately half of the population of the argos2⁵⁷ embryos, the cephalic region failed to invaginate into a dorsal pouch.

**FIG. 4.** argos2⁰⁰ lacZ expression in terminal sensory organs. Stage 15 argos2⁰⁰/+ embryos are double-immunostained with anti-β-galactosidase antibody (blue) and Mab22C10 neuron-specific marker (brown). The lateral view of the head region is shown (A). The dorsal organ (do) and the terminal organ (to) of the antenno-maxillary complex are shown. The lateral view (B) and the dorsal view (C) of the telson are shown. The spiracular sensory organ (sso), the dorsocaudal sensory organ (dcsc), and the caudal sensory organ (csc) can be seen. Dendrites of these sensory neurons are indicated by arrowheads in (C). argos-expressing cells surround the dendrites of sensory neurons. These results clearly demonstrate that argos is expressed in the outer support cells of these terminal sense organs.
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A single Ch organ precursor by three rounds of asymmetric cell division (Bodmer et al., 1989).

To investigate the role of argos in the development of embryonic PNS, we examined Ch organs and Es organs in the argos mutant embryo. An increase in Ch organs was observed in the PNS of argos mutants. One or two additional Ch neurons were identified in each DCh3 (Fig. 6D) and LCh5 using Mab22C10 (Fig. 6E). Examination using anti-Crumbs (Crb) antibody, which labels scolopale at the tip of the Ch dendrite as well as a dot at the tip of the Es dendrite (Tepaû et al., 1990), revealed that the mutant embryos had an additional scolopale cell in DCh3 and LCh5 (Fig. 6F). The number of dots and neurons of Es organs, however, were unchanged in the mutant PNS (data not shown). In order to characterize the Ch organs of argos mu-

**FIG. 5.** The deformed head region of the argos mutant. Anti-Crb immunostaining of wild-type (A, B) and argos257/argos257 (C-E) stage 16 embryos, and an argos257/argos257 embryo stained with anti-β-galactosidase antibody (F). (A, C, E) Dorsal views. The brain hemispheres are sometimes fused (C) and sometimes divided into lobes (arrows in E). The optic lobe primordia (arrowheads) occasionally fail to migrate into their proper position. (B, D, F) Lateral views. During late embryogenesis in the wild type, the dorsal regions of the head segments become incorporated into the dorsal pouch (arrowheads) and the ventral regions, including the salivary duct (arrow), are simultaneously incorporated into the atriopharyngeal cavity (B). In the moderate argos phenotype (D), the shallow dorsal pouch (arrowheads) and the salivary duct have opened to the outside (arrow), resulting in the arrest of head involution. In the severe argos phenotype (F), the dorsal epidermis, which is normally incorporated into the dorsal pouch, is sliding into the caudal side of the brain hemisphere (arrowheads). Formation of the dorsal pouch and atriopharyngeal cavity is arrested, and the salivary duct remains at the ventral surface of the embryo in late embryogenesis (arrow).

**FIG. 6.** Chordotonal neurons and scolopales in argos null-type mutant embryos. The clusters of chordotonal (Ch) organs in the PNS of both wild type (A-C) and argos257/argos257 (D-F) stained with the neuron-specific marker MAb22C10 (A, B, D, E) and anti-Crb antibody, which labels the scolopale at the tip of the Ch dendrite (C, F). Ch neurons are indicated by arrowheads. Each thoracic hemisegment in the wild type has a Ch organ cluster with three inverted Ch organs (Ch neurons), referred to as DCh3 (A), and each abdominal hemisegment has a LCh5 cluster which includes five Ch organs (B). In argos mutants, however, these Ch organ clusters have an additional Ch neuron in both the thoracic (D, four Ch organs) and the abdominal segments (E, six Ch organs). (C, F) Anti-Crb antibody labels five scolopales at the tips of the Ch dendrites of LCh5 in the wild type (C). A total of six scolopales, including an additional scolopale, can be observed in argos mutant embryos (F).
tants further, mutant embryos were stained with a battery of cell-type specific markers (Anti-Prospero antibody, the scolopale cell (Vaessen et al., 1991); anti-RK2 antibody, the ligament cell (Campbell et al., 1994); and anti-Couch potato (Cpo) antibody, nuclei of all four cells of each Ch organ (Bellen et al., 1992)). These marker stainings showed that 46.4% of LCh5s, 34.6% of DCh3s, and 9.4% of abdominal ventral Ch organs, but not V'Ch1, included an additional Ch neuron, as well as three support cells composing an additional Ch organ in argos null mutant embryos (n = 192) (Figs. 7A - 7F).

As the increase in Ch neurons in argos embryos was associated with a parallel increase in Ch organs, the presence of additional Ch neurons was unlikely to have resulted from a transformation of support cells into cells possessing neuronal properties. To address whether the argos mutant embryos have extra Ch organ precursors, we assessed ato gene expression in argos mutants. We examined mutant embryos between stage 8 and stage 11, wherein each Ch organ precursor is singled out from a proneural cluster through the action of neurogenic genes. In the wild type, ato is expressed transiently in five of eight Ch organ precursors in each abdominal segment (Jarman et al., 1993, 1995). We identified a group of four or five ato-expressing Ch organ precursors located in the posterior compartment of abdominal segments, but we did not detect obvious changes in number between wild-type and argos mutant embryos (data not shown).

Overexpression of argos Causes a Loss of Ch Organs

To test the hypothesis that argos negatively regulates the number of Ch organ precursors, we characterized the effect of ubiquitous overexpression of argos gene products in the wild-type background during Ch organ development. The overexpression experiment was carried out using the hs-argos 4 transformant line that regulates a full-length argos cDNA under the hsp70 promoter (Sawamoto et al., 1994). To determine the effects of argos overexpression, wild-type embryos and hs-argos 4 embryos were subjected to brief heat-shock treatments prior to the time when Ch organ precursors appear. The embryos were allowed to develop until the late embryonic stage (stage 16) when differentiation of the PNS was complete so that they could be identified immunohistochemically (Fig. 8A). The heat-shock treatment had no apparent effect on the pattern of sensory organ development in the wild type. The hs-argos 4 embryos subjected to heat shock showed a loss of one or two Ch organs at LCh5 (Figs. 8B and 8C), although no clear abnormalities were observed without heat shock. Based on the Cpo staining pattern, the surviving Ch organs retained their morphology, and the cell identities of all four cells of which each surviving Ch organ is composed were unaffected. The hs-argos 4 transformant embryos heat-shocked after 6 hr of development did not show any obvious abnormalities in the pattern of the PNS (data not shown). Therefore, the overexpression of the Argos protein leads to a loss of Ch organs, if it occurs at an early stage of Ch organ development.

Ch Organ Phenotype of argos in rhomboid Background

Mutations in some spitz group genes affect the number of Ch organs, but not the number of Es organs. One of the spitz group genes, rhomboid (rho), which encodes a transmembrane protein, is expressed in Ch organ precursors (Bier et al., 1990). The rho(del1) null mutant embryo showed the absence of two of five Ch organs at LCh5 and one ventral Ch organ (VChB) in all abdominal segments (Figs. 9A and 9B), but never changed the number of V'Ch1 as well as argos null mutants (Bier et al., 1990). It seems that these two genes act upon a common signaling pathway to regulate the number of Ch organs, because rho and argos affect the development of the same subsets of peripheral sensory organs. In order to confirm this hypothesis, we prepared argos(del1) and rho(del1) double null mutants and examined PNS in this mutant embryo. The rho(del1) argos(del1) double null mutant embryo exhibited the same phenotype as the rho(del1) single mutation, i.e., a lack of two Ch organs at LCh5 and VChB, and had no effect on the number of Es organs or V'Ch1 (Figs. 9C and 9D). The fact that the phenotype of the argos null mutant was not observed in the rho mutant background indicates that rho acts epistatically to argos in Ch organ development.

DISCUSSION

The present findings regarding the localization of argos transcripts and the phenotypes of loss and gain of function mutants suggested that the argos gene product has multiple functions in the embryonic nervous system. In the PNS, for example, argos seemed to regulate the number of Ch organ precursors by functioning as a diffusible inhibitor.

Correlation between argos Expression and Mutant Phenotypes

We previously reported that there are one or two additional large and small sensilla in the antennal-maxillary complex, a prominent sense organ in the head, in the argos loss of function mutant (Okano et al., 1992). Expression of the argos transcripts was detected in prominent structures in mandibular and maxillary segments which give rise to the antennal-maxillary complex. The Mab22C10 antibody and argos enhancer-trap double staining revealed that argos-expressing cells were located around the dendrites of sensory neurons of the antennal-maxillary complex in late embryogenesis. Although this sensory organ is distinct from Ch organs, argos may regulate the development of this sensory organ as an inhibitory signal as well.

The optic lobe placode expressed the argos transcripts...
FIG. 7. Chordotonal organs in argos null type mutant embryos. Clusters of chordotonal (Ch) organs in the PNS of both wild type (A, C, E) and argos<sup>257/257</sup> (B, D, F) stained with anti-Cpo antibody, which labels the nuclei of all four cells constituting each Ch organ with differing intensities (A–F). (A–F) Tracings of Ch organs in these anti-Cpo staining photographs, respectively. Cpo-expression in the wild-type DCh3 (A, three Ch organs) and argos DCh3 (B, four Ch organs), and in wild-type LCh5 (C, five Ch organs) and argos LCh5 (D, six Ch organs) are shown. (E) The wild-type ventral group of PNS has two Ch organs, VChA and VChB. (F) Three Ch organs (two VChAs and VChB) can be seen in the argos ventral group. Mutant Ch organ clusters (LCh5, DCh3, and VChA,B) include not only an additional Ch neuron, but its other three support cells constitute an additional Ch organ. c, cap cell; s, scolopale cell; n, neuron; l, ligament cell.

weakly, throughout late embryogenesis. The signal became more intense after the optic lobe primordia detached from the head ectoderm. argos expression in the optic lobes continued throughout larval development and is required for the development of adult optic lobes (Sawamoto et al., 1996). We occasionally observed failure of head involution, deformed brain hemispheres, and the failure of developing optic lobe migration in argos mutants. The mechanisms responsible for these morphological abnormalities in the head region have not been elucidated. However, one possibility is that the anterodorsal expression of the argos transcripts at the cellular blastoderm stage (Fig. 1A) may play a role in the establishment of embryonic terminal identity or the area of the procephalic neurogenic region, as this anterior-most domain corresponds to the hückebäin expression which is required for the determination of the terminal

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FIG. 8. Overexpression of argos caused a loss of Ch organs. (A) Diagrammatic representation of the experimental protocols for overexpression of argos. The overexpression experiment was carried out using the hs-argos 4 transformant line that regulates full-length argos cDNA under the hsp70 promoter. Eggs were collected 0–2 hr after egg laying and incubated at 25°C throughout embryogenesis. Heat shock treatment (37°C for 30 min (represented by the shaded box)) was delivered 1 hr before DNA replication in Ch organ precursors. After heat-shock treatment, embryos were further incubated to allow sufficient differentiation of the PNS cells. The embryos were fixed for immunohistochemistry at 13.5–15.5 hr of development (stage 16 indicated by the hatched box). (B, C) The effect of argos overexpression decreased the number of Ch organs. (B) The LCh5 in hs-argos 4 transformant line with heat-shock treatment. Only three Ch organs are observed in this LCh5 (normally five), and the cell identities in these three Ch organs remain unchanged. (C) Drawings of Ch organs from the photograph in B. c, cap cell; s, scolopale cell; n, neuron; l, ligament cell.

identity (data not shown). Another possibility is that the presence of extra cells in the head region before head involution resulted in morphological abnormalities. Programmed cell death (PCD) evidently plays an important role in head involution. Most of engrailed-expressing cells in each head segment degenerate prior to head involution and are taken up by macrophages (Younossi-Hartenstein et al., 1993). The occurrence of some large clusters of degenerating cells in the head region seems to be involved in the reshaping of the head region during head involution (Jürgens and Hartenstein, 1993). Furthermore, a recent report claimed that loss of function of the head involution defective (hid) gene, which encodes an essential molecule for PCD, results in failure of head involution, the same as in argos mutants (Abbott and Lengyel, 1991; Grether et al., 1995). The argos loss of function mutant generates extra cells in many tissues such as extra photoreceptor cells, pigment cells, and cone cells in the adult eye and extra sensilla in the larval sensory organs, and also has been shown to reduce PCD in the pupal eye disc (Brunner et al., 1994). In contrast, the overexpression of argos increased apoptotic cells in the pupal eye disc (Freeman, 1994a). Thus, similar to the hid mutant, the presence of extra cells caused by the argos mutation in the head region may interfere with head involution.

In the ventral nerve cord, the argos transcripts were localized in the developing midline glia throughout late embryogenesis. Although the midline glia are essential for formation of commissure axon tracts (Klämbt et al., 1991), we were unable to identify any abnormality in the formation of commissure axon tracts in mutant embryos immunohis-

FIG. 9. rho is epistatic to argos. (A–D) Anti-Cpo immunostaining of the LCh5 of stage 16 embryos. (A, B) rho⁰¹/rho⁰¹; (C, D) rho⁰¹ argos²⁵⁷/rho⁰¹ argos²⁵⁷. (A, B) The rhomboid (rho) null mutant (rho⁰¹/rho⁰¹) embryo showed the absence of two of the five Ch organs in LCh5 in all abdominal segments. (C, D) The rho⁰¹ argos²⁵⁷ double null mutant embryo showed the same phenotype as the rho single mutation, i.e., the lack of two Ch organs in LCh5. The phenotypes of the argos null mutant (ectopic Ch organs) were not observed in the rho mutant background.
tochemically with MAAb22C10, FaslII (Grenningloh et al., 1991), FaslII (Patel et al., 1987) or anti-HRP antibodies (Jan and Jan, 1982) (data not shown).

No significant morphological changes were observed in many other tissues which expressed argos. For example, in the tracheal system, although apical accumulation of argos transcripts was observed after germband retraction, no obvious morphological change was observed by anti-Crb antibody staining. Thus, argos function seems to be compensated for by other factors in the development of these tissues.

argos Function in the Development of Chordotonal Organs

It is known that loss of function of any neurogenic gene leads to overproduction of PNS neurons at the expense of epidermal cells, owing to the lack of lateral inhibition within proneural clusters (Hartenstein and Campos-Ortega, 1986). In the case of the argos mutation, however, only one or two extra Ch neurons were observed in each Ch organ cluster, and each extra Ch neuron had three other outer support cells composing an extra Ch organ. Although an additional Ch organ emerged in DCh3, LCh5, and in abdominal ventral Ch organs, no obvious abnormality was observed in mutant V*Ch1 or any Es organs. Accordingly, argos transcripts were not detected in the anterior compartment. Where Es organ precursors originate. In contrast to the phenotype of the null mutant, overexpression of argos induced a loss of Ch organs. Despite the difference in number of Ch organs in the argos loss or gain of function mutants, the cell lineages of Ch organs in both argos mutants were unaffected. The argos transcripts started to be expressed in cells surrounding Ch organ precursors after they had already delaminated from the proneural cluster in the epidermal layer. Proneural clusters appeared to occur normally in argos embryos (data not shown). Therefore, the overproduction of Ch neurons in argos null mutants seems to be caused by a mechanism different from the loss of lateral inhibition through the Notch signaling pathway. How does argos regulate the number of Ch organs? We propose that argos may act negatively on the signaling cascade which is common to some spitz group genes to regulate the final number of Ch organ precursors (Fig. 10). This possibility is based on following circumstantial evidence.

The development of Ch organs begins at the germband extended stage with a cluster of cells in the dorsal epidermis expressing the ato gene. Ato is known to be a proneural gene for the formation of Ch organs and photoreceptor cells (Jarman et al., 1993, 1994, 1995). Ch organs are eliminated in embryos carrying chromosomal deficiencies that remove ato, and ectopic expression of ato promotes the formation of extra Ch organs. This gene is expressed in the proneural clusters and Ch organ precursors in the posterior compartment of each hemisegment. However, only five Ch organ precursors express ato mRNA in each abdominal hemisegment (Jarman et al., 1993), although eight Ch organs (LCh5, VChA, VChB, and V*Ch1) are usually observed in the late embryonic stage. The cell-lineage explaining how these five ato-expressing Ch organ precursors ultimately supply eight precursors remains unknown. spitz group genes, such as rho, spitz (spi), and Star (S), probably play roles in the process of generating the other three Ch organ precursors from five ato-expressing Ch organ precursors, because the loss of function mutants of rho, spi, and S have only five Ch organs in each abdominal hemisegment due to loss of two Ch organs from LCh5 and VChB and resembles overexpression of the argos. The phenotype of the argos and rho double mutant is same as that of the rho single mutant. It is a common feature of both argos and rho mutants that the numbers of V*Ch1 and Es organs are unaffected.

FIG. 10. Summary in abdominal Ch organ phenotypes in argos. In the wild-type, each abdominal segment has eight Ch organs, i.e., LCh5 contains five units in a lateral group, VChA and VChB are individually located in a ventral group, and V*Ch1 is located between the lateral group and the ventral group. The argos loss of function mutant has extra Ch organs in LCh5 and the ventral group (hatched Ch organs), whereas overexpression of the argos gene product causes loss of Ch organs in same groups (white Ch organs). The loss of function mutant of rho has only five Ch organs in each abdominal segment due to loss of two Ch organs from LCh5 and VChB and resembles overexpression of the argos. The phenotype of the argos and rho double mutant is same as that of the rho single mutant. It is a common feature of both argos and rho mutants that the numbers of V*Ch1 and Es organs are unaffected.
berlein et al., 1993), and in contrast, argos, which encodes a diffusible factor, is expressed in neighboring cells, argos may act on the neighboring Ch organ precursor as a diffusible factor to regulate the final number of Ch organs in the common pathway with spitz group genes. We also showed that the phenotype of argos and rho double mutants is the same as that of the rho single mutation, indicating that rho is epistatic to argos. These results suggest that rho acts downstream to argos or that the function of rho is necessary for the induction of argos in this developmental step.

In addition to Ch organs, the argos mutant and the spitz group mutants produced opposite phenotypes in other tissues, such as adult eye and wing veins (Freeman et al., 1992a; Sturtevant et al., 1993; Freeman, 1994a, 1994b; Tio et al., 1994; Sawamoto et al., 1994). These spitz group genes are also known to amplify DER signaling during the development of these organs (Heberlein et al., 1993; Raz and Shilo, 1993; Sturtevant et al., 1993; Kolodkin et al., 1994; Freeman, 1994b; Tio et al., 1994; Sturtevant and Bier, 1995). Although the functions of rho and Spiz genes in DER signaling have not been elucidated, a recent study using a cell culture system elegantly demonstrated that the spiz gene product, which is a Drosophila homolog to mammalian TGF-α, functions as a ligand of DER (Schwetz et al., 1995). Because DER is also expressed in Ch organ precursors (Sturtevant et al., 1994) and its loss of function mutation causes a reduction in the number of Ch organs just as in the spitz group mutations (M.O. and H.O., unpublished results), the spiz gene product may act on Ch organ precursors through the DER signaling pathway as well as adult photoreceptor development. argos also acts as a negative regulator for downstream components in the DER signaling pathway, such as Ras1 and rolled (Simon et al., 1991; Biggs et al., 1994; Brunner et al., 1994) during adult eye and wing vein development (K. Sawamoto, M. Okabe, T. Tanimura, K. Mikoshiba, Y. Nishida, and H. Okano, submitted for publication). This suggests that argos, spitz group genes, and DER probably closely interact during the regulation of the number of Ch organ precursors.

In conclusion, spitz group genes act to generate three Ch organ precursors in each abdominal segment, whereas argos may inhibit the signaling pathway mediated by spitz group genes to regulate the final number of Ch organ precursors.

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