Development 104, 533–548 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

Expression of intermediate filament proteins during development of *Xenopus laevis*

III. Identification of mRNAs encoding cytokeratins typical of complex epithelia

BERNADETTE FOUQUET, HARALD HERRMANN, JÜRGEN K. FRANZ and WERNER W. FRANKE

Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany

Summary

A *Xenopus laevis* mRNA encoding a cytokeratin of the basic (type II) subfamily that is expressed in postgastrulation embryos was cDNA-cloned and sequenced. Comparison of the deduced amino acid sequence of this polypeptide (513 residues, calculated mol. wt 55 454; $M_r \sim 58\,000$ on SDS–PAGE) with those of other cytokeratins revealed its relationship to certain type II cytokeratins of the same and other species, but also remarkable differences. Using a subclone representing the 3′-untranslated portion of the 2·4 kb mRNA encoding this cytokeratin, designated XenCK55(5/6), in Northern blot experiments, we found that it differs from the only other *Xenopus* type II cytokeratin known, i.e. the simple epithelium-type component XenCK1(8), in that it is absent in unfertilized eggs and pregastrulation embryos. XenCK55(5/6) mRNA was first detected at gastrulation (stage 11) and found to rapidly increase during neurulation and further development. It was also identified in *Xenopus laevis* cultured kidney epithelial cells of the line A6 and in the adult animal where it is a major polypeptide in the oesophageal mucosa but absent in most other tissues examined. The pattern of XenCK55(5/6) expression during embryonic development was similar to that reported for the type I polypeptides of the 'XK81 subfamily' previously reported to be embryo-specific and absent in adult tissues. Therefore, we used a XK81 mRNA probe representing the 3′-untranslated region in Northern blots, S1 nuclease and hybrid-selection–translation assays and found the ~1·6 kb XK81 mRNA and the resulting protein of $M_r \sim 48\,000$ not only in postgastrula embryos and tadpoles but also in the oesophagus of adult animals. Our results show that both these type II and type I cytokeratins are synthesized only on gastrulation and are very actively produced in early development. However, their synthesis is not restricted to developmental stages but is continued in at least one epithelium of the adult organism. These observations raise doubts on the occurrence of *Xenopus* cytokeratins that are strictly specific for certain embryonic or larval stages and absent in the adult. They rather suggest that embryonically expressed cytokeratins are also produced in some adult tissues, although in a restricted pattern of tissue and cell type distribution.

Key words: *Xenopus laevis*, intermediate filament protein, mRNA, cytokeratin, amino acid sequence.

Introduction

In studies of the synthesis of intermediate filament (IF) proteins during human and murine development, it has been noted that the expression of the individual cytokeratin genes in the diverse epithelia is developmentally regulated, in position and in time, but that all cytokeratins found in embryonic or fetal tissues are also synthesized in one or several tissues of the adult animals. While both early embryonic epithelia, i.e. endoderm and embryonic ectoderm, contain only IFs formed by cytokeratins of the 'simple epithelium type', i.e. cytokeratins 8 and 18 and their equivalents in non-human species (Brûlet *et al.* 1980;

Jackson *et al.* 1980, 1981; Franke *et al.* 1982*a*,*c*; Lehtonen *et al.* 1983; Oshima *et al.* 1983; Regauer *et al.* 1985; Chisholm & Houliston, 1987), apparently also with low amounts of cytokeratin 19 (see Jackson *et al.* 1981), the formation of stratified, pseudostratified and complex glandular epithelia is accompanied by the synthesis of other members of the cytokeratin family (see Moll *et al.* 1982*a*,*b*; Dale *et al.* 1985; Regauer *et al.* 1985). In summary, all cytokeratins found in mammalian embryos and fetuses have also been detected in one of the epithelia of the adult, unlike expression patterns of other multigene families such as the globins in which specific members are synthesized only in certain embryonal stages (for review, see Collins & Weismann, 1984).

Studies of amphibian embryogenesis have suggested a more complicated situation. In oocytes and early embryos of the African clawed toad, *Xenopus laevis*, three cytokeratins have been identified that are equivalent to human cytokeratins 8, 18 and 19 and are also expressed in several organs of the adult animal such as liver and intestine (Franz *et al.* 1983; Franz & Franke, 1986; for localization see also Gall *et al.* 1983; Godsave *et al.* 1984; Wylie *et al.* 1986; Klymkowsky *et al.* 1987). Similarly, advanced larval stages, including tadpoles, express some cytokeratins that are also found in the epidermis of adult animals (Franz & Franke, 1986; Hoffmann & Franz, 1984; Hoffmann *et al.* 1985; Ellison *et al.* 1985). In contrast, Dawid and colleagues have described two different groups of cytokeratins, the 'XK81' subfamily comprising at least four different genes and the 'XK70' subfamily with two genes identified so far, which are expressed on gastrulation, continually synthesized during embryonal and larval development but have not been detected in significant amounts after metamorphosis (Dawid *et al.* 1985; Jonas *et al.* 1985; Winkles *et al.* 1985; Dawid & Sargent, 1986; Miyatani *et al.* 1986; Sargent *et al.* 1986; Jamrich *et al.* 1987). From their mRNA analyses using 'dot blot' techniques, these authors have concluded that the genes for cytokeratins of the XK81 and XK70 subfamilies belong to those expressed only during certain developmental stages.

Because of the fundamental importance of the concept of embryonic stage-specific IF proteins in relation to patterns and mechanisms of tissue formation and morphogenesis and, in view of the apparent variance in this respect between mammalian and amphibian embryogenesis, we have examined the expression of cytokeratin genes in *Xenopus* in greater detail. Therefore, we have cDNA-cloned certain cytokeratin mRNAs that are expressed at defined embryonic stages and examined their expression during subsequent development and in various adult tissues. In our analyses, we have also taken into account observations made in mammals that certain cytokeratins are synthesized only in one or a few cell types (Moll *et al.* 1982*a*; Tseng *et al.* 1982; Quinlan *et al.* 1985) so that the amounts of a given polypeptide produced in an animal or a given tissue may represent only a minuscule fraction of the total cytokeratins present. Here we describe a novel basic type II cytokeratin of *Xenopus laevis* expressed at high levels in early embryogenesis, and we show that this protein and cytokeratin XK81, the prototype of one of the embryo-specific cytokeratin groups described by Jonas *et al.* (1985), are synthesized in at least one tissue of the adult, i.e. the oesophagus.

Materials and methods

Animals and cells

Females of *Xenopus laevis* were kept as described (Krohne *et al.* 1981). After injection of human chorion gonadotropin, eggs were stripped and fertilized *in vitro*; embryos were incubated in 5 % DeBoers medium (Herrmann *et al.* 1989*a*) and staged according to Nieuwkoop & Faber (1967).

Various tissues from adult animals were obtained as described (Franz *et al.* 1983; Benavente *et al.* 1985; Franz, 1987; Herrmann *et al.* 1989*a*).

For enrichment of oesophageal epithelium, the oesophagus was removed, opened by a longitudinal incision, the mucosal tissue was scraped off and collected in phosphate-buffered saline (PBS).

Conditions for growing XLKE-A6 cell cultures have been given (for refs see Franke *et al.* 1979; Herrmann *et al.* 1989*a*).

Characterization of cytoskeletal proteins

Cytoskeletal proteins were prepared from various adult tissues and whole embryos and analysed by two-dimensional gel electrophoresis as described (Franz *et al.* 1983; Herrmann *et al.* 1989*a*). In addition, in several cases, immunoblotting using a monoclonal antibody of broad specificity and interspecies cross-reactivity ($K_s$pan 1-8.136; Achtstätter *et al.* 1986; Jahn *et al.* 1987), and cytokeratin-binding assays on nitrocellulose paper, using ^{125}I-labelled purified rat cytokeratins 8 and 18 (Hatzfeld *et al.* 1987) were performed.

Isolation of cDNA clones and DNA sequencing

A stage-17 *Xenopus* cDNA library in λgt10 (kindly provided by Dr D. A. Melton, Harvard University, Cambridge, MA, USA) was screened with a hamster vimentin DNA probe under conditions of reduced stringency (for details see the companion paper by Herrmann *et al.* 1989*a*). Two clones with significant cross-hybridization, although less intense than that obtained with the authentic *Xenopus* vimentin clones, were selected, sequenced and shown, by comparison with the XenCK1(8) sequence (Franz & Franke, 1986), to code for a basic (type II) cytokeratin. The complete sequence was determined according to Sanger *et al.* (1977). In addition, one strand was sequenced using the chemical modification method (Maxam & Gilbert, 1977).

RNA preparations

Total RNA was obtained from eggs, whole embryos, tadpoles and adult tissues as previously described (Magin *et al.* 1983). Alternatively, the method of Chirgwin *et al.* (1979) was used. In brief, the material was homogenized in 5 M-guanidinium thiocyanate (100 mM-Tris–HCl, pH 7·5, 10 mM-EDTA, 20 mM-dithiothreitol, 1 % Sarkosyl) with a Dounce homogenizer and RNA was pelleted by ultracentrifugation (180000*g* for 17 h) through a 6 M-CsCl gradient. The pelleted RNA was redissolved in 10 mM-Tris–HCl (pH 7·5, 0·1 mM-EDTA, 0·1 % SDS), extracted three times with phenol/chloroform, and finally precipitated in 0·3 M-sodium acetate, followed by addition of 2 vols of ethanol. Poly(A)$^+$RNA was prepared with Hybond mAP paper (Medac, Hamburg, FRG) as described by Werner *et al.* (1984).

RNA blot analysis

Total RNA or poly(A)$^+$RNA was analysed by electrophoresis on agarose gels after denaturation with glyoxal or on formaldehyde/agarose gels (Herrmann *et al.* 1989*a*), followed by RNA blot hybridization as described (Jorcano *et al.* 1984). For detection of specific mRNAs, the following ^{32}P-labelled probes were used: (*i*) an antisense RNA prepared from the cDNA clone pKXl1/8 (Franz & Franke, 1986) using the Bluescribe system (Stratagene, La Jolla, CA, USA) and [α-^{32}P]UTP; (*ii*) a random-primed probe of a 800 bp *Hin*dIII fragment at the 3′ end of the clone pXenCK55(5/6) using [α-^{32}P]dATP (for details see Results) and (*iii*) a synthetic 60-mer polynucleotide complementary to a large portion of the 3′ noncoding region (ATCCAACAGAATGCGAAATAAATGCACAAA-TAAGCAGAAATCTTCCCTGAGATCCTGAAG) of clone pC8128 (Jonas *et al.* 1985) encoding the *Xenopus* cytokeratin XK81 (this is the region in which mRNAs of different cytokeratins, even those of the same subfamily, do not reveal sequence homologies). This latter probe was end-labelled with [γ-^{32}P]ATP.

Hybrid selection–translation

In order to isolate mRNA coding for the XK81 polypeptide, the 60-mer polynucleotide of pC8128 was cloned into the Bluescribe vector (Stratagene, La Jolla, CA, USA). For the newly identified cytokeratin, XenCK55(5/6), a 3′ untranslated *Hin*dIII fragment of the sequenced clone (see Fig. 1) was subcloned into the Bluescript vector (Stratagene, La Jolla, USA), and this construct was used for hybrid selection. 300 μg of total RNA were hybridized to the immobilized probe at 37°C, washed with 2 × SSC, 0·2 % SDS at room temperature and with 0·1 × SSC, 0·2 % SDS (at 37°C for the polynucleotide and at 65°C for the cDNA clones). The selected mRNAs were used for translation *in vitro* in the reticulocyte lysate system and the products obtained were examined by gel electrophoresis (Jorcano *et al.* 1984).

S1 nuclease protection assay

RNA was also examined by the S1 nuclease protection assay (Berk & Sharp, 1977). 5 μg poly(A)$^+$RNA were hybridized with 6×10^4 cts min^{-1} 5′-labelled synthetic polynucleotide in 20 μl of formamide hybridization buffer (80 % formamide, 0·4 M-NaCl, 40 mM-Pipes buffer, pH 7·4, 1 mM-EDTA) at 36°C for 18 h. Digestion was performed by adding 250 μl S1 nuclease buffer (250 mM-NaCl, 30 mM-sodium acetate, 5 mM-$ZnCl_2$, 400 i.u. ml^{-1} S1 nuclease (Sigma, St Louis, MO, USA)) and protected nucleotides were recovered by ethanol precipitation with 20 μg yeast tRNA added as carrier. Pellets were taken up in 3 μl formamide sample buffer and radioactivity was determined by Cerenkov counting. 1 μl of each sample was analysed on 8 % sequencing gels. To control stability of the probe, samples were processed in parallel except that the S1 nuclease was omitted from the S1 nuclease buffer. After determination of radioactivity the samples were taken up in formamide sample buffer to 300 cts min^{-1} μl^{-1}.

Microscopy

Cryostat sections through snap-frozen tissues or embryos were processed for immunofluorescence microscopy (Jahn *et al.* 1987) using monoclonal murine antibodies to cytokeratins such as KL1 (Viac *et al.* 1983), lu-5 (Franke *et al.* 1987), $K_s$pan1-8.136 (Jahn *et al.* 1987) or the vimentin antibody VIM-3B4 (Herrmann *et al.* 1989*a*,*b*). Alternatively, we used guinea pig antibodies to cytokeratins or vimentin (Franke *et al.* 1979).

For electron microscopy, small pieces of tissue were fixed with sodium-cacodylate-buffered 2·5 % glutaraldehyde and processed as described (Franke *et al.* 1976).

Results

Isolation of a cDNA clone for a new type II cytokeratin

In the course of screening an embryonic stage-17 *Xenopus* cDNA library for *Xenopus* vimentin cDNA clones, we noted two clones that hybridized less intensely to the hamster vimentin cDNA probe used originally and to the identified *Xenopus* vimentin and desmin cDNAs (for details see Herrmann *et al.* 1989*a*,*b*) and had restriction maps completely different from those of *Xenopus* vimentin and desmin. In hybrid selection experiments, mRNA was enriched, which directed the synthesis of a polypeptide that, in SDS–PAGE, showed a mobility slightly lower than that of hamster vimentin (data not shown) and which, on two-dimensional gel electrophoresis, appeared as a pair of polypeptide spots slightly more basic than the known simple epithelial equivalent to human cytokeratin 8, i.e. component XenCK1(8) (Franz & Franke, 1986). However, the *Pst*I restriction map of these clones was different from that of XenCK1(8). Sequencing of the four *Pst*I fragments obtained then showed, besides a considerable sequence similarity to XenCK1(8), that both clones coded for the same polypeptide of the basic (type II) cytokeratin subfamily different from all known cytokeratins.

Nucleotide sequence and deduced amino acid sequence

Fig. 1 shows the nucleotide sequence of the cloned mRNA and the amino acid sequence deduced therefrom. The sequence of 2329 nucleotides contains the polyadenylation signal of the corresponding mRNA but the 5′-untranslated region contains only 14 nucleotides. From comparison with the mRNA size, estimated in Northern blots to be 2·4 kb (see below), we suspect that approximately 200 nucleotides of the mRNA are not represented in this clone, and we assume that the first methionine codon, which is preceded at −3 with A and followed in +4 with G, thus meeting the requirement for a ribosome initiation site (Kozak, 1986), defines the translation start. The sequence TCACT preceding the start codon is not an 'ideal' consensus sequence CCACC, but it conforms to the more general scheme of PyCACPy that seems to provide a sufficient environment for an initiation site in many other mRNAs (Kozak, 1986).

The corresponding amino acid sequence represents a polypeptide of 513 amino acids, including the initial methionine which is probably lost in the mature protein, corresponding to a total molecular weight (mol. wt.) of 55 454 and an estimated pI of 6·0. These data have to be compared to the 502 amino acids, mol. wt. 55 688, and pI 5·8 determined for the only other *Xenopus* type II cytokeratin sequenced, i.e. the simple epithelial protein XenCK1(8) as described by Franz & Franke (1986).

In Fig. 2, the deduced amino acid sequence is compared with those of some other type II cytokeratins selected because of certain similarities. Since the corresponding *in vitro* translation product migrates, on SDS–PAGE, with an apparent M_r of 58 000 and contains some sequence features characteristic of human cytokeratins 5 and 6 (see below), we desig-

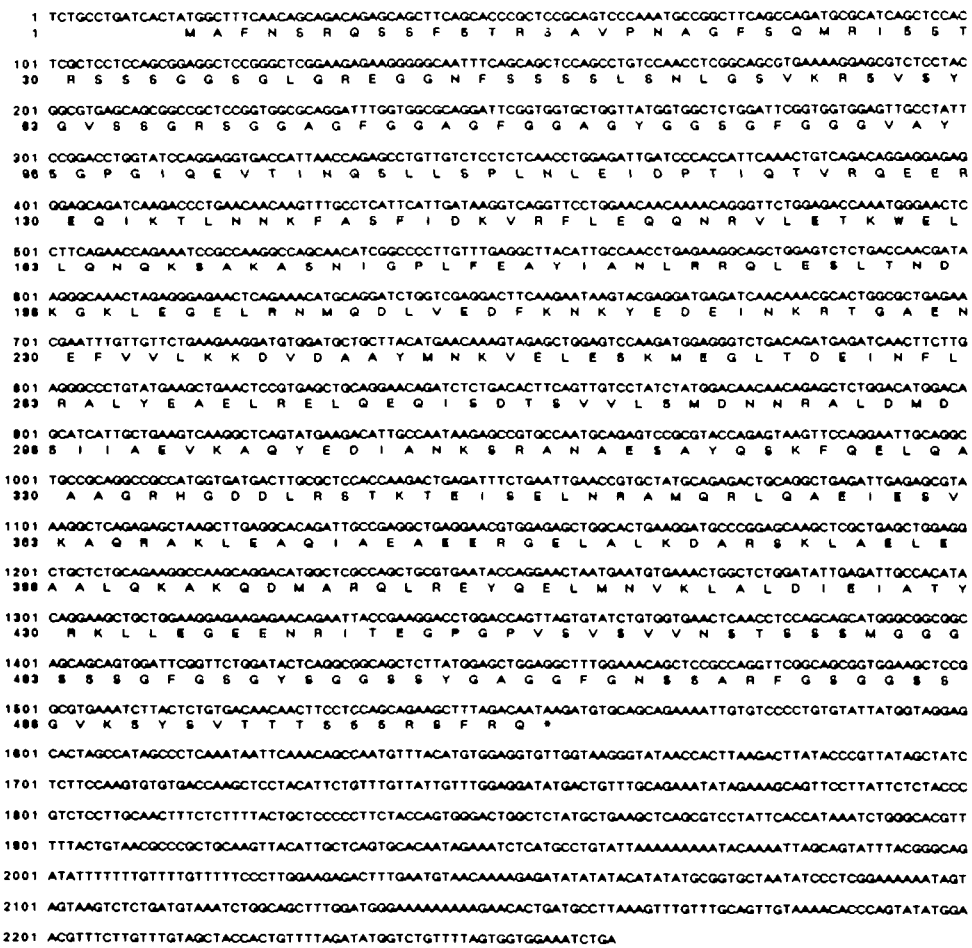

Fig. 1. Nucleotide sequence and deduced amino acid sequence of *Xenopus laevis* cDNA clone pXenCK55(5/6) encoding a type II cytokeratin. Asterisk denotes stop codon. The poly(A) region and the portion of the 5′-untranslated region of the mRNA are not represented in the clones.

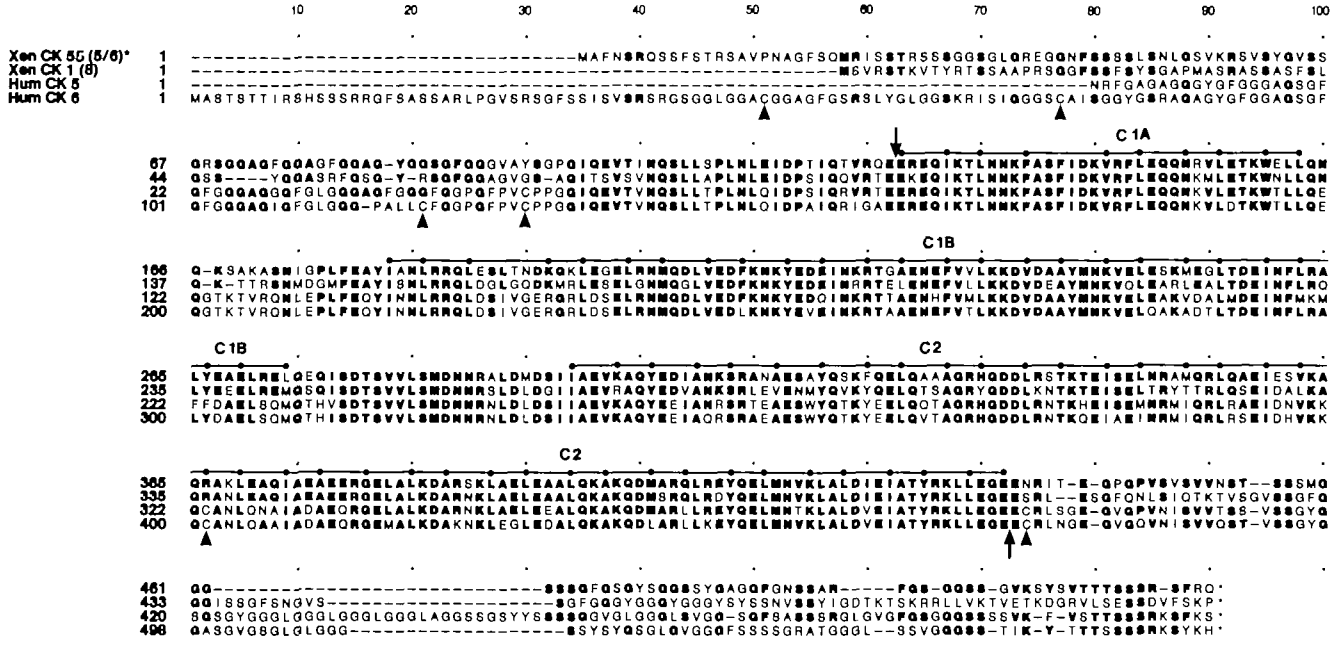

Fig. 2. Amino acid comparison of *Xenopus* cytokeratin Xen55(5/6), *Xenopus* cytokeratin XenCK1(8) (taken from Franz & Franke, 1986), the partial sequence of human cytokeratin K5 (from Lersch & Fuchs, 1988) and human cytokeratin K6 (from Tyner *et al.* 1985). The asterisk in the XenCK55(5/6) sequence indicates the problem of designation in relation to the human cytokeratin catalogue of Moll *et al.* (1982*a*). This protein migrates with an apparent M_r of 58000 on SDS–PAGE and shares sequence features in the head and tail region with human cytokeratins K5 and K6.

Sequences are aligned to obtain maximal homology, deletions introduced for this purpose are denoted by horizontal bars. Bold-faced letters denote amino acids identical in *Xenopus* cytokeratin XenCK55(5/6) and at least one of the other basic cytokeratins. The downward arrow indicates the start and the upward arrow the end of the α-helical rod. The lines and dots above the sequence blocks indicate the extent of the coiled-coil subdomains C1A, C1b and C2 of the rod domain. The dots represent positions *a* and *d* of the heptade convention to maximize coiled-coil configuration. Note the absence of cysteines in both *Xenopus* cytokeratins, whereas 6 are present in human cytokeratin 6 and 3 in the partial sequence of human cytokeratin 5 (indicated by arrowheads).

nated this polypeptide XenCK55(5/6). In its central α-helical rod region, it is highly homologous to other type II cytokeratins such as *Xenopus* component cytokeratin 1(8) and human cytokeratins 5 and 6 (Hanukoglu & Fuchs, 1983; Tyner *et al.* 1985; Lersch & Fuchs, 1988), except for the short first 'spacer' region between coils 1A and 1B. Altogether, the degree of amino acid homology of XenCK55(5/6) in the rod domain is 79% with the *Xenopus* protein XenCK1(8) and approximately 75% with both human cytokeratins 5 and 6. Shortly after the start of the α-helical rod, XenCK55(5/6) shows the FASFI motif characteristic for type II cytokeratins, which is replaced by LASYL in the type I cytokeratins.

Both the head and the tail domains are considerably shorter than in human cytokeratins 5 and 6. Especially, the repeated GGGX tetrapeptides found in the head and tail domains of many other type II cytokeratins, though not in all (see the tails of human and bovine cytokeratins 4, 7 and 8; Glass *et al.* 1985; Magin *et al.* 1986; Leube *et al.* 1986, 1988; Sémat *et al.* 1988), do not exist in XenCK55(5/6). In the head portion, however, $GG^{S}_{A}G^{F}_{Y}$ motifs occur in tandem, similar to those in bovine epidermal cytokeratin III (M. Blessing & W. W. Franke, unpublished data), the murine equivalent to cytokeratin 4 (Knapp *et al.* 1986) and XenCKIII (Hoffmann *et al.* 1985). Both the head and tail domain are very rich in hydroxyamino acids, most strikingly in the tail (32 out of a total of 77 residues), notably its end (12 out of the terminal 20 residues are hydroxyamino acids). Interestingly, neither XenCK55(5/6) nor XenCK1(8) contain any cysteine residue, compared to six in human cytokeratin 6 and at least three in human cytokeratin 5.

Remarkably, in the otherwise rather diverged sequences of the head and tail domains, the *Xenopus* type II cytokeratin XenCK55(5/6) also shows some sequence similarities with certain epidermal cytokeratins of other species. Three amino acids after the initial methionine a decapeptide sequence (SRQSSFSTRS) occurs which is very similar to the aminoterminus of bovine cytokeratin III (SRQSTVSFRS; see M. Blessing & W. W. Franke, unpublished data). At the carboxyterminus,

XenCK55(5/6) contains several motifs that are also present in human cytokeratins 5 and 6 (Fig. 2), and partly also in human cytokeratin I and bovine epidermal cytokeratins III and IV, but are absent in several other type II cytokeratins (Fig. 3). This and the pattern of its expression in adult animals suggest that XenCK55(5/6) is related to human cytokeratins 5 and 6.

Identification of polypeptide XenCK55(5/6)

Because of the high degree of homology between XenCK55(5/6) and XenCK1(8) in large parts of their mRNAs, it was necessary to use, in mRNA hybrid selection experiments, a subclone representing a sequence-divergent portion of the 3′-untranslated region probe. With this specific probe we selected mRNA coding for a polypeptide that, on two-dimensional gel electrophoresis, migrated at a position corresponding to $M_r \sim 58\,000$ and a pH almost identical to that of bovine serum albumin (Fig. 4A–C). In such translation experiments, the same polypeptide was obtained with mRNA from embryonic stage 18 (Fig. 4B) and from adult oesophageal mucosa (Fig. 4C).

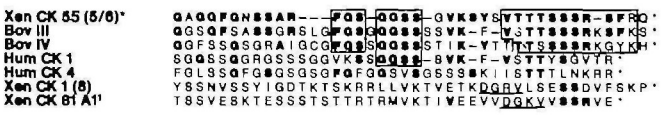

Fig. 3. Amino acid sequence comparison of the carboxyterminal region of *Xenopus* cytokeratin XenCK55(5/6) with that of various other cytokeratins of the same (type II) subfamily. *Bov*III and *Bov*IV are the respective bovine analogues to human cytokeratins 5 and 6. Human cytokeratins 1 (Johnson *et al.* 1985) and 4 (Leube *et al.* 1988) as well as the *Xenopus* type II cytokeratins 1(8) (Franz & Franke, 1986) and the type I cytokeratin XK81, here named HXenCK81A1[1] (Jonas *et al.* 1985) are shown for comparison. Sequences are aligned to achieve maximal homology for cytokeratins XenCK55(5/6) with *Bov*III and *Bov*IV. Boxes denote extensive sequence homology. Conservative exchanges have been included into the boxed area. The consensus sequence DGRKV found in certain type I and type II cytokeratins as well as in several nonkeratinous IF proteins has been underlined.

Differential expression of the type II cytokeratins XenCK1(8) and XenCK55(5/6) during embryogenesis and in adult animals

When we examined, by Northern blot analysis, the expression of different cytokeratins in unfertilized eggs and in various stages of development, we observed drastically different patterns of mRNA syn-

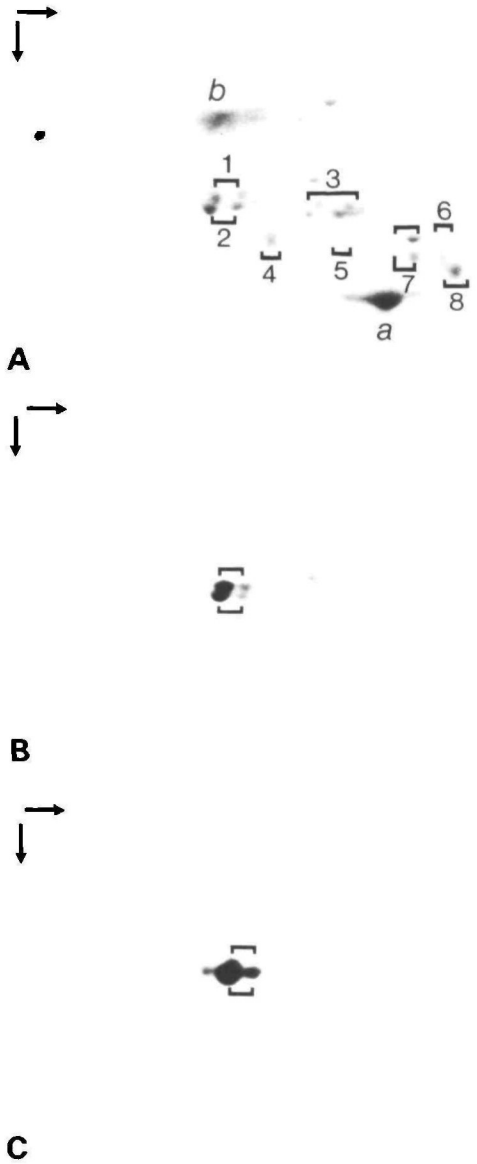

Fig. 4. Identification of the polypeptide encoded by the XenCK55(5/6) mRNA, using *in vitro* translation of mRNA selected by hybridization to the cDNA clone pXenCK55(5/6), followed by two-dimensional co-electrophoresis of the radioactively labelled translation product with cytoskeletal proteins of oesophageal mucosa from adult *Xenopus* (first dimension: isoelectric focusing, direction denoted by horizontal arrow; second dimension: SDS–PAGE, vertical arrow). (A) Coomassie-blue-stained gel, major cytoskeletal proteins of oesophageal mucosa are denoted by brackets. Cytokeratins are numbered, the bracket without a number denotes an as yet unidentified cytoskeletal protein. a, actin; b, bovine serum albumin. (B) Autoradiograph of the gel shown in A, showing that the ^{35}S-methionine-labelled products obtained after translation *in vitro* of the hybrid-selected embryonal stage-18 mRNA comigrate with the unlabelled oesophageal cytokeratin polypeptides 1 and 2. (C) Autoradiograph of a gel in parallel, showing the ^{35}S-methionine-labelled product obtained after translation *in vitro* of the hybrid-selected mRNA from total RNA of oesophageal mucosa of the adult animal which comigrates with the oesophageal polypeptides 1 and 2.

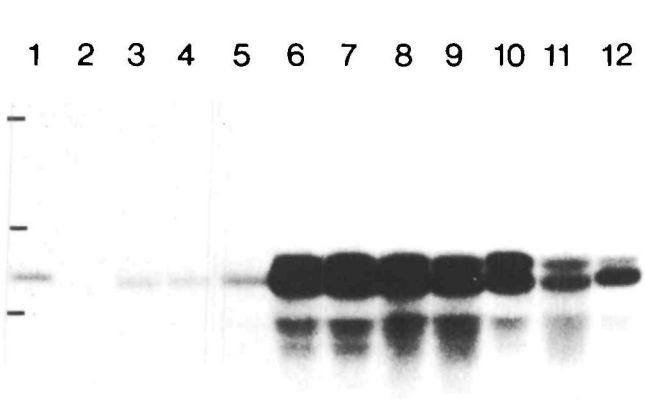

Fig. 5. Detection of mRNA encoding cytokeratin XenCK1(8) in various stages of development of *Xenopus laevis* by Northern blot hybridization. Lane 1, unfertilized eggs (5 μg poly(A)$^+$RNA); lane2, not loaded to control background; lane 3, stage 6·5 (morula); lane 4, stage 9 (fine cell blastula); lane 5, stage 11 (gastrula); lane 6, stage 14 (neural plate stage); lane 7, stage 18 (neural groove stage); lane 8, stage 28; lane 9, stage 34; lane 10, stage 36; lane 11, stage 39; lane 12, stage 42 (swimming tadpole). 50 μg of total RNA were loaded, if not indicated otherwise. RNAs were hybridized with an antisense RNA probe synthesized from clone pKXL1/8 (Franz & Franke, 1986). Note reaction with an ~2·2 kb RNA in unfertilized eggs and all developmental stages, with an abrupt increase at stage 14 (lane 6). Horizontal bars indicate positions of rRNAs run for reference in the same gel as size markers (from top to bottom: bovine 28S, *E. coli* 23S and bovine 18S rRNAs).

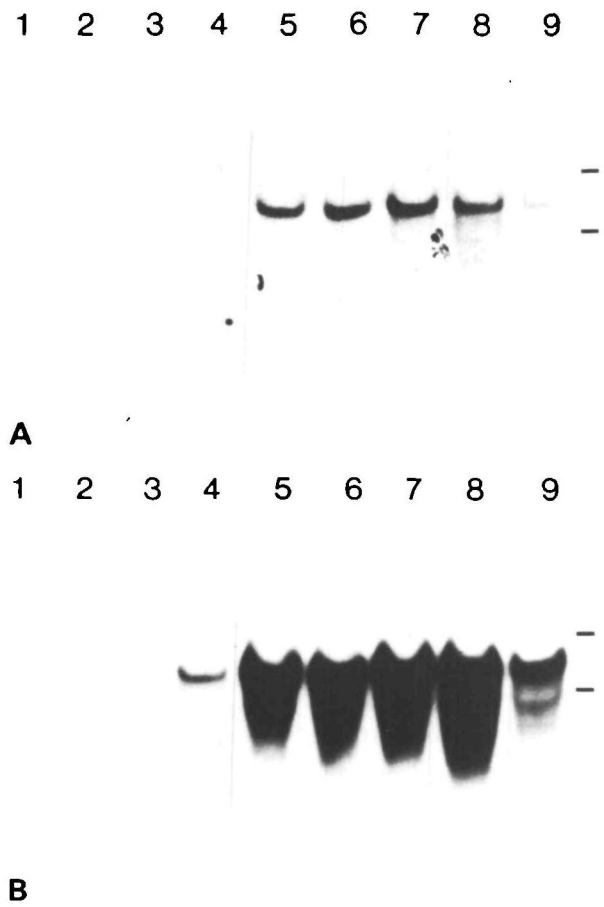

Fig. 6. Detection of mRNA encoding cytokeratin XenCK55(5/6) in various stages of development of *Xenopus laevis* by Northern blot hybridization. Lane 1, unfertilized eggs; lane 2, stage 6·5 (morula); lane 3, stage 9 (fine cell blastula); lane 4, stage 11 (gastrula); lane 5, stage 14 (neural plate stage); lane 6, stage 18 (neural groove stage); lane 7, stage 28; lane 8, stage 39; lane 9, stage 42 (swimming tadpole). 20 μg of total RNA were loaded on a formaldehyde/agarose gel. RNAs were hybridized with a random-primed, ^{32}P-labelled, 3′-specific probe synthesized from clone pXenCK55(5/6). Note reaction with a ~2·4 kb RNA (lanes 5 to 8). The mRNA is first detected in gastrulae (stage 11, lane 4), as revealed after prolonged exposure and drastically increases in neurulae. There is no specific mRNA detectable in unfertilized eggs and pregastrulation stage (lanes 1–3 RNA loading and blot was controlled by photography).

thesis and accumulation. Fig. 5 presents the mRNA contents of cytokeratin XenCK1(8), i.e. the equivalent to human cytokeratin 8, which is already present in unfertilized eggs and early blastulae, although in relatively low concentrations, and shows an increase at neurulation (e.g. stage 14; Fig. 5, lane 6). Because we had previously shown that this mRNA also occurs in adult tissues such as liver and intestine (Franz & Franke, 1986; Franz, 1987), we used this probe as a general positive cytokeratin expression control in our studies.

The expression of XenCK55(5/6) during embryogenesis, however, showed a different situation (Fig. 6A,B). Unfertilized eggs were completely negative (see Fig. 6B for an autoradiograph after prolonged exposure), and the first positive signal was seen at gastrulation (lane 4, stage 11), followed by a strong increase at neurulation (lane 5, stage 14). Thus, the time course of expression of the type II cytokeratin XenCK55(5/6) appeared to be similar to that of the type I cytokeratins XK70 and XK81 (Dawid *et al.* 1985; Jonas *et al.* 1985; Winkles *et al.* 1985; Dawid & Sargent, 1986; Miyatani *et al.* 1986).

When the expression of cytokeratin XenCK55(5/6) in adult animals was examined using RNA from various tissues, only oesophageal mucosa was positive whereas ovary, liver, skeletal and cardiac muscle were negative (Fig. 7). Cultured XLKE-A6 cells were also positive but gave a much weaker signal (Fig. 7,

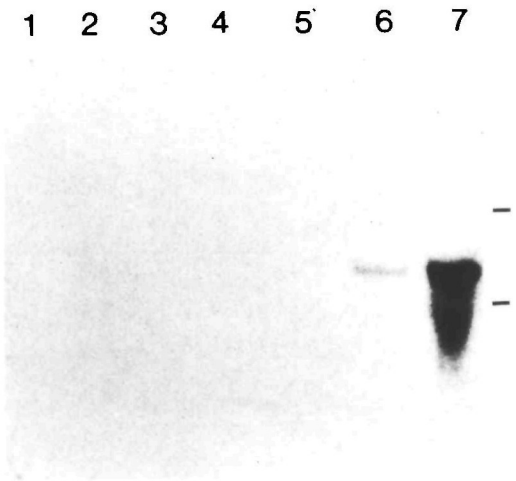

Fig. 7. Detection of mRNA encoding cytokeratin XenCK55(5/6) in different tissues of adult *Xenopus laevis*. Lane 1, ovary; lane 2, liver; lane 3, cardiac muscle; lane 4, skeletal muscle; lane 5, skin; lane 6, XLKE-A6 cell cultures; lane 7, oesophagus. 20 μg total RNA (lanes 3, 4 and 7) or 1 μg poly(A)$^+$RNA (lanes 1, 2, 5 and 6) were loaded on a formaldehyde/agarose gel. RNAs were hybridized with a random-primed, ^{32}P-labelled fragment of clone pXenCK55(5/6) representing the 3'-untranslated region of the mRNA. Bars indicate positions of 28S and 18S rRNA of *Xenopus laevis*. RNA of negative samples (lanes 1–4) has been controlled by reaction with other cDNA probes such as pXenVim1, pXenDes1, pXL1/8 (Franz & Franke, 1986; Herrmann *et al.* 1988*a*,*b*).

lane 6), and an even weaker signal was obtained with RNA from skin on prolonged exposure.

Expression of cytokeratin XK81

Because the pattern of expression of the type II cytokeratin XenCK55(5/6) during development resembled that described for the type I cytokeratin XK81 (Jonas *et al.* 1985; Dawid & Sargent, 1986; Miyatani *et al.* 1986), we used a cloned synthetic polynucleotide as a probe for XK81 mRNA in Northern blot experiments. The results obtained confirmed those of Jonas *et al.* (1985; Miyatani *et al.* 1986) in that this ~1·6 kb mRNA was detected at gastrulation and in postgastrulation stages, including tadpoles (Fig. 8A), but was absent in adult epidermis. However, in contrast to Dawid and colleagues (Jonas *et al.* 1985; Winkles *et al.* 1985; Dawid & Sargent, 1986; cf. Sargent *et al.* 1986) we observed a positive, albeit relatively weak, reaction in an adult tissue, i.e. oesophageal mucosa (Fig. 8A, lane 5). Other internal tissues such as liver (data not shown) and muscle, as well as cultured epithelial cells of line XLKE-A6, were negative (Fig. 8A).

Because of the importance of the identification of a cytokeratin previously believed to be embryo-specific, we used a sensitive S1 nuclease protection assay to further characterize the mRNA detected in oesophageal epithelium. As shown in Fig. 8B, XK81 mRNA was not detected in embryonal stages prior to stage 9 but was already abundant in the stage 18 (neural groove stage; lane 6). It was also found in tadpoles (Fig. 8B, lane 7) and in lower, but significant, concentrations in the oesophageal epithelium of adult animals (Fig. 8B, lane 8). It was not detected in adult epidermis (lane 9), XLKE-A6 cells (lane 10), ovarian tissue including follicular epithelium (lane 12), or in skeletal muscle tissue (lane 11).

Microscopy

The finding that both the type II cytokeratin XenCK55(5/6) and the type I cytokeratin XK81 mRNA are selectively expressed in the oesophageal epithelium of the adult animal stimulated our interest in the morphology and the cytoskeletal composition of this tissue. Immunofluorescence microscopy of frozen sections of the oesophagus of adult toads showed that the mucosal epithelium of this organ is a complex ('pseudostratified') epithelium of mostly columnar cells which are rich in IFs of the cytokeratin type (Fig. 9) but are negative for vimentin and desmin (data not shown; see also Jahn *et al.* 1987).

Detailed light and electron microscopy (Fouquet, 1987) revealed a remarkable cell-type complexity of this epithelium which histologically differs considerably from the organization of mammalian oesophageal epithelium (Bronn & Hoffmann, 1878). Four major cell types were readily distinguished.

(i) The most abundant cells are the fundamental columnar epithelial cells which are rich in cytokeratin IFs, desmosomes and mitochondria, form numerous intercellular bridges with desmosomes and attached IF bundles (tonofibrils) resembling spinous cell layers of epidermis, as well as 'hemidesmosomes'. In these cells, the cytokeratin is not exclusively arranged into regular IF bundles but is also found in cytoplasmic aggregates of thinner filaments, which are reminiscent of the spheroidal aggregates transiently formed during mitosis in diverse cell cultures (Franke *et al.* 1982*b*; Lane *et al.* 1982) and in certain normal and tumorous tissues (e.g. Brown *et al.* 1983; Geiger *et al.* 1984). Such spheroidal aggregates of cytokeratin material seem to be more common in amphibian tissues as they have been reported for larval epidermis ('figures of Eberth'; Fox, 1986; Fox & Whitear, 1986) and for endothelia (Jahn *et al.* 1987; Fouquet, 1987).

(ii) Mucous cells containing subapical aggregates of secretory vesicles are occasionally met in situations suggestive of apical discharge.

(iii) Small, dark staining, basally located, cells,

resembling the 'reserve cells' of diverse complex mammalian epithelia are seen.

(iv) Neuroendocrine cells, which are characterized by 'dense-core' as well as 'empty-looking' neurotransmitter vesicles, are less frequent and mostly located in basal positions.

Identification of cytokeratins XenCK55(5/6) and XK81 in oesophageal cells

On two-dimensional gel electrophoresis of cytoskeletal proteins from oesophageal mucosa of adult animals, nine major polypeptides were resolved (Fig. 10A), eight of which were positively identified as cytokeratins by immunoblotting (Fig. 10B) and complementary cytokeratin binding *in vitro* (Fig. 10C). Of these eight oesophageal cytokeratins, four (nos 1–4) showed reactions typical of type II cytokeratins (not shown) whereas the other four reacted in a mode typical of type I cytokeratins (Fig. 10C).

In order to identify unequivocally the oesophageal cytokeratin polypeptides encoded by the XenCK55(5/6) and XK81 mRNAs, we performed hybridization-selection experiments, followed by *in vitro* translation of the selected mRNAs. The polypeptides obtained after translation of the mRNA selected by the specific 3′-untranslated region of clone pXenCK55(5/6) comigrated with the polypeptides numbered 1 and 2 in the cytoskeletal preparation of oesophageal mucosa (compare Fig. 4B,C). At present we cannot decide whether (i) the oesophageal cells contain only cytokeratin 1, i.e. cytokeratin XenCK55(5/6), as the only genuine product whereas component 2 is a secondary degradation or modification, or (ii) whether these results reflect the presence of two very similar, but not identical, cytokeratins (for 'microheterogeneity' or possible allelic differences of cytokeratins see Hoffmann *et al.* 1985; Tyner & Fuchs, 1986; Franz, 1987).

The mRNA selected by the XK81 probe, i.e. the synthetic 60-mer polynucleotide cloned into Blue-

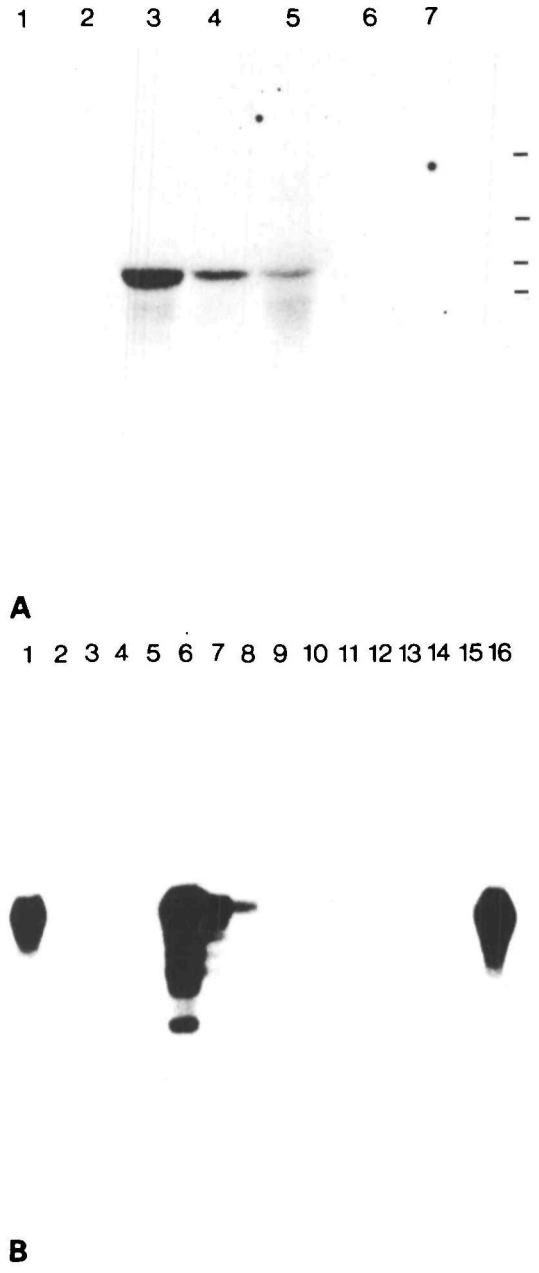

Fig. 8. Identification of cytokeratin XenCK81[1] mRNA in developmental stages and adult tissues of *Xenopus laevis*. (A) RNA blot analysis (Northern blot) of gel electrophoretically separated total RNA (50 μg each) from different tissues and embryonic stages: Lane 1, oocytes; lane 2, unfertilized eggs; lane 3, stage 18 (neural groove stage); lane 4, stage 42 (swimming tadpole); lane 5, adult oesophageal mucosa; lane 6, adult skeletal muscle; lane 7, cultured kidney epithelial cells of line A6. RNAs were hybridized with radioactively labelled, XenCK81-specific 60-mer polynucleotide (see Materials and Methods). There is a reaction with an approximately 1·6 kb RNA in stages 18 and 42 (lanes 3 and 4) and in the oesophageal epithelium (lane 5). Horizontal bars indicate positions of rRNAs co-electrophoresed for reference (from top to bottom: bovine 28S, *E. coli* 23S, bovine 18S and *E. coli* 16S rRNA). (B) S1 nuclease protection analysis using poly(A)$^+$RNA (5 μg each) from different embryonic stages and adult tissues and the radioactively labelled polynucleotide probe for XenCK81A. Lane 1, 60-mer polynucleotide alone; lane 2, not loaded; lane 3, unfertilized eggs; lane 4, stage 6·5 (morula); lane 5, stage 9 (fine cell blastula); lane 6, stage 18 (neurula groove stage); lane 7, stage 42 (swimming tadpole); lane 8, adult oesophageal epithelium; lane 9, adult epidermis; lane 10, cultured kidney epithelial cells of line XLKE-A6; lane 11, adult skeletal muscle; lane 12, ovary; lane 13, yeast tRNA; lane 14, assay without RNA added; lane 15, not loaded; lane 16, assay without S1 nuclease, 300 cpm loaded. Note the extremely strong reaction in lane 6 (stage 18) but also positive, although weaker signals in lanes 7 (tadpole) and 8 (adult oesophagus).

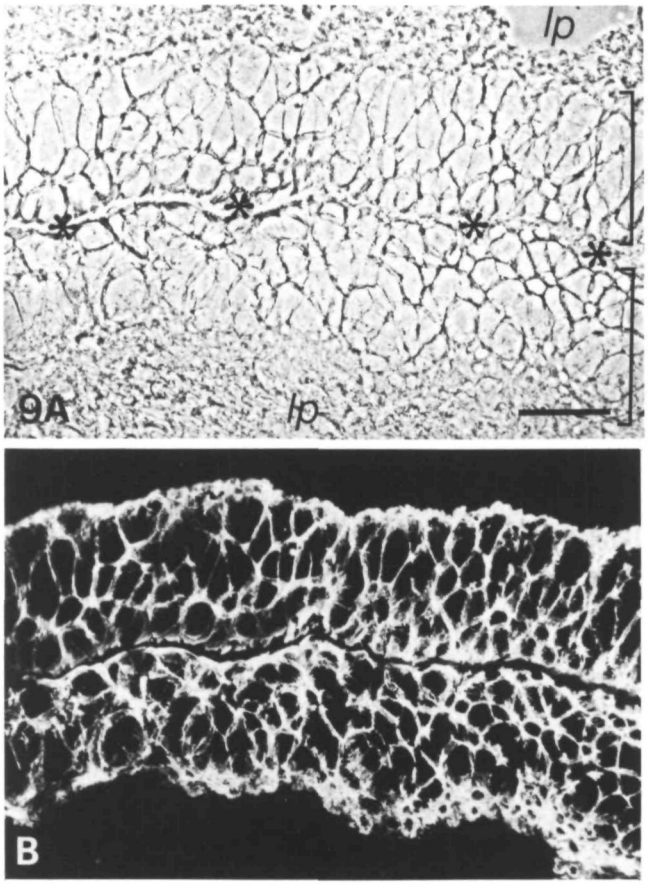

Fig. 9. Immunofluorescence microscopy (A, phase-contrast optics; B, epifluorescence) of section through frozen oesophagus of adult *Xenopus laevis*, showing positive reaction with monoclonal cytokeratin antibody (KL1) in the epithelium (brackets) whereas the *lamina proparia* (*lp*) is negative. Asterisks denote the oesophageal lumen. Bar, 50 μm.

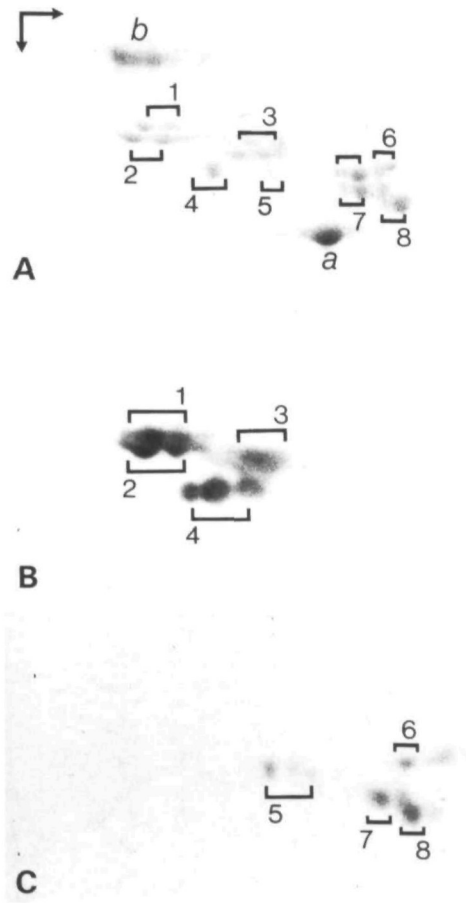

Fig. 10. Two-dimensional gel electrophoresis of cytoskeletal proteins from oesophageal mucosa of adult *Xenopus laevis* as seen after staining with Coomassie blue (A), after immunoblot reaction with monoclonal antibody $K_s$pan1-8.136 reactive with type II cytokeratins (B, autoradiograph), or after identification of type I cytokeratins by specific binding of purified, [^{125}I]-labelled cytokeratin 8 (A) from rat liver (C, autoradiograph). Separation of proteins was as described in Fig. 4. *a*, actin; *b*, bovine serum albumin. Brackets with numbers designate the polypeptides identified as cytokeratins, the bracket without a number denotes a yet unidentified component. Type II cytokeratin polypeptides are collectively identified by the reaction shown in B, type I cytokeratins by the cytokeratin binding assay (C).

scribe vector, was translated into a polypeptide that comigrated with the component designated 6 in the cytoskeletal preparation of oesophageal mucosa (Fig. 11A,B).

Discussion

The various polypeptides of epithelial cytokeratins are differentially expressed in the various epithelial cell types. In mammals, four major cytokeratin categories can be distinguished according to their histological distribution (Moll *et al.* 1982*a*; Sun *et al.* 1985). (i) The 'simple epithelial cytokeratins', i.e. components 7, 8, 18 and 19 of the human cytokeratin catalogue, are the only cytokeratins present in certain one-layered epithelia, and at least cytokeratins 8 and 18 can also be expressed in certain cell types or layers of complex and stratified epithelia (Moll *et al.* 1984; Lane *et al.* 1985; Bartek *et al.* 1986; Bosch *et al.* 1988) and in some non-epithelial tissues (Quinlan *et al.* 1985; Franke & Moll, 1987; Jahn *et al.* 1987). (ii) Cytokeratins typical of certain epithelia of high cell complexity, i.e. glandular, ductal, pseudostratified and non-epidermal stratified epithelia as well as the 'transitional epithelium' of the urinary tract, include components 4–6, 13–15 and 17 of the human catalogue (Moll *et al.* 1982*a*; Banks-Schlegel & Harris, 1983; Achtstätter *et al.* 1985; Grace *et al.* 1985; Nagle *et al.* 1985; Quinlan *et al.* 1985; Sun *et al.* 1985; Van Muijen *et al.* 1986). (iii) Human cytokeratins 1, 2, 5,

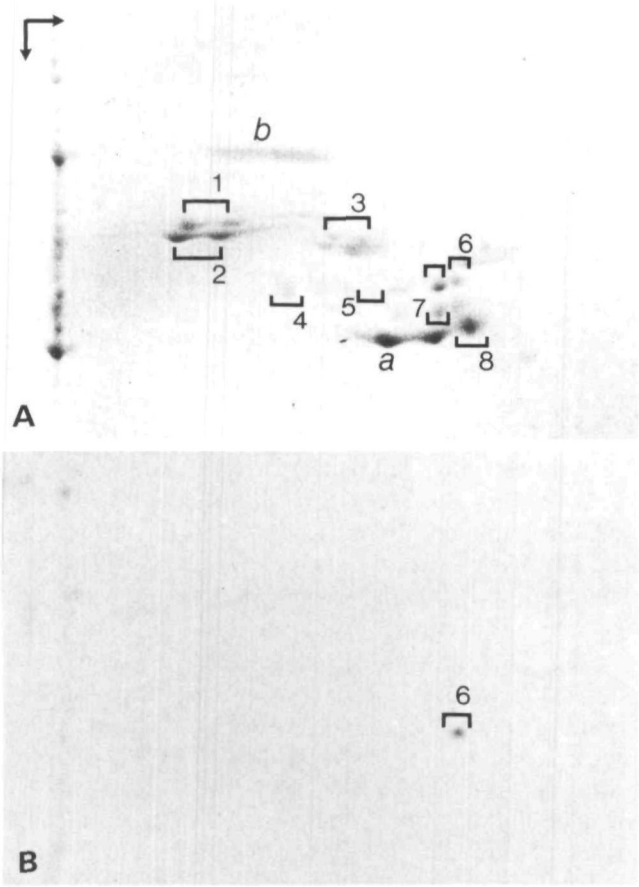

Fig. 11. Identification of the polypeptide encoded by XenCK81A mRNA, using *in vitro* translation of mRNA selected by hybridization to the cloned 60-mer polynucleotide, followed by two-dimensional coelectrophoresis of the translation product with cytoskeletal proteins of oesophageal mucosa from adult *Xenopus*. Separation of polypeptides was as described in Fig. 4. (A) Coomassie blue-staining. (B) Autoradiograph of the gel shown in A. Note that the [^{35}S]methionine-labelled product of the *in vitro* translation from hybrid-selected embryonal stage-18 mRNA comigrates with the unlabelled oesophageal cytokeratin polypeptide 6 as seen in A.

9–11 and 16 are typical of the suprabasal differentiation of epidermis (e.g. Fuchs & Green, 1980; Steinert *et al.* 1985; Sun *et al.* 1985; Knapp *et al.* 1986; Roop *et al.* 1987) and certain other stratified epithelia such as gingiva, vagina, exocervix, penile mucosa and certain parts of the amnion epithelium (Moll *et al.* 1983; Ouhayoun *et al.* 1985; Quinlan *et al.* 1985; Regauer *et al.* 1985; Lane *et al.* 1985; Morgan *et al.* 1987). (iv) Cytokeratins 3 and 12 have so far only been found in corneal epithelium (Moll *et al.* 1982*a*; Schermer *et al.* 1986).

The specific cytokeratins of these different categories are also characterized by certain features of their amino acid sequences (e.g. Steinert *et al.* 1985; Leube *et al.* 1986, 1988; Oshima *et al.* 1986; Fuchs *et al.* 1987; Sémat *et al.* 1988), and certain species differences between orthologous cytokeratins have also been noted (e.g. Franz & Franke, 1986). Our present study on *Xenopus laevis* cytokeratin XenCK55(5/6) presents the first sequence of a non-mammalian type II cytokeratin typical of complex epithelia, corresponding to the group (ii) of human cytokeratins, i.e. components 4–6. It is difficult, however, to relate precisely this *Xenopus* cytokeratin to a specific human, bovine or murine cytokeratin of this category. While the XenCK55(5/6) rod domain displays similarly high homologies to the human cytokeratins 4 (Leube *et al.* 1988), 5 (Lersch & Fuchs, 1988) and 6 (Hanukoglu & Fuchs, 1982; Tyner *et al.* 1985) as well as to the murine cytokeratin of M_r 57 000 (Knapp *et al.* 1986), its head and tail regions differ markedly from all these mammalian counterparts (see Results). Clearly, in the case of cytokeratin XenCK55(5/6) these interspecies differences are greater than those observed between the *Xenopus* (Franz & Franke, 1986), murine (Sémat *et al.* 1988) and bovine (Magin *et al.* 1986) equivalents of human cytokeratin 8 (Leube *et al.* 1988) which show homologous sequences of considerable lengths in the head and the tail, although the overall homology is lower in these domains than that in the rod piece.

In mammalian embryogenesis, the first tissues formed are polar simple epithelia containing cytokeratins only of category (i), i.e. cytokeratins 8 and 18, sometimes in combination with some cytokeratin 19 (for refs see Introduction). Subsequently in development, some epithelia remain at this level of organization whereas others differentiate into complex pseudostratified or stratified epithelia, concomitant with the advent of cytokeratins of the second category (Moll *et al.* 1982*b*; Regauer *et al.* 1985; Quinlan *et al.* 1985). Many of the cytokeratins of this category are also found in certain cell layers of fetal epidermis which, however, then produces additional cytokeratins of category (iii) and restricts its cytokeratin pattern to that typical of mature epidermis (e.g., Banks-Schlegel, 1982; Moll *et al.* 1982*b*; Dale *et al.* 1985; Lane *et al.* 1985). Important in the context of the present study is the fact that all cytokeratins identified in some embryonic or fetal stage also occur in one of the adult tissues.

In contrast, Winkles *et al.* (1985) have proposed that in *Xenopus* development three groups of cytokeratins can be classified according to their developmental pattern of synthesis: (i) egg- and embryo-specific; (ii) embryo-specific; and (iii) adult-specific. Moreover, Ellison *et al.* (1985) have shown that the *Xenopus* epidermis expresses different combinations of cytokeratins in embryonic stages, tadpoles and metamorphosed animals. However, our previous

(Franz & Franke, 1986) and present data lead to a different concept of expression of cytokeratin genes during *Xenopus* development, which is not too dissimilar to the patterns of cytokeratin synthesis during epithelial differentiations in mammalian development. To correspond with the grouping of mammalian cytokeratins according to their expression in histogenesis, we classify the *Xenopus* cytokeratins in relation to their patterns of developmental appearance into three major categories.

(i) Oocytes, eggs, blastula, gastrula and postgastrulation epithelia all synthesize cytokeratins of the simple epithelium type, i.e. the amphibian equivalents of human cytokeratins 8, 18 and 19, and these are continued to be expressed in various simple epithelia of the adult animal (Franz *et al.* 1983; Franz & Franke, 1986; this study), in endothelium (Jahn *et al.* 1987; compare with Godsave *et al.* 1986), retinal pigment epithelium (Owaribe *et al.* 1988), and certain types of smooth muscle (Jahn *et al.* 1987).

(ii) During and after gastrulation another category of cytokeratins is newly synthesized in some embryonic epithelia, most prominently in the ectoderm and embryonic epidermis. This category includes the type I cytokeratins of the XK81 and XK70 'subfamilies' (Jonas *et al.* 1985; Miyatani *et al.* 1986) as well as the type II cytokeratin XenCK55(5/6) described in the present study. The latter protein may be related to the type II cytokeratin encoded by clone DG76 mentioned by Dawid & Sargent (1986; see also Jamrich *et al.* 1987) but a direct comparison is not possible because of the lack of sequence information. The XK81 and XK70 cytokeratins resemble human cytokeratins of the group 14–16 (Hanukoglu & Fuchs, 1982; Tyner *et al.* 1985; Leube *et al.* 1988), and *Xenopus* cytokeratin XenCK55(5/6) shows a relatively close relationship to human cytokeratins 5 and 6. It is probable that cytokeratin XenCK55(5/6) is a natural 'partner' of cytokeratins XK81 and XK70, forming the typical type I–type II heterotypic tetramer subunits with each other (Hatzfeld & Franke, 1985; Quinlan *et al.* 1985; Sun *et al.* 1985; Fuchs *et al.* 1987).

Our detection of both XenCK55(5/6) and XK81 in the oesophageal mucosa of the adult animal shows that the genes encoding these proteins are not totally inactivated during – or after – metamorphosis. Rather, their expression is only restricted to certain cell types and tissues. Clearly, these cytokeratins are not 'embryo-specific' in general terms. Restriction of expression of XK81 and XK70 cytokeratins to a small subpopulation of cells has already been discussed by Sargent *et al.* (1986) as one of the possible alternative explanations for the drastic decrease of XK81 mRNA synthesis upon metamorphosis.

(iii) The third cytokeratin category of *Xenopus* comprises those epidermal cytokeratins that appear later in development, concomitant with epidermal differentiation (for examples, see Franz *et al.* 1983; Hoffmann & Franz, 1984; Ellison *et al.* 1985; Hoffmann *et al.* 1985; Franz & Franke, 1986). Like the corresponding mammalian cytokeratins, many of the *Xenopus* cytokeratins of this category are also characterized by repeated oligoglycine clusters in the head and tail domains (Hoffmann *et al.* 1985; Steinert *et al.* 1985).

We agree with Dawid and colleagues (Dawid & Sargent, 1986; Sargent *et al.* 1986) that the type I cytokeratins of the XK81 group are among the early expressed IF protein genes showing a rapid increase of synthesis after stage 11 (for later stages such as neurulae see also Slack, 1984), and now we add the type II cytokeratin XenCK55(5/6) to this list. Using *in situ* hybridization, Jamrich *et al.* (1987) have detected newly synthesized mRNA for certain cytokeratins of this group in the ectoderm of blastulae of stages 9 and 10. Only a few other zygotic genes are detectably induced in such early stages (e.g. Jonas *et al.* 1985; Akers *et al.* 1986; Dawid & Sargent, 1986; Dworkin-Rastl *et al.* 1986; Gurdon, 1987; Kintner & Melton, 1987; Sharpe *et al.* 1987). It is hoped that the availability of probes for IF protein mRNAs such as those described in this study will help to elucidate the mechanisms that control these early expression programs of such cell architectural elements and also to identify, in combination with *in situ* hybridization methods, the cell types in which certain cytokeratins continue to be synthesized in the adult tissues.

We thank Dr Martina Schnoelzer-Rackwitz for the synthesis of the polynucleotide, Irmgard Purkert for carefully typing the manuscript, and we gratefully acknowledge the expert technical assistance of Monika Brettel and Ralf Zimbelmann. The work has been supported in parts by the Deutsche Forschungsgemeinschaft.

References

Achtstätter, T., Hatzfeld, M., Quinlan, R. A., Parmelee, D. C. & Franke, W. W. (1986). Separation of cytokeratin polypeptides by gel electrophoretic and chromatographic techniques and their identification by immunoblotting. *Meth. Enzymol.* **134**, 355–371.

Achtstätter, T., Moll, R., Moore, B. & Franke, W. W. (1985). Cytokeratin polypeptide patterns of different epithelia of human male urogenital tract: immunofluorescence and gel electrophoresis studies. *J. Histochem. Cytochem.* **33**, 415–426.

Akers, R. M., Phillips, C. R. & Wessells, N. K. (1986). Expression of an epidermal antigen used to study tissue induction in the early *Xenopus laevis* embryo. *Science* **231**, 613–616.

Banks-Schlegel, S. P. (1982). Keratin alterations during

embryonic epidermal differentiation: a presage of adult epidermal maturation. *J. Cell Biol.* **93**, 551–559.

BANKS-SCHLEGEL, S. P. & HARRIS, C. C. (1983). Tissue-specific expression of keratin proteins in human esophageal and epidermal epithelium and their cultured keratinocytes. *Expl Cell Res.* **146**, 271–280.

BARTEK, J., BARTKOVA, J., TAYLOR-PAPADIMITRIOU, J., REJTHAR, A., KOVARIK, J., LUKAS, Z. & VOJTESEK, B. (1986). Differential expression of keratin 19 in normal human epithelial tissues revealed by monospecific monoclonal antibodies. *Histochem. J.* **18**, 565–575.

BENAVENTE, R., KROHNE, G. & FRANKE, W. W. (1985). Cell type-specific expression of nuclear lamina proteins during development of Xenopus laevis. *Cell* **41**, 177–190.

BERK, A. J. & SHARP, P. A. (1977). Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. *Cell* **12**, 721–732.

BOSCH, F. X., LEUBE, R. E., ACHTSTÄTTER, T., MOLL, R. & FRANKE, W. W. (1988). Expression of simple-epithelial type cytokeratins in stratified epithelia as detected by immunolocalization and hybridization in situi. *J. Cell Biol.* **106**, (in press).

BRONN, H. G. & HOFFMANN, C. K. (1878). Die Klassen und Ordnungen des Thier-Reiches. C.F. Winter'sche Verlagshandlung, Leipzig.

BROWN, D. T., ANDERTON, B. H. & WYLIE, C. C. (1983). Alterations in the organisation of cytokeratin filaments in normal and malignant human colonic epithelial cells during mitosis. *Cell Tiss. Res.* **233**, 619–628.

BRÛLET, P., BABINET, C., KEMLER, R. & JACOB, F. (1980). Monoclonal antibodies against trophectoderm-specific markers during mouse blastocyst formation. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4113–4117.

CHIRGWIN, J. M., PRZYBYLA, A. E., MACDONALD, R. & RUTTER, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5295–5299.

CHISHOLM, J. C. & HOULISTON, E. (1987). Cytokeratin filament assembly in the preimplantation mouse embryo. *Development* **101**, 565–582.

COLLINS, F. C. & WEISSMAN, S. M. (1984). The molecular genetics of human hemoglobin. In *Nucleic Acid Research and Molecular Biology*, vol. 31 (ed. W. E. Cohn & K. Moldave), pp. 317–462. New York: Academic Press.

DALE, B. A., HOLBROOK, K. A., KIMBALL, J. R., HOFF, M. & SUN, T.-T. (1985). Expression of epidermal keratins and filaggrin during human fetal skin development. *J. Cell Biol.* **101**, 1257–1269.

DAWID, I. B., HAYNES, S. R., JAMRICH, M., JONAS, E., MIYATANI, S., SARGENT, T. D. & WINKLES, J. A. (1985). Gene expression in *Xenopus* embryogenesis. *J. Embryol. exp. Morph.* **89 Supplement**, 113–124.

DAWID, I. B. & SARGENT, T. D. (1986). Molecular embryology in amphibians: new approaches to old questions. *Trends in Genet.* **2**, 47–50.

DWORKIN-RASTL, E., KELLEY, D. B. & DWORKIN, M. B. (1986). Localization of specific mRNA sequence in *Xenopus laevis* embryos by *in situ* hybridization. *J. Embryol. exp. Morph.* **91**, 153–168.

ELLISON, T. R., MATHISEN, P. M. & MILLER, L. (1985). Developmental changes in keratin patterns during epidermal maturation. *Devl Biol.* **112**, 329–337.

FOUQUET, B. (1987). Expression von Cytokeratinen in der Embryogenese von *Xenopus laevis*, Diploma thesis. Faculty of Biology, University of Heidelberg, FRG, pp. 1–129.

FOX, H. (1986). Epidemis. In *Biology of the Integument*, Part 2, *Vertebrates*. (ed. J. Bereiter-Hahn, A. G. Matoltsy & K. S. Richards), pp. 678–735. Berlin: Springer-Verlag.

FOX, H. & WHITEAR, M. (1986). Genesis and regression of the figures of Eberth and occurrence of cytokeratin aggregates in the epidermis of anuran larvae. *Anat. Embryol.* **174**, 73–82.

FRANKE, W. W., GRUND, C., KUHN, C., JACKSON, B. W. & ILLMENSEE, K. (1982*a*). Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin filaments. *Differentiation* **23**, 43–59.

FRANKE, W. W. & MOLL, R. (1987). Cytoskeletal components of lymphoid organs. I. Synthesis of cytokeratins 8 and 18 and desmin in subpopulations of extrafollicular reticulum cells of human lymph nodes, tonsils, and spleen. *Differentiation* **36**, 145–163.

FRANKE, W. W., RATHKE, P. C., SEIB, E., TRENDELENBURG, M. F., OSBORN, M. & WEBER, K. (1976). Distribution and mode of arrangement of microfilamentous structures and actin in the cortex of the amphibian oocyte. *Eur. J. Cell Biol.* **14**, 111–130.

FRANKE, W. W., SCHMID, E., GRUND, C. & GEIGER, B. (1982*b*). Intermediate filament proteins and nonfilamentous structures: transient distinegration and inclusion of subunit proteins in granular aggregates. *Cell* **30**, 103–113.

FRANKE, W. W., SCHMID, E., SCHILLER, D. L., WINTER, S., JARASCH, E. D., MOLL, R., DENK, H., JACKSON, B. W. & ILLMENSEE, K. (1982*c*). Differentiation-related patterns of expression of proteins of intermediate-size filaments in tissue and cultured cells. *Cold Spring Harbor Symp. quant. Biol.* **46**, 431–453.

FRANKE, W. W., SCHMID, E., WINTER, S., OSBORN, M. & WEBER, K. (1979). Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Expl Cell Res.* **123**, 25–46.

FRANKE, W. W., WINTER, S., VON OVERBECK, J., GUDAT, F., HEITZ, P. U. & STÄHLI, C. (1987). Identification of the conserved, conformation-dependent cytokeratin epitope recognized by monclonal antibody (lu-5). *Virchows Arch. A (Path. Anat.)* **411**, 137–147.

FRANZ, J. K. (1987). Charakterisierung und Klonierung von Cytokeratinen aus Oocyten von *Xenopus laevis*. Ph.D. Thesis. Faculty of Biology, University of Heidelberg, pp. 1–220.

FRANZ, J. K. & FRANKE, W. W. (1986). Cloning of cDNA and amino acid sequence of a cytokeratin expressed in oocytes of *Xenopus laevis*. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6475–6479.

FRANZ, J. K., GALL, L., WILLIAMS, M. A., PICHERAL, B. & FRANKE, W. W. (1983). Intermediate-size filaments

in a germ cell: Expression of cytokeratins in oocytes and eggs of the frog *Xenopus*. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6254–6258.

FUCHS, E. & GREEN, H. (1980). Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell* **19**, 1033–1042.

FUCHS, E., TYNER, A. L., GIUDICE, G. J., MARCHUK, D., RAYCHAUDHURY, A. & ROSENBERG, M. (1987). The human keratin genes and their differential expression. *Curr. Top. devl Biol.* **22**, 5–34.

GALL, L., PICHERAL, B. & GOUNON, P. (1983). Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell* **47**, 331–342.

GEIGER, B., KREIS, T. E., GIGI, O., SCHMID, E., MITTNACHT, S., JORCANO, J. L., VON BASSEWITZ, D. B. & FRANKE, W. W. (1984). Dynamic rearrangements of cytokeratins in living cells. In *Cancer Cells 1, The Transformed Phenotype* (ed. A. J. Levine, G. F. Vande Woude, W. C. Topp & J. D. Watson), pp. 201–215. Cold Spring Harbor: Cold Spring Harbor Laboratory.

GLASS, C., KIM, K. H. & FUCHS, E. (1985). Sequence and expression of a human type II mesothelial keratin. *J. Cell Biol.* **101**, 2366–2373.

GODSAVE, S. F., ANDERTON, B. H. & WYLIE, C. C. (1986). The appearance and distribution of intermediate filament proteins during differentiation of the central nervous system, skin and notochord of *Xenopus laevis*. *J. Embryol. exp. Morph.* **97**, 201–223.

GODSAVE, S. F., WYLIE, C. C., LANE, E. B. & ANDERTON, B. H. (1984). Intermediate filaments in the *Xenopus* oocyte: the appearance and distribution of cytokeratin-containing filaments. *J. Embryol. exp. Morph.* **83**, 157–167.

GRACE, M. P., KIM, K. H., TRUE, L. D. & FUCHS, E. (1985). Keratin expression in normal esophageal epithelium and squamous cell carcinoma of the esophagus. *Cancer Res.* **45**, 841–846.

GURDON, J. B. (1987). Embryonic induction – molecular prospects. *Development* **99**, 285–306.

HANUKOGLU, I. & FUCHS, E. (1982). The cDNA of a human epidermal keratin: divergence of sequence but conservation of structure among intermediate-filament proteins. *Cell* **31**, 243–252.

HANUKOGLU, I. & FUCHS, E. (1983). The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. *Cell* **33**, 915–924.

HATZFELD, M. & FRANKE, W. W. (1985). Pair formation and promiscuity of cytokeratins: formation in vitro of heterotypic complexes and intermediate-sized filaments by homologous and heterologous recombinations of purified polypeptides. *J. Cell Biol.* **101**, 1826–1841.

HATZFELD, M., MAIER, G. & FRANKE, W. W. (1987). Cytokeratin domains involved in heterotypic complex formation determined by *in vitro* binding assays. *J. molec. Biol.* **197**, 237–255.

HERRMANN, H., FOUQUET, B. & FRANKE, W. W. (1989*a*). Expression of intermediate filament proteins during development of *Xenopus laevis*. I. cDNA clones encoding different forms of vimentin. *Development* **105**.

HERRMANN, H., FOUQUET, B. & FRANKE, W. W. (1989*b*). Expression of intermediate filament proteins during development of *Xenopus laevis*. II. Identification and molecular characterization of desmin. *Development* **105**.

HOFFMANN, W. & FRANZ, J. K. (1984). Amino acid sequence of the carboxy-terminal part of an acidic type I cytokeratin of molecular weight 51 000 from *Xenopus laevis* epidermis as predicted from the cDNA sequence. *EMBO J.* **3**, 1301–1306.

HOFFMANN, W., FRANZ, J. K. & FRANKE, W. W. (1985). Amino acid sequence microheterogeneities of basic (type II) cytokeratins of *Xenopus laevis* epidermis and evolutionary conservativity of helical and nonhelical domains. *J. molec. Biol.* **184**, 713–724.

JACKSON, B. W., GRUND, C., SCHMID, E., BÜRKI, K., FRANKE, W. W. & ILLMENSEE, K. (1980). Formation of cytoskeletal elements during mouse embryogenesis. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation mouse embryos. *Differentiation* **17**, 161–179.

JACKSON, B. W., GRUND, C., WINTER, S., FRANKE, W. W. & ILLMENSEE, K. (1981). Formation of cytoskeletal elements during mouse embryogenesis. II. Epithelial differentiation and intermediate-sized filaments in early post-implantation embryos. *Differentiation* **20**, 203–216.

JAHN, L., FOUQUET, B., ROHE, K. & FRANKE, W. W. (1987). Cytokeratins in certain endothelial and smooth muscle cells of two taxonomically distant vertebrate species, *Xenopus laevis* and man. *Differentiation* **36**, 234–254.

JAMRICH, M., SARGENT, T. D. & DAWID, I. B. (1987). Cell-type-specific expression of epidermal cytokeratin genes during gastrulation of *Xenopus laevis*. *Genes and Development* **1**, 124–132.

JOHNSON, L. D., IDLER, W. W., ZHOU, X.-M., ROOP, D. R. & STEINERT, P. M. (1985). Structure of a gene for the human epidermal 67-kDa keratin. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1896–1900.

JONAS, E., SARGENT, T. D. & DAWID, I. B. (1985). Epidermal keratin gene expressed in embryos of *Xenopus laevis*. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5413–5417.

JORCANO, J. L., MAGIN, T. M. & FRANKE, W. W. (1984). Cell type-specific expression of bovine keratin genes as demonstrated by the use of complementary DNA clones. *J. molec. Biol.* **176**, 21–37.

KINTNER, C. R. & MELTON, D. A. (1987). Expression of *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. *Development* **99**, 311–325.

KLYMKOWSKY, M. W., MAYNELL, L. A. & POLSON, A. G. (1987). Polar asymmetry in the organization of the cortical cytokeratin system of *Xenopus laevis* oocytes and embryos. *Development* **100**, 543–557.

KNAPP, B., RENTROP, M., SCHWEIZER, J. & WINTER, H. (1986). Nonepidermal members of the keratin multigene family: cDNA sequences and *in situ* localization of the mRNA. *Nucleic Acids Res.* **14**, 751–763.

KOZAK, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44**, 283–292.

KROHNE, G., DABAUVALLE, M.-C. & FRANKE, W. W. (1981). Cell type-specific differences in protein composition of nuclear pore complex-lamina structures in oocytes and erythrocytes of *Xenopus laevis*. *J. molec. Biol.* **151**, 121–141.

LANE, E. B., BARTEK, J., PURKIS, P. E. & LEIGH, I. M. (1985). Keratin antigens in differentiating skin. *A. New York Acad. Sci.* **455**, 241–258.

LANE, E. B., GOODMAN, S. L. & TREJDOSIEWICZ, L. K. (1982). Disruption of the keratin filament network during epithelial cell divisions. *EMBO J.* **1**, 1365–1372.

LEHTONEN, E., LEHTO, V.-P., VARTIO, T., BADLEY, R. A. & VIRTANEN, I. (1983). Expression of cytokeratin polypeptides in mouse oocytes and preimplantation embryos. *Devl Biol.* **100**, 158–165.

LERSCH, R. & FUCHS, E. (1988). Sequence and expression of a type II keratin, K5, in human epidermal cells. *Molec. Cell Biol.* **8**, 486–493.

LEUBE, R. E., BADER, B. L., BOSCH, F. X., ZIMBELMANN, R., ACHTSTÄTTER, T. & FRANKE, W. W. (1988). Molecular characterization and expression of the stratification-related cytokeratins 4 and 15. *J. Cell Biol.* **106**, 1249–1261.

LEUBE, R. E., BOSCH, F. X., ROMANO, V., ZIMBELMANN, R., HÖFLER, H. & FRANKE, W. W. (1986). Cytokeratin expression in simple epithelia. III. Detection of mRNAs encoding human cytokeratins nos. 8 and 18 in normal and tumor cells by hybridization with cDNA sequences in vitro and in situ. *Differentiation* **33**, 69–85.

MAGIN, T. M., JORCANO, J. L. & FRANKE, W. W. (1983). Translational products of mRNAs coding for non-epidermal cytokeratins. *EMBO J.* **2**, 1387–1392.

MAGIN, T. M., JORCANO, J. L. & FRANKE, W. W. (1986). Cytokeratin expression in simple epithelia. II. cDNA cloning and sequence characteristics of bovine cytokeratin A (no. 8). *Differentiation* **30**, 254–264.

MAXAM, A. M. & GILBERT, W. (1977). A new method for sequencing DNA. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564.

MIYATANI, S., WINKLES, J. A., SARGENT, T. D. & DAWID, I. B. (1986). Stage-specific keratins in *Xenopus laevis* embryos and tadpoles: The XK81 gene family. *J. Cell Biol.* **103**, 1957–1965.

MOLL, R., FRANKE, W. W., SCHILLER, D. L., GEIGER, B. & KREPLER, R. (1982*a*). The catalog of human cytokeratin polypeptides: Patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. *Cell* **31**, 11–24.

MOLL, R., LEVY, R., CZERNOBILSKY, B., HOHLWEG-MAJERT, P., DALLENBACH-HELLWEG, G. & FRANKE, W. W. (1983). Cytokeratins of normal epithelia and some neoplasms of the female genital tract. *Lab. Invest.* **49**, 599–610.

MOLL, R., MOLL, I. & FRANKE, W. W. (1984). Identification of Merkel cells in human skin by specific cytokeratin antibodies: Changes of cell density and distribution in fetal and adult plantar epidermis. *Differentiation* **28**, 136–154.

MOLL, R., MOLL, I. & WIEST, W. (1982*b*). Changes in the pattern of cytokeratin polypeptides in epidermis and hair follicles during skin development in human fetuses. *Differentiation* **23**, 170–178.

MORGAN, P. R., LEIGH, I. M., PURKIS, P. E., GARDNER, I. D., VAN MUIJEN, G. N. P. & LANE, E. B. (1987). Site variation in keratin expression in human oral epithelia – an immunocytochemical study of individual keratins. *Epithelia* **1**, 31–43.

NAGLE, R. B., MOLL, R., WEIDAUER, H., NEMETSCHEK, H. & FRANKE, W. W. (1985). Different patterns of cytokeratin expression in the normal epithelia of the upper respiratory tract. *Differentiation* **30**, 130–140.

NIEUWKOOP, P. D. & FABER, J. (1967). *Normal Table of* Xenopus laevis *(Daudin)*. pp. 1–252. Second Edition, Amsterdam: North-Holland Publishing Company.

OSHIMA, R. G., HOWE, W. E., HOWE, W. E., KLIER, F. G., ADAMSON, E. D. & SHEVINSKY, L. H. (1983). Intermediate filament protein synthesis in preimplantation murine embryos. *Devl Biol.* **99**, 447–455.

OSHIMA, R. G., MILLAN, J. L. & CECENA, G. (1986). Comparison of mouse and human keratin 18: A component of intermediate filaments expressed prior to implantation. *Differentiation* **33**, 61–68.

OUHAYOUN, J.-P., GOSSELIN, F., FOREST, N., WINTER, S. & FRANKE, W. W. (1985). Cytokeratin patterns of human oral epithelia: differences in cytokeratin synthesis in gingival epithelium and the adjacent alveolar mucosa. *Differentiation* **30**, 123–129.

OWARIBE, K., KARTENBECK, J., RUNGGER-BRÄNDLE, E. & FRANKE, W. W. (1988). Cytoskeletons of retinal pigment epithelial cells: Interspecies differences of expression patterns indicate independence of cell function from the specific complement of cytoskeletal proteins. *Cell Tissue Res.* (in press).

QUINLAN, R. A., SCHILLER, D. L., HATZFELD, M., ACHTSTÄTTER, T., MOLL, R., JORCANO, J. L., MAGIN, T. M. & FRANKE, W. W. (1985). Patterns of expression and organization of cytokeratin intermediate filaments. *A. N.Y. Acad. Sci.* **455**, 282–306.

REGAUER, S., FRANKE, W. W. & VIRTANEN, I. (1985). Intermediate filament cytoskeleton of amnion epithelium and cultured amnion epithelial cells: Expression of epidermal cytokeratins in cells of a simple epithelium. *J. Cell Biol.* **100**, 997–1009.

ROOP, D. R., HUITFELDT, H., KILKENNY, A. & YUSPA, S. H. (1987). Regulated expression of differentiation-associated keratins in cultured epidermal cells detected by monospecific antibodies to unique peptides of mouse epidermal keratins. *Differentiation* **35**, 143–150.

SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467.

SARGENT, T. D., JAMRICH, M. & DAWID, I. B. (1986). Cell interactions and the control of gene activity during early development of *Xenopus laevis*. *Devl Biol.* **114**, 238–246.

SCHERMER, A., GALVIN, S. & SUN, T.-T. (1986). Differentiation-related expression of a major 64K

corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J. Cell Biol.* **103**, 49–62.

SÉMAT, A., VASSEUR, M., MAILLET, L., BRÛLET, P. & DARMON, Y. M. (1988). Sequence analysis of murine cytokeratin endo A (no. 8) cDNA. Evidence for mRNA species initiated upstream of the normal 5′ end in PCC4 cells. *Differentiation* **37**, 40–46.

SHARPE, C. R., FRITZ, A., DEROBERTIS, E. M. & GURDON, J. B. (1987). A homeobox-containing marker of posterior neural differentiation shows the importance of predetermination in neural induction. *Cell* **50**, 749–758.

SLACK, J. M. W. (1984). Regional biosynthetic markers in the early amphibian embryo. *J. Embryol. exp. Morph.* **80**, 289–319.

STEINERT, P. M., PARRY, D. A. D., IDLER, W. W., JOHNSON, L. D., STEVEN, A. C. & ROOP, D. R. (1985). Amino acid sequence of mouse and human epidermal type II keratins of M_r 67 000 provide a systematic basis for the structural and functional diversity of the end domains of keratin intermediate filament subunits. *J. biol. Chem.* **260**, 7142–7149.

SUN, T.-T., TSENG, S. C. G., HUANG, A. J.-W., COOPER, D., SCHERMER, A., LYNCH, M. H., WEISS, R. & EICHNER, R. (1985). Monoclonal antibody studies of mammalian epithelial keratins: A review. *A. New York Acad. Sci.* **455**, 307–329.

TSENG, S. C. G., JARVINEN, M. J., NELSON, W. G., HUANG, J.-W., WOODCOCK-MITCHELL, J. & SUN, T.-T. (1982). Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. *Cell* **30**, 361–372.

TYNER, A. L., EICHMAN, M. J. & FUCHS, E. (1985). The sequence of a type II keratin gene expressed in human skin: conservation of structure among all intermediate filament genes. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4683–4687.

TYNER, A. L. & FUCHS, E. (1986). Evidence for posttranscriptional regulation of the keratins expressed during hyperproliferation and malignant transformation in human epidermis. *J. Cell Biol.* **103**, 1945–1955.

VAN MUIJEN, G. N. P., RUITER, D. J., FRANKE, W. W., ACHTSTÄTTER, T., HAASNOOT, W. H. B., PONEC, M. & WARNAAR, S. O. (1986). Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. *Expl Cell Res.* **162**, 97–113.

VIAC, J., REANO, A., BROCHIER, J., STAQUET, M.-J. & THIVOLET, J. (1983). Reactivity pattern of monoclonal antikeratin antibody (KL1). *J. Invest. Dermatol.* **81**, 351–354.

WERNER, D., CHEMLA, Y. & HERZBERG, M. (1984). Isolation of poly(A)$^+$RNA by paper affinity chromatography. *Anal. Biochem.* **141**, 329–336.

WINKLES, J. A., SARGENT, T. D., PARRY, D. A. D., JONAS, E. & DAWID, I. B. (1985). Developmentally regulated cytokeratin gene in *Xenopus laevis*. *Molec. Cell Biol.* **5**, 2575–2581.

WYLIE, C. C., HEASMAN, J., PARKE, J. M., ANDERTON, B. & TANG, P. (1986). Cytoskeletal changes during oogenesis and early development of *Xenopus laevis*. *J. Cell Sci. Suppl.* **5**, 329–341.

(Accepted 31 August 1988)