The poly-adenylated (A) tail on nearly all eukaryotic mRNAs plays a number of important roles in mRNA metabolism including enhancing translation, mRNA stability and transport from the nucleus. Recent studies in a variety of model organisms have revealed a pivotal role for regulated deadenylation as being rate limiting for mRNA degradation and deadenylation is now recognized as a mechanism of mRNA mediated gene regulation. Current techniques for analysis of poly(A) tail length include ligation-mediated PCR (LM-PAT) and Northern blotting, both of which are limited to analysis of abundant mRNAs. These method limitations coupled with the heightened interest in poly(A) metabolism encouraged us to develop a convenient and powerful assay to measure poly(A) tail length.

We have taken advantage of the fact that poly(A) polymerase can add a limited number (~25) of guanosine residues to an existing poly(A) tail. We have found that the same polymerase also can add inosine. This is an advantage because inosine forms less self-pairing structure than guanosine. Accordingly, we extend total RNA with a mixture of guanosine and inosine. This tail then provides a priming site for reverse transcription. When coupled with a gene specific internal primer, we can quickly and accurately determine the length distribution of poly(A) tails in any mRNA. This novel method should be of broad utility in the currently very vibrant area of poly(A) tail metabolism.

CONCLUSIONS

We have demonstrated that poly(A) tail length determination can be performed by adding a limited number of guanosine and inosine with poly(A) polymerase and PCR amplification.

Major advantages of this approach are:

- improved efficiency using poly(A) polymerase,
- improved sensitivity and specificity by PCR, and
- improved precision using a priming site located immediately downstream of the poly(A) tail.

REFERENCES