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NOTES

Localization of the *Escherichia coli rnt* Gene Encoding RNase T by Using a Combination of Physical and Genetic Mapping

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The *rnt* gene encoding RNase T was cloned on a 13-kilobase *Bam*HI fragment. Restriction analysis of the fragment and comparison of it with the *Escherichia coli* restriction map localized *rnt* to kilobase coordinates 1733 to 1746, corresponding to about 36 min on the genetic map. The map location was confirmed by cotransduction with the nearby *zdg-229*::Tn*10* and *ksgB1* markers.

RNase T is one of seven exoribonucleases that have been identified in extracts of *Escherichia coli* (2). It has been purified to homogeneity and is an α_2 dimer with a molecular weight of 50,000 (3). In vitro RNase T is highly specific for mature tRNA, releasing AMP and tRNA-C-C as products (3, 4). Studies of a mutant strain that lacked 70% of the RNase T activity indicated that RNase T is responsible for the end-turnover of tRNA that occurs in all cells (5). This process, the physiological role of which is unclear, consists of the removal and readdition of the terminal AMP residue of tRNA and requires RNase T and tRNA nucleotidyltransferase (1).

Since the *rnt* mutant was isolated in a background that lacked several other RNases (5), it has not been possible to study the effect of this mutation in isolation. For this reason, and also to obtain a more defective mutant than the one available, we wished to map the location of the *rnt* gene on the *E. coli* chromosome. Our recent cloning of the *rnt* gene has given us the opportunity to do this simply by comparing its restriction map with that of the restriction map of the total *E. coli* chromosome (8).

The *rnt* gene was cloned by screening a cosmid library containing large fragments of the *E. coli* genome (~40 to 50 kilobases [kb]) in plasmid pHC79 (7) for transformants with elevated levels of RNase T. For this purpose, an *E. coli* strain, 20-12E/18-11 (RNase I^-, II^-, D^-, BN^-, T^-), that was deficient in multiple RNase activities (5), including RNase T, was used as the recipient; and extracts of transformants were initially combined in groups of three for RNase assays. A similar strategy was used previously for the cloning of the *rnd* gene encoding RNase D (11).

RNase T activity was determined by measuring the release of [^{14}C]AMP from tRNA-C-C-[^{14}C]A as described previously (4). Assays for RNase D and RNase II, two other known *E. coli* exoribonucleases that act on tRNA, were carried out by using ^{32}P-labeled diesterase-treated tRNA and ^{3}H-labeled poly(A), respectively, as described earlier (10).

By using these procedures, 200 transformants were screened; and 2 transformants with RNase T activity elevated to about twice the wild-type levels were identified. To ensure the presence of elevated activity in these clones, plasmid DNA was isolated from each and was used to

TABLE 1. Substrate specificity of extracts from putative *rnt* clones

Substrate	Relative activity of extract from strain[a]		
	20-12E/18-11	Clone 1	Clone 2
tRNA-C-C-[^{14}C]A	33	167	157
^{32}P-labeled diesterase-treated tRNA	2	2	13
^{3}H-labeled poly(A)	8	16	7

[a] Activities in extracts of wild-type strain CA265 were set at 100. Hydrolysis of tRNA-C-C-[^{14}C]A is diagnostic for RNase T, hydrolysis of ^{32}P-labeled diesterase-treated tRNA is diagnostic for RNase D, and hydrolysis of ^{3}H-labeled poly(A) is diagnostic for RNase II.

TABLE 2. Restriction pattern of the *rnt* clone and comparison with the *E. coli* map

Restriction nuclease	Measured size (kb) of restriction fragment[a]	Expected size (kb) of restriction fragment[b]
*Bam*HI	13.0	12.6
	7.2	6.4
*Hin*dIII	~19	~19
*Eco*RI	~19	~19
*Eco*RV[c]	6.1	
	6.1	
	3.9	
	2.4	2.3
*Kpn*I	~19	~19
*Pst*I	8.4	8.0
	4.3	4.4
	4.3	4.1
	2.3	2.3
	0.8	0.7
*Pvu*II	6.0	5.6
	4.5	4.2
	2.3	2.2
	2.0	2.1
	Several other small fragments	Several other small fragments

[a] Determined on a 0.8% agarose gel and compared with lambda DNA digested with *Hin*dIII; it includes nucleotides from the pHC79 vector.
[b] Based on the region between positions 1733 and 1746 in kilobase coordinates from the map of Kohara et al. (8).
[c] Incomplete map in this region (8).

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TABLE 3. Cotransduction of markers in the *rnt* region

Cross	Strains and relevant markers[a]		Selected marker	Unselected marker	No. of colonies with unselected marker/ no. of colonies with selected marker	Cotransduction frequency (%)
	P1 *vir* donor	Recipient				
1	FS173 (Ksg^r)	18-11 Ksg^s	Ksg^r	*rnt*$^+$	8/14	57
2	G176 (*zdg-299*::Tn*10*)	18-11 (Tet^s)	Tn*10*	*rnt*$^+$	6/10	60
3	18-11 (*zdg-299*::Tn*10 rnt*$^+$)	18-11 (Tet^s)	Tn*10*	*rnt*$^+$	6/10	60
4	18-11 (*zdg-299*::Tn*10 rnt*)	CA265 (Tet^s)	Tn*10*	*rnt*	6/10	60

[a] Ksg^r, Kasugamycin resistant; Tet^s, tetracycline sensitive; 18-11, strain 20-12E/18-11, which was RNase T$^-$.

retransform strain 20-12E/18-11. The five colonies picked from each transformation all showed elevated RNase T activities, confirming that they carried the appropriate gene. That the elevated RNase activity was RNase T was verified by determining the specificities of extracts prepared from the two clones (Table 1). Only RNase T activity was significantly elevated (~fivefold) in extracts of the cosmid clones compared with the residual activity in extracts of the recipient strain 20-12E/18-11; the RNase T activities of the clones were also elevated compared with that in the wild-type parental strain CA265, indicating the presence of about two cosmid copies of the *rnt* gene. There was no increase in either RNase D or RNase II activity in these clones. Furthermore, upon subcloning of the *rnt* gene as a 2.5-kb fragment in pBR328, we obtained levels of RNase T equal to 10 to 15 times that in the wild type (data not shown).

One difficulty in the study of strains carrying the cloned *rnt* gene has been their slow growth. Although this has not yet been studied in detail, it is clear that elevated levels of RNase T are deleterious to *E. coli* and that, with continued growth, cultures become enriched for cells in which the elevation of RNase T activity is decreased. This was already suggested from the data in Table 1, in which the elevation of RNase T activity compared with that in the wild type was less than twofold, although plasmid pHC79 with a large insert should be present at about five copies (7). Nevertheless, despite these difficulties, it was possible to subclone the *rnt* gene in pHC79 by using elevated RNase T activity as the assay.

The *rnt* gene from clone 1 was subcloned by digestion with *Bam*HI and religation into pHC79. Elevated RNase T activity was associated with an ~13-kb fragment that was also resistant to cleavage by *Eco*RI and *Hin*dIII. The fact that the fragment was insensitive to a variety of restriction nucleases suggested that a more detailed analysis and comparison with the map of Kohara et al. (8) of the total *E. coli* chromosome might provide the location of the *rnt* gene. The *Bam*HI fragment in pHC79 was digested with seven of the eight restriction nucleases used by Kohara et al. (8) to give the pattern listed in Table 2. Comparison with the *E. coli* restriction map revealed that there was only a single region of the genome that would be expected to generate the pattern observed (Table 2) and that the match was extremely close. This region was located between positions 1733 and 1746 in kilobase coordinates on the restriction map, corresponding to about 36 min on the genetic map (8).

To ensure the accuracy of this assignment, transductional analysis was carried out by using nearby markers. The *rnt* gene was cotransducible at about 60% frequency with both *zdg-299*::Tn*10* (6) and *ksgB1* (9) (Table 3, crosses 1 and 2). Furthermore, placement of the Tn*10* transposon near *rnt* allowed transfer of the mutant and wild-type alleles to other strains with identical cotransduction frequencies (Table 3, crosses 3 and 4). These results confirm the map location of *rnt* at about 36 min and allowed us to separate the known *rnt* mutation from the RNase-deficient background so that it might be studied in isolation. The *rnt* gene was also subcloned to a smaller restriction fragment for further analysis. The availability of the *rnt* clone and knowledge of its map position should permit us to isolate a more defective RNase T mutant and to transfer it to various genetic backgrounds.

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