Psr1, a nuclear localized protein that regulates phosphorus metabolism in *Chlamydomonas*

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Understanding the ways in which phosphorus metabolism is regulated in photosynthetic eukaryotes is critical for optimizing crop productivity and managing aquatic ecosystems in which phosphorus can be a major source of pollution. Here we describe a gene encoding a regulator of phosphorus metabolism, designated *Psr1* (phosphorus starvation response), from a photosynthetic eukaryote. The Psr1 protein is critical for acclimation of the unicellular green alga *Chlamydomonas reinhardtii* to phosphorus starvation. The N-terminal half of Psr1 contains a region similar to myb DNA-binding domains and the C-terminal half possesses glutamine-rich sequences characteristic of transcriptional activators. The level of Psr1 increases at least 10-fold upon phosphate starvation, and immunocytochemical studies demonstrate that this protein is nuclear-localized under both nutrient-replete and phosphorus-starvation conditions. Finally, Psr1 and angiosperm proteins have domains that are similar, suggesting a possible role for Psr1 homologs in the control of phosphorus metabolism in vascular plants. With the identification of regulators such as Psr1 it may become possible to engineer photosynthetic organisms for more efficient utilization of phosphorus and to establish better practices for the management of agricultural lands and natural ecosystems.

Phosphorus (P) is a major component of nucleic acids and phospholipids and is present in the biosphere as the oxidized anion, phosphate (P_i). P_i is not easily accessible to most plants and microbes because it forms insoluble precipitates with common cations or is covalently bound to organic molecules (1–3). Crop yields are limited by P availability, and, consequently, P, in the form of P_i, is an important component of commercial fertilizers. A considerable proportion of this "supplementary" P_i is leached from agricultural fields and deposited into aquatic ecosystems, triggering rapid algal proliferation (algal blooms), which leads to eutrophication and fish kills (2). The sustainability of agricultural yields and quality of aquatic ecosystems would benefit from more efficient acquisition and utilization of P. Efficient P utilization by crop plants would decrease our dependence on rock P_i reserves, the mining of which has serious economic and ecological consequences (3). Thus, a more complete understanding of P utilization in plants has significant implications with respect to both the environment and world agriculture.

P limitation triggers a suite of "starvation responses" in most organisms. These responses can be divided into two categories, the P-specific responses and the general responses (4–6). The P-specific responses promote efficient mobilization and acquisition of P from extracellular and intracellular stores (e.g., synthesis and secretion of phosphatases with broad substrate specificity, accumulation of high affinity P_i transporters) (5, 7). The general responses allow for long-term survival by coordinating the metabolism of the cell to nutrient availability and growth potential. P starvation responses in *Escherichia coli* are regulated primarily at the level of transcription by a two-component regulatory system (PhoB and PhoR) (8). In *Saccharomyces cerevisiae*, a cyclin-dependent kinase complex (Pho80-Pho85) that modulates the activity of a specific transcription factor (Pho4) controls P starvation responses (9).

Recent work with vascular plants, especially with *Arabidopsis thaliana*, has centered on the characterization of P_i uptake, P_i translocation, and P-accessing enzymes (such as phytases and phosphatases) (10–13). Studies with vascular plants have been complicated by relatively long generation times, the presence of multiple tissue types, and complex developmental regulation. These features have made it difficult to identify regulatory elements important for the acclimation of plants to P starvation. We have exploited the unicellular green alga *Chlamydomonas reinhardtii* to examine P starvation responses in a photosynthetic eukaryote (5–7). Many of the powerful genetic and molecular tools used to elucidate biological processes in *S. cerevisiae* also can be applied to *C. reinhardtii* (14, 15). Furthermore, the identification of *C. reinhardtii* regulatory elements is likely to provide clues important for defining analogous elements in the angiosperm cDNA and genomic sequence databases.

We recently have characterized several aspects of P starvation responses in *C. reinhardtii* and have screened for mutants aberrant for these responses (7). One of the mutants, *psr1* (phosphorus starvation response), is defective in a number of specific responses to P starvation (such as the development of a high-affinity P_i uptake system, or secretion of alkaline phosphatase), but has normal induction of the general starvation responses. We cloned the *PSR1* locus to understand the molecular basis of the *psr1* phenotype, to determine how P stress responses in photosynthetic eukaryotes relate to those of nonphotosynthetic eukaryotes, and to acquire clues concerning the regulation of P starvation responses in vascular plants.

Materials and Methods

Growth Conditions and Strains. CC125 (*Chlamydomonas* stock center, Duke University, Durham, NC) was the wild-type strain and the *psr1–1* and *psr1–2* mutants (7) were outcrossed to CC125 at least four times to ensure genetic homogeneity. *cw15* and *nit1* mutations were introduced into the genetic backgrounds to increase the efficiency of and to serve as a marker for transformation, respectively. Liquid cultures were grown on a rotary shaker (120 rpm) in Erlenmeyer flasks under 80 μmol photon $m^{-2}{\cdot}s^{-1}$ illumination supplied from cool, white fluorescent lamps at 27°C. Replete growth medium was TAP (tris-acetate-phosphate) (16). To impose P starvation, cells were centrifuged (5,000 $\times$ *g*, 5 min), washed twice with TA (TAP medium lacking phosphate; ref. 5), and resuspended in TA. Selection for growth on glucose-1-phosphate was performed with TA agarose medium supplemented with 100 μM glucose-1-phosphate (Sigma). All measurements were performed on cells in their midlogarithmic phase of growth (1–5 $\times$ 10^6 cells ml^{-1}).

Transformation and Complementation of *psr1–1*. The *NIT1* selection was used to determine optimal, high-efficiency electroporation conditions (17) of a recipient *psr1–1 cw15 nit1* strain. This strain then was transformed with pools of 196 cosmid clones (two 96-well

Abbreviations: HA, hemagglutinin; TA, tris-acetate.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF174532 and AF174480).

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plates) from an indexed cosmid library (18). Recipient cells were resuspended in TA + 60 mM sucrose, electroporated (25 μF, 1,875 V/cm) in the presence of the cosmid pools, and plated with 20% cornstarch on TA + glucose-1-phosphate medium. For physiological analyses, the complemented strain was outcrossed to CC125 twice.

Physiological Measurements. Alkaline phosphatase, cell density, and P_i uptake measurements were performed as described (7).

Manipulation of *Psr1* Genomic Clone. The complementing *Xba*I–*Spe*I 5.6-kbp fragment was cloned into the *Xba*I site of pUC119, destroying the *Spe*I site. To obtain the 4.3-kbp fragment (see Fig. 2*A*), the resulting plasmid was digested with *Hin*dIII (in the insert) and *Kpn*I (in the polylinker).

DNA and RNA Hybridization. DNA and RNA blot hybridizations were performed as described (20). For RNA analyses, polyadenylated RNA was isolated from total RNA by using oligo(dT) Sephadex (Stratagene). The RNA samples were from cells grown in complete medium or exposed to P deprivation for 8, 16, 24, and 48 h. Four micrograms of mRNA was resolved by electrophoresis on a formaldehyde-agarose gel, blotted onto supported nitrocellulose (Schleicher & Schuell), and probed with the 2.6-kbp 3′ end of the cDNA. After hybridization, the membranes were exposed to film for 6 days.

RNase Protection Assay. RNase protection assays were performed with the Hyspeed RPA kit (Ambion, Austin, TX). The DNA insert was synthesized by PCR using the primers 5′-TATGAACGCGGCGCAGCAGTCCTCAGAGTA and 5′-TGAGGCGGTACTTCTGCAGGTGCGACTTGA. The PCR product was ligated into pGEM-T easy (Promega) and the labeled RNA (antisense) probe synthesized with a Stratagene RNA synthesis kit by using the T7 promoter. The riboprobe (502 bases) spanned an intron to confirm that RNase protection was not a consequence of DNA contamination, and it contained non-*Psr1* sequences on the 5′ and 3′ ends to confirm RNase digestion. The protection assay was performed with 30 μg of total RNA that was treated previously with DNase I (Boehringer Mannheim). A portion of each RNA sample was subjected to electrophoresis on a formaldehyde-agarose gel to confirm RNA concentrations determined spectrophotometrically. The intensity of the 395-base protected fragment was quantified by using a PhosphorImager (Molecular Dynamics).

Isolation of *Psr1* cDNA. The cDNA was isolated from a recombinant library constructed with mRNA from cells starved of P for 8, 16, 24, and 48 h by using a Stratagene cDNA synthesis kit. cDNA clones also were isolated from a library constructed from cells grown on complete medium, kindly provided by M. Goldschmidt-Clermont (University of Geneva). The *Psr1* cDNA was isolated at a frequency of 1 in 60,000 from the library derived from P-starved cells and was less abundant in the library derived from cells grown in complete medium. Because none of the cDNAs appeared to be full-length, nested primers were used to perform reverse transcription–PCR and to isolate the 5′ end of the transcript. The loss of the 5′ intron demonstrated that the product of the reverse transcription reaction served as the template for the PCR.

Construction of Psr1 with a 3× Hemagglutinin (HA) Epitope. Using pTS515 kindly provided by Becket Feierbach from the Stearns Laboratory (Stanford University), we used primers 5′-GGTGGCGGCCGCATCTTTTTACCCATACGAT-3′ and 5′-CGGTGCGCGGCCGCATGAGCAGCGTAATCTGGAACGTC-3′ to generate a PCR product that, when digested with *Not*I, would insert in-frame into the *Psr1* genomic clone. Clones were sequenced to confirm insertion and orientation of the 3×HA DNA tag and then transformed into the *psr1–1* mutant. The position of the 3×HA peptide in Psr1 is shown in Fig. 2*B*.

Western Analysis. Total protein was precipitated with 90% ice-cold acetone, resuspended in SDS loading buffer (6), and subjected to electrophoresis through a 10% SDS-polyacrylamide gel. After electroblotting, Psr1::3×HA was detected by using an anti-HA high-affinity rat mAb (1:100 dilution) (Boehringer Mannheim). The secondary antibody was a biotinylated goat anti-rat antibody

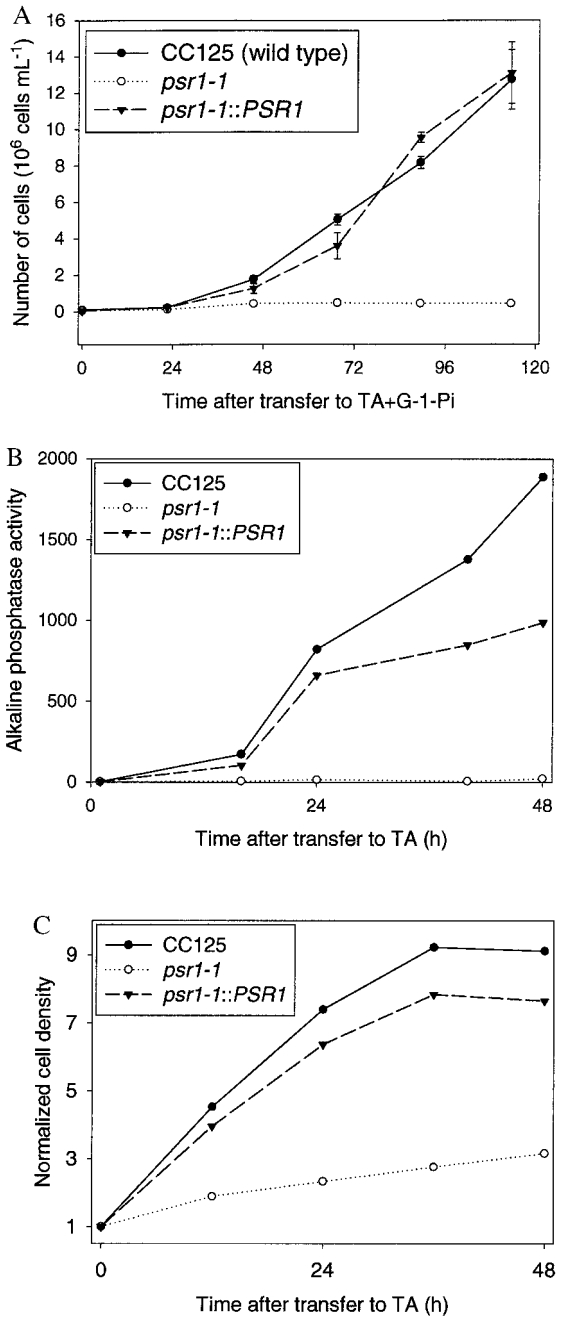

Fig. 1. Multiple phenotypes of *psr1* mutant are complemented by 5.6-kbp genomic fragment. (*A*) Growth of cells in liquid TAP medium with P_i replaced by 100 μM glucose-1-phosphate. (*B*) Extracellular alkaline phosphatase activity at various times after the transfer of cells to TA medium. (*C*) Growth of cells after transfer to TA medium. Measurements were conducted as described previously (7). All experiments were repeated three times; in all cases the trends were identical.

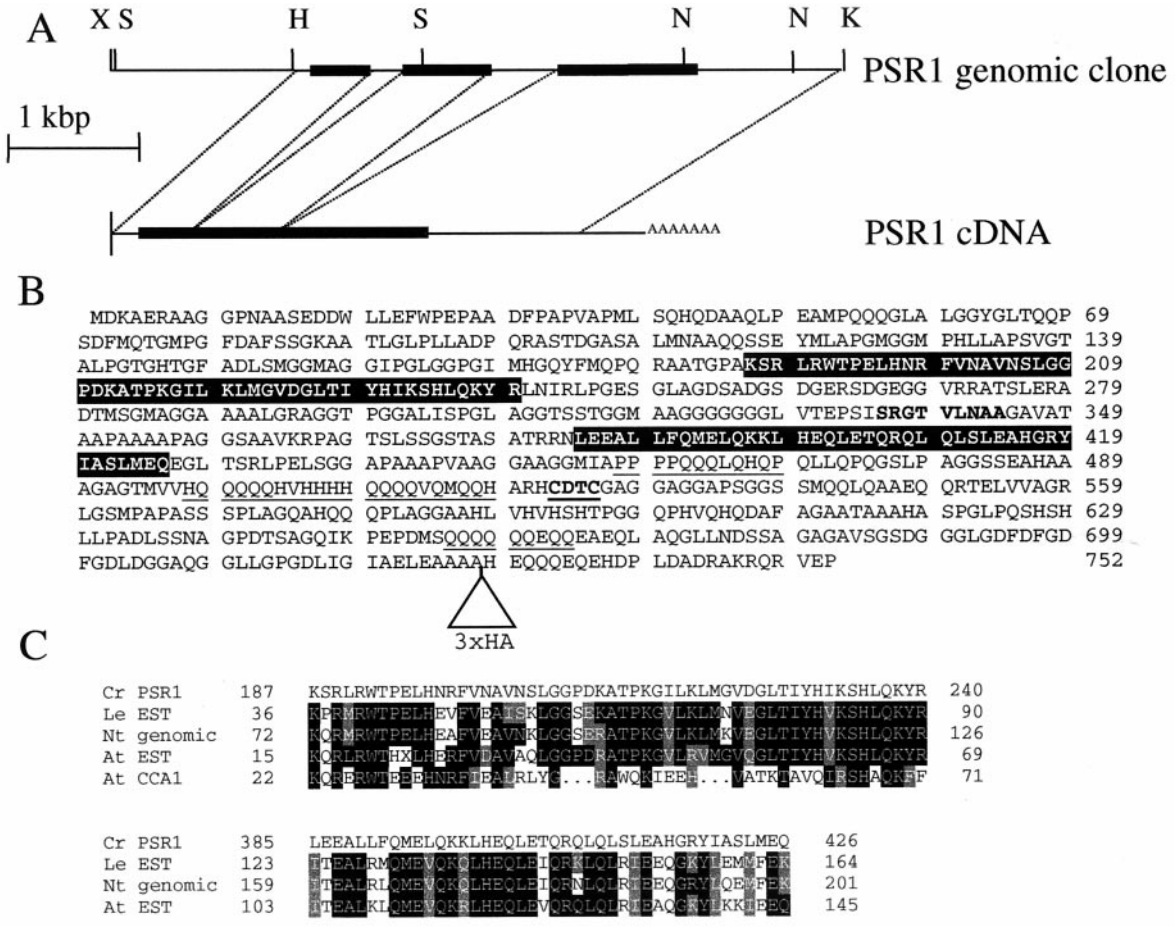

Fig. 2. *Psr1* gene structure and sequence. (*A*) Structure of *Psr1* cDNA and genomic clones. Abbreviations for restriction enzyme sites are X, *Xba*I; H, *Hin*dIII; S, *Sal*I; N, *Not*I; and K, *Kpn*I. The solid rectangle indicates the position of the ORF, and dashed lines indicate the sites of intron splicing. (*B*) Predicted amino acid sequence of Psr1. Amino acid sequence begins with the first in-frame methionine after a stop codon in the cDNA. Features of Psr1 include: a helix–turn–helix dimerization domain (bold), a putative metal binding site (bold, underlined), three glutamine-rich regions that share similarity to activation domains of homeobox proteins in various organisms (underlined; ref. 19), and two regions that are similar to deduced sequences in vascular plant proteins (highlighted in a black background). The triangle near the C terminus indicates an in-frame insertion of the 3×HA (HA epitope) tag. (*C*) Alignment of predicted Psr1 and vascular plant amino acid sequences. The two domains of the predicted protein sequence of Psr1 (amino acids 187–240 and 386–428) are aligned with proteins deduced from cDNA sequences of *Lycopersicon esculentum* (Le EST, accession no. AI485303), *A. thaliana* (At EST, combination of accession nos. H76190, T45758, T04440, T76484, and T41999), a genomic clone of *Nicotiana tabacum* (NT genomic, accession no. AB017693), and the distantly related myb DNA-binding domain of Cca1 (only in the upper alignment, At CCA1, accession no. U28422). Identical residues are in black and conserved residues are in gray.

(1:5,000 dilution) (Boehringer Mannheim) and was detected by using Streptavidin conjugated to horseradish peroxidase (1:5,000 dilution) (Molecular Probes) and an ECL chemiluminescence kit (Amersham Pharmacia). The predicted molecular mass of Psr1 is 75,799 Da, with the 3×HA insert adding 4,102 Da.

Immunocytochemistry. Cells transformed with *Psr1* or with *Psr1*::3×HA were adhered to eight-well Teflon-coated glass slides that were pretreated with 0.1% polyethylenimine (Sigma). To fix the cells, the slides were submerged in 100% methanol (−20°C) for 5 min and then allowed to air-dry. Slides were rehydrated with 1× PBS + 5% goat serum and then incubated with the high-affinity HA antibody at a dilution of 1:100. The slides were washed with 1× PBS + 5% goat serum, incubated with an Alexa-488-anti-rat conjugate (Molecular Probes), rinsed with 1 μg/ml propidium iodide in the final wash, mounted in 1% propylgallate/50% glycerol/49% 1× PBS, and observed with a ×60 oil objective in a Bio-Rad MRC 1024 confocal microscope.

Results

To complement the *psr1* mutant, we transformed the mutant (*psr1–1* allele) with an indexed cosmid library (17, 18) and selected for growth of the cells on medium in which P_i was replaced with glucose-1-phosphate. Because the *psr1* mutant was unable to accumulate periplasmic phosphatase activity in response to P starvation, this strain could not assimilate esterified P and, therefore, could not grow on glucose-1-phosphate (Fig. 1*A*). In contrast, the growth rate of wild-type cells in medium containing glucose-1-phosphate as the sole P source was similar to that of cells maintained in medium with P_i. This discrimination against the *psr1* mutant provided an effective selection for complementation. A cosmid (cHC2:4C9) and, subsequently, a 5.6-kbp *Xba*I–*Spe*I fragment were isolated that, when transformed into the *psr1* mutant, enabled it to grow on glucose-1-phosphate. Using a subcloned 4.3-kbp *Hin*dIII–*Kpn*I fragment derived from the original 5.6-kbp fragment as a hybridization probe, we demonstrated that this region of the genome was polymorphic in a second, insertional allele of *psr1* (*psr1–2*, data not shown). To confirm complementation, we characterized the responses of the *psr1* strain harboring the 5.6-kbp fragment to P starvation. The original *psr1* mutant divides only once and does not accumulate extracellular phosphatase activity or develop a high-affinity P_i uptake system in response to P starvation. The

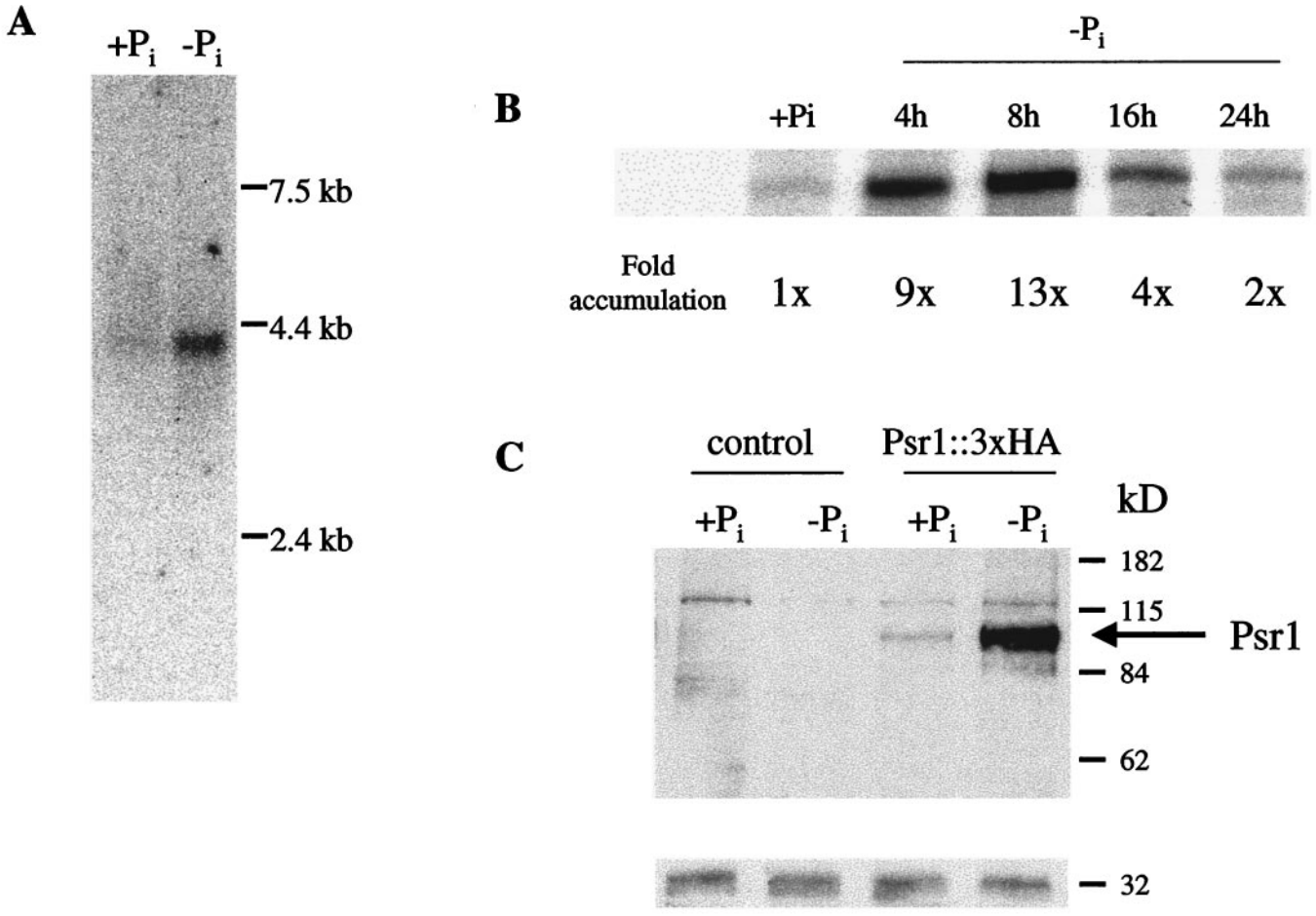

Fig. 3. Northern blot hybridization, RNase protection assays, and Western blot analysis of the Psr1 protein. (*A*) Northern blot hybridization of a *Psr1* gene-specific probe to RNA from the wild-type strain CC125. Polyadenylated RNA from cells grown in nutrient-replete medium ($+P_i$) and from cells starved for 8–48 h and pooled ($-P_i$) was analyzed. (*B*) RNase protection of a 395-base riboprobe by 30 μg of total RNA. The level of accumulation of the transcript (fold change relative to $+P_i$ conditions) is indicated beneath each lane. This experiment was repeated on separately isolated RNA samples, and the trend was identical. (*C*) Western blot analysis of the Psr1 protein containing the 3×HA tag. The first two lanes contain protein that was extracted from cells in which Psr1 did not contain the 3×HA tag. The second two lanes contain protein that was extracted from the *psr1–1* mutant containing the PSR1::3×HA construct. Protein extracts were from either cells grown in complete medium ($+P_i$) or cells starved for P for 24 h ($-P_i$). Three independent transformants were examined, and all three gave essentially identical results. To control for loading, the blot was stripped and reprobed for D1 protein (32-kDa polypeptide), which was shown previously not to change in abundance after 24 h of phosphorus starvation (6).

putative complemented strain, like wild-type cells, grew on medium with glucose-1-phosphate as the sole P source, accumulated extracellular alkaline phosphatase activity (and failed to do so in P-replete medium), and divided 3–4 times after the imposition of P starvation (Fig. 1). The complemented strain also developed high-affinity P_i uptake to the same extent as wild-type cells during P starvation (data not shown). These results demonstrate that the 5.6-kbp fragment contains the *Psr1* gene.

Using the 4.3-kbp *Hin*dIII–*Kpn*I genomic fragment, which is able to complement the mutant phenotype (data not shown), as a probe, we isolated a 2.6-kbp cDNA clone and used reverse transcription–PCR to isolate the remainder of the 4.2-kb transcript. Fig. 2*A* diagrams the cDNA and the intron/exon structure of the genomic clone. Although the genomic clone complements the mutant phenotype, it lacks 500 bp of the 1,733-bp 3′ untranslated region of the cDNA. The sequence of the 1.3-kbp *Xba*I–*Hin*dIII genomic fragment did not contain any large ORF. The Psr1 protein, as deduced from the cDNA sequence, is 752 aa with no apparent transmembrane domains (Fig. 2*B*). Comparisons of Psr1 with proteins represented in the databases revealed no homologs similar to Psr1 over its entire length. However, there are domains in Psr1 that are characteristic of transcription regulators. The region from amino acid 187 to 240 has similarity to CCA1, a transcription factor in *A. thaliana* that is responsible for phytochrome-mediated regulation of the light-harvesting genes (Fig. 2*C*) (21). This domain is required for sequence-specific binding of CCA1 to DNA and is postulated to be related to the domain of myb proteins that binds DNA and activates transcription (22). The myb-like domain of CCA1 is 31% identical to the third repeat of the human c-myb DNA-binding domain whereas the analogous domain in Psr1 is 40% identical to that of CCA1 and 28% identical to that of human c-myb. The presence of a myb-like domain in Psr1 suggests that Psr1 may activate the transcription of specific genes in response to P starvation. Some support for this hypothesis comes from the finding that Psr1 also contains three stretches rich in glutamine (Fig. 2*B*, underlined); these regions are critical for the positive regulatory activity of several different eukaryotic transcription factors (19, 23, 24). Another domain common to eukaryotic helix–loop–helix transcription factors and Psr1 is a structure that facilitates protein dimerization (Fig. 2*B*, bold). Finally, the only two cysteines in Psr1 are separated by 2 aa and may be involved in metal binding (Fig. 2*B*, bold and underlined). Vascular plants (tomato, tobacco, and *A. thaliana*) contain genes encoding proteins with considerable simi-

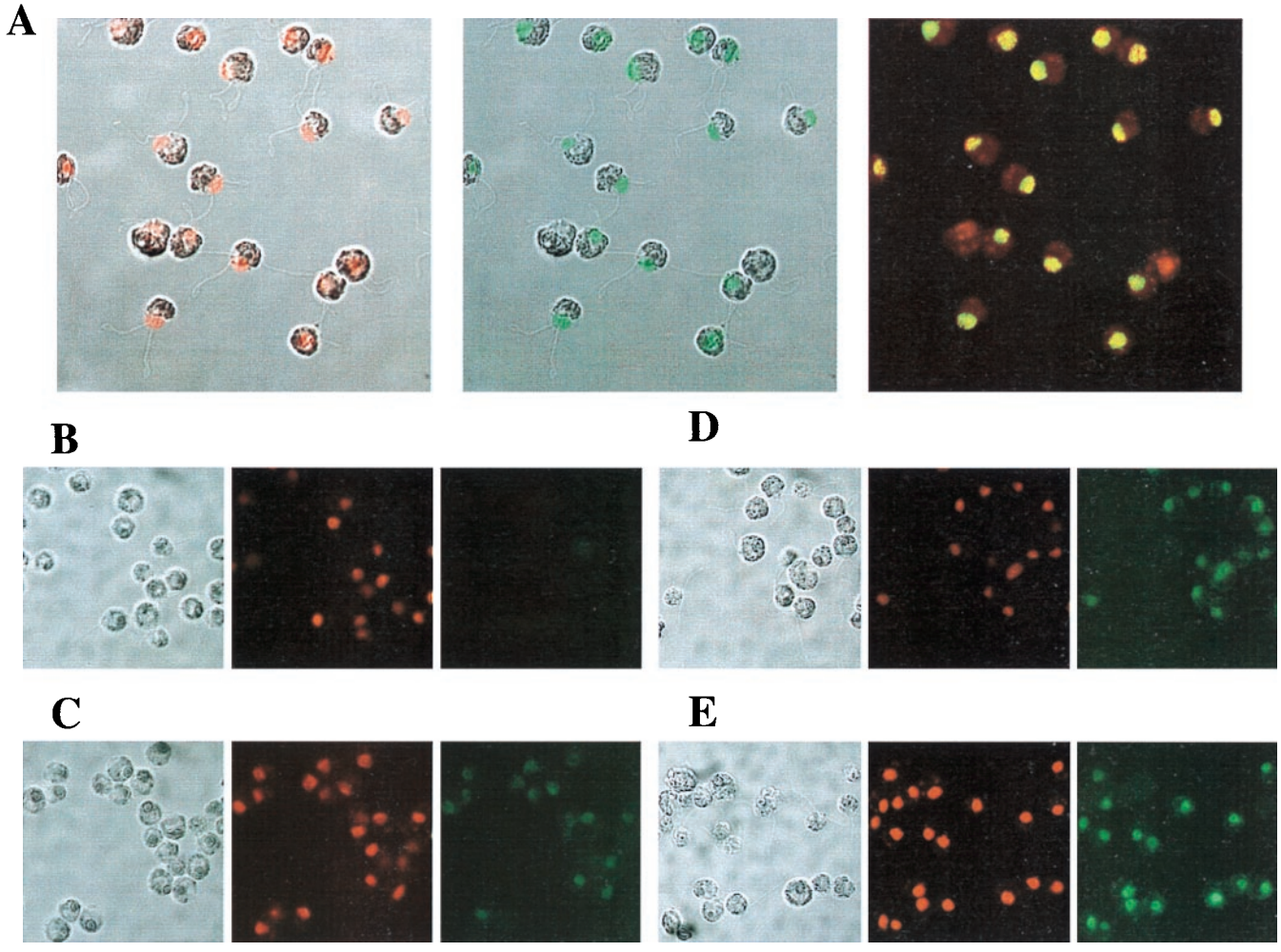

Fig. 4. Immunolocalization of Psr1::3xHA. (*A*) Localization of DNA (red with transmission overlay, *Left*), Psr1::3×HA (green with transmission overlay, *Center*), and both DNA and Psr1::3×HA (yellow is coincident localization, *Right*). (*B*–*E*) Transmission microscopy (*Left*) after staining with propidium iodide for DNA (red, *Center*), or after incubation with antibodies to the HA tag and the fluorescent Alexa 488-anti rat conjugate (green, *Right*). *B* is a *psr1* mutant complemented with the wild-type copy of Psr1 and starved for P for 24 h. *C* is the Psr1::3×HA strain grown under nutrient replete conditions. *D* is the Psr1::3×HA strain starved for P for 8 h. *E* is the Psr1::3×HA strain starved for P for 24 h.

larity to Psr1 in the putative myb region and a second region of 42 aa (amino acids 385–426) (Fig. 2*C*). The function of this second region is not known, although it is predicted to form an α-helix (using NNPREDICT at www.expasy.ch) and may be required for protein–protein or protein–DNA interactions.

We have examined *Psr1* transcript accumulation under P-replete and P-starvation conditions. There is a significant increase in the level of the *Psr1* transcript after starvation of cells for P (compare $+P_i$ and $-P_i$, Fig. 3*A*). RNase protection assays were used to quantify *Psr1* transcript levels during growth in P-replete and P-starvation conditions. There was a significant increase in the level of *Psr1* transcript when wild-type cells were exposed to P starvation for 4 h, with a peak in accumulation after 8 h. After 24 h of starvation the transcript level declined to near that observed in nutrient-replete cells (Fig. 3*B*). No transcript was detected in the *psr1–1* and *psr1–2* mutant alleles. Induction of the high-affinity P_i transporter and secretion of phosphatase activity can be detected only after 8 and 16 h of starvation, respectively. Hence, the increase in *Psr1* transcript precedes the induction of the P-starvation responses.

To quantify Psr1 protein levels and to define its subcellular localization, we inserted a sequence encoding three copies of a HA peptide, which is used widely for epitope tagging, into the genomic sequence of *Psr1* (*Psr1*::3×HA) (Fig. 2*B*). The tagged protein complemented all of the *psr1* mutant phenotypes and had no aberrant phenotypes under P-replete conditions. The migration of the tagged Psr1 in a denaturing polyacrylamide gel (Fig. 3*C*) was retarded relative to its predicted molecular mass (100 kDa vs. the predicted mass of 80 kDa). This decreased mobility may be a consequence of protein modification; Psr1 has 12 putative phosphorylation sites and one putative glycosylation site and many putative myristylation sites. Aberrant SDS binding also may explain reduced Psr1 mobility. The level of the Psr1 protein increased by more than 10-fold after 1 day of P_i starvation. This increase in the level of protein reflects the accumulation of the *Psr1* transcript observed during P starvation (Fig. 3*B*). Hence, the primary control of Psr1 protein levels is probably transcriptional.

To determine the subcellular localization of the Psr1 protein, transformed cells expressing Psr1::3×HA were fixed, incubated with HA antibodies and a secondary antibody conjugated to a fluorophore (Alexa-488), and viewed by confocal microscopy. P-starved (24 h) cells with the 3×HA construct exhibited green fluorescence that colocalized with DNA staining (red fluorescence) (Fig. 4*A*); cells that were starved for P but did not contain the 3×HA construct exhibited little green fluorescence (Fig. 4*B*). A time course in which immunocytochemical staining was used to

localize the Psr1::3×HA polypeptide after P starvation is shown in Fig. 4 *C–E*. Cells from nutrient-replete cultures exhibited a low level of green fluorescence, most of which was localized to the nucleus (Fig. 4*C*). After 8 h of P starvation the nuclei became significantly more fluorescent, although a small amount of fluorescence was also present in the cytoplasm (Fig. 4*D*). After 24 h of starvation the nuclei became highly fluorescent (Fig. 4*E*). As in the Western blot analyses, these results demonstrated that the Psr1 protein accumulates during P starvation. They also showed that newly synthesized Psr1 was localized primarily to the nucleus. Because the same low-level green fluorescence is observed in the cytoplasm of starved and unstarved cells (1–5% of the fluorescence emanates from the cytoplasm), the translocation of Psr1 to the nucleus is not likely to be a key regulatory step in the P_i starvation response in *C. reinhardtii*. This is unlike the situation in *S. cerevisiae* or *Neurospora crassa*, where certain regulatory factors localize only to the nucleus when the cells are starved of P (25–27). Although Psr1 does not contain a recognizable nuclear localization sequence, transcription factors can be carried into the nucleus via associations with other proteins (28).

Discussion

Several lines of evidence indicate that Psr1 is involved in regulating the acclimation responses of *C. reinhardtii* to P_i limitation, probably by altering transcription of a specific set of genes. First, *psr1* mutant strains are defective in multiple, specific responses to P deprivation, including the accumulation of extracellular phosphatase activity, the induction of a high-affinity P_i uptake system, and the secretion of P starvation-specific polypeptides (7). Second, Psr1 and transcription factors share domains that are required for DNA binding, transcription factor dimerization, and transcriptional activation. Third, both the *Psr1* transcript and the Psr1 protein accumulate in response to P starvation. Finally, Psr1 is nuclear-localized.

Psr1 is the first regulator of P metabolism in a photosynthetic eukaryote that has been identified. There have been extensive studies using *S. cerevisiae* to dissect the mechanisms that control the acquisition and assimilation of P, and several regulatory elements have been characterized. Psr1 does not resemble any of these *S. cerevisiae* proteins, nor does it resemble any other proteins encoded by the *S. cerevisiae* genome. These findings suggest that there are substantive differences in the regulation of P_i metabolism in yeast and photosynthetic eukaryotes.

Significantly, Psr1 has similarity to several deduced proteins represented by plant expressed sequence tags. These deduced plant protein sequences contain both the myb-like domain and the conserved α-helix domain of Psr1. Although still speculative, these findings suggest that the mechanism associated with Psr1 control of gene regulation in *C. reinhardtii* also is used by vascular plants and raise the possibility that similar regulatory elements in green algae and vascular plants control P metabolism. The *C. reinhardtii* Psr1 protein has a C-terminal extension relative to the vascular plant Psr1-like proteins. If the C-terminal half of the Psr1 polypeptide functions in transcriptional activation, it is not surprising that it is not conserved in vascular plants because sequence similarity often is low among transcriptional activation domains (28). Extending the analysis of the Psr1 protein and studies of vascular plant proteins with similar sequences may help elucidate the control of P metabolism in vascular plants. Furthermore, a more thorough understanding of how vascular plants acclimate to P deprivation should permit the development of logical strategies to engineer plants for more efficient utilization of P; increased efficiency of P utilization might help minimize P supplementation in agriculture and lessen the potential for polluting lakes and rivers.

A number of different models can be generated to describe how Psr1 regulates P metabolism in *C. reinhardtii.* Below we describe a relatively simple, speculative model that could explain how Psr1 may regulate P metabolism in *C. reinhardtii*. Under nutrient-replete conditions Psr1 is present, but the protein exists in an inactive form. During P starvation it is activated by an unknown mechanism and temporally regulates the transcription of specific genes. Temporal regulation of specific genes can be achieved if the different P starvation-activated promoters bind Psr1 with different affinities. The promoters for genes encoding Psr1 and the P_i transporter would be activated rapidly. As active Psr1 accumulates in the nucleus, the lower-affinity promoters (such as the alkaline phosphatase promoter) would bind Psr1. Psr1 activity may depend on the formation of Psr1 dimers. Hence, the accumulation of Psr1 in the nucleus during P starvation also could shift the equilibrium toward dimer formation, which would further promote gene transcription. After 24 h of P deprivation, cell growth rates would decline along with the level of the Psr1 transcript. This decline may reflect other regulatory processes that become prominent only at later stages in the acclimation process. With further characterization of the Psr1 protein and of mutants aberrant in P metabolism, we will be able to test this model and to define the regulatory elements required for control of P metabolism in *C. reinhardtii*.

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