All components of mPSF subcomplex, but not symplekin, participate in polyadenylation of transcripts generated by RNA polymerase III

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I. Ustiantsev[I], O. Borodulina[I], D. Kramerov[I]

[I]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991, Moscow, Russia

Most RNAs synthesized by RNA polymerase II (Pol II) are subject to polyadenylation (PA). The main cis-signal directing PA is the AAUAAA hexamer near the 3'-end of mRNA. A few multiprotein complexes are involved in poly(A) tail synthesis by poly(A)-polymerase (PAP). The main of these complexes is CPSF consisting of two subcomplexes: mPSF directly binding the AAUAAA signal and mCF responsible for the cleavage of RNA downstream of this signal. MPSF consists of CPSF160, CPSF30, hFip1, and WDR33 subunits, whereas mCF includes CPSF73, CPSF100, and symplekin. Previously, we discovered that not only Pol II transcripts but also certain transcripts generated by RNA polymerase III (Pol III) could be polyadenylated in an AAUAAA-dependent manner. Namely, this unique feature is inherent to Pol III-synthesized transcripts of some mammalian SINEs, short mobile genetic elements. Here we established a group of proteins responsible for the PA of Pol III transcripts of SINEs. We performed individual knockdowns (KDs) of proteins of interest by siRNA in HeLa cells, transfected cells by a SINE-containing plasmid, and estimated the PA of SINE RNA by Northern blotting. We found that the KD of each of the four components of the mPSF subcomplex, as well as PAP, decreased the PA of SINE transcripts, whereas the KD of symplekin had no such effect. Since symplekin maintains the stability of the mCF subcomplex, it is very unlikely that mCF is involved in the PA of SINE transcripts. Thus, mPSF and canonical PAP but not mCF are involved in the PA of RNA transcribed by Pol III. The analysis of B2 (mouse) and Ves (bat) SINEs allowed us to identify the nucleotide sequences of two additional cis-signals (β and τ) contributing to the PA of their transcripts. Finally, the mutation analysis and KD showed that an auxiliary factor CFIm interacts with the τ signal of the B2 transcript and enhances its polyadenylation. The study was funded by the Russian Scientific Foundation (grant no. 19-14-00327).