labeling pattern in zebrafish is in preparation (K. Hatta *et al.*, in preparation). Recent molecular studies have shown that there are two *engrailed* homeobox genes in zebrafish (M. Westerfield, personal communication). It is likely that 4D9 reacts with proteins encoded by both of them, and whether one or both of these genes is expressed in the jaw muscles is currently unknown.
9. C. A. Davis *et al.*, *Genes Dev.* **2**, 361 (1988).
10. D. Davidson, E. Graham, C. Sime, R. Hill, *Development* **104**, 305 (1988).
11. Hours after fertilization, development at 28.5°C.
12. This labeling pattern is similar to that previously described for the cranial mesenchyme of mouse (*En-1*) (*10*) and chicken (*6*) embryos, although the eventual fates of those cells were not characterized.
13. The two muscles cooperate to enlarge the buccal cavity and to cause the mouth to protrude (the mechanical advantage of DO is increased by LAP action; DO also enlarges the opercular cavity [R. Chiasson, *Laboratory Anatomy of the Perch* (Brown, Dubuque, IA, ed. 3, 1980)].
14. Sometimes we saw a faintly stained mesenchymal aggregate just proximal to the LAP and DO precursors. We did not further examine the origin and fate of these cells, but, because they appear similar to the LAP and DO precursor cells, it is possible that their fate is to form jaw muscles proximal to LAP and DO. Expression disappears by 72 h.
15. D. M. Noden, *Am. J. Anat.* **168**, 257 (1983).
16. Neural crest in zebrafish, as in other vertebrates, derives from ectoderm [C. B. Kimmel *et al.*, *Development* **108**, 581 (1990)].
17. In the chicken, mesenchyme derived from both cranial neural crest and mesoderm appears to express Eng [(*6*); C. A. Gardner and K. F. Barald, personal communication].
18. A similar association, between trigeminal axons and a patch of mesenchyme that accumulates laminin, has been reported in the chicken, where the cells were thought to be muscle precursors [S. A. Moody *et al.*, *J. Comp. Neurol.* **283**, 38 (1989)].
19. W. K. Metcalfe, *ibid.* **238**, 218 (1985).
20. A consequence of phylogenetic conservation of the homeobox genes, their regulation, and their functions during development is that their expression patterns will permit identification of homologous cells in diverse organisms. The homologs of LAP and DO in tetrapods are presently unknown but could be identified by their expression of Eng.
21. For example, jaw muscle–conditioned culture medium, but not forelimb muscle–conditioned medium, enhances axonal extension by trigeminal motoneurons in vitro [M. B. Heaton and D. B. Wayne, *J. Comp. Neurol.* **243**, 381 (1986)].
22. In the mouse, an insertional mutation destroying the *En-2* gene was produced by homologous recombination in embryonic stem cells, although the phenotype this mutation produces in embryos has not yet been reported [A. L. Joyner, W. C. Scarnes, J. Rossant, *Nature* **338**, 153 (1989)].
23. G. Streisinger *et al.*, *ibid.* **291**, 293 (1981).
24. B. Trevarrow *et al.*, *Neuron* **4**, 669 (1990).
25. E. Hanneman *et al.*, *Development* **103**, 49 (1988).
26. We thank K. Coleman, T. Kornberg, N. Patel, and C. Goodman for the 4D9 antibody; B. Trevarrow for the zn-1 antibody; P. Z. Myers for imaging software; D. A. Kane for his help with computer image composition; M. McDowell and H. Howard for technical assistance; and M. Westerfield for comments on the manuscript. Supported by grants from the NIH (HD22486, NS17963) and the Naitoh Foundation.

23 March 1990; accepted 12 July 1990

cdc2 Gene Expression at the G_1 to S Transition in Human T Lymphocytes

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The product of the *cdc2* gene, designated $p34^{cdc2}$, is a serine-threonine protein kinase that controls entry of eukaryotic cells into mitosis. Freshly isolated human T lymphocytes (G_0 phase) were found to have very low amounts of $p34^{cdc2}$ and *cdc2* messenger RNA. Expression of *cdc2* increased 18 to 24 hours after exposure of T cells to phytohemagglutinin, coincident with the G_1 to S transition. Antisense oligodeoxynucleotides could reduce the increase in *cdc2* expression and inhibited DNA synthesis, but had no effect on several early and mid-G_1 events, including blastogenesis and expression of interleukin-2 receptors, transferrin receptors, c-*myb*, and c-*myc*. Induction of *cdc2* required prior induction of c-*myb* and c-*myc*. These results suggest that *cdc2* induction is part of an orderly sequence of events that occurs at the G_1 to S transition in T cells.

THE SERINE-THREONINE PROTEIN kinase $p34^{cdc2}$ plays a key role in the regulation of the eukaryotic cell cycle. Originally identified as the product of the *cdc2* gene of *Schizosaccharomyces pombe* and the *CDC28* gene of *Saccharomyces cerevisiae*, $p34^{cdc2}$ is now known to be the catalytic subunit of the mitosis-regulating protein kinase complex known as maturation promoting factor (MPF) or growth-associated H1 kinase (*1*). In growing HeLa cells, the amount of $p34^{cdc2}$ remains constant throughout the cell cycle, but $p34^{cdc2}$ H1 kinase activity increases dramatically as cells progress from G_1 to mitosis (*2*). The regulation of $p34^{cdc2}$ function is incompletely understood but is believed to be modulated by associated proteins, such as $p13^{suc1}$ and cyclins. Also, the phosphorylation of $p34^{cdc2}$ fluctuates as a function of the cell cycle (*2–4*) and full activation of $p34^{cdc2}$ in mitotic metaphase requires that residues in the adenosine triphosphate (ATP) binding site be dephosphorylated (*2, 5*).

In fission yeast, *cdc2* is required both at the G_2 to M (G_2-M) transition and in G_1 at "Start" (*6*). The requirement, if any, for $p34^{cdc2}$ in the G_1 phase of the cell cycle in higher eukaryotes has been difficult to assess, partly because of the lack of suitable cell cycle control mutants, although a potential role for $p34^{cdc2}$ at the G_1-S transition has been postulated (*7*). Microinjection of antibodies to $p34^{cdc2}$ into serum-stimulated rat fibroblasts caused cells to arrest in G_2 but had no effect on DNA replication (*8*). However, the function of $p34^{cdc2}$ may be different in G_1 than G_2, and $p34^{cdc2}$ could be less susceptible to antibody neutralization in G_1 and S because of a difference in intracellular localization or associated proteins.

Early events in cell cycle control have been studied in hematopoietic cells because these cells spontaneously arrest in G_0 during the process of differentiation. T and B lymphocytes can be induced to reenter the cell cycle in response to specific antigen or mitogenic lectins, whereas myeloid cells are terminally differentiated and remain in G_0-G_1 (*9*). To investigate the role of $p34^{cdc2}$ in G_1 cell cycle control in human cells, we have examined the expression of *cdc2* in T cells after lectin stimulation and have used antisense oligodeoxynucleotides to modulate *cdc2* expression.

The DNA content of freshly isolated blood T cells was analyzed by flow cytometry after staining with propidium iodide. As described previously (*10*), all T cells were found to be in G_0, but in response to the mitogenic lectin phytohemagglutinin (PHA) they could be synchronously induced to undergo blastogenesis (the morphological changes of the G_0-G_1 transition) (after 8 to 12 hours), begin DNA synthesis (after 18 to 24 hours), and initiate mitosis (after 24 to 48 hours). The expression of *cdc2* mRNA (*11*) was evaluated by Northern blotting (Fig. 1A) and reverse PCR (polymerase chain reaction) (Fig. 1B) in T cells for up to 96 hours after stimulation. Before PHA stimulation, *cdc2* mRNA was undetectable, and it first became detectable at 15 to 18 hours by reverse PCR and at ~24 hours by Northern blot. The expression of $p34^{cdc2}$ was simultaneously evaluated by immunoblotting with a monospecific polyclonal antibody to a human $p34^{cdc2}$ fragment (*12*).

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Resting T cells contained very low concentrations of $p34^{cdc2}$ (Fig. 1C). In some experiments, an immunoreactive band migrating at a position corresponding to 34 kD was observed in resting T cells (Fig. 1C); however, this band was inconsistently detected in different experiments and was not detected with some batches of the specific antiserum. In all experiments, $p34^{cdc2}$ migrated as a doublet or triplet at a position corresponding to 32 to 34 kD. Increased amounts of $p34^{cdc2}$ were detected after 24 hours, and by 36 hours the amount of $p34^{cdc2}$ was ten times as great as that present in cells before stimulation (Fig. 1C). The identity of these 32- to 34-kD proteins as authentic human $p34^{cdc2}$ was supported by their specific precipitation with $p13^{suc1}$ coupled to Sepharose beads (*13*) and comigration with recombinant human $p34^{cdc2}$ (*14*). Thus, in quiescent G_0 phase T cells, expression of *cdc2* was either low or nonexistent, but after stimulation, *cdc2* mRNA and $p34^{cdc2}$ were specifically induced at a time corresponding to late G_1 or early S phase. After 15 to 24 hours, <5% of cells had entered S phase under these experimental conditions. In order to confirm the expression of *cdc2* at G_1-S, T cells were stimulated with PHA in the presence of 1 mM hydroxyurea, which causes cell cycle arrest at G_1-S through inhibition of DNA synthesis (*15*). Hydroxyurea had no effect on the PHA-induced expression of *cdc2* RNA or $p34^{cdc2}$.

To assess the functional significance of the induction of $p34^{cdc2}$ in lectin-stimulated T cells, we attempted to inhibit $p34^{cdc2}$ expression with an 18-base antisense oligodeoxynucleotide complementary to codons −3 to +3 of the *cdc2* mRNA. As controls, sense and nonsense *cdc2* 18-base oligomers were used, and for comparative purposes, antisense, sense, or nonsense oligomers for codons 2 to 7 of the c-*myb* mRNA, codons 1 to 5 of the c-*myc* mRNA, codons −5 to +1 of tubulin mRNA, codons −1 to +5 of cyclin B mRNA, and codons −1 to +5 of *CD5* mRNA were also evaluated. Both c-*myb* and c-*myc* antisense oligomers have been shown to block entry into S phase of T cells in suspension culture, whereas sense or nonsense oligomers have no effect (*16*). Antisense *cdc2* oligomers, but not sense or nonsense oligomers, reduced the induction of $p34^{cdc2}$ at 24 to 36 hours by 47 to 75% (estimated by scanning densitometry) in three experiments (Fig. 2A). Similarly, antisense c-*myc* and antisense c-*myb* oligonucleotides, but not sense or nonsense oligomers, reduced induction of c-*myc* and c-*myb* by 55 and 89%, respectively, in the experiment shown in Fig. 2A. Induction of $p34^{cdc2}$ expression was at least partly dependent on expression of c-*myc*, c-*myb*, or both (Fig. 2A), because treatment of T cells with either antisense c-*myb* or c-*myc* oligomers reduced $p34^{cdc2}$ induction by 40 and 57%, respectively. Antisense c-*myc* oligomers had a minimal effect on induction of c-*myb* (17% reduction in the experiment shown in Fig. 2A), and antisense c-*myb* reduced expression of c-*myc* by 35%, suggesting that expression of these two proteins in T cells may be interrelated to a small degree.

The effects of blocking $p34^{cdc2}$ up-regulation on a series of G_1 or S phase–associated events, including blastogenesis (G_0-G_1), induction of interleukin-2 (IL-2) receptor and transferrin receptor expression (early G_1), induction of c-*myb* and c-*myc* (early to mid-G_1), and DNA synthesis (S phase), were determined. Antisense *cdc2* oligomer at a concentration of 200 μg/ml (~35 μM) had no effect on blastogenesis, cell viability, induction of the transferrin receptor, or induction of the IL-2 receptor. Similarly, antisense *cdc2* oligomer had no effect on induction of proteins encoded by c-*myc* and c-*myb* (Fig. 2A). However, antisense *cdc2* oligomer, but not sense or nonsense *cdc2* oligomers, reduced T cell entry into S phase when assessed at 36 or 72 hours and were as effective as antisense c-*myb* or c-*myc* oligomers (Fig. 2C). Further, antisense or sense oligomers to cyclin B (Fig. 2C), tubulin, and *CD5* mRNAs had no effect. Antisense *cdc2*, but not nonsense *cdc2*, reduced DNA synthesis ([^{3}H]thymidine uptake) in stimulated T cells (Fig. 2B). Thus, a function associated with induction of $p34^{cdc2}$ is required in late G_1 or early S phase for DNA synthesis in T cells, but is not required for earlier G_1 events including expression of the IL-2 receptor, the transferrin receptor, c-*myc*, or c-*myb*. Although the antisense oligonucleotide studies demonstrate an association between induction of *cdc2* expression and entry into S phase in T cells, proof that $p34^{cdc2}$ itself regulates the G_1-S transition will require additional studies, such as demonstrating that overexpression of *cdc2* makes the cell resistant to the oligonucleotide effect. As expected from previous studies (*16*), antisense c-*myc* and c-*myb* oligomers had no effect on blastogenesis or IL-2 receptor expression, but inhibited entry into S phase (Fig. 2C).

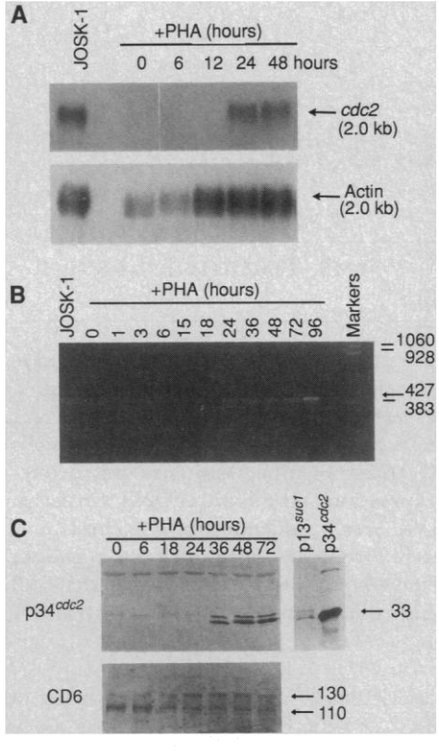

Fig. 1. Expression of *cdc2* mRNA and protein in T cells after PHA stimulation. T cells were prepared from peripheral blood by E-rosetting and plastic adherence. Cells were cultured at 2×10^6 to 5×10^6 cells/ml in RPMI 1640 medium (Gibco) with 10% heat-inactivated fetal bovine serum (FBS) and phytohemagglutinin-P (PHA) (Wellcome) at a concentration of 1 μg/ml. (**A**) Induction of *cdc2* RNA. Total cellular RNA was isolated at various times and 15-μg samples were subjected to 1.2% agarose formaldehyde gel electrophoresis and blotted onto nitrocellulose. The *cdc2* mRNA was detected with a 0.9-kb Kpn I–Pvu II fragment of human *cdc2* cDNA as a probe (*11*). A murine β-actin cDNA was used to reprobe blots to control for variations in amount of RNA loaded. JOSK-I is a human monocytic leukemic cell line (*25*). (**B**) Detection of *cdc2* mRNA by PCR. RNA from 2.5×10^4 cells was reverse transcribed (1 hour, 37°C) with 200 U of Moloney murine leukemia virus reverse transcriptase and a 3′ antisense primer (corresponding to nucleotides 574 to 597) as template. Resulting cRNA fragments were amplified by PCR (40 cycles) with 2.5 U of Taq polymerase and 24-base primers flanking a 427-bp sequence coding for amino acids 11 to 153 (*26*). A portion (10 μl) of the 100-μl reaction volume was separated in a 2% agarose gel and stained with ethidium bromide. Molecular size markers (expressed in bases) were a Bst NI digest of pBR322. (**C**) Induction of $p34^{cdc2}$. Protein extracts (25 μg per lane) were separated by 10% SDS–polyacrylamide gel electrophoresis on a minigel apparatus (Schleicher & Schuell) and transferred to a nitrocellulose membrane. Immunoblotting was performed with a 1:1000 dilution of monospecific rabbit antiserum to recombinant human $p34^{cdc2}$ (*12*), and blots were developed with alkaline phosphatase–conjugated goat antibodies to rabbit immunoglobulin G (Promega). Human $p34^{cdc2}$ from Sf9 cells infected with a baculovirus encoding $p34^{cdc2}$ was used as a positive control ($p34^{cdc2}$ lane) (*14*). The identification of the 32- to 34-kD bands as authentic human $p34^{cdc2}$ was supported by their disappearance from the 72-hour sample after treatment with $p13^{suc1}$-coupled agarose beads as described (*13*) ($p13^{suc1}$ lane) (*13*). As a control for protein loading, the upper half of the blot was separately stained for expression of the T cell membrane protein CD6 with monoclonal antibody 2H1, which detects a 110- to 130-kD protein (*27*). Sizes are shown in kilodaltons.

Finally, we examined the possible requirement for $p34^{cdc2}$ in a known G_1-S–associated phosphorylation event, the phosphorylation of the product of the retinoblastoma gene, Rb (*17, 18*). We have previously shown that lymphocytes express a G_1-associated kinase that can phosphorylate Rb, whereas myeloid cells do not (*18*). The phosphorylation of Rb at G_1-S occurs in a wide variety of human cells and has been suggested to be involved in the regulation of the G_1-S transition (*17, 18*). The identities of the Rb kinases are unknown, but Rb contains six consensus sites for phosphorylation by $p34^{cdc2}$ (*19*). Phosphorylated species of Rb (Rb-phos) can be readily identified by their slower migration on SDS–polyacrylamide gel electrophoresis (*20*). The induction of $p34^{cdc2}$ (Fig. 1) thus coincides with the phosphorylation of Rb and the increase in the amount of Rb that occurs at G_1-S (*18*). When cells were treated with antisense *cdc2* oligomers, the amount of Rb-phos, particularly the most slowly migrating forms, was reduced (Fig. 3). The characteristic up-regulation of total Rb protein that occurs in T cells (*18*) was also diminished. Nonsense oligomers had no effect. Treatment of T cells with antisense c-*myb* or c-*myc* oligomers also reduced the amount of Rb-phos, whereas sense or nonsense oligomers had no effect (Fig. 3). Thus, c-*myb*, c-*myc*, and $p34^{cdc2}$ are directly or indirectly required for some of the phosphorylation of Rb. However, the reduced amount of Rb-phos that occurs in response to antisense *cdc2* oligomers does not indicate that $p34^{cdc2}$ itself is the kinase responsible for Rb phosphorylation. With histone H1 as a substrate, $p34^{cdc2}$ has been shown to be relatively inactive as a kinase in G_1 compared to G_2-M (*2*). However, it has been suggested that the substrate specificity of $p34^{cdc2}$ may vary in different phases of the cell cycle (*7*).

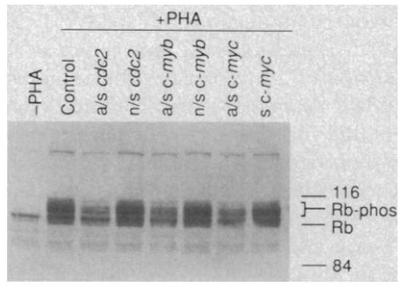

Fig. 3. Expression and phosphorylation of Rb protein in stimulated T cells. Unphosphorylated Rb and phosphorylated Rb (Rb-phos) were detected by immunoblotting with monoclonal antibody RB-PMG3-245 (Pharmingen) as described (*17, 18*). T cells were cultured with PHA (1 μg/ml) for 48 hours with antisense (a/s), nonsense (n/s), or sense (s) oligomers as indicated, and 120 μg of protein lysate was applied to each lane. Molecular sizes are shown in kilodaltons.

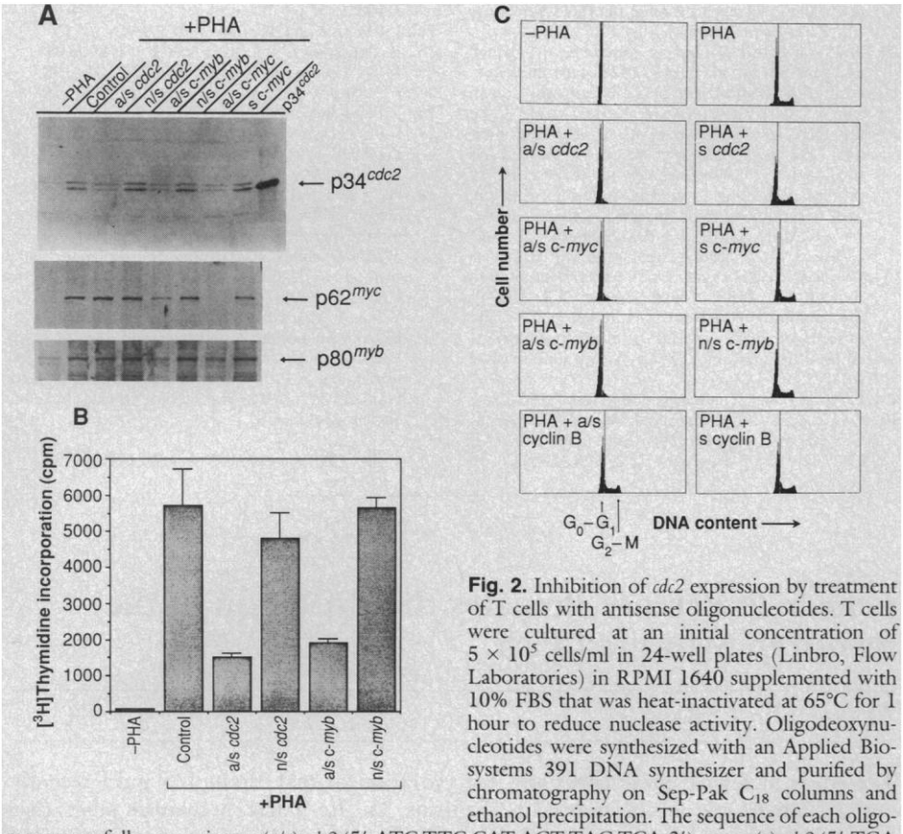

Fig. 2. Inhibition of *cdc2* expression by treatment of T cells with antisense oligonucleotides. T cells were cultured at an initial concentration of 5×10^5 cells/ml in 24-well plates (Linbro, Flow Laboratories) in RPMI 1640 supplemented with 10% FBS that was heat-inactivated at 65°C for 1 hour to reduce nuclease activity. Oligodeoxynucleotides were synthesized with an Applied Biosystems 391 DNA synthesizer and purified by chromatography on Sep-Pak C_{18} columns and ethanol precipitation. The sequence of each oligomer was as follows: antisense (a/s) *cdc2* (5′-ATC TTC CAT AGT TAG TCA-3′), sense (s) *cdc2* (5′-TGA CTA ACT ATG GAA GAT-3′), nonsense (n/s) *cdc2* (5′-TGG CAA ACT TAT TCT ACT-3′), a/s c-*myb* (5′-GTG CCG GGG TCT TCG GGC-3′), s c-*myb* (5′-GCC CGA AGA CCC CGG CAC-3′), n/s c-*myb* (5′-GCG GGT TGT CGC GCG GTC-3′), a/s c-*myc* (5′-AAC GTT GAG GGG CAT-3′), s c-*myc* (5′-ATG CCC CTC AAC GTT-3′), a/s cyclin B (*28*) (5′-GAC TCG GAG CGC CAT GGC-3′), s cyclin B (5′-GCC ATG GCG CTC CGA GTC-3′). The cells were stimulated with PHA (0.5 μg/ml) and the oligonucleotides were added to the cultures 6 hours before and 0, 24, and 48 hours after PHA stimulation [at a concentration of 200 μg/ml (~35 μM) for the first addition and 20 μg/ml for the subsequent additions (total = 260 μg/ml)]. Oligonucleotide uptake, stability, and S1 nuclease resistance were examined as described (*29*). Approximately 2% of the added oligomers were taken up by the cells after 24 hours. S1 nuclease–resistant hybrid structures appeared after 24 hours. The figure shows results from one of four similar experiments. (**A**) Effect of antisense oligodeoxynucleotides on $p34^{cdc2}$, c-*myc*, and c-*myb* induction. The expression of $p34^{cdc2}$ was evaluated by immunoblot before (−PHA) and 48 hours after PHA stimulation. The protein encoded by c-*myc* ($p62^{myc}$) was detected with antibody OM-11-902 (Cambridge Research Biochemicals, Cambridge, Massachusetts) and that encoded by c-*myb* ($p80^{myb}$) was detected with antibody OA-11-841 (Cambridge Research Biochemicals). Equal amounts of protein (25 μg) were loaded in each lane. Control samples received treatment with PHA but not oligonucleotides. (**B**) [^{3}H]Thymidine incorporation (mean ± SD) was measured after incubating 200-μl cultures (5×10^4 cells per culture) with 0.2 μCi of [^{3}H]thymidine (2 Ci/mmol; Dupont Biotechnology Systems) for 16 hours in triplicate (*18*). Nonsense oligomers were used to control for any nonspecific inhibition of [^{3}H]thymidine uptake due to the thymidine in the antisense synthetic oligomers (*16*). (**C**) Cell cycle analysis was performed by flow cytometry after staining DNA with propidium iodide. An equal number of cells (10^4) was analyzed for each treatment. Approximately 50% of the cells entered S, G_2, or M phase after 72 hours of culture with PHA (1 μg/ml).

If $p34^{cdc2}$ does participate in Rb phosphorylation, it is possible that it is not the only such kinase, because of the multiple species of phosphorylated Rb observed in proliferating T cells (Fig. 3). Also, these studies do not address whether all of the phosphorylation of Rb is taking place at G_1-S. It is possible that the antisense oligomers reduce Rb phosphorylation that occurs later in the cell cycle. The effect of antisense c-*myc* and c-*myb* on Rb-phos could be mediated through their effects on $p34^{cdc2}$ expression.

The progression of T cells through the cell cycle is believed to require the sequential expression of a series of cell cycle control genes. Our results support this notion and suggest that the expression of c-*myc*, c-*myb*, and *cdc2* are interrelated. Expression of c-*myc* has been shown to be biphasic, with an early peak at 2 to 5 hours, and a later peak at 12 to 24 hours (*21*). The expression of c-*myb* peaks at 16 to 20 hours (*22*). Our results show that increased expression of $p34^{cdc2}$ is detectable by 24 to 36 hours. The expression of c-*myc* and c-*myb* was required for

subsequent induction of $p34^{cdc2}$, but $p34^{cdc2}$ expression was not required for induction of c-*myb* or c-*myc*; the inhibition of $p34^{cdc2}$ expression with antisense oligomers did not affect induction of either proto-oncogene. Thus, $p34^{cdc2}$ expression occurs relatively late in the sequence of G_1-S control genes, subsequent to the induction of c-*myb* and c-*myc*. These results suggest that some of the known requirement for c-*myc* and c-*myb* for the G_1-S transition in lectin-stimulated T cells (*16*) could be mediated through induction of $p34^{cdc2}$, because reduction of $p34^{cdc2}$ expression, either directly by antisense *cdc2* oligomers or indirectly by blocking either c-*myc* or c-*myb* induction, produces equivalent inhibition of DNA synthesis. The observed degree of inhibition of $p34^{cdc2}$ expression after treatment of cells with antisense c-*myb* or c-*myc* could, by itself, be sufficient to block cells at G_1-S. However, c-*myb* and c-*myc* likely regulate the expression of many genes in G_1 and may control several parallel events that are also required for the G_1-S transition.

Our antisense oligomer experiments indicate that $p34^{cdc2}$ is not likely to have a regulatory function at G_0-G_1 in lectin-activated T cells. The small amount of $p34^{cdc2}$ present in resting T cells may be characteristic of G_0 cells, and a similar finding has also been reported for serum-starved fibroblasts (*4*) and quiescent baby rat kidney cells (*23*). It is possible, however, that $p34^{cdc2}$ plays some role in G_0 cells, because expression of *cdc2* has been described in nonproliferating brain cells (*22*).

Finally, it is worth considering that aberrant expression of c-*myc* and c-*myb* in hematopoietic cells can result in leukemia and that loss of expression of Rb is associated with transformation of many cell lineages, including T cell leukemia (*24*). It is therefore possible that $p34^{cdc2}$ itself or regulators of $p34^{cdc2}$ function at G_1-S could contribute to transformation of hematopoietic cells.

REFERENCES AND NOTES

1. D. Arion *et al.*, *Cell* **55**, 371 (1988); J. Gautier *et al.*, *ibid.* **53**, 433 (1988); J.-C. Labbe *et al.*, *Nature* **335**, 251 (1988).
2. G. Draetta and D. Beach, *Cell* **54**, 17 (1988).
3. G. Draetta *et al.*, *Nature* **336**, 738 (1988); V. Simanis and P. Nurse, *Cell* **45**, 261 (1986).
4. M. G. Lee *et al.*, *Nature* **333**, 676 (1988).
5. W. Dunphy and J. Newport, *Cell* **58**, 181 (1989); J. Gautier, T. Matuskawa, P. Nurse, J. Maller, *Nature* **339**, 626 (1989); K. Gould and P. Nurse, *ibid.* **342**, 39 (1989); A. O. Morla, G. Draetta, D. Beach, J. Y. J. Wang, *Cell* **58**, 193 (1989).
6. P. Nurse and Y. Bissett, *Nature* **292**, 558 (1981); S. I. Reed, J. A. Hadwiger, A. T. Lorincz, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4055 (1985).
7. A. Giordano *et al.*, *Cell* **58**, 981 (1989).
8. K. Riabowol *et al.*, *ibid.* **57**, 393 (1989).
9. S. A. Cannistra and J. D. Griffin, *Sem. Hematol.* **25**, 173 (1988); T. P. Stossel, *N. Engl. J. Med.* **290**, 717 (1974).
10. G. R. Crabtree, *Science* **243**, 355 (1989).
11. A 2.0-kb Bam HI fragment containing the human *cdc2* gene was excised from pOB231 (*26*) and inserted into the polylinker of pBD52 (B. Druker, unpublished data) to generate pSAF1. pBD52 is a derivative of pGC1 [R. M. Myers, L. S. Lerman, T. Maniatis, *Science* **229**, 242 (1985)]. pSAF1 was digested with Kpn I and Pvu II and the 0.9-kb fragment of human *cdc2* was used as a probe for Northern blotting after labeling with ^{32}P [A. P. Feinberg and B. Vogelstein, *Anal Biochem.* **132**, 6 (1983)].
12. A 23.5-kD protein fragment encompassing the COOH-terminus of $p34^{cdc2}$ (beginning at methionine residue 85) was expressed in bacteria from the *tac* promoter. Protein was isolated by SDS–polyacrylamide gel electrophoresis and used to inoculate New Zealand White rabbits (M. Lee and H. Piwnica-Worms, unpublished data).
13. Purified p13 was isolated from an overexpressing strain of *Escherichia coli* as described [L. Brizuela, G. Draetta, D. Beach, *EMBO J.* **6**, 3507 (1987)] and coupled to cyanogen bromide–activated Sepharose 6B (5 mg per milliliter of gel) (Pharmacia).
14. A 2.0-kb Bam HI fragment containing the human *cdc2* gene was excised from pOB231 (*10*) and inserted into the Bam HI site of the baculovirus expression vector pVL941 [A. V. Luckow and M. D. Summers, *Bio/Technology* **6**, 47 (1988)]. Recombinant virus encoding $p34^{cdc2}$ was plaque-purified and used to infect *Spodoptera frugiperda* (Sf9) cells. All procedures relating to viral propagation, isolation, and infection were performed essentially as described [M. D. Summers and G. E. Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agricultural Experiment Station Bulletin No. 1555, College Station, TX, 1987)]. Five percent of total cell protein was $p34^{cdc2}$, the majority of which was soluble and unphosphorylated (L. Parker and H. Piwnica-Worms, unpublished data).
15. P. Kurki *et al.*, *J. Immunol.* **138**, 4114 (1987).
16. R. Heikkila *et al.*, *Nature* **328**, 445 (1987); A. M. Gewirtz *et al.*, *Science* **245**, 180 (1989); A. M. Churilla *et al.*, *J. Exp. Med.* **170**, 105 (1989).
17. J. A. DeCaprio *et al.*, *Cell* **58**, 1085 (1989); K. Buchkovich, L. A. Duffy, E. Harlow, *ibid.*, p. 1097; P.-L. Chen *et al.*, *ibid.*, p. 1193; K. Mihara *et al.*, *Science* **246**, 1300 (1989); H.-J. Xu *et al.*, *Oncogene* **4**, 807 (1989).
18. Y. Furukawa *et al.*, *Proc. Natl. Acad Sci. U.S.A.* **87**, 2770 (1990).
19. Y. Taya *et al.*, *Biochem. Biophys. Res. Commun.* **164**, 580 (1989).
20. J. A. DeCaprio *et al.*, *Cell* **54**, 275 (1988); J. W. Ludlow *et al.*, *ibid.* **56**, 57 (1989); J. W. Ludlow *et al.*, *ibid.* **60**, 387 (1990).
21. K. Kelley, B. H. Cochran, C. D. Stiles, P. Leder, *ibid.* **35**, 603 (1983); J. C. Reed, J. C. Alpers, P. C. Nowell, R. G. Hoover, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3982 (1986); M. A. Shipp and E. L. Reinherz, *J. Immunol.* **139**, 2143 (1987).
22. J. B. Stern and K. A. Smith, *Science* **233**, 203 (1986).
23. G. Draetta, D. Beach, E. Moran, *Oncogene* **2**, 553 (1988).
24. J. Cheng *et al.*, *Blood* **75**, 730 (1990).
25. Y. Furukawa *et al.*, *Cancer Res.* **47**, 2589 (1987).
26. M. G. Lee and P. Nurse, *Nature* **327**, 31 (1987).
27. J. A. Swack *et al.*, *Mol. Immunol.* **26**, 1037 (1989).
28. J. Pines and T. Hunter, *Cell* **58**, 833 (1989).
29. E. L. Wickstrom *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1028 (1988); J. T. Holt, R. L. Redner, A. W. Nienhuis, *Mol. Cell. Biol.* **8**, 963 (1988).

30. We thank J. DeCaprio and B. Druker for critical review of the data and the manuscript; G. Draetta for the bacterial p13 overproducer; P. Nurse for pOB231; and C. Morimoto for the monoclonal antibody to CD6. Supported in part by PHS grants CA36167, CA47843, CA50767, and CA34183. Y.F. is supported in part by a fellowship from the Uehara Memorial Foundation and the Mochida Foundation for Medical and Pharmaceutical Research, and H.P.-W. by a Whitaker Health Sciences Fund Award. J.D.G. is a Scholar of the Leukemia Society of America.

8 May 1990; accepted 29 August 1990

Female Preference Predates the Evolution of the Sword in Swordtail Fish

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The study of female preferences and the evolution of male traits has until recently centered on genetic coevolutionary mechanisms. An alternative mechanism posits that a preference results from a preestablished bias in the female information-processing system arising from sources independent of sexual selection. Male traits then arise that are selected by this preexisting preference. The genus *Xiphophorus* consists of swordless platyfish and swordtails. Swordlessness is the primitive state. In this study, female platyfish, *X. maculatus*, were found to prefer conspecific males with artificial swords over those without swords, despite evidence that the common ancestor of platyfish and swordtails was swordless. These results suggest that the evolution of the sword in the swordtail clade was a consequence of selection arising from a preexisting bias.

SEXUAL SELECTION WAS FIRST PROposed by Darwin (*1*) as a mechanism to explain the evolution of elaborate traits in males that appear to decrease their survival. One type of sexual selection, female choice, involves a preference by females for traits in males. Until recently, models that stress the coevolution of a female preference and a male trait have dominated theoretical and empirical treatments (*2, 3*). Few data have been produced that support one of these models to the exclusion of another, and alternative, testable models have been proposed [see (*4*) for review]. A non-coevolutionary explanation for the evolution of a female preference and a male trait proposes that biases in the female sensory or cognitive system, or both, arise and increase to a high

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