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cDNA cloning of a novel cdc2⁺/CDC28-related protein kinase from rice

Shingo Hata

National Institute of Agrobiological Resources, 2-1-2 Kannondai, Tsukuba, Ibaraki 305, Japan

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A cDNA clone, named R2, has been isolated by screening a rice cell cDNA library with a redundant oligonucleotide probe derived from the conserved ATP binding site of cdc2⁺/CDC28 protein kinases. The cDNA contained the entire coding sequence for a 424 amino acid polypeptide with a molecular mass of 47.6 kDa. The R2 mRNA, 2.1 kb in size, was expressed in both cultured rice cells and rice seedlings at similar levels. The predicted R2 protein has canonical motifs for ATP binding and catalysis, and is significantly homologous (up to 47%) to members of the cdc2⁺/CDC28 subfamily of serine/threonine protein kinase. The R2 protein is a novel member of the subfamily.

Serine/threonine kinase; cdc2⁺; CDC28; cDNA cloning; *Oryza sativa*

1. INTRODUCTION

The protein kinases constitute a large family which is classified into a number of subfamilies based upon the results of amino acid sequence analysis [1]. Among them the cdc2⁺/CDC28 subfamily of serine/threonine kinases is attracting much attention, because recent studies on cdc2⁺/CDC28 kinases and their homologs in vertebrates have shown that they are the key enzymes for cell cycle regulation in eukaryotes [2–4]. Some other members of this subfamily were also reported to play important roles in such as signal transduction in the morphogenesis of *Drosophila* [5,6], the pheromone-induced mating process of budding yeasts [7,8] and the response to phosphate depletion of yeasts [9]. The subfamily is growing at an accelerated pace. In higher plants, however, little is known about protein kinases of this type. Here I report the cDNA cloning and sequencing of a new member of the subfamily from rice.

2. MATERIALS AND METHODS

Poly(A)⁺ RNA of cultured cells of rice (*Oryza sativa* L., cv. Nipponbare) [10] was used to construct an oligo(dT)-primed cDNA library. Double-stranded cDNA was prepared with a cDNA synthesis kit (Pharmacia LKB), ligated with the λZAPII vector (Stratagene) and then packaged into phage particles using Gigapack Gold (Stratagene). The details of the library construction will be published elsewhere.

A 16-fold degenerate 30-mer of an antisense oligonucleotide (5′-(T/C)TT(A/G)TAIACIACICC(A/G)TAIGTICC(T/C)TCICC-3′) was synthesized, based on the highly conserved amino acid sequence (GEGTYGVVYK) around the ATP binding site of cdc2⁺/CDC28 kinases [1,11]. Deoxyinosine was included to reduce degeneracy [12]. The oligonucleotide was labeled with [γ-³²P]ATP and polynucleotide kinase. This probe was used to screen plaques blotted onto Hybond-N membranes (Amersham). Phage DNAs in the positive clones were excised in vivo to recover pBluescript plasmids according to Stratagene's instructions. The nucleotide sequences of both strands were determined after deletion of the plasmids with exonuclease III (Promega), as described [13]. The secondary structures of polypeptides were predicted by the method of Chou and Fasman [14] using the computer software, GENETYX (Software Development).

Northern blot analysis was performed as described [13] using 2 μg each of poly(A)⁺ RNA isolated from the above mentioned rice cells and rice seedlings (cv. Tan-ginbozu).

Correspondence address: S. Hata, National Institute of Agrobiological Resources, 2-1-2 Kannondai, Tsukuba, Ibaraki 305, Japan

3. RESULTS AND DISCUSSION

3.1. *Isolation of the cDNA clones*

To look for cDNAs for plant protein kinases in the cdc2⁺/CDC28 subfamily, about 1.2×10^5 clones of the library were screened with the oligonucleotide probe. Six positive spots were detected. The cross-hybridization among them has not been checked. Two recombinant phages were picked up at random, purified and further analyzed. Partial sequencing showed that both cDNAs define the same gene.

3.2. *Nucleotide and deduced amino acid sequences*

One of the cDNAs, designated R2, is 1764 bp in length and has an open reading frame for a 424 amino acid polypeptide (Fig. 1). Although no in-frame stop codon was found upstream of the ATG codon at positions 173–175, I assume that it represents the translation initiation site for the following reasons. First, the other overlapping clone, which was truncated at the 5′ portion of the gene, had 160 bp more (besides the polyA tail) at the 3′ end (data not shown). Thus R2 cDNA lacks 3′ part of its mRNA. Secondly, primer-extension analysis showed that R2 cDNA lacks only the 10

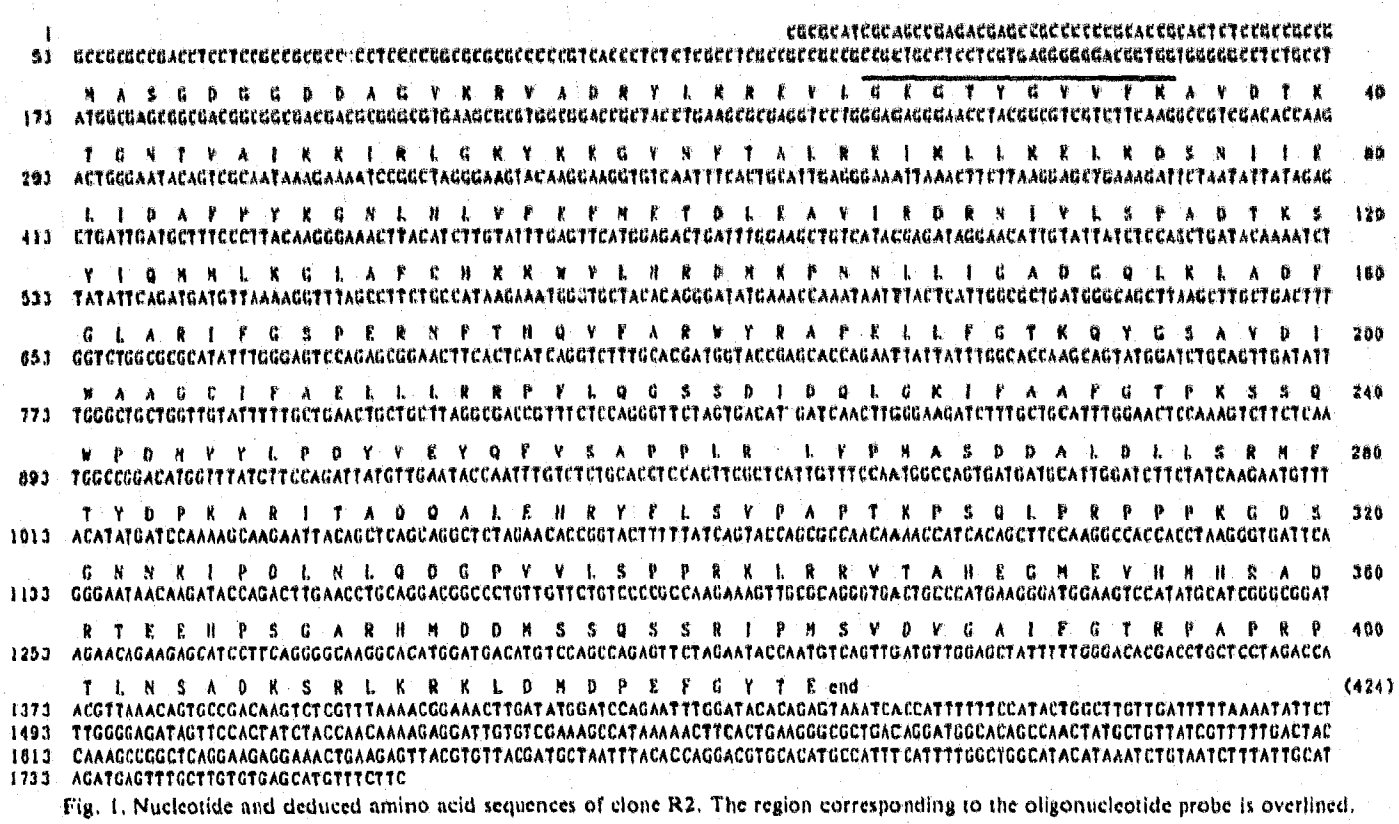

Fig. 1. Nucleotide and deduced amino acid sequences of clone R2. The region corresponding to the oligonucleotide probe is overlined.

nucleotides from the 5′ end of mRNA (data not shown). The calculated molecular mass of the R2 protein is 47 622 Da. It is larger than cdc2⁺/CDC28 kinases (~34 kDa) in various other species, including those of some plants [15,16].

3.3. *Northern blot analysis*

On Northern blot analysis an R2 mRNA species of approximately 2.1 kb in size was detected in both cultured cells and seedlings of rice (Fig. 2). A second fainter band corresponding to 0.8 kb was also observed for an unknown reason. Fig. 2 also shows that the mRNA contents of the two materials are similar.

3.4. *Relationships among the protein kinases*

As shown in Fig. 3, 15 invariant amino acids characteristic of all protein kinases [1] were conserved in R2. Of note among them are the GXGXXG motif in subdomain I and the invariant amino acids in subdomains VI and VIII (implicated in ATP binding), and the K residue in subdomain II (involved in phosphotransfer) [1]. The indicator sequences in subdomains VI and VIII of R2 were more homologous to those of serine/threonine kinases than those of tyrosine kinases

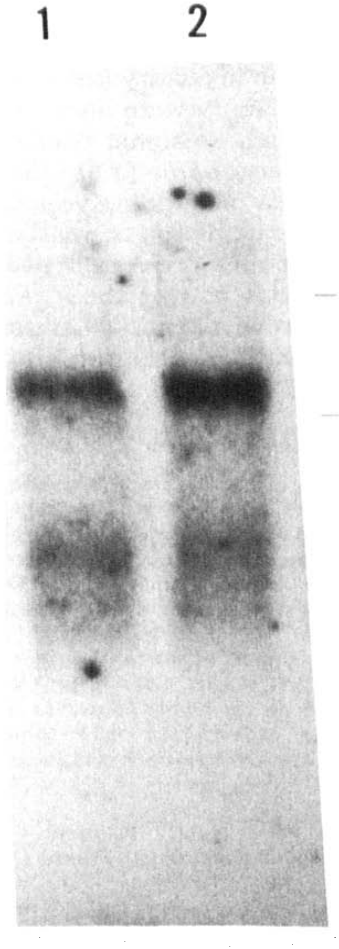

Fig. 2. Northern blot analysis. Poly(A)⁺ RNAs of cultured rice cells (lane 1) and rice seedlings (lane 2) were probed with ^{32}P-labeled R2 cDNA. 25 S and 17 S ribosomal RNA served as markers.

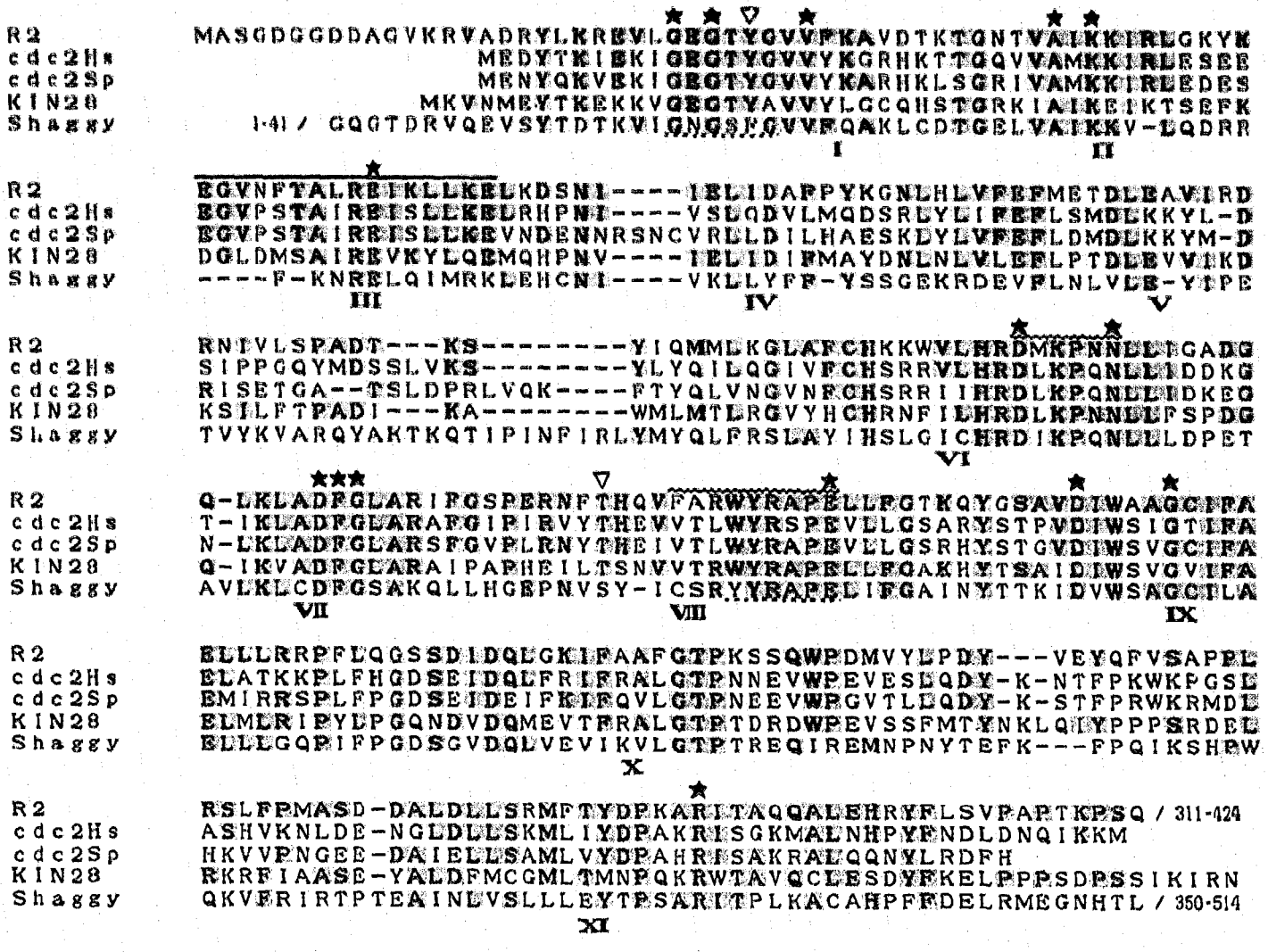

Fig. 3. Amino acid sequence alignment of the R2 protein and some other members of the cdc2$^+$/CDC28 subfamily. Amino acids identical to those in R2 are shaded. Division into subdomains I–XI is according to [1]. cdc2Hs, a human cdc2$^+$/CDC28 homolog; cdc2Sp, cdc2$^+$ of fission yeast; KIN28, an uncharacterized gene of budding yeast; Shaggy, segment-polarity gene of fly; ★, invariant amino acids in protein kinases [1]; ▽, phosphorylation sites of cdc2$^+$/CDC28 homologs; solid overline, the region of the 16-amino acid 'PSTAIR' motif reported to be perfectly conserved in cdc2$^+$/CDC28 homologs; wavy overline, indicator sequence used to discriminate serine/threonine kinases and tyrosine kinases [1]; dashed underline, amino acid sequence used to amplify a plant cdc2$^+$/CDC28 homolog by means of the polymerase chain reaction [16].

[1]. Significant homology was observed between R2 and the human homolog of cdc2$^+$/CDC28 [11] (46% identity over subdomains I–XI), R2 and cdc2$^+$ of fission yeast [17] (45%), and R2 and KIN28 of budding yeast [18] (47%). Other members of the cdc2$^+$/CDC28 subfamily exhibited lower homology; e.g. for PHO85 [9], PSK-J3 [1,19] and Shaggy [6], it was 40%, 34% and 31%, respectively. Protein kinases of other subfamilies were even less homologous. Thus, R2 is a novel member of the cdc2$^+$/CDC28 subfamily of serine/threonine protein kinases.

All cdc2$^+$/CDC28 functional homologs reported to date have the hallmark motif in subdomain III [2–4,11,16 and the references therein]. R2 has a similar sequence at the same position (Fig. 3). Moreover, the phosphorylation sites of cdc2$^+$/CDC28 homologs [4,20] and the amino acid sequences used to amplify the gene for a cdc2$^+$/CDC28 homolog in pea [16] are also conserved in R2 (Fig. 3). These observations raised a possibility that R2 might have the same function as cdc2$^+$/CDC28. To examine this possibility, the secondary structures predicted for subdomains III and IV were compared (Fig. 4). The region in cdc2$^+$ of fission yeast contained two α helices separated by a short stretch of polypeptides and so this structure was proposed to be important for cell cycle regulation [21]. On the other hand, the same region in R2 contained only one helix at another position. This result, together with the large molecular mass, strongly suggests that the R2 protein plays a different physiological role to that of

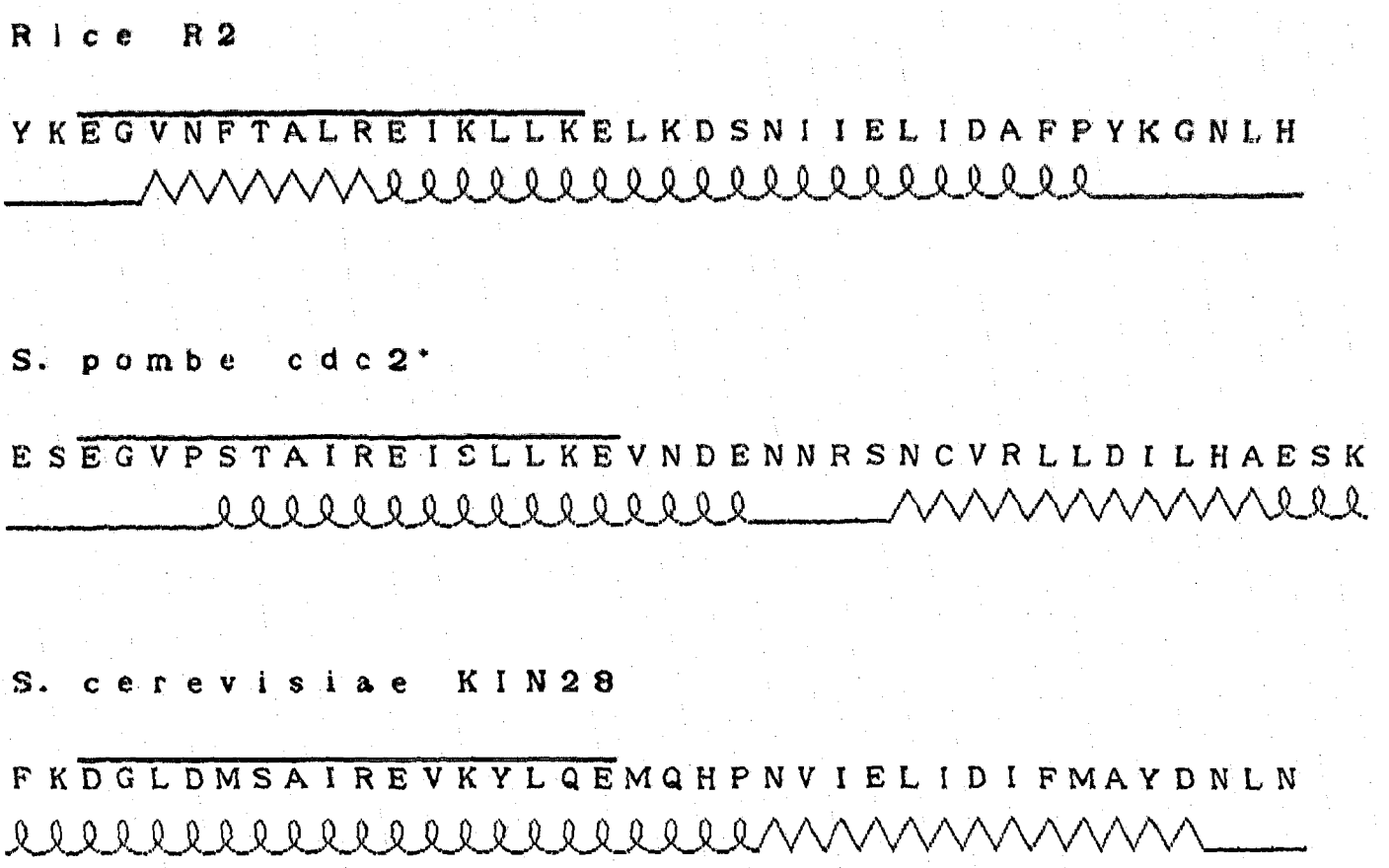

Fig. 4. Predicted secondary structures. The overline indicates the region of the invariant 'PSTAIR' motif in cdc2*/CDC28 kinases. Helical line, α-helix; zigzag line, β-sheet; straight line, random coil.

cdc2*/CDC28 kinases. The secondary structure of R2 was also different from that of KIN28, the function of which is unknown [18].

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