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Complementation of *snf1*, a mutation affecting global regulation of carbon metabolism in yeast, by a plant protein kinase cDNA

(cereals/seeds/development/carbon partitioning)

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ABSTRACT **A cDNA, cRKIN1, encoding a putative homologue of the yeast (*Saccharomyces cerevisiae*) *SNF1*-encoded protein-serine/threonine kinase, has been isolated from a library prepared from rye endosperm mRNA. Northern blot analysis demonstrated the presence of cRKIN1-related transcripts in developing endosperms but not in shoots, and Southern blot analysis showed the presence of a small gene family. *SNF1* plays a central role in carbon catabolite repression in yeast and expression of the RKIN1 sequence in yeast *snf1* mutants restored *SNF1* function. This suggests that the RKIN1 protein has a role in the control of carbon metabolism in endosperms of rye.**

Protein kinases are known to play key roles in animals and yeasts in the control of cell division (1) and in cellular response to external stimuli and in metabolic regulation (2). For example, the role of insulin in controlling glycogen synthesis in mammals is mediated by an array of phosphorylation and dephosphorylation steps, the former catalyzed by protein kinases (3). Although it has been suggested that protein kinases play similar roles in plants (4), this remains to be demonstrated. However, recent studies have shown that protein kinases related to the cdc2 cell cycle protein of fission yeast (5) and its homologues in budding yeast (6), human cells (7), and *Xenopus* (8) are also present in pea (9) and *Arabidopsis* (10). All protein kinases that have been characterized in detail contain a number of key residues and conserved regions in the catalytic domain (11) but can be divided into two classes: those that phosphorylate serine/threonine residues and those that phosphorylate tyrosine residues. In the present report, we present the nucleotide sequence of a cDNA, cRKIN1,** isolated from a rye endosperm cDNA library. The cRKIN1-encoded protein contains all the invariant residues and conserved domains characteristic of eukaryotic protein-serine/threonine kinases. It is particularly similar to the product of the *SNF1* gene of yeast (*Saccharomyces cerevisiae*) (12), a protein affecting global regulation of carbon metabolism, and the expression of cRKIN1 in yeast *snf1* mutants restores *SNF1* function.

MATERIALS AND METHODS

Construction of a cDNA Library from Rye Endosperm mRNA and Isolation of λcRKIN1. Free and membrane-bound polysomes were prepared from rye endosperms 20 days after anthesis. Poly(A)$^+$-rich RNA was purified by affinity chromatography using oligo(dT)-cellulose. cDNA was prepared from this material using the Amersham cDNA synthesis kit and was cloned into the vector λgt10. The resulting cDNA library contained 3×10^5 recombinants. λcRKIN1 was isolated from this library in the course of an unrelated experiment.

DNA Sequencing. The cDNA insert from λcRKIN1 was excised and ligated into pUC18 to produce the plasmid pcRKIN1. Sequential overlapping deletions of the insert were generated by linearization followed by digestion with exonuclease III. These were excised and cloned into M13mp18 for sequencing. The entire insert was sequenced in both directions and the sequences were analyzed using the programs of the University of Wisconsin Genetics Computer Group (13).

Southern and Northern Blot Analyses. A Southern blot of rye genomic DNA and a Northern blot of rye endosperm and shoot mRNA were probed with the full-length insert from pcRKIN1 using the methods described by Sabelli and Shewry (14). The blots were washed at high stringency (65°C in 0.5× standard saline citrate) before autoradiography.

Construction of Plasmid pDC1. The yeast expression plasmid pMA99 was kindly provided by S. M. Kingsman and A. J. Kingsman (University of Oxford, U.K.). It includes a *Bam*HI fragment containing the *Escherichia coli lacZ* gene downstream of the yeast phosphoglycerate kinase (*PGK1*) gene promoter. This fragment was removed and replaced with a *Stu* I–*Eco*RI fragment of cRKIN1 ligated to Pharmacia *Bam*HI 5′-phosphorylated linkers. This fragment contains 84 base pairs of the leader sequence, the whole coding region, and the 3′ flanking region.

Yeast Strains. Yeast strain MCY1845 (*MATα ade2 ura3 snf1Δ10*) kindly provided by M. Carlson (Columbia University, New York) was mated to strain DBY747 (*MATa his3 leu2 ura3 trp1*) and the resulting diploid was sporulated to yield a variety of segregants including strain 30.1.6 (*MATα his3 ura3 trp1 snf1Δ10*).

RESULTS AND DISCUSSION

A cDNA clone, pcRKIN1, was isolated from a rye endosperm cDNA library in the course of an unrelated experiment. The cDNA insert, consisting of 1809 base pairs plus a poly(A) tail, was completely sequenced in both directions (Fig. 1). It contains an open reading frame from the start of the sequence to a stop codon (TAG) at position 1616. The length of the transcript seen in Northern blot analysis (see

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M74113).

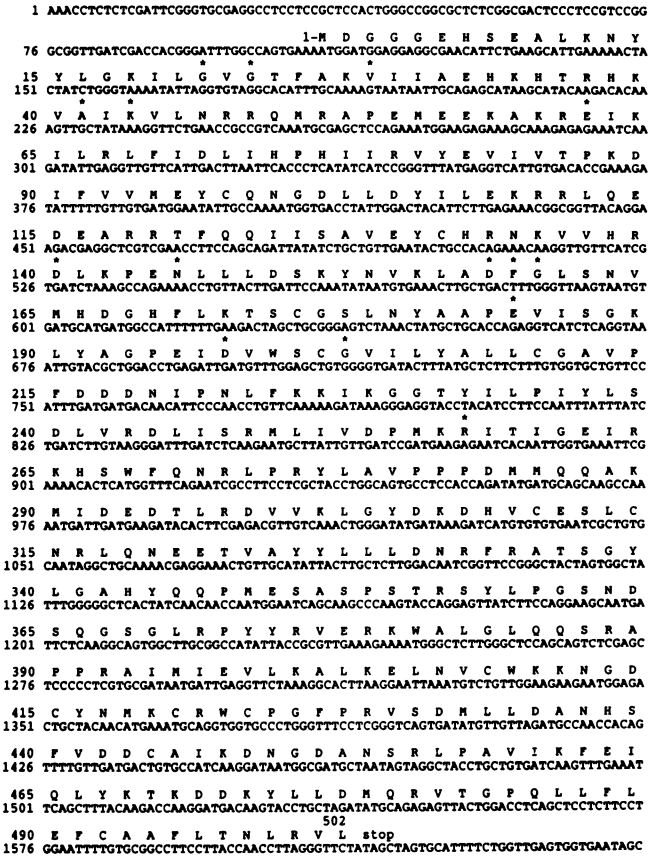

FIG. 1. Nucleotide and derived amino acid sequence of cRKIN1. Invariant residues characteristic of eukaryotic protein-serine/threonine kinases are indicated (*).

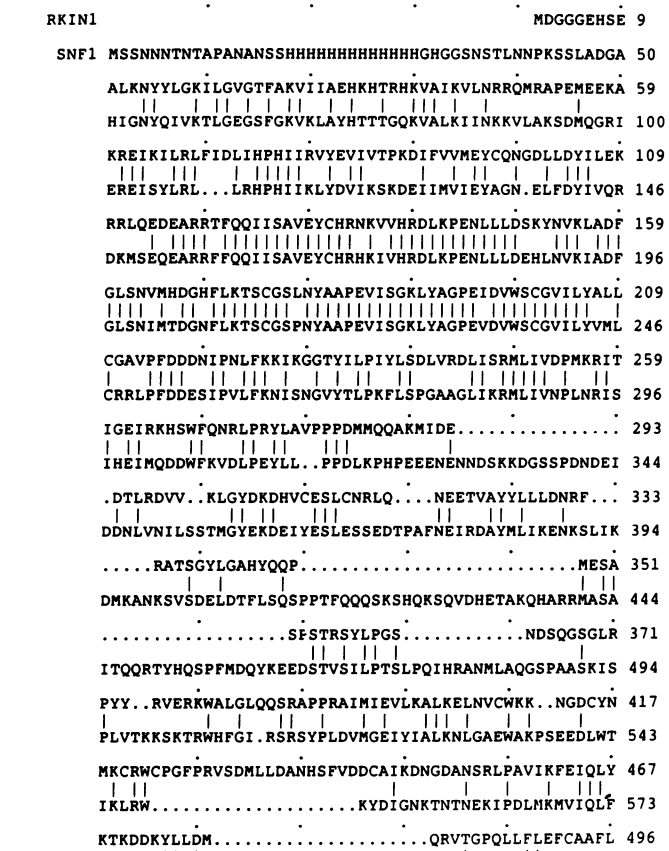

FIG. 2. Derived amino acid sequence of the protein encoded by cRKIN1 aligned with that encoded by the *SNF1* gene of yeast. The alignment was made using the program GAP (13) with a gap weight of 3.0 and a gap length weight of 0.1.

below) and sequences obtained for the 5′ ends of genomic clones of homologous genes from barley and *Arabidopsis* (unpublished data) suggests that the cRKIN1 cDNA is complete or nearly complete and that the ATG sequence at position 110 is the translation start site. The open reading frame is, therefore, 1506 base pairs long and encodes a protein of 502 amino acid residues (Fig. 1) with a calculated molecular weight of 57,710. The protein is particularly rich in leucine (11.6 mol %) and lysine (7.2 mol %). It contains all the invariant or nearly invariant residues and all the conserved domains characteristic of eukaryotic protein-serine/threonine kinases (11). The invariant residues are Gly^{21}, Gly^{23}, Val^{28}, Ala^{41}, Lys^{43}, Glu^{62}, Asp^{140}, Asn^{145}, Asp^{158}, Phe^{159}, Gly^{160}, Glu^{184}, Asp^{197}, Gly^{202}, and Arg^{257} (these are indicated in Fig. 1). The 11 conserved domains lie between Leu^{20} and Arg^{257}, with the core catalytic domain (11) consisting of residues Ala^{127} to Tyr^{206}. The sequence Asp^{140}-Leu-Lys-Pro-Glu-Asn is indicative of serine/threonine specificity, tyrosine kinases having a different consensus in this region.

Searches of the GenBank and EMBL nucleotide sequence data bases and the National Biomedical Research Foundation protein sequence data base (January 4, 1991) matched the RKIN1 amino acid sequence most closely with that of the *SNF1* gene product of the budding yeast (*S. cerevisiae*) (12). The *SNF1* gene product is a protein-serine/threonine kinase required for the derepression of several glucose-repressible genes, including *SUC2* (invertase). The RKIN1 and SNF1 amino acid sequences are aligned in Fig. 2. The catalytic domains are most highly conserved but conserved regions and residues are present throughout the sequences. The GAP program (13) calculates a similarity of 66.9% between the RKIN1 and SNF1 amino acid sequences. Of the 80 residues that constitute the core catalytic region of each protein, 69 are identical and 6 of the 11 substitutions are conservative.

Southern blot analysis of rye genomic DNA probed with the pcRKIN1 cDNA insert showed several strongly hybridizing bands (Fig. 3*A*), indicating a small multigene family. The presence of other more weakly hybridizing bands showed that less closely related sequences were also present. Strongly hybridizing sequences are also present in *Arabidopsis*, barley, and wheat (unpublished data). Northern blot analysis showed that RKIN1-related transcripts of ≈1800 nucleotides in length were present in developing endosperms but not in shoots (Fig. 3*B*).

To determine the function of the RKIN1 protein, its ability to restore SNF1 activity in yeast (*S. cerevisiae*) *snf1* mutants was tested. Strains of *S. cerevisiae* carrying *snf1* mutations fail to derepress many of the enzymes of oxidative metabolism. Consequently, they are unable to utilize substrates such as glycerol and ethanol. Yeast 30.1.6, carrying the *snf1* mutation, was transformed with plasmid pDC1 (Fig. 4*A*). pDC1 is maintained at low copy number (1–2 copies per cell) because of the presence of the CEN3 sequence, behaving like a tiny natural chromosome in terms of replication and partitioning. It contains the yeast phosphoglycerate kinase (*PGK1*) gene promoter up to nucleotide −2 (15), downstream of which a fragment containing the RKIN1 sequence from 84 base pairs upstream of the ATG to the 3′ end has been

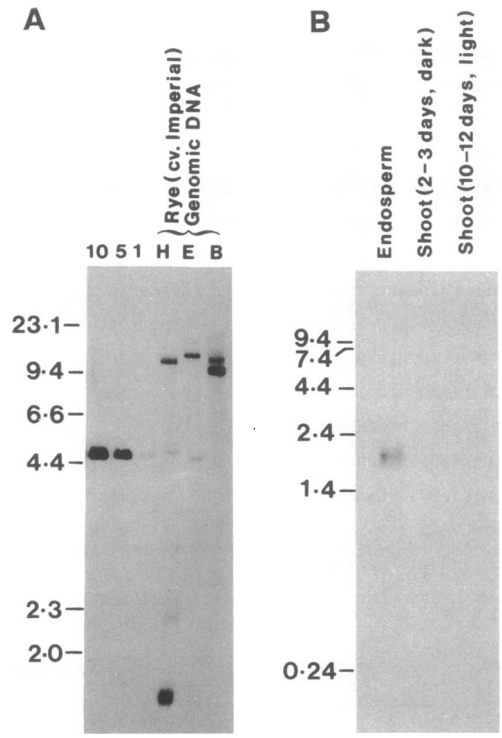

FIG. 3. (*A*) Southern blot analysis of genomic DNA from rye cv. Imperial, digested with restriction enzymes *Hin*dIII (H), *Eco*RI (E), and *Bam*HI (B), with linearized pcRKIN1 plasmid representing 10, 5, and 1 copies of the hybridizing sequence per haploid genome, as indicated. (*B*) Northern blot analysis of RNA from endosperms and shoots of rye cv. Imperial. The relative mobilities of size markers (in kilobases) are also shown. Both filters were probed with the full-length pcRKIN1 insert.

inserted. The resulting chimeric gene (Fig. 4*B*) contains all the yeast signals required for efficient initiation of transcrip-

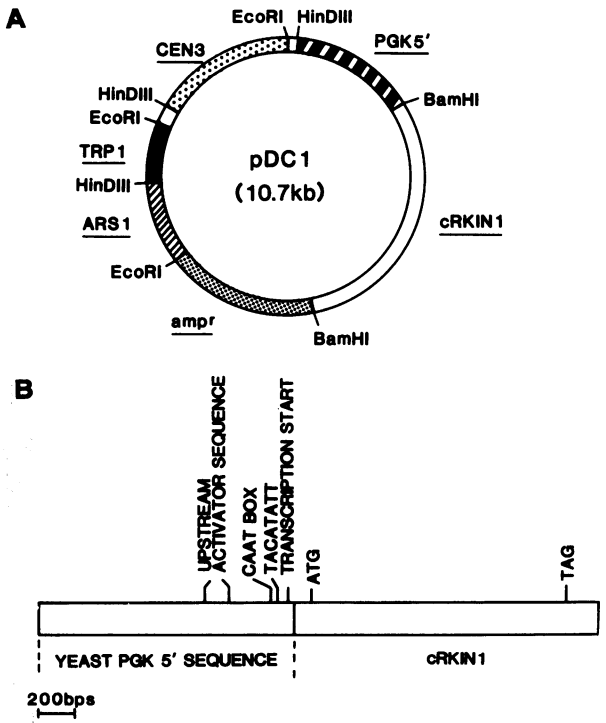

FIG. 4. (*A*) Plasmid pDC1 used for the expression of the RKIN1 protein in yeast. (*B*) The chimeric gene resulting from the ligation of the cRKIN1 sequence downstream of the *PGK1* gene promoter.

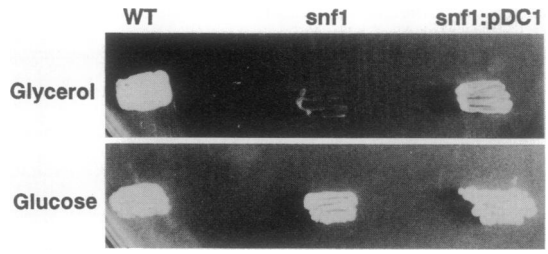

FIG. 5. Growth of yeast (*S. cerevisiae*) wild type (WT), *snf1* mutant strain 30.1.6 (snf1), and strain 30.1.6 transformed with plasmid pDC1 (snf1:pDC1) on glycerol and glucose media.

tion and uses the RKIN1 signals for the start and termination of translation. Transformants containing pDC1 were sought by plating-out on glycerol and ethanol minimal medium lacking tryptophan (the selectable marker in plasmid pDC1 is *TRP1*). *Bona fide* transformants grew on the glycerol and ethanol medium (Fig. 5). This indicates that the rye RKIN1 protein is able to perform the same function in yeast as the *SNF1* gene product.

SNF1 plays a central role in carbon catabolite repression in yeast (12). It was identified by screening for genes involved in the regulation of invertase gene expression and is required for the expression of several glucose-repressible genes in response to glucose deprivation. The *snf1* mutants are unable to utilize sucrose, melibiose, galactose, or nonfermentable carbon sources. *SNF1* controls *SUC2* (invertase) expression at the transcriptional level (16), *snf1* mutants failing to produce SUC2 mRNA (17).

Although cereals are the major food crops in the world and starch is their major storage product (accounting for 70–80% of the grain dry weight), nothing is known of the factors controlling the partitioning of assimilated carbon between starch and other storage products (mainly oil and proteins). Similarly, the role of invertase in the developing endosperm is unclear. Doehlert and Felker (18) demonstrated that invertase activity was restricted to the basal and pedical tissues in developing maize endosperms and consisted of soluble and cell wall-associated forms. They proposed that the location of the latter form is consistent with a role in phloem unloading, sucrose hydrolysis maintaining a concentration gradient between the phloem and apoplast. In contrast, Donovan *et al.* (19) demonstrated that sucrose was not metabolized during its movement along the wheat grain, suggesting that sucrose hydrolysis is not essential for phloem unloading.

Our isolation of the RKIN1 cDNA indicates that protein phosphorylation may play a regulatory role in carbohydrate metabolism in developing cereal endosperms, as in mammalian tissues. It also provides an opportunity to explore the control of carbohydrate metabolism and in particular the role of invertase *in vivo* in transgenic plants. Finally, our success in complementing the *snf1* mutation suggests that complementation of yeast mutations may prove to be a useful tool for isolating other genes that regulate plant metabolism.

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