ALTERATIONS IN POLYRIBOSOMES DURING ERYTHROID CELL MATURATION

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ABSTRACT

This communication presents a morphological study of the changes in ribosome content and organization which occur during the maturation of erythroid cells of the phenylhydrazine-treated rabbit. Electron micrographs of thin sectioned nucleated and non-nucleated erythroid cells have been subjected to a quantitative analysis of the distribution of ribosomes as polyribosomes of various sizes and as single ribosomes. The ribosomes of nucleated erythroid cells of marrow are virtually all arranged in the polyribosome configuration consisting of clusters of 2 to 6 individual ribosomes. These cells are the most active in the erythroid series in protein biosynthesis. During maturation to the non-nucleated reticulocyte stage, found in the circulating blood, there is a decrease in protein synthesizing capacity, a fall in total ribosome content, and, more significantly, a decrease in the number and size of polyribosomes. Maturation to the ribosome-free erythrocyte, either under *in vitro* or *in vivo* conditions, entails a further decrease in protein synthesis which correlates with a progressive disaggregation of the biosynthetically active polyribosomes into smaller clusters and inactive single ribosomes. Possible models which may account for the stability of the polyribosome and for the mechanism of polyribosome dissociation are discussed.

INTRODUCTION

Ribosomes, either membrane bound (1-3) or free (4-6) have been established as sites of protein biosynthesis. In mammalian reticulocytes, protein synthesis has been shown to proceed predominantly on ribosomes with sedimentation coefficients in excess of 110S (7-9). Electron micrographs of ribosome fractions isolated from lysed reticulocytes revealed that the rapidly sedimenting ribosomes (110S or greater, termed polyribosomes) are probably clusters of two or more 78S ribosomes (8). Polyribosomes have been seen in thin sections of fixed whole reticulocytes (10, 11). Previous studies from this laboratory demonstrated that the capacity of intact reticulocytes to synthesize protein is related to their content of polyribosomes (11). A role of polyribosomes in protein biosynthesis has also been demonstrated in bacteria

(12-14), Hela cells (15), and liver (16, 17). Moreover, a variety of cells contain ribosomes organized in clusters, as demonstrated by electron microscope studies (18-20).

In the process of cell maturation, a loss of cytoplasmic ribonucleoprotein has been noted in experiments with a number of different cells (20). During the *in vitro* maturation of reticulocytes there is a profound decrease in polyribosome content associated with a loss in protein synthesizing capacity (11). The present investigation concerns a detailed analysis of the alterations in polyribosome content and structure which occur as erythroid cells mature under *in vivo* as well as *in vitro* conditions. In the intact cell, polyribosomes appear predominantly as rows of paired ribosomes. In immature cells, synthesizing protein at a relatively

rapid rate, more than 99 per cent of the ribosomes appear in the polyribosome configuration. As maturation proceeds, there is an orderly shift in polyribosome size toward smaller clusters and single ribosomes, with an over-all decrease in the cell content of ribosomes.

MATERIALS AND METHODS

PREPARATION OF RETICULOCYTES: Reticulocytes were prepared from the blood of phenylhydrazine-treated rabbits by the method of Borsook et al. (21).

CONDITIONS FOR MATURATION OF RETICULOCYTES IN VITRO: The conditions for in vitro maturation of cells have been previously described (11). Cells were suspended in a buffered medium containing plasma and antibiotics, and placed in a siliconized, water-jacketed, incubation flask in which temperature, pH, substrate concentration, and gas phase were maintained constant. At the onset and at various times during incubation, aliquots were removed for electron microscope study.

CONDITIONS FOR MATURATION OF RETICULOCYTES IN VIVO: For the study of reticulocyte maturation under in vivo conditions, heparinized reticulocyte-rich blood was isolated within a diffusion chamber. The diffusion chamber was prepared by a procedure similar to that of Algire et al. (22). Lucite rings, 2 mm thick with an internal diameter of 15 mm, were closed with two membrane filters of an average pore size of 0.4 micron (maximal pore size, 0.8 micron). Blood was introduced into the sterilized chamber through a hole in the ring which was subsequently sealed with cement, and the chamber implanted in the peritoneal cavity of the same rabbit which had donated the reticulocytes. After various intervals, indicated below, the chamber was removed, opened, and the blood prepared for electron microscopy.

MORPHOLOGICAL STUDIES: Red blood cell and reticulocyte counts were performed according to standard techniques (23, 24). Although the reticulocyte count proved to be a guide to the progress of maturation, it provided an unreliable quantitation when compared with electron microscope data. For the purposes of this study, therefore, the reticulocyte is defined by its electron microscopic appearance, as a non-nucleated erythroid cell containing ribosomes in any observable number.

Cells from bone marrow, circulating blood, or the maturing cell suspensions were fixed for 1 to 2 hours at 4°C in 1 per cent glutaraldehyde in 0.067 M phosphate buffer at pH 7.3 (25). Glutaraldehyde-fixed cells were then washed thoroughly in 0.1 M phosphate buffer and post-fixed with 1 per cent osmium tetroxide in 0.1 M phosphate buffer. Pellets of fixed and dehydrated cells were embedded in epoxy resin, sectioned, stained with 1 per cent uranyl acetate in 50

per cent ethanol or lead citrate (26), and examined in an RCA EMU 2.

Electron micrographs were routinely obtained at an initial magnification of 5,000. At this magnification, portions of several cells were included in each field. Since ribosomes could not be recognized on the fluorescent screen, it was impossible to bias the selection of cells. In general, all cells within sections of adequate quality were micrographed and all technically satisfactory micrographs were analyzed. Within an arbitrary and standard area inscribed onto each cell (one-inch square at a final magnification of 25,000), the ribosomes were counted and classified according to the size of the ribosomal aggregates to which they belonged. For each experimental sample at least 50 cells were analyzed in this manner and the results were expressed as ribosomes counted per 50 cells. Since not all of the cells contain ribosomes, the percentages of ribosome-containing (i.e. reticulocytes) and ribosome-free cells (i.e. mature erythrocytes) were also determined. No significant differences were observed between the data on ribosome distribution obtained from duplicate samples, as determined by the chi square test for the distribution of frequencies.

Single ribosomes, as observed in these preparations, have a diameter of approximately 200 A. Aggregates are frequently too large to be included within the thickness of one section (approximately 600 A). Clearly, a certain fraction of the aggregates will be transected by the plane of sectioning, resulting in an apparent loss of large aggregates and a spurious increase in the observed number of single ribosomes and small clusters. Two methods have been employed to evaluate the magnitude of this discrepancy between the observed and actual distribution of ribosomes in clusters. On the one hand, ribosomes were counted in a standard area as described above. Subsequently the same area was overlaid with a grid consisting of parallel lines spaced to represent the estimated distance between adjacent planes of sectioning (600 A, $\times$ 25,000; Fig. 1). Those clusters now transected by the simulated section planes were noted and a new distribution of single and clustered ribosomes calculated. The difference in the distribution of ribosome clusters as calculated from the open and the gridded area was used to estimate the magnitude of the error introduced by sectioning. To avoid certain theoretical objections to this empirical correction,[1] as well as the laborious and repetitious task of correction by the grid method, a more general solution for the problem was devised

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[1] It is recognized that the grid-derived estimate assumes that the degree of error introduced by the simulated sectioning reflects that introduced by the initial physical sectioning. This assumption is only approximately true, as the polyribosomes have already been sectioned once when subjected to the grid analysis.

(27). A mathematical formulation was employed which expresses the probability that clustered ribosomes would be transected and observed as single ribosomes or clusters of smaller size. The agreement between the mathematical and empirical grid methods is sufficiently close (27) to justify the use of the calculated correction for the data in these studies.

RESULTS

Structure of the Polyribosome

Electron microscopy of thin sections of reticulocytes reveals the presence of clusters of from two to six ribosomes. Larger aggregates are rare. The most commonly observed pattern is a double row of ribosomes. For example, a cluster of six ribosomes consists of a file of three rows of two ribosomes each (Fig. 1, f). Examples of some polyribosome patterns are illustrated in Fig. 2. The diameter of individual ribosomes is approximately 200 A, while the center-to-center distance between adjacent clustered ribosomes averages 250 A, measured on those clusters whose planar surfaces appear to lie parallel to that of the section. The only variant which is observed with any frequency
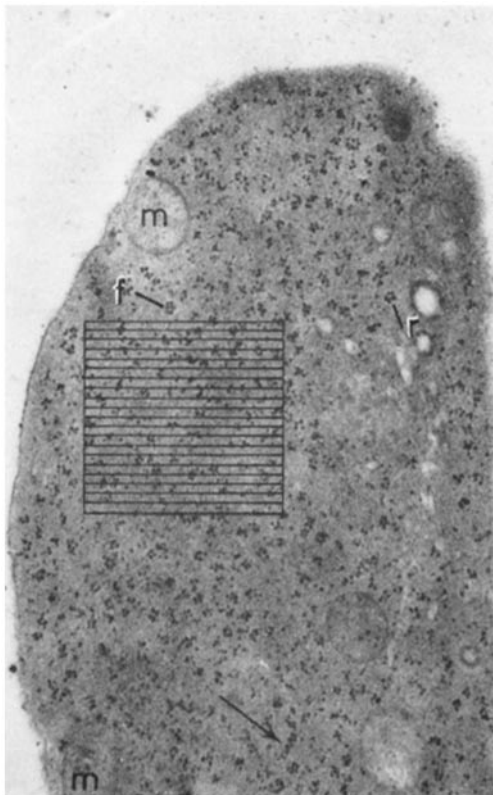

FIGURE 1 A portion of a reticulocyte from a rabbit rendered anemic by repeated injections of phenylhydrazine. Several mitochondria (m) and small vesicles are apparent. Most ribosomes are arranged in clusters of two or more single particles. Double files (f) as well as ring-like (r) patterns are seen. A long chain of ribosomes is indicated by an arrow. A standard area has been ruled with lines representing adjacent planes of sectioning, approximately 600 A apart. The error in ribosome distribution introduced by the sectioning process may be estimated by recounting the ribosomes within the simulated sections (see Methods). X 25,000.
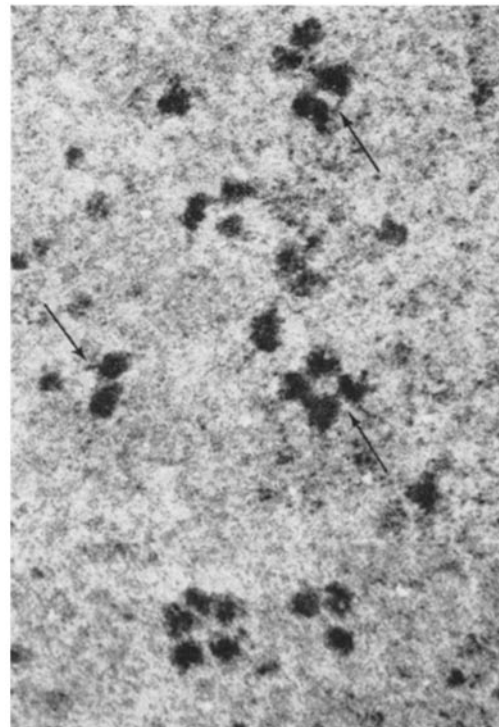

FIGURE 2 Single ribosomes and ribosomes organized in clusters of from two to five. The five-membered polyribosome is arranged in a ring-like configuration. Filamentous structures extending from ribosomes are indicated by arrows. Lead citrate stain. X 212,000.

is an arrangement of five ribosomes in a pentagonal structure (Fig. 2). This pattern is most frequently seen in preparations of immature reticulocytes and in bone marrow cells. More uncommon are somewhat longer, more irregular structures (Fig. 1, arrow) similar to those patterns interpreted as helices by Behnke (28) and by Waddington and

Perry (29). The rarity of this observation makes it more likely that these represent larger but typically arrayed clusters passing obliquely through the section.

Few reticulocyte ribosomes are associated with membranous structures of the type defined as endoplasmic reticulum, although small cisternae are seen in nucleated red cells of the marrow. Short, ribosome-associated filaments, some appearing to lie between adjacent ribosomes in the polyribosome structure, are observed (Fig. 2). Such fine structures may represent artifacts of tissue fixation or staining. Nevertheless, similar structures have been seen in preparations of isolated ribosomes examined by the negative contrast technique (8).

In order to demonstrate that ribosome clustering reflects a biologically significant aggregation and

FIGURE 3   Portion of a reticulocyte after 60 minutes of incubation with 10^{-3}M sodium fluoride. Virtually all the ribosomes appear to be single, unclustered particles. Uranyl acetate stain. $\times$ 37,000.

not merely the random approximation of closely packed ribosomes, intact reticulocytes were incubated in the presence of 10^{-3} M sodium fluoride. This agent is an effective inhibitor of protein biosynthesis and results in a prompt conversion of rapidly sedimenting polyribosomes to the 78S form, as determined by sucrose-density gradient ultracentrifugation (30). A pronounced increase in the proportion of single, non-clustered ribosomes without any significant change in the total number of ribosomes was observed in electron micrographs of reticulocytes incubated for 1 hour with sodium fluoride (Table I, Fig. 3). This observation sug-

TABLE I

Effect of Sodium Fluoride on Polyribosomes of Intact Reticulocytes

| | Ribosomes‡ | | | | | |
| | | | Polyribosomes of cluster size | | | |
Experiment	Total	Single	2	3	4	5
	Number		Per cent			
Control	1076	24	24	30	19	3
Sodium fluoride*	940	75	23	2		
Control	773	28	24	29	19	
Sodium fluoride*	896	83	15	2		

* Cells incubated at 37°C with 10^{-3}M sodium fluoride for 60 minutes (30).
‡ Ribosomes counted within the standard unit area in 50 cells; see Materials and Methods.

gests that the appearance of single and clustered ribosomes is not an artifact dependent upon their density, but, rather, reflects their structural organization in the cell.

Ribosomes in Peripheral Blood Cells

Peripheral blood from anemic rabbits contains reticulocytes at various stages of maturity, since at any moment the blood contains cells newly released from the marrow as well as those on the verge of becoming erythrocytes. Some reticulocytes, from the peripheral blood of a phenylhydrazine-treated rabbit, contain large numbers of ribosomes, most of which are in clusters of from two to six (Fig. 1). Mitochondria and small vacuoles are commonly observed in such cells. Other reticulocytes contain ribosomes predominantly or-
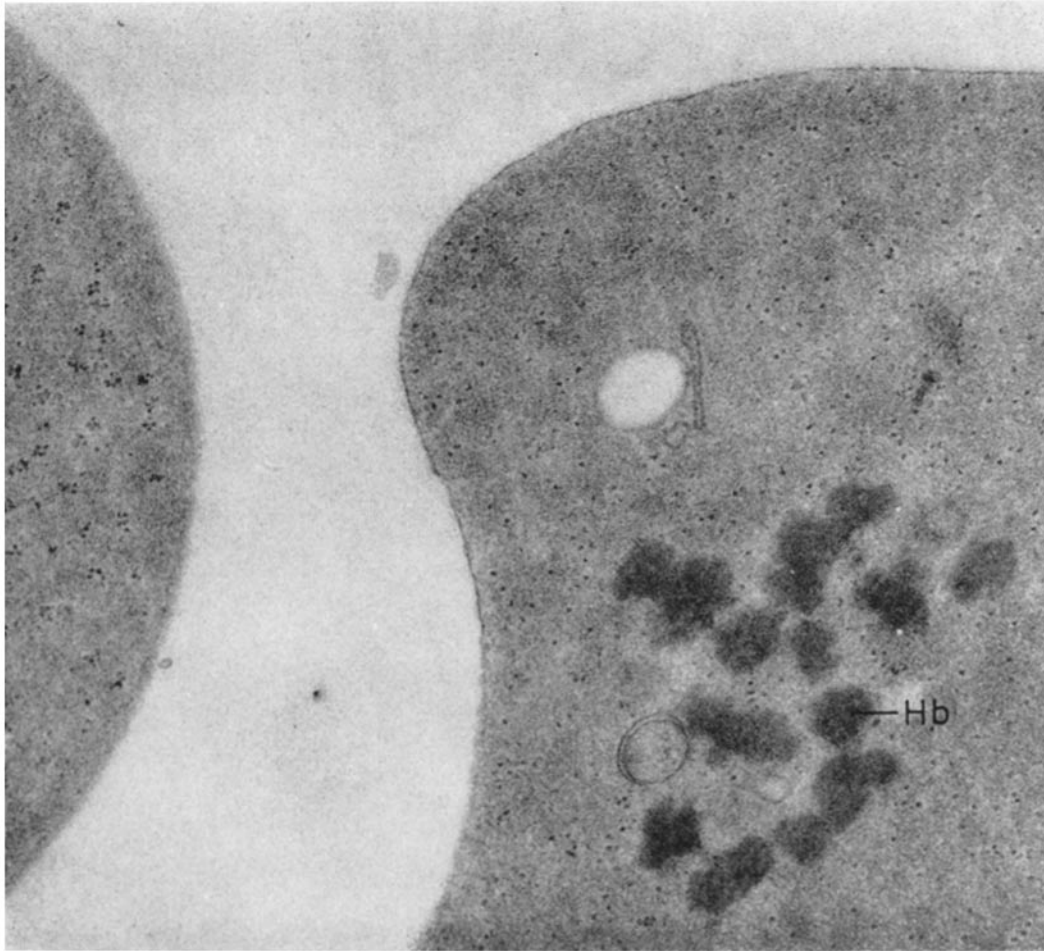
FIGURE 4 Two adjacent reticulocytes from the peripheral blood of a phenylhydrazine-treated, anemic rabbit. At the left is a relatively immature reticulocyte containing clusters of from 2 to 4 ribosomes. The cell at the right contains predominantly single ribosomes. Numerous dense, finely granular Heinz bodies (*Hb*) are also observed in this more mature reticulocyte. Uranyl acetate stain. × 42,000.

ganized in smaller clusters of two to four, while others contain mainly single ribosomes (Fig. 4). Such cells may also display dense bodies composed of amorphous or finely granular material (Fig. 4, *Hb*). The preparation from which these reticulocytes were taken included a large proportion of cells containing Heinz bodies, presumed to be aggregates of oxidized hemoglobin consequent to an effect of phenylhydrazine on cellular metabolism (31). It is assumed that the dense bodies observed in the electron micrographs represent Heinz bodies. Most Heinz bodies were found in mature erythrocytes. This is consistent with the fact that as reticulocytes mature to erythrocytes a number of

enzyme systems are lost (32). As a consequence, the maturing red cell has a decreasing capacity to maintain hemoglobin in a reduced state. The only reticulocytes in which Heinz bodies are observed are those, as illustrated by Fig. 4, containing predominantly single, unclustered ribosomes. These reticulocytes are considered to be the most mature (see below).

An analysis of the distribution of ribosomes as singles and clusters in the population of circulating cells in seven anemic rabbits is summarized in Table II. The over-all percentage of ribosomes in polyribosomes was found to be remarkably constant (72 to 79 per cent) in blood in which the

Circulating Blood From Phenylhydrazine-Treated Rabbits

Reticulocytes*	Ribosomes‡				
	Total	Single		Polyribosomes	
Per cent	Number	Number	Per cent	Number	Per cent
I 68	2744	584	21	2160	79
II 60	3164	744	24	2420	76
III 30	684	144	21	540	79
IV 30	528	111	21	417	79
V 30	698	150	21	548	79
VI 68	1078	260	24	816	76
VII 39	773	216	28	557	72

* Determined by electron microscopy, as non-nucleated erythroid cells containing ribosomes in the unit area; expressed as per cent of the total red blood cell count.
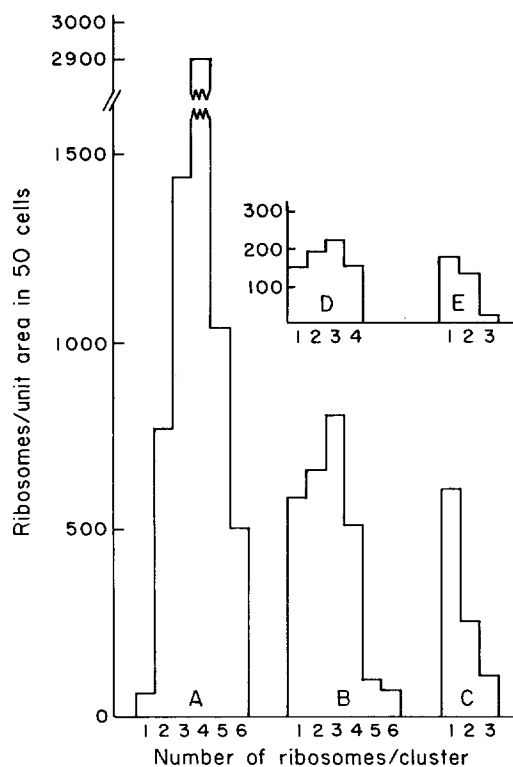‡ Counted within the standard unit area in 50 cells.

FIGURE 5 The distribution of ribosomes as singles and as clusters (polyribosomes) of various sizes, counted in 50 erythroid cells of the marrow (A), circulating blood (B and D) and blood matured for 24 hours under in vitro (C) and in vivo (E) conditions. Samples, A, B, and C are from a rabbit whose circulating reticulocytes comprise 68 per cent of the total red blood cells; D and E are derived from a rabbit with only 30 per cent circulating reticulocytes.

reticulocyte count ranged from 30 to 68 per cent. On the basis of data to be presented below, it appears likely that the proportion of ribosomes which appear as polyribosomes within a reticulocyte is a reflection of the degree of maturity of the cell. These data suggest that in these phenylhydrazine-treated rabbits, despite a considerable variation in the proportion of cells which are reticulocytes, the populations of reticulocytes may be comparable with respect to their mean age.

In Fig. 5 (B and D) are illustrated the patterns of distribution of ribosomes from reticulocytes of two rabbits with reticulocyte counts of 68 per cent and 30 per cent, respectively. More ribosomes appear in clusters of 3 than in aggregates of any other size. It has been reported (8) that clusters of 5 ribosomes account for the largest share of the polyribosome population isolated from rabbit reticulocytes by sucrose-density gradient ultracentrifugation. The reason for this discrepancy in the size of the predominant polyribosomes, as determined by electron microscopy of thin sections and by sedimentation analysis, is not apparent.

Analysis of Ribosome Distribution in Individual Cells

The present techniques permit an examination of the arrangement of ribosomes within individual cells. Analysis at the level of single cells rather than cell populations should provide a clearer picture of the alterations in polyribosomes associated with maturation. For this purpose a total of 288 reticulocytes from a sample of anemic rabbit blood was

TABLE III

The Distribution of Reticulocytes Grouped According to the Size of the Largest Polyribosome Observed in Each Cell

| Experiment	Group (largest polyribosome*)	Reticulocytes		Ribosomes/cell‡						
				Total	Single	Polyribosomes of cluster size				
						2	3	4	5	6
		(Number)	(Per cent)							
A. Circulating blood	6	8	3	111	14	22	26	28	3	18
	5	30	10	90	10	20	25	21	14	
	4	141	49	63	10	17	20	16		
	3	63	22	40	14	13	13			
	2 or 1	46	16	23	14	9				
B. *In vitro*, matured§	6	0	0	—	—	—	—	—	—	—
	5	10	11	72	7	13	32	9	11	
	4	27	28	65	17	21	15	12		
	3	33	35	47	23	14	10			
	2 or 1	25	26	28	18	10				
C. *In vivo*, matured§	6	0	0	—	—	—	—	—	—	—
	5	0	0	—	—	—	—	—	—	
	4	0	0	—	—	—	—	—		
	3	8	29	37	15	15	7			
	2 or 1	20	71	7	6	1				

* Observed within the standard unit area.
‡ Mean value for the cells in each group, as counted within the standard unit area.
§ For 24 hours, either in the incubation flask or the intraperi oneal diffusion chamber.

analyzed. The cells were grouped on the basis of the size of the largest aggregate of ribosomes observed in the standard area in each cell (Table III, Experiment A). Over 60 per cent of the cells contain clusters of four or more ribosomes. When these cells are ordered as in Table III, a progressive decrease in average number of ribosomes per cell is observed as the maximal size of the polyribosomes decreases. Previous studies have indicated that as reticulocytes mature to erythrocytes a decrease in the polyribosome content is associated with a fall in capacity to synthesize protein (11). Taken as a whole, the data suggest that maturation of individual cells might be characterized by an orderly shift in polyribosome size toward smaller clusters and single ribosomes. This hypothesis was directly tested by examining the alterations in ribosome arrangement within individual cells during maturation under *in vitro* and *in vivo* conditions.

Reticulocyte Maturation Under In Vitro Conditions

It has been previously demonstrated that as reticulocytes mature there is a fall in the number of polyribosomes which is proportionately greater than the decrease in the total number of ribosomes. The decrease in the capacity for protein synthesis which accompanies reticulocyte maturation was observed to be more closely correlated with their residual content of clustered ribosomes than with the total or the single ribosome complement (11). This observation is consistent with the established role of the polyribosome in protein biosynthesis (7–9, 11–17). Figure 5 (*B* and *C*) illustrates the alteration in distribution of ribosomes in a population of cells which were matured *in vitro* for a period of 24 hours. The effect of *in vitro* maturation upon the polyribosomes of individual cells becomes apparent when the reticulocytes are grouped according to the size of the largest polyribosome in each cell (Table III, Experiment B). With
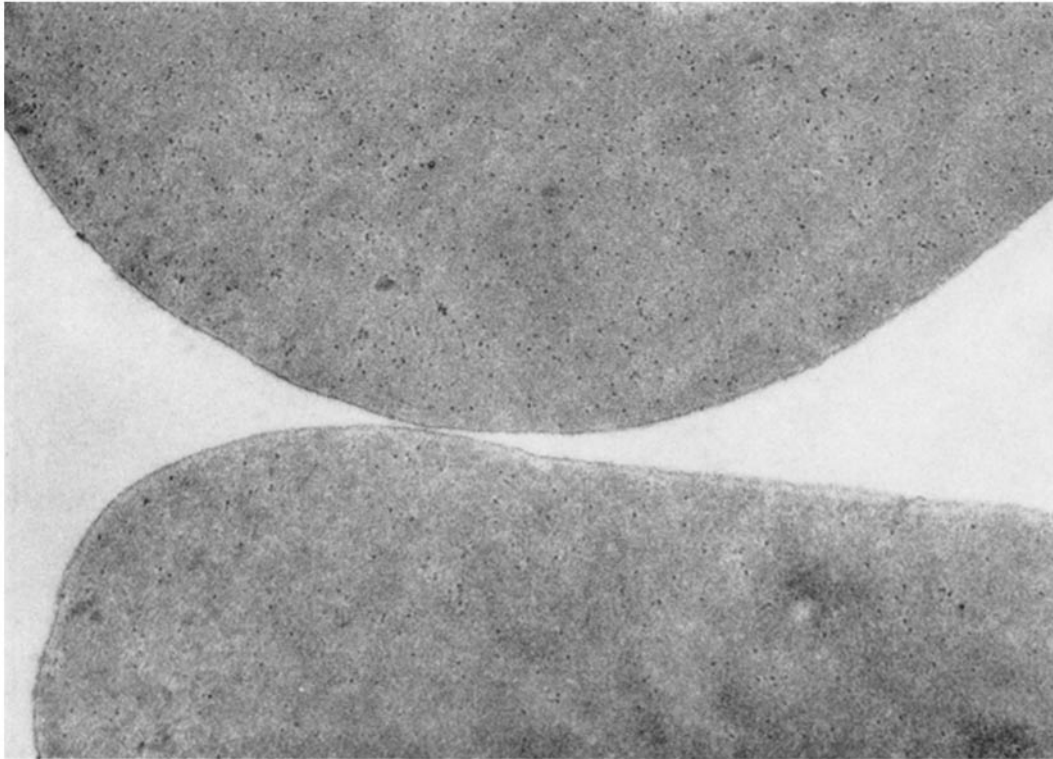
FIGURE 6   Two relatively mature reticulocytes examined after 24 hours in the intraperitoneal diffusion chamber. The cell at top contains numerous ribosomes, a few of which are in small clusters. The lower and more mature reticulocyte contains only a few, single, ribosomes. Section stained with uranyl acetate. × 33,000.

in vitro maturation there occurs an increasing proportion (over 60 per cent) of cells which contain only small clusters (3 or less ribosomes) or single ribosomes.

Maturation of Reticulocytes in Vivo

In order to approximate physiological conditions, the maturation of reticulocytes was studied *in vivo*. The constant influx of new reticulocytes from marrow makes it impossible to study the maturation of reticulocytes by sequential sampling of the circulating blood. Therefore, reticulocytes were isolated from the peripheral circulation by intraperitoneal implantation of reticulocyte-rich blood within a diffuse chamber. Under these conditions a significant maturation of reticulocytes to mature erythrocytes is observed within 17 to 24 hours after implantation. The shift in ribosome distribution towards singles and small clusters is similar to that which occurs during *in vitro* maturation (Fig. 5 *D* and *E*). In Table III, Experi-

ment C, reticulocytes matured for 24 hours *in vivo* are grouped according to the size of their largest polyribosome. No reticulocytes remain having clusters composed of more than three ribosomes. This lends further support to the hypothesis that maturation of reticulocytes is associated with an orderly and progressive decrease in the size of polyribosomes.

Fig. 6 is an electron micrograph of two adjacent reticulocytes removed from an implanted peritoneal diffusion chamber after 24 hours. The upper cell contains predominantly single ribosomes with an occasional cluster of two or three. The lower cell is characteristic of the most mature class of reticulocytes, having only a scattering of ribosomes, virtually all of which are single particles.

Nucleated Erythroid Cells of Marrow

Samples of bone marrow from phenylhydrazine-treated, anemic rabbits were prepared for electron microscopy and the nucleated red blood cells
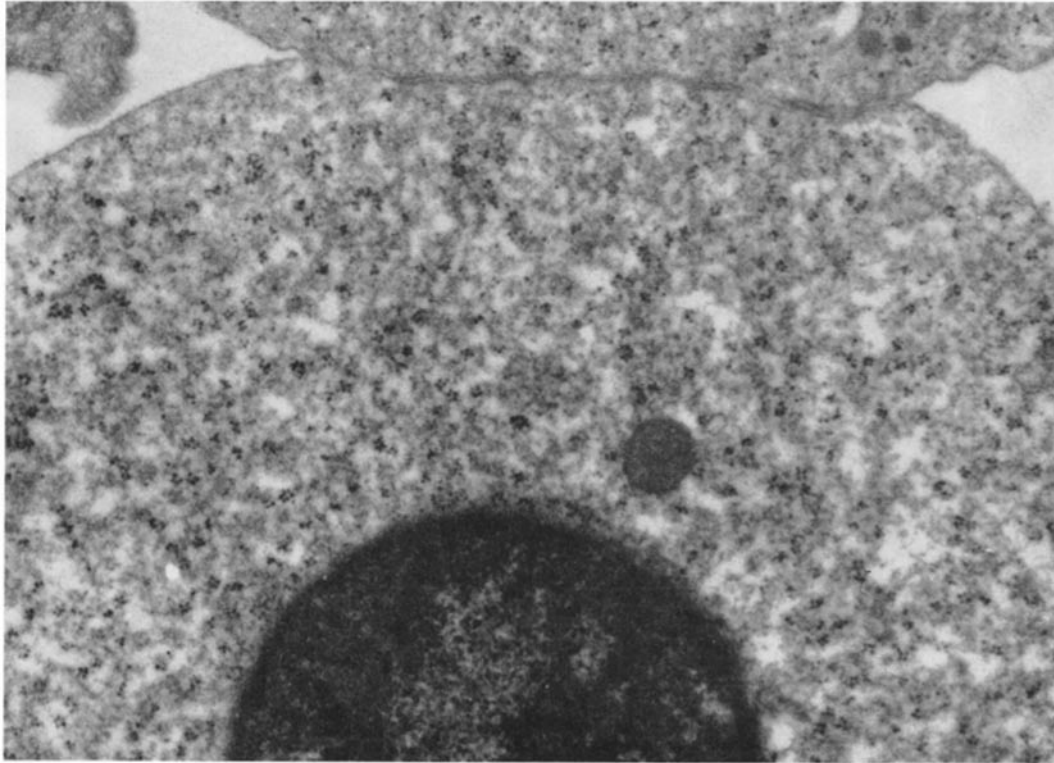
FIGURE 7   A nucleated erythroid cell from the marrow of an anemic rabbit. The cytoplasm is partially filled with a dense, finely granular material presumed to be hemoglobin. Many ribosome clusters are seen. The nuclear membrane is tangentially sectioned, hence it is not clearly displayed. Section stained with uranyl acetate. × 30,000.

examined for their content of ribosome clusters (Fig. 7). The capacity of marrow erythroid cells to incorporate C^{14}-labeled amino acids has been determined by Lingrel and Borsook (33). Their studies reveal that the rate of incorporation is markedly higher in marrow than in reticulocytes. The total ribosome content of 50 nucleated erythroid cells is also considerably greater than that of reticulocytes from the circulating blood (Fig. 5, A and B). This difference, consistent with that reported by Lingrel and Borsook (33), is greater than can be accounted for by the fact that 100 per cent of the marrow cells contain ribosomes as compared with only 68 per cent of the circulating red blood cells in this rabbit. Moreover, since primitive nucleated red blood cells have considerably larger cytoplasmic volumes than reticulocytes (34), the technique of counting ribosomes in a standard area minimizes the actual differences in ribosome content between nucleated cells and

reticulocytes. The distribution of ribosomes into clusters of various sizes is not, however, influenced by these factors which distort the comparison of total ribosome content. The peak incidence of ribosomes is in clusters of four, indicating a higher order of aggregation than is observed in reticulocytes. Even more striking is the virtual absence of single, unclustered ribosomes, which, in marrow, account for only 0.8 per cent of the total ribosome population. Since currently available techniques and criteria for independently assessing the degree of maturity of marrow cells as observed in electron micrographs are not entirely adequate, no attempt has been made to arrange these cells in a maturational sequence. Polyribosomes consisting of as many as 5 or 6 ribosomes are encountered in 92 per cent of nucleated erythroid cells, whereas only 13 per cent of circulating reticulocytes contain polyribosomes of this size. For the purpose of comparison, the distribution of ribosome-con-

taining erythroid cells from marrow, peripheral blood, and blood after maturation, classified according to the size of their largest polyribosome is illustrated in Fig. 8. It is evident that with increasing degrees of maturation there is a progressive increase in the proportion of cells containing the smallest clusters or only single ribosomes, in addition to the growing proportion of mature, ribosome-free erythrocytes.
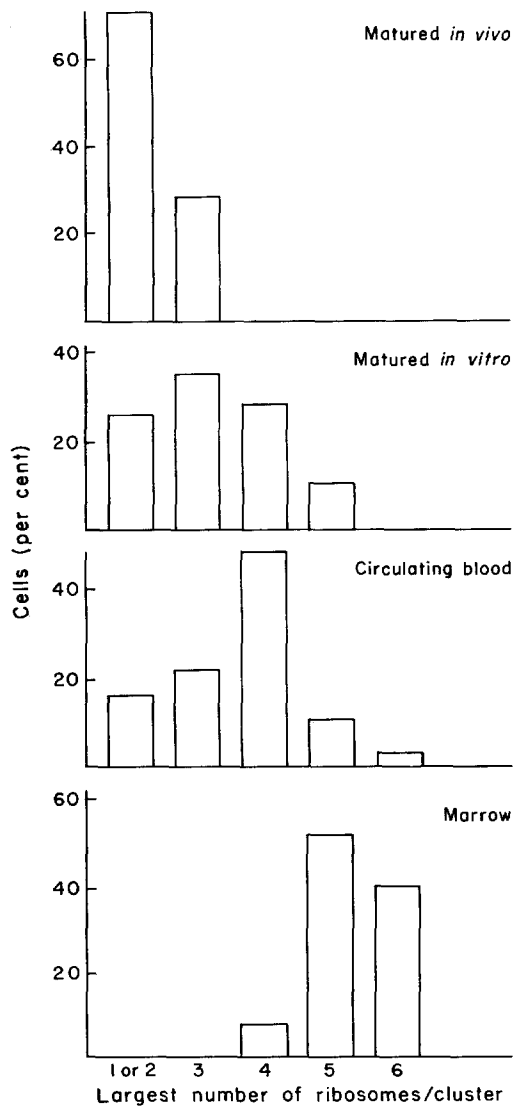
Fate of the Ribosome Cluster

Analysis of the relationship between mean total and clustered ribosomes per unit area per cell for reticulocytes of all degrees of maturity indicates that polyribosomes disappear completely at a stage when single ribosomes are still present (Fig. 9). This stage is illustrated by the lower cell in Fig. 6. In brief, in the marrow there exists a high order of ribosome aggregation with virtually no single ribosomes. In reticulocytes there is a lesser
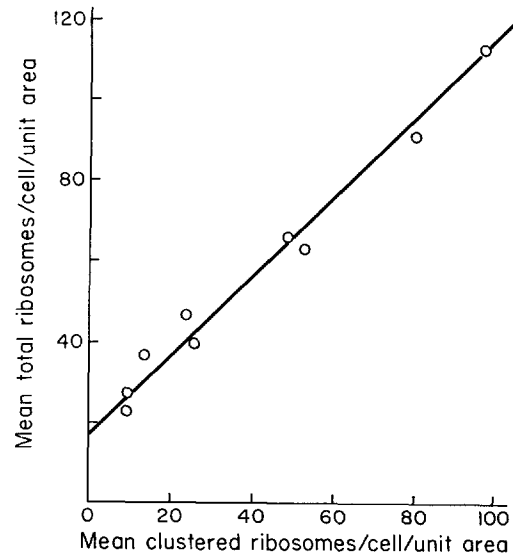

FIGURE 9 The relationship between total and clustered ribosomes (polyribosomes) plotted from an analysis of the ribosome distribution in 411 reticulocytes classified and averaged as in Table III. Reticulocytes from the experiments summarized in Table III are considered as one population. These data therefore reflect reticulocytes which range from highly immature to practically erythrocytes.

FIGURE 8 The distribution of reticulocytes from marrow, circulating blood, as well as after *in vitro* and *in vivo* maturation, classified according to the size of the largest polyribosome observed in the standard unit area in each cell.

degree of ribosome aggregation while significant numbers of single ribosomes first make their appearance and are, in turn, the last to disappear. A likely explanation of these data is that single ribosomes are a product of the dissociation of clusters and that they are a transient form in the process of complete ribosomal disintegration.

DISCUSSION

The present studies indicate that as erythroid cells mature there occurs a progressive dissociation of polyribosomes into smaller aggregates and single ribosomes as well as a gradual fall in total ribosome

content. Numerous studies (7–9, 11–17) implicate the polyribosome as the site of protein synthesis. The loss in protein-synthesizing capacity which accompanies erythroid cell maturation appears to be related to the observed fall in polyribosome content. The maturation of a variety of cells is accompanied by an analogous loss of cytoplasmic ribonucleoprotein (20). It appears likely that the process of polyribosome dissociation is a more general phenomenon which accompanies cellular maturation when the mature cell is relatively inactive in protein synthesis.

Previous studies (35) have indicated that reticulocyte maturation is accompanied by a degradation of cellular RNA to purines and pyrimidines which diffuse from the cell. The present observations indicate that the ultimate disintegration of ribosomal RNA into its component bases is preceded by the dissociation of the biosynthetically active polyribosomes into the inactive 78S ribosomes and, possibly, smaller subunits.

Information as to the mode of dissociation of polyribosomes in the course of cell aging is, in principle, contained in the polyribosome size distributions observed at different stages of reticulocyte maturation (Table III); i.e., different models of the dissociation process predict different distributions which may be compared with those actually observed. Of the many models possible, two are considered, in which the elementary act of dissociation of a polyribosome occurs by:

I. Fragmentation into a pair of smaller polyribosomes of any size; and II. Loss of one ribosome from the polyribosome (this is actually a special case of Model I). The data of Table III, Experiment A (which is the largest population and least subject to small sample variation) indicate a preference for Model II, that is, polyribosome dissociation by sequential loss of single ribosomes.[2] It has been hypothesized (8, 9, 13, 16, 36) that the individual 78S ribosomes in the polyribosome structure are held together by a strand of messenger RNA, along which the ribosomes move as the nascent protein chain grows. It is assumed that as a peptide chain is completed a 78S ribosome and its attached, newly synthesized protein are released from the terminal end of the messenger RNA strand and hence dissociated from the polyribosome. Model II is compatible with this hypothesis. The present observations and analysis are not,

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[2] The mathematical analysis of these models is described in the Appendix.

however, regarded as more than suggestive on this point, because of the relatively limited amount of experimental data for this type of model analysis.

The presence of short filamentous structures between the constituent ribosomes of a polyribosome suggests that a structural unit maintains the 78S ribosomes in the polyribosomal configuration. There is evidence that the integrity of the polyribosome is dependent upon an RNA-containing structure or structures (8, 9, 13, 15, 16, 30, 37). As previously noted, current speculation has focused upon messenger RNA for this role, but there is also evidence that both ribosomal (37) and transfer RNA (30) may contribute to the stability of the polyribosome.

The erythroid cells most active in protein synthesis are the nucleated cells of the marrow (33). Ribosomes in this population display the highest order of aggregation observed in the erythroid series. Moreover, less than 1 per cent of the ribosomes are single, unclustered units. In order for this observation to be consistent with the hypothesis that single ribosomes are released during the synthesis of protein and are capable of re-attachment to polyribosomes with the initiation of new polypeptide chains (36, 38, 39), it must be assumed that the rate of re-attachment is sufficiently great that only a small pool of single ribosomes can be demonstrated. On the other hand, it is possible that the function of polyribosomes in protein synthesis does not require the release of single ribosomes. It has been demonstrated that the dissociation of polyribosomes can proceed at similar rates in reticulocytes actively synthesizing protein and in cells, under conditions of amino acid starvation, in which no peptide bond formation occurs (40). Although the rate of polyribosome dissociation may not be dependent upon active protein synthesis, it is clear that the integrity of the polyribosome is essential for protein formation. This study suggests that elucidation of the structure and function of polyribosomes and the factors which control the process of polyribosome dissociation will be of fundamental importance to an understanding of cellular maturation.

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