Isolation and Mapping of a Mutation in *Escherichia coli* with Altered Levels of Ribonuclease H

PHILIP L. CARL,¹ LEONARD BLOOM,² AND ROBERT J. CROUCH^{2*} Department of Microbiology. University of Illinois. Urbana. Illinois 61801.¹ and Laboratory of Molecular.

Genetics, National Institute of Child Health and Human Development. National Institutes of Health. Bethesda. Maryland 20205² was isolated after mutagenesis with ethyl methane sulfonate. A procedure for

Vor. 144 1980	MUTATION IN E. COLI WITH	H ALTERED RNase H29
	resulting [³² P]bolv(A	A) was separated from unincorpo-
		м- с. 4 0-
₩		
· · · · · · · · · · · · · · · · · · ·		
Recterial and phage strains. Stra	ins of <u>E_coli</u> column (1 bv 60 cm)	which had been previously equil-
	· · · · · ·	
		1 6 1
t		
·		

30 CARL, BLOOM, AND CROUCH

J. BACTERIOL.

. . . .

e"	then added. and incubation was continued for 10 min.	terminations were used to adjust the size of aliquot to
		and the second s
. <u>.</u> .	To freeze-thaw the extracts, the travs were placed in	be assaved (0.2 ug of protein per 25-ul assav). Acid
žer.		
	a slurry of dry ice-ethanol and then placed in a water	solubility was determined as described previously (13).
-	bath at room temperature. When thawing was com-	Type C was based on the reconstitution of
é <u>. </u>		
20.		
	plete. the travs were returned to an ice-water bath.	RNase H in SDS-polvacrylamide gels containing
4 		
· · · · · · · · · · · · · · · · · · ·		
)E		
1		
Li cin Ita		
₩		
<u>1</u>		
<u>.</u>		

		vme-EDTA extracts of 14 metD ⁺ transductants
• • • •		
and KS351 (parent) an	d FP9 (mutant) strains were electron	oresed in a 15% SDS-polvacrylamide gel which
<u>una Kös</u> on (parenti an	a EB2 (matant) strains were electronn	oresea in a 13% SUS-Robact Viamute vel uniter
·		
· · · · · · · · · · · · · · · · · · ·		
1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1		
•		

chromosome. P1 transduction experiments	
۹ جناب می است	
t	_
1	
	1
A	_
<u> </u>	
<u>,</u>	_
-	_/
•	
	ب

i

<u>د.</u>		4
- ž		21.1. ty
- 5		
ч ,		
J 4		
		A state of the sta
L	** ****	
-		
	. 11	norma (17). Our nomita with a CalE1 time
	. 11	nome (17). <u>Our nomite with a Ca</u> IE1 time
	1	nonna (17). Our manita mith a CalEl tima
		nomo (17) <u>. Our nomite with o Co</u> IFI timo
· · · · · · · · · · · · · · · · · · ·	. 11	nome (17). <u>Our nomite mith e Co</u> IE1 +ma
· · · · · · · · · · · · · · · · · · ·	. 11	nome (17). Our nomite with a CalEl time
· · · · · · · · · · · · · · · · · · ·	. 11	nonno (17). Our manita mith o ColD1 timo
	. 11	nome (17)_ <u>Our nomite mith o</u> ColEl time
	. 11	
	1	noma (17)_ <u>Ouw somiles with a Cal</u> El tima
	. 11	nome (17)_ <u>Our nomite mith o</u> ColEl +ime
	- 11	nome (17). Our nomite mith o ColEl time
	- 41	
		nome (17)

<u></u>	34	CARL, BLOOM. AND CRO	UCH		J. BACTERIOL.
· · ·					
······································		&) »			
		-			
<u></u> -					
Ŧ					
<u>);</u>		<u></u>			
# 					
_	117 A	hangh Mirkaal Drawn far his darrata		ase H, and DNA polymerase	I. Cold Spring Harbor
			•		
na kana na s					
·····					
Å					
				<u></u> }	
, <u> </u>					
×					
i)a					
-					

gene for exonuclease III in Escherichia coli K-12. J. ribonucleases H from yeast cells. Eur. J. Biochem. 69:

Bacteriol. 126:1082-1088. Z. Wyers. F. A. Sentenac. and P. Fromagoet. 1973. Role	377-383. <u>39. Zipkas. D., and M. Rilev. 1975. Proposal concernin</u>
•	
of DNA-RNA hybrids in eukarvotes: ribonuclease H in	mechanism of evolution of the genome of Escherichi
-	

yeast. Eur. J. Biochem. **36:**270-281. 38. Wyers, F., A. Sentenac, and P. Fromagoet. 1976. Role of DNA-RNA hybrids in eukaryotes: purification of two coli. Proc. Natl. Acad. Sci. U.S.A. 72:1354-1358.

Zubay, G. 1973. In vitro synthesis of protein in microbial systems. Annu. Rev. Genet. 7:267-287.