# STRUCTURAL CHANGES IN RIBOSOMES DURING DEVELOPMENT

William DeWitt and Richard P. Price*

Department of Biology
Williams College
Williamstown, Massachusetts 01267

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Summary: Basic ribosomal proteins, extracted from immature erythrocytes of both pre-metamorphic and adult bullfrogs (*Rana catesbeiana*), were characterized on urea-polyacrylamide gels. Ribosomes from larval and adult organisms display quantitative and qualitative differences in the banding patterns of their basic ribosomal proteins. Such alterations in ribosomal structure may be important in the control of gene expression during development.

Sussman (1) has proposed a model for qualitative control of protein synthesis in which differential gene expression is a result of the selective attachment of ribosomes to messenger RNA. According to this hypothesis, messenger RNA will not be translated unless it possesses a redundant base sequence at the 5'-terminus that is recognized by the ribosomes present in the nucleus. Thus, control of gene expression during cellular differentiation operates through the mechanism which determines the species of ribosomes synthesized in a cell at a given point in development.

In a test of this hypothesis, we have looked for structural differences in ribosomes of immature erythrocytes from premetamorphic and adult *Rana catesbeiana*, and have examined changes in ribosomal structure at the time of metamorphosis. We conclude that the basic ribosomal proteins of the larval bullfrog differ

*Present Address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

both quantitatively and qualitatively from those of the adult organism, and that this change appears during thyroxin-induced metamorphosis of tadpoles.

## MATERIALS AND METHODS

*Rana catesbeiana* tadpoles and frogs were purchased from Mogul-ED Corporation, Oshkosh, Wisconsin. To obtain immature red blood cells, tadpoles and frogs were injected on two consecutive days with phenylhydrazine at a concentration of 25 µg per gram of body weight. For each experiment blood cells were collected from 10-12 tadpoles at 18 days after the initial injection; at that time, the peripheral blood contains a homogeneous population of polychromatophilic erythroblasts (2). In the case of the adult frogs it is sometimes necessary to lengthen the time of recovery from phenylhydrazine-induced anemia in order to obtain sufficient quantities of erythroblasts.

Red blood cells were freed of contaminating white cells by filtration through short columns of lamb's wool (2), and were subsequently lysed in 10 volumes of cold TMA (0.03 M $NH_4Cl$, 0.01 M $MgCl_2$, 0.01 M Tris, pH 7.8) by gentle homogenization in an all-glass, manually-operated homogenizer or by the addition of saponin at a final concentration of 0.5%. Nuclei and cellular debris were removed by centrifuging the lysate at 25,000 x g for 10 minutes. The top three-quarters of the supernatant was carefully removed and recentrifuged at 25,000 x g for 15 minutes. The resultant supernatant was layered on a discontinuous sucrose gradient containing 2 ml of 40% sucrose, 2 ml of 30% sucrose and 1.5 ml of 15% sucrose, all in TMA. Ribosomes were pelleted by centrifugation for 2.5 hours at 105,000 x g, and washed twice by suspension in TMA. The final pellet was resuspended in 0.5 M $NH_4Cl$, 0.01 M Tris, 1.0 mM $MgCl_2$, pH 7.4, and incubated for 5 hours at 3°C (3). The suspension

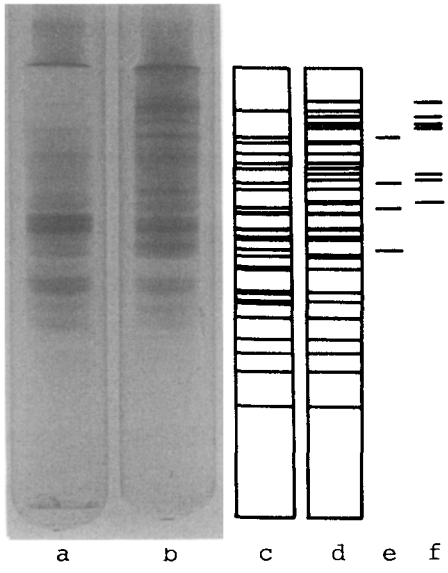


Figure 1: Polyacrylamide gel electrophoresis of basic ribosomal proteins from red blood cells of (a) larval and (b) adult *Rana catesbeiana*. Diagrammatic representation of the larval and adult gels is shown in (c) and (d), respectively. Bands which are present in only tadpole or frog ribosomes are indicated in Figure 1 (e) and (f), respectively.

was then centrifuged at 15,000 x g for 10 minutes, the pellet was discarded and the ribosomal subunits were pelleted by centrifugation. The final pellet was suspended in a small volume of 66% acetic acid, thoroughly dispersed, and incubated at 3°C for 2.5 hours (4). Alternately, the pellet was suspended in a small volume of 2M LiCl, 4M urea and incubated in the cold for 16 hours (5). Following incubation, precipitated RNA was removed by centrifugation at 15,000 x g for 10 minutes. The supernatant was dialyzed for 6 hours against 8M urea in β-alanine - acetic acid buffer, pH 4.5, and 80 µg of ribosome protein were layered on to urea-polyacrylamide gels (6). Following electrophoresis for 4.5 hours at 2.5 ma per tube, gels were stained with 1% amido black in 7% acetic acid and destained electrophoretically. Protein was determined by the method of Lowry (7).

## RESULTS AND DISCUSSION

Electrophoresis on urea-polyacrylamide gels at pH 4.5 clearly demonstrates both qualitative and quantitative differences in the banding patterns of larval (pre-metamorphic) and adult basic ribosomal proteins (Figure 1). Gels from the acetic acid and LiCl-urea extractions were identical. Larval ribosomes contain 22 basic protein bands, while 25 bands are clearly distinguishable from ribosomes of the adult frog. Both types of ribosomes possess 18 bands in common, although quantitative differences in these bands are clearly apparent between the two gels. In addition, protein bands which are present only in tadpole or frog ribosomes are indicated in Figure 1 e & f, respectively.

The banding patterns of gels from both adult and premetamorphic animals were entirely reproducible in four separate experiments, both in the relative intensity and color of the bands when stained with amido black. Identical patterns are obtained when red cells are lysed by saponin or by gentle homogenization in a manually-operated all-glass homogenizer. These differences are not due to contamination by hemoglobin or histone proteins, since both larval and adult hemoglobins and histones were shown to possess mobilities different from those of any of the ribosomal proteins, under the conditions of electrophoresis employed here. Similar results were obtained using ribosome preparations which had not been washed with ammonium chloride. It therefore appears that there is no differential sensitivity of the larval and adult ribosomes to the wash. In addition, the electrophoretic band patterns are not affected by carrying out the extraction procedure and running the gels in the presence of β-mercaptoethanol. It is thus unlikely that

the observed differences arise from random disulfide bond formation or aggregation of proteins.

The observed switch in ribosomal structure should be evident at the time of metamorphosis when erythroid cells characteristic of adult bullfrogs begin to replace larval erythrocytes in the peripheral circulation. Consequently, normal tadpoles were immersed in water containing $2.5 \times 10^{-8}$M L-thyroxin and allowed to metamorphose into froglets for 45 days. The ribosomal proteins from erythroid cells of these organisms demonstrated a banding pattern identical to that obtained from adult frog erythroblasts, even though approximately 80% of the circulating erythroid cells present in these animals consists of mature erythrocytes containing hemoglobin characteristic of the larval organism. Earlier ultrastructural experiments (8) have demonstrated that mature amphibian erythrocytes contain very few ribosomes; hence, ribosomes obtained from circulating red blood cells of metamorphosing animals will be largely from newly-released, immature frog erythroid cells, which contain an abundance of ribosomes. The result from this experiment substantiates the conclusion that structural differences exist between ribosomes of tadpole and frog erythroid cells, and appears to rule out the possibility that genetic differences between the tadpoles and frogs can account for the observed dissimilarities in banding patterns.

The electrophoretic method which we used in these experiments undoubtedly does not resolve all of the basic ribosomal proteins present in the mixture, as two-dimensional polyacrylamide gel electrophoresis has been shown to resolve 21 proteins in the 30s subunit and 34 proteins in the 50s subunit of *E. coli* ribosomes (9,10). Unfortunately, we could obtain only small

amounts of ribosomes from tadpole and frog erythroid cells, and hence did not have sufficient material to separate the proteins by two-dimensional gel electrophoresis or to examine the protein composition of the individual subunits. Thus, ribosomes from the two sources may well have additional differences in their protein composition.

Many workers have suggested that alterations in ribosomal structure during development may play an important role in the control of gene expression. The changes in ribosomal structure reported here may thus be significant in the differentiation of a new population of adult erythroid cells during amphibian metamorphosis.

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