CC/NUMBER 28
JULY 11, 1988

This Week's Citation Classic®

Darnell J E, Philipson L, Wall R & Adesnik M. Polyadenylic acid sequences: role in conversion of nuclear RNA into messenger RNA. *Science* 174:507-10, 1971.
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A 200-250 nucleotide stretch of polyadenylic acid was found to be added first to heterogeneous nuclear RNA and then to appear in messenger RNA (mRNA). Experiments with metabolic inhibitors suggested a post-transcriptional addition that was required for the accumulation of stable mRNA. [The *SCI*® indicates that this paper has been cited in over 545 publications.]

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December 9, 1987

At the time of this article it was known that mammalian cells processed high molecular weight precursor RNA molecules for ribosomal RNA and transfer RNA.[1] The evidence included identification of chemical markers such as similar base composition, methyl group additions, and pseudouridine content of the product, so we were very much on the lookout for some chemical marker to relate the high molecular weight heterogeneous nuclear RNA (hnRNA) to messenger RNA (mRNA). The only evidence that connected the two at the time was a similar average base composition[1] and the fact that SV40-infected cells produced nuclear SV40-specific RNA that was larger than polysomal SV40-specific mRNA.[2]

In the spring of 1970, while studying radiolabeled HeLa hnRNA and mRNA, I noticed a greater resistance to RNase digestion of ^{32}P-labeled mRNA compared to ^{3}H uridine-labeled mRNA, and the ^{32}P-labeled resistant core was almost all adenylic acid. I then tested ^{3}H adenosine-labeled mRNA and found even greater RNase resistance than with ^{32}P or ^{3}H uridine-labeled RNA. Both Mary Edmonds and George Brawerman had detected polyadenylic acid, poly(A), in mammalian cells in the 1960s, but no biologic significance had been assigned to the poly(A). Joe Kates heightened interest in poly(A) when he found it as part of the mRNA made by the vaccinia virion.[3] By the spring of 1971, poly(A) had been clearly identified in both hnRNA and in the mRNA fraction of cultured cells.[3-6]

In the *Science* paper that is the subject of this commentary, several additional experiments helped us to clearly connect the poly(A) found in the hnRNA and that in the mRNA. We also suggested for the first time a precursor-product relationship between hnRNA and mRNA that was based on the presence in both of an identifiable marker. By gel electrophoresis both the briefly labeled poly(A) in the nucleus and the first appearing poly(A) in the cytoplasm were shown to be 200-250 nucleotides long. About 1 A was found in poly(A) for every 3 A's in mRNA suggesting one unit of about 250 A's in *every single* mRNA molecule. However, the poly(A) seemed to be made in the nucleus: after brief labels it was mainly in the nucleus, while after longer label times more was found in the cytoplasm. The poly(A) seemed to be a posttranscriptional product because actinomycin, which stops RNA polymerase progression, stopped total RNA synthesis for at least a minute or two before it stopped poly(A) synthesis. Finally, 3'-deoxyadenosine (cordycepin) stopped poly(A) synthesis but not hnRNA labeling; however, little or no labeled polysomal mRNA accumulated in cordycepin-treated cells. Thus we proposed that poly(A) was a nuclear RNA addition to certain hnRNA molecules that were to be successfully processed and then accumulate in stable mRNA, a proposal that has stood the test of time.

Why was this article, which was a strong suggestion but not a proof of the hnRNA to mRNA pathway, referenced heavily? First, if it were a correct proposal that processing was the rule in mRNA formation, it was of obvious importance to learn these details so mRNA regulation could eventually be studied. Many references were made with this prospect in mind. Second, this article was not by any means the final step in establishing a processing pathway, and many authors took a shot at disproving mRNA processing, referencing this paper along the way. While we still do not know exactly how poly(A) stabilizes mRNA (its most likely role in RNA metabolism[7]) this work did help a great deal to correctly direct attention to the hnRNA-mRNA pathway.

1. **Darnell J E.** Ribonucleic acids from animal cells. *Bacteriol. Rev.* 32:262-90, 1968. (Cited 520 times.)
2. **Lindberg U & Darnell J E.** SV40-specific RNA in the nucleus and polyribosomes of transformed cells. *Proc. Nat. Acad. Sci. USA* 65:1089-96, 1970. (Cited 130 times.)
3. **Kates J & Beeson J.** Ribonucleic acid synthesis in vaccinia virus II. Synthesis of polyriboadenylic acid. *J. Mol. Biol.* 50:19-33, 1970. (Cited 110 times.)
4. **Edmonds M, Vaughn M H & Nakazato H.** Polyadenylic acid sequences in the heterogeneous nuclear RNA and rapidly-labeled polyribosomal RNA of HeLa cells: possible evidence for a precursor relationship. *Proc. Nat. Acad. Sci. USA* 68:1336-80, 1971. (Cited 230 times.)
5. **Lee Y, Mendecki J & Brawerman G.** A polynucleotide segment rich in adenylic acid in the rapidly-labeled polyribosomal RNA component of mouse sarcoma 180 ascites cells. *Proc. Nat. Acad. Sci. USA* 68:1331-5, 1971.
6. **Darnell J E, Wall R & Tushinski R J.** An adenylic acid-rich sequence in messenger RNA of HeLa cells and its possible relationship to reiterated sites in DNA. *Proc. Nat. Acad. Sci. USA* 68:1321-5, 1971. (Cited 465 times.)
7. **Zeevi M, Nevins J R & Darnell J E.** Newly formed mRNA lacking polyadenylic acid enters the cytoplasm and the polyribosomes but has a shorter half-life in the absence of polyadenylic acid. *Mol. Cell. Biol.* 2:517-25, 1982.