Supplementary data

GST fusion proteins

cDNAs encoding *Xenopus* Cdc25A peptides (S120, residues 91-136; S137, 120-168; S168, 138-189; S190, 166-215; S295, 265-316; S502, 472-521), a human Cdc25B peptide (S549, 516-566), *Xenopus* Cdc25C peptides (S287, 254-316; T533, 500-550), a *Drosophila* String peptide (S457, 424-474), or their Ser/Thr → Ala mutant peptides, were subcloned into the pGEX-3X plasmid vector, and the GST-fused peptides were bacterially expressed and purified by standard methods.

In vitro Cdc25A phosphatase assays

For *in vitro* Cdc25A phosphatase assays (see Figure 4A in text), GST-Cdc25A fusion protein, GST-Δ60-Chk1 protein, or GST-cyclin A1 protein was overexpressed in activated eggs for 2 h (by injection of 2 ng of their mRNA per egg), pulldowned with glutathione-Sepharose beads (Amersham) in a pulldown buffer (20 mM sodium phosphate [pH 8.0], 80 mM β-glycerophosphate, 0.2% Tween-20, 1 mM EDTA, 1 mM dithiothreitol (DTT), 2 μM pepstatin A, 10 μg/ml aprotinin and 0.2 mM PMSF), and then eluted from the beads either with a kinase assay buffer (50 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 2 μM pepstatin A, 10 μg/ml aprotinin, 0.2 mM PMSF and 10 mM glutathione: for GST-Cdc25A and GST-Δ60-Chk1) or with a phosphatase assay buffer (50 mM Tris-HCl [pH 8.0], 20 mM EDTA, 50 mM NaCl, 1 mM DTT, 0.1 mg/ml BSA, 2 μM pepstatin A, 10 μg/ml aprotinin, 0.2 mM PMSF and 10 mM glutathione: for GST-cyclin A1). [In the case of GST-cyclin A1, Xe-Wee1B (Okamoto *et al*, 2002) was co-expressed in eggs to produce Tyr15-phosphorylated Cdk1-cyclin A1 complexes.] Purified GST-Cdc25A protein (equivalent to 3 eggs) was first incubated with 3 mM ATP and purified GST-Δ60-Chk1 protein (equivalent to 15 eggs) in 30 μl of the kinase buffer for 1 h at 23 ˚C, and the reaction was stopped by the addition of 10 mM EDTA. The phosphorylated GST-Cdc25A protein (equivalent to one egg) was then incubated with purified Tyr15-phosphorylated Cdk1-cyclin A1 complexes (equivalent to 10 eggs) in 20 μl of the phosphatase assay buffer for 30 min at 23 ˚C. Tyr15 phosphorylation of the Cdk1-cyclin A1 complexes was then detected by immunoblotting using

anti-Cdk1 phospho-Tyr15 antibody.

Reference

Okamoto K, Nakajo N, Sagata N (2002) The existence of two distinct Wee1 isoforms in *Xenopus*: implications for the developmental regulation of the cell cycle. *EMBO J*: 2472- 2484