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This Week's Citation Classic ®

Aviv H & Leder P. *Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Nat. Acad. Sci. USA* 69:1408-12, 1972.
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A convenient technique for the partial purification of large quantities of functional, poly(adenylic acid)-rich mRNA is described. Biologically active rabbit globin mRNA was purified by this procedure and assayed for its ability to direct the synthesis of rabbit globin in a cell-free extract of ascites tumor. [The *SCI*® indicates that this paper has been cited in more than 6,150 publications.]

How to Win Friends and Advance Science

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I came to Philip Leder's laboratory at the National Institute of Child Health and Human Development in 1970 after finishing my doctorate at the Weizmann Institute of Science in Israel. The main research direction of the lab was to study immunoglobulin biosynthesis using molecular biology approaches. One of the first steps in this endeavor was to isolate the immunoglobulin mRNA from myeloma cells (MOPC-41). The isolation of mRNA was thought to be essential in order to have a handle on the biosynthesis of immunoglobulins.

After establishing a cell-free translation system using an extract from a murine ascites cell line,[1] we set up to establish an extraction procedure for mRNA from the myeloma cells. However, for about six months we could not get any counts incorporated from labeled amino acids, whatever we tried. It was a very frustrating experience. Then I suggested that we check out our extraction procedures (particularly the quality of freshly distilled phenol) using mRNA extracted from rabbit reticulocytes for which there were a number of published procedures. This was done just as a control. It worked fine when mRNA was extracted from retics, but failed when extracted from myeloma tissue.

At that time, a number of papers were published showing that globin mRNA has a stretch of adenosines at the 3' end whose function was not clear.[2] It occurred to me that we might be able to separate globin mRNA from ribosomal RNA and tRNA by hybridizing mRNA to a solid phase polymer containing a short stretch of thymidines (oligo dT). It was not obvious, however, that such a short A:T hybrid would be stable. To synthesize oligo dT chemically hooked to cellulose, I found a procedure used previously[3] that involved condensation using DCC, which is very sensitive to water. Knowing very little organic chemistry, this was not encouraging for me. I sought advice from Israel Schechter of the Weizmann Institute who spent a sabbatical in the lab. I set up the synthesis which involved a whole week of shaking. I did this with great hesitation, sure that it wouldn't work. This was probably the only organic synthesis that I ever did in my career, and it worked.

Having lots of extracted reticulocyte RNA, I set up the procedure that is described in this most-cited paper, and it worked right away. We got plenty of labeled amino acids incorporated into protein directed by mRNA.

I immediately followed this experiment by running through this little column of oligo dT, using RNA preparations extracted from myeloma cells, which, luckily, I had not discarded. This opened up many studies on immunoglobin mRNA, which were subsequently published.[4]

Having lots of pure globin mRNA, we joined forces with Jeff Ross and Ed Scolnick to synthesize complementary DNA[5]—an important milestone in the development of gene cloning and molecular biotechnology.

My procedure for mRNA purification made me lots of friends. Many colleagues have asked for samples of this precious oligo dT cellulose, which I was willing to share with them provided they made their own batch under my guidance and we split it.

I also had complaints that the column did not work. It mostly turned out that the column was not recycled properly.

In the nearly 20 years since the procedure was published, it has been cited in thousands of publications and is still the most common technique of mRNA purification. Very small changes in the procedure subsequently have been introduced. Some save on the washing step when purity is not crucial, some recycle the unbound material if a higher yield is desired. But to the best of my knowledge, most researchers have been happy with the procedure as originally described.

1. **Aviv H, Boime I & Leder P.** Protein-synthesis directed by encephalomyocarditis virus-RNA—properties of a transfer RNA-dependent system. *Proc. Nat. Acad. Sci. USA* 68:2303-7, 1971. (Cited 135 times.)
2. **Edmonds M & Caramela M G.** Isolation and characterization of adenosine monophosphate-rich polynucleotides synthesized by Ehrlich ascites cells. *J. Biol. Chem.* 244:1314-24, 1969. (Cited 230 times.)
3. **Gilham P T.** Synthesis of polynucleotide-celluloses and their use in fractionation of polynucleotides. *J. Amer. Chem. Soc.* 86:4982-5, 1964. (Cited 190 times.)
4. **Swan D, Aviv H & Leder O.** Purification and properties of biologically active messenger-RNA for a myeloma light chain. *Proc. Nat. Acad. Sci. USA* 69:1967-71, 1972. (Cited 245 times.)
5. **Ross J, Aviv H, Scolnick E & Leder P.** In-vitro synthesis of DNA complementary to purified rabbit globin messenger-RNA. *Proc. Nat. Acad. Sci. USA* 69:264-8, 1972. (Cited 230 times.)

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