Molecular mechanisms of viral DNA packaging initiation: recognition and cleavage of the pac site by bacteriophage SPP1 terminase

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Résumé

Specific recognition of the viral genome is an essential step in the assembly of most viruses, including tailed bacteriophages. In numerous tailed phages, such as SPP1, genome packaging is initiated by recognition and cleavage of a specific sequence *pac*, by the small (TerS) and large (TerL) terminase subunits. Cleavage at *pac* occurs only once, initiating a packaging series along a substrate concatemer. The packaging cycle is terminated by a non-specific sequence cleavage determined by the amount of DNA inside the capsid, yielding packaged molecules longer than the phage genome (headful packaging mechanism). It was previously shown that the SPP1 *pac* region has two sequences where TerS binds (*pac*R and *pac*L) flanking the segment where TerL cleaves the SPP1 DNA (*pac*C). However, the *pac* specific sequences required to achieve this endonucleolytic cut were not established. Their characterization is essential to understand the underlying mechanism. In this study we used a plasmid minimal system encoding SPP1 *pac*, TerS and TerL that mimics specific *pac* recognition and its auto-regulated cleavage in *Bacillus subtilis*. the SPP1 host. We show that the *pac*R sequence localized within 35 bp downstream of the *pac* cut can be extensively degenerated, including its c1 and c2 repeats, and that only disruption of a 5 bp polyadenine tract impairs *pac* cleavage. This result together with deletion analysis of *pac*L shows that the specific DNA sequences required for targeting the terminase for *pac* cleavage are considerably shorter than the large region bound by TerS. Furthermore, extensive degeneration of the 6 bp target sequence within *pac*C where *pac* cleavage occurs, reveals that TerL maintains, remarkably, its precise position of cleavage. Studies with SPP1-related phages show conservation of the cut position, irrespectively of sequence variation in *pac*C, in *pac*R or changes in *pac*L-*pac*C distance. Mechanistically our data are compatible with a model in which TerS interactions with part of the *pac*L sequence and a poly-A tract in *pac*R are sufficient to orient very accurately the TerL nuclease to a defined *pac*C position. They also demonstrate that the resulting precise cut at *pac*C is independent of the targeted DNA sequence.

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