L-21

RELATIONSHIP BETWEEN YEAST VIRUS LIKE ELEMENTS AND POXVIRUSES AT THE TRANSCRIPTIONAL LEVEL

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Yeast cytoplasmic double-stranded DNA virus-like elements (VLEs, also known as linear plasmids) were found in a number of yeast species belonging to nine genera. The *Kluyveromyces lactis* pGKL1/2 plasmids, which serve as archetypes of yeast linear plasmids, are peculiar in many respects. Both plasmids are cytoplasmically localized, possess proteins covalently linked to their terminal inverted repeats and their compact genomes code for 15 genes in total including a killer toxin, two DNA polymerases, an RNA polymerase, a DNA helicase and a capping enzyme. Functions of most of the genes are putative and have not been assigned to them experimentally yet. We and others have found, that the RNA polymerase encoded by pGKL plasmids, as well as the capping enzyme and RNA helicase, are similar to the corresponding enzyme encoded by vaccinia virus (VACV). We have recently described unique structures of yeast VLEs mRNAs, we found that although these VLEs encode their own putative capping enzyme, only a few VLEs genes code for 5'-capped transcripts and that all of the VLE specific mRNAs are not 3'-polyadenylated. Surprisingly, the majority of VLE promoters give rise to uncapped mRNAs starting with short poly(A) sequences at their 5' ends that are not complementary to the plasmid DNA.

Because VLEs show a high similarity of their transcriptional apparatus with the transcriptional apparatus of poxviruses, we focused on the detailed analysis of poxviral mRNA molecules. Vaccinia virus is a prototypical poxvirus originally used for eradication of smallpox. Investigation into VACV mRNAs carried out almost half a century ago substantially contributed to the fundamental discovery of the 5' mRNA cap, a hallmark of all eukaryotic and many viral mRNAs. VACV research also facilitated the identification and understanding of the general mechanism of 5' mRNA cap synthesis. We characterized the VACV transcripts at the individual mRNA molecule level and found that vaccinia postreplicative mRNAs, containing nontemplated 5' poly(A) leaders, surprisingly lack the 5' cap structure *in vivo*. We showing that 5' cap occurrence in viral mRNAs gradually decreases in each successive gene time classes, in contrast to the reciprocal increase in 5' poly(A) leader lengths, and that these two variables are mutually negatively correlated. We also demonstrate that the initiator region element (INR) directly or indirectly influences both the frequency of 5' mRNA capping and the occurrence of 5' poly(A) leaders, including their lengths in postreplicative VACV mRNAs. Considering all the results together, we can speculate that the degree of 5' mRNA polyadenylation can directly affect the synthesis of the 5' cap by some hitherto unknown mechanism. This idea is further supported by our observation that 5' poly (A) leaders in m^7G cap-containing VACV late transcripts are significantly shorter than the 5' mRNA leaders, these lengths of which were calculated from the unbiased set of all VACV late mRNAs. Collectively, our results support the hypothesis that VACV transcription regulation ensures a gradual shift in viral mRNA translation initiation from a cap-dependent to cap-independent mechanism, which is accompanied by virus-induced modification of the host translation machinery.

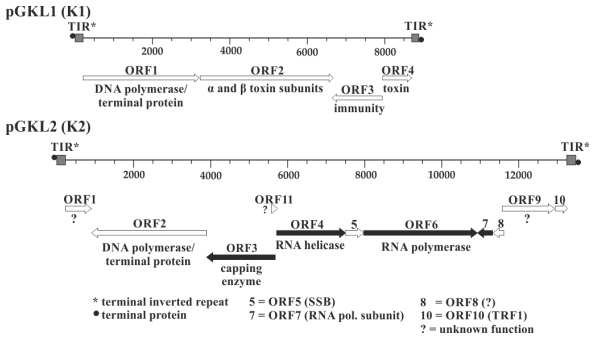

Fig. 1. **Genetic organization of pGKL VLEs (linear cytoplasmic plasmids) with indicated genes encoding components of the transcription apparatus**

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