cyanobacteria were separated as described [S. A. Nierzwicki-Bauer and R. Haselkorn, *EMBO J.* **5**, 29 (1986)]. The isolated cyanobacteria were examined microscopically to ensure the purity of the preparation. Total RNA and DNA was isolated from free-living and symbiotic cyanobacteria as described [J. W. Golden, S. J. Robinson, R. Haselkorn, *Nature* **314**, 419 (1985)]. RNA was separated from DNA by CsCl density gradient centrifugation, with a 5.7 M CsCl cushion. The DNA was centrifuged as described by Maniatis *et al.* (*22*). RNA samples extracted from isolated *A. azollae* lacked eukaryotic rRNA bands, providing additional confirmation that these preparations were free of contaminating eukaryotic organelles.

35. We thank M. G. Kuhsel and J. D. Palmer for communicating their manuscript (*25*) prior to submission, and for comments on our manuscript; and H. Ozeki for providing pT7-23. Supported by NIH grant GM37746 (D.A.S.), NSF grant DMB-9011698 (S.A.N.-B.), and, in part, by a Burke Graduate Fellowship (H.G.B.).

17 July 1990; accepted 17 October 1990

An Ancient Group I Intron Shared by Eubacteria and Chloroplasts

MARIA G. KUHSEL, RODERICK STRICKLAND, JEFFREY D. PALMER*

Introns have been found in the genomes of all major groups of organisms except eubacteria. The presence of introns in chloroplasts and mitochondria, both of which are of eubacterial origin, has been interpreted as evidence either for the recent acquisition of introns by organelles or for the loss of introns from their eubacterial progenitors. The gene for the leucine transfer RNA with a UAA anticodon [tRNA^{Leu}(UAA)] from five diverse cyanobacteria and several major groups of chloroplasts contains a single group I intron. The intron is conserved in secondary structure and primary sequence, and occupies the same position, within the UAA anticodon. The homology of the intron across chloroplasts and cyanobacteria implies that it was present in their common ancestor and that it has been maintained in their genomes for at least 1 billion years.

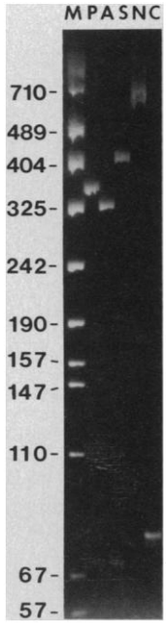

Fig. 1. Size determination of tRNA^{Leu} genes from cyanobacteria and chloroplasts. PCR products from *Phormidium* N182 (P), *Anacystis* R2 (A), *Scytonema* PCC7110 (S), *Nicotiana tabacum* (N), and *Chara hispida* (C), and Hpa II fragments from Bluescript SK+ (M) (sizes marked in base pairs), were subjected to electrophoresis on a 5% polyacrylamide gel and stained with ethidium bromide. Sequence of the 5′ PCR primer (exon positions 8 to 33): TGG(CT)GAAAT (CT)GGTAGACGC(AT) (AG)CGGAC. Sequence of the 3′ PCR primer (positions 51 to 85): TGGG-GATAGAGGGACTTGA-ACCCTCACGATTTTTA. PCR was performed with 100 ng of template DNA, 100 pmol of each primer, 20 nmol of each deoxynucleotide triphosphate, 2 units of Taq polymerase (Promega), and 1× Taq buffer (Promega). The reaction mixture was heated at 94°C for 2.0 min and then subjected to 40 cycles consisting of 1.5 min at 92°C, 1.5 min at 50°C, and 2.0 min at 72°C, with the last step being increased by 5 s at each cycle and the final 72°C step lasting for 12 min.

INTRONS ARE WIDESPREAD, OCCURring in nuclear, chloroplast, and mitochondrial genomes of eukaryotes (*1*), in archaebacterial genomes (*2*), and in viruses of eukaryotes and eubacteria (*3*). The "introns early" view of intron evolution maintains that most introns were present in the common ancestor of eukaryotes, eubacteria, and archaebacteria, and that the general direction of their evolution has been toward loss (*4*). These arguments are based on the self-splicing properties displayed by certain introns—which are interpreted to be remnants of an ancient "RNA world"—on the broad distribution of certain nuclear introns among diverse lineages of eukaryotes, and on the conserved structure among eukaryotes and bacteria of protein genes thought to have been assembled by the intron-mediated process termed "exon shuffling" (*5*). The "introns late" view holds that primordial genes lacked introns, which were instead acquired independently, and relatively recently, in various lineages of life (*6*). This view is based on the lateral mobility of certain types of introns (*7*) and on the sporadic distribution of introns across all of life (*1–3*).

Critical to the debate over intron evolution is the complete absence of introns from eubacterial genes. Although introns have been found in several eubacteriophage genes, these are mobile group I introns that are thought to be derived from lateral gene transfer (*3, 7*). Introns-late advocates interpret the absence of introns from eubacterial genes as evidence that their common ancestor lacked introns (*6*), whereas introns-early proponents argue that selection for rapid bacterial growth has led to a streamlining of the genome and the elimination of introns (*4*). Chloroplasts and mitochondria are derived from eubacteria by endosymbiosis (*8*), yet in many lineages they contain numerous introns. Introns-early adherents view this as evidence that the selection to eliminate introns and streamline the genome is lessened in organelles compared to bacteria (*4*), whereas introns-late adherents contend that organelles acquired their introns after endosymbiosis (*6, 9, 10*).

A nearly identical set of 20 introns has been conserved throughout the evolution of land plant chloroplasts (*11*); however, these introns are largely absent from the few algae that have been examined (*10*), and phylogenetic survey shows that two tRNA introns were acquired specifically in the green algal ancestors of land plants (*12*). In contrast, a third tRNA intron, a group I intron in a gene for the leucine tRNA with a UAA anticodon [tRNA^{Leu}(UAA)], is found in chloroplasts of land plants and the distantly related alga *Cyanophora paradoxa* (*13*). This is therefore the best candidate for being an ancient intron potentially of eubacterial origin.

The distribution of this group I intron in tRNA^{Leu}(UAA) genes of chloroplasts and cyanobacteria—the progenitors of chloroplasts (*8*)—was assessed by determining the size and sequence of the products of polymerase chain reaction (PCR) amplification that was performed with primers specific for the exons of sequenced tRNA^{Leu}(UAA) genes of land plants and *Cyanophora* (*13, 14*). The group I intron splits these genes into exons of 35 and 50 base pairs (bp). Amplification with a 26-nucleotide (nt) 5′ primer (positions 8 to 33) and a 35-nt 3′ primer (positions 51 to 85) should yield a PCR product of ~80 bp for tRNA^{Leu} genes lacking introns, and for genes containing an intron, a product larger than 80 bp by the size of the intron. Control amplification with DNA from *Nicotiana tabacum* gave a product estimated to be 640 bp in size (Fig. 1), in reasonable agreement with the expected size of 581 bp (*14*). The DNA from three phylogenetically diverse (*15*) cyanobacteria—*Phormidium* N182, *Anacystis* R2, and *Scytonema* PCC7110—also yielded PCR products substantially larger (330 to 410 bp) (Fig. 1) than the 80 bp expected for an

Department of Biology, Indiana University, Bloomington, IN 47405.

*To whom correspondence should be addressed.

uninterrupted gene. In contrast, the green alga *Chara hispida* gave a product indicative of an unsplit gene. Overall, each of five cyanobacteria tested and 7 of 12 algae tested gave PCR products of a size expected for an intron-containing gene (*16*). Four observations suggest that the PCR products result from amplification of the stated genome and not of any contaminant genome: (i) Only a single PCR band was detected in each of the five amplifications shown (Fig. 1); (ii) each species yielded a PCR product of a different size (Fig. 1); (iii) all amplifications yielded similar amounts of product (Fig. 1); (iv) each of several PCR products used as probes in Southern (DNA) blot experiments hybridized strongly and specifically to the appropriate source DNA (*17*).

Nine different PCR products were cloned and sequenced (Table 1) (*18*). Sequence comparisons revealed that all nine products correspond to $tRNA^{Leu}$(UAA) genes containing a single intron of 204 to 345 bp located between the U and A of the UAA anticodon (Table 1; Figs. 2 and 3). In addition to sequencing cloned PCR products, we isolated a clone from a phage library of *Anacystis* DNA and showed that it contains a segment of a $tRNA^{Leu}$(UAA) gene that includes an intron identical in sequence to that of the *Anacystis* PCR product (*19*).

All of the $tRNA^{Leu}$(UAA) introns can be

Table 1. Size and conserved sequence elements of the $tRNA^{Leu}$(UAA) introns of cyanobacteria and chloroplasts. Within the tRNA intron sequences, a dash indicates identity to the *Anacystis* sequence, a letter indicates a nucleotide difference, and an asterisk indicates a nucleotide deletion. Within the consensus sequence, which is based on 66 group I introns (*20*), an uppercase letter designates >90% conservation of a particular nucleotide, a lowercase letter designates 70 to 90% conservation, a pair of lowercase letters indicates that two nucleotides account for >90% of the sequences, and an "n" indicates that no nucleotide is conserved at the level of these criteria. Underlined nucleotides are proposed to be base paired in the intron secondary structure (*20*). The *Cyanophora* and *Nicotiana* sequences are published (*13, 14*); the rest are from this work.

Phylum	Organism	Intron size (bp)	Conserved intron sequences			
			P	Q	R	S
cyanobacterium	*Anacystis* R2	239	AACUCAGGGAAAC	AAUCCUGAGC	GUGCAGAGACUAGA	AAGGGAUAGUCC
cyanobacterium	*Phormidium* N182	269	------------	----------	--------G-CC--	------GG----
cyanobacterium	*Scytonema* PCC7110	307	------------	----------	--------G-CC--	------GG----
glaucophyte	*Cyanophora paradoxa*	232	--U----------	----------	----------C--	----U-G-----
chrysophyte	*Ochromonas danica*	304	--U----A-----	----------	----------CA-	---AU-G-----
xanthophyte	*Vaucheria bursata*	223	--U---A----*-	--C-------	----------CA-	---A--G-----
phaeophyte	*Costaria costata*	231	--U---A----*-	----U-----	----------C--	---A--G-----
phaeophyte	*Dictyota dichotoma*	345	--U----A-----	G---A-----	----------CA-	---AU-G-----
chlorophyte	*Chlorella vulgaris*	225	--U----------	---------U	----------CA-	---A--G-----
chlorophyte	*Bryopsis plumosa*	204	--A--GA------	--C-UC----	---U-------UU-	---A--A-----
metaphyte	*Nicotiana tabacum*	503	--U----A-----	----------	----------CA-	---AU-G-----
consensus group I			auauuncnnngaAn	c aAunngnagg	guu ga ccgcAacGAcUana	c AaGauAuAgUCu

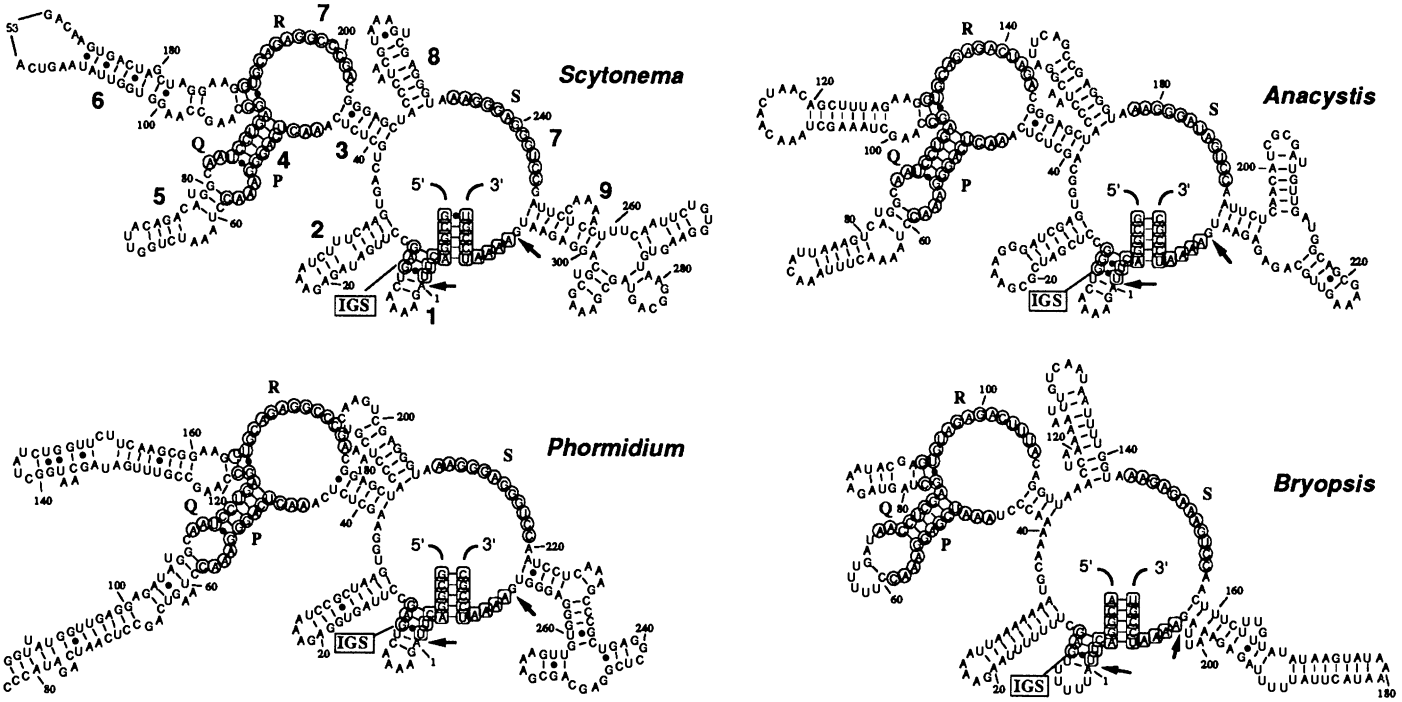

Fig. 2. Sequences and secondary structure models of the $tRNA^{Leu}$(UAA) introns of three cyanobacteria and the green alga *Bryopsis*. A-U and G-C base pairs are indicated with dashes and G-U base pairs with solid dots. The 5′ and 3′ exon sequences are boxed and putative splice sites are marked with arrows. Group I intron conserved sequences P, Q, R, and S, and IGS (internal guide sequence) are circled (*20*). Stem regions P1 to P9 of *Scytonema* are marked with numerals 1 to 9. Sequence of the central 53-nucleotide segment of stem 6 of *Scytonema*: UUUUUGACUUGUCC-UUCGGUUAAUUAUCAAGAACAUAAGGACAAACAACAAAU. Secondary structures were constructed with the program "Loop-D-Loop."

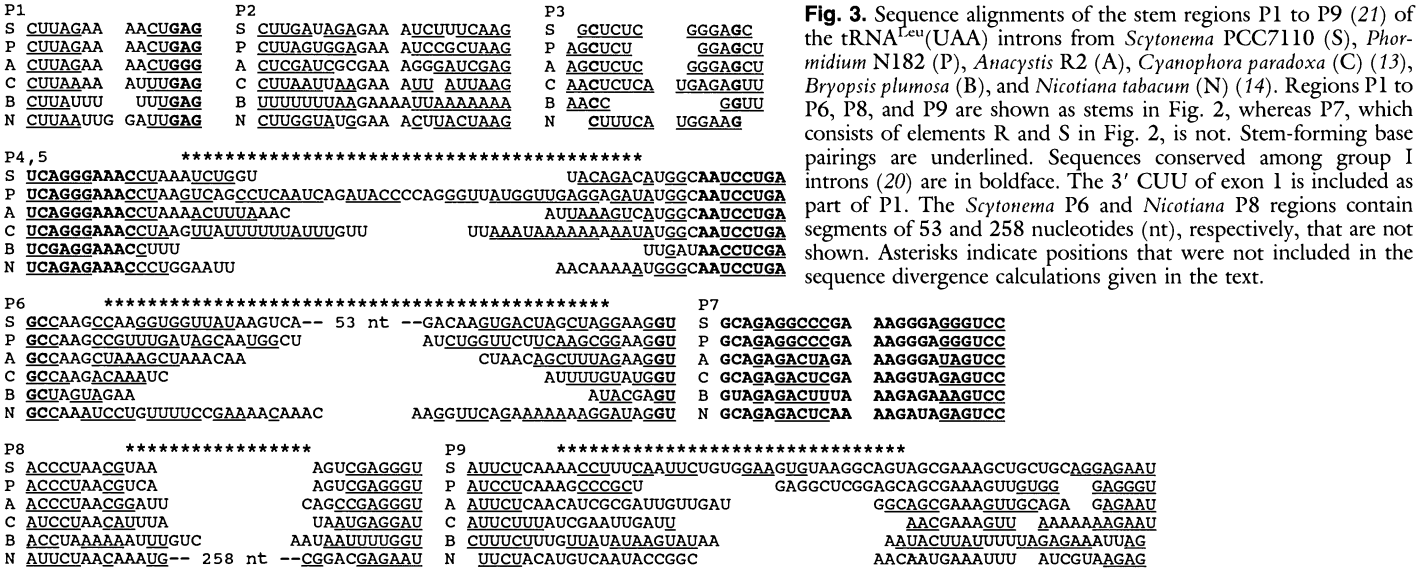

Fig. 3. Sequence alignments of the stem regions P1 to P9 (*21*) of the tRNA^{Leu}(UAA) introns from *Scytonema* PCC7110 (S), *Phormidium* N182 (P), *Anacystis* R2 (A), *Cyanophora paradoxa* (C) (*13*), *Bryopsis plumosa* (B), and *Nicotiana tabacum* (N) (*14*). Regions P1 to P6, P8, and P9 are shown as stems in Fig. 2, whereas P7, which consists of elements R and S in Fig. 2, is not. Stem-forming base pairings are underlined. Sequences conserved among group I introns (*20*) are in boldface. The 3′ CUU of exon 1 is included as part of P1. The *Scytonema* P6 and *Nicotiana* P8 regions contain segments of 53 and 258 nucleotides (nt), respectively, that are not shown. Asterisks indicate positions that were not included in the sequence divergence calculations given in the text.

folded into secondary structures (Fig. 2) that conform to the consensus group I intron structure (*20*). This consensus structure contains regions of complementary sequences that form nine stem-loop structures (P1 to P9; Figs. 2 and 3). These stem regions include four short domains that are conserved in primary sequence among all group I introns (P, Q, R, and S; Table 1 and Fig. 2). In addition, the tRNA^{Leu} introns possess an unusually high degree of sequence similarity in the regions surrounding these four domains (*21*); 83 and 76% of the core 162 nucleotides in which the domains are embedded are conserved between the cyanobacteria *Anacystis* and *Phormidium* and between *Anacystis* and the alga *Cyanophora,* respectively (Fig. 3). The major differences among the tRNA^{Leu} introns are size variations in stem regions P5, P6, P8, and P9. The smallest intron, from *Bryopsis*, is only 204 nt and contains the shortest P5 and P6 stems, whereas the 307- and 503-nt introns of *Scytonema* and *Nicotiana* contain insertions of approximately 50 nt in stem P6 and 250 nt in stem P8, respectively (Figs. 2 and 3). Differences among taxa in the consensus sequences P, Q, R, and S are most notable in *Scytonema* and *Phormidium*, which differ from *Anacystis*, all chloroplasts, and the consensus itself at the nearly invariant positions 9 and 11 in region R and position 8 in region S (Table 1). However, these changes preserve base pairing in the stem regions, and the same changes (often occurring in concert) have been observed in other group I introns (*20*).

The similarity of the tRNA^{Leu} intron to other group I introns, several of which self-splice in vitro (*20*), suggests that it may share this ability. Indeed, self-splicing has been demonstrated for this intron in certain cyanobacteria (*21*). The tRNA^{Leu}(UAA) intron is small and lacks any open reading frames, whereas several group I introns encode sequence-specific endonucleases, which enable them to insert themselves into genes lacking the intron and thereby to spread laterally (*3, 7*). This lack of "automobility" for the tRNA^{Leu}(UAA) intron reduces the likelihood that it was acquired independently by horizontal evolution in the various chloroplast and cyanobacterial lineages that possess it.

The presence of this intron in all major groups of chloroplasts examined and in cyanobacteria is strong evidence that it resided in the tRNA^{Leu}(UAA) gene of the cyanobacterial ancestor of plastids. If we accept this phylogenetic continuity across the organelle-bacterial boundary, we can estimate a minimum age for this intron of 1 billion years, the putative age of plastids (*22*). Because the intron is present in all five cyanobacteria examined here and in three other cyanobacteria (*21*), it may be much older, perhaps as old as the 3.5 billion–year-old fossils that have been suggested to represent cyanobacteria (*23*).

The discovery of introns in eubacterial genes closes the last major gap in the phylogenetic distribution of introns. It also provides a starting point to help resolve the controversy concerning the origin of introns—early or late—at least as it pertains to group I introns. Cyanobacteria are only one of many eubacterial phyla (*24*), and thus an important question is whether tRNA^{Leu} (UAA) genes of other eubacterial lineages also contain a group I intron. Although four previously studied eubacterial tRNA^{Leu} (UAA) genes lack introns (*25, 26*), we have found that some members of other eubacterial phyla do contain tRNA^{Leu}(UAA) introns (*17*). The investigation of eukaryotic and archaebacterial tRNA^{Leu}(UAA) genes, which are currently terra incognita (*26*), is thus now called for.

REFERENCES AND NOTES

1. T. R. Cech, *Cell* **44**, 207 (1986); F. Michel, K. Umesono, H. Ozeki, *Gene* **82**, 5 (1989).
2. B. P. Kaine, *J. Mol. Evol.* **25**, 248 (1987); J. Kjems, J. Jensen, T. Olesen, R. A. Garrett, *Can. J. Microbiol.* **35**, 210 (1989).
3. M. Belfort, *Trends Genet.* **5**, 209 (1989); S. M. Quirk, D. Bell-Pederson, M. Belfort, *Cell* **56**, 455 (1989).
4. J. E. Darnell and W. F. Doolittle, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1271 (1986); W. Gilbert, M. Marchionni, G. McKnight, *Cell* **46**, 151 (1986).
5. W. Gilbert, *Nature* **271**, 501 (1978).
6. T. Cavalier-Smith, *ibid.* **315**, 283 (1985); N. J. Dibb and A. J. Newman, *EMBO J.* **8**, 2015 (1989); J. H. Rogers, *Trends Genet.* **5**, 213 (1989).
7. A. M. Lambowitz, *Cell* **56**, 323 (1989); P. S. Perlman and R. A. Butow, *Science* **246**, 1106 (1989); B. Dujon, *Gene* **82**, 91 (1989).
8. M. W. Gray, *Trends Genet.* **5**, 294 (1989).
9. J. C. Gingrich and R. B. Hallick, *J. Biol. Chem.* **260**, 16156 (1985).
10. J. D. Palmer, in *The Molecular Biology of Plastids*, L. Bogorad and I. K. Vasil, Eds. (Academic Press, Orlando, FL, in press).
11. K. Ohyama *et al.*, *J. Mol. Biol.* **203**, 281 (1988); J. Hiratsuka *et al.*, *Mol. Gen. Genet.* **217**, 185 (1989).
12. J. R. Manhart and J. D. Palmer, *Nature* **345**, 268 (1990).
13. J.-L. Evrard, M. Kuntz, N. A. Straus, J.-H. Weil, *Gene* **71**, 115 (1988).
14. K. Yamada, K. Shinozaki, M. Sugiura, *Plant Mol. Biol.* 6, 193 (1986).
15. S. J. Giovannoni *et al.*, *J. Bacteriol.* **170**, 3584 (1988).
16. The five cyanobacteria include the three in Fig. 1, plus *Plectonema* L2 and *Gloeothece* PCC6501. The algae with introns include the six listed in Table 1, plus *Gracilaria lemaneiformis*. The algae without introns include *Chara hispida* (Fig. 1) and *Euglena gracilis* [R. B. Hallick, M. J. Hollingsworth, J. A. Nickoloff, *Plant Mol. Biol.* **3**, 169 (1984)], plus *Cladophora fracta*, *Pandorina* clone 854, *Chlamydomonas reinhardtii*, and *Smithora naiadum*.
17. M. G. Kuhsel and J. D. Palmer, unpublished data.
18. PCR products were purified by preparative electrophoresis, cloned into the Eco RV site of phagemid Bluescript SK+, and sequenced by the dideoxy chain termination method. Five independent clones (resulting from one to three independent PCR amplifications) were sequenced for each species, and both strands of each product were sequenced. Except for the primer-binding regions, all five clones

were identical for any one species. The GenBank accession numbers of the nine intron sequences are M36884 to M36886, M36888 to M36890, M36892, M36893, and M55288.
19. The *Anacystis* clone was isolated from a library supplied by A. Grossman and contains the 3′ exon of $tRNA^{Leu}$(UAA) and 90% of the intron; within this region, the PCR product and the genomic clone are identical.
20. T. R. Cech, *Gene* **73**, 259 (1988).
21. M.-Q. Xu, S. D. Kathe, H. Goodrich-Blair, S. A. Nierzwicki-Bauer, D. A. Shub, *Science* **250**, 1568 (1990).
22. A. C. Wilson, H. Ochman, E. M. Prager, *Trends Genet.* **5**, 294 (1989); T. Cavalier-Smith, *Ann. N. Y. Acad. Sci.* **503**, 55 (1987).
23. Microfossils of age 3.5 billion years have been controversially (*15*) interpreted as cyanobacteria [J. Schopf and B. M. Packer, *Science* **237**, 70 (1987)].
24. C. R. Woese, *Microbiol. Rev.* **51**, 221 (1987).
25. P. Simoneau, R. Wenzel, R. Herrmann, P. Hu, *Nucleic Acids Res.* **18**, 2814 (1990); Y. Komine, T. Adachi, H. Inokuchi, H. Ozeki, *J. Mol. Biol.* **212**, 579 (1990).
26. M. Sprinzl *et al.*, *Nucleic Acids Res. Seg. Suppl.* **17**, r1 (1989).
27. We thank R. Butow, J. Logsdon, R. Olmstead, N. Pace, P. Perlman, D. Shub, K. Wolfe, and M. Zolan for comments on the manuscript; D. Shub for communication of manuscript (*21*) prior to submission; D. Gilbert for providing the program "Loop-D-Loop"; A. Grossman for *Anacystis* DNA and library; and A. Coleman, S. Fain, L. Goff, R. Meints, K. Kowallik, N. Pace, S. Turner, A. Wilmotte, and K. Zetsche for DNA's and cell pellets. Supported by NIH grant 35087 (J.D.P.) and Deutsche Forschungsgemeinschaft grant KU721/1-1 (M.G.K.).

16 July 1990; accepted 16 October 1990

Complementation of the Mitotic Activator, p80^{cdc25}, by a Human Protein-Tyrosine Phosphatase

KATHLEEN L. GOULD,* SERGIO MORENO, NICHOLAS K. TONKS,† PAUL NURSE

The onset of M phase requires the activation of the pp34 protein kinase in all eukaryotes thus far examined. In *Schizosaccharomyces pombe*, pp34 is phosphorylated on Tyr^{15}, and dephosphorylation of this residue regulates the initiation of mitosis. In this study, it is shown that dephosphorylation of Tyr^{15} triggered activation of the pp34-cyclin complex from fission yeast, that a human protein-tyrosine phosphatase can catalyze this event both in vitro and in vivo, and that activation of fission yeast pp34 does not require threonine dephosphorylation. The complementary DNA that encoded the tyrosine phosphatase replaced the mitotic activator p80^{cdc25}, closely associating the *cdc25*$^{+}$-activating pathway with tyrosine dephosphorylation of pp34.

THE FISSION YEAST *CDC2*$^{+}$ GENE IS required for cell cycle progression at both the G_1/S and G_2/M phase transitions (*1*). The product of the *cdc2*$^{+}$ gene is a 34-kD protein–serine/threonine kinase (pp34) that has been highly conserved throughout evolution (*2*). Homologs of this protein kinase appear to regulate the onset of mitosis in all eukaryotes examined, as well as meiotic M phase in amphibian, sea urchin, and mollusk oocytes (*2*). Activation of pp34 and entry into mitosis is dependent on physical association between cyclin, which in *S. pombe* is the product of the *cdc13*$^{+}$ gene (*2*), and pp34. Activation of pp34 in fission yeast also requires the product of the *cdc25*$^{+}$ gene, p80^{cdc25}(*3*), which accumulates during the G_2 period of the cell cycle (*4*).

Dephosphorylation of pp34 on tyrosine, threonine, or both residues has been correlated with activation of the pp34-cyclin complex in many systems (*5*–*7*). *S. pombe* pp34 is phosphorylated in a cell cycle–dependent manner on a single detectable threonine residue (*8*) and on a single tyrosine residue, Tyr^{15} (*5*). We expressed a mutant pp34 in which Tyr^{15} had been replaced with phenylalanine in *S. pombe*, and concluded that phosphorylation of Tyr^{15} normally inhibits pp34 activation and that Tyr^{15} dephosphorylation is a rate-limiting step for the initiation of mitosis (*5*). Here we show that in *S. pombe*, tyrosine dephosphorylation is the trigger for pp34-cyclin activation. Moreover, we show that a human protein-tyrosine phosphatase (PTPase) expressed in *S. pombe* can replace the mitotic activator p80^{cdc25} and drive entry into mitosis.

Prevention of Tyr^{15} phosphorylation causes advancement of fission yeast cells into M phase prematurely, and thus, probably represents the last step in the activation of the pp34-cyclin complex. However, it was possible that although tyrosine dephosphorylation was rate-limiting, other downstream events were required before activation of pp34 protein kinase activity. If tyrosine dephosphorylation alone is sufficient to activate the pp34-cyclin complex, then protein kinase activation should be achieved by enzymatically removing phosphate from Tyr^{15} in vitro. First we tested whether a purified PTPase, termed the T cell PTPase (*9*), could dephosphorylate ^{32}P-labeled pp34. We isolated ^{32}P-labeled pp34

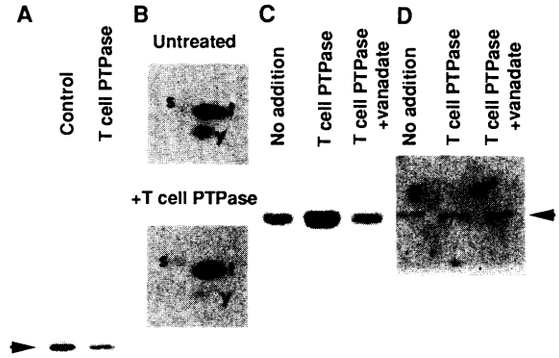

Fig. 1. Activation of pp34 by tyrosine dephosphorylation. (**A**) Treatment of pp34 with T cell PTPase. pp34 was immunoprecipitated with antiserum to pp34 (Ab 4711) (*5*) from nondenatured lysates (*16*) of *cdc25-22* cells labeled with [^{32}P]orthophosphate and growth-arrested at 36°C as described *17*). One-half of the immunoprecipitate was treated with PTPase buffer alone (*18*) and the other half with buffer and T cell PTPase for 30 min at 30°C (*19*). The arrow indicates pp34. The autoradiograph was exposed for 16 hours. (**B**) Phosphoamino acid analyses (*20*) of pp34 from ^{32}P-labeled–untreated and PTPase-treated immunoprecipitates similar to those in (A) (6-day exposure). S, phosphoserine; t, phosphothreonine; y, phosphotyrosine. (**C**) Histone H1 kinase assays. pp34 was immunoprecipitated with Ab 4711 from unlabeled nondenatured lysates of *cdc25-22*–arrested cells and split into three equal portions. One-third was treated with PTPase buffer, one-third with buffer and T cell PTPase, and one-third with buffer, T cell PTPase, and sodium orthovanadate (1 mM). After washing with kinase buffer (*16*), one-half of these immunoprecipitates was assayed for histone H1 kinase activity as described (*16*). The protein bands on the autoradiograph were quantified by densitometry. (**D**) pp34 immunoblot with antibody to pp34 COOH-terminal peptide, PN24, (*21*) of the remaining halves of the immunoprecipitates described in (C). The arrow indicates pp34.

K. L. Gould, S. Moreno, P. Nurse, Imperial Cancer Research Fund Cell Cycle Group, Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom.
N. K. Tonks, Department of Biochemistry, SJ-70, University of Washington, Seattle, WA 98195.

*To whom correspondence should be addressed.
†Present address: James Laboratory, Cold Spring Harbor Labs, P.O. Box 100, Bungtown Road, Cold Spring Harbor, NY 11724.