Role of Total Ribonucleic Acid Concentration and the Ratio of Translating and Nontranslating Ribosomes in Development of Compensatory Hypertrophy of the Heart

By F. S. Meerson, M. P. Iavich, and M. I. Lerman

ABSTRACT

Initial adaptation of the myocardium to continuous stress produced by a pressure overload was found to involve increased biosynthesis of ribonucleic acid (RNA) accompanied by an increased ratio of translating to nontranslating ribosomes. Following two days of compensatory hyperfunction, ratios of translating polysomes to nontranslating ribosomal monomers and subunits increased from 1.0 to 1.5. A mathematical model is presented, based on these and other data, which predicts that the increased heart mass observed during hypertrophy cannot be explained solely by the increased concentration of myocardial RNA. It is suggested that an increase in heart mass during compensatory hypertrophy depends upon both an increased level of myocardial RNA and an increase in the proportion of the total population of heart ribosomes involved in translation of messenger RNA.

KEY WORDS

myocardium cardiac hypertrophy protein synthesis translation transcription mathematical model

As the heart adapts to continuous stress caused by constriction of the aorta, ribonucleic acid (RNA) and protein synthesis are activated in the myocardium. As a result, cardiac muscle hypertrophies, and the weight of the heart increases 1.5 to 2 times. In this case, a close parallelism is observed between the accumulation of RNA in the myocardium, its protein-synthesizing ability, and increase in heart mass. There are a number of unsolved problems in the dynamics of this adaptation process. In particular, mechanisms regulating protein synthesis in the cardiac muscle during hyperfunction and the nature of the compensatory growth remain obscure. Normally, increased protein biosynthesis is connected with an increase in polyribosomes in the cells. The latter may be caused by activation of messenger RNA (mRNA) synthesis compared with ribosomal RNA (rRNA) synthesis, as well as mobilization of nontranslating ribosomes resulting from an increased frequency of peptide-chain initiation. Moreover, in higher animals an increase in protein synthesis can be regulated by an increase in ribosome content in the cells. Zak et al., as well as Moroz,

Zak et al., have described a sharp increase in rRNA and transfer RNA (tRNA) contents in hearts undergoing hypertrophy. However, they did not succeed in detecting a difference in the size distribution within the polysomes of cardiac muscle. These authors came to the conclusion that the increase in protein synthesis in the hypertrophying heart was achieved by an overall increase in the number of ribosomes per unit mass of the organ.

Schreiber et al., studying RNA and protein synthesis in the isolated heart subjected to increased stress, concluded that an increase in protein synthesis in the heart is regulated by an increase in the formation of mRNA. Moroz and Zak et al. have studied size distribution of a small portion of the total ribosomes. The ribosomes they isolated contained only 10% to 15% of the total cellular RNA and obviously did not reflect the size distribution of the total population. Thus, the question of the ratio of polyribosomes and nontranslating ribosomes in the cardiac muscle during the development of hypertrophy remains obscure.

In connection with this, the present work investigated the ratio of translating and nontranslating ribosomes at early stages of compensatory hyperfunction of the heart, and then, by means of a mathematical model, described interactions between RNA content and increase in heart mass in the process of compensatory hyperfunction of the heart.

Materials and Methods

Male white rats weighing 220 to 240 g were used in the experiments. Compensatory hyperfunction of the heart was caused by establishing a coarctation of the abdominal aorta by the method of Beznak as modified by
Kogan. This provided a two-thirds to three-fourths decrease in cross section of the subdiaphragmatic aorta.

For determination of RNA content, cardiac muscle was processed according to the scheme of Schmidt and Thannhauser. The amount of RNA was measured spectrophotometrically by the method of Spirin. In examination of the size distribution of cardiac muscle ribosomes, sedimentation through saccharose gradients was used in combination with labeling of the RNA. RNA was labeled by administration of 100 mCi per rat of [14C] orotic acid. The radioactivity was given 48 hours before the animals were killed by decapitation. The method of Heywood et al. was used for separation of ribosomes. The hearts were homogenized in 3 volumes of a buffer containing 0.250 M KCl, 0.010 M MgCl₂, and 0.01 M Tris-HCl, pH 7.4. In addition, 20% Triton X-100 was added to this medium to give a final concentration of 1%. The muscle was first ground in a Waring-type homogenizer (30 seconds at 8,000 rpm) and then in a glass homogenizer with a Teflon pestle. A homogenizer with a slightly ground piston was used first (5 to 6 strokes of the piston), followed by a more closely ground one. Hearts of 10 to 12 animals (about 7 to 10 g of muscle tissue) were normally used in each experiment.

The resulting homogenate was filtered through a double layer of gauze and centrifuged for 15 minutes at 12,000 rpm to remove large fragments of cells, nuclei, and mitochondria. The postmitochondrial supernatant (4 ml) was layered onto a linear saccharose gradient (50 ml; 10% to 40% saccharose) prepared in the homogenizing medium. The gradients were spun for 2 hours at 25,000 rpm in an SW-25 bucket rotor (4°C). All preliminary procedures were also carried out at 4°C.

In one series of experiments designed to determine the size distribution of the ribosomes, ethylenediaminetetraacetate (EDTA) was added to give a final concentration of 0.02 M before the postmitochondrial supernatant was layered on the saccharose gradient. In this case, the gradients were prepared in a buffer containing only 0.010 M Tris-HCl solution, pH 7.4, and 0.250 M KCl. In other experiments the postmitochondrial supernatant was treated with ribonuclease. Incubation was for 15 minutes at 37°C with 20 μg of bovine ribonuclease. The supernatant was layered on a gradient that was prepared in a homogenizing medium. All solutions were prepared with deionized water.

Upon completion of centrifugation of the gradients, the tubes were punctured and 45-drop fractions were collected (42 to 44 fractions in all). The optical density of the fractions was measured in an SF-4 spectrophotometer. For determination of radioactivity of the individual ribosome fractions, 0.2 μg of yeast RNA was added as a carrier, and the RNA was precipitated with 10% trichloroacetic acid (TCA). The precipitate was deposited on Millipore filters (A and FS); washed with a cold 5% TCA solution, alcohol, and ether; dried; and counted in a Mark II scintillation counter.

Analysis of the labeled ribosomes after their centrifugation in saccharose density gradient was carried out by calculation of the total radioactivity of the gradient fractions containing polyribosomes and nontranslating materials (dimers, monomers, and subunits). Localization of the subunits was calculated mainly by proceeding from the distribution of the monomer peak. A similar analysis was used for estimating the quantitative changes in the polyribosome profile of control hearts and those undergoing hypertrophy.

In some experiments the ribosome preparations were separated from the postmitochondrial supernatant. For this, the supernatant was diluted fourfold with a buffer containing 0.01 M MgCl₂ and 0.01 M Tris-HCl, pH 7.4, and left in an ice bath for 15 minutes. With this reduction in ionic strength the ribosomes could be collected as a pellet after 10 minutes of centrifugation at 10,000 rpm. The pellets were carefully suspended in a small volume of buffer containing 0.5 M KCl, 0.01 M Tris-HCl, and 0.01 M MgCl₂, and their optical densities were determined in the SF-4 spectrophotometer.

The RNA content of ribosome preparations, as well as that in the postmitochondrial supernatants, was determined by the method of Spirin. Proteins were determined by absorption at 280 nm, as well as by the method of Lowry with bovine albumin as the standard.

**Reagents and isotopes**

Potassium chloride, magnesium chloride, saccharose, trichloroacetic acid, and [14C] orotic acid (60 mCi/mM) were obtained from sources within the U.S.S.R.; Tris-HCl and ribonuclease from Merck; EDTA from Reanal (Hungary); and Triton X-100 from Ferak (Federal Republic of Germany).

**Results**

The data presented in Table 1 reflect the changes in heart weight and RNA content. Heart weights, RNA concentration, and total RNA content in control hearts did not change significantly during the period of observation.

With compensatory hyperfunction of the heart, the weight of the cardiac muscle increased 20% within 3 days, and by 36% and 40% after about 10 days. RNA concentration increased 27% to 30%. Accordingly, the RNA content of the entire heart increased initially by 50% and subsequently by 65%. Between the 13th and 19th days of compensatory hyperfunction of the heart, the heart weight was maintained at a constant value that was 50% above that of the controls. RNA concentration was the same as in control hearts, resulting in an increase in total RNA content of the heart that was proportional to the increase in heart mass.

Estimations of the RNA content of the postmitochondrial supernatants showed that 5% of the total RNA of the homogenate, which amounted to 1.5 mg of RNA per gram of tissue, was recovered in it. A portion of this RNA, about 25%, was tRNA; consequently, the postmitochondrial supernatant obtained by our method of homogenization contained about 1.15 mg rRNA per gram of cardiac muscle, which corresponded to about 2.3 mg of ribosomes.

In estimating the amount of RNA in the ribosome preparations separated from the supernatant...
fraction by centrifugation in low-ionic-strength buffer, it was found that 0.5 to 0.55 mg of rRNA per gram of tissue was recovered. This amounted to 1 to 1.1 mg of ribosomes. This recovery is approximately 4 times greater than the recovery of ribosomes which Zak et al., Köbbel et al., and Earl et al. were able to achieve from heart muscle.

The ribosomes obtained were contaminated with protein, apparently myosin. This interfered with the RNA analysis by absorption in the ultraviolet range. To aid in the identification of RNA, we used a labeled RNA precursor, [14C] orotic acid, for characterization of the ribosome sedimentation profile. When the isotope was administered 48 hours prior to the experiment, it was concentrated mainly in rRNA and tRNA; tRNA was localized in the upper fractions of the gradient, and consequently radioactivity in the main portion of the gradient represented rRNA (i.e., by ribosomes).

The sedimentation profile of ribosomal structures separated from the hearts of control animals is shown in Figure 1. The radioactivity distribution is evidence that the ribosome profile has a characteristic distribution, with a sharp peak in the monomer region (80 S) and a less pronounced one in the dimer region.

Treatment of the postmitochondrial supernatant with ribonuclease (RNase) led to disappearance of radioactivity in the heavy zone of the gradient and the appearance of marker in the monomer region. This result is consistent with the assumption that mild treatment of polyribosomes with low concentrations of ribonuclease leads to hydrolysis of mRNA and fragmentation of polyribosomes into monomers. On the other hand, treatment of the postmitochondrial supernatant with EDTA (Fig. 1) removed Mg ions from the ribosomes and caused their dissociation into subunits. The marker then appeared in the slowly sedimenting fractions at the top of the gradient. These characteristic effects of RNase and EDTA on distribution of radioactivity in the saccharose density gradients are evidence that the initial sedimentation profile was due to the polyribosomes of cardiac muscle.

The ribosome profiles in Figure 2 permit comparison of a normal heart with a heart undergoing compensatory hyperfunction for 48 hours, and reflect the principal finding of this paper. It can be

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**TABLE 1**

*Dynamics of Myocardial RNA Content in Compensatory Hyperfunction of the Heart*

<table>
<thead>
<tr>
<th>Time after start of CHH (days)</th>
<th>Control</th>
<th>Compensatory hypertrophy of the heart (CHH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart weight (mg)</td>
<td>RNA content mg/100 mg tissue</td>
</tr>
<tr>
<td>0</td>
<td>580</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>0.29</td>
</tr>
<tr>
<td>5</td>
<td>600</td>
<td>0.32</td>
</tr>
<tr>
<td>7</td>
<td>610</td>
<td>0.30</td>
</tr>
<tr>
<td>9</td>
<td>620</td>
<td>0.29</td>
</tr>
<tr>
<td>11</td>
<td>620</td>
<td>0.31</td>
</tr>
<tr>
<td>13</td>
<td>610</td>
<td>0.31</td>
</tr>
<tr>
<td>15</td>
<td>600</td>
<td>0.30</td>
</tr>
<tr>
<td>17</td>
<td>620</td>
<td>0.29</td>
</tr>
<tr>
<td>19</td>
<td>620</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Each number is the mean value obtained in determination of heart weight and amount of RNA in five animals.
seen that 48 hours after the start of hyperfunction, not only did the specific radioactivity of the RNA in fractions of the gradient increase sharply, but the relative content of polyribosomes in the total ribosome preparations increased in comparison with the controls. The fraction of polysomes in normal hearts was about 50% of ribosomes; in hyperfunction this fraction increased to 60%, and only 40% of the ribosomes did not participate in translation. In other words, the ratio between translating and nontranslating materials (dimers, monoribosomes, subunits) in the control was about 1, but increased to about 1.5 in hyperfunction.

Further experiments have shown that the relative increase in polyribosome content of the myocardium observed in the initial stages of compensatory hyperfunction levels off completely eight days after the start of compensatory hyperfunction. At this time, synthesis of rRNA is activated as before, and the content of ribosomal structures remains elevated (Fig. 3).

Comparison of the sedimentation profiles of the postmitochondrial supernatants from control and hypertrophying hearts, both on the second and on the eighth day after the start of hyperfunction, discloses increased levels of radioactivity in the upper fractions of the gradient. This is evidently caused by increased tRNA synthesis.

**Discussion**

The principal finding of these experiments is that in hyperfunction of the heart, activation of transcription and increase in RNA content are accompanied by an increase in the number of polyribosomes in the total ribosome population of the heart. These findings contradict the data of Moroz* and those of Zak et al.* This disagreement is apparently explained by the fact that these authors used a saccharose-salt medium, which permits recovery of a negligible portion of all the ribosomes of the heart (10% to 15%). Evidently the ribosomes obtained in this way did not reflect the state of functional activity of the entire population.

Using the method of Heywood et al.,15,16 we succeeded in separating relatively large numbers of ribosomes from the cardiac muscles of adult rats. In this case, the population of ribosomes is apparently representative of the ribosome structures of the myocardium, synthesizing different classes of heart proteins. The increased numbers of polyribosomes that we found in the total ribosome population of hypertrophying hearts may depend on two different factors:

1. In the generalized activation of transcription caused by compensatory hypertrophy of the heart, mRNA synthesis may increase to a greater extent than rRNA synthesis, and could account for the increase in ratio between polyribosomes and non-translating ribosomes.

2. Mobilization of nontranslating ribosomes may take place in the hypertrophying heart, and
the frequency of initiation may increase. These processes could also account for the change in the ribosome sedimentation profile. Of course, this proposition requires experimental verification.

The increase in the ratio of polyribosomes to nontranslating ribosomes indicates that in the initial stages of compensatory hypertrophy of the heart, the available RNA per unit mass forms a larger quantity of protein than in normal heart. In other words, if our results correspond to the real situation, the heart mass must increase to a greater extent in the initial stages of compensatory hyperfunction than the concentration of RNA in the myocardium. With this in mind, we have devised a quantitative characterization of the interrelations between RNA concentration in the myocardium and the increase in heart mass, using the data of the experiments presented in Table 1.

We have introduced the following designations for description of the interrelations referred to:

- \( M \) = mass of the heart during development of hypertrophy
- \( M_0 \) = mass of the heart at start of hyperfunction
- \( W \) = relative weight of the heart
- \( R_e \) = total RNA concentration of myocardium
- \( R \) = RNA content of myocardium
- \( P \) = RNA concentration of polyribosomes during compensatory hypertrophy
- \( P_o \) = RNA concentration in the polyribosome population in which protein synthesis and degradation are equal and the mass of the heart does not change
- \( R_o \) = total RNA concentration corresponding to\( P \)
- \( C_1 \) = coefficient characterizing the time necessary for synthesis of one polypeptide chain on the ribosomes
- \( C_2 \) = ratio of the number of translating ribosomes to their total number.

All variables are taken to be functions of time \( t \).

It is evident that the rate of change in mass of the heart is determined by the difference between the RNA concentrations of translating polyribosomes at a given moment of time and the concentration of the same RNA at which no change in organ mass would take place. Therefore, the dynamics of change in mass of the heart can be described by the following equation:

\[
\frac{dM}{dt} = C_1(P - P_o)M
\]  

(1)

Letting \( C = C_1C_2 \), equation (1) can be rewritten in the form:

\[
\frac{dM}{dt} = (CR_e - \beta CR_o)M
\]  

(2)

In this expression, \( C \) serves as an index of the rate of mRNA translation by the ribosomes, encompassing both the time for synthesis of one polypeptide chain and the ratio of the number of translating ribosomes to their total number.

The relationship of change in heart weight to stability of the proteins that are formed is not considered in explicit form in equation (2). It is evident that a reduction in the rate of protein degradation, an increase in their stability, under otherwise equal conditions would cause an additional increase in heart mass; reduction in protein stability should lead to the opposite result.

If the protein degradation rate is increased or decreased by \( \beta \), the concentration of translating RNA at which the mass of the organ does not change is increased or decreased by the same number of times. Taking this possibility into account, equation (2) can be written in the form:

\[
\frac{dM}{dt} = (CR_e - \beta CR_o)M
\]  

(3)

The coefficient \( \beta C \) is the ratio of protein degradation at time \( t \) to the value at \( t_0 \).

Integrating equation (3) from \( t_0 \) to \( t \), we obtain

\[
W = M/M_0 = e\int_0^t[(CR_e - \beta CR_o)]dt
\]  

(4)

Expression (4), in general form, describes the relationship between the relative weight of the heart and the RNA concentration. Using this expression, the effect of total RNA concentration, the ratio of translating-to-nontranslating RNA, and the rates of synthesis and degradation of protein on the mass of the heart can be evaluated differentially. For this analysis it is advisable to assume initially that all these factors are constant in compensatory hyperfunction of the heart, with the exception of total RNA concentration, which increases progressively and results in faster protein synthesis and an increase in heart mass.

The "theoretical" curve in Figure 4 reflects the changes of heart weight calculated by means of expression (4) on the basis of the measurements of total RNA concentration in compensatory hyperfunction of the heart (see Table 1) and the assumption that all other parameters remain constant. Determination of coefficient \( C \) in (4) and the plotting of this theoretical curve were carried out so as to result in a 50% increase in mass at the end of the period investigated.

The "experimental" curve in the same figure reflects the actual changes of heart weight in compensatory hyperfunction (see Table 1). It is obvious that the experimental curve is significantly higher than the theoretical one. This indicates that the factors which (aside from the total RNA concentration) can affect the heart weight do not remain constant and that they have a significant
effect on heart weight under conditions of compensatory hyperfunction. These factors may be an increase in protein stability, a reduction in the time for synthesis of a polypeptide chain on the polyribosomes, and, finally, an increase in the ratio of translating ribosomes to nontranslating ribosomes.

In evaluating the probable role of protein degradation, it should be kept in mind that according to some data obtained in experiments in vivo, labeled amino acids leave the heart proteins more slowly during hyperfunction than under resting conditions. This result can be explained not only by a decrease in the rate of protein degradation, but by an increase in reutilization of labeled amino acids from the blood. These amino acids may originally have been incorporated into the protein of other organs. However, in our opinion the recent work of Schreiber et al. indicates that in isolated hearts hyperfunction does not affect the decay rate of myocardial proteins. The effect of different factors on the rate of protein synthesis by the ribosomes has been studied insufficiently thus far, although it is known that a reduction in myoglobin concentration in the medium stimulates its synthesis in the ribosome system.

The third of the possible factors, the ratio of translating ribosomes to nontranslating ribosomes, was the subject of study in experiments described above. It was determined that this ratio increases from 1.0 to 1.5 on the second day of compensatory hyperfunction of the heart. As discussed above, the increase in the ratio of the number of translating ribosomes to nontranslating ones is the most probable cause for the increase in heart mass in compensatory hypertrophy beyond the increase that would have been expected from the increase in RNA concentration.

In this manner, the signal which arises during an increase in heart function activates the genetic apparatus of the myocardial cells and not only increases the synthesis of all types of RNA and their concentrations in the myocardium, but leads to an increase in the number of polyribosomes in the total heart ribosome population.

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