**giant nuclei** is essential in the cell cycle transition from meiosis to mitosis

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SUMMARY

At the transition from meiosis to cleavage mitoses, *Drosophila* requires the cell cycle regulators encoded by the genes, *giant nuclei (gnu)*, *plutonium (plu)* and *pan gu (png)*. Embryos lacking Gnu protein undergo DNA replication and centrosome proliferation without chromosome condensation or mitotic segregation. We have identified the *gnu* gene encoding a novel phosphoprotein dephosphorylated by Protein phosphatase 1 at egg activation. Gnu is normally expressed in the nurse cells and oocyte of the ovary and is degraded during the embryonic cleavage mitoses. Ovarian death and sterility result from *gnu* gain of function. *gnu* function requires the activity of *pan gu* and *plu*.

Key words: *Drosophila melanogaster*, Mitosis, DNA replication, Fertilization, Oogenesis, Protein phosphatase 1.

INTRODUCTION

Natural developmental mechanisms ensure that oocytes and eggs arrest to await fertilization. These exhibit remarkable evolutionary flexibility, with different species using a variety of discrete arrest points, illustrating the diversity of regulatory mechanisms that have evolved to arrest the fundamental cell cycle oscillator (Sagata, 1996). The *Drosophila* oocyte is normally arrested by its chiasmate chromosomes at metaphase of the first meiotic division (Jang et al., 1995). Movement of the oocyte into the oviduct is accompanied by its hydration and activation. Gnu is normally expressed in the nurse cells and oocyte of the ovary and is degraded during the embryonic cleavage cycles (Freeman et al., 1986; Freeman and Glover, 1987; Shamanski and Orr-Weaver, 1991; Axton et al., 1994; Elfring et al., 1997; Fenger et al., 2000). Regardless of embryonic genotype, oocytes, eggs and embryos derived from *plu*, *png* or *gnu* homozygous females will be referred to here as *plu*, *png* or *gnu* oocytes, eggs or embryos.

Pan gu and Plu co-immunoprecipitate from egg and embryo extracts suggesting they act in a complex. However, the level of Plu is reduced in null *png* mutants (Elfring et al., 1997) leaving open the possibility that Plu is a downstream effector of Pan gu. The levels of the mitotic Cyclins A and B and Cdk1 kinase activity are decreased in embryonic extracts mutant for *png*, *gnu* or *plu* (Fenger et al., 2000) providing a link between the giant nuclei phenotype with known cell cycle regulators. In addition, a genetic screen for *png* genetic interactors identified enhancement by *cyclin B*. Experimental restoration of Cyclin B levels in a *png* background was able to restore polar body chromosome condensation, though the zygotic nuclei eventually became polyploid (Lee et al., 2001). Thus Cyclin B is a critical, but probably not the sole, target of *plu*, *plu* and *gnu* action. Here we describe the cloning of the *gnu* gene. We also describe an analysis of the *gnu* over-replication phenotype and investigate *gnu* function using epitope-tagged constructs.

MATERIALS AND METHODS

*Drosophila* stocks and libraries

Stocks (Lindsley and Zimm, 1992) were maintained at 25°C under
standard conditions (Roberts and Standen, 1998). The single extant gnu mutation ([Freeman et al., 1986] Tübingen stock gnuB536) was produced in a screen for female steriles by EMS mutagenesis of ru th kni rae pδ eδ ca. gnu complemented Df(3L);JfD21 and Df(3L);BrdR15 but was uncovered by Df(3L);JfM21 and Df(3L);D5rv5. The phage library was constructed by C. González in BamHI-cleaved ADASH (Stratagene). The cosmids were in NotBamNot CosPer vector (Tamkun et al., 1992) and Loristü (Siden-Kiamos et al., 1990).

**P-element induced male recombination**

gnu was mapped by P-element induced male recombination (Chen et al., 1998) relative to Trp2322 (a P-element insertion verified by inverse PCR). The gnu stock ru gnu kni ru th pδ eδ /TM3 Sb has visible flanking markers ru and kni[r]. The source of transposase was Delta2-3 CyO. Six independent recombinant chromosomes were recovered and all indicated that gnu is proximal relative to Trp2322.

**Transgenes**

A 3.5 kb XhoI fragment from phage clone 23.13.3 was transferred into the XhoI site of pcAspR4 (Sambrook et al., 1989). This comprised the complete ORF of CG5272, with 1.6 kb of 5′ and 3′UTR sequence and the CG5258 (NHP2) coding region including the stop codon but lacking the 3′ UTR. This construct, inserted (Spradling, 1986) on the second chromosome (F1I) and an independent insertion (M2A) restored fertility to homozygous females such that they produced fertile adult progeny. The premature stop codon and SpeI site were introduced by site-directed mutagenesis using the QuikChange™ (Stratagene) strategy with the primers CGGGA TCCAAAGGAGAAGAACTTTTCACTG and CGC-(Cormack et al., 1996) was amplified using the restriction site-tagged restriction enzyme-tagged primers GCTCTAGAGCTCAGCTGTTC and the genomic AAAAGCGGCCGCA TTA TTTGTAAAA TTACCG and GCTCTAGAGCTCAGCTGTTC. The mutated fragment was cloned into EcoRI/XhoI-cut pcAspR4. This construct inserted on the second chromosome (stock GS3A) and independent insertion (GS2B) did not rescue the sterility of homozygous gnu females. The eggs laid by such females failed to undergo any normal cleavage cycles and all developed giant nuclei.

**Production of GFP-tagged gnu constructs**

The 3.5 kb XhoI fragment from pBluescript SK was treated to remove the downstream CG5258 (NHP2) gene and destroy a vector BamHI site, by Pmel/BamHI digestion, end filling and re-ligation. A gnu 3′ BamHI site was created by site-directed mutagenesis with the primers GCCAAGCAATTCCTGGATCTATCTGTTAGG and CCTAGGATATAAGATCGGAATGTTGCTGTG. The transgene and imaged with identical confocal settings. For Gnu-GFP mis-expression, a UASp gnu-GFP construct was produced by PCR using the restriction-tagged primers GCCTAGAAGCTCATGTT-TCTTAGCC and GGAATCTAAGCATACTAGCTGCGCCG and the product was inserted into EcoRI/XhoI-digested pcAspR4 to create a genomic gnu-GFP construct. This construct, inserted on the second chromosome, restored fertility to homozygous gnu females (GG4c).

**RESULTS**

**gnu eggs and embryos develop giant polyploid nuclei in which DNA replication is uncoupled from nuclear division**

DNA replication in gnu eggs and embryos might be continuous or cyclic, with gaps in which there is no replication. To determine between these possibilities, we examined the distribution of the DNA polymerase-δ processivity factor, PCNA (Yamaguchi et al., 1991) and nuclear lamins in gnu embryos. In gnu embryos, the majority of giant nuclei stained for PCNA indicating that they were in S phase. However PCNA was excluded from a number of nuclei (Fig. 1A,B) even in the presence of a nucleus containing PCNA within the same embryo, suggesting that giant nuclei exit S phase and that the nuclei in a single gnu embryo do not always cycle in synchrony. The majority of the nuclei were surrounded by an intact lamina but occasionally, giant nuclei were observed in which the nuclear lamins had dissociated (data not shown).

When BrdU incorporation was used to detect DNA
replication in gnu embryos, some, but not all, of the giant nuclei incorporated BrdU (Fig. 1C,D). Taken together, the results from these cell cycle markers suggest that some nuclei were in S phase whilst others in the same embryo were not and that DNA replication in gnu embryos is cyclic or of limited duration.

It was previously reported that, in gnu eggs and embryos, the nuclei neither condense chromosomes nor divide but the centrosomes replicate and organise asters apparently normally (Freeman and Glover, 1987). We examined microtubule asters centrosomes replicate and organise asters apparently normally the nuclei neither condense chromosomes nor divide but the asters were observed (Fig. 1E,F).

**Gnu is a small novel protein**

gnu lies between the distal breakpoint of Df(3L)fzD21 at 70E5-6 and the distal breakpoint of Df(3L)BrdR15 at 71A1-2. Microdissected clones of polytene chromosome DNA (Saunders, 1990) from the region were used as starting points to construct a genomic walk. By sequencing the ends of the inserts of phage and cosmid clones, we anchored the walk to the sequence of the Drosophila genome (Adams et al., 2000). gnu was mapped proximal to Trl by P-element-induced male recombination, placing gnu within a region of 131 kb and 10 predicted genes between Trl and the distal breakpoint of Df(3L)BrdR15. Sequencing genes from the gnu chromosome in this region revealed a C to T mutation in gene CG5272 resulting in a premature stop codon (Fig. 2). This mutation was not present on other lines (fs(3)131-19 and fs(3)135-17, Tübingen stock centre) made in the same mutagenesis screen as gnu (data not shown).

In transgenic Drosophila, a 3.5 kb XbaI fragment from a phage containing CG5272, complemented the female sterility of the gnu mutation, however, transformants containing the same construct, except for a premature stop codon introduced into CG5272 by site-directed mutagenesis, did not rescue the gnu mutation. Therefore gnu is CG5272.

cDNAs GM10421 and LD12084, corresponding to ESTs in the BDGP database (http://www.fruitfly.org) that matched CG5272 were sequenced, confirming that gnu is a small gene with a single intron encoding a 240 amino acid protein with a predicted molecular mass of 27 kDa (Fig. 2). The premature stop codon in gnu mutants would produce a truncated protein lacking the C-terminal 94 residues. The deduced Gnu sequence was used to search the protein databases. No close matches were found, therefore Gnu is a novel protein.

**Gnu is specific for early development**

Rabbit anti-Gnu antiserum Rb86, raised against a synthetic peptide comprising aa117-131 is specific for Gnu and for Gnu-GFP but does not recognise a truncated product of the gnu mutant (Fig. 3A). The expression profile of a functional Gnu-GFP fusion protein under the control of the gnu promoter was examined by immunoblotting and detection with a monoclonal antibody against GFP. Gnu-GFP was expressed in ovaries and 0- to 3-hour embryos (Fig. 3A,B) and in unfertilised eggs, but not in larval tissues or in adult testes (not shown). The epitope-tagged protein had very similar expression to native Gnu detected with an anti-peptide antiserum, but had a somewhat longer half-life in cleavage embryos. We did not detect Gnu in embryos more than 1 hour after egg deposition (Fig. 3B), in larvae or in adult testes (not shown). The mobility of Gnu and of Gnu-GFP from ovaries was slower than from unfertilised eggs or embryos suggesting Gnu is post-translationally modified. The mobility of the embryonic isoform matched the predicted size of the fusion protein (54 kDa). GFP mobility in extracts from ovaries and embryos from a ubiquitin-driven GFP line were identical (data not shown), therefore it is only the Gnu moiety of Gnu-GFP that is modified.

**Gnu is dephosphorylated upon egg activation**

In ovary extracts with phosphatase inhibitors, the slow moving form of Gnu-GFP was observed (Fig. 3A,C). If phosphatase inhibitors were omitted from the ovary extraction, the amount of slow moving form was reduced in favour of the fast moving form with the same mobility as Gnu-GFP from embryos (Fig. 3C). To ascertain which protein phosphatases (PPs) are involved, specific inhibitors of serine/threonine protein phosphatases were tested for their ability to stabilise the slow moving form (Fig. 3C). Okadaic acid (OA) at low concentration (1 nM), sufficient to inhibit PP2A, did not
stabilise slow moving Gnu-GFP, however OA at a higher concentration (50 nM), sufficient to inhibit PP1 (Mackintosh and Mackintosh, 1994), stabilised slow moving Gnu-GFP. I-2Dm, a specific inhibitor of PP1 (Bennett et al., 1999), also stabilised slow moving Gnu-GFP. We conclude that PP1 can dephosphorylate Gnu in ovary extracts.

To determine the developmental time-point at which Gnu dephosphorylation occurs, we crossed the genomic gnu-GFP transgene into mutant backgrounds that cause the oocyte to arrest development during meiosis [cortex and grauzone (Page and Orr-Weaver, 1996)], or immediately following meiosis but prior to the first zygotic mitosis deadhead (Pellicena-Palle et al., 1997)]. Gnu-GFP mobility in ovaries and eggs in these mutant backgrounds was indistinguishable from wild-type (Fig. 3D) indicating that Gnu is dephosphorylated before meiotic arrest induced by cortex and grauzone, most likely at egg activation.

Gnu accumulates in oocytes of stage 11 egg chambers and is cytoplasmic in eggs and embryos

In ovaries, Gnu-GFP was first observed in fixed oocytes of stage 11 egg chambers (Fig. 4A). In subsequent stages it accumulated in the oocyte but was not observed in nurse cells (Fig. 4B). In eggs, Gnu-GFP was cytoplasmic and showed no association with the replicatively inactive polar body chromosomes (Fig. 4C,D). In syncytial embryos Gnu-GFP was again cytoplasmic at all stages of the cell cycle. Although the nuclear envelope stains somewhat more distinctly, Gnu is neither strongly localised within, nor excluded from zygotic nuclei (Fig. 4E,F).

Gnu post-translational modifications are not dependent on pgn

Gnu-GFP mobility in ovaries and eggs in both null and weak pgn backgrounds was indistinguishable from wild-type (Fig. 3E). Therefore, Pan gu is neither the kinase that phosphorylates Gnu nor part of a pathway leading to Gnu dephosphorylation. Even if Pan gu does not influence Gnu modification, it might regulate Gnu localisation. To test this possibility we examined Gnu-GFP localisation in null background. We found that Gnu was cytoplasmic in embryos and it was excluded from the giant nuclei (Fig. 4G-H).
However, subsequent stages were characterised by large amounts of irregularly localised, often fragmented, chromatin resulting from the degeneration of nurse cell nuclei. No stage 14 egg chambers could be distinguished. Surprisingly, given that Gnu-GFP, expressed from its own promoter, was unlocalised in embryos, mis-expressed Gnu-GFP was exclusively nuclear in nurse cells (Fig. 5D-F).

Control females containing the same driver and a UASp GFP construct had wild-type ovarian morphology and were fertile (Fig. 5B). GFP was present throughout their nurse cells, though the nuclei had slightly higher levels than the cytoplasm (Fig. 5B). We concluded that GFP does not show particular affinity for nurse cell nuclei or impede oogenesis, therefore the nuclear accumulation and sterility arising from Gnu-GFP mis-expression resulted from the Gnu moiety.

We compared the mobility of ectopic Gnu-GFP from ovaries to that of Gnu-GFP from ovaries expressing the genomic construct. Mis-expressed Gnu-GFP from ovaries was identical to the dephosphorylated embryonic form (Fig. 5G). Thus nurse cells do not phosphorylate Gnu, suggesting that the Gnu kinase activity is restricted to the oocyte.

To test whether the sterility associated with Gnu misexpression was a consequence of Gnu alone, we mis-expressed Gnu in ovaries in a png mutant background. Females homozygous for png1058 and containing the maternal alpha4tubulin>GAL4:VP16 and UASp gnu-GFP constructs laid eggs (Table 1). Staining of their ovaries revealed they were morphologically normal with no abnormal egg chambers or fragmented DNA (Fig. 5C). The egg laying rates for such
females were similar to wild type (Table 1) indicating that the restoration of ovarian function was complete. The eggs did not hatch but, when stained for chromatin, exhibited a giant nuclei phenotype identical to that in Fig. 4G-I, typical of embryos. The earliest mis-expression and amount of Gnu-GFP fluorescence in a background was the same as in a wild-type background indicating that the restoration of ovary function is not caused by an effect of png on Gnu-GFP mis-expression levels or timing. The effect of a homozygous null plu mutation in combination with Gnu-GFP mis-expression was indistinguishable from that of the null png mutation (Table 1).

Since the Gnu gain-of-function phenotype resembles that of Profilin mutants (Cooley et al., 1992) we examined the actin cytoskeleton of egg chambers (Fig. 6). Defects were first seen at stage 10, when nurse cells mis-expressing Gnu failed to assemble the actin meshwork and did not dump their cytoplasmic contents into the oocyte. We do not know whether the disruption of actin reorganisation is the primary consequence of excess Gnu, or whether premature egg chamber death results in the dramatically abnormal aggregates of F-actin. What is clear from our epistasis experiments is that this ‘dump-less’ phenotype is specific, in that it also requires Pan gu and Plu.
We have mapped the phosphatase. A specific inhibitor of PP1, I-2, stabilised phosphorylation is independent of Pan gu protein kinase is dephosphorylated upon egg activation, and that this Gnu is phosphorylated before it is needed in oocytes, We deduce from its mobility shift on immunoblots that Gnu phosphorylation other organisms. protein with no obvious domains or homologues shared with gnu is therefore gene CG5272 and encodes a novel 27 kDa transformants with the same fragment, but in which the ORF on the C to T nonsense mutation in gene CG5272 (Adams et al., 2000) resulted in the mutant phenotype in transgenic gnu, whereas transformants with the same fragment, but in which the ORF had been mutagenised, did not complement the gnu mutation. gnu is therefore gene CG5272 and encodes a novel 27 kDa protein with no obvious domains or homologues shared with other organisms.

Gnu phosphorylation
We deduce from its mobility shift on immunoblots that Gnu is phosphorylated before it is needed in oocytes, is dephosphorylated upon egg activation, and that this phosphorylation is independent of Pan gu protein kinase activity. A specific inhibitor of PP1, I-2, stabilised phosphorylated Gnu in vitro, implicating PP1 as the relevant phosphatase. Drosophila contains four PP1 genes of which PP1 87B provides 80% of total PP1 activity (Dombrádi et al., 1989) and is required for mitotic progression (Axton et al., 1990), though none have previously been ascribed a role in egg activation or fertilisation.

PP1 87B and PP2A28D mutations genetically suppress a weak png allele (Lee et al., 2001) However, these data are not consistent with our finding that PP1 activates Gnu. If unphosphorylated embryonic Gnu is the active form and Gnu and Pan gu act in the same pathway, then reducing the dose of the activating phosphatase should enhance a png phenotype. It may be that these phosphatases oppose png action directly by dephosphorylating the Pan gu substrate (Lee et al., 2001) or that they are the cell cycle regulators targeted by Gnu, Plu and PnP as discussed below.

Gain-of-function phenotype
Gnu mis-expression using the UAS-Gal4 system (Rørth, 1998) in Drosophila ovaries resulted in sterility due to an inability to lay eggs. Dissection of the ovaries revealed that early oogenesis was unaffected. Gnu was expressed in egg chambers from stage 5 onwards and was localised solely to the nurse cell nuclei. No normal egg chambers could be discerned at stage 10 or later when gross aberrations in the organisation of the actin cytoskeleton resulted in failure to transfer nurse cell cytoplasm into the oocyte. Gnu mobility from such ovaries was identical to the dephosphorylated form suggesting that the protein kinase that phosphorylates Gnu is not present or active in the nurse cells.

Since gnu, plu and png mutations have the same phenotype, we were previously unable to determine whether the gene products act in series or in parallel. Here we have used the dominant ovarian phenotype resulting from Gnu mis-expression to investigate the epistasis of gnu, png and plu. Loss of png or plu function blocked the ovarian phenotype caused by Gnu mis-expression. This result implies that ectopic Gnu destroys egg chambers only through Pan gu and Plu. It is therefore likely that wild-type Gnu function in the egg and embryo also requires Pan gu. Although Gnu-GFP is more obviously excluded from the larger png giant nuclei (Fig. 4H) than from zygotic nuclei (Fig. 4E) we do not favour the explanation that Gnu requires Pan gu for nuclear localisation. Firstly, Gnu-GFP is not specifically nuclear in wild-type embryos (Fig. 4E) and secondly, in a png null ovary, ectopic Gnu-GFP is able to concentrate in the polytene nurse cell nuclei (Fig. 6C). The remaining possibilities are that Gnu acts upstream of Pan gu and Plu or that it acts in a complex with Pan gu and Plu.

We have mis-expressed Gnu in polytene salivary glands and ovarian follicle cells (data not shown). In both cases Gnu was exclusively nuclear, but its expression had no obvious effect on tissue morphology. However, not all nurse cell nuclei in an egg chamber contained Gnu-GFP, suggesting that the presence of Gnu-GFP reflects the transcriptional activity of the nucleus or depends upon its cell cycle status. Why is Gnu nuclear in polytene cells and cytoplasmic in the diploid syncytial blastoderm and larval neuroblasts (data not shown)? Gnu contains no obvious nuclear import sequence, suggesting that Gnu binds a factor that is cytoplasmic in eggs and embryos (including those with giant nuclei; Fig. 4H), and nuclear in polytene tissues. Polytene and diploid tissues have different Cyclin profiles. Embryos are replete with maternal Cyclins A, B and E whereas polytene tissues have no Cyclin A or B (Lehner and O’Farrell, 1989; Lehner and O’Farrell, 1990; Richardson et al., 1993) and Cyclin E is expressed periodically in nurse cell nuclei and constantly in the

**DISCUSSION**

We have mapped the gnu gene using chromosome walking and P-element-mediated male recombination and have identified a C to T nonsense mutation in gene CG5272 (Adams et al., 2000) on the gnu chromosome. A wild-type copy of this gene rescued the gnu mutant phenotype in transgenic Drosophila, whereas transformants with the same fragment, but in which the ORF had been mutagenised, did not complement the gnu mutation. gnu is therefore gene CG5272 and encodes a novel 27 kDa protein with no obvious domains or homologues shared with other organisms.

**Table 1. Epistasis of gnu with png and plu**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Eggs/fly/day</th>
<th>Eggs</th>
<th>Phenotype</th>
<th>% surviving females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>s.d.</td>
<td>Hydrated</td>
<td>Hatched</td>
</tr>
<tr>
<td>OregonR</td>
<td>33.2</td>
<td>13.7</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>png(1058);png(1058)</td>
<td>21.5</td>
<td>10.6</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>w;matc4T GAL4VP16/UASp gnuEGFP</td>
<td>0.1</td>
<td>0.4</td>
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<td>No</td>
</tr>
<tr>
<td>png(1058);png(1058); matc4T GAL4VP16/UASp gnuEGFP</td>
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<td>12.0</td>
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<td>No</td>
</tr>
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<td>orp(1058);png(1058); matc4T GAL4VP16/UASp gnuEGFP</td>
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<td>0.8</td>
<td>No</td>
<td>No</td>
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<td>24.8</td>
<td>7.8</td>
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<td>No</td>
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<tr>
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<td>8.0</td>
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<td>Yes</td>
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<td>30.7</td>
<td>9.4</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
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<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>matc4T GAL4VP16/w; UASp gnuEGFP plu[6]/plu[6]</td>
<td>Many</td>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The gnu gain-of-function (gain) phenotype consisted of reduced lifespan, disintegrating ovary (Fig. 5A, Fig. 6D-F), few flaccid eggs were laid and these did not hatch. The png null (loss) phenotype consisted of normal viability and ovarian morphology (Fig. 5C), many hydrated eggs with giant nuclei (similar to Fig. 4G-I) were laid and these did not hatch. The double mutant combination exhibited the png loss phenotype. Gnu overexpression combined with a null allele of plu resulted in the plu loss phenotype.
germinal vesicle (Lilly and Spradling, 1996). Our observations fit the Cyclin E pattern, ectopic Gnu was not present in all nurse or follicle cell nuclei and was concentrated in germinal vesicles.

**Downstream targets of giant nuclei genes**

The DNA replication in gnu embryos resembles the endoreduplicated observed in *Drosophila* ovarian nurse cells and larval salivary glands and this raises the question of how the normal mechanisms that license DNA replication once per cell cycle are subverted in gnu embryos. In yeast, Cdk1 activity, modulated by Cyclin levels, is responsible for resetting replication origins (Hayles et al., 1994) raising the possibility that over-replication in gnu embryos may result from inappropriate Cdk1 activity. Indeed, in gnu, plu or png embryos, levels of Cyclin A and B proteins and Cdk1 activity are reduced (Fenger et al., 2000). Restored Cyclin B levels can suppress a weak png mutation (Lee et al., 2001).

Several features of early *Drosophila* embryogenesis may have necessitated the evolution of these specialised regulators of the cell cycle. Firstly, distinct cell cycle fates befall the polar body and zygotic nuclei within a common cytoplasm. Secondly, many cell cycle regulators are in excess, so that the first 13 cycles are rapid and lack G1 or G2 phases, but S and M phases must alternate accurately. Finally, correct cell cycle regulation is achieved by a small subset of the available cell cycle control proteins (e.g. Cyclins A and B) (Edgar et al., 1994). In this context, Gnu, Plu and Pan gu act coordinately to ensure that the cell cycle oscillations experienced by the nuclei are temporally and locally apt.

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