ANALYSIS OF THE YEAST LINEAR PLASMID mRNAs REVEALED UNUSUAL ADDITION OF THE NON-TEMPLATE NUCLEOTIDES TO THEIR 5' ENDS AND SUGGESTED A NOVEL MECHANISM OF TRANSLATION INITIATION

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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 analysed pGKL plasmid mRNAs using 5' and 3' RACE techniques accompanied by digestion with the purified hDcp2 decapping enzyme. Even if the pGKL plasmids encode their own putative capping enzyme, these experiments revealed that only a few pGKL1/2 genes code for 5'-capped transcripts and that all of the plasmid specific mRNAs are not 3'-polyadenylated. Surprisingly, the majority of pGKL promoters give rise to uncapped mRNAs starting with short poly(A) sequences at their 5' ends that are not complementary to the plasmid DNA. To verify the hypothesis that either addition of the 5' non-template adenylates or translation initiation of such mRNAs can be influenced by the poly(A) binding protein (Pab1), we created the *K. lactis* strain bearing double-deletion in *PAB1* and *PBP1* genes. However, we did not observe any changes in the 5' UTR non-template sequences, pGKL plasmid stability and/or killer toxin production. The results are further strongly supported by our findings that mRNAs encoded by some of the pGKL genes do not bind to the yeast cap-binding translation initiation factor 4E *in vitro* while cellular mRNAs do. Moreover, production of the killer toxin, naturally encoded by pGKL plasmids, is unaltered in yeast strains conditionally deprived of the eIF4E in contrast to ceased production of the same killer toxin artificially expressed under the control of the strong Pol II driven promoter, thus suggesting cap-independent translation of the toxin-coding pGKL mRNAs. Moreover, we found that the RNA polymerase encoded by pGKL plasmids, as well as the capping enzyme, are similar to the corresponding enzyme encoded by vaccinia virus, which is surprisingly also the same for the plasmid-specific mRNAs that contain non-template poly(A) sequences at their 5′ ends, which are found specifically in the intermediate and late vaccinia virus mRNAs.
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References
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