Sec61β, a subunit of the protein translocation channel, is required during Drosophila development

Rafael Valcárcel¹, Ursula Weber¹, David B. Jackson¹, Vladimir Benes², Wilhelm Ansorge², Dirk Bohmann¹ and Marek Mlodzik¹,*

¹Developmental Biology and ²Biochemical, Instrumentation Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany
*Author for correspondence (e-mail: mlodzik@embl-heidelberg.de)

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SUMMARY

We have identified and isolated mutations in the first Drosophila gene encoding a subunit of the Sec61 protein translocation channel, DSec61β. While neither the Saccharomyces cerevisiae Sec61β nor its functional Escherichia coli homologue are essential for viability or for protein translocation, we show that DSec61β is essential for embryonic development. Homozygous mutant embryos die at the end of embryogenesis and are impaired in the secretion of cuticle proteins from the epidermis. DSec61β germ line clones, result in defects in dorso-ventral patterning of the egg and are consistent with affected secretion of the protein Gurken from the oocyte to the follicle cells. Clonal analyses in the imaginal discs reveal defects in adult structures, including rhabdomere morphogenesis and a reduction of the size of tarsal segments in the leg. This is the first in vivo study of a component of the protein translocation machinery in higher eukaryotes, and illustrates how a protein that has an inessential, kinetic function in single-cell organisms can become critical for the complex development of a multicellular organism.

Key words: Drosophila, Sec61β, Protein translocation, Secretion, Cuticle, Oogenesis, Gurken

INTRODUCTION

The initial step in the biogenesis of most extra-cellular (and many organellar proteins) in eukaryotic cells is their translocation into the endoplasmic reticulum (ER). Translocation occurs through a hydrophilic channel that has been conserved throughout evolution, and which is part of a protein translocation machinery (for review see Matlack et al., 1998; Zimmermann, 1998; Rapoport et al., 1996). The channel itself has been proposed to be a passive conduit for polypeptides. Other proteins that associate with the channel are thought to provide the driving force for translocation and to determine its directionality.

The protein-conducting channels of the ER in eukaryotes are composed of oligomers of the Sec61p complex, consisting of three transmembrane subunits: Sec61α, Sec61β and Sec61γ (Görlich and Rapoport, 1993; Hanein et al., 1996). Sec61α is the cognate of Sec61p of S. cerevisiae (Görlich et al., 1992), and Sec61γ is homologous to the yeast protein SSS1. Sec61α and yeast Sec61p show significant similarity to SecY, a key component of the protein export apparatus of E. coli. Like Sec61α, SecY is associated with two other proteins, SecE and SecG, which are the functional homologues of Sec61γ/SSS1 and Sec61β, respectively.

The high conservation of Sec61α/SecY and Sec61γ/SecE homologues suggests that these are critical components of the Sec61p complex (Hartmann et al., 1994). Similarly, oligomeric ring-like assemblies (likely representing protein-conducting channels) in Bacillus subtilis consist of SecY and SecE and are similar to those formed by eukaryotic Sec61p complexes (Meyer et al., 1999). SecY and SecE are sufficient to form a translocationally active complex. These two subunits of the Sec61p/SecYp complexes are thus key components of the protein translocation machinery in all organisms studied. Consistent with this, Sec61p, the S. cerevisiae Sec61α homologue, is essential for cell viability: null mutations in this gene are lethal (Deshaies and Schekman, 1987). A second Sec61α yeast homologue (Ssh1p), however, is only required for normal growth rates, but is not essential (Finke et al., 1996). Sss1, the Sec61γ homologue, is an essential gene whose overexpression restores translocation in Sec61p mutants (Esnault et al., 1993). Depletion of the Sss1 protein results in rapid accumulation of a variety of secretory or membrane proteins devoid of post-translational modifications. Thus, Sss1 is required for continued translocation of secretory pre-proteins.

Genetic and biochemical studies of Sec61β in S. cerevisiae suggest that it is required to facilitate translocation of proteins into the ER, but it is not essential for the process (Finke et al., 1996; Panzner et al., 1995; Toikkanen et al., 1996; Kalies et al., 1998). Genetic screens in yeast have failed to identify either of the two Sec61β homologues via a mutant phenotype. The first homologue (Shb1/Shb1) was identified biochemically (Panzner et al., 1995), and the second (Shb2/Shb2) was
identified by heterologous hybridization (Toikkanen et al., 1996) and a homology search (Finke et al., 1996). Both Sec61β homologues can be simultaneously disrupted without an effect on viability. The only phenotype exhibited by such double mutants is a temperature sensitive growth defect (Finke et al., 1996; Toikkanen et al., 1996). Closer analysis revealed that at the restrictive temperature the mutant cells accumulate precursors of secretory proteins, and microsomes isolated from these cells exhibit a reduced rate of post-translational protein transport.

Biochemical studies have also suggested a non-essential role for Sec61β in mammals. In a reconstituted system, if sufficient time is given for the interaction of the nascent polypeptide with the mutant Sec61 complex, translocation can be almost normal in the absence of Sec61β. However, a Sec61 complex lacking Sec61β is largely inactive when elongation and membrane targeting of a nascent polypeptide chain have to happen at the same time. The translocation process is perturbed at a step where the nascent chain would be inserted into the translocation channel. Thus Sec61β kinetically facilitates cotranslational translocation but it is not essential for the process (Kalies et al., 1998). Although there is no amino acid sequence conservation between eukaryotic Sec61β and bacterial SecG, these seem to be functionally homologous components of protein-conducting channels. SecG from E. coli stimulates the protein translocation activity when reconstituted in proteoliposomes with SecY, SecE, and SecA (the ATP-dependent motor protein for translocation; Nishiyama et al., 1999). Thus, in bacteria and eukaryotes the Sec61 homologues can be simultaneously disrupted without an effect on viability. The only phenotype exhibited by such double homozygous embryos (both at stage 17; Campos-Ortega and Hartenstein, 1985) were dechorionated, the vitelline membrane was dissected manually and embryos were punctured to facilitate fixation, which was carried out overnight at RT (PBS containing 1% glutaraldehyde). Embryos were postfixed with 1% osmium tetroxide, which was carried out overnight at RT (PBS containing 1% glutaraldehyde). Embryos were postfixed with 1% osmium tetroxide, dehydrated in a standard series up to 70% ethanol and incubated with 1% glutaraldehyde for 1 hour, followed by a second fixation with 1% osmium tetroxide for 1 hour. Embryos were then dehydrated in a standard series of acetone and embedded in Epon812. Thin sections were stained with Reynold's lead citrate.

**Fly strains**

The P-element induced mutation DSec61β was identified in a P-element collection of second and third chromosome lethal inserts (Guichet et al., 1997; Erdelyi et al., 1995), as line 14448. To confirm that the lethal phenotype of DSec61β was linked to the P-element insertion point in the Drosophila Sec61β gene, DSec61β (the only Sec61 homologue described in Drosophila so far), and analyzed their phenotypic effects in distinct developmental processes. DSec61β mutant embryos die at the end of embryogenesis with a severe defect in the deposition of cuticle proteins. Germ line clones of DSec61β result in defects in dorsal-ventral patterning of the egg, a phenotype that closely resembles that of hypomorphic DSec61β alleles. Mutant clones of DSec61β in the eye and the leg show morphological defects. The DSec61β mutant phenotypes demonstrate the importance of this Sec61 subunit in the context of the development of multicellular organisms.

**MATERIALS AND METHODS**

**Fly strains**

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**Molecular analysis**

DNA sequences flanking the P-element insertion were recovered using the plasmid rescue method (Wilson et al., 1989). The cDNA was obtained as EST clone LD09383 from the BDGP. DNA and protein sequences were analyzed using the Lasergene Navigator DNA STAR program and the BLAST service of the BDGP. For rescue, the LD09383 cDNA was cloned behind the tubulin promoter (tub-β) in a standard Drosophila transformation vector (Casper, kindly provided by Stephen Cohen). The genomic sequence of the DSec61β gene is available from the BDGP (accession number AC005646). By comparing the DSec61β cDNA with the genomic sequence from the BDGP, a map of the intron-exon organization of the DSec61β gene can be drawn as in Fig. 1A. Sequence analysis suggest a transcription start site 29 bp upstream of the P-element insertion point in the DSec61β allele. The insertion point of DSec61β maps upstream of this putative start site, which might explain the weaker phenotypes associated with this allele (see below).

**Clonal analysis**

Homozygous clones of the DSec61β allele were generated with the FLP/FRT system (Xu and Rubin, 1993). Eye clones were marked by the increased levels of pigment (cells containing two copies of P{lac-W} (Bier et al., 1989) can be distinguished from the surrounding tissue). Wing and leg clones were marked by loss of the cell autonomous bristle and trichome marker forked. The clones were then scored for eye phenotypes in adult flies. The clones were marked by the increased levels of pigment (cells containing two copies of P{lac-W} (Bier et al., 1989) can be distinguished from the surrounding tissue). Wing and leg clones were marked by loss of the cell autonomous bristle and trichome marker forked. The clones were given a growth advantage using the Minute technique (Morata and Ripoll, 1975). Standard histological methods were used to section adult eyes (Tolmach and Read, 1987). Mounting of legs and wings was performed by standard methods (Wieschaus and Nüsslein-Volhard, 1986).

**Germ line mosaics**

The germ line clones of DSec61β were generated using the FLP recombinase dominant female sterile technique adapted for the second chromosome (Chou and Perrimon, 1996). Females with germ line clones were mated either with Ore-R or with DSec61β/CyO, Wg-lacZ males. The distribution of the egg shell phenotypes was very similar in both cases.
RESULTS

Identification of the DSec61β alleles

Analysis of embryonic cuticles from a P-element collection of second and third chromosome lethal inserts (Guichet et al., 1997; Erdelyi et al., 1995), directed our attention to line 14448, a late embryonic lethal resulting in almost transparent cuticles (see below). The lethality and cuticle phenotype is revertible by P-element excision.

The sequences flanking the P-element (inserted at 51B10-11 on chromosome 2R) were obtained by plasmid rescue, and their conceptual translation showed 97% amino acid identity to the human Sec61β protein (henceforth the P-insertion is referred to as DSec61βP1). Several corresponding ESTs were detected in the BDGP EST database. The full-length cDNA was sequenced and the predicted amino acid composition is highly homologous to the human Sec61β protein (90.9% amino acid similarity and 67.7% identity; Fig. 1B). A phylogenetic tree showing the relationship of DSec61β with its homologues in different organisms is shown in Fig. 1C.

Functional prokaryotic homologues do not show significant sequence conservation.

Additional P-element insertions located in the same chromosomal area were tested for complementation with DSec61βP1. Two P-insertions, l(2)07214 and l(2)k07836 did not complement DSec61βP1 (subsequently referred to as DSec61βP2 and DSec61βP3, respectively). In addition, Df(2R)03072, which removes DSec61β genomic sequence (see Materials and Methods), does not complement any of the three P-alleles.

The lethal phenotype of the three DSec61β P-alleles is rescued by ubiquitous expression of DSec61β cDNA under the control of the tubulin promoter (tub-DSec61β), demonstrating that the lethality associated with the P-insertions is caused by a reduction or loss of DSec61β function (data not shown).

DSec61β is embryonic lethal

Embryos homozygous for DSec61βP1 die at the end of embryonic development (stage 17 according to Campos-Ortega and Hartenstein, 1985) with a very thin cuticle in which the denticle belts are hardly visible (Fig. 2). Even when the vitelline membrane from late homozygous DSec61βP1 embryos was removed, the embryos (which could hardly move) died after few minutes. Mendelian distribution analysis revealed that all homozygous embryos die. Despite the almost
transparent cuticle, careful microscopic analysis did not reveal any obvious patterning defects.

The DSec61βP1 allele is a strong allele (possibly a null) because its phenotype over the deficiency (DSec61βP1/Df(2R)03072) is very similar to that of homozygous DSec61βP1 embryos (compare Fig. 2B and C). Embryos homozygous for two other alleles, DSec61βP2 and DSec61βP3, can also show cuticles similar to that of DSec61βP1 embryos, but in many cases their defects are moderate (not shown).

**DSec61β mutants show severe defects in the secretion of cuticle proteins**

To assess the morphological defects at a cellular level, electron microscopic analysis of the cuticle of DSec61βP1 mutant embryos was performed. Secretion of cuticle from the epidermis starts around 12 hours after egg laying (AEL), and the final form of larval cuticle is complete by 16 hours AEL (Harrison et al., 1994; Poodry, 1980; Hillman and Lesnik, 1970). Drosophila epidermis actively secretes cuticle at the end of stage 15 (11:20 to 13 hours AEL) and during stage 16 (13 to 16 hours AEL; Campos-Ortega and Hartenstein, 1985). At the time of hatching, the wild-type cuticle is characterized by three distinct layers clearly discernible by electron microscopy: two layers of epicuticle and the endocuticle (Fig. 3A). The epicuticle is composed of cuticulin (labeled ‘c’ in Fig. 3), a trilaminar structure possibly made up of cross-linked hydrocarbons, and underneath it the protein epicuticle, which is densely granular (labeled ‘pe’ in Fig. 3). The protein epicuticle is secreted by exocytosis from the Golgi complex. Further below, a layer of endocuticle (labeled ‘e’ in Fig. 3) is composed of a thick complex of chitin fibers and associated proteins.

DSec61βP1 homozygous embryos display a dramatically different cuticle compared to wild type. The protein epicuticle layer is absent in the mutants and the thickness of the endocuticle is reduced (Fig. 3B). Microvilli (m) are seen at this late developmental stage (stage 17 according to Campos-Ortega and Hartenstein, 1985) only in the DSec61βP1 mutant embryos (Fig. 3B). Taken together with the high homology to mammalian Sec61β, these defects probably arise as a consequence of secretion defects in the DSec61β mutant (see Discussion).

Other defects (probably also related to failure in secretion of cuticle proteins) can be detected in DSec61βP1 embryos. The characteristic distribution of the tracheal conduits in wild-type embryos (Manning and Krasnow, 1993) is dramatically affected in homozygous DSec61βP1 embryos (data not shown). However, there are secreted proteins which do not seem to be modulated by the secreted factors proposed in which Wingless is largely unaffected (data not shown). It is possible that maternal DSec61β is responsible for the survival of the DSec61βP1 embryos until stage 17. To study the developmental requirements for DSec61β in the absence of the maternal contribution we analyzed DSec61βP1 germ line clones (see below).

**Eggs derived from DSec61β germ line clones show dorso-ventral patterning defects**

The dorsal-ventral polarity of the Drosophila egg chamber depends on the localization of the oocyte nucleus and the gurken (grk) RNA to the dorsal-anterior corner of the oocyte. Grk protein belongs to the transforming growth factor a (TGFa) family of secreted growth factors and initiates, during mid stages of oogenesis, a cell-cell communication process between the oocyte and the surrounding layer of somatic follicle cells. The follicle cells provide yolk to the oocyte, secrete the egg shell, and transmit patterning information required for the development of the future embryo. Grk protein acts as a ligand for the Drosophila EGF receptor (torpedo/Egfr) expressed in the somatic follicle cells surrounding the oocyte. A model for dorsal specification and patterning has been proposed in which Egfr is activated by Grk. This effect is modulated by the secreted factors Spitz and Argos (Wasserman and Freeman, 1998; Neuman-Silberberg and Schupbach, 1996; Peri et al., 1999). cornichon (cni) is a gene required in the germ line for dorsal-ventral signaling (Roth et al., 1995). In light of the presumed role of DSec61β as a component of the protein translocation machinery, it is interesting that cni has a homologue in yeast Erv14p (ER-vesicle protein of 14 kDa), which has been proposed to be required for the export of specific secretory cargo from the ER (Powers and Barlowe, 1998).

Grk-induced activation of the Egfr results (among other developmental readouts) in the formation of dorsal appendages (Roth et al., 1995; Neuman-Silberberg and Schupbach, 1996; Wasserman and Freeman, 1998; Peri et al., 1999). Two clearly separated dorsal appendages (da) form in wild-type eggs (Wasserman and Freeman, 1998). Females homozygous for grk, cni, top, spi or argos hypomorphic alleles lay eggs with shells that have either partially or completely fused dorsal appendages (Roth et al., 1995; Wasserman and Freeman, 1998). When Argos or Spitz are completely removed from the
Sec61β is required for Drosophila development

**Table 1. Distribution of eggshell phenotypes in DSec61β germ line clones**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Wt</td>
<td>1.7%</td>
</tr>
<tr>
<td>Interspace reduced</td>
<td>7.6%</td>
</tr>
<tr>
<td>Partially fused</td>
<td>27.9%</td>
</tr>
<tr>
<td>Totally fused</td>
<td>62.8%</td>
</tr>
<tr>
<td>Total defects</td>
<td>98.3%</td>
</tr>
<tr>
<td>Number of embryos scored</td>
<td>248</td>
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</table>

The percentages shown correspond to the crossing of Ore-R males to females with DSec61β germ line clones. Dorsal appendages are separated by a reduced distance (interspace reduced), partially fused or completely fused. This high frequency indicates that these are germine defects. Occasional clones in the follicle cells could not account for such a high frequency.

DSec61β2 had previously been identified in a screen for zygotic lethal mutations with maternal effect phenotypes in Drosophila (Perrimon et al., 1996). Embryos derived from germ line clones for this allele showed also poor cuticle development. DSec61β2 has been reported to be lethal at the pupal stage (Perrimon et al., 1996), whereas the DSec61β1 allele is embryonic lethal in all homozygous embryos.

Strikingly, the oogenesis/egg shell phenotypes of DSec61β mutants are reminiscent of hypomorphic grk and cni alleles as described above (see also Discussion). In conclusion, the patterning of the dorsal-ventral axis of the egg shell, which depends on the secretion of Grk from the oocyte to the follicle cells, is deficient in DSec61β mutants. This observation is consistent with a reduction of the amount of Grk secreted in DSec61β1 germ line clones.

**Analysis of developmental defects in adult structures induced by DSec61β1 mitotic clones**

In order to elucidate whether DSec61β is required during the development of adult structures, mutant clones of DSec61β1 were analyzed in adult flies. The FRT-FLP recombination technique was used (Xu and Rubin, 1993) to generate clones marked either by differences in eye pigment or by forked bristles in the cuticle. Approximately 5% of the ommatidia either lack photoreceptors or show morphological defects in the mutant eye clones. Often the number of rhabdomeres detected is lower in apical sections than in more basal sections (Fig. 5, compare A and C with B and D), suggesting defects in the differentiation of mutant photoreceptors that cause the rhabdomeres not to form over the entire apical-basal extent of the retina. Only rarely are photoreceptors completely missing. To determine if DSec61β plays a role in early cell fate determination during eye development, DSec61β1 mutant clones were analyzed in the eye imaginal discs with several markers (data not shown). No defects were observed, indicating that the phenotype seen in mutant adult tissue reflects problems during photoreceptor differentiation.

DSec61β1 clones in the wing did not display any visible defects (not shown). Homozygous clones in the leg, however, were associated with a severe size-reduction of the tarsal segments (Fig. 6). The reduction in tarsal segment size often occurred distal to the clonal area, which would be consistent with the mutant cells preventing a secreted factor or a signaling event from reaching the more distal part of the leg. A similar size reduction was seen in transheterozygous individuals of the weaker DSec61β alleles (Fig. 6D), and in homozygous

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Fig. 4. The egg shell phenotypes of embryos derived from germ line clones of the DSec61β1 mutation. Dark-field photographs are shown. (A) Wild-type egg with two well separated dorsal appendages (da). (B-D) Eggs derived from germ line clones of DSec61β1 showing dorsal appendages separated by a reduced distance (B), or with either partially (C) or completely (D) fused dorsal appendages. Similar fusions are observed in eggs from DSec61β1/DSec61β3 escapers (E).

Females with germ line clones were mated either with Ore-R or DSec61β1/CyO,Wg-lacZ males. The distribution of the eggshell phenotypes (see Table 1) was very similar in both cases. The high frequency of defects (98.3% in total) indicates that the eggshell phenotypes are associated with the germ line clones, rather than with mutant clones in other cell types.

No embryos hatched in either the control Ore-R, or the experimental DSec61β1/CyO,Wg-lacZ, crosses. Embryos died at a variety of developmental stages, and only few (2.1% when embryos were grown at 25°C) reached stages 16 or 17 (Campos-Ortega and Hartenstein, 1985), showing a very faint cuticle. Thus, there is no paternal rescue of the ‘thin cuticle’ phenotype, and the lack of DSec61β maternal contribution causes the DSec61β mutant embryos to die at earlier developmental stages. However, the stage of lethality of embryos derived from DSec61β germ line clones varied substantially with the temperature. At 18°C the percentage of embryos that reached late embryonic stages (16 and 17) increased to 34.4%. Such embryos showed no obvious patterning defects, in agreement with the lack of defects in Wingless secretion in the zygotic DSec61β1 embryos (not shown). This temperature effect could be related to the catalytic function of Sec61β as proposed for the yeast homologue (see Discussion).
DISCUSSION

The essential components of the translocation machinery have been identified in different organisms, and the molecular mechanisms by which these core components function and regulate the translocation process are being studied intensively (for review see Hegde and Lingappa, 1999). The identification of components of the protein translocation machinery in a developmentally complex but genetically and biochemically approachable organism such as Drosophila are likely to be instrumental in the analysis of its regulation. We have identified the gene encoding the Sec61β subunit of the protein translocation channel into the endoplasmic reticulum in Drosophila. In contrast to the non-essential character of the Sec61β homologue in yeast and of its functional homologues in bacteria, mutations in the DSec61β gene are embryonic lethal and they show a variety of phenotypic effects in the adult revealed by clonal analysis. Below we discuss how this may be explained in terms of defects in protein secretion, and how it might help to understand the function of Sec61β during Drosophila development.

**DSec61β and cuticle secretion**

The flexible cuticle of Drosophila larvae is secreted from the epidermis during embryonic development (Harrison et al., 1994; Poodry, 1980; Hillman and Lesnik, 1970; Martínez Arias, 1993). At the time of hatching, the cuticle is formed by two layers of epicuticle and an endocuticle. The internal layer of the epicuticle is densely granular and accumulates proteins secreted from the Golgi complex. The endocuticle is also composed of secreted proteins and chitin fibers. Protein secretion is therefore a key aspect of the formation of the cuticle.

Several lines of evidence indicate that cuticle protein secretion is abnormal in homozygous DSec61βP1 embryos. First, the protein epicuticle is absent and the thickness of the endocuticle is reduced. Cuticle proteins are expressed from genes located at the 65A cluster and show quantitative and qualitative differences in expression at different developmental stages (Snyder et al., 1982; Chihara et al., 1982; Charles et al., 1997, 1998). A defect in the secretion of these proteins might explain the absence of protein epicuticle layer in DSec61β mutant embryos. Second, the cuticle defects resemble the phenotype associated with mutants in a gene involved in exocytosis, Rop (Ras opposite) (Harrison et al., 1994). A third indication comes from the morphology of the microvillar tips at the surface of the epidermis. These have been proposed to be the points of secretory activity in hypodermal cuticle formation.

**Fig. 5. DSec61β eye phenotype.** Tangential sections through adult eyes containing two different homozygous clones. A/B and C/D are corresponding sections at an apical level (A and C) and a more basal level (B and D). The clones are marked by the increased amount of pigments (cells containing two copies of P{lac-W} can be distinguished from the surrounding tissue). Anterior is left, dorsal is up. Note that the phenotype is more pronounced at the apical level. Ommatidia with a reduced number of rhabdomeres are indicated in red numbers.

**Fig. 6. DSec61β leg phenotypes.** Homozygous DSec61βP1 leg clones (B and C) result in tarsal segments of a reduced size as compared to wild type (A). The clonal tissue is marked forked- bristles (arrows). Note that size reduction in tarsal segments is located distally to clone. Similar leg defects are also observed in DSec61βP2/DSec61βP3 escapers (D).

DSec61βP1 flies, which carry only one copy of the rescue construct tub-DSec61β (not shown).
(Hillman and Lesnik, 1970), and are completely retracted by the end of embryonic development (late stage 17; Campos-Ortega and Hartenstein, 1985). Consistent with alterations in the protein secretion pathway in DSec61β mutants, microvilli (m) are still present at the surface of the epidermis at embryonic stage 17 in mutant embryos, and the thinner endocuticle layer resembles the endocuticle of wild-type embryos at stage 16.

Finally, other cells with active cuticle secretion during Drosophila development are also affected in the DSec61β mutants (Hillman and Lesnik, 1970). During tracheal development cuticle is secreted from the tracheal epithelium (for review see Manning and Krasnow, 1993). The very characteristic distribution of the tracheal conduits in a wild-type embryo is severely affected in homozygous DSec61βP1 embryos (not shown) probably as a consequence of the impaired cuticle secretion in these mutants. Furthermore, while in wild-type embryos the trachea begins to fill with air about 18 hours after egg laying, the trachea of DSec61βP1 embryos failed to fill with air, possibly because they collapsed due to insufficient cuticle deposition.

**DSec61β and Grk secretion**

Gurken protein secretion from the oocyte is required to start a chain of events in the follicle cells which will result in the dorsal-ventral patterning of the egg and the associated formation of the dorsal appendages (Wasserman and Freeman, 1998; Roth et al., 1995; Neuman-Silberberg and Schupbach, 1996; Peri et al., 1999). Hypomorphic grk alleles, as well as hypomorphic alleles of coronichon in which Grk secretion is presumably affected (Roth et al., 1995; Powers and Barlowe, 1998) show fused dorsal appendages. The egg shells derived from DSec61βP1 germ line clones also show fusion of the dorsal appendages.

Grk is secreted from the oocyte (germ line) to the follicle cells. In agreement, the DSec61βP1 defect is detected in germ line clones. Although we cannot rule out at present that other defects in protein secretion also contribute to dorsal appendage fusion, the similarity between the DSec61β and grk phenotypes is striking. Moreover, Cni is homologous to a yeast protein required for secretion, strongly supporting the notion that full extent of Grk secretion is essential for dorsal-ventral patterning of the egg.

**Function of DSec61β**

The yeast Sec61β homologue is thought to facilitate cotranslational protein translocation into the ER (Kalies et al., 1998), and its functional bacterial homologue, SecG, also stimulates protein translocation (Hanada et al., 1994, 1996; Nishiyama et al., 1993, 1994). In neither of these organisms, however, the genes are essential for cell viability. These and other biochemical data suggest that Sec61β-like activities are not strictly required for protein translocation, but they could play a kinetic role in the process. Our observation that an increased percentage of mutant embryos (derived from DSec61βP1 germ line clones) develop further at low temperature (18°C) could be related to the proposed catalytic function of Sec61β. Possibly, deficiencies in DSec61β function are less deleterious at lower temperatures because of the additional time given for the protein translocation process to happen.

Appropriate translocation rates may be critical for the function of particular proteins. The phenotypes of DSec61β could be consistent with failures to cope with high rates of protein export required in specific cells at defined times during Drosophila development. Secretion of proteins during embryonic cuticle formation could be one such process. A lower secretion rate in DSec61β germ line clones would be also consistent with a reduced amount of Grk secreted from the oocyte to the follicle cells, which would result in a phenotype reminiscent to a grk hypomorph (i.e. fusion of dorsal appendages). The lower phenotypic penetrance of DSec61β mutants in the generation of adult organs could be explained by a lower requirement in terms of secretion rates. Alternatively, it is possible that a second, not yet identified gene exists for a related DSec61β protein, which could be responsible for protein translocation in other developmental processes. Similarly, Sec61β could be required for the translocation into the ER of only a particular subset of proteins, like the embryonic cuticle proteins and Grk.

In addition to the effects on the amount of protein secreted, changes in the rate of translocation can also have qualitative effects in the structure, function and topological fate of the translocated protein (for review see Hegde and Lingappa, 1999). Regulation of the translocation rate is known to affect the accessibility of the nascent chain to chaperones and protein modification enzymes. It is therefore conceivable that changes in DSec61β function result in changes in the kinetics of cotranslational folding and/or in different extents of post-translational modification that differentially affect distinct protein substrates. As a consequence, the activity and function of particular polypeptides could be compromised even if their total amount produced is not changed.

Although other components of the protein translocation machinery have been identified in Drosophila, like the homologue of the 19 Kda subunit of SRP (Lai and Langley, 1997) or the homologue of Sec62p (Noel and Cartwright, 1994), no mutants in these genes have been reported. With the identification of DSec61β and the analysis of the phenotypes generated by mutants of this gene, we report the first genetic study of a component of the protein translocation machinery in Drosophila. Our results reveal the important contribution of factors that, without playing an essential role in basic processes or in the viability of unicellular organisms, become critical for the complex development of multicellular organisms. They also underscore the value of experiments in whole organisms to understand the function of the different components of complexes performing basic cellular functions.

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