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ON THE EXISTENCE OF ARRESTED TRANSCRIPTIONAL MACHINERY IN LATE STAGES OF AVIAN ERYTHROPOIESIS

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A large number of cell differentiation processes is characterized by the predominance of the synthesis of one or few special proteins, and such a "specialization" is often concomitant with the switching off of most other genes. This progressive inactivation of transcriptional activity may then proceed even further and finally result in a highly differentiated cell which shows little, if any, transcription and translation. The erythropoiesis of animals with nucleated erythrocytes such as amphibia, reptiles and birds is a particularly well defined differentiation process which shows dramatic changes in cellular and nuclear function. During the formation of the red blood cell the RNA synthesis decreases at the same time as the pattern of transcription and protein biosynthesis changes[1]. In the end product of this differentiation, the mature avian erythrocyte, nearly all cytoplasmic structures, including ribosomes and mitochondria, have disappeared and most, if not all, chromatin is in a highly condensed form[2,3]. The disappearance of polyribosomes and nucleoli and the vast decrease in the synthesis of RNA led to the general concept that the fully mature avian erythrocyte is completely inactive in RNA synthesis[4-7]. Recently, however, there have been indications in the literature[8-10] of some continued RNA synthesis even in fully mature erythrocytes. Therefore, we have examined the question as to the presence and formation of some, perhaps specific, RNA in the hen erythrocyte in more detail. The results can be summarized as follows (for details and experimental procedures see[11]).

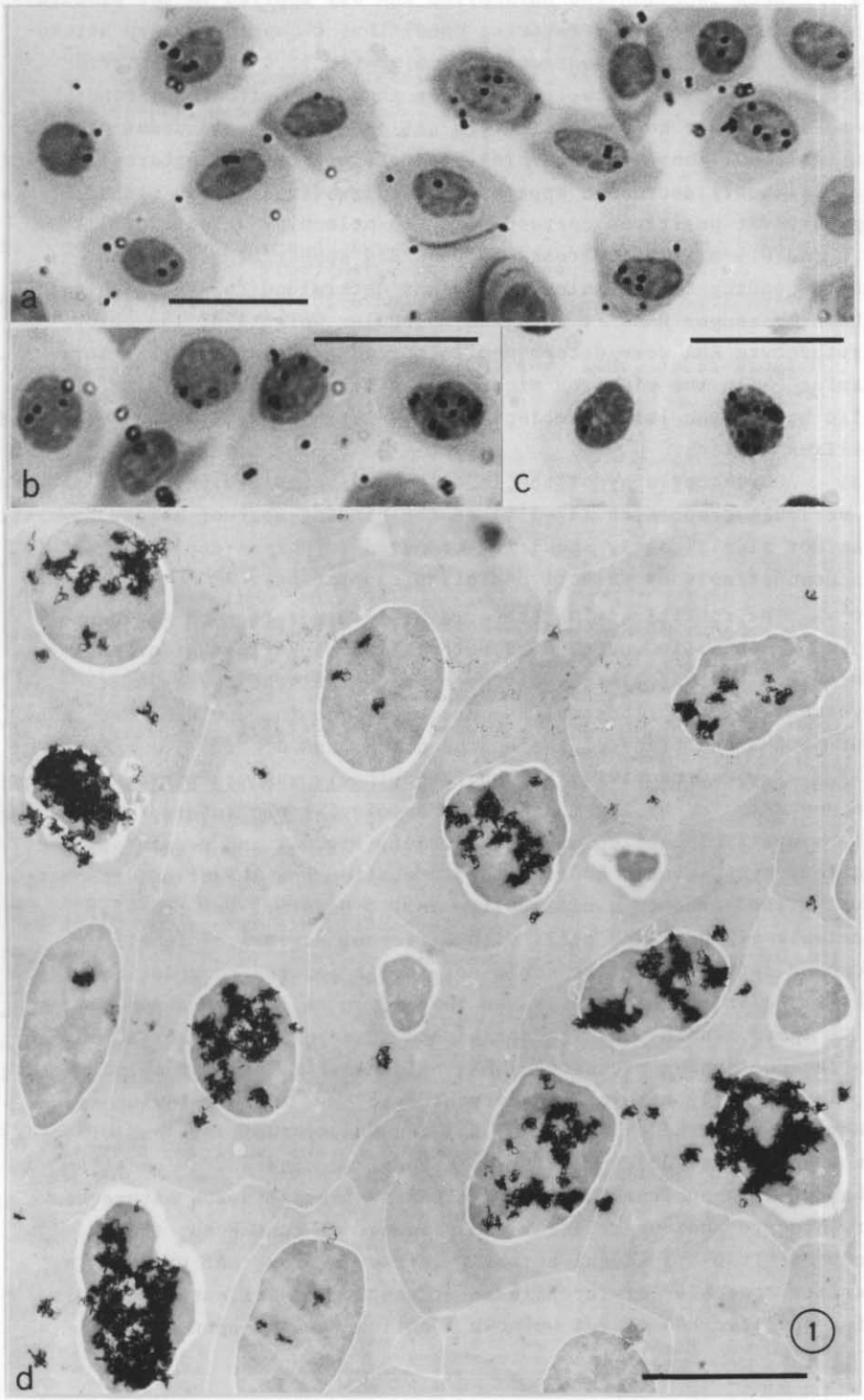
(1) In order to estimate the minimal amount of RNA in the mature hen erythrocyte the total nucleic acids were extracted from large numbers (10^{10}-10^{11}) of purified red blood cells and isolated

erythrocyte nuclei[12-15], and RNA and DNA was determined by various procedures. The minimal amount of RNA in the mature hen erythrocyte is 0.02 to 0.04 pg, that is about 1 to 2% compared to the 1.7 pg of DNA present in the nuclei. As demonstrable by cytochemistry[16] most of this RNA is located in the interchromatinic regions of the nucleus.

(2) As previous authors[8,9,17] we found that radioactive precursors are incorporated into TCA-precipitable RNA of mature erythrocytes. Incorporation of ^{3}H-uridine was noted in vivo after intravenous injection of the precursor as well as in vitro after the addition of the precursor to the freshly prepared blood or after incubating the purified erythrocytes in artificial medium[11]. The incorporated radioactivity was stable against cold TCA and pronase but was hydrolyzed by ribonucleases and with 0.4 N alkali.

(3) When the labelled nucleic acids were extracted from erythrocytes and centrifuged in cesium sulfate gradients, with or without dimethylsulfoxide, DNA and RNA (ca. 1% of the total UV light absorbing material) were clearly separated. The incorporated radioactivity was exclusively recovered in the position of the RNA, demonstrating that the RNA synthesized in the mature hen erythrocyte is not stably associated with DNA.

Fig. 1. Localization of ^{3}H-uridine incorporated in vivo (a-c) and in vitro (d) in hen erythrocytes as revealed by light (a-c) and electron (d) microscopic autoradiography. Figs. a-c. Light microscopic autoradiographs of 1 µm thick sections through mature hen erythrocytes taken from blood that has been collected at 1 hour after injection of 3 mCi ^{3}H-uridine (45 Ci/mmol) into the wing vein. Most of the cells are labelled; the majority of the silver grains is located over the nuclei. Scales indicate 10 µm. Fig. d. Electron microscopic autoradiograph of an ultrathin section of tightly pelleted mature hen erythrocytes. The cells had been prepared as described[11] and incubated for three hours in vitro with 0.25 mCi/ml ^{3}H-uridine contained in minimal essential medium containing Hanks salts. The majority of the silver grains is located over nuclear areas. In some nuclei the labelling seems to be restricted to interchromatinic regions. Cells with different degrees of labelling occur side by side. Cytoplasmic labelling is hardly above background, indicating that considerable amounts of labelled substance have not been transported to the cytoplasm during the incubation time. Scale indicates 5 µm.


a
b
c
d
1

(4) When labelled and unlabelled RNA was applied to gel electrophoresis[11] under non-denaturing conditions it appeared very heterogeneous. Most of the radioactivity migrated in a position corresponding to molecular weights higher than 10^6 daltons. Distinct peaks could not be recognized. In gel electrophoresis under denaturing conditions[11] about three quarters of the RNA appeared with significantly decreased apparent molecular weight. Some peaks appeared at positions corresponding to molecular weights of 1.8, 1.0 and 0.5 million daltons. No peak did appear at a position corresponding to the molecular weight determined for the globin chain messenger RNAs[18]. When the molecular weights of the chick erythrocyte RNA were determined in spread preparations (95% formamide[19]) in the electron microscope a heterogeneity in size was also noted; the largest molecules found were between two and three million daltons.

(5) Chromatography of the labelled RNA on poly(U)sepharose showed a variable proportion (17-33%) of the binding most of which, however, was not significantly specific, compared to poly-A-containing mRNAs, as demonstrable by selective elution experiments.

(6) The sensitivity of the uridine incorporation to Actinomycin D and α-Amanitin revealed charcteristics close to that of mRNA formation but was clearly different from the sensitivity of the synthesis of the ribosomal RNA precursors on the one hand and those for 5S RNA and transfer RNA on the other hand[11,20].

(7) The radioactivity incorporated in vivo and in vitro can also be demonstrated by light and electron microscopic autoradiography. It is mostly localized over the nuclei (Fig. 1) and remains in the nucleus even several hours after incubation and injection, respectively. The percentage of labelled cells, however, was somewhat variable (70-95%) and cells with different degrees of labelling occured side by side. We could not see an inverse correlation of the stage in erythropoiesis and the degree of incorporation.

These findings, which correspond to reports of other authors on the continuing presence of RNA-polymerase B, but not of polymerase A, in the fully mature avian erythrocyte[21-23] are perplexing since they point to the existence of RNA and RNA synthesis in nuclei even in the complete absence of translational machinery. The question whether this nuclear RNA and this RNA synthesis in the mature hen erythrocyte nucleus reflects the presence of conserved, though reduced, transcriptional activity left over from that present in earlier stages of erythropoiesis or whether it reflects synthesis of a specific RNA of yet unknown function remains open.

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