Int. J. Dev. Biol. 37: 589-594 (1993)

Original Article

Microinjection of suc1 transcripts delays the cell cycle clock in *Patella vulgata* embryos

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ABSTRACT The suc1 protein is a cell cycle regulator whose precise function remains to be elucidated. The suc1 cDNA of the mollusk *Patella vulgata* was cloned and sequenced. It encodes a 9 kD protein showing a strong similarity with its human counterparts and to a lesser extend with its yeast counterparts. The expression of suc1 in maturing oocytes was shown to be tightly cell cycle-regulated. The abundance of the suc1 transcripts is high in prophase- and metaphase-arrested oocytes but drops dramatically upon exit from M-phase, after fertilization. The microinjection of suc1 synthetic messengers into embryonic blastomeres delayed the cell cycle clock, thus disrupting the perfect cell cycle synchrony exhibited by the blastomeres of early *Patella* embryos. Interestingly, this suc1 delaying effect was significantly reversed when cyclin B messengers were co-injected with suc1 messengers. These results show that *Patella* embryos offer a quite valuable model to study cell cycle regulation. Moreover, they support the existence of a negative control exerted by suc1 on the cell cycle traverse in a higher eukaryote.

KEY WORDS: *suc1, cell cycle, early cleavages*

Introduction

The molecular regulation of eukaryotic cell cycle traverse has been unravelled during the past five years (for an overview, Kirschner 1992). In particular, the understanding of the control of mitosis has undergone spectacular advancements. It is now clearly established that the entry into M-phase is driven by the universal M-phase kinase whose active form is generally described as a heterodimer of cdc2 and cyclin B proteins, cdc2 being phosphorylated on Thr 161/167 and dephosphorylated on Tyr15 and Thr14 by the Cdc25 phosphatase (reviewed by Clarke and Karsenti, 1991).

The suc1 gene was initially identified in *S. pombe* on the basis of its capacity to suppress cdc2 mutants (Hayles *et al.*, 1986b). By virtue of its ability to associate with cdc2, the suc1 gene product has been widely used as a tool to isolate and study the different subunits of the M-phase kinase. Both genetic and biochemical evidence suggest that the suc1 protein is a component of the active kinase (Hayles *et al.*, 1986a; Brizuela *et al.*, 1987; Draetta *et al.*, 1987; Hadwiger *et al.*, 1989). Many observations obtained both in *S. pombe* and *S. cerevisiae* confer to the suc1 gene product an important role in the regulation of the cell cycle. The disruption of the suc1 gene arrests the cell cycle and overexpression delays the division, mitosis occurring at an increased cell size (Hayles *et al.*, 1986a; Hindley *et al.*, 1987; Hadwiger *et al.*, 1989). More precisely, Moreno *et al.* (1989) showed that *S. pombe* cells deleted on suc1 are arrested in M-phase with a high level of histone kinase activity. Riabowol *et al.* (1989) prevented the normal execution of mitosis in rat fibroblasts by microinjecting either the suc1 protein or the antibodies directed against this protein. Ducommun *et al.* (1991) showed that cdc2 mutant proteins which cannot bind suc1 but retain their ability to associate with cyclins are nonfunctional. However, many authors using acellular systems reported an inhibitory effect of suc1 on the cdc25 phosphatase activity and thus on the activation of the cdc2 kinase (Dunphy and Newport, 1989; Dunphy and Kumagai, 1991; Galaktionov and Beach, 1991; Gautier *et al.*, 1991; Kumagai and Dunphy, 1991). Therefore, although the importance of the suc1 gene product in the regulation of mitosis is clearly established, the precise function of this molecule remains completely mysterious.

As with many marine invertebrates, the mollusk *Patella vulgata* offers a valuable model for the study of cell cycle regulation (Guerrier *et al.*, 1990 for a review). The oocytes undergo a two-step maturation with a first block at the germinal vesicle stage, which can be released by increasing the intracellular pH, and a second block at the first meiotic metaphase which is released upon fertilization (Guerrier *et al.*, 1986). Such a maturation pathway allows the study of the mechanisms controlling entry into M-phase and the maintenance of this phase. Furthermore, the cell cycles of the embryonic

Abbreviations used in this paper: LY, Lucifer Yellow; nt, nucleotide; PCR, polymerase chain reaction.

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0214-6282/93/$03.00

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CTTTTTTTTTTTTTTTTTTATTTTATTTTTTTACTTATTTTTTAAAGTT
TATATTGCCTATATTTTATTATAGATAATAACAACACTATCTTACATCAC
TTGTTGAAAGTTTTTGTCGAATGAAGCTGTGTTACTTTTTCAGCTGTAATC

ATG TCT GCC CGA CAA ATC TAC TAT TCT GAC AAA TAT
 M   S   A   R   Q   I   Y   Y   S   D   K   Y  12

TTT GAT GAA GAC TTT GAG TAC AGG CAT GTG ATG CTC
 F   D   E   D   F   E   Y   R   H   V   M   L  24

CCC AAA GAT ATT GCT AAA ATG GTG CCA AAA AAT CAT
 P   K   D   I   A   K   M   V   P   K   N   H  36

CTG ATG TCA GAA GCA GAA TGG AGA AGC ATC GGA GTA
 L   M   S   E   A   E   W   R   S   I   G   V  48

CAA CAA AGT CAT GGC TGG ATC CAT TAT ATG AAA CAT
 Q   Q   S   H   G   W   I   H   Y   M   K   H  60

GAA CCA GAA CCT CAT ATA CTT TTA TTT AGA AGA AAA
 E   P   E   P   H   I   L   L   F   R   R   K  72

GTG ACA GGC CAG TGATTTGATGTGTAATAACTTAAATAACAATTCA
 V   T   G   Q 76

AAGTGAATATGGATTGGAAATGGTGCCTAAATATGTGTTACTAGAATGAT
GCCAGTGGTCTTTCAATATTTGTGACTAACGGAGAACTCCCACTTGATGC
CGATAGTAAAAATAATATAATCAGTGCACGGACAAAACCTAGTTCTTTTT
GTATCTTTCATTTCTATCGTCTGTCAATCTATTTCTTCTTCTATAGTGTG
AATAATTGACTTTATGAATGAATGATTTGTAAGTATAATAAAGTGTTGat
aaataaaAAAAAAG 676
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Fig. 1. cDNA and deduced amino acid sequences of *Patella* suc1. *The underlined subsequences correspond to the primers used for the PCR reaction. In the 3' untranslated region, the polyadenylation signal is shown in bold and the putative degradation signals in small letters. These sequence data are available from EMBL/GenBank/DDBJ under accession number Z28352.*

blastomeres exhibit a perfect synchrony during early development (Van den Biggelaar, 1977). For this reason, we thought that early *Patella* embryos may constitute an excellent system for examining the possible effects of various compounds on the cell cycle clock.

We cloned the suc1 homolog of *Patella vulgata* and examined its expression at the messenger level during oocyte maturation. We then examined the effects of microinjecting in early embryos suc1 messengers, either alone or in combination with cyclin B messengers.

Results

In a first step, a fragment of the *Patella* suc1 was amplified by PCR from a cDNA preparation of trochophoras (free swimming larvae), using degenerated primers designed from the alignment of the sequences already described in *S. pombe*, *S. cerevisiae* and human (Hindley *et al.*, 1987; Hadwiger *et al.*, 1989; Richardson *et al.*, 1990). A fragment of about 150 nt was obtained in low abundance, was re-amplified by a second PCR run, and subcloned. Partial sequencing further confirmed that this fragment corresponded to a region of a suc1 homolog of *Patella*. In order to obtain a full length cDNA, a λgt 10 cDNA library prepared from trochophora mRNAs (Van Loon *et al.*, 1993) was screened with the amplified fragment as a probe. One positive plaque out of 10^6 screened was detected, replated, and confirmed with a second screening. The insert was isolated and subcloned in pGEM7.

The complete cDNA sequence of this insert is presented in Fig. 1. This sequence is 676 nt long. The long stretch of T's at the beginning of the sequence corresponds to a cloning artefact observed previously with other cDNAs cloned from the same library (our unpublished observations). This sequence exhibits only one open reading frame of significant length (228 nt), which localizes the initiator at nt 152 and the terminator at nt 380. This initiator fulfils Kozak's rule for the initiation of translation (Kozak, 1986). The predicted molecular weight of the protein encoded by this open reading frame is 9.2 kD and is thus in the same range as its human counterparts. The 3' untranslated region shows a polyadenylation signal at nt 650 followed by a short stretch of poly[A] beginning at nt 668.

The alignment of the deduced amino acid sequence of the *Patella* suc1 with the yeast and human sequences is shown in Fig. 2. The *Patella* protein is more similar to the human than to the yeast proteins, which present an amino-terminal and a central region not found in human and *Patella*. Except for a conservative replacement at residue 46 (an isoleucine instead of a leucine), the *Patella* sequence does not break the consensus derived from the alignment of the yeast and human sequences (Richardson *et al.*, 1990). The *Patella* protein shows a slightly stronger similarity with the human CKShs1 (82%) than with the human CKShs2 (78%). These similarity scores show the high degree of conservation of the suc1 gene products throughout evolution.

We examined the steady-state level of suc1 messengers in maturing oocytes by Northern analysis (Fig. 3). Two signals were detected reproducibly, the lower one corresponding to the size of the cDNA and the upper one (not shown) corresponding probably to an unspliced precursor also detected in *S. pombe* (Hayles *et al.*, 1986a; Hindley *et al.*, 1987). A slight increase in the abundance of the suc1 messengers could be observed between the prophasic and the metaphasic blocks. In contrast with cyclin messengers (Van Loon *et al.*, 1991), no significant difference in the polyadenylation state of the suc1 messengers could be detected between these two stages of maturation (data not shown). However, the most striking observation is the dramatic decrease in the abundance of the suc1 messengers after fertilization. It should be stressed that this decrease is probably under-estimated due to the presence of unfertilized oocytes representing 30% of the batch (70% is an excellent fertilization rate in *Patella*). Therefore, it is quite reasonable to conclude that upon fertilization, and thus exit from M-phase, oocytes contain very low levels of suc1 messenger if at all.

In order to examine the effect of deregulating the steady-state level of suc1 mRNAs, blastomeres of early embryos were microinjected with synthetic suc1 messengers transcribed from the cDNA. The *in vitro* translated product of these transcripts exhibited the molecular weight expected from the cDNA sequence (data not shown). Up to the fifth cleavage, the blastomeres of *Patella* embryos exhibit perfectly synchronous cell cycles (Fig. 4, top row). Our hypothesis was that any effect of a deregulated expression of suc1 on the cell cycle traverse would translate into the disruption of synchrony between the blastomeres containing the suc1 messengers and the control blastomeres.

Microinjection is a powerful but delicate technique which can be responsible *per se* for pleiotropic cellular effects, due for instance to a mechanical trauma. As the regulation of the cell cycle traverse is a very sensitive integrator of cellular information (see Murray,

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CKS1    MYHHYHAFQGRKLTDQERARVLEFQDSIHYSPRYSDDNYEYRHVMLPKAMLKVIPSDYF
SUC1    M SK SG VPRLLTASERERLEPFIDQIHYSPRYADDEYEYRHVMLPKAMLKAIPTDYF
CKShs1  MSHK                        QIYYSDKYDDEEFEYRHVMLPKDIAKLVPKT
CKShs2  MAHK                        QIYYSDKYFDEHYEYRHVMLPRELSKQVPKT
Patsuc  MSAR                        QIYYSDKYFDEDFEYRHVMLPKDIAKMVPKN

Cons.   M                            I YS  Y D   EYRHVMLP    K  P

CKS 1   NSEVGTLRILTEDEWRGLGITQSLGWEHYECHAPEPHILLFKRPLNYEAELRAAT....
SUC 1   NPETGTLRILQEEEWRGLGITQSLGWEMYEVHVPEPHILLFKREKDYQ  MK FS....
CKShs1        HLMSESEWRNLGVQQSQGWVHYMIHEPEPHILLFRRPL     PKK......
CKShs2        HLMSEEEWRRLGVQQSLGWVHYMIHEPEPHILLFRRPL     PKD......
Patsuc        HLMSEAEWRSIGVQQSHGWIHYMKHEPEPHILLFRRKVTGQ.

Cons.               E EWR  G  QS GW  Y  H PEPHILLF R
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Fig. 2. Amino acid sequence alignment of *Patella* suc1 with the yeast and human suc1 proteins. *The following sequences were aligned by eye (from top to bottom): the S. cerevisiae CKS1 (Hadwiger et al., 1989), the S. pombe suc1 (Hindley et al., 1987), the human CKShs1 and CKShs2 (Richardson et al., 1991) and the Patella Patsuc. Consensus residues are derived from the alignment.*

1992 for a review), the interpretation of the effects of microinjections on cell cycle can easily be misleading. Because our aim was to compare the cell cycle of injected blastomeres with the cell cycle of non-injected blastomeres within a single embryo, we were very concerned about the risks inherent in the microinjection itself. For this reason, we decided to perform the injections on early 4-cell stage embryos when cytoplasmic bridges are still present between daughter cells. Such a strategy defined three types of blastomeres within the late 4-cell stage embryos which were observed: two blastomeres which did not contain the injected messengers and which provided the cell cycle clock reference, and two blastomeres which contained the exogenous messengers. Importantly, only one of these two blastomeres had undergone the microinjection whereas the other one had received the messengers by diffusion through cytoplasmic bridges. Both of these blastomeres were easily identified thanks to the addition of the fluorochrome Lucifer Yellow (LY) in all the messenger solutions. Therefore, in order to ensure that the asynchrony between LY^+ and LY^- blastomeres was attributable to the deregulated expression of suc1 and not to a mechanical trauma, we only considered the 4-cell stage embryos exhibiting synchronous LY^+ blastomeres.

Microinjection of suc1 transcripts disrupted blastomere synchrony in most of the injected embryos: 85% of the embryos exhibited delayed LY^+ blastomeres compared with the LY^- control blastomeres, whereas the remaining 15% retained synchronous cell cycles. Fig. 4 shows an asynchronous embryo injected with suc1 messengers in which the two LY^+ blastomeres exhibit a metaphase plate whereas the two LY^- blastomeres are already in ana/telophase. In contrast, all the embryos injected with buffer-LY alone showed synchronous blastomeres (not shown).

Early data obtained in yeasts strongly indicate that an overexpression of suc1 delays the transition from G2 to M-phase (Hayles *et al.*, 1986a; Hindley *et al.*, 1987). Although we cannot determine the delayed transition from our observations, it is likely that the microinjection of suc1 transcripts delays the entry of the blastomeres into M-phase. In an attempt to test this hypothesis, we co-injected suc1 transcripts with transcripts encoding cyclin B, a positive regulator of the G2/M transition (van Loon *et al.*, 1991). These co-injections significantly counteracted the delaying effect of suc1, 78% of the injected embryos exhibiting synchronous blastomeres. When a diluted preparation of suc1 messengers was used as a control, synchrony was observed in only 25% of the embryos (Table 1). Fig. 4 shows a typical suc1/cyclin B-injected embryo in which synchronous blastomeres were observed.

Discussion

We used the same cloning strategy as Richardson *et al.* (1990) who identified and cloned two human homologs of suc1. We carried out numerous PCR reactions varying different parameters and always obtained one discrete amplified product in the range of the expected size. The partial sequencing of different subclones of the amplification product did not show any difference and the screening of the trochophora library resulted in the isolation of one single positive plaque. From these observations, it is very likely that there is only one suc1 homolog expressed in *Patella* embryos. However, we cannot rule out the existence of a second molecule expressed in adults. Considering the growing number of subfamily members identified for many cell cycle regulators such as cdc2-like molecules (Meyerson *et al.*, 1992), cyclins (Xiong and Beach, 1991), or cdc25 molecules (Galaktionov and Beach, 1991; Nagata *et al.*, 1991; Alphey *et al.*, 1992), it is reasonable to suspect in higher eukaryotes the existence of a subfamily of several suc1 molecules whose respective expressions would be differentially controlled throughout the cell cycle (as already noted by Richardson *et al.*, 1990) and/

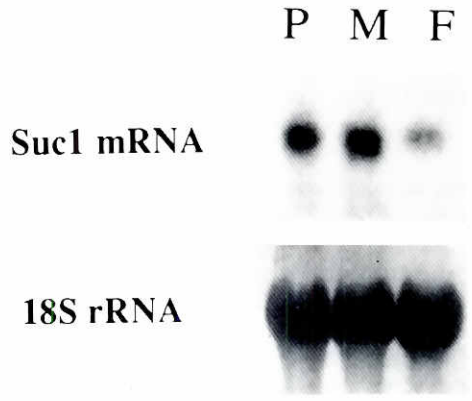

Fig. 3. Steady-state level of suc1 mRNA in maturing oocytes. *Northern blot analysis of suc1 mRNA in prophase I (P), metaphase I (M), and fertilized (F) oocytes. Upper panel: hybridization with labeled suc1 cDNA. Lower panel: 18S rRNA stained on the blot with methylene blue.*

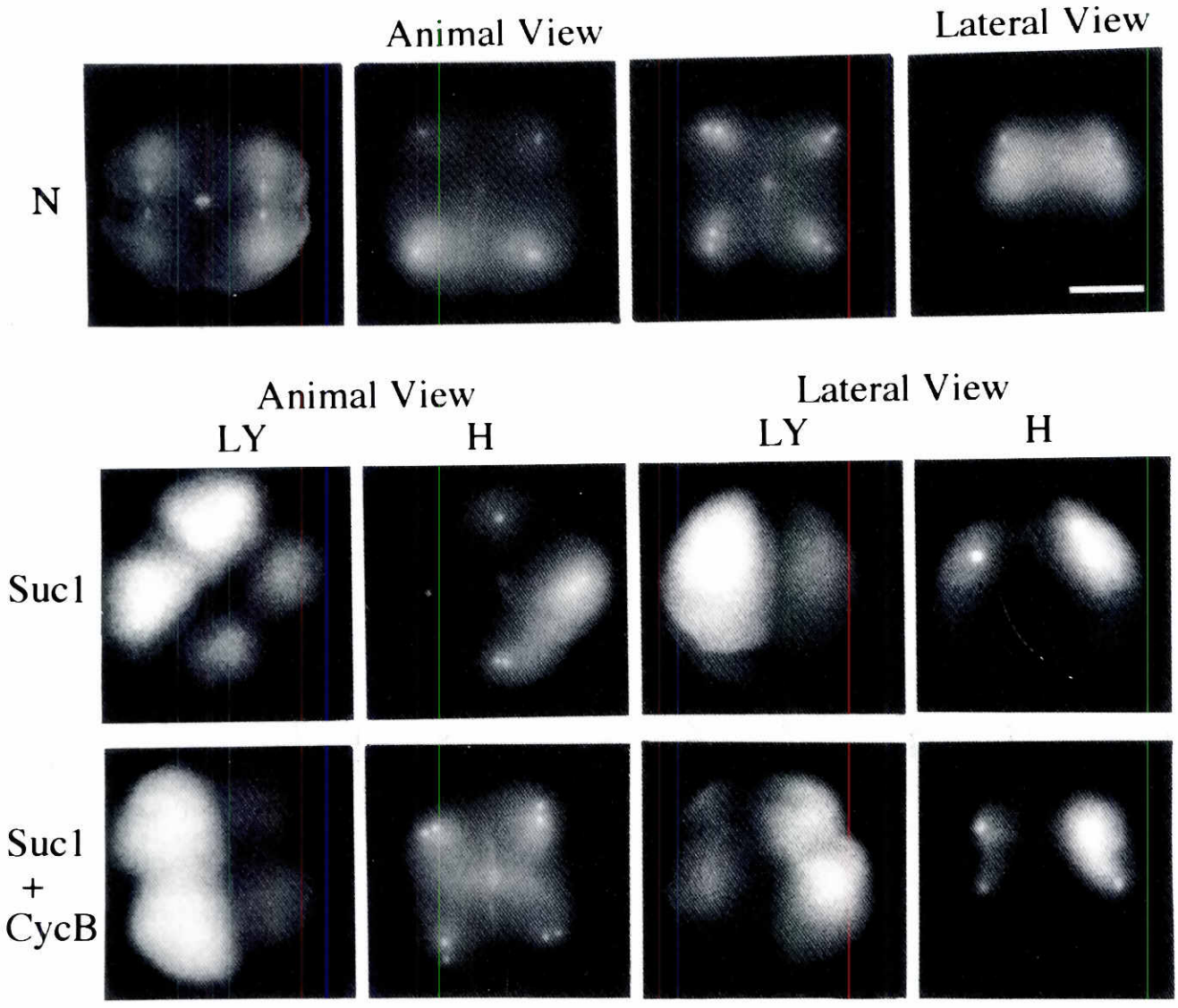

Fig. 4. Effect of microinjection of mRNAs on the cell cycle synchrony of embryonic blastomeres. (Top row) *Cell cycle synchrony between the blastomeres of a non-injected embryo stained with Hoechst 33258. From left to right: late 2-cell stage , 4-cell stage metaphases, 4-cell stage anaphases (animal and lateral views).* **(Suc1 row)** *Typical asynchronous blastomeres in a 4-cell stage embryo observed after microinjection of suc1 mRNA.* **(Suc1+CycB row)** *Typical synchronous blastomeres in a 4-cell stage embryo observed after microinjection of suc1+cyclin B mRNAs. Bar, 50 μ.*

or throughout development (as already observed for cdc25 by Kakizuka *et al.*, 1992).

The examination of the steady-state level of suc1 messengers in maturating oocytes revealed a marked regulation of expression through the cell cycle, the messenger level reaching a peak in metaphase and dropping dramatically after fertilization (G1 phase). This finding is consistent with the observations of Richardson *et al.* (1990), who described a peak of expression of both suc1 molecules in the G2/M population of Hela cells. In light of the rapid diminution of the level of the *Patella* suc1 messengers after fertilization, it may be relevant to note the presence of two overlapping motifs ATAAA starting respectively at nt 662 and 666 in the 3' untranslated region of the cDNA (Fig. 1). This motif is indeed associated with the susceptibility of mRNA to degradation in human cells (Shaw and Kamen, 1986). It is found twice in the 3' untranslated regions of the two human suc1 cDNAs (Richardson *et al.*, 1991), the second motif of the CKShs2 cDNA being located just upstream the poly[A] region, like the motif present in the *Patella* cDNA. Also noted in cyclin cDNAs (Labbé *et al.*, 1989; Van Loon *et al.*, 1991), the relevance of these degradation motifs in the expression pattern of the cyclin and suc1 messengers remains to be established.

The cell cycle regulation of the steady-state level of suc1 mRNAs prompted us to microinject suc1 transcripts into embryonic blastomeres and to observe the effect on cell cycle traverse. The LY^+ blastomeres exhibited a significant delay in 85% of the injected embryos. The microinjection of suc1 transcripts is quite likely to

TABLE 1

RESULTS OF mRNA MICROINJECTIONS

Messengers injected	Number of injected embryos (n)	Injected embryos exhibiting synchronous blastomeres (%)
Suc1	19	15
Suc1/ buffer (1:3)	16	25
Suc1/ CycB (1:3)	18	78
Buffer	ND*	100

*: Microinjections of buffer-LY alone were performed before starting every experiment in order to check the quality of the batch of embryos as well as all the parameters of the microinjection. At least 15 embryos were microinjected.

result in a large increase in the steady-state level of suc1 protein in the blastomeres. At present, the extent of this increase cannot be determined in the absence of a suitable antibody. Interestingly, the LY^+ blastomeres managed to go through many rounds of cell division and maintained a constant delay compared with LY^- blastomeres (not shown). The fact that the delay did not increase strongly suggests a limited half-life of the injected messengers, which probably undergo a degradation upon exit from M-phase as observed in fertilized oocytes. Our experimental procedure does not make it possible to determine directly the phase of the cell cycle during which suc1 exerts its delaying effect. In *S. pombe* , the overexpression of suc1 reduces the growth rate by delaying the G2/M transition (Hayles *et al.*, 1986a; Hindley *et al.*, 1987). When added to cytoplasmic extracts of *Xenopus* prophase oocytes, $p13^{suc1}$ inhibits the amplification of MPF. Moreover, the injection of $p13^{suc1}$ into *Xenopus* oocytes inhibits MPF-induced maturation (Dunphy *et al.*, 1988). Therefore, it is quite likely that the injected blastomeres are delayed in the completion of their G2 phase. The significant reversion of the suc1 delaying effect by the co-injection of cyclin B and suc1 messengers strongly supports this view since cyclin B plays a pivotal role in driving the G2/M transition. If our results extend to a higher eukaryote the effects of an overexpression of suc1 already described in yeasts, they contradict the observations of Riabowol *et al.* (1989) which seem delicate to interpret (see Introduction).

The inhibitory effect of suc1 on the entry into M-phase and thus on the activation of the cdc2 kinase *in vitro* has been largely documented by many authors during the past few years. Dunphy and Newport (1989) showed for the first time that $p13^{suc1}$ inhibits the tyrosine dephosphorylation of cdc2 when added to a pre-MPF fraction of *Xenopus* oocytes. After the identification of cdc25 as the phosphatase responsible for the tyrosine dephosphorylation of cdc2, $p13^{suc1}$ was shown to be an inhibitor of the phosphatase activity of cdc25 in acellular systems (Dunphy and Kumagai, 1991; Galaktionov and Beach, 1991; Gautier *et al.*, 1991; Kumagai and Dunphy, 1991). However, the inhibitory effect of $p13^{suc1}$ on the cdc25 activity was only seen when acellular extracts or partially purified molecules were used. Therefore, as already discussed by Gautier *et al.* (1991), it appears that $p13^{suc1}$ does not act directly at the level of the cdc25 phosphatase but may exert its inhibitory effect in an indirect manner which remains to be determined.

Materials and Methods

Handling of Patella oocytes and embryos

Adult specimens of *Patella vulgata* were collected at Roscoff (Bretagne, France) and kept in tanks of recirculated 16°C sea water. Oocytes arrested in prophase I and in metaphase I were obtained and labeled with [^{35}S]Methionine as previously described (Guerrier *et al.*, 1986; Van Loon *et al.*, 1991). *In vitro* fertilization and culture of the embryos were performed according to Van den Biggelaar (1977).

PCR amplification and suc1 cDNA cloning/sequencing

Poly(A)$^+$ RNA of trochophoras (swimming *Patella* larvae, 16 h after fertilization) underwent reverse transcription and PCR using the GeneAmp RNA PCR kit from Perkin Elmer Cetus essentially according to the instructions of this manufacturer. The primers used were designed from the sequence alignment of Richardson *et al.* (1990) and had the following sequences: 5' primer, 5'-ATACATATGGARTAYMGNCAYGTNATGYT-3'; 3'primer, 5'ATAACTAGTARN ARXATRTGNGGYTCNGG-3'. (Y=C+T; R=A+G; M=A+C; X=A+G+T; N=A+C+G+T). The degeneracy of these primers is 512-fold and 3072-fold respectively. The underlined subsequences correspond to the restriction sites NdeI and SpeI. In order to avoid an amplification from the interaction betwen these two primers, a "hot start" procedure was carried out, the Taq polymerase being added during the initial denaturation step. The PCR incubations consisted of 40 cycles of 45 s at 94°C, 90 s at 37°C, 30 s at 72°C , followed by a 10 min incubation at 72°C. The amplified product of the expected size was isolated from a 2% agarose gel by a QIAEX kit from Qiagen, re-amplified for confirmation, digested with NdeI and SpeI, and subcloned in pGEM-5Zf(+) from Promega. The cloned product was then labeled with [^{32}P]dATP using a random-priming kit from Boehringer and used as a probe to screen a trochophora λgt10 library (Van Loon *et al.*, 1993) according to the instructions of Amersham (membrane transfer and detection methods). The positive plaque was re-plated, re-screened and isolated. Its DNA was extracted according to Maniatis (1982) and digested with EcoRI. The insert was subcloned in pGEM-7Zf(+) from Promega and sequenced from both sides using a T7 DNA sequencing kit from Pharmacia. In order to sequence both strands of the total cDNA, the insert was split in two fragments by a double-digestion with EcoRI and BamHI and the two fragments were subcloned and sequenced.

RNA techniques

Total RNA was isolated from oocytes and embryos according to Rosenthal and Wilt (1986). Poly(A)$^+$ RNA was obtained by chromatography on oligo(dT)-cellulose type 7 from Pharmacia essentially according to Maniatis *et al.* (1982). Northern Blots were performed according to Amersham (membrane transfer and detection methods). The blots were probed with the *Patella* suc1 cDNA labeled with [^{32}P]dATP. The *in vitro* transcriptions of suc1 and cyclin cDNAs were performed using a Riboprobe Gemini System from Promega.

Microinjections, staining and observations

The suc1 and cyclin B messengers transcribed from 5 µg of DNA template were resuspended in 8 µl of 10 mM Hepes/0.4% Lucifer Yellow. Glass micropipettes pulled with a Mecanex micropipette puller were back-filled with the microinjection solution. Blastomeres were injected by pressure using a Narishige microinjector. Injected cells were traced by monitoring the fluorescence. Those which showed cytoplasmic leakage, extrusions or overloading were discarded. The selected embryos were transfered into sea water containing 5 µg/ml propidium iodine to check cell viability. 30 min after microinjection, the embryos were fixed 1 h at 4°C in 3.7% formaldehyde in PBS, rinsed 10 min in PBS, stained 15 min with Hoechst 33258 (0.5 µg/ml in PBS), rinsed 30 min in PBS and mounted on depression object glasses in a glycerol-PBS solution (9:1). Successfully injected embryos were observed under a Zeist Axiovert fluorescence microscope.

Acknowledgments

We wish to thank Drs. P. de Bagnols, J. Baguñà, J. A.M. van den Biggelaar, J. Daillie, and B.B. Rudkin for their valuable comments on this

manuscript. We are grateful to Leo van Grunsven for the gift of the Trochophora cDNA. P.C. has been supported by post-doctoral fellowships from l' Association pour la Recherche contre le Cancer (ARC) and la Ligue Nationale contre le Cancer.

References

ALPHEY, L., JIMENEZ, J., WHITE-COOPER, H., DAWSON, I., NURSE, P. and GLOVER, D.M. (1992). twine, a cdc25 homolog that functions in the male and female germline of *Drosophila*. *Cell 69:* 977-988.

BRIZUELA, L., DRAETTA, G. and BEACH, D. (1987). $p13^{suc1}$ acts in the fission yeast cell division cycle as a component of the $p34^{cdc2}$ protein kinase. *EMBO J. 6:* 3507-3514.

CLARKE, P.R. and KARSENTI, E. (1991). Regulation of $p34^{cdc2}$ protein kinase: new insights into protein phosphorylation and the cell cycle. *J. Cell Sci. 100:* 409-414.

DRAETTA, G., BRIZUELA, L., POTASHKIN, J. and BEACH, D. (1987). Identification of p34 and p13 homologs of the cell cycle regulators of fission yeast encoded by cdc2+ and suc1+. *Cell 50:* 319-325.

DUCOMMUN, B., BRAMBILLA, P. and DRAETTA, G. (1991). Mutations at sites involved in Suc1 binding inactivate Cdc2. *Mol. Cell. Biol. 11:* 6177-6184.

DUNPHY, W.G. and KUMAGAI, A. (1991). The cdc25 protein contains an intrinsic phosphatase activity. *Cell 67:* 189-196.

DUNPHY, W.G. and NEWPORT, J.W. (1989). Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the *Xenopus* cdc2 protein kinase. *Cell 58:* 181-191.

DUNPHY, W.G., BRIZUELA, L., BEACH, D. and NEWPORT, J. (1988). The *Xenopus* cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell 54:* 423-431.

GALAKTIONOV, K. and BEACH, D. (1991). Specific activation of cdc25 tyrosine phosphatase by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell 67:* 1181-1194.

GAUTIER, J., SOLOMON, M.J., BOOHER, R.N., BAZAN, J.F. and KIRSCHNER, M.W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates $p34^{cdc2}$. *Cell 67:* 197-211.

GUERRIER, P., BRASSART, M., DAVID, C. and MOREAU, M. (1986). Sequential control of meiosis reinitiation by pH and Ca2+ in oocytes of the prosobranch mollusk *Patella vulgata*. *Dev. Biol. 114:* 315-324.

GUERRIER, P., COLAS, P. and NÉANT, I. (1990). Meiosis reinitiation as a model system for the study of cell division and cell differentiation. *Int. J. Dev. Biol. 34:* 93-109.

HADWIGER, J.A., WITTENBERG, C., MENDENHALL, M.D. and REED, S.I. (1989). The Saccharomyces cerevisiae CKS1 gene, a homolog of the Schizosaccharomyces pombe suc1+ gene, encodes a subunit of the Cdc28 protein kinase complex. *Mol. Cell. Biol. 9:* 2034-2041.

HAYLES, J., AVES, S. and NURSE, P. (1986a). suc1 is an essential gene involved in both the cell cycle and growth in fission yeast. *EMBO J. 5:* 3373-3379.

HAYLES, J., BEACH, D., DURKACZ, B. and NURSE, P. (1986b). The fission yeast cell cycle control gene cdc2: isolation of a sequence that supresses cdc2 mutant function. *Mol. Gen. Genet. 202:* 291-293.

HINDLEY, J., PHEAR, G.A., STEIN, M. and BEACH, D. (1987). Suc1+ encodes a predicted 13-kilodalton protein that is essential for cell viability and is directly involved in the division cycle of *Schizosaccharomyces pombe*. *Mol. Cell. Biol. 7:* 504-511.

KAKIZUKA, A., SEBASTIAN, B., BORGNEYER, U., HERMANS-BORGMEYER, I., BOLADO, J., HUNTER, T., HOEKSTRA, M.F. and EVANS, R.M. (1992). A mouse cdc25 homolog is differentially and developmentally expressed. *Genes Dev. 6:* 578-590.

KIRSCHNER, M. (1992). The cell cycle then and now. *TIBS. 17:* 281-285.

KOZAK, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell 44:* 283-292.

KUMAGAI, A. and DUNPHY, W.G. (1991). The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. *Cell 64:* 903-914.

LABBÉ, J.C., CAPONY, J.P., CAPUT, D., CAVADORE, J-C., DERANCOURT, J., KAGHAD, M., LELIAS, J.M., PICARD, A. and DORÉE, M. (1989). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J. 8:* 3053-3058.

MANIATIS, T., FRITSCH, E.F. and SAMBROOK, J. (1982). *Molecular Cloning*. Cold Spring Harbor Laboratory, New York.

MEYERSON, M., ENDERS, G.H., WU, C.L., SU, L.K., GORKA, C., NELSON, C., HARLOW, E. and TSAI, L.H. (1992). A family of human cdc2-related protein kinases. *EMBO J. 11:* 2909-2917.

MORENO, S., HAYLES, J. and NURSE, P. (1989). Regulation of $p34^{cdc2}$ protein kinase during mitosis. *Cell 58:* 361-372.

MURRAY, A.W. (1992). Creative blocks: cell-cycle checkpoints and feedback controls. *Nature 359:* 599-604.

NAGATA, A., IGARASHI, M., JINNO, S., SUTO, K. and OKAYAMA, H. (1991). An additional homolog of the fission yeast cdc25+ gene occurs in humans and is highly expressed in some cancer cells. *New Biologist 3:* 959-968.

RIABOWOL, K., DRAETTA, G., BRIZUELA, L., VANDRE, D. and BEACH, D. (1989). The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells. *Cell 57:* 393-401.

RICHARDSON, H.E., STUELAND, C.S., THOMAS, J., RUSSELL, P. and REED, S.I. (1990). Human cDNAs encoding homologs of the small $p34^{Cdc28/cdc2}$-associated protein of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Genes Dev. 4:* 1332-1344.

ROSENTHAL, E.T. and WILT, F.H. (1986). Patterns of maternal RNA accumulation and adenylation during oogenesis in *Urechis caupo*. *Dev. Biol. 117:* 55-63.

SHAW, G. and KAMEN, R. (1986). A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell 46:* 659-667.

VAN DEN BIGGELAAR, J.A.M. (1977). Development of dorsoventral polarity and mesentoblast determination in *Patella vulgata*. *J. Morphol. 154:* 157-186.

VAN LOON, A.E., COLAS, P., GOEDEMANS, H.J., NÉANT, I., DALBON, P. and GUERRIER, P. (1991). The role of cyclins in the maturation of *Patella vulgata* oocytes. *EMBO J. 10:* 3343-3349.

VAN LOON, A.E., GOEDEMANS, H.J., DAEMEN, A.J.J.M., VAN DEN KAMP, A.J. and VAN DEN BIGGELAAR, J.A.M. (1993). Actin genes expressed during early development of *Patella vulgata*. *Roux Arch. Dev. Biol. 202:* 77-84.

XIONG, Y. and BEACH, D. (1991). Population explosion in the cyclin family. *Curr. Biol. 1:* 362-364.

Accepted for publication: November 1993