THE UTILIZATION OF GENES FOR RIBOSOMAL RNA, 5S RNA, AND TRANSFER RNA IN LIVER CELLS OF ADULT RATS*

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Abstract.—The rates of synthesis of ribosomes, 5*S* RNA, and tRNA necessary to maintain the steady-state concentrations of these entities in liver cytoplasm of adult rats were determined. On the average, each liver cell in the adult rat synthesizes 650 ribosomes, 650 molecules of 5*S* RNA, and 11,000 molecules of tRNA each minute. The numbers of genes per liver cell for rRNA, 5*S* RNA, and tRNA were 330, 1660, and 13,000, respectively, as determined by RNA: DNA hybridization experiments. Thus, on the average, individual genes for rRNA, tRNA, and 5*S* RNA are transcribed twice a minute, once a minute, and once every 2.5 minutes, respectively, in the adult rat liver.

Introduction.—Various quantitative aspects of the metabolism of ribosomes, 5*S* RNA, and tRNA in liver cells of adult rats have been investigated.[1–4] However, no account has yet appeared describing in molecular terms the rates of synthesis necessary to maintain the steady-state concentrations of these entities within the cell, or of the load this turnover imposes upon the genes. This paper presents such an account. The net rates of synthesis, and the numbers of genes coding for rRNA, 5*S* RNA, and tRNA were determined, and average values for the frequencies of transcription were calculated.

Materials and Methods.—Administration of radioactive orotic acid to rats: Female white rats (wt. 170 ± 15 gm) used for decay-turnover experiments received 2.5 μci of orotic acid-6-^{14}C (30 mci/mmole) by tail vein or intraperitoneal injection. To minimize the period of labeling, each rat received 25 μmoles unlabeled sodium orotate by intraperitoneal injection 2 hr after administration of the radioactive orotic acid.[5] Rats from which RNA of high spec. act. (0.71–1.54 × 10^5 dpm/μg) was obtained for use in hybridization experiments received 3–5 mci orotic acid 5-^{3}H (14.4 ci/mmole) by intraperitoneal injection on 5 successive days and were sacrificed on the sixth. Feeding was *ad libitum*.

Preparation of RNA from rat liver: Ribosomes were prepared from the 15,000 *g* supernatant by treatment with 1.3% sodium deoxycholate and isolated by sedimentation through zones of 0.5 and 1.0 *M* sucrose.[6] tRNA was obtained from material remaining in the upper two thirds of the supernatant after centrifugation of the 15,000 *g* supernatant at 165,000 *g* for 3 to 7 hr. Extraction of RNA was as previously described.[6] RNA for use in hybridization experiments was purified as follows. 30*S* rRNA was obtained from the RNA of ribosomes by density gradient centrifugation in sucrose (17–34%) containing 0.25% SDS,‡ and, after precipitation with ethanol: potassium acetate, was subjected to MAK chromatography.[7] The leading portion of the 30*S* rRNA peak was collected and again subjected to density gradient centrifugation in sucrose and 0.25% SDS to remove any traces of methylated albumin. 5*S* RNA was obtained from the RNA of ribosomes by chromatography on Sephadex G-200[6] and was further purified by MAK chromatography. The 5*S* RNA thus obtained was extracted with SDS and phenol to ensure the absence of methylated albumin. tRNA was purified by chromatography on Sephadex G-200 of RNA extracted from the 165,000 *g* supernatant. All RNA for use in hybridization experiments was dialyzed at 4° against 0.01 × SSC.

Preparation of DNA: Rat liver tissue in 10 vol of medium (0.25 *M* sucrose, 0.05 *M* Tris, pH 7.6 at 0°, 0.0035 *M* $CaCl_2$ at 3°C) was briefly homogenized in a loose-fitting Potter-Elvehjem homogenizer and filtered through 3 layers of cheesecloth. Nuclei were sedimented by centrifugation at 700 *g* for 10 min, suspended in 4 vol of 0.15 *M* NaCl, 0.1 *M* EDTA (pH 8.0), and 0.5% SDS, and lysed by incubation at 37° for 4 min. The resulting solution was extracted twice for 10 min at 0° with an equal vol of water-saturated phenol, and the DNA was precipitated with 2 vol of ethanol at −20°. Subsequent steps were according to the technique of Marmur[8] except that treatment with heat-treated pancreatic ribonuclease (5× recrystallized, Calbiochem) was with 150 μg/ml for 2 hours at 37°. Treatment with self-digested pronase (50 μg/ml) for 2 hr at 37° was also included.[9] DNA from *Escherichia coli* (as a frozen paste from General Biochemicals) was obtained by the method of Marmur,[8] except that the initial deproteinization was with water-saturated phenol and treatment with pronase and ribonuclease was as described above. Purity of the DNA was determined by spectral analysis and by the absence of RNase or other protein.

DNA in 0.01 × SSC at a concentration of 100 to 150 μg/ml was denatured by treatment with 0.1 *N* NaOH for 1 hr at room temperature. Denaturation was complete as measured by hyperchromicity and by adherence to nitrocellulose filters (B-6, 25 mm from Schleicher and Schuell). To each filter, 20–30 μg DNA in 6 × SSC was applied.[10]

Hybridization: The method of Gillespie and Spiegelman[9] was used. To each tube, 2–3 filters containing rat liver DNA, 1–2 filters containing *E. coli* DNA, and 1 filter lacking DNA were added. The filters were immersed in 3.0 ml of 4 × SSC containing the required amounts of RNA and incubated for 18 hr at 69°. The filters were washed with 4 × SSC and treated with ribonuclease (20 μg/ml) for 90 minutes at 29°. RNA bound to rat liver DNA was determined after correction for the amount bound to *E. coli* DNA. The amounts of RNA bound to filters lacking DNA and to those containing *E. coli* DNA were similar and less than 0.01% of the input RNA; this always corresponded to less than one third of the amount bound to filters containing rat liver DNA. Loss of DNA from filters during incubation and subsequent washing was negligible.

Analytical methods: DNA was estimated by absorption of 268 mμ after hydrolysis in perchloric acid for 30 min at 70°. An ϵ(P) of 8782 and a phosphorous content of 9.2% was assumed.[11] RNA was estimated by absorption at 260 mμ (A_{260} 1% 1 cm = 250). Radioactivity was measured by a Packard Tricarb liquid scintillation spectrometer using the channel ratio method for determining efficiency. Protein was determined by the Lowry method.[12] Ribonuclease activity was measured by appearance of acid-soluble radioactivity after incubation of the sample in SSC with radioactive rRNA for 16 hr at 37°.

Results.—The turnover of ribosomes, 5S RNA, and tRNA in liver cytoplasm of adult rats: The rates of synthesis necessary to maintain the steady-state amounts of ribosomes, ribosome-associated 5*S* RNA, and tRNA in rat liver cytoplasm were determined from knowledge of their abundance and half-lives in cytoplasm according to the equation:

$$\text{rate of synthesis} = \text{rate of turnover} = \frac{\text{Ln } 2 \times \text{pool-size}}{\text{half-life}}.$$

Half-lives were determined by measurement of the specific radioactivity of the RNA components of the ribosomes (30*S* and 18*S* rRNA) and of 5*S* RNA and tRNA obtained from the livers of rats sacrificed at various intervals after the administration of orotic acid-6-$^{14}_{c}$ (Fig. 1). Two experiments were performed. In the first, rRNA, 5*S* RNA, and tRNA were separated by Sephadex G-200 chromatography of RNA extracted from ribosomes,[6] and in the second, tRNA was obtained from the 165,000 *g* supernatant. The value for the half-life of ribosomes ($t_{1/2}$ of 107 hr) in cytoplasm agrees well with values reported previ-

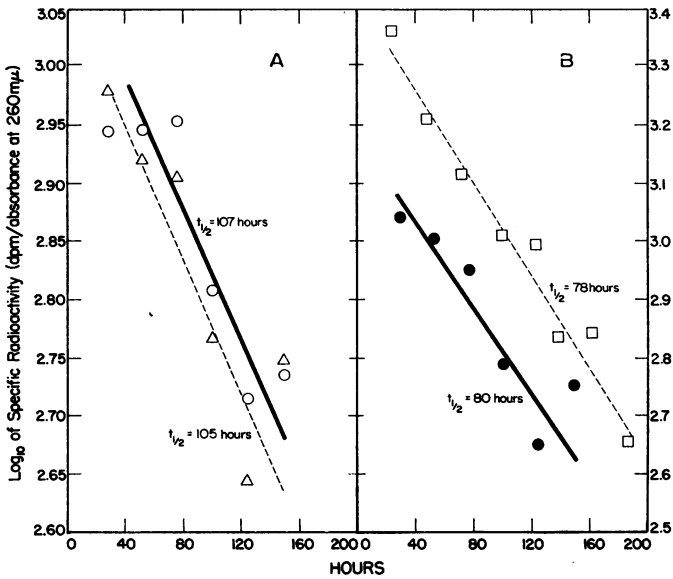

FIG. 1.—Decay-turnover of ribonucleic acids in liver cytoplasm of adult rats. (*A*) 30*S* and 18*S* rRNA (—○—) and 5*S* RNA (—△—). (*B*) tRNA from ribosomes (—●—) and from the 165,000 *g* supernatant (—□—). Half-lives were computed from a least squares fit of the data.

ously[1, 2] and was similar to that of 5*S* RNA. Transfer RNA turned over more rapidly than rRNA and 5*S* RNA and had a half-life in cytoplasm of about 80 hours.

Values for the half-lives thus obtained accurately reflect turnover only if the specific radioactivity of nucleotides in the nucleotide pool is low relative to the specific radioactivity of nucleotides in RNA throughout the period of measurement. After a "chase" injection of unlabelled orotic acid, as administered in these experiments, radioactivity is eliminated from the nucleotide pool and is negligible in amount after about 20 hours.[5] Thus, estimates of turnover obtained in this way are meaningful. Similar conclusions were reached by Hirsch and Hiatt[1] who found that the kinetics of ribosome decay were similar after labeling either with ^{3}H-orotic acid or the metabolically labile L-arginine-guanido-^{14}C.

The number of ribosomes and, hence, also of molecules of 5*S* RNA[3, 4, 6, 13] in the cytoplasm of an average liver cell of a normal adult rat is 6×10^6 and the number of tRNA molecules is 7.5×10^7.[3] Assuming half-lives in cytoplasm of 106 hours for ribosomes and 5*S* RNA, and of 79 hours for tRNA, each cell requires 650 ribosomes, 650 molecules of 5*S* RNA, and 11,000 molecules of tRNA each minute to maintain the cytoplasmic pools.

Hybridization of rat liver 30S rRNA, 5S RNA, and tRNA to homologous DNA.—30S rRNA and tRNA: By chromatographic criteria both ^{3}H-30S rRNA and ^{3}H-tRNA were free of detectable contamination by other ribonucleic acids. ^{3}H-tRNA contained no messenger-like RNA or rRNA as judged by the absence of competition with unlabeled RNA of high molecular weight from ribosomes.

The absence of messenger-like RNA from the preparation of ^{3}H-rRNA was not demonstrated experimentally. Values for the number of genes for 30*S* rRNA and tRNA were obtained by analysis of hybrid data in double-reciprocal form (Fig. 2), as described in the Appendix. On the average, each rat liver cell contains 330 genes for 30*S* rRNA, and 13,000 genes for the approximately 40–50 different species of tRNA within the cell. The amount of DNA complementary to 30*S* rRNA (0.0095%) is within the range established for several vertebrates,[14–17] but is somewhat lower than previous estimates for rat liver.[16, 17] The amount of DNA complementary to tRNA (0.0058%) is about half that found in *Xeonpus laevis*.[14]

The dissociation constants for the binding of 30*S* rRNA and tRNA were compared with the corresponding constants for RNA:DNA interactions with nucleic acids from *Bacillus subtilis* and *Drosophila melanogaster* (Table 1). The latter constants were computed from previously published data. The dissociation constants for the ribosomal RNA:DNA interactions with nucleic acids from the three organisms are similar and are much lower than the values for the tRNA:DNA interactions.

5S RNA: Competition experiments showed that the ^{3}H-5*S* RNA was contaminated by RNA also present in high molecular weight and tRNA. However, an approximate value for the number of genes for 5*S* RNA was obtained from competition experiments utilizing near-saturating amounts of ^{3}H-5*S* RNA and various unlabeled ribonucleic acids. The experiments were performed with an input concentration of ^{3}H-5*S* RNA of 1.33 μg/ml, at which point 80 per cent of the available binding sites were occupied. Addition of unlabeled RNA from ribosomes (freed of 5*S* RNA by chromatography on Sephadex G-200) and from the 165,000 *g* supernatant eliminated about 75 per cent of the radioactivity in hybrid (Fig. 3). An apparent plateau was reached after addition of an 89-fold excess of

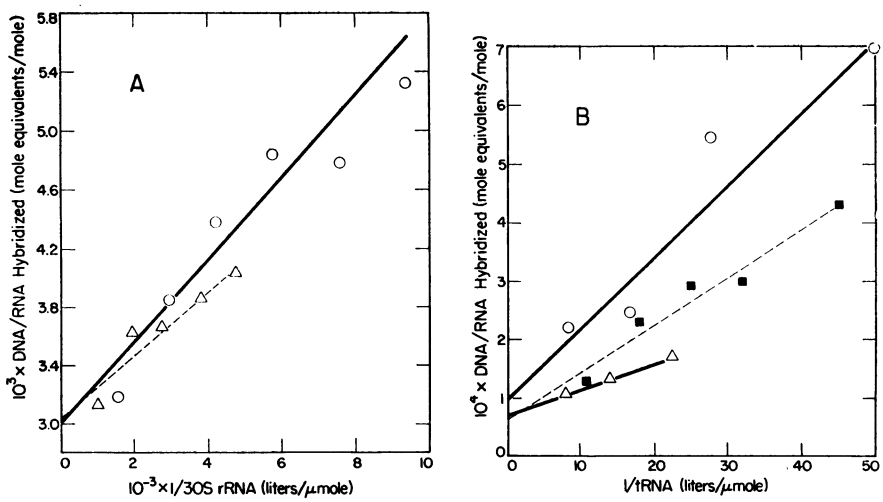

FIG. 2.—Hybridization to DNA from rat liver of (*A*) ^{3}H-30*S* rRNA and (*B*) ^{3}H-tRNA. The various symbols show the results of separate experiments. The calculations are described in the Appendix. Each point represents the average of 2 to 3 filters.

TABLE 1. *Binding constants for interactions between homologous ribo- and deoxyribonucleic acids.*

Origin of reactants	Dissociation Constants (10^3 × μmoles/liter) rRNA:DNA	tRNA:DNA
Rat liver	0.082	101
*B. subtilis**	0.173	7.74
D. melanogaster†	0.054	14.8

* Computed from data in ref. 18.
† Computed from data in ref. 10 and 19.
Values for the molecular weights of the reactants from rat liver are as specified in the Appendix, and from *B. subtilis* and *D. melanogaster* are as quoted in the papers referred to above.

unlabeled RNA of high molecular weight from ribosomes and a 300-fold excess of RNA from the 165,000 *g* supernatant. However, not all the radioactivity remaining bound to DNA after these additions of unlabeled RNA could be attributed to components unique to the preparation of ^{3}H-5*S* RNA, because the isotopic dilution of the contaminants was finite.

To determine the contribution of the contaminants to the residual hybrid, it was necessary to know the relative concentrations of the components common to

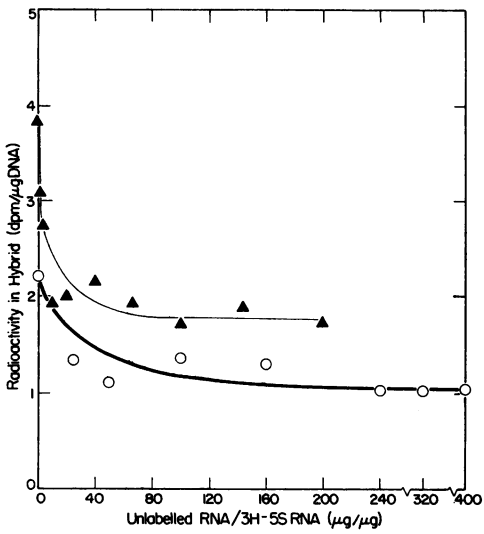

FIG. 3.—Hybridization of ^{3}H-5*S* RNA to rat liver DNA: the effect of addition of unlabeled ribonucleic acids free of 5*S* RNA. (—▲—) addition of RNA of high molecular weight from rat liver ribosomes: (—○—) addition of 165,000 *g* supernatant RNA from rat liver to ^{3}H-5*S* RNA containing an 89-fold excess of RNA of high molecular weight from rat liver ribosomes. The concentration of ^{3}H-5*S* RNA (specific radioactivity of 7.09×10^4 dpm/μg) in all tubes was 1.33 μg/ml. Each point represents the average of 2 to 3 filters. In this experiment, binding of radioactive RNA to filters containing *E. coli* DNA was negligible.

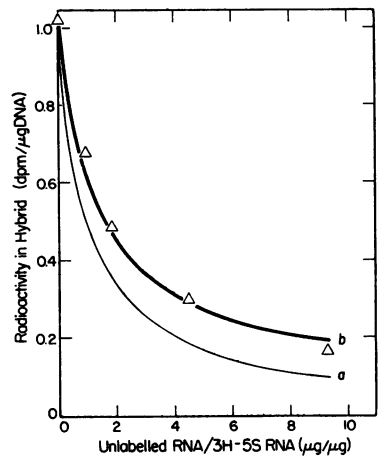

FIG. 4.—Hybridization of ^{3}H-5*S* RNA to rat liver DNA: the effect of addition of unlabeled 5*S* RNA on hybrid remaining after addition of an 89-fold excess of RNA of high molecular weight from rat liver ribosomes and a 300-fold excess of 165,000 *g* supernatant RNA from rat liver. Curves (*a*) and (*b*) are theoretical curves (see text) and (Δ) are the experimental values. The concentration of ^{3}H-5*S* RNA (specific radioactivity of 7.09×10^4 dpm/μg) in all tubes was 1.18 μg/ml. Each point represents the average of 2 to 3 filters.

the labeled and unlabeled RNA preparations, because the actual isotopic dilution achieved is the product of this factor and of the relative excess of the unlabeled RNA which was added. The relative concentrations of common components were determined by replotting the data according to the methods of Mangiarotti *et al.*,[20] and the actual isotopic dilution was calculated (Table 2). In the presence of the indicated amounts of unlabeled ribonucleic acids, 0.164 of a total of 1.0 dpm in hybrid per microgram of DNA was attributable to the contaminants.

To confirm this estimate, an additional competition experiment was performed in which various small amounts of unlabeled 5*S* RNA were added to tubes each containing ^{3}H-5*S* RNA, and 89-fold excess of unlabeled RNA of high molecular weight from ribosomes, and a 300-fold excess of unlabeled RNA from the 165,000 *g* supernatant. Theoretical competition curves were constructed based on the isotopic dilution which would be expected if: (a) all the radioactivity in the residual hybrid was from RNA unique to the preparations of 5*S* RNA (curve *a*, Fig. 4); (b) the radioactivity in the residual hybrid was largely from RNA unique to the preparations of 5*S* RNA but, to the extent predicted in Table 2,

TABLE 2. *Hybridization of ^{3}H-5S RNA: the contribution of contaminants to radioactive hybrid remaining after addition of unlabeled RNA (free of 5S RNA) from ribosomes and the 165,000 g supernatant.*

Unlabeled RNA	Relative content in unlabeled RNA of components present also in the ^{3}H-5*S* RNA preparation (μg/μg)	Unlabeled RNA: ^{3}H-5*S* RNA (μg/μg)	Actual isotopic dilution	Radioactivity in hybrid due to remaining contaminant (dpm/μg DNA)
High molecular weight RNA from ribosomes	0.20	89	1 in 18.8	0.112*
165,000 g supernatant RNA	0.08	300	1 in 25	0.052†

* Radioactivity contributed to hybrid by contaminant before dilution was 2.10 dpm/μg DNA.
† Radioactivity contributed to hybrid by contaminant before dilution was 1.27 dpm/μg DNA.

was also from those RNA species common to the preparations of 5*S* RNA and the unlabeled ribonucleic acids that were added (curve *b*, Fig. 4).

The experimental values (Fig. 4) fitted curve *b* and support the estimate for the radioactivity in the residual hybrid due to contaminants, and to components unique to the ^{3}H-5*S* RNA. The amount of hybrid contributed by the unique components was bound to 0.0012 per cent of the DNA, which would correspond to 1660 genes for 5*S* RNA per rat liver cell, if the only component unique to the preparation of ^{3}H-5*S* RNA were 5*S* RNA.

Evidence of the avoidance of artifacts was provided by the absence of significant elimination of radioactivity in hybrid on addition of large amounts of RNA from *E. coli* ribosomes, and by the quantitative elimination of radioactivity in hybrid caused by addition of small amounts of unlabeled 5*S* RNA to tubes containing ^{3}H-5*S* RNA and large amounts of other unlabeled ribonucleic acids (Fig. 4).

Discussion.—The rates of synthesis of ribosomes, 5*S* RNA, and tRNA which were measured were those required to replenish the cytoplasmic pools. The actual rates of synthesis would be different if significant intranuclear turnover

occurred. Of these ribonucleic acids, only 5*S* RNA is present in the nucleus in appreciable amount; however, turnover there of 5*S* RNA is slow.[13] Thus, the measured rates probably closely approximate the actual rates of synthesis. The number of genes for rRNA was estimated by measuring the number of binding sites on DNA for the 30*S* rRNA component of ribosomes, although the primary transcription product of rRNA genes is a 45*S* RNA molecules.[21] However, since 45*S* RNA is precursor to one molecule of 30*S* rRNA and one molecule of 18*S* rRNA,[21] measurement of the number of binding sites for 30*S* rRNA is equivalent to measurement of the minimum number of genes for rRNA.

The frequency of transcription of individual genes cannot be determined without knowledge of the proportion of genes which are active at any one time. However, average values for frequencies of transcription are readily calculated from knowledge of the rates of synthesis and the total number of genes. Each 45*S* provides sufficient 30*S* rRNA and 18*S* rRNA for the synthesis of one ribosome.[21] Thus, the rate of synthesis of 45*S* RNA must at least equal the rate of synthesis of ribosomes, and might be greater if wastage of 45*S* RNA occurs. In the livers of adult rats the rate of synthesis of 45*S* RNA is, therefore, at least 650 molecules per minute per cell, and the average frequency of transcription is at least two molecules per minute per gene. The average frequencies of transcription of tRNA and 5*S* RNA are 0.85 and 0.4 molecules per minute per gene, respectively.

The frequency of transcription of any particular gene depends on the rate of extension of nascent RNA chains and the spacing of RNA polymerase molecules on that gene. In *E. coli* growing under optimal conditions, the average spacing of RNA polymerase molecules on genes for stable RNA is about 54 nucleotides, which is about as close as is physically possible.[22] In HeLa cells, 2.5 minutes are required for the synthesis of a 45*S* RNA molecule of about 13,000 nucleotides.[23] The rate of chain extension in HeLa cells is thus 87 nucleotides per second, which is twice the rate in rapidly growing *E. coli*.[22] If it is assumed that maximum gene utilization in rat liver cells involves a chain extension rate of 87 nucleotides per second and an RNA polymerase density of one molecule per 54 nucleotides, each nucleotide of a gene would be transcribed $(87 \times 60)/54$, or 97 times per minute. Gene products would be formed at the same rate, regardless of the size of the gene. On this basis, genes for rRNA, 5*S* RNA, and tRNA appear to be utilized at less than 5 per cent of their capacity in liver cells of adult rats.

Appendix.—For a ligand (DNA) with n independent binding sites each with the same intrinsic binding constant (K_d)

$$\frac{1}{r} = \frac{K_d}{n} \cdot \frac{1}{(\mathrm{A})} + \frac{1}{n}$$

where (A) is the molar concentration of the unbound moiety (RNA) and r is moles of A bound per mole equivalent of ligand.[24] K_d and n were obtained from a least-squares fit of the data plotted in the form of $1/r$ against $1/(A)$.

A mole equivalent of rat liver DNA was considered to be 5.77×10^{12} daltons, which is the amount of DNA per liver cell.[25] Thus, values of n are equal to

genes per cell, but are greater than genes per diploid genome because some cells contain polyploid nuclei. The molecular weights of 30*S* rRNA, and tRNA are 1.65×10^6, 4.1×10^4, and 2.5×10^4, respectively.[26]

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‡ Abbreviations are: SDS for sodium dodecyl sulfate; SSC for 0.15 *M* NaCl, 0.015 *M* sodium citrate buffer, pH 7.0.

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