

# This Week's Citation Classic

CC/NUMBER 27  
JULY 5, 1982

Hardy S J S, Kurland C G, Voynow P & Mora G. The ribosomal proteins of *Escherichia coli*. I. Purification of the 30S ribosomal proteins. *Biochemistry* 8:2897-905, 1969.  
[Dept. Zool. and Lab. Molec. Biol., Univ. Wisconsin, Madison, WI]

This paper demonstrates that there are approximately 20 proteins in the 30S ribosomal subunits of *Escherichia coli* and describes their purification. It shows that the multiplicity of components is not due to contamination with nonribosomal proteins, nor to random disulfide bridge formation, nor to proteolytic degradation of fewer components. It also demonstrates that a similar multiplicity is found in intact cells. [The SCI® indicates that this paper has been cited over 590 times since 1969.]

Simon Hardy  
Department of Biology  
University of York  
Heslington, York YO1 5DD  
England

March 15, 1982

"The work described in this paper was the culmination of my career as a graduate student of Chuck Kurland. We were interested in the structure and function of the ribosomes of *E. coli*. Our earlier analysis of a ribosome-associated enzyme had forced on us the realization that mere association was, at best, only a weak indication of a protein's role in the structure or function of ribosomes. We therefore proposed several more rigorous criteria for identifying ribosomal proteins.<sup>1</sup> Then we wondered whether the pioneering work of Waller<sup>2</sup> showing a great multiplicity of ribosomal proteins was correct. Could it be instead a multiplicity of contaminants and other artifacts? Indeed, the idea that ribosomes were very simple structures similar to small viruses, containing only a few proteins, was both attractive and prevalent at that time.

"So we began the daunting task of purifying and characterizing all the ribosomal proteins, at each stage attempting to rule out

all artifacts which would increase the number. Thus we began with rigorously washed particles, eliminated disulfide bridges, checked for proteolytic degradation, and showed that most of the proteins that we purified were present in intact cells. The purified proteins were then shown to be both physically and chemically distinct in the following paper.<sup>3</sup> Kurland's simple idea that phosphocellulose, being an analogue of ribosomal RNA, would be the ideal ion exchange resin for separating ribosomal proteins proved to be splendidly correct and was the greatest single contribution to the experimental work. Kurland, in fact, provided most of the ideas and direction. I worked out the purification schemes and Paul Voynow, in between measuring the molecular weights of the proteins,<sup>3</sup> provided large quantities of ribosomal subunits by developing our use of the then new zonal rotor. Guido Mora, a later arrival whose day was to come with the 50S subunit proteins, helped in the final purifications.

"This work and similar work from other laboratories destroyed the simple theory of ribosome structure. As a result, at the Nucleic Acids Gordon Conference of 1968, Jim Watson collected the two bottles of champagne he had bet Howard Dintzis at an earlier Gordon Conference, and I, along with many other contributors, was invited to share them. That thimbleful of champagne is still listed in the honors section of my C.V.

"Now comes the embarrassing part. Although the paper was an important and good contribution to the field at the time, it has been cited so many times only because I altered a magnesium concentration. Since we wanted to be sure that we were purifying all the ribosomal proteins, it was important to get the highest yields of protein in separating it from the ribosomal RNA. I discovered that by raising the magnesium concentration of the standard acetic acid extraction<sup>4</sup> from 0.01 M to 0.1 M, the recovery of protein increased from 80 percent to better than 95 percent. Since the acetic acid procedure is still regularly used and nobody has improved it, our paper has become a *Citation Classic*. A more recent review of this field may be found in 'Structure and function of the bacterial ribosome.'<sup>5</sup>

1. Hardy S J S & Kurland C G. The relationship between poly A polymerase and the ribosomes. *Biochemistry* 5:3676-84, 1966.
2. Waller J P. Fractionation of the ribosomal protein from *Escherichia coli*. *J. Mol. Biol.* 10:319-36, 1964.
3. Craven G R, Voynow P, Hardy S J S & Kurland C G. The ribosomal proteins of *Escherichia coli*. II. Chemical and physical characterization of the 30S ribosomal proteins. *Biochemistry* 8:2906-15, 1969.
4. Waller J P & Harris J. Studies on the composition of the protein from *Escherichia coli* ribosomes. *Proc. Nat. Acad. Sci. US* 47:18-23, 1961.
5. Kurland C G. Structure and function of the bacterial ribosome. *Annu. Rev. Biochem.* 46:173-200, 1977.