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Isolation of two closely related vitellogenin genes, including their flanking regions, from a *Xenopus laevis* gene library

(recombinant DNA/restriction endonuclease mapping/gene family)

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ABSTRACT **A gene library of *Xenopus laevis* was constructed from embryonic DNA partially digested with restriction endonucleases *Hae* III and *Alu* I and joined to the phage λ Charon 4 cloning vector with *Eco*RI linkers. Nucleotide sequences from three of the four related vitellogenin genes have been isolated. Two of the genes (called A1 and A2) were isolated in their entirety together with long stretches of flanking sequences. These two closely related vitellogenin genes have lengths of about 21 and 16 kilobases, but both produce a vitellogenin mRNA of 6.3 kilobases.**

In amphibians and other oviparous vertebrates, vitellogenin, the precursor of the yolk proteins, is synthesized in the liver under the control of estrogen. We and others have shown earlier that, in *Xenopus laevis*, hormonal induction is followed by extensive accumulation of stable vitellogenin mRNA in the hepatocytes (1, 2); in fully induced liver, vitellogenin mRNA accounts for almost 50% of the poly(A)-containing RNA (3, 4). Consequently, 70–90% of the newly synthesized protein is vitellogenin (refs. 5 and 6; for reviews see refs. 7 and 8). Cloning experiments involving cDNA derived from purified vitellogenin mRNA have led us to the conclusion that the vitellogenin mRNA population is composed of four related mRNAs of identical length (9, 10). We called the four mRNAs A1, A2, B1, and B2. The A and B groups exhibit a sequence difference of about 20%; the sequence difference between A1 and A2 or B1 and B2 mRNA sequences is only about 5%. All four mRNAs code for vitellogenins of similar molecular weights and are expressed simultaneously upon hormone treatment in individual animals (10, 11). Hybridization of cloned cDNA to Southern blots of uncloned genomic DNA digests showed that the different RNAs are transcribed from distinct genes (10). The biological implications of this "variant repetition" (12) are not known. We now report on the construction of a *X. laevis* gene library and on the isolation of the two A genes, including their flanking sequences.

MATERIALS AND METHODS

Preparation of a *X. laevis* Gene Library. The *Xenopus* library was constructed by using procedures described by Maniatis et al. (13). *X. laevis* embryonic DNA with a mean length of over 100 kilobases (kb) (gift from Steven McKnight) was prepared from embryos obtained from three independent matings. The DNA was fragmented by partial digestion with restriction endonucleases *Alu* I and *Hae* III. The DNA pooled from four independent *Alu* I and four independent *Hae* III digests was fractionated on 10–35% sucrose gradients (13), and DNA fragments of 15–24 kb were collected. This DNA was treated with *Eco*RI methylase (gift from Robert Rubin and Paul Modrich; see ref. 14) in 0.1 M Tris·HCl, pH 8.0/10 mM EDTA/6 μM *S*-adenosyl-L-methionine/5 mM dithiothreitol/200 μg of bovine serum albumin per ml/105 μg of DNA per ml/77 ng (2 nM) of *Eco*RI methylase per ml for 80 min at 37°C (14). After two phenol extractions and two ethanol precipitations, the methylated DNA was dissolved in 5 mM Tris·HCl, pH 7.5, and dialyzed for 7 hr against the same buffer. Before joining to the *Xenopus* DNA, octameric *Eco*RI linkers (Collaborative Research, Waltham, MA) were phosphorylated to convert the 5′-hydroxyl ends to 5′-phosphate ends (13). After the kinase reaction at 37°C, the reaction mixture was cooled to 16°C over 6 hr to allow renaturation of the linkers. The reaction mixture was then added to the *Xenopus* DNA and the ligation reaction was conducted at 8°C for 27 hr in 66 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$/1 mM spermidine/15 mM dithiothreitol/1 mM ATP/12 μg of *Eco*RI linkers per ml/130 μg of *Xenopus* DNA per ml/20 Weiss units of phage T4 ligase (New England BioLabs) per ml. The reaction mixture was extracted with phenol, and the DNA was precipitated with ethanol, dissolved in 100 mM Tris·HCl, pH 8.0/10 mM EDTA, heated 10 min at 60°C, loaded on 10–35% sucrose gradients, and centrifuged as described (13). DNA fragments bigger than 15 kb were collected and digested with a large excess of *Eco*RI. The left and right arms of the Charon 4 vector were prepared as described (13), and a reaction mixture containing 300 μg of arms and 125 μg of *Xenopus* DNA per ml in ligase buffer without ATP, dithiothreitol, and ligase was heated for 1 hr at 42°C to anneal the phage cohesive ends. After cooling down to 8°C, ATP, dithiothreitol, and ligase were added to 1 mM, 15 mM, and 100 Weiss unit/ml, respectively, and incubated for 24 hr. The *in vitro* packaging extracts were prepared and handled as described (15). The packaging reaction (2 μg DNA per tube) was performed exactly as described (15) and the mixture was plated on a fresh overnight culture of *Escherichia coli* K802 at a density of 7–10 × 10^3 plaque-forming units (PFU) per 15-cm-diameter L broth plate. After an overnight incubation at 37°C the top agar containing the amplified phages was scraped and handled as described (13). The experiments were carried out under P2–EK1 conditions in accordance with the *NIH Guidelines for Recombinant DNA Research*.

Screening of the Library. Screening was done on 150-mm L broth or NZCYM plates (13) containing 10^4 PFU by the *in situ* hybridization method (16). Plaques were lifted by two sequential applications of 132-mm BA85 Schleicher and Schuell nitrocellulose filters to plates precooled to 4°C. The first application was for 10–15 min and the second for 20–25 min, both at room temperature. The filters were dried for 1 hr at room temperature, treated with alkali, neutralized, and baked for 6 hr at 80°C. The filters were preincubated at room temperature

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Abbreviations: NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate; kb, kilobase(s) or kilobase pairs; PFU, plaque-forming unit.

for 10–15 hr in 50% formamide/0.6 M NaCl/200 mM Tris·HCl, pH 8.0/20 mM EDTA/0.1% sodium pyrophosphate/5 × Denhardt solution (17)/250 μg of *E. coli* tRNA per ml/100 μg of sheared denatured salmon sperm DNA per ml/0.2% sodium dodecyl sulfate (4 ml per filter). Vitellogenin cDNA clones (9, 10) were used as hybridization probes after nick translation to a specific activity of 0.5–1 × 10^8 cpm/μg (18). Hybridization was performed with 1.5–2 × 10^6 cpm of probe per 132-mm filter for 24 hr at 37°C, in the same solution as above (2.7 ml per filter) in a sealed plastic bag containing up to 25 filters. The filters were soaked sequentially in the hybridization solution in a petri dish before being introduced into the plastic bag. After hybridization, the filters were washed three times for 75 min each in 50% formamide/0.30 M NaCl/0.03 M sodium citrate/0.1% sodium dodecyl sulfate at room temperature, and one time for 45–50 min at 37°C in the same solution, followed by another wash of 75 min at room temperature. After being rinsed in 0.30 M NaCl/0.03 M sodium citrate (= 2× standard saline/citrate, NaCl/Cit) the filters were dried and exposed with an intensifying screen without preflashing of the films. An exposure of 10–15 hr resulted in strong positive signals from the plaques containing vitellogenin sequences. Positive plaques were purified by two or three further screening cycles with platings at much lower density. Phages containing vitellogenin sequences were grown in liquid cultures and DNA was prepared essentially as described (13).

Complexity Analysis of the Library. The procedures described by Britten et al. (19) and Galau et al. (20) were used as follows. DNA from *X. laevis* blood cells or from total library was sheared to an average length of 300 nucleotides in a Virtis blender. Single-copy tracer was prepared from blood cell DNA by 68°C incubation of the denatured DNA to a C_0t (concentration of DNA in mol of nucleotide per liter × incubation time in sec) of 1500 followed by isolation of the single-stranded material on hydroxylapatite. This DNA was then annealed to a C_0t of 13,000, at 68°C, and the reassociated material was collected on hydroxylapatite, recovered by melting at 98°C, and again annealed to a C_0t of 13,000. A sample of this DNA was labeled with [^{3}H]ATP and [^{3}H]CTP by gap translation. For measurement of reassociation rates, labeled DNA was mixed with at least a 1000-fold excess of sheared unlabeled blood cell DNA or library DNA and annealed in 0.4 M phosphate buffer at 68°C to the desired C_0t. The samples were then fractionated on hydroxylapatite columns. C_0t values were corrected to standard salt concentration (19). C_0t values for reassociation of library DNA are expressed in terms of the concentration of *Xenopus* DNA inserts. The extent of reaction was normalized for reactivity of the tracer, which ranged from 57% to 78% in different experiments.

Restriction Endonuclease Analysis. Digestions with the different restriction endonucleases were done as recommended by the supplier (Bethesda Research Labs, Bethesda, MD).

Table 1. *X. laevis* DNA library

DNA: embryonic, *Alu* I and *Hae* III nonlimit digests, octameric *Eco*RI linkers
Cloning vector: λ Charon 4
Packaging efficiency of extracts for
λ wild type: 4 × 10^7 PFU/μg
λ Charon 4: 4 × 10^6 PFU/μg
Packaging efficiency of *Xenopus* DNA: 1.5 × 10^5 PFU/μg
Number of independent recombinant phage obtained: 0.9–1 × 10^6
Number of recombinant phage needed for a complete library: 8.85 × 10^5
Complexity of cloned DNA: similar to uncloned DNA (see Fig. 1)
Mean length of the cloned *Xenopus* DNA fragments: 16 kb

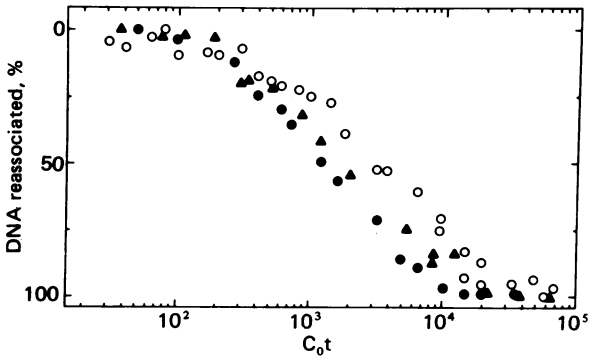

FIG. 1. Reassociation kinetics of *X. laevis* and library DNA. Labeled single-copy DNA was prepared from *X. laevis* blood cell DNA and annealed with an excess of unlabeled blood cell DNA (●, ○) or library DNA (▲). ○ and ● represent two separate experiments.

Heteroduplex Analysis. The DNAs (1.5 μg/ml of each clone) were denatured at 65°C for 5 min in 70% formamide/0.3 M NaCl/5 mM EDTA/10 mM Tris·HCl, pH 8.0 and subsequently reannealed in the same buffer for 90 min at 35°C. A sample was diluted 1:10 to a final concentration of 40% (vol/vol) formamide/1.8 M urea/0.1 M Tris·HCl, pH 8.5/ 10 mM EDTA/50 μg of cytochrome *c* per ml and spread on 5% formamide. Simian virus 40 and phage φX174 DNAs were included as double- and single-stranded length markers. Pictures were taken in a Philips EM 400 electron microscope.

RESULTS

Construction and Characterization of a Gene Library from *X. laevis*. The construction of this *Xenopus* library followed the strategy described by Maniatis et al. (13) and was made

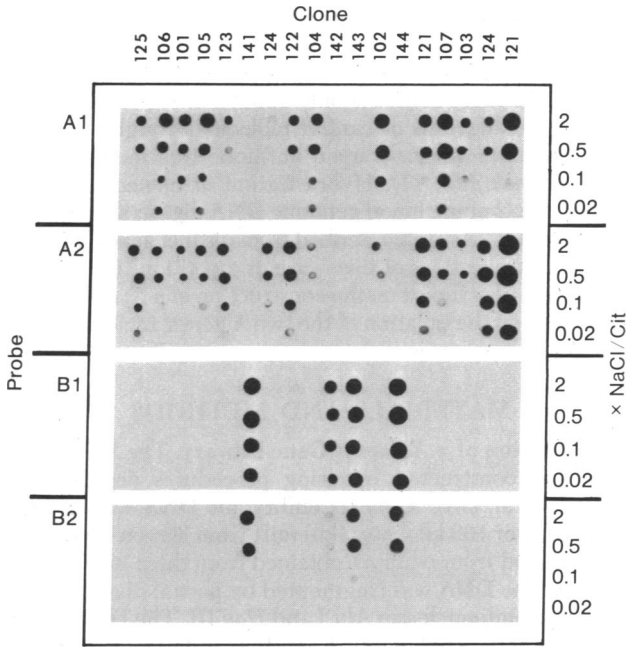

FIG. 2. Melting of hybrids between vitellogenin cDNA clones and genomic clones. Spots of 0.5 μg of DNA of different genomic clones were applied to a nitrocellulose filter sheet and hybridized under the conditions given in *Materials and Methods* with nick-translated vitellogenin cDNAs representing the four vitellogenin mRNAs A1, A2, B1, and B2 (10). Strips of the filters were washed at 37°C in 50% formamide/0.1% sodium dodecyl sulfate and different concentrations of NaCl/Cit as indicated in the figure, for 10 hr with six changes. The strips were dried, reassembled, and autoradiographed.

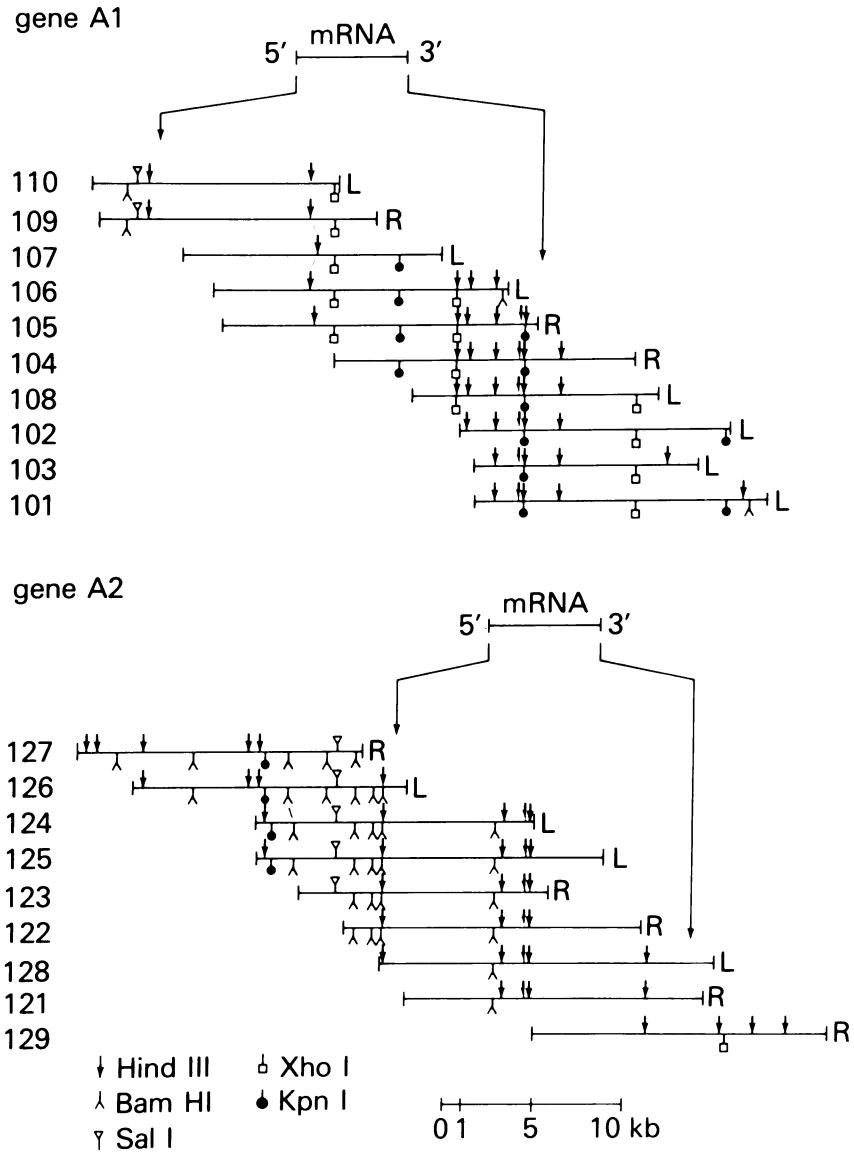

FIG. 3. Maps of overlapping cloned fragments of two vitellogenin genes. Overlapping clones were aligned by heteroduplex mapping in the electron microscope and by restriction endonuclease analysis. Restriction digests were run on 0.8% agarose gels. Small fragments may therefore have been missed. The vitellogenin mRNA is shown at the top and is drawn at the same scale as the DNA maps. The basis for the alignment of 5′- and 3′-terminal segments of the RNA with specific regions of the DNA is explained in the text. L and R refer to the left and right arms of the Charon 4 vector; a knowledge of the polarity of the insertion of the *Xenopus* DNA into the vector is important in designing heteroduplex experiments.

possible by the development of convenient λ cloning vectors (21) and *in vitro* packaging systems (22). In addition, the rapid *in situ* plaque hybridization procedure of Benton and Davis (16) allows the screening for single copy sequences in the complex *Xenopus* genome. The characteristics of our library are given in Table 1. The mean length of the cloned *Xenopus* DNA fragments was determined by electron microscopy from total library DNA and is 16 kb. This agrees well with the mean length of 15.9 kb obtained from 23 different inserts containing vitellogenin sequences (see below). Given the size of the *X. laevis* genome of 3.1×10^9 base pairs per haploid set (23) and the mean length of the cloned fragments, a library of 8.85×10^5 independent recombinant phages is needed to find any single copy sequence with a probability of 99% (24). Because we obtained $0.9–1 \times 10^6$ independent recombinant phages, and because the single copy complexity of the library is similar to that of uncloned *Xenopus* DNA (Fig. 1), the library can be considered as complete. However, due to the assumptions made in this kind of analysis and the limited sensitivity of the measurements, we cannot exclude the possibility that certain regions of the genome are underrepresented in our library.

Isolation of Vitellogenin Gene Sequences. Our cloning experiments of vitellogenin cDNA (9) have led to the conclusion that vitellogenin in *X. laevis* is encoded in four related genes (10). In a first screening of the library a mixed probe of cDNA clones representing all four genes was used to isolate sequences from the different genes. Eighteen recombinant phages were obtained in the first screen, which tested 4×10^5 phages. The clones were named λXlv (λ *X. laevis* vitellogenin) and numbered. To attribute each cloned genomic sequence to one of the four genes, we used the spotting technique applied earlier to demonstrate sequence divergence between different cDNA clones (10). The cloned genomic DNAs were spotted onto a nitrocellulose filter (16 spots per clone), and the filter was cut into four parts containing 4 spots per clone and hybridized separately to labeled cDNA probes representing the four genes. The filter sections were cut again into four parts and these strips were washed under conditions of increasing stringency. After washing the whole filter was assembled again and autoradiographed. Thus, each cloned DNA was tested with each of the four labeled probes at four levels of stringency. The results are shown in Fig. 2. Under the least stringent conditions only a few if any A/B crosshybrids are seen due to the presence of a 20% difference between the A and B structural sequences. Thus,

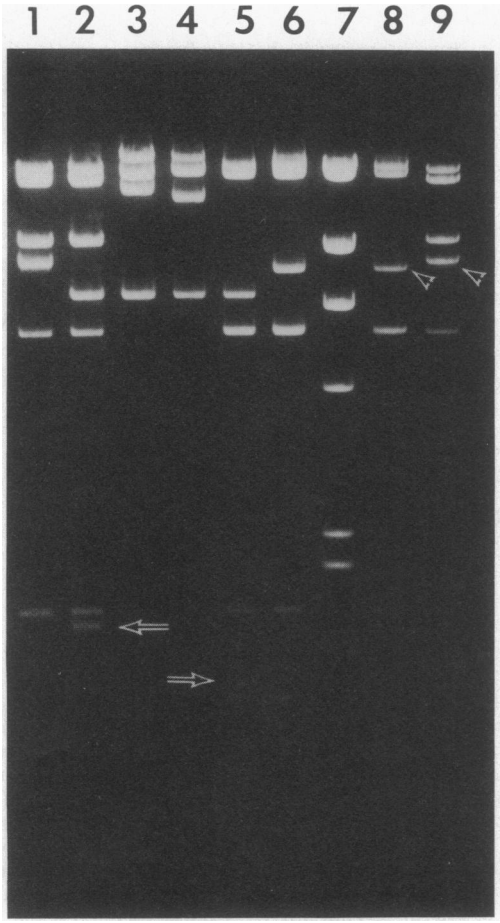

FIG. 4. Length polymorphism in the A1 gene. The figure shows restriction endonuclease digests of several cloned DNAs separated on an agarose gel. Lane 1, λXlv106, *Hin*dIII; lane 2, 106, *Hin*dIII-*Xho* I double digest; lane 3, 106, *Xho* I; lane 4, λXlv105, *Xho* I; lane 5, 105, *Hin*dIII-*Xho* I double digest; lane 6, 105, *Hin*dIII; lane 7, λDNA digested with *Hin*dIII, marker; lane 8, 105, *Hin*dIII; lane 9, 106, *Hin*dIII (see also Fig. 3). Complete arrows point to homologous *Hin*dIII-*Xho* I double digest fragments showing length heterogeneity: 1.55 kb in λXlv106, lane 2, and 1.2 kb in λXlv105, lane 5. Arrowheads indicate homologous intragenic *Hin*dIII fragments showing length heterogeneity: 8.3 kb in λXlv106 and 7.95 kb in λXlv105.

distinction between A and B gene sequences is obvious immediately. For example, λXlv125 contains an A gene sequence, whereas λXlv141 contains a B gene sequence. Within the A clones, distinction between A1 and A2 gene sequences can also be made on the basis of the stability of the hybrids; e.g., λXlv125 contains an A2 sequence and λXlv106 an A1 sequence. Within the B group, the result is also clear. All the B clones obtained in this screening contain B1 gene sequences.

Characterization of the Two A Genes. From initial experiments it became clear that the genomic vitellogenin sequences were much longer than the mRNA itself and that each of the recombinant phages recovered in the first screen contained only a portion of the gene. Thus it became necessary to obtain overlapping series of cloned DNA segments to cover the entire length of any vitellogenin gene and some of its flanking regions. At that point we decided to focus our experiments on the complete isolation and characterization of the two A genes only. Fig. 3 shows the results of an analysis that included hybridization of 3′-terminal and internal cDNA clones to the genomic clones, mapping of cleavage sites for several restriction endonucleases, and mapping of heteroduplex molecules between different clones in the electron microscope. Fig. 3 contains some

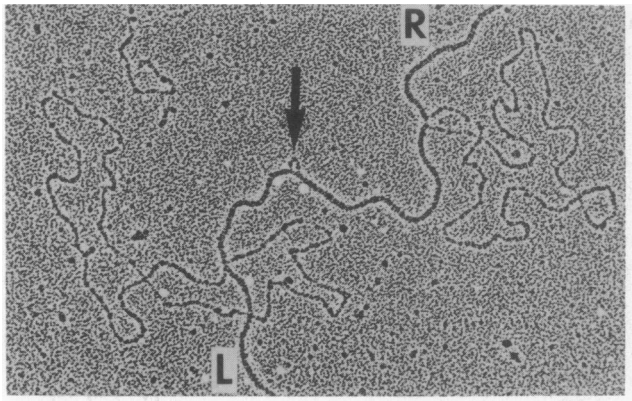

FIG. 5. Heteroduplex between λXlv123 and λXlv127. L and R indicate the left and right arms of the phage vector, which continue beyond the figure. The two large deletion loops at the boundary between phage and *Xenopus* DNA are due to the fact that the two clones overlap in only part of their length. The arrow points to the small deletion loop caused by length heterogeneity within the cloned *Xenopus* sequences.

additional clones not shown in Fig. 2. λXlv108, 109, 110, 128, and 129 were found in later screenings with labeled cDNA clones as probe, and λXlv126 and 127 were found by screening the library with the 5′ proximal *Hin*dIII fragment of λXlv125; the latter screen is an example of "chromosomal walking" along the DNA strand in the 5′ flanking region. The ten A1 clones and the nine A2 clones cover 38 and 42.5 kb, respectively. The vitellogenin mRNA of 6.3 kb (9, 25) is drawn in the same scale at the top of the figure. The arrows originating at the 5′ and 3′ ends of the mRNA point to the positions on the genomic clones beyond which A1 and A2 gene sequences diverge sufficiently so that no A1·A2 heteroduplexes form. The results of this heteroduplex mapping as well as R-loop mapping with mRNA that confirmed these positions will be presented in detail elsewhere. Considering the sequence divergence points as tentative 5′ and 3′ ends of the structural genes, the lengths of the A1 and A2 genes are 21 and 16 kb, respectively. λXlv128 was found in a round of screening with a cDNA clone corresponding to the 3′ end of the A2 gene. This recombinant phage turned out to be a fortunate find because it contains a complete copy of the A2 gene, assuming that our tentative assignments of the ends of the gene are correct.

As shown in Fig. 3, the restriction maps derived from different clones agree well in their overlapping regions, with some exceptions. λXlv103 and 106 have an additional *Hin*dIII and *Bam*HI site, respectively. Also in λXlv106, the large intragenic *Hin*dIII fragment (8.3 kb) is 350 base pairs longer than the corresponding fragment (7.95 kb) in λXlv105 (Fig. 3 and Fig. 4, arrows in lanes 8 and 9). *Xho* I/*Hin*dIII double digests of these two clones show that the resulting small *Xho-Hin*d fragment measures 1.55 kb in λXlv106 and 1.2 kb in λXlv105 (Fig. 4, arrows in lanes 3 and 5). λXlv109 and λXlv110 are identical to λXlv106 in this region, and λXlv107 is identical to λXlv105. Electron microscopic analysis of heteroduplexes between these two types of clones shows a small deletion loop in the region corresponding to the small *Xho* I-*Hin*dIII fragment. This length difference appears to occur within an intron (unpublished data). A similar kind of observation was made in the 5′ flanking sequence of the A2 gene. λXlv126 and 127 have an additional *Bam*HI site to the left of the *Sal* I site. Due to this additional site the 3.35-kb *Bam*HI fragment from this region in λXlv124 and 125 is replaced by two *Bam*HI fragments of 1.4 kb and 2.3 kb (total 3.7 kb) in λXlv126 and 127. Heteroduplex analysis between the two types of clones revealed, in analogy to the sit-

uation described for the A1 gene, a small deletion loop at the position of the additional *Bam*HI site in λXlv126 and 127 (Fig. 5).

DISCUSSION

This study illustrates some of the advantages of the gene library approach in the isolation of large genes forming a small family. In a single screening experiment with a mixed cloned cDNA probe we isolated sequences of three of the four members of the family. We then focused our work on the complete isolation of the two A genes, including their flanking sequences. The isolation and characterization of two large sets of overlapping clones demonstrate that, in the region studied, our library behaves as expected and appears to be effectively complete. Also, the identity of the restriction maps in their overlapping parts suggests that the cloned sequences are indeed representative of uncloned genomic DNA. Because the A1 and A2 structural gene sequences are closely related (10), it would have been more difficult because of crosshybridization to reach such a strong conclusion from hybridization experiments using restriction digests of total uncloned genomic DNA. Taking into account that the cloned DNA was derived from embryos pooled from three matings, the level of heterogeneity in the restriction pattern that could be due to population polymorphism is relatively low. Such polymorphism may become more apparent as the sites of more enzymes are mapped. The length heterogeneity found at a specific position within the A1 gene and in the 5′ flanking region of the A2 gene is not fully understood. At the present time we cannot decide with certainty whether we deal with population polymorphism or with a cloning artifact. If the latter were true, the results would strongly suggest localized deletion (or insertion) hot spots, because these length variations occur in several cloned DNAs at the same position. Because of the above requirement and because both length differences appear to occur in regions that do not code for protein, we believe that the effect is most likely due to polymorphism in the *Xenopus* population.

We had suggested earlier by blotting experiments in which uncloned DNA was hybridized with cDNA clones that the vitellogenin genes are split (10), as are numerous other eukaryotic genes (for a review, see ref. 12). The finding that the A1 and A2 genes are 3.3 and 2.5 times longer than the mRNA supports and extends these observations. Studies on nuclear RNA molecules that are interpreted as precursors to vitellogenin mRNA have shown the presence of 12 introns in a portion of the A1 gene (26). The arrangement and sizes of the many introns in the two A genes will be presented in a future publication. The vitellogenin mRNA population is homogenous in size (1, 25), and R-loop experiments in the electron microscope with A1 and A2 cDNA clones have more directly demonstrated that A1 and A2 mRNAs are of similar length (9, 10). The length difference of 5 kb found between the two genes must therefore be accounted for by differences in the introns. It is not yet known if the vitellogenin genes are linked. In analogy to some other multigene families (27, 28), it would not be surprising if they are linked. From the present data we can already conclude that, if the two genes are linked, the intergene distance must be larger than 31 kb if the order is (5′)A1-A2(3′) and larger than 11.5 kb if the order is (5′)A2-A1(3′).

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1. Wahli, W., Wyler, T., Weber, R. & Ryffel, G. U. (1976) *Eur. J. Biochem.* **66,** 457–465.
2. Shapiro, D. J., & Baker, H. J. (1977) *J. Biol. Chem.* **252,** 5244–5250.
3. Ryffel, G. U., Wahli, W. & Weber, R. (1977) *Cell* **11,** 213–221.
4. Baker, H. J. & Shapiro, D. J. (1977) *J. Biol. Chem.* **252,** 8428–8434.
5. Clemens, M. J., Lofthouse, R. & Tata, J. R. (1975) *J. Biol. Chem.* **250,** 2213–2218.
6. Shapiro, D. J., Baker, H. J. & Stitt, D. T. (1976) *J. Biol. Chem.* **251,** 3105–3111.
7. Tata, J. R. (1976) *Cell* **9,** 1–14.
8. Ryffel, G. U. (1978) *Mol. Cell. Endocrinol.* **12,** 237–246.
9. Wahli, W., Ryffel, G. U., Wyler, T., Jaggi, R. B., Weber, R. & Dawid, I. B. (1978) *Dev. Biol.* **67,** 371–383.
10. Wahli, W., Dawid, I. B., Wyler, T., Jaggi, R. B., Weber, R. & Ryffel, G. U. (1979) *Cell* **16,** 535–549.
11. Felber, B. K., Maurhofer, S., Jaggi, R. B., Wyler, T., Wahli, W., Ryffel, G. U. & Weber, R. (1980) *Eur. J. Biochem.*, in press.
12. Dawid, I. B. & Wahli, W. (1979) *Dev. Biol.* **69,** 305–328.
13. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
14. Rubin, R. A. & Modrich, P. (1977) *J. Biol. Chem.* **252,** 7265–7272.
15. Enquist, L. & Sternberg, N. (1979) *Methods Enzymol.* **68,** in press.
16. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
17. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–646.
18. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
19. Britten, R. J., Graham, D. E. & Neufeld, B. E. (1974) *Methods Enzymol.* **29,** 363–418.
20. Galau, G. A., Klein, W. H., Davis, M. M., Wold, B. J., Britten, R. J. & Davidson, E. H. (1976) *Cell* **7,** 487–505.
21. Blattner, F. R., Williams, B. G., Blechl, A. E., Denniston-Thompson, K., Faber, H. E., Furlong, L. A., Grunwald, D. J., Kiefer, D. O., Moore, D. D., Schumm, J. W., Sheldon, E. L. & Smithies, O. (1977) *Science* **196,** 161–169.
22. Sternberg, N., Tiemeier, D. & Enquist, L. (1977) *Gene* **1,** 255–280.
23. Dawid, I. B. (1965) *J. Mol. Biol.* **12,** 581–599.
24. Clarke, L. & Carbon, J. (1976) *Cell* **9,** 91–99.
25. Wahli, W., Wyler, T., Weber, R. & Ryffel, G. U. (1978) *Eur. J. Biochem.* **86,** 225–234.
26. Ryffel, G. U., Wyler, T., Muellener, D. B. & Weber, R. (1980) *Cell* **19,** 53–61.
27. Little, P. F. R., Flavell, R. A., Kooter, J. M., Annison, G. & Williamson, R. (1979) *Nature (London)* **278,** 227–231.
28. Fritsch, E. F., Lawn, R. M. & Maniatis, T. (1979) *Nature (London)* **279,** 598–608.