A Deduced Thermomonospora curvata Protein Containing Serine/Threonine Protein Kinase and WD-Repeat Domains

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The gene pkwA coding for a typical WD-repeat protein was found in the chromosome of the bacterium Thermomonospora curvata CCM 3352. Until now WD-repeat proteins were thought to be confined to eukaryotes.

Neer et al. (6) have recently reviewed and defined the family of WD-repeat proteins, so called because they carry four to eight repeats of a conserved motif which usually ends in the amino acids tryptophan and aspartate (W and D in the single-letter code). The members of the family (insofar as their functions are known) are regulatory proteins, and WD repeats are assumed to function in protein-protein interactions. Until now these proteins were thought to be confined to eukaryotes.

During work on the isolation and cloning of genes coding for the components of the maltose regulon in Thermomonospora curvata CCM 3352 with the help of degenerate oligonucleotide probes (4a), we isolated a DNA fragment carrying a part of a gene that was apparently not a member of this regulon. However, its deduced amino acid sequence displayed interesting features, predicting a role for this gene product in global regulatory mechanisms of this organism. We have sequenced the whole pkwA gene (pkwA stands for protein kinase with WD-repeat domain) (Fig. 1) from this facultatively thermophilic actinomycete. The gene was cloned on a 3.2-kb SalI-BamHI fragment of chromosomal DNA and is predicted to code for a 742-amino-acid polypeptide. The C terminus of the putative protein has a typical WD structure (Fig. 2): the number of repeats is seven (the typical number of repeats is between four and eight); the spacing between repeats is 11 amino acids (the typical number of amino acids is between 6 and 94); the consensus N-terminal amino acids (GH), C-terminal amino acids (WD), and length of the constant-length core of 26 to 27 amino acids (the typical number is between 23 and 41) are all typical features; and the amino acid composition is also in agreement with the consensus sequence published by Neer et al. (6). Thus, there seems to be no doubt that pkwA encodes a typical WD-repeat protein.

An inspection of the complete amino acid sequence of PkwA revealed the presence of three distinct protein regions. The N-terminal sequence is highly similar to protein kinases of a serine/threonine type and encompasses all 11 domains conserved among this class of enzymes (3). The central spacer region of PkwA is formed by a stretch rich in proline and glutamic acid. In this protein section confined by Glu-301 and Pro-394 there are 46 residues of either Pro or Glu. The C-terminal portion of PkwA is composed of seven recurring WD repeats.

The amino acid sequence of the N-terminal region of the PkwA protein (267 amino acids) shows the highest similarity with the N-terminal portion of the protein kinase AfsK sequence (268 amino acids) from Streptomyces coelicolor A3(2) (5). These regions, which contain all 11 protein kinase conserved domains, are identical in 50% of residues. Both sequences are also similar with respect to their organization into three distinct domains in the protein molecule. AfsK also possesses a spacer domain of a length similar to that of PkwA that contains a number of Pro residues which are accompanied by Ala. The C-terminal portion of AfsK, whose function has not yet been assessed, does not contain any WD motifs; however, we found that a clearly observable recurring stretch of amino acids containing the consensus sequence (L/V)X(A/T)L(D/E/K)(A/V)XGX(R/K)W occurs here five times and that the less conserved motif with L/W at the fourth position even occurs seven times.

Thermomonospora and Streptomyces are related genera (2, 7, 8) characterized by complex morphologies resembling those of filamentous fungi and by their ability to produce a wide variety of secondary metabolites (1). AfsK protein kinase is a part of a control cascade involved in the control of secondary metabolite production. It is likely that PkwA also plays a regulatory role during the organism’s complex growth cycle and in secondary metabolite production.

Two of three domains (kinase and WD-repeat domains) linked in the PkwA protein also resemble the complex of two eukaryotic proteins (protein kinase [PKC] and RACK1) (9). In this complex, the activated protein kinase C is bound to the receptor protein RACK1, composed almost entirely of seven WD repeats. While bound to RACKs, the activated PKCs are translocated from cytosolic into particulate (membrane) fractions. Therefore, the function of the putative PkwA protein could be to interact with a subset of proteins and mediate signal relay by phosphorylation.

The data presented here should be supported by results from the isolation and characterization of the PkwA protein; however, they strongly indicate that WD proteins are also present in prokaryotes. So far only a single example of a protein with a WD-repeat domain and a catalytic domain (protein kinase) in one polypeptide has been described (the Vps15p protein found in Saccharomyces cerevisiae [4]). The resemblance of the domain organization of PkwA protein with that of AfsK from Streptomyces coelicolor A3(2) indicates that these proteins could represent a novel family of bacterial regulators similar to those found so far only in eukaryotes.

While this paper was being reviewed, another example of a prokaryotic WD-repeat protein gene from Synechocystis spp. was released by GenBank under accession number D63999 as the 44th CDS in this entry.

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FIG. 1. Nucleotide and amino acid sequences of the *T. curvata* pkwA gene. The deduced amino acids are aligned below the second nucleotide of each codon of the nucleotide sequence. A putative ribosomal binding site (RBS) is underlined. Restriction endonuclease sites are indicated.

![Image of nucleotide and amino acid sequences](image-url)
The nucleotide sequence of the \( \text{pkwA} \) gene is recorded in GenBank under accession number U72320.

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