The EMBO Journal Vol.18 No.7 pp.1869–1877, 1999

Speedy: a novel cell cycle regulator of the G_2/M transition

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Stage VI *Xenopus* oocytes are suspended at the G_2/M transition of meiosis I, and represent an excellent system for the identification and examination of cell cycle regulatory proteins. Essential cell cycle regulators such as MAPK, cyclins and *mos* have the ability to induce oocyte maturation, causing the resumption of the cell cycle from its arrested state. We have identified the product of a novel *Xenopus* gene, Speedy or Spy1, which is able to induce rapid maturation of *Xenopus* oocytes, resulting in the induction of germinal vesicle breakdown (GVBD) and activation of M-phase-promoting factor (MPF). Spy1 activates the MAPK pathway in oocytes, and its ability to induce maturation is dependent upon this pathway. Spy1-induced maturation occurs much more rapidly than maturation induced by other cell cycle regulators including progesterone, *mos* or Ras, and does not require any of these proteins or hormones, indicating that Spy1-induced maturation proceeds through a novel regulatory pathway. In addition, we have shown that Spy1 physically interacts with cdk2, and prematurely activates cdk2 kinase activity. Spy1 therefore represents a novel cell cycle regulatory protein, inducing maturation through the activation of MAPK and MPF, and also leading to the premature activation of cdk2.

Keywords: cdk2/G_2–M transition/MAPK/MPF/*rad1*

Introduction

Resting (stage VI) oocytes are naturally arrested at the G_2/M boundary of the first meiotic division. This arrest is normally overcome by stimulation with progesterone and results in progression of oocytes through meiosis. During this maturation process, germinal vesicle breakdown (GVBD) and spindle formation occur, immediately followed by a short interkinesis and entry into meiosis I. Mature oocytes are arrested again at metaphase of meiosis II until fertilization. Studies have shown that introduction of certain cell cycle proteins, such as cyclin A and B, activated cdc2 or some Ser/Thr kinases (i.e. activated Raf-1 or *mos*), can also induce oocytes to resume the cell cycle (reviewed in Sagata, 1997).

The *mos* proto-oncogene encodes a Ser/Thr kinase that is specifically expressed and functions during meiotic maturation (or G_2/M progression) of vertebrate oocytes (Sagata *et al*., 1988, 1989; Freeman *et al*., 1989; O'Keefe *et al*., 1989). *mos* is expressed at a very low level in fully grown *Xenopus* oocytes that are arrested in prophase of meiosis I (Sagata *et al*., 1989). Progesterone, secreted by the surrounding follicle cells, induces *mos* protein synthesis and thereby stimulates oocytes to resume meiosis (Sagata *et al*., 1989). This maturation previously has been shown to be mediated by the MAPK signal transduction pathway (Posada *et al*., 1993; Nebreda and Hunt, 1993).

mos and MAPK have both been shown to be essential for oocyte maturation. Inhibition of *mos* translation by injection of antisense oligonucleotides directed against *mos* mRNA blocks progesterone-induced oocyte maturation (Sagata *et al*., 1988; Freeman *et al*., 1989; Muslin *et al*., 1993). Similarly, inhibition of MAPK activation by injection of neutralizing antibodies directed against MAPKK blocks *mos*-induced maturation and, consequently, progesterone-induced maturation (Kosako *et al*., 1992, 1996). Thus, *mos* and MAPK activity are required for natural oocyte maturation.

mos functions by directly binding to and activating MAPKK (Chen and Cooper, 1995), which in turn activates MAPK. MAPK subsequently activates M-phase-promoting factor (MPF; the universal M-phase regulator that controls meiotic progression). MPF is composed of a regulatory subunit, cyclin B, and a catalytic subunit, the cdc2 protein kinase. This activation of MPF, involving dephosphorylation on Thr14 and Tyr15 along with phosphorylation of Thr161 of cdc2, leads to GVBD. Following the completion of GVBD and meiosis I, oocytes enter interphase and subsequently meiosis II, where they are arrested as unfertilized eggs (reviewed in Sagata, 1997).

The *ras* proto-oncogene product can also induce oocyte maturation. This maturation appears to be similar to *mos*-induced maturation. In this case, Ras recruits and activates Raf-1, which can bind and activate MAPKK, resulting in the activation of the MAPK pathway. Subsequently, maturation continues through MPF activation and GVBD in the same manner as *mos*-induced maturation (Barrett *et al*., 1990).

This extensively studied mechanism of *Xenopus* oocyte maturation remains to be understood fully. For example, little is known about the initial steps of maturation. Events that occur upstream of *mos* activation are unclear. The Ser/Thr kinase Eg2 has been shown to become phosphorylated within minutes of progesterone stimulation, and the presence of Eg2 protein can accelerate progesterone-induced oocyte maturation (Andresson and Ruderman, 1998). Thus, Eg2 has been suggested to act in the initial steps of oocyte maturation. Furthermore, v-*mos* (the viral homolog) is known to be regulated by protein kinase A in mammalian

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cells (Yang *et al*., 1996). Moreover, the physiological significance of these interactions remains to be elucidated fully.

Interestingly, the NIMA protein kinase of *Aspergillus nidulans* was shown to bypass this well-documented system of *Xenopus* oocyte maturation altogether. Specifically, NIMA was able to induce GVBD without activating *mos*, cdc2 or MAPK (Lu and Hunter, 1995). This suggests the existence of additional cell cycle regulators of the G_2/M transition which may induce oocyte maturation by activation of alternative pathways. This intriguing result, together with the lack of understanding regarding the initiation of oocyte maturation, prompted us to screen for novel cell cycle regulator genes that function in *Xenopus* meiotic maturation.

Towards this end, we screened a *Xenopus laevis* ovary cDNA library using a yeast cell line deficient in *rad1*. In the fission yeast, *Schizosaccharomyces pombe*, the cell cycle checkpoint gene *rad1* is required to ensure that mitosis does not occur in the presence of DNA damage, or in the absence of complete DNA replication (Al-Khodairy and Carr, 1992; Enoch *et al*., 1992; Rowley *et al*., 1992). Loss of *rad1* function results in cell death following DNA damage or blocks replication due to failure to arrest at G_2/M (Hartwell and Weinert, 1989). Thus, by selecting for *Xenopus* cDNA clones that could overcome this G_2/M checkpoint deficiency in yeast, we were able to isolate cell cycle control genes.

In this study, we report the identification and characterization of a novel *Xenopus* gene product, designated Speedy (Spy1), which shares no significant sequence homology with any known protein. Spy1 expression in *Xenopus* oocytes is sufficient to stimulate meiotic maturation. Furthermore, the rate of Spy1-induced maturation is significantly faster than that of *mos* or progesterone. This ability of Spy1 to initiate meiosis appears to be independent of *mos* or Ras. However, similarly to *mos* and Ras, Spy1-induced maturation leads to activation of the MAPK pathway and is dependent on MAPK activation. Expression of Spy1 also leads to a rapid activation of MPF and premature activation and association with cdk2. Taken together, these results show that Spy1 represents a novel cell cycle gene product regulating the G_2/M transition in *Xenopus* oocytes.

Results

A Xenopus cDNA clone is able to confer resistance to radiation in fission yeast

In this study, we used genetic screening in fission yeast to search for cell cycle regulatory proteins in *Xenopus* which act at the G_2/M transition. A similar approach was used to isolate *Xenopus* genes which are able to prevent mitotic catastrophe in a *wee1*/*mik1*-deficient mutant of *S.pombe* (Su and Maller, 1995). Using a *rad1*-deficient strain of *S.pombe*, K1 (*h*$^-$ *rad1-1 leu1-32 ura4-D38*), which fails to arrest normally at the G_2/M boundary upon DNA damage (Hartwell and Weinert, 1989; Al-Khodairy and Carr, 1992; Enoch *et al*., 1992; Rowley *et al*., 1992), we screened a *X.laevis* total ovary cDNA library for plasmids that conferred resistance to UV radiation. The *X.laevis* total ovary cDNA library contains cDNAs cloned under the control of a constitutively expressed *S.pombe*

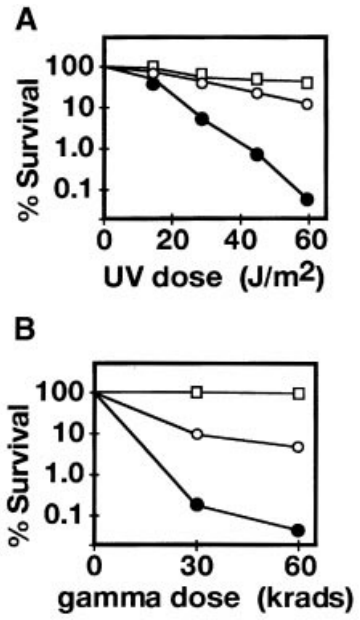

Fig. 1. A new *Xenopus* gene confers radiation resistance to fission yeast. Transformant partially rescues the sensitivity of *rad1-1* to UV radiation (**A**) and γ radiation (**B**). K1 (*h*$^-$*rad1-1 leu1-32 ura4-D38*) cells transformed with vector alone (●), K1/*X.laevis* transformant pSpy1 (○) and wild-type *rad1*$^+$-972 (□).

adh promoter (Su and Maller, 1995). From a screening of ~5.5×10^4 transformants, we isolated one clone which partially rescued the UV sensitivity of the K1 strain (Figure 1A). As a control, wild-type *rad*$^+$-972 showed normal resistance to UV radiation whereas the K1 strain carrying the parental vector alone was unable to confer resistance (Figure 1A). We then tested whether this *Xenopus* clone was also able to impart resistance to ionizing radiation. Upon exposure to γ radiation, cells carrying the *Xenopus* clone showed significant resistance to ionizing radiation, similar to the wild-type *rad1*$^+$-972 strain (Figure 1B). Taken together, these results demonstrated that expression of the *Xenopus* cDNA in fission yeast resulted in resistance to irradiation damage.

Isolation of a cDNA clone that encodes Speedy

To investigate the function of this *Xenopus* clone, hereafter designated Speedy (Spy1), the sequence of this transformant was determined. A single open reading frame (ORF) of 1260 nucleotides was identified which encodes a predicted polypeptide of 298 residues (Figure 2). A database search yielded no informative homology to any known protein, although this search did reveal a potential nuclear export signal (NES) motif (amino acids 51–59), an acidic amino acid domain (amino acids 127–132) and two putative PEST sequences (amino acids 238–249 and 253–277) (Figure 2A). The importance of the PEST sequences in the regulation of the protein stability and rapid turnover of some cell cycle proteins suggests that the Spy1 protein could have a short half life. Northern blot analysis with a radiolabeled Spy1 probe revealed expression in *Xenopus* stage VI oocytes of a single mRNA species of ~1.3 kb (data not shown).

Using quantitative RT–PCR, we examined Spy1 RNA expression in stage VI oocytes, eggs and early embryos from different developmental stages after fertilization. As

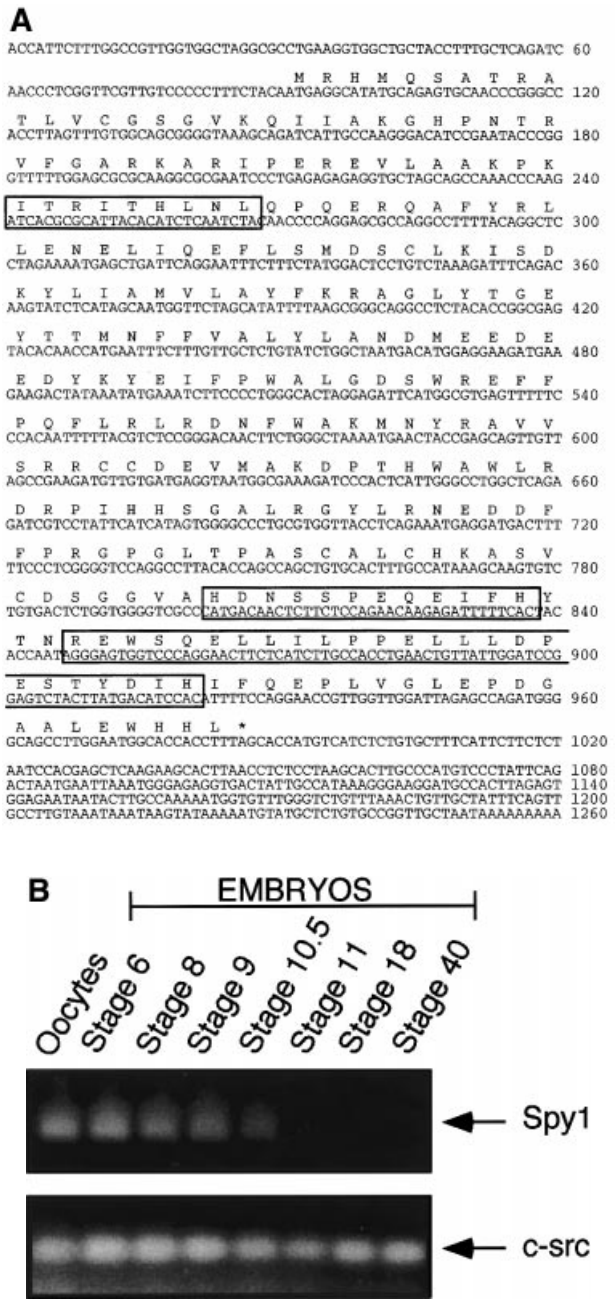

Fig. 2. Speedy; a novel *Xenopus* gene. (**A**) Nucleotide sequence and predicted amino acid sequence of *Xenopus* Spy1. Potential NES (amino acids 51–59) and PEST sequences (amino acids 238–249 and 253–277) are boxed. The DDBJ/EMBL/GenBank accession number for Spy1 is AJ133117. (**B**) Expression of Spy1 RNA during *Xenopus* development. For RT–PCR analysis, total RNA was isolated from stage VI oocytes or staged embryos, and 200 ng of RNA were assayed either for the presence of Spy1 (upper panel) or, as an internal control, c-*src* RNA (lower panel). The products of RT–PCR were analyzed by 1.5% agarose gel electrophoresis in the presence of ethidium bromide. The level of c-*src* RNA serves as a control for RNA loading as previously described (Rempel *et al*., 1995).

shown in Figure 2B, the level of speedy transcript was essentially constant from stage VI oocytes to the end of blastulation (stage 9), but decreased at the onset of gastrulation (stages 10–11) and became undetectable at later stages. As an internal control for RNA recovery, RT–PCR analysis of c-*src* RNA expression was evaluated. As previously shown (Rempel *et al*., 1995), the level of c-*src* RNA expression remains constant throughout oogenesis and early embryogenesis (Figure 2B). Taken together, these data indicate that Spy1 is expressed as a characteristic maternal mRNA in *Xenopus*.

Having demonstrated the presence of speedy mRNA throughout oogenesis and early embryogenesis, we sought to determine whether Spy1 protein is translated specifically during all or part of this period. Lysates prepared from resting oocytes, from oocytes induced to mature *in vitro* with progesterone or from early embryos were analyzed using two different affinity-purified C-terminal peptide antisera. Analysis by immunoblotting, immunoprecipitation, or immunoprecipitation followed by immunoblotting all revealed that Spy1 protein, if expressed at all, is at a level below detection (data not shown). These results suggest that either Spy1 protein is not expressed at all, despite the presence of Spy1 mRNA as a maternal message, or, more likely, that its translation results in an accumulation of Spy1 protein below our current level of detection.

Spy1 induces GVBD in Xenopus oocytes

To investigate the role of Spy1 protein during the cell cycle, we first examined the ability of Spy1 to regulate the G_2/M transition in *X.laevis* oocytes. Microinjection of *in vitro* synthesized mRNA encoding Spy1 into stage VI *Xenopus* oocytes was performed. At various times after microinjection, oocytes were collected and analyzed for their capacity to promote the G_2/M transition. As controls, oocytes were incubated with progesterone or injected in parallel with mRNA encoding the *Xenopus* Ser/Thr kinase *mos*, which are both natural inducers of oocyte maturation (Masui and Clarke, 1979; Freeman *et al*., 1990). Surprisingly, injection of Spy1 mRNA resulted in a release of oocytes from G_2 arrest and led to rapid oocyte maturation characterized by formation of a white spot on the animal pole and GVBD. These results demonstrated a cell cycle regulatory activity associated with Spy1 (Figure 3B). In progesterone-induced or *mos*-injected oocytes, 50% of the oocytes reached GVBD between 5 and 6 h after treatment. Significantly, Spy1-induced oocyte maturation was ~2-fold faster than maturation induced by progesterone or *mos* (Figure 3B). To confirm the breakdown of the nuclear envelope in oocytes injected with Spy1 mRNA, oocytes were fixed by trichloroacetic acid (TCA) and dissected. Analysis of these oocytes revealed the absence of nuclei, indicating completion of GVBD. Thus, expression of Spy1 is sufficient to overcome a G_2 arrest and can induce rapid oocyte maturation.

To detect Spy1 protein, we produced specific antibodies directed against the C-terminus of Spy1. The specificity of these antibodies is illustrated in Figure 3A. As determined by immunoprecipitation, these affinity-purified antisera detected a single protein band of ~43 kDa in an SDS–PAGE using *in vitro* translated Spy1 (Figure 3A, lane 4). This immunoreactive band was blocked in competition experiments using the corresponding peptide antigen (Figure 3A, lane 5). Injection of RNA encoding speedy into oocytes led to the expression of a 43 kDa protein, consistent with the *in vitro* translated Spy1 (Figure 3B, inset).

Spy1 activates a novel signal transduction pathway in Xenopus oocytes

To examine further the mechanism of rapid Spy1-induced maturation of oocytes, we tested whether Spy1 participates

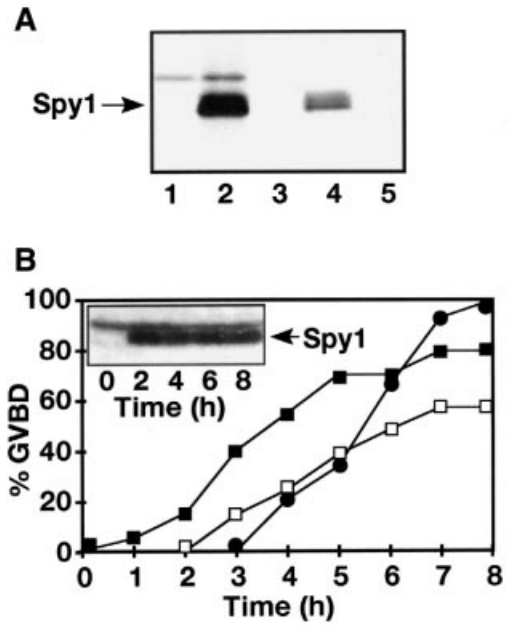

Fig. 3. Spy1 expression induces rapid oocyte maturation. (**A**) *In vitro* translation and immunoprecipitation of Spy1. Mock translation (lane 1); *in vitro* translation of Spy1 RNA (lane 2); Spy1 *in vitro* translation immunoprecipitated with pre-immune serum (lane 3), with affinity-purified antiserum (lane 4) or with affinity-purified antiserum with the addition of the cognate peptide (lane 5). (**B**) Time course of oocyte maturation. Oocytes were either stimulated with progesterone (●) or injected with Spy1 (■) or *mos* (□) mRNA and analyzed for GVBD at the indicated time points. The inset shows expression of Spy1, following microinjection of Spy1 RNA into oocytes. Spy1 protein was recovered at the indicated times from oocyte lysates by immunoprecipitation, and detected by immunoblotting and ECL.

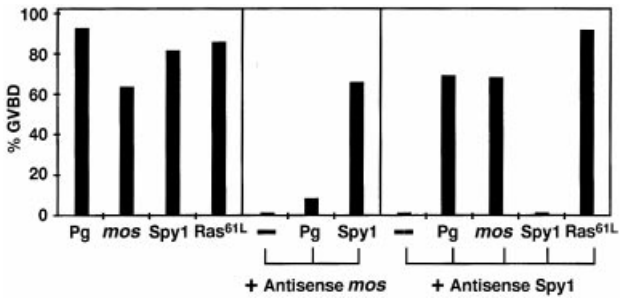

Fig. 4. Spy1 induces GVBD in Xenopus oocytes independently of *mos* or Ras. Oocytes were injected with the indicated mRNAs or incubated with progesterone (Pg) and scored for GVBD 8 h later. For antisense experiments, oocytes were first injected with the appropriate antisense oligonucleotides and, 4 h later, either injected with the indicated mRNA or incubated in progesterone. Oocytes were scored for GVBD by the formation of a white spot on the animal pole 8 h after secondary treatment. This graph is the composite of several independent experiments; for each condition, 40–130 oocytes were examined.

in the maturation pathways activated by the *mos* proto-oncogene product or by an oncogenic derivative of Ras (Korn *et al*., 1987; Sagata *et al*., 1988; Freeman *et al*., 1989; Barrett *et al*., 1990; Fabian *et al*., 1993; Muslin *et al*., 1993). It has been shown previously that injection of specific antisense *mos* oligonucleotides into oocytes results in a blockage of both progesterone-induced and insulin-stimulated pathways (Sagata *et al*., 1988; Freeman *et al*., 1989; Barrett *et al*., 1990; Fabian *et al*., 1993). Antisense oligonucleotides directed against either *mos* or Spy1 were microinjected into oocytes, and 4 h later *mos* or Spy1 mRNA was microinjected or progesterone was added as indicated (Figure 4, center). As reported previously (Sagata *et al*., 1988; Freeman *et al*., 1989; Muslin *et al*., 1993), antisense *mos* oligonucleotides were able to block progesterone-induced GVBD. However, antisense *mos* oligonucleotides failed to block Spy1-induced GVBD, indicating that *mos* is not required for Spy1-induced maturation (Figure 4, center). Reciprocally, antisense Spy1 oligonucleotides were unable to prevent oocyte maturation in response to *mos* or progesterone, although they did block Spy1-induced maturation (Figure 4, right). We next examined whether Spy1 plays a role in oocyte maturation induced by activated Ras. We used an oncogenic form of the H-*ras* product in which a glutamine residue at position 61 was replaced by a leucine (Hart and Donoghue, 1997). Microinjection of *in vitro* synthesized mRNA encoding Ras 61L resulted in induction of oocyte maturation (Figure 4, left). In the presence of antisense Spy1 oligonucleotides, activated Ras was still able to induce oocyte maturation (Figure 4, right).

Taken together, these results suggest that *de novo* translation of Spy1 is not required during oocyte maturation in response to progesterone, *mos* or Ras. Nonetheless, endogenous Spy1 protein may exist in the resting stage VI oocyte at a level below our current limit of detection, and the activity of this endogenous Spy1 protein would not be susceptible to inhibition or ablation by microinjection of Spy1 antisense oligonucleotides.

Expression of Spy1 in oocytes results in MAPK and MPF activation

Previous work established that overexpression of NIMA protein from *A.nidulans* resulted in a release of *Xenopus* oocytes from metaphase arrest and progression from G_2 into meiosis. However, the ability of NIMA to induce GVBD in *Xenopus* oocytes is independent of *mos*, MAPK or cdc2 activation and results in an unusual oocyte maturation characterized by nuclear envelope breakdown and chromatin condensation without spindle formation and nuclear movement. These results suggested alternative pathways to regulate cell cycle progression in maturing oocytes (Lu and Hunter, 1995).

To determine whether Spy1-induced oocyte maturation proceeds through the normal meiotic pathway or possibly a NIMA-like pathway, we tested whether overexpression of Spy1 in oocytes resulted in MAPK and cdc2 activation. Activation of cdc2 and MAPK was examined in oocytes undergoing meiotic maturation in response to progesterone, *mos* mRNA or Spy1 mRNA. Cdc2 activity was assayed by specific immunoprecipitation with an anti-cdc2 antibody followed by a histone H1 kinase assay. In Spy1-injected oocytes, cdc2 kinase activity was found to be stimulated as efficiently as in *mos*-injected or progesterone-stimulated oocytes (Figure 5A). In addition, injection of Spy1 into oocytes led to MAPK activation, as indicated by the electrophoretic mobility shift of endogenous MAPK protein (Figure 5B). Moreover, in Spy1-injected oocytes, cdc2 kinase and MAPK activities were found to be stimulated rapidly, as early as 2 h. In comparison, cdc2 and MAPK activation in *mos*-injected or progesterone-induced oocytes occurred between 4 and

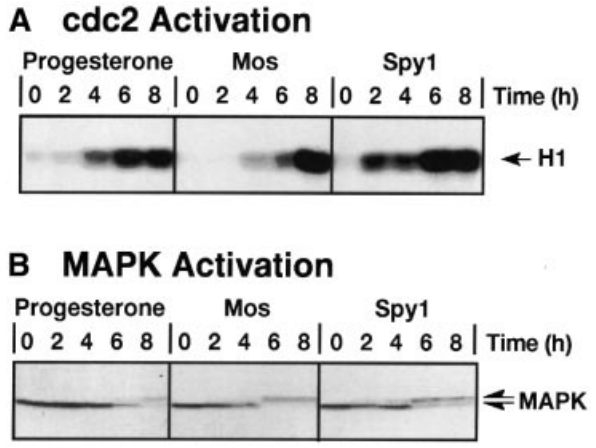

Fig. 5. MPF and MAPK activation by Spy1. Oocytes were incubated with progesterone, or injected with *mos* or Spy1 mRNA. Oocyte lysates were obtained at the indicated times. (**A**) Following immunoprecipitation of cdc2, samples were assayed for phosphorylation of histone H1. (**B**) Analysis of the MAPK mobility shift due to phosphorylation.

6 h after stimulation (Figure 5). Thus, the rapid activation of the MAPK pathway and MPF in Spy1-injected oocytes correlates with the rapid induction of GVBD. Taken together, these results suggest that in contrast to the NIMA-like pathway, Spy1 induces oocyte maturation by an alternative pathway, resulting in normal meiotic morphological changes.

Induction of meiosis by Spy1 requires the activation of the MAPK pathway

Previous studies have described that hormonal activation of prophase-arrested oocytes leads to activation of the MAPK pathway (Nebreda and Hunt, 1993; Posada *et al*., 1993). Under normal physiological activation, progesterone induces oocytes by stimulating the translation of *mos* protein, which is a MAP kinase kinase kinase. Then, *mos* leads to the stimulation of MAPK activity through the direct activation of MEK (for a review see Sagata, 1997). To elucidate whether activation of MAPK was required for Spy1 activity in oocytes, an antibody directed against the N-terminus of MEK was microinjected into *Xenopus* oocytes specifically to block MEK activation (Kosako *et al*., 1992, 1996). These pre-injected oocytes were then treated 4 h later with either progesterone or okadaic acid, or injected with *mos* or Spy1 mRNAs. The MEK antibody was able to inhibit oocyte maturation induced by Spy1, as well as that induced by progesterone or *mos* (Figure 6A). However, maturation in response to okadaic acid, a PP2A phosphatase inhibitor which acts independently of the MAPK pathway (Kosako *et al*., 1994; Huang and Ferrell, 1996), was not inhibited by the MEK antibody (Figure 6A).

Alternatively, *Xenopus* oocytes were incubated in the presence or absence of PD98059, a selective inhibitor of MEK (Pang *et al*., 1995), and then microinjected with Spy1 mRNA or stimulated with progesterone. A threshold concentration of PD98059 was used to avoid any toxicity of the inhibitor on the cells, but which would still result in inhibition of MEK activity. At 50% of GVBD, a delay of ~2 h was observed in both Spy1-injected and progesterone-induced oocytes treated with the drug (Figure

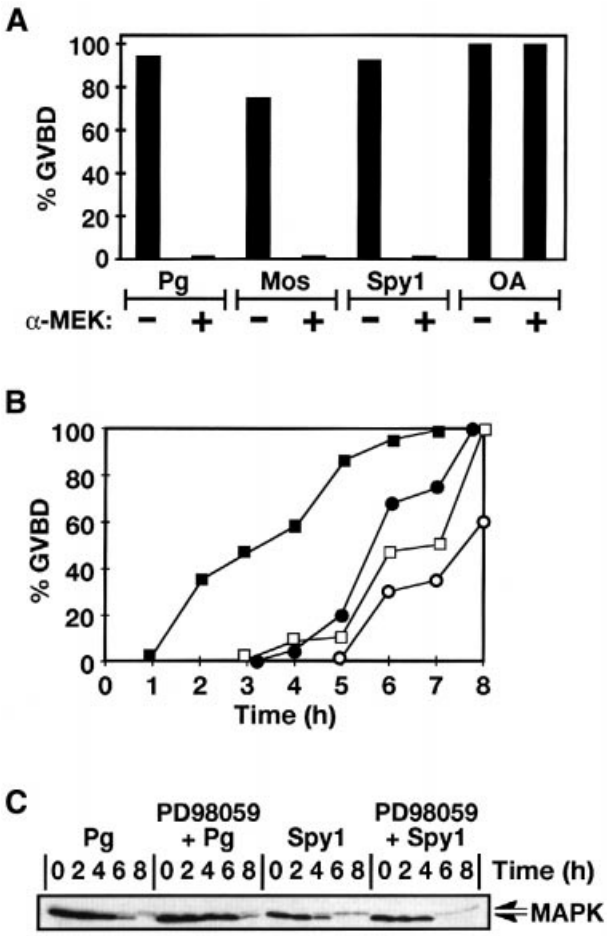

Fig. 6. Spy1-induced oocyte maturation is dependent on MAPK. (**A**) Oocytes were stimulated with progesterone (Pg), injected with *mos* or Spy1 mRNAs or treated with okadaic acid (OA). For double injections, the anti-MEK antibody was injected, followed by the indicated secondary treatment 4 h later. (**B**) Kinetics of oocyte maturation following Spy1 mRNA injection (■), progesterone stimulation (●), overnight incubation in PD98059 (50 mM) followed by Spy1 injection (□) or overnight incubation in PD98059 followed by incubation in progesterone (○). (**C**) Immunoblot of MAPK activation at the indicated times and conditions. For PD98059 treatment, oocytes were incubated in PD98059 (50 mM) overnight followed by secondary treatment.

6B). When examined for the electrophoretic mobility shift of activated MAPK, this delay in maturation correlated with a delay in the phosphorylation of MAPK in both progesterone-induced and Spy1-injected oocytes incubated with PD98059 (Figure 6C). Taken together, these results clearly indicate that MEK-mediated MAPK activation is required for Spy1-induced oocyte maturation, and distinguishes Spy1 from MEK-independent phosphatase inhibitors such as okadaic acid.

Physical interaction between Spy1 and cdk2

Considering that Spy1 represents a new cell cycle regulatory protein, we inquired whether Spy1 interacts directly with known cell cycle regulatory proteins. Using ^{35}S-labeled lysates from progesterone-induced *Xenopus* oocytes, we found three proteins which specifically bound to GST–Spy1, one of which appeared to correspond to cdk2 by immunoblotting (data not shown). To confirm this interaction, oocytes were microinjected with either

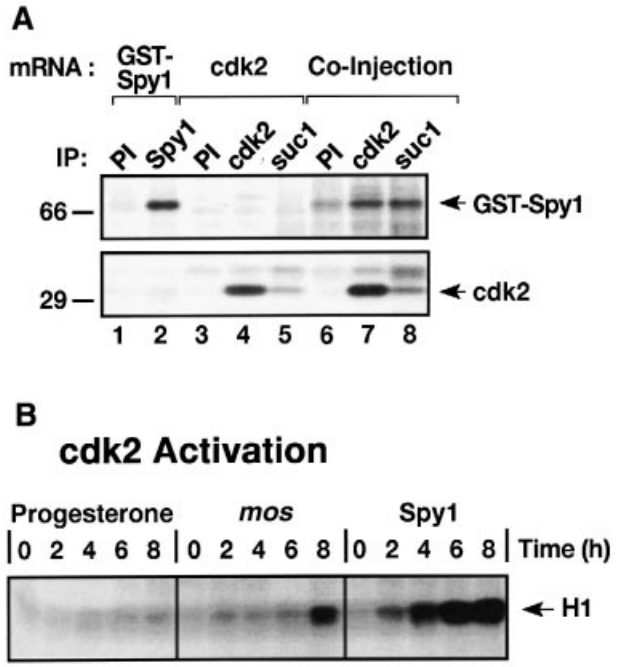

Fig. 7. Premature activation of cdk2 and association with Spy1. (**A**) Co-immunoprecipitation of cdk2 with GST–Spy1 from *Xenopus* oocytes labeled with [^{35}S]cysteine and methionine. Lanes 1, 3 and 6, pre-immune serum (PI); lane 2, immunoprecipitation with anti-Spy1 antiserum; lanes 4 and 7, immunoprecipitation with anti-cdk2 antiserum; lanes 5 and 8, pull-down with p13^{suc1}–beads. (**B**) Oocytes were incubated with progesterone, or injected with *mos* or Spy1 mRNA. Oocyte lysates were obtained at the indicated times and cdk2 was immunoprecipitated and assayed for phosphorylation of histone H1.

GST–Spy1 mRNA or cdk2 mRNA, or co-injected with both mRNAs. In doubly injected oocytes, GST–Spy1 co-immunoprecipitated with cdk2 using antisera directed against cdk2 (Figure 7A, lane 7), and was shown to associate with cdk2 using p13^{suc1}–beads (Figure 7A, lane 8) which specifically bind cdk2 and cdc2 (Pondaven *et al*., 1990). Moreover, it was possible to demonstrate a co-immunoprecipitation interaction between Spy1 and cdk2 in lysates from a stably transfected murine cell line expressing GST–Spy1 (data not shown).

Recently, new substrates for cdk2 protein kinase were found using an expression screening system (Zhao *et al*., 1998). To determine whether Spy1 could represent a substrate for cdk2, a cdk2 kinase assay using GST–Spy1 was performed. The result from this assay indicated that Spy1 is not phosphorylated directly by cdk2 *in vitro* (data not shown). In contrast to the binding interaction with cdk2, we were unable to detect a specific binding interaction of Spy1 with cdc2 (data not shown), suggesting that the rapid induction of meiosis by Spy1 may not result from a direct activation of pre-MPF complexes in resting oocytes.

Despite a controversial role for cdk2 during meiotic maturation of *Xenopus* oocytes, cdk2 kinase activity remains low in meiosis I and peaks during meiosis II (Rempel *et al*., 1995; Furuno *et al*., 1997). To investigate if cdk2 kinase activity is affected in Spy1-injected oocytes, cdk2 was immunoprecipitated specifically from oocytes induced by progesterone, *mos* or Spy1, and its activation was assayed by phosphorylation of histone H1. In comparison with progesterone- or *mos*-induced oocytes, Spy1-induced maturation resulted in a dramatic and rapid appearance of cdk2 activity (Figure 7B), reaching maximal levels by 4–6 h. This premature activation of cdk2 also suggests the possibility that meiosis I has been shortened during Spy1-stimulated oocyte maturation.

Taken together, these results showed that Spy1 induces meiotic progression in *Xenopus* oocytes by a rapid activation of the MAPK pathway and MPF and, additionally, activates cdk2 kinase prematurely. However, blocking cdk2 kinase activity with p21^{CIP} kinase inhibitor did not interfere with Spy1-induced oocyte maturation (data not shown), demonstrating that the cdk2 activation by Spy1 is not involved directly in the rapid maturation process.

Discussion

We have described a novel *Xenopus* gene, named Speedy, having important functional properties in the regulation of the G_2/M progression in *Xenopus* oocytes. First, we demonstrated that Spy1 represents a new class of inducer of G_2/M transition in *Xenopus*. Microinjection of Spy1 mRNA into oocytes resulted in a rapid induction of oocyte maturation which led to MAPK and cdc2 activation. Similar results have been obtained for *mos* and Ras oncogenic proteins (Nebreda and Hunt, 1993; Shibuya and Ruderman, 1993). The ability of Spy1 to trigger the appropriate activation of MAPK and MPF in oocytes suggests that Spy1 could be a component of a normal meiotic pathway. Moreover, our results demonstrated a requirement for the MAPK pathway in Spy1 induction of oocyte maturation (see Figure 6). Does Spy1, similarly to *mos* or Raf-1 protein kinases, function as a direct activator of MEK or does it act further upstream? The idea that Spy1 might represent a new MAP kinase kinase kinase is contradicted by several observations: (i) the Spy1 amino acid sequence does not contain any homology with other kinases; and (ii) the addition of Spy1 to reticulocyte lysate did not result in activation of p44^{MAPK} and p42^{MAPK} (data not shown). One possibility is that Spy1, in a *mos*-independent fashion, leads directly or indirectly to activation of some as yet unidentified MAPKKK. This hypothesis is supported by our observation that Spy1-induced maturation is MAPK dependent, but it is unlikely that Spy1 directly activates MEK.

Lastly, the rapid activation of MAPK and cdc2 activity detected in Spy1-induced oocytes correlates with the induction of oocyte maturation by Spy1. In contrast, the premature activation of cdk2 kinase activity seen in Spy1 oocyte lysates suggests the possibility that this cdk2 activation results in a shortening of meiosis I. Reduction in the timing of some somatic cell phases has been observed after overexpression, for example, of cyclin E or D (Resnitzky *et al*., 1994).

In this study, we demonstrated that Spy1 is a potent regulator of the G_2/M checkpoint in *Xenopus* oocytes and in yeast. Genetic studies in fission yeast recently have identified a number of checkpoint proteins that prevent mitosis when DNA is damaged and the integrity of the genome is compromised, including the protein kinase Chk1 isolated by its ability to arrest the cell cycle in response to DNA damage (Walworth *et al*., 1993; Murray, 1994; reviewed in Nurse, 1997). Similarly to Spy1, Chk1 confers resistance to radiation in a *rad1*-deficient yeast

cell line. Specifically, both proteins are able to arrest cells at the G_2/M transition following irradiation, resulting in survival of yeast cells. Interestingly, Spy1 and Chk1 appear to play opposite roles in the control of the cell cycle. In *Xenopus* eggs, Chk1 has been shown to block the cell cycle through phosphorylation of cdc25 in response to unreplicated or damaged DNA (Kumagai *et al*., 1998), while we have shown here that Spy1 positively regulates the meiotic cell cycle.

In this study, Spy1 was identified due to its ability to rescue a *rad1* mutant in *S.pombe*, suggesting a possible role for Spy1 in replication or repair checkpoints. Since cdk2 complexes previously have been implicated in DNA damage responses (Walker *et al*., 1995; Poon *et al*., 1996), our demonstration of a Spy1–cdk2 interaction would be consistent with such a role for Spy1 in these checkpoints. Thus, the interaction with cdk2 may provide a link between speedy and the DNA damage pathway. However, further work will be required to understand fully this role of Spy1, and to integrate a potential checkpoint function with its activity in G_2/M regulation and oocyte maturation as described in this study.

One of our interesting findings is the physical association between Spy1 and cdk2 in *Xenopus* oocytes. It is difficult to reconcile the rapid induction of oocyte maturation by Spy1 and its interaction with cdk2. It is noteworthy that microinjection of cdk2 mRNA into oocytes does not result in G_2/M transition (Gabrielli *et al*., 1992; Furuno *et al*., 1994; our unpublished results). Expression of cdk2 protein previously has been shown to occur at low levels in resting oocytes, whereas cdk2 synthesis during oocyte maturation reaches a maximum level around GVBD (Gabrielli *et al*., 1992). Despite high expression of cdk2 in *Xenopus* oocytes, and despite histone H1 kinase activity associated with cdk2–cyclin E complexes, no clear function has been demonstrated for cdk2 during oocyte maturation (Gabrielli *et al*., 1992; Furuno *et al*., 1997). The original study on the possible role of cdk2 in *Xenopus* oocytes reported by Gabrielli *et al*. (1993) demonstrated cytostatic activity (CSF) associated with this kinase. However, recent studies using a specific inhibitor of cdk2 kinase activity, $p21^{CIP}$, have shown no functional role for cdk2 in metaphase II arrest (Furuno *et al*., 1997).

Once again, these results raise the question of a normal function for cdk2 in maturing oocytes. In fact, it was suggested that the storage of cdk2 kinase in *Xenopus* oocytes resulted in the activation of DNA replication (Fang and Newport, 1991) and MPF (Guadagno and Newport, 1996) during the early embryonic cell cycle. However, maturing oocytes possess the ability to induce DNA replication shortly after GVBD near the end of the meiosis I phase (Gurdon, 1967; Furuno *et al*., 1994). This ability is suppressed during the meiosis I/meiosis II transition by the *mos* protein kinase, together with a newly synthesized unknown protein 'X' and MPF (Furuno *et al*., 1994). However, Akamatsu *et al*. (1998) recently have reported that cyclin E–cdk2 complexes associated with the transcription factor E2F develop the ability to induce DNA replication in *Xenopus* oocytes, reinforcing a putative role for cdk2 in the activation of DNA replication in *Xenopus* oocytes. Indeed, by a *mos*-independent pathway, Spy1 could act directly to suppress the DNA replication-inducing ability of the cyclin E–cdk2 complex by binding to the cdk2 subunit. We were unable to detect endogenous Spy1 protein during oocyte maturation and early embryogenesis, although we were able to detect speedy mRNA present as a maternal mRNA in oocytes and through blastulation. This may reflect the technical difficulty in detecting Spy1 arising from the apparent instability of Spy1 protein during the maturation process. This last hypothesis is supported by the presence of PEST domains in the Spy1 amino acid sequence (see Figure 2). It will be interesting to investigate whether Spy1 represents the labile protein 'X' described by Furuno *et al*. (1994) and which is involved in the suppression of DNA replication. Work currently is underway to characterize a potential role for Spy1 in the control of DNA replication either in *Xenopus* oocytes or during early embryo development.

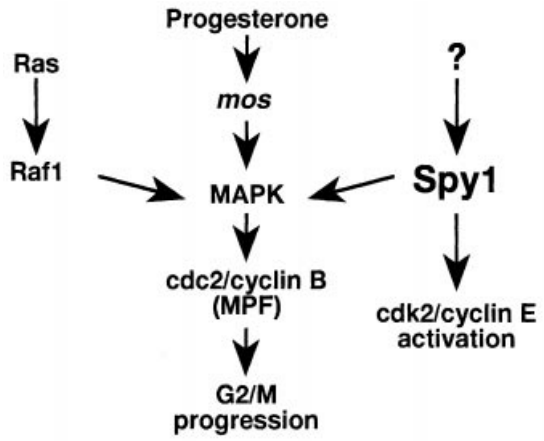

Fig. 8. A model for Spy1 in the activation of the G_2/M transition in *Xenopus* oocytes.

Taken together, our results lead to a model (Figure 8) in which activation of the Spy1 pathway has two significant effects: first, activation of the MAPK pathway and MPF, leading to rapid MAPK-dependent meiotic maturation which is cdk2 independent; and secondly, premature activation of cdk2, which may be mediated by the direct interaction between Spy1 and cdk2 that we observed. Our findings also demonstrate that Spy1 triggers G_2/M progression independently of *mos*. Spy1 evidently represents a new class of cell cycle regulatory protein which can activate MAPK and induce rapid progression through G_2/M.

Materials and methods

Xenopus cDNA library and UV irradiation experiments

The *X.laevis* total ovary cDNA library (from J.Maller) was described previously (Su and Maller, 1995). UV irradiation was carried out using a Stratalinker 2400 source (Stratagene). Cells were first grown to mid-logarithmic phase, plated on solid agar and irradiated on the plates. For each dose, several dilutions of cells were used to determine the percentage survival following colony formation. The number of colonies surviving was recorded after 3 days incubation at 30°C. For experiments requiring ionizing radiation, cells were grown to mid-logarithmic phase in the appropriate selective liquid medium and diluted to 5×10^6 cells/ml. Cells were then irradiated at 2 Krads/min with a ^{137}Cs source. Following irradiation, cells were plated immediately at several dilutions per dose in duplicate on YES solid medium. Surviving colonies were counted following 3 days incubation at 30°C.

Oocyte microinjections

Stage VI *Xenopus* oocytes were dissected manually after treatment with collagenase (2 mg/ml, type II, Sigma) and treated with progesterone

(30 μM) or microinjected with 50 nl of RNA at 1.0 mg/ml (Freeman *et al*., 1989). In some cases, oocytes were incubated overnight with PD98059 inhibitor (50 μM, Calbiochem) in MBS-H buffer or microinjected with anti-MEK antibody (Kosako *et al*., 1992, 1996) 4 h prior to secondary microinjection with the indicated samples. For the antisense oligonucleotide microinjections, 50 nl of the appropriate oligonucleotides at 1 mg/ml were microinjected, followed by subsequent treatment 4 h later. For double injection experiments, oocytes were microinjected first with 50 nl of *Xenopus* cdk2 mRNA followed by secondary microinjections with Spy1 mRNA 4 h later.

Antisera

Affinity-purified rabbit antisera were prepared against two different C-terminal Spy1 peptides, LEWHHL and EPDGAALEWHHL. Anti-MAPK (ERK1+ERK2) antibody was purchased from Zymed. Antiserum against *Xenopus* cdk2 was prepared in this laboratory and was described previously (Fang and Newport, 1991).

H1 kinase assays

To assay MPF activity in oocytes, two oocytes per time point were lysed in extraction buffer as described (Freeman *et al*., 1989). Cdc2 was immunoprecipitated from oocyte lysates using anti-cdc2 antibody (Santa Cruz Biotechnology) followed by *in vitro* kinase assay using histone H1 as a substrate. Briefly, immunoprecipitates from clarified lysates were resuspended into 40 μl of kinase buffer {40 mM HEPES pH 7.5, 10 mM EGTA, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 100 μM ATP, 15 μCi of [γ-^{32}P]ATP (NEN) and 0.8 μg of histone H1 (Sigma)}. Reactions were stopped by the addition of sample buffer and analyzed by 10% SDS–PAGE followed by autoradiography. Cdk2 kinase activity was assayed as described previously (Rempel *et al*., 1995).

RT–PCR assays

Total RNA was isolated from oocytes and staged embryos by the TRIzol reagent as described by the manufacturer (Gibco-BRL, Life Technologies). RT–PCR was performed using 200 ng of total RNA and 50 pmol of sense primer (ATGGAGGAAGATGAAGAAGACTA) and antisense primer (GAAAAAGTCATCCTCATT) for the Spy gene, and 50 pmol of sense primer (TACATCACGTCTCGAACTC) and antisense primer (ACAGCATATAACTGTACCAG) for the c-*src* gene. RT–PCR assays were carried out as recommended by the manufacturer (Promega).

Plasmid construction, oligonucleotide and mRNA synthesis, and in vitro translation

The *Xenopus mos*^{wt} construct was described previously (Freeman *et al*., 1989). For synthesis of Ras 61L mRNA, pCDNA1-ras61L (Hart and Donoghue, 1997) was cut with *Hin*dIII and *Xba*I and inserted into pSP64(polyA) (Promega). To generate *Xenopus* Spy1/pSP64(polyA), the Spy1 plasmid derived from the *Xenopus* cDNA library (pKL28) was cut with *Pst*I and *Sac*I and ligated into pSP64(polyA). RNAs were synthesized from these constructs using SP6 polymerase as described previously (Freeman *et al*., 1989). To construct GST–Spy1, the Spy1 cDNA was digested with *Nde*I and *Not*I and inserted using adaptor oligonucleotides into the *Bam*HI and *Not*I sites of the eukaryotic GST fusion protein expression vector pEBG. To generate GST–Spy1/pSP64(polyA), the pEBG-Spy1 plasmid was cut with *Xba*I and *Eco*RI and inserted into the *Xba*I and *Eco*RI sites of pSP64(polyA) vector. An antisense oligonucleotide that spans the Spy1 start codon (GCCTCATTGTAGAAAGGGGGACAAC) was synthesized on an Applied Biosystems 381A DNA synthesizer, and the antisense *mos* oligonucleotide was described previously (Freeman *et al*., 1989). Coupled *in vitro* transcription–translation (Promega) using [^{35}S]methionine was carried out as described by the manufacturer.

Immunoprecipitations and immunoblotting

For immunoprecipitation, oocyte lysates were clarified with protein A–Sepharose beads (Sigma) and incubated with primary antisera against cdc2, cdk2 or Spy1 for 3 h at 4°C, followed by the addition of protein A–Sepharose and incubation at 4°C with gentle rotation for an additional 1 h. For p13^{suc1} pull-down experiments, p13^{suc1}–beads (Calbiochem) were added to oocyte lysates and incubated with rotation at 4°C for 1 h. These complexes were washed extensively, after which they were resolved by 10% SDS–PAGE and detected by autoradiography. As indicated, immunoprecipitation of Spy1 was blocked by pre-incubation of Spy1 antisera with the cognate peptide at 2 μg of peptide/μg of antibody. MAPK was detected by immunoblotting with anti-MAPK antibody (Zymed) followed by enhanced chemiluminescence (Amersham).

Accession number

The sequence for Spy1 has now been deposited in the DDBJ/EMBL/GenBank databases under accession number AJ133117.

Acknowledgements

We thank J.Maller for the kind gift of the *Xenopus laevis* cDNA library; E.Nishida for kindly providing anti-MEK antibody; and K.Hart and other members of the D.J.D. laboratory for critical review of the manuscript. This work was supported by grants from the National Institutes of Health to D.J.D. (R01-CA34456) and S.S. (R01-GM31253). R.W.D. gratefully acknowledges support from a National Institutes of Health NRSA grant (T32-CA09523).

References

Akamatsu,E., Tanaka,T. and Kato,J.Y. (1998) Transcription factor E2F and cyclin E–Cdk2 complex cooperate to induce chromosomal DNA replication in *Xenopus* oocytes. *J. Biol. Chem.*, **273**, 16494–16500.

Al-Khodairy,F. and Carr,A.M. (1992) DNA repair mutants defining G_2 checkpoint pathways in *Schizosaccharomyces pombe*. *EMBO J.*, **11**, 1343–1350.

Andresson,T. and Ruderman,J.V. (1998) The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated pathway. *EMBO J.*, **17**, 5627–5637.

Barrett,C.B., Schroetke,R.M., Van der Hoorn,F.A., Nordeen,S.K. and Maller,J.L. (1990) Ha-ras^{val12,thr59} activates S6 kinase and p34^{cdc2} kinase in *Xenopus* oocytes: evidence for c-*mos*^{xe}-dependent and -independent pathways. *Mol. Cell. Biol.*, **10**, 310–315.

Chen,M. and Cooper,J.A. (1995) Ser-3 is important for regulating *mos* interaction with and stimulation of mitogen-activated protein kinase kinase. *Mol. Cell. Biol.*, **15**, 4727–4734.

Enoch,T., Carr,A.M. and Nurse,P. (1992) Fission yeast genes involved in coupling mitosis to completion of DNA replication. *Genes Dev.*, **6**, 2035–2046.

Fabian,J.R., Morrison,D.K. and Daar,I. (1993) Requirement for Raf and MAP kinase function during the meiotic maturation of *Xenopus* oocytes. *J. Cell Biol.*, **122**, 645–652.

Fang,F. and Newport,J.W. (1991) Evidence that the G_1–S and G_2–M transitions are controlled by different cdc2 proteins in higher eukaryotes. *Cell*, **66**, 731–742.

Freeman,R.S., Pickham,K.M., Kanki,J.P., Lee,B.A., Pena,S.V. and Donoghue,D.J. (1989) *Xenopus* homolog of the *mos* protooncogene transforms mammalian fibroblasts and induces maturation of *Xenopus* oocytes. *Proc. Natl Acad. Sci. USA*, **86**, 5805–5809.

Freeman,R.S., Kanki,J.P., Ballantyne,S.M., Pickham,K.M. and Donoghue,D.J. (1990) Effects of the v-*mos* oncogene on *Xenopus* development: meiotic induction in oocytes and mitotic arrest in cleaving embryos. *J. Cell Biol.*, **111**, 533–541.

Furuno,N., Nishizawa,M., Okasaki,K., Tanaka,H., Iwashita,J., Nakajo,N., Ogawa,Y. and Sagata,N. (1994) Suppression of DNA replication via *mos* function during meiotic divisions in *Xenopus* oocytes. *EMBO J.*, **13**, 2399–2410.

Furuno,N., Ogawa,Y., Iwashita,J., Nakajo,N. and Sagata,N. (1997) Meiotic cell cycle in *Xenopus* oocytes is independent of cdk2 kinase. *EMBO J.*, **16**, 3860–3865.

Gabrielli,B.G., Roy,L.M., Gautier,J., Philippe,M. and Maller,J.L. (1992) A cdc2-related kinase oscillates in the cell cycle independently of cyclins G_2/M and cdc2. *J. Biol. Chem.*, **267**, 1969–1975.

Gabrielli,B.G., Roy,L.M. and Maller,J.L. (1993) Requirement for cdk2 in cytostatic factor-mediated metaphase II arrest. *Science*, **259**, 1766–1769.

Guadagno,T.M. and Newport,J.W. (1996) Cdk2 kinase is required for entry into mitosis as a positive regulator of Cdc2–cyclin B kinase activity. *Cell*, **84**, 73–82.

Gurdon,J.B. (1967) On the origin and persistence of a cytoplasmic state inducing nuclear DNA synthesis in frogs' eggs. *Proc. Natl Acad. Sci. USA*, **58**, 545–552.

Hart,K.C. and Donoghue,D.J. (1997) Derivatives of activated H-ras lacking C-terminal lipid modifications retain transforming ability if targeted to the correct subcellular location. *Oncogene*, **14**, 945–953.

Hartwell,L.H. and Weinert,T.A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science*, **246**, 629–634.

Huang,C.F. and Ferrell,J.E. (1996) Dependence of *mos*-induced cdc2 activation on MAP kinase function in a cell-free system. *EMBO J.*, **15**, 2169–2173.
Korn,L.J., Siebel,C.W., McCormick,F. and Roth,R.A. (1987) *ras* p21 as a potential mediator of insulin action in *Xenopus* oocytes. *Science*, **236**, 840–843.
Kosako,H., Gotoh,Y., Matsuda,S., Ishikawa,M. and Nishida,E. (1992) *Xenopus* MAP kinase activator is a serine/threonine/tyrosine kinase activated by threonine phosphorylation. *EMBO J.*, **11**, 2903–2908.
Kosako,H., Gotoh,Y. and Nishida,E. (1994) Requirement for the MAP kinase kinase/MAP kinase cascade in *Xenopus* oocyte maturation. *EMBO J.*, **13**, 2131–2138.
Kosako,H., Akamatsu,Y., Tsurushita,N., Lee,K.K., Gotoh,Y. and Nishida,E. (1996) Isolation and characterization of neutralizing single-chain antibodies against *Xenopus* mitogen-activated protein kinase kinase from phage display libraries. *Biochemistry*, **35**, 13212–13221.
Kumagai,A., Guo,Z., Emami,K.H., Wang,S.X. and Dunphy,W.G. (1998) The *Xenopus* Chk1 protein kinase mediates a caffeine-sensitive pathway of checkpoint control in cell-free extracts. *J. Cell Biol.*, **142**, 1559–1569.
Lu,K.P. and Hunter,T. (1995) Evidence for a NIMA-like mitotic pathway in vertebrate cells. *Cell*, **81**, 413–424.
Masui,Y. and Clarke,H.J. (1979) Oocyte maturation. *Int. Rev. Cytol.*, **57**, 185–282.
Murray,A. (1994) Cell cycle checkpoints. *Curr. Opin. Cell, Biol.*, **6**, 872–876.
Muslin,A.J., MacNicol,A.M. and Williams,L.T. (1993) Raf-1 protein kinase is important for progesterone-induced *Xenopus* oocyte maturation and acts downstream of *mos*. *Mol. Cell. Biol.*, **13**, 4197–4202.
Nebreda,A.R. and Hunt,T. (1993) The c-*mos* proto-oncogene protein kinase turns on and maintains the activity of MAP kinase, but not MPF, in cell-free extracts of *Xenopus* oocytes and eggs. *EMBO J.*, **12**, 1979–1986.
Nurse,P. (1997) Checkpoint pathways come of age. *Cell*, **91**, 865–867.
O'Keefe,S.J., Wolfes,H., Kiessling,A.A. and Cooper,G.M. (1989) Microinjection of antisense c-*mos* oligonucleotides prevents meiosis II in the maturing mouse egg. *Proc. Natl Acad. Sci. USA*, **86**, 7038–7042.
Pang,L., Sawada,T., Decker,S.J. and Saltiel,A.R. (1995) Inhibition of MAP kinase kinase blocks the differentiation of PC-12 cells induced by nerve growth factor. *J. Biol. Chem.*, **270**, 13585–13588.
Pondaven,P., Meijer,L. and Beach,D. (1990) Activation of M-phase-specific histone H1 kinase by modification of the phosphorylation of its $p34^{cdc2}$ and cyclin components. *Genes Dev.*, **4**, 9–17.
Poon,R.Y.C., Jiang,W., Toyoshima,H. and Hunter,T. (1996) Cyclin-dependent kinases are inactivated by a combination of p21 and Thr-14/Tyr-15 phosphorylation after UV-induced DNA damage. *J. Biol. Chem.*, **271**, 13283–13291.
Posada,J., Yew,N., Ahn,N.G., Vande Woude,G.F. and Cooper,J.A. (1993) *mos* stimulates MAP kinase in *Xenopus* oocytes and activates a MAP kinase kinase *in vitro*. *Mol. Cell. Biol.*, **13**, 2546–2553.
Rempel,R.E., Sleight,S.B. and Maller,J.L. (1995) Maternal *Xenopus* Cdk2–cyclin E complexes function during meiotic and early embryonic cell cycles that lack a G_1 phase. *J. Biol. Chem.*, **270**, 6843–6855.
Resnitzky,D., Gossen,M., Bujard,H. and Reed,S.I. (1994) Acceleration of the G_1/S phase transition by expression of cyclins D1 and E with an inducible system. *Mol. Cell. Biol.*, **14**, 1669–1679.
Rowley,R., Subramani,S. and Young,P.G. (1992) Checkpoint controls in *Schizosaccharomyces pombe*: *rad1*. *EMBO J.*, **11**, 1335–1342.
Sagata,N. (1997) What does *mos* do in oocytes and somatic cells. *BioEssays*, **19**, 13–21.
Sagata,N., Oskarsson,M., Copeland,T., Brumbaugh,J. and Vande Woude,G.F. (1988) Function of c-*mos* proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature*, **335**, 519–525.
Sagata,N., Watanabe,N., Vande Woude,G.F. and Ikawa,Y. (1989) The c-*mos* proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature*, **342**, 512–518.
Shibuya,E.K. and Ruderman,J.V. (1993) *mos* induces the *in vitro* activation of mitogen-activated protein kinases in lysates of frog oocytes and mammalian somatic cells. *Mol. Biol. Cell*, **4**, 781–790.
Su,J.Y. and Maller,J.L. (1995) Cloning and expression of a *Xenopus* gene that prevents mitotic catastrophe in fission yeast. *Mol. Gen. Genet.*, **246**, 387–396.
Walker,D.H., Adami,G.R., Dold,K.M. and Babiss,L.E. (1995) Misregulated expression of the cyclin dependent kinase 2 protein in human fibroblasts is accompanied by the inability to maintain a G_2 arrest following DNA damage. *Cell Growth Differ.*, **6**, 1053–1061.
Walworth,N., Davey,S. and Beach,D. (1993) Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. *Nature*, **363**, 368–371.
Yang,Y., Herrmann,C.H., Arlinghaus,R.B. and Singh,B. (1996) Inhibition of v-*mos* kinase activity by protein kinase A. *Mol. Cell. Biol.*, **16**, 800–809.
Zhao,J., Dynlacht,B., Imai,T., Hori,T. and Harlow,E. (1998) Expression of NPAT, a novel substrate of cyclin E–CDK2, promotes S-phase entry. *Genes Dev.*, **12**, 456–461.

Received October 12, 1998; revised and accepted February 11, 1999