Developmental pattern and molecular identification of globin chains in *Xenopus laevis*


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**Summary.** High-resolution electrophoresis of larval and adult hemoglobins of *Xenopus laevis* reveals stage-specific differences in the number and mobility of the globin chains. To establish the relationship between the globin chains and the previously described globin genes, the corresponding mRNAs were hybrid-selected from total erythroblast RNA by representative cDNA clones, and translated in vitro. Electrophoretic separation of the translation products allowed identification of a major and a minor α-globin chain in the larval and adult stages. This also holds for the adult β-chains, however in the larval stage a difference in abundance is only detectable in the β-mRNAs, but not in the translation products, because they comigrate. The fact that major and minor globin chains can be assigned to genes, which are located in two clusters, suggests that the related genes are expressed coordinately, but at different levels. Analysis of the globin patterns during development reveals that transition from the larval to the adult globin chains coincides with metamorphosis. Moreover, there is evidence of two globin chains that are only expressed in early larval stages and hence might be related to additional larval β-globin genes of as yet unknown genomic location.

**Key words:** Globin transition – Positive selection of mRNAs – In vitro translation – High-resolution electrophoresis

**Introduction**

Sequential changes in hemoglobin synthesis are a common feature in vertebrate development and reflect differential expression of the genes coding for the polypeptide subunits of hemoglobin. In the anuran *Xenopus laevis* transition of hemoglobins occurs at metamorphosis (Just et al. 1977) and is characterized by the complete replacement of the larval by adult hemoglobins, which are immunologically distinct (Just et al. 1980) and differ in their globin subunits (Hentschel et al. 1979; Hosbach et al. 1982). Moreover, Kobel and Wolff (1983) have discovered globin chains that are restricted to early larval development, suggesting a two-step transition in hemoglobin phenotypes.

Analysis of cDNA clones, derived from poly(A)⁺ RNA of erythroblasts, has shown that in both larval and adult *Xenopus laevis* four abundant sequences are expressed, comprising at each stage two pairs of closely related α- and β-sequences. From melting curves, divergence in either pair of related sequences has been estimated as 13%–14% for the larval, and 6%–8% for the adult sequences (Widmer et al. 1981). Two additional β-sequences, restricted to early larval stages, and three more α-sequences, present throughout larval life, have been reported by Banville and Williams (1985a, b).

The globin genes of *Xenopus laevis* are characterized by a unique organization, in that α- and β-genes are linked and arranged in two clusters, each one containing an adult α- and an adult β-gene, which are flanked at the 5' side by two larval α- and at the 3' side by two larval β-genes (Jeffreys et al. 1980; Hosbach et al. 1983). Moreover, it has been suggested that *Xenopus laevis* might contain additional adult α- and β-genes (Patient et al. 1982) as well as more larval genes (Banville and Williams 1985b), but these genes have not yet been isolated, nor do we know their genomic location.

Although the cDNA sequences encoding globin chains are already well characterized, their relationship to the stage-specific patterns of globin chains are as yet not fully established. So far hybrid-arrested and in vitro translation of positively selected mRNAs have allowed the identification of the major adult α- and β-globin chains (Kay et al. 1980) and to discriminate three distinct adult α- and three putative adult β-chains (Patient et al. 1982). However, assignment of the larval globin chains to the corresponding mRNA sequences is not known. Yet this information is essential for elucidating regulatory steps in the stage-specific expression of the globin genes.

In this study we have analysed the developmental changes in the pattern of globin chains of *Xenopus laevis*, using high-resolution electrophoresis, which allows unambiguous separation of larval and adult globin chains (Hosbach et al. 1982). Moreover, we have attempted to identify individual globin chains by in vitro translation of hybrid-selected mRNAs and to correlate the globin chains with the corresponding members of the globin gene family. Preliminary data have been presented previously (Sandmeier et al. 1986).

**Materials and methods**

**Animals.** Adult *Xenopus laevis* were purchased from the South African Snake Farm, Fish Hoek (Cape Province). Larval stages were obtained from heart puncture, washed under standard conditions and designated according to Nieuwkoop and Faber (1967).

**Separation of globin chains.** Erythrocytes were collected from adult and larval stages by heart puncture, washed...
in 3.2% sodium citrate and lysed in 10 mM MgCl₂. Hemoglobin was determined photometrically at 410 nm.

For analysis of globin chains hemoglobins were denatured in 4 M urea, 5% acetic acid and 5% mercaptoethanol. Electrophoretic separation was 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. The gels were stained with Coomassie Brilliant Blue R 250 (Merck).

In vivo labelling of hemoglobin. For comparison of the in vitro translation products of globin mRNAs with the native globin polypeptides, radiolabelled hemolysates of anemic animals were prepared. For induction of anemia, adult Xenopus were injected twice intraperitoneally with 0.2 ml 1% phenylhydrazine, and larvae, kept in a thyreostatic condition, were collected 24 h after the last injection.

Electrophoretic separation was on gels containing 12% acrylamide, 0.07% bisacrylamide, 2.5 M urea and 1% Tri- urea (Ultrapure, Schwarz/Mann), 5% acetic acid and 0.7% acrylamide (Sigma), 0.08% bisacrylamide (Sigma), 6 M urea, 5% acetic acid and 5% mercaptoethanol. The hybridized RNA was eluted by boiling the filters for 1 min in distilled water. As judged from the yield of radiolabelled translation products (see below) maximum hybridization was reached after 12 h.

In vitro translation of RNAs and identification of translation products. In vitro translation of total cellular RNA, 9 S mRNA or hybrid-selected mRNAs was done in the wheat germ system (BRL), using L-[4,5-3H]leucine (specific activity: 120-190 Ci/mm mol Amersham) as radioactive amino acid. The translation products were analysed by polyacrylamide gel electrophoresis in acid urea-Triton X-100 as described above and detected by fluorography using Amplify (Amersham).

Discrimination of hybrid-selected larval βr- and βt-mRNAs by Northern blotting. Total cellular RNA and mRNAs, selected with βr- and βt-cDNA, were denatured by glyoxal, subjected to electrophoresis on a 1.5% agarose gel (McMaster and Carmichael 1977) and transferred to nitrocellulose filters as described by Thomas (1980). The filters were hybridized with nick-translated βr- and βt-cDNAs (Rigby et al. 1977) in a buffer containing 0.3 M NaCl and 50% formamide (Wahl et al. 1981) at 37 °C for 18 h and washed in 3 x SSC, 0.1% SDS (non-stringent conditions) followed by 0.1 x SSC, 0.1% SDS at 65 °C (stringent conditions).

R-loop formation for electron microscopy. Adult αr, αt, βr- and βt-cDNAs, cloned in pBR322, were linearized with EcoRI, AatI, AatII and HindIII, respectively, and precipitated with ethanol. The selected αr-, αt-, βr- and βt-mRNAs were treated with DNase I (5 µg/ml Worthington) in the presence of 5 Mm diithiothreitol (DTT), 5 Mm MgCl₂ and 1 unit/µl of RNasin (Promega Biotec) to remove the plasmid DNA released from nitrocellulose filters during mRNA elution. After extraction with phenol/chloroform the mRNAs were precipitated with ethanol.

For R-loop formation, aliquots (0.3 µg) of either hybrid-selected αr- or αt-mRNA were incubated for 3 h at 48 °C in a sealed 10-µl capillary containing 0.1 µg each of αr- and αt-cDNA plasmids in 70% formamide, 0.3 M NaCl, 5 mM EDTA, 10 mM Hepes, pH 7.6. The selected βr- or βt-mRNAs were hybridized with a mixture containing 0.1 µg each of βr- and βt-cDNA plasmids in 80% formamide, 0.3 M NaCl, 5 mM EDTA, 10 mM Hepes pH 7.6 for 3 h at 54 °C. The samples were diluted 200-fold in 75% formamide, 3 M urea, 30 M Tris-HCl, 1 mM EDTA and 40 µg/ml cytochrome c and spread onto distilled water as hypophase. The specimens were processed for electron microscopy, as described previously (Wahl et al. 1976).

Results

Developmental changes in abundance and types of globin chains

To obtain information on the appearance of individual globin chains during development, hemolysates of larval stages