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DRpp20 and DRpp40: Two protein subunits involved in *Dictyostelium discoideum* ribonuclease P holoenzyme assembly

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Abstract

Ribonuclease P is an essential enzyme that matures the 5′ ends of all primary tRNA transcripts. RNase P enzymes contain a similar in size RNA subunit which is absolutely required for catalysis. The holoenzyme from *Dictyostelium discoideum* possesses an essential for activity RNA subunit but the exact protein composition is still under investigation. Bioinformatic analysis of *D. discoideum* sequencing data returned seven ORFs homologous to previously characterized RNase P protein subunits from human. In the present study, DRpp20 and DRpp40 were cloned and characterized. These proteins apart from the noted similarity possess idiosyncratic regions. Immunobiochemical analysis presented herein indicates their direct involvement in the formation of the ribonucleoprotein complex of *D. discoideum* RNase P holoenzyme.

Keywords: RNase P; RNA processing; tRNA; Ribonucleoprotein

1. Introduction

Ribonuclease P (RNase P) is one of the few ribozymes of ancestral origin still represented in contemporary cells. RNase P is a ubiquitous and essential ribonucleoprotein that catalyzes a specific endonucleolytic cleavage of a phosphodiester bond thus removing the 5′ leading sequence from all precursor tRNA molecules. It has been found in organisms representing the three kingdoms of life – Bacteria, Archaea and Eukaryotes – as well as in the major subcellular organelles, mitochondria and chloroplasts (Frank and Pace, 1998). RNase P RNA is one of the first catalytic RNA molecules identified (Xiao et al., 2002). Under elevated ionic conditions *in vitro*, the RNA subunit from Bacteria (Guerrier-Takada et al., 1983), some Archaea (Pannucci et al., 1999) is catalytically active in the absence of the protein fraction of RNase P and together with the ribosome is the only known RNA catalysts naturally devoted to act *in trans*. Very recently, evidence have been put forward indicating catalytic activity of the RNase P RNA subunit from human and *Giardia lamblia* (Kikovska et al., 2007).

RNase P enzymes contain a similar in size RNA subunit which is absolutely required for catalysis. Contrary to the RNA, the size and number of protein subunits of the holoenzyme varies significantly, from one small subunit in Bacteria (~10% by mass) (Frank and Pace, 1998), to at least four protein subunits in Archaea (~45% by mass) (Hall and Brown, 2002; Kouzuma et al., 2003; Boomershine et al., 2003; Kawano et al., 2006; Wilson et al., 2006), and up to ten protein subunits in Eukarya (~70% by mass) (Chamberlain et al., 1998; Xiao et al., 2001). This enrichment of the protein fraction in eukaryotes, is thought to represent an evolutionary change in the holoenzyme's structure and function, which still remains not well understood.

Abbreviations: RNase P, ribonuclease P; DRpp20/40, Dictyostelium RNase P, Protein 20/40; Pre-tRNA, precursor transfer RNA; EST, expressed sequence tags; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; IPTG, isopropyl-β-D-thiogalactopyranoside; IMAC, immobilized metal affinity chromatography; Aas, amino acids; MALDI-TOF, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

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The only fully characterized eukaryotic protein complements concern *Saccharomyces cerevisiae* and human RNase P holoenzymes. The first is comprised of nine protein subunits (Pop1, Pop3, Pop4, Pop5, Pop6, Pop7 Pop8, Rpp1 and Rpr2) which show large variations in size (15.5–100.5 kDa) (Chamberlain et al., 1998) and are essential for yeast viability and enzymatic activity (Xiao et al., 2001). Similarly, studies on human RNase P from HeLa cells have revealed the existence of ten protein subunits (hPop1, hPop5, Rpp14, Rpp20, Rpp21, Rpp25, Rpp29, Rpp30, Rpp38 and Rpp40) ranging from 14 to 115 kDa (Jarrous, 2002). At least six of human proteins appear to be homologous to the subunits of *S. cerevisiae* RNase P (hPop1/Pop1, Rpp30/Rpp1p, Rpp29/Pop4, Rpp21/Rpr2, Rpp20/Pop7p, hPop5/Pop5). The relationships between Rpp14, Rpp25 and Rpp40 with the yeast proteins are not clear. The set of eukaryotic protein subunits contains only two acidic proteins, Pop8 (p*I* 4.6) from yeast and Rpp40 (p*I* 5.2) from human while the rest are quite basic (p*I*>9) (Walker and Engelke, 2006). The yeast Pop7, the human Rpp20 and Rpp25 have predicted sequence similarity to the Alba superfamily of proteins which appear to have originated as RNA-binding proteins that formed various ribonucleoprotein complexes (Aravind et al., 2003). The homology of Rpp20 and Pop7 proteins was further supported by Rosenblad et al. (2006). Recently it was shown that Rpp20 and Rpp25 form a heterodimer which regulates their RNA-binding activity, subcellular localization and expression. The Rpp20–Rpp25 heterodimerization is resistant to both high ionic conditions and a nonionic detergent (Welting et al., 2007). Furthermore, Rpp20 was reported to exhibit ATPase activity and to interact with the proteins SMN, Hsp27 and KIAA0065 (Li and Altman, 2001; Jiang and Altman, 2001; Hua and Zhou, 2004).

Dictyostelium discoideum RNase P holoenzyme is a ribonucleoprotein complex, consisted of RNA and proteins essential for catalytic activity. Considering its buoyant density, *D. discoideum* RNase P appears to have the higher protein content among the characterized holoenzymes of eukaryotic origin (Frank and Pace, 1998; Stathopoulos et al., 1995). Although it has been established that this enzyme contains both essential RNA and protein components, very little is known on the exact composition of the ribonucleoprotein complex. Bioinformatics analysis of the *D. discoideum* sequencing data returned seven open reading frames homologous to previously characterized RNase P protein subunits from human. The encoded proteins (Pop1, Pop5, DRpp20, DRpp25, DRpp29, DRpp30 and DRpp40) apart from the noted similarity with their human counterparts bear regions that distinct *D. discoideum* RNase P protein subunits from all others, such as low complexity motifs. The gene of *D. discoideum* RNase P RNA subunit has been identified through phylogenetic comparative analysis by Marquez et al. (2005). Recently we reported the cloning and initial characterization of *D. discoideum* RNase P protein subunit DRpp30 giving a first insight of its structure and possible role in the holoenzyme functions (Vourekas et al., 2007). In this study we describe our experimental approach to investigate the association of the polypeptides encoded by the *drpp20* and *drpp40* ORFs with the RNase P holoenzyme.

2. Materials and methods

2.1. Growth of D. discoideum and partial RNase P purification

Growth of *D. discoideum* cells, cell homogenization, RNase P activity recovery and enzyme assays were essentially carried out as previously described (Stathopoulos et al., 1995). The purification scheme included two steps of anion exchange chromatography (DEAE cellulose, Whatman) and a final purification step by cesium sulfate density gradient centrifugation of concentrated RNase P sample.

2.2. Molecular cloning of drrp20 and drpp40

D. discoideum cDNA encoding proteins homologous to known RNase P protein subunits were identified through keyword searches (Altschul et al., 1997) of the EST data base of the cDNA sequencing project in Japan (http://dictycdb.biol.tsukuba.ac.jp/cDNAproject.html) or by BLAST searches of raw sequencing data at dictyBase (http://dictybase.org/db/cgi-bin/blast.pl) using human homologues. The genes were named according to the human homologues, and the prefix D (*D*ictyostelium) was added to indicate their distinctiveness.

The *drpp20* gene was amplified by PCR with the SSF189 clone containing the complete ORF (acquired from cDNA project in Japan) as a template using the primers FLdrpp20F (5′-CATATGAGCGATACTGAATTCGA-3′) and FLdrpp20R (5′-CTCGAGACATGTTTCTTGAACTTTTAATTGTTG-3′). *Nde* I and *Xho* I sites (underlined) were incorporated to the 5′- and 3′-ends, respectively. The sequence of *drpp20* was deposited in NCBI database (GenBank accession number DQ295794).

The clone CFG414 (ddc13l04) containing an open reading frame of 1272 bp similar to human Rpp40 was likewise identified, and acquired from the same source. The *drpp40* gene was amplified by PCR using the following primer combination: FLdrpp40F (5′-CATATGAGTATTATAAATAATGAAGTACC-3′) and FLdrpp40R (5′-CTCGAGA CAATAATAATCATAAG-TACC-3′). The sequence of *drpp40* was also deposited in NCBI database (GenBank accession number DQ295793). A part of this ORF (591–1161 nts) encoding a polypeptide named Δ*drpp40* with strong antigenic epitopes was amplified by means of PCR using *D. discoideum* genomic DNA as a template and the following set of primers subrpp40F: 5′-CATATGCAAAGATACATCATCACATTGG-3′ and subrpp40R: 5′-CTCGAGAGAGATTGGAGTATCAGCG-3′. *Nde* I and *Xho* I sites (underlined) were incorporated to the 5′- and 3′-ends, respectively.

The PCR products were directly cloned in pCRII-TOPO vector and both strands were sequenced.

2.3. Overexpression and purification of recombinant proteins

The putative ORFs were subcloned into pET29a(+) expression vector (Novagen) carrying C-terminal histidine tag. The recombinant plasmids were verified by restriction digestion and sequencing to ensure that no mutations had taken place.