The So-called Chromosomal Verotoxin Genes are Actually Carried by Defective Prophages

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We have carried out an exhaustive search of the literature and databases on DNA sequences of verotoxin genes, most of which are carried by prophages and only two described as chromosomal: one is from Shigella dysenteriae,1 and the other from Escherichia coli.2

A common feature of these cases is the existence of an insertion sequence (IS) upstream of the toxin genes.1-3 The authors of these papers speculate that the IS is probably a component of a composite transposon bracketing the verotoxin genes, and is responsible for moving about of the verotoxin gene within the Shigella or E. coli genome, resulting in many copies of the verotoxin gene within a single genome. However, we think there is another way to look at this close location of an insertion element upstream of the toxin genes (Fig. 1).

On close scrutiny, the region between the IS and the toxin genes has high homology with a phage sequence, strongly suggesting that it is derived from a prophage that included the toxin genes. The region downstream of the toxin genes is also homologous to a phage sequence (Fig. 2). We propose that the IS's were responsible for the defectiveness of the prophage; either by disruption of the prophage by insertion within the prophage, or by DNA deletion starting from one end of the IS outward, extending into part of the prophage.

In other cases in Shigella and E. coli, where the verotoxin genes are believed to reside in the chromosome rather than in the prophage although the DNA sequence of their neighborhood is not yet available, they are unusual in that they are all sensitive to the so-called SOS signals.5-8 This indicates that they are subject to controls similar to a prophage such as the SOS system and the Q gene. Of course, our result does not exclude the possibility that a truly chromosomal verotoxin gene exists in Shigella or elsewhere. But there are currently no such cases in the literature.

References


Figure 1. Location of IS's in *Shigella dysenteriae*, *Escherichia coli* O111:H~ strain PH, and homology of toxin gene neighborhood with phages P22, H-19B and λ. IS2 is the coding region of Shiga toxin, and VT I is that of verotoxin. The region between broken lines A and B shows homology between *S. dysenteriae* and strain PH; the region between C and D is that of strain PH and phage H-19B. Homology between *Shigella* and H-19B includes an overlap of 3 bp in the “right” end of IS. In strain PH a gap of 4 bp is seen from the end of IS to the sequence homologous to P22. According to Neely and Friedman, the region of H-19B and λ shown has high sequence homology (> 90%) except genes Q and S, which, however, have been shown to have homologous functions. Further, they have recognized 41% sequence homology between the Q genes of H-19B and phage 21. The DNA sequence data are taken from GenBank/EMBL/DDBJ database. Their Accession Numbers are: *Shigella dysenteriae*, M24352; *Escherichia coli* O111:H~ strain PH, L0418; P22, X78401; H-19B, AF039873 and λ, J02459, respectively.

Figure 2. Matching between the toxin genes and their neighborhood of *S. dysenteriae* and phage H-19B. Open boxes show the coding sequence of Shiga toxin or verotoxin genes. “S.D.” is Shine-Dalgarno sequence. The 3' end of the *Shigella* sequence shown is the end of the sequence available at present.

Figure 3. The qut genes near the purf promoters of H-19B and *Shigella*. a) Putative qut site near the purf promoter of H-19B. b) Putative qut site and the “toxin” promoter of both H-19B VT I gene and SHT gene. Homology between the DNA sequences just downstream to the respective Q genes of phage H-19B and λ are shown. The purf promoter was assigned using Genetyx, on the assumption that it is located just downstream of the Q gene. The “toxin promoter” of H-19B was inferred from that of *S. dysenteriae* (Fig. 2) identified by Kozlov et al. The two putative H-19B qut sites have 68% and 61% sequence homology with that of λ, respectively.