Analysis of globin transition in *Xenopus laevis* and identification of globins by in vitro translation of hybrid-selected mRNA

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The switch from larval to adult globin synthesis during amphibian metamorphosis was analyzed by polyacrylamide gel electrophoresis in acid urea-Triton X-100. 4–6 slowly migrating components were detected in larvae and at least 4 faster components appear at metamorphosis and persist in the adult stage. To relate the globin subunits to the mRNA sequences, isolated as cDNA clones, mRNAs were hybrid-selected from total RNA of larval and adult erythrocytes and translated in a wheat germ system. The translation products were identified by comparison with the globin pattern of hemolysates

(Xenopus laevis) *Globin* *Electrophoresis* *Hybrid selection* *Translation*

1. INTRODUCTION

The transition of hemoglobins during anuran metamorphosis represents an attractive model for developmental gene regulation. In *Xenopus laevis* this transition has been shown to involve the complete replacement of larval hemoglobins by adult ones [1,2]. Using highly resolving polyacrylamide gel electrophoresis in the presence of the nonionic detergent Triton X-100 [3] a clear separation of larval and adult globin chains has been obtained, thus making it possible to follow developmental changes of individual globin chains [4]. Using hybrid-arrested translation and acid-urea polyacrylamide gel electrophoresis of the translation products, Hentschel et al. [5] assigned a major and a minor adult globin chain. With the same technique Patient et al. [6] identified three of the adult proteins as α-globin polypeptides and three others as β-globin chains.

Analysis of cDNA clones, derived from poly(A)$^+$ RNA, isolated from erythroblasts of anemic *X. laevis*, has revealed that in both the larval and the adult stage four abundant globin mRNA species are expressed, comprising at each stage two closely related α- and two closely related β-sequences. From melting curves divergence of both types of related sequences was estimated to be 13–14% for the larval and 6–8% for the adult stage [7].

We have taken advantage of these cDNA clones, representing abundant and stage-specific globin mRNA species, to identify individual components of the rather complex globin patterns using hybrid selection and in vitro translation of mRNAs.

2. MATERIALS AND METHODS

2.1. *Analysis of globin chains*

Erythrocytes were collected from larval and adult *X. laevis* (Daud.) Larval stages were designated according to Nieuwkoop and Faber [8]. The blood cells were washed in 3.2% sodium citrate and lysed in 10 mM $MgCl_2$. Hemoglobin was denatured in 4 M urea, 5% acetic acid and 5% β-mercaptoethanol. Electrophoresis was carried

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out on gels containing 12% acrylamide (Sigma), 0.08% bisacrylamide (Sigma), 6 M urea (ultrapure, Schwarz/Mann), 5% acetic acid and 0.7% Triton X-100 (Sigma). The stacking gel consisted of 5% acrylamide, 0.07% bisacrylamide, 2.5 M urea and 1% Triton X-100. As running buffer 5% acetic acid with 15 mM cysteamine was used.

2.2. *In vitro translation of hybrid-selected mRNAs*

50 μg globin cDNA cloned in pBR322 [7] were denatured in 0.5 M NaOH and immobilized on nitrocellulose filters of 1.8 cm diameter. Total cellular RNA (50 μg), extracted with guanidinium chloride [9], was hybridized to the filters for 14 h in a mixture of 80% formamide, 0.4 M NaCl, 10 mM Hepes (pH 7.0), 1 mM EDTA and 1% SDS either at 50°C (stringent conditions) or 30°C (non-stringent conditions). The filters were washed three times in the hybridization solution followed by three cycles in 0.5 × SSC at either 30 or 50°C. The hybridized RNA was eluted by boiling the filters for 1 min in distilled water. The ethanol-precipitated mRNA was translated in a wheat germ system (BRL) and the translation products were analysed by polyacrylamide gel electrophoresis.

3. RESULTS AND DISCUSSION

Highly resolving acid urea electrophoresis in the presence of Triton X-100 allowed unequivocal separation of the larval and adult globin polypeptide chains. With this technique it was possible to trace the developmental changes in the globin pattern of *X. laevis*. As shown in fig.1, the larval globins are resolved into 4–6 relatively slowly migrating components, which disappear after metamorphosis. About 4 adult globin chains of higher mobility appear during metamorphosis and persist in adult frogs. There is evidence of an even earlier change in the larval globin pattern, as shown by the disappearance of 2 components between stages 45 and 56. Such an early switch in globin gene expression was also described by Kobel and Wolff [4]. Since it is difficult to obtain sufficient amounts of blood cells from embryonic stages (< stage 45), it has not yet been possible to characterize the early disappearing globin chains, which might represent embryo-specific components. The occurrence of early, transiently expressed globin mRNA species has been reported by Banville and Williams [10].

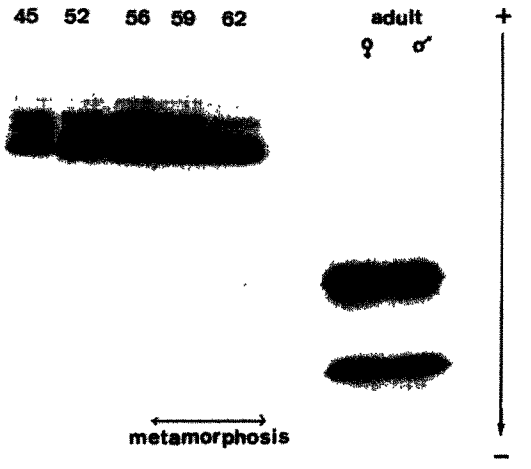

Fig.1. Developmental changes in the pattern of globin polypeptide chains from erythrocytes of different larval stages and adult *X. laevis* (stages according to [8]).

For identification of the different globin chains the respective mRNA species were isolated by hybrid selection and translated in vitro. cDNA clones corresponding to the two related α- and two related β-sequences of the larval and adult stage [7] were immobilized on nitrocellulose filters and hybridized to total cellular RNA at low and high stringency. As shown in fig.2, the translation products obtained from mRNA, selected by hybridization with either adult α_I- or α_{II}-cDNA under non-stringent conditions, are resolved by polyacrylamide gel electrophoresis into two proteins in both cases. mRNA, hybrid-selected with the related adult β_I- or β_{II}-cDNA clone under non-stringent conditions, also yields two proteins, but of lower mobility than the α-globin chains.

As the melting temperatures of the related adult α_I/α_{II}- and β_I/β_{II}-cDNA sequences [7] differ only by 6 and 8°C, respectively, high stringency is required for hybrid selection of single globin mRNA species. Fig.3 demonstrates that the RNAs selected by the adult α_I- and α_{II}-cDNA clone, respectively, were translated each into a single component of different electrophoretic mobility. Using the related adult β_I- and β_{II}-cDNA clone for hybrid selection an analogous result was obtained. Thus it was possible to identify in the adult globin pattern

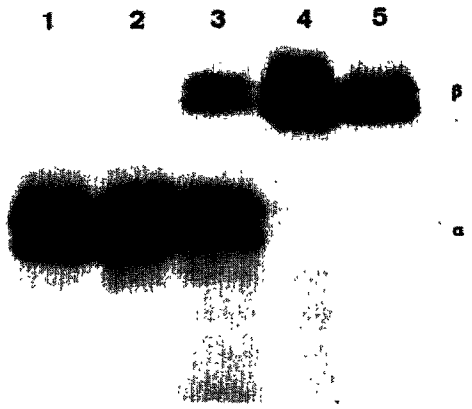

Fig.2. Translation products of adult mRNA hybrid-selected under non-stringent conditions. Translation products of mRNA selected with adult α_I- (1) and α_{II}- (2), β_I- (4) and β_{II}- (5) cDNA, respectively, as well as of total adult mRNA (3) as control.

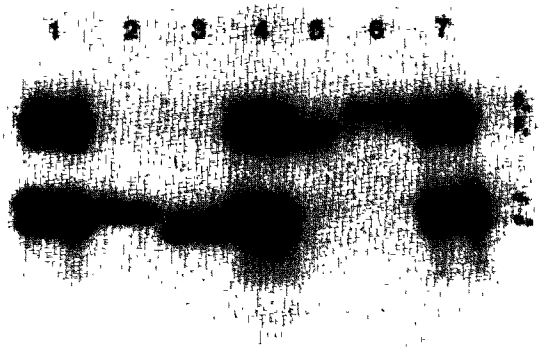

Fig.3. Translation products of adult mRNAs hybrid-selected under stringent conditions. Translation products of total adult mRNA (1,4,7) as control, of mRNA selected with adult α_I- (2) and α_{II}- (3), β_I- (5) and β_{II}- (6) cDNA, respectively.

two major components, corresponding to the α_I- and β_I-cDNA sequences, respectively, as well as two minor components, specific for the α_{II}- and the β_{II}-cDNA, respectively.

Extending hybrid selection under stringent conditions to total RNA from larval erythrocytes, using cDNA clones corresponding to the abundant larval globin mRNA species, it was possible to correlate the translation products with specific components of the larval globin pattern.

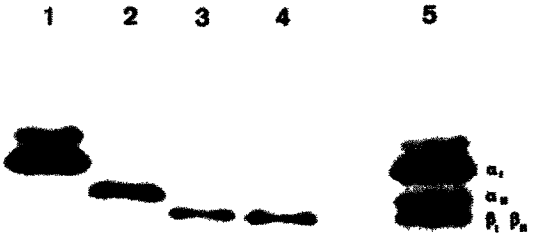

Fig.4. Translation products of larval mRNAs hybrid-selected under stringent conditions. Translation products of mRNA selected with larval α_I- (1) and α_{II}- (2), β_I- (3) and β_{II}- (4) cDNA, respectively, as well as of total larval mRNA (5) as control.

As shown by fig.4, mRNA selected with the larval α_I-cDNA clone directs synthesis of two slowly migrating globin chains. The minor component presumably represents an allelic variant corresponding to the polymorphism of α-mRNA sequences, shown by Banville and Williams [11]. The translation products of the selected larval β_I- and β_{II}-mRNA are not resolved in our electrophoresis system. That the larval β_I- and β_{II}-cDNA, in fact, select two different mRNA species was confirmed by Northern blot analysis.

In summary, our results demonstrate that the cDNA clones, derived from abundant and stage-specific mRNA species, correspond to specific components of the larval and adult globin pattern.

ACKNOWLEDGEMENT

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