Differential Regulation of the Levels of Three Gap Junction mRNAs in Xenopus Embryos

R. L. Gimlich,* N. M. Kumar, and N. B. Gilula

Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037; and *Department of Zoology, Oregon State University, Corvallis, Oregon 97331-2914

Abstract. Xenopus mRNAs that potentially encode gap junction proteins in the oocyte and early embryo have been identified by low-stringency screening of cDNA libraries with cloned mammalian gap junction cDNAs. The levels of these mRNAs show strikingly different temporal regulation and tissue distribution. Using a nomenclature designed to stress important structural similarities of distinct gap junction gene products, the deduced polypeptides have been designated the *Xenopus* α_1 and α_2 gap junction proteins. The α_2 gap junction mRNA is a maternal transcript that disappears by the late gastrula stage. It is not detected in any organ of the adult except the ovary, and resides primarily, if not exclusively, in the oocytes and early embryos. The α_1 gap junction mRNA appears during organogenesis, and is detected in RNA from a wide variety of organs. It is also found in full-grown oocytes, but is rapidly degraded upon oocyte maturation, both in vivo and in vitro. The α_1 and α_2 mRNAs encode proteins with different degrees of amino acid sequence similarity to the predominant gap junction subunit of the mammalian heart (connexin 43). Together with our earlier report of a mid-embryonic (β_1) gap junction mRNA, the results suggest that intercellular communication during oocyte growth and postfertilization development is a complex phenomenon involving the coordinated regulation of several genes.

INTERCELLULAR communication, mediated by the passage of ions and small molecules via cell–cell gap junctional channels, is thought to contribute to the regulation of pattern during early development (see recent reviews by Caveney, 1985; Guthrie and Gilula, 1989). Most of the evidence for this supposition is indirect, relying on correlations between the apparent amount of gap junctional conductance and the timing of determinative events (de Laat et al., 1980) or the placement of developmental boundaries such as compartment borders (Warner and Lawrence, 1982; Blennerhasset and Caveney, 1984; Weir and Lo, 1984; see also Fraser and Bryant, 1985). In more direct experiments polyclonal antisera directed against purified mamalian gap junction proteins have been used to inhibit conductance from cell to cell. Microinjection of affinity-purified antibodies to the 32-kD rat liver gap junction protein into particular blastomeres in the *Xenopus laevis* embryo causes both a reduction of junctional conductance and pronounced defects in the development of dorsoanterior structures, including derivatives of the neural tube (Warner et al., 1984). The morphogenetic gradient controlling bud position in the body column of *Hydra attenuata* is altered after permeabilized epithelial cells take up these antibodies, resulting in an increased frequency of budding near the head (Fraser et al., 1987). Finally, compaction of blastomeres in the mouse morula is inhibited when they are microinjected with gap junction–directed antisera (Lee et al., 1987).

In each of these cases, loss or reduction of gap junctional communication among treated cells is closely correlated with specific developmental effects. The antibodies used have been shown to bind specifically to the cytoplasmic side of gap junctional membranes in immunocytochemical studies of rat liver junctional complexes (Zimmer et al., 1987; Milks et al., 1988). They will also block the conductance of reconstituted gap junctional channels in vitro (Young et al., 1987). It is difficult, however, to rule out the possibility that developmental effects and communication defects in vivo are mediated by different specificities in polyclonal antibody preparations.

Further progress in deciphering the role of gap junctional communication in early developmental specification would be aided by an understanding of the structure and pattern of expression of genes which encode gap junction proteins in the embryo under study. We are pursuing this approach in the frog *Xenopus laevis* for several reasons. These embryos show some of the most striking developmental perturbations after antibody-mediated reduction of junctional communication (Warner et al., 1984). The spatial pattern of communication has been described at several stages of development by dye transfer and electrophysiological techniques (Blackshaw and Warner, 1976; Ito and Ikematsu, 1980; Guthrie, 1984; Guthrie et al., 1988). Regulated changes in the number of gap junctional structures at several developmental stages have been described (Decker and Friend, 1974;

597

Browne et al., 1979), and the physiological properties of *Xenopus* embryonic gap junctions have been extensively studied (Slack and Warner, 1973; Spray et al., 1979; Turin and Warner, 1980). Here, we describe several *Xenopus* embryonic mRNA sequences similar to those encoding gap junctions in mammalian organs, and present a study of their developmental regulation. One of these mRNAs (for connexin 38, or the α_2 gap junction protein) has been described recently and reported to stimulate gap junctional conductance upon injection into the *Xenopus* oocyte (Ebihara et al., 1989).

Materials and Methods

Collection, Culture, and Staging of Oocytes and Embryos

Adult *Xenopus laevis* were maintained at 22°C on a 12-h light/dark cycle and a diet of Purina Trout Chow (Ralston-Purina Corp., St. Louis, MO). Ovulation was induced by injection of 400–700 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO) into the dorsal lymph sac, and stripped eggs were fertilized in vitro with minced testis. Fertilized eggs were dejellied in a solution of 0.1 M NaCl, 2.5% (wt/vol) L-cysteine hydrochloride (Sigma Chemical Co.), pH 7.9. Embryos were cultured at 18–22°C in 33% modified Ringers' solution (33% MR; MR is 0.1 M NaCl, 0.002 M KCl, 0.001 M MgCl₂, 0.002 M CaCl₂),[1] and staged according to the normal table of *Xenopus* development (Nieuwkoop and Faber, 1975). Oocytes were removed from follicles manually for maturation experiments, or by gentle agitation for 1 h at 22°C in a solution of 0.1 mg/ml collagenase (Sigma Chemical Co.; type II) in MR, for bulk RNA isolations. After collagenase digestion, isolated oocytes were separated from residual follicular cells by repeated washing and settling in MR. All defolliculated oocytes and oocyte suspensions were examined microscopically to ensure that residual follicular cells were removed. Oocytes were cultured in modified Barth's medium (Colman, 1985) and staged according to Dumont (1972). Maturation was induced in vitro with progesterone (Eli Lilly & Co., Indianapolis, IN) at 5 g/ml in modified Barth's solution.

Screening of cDNA Clone Libraries

A 1.4-kb coding sequence subclone of the rat heart gap junction cDNA (Beyer et al., 1987) was kindly provided by Dr. Eric Beyer (Harvard Medical School, Cambridge, MA). A λgt10 cDNA library made with *Xenopus* ovary poly (A)+ RNA was kindly provided by Dr. Harold Weintraub (Fred Hutchinson Cancer Research Institute, Seattle, WA). Hybridization screening of this library was done in 40% formamide, 6× SSC, 37°C, with washing in 2× SSC, 37°C. Hybridization probes were synthesized from double-stranded DNA using the large fragment of DNA polymerase I and random hexameric oligonucleotides (Pharmacia Fine Chemicals, Piscataway, NJ) as primers (Feinberg and Vogelstein, 1983).

DNA Sequence Analysis

Sequencing of cDNA fragments subcloned in M13mp19 was done using the 17-mer universal sequencing primer (New England Biolabs, Beverly, MA), or clone-specific DNA oligonucleotides as described (Gimlich et al., 1988). Oligonucleotides were synthesized with a synthesizer (model 380A; Applied Biosystems, Foster City, CA). DNA and protein sequences were compiled and analyzed using PCGene software (Intelligenetics, Palo Alto, CA).

RNA and DNA Blot Analyses

Genomic DNA and RNA from oocytes, embryos, and adult organs was purified as described in Gimlich et al. (1988). RNAs were separated by electrophoresis through 1% agarose gels containing 0.66 M formaldehyde and transferred to nylon filters (Micron Separations, Inc., Westboro, MA) for hybridization with radiolabeled fragments of cloned *Xenopus* cDNA. Hy-

bridization to RNA was done at 37°C in 50% formamide, 6× SSC, washing in 0.2× SSC at 65°C. Actin mRNAs were detected by reduced stringency hybridization with a *Xenopus* skeletal actin cDNA (Sargent et al., 1986), as described (Gimlich et al., 1988). Heart polyadenylated RNA was probed with connexin 43 cDNA (Beyer et al., 1987) under the same reduced stringency conditions. Genomic DNA was digested with several restriction endonucleases and fractionated on 0.7% agarose gels for transfer to nylon filters and hybridization with digoxygenin labeled cDNA probes. Hybridizations were done at 37°C in 50% formamide, 6× SSC, washing in 0.2× SSC at 65°C. Hybrids were detected using a commercially supplied immunochemical system (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Nuclease Protection Analysis

Single-stranded DNA probes for nuclease protection assays were synthesized from (+)-strand M13 subclones and purified from alkaline agarose gels or from 5% polyacrylamide, 6 M urea sequencing gels by electroelution. After hybridization with RNA (200–1,000 g/ml) in 80% formamide, 0.4 M NaCl, residual single-stranded probe was digested with 50 IU of S1 nuclease (Bethesda Research Laboratories) as described (Berk and Sharp, 1977). DNA fragments protected by annealing with RNA were purified, separated by electrophoresis in 6% polyacrylamide gels containing 6 M urea, and detected by autoradiography at −70°C using Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) with an intensifying screen. Optimal hybridization and digestion temperatures were empirically determined for each probe.

Results

Isolation of Ovary cDNA Clones

In an earlier report, cDNA clones representing a putative *Xenopus* gap junction (GJ) protein mRNA were described (Gimlich et al., 1988). This mRNA encodes a product with 74% amino acid sequence identity to the human 32-kD liver GJ protein (Kumar and Gilula, 1986). It was undetectable in the frog ovary and in oocytes, and began to accumulate late in gastrulation. Thus it was expected that mRNAs encoding other embryonic GJ proteins would account for maternal and early embryonic gap junctional structures and activity. Such transcripts might be more closely related to one of the other sequences that encodes these proteins in mammalian organs (Kumar and Gilula, 1986; Paul, 1986; Beyer et al., 1987). Therefore, a *Xenopus* ovary cDNA library in λgt10 was screened at reduced hybridization stringency, as described in Materials and Methods, with the coding region subfragment of the rat heart-derived "connexin 43" cDNA described by Beyer et al. (1987).

Xenopus cDNA inserts scoring as positive on duplicate filters in this screen were excised and cloned in M13 for sequencing by the chain termination method. Two classes of cDNA clones were obtained. The sequences of the longest clone in each class are presented in Fig. 1. The two sequences were initially designated Ov7 and Ov11. Each contained the coding information for a polypeptide with extensive similarity to the protein encoded by connexin 43 mRNA in rat heart. Two distinct cDNA clones of the Ov7 class differed in their 5'-nontranslating regions (Fig. 1), suggesting the possibility of alternate transcripts containing this coding sequence.

A comparison of the deduced *Xenopus* amino acid sequences with the connexin 43 sequence is shown in Fig. 2. The *Xenopus* Ov11 (α_2, see below) cDNA contains the coding sequence for a 334-amino acid protein of 37,834 D. When the amino acid sequence is optimally aligned with that of the

---

1. *Abbreviations used in this paper*: GJ, gap junction; MR, modified Ringers' solution: 0.1 M NaCl, 0.002 M KCl, 0.001 M MgCl₂, 0.002 M CaCl₂.

Ov11 cDNA (α_2 GJ)

▼GAATTCCGCCCCCCCCCCCCCCCCATTAGTGGAGGAAAGTATTGATACTGTCAGACTTACTGCCAGATGCTGTGAAAC 76

ATGGCAGGATGGGAATTACTAAAGCTCTTACTGGATGATGTCCAGGAACATTCCACACTTATTGGGAAAGTCTGGCTAACCGTACTTTTTATCTTCAGA 175
M A G W E L L K L L L D D V Q E H S T L I G K V W L T V L F I F R (33)

ATATTTATTCTGAGTGTTGCAGGTGAGTCTGTGTGGACAGACGAGCAGTCAGATTTCATCTGTAACACTCAGCAGCCTGGCTGTACCAATGTCTGCTAT 274
I F I L S V A G E S V W T D E Q S D F I C N T Q Q P G C T N V C Y (66)

GATCAGGCCTTCCCTATTTCACATGTTCGCTATTGGGTGCTGCA▼GTTTCTCTTTGTCAGTACACCTACCTTGATCTACTTGGGCCACATGGTTTACCTG 373
D Q A F P I S H V R Y W V L Q F L F V S T P T L I Y L G H M V Y L (99)

TCTAAGAAAGAGGAGAAGGAAAGACAGAAGGAGAATGAATCAAGGATTCTGGTGGCAAATGAAGCACAAACAGAAGTACACTCTTCTGCTACTAAGAAA 472
S K K E E K E R Q K E N E S R I L V A N E A Q T E V H S S A T K K (132)

ATAAGGATTCAAGGGCCCTTGATGTGCACATACACCACCAGTGTAGTCTTCAAAAGTATTTTTGAAGCTGGGTTCCTGCTAGGCCAGTGGTACATATAC 571
I R I Q G P L M C T Y T T S V V F K S I F E A G F L L G Q W Y I Y (165)

GGCTTTGTGATGTCTCCAATATTTGTGTGTGAGCGAATCCCATGCAAACATAAAGTTGAGTGTTTTGTATCAAGGCCCATGGAAAAAACAATATTTATC 670
G F V M S P I F V C E R I P C K H K V E C F V S R P M E K T I F I (198)

ATATTCATGCTAGTCGTTTCTCTCATCTCTCTGTTGTTAAATTTGATGGAACTTATTCATCTAAGCTTTAAGTGTTTCCAACATGGTATAAAAGAAGGT 769
I F M L V V S L I S L L L N L M E L I H L S F K C F Q H G I K E G (231)

GCAACTTGTTCTCCAACAGGGATTCCATTCAATGGGGCCGGAAACCGAATGCCCCCACAGGAATACACCAATCCTCCCTCTAGCAACCAGGACATTGAT 868
A T C S P T G I P F N G A G N R M P P Q E Y T N P P S S N Q D I D (264)

CTGCCATCTTATAACAAAATGTCTGGGGGGCACAATTGGTCACGTATCCAGATGGAGCAGCAAGTTAATGGTCTTGTGAAACCCAAGTGTCAGTGTGAC 967
L P S Y N K M S G G H N W S R I Q M E Q Q V N G L V K P K C Q C D (297)

TGTTGGTCCCAAAGTGCTATTTCTGTGGTGGTTTCTGGAGCACCAGGTATAATCTCAAACATGGATGCAGTCAAAAGTAATCACCAGACCTCTTCCAAG 1066
C W S Q S A I S V V V S G A P G I I S N M D A V K S N H Q T S S K (330)

CAGCAGTATGTTTAA 1081
Q Q Y V   (334)

TAGAATAGTATGATGGTTACCTTTCAAATATGCAGGTGATAGTCCAACTTTGTGTGTTGAGTAGTGAATTGTAGATGCACCTCCAAGGATTTCAGTAAG 1180
TCCATGAATCCATGTGTTGAATCCCTCTTTATCCACAAGATGAATGGGCTTGTCTTAATGATCAAAGTGCATATTTTGATTTTTTTGGGGGGGGAAGAAG 1279
AAGGTATATTGTTTTTTCGCACTCTAGGCTGATAAATGTCTAAACACTAATTTGGTATGTATTAATAAAATAAATAAAATGAGCAAATTATTTACAGTC 1378
TAAAAAAAAAAAAAAAAAAAAAAA 1400

Ov7 cDNA (α_1 GJ)

[CGATGATCCTGCAGATCCTTGTTCAGAGGAGATCATGCT] -
[GAAATTCCCCCCCCCCCCCCCCCCCCAGGACACTCCAGTGCTTTGATTCTTACAAAGAGCTACCATGTGGGCTAAGAGAGTGAGAAG] -GGGGGCGTGA 96
CGTGCAGGTATGCGCCGCTGGAAGCAGAAACGGGTCACATGGATATCGGGCCGGCACAGACATCACTAATGGTGAATAAAGGTAACCACTTTTGAGC 194

ATGGGTGACTGGAGTGCCTTAGGAAGACTTCTTGACAAAGTTCAAGCCTATTCCACTGCAGGAGGGAAGGTGTGGCTGTCTGTCCTATTCATATTTCGA 292
M G D W S A L G R L L D K V Q A Y S T A G G K V W L S V L F I F R (33)

ATCCTTTTATTAGGGACAGCAGTGGAGTCAGCCTGGGGCGATGAACAATCAGCATTTGTCTGCAATACTCAGCAACCAGGTTGTGAGAATGTTTGCTAT 391
I L L L G T A V E S A W G D E Q S A F V C N T Q Q P G C E N V C Y (66)

GATAAGTCATTCCCTATATCTCATGTGCGTTTCTGGGTACTTCAGATCATATTTGTCTCAACACCGACCCTGCTTTATTTAGCCCACGTTTTTTACTTG 490
D K S F P I S H V R F W V L Q I I F V S T P T L L Y L A H V F Y L (99)

ATGAGAAAAGAAGAGAAGCTGAACAGGAAAGAAGAAGAACTTAAAATGGTTCAGAACGAAGGTGGTAATGT▼AGACATGCACCTTAAACAAATCGAAATA 589
M R K E E K L N R K E E E L K M V Q N E G G N V D M H L K Q I E I (132)

AAGAAATTCAAATATGGCCTTGAAGAGCATGGAAAAGTCAAGATGCGAGGTGGACTGCTTCGCACCTACATCATCAGCATTTTGTTTAAATCAGTATTT 688
K K F K Y G L E E H G K V K M R G G L L R T Y I I S I L F K S V F (165)

GAAGTTGGCTTCATTATCATTCAATGGTACATGTATGGGTTTAGCTTAAGTGCCATCTATACATGCAAAAGAGATCCCTGCCCACATCAAGTGGACTGT 787
E V G F I I I Q W Y M Y G F S L S A I Y T C K R D P C P H Q V D C (198)

TTTCTTTCTCGACCCACAGAGAAAACCATTTTTATCTGGTTTATGTTGATCGTATCTATAGTTTCTCTTGCCCTAAATATCATCGAGTTATTCTATGTC 886
F L S R P T E K T I F I W F M L I V S I V S L A L N I I E L F Y V (231)

ACCTACAAAAGTATCAAAGATGGCATCAAAGGGAAAAAAGACCCATTTTCTGCAACAAATGATGCTGTGATTTCTGGAAAGGAA▼TGTGGCTCCCCTAAA 985
T Y K S I K D G I K G K K D P F S A T N D A V I S G K E C G S P K (264)

TATGCCTATTTTAATGGCTGTTCTTCTCCAACAGCTCCCATGTCTCCACCTGGTTACAAGCTTGTTACTGGAGAAAGAAATCCTTCTTCCTGCCGCAAT 1084
Y A Y F N G C S S P T A P M S P P G Y K L V T G E R N P S S C R N (297)

TATAACAAGCAAGCAAGTGAACAAAATTGGGCAAATTACAGTGCAGAACAAAACAGGATGGGACAGGCTGGAAGCACAATTTCTAACACTCATGCACAG 1183
Y N K Q A S E Q N W A N Y S A E Q N R M G Q A G S T I S N T H A Q (330)

CCATTTGATTTTTCTGATGAACATCAGAATACTAAGAAAATGGCACCAGGACATGAAATGCAGCCACTGACTATATTGGACCAAAGACCATCTAGTAGA 1282
P F D F S D E H Q N T K K M A P G H E M Q P L T I L D Q R P S S R (363)

GCAAGCAGTCATGCTAGTAGCAGACCACGACCTGATGATTTAGAGATCTAG 1333
A S S H A S S R P R P D D L E I   (379)

TCAACGTTGATGAATTACCAGAAGGCACATTTTAAGAGTGGAATTGGTACTAAATTCTCTCAAATAAGATTTAACAAACATTTTTGATGTACTTTGATA 1432
TGGCGAGGGAGAATCGCTTTGATATTTAAGTAGCTCATACAAATTTTTACAGTCTTAAATGACTCGTACAATATTTCTTTATAATGTATATCTAAGACA 1531
ACTTATGTGTTTTCAGTTTGCCCAAAAAGTGAAATATTTGGGTGAGCAATGTTGTTTTATGACTGAGCAATTAAGGTCAGCCTACTATGTATAAGACGA 1630
GAGATTTCTTACAGTGATTTTAGAAATTAACTTAAATAATTGTTTGACTGTTAGGAAACTTTTACAACAATTCTGGAGAAGGACACCTGGATGTTTTCT 1729
CAATATGACTAGCATTTTTTACTTGCCCCATTTCTTCTTCTGTTTAAGAAAATTGTGCTTATGTAAATAAATTGTTCGCTCACGTGCCTTTATTAAAAA 1828
TGACCTAAAAGCAACATTAAAAAAAAAAAAAAAAAAAAA 1841

Figure 1. Nucleotide sequences of *Xenopus* Ov11 (α_2) and Ov7 (α_1) cDNAs isolated from a λgt10 ovary cDNA library. Both strands of each clone were sequenced as described in Materials and Methods. The longest open translational reading frames are shown below each sequence, and the numbering in parentheses refers to codons. Single-stranded probes used in nuclease protection assays were complementary to the sequences between arrowheads. Flanking sequences at the 5' ends of two distinct Ov7 DNA clones are shown in brackets. The significance of poly-dC near the 5' ends of two of the cDNA sequences is unknown. These sequence data are available from EMBL/ Genbank/DDBJ under accession numbers X17242 (α_2-GJ) and X17243 (α_1-GJ).

```
α₂  MAGWELLKLL LDDVQEHSTL IGKVWLTVLF IFRIFILSVA
α₁  MGDWSALGRL LDKVQAYSTA GGKVWLSVLF IFRILLLGTA
RH  MGDWSALGKL LDKVQAYSTA GGKVWLSVLF IFRILLLGTA
β₁  .......... .........I G-RIWLSVVF IFRIMVLVAA
HL  .......... .......TAI G-RVWLSVIF IFRIMVLVVA

α₂  GESVWTDEQS DFICNTQQPG CTNVCYDQAF PISHVRYWVL
α₁  VESAWGDEQS AFVCNTQQPG CENVCYDKSF PISHVRFWVL
RH  VESAWGDEQS AFRCNTQQPG CENVCYDKSF PISHVRFWVL
β₁  AESVWGDEKS AFTCNTQQPG CNSVCYDHFF PISHIRLWAL
HL  AESVWGDEKS SFICNTLQPG CNSVCYDQFF PISHVRLWSL

α₂  QFLFVSTPTL IYLGHMVYLS KKEEKERQKE NESRIL----
α₁  QIIFVSTPTL LYLAHVFYLM RKEEKLNRKE EELKMVQNEG
RH  QIIFVSVPTL LYLAHVFYVM RKEEKLNKKE EELKVAQTDG
β₁  QLIIVSTPAL LVAMHVA... ...:...... ..........
HL  QLILVSTPAL LVAMHVA... .......... ..........
                                    *   *
α₂  VANE---AQT EVHSSAT--- --KKIRIQGPL MCTYTTSVV
α₁  GNVDMHLKQI EIKKFKYGLE EHGKVKMRGGL LRTYIISIV
RH  VNVEMHLKQI EIKKFKYGIE EHGKVKMRGGL LRTYIISIL
β₁  .......... .......... ........GTL WWTYISSFF
HL  .......... .......... ........GTL WWTYVISVV
    *   *
α₂  FKSIFEAGFL LGQWYIY-GF VMSPIFVCER IPCKHKVECF
α₁  FKSVFEVGFI IIQWYMY-GF VLSAIYTCKR DPCPHQVDCF
RH  FKSVFEVAFL LIQWYIY-GF SLSAVYTCKR DPCPHQVDCF
β₁  FRIIFEAAFM YIFYLIYPGY SMIRLVYCDA YPCPNTVDCF
HL  FRLLFEAVFM YVFYLLYPGY AMVRLVYCDA YPCPNTVDCF

α₂  VSRPMEKTIF IIFMLVVSLI SLLLNLMELI HLSFKCFQHG
α₁  LSRPTEKTIF IWFMLIVSIV SLALNIIELF YVTYKSIKDG
RH  LSRPTEKTIF IIFMLVVSLV SLALNIIELF YVFFKGVKDR
β₁  VSRPTEKTIF TVFMLVASFV CIVLNVAEVF FLIAQACT..
HL  VSRPTEKTVF TVFMLAASGI CIILNVAEVV YLII......

α₂  IKEGATC-SP T--------- -G-P----FN GAGNRMPPQE
α₁  IKGKKDPFSA TNDAVISGKE CGSPKYAYFN GCSSPTAP--
RH  VKGRSDPYHA TTGPLSPSKD CGSPKYAYFN GCSSPTAPLS

α₂  YTNPPSSNQD -IDL-PS--- -YNKMSGGHN WSRIQMEQQV
α₁  -MSPPGYKLV TGERNPSSCR NYNKQASEQN WANYSAEQNR
RH  PMSPPGYKLV TGDRNNSSCR NYNKQASEQN WANYSAEQNR

α₂  NGLVKPKCQC DCWSQSAISV VVSGAPGIIS N--M------
α₁  MGQAGSTISN THAQPFDFSD EHQNTKKMAP GHEMQPLTIL
RH  MGQAGSTISN SHAQPFDFPD DNQNAKKVAA GHELQPLAIV

α₂  DAVKSNHQ-- -TSSKQQYV-(COOH)
α₁  DQRPSSRASS HASSRPRPDD LEI-(COOH)
RH  DQRPSSRASS RASSRPRPDD LEI-(COOH)
```

Figure 2. Deduced amino acid sequences from the three *Xenopus* cDNAs are compared with mammalian gap junction protein sequences. Complete protein products of the Ov7 (α₁) and Ov11 (α₂) GJ mRNAs were manually aligned for optimal amino acid sequence fit with the deduced rat heart connexin 43 protein sequence (RH; Beyer et al., 1987). Partial products of the *Xenopus* β₁ mRNA (deduced from the XE11a cDNA sequence of Gimlich et al., 1988), and human connexin 32 mRNA (HL), (Kumar and Gilula, 1986) are shown to emphasize the conservation of sequences in the putative transmembrane (underlined) and extracellular domains. The positions of putative transmembrane sequences are as predicted by Milks et al. (1988). Asterisks show the highly conserved residues in the third transmembrane domain. Dashes indicate gaps introduced for optimal sequence alignment.

rat heart protein, 153 of the 334 residues are identical (46%), and 64 of the 182 differences are conservative in charge and polarity. The 379-codon *Xenopus* Ov7 (α₁) cDNA sequence predicts a 42,964 D protein which is identical to connexin 43 in 328 of 379 residues (87%); 32 of 51 amino acid changes are conservative. Other features of this structural comparison will be discussed below.

These *Xenopus* mRNAs are apparently transcribed from different genes. Although their products show extensive amino acid sequence similarity, there are no substantial blocks of nucleotide sequence identity. Genomic DNA was analyzed in Southern blots probed with the Ov11 and Ov7 cDNAs. The two probes hybridized to distinct sets of restriction fragments (Fig. 3). In addition, the hybridization patterns were different from those obtained with the *Xenopus* XE11a cDNA probe (Gimlich et al., 1988). These results suggest that each mRNA is the product of a distinct single-copy or low-copy number gene. Genomic sequences hybridizing with the Ov11 cDNA probe contained several restriction sites not found in the cDNA sequence, suggesting the existence of intervening sequences or of multiple highly conserved copies of this gene.

A Nomenclature for Xenopus Gap Junction Proteins

Because the predicted frog oocyte proteins show strong primary structure similarity to known gap junctional proteins, we will tentatively refer to them as products of *Xenopus* gap junction mRNAs. The present nomenclature for GJ subunits (the "connexin" system) is based primarily on the molecular weights of proteins deduced from cDNA sequences (Beyer et al., 1987). Very closely related proteins from different species can, however, have different predicted molecular weights. For instance, the mammalian liver/endodermal GJ protein is 32 kD, whereas the *Xenopus* homologue is 30 kD (Gimlich et al., 1988). To avoid confusion, we have devised an alternative nomenclature that emphasizes amino acid sequence similarities rather than protein product size (Table I). Thus, rat connexin 43 and the predicted *Xenopus* Ov7 protein fall into the same class, which we will call the "α₁" GJ proteins. The *Xenopus* Ov11 cDNA predicts a similar but more
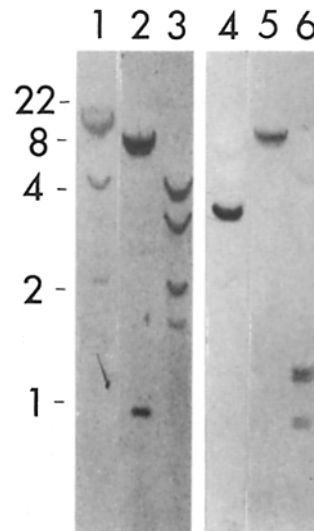


```
      1 2 3   4 5 6
22-
 8-
 4-
 2-
 1-
```

Figure 3. *Xenopus* Ov11 (α₂) and Ov7 (α₁) GJ cDNAs represent different genes. Genomic DNA from liver was digested with restriction endonucleases Bam HI (lanes *1* and *4*), Eco RI (lanes *2* and *5*), or Hind III (lanes *3* and *6*), electrophoresed, and blotted to nylon. DNA on duplicate filters was hybridized with a digoxygenin-labeled Ov11 cDNA probe (lanes *1–3*) or an Ov7 cDNA probe (lanes *4–6*). Size markers were Eco RI+Hind III fragments of λ DNA and a 1-kb pair ladder (Bethesda Research Laboratories, Gaithersburg, MD).

Table I. Nomenclature for Gap Junction Proteins

Reported gene products	References	New designation
Connexin 32; human, rat	Kumar and Gilula, 1986 Paul et al., 1986	β_1
Connexin 30; Xenopus	Gimlich et al., 1988; ("XE11a" cDNA)	β_1
Connexin 26; rat	Zhang and Nicholson, 1989	β_2
Connexin 43; rat	Beyer et al., 1987	α_1
Connexin 43; Xenopus	This report ("Ov7" cDNA)	α_1
Connexin 38; Xenopus	Ebihara et al., 1989; and this report ("Ov11" cDNA)	α_2

divergent protein, which we refer to as an "α_2" GJ protein. The Xenopus XE11a cDNA (Gimlich et al., 1988) predicts a protein classified with human and rat connexin 32 (Paul et al., 1986; Kumar and Gilula, 1986) as "β_1" GJ proteins. Finally, the 26-kD GJ protein described by Zhang and Nicholson (1988), which also falls into the β class, can be termed a "β_2" GJ protein.

Developmental Regulation of GJ mRNA Levels

The α_2 gap junction cDNA insert was excised from M13, radiolabeled, and used in hybridizations to filter-bound polyadenylated RNA from Xenopus ovaries and enzymatically isolated Stage IV-VI oocytes. A single band of 1.4 kb hybridized with this probe (Fig. 4, lanes *1* and *2*). In a hybridization with ovary RNA, the α_1 cDNA probe bound to multiple polyadenylated RNAs of lower abundance than the α_2 transcript. These results suggest that α_1 GJ poly (A)+ RNAs are heterogeneous with respect to transcriptional initiation sites or patterns of RNA processing. At least one of these transcripts was also present at very low abundance in heart polyadenylated RNA (Fig. 4, lanes *3* and *4*). By comparison, probing the same lane of heart RNA at reduced hybridization stringency with the connexin 43 cDNA probe (Beyer et al., 1987) revealed a single 1.7-kb transcript of apparently greater abundance than most of the α_1 RNAs (lane *5*). In addition, low stringency hybridization of this filter with a probe for Xenopus muscle actin (Fig. 4, lanes *6* and *7*; Sargent et al., 1986) revealed abundant intact muscle and cytoskeletal actin mRNAs.

Quantitative S1 nuclease protection analysis provided a detailed picture of the relative timing of accumulation of the putative α_2, β_1, and α_1 GJ mRNAs during early development (Fig. 5). Single-stranded antisense strand DNA probes were synthesized from M13 templates of the three cDNAs (Fig. 5 *a*), and hybridized in probe mass excess with complementary transcripts in total RNA from oocytes and embryonic stages. In such experiments, hybridization with relatively rare mRNAs proceeds essentially to completion (3–4 × $C_0t_{1/2}$), so the amount of S1 nuclease resistant probe provides a direct measure of the transcript abundance at each developmental stage. A probe complementary to 318 nucleotides (nt) at the 5' end of the α_2 mRNA (Fig. 5 *a*) produced four major S1 nuclease-resistant fragments, ranging from 270 to 318 nt, after hybridization with oocyte RNA (Fig. 5 *b*). These may result from multiple sites of transcriptional initiation, alternative RNA splicing events, or from limited degradation at the 5' ends of these RNA molecules. Tran-

scripts complementary to the α_2 cDNA probe were detected in early oocytes and maintained through late oogenesis and fertilization. Thereafter, their abundance decreased gradually through blastula and gastrula stages, and was negligible by the early neurula stage. In contrast, the β_1 mRNA, as shown previously (Gimlich et al., 1988), was undetectable prior to the midgastrula stage and accumulated to a moderate abundance by late neurula (Fig. 5 *c*). In a separate hybridization experiment its abundance was found to decrease somewhat during tailbud and early tadpole stages (Fig. 5 *e*).

The developmental profile of the α_1 GJ mRNA was more complex. The single-stranded α_1 cDNA probe used was complementary to sequences within the protein coding region (Fig. 5 *a*). Nevertheless, hybridization with oocyte RNA often produced a pair of S1-resistant fragments (Fig. 5 *d*). The smaller fragment of this pair was not detected consistently, even when the same RNA samples were assayed; its presence presumably reflected occasional intramolecular S1 nuclease digestion at an A:T-rich region of the RNA:DNA hybrid, a limited sequence polymorphism between the original cDNA source and the Xenopus population used in this laboratory, or the existance of a rarer, alternatively processed RNA. Probe protection was observed after hybridization with early and late stage oocyte RNAs, while fertilized egg and embryo RNAs lacked detectable α_1 transcripts. Gastrula poly(A)+ RNA also lacked α_1 mRNAs (data not
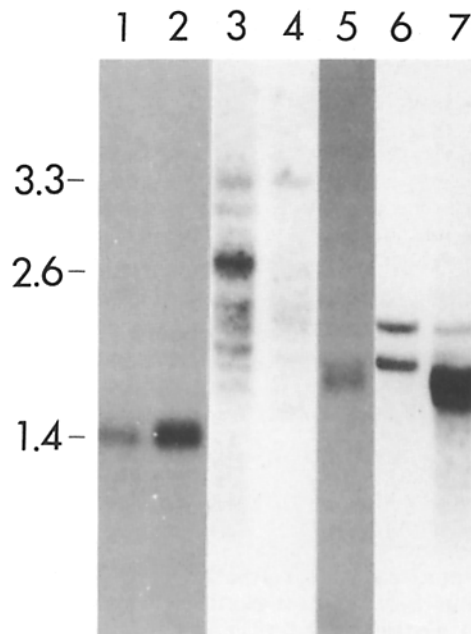
Figure 4. Transcripts complementary to *Xenopus* gap junction cDNA probes. Poly (A)+ RNA (10 μg per lane) from ovaries (lanes *1, 3,* and *5*), enzymatically isolated oocytes (lane *2*), or heart (lanes *4, 5,* and *7*) was electrophoresed, transferred to nylon, and probed with the α_2 cDNA (lanes *1* and *2*, 1 d exposure) or the α_1 cDNA probes (lanes *3* and *4*, 10 d exposure). The heart poly A+ RNA lane was probed at relaxed stringency with connexin 43 cDNA (lane *5*; 2 d exposure), revealing a predominant 1.7-kb hybridizing transcript. Ovary and heart RNA were also probed with a muscle actin cDNA at reduced stringency to verify the intactness of the preparations (lanes *6* and *7*; 8 h exposure). Transcript sizes were determined with reference to RNA markers (Bethesda Research Laboratories).
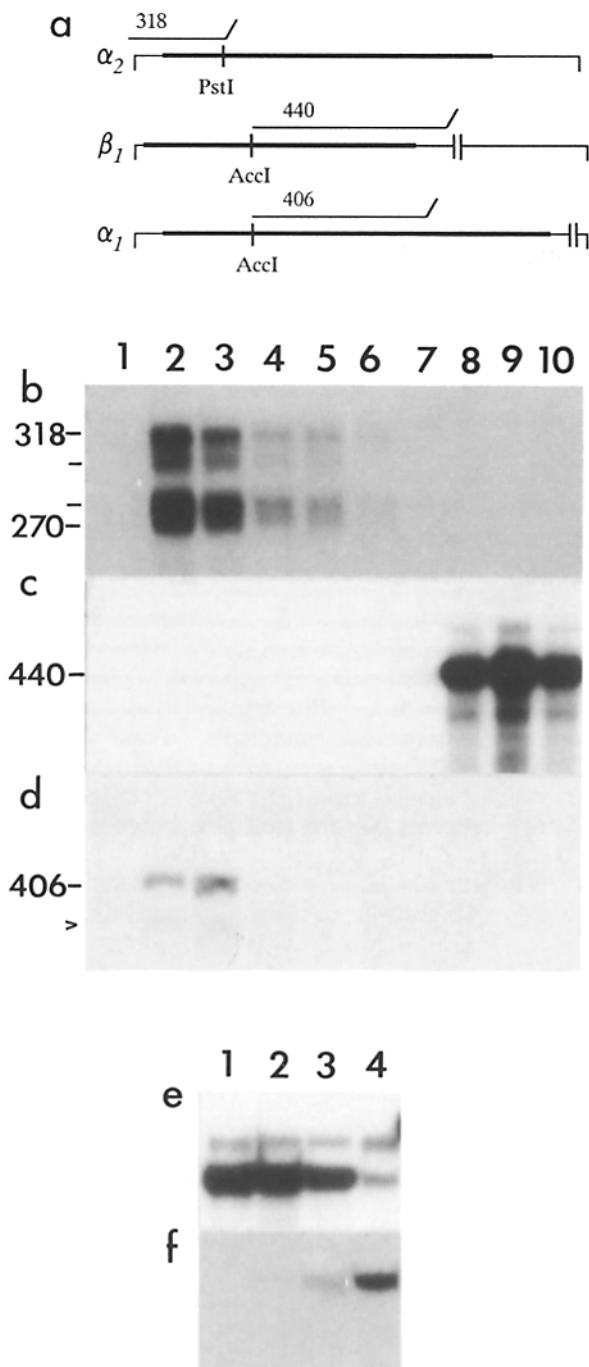
Figure 5. S1 nuclease protection assays of the developmental modulation of GJ mRNA levels. (a) Single-stranded probes produced on M13 templates were complementary to the indicated portions of each mRNA. The sequences of these probes are indicated in Fig. 1. (b–d) 25 μg of total RNA from each developmental stage was hybridized with (b) the α_2 GJ probe, (c) β_1 GJ probe, or (d) the α_1 GJ probe, and the products of S1 nuclease digestion electrophoresed on sequencing gels. Protected probe fragments were sized relative to radiolabeled Hinf I+Eco RI fragments of pGEM1 DNA. Lanes contain assays with yeast tRNA (lane 1), stage I-IV oocyte RNA (lane 2), stage IV-VI oocytes (lane 3), fertilized eggs (lane 4), stage 8 blastulae (lane 5), stage 10 gastrulae (lane 6), stage 13 neurulae (lane 7), stage 16 neurulae (lane 8), stage 20 neurulae (lane 9), and stage 25 early tailbud embryos (lane 10). (e) (f) In a separate experiment, 25 μg of total RNA from tadpole stages was assayed for (e) β_1, and (f) α_1 GJ mRNAs. Samples assayed were

shown). The limit of sensitivity of these assays was previously estimated to be ∼ <0.1 pg/g polyadenylated RNA (Gimlich et al., 1988), corresponding to <4,000 transcripts per oocyte or <0.4 α_1 RNA molecules per cell at gastrulation. The α_1 GJ mRNA began to accumulate late in embryonic development, during tailbud and tadpole stages (about stage 35; Fig. 5 f). Table II summarizes the patterns of accumulation and decay of α_1, α_2, and β_1 mRNA during embryogenesis.

Levels of α_1 and α_2 mRNAs Are Differentially Regulated at Oocyte Maturation

The α_1 and α_2 GJ mRNAs in Xenopus oocytes consistently showed different changes in abundance between late oogenesis and fertilization. To begin to study the mechanism of this differential turnover, full-grown oocytes were manually removed from their follicles after partial ovariectomy of two females, and matured in vitro with progesterone. The levels of α_1 and α_2 mRNA in samples of non-matured and matured oocytes and ovulated eggs were assayed using S1 nuclease protection as described above (Fig. 6). Levels of α_1 mRNA detected in manually defolliculated oocytes were comparable to those found in RNA from enzymatically defolliculated oocytes (data not shown). The α_1 mRNA declined at least 91% in abundance during a three hour period of incubation in progesterone (Fig. 6 a; lanes 4 and 5). Germinal vesicle breakdown had occurred in 50% of these oocytes by 2 1/2 h, and in 100% by 3 h. After partial ovariectomy, the frogs were injected with human chorionic gonadotropin to induce ovulation. Eggs stripped 9 h later contained no detectable α_1 mRNA (Fig. 6 a; lanes 6 and 7). In contrast, the level of the α_2 mRNA declined by only ∼46% during the 3-h progesterone incubation; this decline may have been in part an artifact of in vitro maturation conditions, as ovulated eggs from the same frogs had control levels of α_2 mRNA (Fig. 6 b). Control defolliculated oocytes maintained in culture for 3 h reproducibly showed only a 38% decrease in α_1 mRNA content (Fig. 6 a; lanes 8 and 9). Thus, the disappearance of α_1 mRNA during oocyte maturation in vitro was not exclusively attributable to adverse culture conditions or the possible shedding of contaminating follicle cells. In addition, the abundance of cytoplasmic γ actin mRNAs changed by no more than ∼14% as a result of oocyte maturation in vitro. Therefore, the events of oocyte maturation in vitro, as well as in vivo, resulted in differential effects on the levels of the two putative gap junction protein mRNAs.

Tissue-specific Abundance of α_2, β_1, and α_1 mRNAs in Adults

Total RNA was prepared from adult Xenopus tissues and organs and assayed for the levels of GJ mRNAs by S1 nuclease protection. The results are summarized in Table III, along

from stages 25 (lane 1), 35 (lane 2), 38 (lane 3), and 41 (lane 4). Arrowhead denotes position of smaller protected probe fragment seen in some assays for α_1 GJ mRNA. Star indicates a nuclease-resistant probe DNA fraction seen in this particular assay for β_1 mRNA.

Table II. Developmental Regulation of GJ mRNA Levels

mRNA	Stage I-IV oocyte	Stage IV-VI oocyte	Unfertilized egg	Blastula	Gastrula	Neurula	Tailbud	Feeding tadpole
α_2	+ + +	+ + +	+ + +	+ +	+/−	−	−	−
β_1	−	−	−	−	+/−	+ + +	+ + + +	+ + +
α_1	+	+	−	−	−	−	+/−	+ +

Relative abundances of mRNA in total RNA sampled at various developmental stages were determined by S1 nuclease protection assay as described (Fig. 5). Blastula RNA was isolated at stage 8, gastrula RNA at stage 10, neurula RNA at stage 20, tailbud embryo RNA at stage 25, and feeding tadpole at stage 45 (Nieuwkoop and Faber, 1975).

with the previously reported tissue distribution of the β_1 GJ mRNA (Gimlich et al., 1988). The three mRNAs were detected in overlapping sets of tissues; both the β_1 and α_1 transcripts were found in RNA from lung, liver, intestines, stomach and kidney.

The most striking result of this survey was that the α_2 transcript was detected only in the ovary in adults. As shown

in the Northern blot analysis of Fig. 4 a, when equivalent amounts of total ovary and full-grown oocyte poly (A)+ RNA were assayed, a higher abundance of α_2 GJ mRNA was found in oocytes. This suggested that the bulk of this transcript in the ovary is contained in the oocytes. Further analysis of the cell-type distribution of this mRNA by in situ hybridization in ovary tissue is underway.

Discussion

This is the first detailed study of the developmental regulation of the gap junction gene family. The three Xenopus cDNA sequences described here and previously (Gimlich et al., 1988; Ebihara et al., 1989) represent distinct mRNAs with very different patterns of accumulation and turnover during development and in the adult. Of the α_1 and α_2 GJ mRNAs, both present during oogenesis, only the α_2 transcript can be termed a maternal mRNA, because the α_1 RNAs disappear during oocyte maturation. The α_2 GJ mRNA, on the other hand, persists through maturation and fertilization, and is still detectable at early- to mid-gastrula stages of development. From S1 nuclease protection assays, we estimate that this mRNA is ~10-fold more abundant in the oocyte than the α_1 RNAs. The α_2 mRNA was detectable only in the ovary and oocytes of adults. Because its abundance in oocyte poly (A)+ RNA is apparently higher than
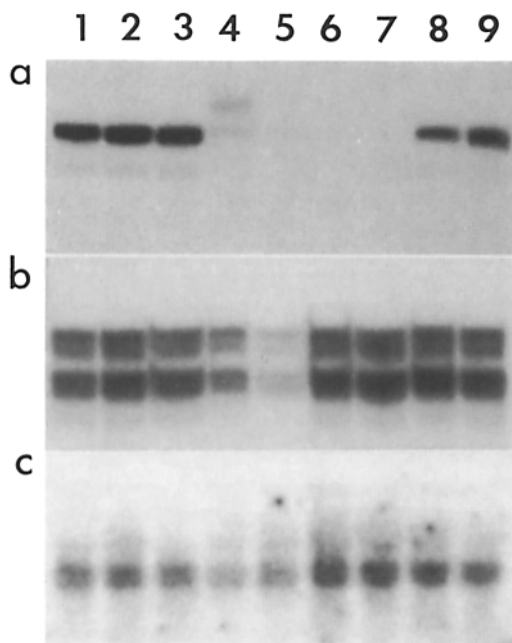
Figure 6. Modulation of α_1 and α_2 mRNA levels during oocyte maturation in vitro. Stage VI oocytes were dissected from their follicles and cultured as described in Materials and Methods, with or without progesterone at 5 μg/ml. Duplicate or triplicate samples were collected immediately after addition of progesterone (lanes 1-3), and at 3 h (100% germinal vesicle breakdown; lanes 4 and 5). Eggs stripped from these frogs 9 h later (lanes 6 and 7) were also assayed. Oocytes cultured in vitro for 3 h without progesterone were assayed (lanes 8 and 9) as a control for the effects of incubation conditions. These control oocytes did not show germinal vesicle breakdown. a shows the levels of α_1 GJ mRNA. The top band in lane 4 represents a small amount of residual full-length probe DNA. In b, the levels of α_2 GJ mRNA are shown. Lanes 1-3 and 6-9 represent transcript levels in RNA from nine oocytes per lane; RNA from seven oocytes per lane was assayed in lanes 4 and 5. c shows an equivalent fraction of each RNA sample assayed for γ-cytoskeletal actin mRNAs by Northern blot hybridization at low stringency with a Xenopus skeletal actin cDNA (Sargent et al., 1986). Changes in the abundance of specific transcripts were quantitated by densitometric scanning of the autoradiographs. Values cited in the text are comparisons of means ± SD of the areas under scan peaks.

Table III. Tissue Distribution of Three Xenopus GJ mRNAs

Organ	α_2 mRNA	β_1 mRNA	α_1 mRNA
Lung	−	+ +	+ +
Liver	−	+ + +	+
Intestines	−	+ + +	+ +
Stomach	−	+ + + +	+
Spleen	−	−	+
Body wall muscle	−	−	+
Laryngeal muscle	−	−	−
Thigh muscle	−	−	+
Heart	−	−	+
Kidney	−	+ +	+
Brain	−	−	+ + +
Ovary	+ + +	−	+ +
Oviduct	−	−	+
Testis	−	−	+
Bladder	−	−	ND

Single-stranded antisense strand cDNA probes (Fig. 5 a) were hybridized in solution with 20 μg of total RNA from each organ, at a concentration of 0.6-1.0 mg RNA/ml. Probe specific activity in each case was ~8 × 10⁸ cpm/μg, and at least 125 pg probe was used in each hybridization. After S1 nuclease digestion, protected probe fragments were electrophoresed on 6% polyacrylamide/urea gels, and the approximate relative abundances were determined by inspection of autoradiographs (represented as −, +, + +, + + +, and + + + +).

that in whole ovary poly (A)+ RNA, it is quite possible that this transcript is unique to the oocyte and early embryo in *Xenopus*.

As described previously, the β_1 GJ mRNA first accumulates during gastrulation, when the α_2 transcripts are quite rare, and becomes moderately abundant by tailbud stages. The overall abundance of this RNA declines by about the time of feeding (after stage 41), though it has been found to be localized by the tailbud stage in prospective endoderm, where it is stably expressed through adulthood (Gimlich et al., 1988).

The last of these three transcripts to accumulate in the embryo is the α_1 GJ mRNA. First detectable at ~2 d of development, it increases in overall abundance through at least stage 41, and is later expressed in a wide variety of adult organs, including muscle tissues, the male and female reproductive tract, and the brain. Like the β_1 mRNA, α_1 transcripts are also found in the mesonephric kidney and alimentary tract. This distribution is similar to that of the closely related connexin 43 protein (Beyer et al., 1989) and mRNA of the rat (Beyer et al., 1987), although that transcript was not detected in the brain or in organs of endodermal origin. However, the abundance of α_1 transcripts in *Xenopus* is generally quite low, and it is possible that connexin 43 mRNA is equally widely distributed, but not detectable in the total RNA Northern blots of Beyer et al. (1987) due to its rarity. Because the α_1 mRNA is so scarce in the *Xenopus* heart, we do not feel that it is likely to represent the only functional *Xenopus* homologue of rat connexin 43. In fact, connexin 43 cDNA (Beyer et al., 1987) hybridizes to a *Xenopus* heart mRNA smaller and apparently more abundant than that detected with the α_1 cDNA probe. The identity of this transcript is currently being investigated.

Polyadenylated ovary RNAs that hybridize to the α_1 GJ cDNA probe are heterogeneous in molecular weight. The largest transcripts are 3.3 kb, whereas the major transcript is 2.6 kb. It is not clear whether these RNAs are products of alternative patterns of splicing, degradation of a single larger RNA, or expression of distinct closely related genes. Analysis of the α_1 GJ gene is underway to address the question of transcript heterogeneity.

Although α_1 GJ mRNA is not detectable in the ovulated unfertilized egg, it is present at early stages of oogenesis, and maintains its abundance through the full-grown oocyte stage. Degradation of the α_1 mRNA at oocyte maturation is largely due to physiological changes in the oocyte in response to hormonal stimulation, since it occurs reproducibly in vitro as well as in vivo. This effect is specific for a small class of oocyte mRNAs (reviewed by Davidson, 1986) that includes the α_1 transcripts; neither γ-cytoskeletal actin mRNAs nor the α_2 GJ mRNA reproducibly declined in abundance between the end of oogenesis and the time of fertilization. Based on this pattern of turnover, we propose that the α_1 GJ gene product participates in hormone-regulated intrafollicular communication events during oogenesis and early in oocyte maturation. Such a function has been suggested for cumulus-oocyte gap junctional communication before ovulation in mammals (Gilula et al., 1978; Larsen et al., 1986). The regulated assembly of this gene product into new gap junction structures may account for the hormone-induced changes in follicular gap junction size and density observed

by Browne et al., (1979; for a discussion of oocyte-follicle communication in amphibia, see Wallace, 1983).

It is crucial to substantiate the claim that these three *Xenopus* gene products are in fact gap junction constituents. In part, the argument rests on the strength of their primary structure similarity to known gap junction channel-forming proteins. The close relationship of the *Xenopus* 30-kD mid-embryonic β_1 gene product to the mammalian channel-forming 32-kD gap junction protein (Young et al., 1987) has been detailed previously (Gimlich et al., 1988). The α_1 and α_2 gene products are closely related to the rat connexin 43 protein, which has also been reported to form intercellular conducting channels (Swenson et al., 1989).

Each of the deduced *Xenopus* proteins shares with the mammalian liver and cardiac gap junction proteins the potential to form a bundle of four helical hydrophobic domains, thought to traverse the plasma membrane (Milks et al., 1988). The primary sequences of each of these domains are highly conserved, as are their positions in each molecule (Fig. 2, *underlined regions*). Of particular interest is the third predicted transmembrane domain (e.g., between residues 153–183 of the *Xenopus* α_1 protein, as predicted by the method of Rao and Argos, 1986). In each putative GJ protein, this region contains an evenly spaced group of polar and charged residues, T---S---K/R---E (see Fig. 2, *asterisks*). Lysine (K) in the third position of this quartet is characteristic of the β class of GJ proteins, while arginine (R) in this position characterizes the α class. Milks et al. (1988) have argued that this helical domain contributes one polar face per junctional subunit to form a hydrated channel through the hexameric connexon. The strong conservation of this feature lends support both to arguments for its functional importance and to the claim that the *Xenopus* sequences encode gap junction proteins.

Other highly conserved features are the putative extracellular domains. Combined immunochemical, proteolytic, and ultrastructural studies (Zimmer et al., 1987; Milks et al., 1988; Goodenough et al., 1988) suggest that the sequences between transmembrane domains 1 and 2 and those between transmembrane domains 3 and 4 lie in the extracellular gap of the hepatocyte junction. Fig. 2 shows that these sequences are very similar in all the putative gap junction proteins. In particular, the spacing of cysteine residues in these domains is stereotypical; this may be important in their folding and in the ability of diverse cell types to form functional heterospecific gap junctions (Michalke and Loewenstein, 1971; Epstein and Gilula, 1977; see also Swenson et al., 1989).

Final verification of the gap junction channel–forming capability of the three *Xenopus* gene products can only be obtained using functional assays. The relative merits of several functional tests of the channel-forming ability of a cloned gene product have been well discussed by Beyer et al. (1987). Recently, a *Xenopus* α_2 cDNA clone (connexin 38) was independently isolated by Ebihara et al. (1989). Sp6 RNA (cRNA) transcribed from this template was capable of promoting an ionic conductance between two cRNA-injected *Xenopus* oocytes, suggesting that the α_2 gene product is indeed a gap junction subunit. No similar analysis has yet been attempted with the α_1 mRNA. We feel that the existence of at least two oocyte mRNAs which potentially encode distinct endogenous gap junction structures complicates interpreta-

tion of such experiments. The timing of translation of these mRNAs is currently being studied to determine whether they might contribute to endogenous conductances seen in the oocyte communication assay (Dahl et al., 1987). In the meantime, we feel that it will be constructive to characterize putative gap junction proteins using artificial lipid bilayers (Young et al., 1987) containing products of the cloned cDNAs produced in vitro or by DNA transformation of unicellular eukaryotes such as yeast, which are not expected to assemble their own gap junctions.

References

Berk, A. J., and P. A. Sharp. 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. *Cell.* 12:721-732.

Beyer, E. C., D. L. Paul, and D. A. Goodenough. 1987. Connexin 43: a protein from rat heart homologous to a gap junction protein from liver. *J. Cell Biol.* 105:2621-2629.

Beyer, E. C., J. Kistler, D. L. Paul, and D. A. Goodenough. 1989. Antisera directed against connexin 43 peptides react with a 43-kD protein localized to gap junctions in myocardium and other tissues. *J. Cell Biol.* 108:595-605.

Blackshaw, S. E., and A. E. Warner. 1976. Low resistance junctions between mesoderm cells during development of trunk muscles. *J. Physiol. (Lond.).* 255:209-230.

Blennerhassett, M. G., and S. Caveney. 1984. Separation of developmental compartments by a cell type with reduced junctional permeability. *Nature (Lond.).* 309:361-364.

Browne, C. L., S. H. Wiley, and J. N. Dumont. 1979. Oocyte-follicle cell gap junctions in *Xenopus laevis* and the effects of gonadotropin on their permeability. *Science (Wash. DC).* 203:182-183.

Caveney, S. 1985. The role of gap junctions in development. *Annu. Rev. Physiol.* 47:19-35.

Colman, A. 1984. Expression of exogenous DNA in Xenopus oocytes. *In* Transcription and Translation: A Practical Approach. B. D. Hames, and S. J. Higgins, editors. IRL Press, Oxford, UK. 49-70.

Dahl, G., T. Miller, D. Paul, R. Voellmy, and R. Werner. 1987. Expression of functional cell-cell channels from cloned rat liver gap junction complementary DNA. *Science (Wash. DC).* 236:1290-1293.

Davidson, E. H. 1986. Gene Activity in Early Development. Academic Press, New York.

de Laat, S. W., L. G. J. Tertoolen, A. W. C. Dorresteijn, and J. A. M. van den Biggelar. 1980. Intercellular communication patterns are involved in cell determination in early molluscan development. *Nature (Lond.).* 287:546-548.

Decker, R. S., and D. S. Friend. 1974. Assembly of gap junctions during amphibian neurulation. *J. Cell Biol.* 62:32-47.

Dumont, J. N. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 36:153-180.

Ebihara, L., E. C. Beyer, K. I. Swenson, D. L. Paul, and D. A. Goodenough. 1989. Cloning and expression of a *Xenopus* embryonic gap junction protein. *Science (Wash. DC).* 243:1194-1195.

Epstein, M. L., and N. B. Gilula. 1977. A study of communication specificity between cells in culture. *J. Cell Biol.* 75:769-787.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.

Fraser, S. E., and P. J. Bryant. 1985. Patterns of dye coupling in the imaginal wing disk of *Drosophila melanogaster. Nature (Lond.).* 317:533-536.

Fraser, S. E., C. R. Green, H. R. Bode, and N. B. Gilula. 1987. Selective disruption of gap junctional communication interferes with a patterning process in *Hydra. Science (Wash. DC).* 237:49-55.

Gilula, N. B., M. L. Epstein, and W. H. Beers. 1978. Cell-to-cell communication and ovulation: A study of the cumulus-oocyte complex. *J. Cell Biol.* 78:58-75.

Gimlich, R. L., N. M. Kumar, and N. B. Gilula. 1988. Sequence and developmental expression of mRNA coding for a gap junction protein in *Xenopus. J. Cell Biol.* 107:1065-1073.

Goodeneough, D. A., D. L. Paul, and L. A. Jesaitis. 1988. Topological distribution of two connexin 32 antigenic sites in intact and split rodent hepatocyte gap junctions. *J. Cell Biol.* 107:1817-1824.

Guthrie, S. C. 1984. Pattern of junctional communication in the early amphibian embryo. *Nature (Lond.).* 311:149-151.

Guthrie, S. C., and N. B. Gilula. 1989. Gap junctional communication and development. *Trends Neurosci.* 12:12-16.

Guthrie, S. C., L. Turin, and A. E. Warner. 1988. Patterns of junctional communication during development of the early amphibian embryo. *Development.* 103:769-783.

Ito, S., and Y. Ikematsu. 1980. Inter and intra-tissue communication during amphibian development. *Dev. Growth Differ.* 22:247-256.

Kumar, N. M., and N. B. Gilula. 1986. Cloning and characterization of human and rat liver cDNAs coding for a gap junction protein. *J. Cell Biol.* 103:767-776.

Larsen, W. J., S. E. Wert, and G. D. Brunner. 1986. A dramatic loss of cumulus cell gap junctions is correlated with germinal vesicle breakdown in rat oocytes. *Dev. Biol.* 113:517-521.

Lee, S., N. B. Gilula, and A. E. Warner. 1987. Gap junctional communication and compaction during preimplantation stages of mouse development. *Cell.* 51:851-860.

Michalke, W., and W. R. Loewenstein. 1971. Communication between cells of different types. *Nature (Lond.).* 232:121-122.

Milks, L. C., N. M. Kumar, R. Houghten, N. Unwin, and N. B. Gilula. 1988. Topology of the 32-kD liver gap junction protein determined by site-directed antibody localizations. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2967-2975.

Nieuwkoop, P. D., and J. Faber. 1975. Normal Table of *Xenopus laevis* (Daudin). 2nd ed. Elsevier/North Holland, Amsterdam.

Paul, D. L. 1986. Molecular cloning of cDNA for rat liver gap junction protein. *J. Cell Biol.* 103:123-134.

Rao, J. K. M., and P. Argos. 1986. A conformational preference parameter to predict helices in integral membrane proteins. *Biochim. Biophys. Acta.* 869:197-214.

Sargent, T. D., M. Jamrich, and I. B. Dawid. 1986. Cell interactions and the control of gene activity during early development of *Xenopus laevis. Dev. Biol.* 114:238-246.

Slack, C., and A. E. Warner. 1973. Intracellular and intercellular potentials in the early amphibian embryo. *J. Physiol. (Lond.).* 232:313-330.

Spray, D. C., A. L. Harris, and M. V. L. Bennett. 1979. Voltage dependence of junctional conductance in early amphibian embryos. *Science (Wash. DC).* 204:432-434.

Swenson, K. I., J. R. Jordan, E. C. Beyer, and D. L. Paul. 1989. Formation of gap junctions by expression of connexins in *Xenopus* oocyte pairs. *Cell.* 57:145-155.

Turin, L., and A. E. Warner. 1980. Intracellular pH in *Xenopus* embryos: its effect on current flow between blastomeres. *J. Physiol. (Lond.).* 300:489-504.

Wallace, R. A. 1983. Interactions between somatic cells and the growing oocyte of *Xenopus laevis. In* Current Problems in Germ Cell Differentiation. A. McLaren, and C. C. Wylie, editors. Cambridge University Press, Cambridge, UK. 285-306.

Warner, A. E., and P. A. Lawrence. 1982. Permeability of gap junctions at the segmental border in insect epidermis. *Cell.* 28:243-252.

Warner, A. E., S. C. Guthrie, and N. B. Gilula. 1984. Antibodies to gap-junctional protein selectively disrupt junctional communication in the early amphibian embryo. *Nature (Lond.).* 311:127-131.

Weir, M. P., and C. W. Lo. 1984. Gap-junctional communication compartments in the *Drosophila* wing imaginal disk. *Dev. Biol.* 102:130-146.

Young, J. D.-E., Z. A. Cohn, and N. B. Gilula. 1987. Functional assembly of gap junction conductance in lipid bilayers: demonstration that the major 27kD protein forms the junctional channel. *Cell.* 48:733-743.

Zimmer, P. B., C. R. Green, H. W. Evans, and N. B. Gilula. 1987. Topological analysis of the major protein in isolated intact rat liver gap junctions and gap junction-derived single membrane structures. *J. Biol. Chem.* 262:7751-7763.

Zhang, J.-T., and B. J. Nicholson. 1989. Sequence and tissue distribution of a second protein of hepatic gap junctions, Cx26, as deduced from its cDNA. *J. Cell Biol.* 109:3391-3402.