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Molecular Cloning and Nucleotide Sequence of *Schizosaccharomyces pombe* Homologue of the Receptor for Activated Protein Kinase C Gene

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Using differential hybridization, we selected the *prk* gene fortuitously from *Schizosaccharomyces pombe* homologous to RACK1 of rat which encodes the receptor for activated protein kinase C. The cDNA sequence of *prk* was determined and its deduced amino acid sequence was 76% homologous to RACK1 and had the feature of trimeric G protein beta subunit. The specific amino acid sequences required for the protein kinase C binding were also present in Prk as in the case of RACK1 protein. From these similarities, we suggest that the Prk is protein kinase C binding protein of *S. pombe*. The involvement of Prk in signal transduction mediated by protein kinase C remained to be studied.

Key words: receptor of activated protein kinase C, protein kinase C, trimeric G protein beta subunit, *Schizosaccharomyces pombe*

Protein kinase C (PKC) plays a key role in signal transduction in response to a variety of extracellular stimuli (15). Immunochemical localization of PKC proteins, as well as the mRNA expression analysis in various tissues, indicates that each number has a distinct pattern of expression (17). PKC-like genes were isolated not only from higher eukaryotes but also from simple eukaryotes such as slime mold and the budding yeast, *Saccharomyces cerevisiae* (11, 19). This suggests that PKCs and the PKC-mediated phosphorylation pathway have fundamental function common to all eukaryotes. The PKC gene family is divided into three group (cPKC, nPKC, and aPKC) (16). Four conserved subdomains (designated C1-C4) have been defined; the C1 and C2 regions reside in the regulatory domain and consist of a cysteine-rich motif and a putative Ca^{2+}-binding region, respectively (16). The cPKC and nPKC proteins have been classified, depending on the presence or absence of the C2 region, respectively (16).

The budding yeast PKC1 containing C2 region was reported to be essential for vegetative growth and *pkc1*-depleted cells arrest as small buds after completion of DNA synthesis (11). Further study, however, indicates that the lethality caused by the cell lysis of *pkc1*-depleted cells can be rescued if the medium contains an osmostabilizer such as 1 M sorbitol (10). In the fission yeast *S. pombe*, two nPKC-like genes, *pck1* and *pck2* were isolated (22). They share an overlapping essential function for cell viability. Cells of a single *pck2* deletion display severe defects in cell shape. In contrast, the induced overexpression of *pck2* is lethal, producing multiseptated and branched cells. The lethal overexpression of *pck2* can be suppressed by staurosporine, a potent protein kinase inhibitor. Loss of *pck1* and *pck2* are not, however, suppressed by an osmostabilizer.

Activation of PKC is associated with its translocation from the cytosolic (soluble) fraction to the particulate fraction (9). This binding to membrane fraction was susceptible to protease trypsin treatment (5). Several data suggest that activated PKC may bind to receptor proteins located at various intracellular sites (2, 4, 6, 7, 8). Mochly-Rosen et al. showed the presence of intracellular receptor proteins for activated protein kinase C and called these proteins "RACKs" (12, 13). The RACKs have the following properties: RACKs are present in the detergent insoluble fraction, binding of PKC to RACKs is dependent on phosphatidylserine, diacylglycerol, and calcium, and these binding are specific and saturable. The

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gene encoding of RACK1 was cloned by the binding capacity of RACK1 to protein kinase C (20). Purified RACK1 inhibited PKC translocation and delayed oocyte maturation (21). Mochly-Rosen *et al.* proposed that the binding site of PKC to RACK is in the C1 and/or C2 regions of PKC, and subsequently showed that the p65 synaptic vesicle-specific protein from rat containing two regions of the C2 homolog of PKC bound to RACK (14). Another protein, phospholipase C-γ1, containing C2 homologous region, found to bind to RACK without phospholipid and calcium (3).

Here, we report that the cDNA sequence encoding the putative receptor for activated protein kinase C of *S. pombe* isolated in the course of the study for cell cycle related genes.

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                                                    CAAC    4
ATGCCAGAACAACTTGTGCTCCGTGCAACTCTCGAAGGTCACTCTGGATGGGTTACATCT   64
 M  P  E  Q  L  V  L  R  A  T  L  E  G  H  S  G  W  V  T  S   20
CTTTCTACTGCCCCCGAGAACCCTGATATTCTTCTTTCCGGTTCTCGTGACAAGTCCATC  124
 L  S  T  A  P  E  N  P  D  I  L  L  S  G  S  R  D  K  S  I   40
ATTTTGTGGAACTTGGTCCGTGATGACGTGAATTATGGAGTCGCACAGAGACGTTTGACC  184
 I  L  W  N  L  V  R  D  D  V  N  Y  G  V  A  Q  R  R  L  T   60
GGCCACAGCCACTTCGTTTCTGACTGTGCCCTTTCCTTCGATAGTCACTATGCCTTGTCT  244
 G  H  S  H  F  V  S  D  C  A  L  S  F  D  S  H  Y  A  L  S   80
GCCTCTTGGGATAAGACCATCCGTTTGTGGGATCTTGAGAAGGGTGAGTGCACTCACCAA  304
 A  S  W  D  K  T  I  R  L  W  D  L  E  K  G  E  C  T  H  Q  100
TTCGTTGGCCACACCAGCGATGTCTTATCTGTCTCCATTTCTCCTGACAACCGCCAGGTT  364
 F  V  G  H  T  S  D  V  L  S  V  S  I  S  P  D  N  R  Q  V  120
GTTTCTGGTTCCCGTGACAAGACCATTAAGATTTGGAACATTATTGGTAACTGCAAGTAC  424
 V  S  G  S  R  D  K  T  I  K  I  W  N  I  I  G  N  C  K  Y  140
ACTATCACCGATGGTGGTCACTCTGACTGGGTTTCTTGTGTGCGCTTCTCTCCTAACCCC  484
 T  I  T  D  G  G  H  S  D  W  V  S  C  V  R  F  S  P  N  P  160
GATAACCTTACCTTCGTCTCTGCTGGTTGGGACAAGGCCGTTAAGGTTTGGGATTTGGAA  544
 D  N  L  T  F  V  S  A  G  W  D  K  A  V  K  V  W  D  L  E  180
ACCTTCTCCCTTCGCACTTCTCACTATGGCCATACTGGTTACGTATCTGCAGTCACCATC  604
 T  F  S  L  R  T  S  H  Y  G  H  T  G  Y  V  S  A  V  T  I  200
TCCCCTGATGGATCTCTTTGTGCTTCCGGTGGAAGAGACGGTACCTTGATGCTTTGGGAT  664
 S  P  D  G  S  L  C  A  S  G  G  R  D  G  T  L  M  L  W  D  220
CTTAACGAGTCTACCCACCTCTACTCTTTGGAAGCCAAGGCTAACATTAATGCCCTTGTT  724
 L  N  E  S  T  H  L  Y  S  L  E  A  K  A  N  I  N  A  L  V  240
TTCTCCCCTAACCGTTACTGGCTTTGTGCCGCCACTGGTTCTTCCATTCGTATCTTCGAT  784
 F  S  P  N  R  Y  W  L  C  A  A  T  G  S  S  I  R  I  F  D  260
TTGGAGACTCAAGAGAAGGTTGATGAACTTACTGTTGACTTTGTTGGTGTTGGCAAGAAG  844
 L  E  T  Q  E  K  V  D  E  L  T  V  D  F  V  G  V  G  K  K  280
AGCTCTGAGCCTGAGTGTATTTCTCTTACCTGGTCTCCTGATGGCCAAACTTTGTTCTCT  904
 S  S  E  P  E  C  I  S  L  T  W  S  P  D  G  Q  T  L  F  S  300
GGCTGGACTGATAATCTCATTCGTGTCTGGCAAGTTACCAAGTAAAAATAAGATTTTAAT  964
 G  W  T  D  N  L  I  R  V  W  Q  V  T  K  *                314
TGTTGTCCCATAAGACGATAATGATGAATGGCTTTAGGGTGCATCGTTTTCTTTAAACTC 1024
TGAATCAAATTCGATTCCCAAAGAAT                                   1049
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of *prk* cDNA. The single letter amino acid code is placed below the second nucleotide of its codon. The termination codon is labeled with an asterisk. This sequence data is available from GenBank under accession number L37885.

Materials and Methods

Strains and media

The *E. coli* strains used in this study were XL1-Blue MRF' and SOLR which are purchased from Stratagene company. The culture medium for *E. coli* was LB (yeast extract 0.5%, trypton 1.0%, sodium chloride 1.0%). The recombinant *E. coli* containing plasmids was cultured in LB with ampicillin 50 μg/ml.

Isolation of cDNA of the *prk* gene

We isolated the cDNA of prk gene fortuitously by differential hybridization (18). A Uni-ZAP XR cDNA library prepared from the mRNA extracted from the S phase arrested *S. pombe* cells. This library was blotted on nitrocellulose filters in duplicate, and then each set of nitrocellulose filter was hybridized with the cDNA probes prepared from mRNAs extracted from the cells arrested at S phase or M phase of cell cycle. We selected phagemid clones showing more intense signals in

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Prk    - MPEQLVLRATLEGHSGWVTSLSTAPENPDILLSGSRDKSIILWNLVRDDV -50
RACK1  - MTEQMTLRGTLKGHNGWVTQIATTPQFPDMILSASRDKTIIMWKLTRDET -50

Prk    - NYGVAQRRLTGHSHFVSDCALSFDSHYALSASWDKTIRLWDLEKGECTHQ -100
RACK1  - NYGIPQRALRGHSHFVSDVVISSDGQFALSGSWDGTLRLWDLTTGTTTRR -100

Prk    - FVGHTSDVLSVSISPDNRQVVSGSRDKTIKIWNIIGNCKYTITDGGHSDW -150
RACK1  - FVGHTKDVLSVAFSSDNRQIVSGSRDKTIKLWNTLGVCKYTVQDESHSEW -150

Prk    - VSCVRFSPNPDNLTFVSAGWDKAVKVWDLETFSLRTSHYGHTGYVSAVTI -200
RACK1  - VSCVRFSPNSSNPIIVSCGWDKLVKVWNLANCKLKTNHIGHTGYLNTVTV -200

Prk    - SPDGSLCASGGRDGTLMLWDLNESTHLYSLEAKANINALVFSPNRYWLCA -250
RACK1  - SPDGSLCASGGKDGQAMLWDLNEGKHLYTLDGGDIINALCFSPNRYWLCA -250

Prk    - ATGSSIRIFDLETQEKVDELTVDFVGVGKKSSEPECISLTWSPDGQTLFS -300
RACK1  - ATGPSIKIWDLEGKIMVDELKQEVISTSSKAEPPQCTSLAWSADGQTLFA -300

Prk    - GWTDNLIRVWQVT---K -314
RACK1  - GYTDNLVRVWQVTIGTR -317
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Fig. 2. Amino acid sequence homology between Prk of *S. pombe* and RACK1 of rat using the single letter amino acid code. Identities between Prk and RACK1 are marked with vertical bar, similarities with single dots. Homologous residues for PKC binding sequences are underlined. Similar residues were defined by the following rules: A=S=T; D=E; N=Q; R=K; I=L=M=V; and F=Y=W.

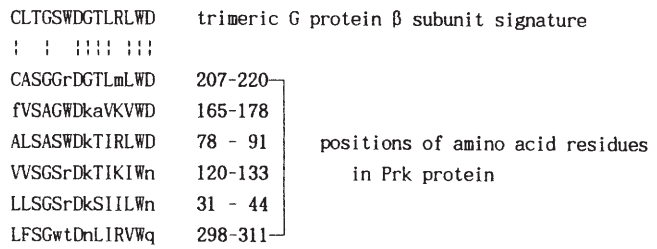

Fig. 3. Trimeric G protein β subunit signature of Prk. Homologous residues to trimeric G protein β subunit signature are marked with capital letters.

one set of filters than the other set of filters.

Phagemid containing the cDNA of *Schizosaccharomyces pombe* was excised from the selected Uni-ZAP recombinant according to the protocol of ZAP-cDNA synthesis kit of Stratagene. The nucleotide sequences of cDNA clones were determined in both directions by the dideoxy sequencing method.

Homology and domain search

The cDNA sequence was compared with the GenBank data base library by homology searching program BLASTP provided by National Institute of Health of USA to compare DNA sequence data with pre-known data base. The presence of trimeric G protein β subunit signatures was identified by the blocks search program provided Fred Hutchinson Cancer Research Center.

Results and Discussion

In the course of study with the genes related to the cell cycle of *S. pombe*, we fortuitously found a cDNA of *prk* gene encoding homologue of the RACK1 of rat from the *S. pombe* cDNA library, which was constructed by inserting the cDNA prepared from cell cycle arrested cells at S phase into phage cloning vector Uni-ZAP XR. Figure 1 shows the nucleotide sequence of the clone we have isolated. The deduced amino acid sequence is 76% homologous to previously reported RACK1 (Fig 2) and also contains the WD motif that are common to β subunit of G protein (20) as shown in Fig. 3. A putative translation start codon (ATG at 8~10) was designated as the methionine start codon, based on the alignment between the single open reading frame of cloned gene and RACK1. RACK1 has the two short peptide sequences homologous to a PKC binding sequence previously identified in annexin I (12) and in the brain PKC inhibitor (KCIP) (1). The two RACK1 peptide sequences, DVLSVAF and DIINALCF, were proven to be directly involved in binding of PKC for its activation (20). The sequences DVLSVSI and ANINALVF homologous to the protein kinase C binding site of RACK1 were also present in Prk at the residues 107~113 and 235~241, respectively (Fig. 2).

Recently two protein kinase C-like genes, *pck1* and *pck2* were cloned from fission yeast. Both proteins lack a putative Ca^{2+} binding domain (C2 region) so that they may belong to the novel protein kinase C group. These genes are essential for cell viability and implicated in cell shape control (22). They showed that *pck2* is allelic to *sts6*, a locus previously identified by its supersensitivity to staurosporine, a potent protein kinase inhibitor. However, since the C2 region is required for the RACK binding (14), we are not sure whether the Prk we have isolated interacts with the Pck1 or Pck2 which does not have C2 region. Therefore, there is possibility that another PKC containing C2 region is present in *S. pombe*. We are in process of examining the function of Prk of *S. pombe* for its possible involvement in PKC mediated signal transduction.

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