Novel mechanism of gene expression control in *Eukaryota* through regulation of protein-coding transcript polyadenosine tail length

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Background

Production of all building blocks of our organisms are defined by our genetic material, called DNA. Execution of each of the many thousand recipes encoded in the DNA requires partial copying (transcription) of the DNA into another, shorter, molecule coined messenger RNA (mRNA). The mRNA contains information about a select building brick, mostly a protein. The RNA ends are modified at the site of its synthesis in the nucleus. At the beginning, the RNA acquires a cap, whereas at the end, a polyadenosine tail (polyA-tail) is added. The polyA-tail regulates the RNAs' function in export to the cytoplasm and subsequent protein production as well as the RNA stability.

The level of mRNA determines the rate of protein production. At the same time, rates of synthesis and degradation determine the levels of mRNA itself. Thus, the fine-tuning of mRNA stability is of crucial importance for the regulation of gene expression. The main factor that defines mRNA stability is the polyA-tail. During the mRNA life span, the polyA-tail is gradually shortened in a process called deadenylation, which is believed to be initiated by PAN2/3 and ended by CCR4-NOT. This sequential polyA-tail shortening by two independent complexes leads eventually to rapid mRNA degradation. Thus, in reality, the speed of poly(A) tail removal determines the half-life of a given mRNA and as follows its availability for protein production. Defining the mechanism of deadenylation is thus key for understanding gene-expression control.

Aim of the project and methodology

This project aims to describe the *in vivo* role of CCR4-NOT and PAN2/3 in mRNA polyA-tail deadenylation. This work will be done using yeast *Saccharomyces cerevisiae* and human cell lines as model organisms to pinpoint evolutionarily conserved mechanisms. We will employ a novel technology, called Direct RNA Sequencing (DRS), to measure the length of polyA-tails on every cellular mRNA. We will generate datasets from wild-type strains and mutants for deadenylase components and tie changes in polyA-tail length to mRNA stability and the rate of protein production.

Expected results

Our preliminary results challenge the so-called sequential deadenylation model and show that in yeast CCR4-NOT and PAN2/3 complexes can regulate polyA-tails of different sets of mRNAs. We thus wish to define the substrate specificity of each complex more thoroughly. This will draw a comprehensive picture of mRNA deadenylation in living cells. Our data also shows that in yeast mRNA polyA-tails are tailored to changing growth conditions and we expect that each deadenylase complex is independently regulated to play a separate and specific role in this process. In conclusion we expect to show that mRNA stability is defined by deadenylation either by PAN2/3 or CCR4-NOT.