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The ε-Subunit of Mitochondrial ATP Synthase Is Required for Normal Spindle Orientation During the Drosophila Embryonic Divisions

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ABSTRACT

We describe the maternal-effect and zygotic phenotypes of null mutations in the Drosophila gene for the ε-subunit of mitochondrial ATP synthase, *stunted (sun)*. Loss of zygotic *sun* expression leads to a dramatic delay in the growth rate of first instar larvae and ultimately death. Embryos lacking maternally supplied *sun* (*sun* embryos) have a sixfold reduction in ATP synthase activity. Cellular analysis of *sun* embryos shows defects only after the nuclei have migrated to the cortex. During the cortical divisions the actin-based metaphase and cellularization furrows do not form properly, and the nuclei show abnormal spacing and division failures. The most striking abnormality is that nuclei and spindles form lines and clusters, instead of adopting a regular spacing. This is reflected in a failure to properly position neighboring nonsister centrosomes during the telophase-to-interphase transition of the cortical divisions. Our study is consistent with a role for Sun in mitochondrial ATP synthesis and suggests that reduced ATP levels selectively affect molecular motors. As Sun has been identified as the ligand for the Methuselah receptor that regulates aging, Sun may function both within and outside mitochondria.

ORGANIZATION of the cytoplasm of eukaryotic cells depends largely on the cytoskeleton. The organizational role of the cytoskeleton is particularly dramatic during the cortical syncytial divisions of the early Drosophila embryo (reviewed in Foe *et al.* 1993; Sullivan and Theurkauf 1995). After 9 divisions in the interior of the embryo, syncytial divisions 10–13 occur in the actin-rich cortex just beneath the plasma membrane. Cortical actin is homogenously distributed prior to the arrival of the nuclei, but undergoes a dramatic redistribution induced by the migrating nuclei and their associated centrosomes. During interphase the actin concentrates into apical caps centered above each cortical nucleus and its apically positioned centrosomes. As the nuclei progress into prophase, the centrosomes migrate toward opposite poles and the actin caps undergo a dramatic redistribution to form an oblong ring outlining each nucleus and its associated separated centrosome pair. The actin rings are structurally and compositionally equivalent to conventional cytokinesis. At metaphase, the furrows invaginate to form a half shell encompassing each spindle. During late anaphase and telophase, the metaphase furrows rapidly regress. Centrosome duplication occurs during late anaphase and newly formed centrosome pairs are again located apically in the next interphase and the actin caps reform (Rothwell and Sullivan 2000).

Mutational analysis has been used to identify components responsible for these cytoskeletal rearrangements (Sullivan *et al.* 1993). A particularly informative class of mutations are those that develop normally through the precortical divisions, but show extensive errors when the nuclei reach the cortex. The rationale for focusing on this class of mutations was that many are likely to disrupt genes involved in the cytoskeletal rearrangements required for proper furrow formation. During the cortical divisions thousands of nuclei are dividing in a confined monolayer and the furrows are required to prevent collisions between neighboring spindles and nuclei. Eleven mutations in this class have been molecularly characterized. They fall into three major groups: centrosome associated (Li and Kaufman 1996; Rothwell *et al.* 1998; Stevenson *et al.* 2001), cortical cytoskeleton components (Zhang *et al.* 1996; Katayoun *et al.* 2000), and cell cycle regulators (Hari *et al.* 1995; Fogarty *et al.* 1997; Sibon *et al.* 1997, 1999; Brodsky *et al.* 2000; Price *et al.* 2000).

Unexpectedly, disruptions of key metabolic processes

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produce very specific defects. Mutations in the gene encoding glutamine synthase I have been shown to disrupt chromosome segregation of the cortical divisions (Frenz and Glover 1996). Hypoxia and nitric oxide induce a rapid, reversible metaphase arrest during the syncytial divisions that is accompanied by a depletion of ATP levels (DiGregorio *et al.* 2001). These examples demonstrate that metabolic processes are important during this time. We report here on the identification and characterization of another gene, *stunted* (*sun*), that specifically disrupts the cortical cell divisions. Our analysis of *sun* shows that it encodes the Drosophila epsilon (ε)-subunit of the mitochondrial ATP synthase. Embryos lacking maternal *sun* display defects in actin furrow formation, spindle orientation, nuclear divisions, and centrosome positioning in the cortical divisions. We discuss the importance of metabolic processes during these dynamic, rapid divisions and discuss why molecular motors should be particularly sensitive to reduced ATP synthase activity.

MATERIALS AND METHODS

Genetics: *y w v FRT101* and *y ovoD1 FRT101/Y; C(1)DX f; F38/F38* stocks were obtained from T.-B. Chou and N. Perrimon. Other stocks were obtained from the Bloomington and Bowling Green stock centers. For EMS mutagenesis, flies were fed 25 mm EMS as a 1% sucrose solution.

Initial screen: y w v FRT101 males were mutagenized with EMS and mated to *runt/FM3* females. *y w v FRT101*/FM3* females (* denotes mutagenized chromosome) were mated individually to *FM7* males. Offspring were screened for the absence of males, which indicated that an X-linked lethal mutation had been induced. *y w v FRT101** females from each line were mated either to *FM7* males to generate a reference stock or to *ovoD1 FRT101/Y; F38/F38* males (*F38* is a heat-shock-inducible FLP recombinase on the second chromosome). Larvae from the latter cross were heat-shocked at 37° for 4 hr to generate germline clones. *FRT101*/ovoD1 FRT101* females were selected and mated to *X/Y; FG2/FG2* males (FG2 is a lacZ reporter gene at the *fushi tarazu* locus). The eggs laid by these crosses were screened for cuticular patterning defects or altered patterns of β-galactosidase staining. The reference lines were examined similarly to distinguish between maternal and zygotic effects of the lethal mutations.

The sun^1 allele was recovered in this screen. The sun^1 chromosome was found to have a mutation in the *runt* (*run*) locus. As *run* is located proximal to the Flipase recombinase target (FRT) on the X chromosome at 19E1–3, it was removed by recombination with a *sc cv ct v g f* stock to generate a *y w v* sun^1 *FRT101* f* line. The recombinant line still displayed the maternal-effect phenotype.

F_2 screen for further sun alleles: y w v FRT101 males were mutagenized with EMS and mated to *run/FM3* females. *y w v FRT101*/FM3* females were recovered and individually mated to two to three *y w v* sun^1 *FRT101 f/Dp(1)sdY#3m* males. The offspring were scored for the absence of *y w v* females, indicating the presence of a mutation on the mutagenized chromosome that fails to complement sun^1. No lethal alleles of *sun* were recovered in the 4636 chromosomes screened, although the *shrinkled* (*shk*) mutation that shows an incompletely penetrant wing phenotype in *trans* was found. The *shk* chromosome was used in an F_1 screen: *y w v FRT101* males were mutagenized with EMS and mated to *shk/shk* females. Of 4035 females examined, 118 had a wing phenotype and these were individually remated to *y w v* sun^1 *FRT101 f/Dp(1)sdY#3m* males. The offspring were scored for the absence of *y w v FRT101*/y w v* sun^1 *FRT101* females. Three lines failed to complement sun^1 of which two were recovered, sun^2 and sun^3. The *shk* stock accumulated modifiers with passaging and was eventually lost.

The zygotic lethal phenotype of the *sun* locus was mapped to 13F/14A by recombination with the multiply marked *sc cv ct v g f* and *m wy sd* os^s chromosomes and with X chromosome deficiencies and duplications. Previous mapping within this region had placed the *sun* lethal complementation group as adjacent and proximal to the *D-Myb* proto-oncogene (A. Katzen, unpublished results).

***In vivo* fluorescence analysis:** For anti-α-tubulin and propidium iodide staining, embryos were fixed in formaldehyde, devitillinized in methanol, gradually rehydrated, and stained as described previously (Rothwell *et al.* 1999; Rothwell and Sullivan 2000). Microscopy was performed using an Olympus IMT2 inverted photoscope equipped with a Bio-Rad (Richmond, CA) MRC 600 laser confocal imaging system. *In vivo* analysis of tubulin behavior was accomplished by microinjection of fluorescently labeled tubulin into embryos and time-lapse images were taken using a fluorescence microscope (Kellogg *et al.* 1988). *In vivo* analysis of histones was performed as described previously (Minden *et al.* 1989). F-actin staining with phalloidin was performed as described previously (Rothwell and Sullivan 2000).

Molecular biology: Genomic fragments and cDNAs were subcloned into Bluescript.SK+ (Stratagene, La Jolla, CA) using standard techniques. The Drosophila stage 10 library used was constructed by J. Tower using a mixture of random and oligo(dT) priming cloned into the *Eco*RI site of λ-gt11. DNA probe preparation was with a Prime-It II random primer kit (Stratagene). Sequencing was with the Sequenase 2.0 kit (United States Biochemical, Cleveland) or the Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) or the AutoRead kit (Pharmacia, Piscataway, NJ) following the manufacturer's instructions. Sequences were compiled using Geneworks (Intelligenetics, Mountain View, CA) or Lasergene software. PCR for mutant allele sequencing was done with rTth DNA polymerase (Perkin Elmer, Norwalk, CT). PCR products were cloned into the Topo vector (Invitrogen, San Diego). Expressed sequence tags (ESTs) from the Berkeley Drosophila Genome project were used to analyze the transcripts in the *sun* region. The sequence obtained for EST GM13815 is identical to the cDNA clone sequences RH48911 and RE19513 in GenBank, but is shorter by 7 bp.

Preparation of DNA from larvae: To sequence mutant alleles, 5–10 mutant larvae were picked by virtue of their retarded development, frozen, crushed in 100 μl buffer A (100 mm Tris/HCl pH 7.6, 100 mm EDTA, 100 mm NaCl, 0.5% SDS), and incubated for 15 min at 65°. The extract was mixed thoroughly with 200 μl of 1.5 m KAc/4.5 m LiCl and chilled for 10 min on ice. The extract was spun at 15,000 rpm for 10 min at room temperature, and the supernatant was transferred to a new tube and respun if necessary. The supernatant was precipitated with 150 μl propanol, and the pellet was washed with 70% ethanol, dried, and resuspended in 50 μl TE. Two microliters were used for PCR.

Mitochondrial ATPase assay: Germline mosaic sun^1 and sun^3 females were generated following a 1-hr heat-shock, and extracts prepared from their embryos were collected for 3-hr intervals over a 3-day period. No embryos were observed from a non-heat-shock control cross between sun^3 females and ovo^{D1} males. Embryos were homogenized with a plastic pestle in 1.5-ml tubes (Kontes) in ~10 volumes of 25 mm Tris (pH 7.5), 0.25 m sucrose, 5 mm EDTA, and a protease inhibitor cocktail

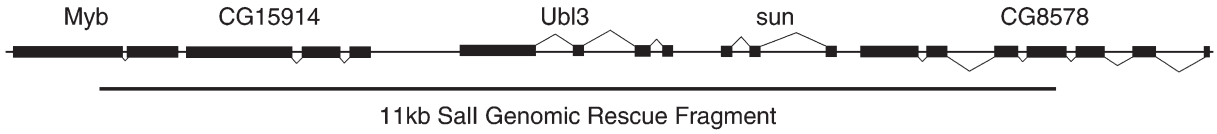

FIGURE 1.—Organization of the *sun* genomic locus. The genomic region around the *sun* locus deduced from restriction mapping, partial sequencing, and genome project data is shown. Identified genes are shown on the chromosome, with the orientation of the gene shown by the lines linking exons above the line (5′ to 3′ is from left to right) or below the line (5′ to 3′ is from right to left). The 11-kb *Sal*I genomic rescue fragment is shown as a bar below the chromosome.

(10 M benzamidine HCl, 1.2 g/ml phenanthroline, 10 g/ml aprotinin, 10 g/ml leupeptin, and 10 g/ml pepstatin A). Homogenates were centrifuged at 100,000 × *g* for 1 hr at 4° and supernatant and pellet fractions were collected, frozen in liquid N_2, and stored at −80° until needed. The pellet fraction contains membrane-associated ATPases, including mitochondrial, lysosomal, and vacuolar ATPases. Protein concentrations were determined by Bradford assay (Bio-Rad), using BSA as a standard prior to freezing. As controls, protein extracts were also made from embryos collected from wild-type and *y w v sun*^{3} *FRT101/FM7c* stocks. ATPase assays were performed according to BOWMAN *et al.* (1978) on 32 and 11 g of total protein from each pellet and supernatant fraction, respectively, in the presence (5 mM) and absence of NaN_3. There was no significant difference in ATPase activity in high-speed supernatant fractions between the different embryo preparations.

RESULTS

The *stunted* maternal-effect locus: The *sun* locus was identified in an EMS screen for X-linked zygotic lethals. The mutagenized chromosomes carried an FRT element, allowing rapid and efficient generation of germline clones and analysis of the maternal requirement of the isolated lethals (CHOU and PERRIMON 1992). Eggs lacking maternal *sun* (*sun*$^{mat-}$; either *sun*^{1} or *sun*^{3}) displayed an almost complete lack of cuticle, and confocal analysis revealed defects in early embryogenesis (see below). An F_2 screen was designed to look for new *sun* alleles, from which we recovered one line that displayed an improperly expanded wing in *trans* to *sun*^{1}. Although we were unable to show conclusively that this mutation (*shrinkled*) was an allele of *sun* (the allele was later lost), we used it to screen for mutations that are unable to complement its wing phenotype. Two mutations that subsequently failed to complement *sun*^{1} were recovered: *sun*^{2} and *sun*^{3}. Mosaic females with germline clones homozygous for either allele produce *sun*$^{mat-}$ embyros with the same embryonic defects as *sun*^{1}, indicating that the new mutations are indeed *sun* alleles, and the same locus is responsible for both zygotic lethality and the maternal phenotype.

We localized the *sun* locus to 13F by recombination and deficiency mapping. *sun* lies within *Df(1)sd*^{72b}, which extends from 13F–14A, an interval previously saturated for lethal mutations (KATZEN and BISHOP 1996). Complementation analysis showed that *sun* corresponds to lethal group XV (KATZEN and BISHOP 1996), which includes two previously recovered alleles. *sun*^{4} (synonym *EM67*) is a zygotic lethal, and attempts to produce germline clones by X-ray-induced mitotic recombination did not lead to any females able to lay eggs. The *sun*^{4} chromosome may therefore carry a second lethal. *sun*^{5} (synonyms *42-3.0B* and *EM69*) is viable, but displays female sterility in *trans* to *sun*^{1} and *sun*^{2} and is lethal in *trans* to *sun*^{3} and *Df(1)sd*^{72b}. As *sun*^{3} is the only allele lethal in *trans* to *sun*^{5}, it is probably a strong hypomorph or a null mutation. *sun*^{4} was found to give fertile females at a rate of 10% when in *trans* to *sun*^{5}, so is probably a weak hypomorph. The maternal effects of *sun*^{1}, *sun*^{2}, and *sun*^{3} were all found to be temperature sensitive, as rescue of cuticle is observed when mosaic females and eggs are kept at 18° (KIDD 1994). The phenotypic experiments described in the rest of this article were all performed using *sun*^{3} and *sun*^{1} at 23°–25°.

Molecular characterization of the stunted locus: The *sun* locus (complementation group XV) was mapped relative to other complementation groups in *Df(1)sd*^{72b} and found to lie adjacent to the *D-Myb* proto-oncogene locus (KATZEN *et al.* 1985, 1998). An 11-kb genomic fragment (Figure 1) was used to generate transgenic flies and found to rescue the zygotic lethal phenotype of *sun* alleles and no other complementation groups in the region. In addition to *D-myb*, other transcripts were identified within the rescue fragment by probing maternal mRNA Northern blots with genomic subfragments and by matching public ESTs to genomic sequence. Transgenes containing the novel ubiquitin-like gene UBL3 (CHADWICK *et al.* 1999) under control of its cognate or a heat-shock promoter failed to rescue *sun* phenotypes. Sequencing of the UBL3 gene in *sun* mutants also failed to detect any changes. The putative protein-coding region of the second gene, FLI-LRR associated protein-1 (CG8578; LIU and YIN 1998), extends beyond the bounds of the genomic rescue fragment. Expression of CG15914 was not detected on ovarian mRNA Northern blots. Thus we focused on a fourth transcription unit identified by the EST GM13815. Sequencing of GM13815 showed it to encode the 61-amino-acid ε-subunit of the mitochondrial ATP synthase (CG9032-RA; Figure 2). The Drosophila ε-subunit is a small basic protein with 48% identity at the amino acid level to the Arabidopsis ε-subunit, 39% homology to the *Saccharomyces cerevisiae* ε-subunit, and 34% homology to the bovine ε-subunit. A second ε-subunit, encoded by CG31477, is present in the fly but as it is not represented in any

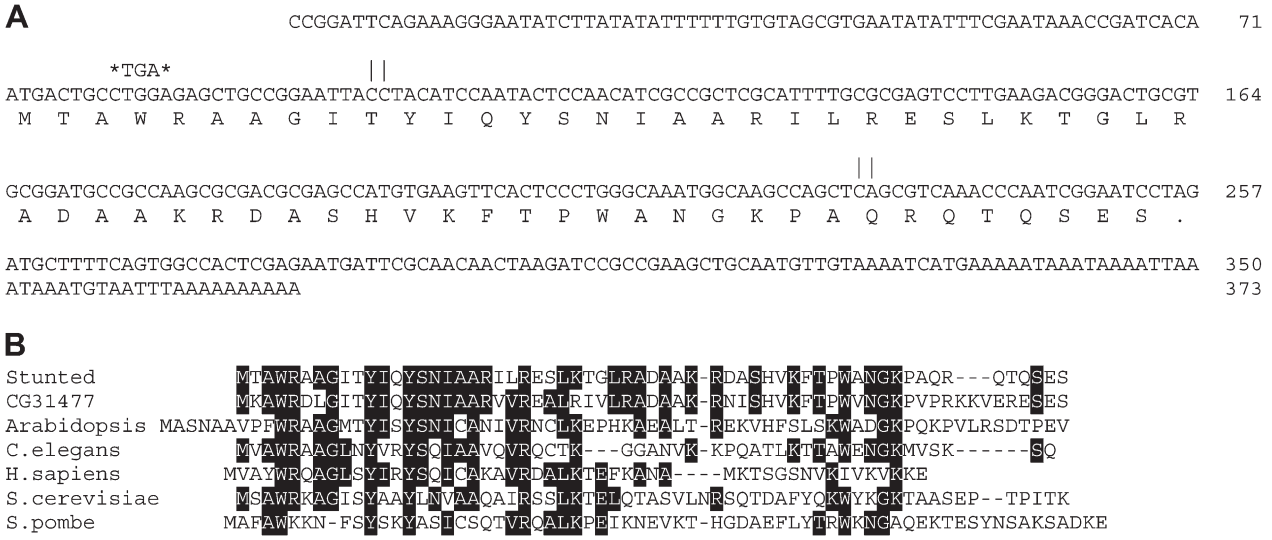

FIGURE 2.—Sequence of the *sun* gene. (A) DNA sequence of the *sun* gene, with the predicted amino acid sequence of the gene product shown at bottom. The mutation found in *sun* alleles 1–3 that changes Trp4 to a stop codon is highlighted with asterisks above the DNA sequence. Vertical bars indicate the location of splice sites; the first intron is 129 bp long, and the second is ~600 bp. The unspliced transcript is predicted to be ~1.3 kb. (B) Alignment of the Sun amino acid sequence with the ε-subunits of ATP synthase from a representative range of organisms. Residues common to three or more sequences are boxed. The amino acid sequence accession numbers are: Drosophila CG31477, NP_731449; Arabidopsis, Q96253; *C. elegans*, P34539; Human, NP_008817; *S. cerevisiae*, NP_015052; and *Schizosaccharomyces pombe*, NP_596577. The *C. elegans* and *S. pombe* sequences are putative proteins identified by analysis of genome projects data.

of the public EST collections, it may be expressed only at a low level or in a tissue not yet sampled. The ε-subunit is duplicated in *Caenorhabditis elegans* and present at three copies in Anopheles; in each case the duplication events appear to have occurred after these species shared a common ancestor with the fly as the ε-subunits are more homologous within each species than between species.

Sequencing of the ε-subunit gene from three mutant alleles ($sun^{1,2,3}$) revealed the same nonsense mutation: TGG (Trp4) to TGA (Stop) (Figure 2A). This change is commonly seen in EMS mutagenesis (ASHBURNER 1989), and as each of the chromosomes displays different complementation characteristics, we believe the three mutations were independent events. The proximity of the nonsense mutation to the start codon indicates that these mutations are null alleles. The differences in complementation characteristics between these mutations are probably due to additional mutations on the chromosomes. This is supported by the ATPase assays described below.

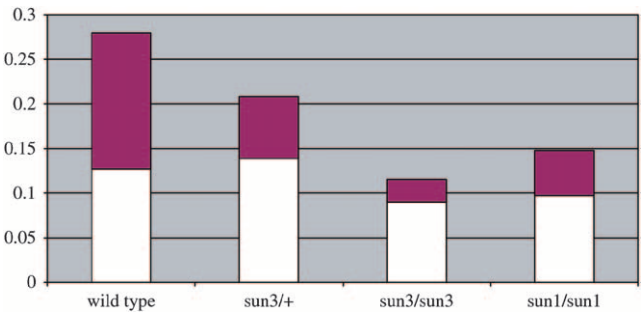

FIGURE 3.—ATPase activity in wild-type and *sun* mutant embryo extracts. Bars indicate ATPase activity in high-speed pellets made from embryos collected from wild-type and sun^{3}/+ stocks or germline mosaic females of sun^{3} and sun^{1}. Optical density (A_{660}) values and genotypes are indicated along the ordinate and abscissa, respectively. The total ATPase activity derived from the mitochondrial ATP synthase is indicated by the purple portion of each bar. ATPase activity was measured in the absence (purple plus white) and presence (white) of the mitochondrial ATP synthase-specific inhibitor NaN_3 (BOWMAN *et al.* 1978). Each protein fraction was assayed twice and the average values are shown.

The ATPase activity of mitochondrial ATP synthase is markedly reduced in *sun* mutants: Mitochondrial ATP synthase both synthesizes and breaks down ATP. The ATPase activity of mitochondrial ATP synthase is directly correlated to the synthetic enzyme's activity, and so we could assay enzyme activity directly by measuring membrane-associated mitochondrial ATPase activity in 0- to 3-hr embryo extracts. There are multiple sources of ATPase activity, and to identify the mitochondrial ATPase, we took advantage of the fact that sodium azide is a specific and potent inhibitor of mitochondrial ATP synthase. This approach is standard for the field. Extracts enriched for mitochondrial proteins were prepared and assayed in parallel and in duplicate for ATPase activity, in the presence and absence of sodium azide. In wild-type embryo extracts roughly one-half of the total ATPase activity detected is sensitive to inhibition by sodium azide and must be derived from mitochondrial ATP synthase (Figure 3).

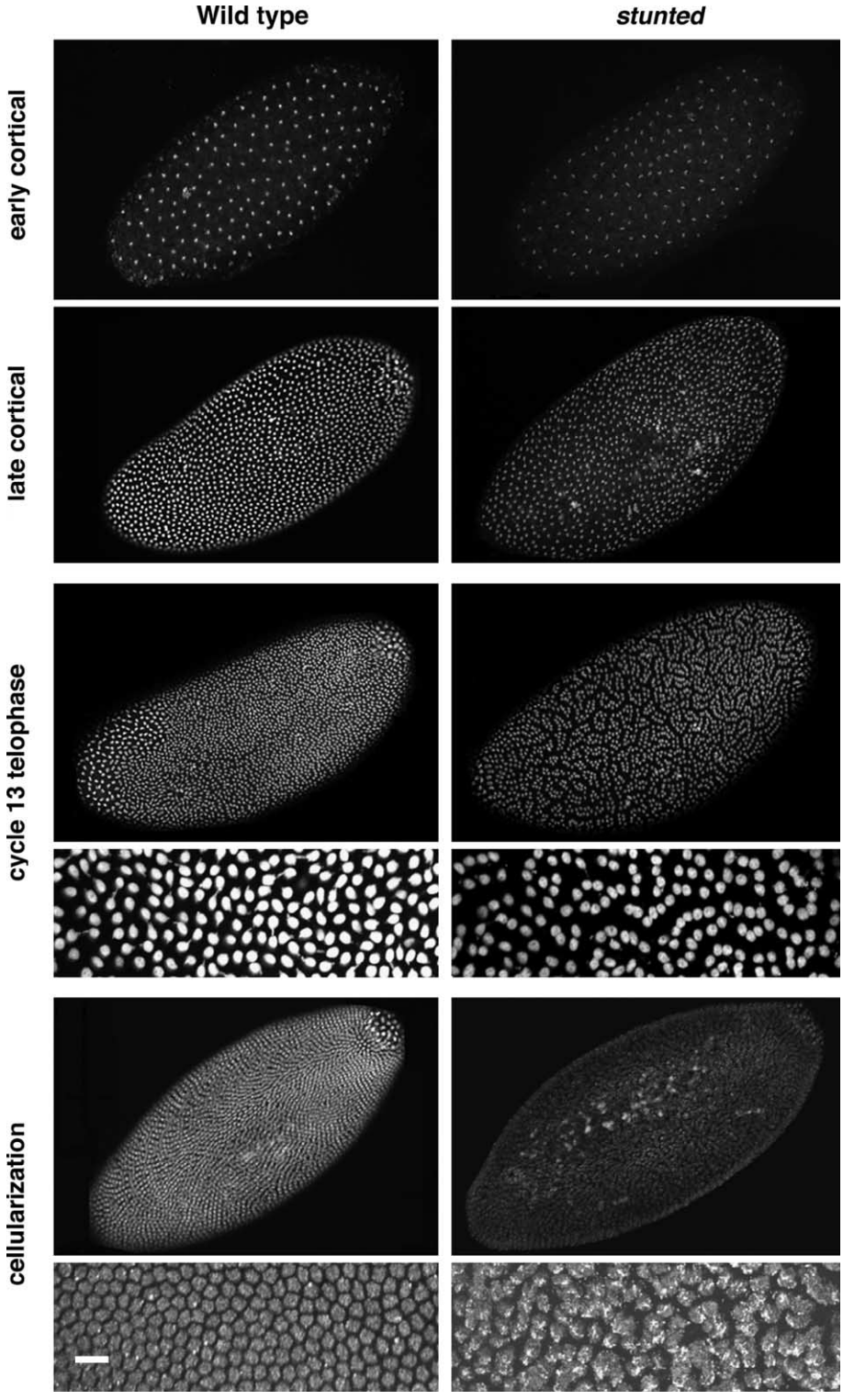

FIGURE 4.—Nuclear behavior of wild-type and stunted embryos. Wild-type (left column) and sun^{mat-} embryos (stunted, right column) were stained with the nuclear dye propidium iodide. During the early cortical divisions, slight abnormalities are observed in sun^{mat-} embryos. During the late cortical divisions, areas in which large numbers of nuclei have fallen back into the center of the embryo are observed and spacing between nuclei has become irregular. At telophase of cycle 13, the spacing defects are readily apparent as nuclei come closer together than in wild type, forming lines of nuclei arranged in clumps in a manner reminiscent of the "paisley" pattern. The 60× insets reveal many nuclei are in contact with their neighbors and form lines of touching nuclei. At cellularization, nuclear fusion has occurred throughout the sun^{mat-} embryos; this is apparent in the 60× inset. Bar, 10 μm.

We also assayed extracts made from embryos derived from sun^{3}/+ adult females, which should have half the normal levels of maternally supplied Sun. As expected, we find mitochondrial ATP synthase activity is reduced twofold compared to wild type, indicating that Sun is a limiting component for mitochondrial ATP synthase activity (Figure 3). In embryo extracts derived from sun^{1} mutant germlines (mosaic females), mitochondrial ATP synthase-specific ATPase activity is reduced sixfold (Figure 3). This major reduction in activity is correlated with the strong sun^{3} embryonic phenotype. Mitochondrial ATP synthase-specific ATPase activity is also significantly compromised in extracts produced by germline mosaic sun^{1} females (Figure 3). The higher levels

of activity in these extracts are consistent with their weaker embryonic phenotype. These results show that *sun* activity is a critical component of mitochondrial ATP synthase activity in Drosophila.

Lack of zygotic *stunted* dramatically reduces larval growth: All four lethal *sun* alleles show a zygotic phenotype of greatly delayed larval growth. Mutant *sun* larvae die stochastically after hatching but some can be maintained in the presence of a yeast food supply for eight days or longer. The mutant larvae undergo little or no growth, and none appear to undergo the first molt to the second instar stage of development. There were no obvious behavioral defects in the larvae, although some individuals can appear sluggish. Maternally supplied *sun* could be responsible for the partial development observed. The lethal phase probably reflects the point at which the maternal supplies run out. Larval growth is driven by DNA replication, which can be measured by the incorporation of BrdU into DNA (Smith and Orr-Weaver 1991). In wild-type larvae deprived of dietary protein, but supplied with sucrose as an energy source, DNA replication is restricted to mushroom body neuroblasts and the gonad (Britton and Edgar 1998). Larvae lacking zygotic *sun* have a BrdU incorporation pattern that closely resembled this pattern (M. Garfinkel and B. A. Edgar, personal communication). A *P* element inserted into the first intron of the α-subunit of mitochondrial ATP synthase also gives a larval growth arrest in the first instar and limited DNA replication (Galloni and Edgar 1999).

The *sun* maternal effect specifically disrupts the arrangement of nuclei of the syncytial blastoderm: sun^{mat-} embryos show defective cuticles indicative of an early requirement for *sun* activity. Preliminary examination of mutant embryos showed that they are already abnormal by the blastoderm stage, so we stained 0- to 4-hr embryos using the nuclear dye propidium iodide (PI; Figure 4). Nuclear migration during cycles 4–10 (axial expansion and cortical migration) appears normal (Table 1). The early cortical divisions (nuclear cycles 10 and 11) are indistinguishable from those in wild-type embryos. However, sun^{mat-} embryos display highly irregular nuclear spacing during the late cortical divisions (Figure 4; Table 1). An increasing tendency of the nuclei to cluster was observed, coincident with the occurrence of nuclear fusion, which is rarely seen in wild-type embryos.

To identify a basis for these phenotypes, we examined nuclear dynamics in sun^{mat-} embryos by injecting fluorescently labeled histone into living embryos (Figure 5). A time-lapse movie revealed that numerous nuclear fusions between nonsister nuclei occur at telophase of cycle 13 (Figure 5, D–F). After fusion, these nuclei drop back into the yolk, often leaving behind free centrosomes (Kidd 1994; R. Abu-Shumays and W. Sullivan, unpublished data). A downstream consequence is the formation of large multinucleate cells just prior to cellularization (cycle 14; Figures 4–6). Syncytial nuclear fusions are the direct result of a failure to properly form metaphase furrows that are generated by the actin cytoskeleton (Schweisguth *et al.* 1990; Simpson and Wieschaus 1990; Sullivan *et al.* 1993).

TABLE 1

Nuclear behavior in wild-type and *sun* embryos

	sun^{mat-}		*sun/FM7*	
Cycle	% abnormal	*N*	% abnormal	*N*
0–9	0	62	0	21
10	6	15	0	9
11	15	34	11	7
12	50	24	14	9
13	86	22	0	7
14	97	33	0	15
Cellularization	97	34	0	9

The nuclei of sun^{mat-} and sun^{mat-}*/FM7* control embryos were stained with propidium iodide and examined for defects in morphology, spacing between nuclei, and position relative to the plasma membrane. The sun^{3} allele was used for this analysis.

The actin cytoskeleton displays defects in sun^{mat-} embryos: In sun^{mat-} embryos, actin caps form normally above interphase nuclei (Figure 6, A and B). However, during metaphase, gaps in the metaphase furrows are common and most frequently found at regions of the metaphase plate most distant from the centrosomes (Figure 6D). This furrow defect is similar to that observed in *nuf* mutant embryos (Rothwell *et al.* 1998). At the onset of cellularization, the normally regular actin network is highly disorganized, and in some areas completely absent, resulting in the formation of multinucleate cells (Figure 6F; Kidd 1994; R. Abu-Shumays and W. Sullivan, unpublished data). A number of zygotic and maternal mutants have this phenotype, *e.g.*, *serendipity*, *nullo*, and *nuf* (Schweisguth *et al.* 1990; Simpson and Wieschaus 1990; Schejter and Wieschaus 1993).

Abnormal centrosome positioning: Previous studies have demonstrated that proper centrosome duplication, segregation, and position are essential for normal furrow formation and nuclear division (Rothwell and Sullivan 2000). To understand the metaphase furrow and nuclear division defects in sun^{mat-} embryos, we examined centrosome behavior during the cortical divisions.

Living sun^{mat-} embryos were injected with fluorescently labeled tubulin to follow centrosome dynamics (Figure 7) (Rothwell and Sullivan 1999). In the late syncytial divisions of wild-type embryos, centrosome duplication occurs during telophase and the sister centrosomes separate to opposite poles in early interphase. Analysis of sun^{mat-} embryos indicates that centrosome duplication and separation occur normally. This is evidenced by the fact that all interphase nuclei contain centrosomes normally positioned at opposite poles (Figure 7B, a). However, the relative position of centrosome pairs on

neighboring nuclei is abnormal in mutant embryos. During the late syncytial divisions of wild-type embryos, mitotic spindles are evenly spaced in an array of orientations that maximize the distance between centrosomes (Valdes-Perez and Minden 1995). In contrast, in metaphase *sun*$^{mat-}$ embryos, the mitotic spindles are often found in parallel arrays (Figure 7, A and B). The parallel arrays arise from the abnormal positioning of neighboring nonsister centrosomes in early interphase (Figure 7B, b, arrows). Since repulsion between overlapping astral microtubules is thought to be the primary factor in positioning neighboring centrosomes, this astral-based process may be compromised in *sun*$^{mat-}$ embryos (see discussion). The abnormal interphase centrosome orientation foreshadows the abnormal orientation of neighboring spindles at metaphase (Figure 7B, c). The abnormal orientation of the neighboring spindles could be due to defects in furrow formation, centrosome positioning, or a combination of both. The variability in the *sun* phenotype made distinguishing between the two possibilities inconclusive.

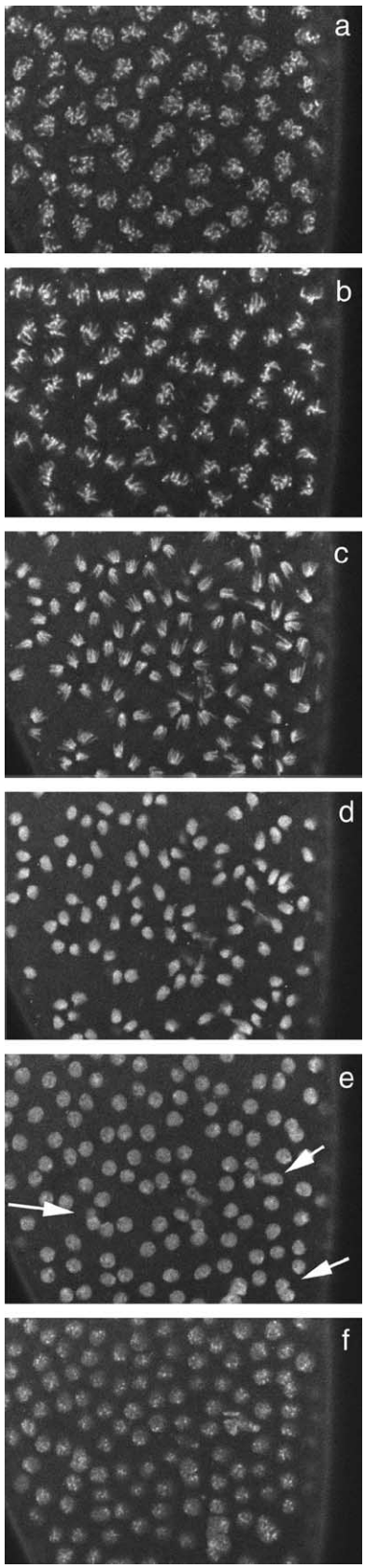

Figure 5.—Nuclear dynamics in a *sun*$^{mat-}$ embryo. Images of living sun^{mat} embryos injected with fluorescently labeled histones at nuclear cycle 13 are shown. Prophase (a), metaphase (b), anaphase (c), telophase (d and e), and interphase (f) are shown. Fusions between dividing nuclei occur at telophase (see arrows in e).

DISCUSSION

The effect of reduced ATP levels in the early embryo: We have described the maternal and zygotic effects of null mutations in the *sun* locus. *sun* encodes the ε-subunit of the mitochondrial ATP synthase, the universal enzyme for cellular ATP synthesis (reviewed in Boyer 1997). In yeast the ε-subunit is a nonessential gene required for dimerization and oligomerization of ATP synthase and is involved in generating the inward foldings of the inner mitochondrial membrane, the cristae (Lai-Zhang *et al.* 1999; Pawnard *et al.* 2003). The ε-subunit is also a potential binding site or target of the natural inhibitor protein IF1 that serves to prevent ATP hydrolysis (Solaini *et al.* 1997; Minauro-Sanmiguel *et al.* 2002). The ε-subunit appears necessary for the maximum efficiency of the ATP synthase complex and it has been proposed to be a molecular clutch regulating the coupling of ATP synthesis to proton flow (Lai-Zhang *et al.* 1999). Expression of the bovine ε-subunit can rescue the growth defect of *S. cerevisiae* carrying a deletion of the ε-subunit gene (Lai-Zhang and Mueller 2000). This result suggests that the molecular function of the ε-subunit within the mitochondrial ATP synthase complex has been conserved throughout eukaryotic evolution. Thus we interpret the *sun* mutant phenotypes as a consequence of reduced levels of ATP. This is consistent with the increased severity of the defects seen at higher temperatures in the *sun* mutants, because ATP requirements and oxygen consumption increase at higher tem-

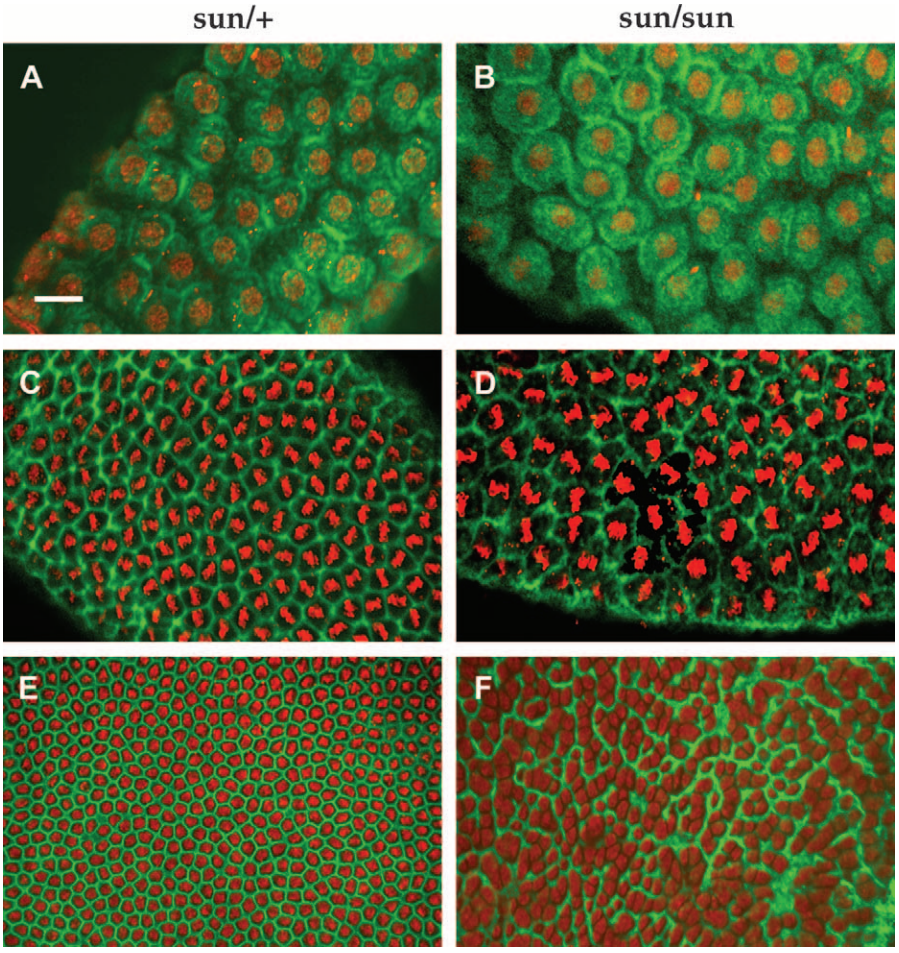

FIGURE 6.—Actin distribution in sun^{mat-} embryos. Wild-type (sun^{mat-}/FM7c) and sun^{mat-} (*stunted*) embryos were stained with fluroscein-labeled phalloidin and propidium iodide. The actin caps appear to form normally in sun^{mat-} (*stunted*) embryos (A and B). However, the metaphase furrows are frequently absent in sun^{mat-} embryos (D). By cellularization, the normal orderly outline of cells (E) is severely disrupted (F). Bar, 10 μm.

peratures. Reducing ATP levels in the embryo by inhibiting oxidative phosphorylation with cyanide or azide induces a cell cycle arrest (DiGregorio *et al.* 2001; W. Sullivan, unpublished observations). We saw no evidence of cell cycle arrests in *sun* maternal-effect embryos.

The *sun* maternal effect is most dramatic during the late cortical cycles, presumably reflecting a greater energetic load at these stages. Lack of *sun* activity disrupts alignment of neighboring spindles and formation of the metaphase and cellularization furrows. A direct consequence of this is fusions of sister nuclei. Computational studies of these mitoses indicate that the even spacing results from interactions of each nucleus with its neighbors (Valdes-Perez and Minden 1995). These interactions most likely arise from centrosome-based astral microtubules repelling one another (de Saint Phalle and Sullivan 1998). The force of their repulsion is inversely proportional to the distance between neighboring centrosomes. The abnormal arrangement of spindles in *sun* embryos is not due to a failure to form astral microtubules, as we do not see a difference between wild-type and *sun* astral microtubules in the light microscope.

Motor proteins and the *sun* phenotype: One interpretation of the *sun* phenotype is that the activity of the motor proteins during the cortical divisions places extra energetic demands on the embryo. In *sun* embryos, while the reduced ATP levels are sufficient for the early divisions, they are insufficient for the cortical divisions. On the basis of the *sun* maternal-effect phenotype, we propose that maintaining regular spacing of the closely packed nuclei is the most ATP-demanding process in the syncytial embryo. The inability to keep nuclei apart leads to inappropriate microtubule interactions, nuclear fusion, and dropping of nuclei back toward the yolk.

The repulsive force between anti-parallel microtubules is generated by microtubule-based motor proteins. Given the number of molecular motors already described, it is highly likely that several contribute to spindle positioning (Scholey *et al.* 2003). The *sun* mutant phenotype could be a failure of the motor proteins to provide this repulsive force. For example, the motor protein KLP61F acts on anti-parallel microtubules to maintain separation of sister centrosomes (Sharp *et al.* 1999). In addition, embryos lacking the motor protein Ncd display centrosomal defects and microtubule spurs between mitotic spindles (Endow *et al.* 1994). Figure 4 of Endow and Komma (1996) shows three aligned spindles

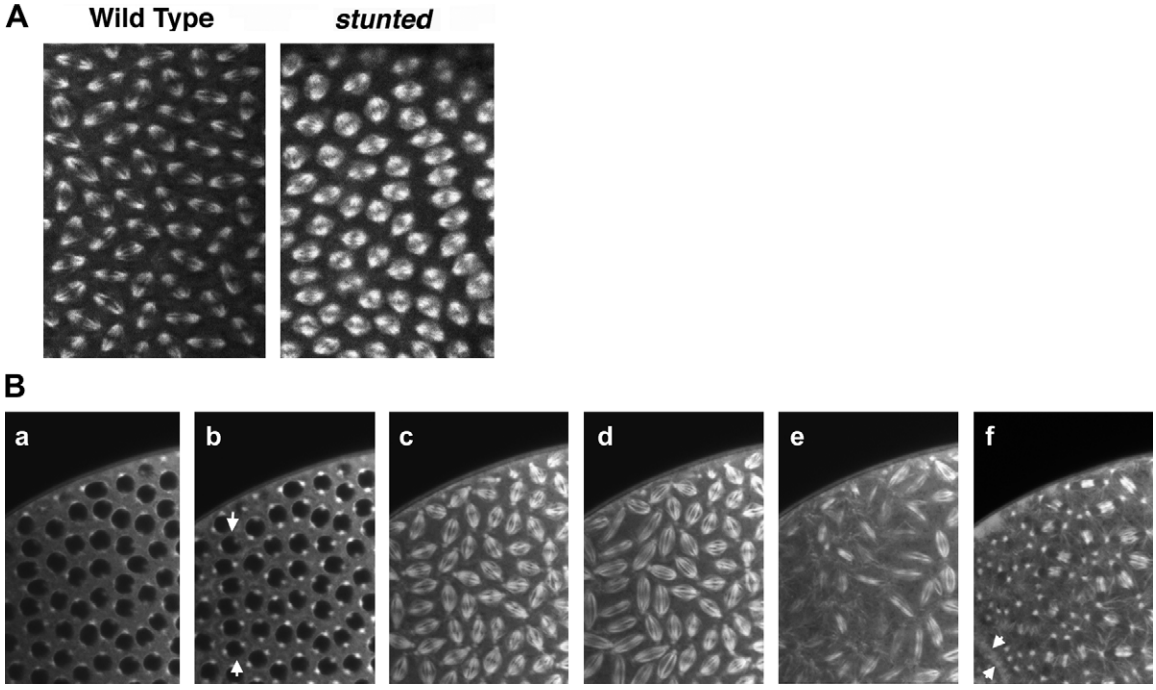

Figure 7.—Analysis of microtubule behavior in a *sun*$^{mat-}$ embryo. (A) Fixed analysis of wild-type *vs.* sun^{mat-} embryos. (B) Time-lapse sequence of a *sun*$^{mat-}$ syncytial blastoderm embryo injected with fluorescently labeled tubulin. (a and b) Prophase. The syncytial nuclei can be seen by an absence of tubulin staining due to exclusion by the nuclear envelope. The position of the centrosomes can be determined from increased density of tubulin on either side of the nuclei. On the left, a line of nuclei (indicated by arrows) in which the centrosomes have aligned can be seen. (c) During metaphase, the mitotic spindles corresponding to the aligned centrosomes can be seen on the left. (d) During late metaphase, irregular mitotic figures become apparent, with inappropriate interactions between neighboring spindles, notably at the bottom left and right corners. (e) Anaphase. A lack of mitotic coordination becomes apparent as some spindles break down more quickly than others, and midbodies form (accumulation of microtubules at the spindle equator; these are more apparent in f). (f) Telophase. The position of the centrosomes can be deduced from small local concentrations of tubulin; midbody formation has occurred at the remaining spindles. A group of tightly apposed abnormal midbodies can be seen in the bottom left (indicated by arrows).

reminiscent of the *sun* maternal effect. A further candidate motor is the Drosophila homolog of MKLP1 kinesin-like protein, which transports oppositely oriented microtubules relative to one another (Schmid and Tautz 1998; Sharp *et al.* 1999).

Intracellular circulation of ATP: One might have expected decreased levels of ATP to have highly pleiotropic effects, and so it is surprising that *sun* has such a specific effect on the syncytial mitoses. Many other mutations affecting the syncytial mitoses turn out to be centrosomal or key regulators of the cell cycle, *e.g.*, *nuclear fallout* (Rothwell *et al.* 1998). The *sun* locus is distinctive by encoding a component of a central metabolic enzyme. Mutations in another essential metabolic enzyme, *Glutamine synthetase 1* (*Gs1*), also specifically affect the syncytial mitoses (Frenz and Glover 1996). Gs1 catalyzes the amination of glutamate in an ATP-dependent manner to produce glutamine, which is required for amino acid, purine, and pyrimidine biosynthesis. Analysis of *Gs1* mutants suggests that delays in syncytial cell cycle progression lead to nuclei being discarded due to a reduction in amino acid and nucleotide availability (Frenz and Glover 1996). Surprisingly, given the requirement of Gs1 for ATP, the *sun* and *Gs1* mutant phenotypes are almost reciprocal, with *Gs1* affecting nuclear events during S phase and *sun* affecting cytoplasmic events during M phase, with little or no effect on DNA segregation.

Why are the *sun* and *Gs1* mutant phenotypes so reciprocal? Gs1 may function at lower ATP concentrations than required for the molecular motors to maintain spindle separation. In the *sun* mutant, there may be sufficient ATP to carry out the functions of Gs1, but not those of the microtubule-associated motors. There may also be distinct biochemical pools from which Gs1 and the motors obtain ATP. Under most conditions, intracellular circulation keeps the ATP concentration perfectly homeostatic, meaning the concentration does not change even when ATP-dependent work is being performed (Hochachka 2003). The *sun* mutant syncytium may resemble a fatigued cell, with local differences in intracellular circulation of ATP to the metabolic pools containing molecular motors and biosynthetic enzymes.

The role of the ε-subunit in multicellular organisms: *S. cerevisiae* deleted for the ε-subunit grow slowly on medium with glycerol as the carbon source, indicating that the ε-subunit is not an essential gene (Lai-Zhang

et al. 1999). In contrast to *S. cerevisiae,* the ε-subunit of ATP synthase is essential for survival of Drosophila. As the bovine ε-subunit can rescue the yeast ε-deletion mutant (Lai-Zhang and Mueller 2000), we believe that the phenotypic differences originate in differences in ATP homeostasis between unicellular and multicellular organisms. No other eukaryotic ε-subunit mutants have been published. Our phenotypic and biochemical observations indicate that ATP levels are reduced but not eliminated, supporting the hypothesis that the ε-subunit is required for maximal efficiency of ATP synthase. The presence of maternal ATP in *sun* mutants allows growth until an energetically demanding process is encountered. In the early embryo, the first defects are seen in the cortical divisions, but the embryos continue to grow and cellularize, albeit abnormally. Embryos lacking maternal *sun* fail to gastrulate (T. Kidd and D. Ish-Horowicz, unpublished observations), suggesting the dynamic cell movements are incompatible with the reduced ATP levels. In the larva, the energetically demanding processes of DNA replication and protein synthesis normally drive a 200-fold increase in mass over 4 days (Galloni and Edgar 1999). The absence of zygotic *sun* causes a larval growth arrest before any significant growth has occurred. Interestingly, the same phenotype is seen for a mutation, *colibri*, in the α-subunit of ATP synthase (Galloni and Edgar 1999). The α-subunit, known as *bellwether* in Drosophila, should be absolutely required for ATP synthesis; a series of alleles have been characterized as recessive lethal, but the exact lethal phase was not determined (Jacobs *et al.* 1998). The *colibri* allele of *bellwether* is a *P*-element insertion in an intron and is most probably a hypomorphic allele allowing some synthesis of ATP in a manner similar to that of *sun* alleles. Mutant clones of *colibri* in the wing show a severe size reduction whereas mutant clones in the eye survive well (Galloni and Edgar 1999), suggesting different energetic requirements in the two tissues. We believe that ε-subunit mutations will be essential in all multicellular organisms, but the effects will vary from tissue to tissue.

The ε-subunit as a mitochondrial subunit and as an extracellular ligand: Sun was unexpectedly identified as the ligand for the Drosophila G-protein-coupled receptor (GPCR), Methuselah (Mth; Cvejic *et al.* 2004). Mutations in *methuselah* (*mth*) extend life span, and the protein is required in motor neurons where it regulates neurotransmitter release (Lin *et al.* 1998; Song *et al.* 2002). A Mth-GFP fusion localizes to the plasma membrane of the presynaptic terminals, although it has not conclusively been shown to be exposed to the extracellular environment (Song *et al.* 2002). Analysis of life span in *sun* mutants (using the alleles generated in this study) revealed extended life span (Cvejic *et al.* 2004).

This diverse function might indicate that the *sun* gene does not encode a genuine homolog of mitochondrial ATP synthase ε-subunits from other species. However, this view appears unlikely. The results from our study strongly suggest Sun indeed participates in ATP synthesis (Figure 3). Rather, the genetic and biochemical analyses suggest that Sun is a bifunctional protein. Such a dual function is reminiscent of another mitochondrial protein, cytochrome C, which functions within the respiratory electron transport chain and is released from the mitochondria to participate in apoptosis (Li *et al.* 2004). A better understanding of Sun function awaits the development of antibody reagents to visualize Sun localization, particularly in Drosophila models of aging. For the moment, there are some intriguing hints as to how Sun might be regulated: *sun* transcription has been shown to be downregulated by oxidative stress, which is thought to limit life span in multicellular organisms (Finkel and Holbrook 2000; Girardot *et al.* 2004). Sun has been shown to bind the regulatory subunit of cAMP protein kinase, suggesting it may be a substrate for phosphorylation (Pka-R1; Giot *et al.* 2003), although it remains to be confirmed *in vivo.*

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LITERATURE CITED

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook*, pp. 345–346. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bowman, B. J., S. E. Mainzer, K. E. Allen and C. W. Slayman, 1978 Effects of inhibitors on the plasma membrane and mitochondrial adenosine triphosphatases of *Neurospora crassa.* Biochim. Biophys. Acta **512:** 13–28.

Boyer, P. D., 1997 The ATP synthase—a splendid molecular machine. Annu. Rev. Biochem. **66:** 717–749.

Britton, J. S., and B. A. Edgar, 1998 Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development **125:** 2149–2158.

Brodsky, M. H, J. J. Sekelsky, G. Tsang, R. S. Hawley and G. M. Rubin, 2000 *mus304* encodes a novel DNA damage checkpoint protein required during Drosophila development. Genes Dev. **14:** 666–678.

Chadwick, B. P., T. Kidd, D. Ish-Horowicz and A. Frischauf, 1999 Cloning, mapping and expression of HCG1, a novel ubiquitin-like gene. Gene **233:** 189–195.

Chou, T. B., and N. Perrimon, 1992 Use of a yeast site-specific recombinase to produce female germline chimeras in Drosophila. Genetics **131:** 643–653.

Cvejic, S., Z. Zhu, S. J. Felice, Y. Berman and X.-Y. Huang, 2004 The endogenous ligand Stunted of the GPCR Methuselah extends lifespan in Drosophila. Nat. Cell Biol. **6:** 540–546.

de Saint Phalle, B., and W. Sullivan, 1998 Spindle assembly and mitosis without centrosomes in parthenogenetic Sciara embryos. J. Cell Biol. **141:** 1383–1391.

DiGregorio, P. J., J. A. Ubersax and P. H. O'Farrell, 2001 Hypoxia and nitric oxide induce a rapid reversible cell cycle arrest of the Drosophila syncytial divisions. J. Biol. Chem. **276:** 1930–1937.

Endow, S. A., and D. J. Komma, 1996 Centrosome and spindle function of the Drosophila Ncd microtubule motor visualized in live embryos using Ncd-GFP fusion proteins. J. Cell Sci. **109:** 2429–2442.

Endow, S. A., R. Chandra, D. J. Komma, A. H. Yamamoto and E. D. Salmon, 1994 Mutants of the Drosophila *ncd* microtubule motor protein cause centrosomal and spindle pole defects in mitosis. J. Cell Sci. **107:** 859–867.

Finkel, T., and N. J. Holbrook, 2000 Oxidants, oxidative stress and the biology of aging. Nature **408:** 239–247.

Foe, V. E., G. M. Odell and B. A. Edgar, 1993 Mitosis and morphogenesis in the Drosophila embryo: point and counterpoint, pp. 149–300 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez-Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fogarty, P., S. D. Campbell, R. Abu-Shumays, B. S. Phalle, K. R. Yu *et al.*, 1997 The Drosophila *grapes* gene is related to checkpoint gene chk1/rad27 and is required for late syncytial division fidelity. Curr. Biol. **7:** 418–426.

Frenz, L. M., and D. M. Glover, 1996 A maternal requirement for glutamine synthetase 1 for the mitotic cycles of syncytial Drosophila embryos. J. Cell Sci. **109:** 2649–2660.

Galloni, M., and B. A. Edgar, 1999 Cell-autonomous and non-autonomous growth-defective mutants of *Drosophila melanogaster*. Development **126:** 2365–2375.

Giot, L., J. S. Bader, C. Brouwer, A. Chaudhuri, B. Kuang *et al.*, 2003 A protein interaction map of *Drosophila melanogaster*. Science **302:** 1727–1736.

Girardot, F., V. Monnier and H. Tricoire, 2004 Genome wide analysis of common and specific stress responses in adult *Drosophila melanogaster*. BMC Genomics **5:** 74.

Hari, K. L., A. Santerre, J. J. Sekelsky, K. S. McKim, J. B. Boyd *et al.*, 1995 The *mei-41* gene of *D. melanogaster* is a structural and functional homologue of the human ataxia telangiectasia gene. Cell **82:** 815–821.

Hochachka, P. W., 2003 Intracellular convection, homeostasis and metabolic regulation. J. Exp. Biol. **206:** 2001–2009.

Jacobs, H., R. Stratmann and C. Lehner, 1998 A screen for lethal mutations in the chromosomal region 59AB suggests that bellwether encodes the alpha subunit of the mitochondrial ATP synthase in Drosophila. Mol. Gen. Genet. **259:** 383–387.

Katayoun, A., B. Stuart and S. A. Wasserman, 2000 Functional analysis of the Drosophila Diaphanous FH protein in early embryonic development. Development **127:** 1887–1897.

Katzen, A. L., and J. M. Bishop, 1996 *myb* provides an essential function during Drosophila development. Proc. Natl. Acad. Sci. USA **93:** 13955–13960.

Katzen, A. L., T. B. Kornberg and J. M. Bishop, 1985 Isolation of the proto-oncogene *c-myb* from D. melanogaster. Cell **41:** 449–456.

Katzen, A. L., J. Jackson, B. P. Harmon, S. M. Fung, G. Ramsay *et al.*, 1998 Drosophila *myb* is required for the G2/M transition and maintenance of diploidy. Genes Dev. **12:** 831–843.

Kellogg, D. R., T. J. Mitchison and B. M. Alberts, 1988 Behavior of microtubules and actin filaments in living Drosophila embryos. Development **103:** 675–686.

Kidd, T., 1994 Genetic and molecular studies of early embryogenesis in Drosophila. Ph.D. Thesis, Oxford University, Oxford.

Lai-Zhang, J., and D. M. Mueller, 2000 Complementation of deletion mutants in the genes encoding the F_1-ATPase by expression of the corresponding bovine subunits in yeast *Saccharomyces cerevisiae*. Eur. J. Biochem. **267:** 2409–2418.

Lai-Zhang, J., Y. Xiao and D. M. Mueller, 1999 Epistatic interactions of deletion mutants in the genes encoding the F_1-ATPase in yeast *Saccharomyces cerevisiae*. EMBO J. **18:** 58–64.

Li, K., and T. C. Kaufman, 1996 The homeotic target gene *centrosomin* encodes an essential centrosomal component. Cell **85:** 585–596.

Li, P., D. Nijhawan and X. Wang, 2004 Mitochondrial activation of apoptosis. Cell **116:** 57–59.

Lin, Y. J., L. Seroude and S. Benzer, 1998 Extended lifespan and stress resistance in the Drosophila mutant *methuselah*. Science **282:** 943–946.

Liu, Y. T., and H. L. Yin, 1998 Identification of the binding partners for flightless I, a novel protein bridging the leucine-rich repeat and the gelsolin superfamilies. J. Biol. Chem. **273:** 7920–7927.

Minauro-Sanmiguel, F., C. Bravo and J. J. Garcia, 2002 Cross linking of the endogenous inhibitor protein (IF1) with rotor (γ, ε) and stator (α) subunits of the mitochondrial ATP synthase. J. Bioenerg. Biomembr. **34:** 433–443.

Minden, J. S., D. A. Agard, J. W. Sedat and B. M. Alberts, 1989 Direct cell lineage analysis in Drosophila melanogaster by time lapse three dimensional optical microscopy of living embryos. J. Cell Biol. **109:** 505–516.

Pawnard, P., J. Vaillien, B. Coulary, J. Schaeffer, V. Soubannier *et al.*, 2003 The ATP synthase is involved in generating mitochondrial cristae morphology. EMBO J. **21:** 221–230.

Price, D., S. Rabinovitch, P. H. O'Farrell and S. D. Campbell, 2000 Drosophila *wee1* has an essential role in the nuclear divisions of early embryogenesis. Genetics **155:** 159–166.

Rothwell, W. F., and W. Sullivan, 1999 Fluorescent analysis of Drosophila embryos, pp. 141–157 in *Drosophila Protocols*, edited by W. Sullivan, A. Ashburner and R. S. Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rothwell, W. F., and W. Sullivan, 2000 The centrosome in early Drosophila development. Curr. Top. Dev. Biol. **49:** 409–447.

Rothwell, W. F., P. Fogarty, C. M. Field and W. Sullivan, 1998 *Nuclear-fallout*, a Drosophila protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization. Development **125:** 1295–1303.

Rothwell, W. F., C. X. Zhang, C. Zelano, T. S. Hsieh and W. Sullivan, 1999 The Drosophila centrosomal protein Nuf is required for recruiting Dah, a membrane associated protein, to furrows in the early embryo. J. Cell Sci. **112:** 2885–2893.

Schejter, E. D., and E. Wieschaus, 1993 Functional elements of the cytoskeleton in the early Drosophila embryo. Annu. Rev. Cell Biol. **9:** 67–99.

Schmid, K. J., and D. Tautz, 1998 Sequence and expression of DmMKLP1, a homolog of the human MKLP1 kinesin-like protein from *Drosophila melanogaster*. Dev. Genes Evol. **208:** 474–476.

Scholey, J. M., I. Brust-Mascher and A. Mogilner, 2003 Cell division. Nature **442:** 746–752.

Schweisguth, F. C., J.-A. Lepesant and A. Vincent, 1990 The *serendipity-alpha* gene encodes a membrane associated protein required for the cellularization of the Drosophila embryo. Genes Dev. **4:** 922–931.

Sharp, D. J., K. R. Yu, J. C. Sisson, W. Sullivan and J. M. Scholey, 1999 Antagonistic microtubule-sliding motors position mitotic centrosomes in Drosophila early embryos. Nat. Cell Biol. **1:** 51–54.

Sibon, O. C., V. A. Stevenson and W. E. Theurkauf, 1997 DNA-replication checkpoint control at the Drosophila midblastula transition. Nature **388:** 93–97.

Sibon, O. C, A. Laurencon, R. Hawley and W. E. Theurkauf, 1999 The Drosophila ATM homologue Mei-41 has an essential checkpoint function at the midblastula transition. Curr. Biol. **9:** 302–312.

Simpson, L., and E. Wieschaus, 1990 Zygotic activity of the *nullo* locus is required to stabilize the actin myosin network during cellularization in Drosophila. Development **110:** 851–863.

Smith, A. V., and T. L. Orr-Weaver, 1991 The regulation of the cell cycle during Drosophila embryogenesis: the transition to polyteny. Development **112:** 997–1008.

Solaini, G., A. Baracca, E. Gabellieri and G. Lenaz, 1997 Modification of the mitochondrial F1-ATPase ε subunit, enhancement of the ATPase activity of the IF1–F1 complex and IF1 binding dependence of the conformation of the ε subunit. Biochem. J. **327:** 443–448.

Song, W., R. Ranjan, K. Dawson-Scully, P. Bronk, L. Marin *et al.*, 2002 Presynaptic regulation of neurotransmission in Drosophila by the g protein-coupled receptor methuselah. Neuron **36:** 105–119.

Stevenson, V., J. Kramer, J. Kuhn and W. E. Theurkauf, 2001 Centrosomes and the Scrambled protein coordintate microtubule-independent actin reorganization. Nat. Cell Biol. **3:** 68–75.

Sullivan, W., and W. E. Theurkauf, 1995 The cytoskeleton and morphogenesis of the early Drosophila embryo. Curr. Opin. Cell Biol. **7:** 18–22.

Sullivan, W., P. Fogarty and W. Theurkauf, 1993 Mutations affecting the cytoskeletal organization of syncytial Drosophila embryos. Development **118:** 1245–1254.

Valdes-Perez, R. E., and J. S. Minden, 1995 *Drosophila melanogaster* syncytial nuclear divisions are patterned: time-lapse images. Hypothesis and computational evidence. J. Theor. Biol. **175:** 525–532.

Zhang, C., M. P. Lee, A. D. Chen, S. D. Brown and T.-S. Hsieh, 1996 Isolation and characterization of a Drosophila gene essential for early embryonic development and formation of cortical cleavage furrows. J. Cell Biol. **134:** 923–934.

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