Summary

RNase J is the only ribonuclease known to possess both endo- and exoribonuclease activities. It is widely distributed in the bacterial world; more than half of sequenced procaryotic genomes contain RNase J orthologues, but this enzyme is not present in *E. coli*. Orthologues of RNase J also exists in Archaea and in plants. During the last three years I have concentrated my efforts on the study of RNase J from the unicellular alga *Chlamydomonas reinhardtii* (CrRNase J).

I overproduced and purified different forms of CrRNase J. Purification of constructs based on the cDNA of *C. reinhardtii* resulted in formation of inclusion bodies. All attempts to resolve this problem were unsuccessful and, in the end, we decided to change strategy and purify CrRNase J under denaturing conditions, followed by renaturation. Obtained that way, CrRNase J was tested for the presence of endonucleolytic and 5'-3' exonucleolytic activity with the *B. subtilis thrS* leader model substrate. This substrate is routinely used to test the double activity of the BsRNase J1. CrRNase J proteins, after denaturation/renaturation, showed high endo- but no significant 5'-3' exonucleolytic activity. However, the weak 5'-3' exonucleolytic activity could have been due to non-optimal renaturation. For this reason we created three new constructs, this time by optimizing the codons for translation in *E. coli* and *B. subtilis*. These proteins were tested with the *thrS* leader sequence as well as with 5' end labelled monophosphorylated oligonucleotides, differing in their first nucleotide (A,C,G or U), to verify if the first nucleotide can influence the 5'-3' exonucleolytic activity of CrRNase J. The results of these activity tests were identical to those obtained with the denatured/renatured enzyme, - strong endonucleolytic activity was present but with very low 5'-3' exonucleolytic activity, compared with that of Bs RNaseJ1.

The lack of strong exonucleolytic activity *in vitro* prompted us to verify if CrRNase J could compensate for a deletion of BsRNase J. We inserted CrRNase J in a single copy on the *B. subtilis* chromosome in strains deleted for BsRNase J1, (*rnjA*, ΔJ1) and/or BsRNase J2 (*rnjB,* ΔJ2) (single and double mutations). The expression of CrRNase J had no effect on growth of a wild type strain, but could not compensate for the severe growth defect caused by the lack of BsRNase J1. The absence of complementation of BsRNase J1 is not proof that it is due to the lack of the 5'-3' exonucleolytic activity. We further analysed two well-studied processing/degradation events that require the 5'-3' exonucleolytic activity of the *B. subtilis* enzyme (16S rRNA and *glmS*) in these recombinant strains. We found that CrRNase J was unable to provide the 5' exonuclease activity required for these processes in *B. subtilis*.

We performed a yeast double-hybrid (Y2H) screen to look for potential partners of the CrRNase J. In this study we tested some of them (FTT2, NDA3, EF3 or HP4), in the *in vitro* test with CrRNase J. None of those proteins was able to stimulate the 5'-3' exonucleolytic activity of *C. reinhardtii* enzyme. Together these results show that, despite our efforts with different substrates, modification of the test system and the presence of potential activating factors, Cr RNase J shows only an endoribonucleolytic activity.

Keywords:
RNase J1, CrRNase J, Endoribonuclease, 5'-3' Exoribonuclease, chloroplasts, mRNA decay