Posterior localization of the Drosophila Giα protein during early embryogenesis requires a subset of the posterior group genes

WILLIAM J. WOLFGANG* and MICHAEL FORTE
Vollum Institute, Oregon Health Sciences University, Portland, Oregon, USA

ABSTRACT  Shortly after fertilization in Drosophila embryos, the G-protein α subunit, Giα, undergoes a dramatic redistribution. Initially granules containing Giα are present throughout the embryonic cortex but during nuclear cleavage they become concentrated at the posterior pole and are lost by the blastoderm stage. Mutations that eliminate anterior structures bicoid, swallow, and exuperantia did not prevent the posterior accumulation of Giα. Likewise, embryos from mothers with dominant gain of function mutations in the Bicaudal D gene show normal polarization of Giα granules. By contrast, a subset of mutations which eliminate posterior structures, cappuccino, spire, staufen, mago nashi, valois, and oskar, prevented the posterior accumulation of Giα. It is important to note that mutations in posterior genes lower in the putative hierarchy vasa, tudor nanos, and pumilio did not affect Giα redistribution. From these results we conclude that Giα redistribution to the posterior pole depends on maternal factors involved in the localization of the posterior morphogen nanos.

KEY WORDS: Drosophila, G-protein, localization, embryo, polarized

Introduction

G protein-coupled signal transduction represents the most diverse and evolutionarily ancient mechanism of transmembrane signaling. This process utilizes a receptor that modulates the activity of intracellular second-messenger systems through the activation of a limited set of intermediary GTP-binding or G proteins. In metazoans, receptors for a wide variety of extracellular signals are all coupled to G proteins and subsequently modulate a variety of effector molecules that synthesize cytoplasmic second messengers or act as ion channels (for review see Neer, 1994, 1995). All G proteins that mediate transmembrane signaling exist as a complex composed of α, β, and γ subunits in their inactive state. In this state, the α subunit contains a bound GDP and the complex associates with a vast array of receptors that have been traditionally characterized as having seven transmembrane domains. When ligand binds to its receptor, the receptor activates one or more G proteins by promoting the exchange of GTP for GDP on the α subunit and the dissociation of α from βγ. Termination of the signal occurs when GTP bound by the α subunit is hydrolyzed to GDP by an enzymatic activity intrinsic to the α subunit (Bourne et al., 1991). Although βγ subunits also have been demonstrated to directly modulate a number of intracellular effector pathways (Clapham and Neer, 1993), the key role of the α subunit of G proteins is to mediate and coordinate signaling from a vast array of receptors to appropriate regulation of effector molecules (Neer, 1995). In addition, since a single receptor activates many G proteins, α subunits are also responsible for amplifying signals from receptors and then directing them to the correct effectors. α subunits can then be thought of as signal integrators and molecular “on/off switches”; central control points regulating and integrating the opening and closing of signaling pathways that result in the modulation of general cellular metabolism, cellular differentiation and cell growth.

In Drosophila 6 known G-protein α subunits have been cloned with an eye to using mutational analysis to reveal novel functions in the context of an intact multicellular organism (reviewed in Forte et al., 1993; Quan et al., 1993). Wolfgang et al. (1991) described the embryonic pattern of expression of three α subunits, Gs, Go, and Gi. Each displayed a specific temporal and spatial pattern in the embryo indicating the potential for unique developmental functions for each subunit. Because of the known importance of polarization of macromolecules and cell signal transduction in establishing embryonic polarity and position (St. Johnston and Nüsslein-Volhard, 1992), we found the rapid polar-

Abbreviations used in this paper: bcd, bicoid; BicD, Bicaudal D; cap, cappuccino; exu, exuperantia; mago, mago nashi; nos, nanos; osk, oskar; pum, pumilio; stau, staufen; sse, swallow; tud, tudor; vas, vasa; vl, valois; Giα, G-protein α subunit; HSP, heat shock protein.

*Address for reprints: Vollum Institute, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park RD, Portland, OR 97201, USA. FAX: 503-4944876.
Fig. 1. Gia distribution in cleavage stage embryos. A 0-3 h collection of embryos were double stained for Gia and nuclei. Each embryo was classified as having a uniform, intermediate or polarized distribution of granules and the number of nuclei noted. The embryo at the top of each histogram illustrates the Gia distribution of the class of embryos counted for the underlying histogram. Among embryos with 1 or 2 nuclei, 66% had uniform, 20% intermediate and 15% polarized granule distribution, whereas, among embryos with 8 or more nuclei, 8% had uniform, 32% intermediate and 60% polarized granule distribution. Thus, initially Gia containing granules are distributed uniformly and then during the early cleavage stage become restricted to the posterior pole. However, this transition is not tightly linked to one nuclear stage and appears to be a gradual process. Polarization is always completed by the syncytial blastoderm stage. Actual number of embryos counted are in the bars.

The localization of gene products lower in the list depends on the normal function of gene products higher in the list (Ephrussi et al., 1991). - , disrupted localization; + , normal localization. * Not all the genes tested in the paper are listed as their position in the hierarchy is unknown or controversial.

**TABLE 1**

<table>
<thead>
<tr>
<th>Maternal Mutant</th>
<th>Gia Localization</th>
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<tr>
<td>cap/spir</td>
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The localizing of granules containing Gia to the posterior pole in early embryos intriguing.

Prior to fertilization, Gia protein is uniformly distributed in the ooplasm but, upon fertilization, becomes concentrated in granules in the cortex of the embryo (Wolfgang et al., 1991). This pattern persists until roughly the 8 nuclei stage when the granules are restricted to the posterior pole. By the syncytial blastoderm stage, the few remaining granules become localized at the boundary between the yolk and the cytoplasm at the posterior pole. After the blastoderm stage the granules are no longer detected. Thus, the restriction of Gia to the posterior pole is rapid, transient and coincides with the time when maternal factors are establishing embryonic position and polarity.

In *Drosophila*, the early embryonic pattern is established by the asymmetric distribution of macromolecules in the oocyte and zygote. Mutational analysis (assaying for the loss of anterior or posterior embryonic structures) has identified many of these molecules and to a certain extent the mechanisms by which they become localized and function (St. Johnston and Nüsslein-Volhard, 1992). Thus, the anterior determinant, bicaudal, will not be properly localized if the mother is mutant for either stau, exuperantia, and swallow (Frohnhöfer and Nüsslein-Volhard, 1987; Berleth et al., 1988; Stephenson et al., 1988). By contrast, the posterior determinant, nanos (Wang and Lehmann, 1991), is mislocalized if the mother carries any one of at least eight different mutations in what are collectively known as posterior group genes (cappuccino, spire, stau, vasa, tudor, valois, mago nashi) (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Manseau and Schüpbach, 1989; Lasko and Ashburner, 1990; Boswell et al., 1991; St. Johnston et al., 1991; Lehmann and Nüsslein-Volhard, 1991). Furthermore, the posterior localization of HSP 83 protein (Ding et al., 1993), cyclin B mRNA (Raft et al., 1990), and components of the posterior group
themseves stau. (St. Johnston et al., 1991) osk, (Ephrussi et al., 1991) tud (Bardsley et al., 1993) and vas (Hay et al., 1990; Lasko and Ashburner, 1990) as well as nos (Wang and Lehmann, 1991) have been shown to require the normal function of posterior group genes.

This sort of mutational analysis, observing the consequences of a single mutation on the distribution or function of a particular macromolecule has begun to reveal the hierarchical organization of biochemical elements in early Drosophila development (Table 1). For this reason we wished to determine the impact, if any, on Gia distribution of the same constellation of gene products known to be required for the correct localization in the early embryo of other molecules.

In this report, we have tested whether Gia localization depends on the same biochemical mechanisms required for posterior localization of the nanos morphogen by examining the Gia distribution in a number of well characterized maternal effect mutants altering the anterior-posterior pattern of the embryo. We find that mutations that eliminate anterior structures (bicoid, swallow, and exuperantia) have no effect on Gia redistribution. By contrast, a subset of the mutations that block formation of posterior structures also block redistribution of Gia containing granules (Table 1). Some of these mutations, cappuccino, spire, and stau/fen are known to be involved in the localization of pole plasm constituents but are not themselves the actual posterior determinants (St. Johnston and Nüsslein-Volhard, 1992). From these results we conclude that the restriction of Gia containing granules to the posterior pole requires a functional system for localizing posterior embryonic determinants.

**Results**

**Gia distribution in wild type embryos**

Previous studies showed that shortly after egg laying, uniformly distributed Gia granules become restricted to the posterior pole early during embryonic cleavage stages (Wolfgang et al., 1991). In order to document the time course of this process more accurately, the distribution of Gia containing granules was detected immunohistochemically followed by propidium iodide staining in the same embryos to allow accurate nuclear counts. In Fig. 1, the number of embryos containing 1 through 8 or more nuclei is shown for each of three classes of embryos containing: (1) uniformly distributed granules; (2) partially polarized granules; and (3) fully polarized granules. The results indicate that in a population of embryos the number of embryos containing polarized granules gradually increases during the first four nuclear divisions but clearly is not tightly regulated with respect to when cleavage stage the polarization occurs. However, after the fourth nuclear division, most embryos (92%) have the polarized or partially polarized appearance. The polarization process is always completed by the syncytial blastoderm stage.

**Anterior class mutants**

Correct localization of the anterior determinant, bicoid, requires the products of at least the exuperantia, and swallow genes (Frohnhofer and Nüsslein-Volhard, 1987). To determine if the redistribution of the Gia protein was affected by mutations in these genes, embryos from mothers homozygous for strong mutations in the bicoid, swallow, or exuperantia genes were collected and stained for Gia containing granules. In each case, the distribution of Gia granules was indistinguishable from wild type mothers or mothers heterozygous for the tested mutation (Fig. 2); i.e. embryos containing both uniform, partially polarized, and polarized distribution of granules were observed and granules were lost by the end of the blastoderm stage. Thus, genes in the pathway responsible for the correct localization and function of the anterior morphogen bicoid do not affect the redistribution of the Gia containing granules.

**Posterior class mutants**

Localization of nanos RNA, the posterior morphogen, requires the function of at least 9 different genes (cappuccino, spire, stau/en, oskar, vasa, tudor, valois, mago nashi, pumilia) (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüppach and Wieschaus, 1986; Manseau and Schüppach, 1989; Lasko and Ashburner, 1990; Boswell et al., 1991; St. Johnston et al., 1991). To test the role of genes in the posterior group in the redistribution of Gia containing granules, embryos collected from females homozygous for strong mutations in each of the genes in this class were examined. Females with mutations in the cappuccino, spire, stau/en, oskar and mago nashi genes produced embryos with uniformly distributed Gia granules which were lost at the blastoderm stage and never became localized to one pole (Fig 3a-d). Heterozygous mothers for these mutations produced embryos with a wild type granule distribution. In syncytial blastoderm stage, embryos from females containing mutations in this group have Gia granules that become concentrated at the boundary of the yolk and cytoplasm around the whole embryo and not just at the posterior pole (particularly evident in mago mutations). By contrast, in embryos from mothers homozygous for vasa, tudor, nanos, and pumilia mutations, the Gia distribution pattern was similar in appearance to embryos from mothers heterozygous or wild type for the tested mutation (Fig. 3e-h). Some oska$i^{64}$ embryos contained weakly polarized Gia granules (Fig. 3i) but in the majority of embryos, the granules were uniformly distributed (Fig. 3d). Since oska$i^{64}$...
disrupts staufen protein localization, we examined a second strong oskar allele, oskar<sup>644</sup>, which does not disrupt staufen localization (St. Johnston et al., 1991). These embryos displayed the same phenotype as osk<sup>644</sup> suggesting that the oskar phenotype results from disruption of osk and not staun mislocalization. Valois embryos contained fewer polarized embryos than controls (Fig. 3i) and like osk<sup>644</sup>, in the few embryos that contained polarized granules, granules were not concentrated at the posterior pole to the same extent as controls. Embryos produced by bicoid/nanos double mutants contained fully polarized granules (Fig. 3k).

**Bicaudal D embryos**

In embryos mutant for the dominant gain of function mutation Bicaudal D, a mirror image duplication of the abdomen is formed from the anterior end of the embryo (Mohler and Wieschaus, 1986). Because Gi<sub>x</sub> containing granules become associated with the posterior pole in cleavage stage embryos, we determined whether the polar localization of Gi<sub>x</sub> would also be duplicated in Bicaudal D embryos. Compared to wild type controls, Bicaudal D embryos showed a wild-type distribution of granules (Fig. 4a). In embryos in which pole cells were present, localization was to the true posterior pole and not the transformed anterior pole.

Recently transformants have been described in which oskar gene sequences have been attached to the bicoid 3' UTR containing sequences required for the anterior localization of the bicoid morphogen (mob transformants, Webster et al., 1994; osk-bcd 3' utr, Ephrussi and Lehmann, 1992). This results in mislocalization of oskar to the anterior pole and the production of bicaudal embryos with anterior pole cells. However, as described above for Bicaudal D mutations, Gi<sub>x</sub> distribution was normal in mob transformant embryos (Fig. 4b).

**Discussion**

In this study we confirmed and extended our initial observation that Gi<sub>x</sub> granules come to reside at the posterior pole during the first 4 nuclear cleavage cycles and then are lost during the blastoderm stage. During this time, maternally provided information is being interpreted to establish the anterior-posterior and dorsal-ventral embryonic axes (St. Johnston and Nüsslein-Volhard, 1992). In this report, we show that restriction of Gi<sub>x</sub> granules to the posterior pole of the embryo depends on a subset of the maternal genes required for localization of posterior determinants (Table 1). This is significant because it links control of the distribution of Gi<sub>x</sub>, a signal transduction molecule, to the biochemical pathway that establishes the embryonic posterior pole.
The mutations, bcd, sww, and exu that cause the loss of anterior structures, had no effect on Giα distribution. By contrast, mutations in a subset of the posterior group genes, (cap, spir, stau, osk, mago, and vis) which eliminate posterior structures, disrupt polarization of Giα. Mutations in other members of this group which eliminate posterior structures (vas, tud, nos, pum), had no effect on Giα redistribution as did mutations in the BicD locus or mob transients. No mutations resulted in the absence of Giα containing granules in early cleavage stage embryos or blocked their loss at the blastoderm stage, indicating that synthesis and degradation are regulated by mechanisms other than polarization.

The posterior class mutations have been divided into two groups, based largely on their interaction with the BicD locus. When cap, spir, or stau are placed in a BicD background, an abdomen forms at the anterior end but no abdomen forms at the posterior end. By contrast, when osk, vas, tud and nos are placed in a BicD background neither anterior nor posterior abdomens form. These observations, as well as the results of other experiments in which osk or nos mRNA is mislocalized (Ephrussi and Lehmann, 1992; Gavis and Lehmann, 1992; Smith et al., 1992; Webster et al., 1994) suggest that cap, spir, and stau are required for the correct transport and/or anchoring of posterior pole plasm components, whereas, osk, vas, tud, vis and nos are required for production of the activities that induce the formation of germ cells and the abdomen. In this context, our results show that Giα polarization depends on genes involved in the localization of pole plasm components (cap, spir, stau) but not on genes required for the production of an active posterior pole plasm, with the exception of osk. This suggests that Giα polarization uses, at least in part, the same machinery as other pole plasm components to become restricted to the posterior pole but does not require an active posterior center. The fact that in bcd/nos double mutants Giα polarization is normal (Fig. 3k) demonstrates that neither the anterior nor posterior morphogen are required. The normal distribution of Giα containing granules in Bic D and mob embryos in which an active posterior center is mislocalized to the anterior pole is consistent with this conclusion.

osk gene products are not believed to participate in global localization processes, yet with two different alleles no or partial polarization of Giα granules is observed. Three plausible explanations are outlined below. 1) The partial localization observed in osk may result from some partial function of the mutant gene product. For osk166, which is a single amino acid substitution, this is possible but for osk1991 this is unlikely as the open reading frame is truncated by a stop codon (Kim-Ha et al., 1991) presumably producing a functionally null gene product. 2) In the absence of osk gene function, transport and localization of Giα is largely blocked with a redundant gene product providing the weak and variable polarizing activity observed. 3) osk may be required for stabilization of Giα granules to the posterior but not the initial transport and localization. A similar stabilizing role for osk has been proposed in the posterior localization of staufen protein (St. Johnston et al., 1991) though the mechanism would be different since osk166 but not osk64 stabilizes staufen whereas neither allele stabilizes Giα to the posterior.

The intermediate phenotype produced in valois embryos (i.e., weak polarization of granules) suggests that polarization may be occurring but cannot be maintained. This is quite similar to the effect of valois mutations on vasa protein which is initially polarized in vis mutants, but the polarization is not maintained (Hay et al., 1990).

Our results demonstrate that the normal restriction of Giα to the posterior pole in cleavage stage embryos depends on a subset of maternal gene products involved in the transport and/or localization of pole plasm components. Furthermore, maintenance of the polarized state may depend on osk and val whose gene products are required for pole plasm activity. Whether there is a direct dependence of Giα localization on these identified maternal gene products, or whether the mislocalization of Giα occurs because of the absence of some other factor whose function in turn depends on maternal gene products we examined, is not known.

Materials and Methods

Stocks

Stocks were maintained and eggs collected and aged at room temperature or 25°C. Canton S strain were used as wild type controls. Anterior class mutants obtained from the stock center were bicoid1, swallow1, and exuperantia2. Posterior group mutants kindly provided by Dr. Ruth Lehmann were cappuccino6, spire6, staufen50, oskar64 (eggs collected from oskaredf / Df (3R)p8083 females), vasaD1, vasaD2 (eggs collected from vasaD1 / vasaD2 females), tudorW2, valois59 (eggs collected from valoisD50 / Df (2R) TW2 females), nanos57, pumilio6600. Dr. Paul MacDonald kindly provided the oskaredf allele and the mob transients. Dr. Robert Boswell kindly provided the mago nashi1 flies. Bicaudal embryos were collected from BicD1 / BicD2 mothers.

Staining

Antibody staining for Giα was as described in Wolfgang et al. (1991). For propidium iodide staining of nuclei, after fixation but prior to initiation of antibody staining, embryos were incubated for 2 h at 37°C in 10 ug/ml RNase. Antibody staining was then completed and embryos mounted in 80% glycerol, 0.02 M Tris (pH 7.7), 1.25 ug/ml propidium iodide. The propidium iodide was viewed with the rhodamine filter set.

Acknowledgments

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References


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