Pelham H R B & Jackson R J. An efficient mRNA-dependent translation system from reticulocyte lysates. *Eur. J. Biochem.* 67:247-56, 1976.
[Department of Biochemistry, University of Cambridge, UK]

A method for destroying endogenous mRNA in reticulocyte lysates while retaining the ability of the extract to translate added mRNA is described. The method involves addition of $CaCl_2$ and a calcium-dependent nuclease to digest the mRNA, followed by ethyleneglycol-bis(2-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) to chelate the calcium and inactivate the nuclease. [The *SCI*® indicates that this paper had been cited in nearly 2,100 publications by the end of 1984.]

Hugh R.B. Pelham
Laboratory of Molecular Biology
Medical Research Council
Cambridge CB2 2QH
England

January 6, 1985

I started work as a graduate student with Richard Jackson at the Department of Biochemistry, University of Cambridge, in October 1975. He and Tim Hunt had been studying protein synthesis in reticulocyte lysates for some years. Initially, I spent some time on an ambitious project to reconstitute coupled transcription and translation by adding rat liver nuclei to reticulocyte lysates. This worked fine except that the nuclei made very little RNA and severely inhibited protein synthesis. After a few weeks, it was clear that even if the nuclei made mRNA, it would be impossible to detect its translation products because of the enormous amounts of endogenous globin mRNA and other minor mRNAs in the reticulocyte extract.

The idea of using micrococcal (staphylococcal) nuclease to get rid of the mRNA came from Tim. It was a bold idea, because normally one goes to great lengths to *avoid* contaminating a translation system with nuclease. It had actually been proposed as an undergraduate project the previous year, but no one had attempted it, partly because it had been tried once without success. I also had difficulty at first, but while Tim was away on a trip to the US, I got the conditions right, and by Christmas it was working well.

The only widely used mRNA-dependent translation system available at the time was the wheat-germ system, which worked well for small RNAs but was notoriously variable and prone to "early quitting" with large RNAs. The nuclease trick made reticulocyte lysate the system of choice for many applications, and I later successfully translated some very large viral RNAs.[1] The same procedure has also been used to make template-dependent translation systems from a number of other cell types and species.[2] I never did get nuclei to do much, but coupled transcription-translation was possible with vaccinia virus core particles.[3]

The frequent citation of the paper reflects the fact that *in vitro* translation has changed from being a specialized technique for studying the mechanism of protein synthesis to being a standard technique both in virology and in the study of gene expression in general, for assaying mRNA and identifying translation products of cloned genes, and so on.[4] Also, there was really only one reference to the nuclease procedure for many years—it is so simple, involving at most three pipetting steps, that it is hard to improve on it. This didn't stop a number of people from writing or calling me and insisting that they had followed the paper to the letter without success! Now, of course, translation kits are available from several manufacturers, and everyone can do it!

1. **Pelham H R B.** Translation of encephalomyocarditis virus RNA in vitro yields an active proteolytic processing enzyme. *Eur. J. Biochem.* 85:457-62, 1978. (Cited 70 times.)
2. **Scott M P, Storti R V, Pardue M L & Rich A.** Cell-free protein synthesis in lysates of *Drosophila melanogaster* cells. *Biochemistry—USA* 18:1588-94, 1979. (Cited 20 times.)
3. **Pelham H R B, Sykes J M M & Hunt T.** Characteristics of a coupled cell-free transcription and translation system directed by vaccinia cores. *Eur. J. Biochem.* 82:199-209, 1978. (Cited 30 times.)
4. **Stueber D, Ibrahimi I, Cutler D, Dobberstein B & Bujard H.** A novel in vitro transcription-translation system: accurate and efficient synthesis of single proteins from cloned DNA sequences. *EMBO J.* 3:3143-8, 1984.

