Nucleotide sequence of a cDNA encoding ribosomal protein P0 in *Dictyostelium discoideum*:

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Ribosomal protein P0 is the eukaryotic equivalent to *E. coli* L10 through which L7/L12 bind to ribosomes (1). Results of a chemical cross-linking study made with *Artemia* ribosomes indicated that P0 forms a pentameric complex by interaction with dimers of P1 and P2 (2). It has been also reported that human P-proteins newly synthesized *in vitro* form similar pentameric complexes which were incorporated by preexisting 60S ribosomes (3). The primary structure of P0 from human ribosomes (3) and from *Saccharomyces cerevisiae* (4) have been already determined, revealing that P0, P1 and P2 share nearly identical carboxy-terminal sequences. This sequence contains an epitope which is recognized by autoantibodies in some patients suffering from systemic lupus erythematosus (5).

We report the nucleotide sequence of a cDNA clone encoding the complete amino acid sequence of *Dictyostelium discoideum* ribosomal protein P0. The cDNA was isolated by immuno screening of a expression library, constructed in lambda gt11 with cDNAs obtained from *Dictyostelium discoideum* (strain AX- 3) 4 hours-starved cells. The screening of the cDNA library was performed using polyclonal antibodies, raised against low molecular weight ribosomal acidic phosphoproteins P1 and P2 from *Dictyostelium*, assuming that these proteins share common epitopes with P0. The insert identified in a lambda phage positive clone was subcloned into pUC plasmid and both strands were sequenced by the chain termination method. The cDNA was 970 nucleotides in length and contained the translation start and stop codons. It also has a 5' noncoding sequence of 10 bases, an open reading frame of 915, and a 3' noncoding sequence of 42. The open reading frame encodes a protein of 305 amino acid residues.

The primary structure predicted from the cDNA sequence reported here was identified to correspond to *Dictyostelium* ribosomal protein P0 by its close relationship to the two eukaryotic equivalent proteins reported to date. A pairwise comparison of identical residues shows that *Dictyostelium* P0 has 50% homology with human P0 (3), and 42% with yeast P0 (4).

Dictyostelium P0 has a molecular weight (33,013) which is smaller than the molecular weight that has been determined for the other species (about 38,000). Like other P proteins, it has a large number of alanines, 38, representing 12.5% of the total amino acid composition; 15 of these residues are clustered within amino acid positions 257–283, constituting an alanyl-rich domain next to the C-terminal region. *Dictyostelium* P0 has an excess of basic residues (32 lysyl, 10 arginyl and 2 histidyl) over the acidic ones (17 glutamyl and 16 aspartyl) and therefore it is actually a basic protein which cannot be included in the ribosomal acidic protein-type, as it seems to be the case in human and yeast (3, 4). P0 is also peculiar in that it lacks tryptophan. In any case,

the alignment of the primary structure predicted for *Dictyostelium* P0 with human P0 and yeast P0 clearly shows a great number of conserved residues which are not dispersed, but rather clustered throughout the protein, revealing the existence of several conserved regions of different lengths. Interestingly, one of these fragments, located between positions 42 and 65 of the *Dictyostelium* P0 sequence, is very rich in basic residues and has been claimed to be involved in the binding to 28S RNA, since it also shows homology with the equivalent region from *E. coli* L10 (4), (see the sequence alignment of this fragment below).

As predicted from the immunological cross-reaction with antibodies raised against *Dictyostelium* P1 and P2, P0 shares common sequences with these two ribosomal acidic proteins. A pairwise comparison with P1 and P2 sequences shows that *Dictyostelium* P0 contains at the carboxyl-end 19 amino acids identical to P2, and 15 identical to P1 (see the alignment of carboxyl-end sequences below). The identity in this region of the sequence implies therefore that *Dictyostelium* P0 also contains the unique C-terminus which is specific for 'A'-type proteins from this organism; it has methionine in position 301, where the other reported P0 have a phenylalanine.

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<i>Dictyostelium</i> P0	N(42)–RKSIRGIGAVLMGKKTMI RKVIR
Human P0	N(43)–RMSLRGKAVVLMGKNTMMRKAIR
Yeast P0	N(41)–RKELRGRAVVLGMKNTMVRRIR
<i>Alignment of putative sequences of interaction with 28S rRNA</i>	
<i>Dictyostelium</i> P0	N(286)–VVEEKKEESDDDDMGMLFD–COOH
<i>Dictyostelium</i> P2	N(87)–VVEEKKEESDDDDMGMLFD–COOH
<i>Dictyostelium</i> P1	N(94)–KE EVKKEESDDDDMGMLFD–COOH
<i>Alignment of carboxy-terminal sequences</i>	