

## Supplementary material

### ***Drosophila grapes*/CHK1 mutants are defective in cyclin proteolysis and coordination of mitotic events**

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#### Supplementary materials and methods

##### *Cyclin A turnover*

Embryos were collected for 30 min and aged for 40 min to 1 h at room temperature to achieve cycles 4–8 (Figure 1a) or 8–12 (Figure 1b). In initial experiments, a fraction of embryos were fixed and stained for DNA (see below) and visualized to confirm that the expected cell-cycle number had been reached. In Figure 1a, embryos were dechorionated and permeabilized with octane using previously published procedures (for example, [S1]). Permeabilized embryos were incubated in Schneider's tissue culture medium containing 20 µg/ml cycloheximide for 30 min, with (+ col, + cyc) or without (+ cyc) a 20 min prior incubation in Schneider's medium containing 50 µg/ml colchicine. For controls, either untreated embryos or embryos that had been permeabilized and incubated in Schneider's media for 30 min were used, with no obvious difference. In both Figure 1a and 1b, approximately equal numbers of embryos were homogenized in HEMG buffer (25 mM HEPES, pH 7.6, 0.1 mM EDTA, 12.5 mM MgCl<sub>2</sub>, 2 mM Na<sub>2</sub>VO<sub>4</sub>, 1 mM benzamide, 0.2 mM PMSF, 2 µg/ml aprotinin, 1.5 mM DTT, 10% glycerol) and boiled in SDS gel loading buffer. Samples were separated on SDS gels and western blotted according to standard procedures. Western blots were probed with rabbit polyclonal antibodies against *Drosophila* cyclin A (1:700 dilution; [S2]) or monoclonal antibodies against *Drosophila* cyclin B (1:2 dilution; [S3]) or rabbit polyclonal antibodies against β-tubulin (Amersham). ECL (Amersham) detection was used for western blots. The blot was also stained with Ponceau (Sigma) to visualize proteins (see Figure 1b) before western blotting.

##### *Antibody staining for PH3*

Embryos were fixed for 20 min in PBS + 10% formaldehyde or 30 min in PBS + 3.7% formaldehyde, using standard procedures. DNA was stained with 10 µg/ml bisbenzamide (Hoechst 33258), and PH3 was detected with a purified rabbit polyclonal antibody (1:1000 dilution; Upstate Biotechnologies) against the epitope ARKS\*TGKAPRKQL (in the single-letter amino-acid code; the asterisk indicates that S is phosphorylated), which is present in three *Drosophila* histone H3 variants. In Figure 2 and Table 1, division cycle was determined from nuclei number ( $n = 2$  for cycle 2;  $n = 4$  for cycle 3, etc.) and nuclear location with respect to the embryo surface (in embryo interior through cycle 8, migration during cycle 9, surface reached at the end of cycle 9).

##### *Fly stocks*

The *grp* stock (*grp*<sup>1</sup>/Cy) has been described before [S4,S5]. The *grp* mutation is a maternal-effect mutation; homozygous mutant mothers are identified by the lack of a CyO balancer and served as a source of *grp* embryos. The *grp*<sup>1</sup> allele was used in all experiments unless otherwise stated. Fly stock carrying the *hs-cyclin A* transgene on chromosome III has been described before [S6]. Flies carrying *grp*<sup>1</sup> and *hs-cyclin A* alleles were constructed using standard *Drosophila* techniques. The deficiency chromosome Df(2L)H20 carries breakpoints at 36A08-09;36E-01-02 (Bloomington Stock Center).

#### Supplementary references

- S1. Edgar BA, O'Farrell PH: **The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by string.** *Cell* 1990, **62**:469-480.
- S2. Lehner CF, O'Farrell PH: **Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression.** *Cell* 1989, **56**:957-968.
- S3. Lehner CF, O'Farrell PH: **The roles of *Drosophila* cyclins A and B in mitotic control.** *Cell* 1990, **61**:535-547.
- S4. Fogarty P, Kalpin RF, Sullivan W: **The *Drosophila* maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13.** *Development* 1994, **120**:2131-2142.
- S5. Fogarty P, Campbell SD, Abu-Shumays R, Phalle BS, Yu KR, Uy GL, et al.: **The *Drosophila grapes* gene is related to checkpoint gene *chk1/rad27* and is required for late syncytial division fidelity.** *Curr Biol* 1997, **7**:418-426.
- S6. Knoblich JA, Lehner CF: **Synergistic action of *Drosophila* cyclins A and B during the G2-M transition.** *EMBO J* 1993, **12**:65-74.