RATIOS OF α- TO β-GLOBIN RNA SEQUENCES IN THE ERYTHROPOIETIC MOUSE SPLEEN

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1. Introduction

The relative expression of murine α- and β-globin genes during erythroid differentiation has been studied using 3 systems. First, cultured erythroleukemia cells have been stimulated by various inducers with conflicting results. The α/β RNA ratio following dimethylsulfoxide (DMSO) induction was 3.7 by hybridization analysis early in erythroid development with a subsequent progression toward 1 [1]. In another study the opposite pattern, an α/β RNA ratio of 0.3 which approached 1 with maximal stimulation, was found [2]. In the latter study, the α/β RNA ratios following maximal induction by butyric acid and hemin were 0.66 and 0.3–0.50 [2]. In the second system, mouse fetal liver erythroid cells stimulated in vitro by erythropoietin, α/β ratios of 1 were found in cells from day 13 fetuses before and after culture [3]. The third system used was in vivo erythroid development of spleen cells following phenylhydrazine injection of mice. The α/β RNA ratios by gel analysis of poly(A)$^+$ total RNA of spleen cells were 0.83 and 0.55 at 66 h and 114 h, respectively [4,5].

Here we report hybridization analysis of the relative number of α to β globin RNA sequences in nuclear, total and poly(A)$^+$ RNA from spleen cells of C57 Bl/6J mice following the same protocol of phenylhydrazine injection. Our results suggest an α/β RNA ratio of 1.3 in total cellular RNA which remains constant during in vivo development. This α/β ratio is similar to that reported previously in total RNA of mouse reticulocytes [1,6]. The results also confirm the decreasing α/β RNA ratio in poly(A)$^+$ RNA observed by gel analysis [5].

2. Materials and methods

The α and β mRNA templates were separated and ^{3}H-labeled cDNA probes were synthesized and isolated as in [6]. The specificities of the α and β cDNA probes, estimated by back hybridization to their respective templates, were 94–97% for the α cDNAs and 89–94% for the β cDNAs. Total cellular RNA was isolated [7] and separated into poly(A)$^+$ and poly(A)$^-$ fractions by oligo(dT)-cellulose chromatography [8]. Total nuclear RNA was prepared from nuclei [9] by hot phenol extraction [10] followed by DNase digestion [11]. Hybridizations in 24 μl mixtures containing a constant amount of cDNA (500 cpm) and varying amounts of RNA were assayed by S_1 nuclease digestion as in [6].

3. Results and discussion

Hybridizations of α and β cDNA probes which are 97% and 94% specific with 66 h and 114 h poly(A)$^+$ spleen total RNA are shown in fig.1A and B, respectively. The amounts of homologous template needed to half saturate the α and β cDNAs used in this experiment were 0.35 ng and 0.33 ng, respectively. Relative α/β globin RNA concentrations were calculated by correcting the ratio of RNA amounts needed to half saturate the α and β cDNAs with the template ratio; e.g., in (B) $\alpha/\beta = (0.82/1.15) \times (0.35/0.33) = 0.76$. Results obtained with different fractions and preparations of spleen RNA are summarized in table 1. These data suggest that in erythropoietic spleen cells the relative expression of α and β genes remains constant

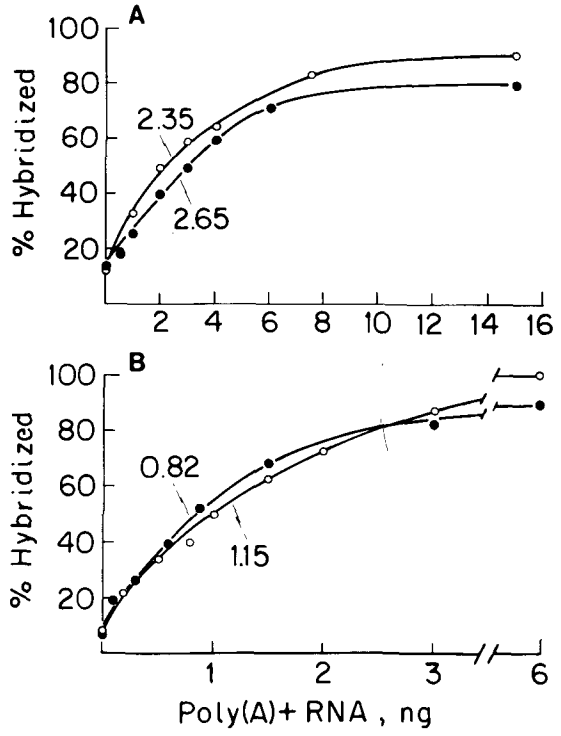

Fig.1. Hybridizations of α (○) and β (●) ^{3}H-labeled cDNA probes with total poly(A)$^+$ spleen RNA obtained 66 h and 114 h after injection (see section 3).

at 1.3 in total RNA at 66 h (1.32) and 114 h (1.28) after injection. This ratio is in agreement with the ratio required to achieve balanced globin chain synthesis in the rabbit [12,13], and it is the same as that observed in the later stage reticulocytes of the mouse [6]. Similar results were also found with total RNA obtained 186 h after injection (α/β RNA = 1.29). The percentages of total RNA that are α and β RNA sequences at 66 h, 114 h and 186 h (α,β = 0.19%, 0.14%; 0.25%, 0.19%; and 0.38%, 0.30%, respectively) are ~4–8-fold greater than the maximum concentrations of globin RNA reported in total RNA of erythroleukemia cells [1]. Others have reported even higher concentrations of globin sequences in cytoplasmic RNA of erythroleukemia cells [2]. The high initial and small subsequent increase in concentration of globin transcripts in erythropoietic spleen cells is surprising and suggests that these cells are in later stages of development than the erythroleukemia cells. Since the increase in globin mRNA active in translation is many fold greater during spleen erythropoiesis [4], our data suggest that certain globin RNA sequences may be inactive in early erythroid cells. The finding of the same α/β RNA ratio in nuclear as in total cellular RNA suggests that the rates of transport from the nucleus are similar for α and β RNAs.

The constant α/β RNA ratio observed in total RNA of erythropoietic spleen cells at various times after phenylhydrazine injection agrees with the report of balanced accumulation of α and β mRNA in day 13 fetal liver erythroid cells before and after culture [3]. In the latter, the reported α/β RNA ratio was 1, but the authors state their methods were not sufficiently sensitive to exclude a somewhat higher ratio.

Finally, the changing α/β RNA ratios reported in poly(A)$^+$ spleen RNA analyzed by gel electrophoresis are confirmed by the present hybridization data [5]. Mouse α and β globin mRNAs are known to have

Table 1
Ratios of α- to β-globin RNA in nuclear, total, and poly(A)$^+$ RNA of mouse spleen cells

Time p injection	Nuclear RNA	Total cellular RNA	Poly(A)$^+$ RNA
66 h (early erythroid phase)	–	1.30[a], 1.17[b], 1.49[c] → 1.32	1.21[a], 1.35[d] → 1.28
114 h (late erythroid phase)	1.36[d], 1.33[d] → 1.35	1.32[a], 1.18[b], 1.35[c] → 1.28	0.76[a], 1.03[d] → 0.90

Specificities of the α and β cDNA probes used are as follows: [a] 97, 94%; [b] 97, 89%; [c] 94, 89%; [d] 97, 93%, respectively (see [6])

different size classes of poly(A) [14,15]. Thus, the different α/β RNA ratios seen in poly(A)$^+$ RNA fractions may indicate that the poly(A) size classes have different distributions in α and β mRNA at different times in cellular development; i.e., on average, the poly(A) of α mRNA is shorter than that of β mRNA in the late erythroid phase. However, while the α/β RNA ratio in the 114 h poly(A)$^+$ RNA (0.90) differs from that of the poly(A)$^-$ RNA (1.42), the calculated α/β ratio derived from these results and the relative amounts of poly(A)$^+$ and poly(A)$^-$ RNA (1.05) do not agree closely with the ratio observed in total RNA (1.28). This discrepancy may be due in part to the lack of complete specificity of the cDNA probes or to residual contamination by DNA of the total and poly(A)$^-$ RNA fractions following DNase digestion.

Acknowledgements

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