Rna processing and synthesis

Processing of t RNA, r RNA andsmall RNAs

To form physiologically active RNA molecules, bacteria process the primary transcripts for r RNA and t RNA.

The newly synthesized RNA molecule i. e the primary transcripts differ from the physiologically active r RNA and t RNA molecules in three important aspects:

- Mature r RNA and t RNA molecules are terminated by a 5’ – monophosphate instead of the expected triphosphate which is found at the ends of all the primary transcripts.
- The primary transcripts are much larger than both the r RNA and t RNA molecules.
- Except the unusual bases (A, G, C and U) which are not present in the original transcript, the t RNA molecules contain all the other bases.

After transcription all these molecular changes are made by processes which are being collectively termed as the post transcriptional modifications or also being as processing.

r RNA processing

Bacterial ribosomes consists of 16s r RNA (1541 nucleotides), 23s r RNA (2904 nucleotides) and 5s r RNA (120 nucleotides) respectively. From a continuous transcript having more than 5000 nucleotides of a known sequence, these molecules plus several t RNA molecules are cleaved. The seven r RNA operons which are present in E. coli differ by the location of the t RNA sequences with respect to the r RNA sequences and also by the

identity of the t RNA molecules. A diagram of one of the transcripts is shown below which contains four different t RNA molecules and the segments are from 5’ to 3’ end respectively.

In the general pattern, 16S-spacer-23S-5S-spacer, in which in the spacer regions is the t RNA, which is being retained in the primary transcripts of the other r RNA operons, but there is variation in the number of t RNAs.

The t RNA transcript as it is being synthesized is cut by several enzymes that act in sequence. The enzyme RNase III ordinarily makes the first cuts, which then cleaves the double stranded RNA molecule in the double stranded stem regions therby making two single strand breaks in complementary sequences but not opposite one another. A 3’-OH group and 5’-P group are being generated but they are not the termini of the r RNA.

To complete the processing several enzymes are being required. The processing sequence is different in all r RNA transcripts or in all bacteria, but the basic excision pattern of all r RNA components from a single precursor is a general phenomenon.

This mechanism provides a constant ratio of the 16S, 23S, and 5S r RNA molecules, although the RNA sequences are discarded which seems somewhat wasteful. If each of these molecules were transcribed separately the efficiency would rather demand some means that would be needed to maintain a 1: 1: 1 ratio of the above mentioned molecules because one molecule of each of these three is present in the ribosome and that these molecules are used nowhere else in the cell.

RNA molecules are very stable once they are present in the ribosomes. The t RNA molecules which are mature are also very stable.

The primary transcripts for t RNA are also being processed by the bacterial cells. From the point of view of synthesis, a well understood t RNA molecule is the E. coli t RNA tyr1 molecule, a molecule containing a known sequence of 85 nucleotides.

The E. coli genome consists of two copies of the t RNA tyr1 gene, the t RNA is transcribed from these two identical adjacent copies of the DNA. Each gene consists of about not 85 but 350bp separated by a 200bp “ spacer”. The single RNA molecule is a combination of two genes that is cut up once after the transcription is complete. Genetic techniques have being used to create a transcription unit containing only a single 350-bp gene, in order to simplify the study of the synthesis of t RNA tyr1.

The transcription start site is present 41bp upstream from the 5’end of the t RNA base sequence and a stop site which is present 22bp downstream from the 3’ terminus end of the t RNA.

The processing of the primary t RNA transcript is done by a series of steps which may be grouped into three major stages:

- Formation of the 3’-OH terminus: this process mainly involves the action of endonuclease which recognises a hairpin loop and a three base sequence CCA which is recognised by an exonuclease.

After digestion at site 1 by endonuclease, the seven bases which are present upstream are being removed by an exonuclease termed as RNase D. Initially

this enzyme stops two bases short of the CCA terminus, though later on it then removes these two bases after the processing of the 5’ terminal is completed. This leaves a molecule which is called pre-t RNA which is easily isolated from E. coli and that which has a structure as follows

5’P-(41 bases) –t RNA-(2 bases)- 3’OH

- Formation of the 5’-P Terminus: an enzyme called as RNase P forms the 5’-P Terminus, which in all E. coli. t RNA molecules is probably responsible for generation of the 5’-P Terminus. The evidence for the above comes from the studies with the E. coli mutant in which at temperatures that of 42 degree Celsius, the enzyme RNase P is inactive. Large RNA molecules accumulate that contains the t RNA sequence and the hairpin loop if at all this mutant is grown at 42 degree Celsius. With the help of an endonucleolytic cleavage, excess of RNA is removed from the 5’ end of the precursor molecule by RNase P that generates a single fragment and a correct 5’ end.

Specific base sequence at the cleavage site or anywhere else is not recognised by RNase P, but instead it still responds to the overall three dimensional conformation of the t RNA molecule with its several hairpin loops and at the right place it makes a cut. RNase D removes the two 3’ terminal nucleotides while leaving the t RNA molecule having correct length, once the 5’ terminus has been formed. An unsual enzyme RNase P contains 14% protein by weight and 86% RNA. Furthermore the protein serves to ensure the correct folding of the RNA and the RNA possesses the catalytic activity, in order to maximize the catalytic activity.

- Production of modified bases: the final modification step includes the production of the altered nucleosides in the t RNA. The necessary changes are being produced by the enzymes that act only on nucleosides at specific positions in t RNA. The following are among the posttranscriptional changes that take place in t RNA: uridines are being converted to pseudouridine, dihydrouridine, ribothymidine and 4-thiouridines (4t U); guanosine being is converted to 2’-O-methylguanosine (2m G); and adenosine is converted to isopentenyladenosine (2pi A).

All the t RNA molecules are being terminated by CCA-3’-OH. The precursor molecule contains the above sequence so that the terminus is generated by an appropriate cut. However , the precursors of some of the t RNA molecules lack a terminal CCA. With the help of these molecules the CCA is added by the enzyme t RNA nucleotidyl transferase.

A single transcription unit contains multiple copies of a particular t RNA molecule; for example, there are presence of four copies of one of the t RNA leu molecules in its precursor molecules. There also a frequent occurrence of different t RNA molecules in a single transcript. Example, one t RNA (thr) and one t RNA (ser) are present in a single unit in E. coli.