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Oligoribonuclease Is Encoded by a Highly Conserved Gene in the 3′-5′ Exonuclease Superfamily

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Oligoribonuclease, a 3′-to-5′ exoribonuclease specific for small oligoribonucleotides, was purified to homogeneity from extracts of *Escherichia coli*. The purified protein is an α2 dimer of 40 kDa. NH_2-terminal sequence analysis of the protein identified the gene encoding oligoribonuclease as *yjeR* (*o204a*), a previously reported open reading frame located at 94 min on the *E. coli* chromosome. However, as a consequence of the sequence information, the translation start site of this open reading frame has been revised. Cloning of *yjeR* led to overexpression of oligoribonuclease activity, and interruption of the cloned gene with a kanamycin resistance cassette eliminated the overexpression. On the basis of these data, we propose that *yjeR* be renamed *orn*. Orthologs of oligoribonuclease are present in a wide range of organisms, extending up to humans.

Oligoribonuclease is one of eight distinct 3′-to-5′ exoribonucleases present in *Escherichia coli* (4, 12). The enzyme has been partially purified and shown to be highly specific for small oligoribonucleotides (3, 11). In this respect, it differs from all the other exoribonucleases, none of which can hydrolyze RNA molecules shorter than about 5 nucleotides (nt) in length (12). Oligoribonuclease is a processive enzyme that initiates attack at a free 3′ hydroxyl group on single-stranded RNAs, releasing 5′ mononucleotides in a sequential manner (3). Apparently, it is the smallest of the *E. coli* exoribonucleases, with a reported molecular weight of approximately 38,000 (11). Despite this information, and the fact that oligoribonuclease has been recognized for over 20 years (11), nothing is known about its cellular role, largely because of the unavailability of mutant strains deficient in the enzyme and the absence of any information about the gene that encodes oligoribonuclease.

As a first step toward elucidating the in vivo function of oligoribonuclease, we have purified the enzyme to homogeneity, obtained partial amino acid sequence information, and used this information to identify and clone the gene that encodes the protein. We report here that oligoribonuclease is encoded by the open reading frame previously designated *yjeR* (Swiss-Prot database accession no. P39287) or *o204a* (2), located at 94 min on the *E. coli* chromosome. This gene, which we have renamed *orn*, is a highly conserved member of the 3′-5′ exonuclease superfamily (9).

Purification of oligoribonuclease. Oligoribonuclease was isolated from the high-speed supernatant fraction prepared from French press extracts of *E. coli* strain CA265II$^-$, which is deficient in RNase II. Oligoribonuclease activity was monitored by the hydrolysis of [^{3}H]oligo(U) (average chain length, ≈3), essentially as described previously (4, 11). Homogeneous oligoribonuclease was obtained by a purification scheme that included ammonium sulfate fractionation, followed by chromatography on DEAE-Sephadex, hydroxylapatite, Ultrogel AcA54, and Affi-Gel Blue. The final preparation obtained with this procedure contained only a single protein with an apparent molecular mass of 20 kDa (Fig. 1, lane 3) based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Inasmuch as the gel filtration chromatography on Ultragel AcA54 indicated that the native enzyme was approximately 40 kDa in size, in close agreement with the size reported earlier (11), these data suggest that oligoribonuclease is an α2 dimer.

A second, larger-scale purification was also carried out to provide sufficient oligoribonuclease for microsequencing. However, this preparation showed two bands on SDS–12% PAGE in the 20-kDa region. Since it was not possible to conclusively determine which of the two proteins corresponded to oligoribonuclease, both bands were transferred to an Immobilon-P membrane and washed and stained according to the procedure of LeGendre and Matsudaira (10).

Although the two purifications of oligoribonuclease carried out as part of this study resulted in either homogeneous or highly purified enzyme, our experience with the procedure is not sufficient to warrant a detailed description here. A more complete description of the purification protocol will be presented elsewhere once it has been refined.

N-terminal sequence analysis. Gas-phase sequencing on an Applied Biosystems 491 protein sequencer was used to determine the amino-terminal residues of each of the two purified proteins. For one band, the sequence was determined to be MTATAQQ which, based on a TFASTA analysis of the GenBank database (6), corresponded to the first seven amino acids of adenine phosphoribosyltransferase, encoded by the *apt* gene at 10 min on the *E. coli* chromosome. The amino-terminal sequence of the second band was found to be SANENNLI WIDLE, which was identical to residues 25 to 37 of a hypothetical protein encoded by *yjeR* (*o204a*) (2), an unidentified open reading frame located at 94 min on the *E. coli* chromosome. Based on this sequence information, we propose that the reading frame of *yjeR* be corrected. It is likely that the true coding region actually begins at the methionine in position 24 rather than at the methionine 23 residues upstream, as originally suggested (2). In addition to the protein sequence data, there is no Shine-Dalgarno sequence upstream of the first potential initiator codon, whereas one is present before the

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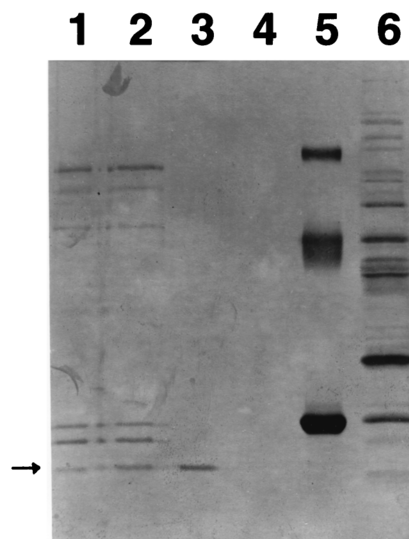

FIG. 1. SDS-PAGE of purified oligoribonuclease. Samples from the Affi-Gel Blue column were concentrated 5- to 10-fold with a Centricon-10 membrane and analyzed by SDS-PAGE on a 12.5% gel. Lane 1, Affi-Gel Blue flowthrough; lane 2, Affi-Gel Blue wash; lane 3, Affi-Gel Blue oligoribonuclease peak activity fraction; lane 4, Affi-Gel Blue fractions before activity peak; lane 5, standards; bovine serum albumin (68,000 Da), ovalbumin (44,000 Da), and chymotrypsinogen (24,500 Da); lane 6, DEAE-Sephadex combined activity peak fractions. The arrow indicates the position of the oligoribonuclease subunit.

codon specifying methionine 24. Assuming the latter translation initiation site, only a single methionine residue would have to be removed to generate the NH_2-terminal sequence actually observed for the purified protein. The resulting 180-amino-acid protein would have a calculated molecular weight of 20,684, in close agreement with the SDS-PAGE data.

Cloning of the gene encoding oligoribonuclease. To determine which of the two genes, *apt* or *yjeR*, is responsible for oligoribonuclease activity, each of them was cloned into plasmid pUC19 for measurement of oligoribonuclease overexpression. To accomplish this, clones 12H5 (no. 152) and 3H6 (no. 651) from the *E. coli* genomic library of Kohara et al. (8), containing *apt* and *yjeR*, respectively, were subjected to PCR amplification. Primers, containing linkers cleavable by the restriction enzymes *Xba*I and *Kpn*I, were designed to flank the *apt* and *yjeR* genes. After 30 cycles with *Taq* DNA polymerase, the resulting *apt* (0.86 kb) and *yjeR* (1.3 kb) PCR amplification products were each digested with the restriction enzymes *Xba*I and *Kpn*I and cloned into the high-copy-number vector pUC19. The resulting plasmids, pAT19, carrying the *apt* gene, and pYJ19, carrying the *yjeR* gene, were each transformed into strain CA265II$^-$ for subsequent measurement of oligoribonuclease activity.

Cells were grown in yeast extract-tryptone medium to an A_{550} of ≈1. Cultures (50 ml each) were centrifuged, and cells were resuspended in a solution of 1.5 ml of Tris-HCl (pH 7.9) (10 mM), $MgCl_2$ (10 mM), NH_4Cl (20 mM), and glycerol (10%). Extracts were prepared by sonication, and cellular debris was removed by centrifugation. An assay of the extracts for oligoribonuclease activity (Table 1) revealed that overexpression was associated only with the presence of plasmid pYJ19 carrying the *yjeR* gene. Overexpression values as much as 50-fold more than those for the pUC19 vector alone were obtained by this procedure. To further substantiate the encoding by *yjeR* of oligoribonuclease, pYJ19 was cleaved with *Bam*HI, and the 4-nt recessed 3′ termini were filled in with the Klenow fragment of DNA polymerase I to produce blunt ends. The kanamycin resistance cassette from plasmid pUC4K (Pharmacia), treated in the same manner, was inserted into *yjeR* by blunt-ended ligation. The resulting plasmid, pYJ-Kan, when grown in strain CA265II$^-$, did not lead to overexpression of oligoribonuclease activity (Table 1). These data show that the 1.3-kb fragment carrying *yjeR* is directly responsible for oligoribonuclease overexpression. On the basis of this finding and on the finding that one of the two proteins in the highly purified oligoribonuclease preparation is YjeR, we conclude that *yjeR* encodes oligoribonuclease and propose that it be renamed *orn.*

TABLE 1. Association of oligoribonuclease activity with the *yjeR* gene

Expt no.	Plasmid present	Oligoribonuclease activity (nmol/20 min/mg of protein)[a]
1		
	pUC19	95
	pAT19	84
	pYJ19	5,500
2		
	None	32
	pYJ19	3,900
	pYJ-Kan	28

[a] Extracts were prepared from strain CA265II$^-$ as described in the text. Assays were carried out for 20 min at 37°C with ≈1 μg of cell extract with [^{3}H]oligo(U) (average chain length, ≈3) as substrate. Assay conditions and analysis of the products were as described previously (3).

Sequence analysis of *orn* and oligoribonuclease. Based on the NH_2-terminal sequence information presented above, the coding region of the *orn* gene encompasses a region of 543 nt that begins with an AUG initiator codon and ends at a UAA termination codon. Four to ten nucleotides upstream of the AUG codon is a purine-rich region that could serve as a Shine-Dalgarno sequence. Immediately upstream of *orn* is an unidentified open reading frame, *yjeQ* or f337 (2), that is transcribed in the opposite direction. Approximately 135 nt of intergenic spacer is present between the *orn* and *yjeQ* coding sequences. Interestingly, no recognizable σ^{70} promoter sequence is found within this region. Whether *orn* might be under the control of a different sigma factor and where its transcription start site is located remain to be determined.

The *orn* gene would encode a polypeptide of approximately 20,700 Da, in excellent agreement with the size of the purified protein by SDS-PAGE (Fig. 1). This subunit is the smallest in the known *E. coli* exoribonucleases. Based on its deduced amino acid sequence, oligoribonuclease is an acidic protein with a pI of approximately 5.0. However, the distribution of acidic and basic residues is distinctly nonrandom. While the N-terminal half of the protein is extremely acidic, the C-terminal half is basic. This observation, coupled with the fact that all the aromatic amino acid residues (F plus Y) are located in the C-terminal portion of the protein, strongly suggests that this part of oligoribonuclease contains its RNA-binding domain.

Of considerable interest, and as recently pointed out by Koonin (9), oligoribonuclease is a highly conserved protein and a member of the superfamily of 3′-5′ exonucleases. Although it is now clear that YjeR is an oligoribonuclease, rather than a DNA exonuclease as suggested previously (9), its function is entirely consistent with those of other members of this superfamily. Based on BLASTP searches (1) of the GenBank and GenBank EST databases, the subfamily of oligoribonuclease orthologs includes members from *Haemophilus influenzae*,

Mycobacterium leprae, *Mycobacterium tuberculosis*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans* (and two other nematodes, *Brugia malayi* and *Pristionchus pacificus*), and *Drosophila melanogaster* and mice, rats, and humans. Orthologs were also found in *Neisseria meningitidis* (7a) and *Neisseria gonorrhoeae* (11a). The degree of identity between the *E. coli* and the higher eukaryotic homologs approaches 50%, suggesting that oligoribonuclease function may have been highly conserved among all of these species.

The cellular role of oligoribonuclease is not yet known. Its catalytic properties in vitro suggest that its function is limited to the removal of small oligoribonucleotides, perhaps those left over during the degradation of mRNA. It is interesting that oligoribonuclease orthologs are not present in the sequenced archael genomes, in mycoplasma, in the cyanobacteria *Synechocystis* species, or in *Bacillus subtilis*. It is known that the mode of mRNA degradation in *B. subtilis* differs from that in *E. coli* (reference 5 and references therein), and this may account for its lack of an oligoribonuclease requirement. Preliminary attempts to incorporate an interrupted *orn* gene into the *E. coli* chromosome by linear transformation with pYJ-Kan have so far been unsuccessful (7), raising the possibility that oligoribonuclease is an essential enzyme in *E. coli*. Studies to conclusively answer this question are currently under way.

Nucleotide sequence accession number. The new information regarding the size of YjeR has been deposited in the Swiss-Prot database (accession no. P39287).

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