Résumé. Nous avons étudié la distribution du RNA 5 S dans le foie du serpent sud-américain *Crotalus durissus terrificus*. Nos résultats ont montré que le RNA 5 S de ce reptile est associé à la sous-unité ribosomique 60 S. D'autre part, la mobilité electrophorétique de ce RNA de faible poids moléculaire est la même que celle d'*Escherichia coli* et de pupe d'*Apis mellifera* L.

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[11] Acknowledgment. We are greatly indebted to Dr. T. Yamane for a supply of 5S RNA of *Escherichia coli*.

De novo RNA Biosynthesis in Isolated Bone Marrow Nuclei

Bone marrow cells are in constant process of proliferation and differentiation, in which the nucleic acids occupy a central role[1]. Furthermore, there is convincing evidence that most of the RNA synthesis takes place in the nucleus of the animal cells[2]. Some of these RNAs are restricted to the nucleus while others are transported to the cytoplasm where they will form part of the biochemical machinery for the synthesis of the proteins[3, 4]. When the synthesis of different types of RNA is high, the requirements for RNA simple precursors of RNA must be very extensive.

In a previous paper we have shown[5] that isolated bone marrow nuclei are capable of synthesizing RNA from ribonucleotide triphosphate precursors, which are likely to be furnished by the cytoplasmic pool. It has also been found that isolated nuclei from both rat liver and Hela cells can incorporate labelled aminoacid into nuclear proteins[6]. These findings suggest that the nuclei of the animal cells have a large degree of autonomy, expressed in their capacity to synthesize complex macromolecules from simple precursors.

This paper describes experiments in which isolated bone marrow nuclei incorporated (^{14}C) formate and (^{14}C) glycine into the RNA purine bases. Using this criterion we show that the isolated rat bone marrow nuclei can synthesize RNA de novo from simple precursors. A preliminary report has already been given in abstract form[7].

Normal male rats of the Wistar strain weighing 230–270 g were used. The bone marrow nuclei were obtained as indicated by Perretta and Bosco[5]. The resulting nuclear pellet was washed[8] with 0.25 *M* sucrose solution containing 10^{-3} *M* MgCl2a and 0.1% Triton X-100 and twice rewashed with 0.25 *M* sucrose solution 10^{-3} *M* MgCl2. All the steps were performed between 0°–4°. The purity of the nuclei fraction was tested by examining the pellet by light and phase microscopy and for the presence of the cytoplasmic marker enzymes glucose 6 phosphate, succinic dehydrogenase, cytochrome oxidase and urate oxidase. In all cases, the nuclei were found to be free of cytoplasmic contaminants.

The purified nuclear fraction was suspended in 0.25 *M* sucrose containing 10^{-3} *M* MgCl2; 5 μCi of (^{14}C) formate or (U-^{14}C) glycine to a final volume of 1.25 ml at pH 6.5 was added and kept at 37 °C for variable periods of time. The reaction was stopped by the addition of cold 2.1 *M* perchloric acid and then the RNA and DNA were isolated following the method described by Smellie, Thomson and Davidson[9]. The DNA and RNA bases were separated by bidimensional chromatography and their concentrations were estimated by measuring the UV-absorption. The radioactivity was counted in a Nuclear Chicago glas-flow counter and expressed as specific activity in count per minute μmole of base.

The pattern of in vitro incorporation of (^{14}C) formate into the nucleic acid bases of isolated marrow nuclei is shown in Table I. Notice that the labelled precursor is extensively incorporated into the RNA purine bases while the DNA bases are not labelled. When the experiment was performed with previously boiled bone marrow nuclei, the RNA and DNA bases did not incorporate radioactivity. It is worth adding since that the thymine in DNA was not labelled, the incubation system was free from bacterial contamination.

In order to confirm de novo RNA synthesis, the uptake of a precursor that is incorporated in the first stages of the metabolic pathway was studied. The incorporation of (U-^{14}C) glycine into the RNA adenine is shown in Table II. As a control another nuclear sample incubated with

Table I. In vitro incorporation of (^{14}C)-formate into the purine bases of the nucleic acids of isolated rat bone marrow nuclei

Base analyzed	Specific activity Counts/min/μmole of bases obtained from RNA	DNA
Adenine	2,065	0
Guanine	1,302	0
Thymine		0

The results are of a typical experiment from a series of 10. Each value represents the average of 2 samples. The nuclei were incubated with 5 μCi of ^{14}C-formate for 2 h. The specific activity of the (^{14}C)-formate was 19 mCi/m*M* (New England Corporation). Other experimental conditions are indicated in the text.

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Table II. In vitro incorporation of (^{14}C) formate and (U–^{14}C) glycine into the RNA adenine of isolated bone marrow nuclei

Labelled precursor	Specific activity Counts/min/μmole of adenine
^{14}C-formate	850
(U–^{14}C) glycine	892

The results are of a typical experiment from a series of 3. 3 μCi of the labelled formate and glycine were used. The specific activity of the (U–^{14}C) glycine was 110 mCi/m*M* (New England Corp.). The other conditions are similar to those mentioned in Table I.

Table III. Effect of the aminopterin on the in vitro incorporation of (^{14}C) formate into the RNA adenine of isolated bone marrow nuclei

Conditions	Specific activity Counts/min/μmole of adenine
Control	3,200
Plus 250 μg aminopterin	720

Typical experiment from a series of 3. The conditions are the same indicated in Table I.

(^{14}C) formate was used. In both samples the incorporation of the respective labelled precursors was roughly the same.

The precursors uptake represents de novo RNA synthesis, because it occurs during the initial steps leading to the formation of the purine ring. The following arguments can be advanced to demonstrate that the results are not due to cytoplasmic contaminations: 1. detergents were employed to remove cytoplasmic proteins from the perinuclear membrane; 2. there is absence of cytoplasmic enzyme activities and 3. the purity and integrity of the nuclear preparation was tested by light and phase microscopy.

It may be argued that the incorporation of the labelled formate into the RNA purines is not a sufficient criterion to measure de novo RNA synthesis, because the precursor is incorporated into the purine nucleus at 2 steps: A) during the formation of the N-formylglycinamide ribonucleotide and B) at the end of the pathway during the formation of the 5-formamidoimidazole-4-carboxamide ribonucleotide. To clarify this point, it would be necessary to evaluate the rate of precursor uptake in both stages. In other words it has to be measured whether the incorporated isotope is fairly equally distributed between the C-2 and C-8 positions of the purine ring. This was done indirectly, analysing the (^{14}C) glycine incorporation into the purines, which contributes with the C-4, C-5 and N-7 atoms, in a reaction that heads the formate participation in the purines pathway. The glycine forms the intermediary glycinamide ribonucleotide. Incorporation of these 2 labelled precursors seems, therefore, to represent de novo biosynthesis of purines from glycine and phosphoribosylamine.

In agreement with this, the folic analog aminopterin has a prominent inhibitory effect (see Table III), indicating that the enzymatic system for the purines synthesis has to be present into the cell nucleus. It has been shown that in liver cell, the folic acid is distributed in the subcellular fractions, corresponding to the nucleus, 20% of the total distribution[10].

With the exception of the mitochondrial RNA, most of the different species of RNA present in the animal cells are synthesized in the cell nucleus. It is well known that the ribosomal RNA is generated by the nucleolus from high molecular weight RNA precursor[11]. In rapidly growing animal tissues, the messenger RNA seems to have a similar origin from the nucleoplasm[3]. These heavy RNAs have a short half-life of minutes[12] and their presence may be exclusively confined to the cell nucleus[4].

The high rate of synthesis of the RNA in eukariotic cells suggests that the nucleus requires for a large pool of nucleotides employed in its synthesis. The experimental evidence reported in this paper supports this assumption, in the sense that bone marrow nuclei can synthesize purines nucleotide, probably to supplement those of the cytoplasmic pool, in order to cover the high demand for these compounds for the synthesis of the several types of RNA.

The characterization and the identification of the RNA types synthesized under the conditions described in this communication and its relationships with specific functions of bone marrow cells are in progress in our laboratory.

Resumen. La biosíntesis de RNA a partir de precursores simples fué estudiada. La incorporación de formiato (C^{14}) y glicina (U–C^{14}) a las bases purícas del RNA fué utilizada como criterio por medir la síntesis de novo de RNA. Estos precursores se incorporaron extensamente a la adenina y guanina del RNA mientras que las del DNA no resultaron marcadas. El antifólico aminopterina produce una marcada inhibición en la incorporación del formiato (C^{14}), indicando con esto que el nucleo posee los sistemas enzimáticos para poder sintetizar el anillo purínico. Se concluye que el nucleo aislado de médula ósea de rata es capaz de sintetizar completamente una molécula de RNA con precursores simples. Las posibles implicaciones biológicas son mencionadas.

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[13] This work was partly supported by the Comisión Nacional de Investigación Cientifica y Tecnológica (Grant No. 63–103) and the Comisión de Investigación de la Facultad de Ciencias Pecuarias y Medicina Veterinaria (Grant No. CF-5 and CF-B2).
[14] We thank Dr. J. FERNÁNDEZ for the critical reading of the manuscript. The technical assistance of Mr. F. GARRIDO is gratefully acknowledged.