Stoperators in Cluster A Bacteriophages

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Bio:

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Abstract:

Short genetic elements called stoperators may be involved in the control of lysogeny which is a process by which some bacterial viruses, also known as phage, can insert themselves into the host’s chromosome. Stoperators have been identified in several Cluster A phage, and this research analyzed the genome of Mycobacteriophage ElTiger69, a subcluster A5 phage isolated and annotated at the University of North Texas, to identify stoperator sequences.
Background

Lysogeny in a temperate bacteriophage is dependent on the ability of the lytic genes in its genome to be switched off. These lytic genes, if transcribed, allow the prophage to go through the process of lysis, ultimately killing the host. On the other hand, if the genes are made transcriptionally silent, then the prophage will experience the lysogenic cycle of growth. In order for these genes to be made silent, the bacteriophage has a specific repressor gene that binds to multiple sites throughout the genome. These sites, which are 13 bp long and located in specific intergenic regions, are called stoperators. When the repressor gene binds to these sites, it exerts a strong polar effect on downstream gene expression, thus making the prophage transcriptionally silent. The bacteriophages of cluster A all have these stoperator sites within their genome, with a consensus sequence varying by only a few nucleotide bases. Most of the research pertaining to stoperators has been done using mycobacteriophage L5, which belongs to cluster A2. Taking a look at relatives of L5 in the A cluster and their potential stoperator sites will perhaps elucidate the mysteries as to how exactly lysogeny works in a temperate phage.

Methods and Materials

ElTiger69, a recently sequenced phage, belongs to the A5 subcluster. The entire phage’s sequence was taken from Phagesdb and blasted against itself using BLASTn. This presented a format in which the entire sequence could be analyzed efficiently. Using the consensus sequence of the cluster A phages as a guide, the searching began. Refer to Figure 1. Out of the 13 bases that make the stoperator sequence, only the last 5 are identical throughout all of the phages in cluster A. This portion of the sequence is TCAAG, and provided a great beginning to the search for stoperators in ElTiger69. Using a simple function on the computer, it was possible to search the entire bacteriophage sequence for the fragment TCAAG, knowing that it was present in the stoperator sequence of all other cluster A phages. This lead to compiling a list of all the possible
stoperator sites in ElTiger69, with all of them ending in TCAAG. This process of searching for these sites continued with many other cluster A5 phages, such as Airmid, Benedict, Theia, LittleCherry, and also a cluster A4 phage, Peaches. Timshel, a cluster A7 phage, was also analyzed. While looking for these stoperator sequences, it was also necessary to examine where they were located, in relation to genes. To do this, the program, DNA Master, was used to examine all of the gene locations in ElTiger69. It was possible to locate and pinpoint the location of these sites in relation to the genes surrounding them. The easiest and simplest form of collecting these data was to compile lists of the potential stoperator sites and their locations in a laboratory notebook.

**Results**

After compiling the list of potential stoperator sites in ElTiger69, the results are clear: there are indeed stoperators present in the phage. There are 27 sites in total, which falls within the range of 20-30, the accepted number in cluster A phages. Out of these 27 sites, there are eight that are identical to one another. The other 19 vary from the identical sequence by only a few bases. The identical sites represent the stoperator sequence for ElTiger69, which is GTCCGTTGTCAAG. This varies slightly from the consensus sequence for cluster A phages, by only a few bases. After analyzing the locations of these sites within the genome, a rather odd conclusion was reached. Most of the identical, or “perfect” stoperator sites lie within the region between gene 88 and gene 1 of ElTiger69. This is the region without any genes. Most of the other locations lie within short intergenic regions. The stoperator sequence was identical in all of the cluster A5 phages as well. Benedict had 27 sites, Airmid 25, Theia 30, and LittleCherry 31. All of these phages contained the identical stoperator sequence, GTCCGTTGTCAAG. After compiling the sites in cluster A4 phage Peaches, we found the stoperator sequence to be
GTGCGATGTCAAG. This sequence differs only by the third and sixth nucleotide. The stoperator sequence of cluster A7 phage Timshel differed slightly as well. Its stoperator sequence was discovered to be GTTCGGTGTCAG, different again only by the third and sixth nucleotide.

**Discussion**

The reason why only 8 of the 27 sites in ElTiger69 match the exact stoperator sequence is puzzling. My guess is that over the evolutionary history of ElTiger69, its code was mutated. Slight variations in the sequence may inhibit the gene repressor from binding correctly to the stoperators, and thus destabilizing lysogeny in the prophage. The fact that the A5 phages all share the same stoperator sequence only strengthens the idea that they are uniquely similar to each other. With the A4 phage Peaches and the A7 phage Timshel varying slightly by only two nucleotides, it also shows that although they are slightly different from the A5 phages, they are all still closely related, and thus in the A cluster together. Much more research is required to fully comprehend the mechanics of these stoperator sequences. I would like to do further work studying the stoperator sites in the rest of the A cluster phages, as well as phages from other clusters. Comparing these stoperators in phages across the spectrum can reveal much more knowledge about them and their functions.
References

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Figure 1. ElTiger69 Data