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This Week's Citation Classic ™

Edmonds M, Vaughan M H. Jr. & 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. Set. US **68**:1336-40, 1971.
[Department of Biochemistry, University of Pittsburgh, PA]

This paper showed that polyA sequences about 200 nucleotides long were covalently bound to messenger RNA (mRNA) and to much larger RNAs in the HeLa cell nucleus. The observation that each mRNA contained a single polyA sequence and the similarity in polyA sequences in the two classes of RNA led us to propose a model in which mRNA molecules are processed from the much longer polyadenylated RNA molecules in the nucleus. [The *SCI*® indicates that this paper has been cited in over 505 publications since 1971]

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"Our discovery of polyA sequences in messenger RNA (mRNA) and heterogeneous nuclear RNA (hnRNA) was the culmination of work begun in the late 1950s at the Institute of Research of Montefiore Hospital in Pittsburgh where Richard Abrams and I were examining extracts of animal cells for RNA synthesis. Within a short time we had found such an activity in extracts from mouse ascites tumor cells.[1] Much to our surprise, however, the enzyme made only polyA and not RNA.[2] Somewhat later we found that the polyA polymerase purified from such extracts actually contained polyA molecules that could be found in boiled extracts of the enzyme.[3] These extracts would stimulate new polyA synthesis when added back to an RNA depleted polyA polymerase.

"These last observations greatly influenced my subsequent thinking about polyA for they suggested that cells must actually contain polyA, and also that such polyA might be relatively easy to isolate. At about that time, Peter Gilham described a method for covalently attaching oligo dT sequences to cellulose that struck us as the ideal way to do it.[4] We quickly prepared oligo dT cellulose by his method and found it would remove polyA from our boiled enzyme extracts. The polyA eluted from this cellulose could then restore polyA synthesis of an RNA depleted polyA polymerase.[5]

"The simplicity and specificity of this method suggested the obvious approach for looking for polyA sequences in animal cells. However, it was not until 1966, after I had moved to the University of Pittsburgh, that I began a systematic search for polyA in ^{32}P-labeled RNAs from mouse ascites tumor cells. I soon found that small quantities of polyA could be recovered from ribonuclease digested RNA from both nuclei and cytoplasm after passage over oligo dT cellulose.[6]

"In 1967 Maurice Vaughan, who had recently joined our department, helped me carry out the first gel electrophoretic analyses of such polyA. To our surprise the polyAs from both nuclear and cytoplasmic RNA were quite homogeneous and rather large (about 200 nucleotides). Having recently come from J.E. Darnell's laboratory in New York, Vaughan was familiar with the very large RNA molecules that turn over rapidly in the nucleus (hnRNA) and suggested we examine different size classes of this RNA for polyA sequences. Indeed, similar polyA sequences turned up in all sizes of hnRNA including the very largest. Although my experiences with polyA polymerases had led me to think of polyA as a distinct set of homopolymers, the association of small polyA sequences with huge RNA molecules suggested a covalent linkage to RNA instead. Since we had already found that small added polyAs tended to cosediment with large hnRNAs in sucrose gradients, it was clear that such artifacts had to be ruled out to establish a covalent attachment to RNA.

"In 1970 Hiroshi Nakazato, who had just arrived in my laboratory from Japan, undertook the hybridization competition experiments described in the paper that effectively ruled out intermolecular hybridization as the source of the polyA sequences in large hnRNA. With this evidence for a covalent linkage firmly established, we submitted our paper on polyA sequences in mRNA and hnRNA and proposed on the basis of the similarity of their polyA sequences that hnRNAs were the precursors of mRNA.

"The frequent citation of this paper may come from its revelation of an unsuspected structural feature of mRNA also shared by many hnRNA molecules. This, of course, provided strong support for the long suspected precursor role for hnRNA, but probably more important was the realization that the polyA sequence could be used to purify mRNA. In fact, Nakazato quickly showed how the mRNA of HeLa cells could be purified on oligo dT cellulose with the techniques now in widespread use."[7]

1. **Edmonds M & Abrams R.** Incorporation of ATP into polynucleotide in extracts of Ehrlich ascites cells. *Biochim. Biophys. Acta* **26**:226-7, 1957.
2. ..... Polynucleotide biosynthesis: formation of a sequence of AMP units from ATP by an enzyme from thymus nuclei. *J Biol. Chem.* **235**:1142-9, 1960.
3. ..... Nature of a polynucleotide required for polyribonucleotide formation from adenosine triphosphate with an enzyme from thymus nuclei. *J. Biol. Chem.* **237**:2636-42. 1962.
4. **Gilham P T.** Complex formation in oligonucleotides and its application to the separation of polynucleotides. *J. Amer. Chem. Soc.* **84**:1311, 1962.
5. **Edmonds M & Abrams R.** Isolation of a naturally occurring polyadenylate from calf thymus nuclei. *J Biol. Chem.* **238**:PCI 186-7, 1963.
6. **Edmonds M & Caramela M G.** The isolation and characterization of AMP-rich polynucleotides synthesized by Ehrlich ascites cells. *J. Biol. Chem.* **244**:1314-24, 1969.
7. **Nakazato H & Edmonds M.** The isolation and purification of rapidly labeled polysome-bound RNA on polythymidylate cellulose. *J. Biol. Chem.* **247**:3365-7. 1972.