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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION: (U)	1b RESTRICTIVE MARKINGS: NA
	3 DISTRIBUTION / AVAILABILITY OF REPORT: Distribution Unlimited
AD-A212 667	5 MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a NAME OF PERFORMING ORGANIZATION: Southern Illinois University at Carbondale	6b OFFICE SYMBOL (If applicable): NA
6c ADDRESS (City, State, and ZIP Code): Dept. of Chemistry & Biochemistry, Carbondale, Illinois 62901-4409	7a NAME OF MONITORING ORGANIZATION: Office of Naval Research
	7b ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000
8a NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research	8b OFFICE SYMBOL (If applicable): ONR
8c ADDRESS (City, State, and ZIP Code): 800 N. Quincy Street, Arlington, VA 22217-5000	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-86-K-0739
	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO: 61153N; PROJECT NO: RR04106; TASK NO: 441d-010; WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)

(U) Structure and expression of various RNAs in the Archaebacteria

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 09/88 TO 08/89	89/09/05	2

16 SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Archaebacteria, Thermophiles, tRNA, Intron, Modified Nucleosides, tRNA-methyltransferases

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

A novel transfer RNA gene of Sulfolobus has been cloned. It has a large "D-loop" containing 27 residues and appears to code for glutamic acid tRNA. The sequence CCGGU occurs twice within the "D-loop". Two symmetrical cuts within or just outside these sequences can produce a typical tRNA and an 18-base "intron". The gene occurs as a single copy in the genome and is transcribed. It is being determined that whether this "intron" is actually spliced out or it remains as a part of the mature tRNA.

In addition, an approximately 9 Kb EcoRI genomic fragment of Sulfolobus, containing genes for two tRNA-methyltransferases has been cloned. The tRNAs isolated from E. coli cells containing this recombinant plasmid contain N^2-methylguanosine (m^2G) and 1-methyladenosine (m^1A), the modified nucleosides not normally present in E. coli tRNAs. It appears that some Sulfolobus genes are expressed when cloned in E. coli and at least some of its enzymes are functional in the E. coli environment.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
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DD Form 1473, JUN 86 — Previous editions are obsolete — SECURITY CLASSIFICATION OF THIS PAGE (U)

S/N 0102-LF-014-6603

ANNUAL REPORT

CONTRACT NO.: N00014-86-K-0739
R&T Code: 4412010

FULL TITLE: Structure and expression of various RNAs in the Archaebacteria.

ABBREVIATED TITLE: RNAs in Archaebacteria

INSTITUTION: The Board of Trustees of Southern Illinois University, Carbondale, IL 62901

PRINCIPAL INVESTIGATOR: Ramesh Gupta
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PERIOD OF REPORT: September 1988 - August 1989

Summary of Project Goals:

1. Separation and sequencing of various RNAs of thermophilic archaebacteria.

2. Sequencing of various tRNA genes of archaebacteria (mainly thermophiles) and their surrounding regions to determine the organization of these genes and to identify the potential transcription control regions and the transcript processing sites.

3. Identification of the transcription initiation and termination sites in tRNA genes of various archaebacteria.

4. Characterization of RNA processing in thermophilic archaebacteria, initially using small transcripts, e.g., tRNA gene transcripts.

Recent Accomplishments

We have partially sequenced specific regions of some of the previously produced clones containing Sulfolobus tRNA genes. Some of these tRNA genes contain introns while others do not. One of these genes appears to be novel. Its transcript can be folded into a tRNA-like structure, in which the "D-loop" contains 27 residues. It appears to code for $tRNA^{Glu}_{CUC}$ and lacks the 3′-terminal CCA sequence of the mature tRNA. The sequence CCGGU occurs twice within the "D-loop"; the first four bases of which can pair with each other to form a stem. Two symmetrical cuts within or just outside these sequences (i.e., at any of the six pairs of positions) can produce a typical tRNA containing all of the invariant and semi-invariant residues at their respective positions and an 18-base "intron". The gene occurs as a single copy in the genome, as revealed by Southern hybridizations, using an "intron" and both the "5′- and 3′-exon" specific probes. The Northern hybridizations by the two "exon" specific probes indicated that the gene is transcribed. For some of the further studies we have subcloned this tRNA gene in pBluescribe vectors

(Stratagene) in such a way that T7 RNA polymerase produces the transcripts which contain the complete sequence of the tRNA from position 2 through 71 (of the mature tRNA), and all of the "intron" and, in addition, some extra sequences at the 5′ and 3′ ends derived from the multiple cloning sites of the vectors.

In an effort to study the synthesis of the modified nucleosides of tRNAs, we are cloning the genes for archaebacterial tRNA-modifying enzymes. We have isolated a recombinant plasmid containing an approximately 9 Kb EcoRI genomic fragment of Sulfolobus in the pUC 19 vector. This fragment seems to contain genes for two tRNA-methyltransferases. The nucleoside composition of the tRNAs isolated from IPTG (isopropylthiogalactoside) induced E. coli cells containing this plasmid, as analysed by Liquid Chromatography/Mass Spectrometry (LC/MS), revealed the presence of N^2-methylguanosine (m^2G) and 1-methyladenosine (m^1A) in these tRNAs. These two modified nucleosides are known to be present in Sulfolobus, but are not normally present in E. coli tRNAs. It appears that at least some of the thermophilic archaebacterial genes are expressed when cloned in E. coli and that at least some of their enzymes are functional in the E. coli environment.

We have also cloned several tRNA genes of Thermococcus. The structures of these genes are being analyzed.

Plans for next year:

During the remaining period of this contract, all of the above mentioned works will be continued.

Initially, we shall determine whether the "intron" sequence (18 bases) in the above mentioned $tRNA^{Glu}$ gene is actually spliced out or whether it exists as part of the mature tRNA. If it is spliced out, then we shall try to determine how its splicing is different from splicing other introns.

Initially the insert of clone carrying m^2G and m^1A modification activities will be gradually deleted from the ends to determine the minimum size of the insert required to express these activities. These genes and their flanking regions will be sequenced. These genes will then be fused, in phase, to the lac Z gene of the pUC 18 or 19 vector with an aim of producing large quantities of the enzymes required for further studies.

In addition, we shall start sequencing mature tRNAs of Sulfolobus. Initially, Glu isoacceptor will be sequenced to determine whether the above mentioned tRNA has the extra 18 base in the mature tRNA.

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