

# The discovery of polyribosomes

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The Watson–Crick DNA structure of 1953 led readily to an understanding of how DNA could be replicated. But how DNA could be used to direct the synthesis of specific proteins was hardly obvious. During the late '50s accumulating evidence implicated RNA in the process of translation, and the Central Dogma, 'DNA makes RNA and RNA makes protein', became generally accepted. To reveal how the various species of RNA carried out translation was the task for the early '60s.

## The setting

By 1962 the major players in protein synthesis had been identified. The ribosome, named just four years previously, was clearly the machine that conducted the reaction (reviewed in [1]). The tRNA (known then as sRNA or 'soluble' RNA) was the adaptor – the intermediate between the nucleotide language and the amino acid language [2,3]. And only the previous year had several laboratories identified mRNA as the carrier of information between genes and the ribosome, presumably serving as the template on which amino-acyl tRNAs were assembled [4,5]. Indeed, a poly U template had just been shown to effect the synthesis of polyphenylalanine [6]. The race to decipher the genetic code was heating up.

Yet, how did these components work together to synthesize a real protein? What were their relative proportions during protein synthesis? Howard Dintzis had just demonstrated that in rabbit reticulocytes the globin chain was assembled stepwise from the N terminus towards the C terminus [7]. But how did an mRNA, thousands of nucleotides in length, interact with a ribosome of only 150–200 Å?

James Watson's group had presented some provocative data, observing that mRNA being translated by *Escherichia coli* ribosomes sedimented more rapidly than 70S ribosomes [8]. They suggested that 'heavy ribosomes [were] sedimenting faster because of associated mRNA', and that 'mRNA-complexed ribosomes

aggregate more easily than free ribosomes'. However, studies of *E. coli* ribosomes were in some confusion because many of the 70S ribosomes dimerized to form 100S complexes in the low salt, high Mg<sup>2+</sup> ionic conditions that were routinely used. Indeed, it was suggested that the '100S ribosomes, not the 70S ribosomes, may be the principal sites of protein synthesis' [8].

## The collaboration

At this time we were graduate students at MIT in the laboratory of Alex Rich, where we had been sharing a bench for several years. Paul had developed a cell-free translation system (CFS) from rabbit reticulocytes and had succeeded in repeating the Dintzis experiment *in vitro*, showing that protein synthesis occurred in the same step-wise fashion (N → C) as found in intact reticulocytes. However, no initiation or completion of new globin polypeptide chains was detectable [9]. He was writing up his thesis and trying to substantiate his observation that the reticulocyte CFS could translate poly U and poly A. Meanwhile, Jon was trying to analyze the interaction of tRNA with *E. coli* ribosomes, running lots of sucrose gradients with *E. coli* extracts. But progress was slow, and he jumped at the opportunity when Jim Darnell, then a new faculty member at MIT whom Jon had just met at a softball game, asked if he would be willing to investigate the ability of poliovirus RNA to serve as mRNA in the *E. coli* extract. Poliovirus RNA did bind to ribosomes and stimulated, albeit weakly, the incorporation of labeled amino acids, some of which immunoprecipitated with polio-specific antibodies [10]. Although it was recognized that bacterial ribosomes were smaller than mammalian ribosomes, at that time there was no suggestion that the mechanism of protein synthesis would differ in the two systems. Nevertheless, the low incorporation suggested that the poliovirus RNA might be a more effective mRNA in a mammalian extract.

So, we struck a deal. Paul would help Jon by translating poliovirus RNA in a

reticulocyte CFS, and Jon would help Paul by running sucrose gradients of poly U bound to reticulocyte ribosomes. But a problem arose. The ribosomes kept sedimenting too far, often into the pellet at the bottom of the tube, even without poliovirus RNA or poly U. As it was easier to follow ribosomes by radioactivity than by ultraviolet absorbance, we decided to label the nascent globin chains on the ribosomes by incubating the reticulocytes briefly with <sup>14</sup>C amino acids. The cells were then lysed osmotically, and their ribosomes were prepared by two cycles of sedimentation and analyzed on a 5–20% sucrose gradient. Much to our surprise we found a complex pattern of several peaks (Fig. 1a). Furthermore, the radioactivity, representing growing globin chains, was not uniformly distributed among the ribosomes, but seemed more abundant in the rapidly sedimenting peaks. Because of reports that active ribosomes tended to 'aggregate' [8], we were concerned that the active ribosomes had stuck together during the initial pelleting. An equally important influence in our thinking, however, was the vocal presence down the hall of Cy Levinthal, who had recently shown that long DNA molecules were subject to breakage by even gentle shear forces [11].

Was the method of ribosome preparation leading to aggregation or was it breaking fragile structures? We decided to determine the arrangement of the ribosomes by direct analysis of a reticulocyte lysate, with only a brief centrifugation to remove cell membranes. A new problem arose: the lysate, with its high concentration of hemoglobin, would not form a stable boundary at the interface with the 5% sucrose then used at the top of gradients. Replacing the 5–20% sucrose gradient with a 15–30% sucrose gradient solved the problem while maintaining a linear rate of sedimentation. Figure 1b is our plot of the first sucrose gradient analysis of reticulocyte lysates that had been briefly labeled with <sup>14</sup>C amino acids. It is difficult to describe the rush of excitement as we plotted the CPM

(counts per min) from the strip of paper that the counter had been generating overnight. The reticulocyte had ribosomes that seemed to be inactive, and sedimented as single 80S ribosomes, but half the ribosomes were in much larger structures – these were the active ones! Both polio and poly U were forgotten as we rushed to analyze just what these structures were.

The next few weeks passed in a flurry of experiments in which we reproduced this result and determined the nature of the larger structures. Electron microscopy (EM) studies of mammalian cells had often revealed clusters of ribosomes on the endoplasmic reticulum [12]. Our experiment showing that the larger structures were insensitive to 0.5% deoxycholate and to 0.5 M NaCl was, therefore, important as it proved that these structures were not held together by membranes. In addition, the large structures were exquisitely sensitive to RNase but unaffected by DNase. They were resistant to lowering of the  $Mg^{2+}$  concentration (an obsession at the time, as Mg ions were recognized as being key to the association–dissociation of ribosomal subunits [4]) but destroyed by chelating  $Mg^{2+}$  ions with EDTA. As we had worried, the structures were sensitive to shear: they were reduced to 80S ribosomes by grinding with alumina (the usual method of preparing bacterial ribosomes at the time) and were partially degraded by repeated sedimentation and resuspension.

The intermediates apparent as partial breakdown products (Fig. 1a) suggested that the structures in the main peak of Fig. 1b were composed of five ribosomes. Indeed, EM analysis of the fractions from a gradient as in Fig. 1b revealed largely penta-ribosome structures in the peak fractions (Fig. 2a), with hexa- and tetra-ribosome structures in the neighboring fractions (this analysis was conducted in collaboration with Cecil Hall's laboratory, where we simply spotted a drop from each fraction on a grid, sucked off the sucrose solution and negatively stained the sample).

Although no individual mRNA had been isolated at that time, one could calculate that ~450 nucleotides were required to encode a globin chain. In a stretched configuration of 3.4 Å per nucleotide, the 1500 Å globin mRNA could accommodate five or six 200-Å ribosomes.

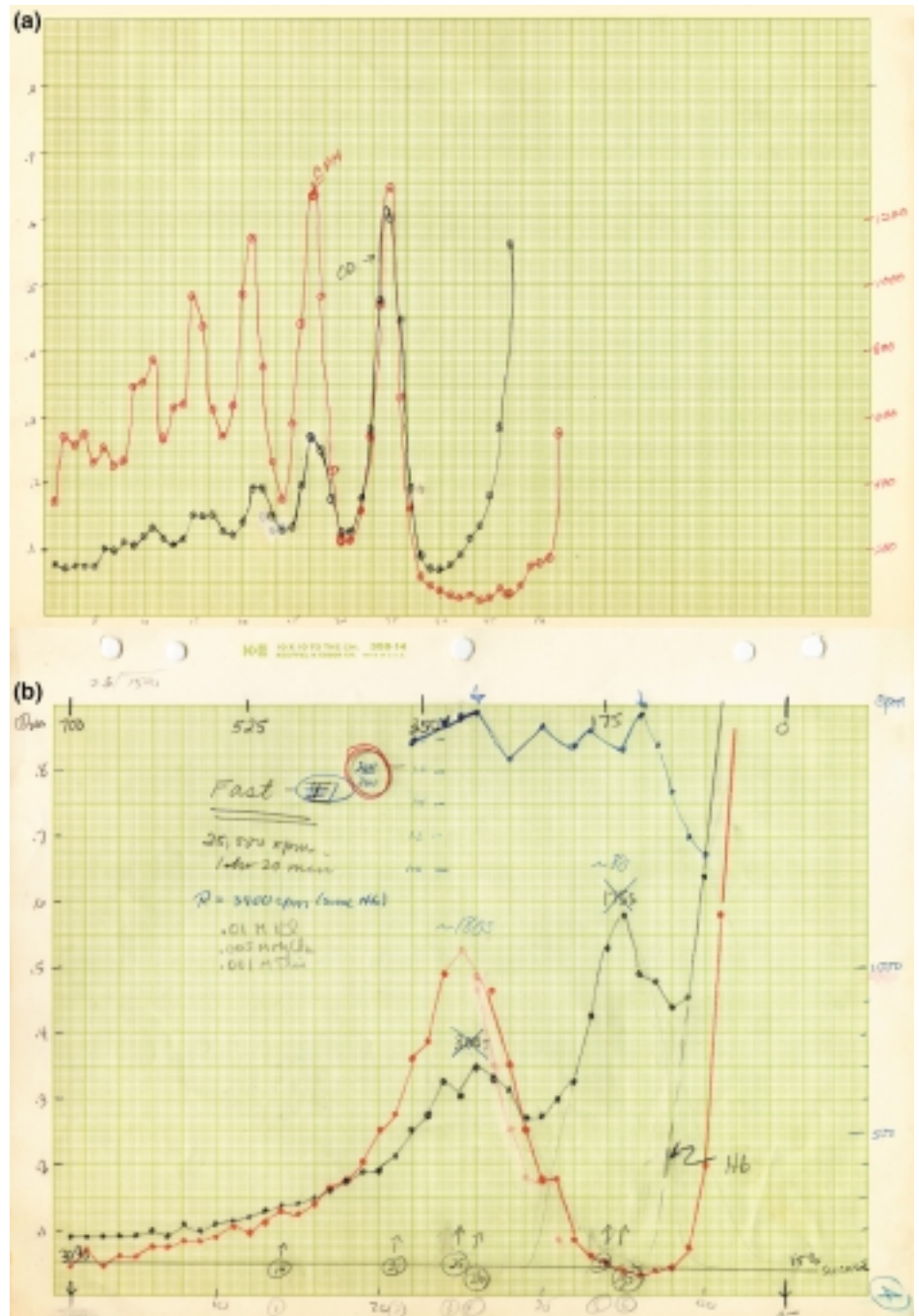


Fig. 1. (a) Analysis on a 5–20% w/w sucrose gradient of ribosomes purified, by two cycles of centrifugation and resuspension, from reticulocytes that had been labeled for 1.5 min with  $^{14}C$  amino acids (24 July 1962). The x-axis represents the 52 fractions from the sucrose gradient: sedimentation from right to left.  $OD_{260}$ , black (left scale); CPM, red (right scale). (b) Our first analysis on a 15–30% w/w sucrose gradient of the clarified lysate of reticulocytes that had been labeled for 0.5 min with  $^{14}C$  amino acids (8 August 1962). (Note the original, short-lived confusion as to the S values of the peaks.) Colors and sedimentation are as in (a). Abbreviations: CPM, counts per min; OD, optical density.

This was subsequently shown to be the case by the exquisite electron micrographs of Henry Slayter [13] (Fig. 2b).

From the moment we saw Fig. 1b, we knew that we were onto a fundamentally new way of thinking about protein synthesis, but it was Alex Rich who was able to put the model in a clear perspective. The rapidly sedimenting

component comprised several ribosomes associated with a single mRNA, each ribosome presumably scanning the mRNA from one end to the other as it synthesized a complete polypeptide. What the microscopists had been seeing for years finally became clear [12]. With the advice of Richard Roberts, who had coined the name 'ribosome' in 1958, we termed the

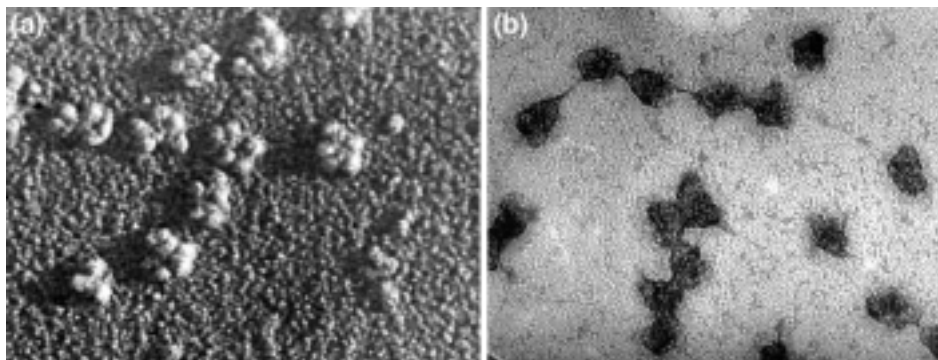


Fig. 2. (a) Negatively stained [15] and (b) positively stained [13] samples from the polysome peak of a gradient such as that of Fig. 1b. In Fig. 2b, the mRNA connecting the ribosomes is clearly visible.

structure a 'polyribosome', or 'polysome' for short. Although in retrospect this concept seems obvious, at the time groups that observed similar phenomena had different ideas; for example, '...the ability of the heavy ribosome particles to incorporate amino acid may be dependent not on their content of 'messenger' RNA alone but on some other factor which is a determinant of the stability of the heavy ribosomes' [14].

#### Going public

It was an exciting time in a graduate student's life, and Jon gave his first public talk at a small meeting organized by MIT in October 1962. Unfortunately, as the meeting coincided with the Cuban missile crisis, the audience was rather distracted. Paul had left for a post-doc in Cambridge with Francis Crick in September, and enjoyed a more attentive audience for his presentation on polysomes at the Medical Research Council, fortunately just before the excitement caused by the announcement of Crick's Nobel prize.

Alex and Jon wrote up the work and sent the paper off to John Edsall at Harvard for submission to *Proc. Natl. Acad. Sci. USA*. After some grumbling from reviewers about who had originated the idea of polyribosomes, the paper was published in January 1963 [15]. This was clearly an observation whose time had come; independently, Alfred Gierer in Tubingen, also using reticulocytes [16], and Hans Noll in Pittsburgh, using rat liver [17], had reached the conclusion that protein synthesis was conducted on structures with multiple ribosomes scanning a single mRNA. Although Noll coined the term 'ergosome' (working ribosome) to describe them, 'polysome' stuck.

It is interesting that, at a time when the attention of molecular biologists was fixed on *E. coli* and its phages, the most compelling evidence for polyribosomes came from mammalian systems. In part, this was because of the harsh methods needed to open bacterial cells. But another important element was the separation of transcription from translation that occurs in eukaryotic but not prokaryotic cells. Dealing with the cytoplasm and, in the case of reticulocytes, only the cytoplasm, made a much clearer analysis possible. Indeed, the subsequent studies of polyribosome function were conducted almost exclusively in mammalian cells (see [18–20] for examples). One could argue that the discovery of polyribosomes was one (of several) turning points that led molecular biologists to realize that mammalian cells were a suitable subject for study.

In Cambridge, Paul and Hildegard Lamfrom continued to develop the reticulocyte CFS, using the membrane-free lysate without further fractionation. They showed that its high activity reflected the recruitment of new ribosomes to the globin mRNA, with completed globin chains being continuously released from the active ribosomes [21]. The lack of translation initiation seen in Paul's thesis work had been a consequence of mRNA fragmentation in the fractionated CFS.

#### How the pieces fit

Whereas the concept of polysomes provided a picture of the ribosome flowing along an mRNA molecule, the way in which this occurred remained obscure. In particular, how many polypeptides were made by a ribosome? How many tRNA molecules could a ribosome accommodate?

Although J-P. Waller had already shown that the protein complement of a ribosome was extremely complex [22], unlike that of a virus, many still thought of the ribosome as a quasi symmetrical structure that might have several active sites (see example in [23]).

At this point it was known that the growing polypeptide chain is covalently attached to a tRNA molecule [24], and that the incoming amino acid is attached to another tRNA. Wally Gilbert had found that a single molecule of tRNA bound to a ribosome, and he proposed a model by which the growing polypeptide chain was passed off from one tRNA to another [25]. However, because it was difficult to determine the fraction of active ribosomes in these preparations, the numerology was somewhat uncertain. The reticulocyte system, in which the active ribosomes could be isolated, offered the possibility of solving this problem.

Jon attempted first to determine the number of growing polypeptide chains by labeling reticulocytes with  $^{14}\text{C}$  leucine or phenylalanine, and measuring the specific activity of the polyribosomes and of the soluble amino acid pool. Knowing the number and location of the leucine and phenylalanine residues in the  $\alpha$  and  $\beta$  globin chains, we could calculate the number of growing chains per ribosome. In numerous experiments, the value came out to be  $\sim 0.8$ . Use of a convenient 'fudge factor' (based on the demonstration that the rate of translation appeared slower at the beginning of translation [26]) raised that value to  $\sim 0.9$  growing chains per ribosome, close enough to 1.0 to conclude that each ribosome carried a single growing chain, a gratifyingly simple number [27].

With a single growing chain, how many tRNA molecules were associated with each ribosome? Although the reticulocyte, without a nucleus, does not transcribe RNA, it does repair the 3'-CCA portion of its tRNA, a process that was being studied in the neighboring laboratory by Ed Herbert [28]. We reasoned that by providing reticulocytes with  $^3\text{H}$  adenine, we might label the tRNAs sufficiently to detect them on functioning ribosomes. Although the labeling was not great, we could measure the tRNAs on polyribosomes, though each experiment tied up the scintillation counter for a week. By determining the specific activity of the tRNA fraction and

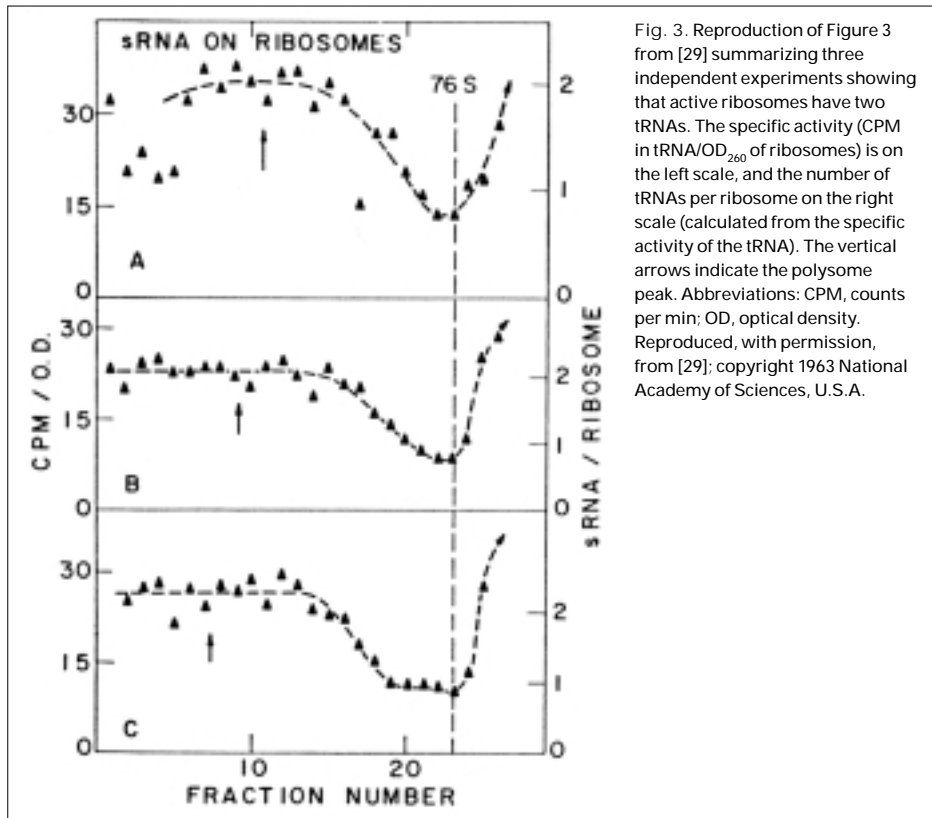


Fig. 3. Reproduction of Figure 3 from [29] summarizing three independent experiments showing that active ribosomes have two tRNAs. The specific activity (CPM in tRNA/OD<sub>260</sub> of ribosomes) is on the left scale, and the number of tRNAs per ribosome on the right scale (calculated from the specific activity of the tRNA). The vertical arrows indicate the polysome peak. Abbreviations: CPM, counts per min; OD, optical density. Reproduced, with permission, from [29]; copyright 1963 National Academy of Sciences, U.S.A.

knowing the molecular weight ratio of the ribosome to the tRNA, we calculated that each active ribosome had two molecules of tRNA, whereas the inactive single ribosomes had only one. The data were robust, as the specific activity was constant throughout the polysome peak (Fig. 3).

Thus, we concluded that an active ribosome has not one but two sites for tRNA. One holds the growing chain, which Alex and Jon termed the 'P' site for peptidyl tRNA, and the other, which we termed the 'A' site, holds the incoming amino-acyl tRNA [29]. This seemed to make sense as it permitted the two tRNAs to reside on the ribosome simultaneously while the peptide bond was formed. A similar conclusion was also reached by the Schweet group, based on dissecting, *in vitro*, the steps of peptide bond formation by reticulocyte ribosomes [30].

In retrospect, however, this two-site model suggests that the formation of the peptide bond is the rate limiting step, whereas we would expect it to be the selection of the amino-acyl tRNA. This discrepancy was resolved a few years later, when Knut Nierhaus showed that the ribosome actually has three sites, the additional one being the 'E' or exit site, which retains the spent tRNA until the A

site is filled [31]. Consequently, a translating ribosome has exactly two tRNAs at all times.

#### Postscript

Thus, in little more than a year and a half our views of protein synthesis had evolved from a vague idea of the triple interaction of mRNA, tRNA and ribosomes, to a model very close to the current one: ribosomes flow from one end of an mRNA to another, and each has a site for a tRNA attached to the growing polypeptide and a site for the incoming tRNA attached to an amino acid.

For each of us, this experience was the coming-of-age as a scientist because we had the thrill of learning something that *no-one* else knew, the essence of conducting science, coupled with the responsibility to see that we got it right. It was also a learning experience. It taught us the importance of scraping the pellets from the bottom of tubes, that is, of keeping our eyes open. And it taught us the importance of colleagues and of comfortable communication between faculty and students, as not only Alex Rich, but also Howard Dintzis, Jim Darnell, Cy Levinthal, Cecil Hall, Henry Slayter and Ed Herbert, all played a direct role in our thinking and our experiments.

#### References

- 1 Siekevitz, P. and Zamecnik, P. (1981) Ribosomes and protein synthesis. *J. Cell Biol.* 91, 53s–65s
- 2 Hoagland, M.B. *et al.* (1958) A soluble ribonucleic acid intermediate in protein synthesis. *J. Biol. Chem.* 231, 241–257
- 3 Crick, F.H.C. (1958) On protein synthesis. *Soc. Exptl. Biol. Symposium XII*, 138–163
- 4 Brenner, S. *et al.* (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* 190, 576–581
- 5 Gros, F. *et al.* (1961) Unstable ribonucleic acid revealed by pulse-labelling of *Escherichia coli*. *Nature* 190, 581–585
- 6 Nirenberg, M. and Matthai, J.H. (1961) The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polynucleotides. *Proc. Natl. Acad. Sci. U. S. A.* 47, 1588–1602
- 7 Dintzis, H.M. (1961) Assembly of the peptide chains of hemoglobin. *Proc. Natl. Acad. Sci. U. S. A.* 47, 247–261
- 8 Risebrough, R.W. *et al.* (1962) Messenger RNA attachment to active ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 48, 430–436
- 9 Knopf, P.M. and Dintzis, H.M. (1965) Hemoglobin synthesis in a cell-free system. *Biochemistry* 4, 1427–1434
- 10 Warner, J.R. *et al.* (1963) The interaction of poliovirus RNA with *Escherichia coli* ribosomes. *Virology* 19, 393–399
- 11 Davison, P.F. *et al.* (1961) The structural unit of the DNA of bacteriophage T4. *Proc. Natl. Acad. Sci. U. S. A.* 47, 1123–1129
- 12 Palade, G.E. and Siekevitz, P. (1956) Pancreatic microsomes; an integrated morphological and biochemical study. *J. Biophys. Biochem. Cytol.* 2, 671–689
- 13 Slayter, H.S. *et al.* (1963) The visualization of polyribosomal structure. *J. Mol. Biol.* 7, 652–657
- 14 Marks, P.A. *et al.* (1962) Protein synthesis in erythroid cells. I. Reticulocyte ribosomes active in stimulating amino acid incorporation. *Proc. Natl. Acad. Sci. U. S. A.* 48, 2163–2171
- 15 Warner, J.R. *et al.* (1963) A multiple ribosomal structure in protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 49, 122–129
- 16 Gierer, A. (1963) Function of aggregated reticulocyte ribosomes in protein synthesis. *J. Mol. Biol.* 6, 148–157
- 17 Wettstein, F.O. *et al.* (1963) Ribosomal aggregate engaged in protein synthesis; characterization of the ergosome. *Nature* 197, 430–435
- 18 Penman, S. *et al.* (1963) Polyribosomes in normal and poliovirus-infected HeLa cells and their relationship to messenger-RNA. *Proc. Natl. Acad. Sci. U. S. A.* 49, 654–661
- 19 Goodman, H.M. and Rich, A. (1963) Mechanism of polyribosomal action during protein synthesis. *Nature* 199, 318–322
- 20 Noll, H. *et al.* (1963) Ribosomal aggregates engaged in protein synthesis: ergosome breakdown and messenger ribonucleic acid transport. *Nature* 198, 632–638
- 21 Lamfrom, H. and Knopf, P.M. (1964) Initiation of hemoglobin synthesis in cell-free systems. *J. Mol. Biol.* 9, 558–575
- 22 Waller, J-P. and Harris, J.I. (1961) Studies on the composition of the protein from *Escherichia coli* ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 47, 18–23

- 23 Davis, B.D. (1963) Discussion in *Cold Spring Harb. Symp. Quant. Biol.* XXVIII, 294–296
- 24 Gilbert, W. (1963) Polypeptide synthesis in *Escherichia coli* III. *J. Mol. Biol.* 6, 389–403
- 25 Cannon, M. *et al.* (1963) The binding of sRNA by *Escherichia coli* ribosomes. *J. Mol. Biol.* 7, 360–370
- 26 Naughton, M.A. and Dintzis, H.M. (1962) Sequential synthesis of the peptide chains of hemoglobin. *Proc. Natl. Acad. Sci. U. S. A.* 47, 1822–1830
- 27 Warner, J.R. and Rich, A. (1964) The number of growing polypeptide chains on reticulocyte polyribosomes. *J. Mol. Biol.* 10, 202–211
- 28 Holt, C.E. *et al.* (1966) Turnover of terminal nucleotides of soluble ribonucleic acid in intact reticulocytes. *J. Biol. Chem.* 241, 1819–1829
- 29 Warner, J.R. and Rich, A. (1964) The number of soluble RNA molecules on reticulocyte polyribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 51, 1134–1141
- 30 Arlinghaus, R. *et al.* (1964) Mechanism of peptide bond formation in polypeptide synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 51, 1291–1299
- 31 Rheinberger, H.J. *et al.* (1981) Three tRNA binding sites on *Escherichia coli* ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 78, 5310–5314

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**Errata**

The obituary of Max Perutz by Kiyoshi Nagai [TIBS (2002) 27, 322–323] was copyright of The Biochemical Society. More information about the Society can be found at <http://www.biochemist.org/>

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In the book review by David J. Galas [TIBS (2002) 27, 324–325], the book details should have read *The Common Thread: A Story of Science, Politics, Ethics and the Human Genome* by John Sulston and Georgina Ferry. Bantam Press, 2002. £17.99 (310 pages) ISBN 0 593 04801 6. *TIBS* apologizes for any confusion caused by this error.

PII: S0968-0004(02)02088-1

**Corrigendum**

On p. 286 of the article by Cromer, B.A. *et al.* [TIBS (2002) 27, 280–287], the sentence 'This loop also includes a His conserved in GABA<sub>A</sub> receptors and GABA<sub>C</sub> receptors that has been shown to be essential for Zn<sup>2+</sup>-mediated inhibition of GABA<sub>C</sub> receptors [46], indicating that increased positive charge in this region is detrimental to anion permeation.' should have read 'This loop also includes a His, conserved in GABA<sub>A</sub> receptors, GABA<sub>C</sub> receptors and glycine receptors, that has been shown to be essential for Zn<sup>2+</sup>-mediated inhibition of glycine receptors [46], indicating that increased positive charge in this region is detrimental to anion permeation.', and Ref. 46 should have been Harvey, R.J. *et al.* (1999) Identification of an inhibitory Zn<sup>2+</sup> binding site on the human glycine receptor  $\alpha$ 1 subunit. *J. Physiol. (Lond.)* 520, 53–64.

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