The cohesive ends of mycobacteriophage L5 DNA

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Mycobacteriophage L5, a temperate phage of the mycobacteria, forms stable lysogens in *Mycobacterium smegmatis* (1). These lysogens contain an L5 prophage integrated into a specific attachment site in the bacterial chromosome (2) and are immune to superinfection by L5. L5 phage particles contain a linear double strand DNA genome which is approximately 50 kb in length (2) which we show here contains cohesive ends.

Gel electrophoresis of endonuclease digested L5 DNA produced altered patterns according to treatment of the sample prior to electrophoresis; if heated to 80°C a 4.75 kb fragment diminished in intensity and fragments of 3.5 kb and 1.25 kb increased in intensity relative to unheated samples (Figure 1). Ligation of L5 DNA prior to digestion eliminated the appearance of the 3.5 kb and 1.25 kb fragments (Figure 1). This behavior is similar to that of lambda DNA (Figure 1) and we conclude that L5 DNA has cohesive termini.

The DNA sequence of the L5 ends was determined by extension of 5' radiolabelled primers annealed to an L5 DNA template. One primer was used for each end of the genome and was extended by addition of deoxyribonucleotide triphosphates and either Klenow, Taq or Sequenase DNA polymerases. Each primer produced one major extension product corresponding to termination at a specific nucleotide position, as identified from an adjacent dideoxy sequencing ladder. Less abundant products were also observed (particularly at the -1 position) presumably resulting from incomplete extension and which varied in intensity depending on the DNA polymerase used. Examination of the DNA sequence at the sites of termination suggested that L5 DNA contains termini with 9-base 3' single strand extensions (Figure 2).

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REFERENCES

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Figure 1. Annealing and ligation of L5 DNA. L5 or lambda (λ) DNA's were digested with Kpn I and BstE II respectively and the DNA fragments separated by agarose gel electrophoresis. DNA's were either ligated (+lig) or not ligated (-lig) prior to digestion and heated at either 37°C or 80°C immediately prior to electrophoresis as indicated. Sizes of the annealed and two denatured terminal fragments respectively are; L5: 4.75 kb, 3.5 kb and 1.25 kb, λ : 14.1 kb, 8.5 kb, 5.6 kb.

Figure 2. DNA sequence of L5 cohesive ends. The left and right ends of the L5 genome are shown, each of which contain complimentary 9-base 3' single strand extensions.

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