INCREASED YIELD OF RIBOSOME DIMERS FROM THE RAT SEMINAL VESICLE FOLLOWING CASTRATION*

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1. Introduction

It has been reported by Reader and Stanners [1] that free ribosomes from certain animal cells which are inactive in protein synthesis can associate, through a Mg^{2+} and temperature dependent reaction, to form dimers which are relatively resistant to the action of ribonuclease. They have provided evidence that this type of dimer is an artefact which is formed under certain conditions from ribosomes not bound to messenger RNA. In actual fact, these ribosomes exist in the tissues as monomers.

Following castration of normal adult rats, the RNA/DNA ratio in their seminal vesicles drops from 2.0–3.0 to a value of about 0.5 within about a week. After testosterone administration to castrated rats, the same ratio is increased about sixfold and attains its maximum within a week [2]. These data obviously reflect a dramatic change in the polyribosome content of the vesicular cells following castration or after replacement of testosterone, since the major portion of total cellular RNA is comprised in this particulate material [3]. Provided that the disappearance of various messenger RNAs after orchiectomy is more rapid than that of the ribosomes, a relative accumulation of ribosomes not linked to messenger RNA may be expected. The results reported by Reader and Stanners offered a useful tool for the detection of such ribosomes.

2. Experimental procedure

The mince of the seminal vesicles of adult Wistar rats was homogenized with a 20 mM tris-HCl buffer, pH 7.6, containing unless otherwise stated 5 mM Mg acetate or $MgCl_2$, 25 mM KCl, 5 mM mercaptoethanol and 0.7 M sucrose. This homogenate was adjusted to 0.5% with respect to Na deoxycholate and centrifuged then at 18,000 *g* to remove cell debris, nuclei and mitochondria. Ribosomes were sedimented from the supernatant fluid at 150,000 *g*. The ribosome pellet was suspended in a solution differing from the homogenizing medium only in the sucrose concentration (0.25 M). Distribution of polysomes was analyzed by sucrose density gradient centrifugation [4]. The Mg^{2+} and K^+ concentrations of the gradients were the same as in the suspending medium. Ultraviolet absorption profiles were determined by collection from the top of the tubes with continuous recording in an ISCO model D density gradient fractionator.

Ribosomes were labelled by incubating 600–800 mg of a mince of the seminal vesicles [5] in 12 ml Krebs-phosphate buffer containing 5.4% (w/v) glucose with 15 μCi of ^{14}C-L-valine (91 mCi/mmole) for 30 min in O_2 atmosphere at 37° with continuous shaking. Following incubation ribosomes were prepared and fractionated as indicated above. About 50 fractions were collected and ribosomes in each fraction were precipitated by means of 7.5% TCA (final concn.) using horse serum albumin as carrier. The precipitates were filtered onto paper disks, dried under an infrared lamp and counted in a Frieseke-Hoepfner methane gas flow counter.

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3. Result and discussion

Following castration, the amounts of the longer polysomes were markedly reduced in the vesicular ribosome preparations, while the proportion of dimers was increased and this increase correlated with the time of castration (fig. 1). Administration of testosterone to the castrated animals resulted in a reversion of these alterations (fig. 2). It is conceivable that the accumulation of ribosome dimers observed after orchiectomy resulted from the action of some RNAase during the preparation of ribosomes. Data in table 1 suggest, however, that this type of breakdown of vesicular polysomes produces monomers rather than dimers.

When the Mg^{2+} concentrations in the homogenizing medium and in the gradient were kept at 2 mM, the accumulation of dimers could not be observed. When the Mg^{2+} concentrations in the ribosome suspensions and in the gradients were restored to 5 mM, some of the monomers from castrated animals, but not from normal or testosterone treated rats, were recovered as dimers.

Thus the ribosome dimers prepared at 5 mM Mg^{2+}

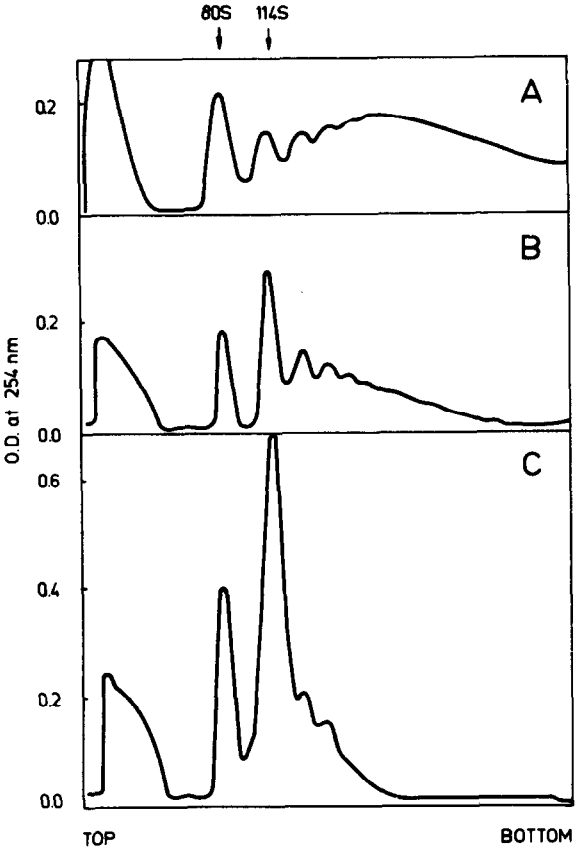

Fig. 1. Ultraviolet absorption profiles of polysomes prepared from the seminal vesicles of normal and castrated rats. Vasicular ribosomes were prepared simultaneously from three groups of 7–12 rats each. The castrated animals were two (B) or six (C) days following orchiectomy. A: ribosomes from normal animals.

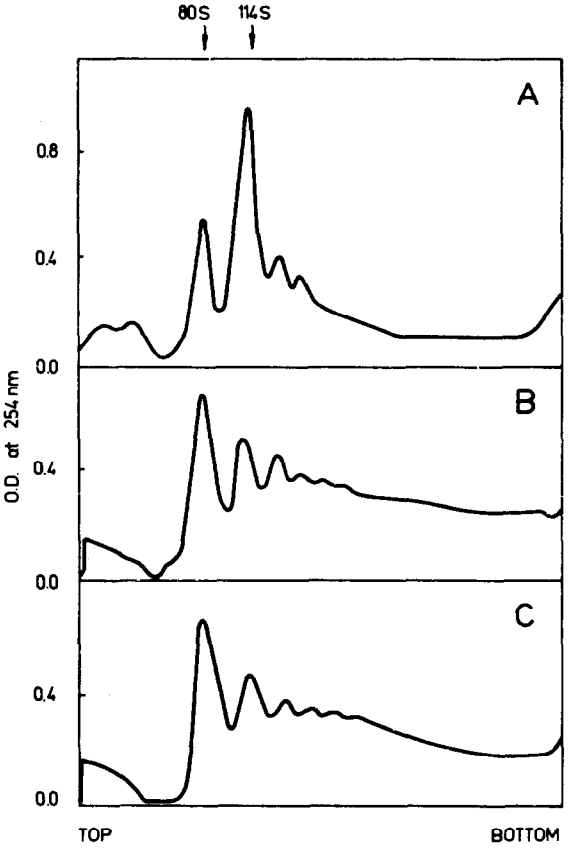

Fig. 2. Effect of administration of testosterone to rats castrated one week prior to killing on the distribution of vesicular ribosomes. Two groups of 8 castrated rats each were treated with daily injections of 2 mg testosterone phenylpropionate (Retandrol, Richter) for two (B) and six (C) days respectively, prior to killing. 12 Castrates were not treated at all (A). Vesicular ribosomes were prepared simultaneously as described in Experimental procedure.

from castrated rats appear to contain a substantial amount of mRNA-free inactive ribosomes, dimerized just as described by Reader and Stanners for inactive liver ribosomes. This presumption was further confirmed by studying the relative rate of labelling of the monomers and of the various ribosome aggregates with ^{14}C-valine (table 2). When the conditions favoured dimerization (i.e. at 5 mM Mg^{2+}), the ribosome dimers of castrated rats, but not of normal rats, were distinguished by a relatively low specific radioactivity. In fact this is expected if ribosomes to which nascent polypeptides were not attached combine to form dimers.

These results suggest that, following castration simultaneously with the progressive decrease of the total RNA content of the vesicular cells, a relative accumulation of ribosomes which are not linked to mRNAs is taking place. These processes lead, within about a week, to a 4–5 fold decrease of the RNA/DNA ratio and to the conversion of nearly half of the residual polysomes to monosomes. It is reasonable to suppose that the rate at which the levels of mRNA are decreasing exceeds the rate of reduction of the cytoplasmic concentration of ribosomes which in turn underlies the relative enrichment of vesicular cells in monosomes. After replacement of testosterone, these monosomes are eliminated from the vesicles, perhaps in consequence of their combination with newly formed messengers. Available data suggest that the deprivation of testosterone brings about, in the rat prostate, a selective 6–7 fold decrease in the rate of transcription of genes coded for the synthesis of ribosomal RNA precursors [6–8]. Furthermore, evidence has been provided that prostatic nuclei and ribosomes prepared from testosterone treated rats are richer in messenger RNAs than those obtained from control castrates [9–11]. Our interpretation of the

Table 1
Effect of RNAase treatment on the distribution of ribosomes in mono-, di- and polymeric forms.

		Percentage distribution of ribosomes from		
		Castrates	Castrates treated with testosterone	
			for 2 days	for 6 days
Monomers	A	18.2	15.5	20.1
	B	32.0	48.5	45.0
Dimers	A	43.8	11.2	13.1
	B	41.5	25.0	23.0
Polymers	A	37.7	73.3	66.7
	B	26.5	26.5	32.0

Ribosomes prepared from the seminal vesicles of castrated rats and of castrates to which testosterone was administered were incubated for 5 min at 0° with 5 μg of pancreatic RNAase and fractionated immediately in sucrose gradients. The proportions of the individual ribosomal components were determined by planimetric analysis of the O.D.$_{254}$ tracings. A: controls; B: RNAase treated ribosomes.

Table 2
Label attached to ribosomes following incubation of the vesicular mince with ^{14}C-valine.

Source of the vesicles	Concn. of Mg^{2+} in the gradients (mM)	cpm/O.D. ratio in			
		monomers	dimers	trimers	tetramers
Normal	5	98	113	162	180
Castrates on the 7th day after operation	5	194	142	229	305
Castrates* A	2	192	244	–	–
B	5	250	171	–	–

Ultraviolet absorption and radioactivity profiles were determined as described in Experimental procedure and the specific radioactivities at the peaks of the absorption profiles were calculated. (Peaks in the radioactivity profiles coincided with those of the absorption profiles).

* Ribosomes prepared at 2 mM Mg^{2+} were divided into two parts: one (A) was suspended and analyzed at 2 mM Mg^{2+}; the other (B) at 5 mM Mg.

experiments described in this paper is compatible with these data. Considering that testosterone appears to regulate the rate of the overall RNA synthesis in the seminal vesicles [12, 13], we may speculate that any discrepancy between the actual cytoplasmic concentrations of ribosomes and messengers might originate from a difference in the rate of breakdown of these entities, rather than from a difference in the rate of their production.

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