Xp54, the *Xenopus* homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes

Michael Ladomery*, Eleanor Wade and John Sommerville*

School of Biological and Medical Sciences, Bute Buildings, University of St Andrews, St Andrews, Fife KY16 9TS, UK

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ABSTRACT

In investigating the composition of stored (maternal) mRNP particles in *Xenopus* oocytes, attention has focussed primarily on the phosphoproteins pp60/56, which are Y-box proteins involved in a general packaging of mRNA. We now identify a third, abundant, integral component of stored mRNP particles, Xp54, which belongs to the family of DEAD-box RNA helicases. Xp54 was first detected by its ability to photocrosslink ATP. Subsequent sequence analysis identifies Xp54 as a member of a helicase subfamily which includes: human p54, encoded at a chromosomal breakpoint in a member of a helicase subfamily which includes: human p54, found at a chromosomal breakpoint in the cell line RC-K8, derived from a diffuse large B-cell lymphoma (16) and now described as a putative proto-oncogene (8,9). More recently, the masking proteins have been identified as belonging to the family of Y-box proteins (10–12), a family with dual roles in transcription regulation and mRNA packaging (13). However, the other abundant mRNP proteins have remained unidentified.

In this report we describe a 54 kDa protein, which is an abundant component of mRNP particles. It turns out to belong to the family of DEAD-box RNA helicases, proteins which can regulate RNA secondary structure in translation initiation, splicing and ribosome biogenesis (14,15). DEAD-box proteins contain a set of conserved motifs necessary for RNA helicase activity, including the NTPase ‘A’ (AXXGXXGKT) and ‘B’ (DEAD) motifs, involved in ATP binding and hydrolysis. Within this increasingly large group of proteins, there are distinct subfamilies. *Xenopus* Xp54 belongs to a subfamily which includes: human p54, found at a chromosomal breakpoint in the cell line RC-K8, derived from a diffuse large B-cell lymphoma (16) and now described as a putative proto-oncogene (17); a mouse equivalent (18); *Drosophila* ME31B, maternally-expressed in oocytes and nurse cells (19); *Schizosaccharomyces pombe* Ste13 cloned by functional complementation of the sterility mutant ste13 (20) and *Saccharomyces cerevisiae* DHH1 (21). We describe the structure of Xp54, its developmental expression, and its presence and activity in mRNP particles. With reference to what is known about its putative homologues p54, ME31B and Ste13, we suggest possible functions for Xp54.

MATERIALS AND METHODS

Cells and tissues

Oocyte stages of *Xenopus laevis* are listed according to Dumont (22), and developmental stages according to Nieuwkoop and Faber (23). XTC cells were cultured as described previously (24).

*To whom correspondence should be addressed. Tel: +44 1334 463583; Fax: +44 1334 463600; Email: js15@st-and.ac.uk

*Present address: MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK
cDNA cloning
Ovary containing only previtellogenic (stage I) oocytes was dissected from an immature female X. laevis. cDNA was synthesized from poly(A)+ RNA extracted from glycerol gradient fractions enriched for polysomes (9). Double-stranded cDNA was cloned directionally into the bacteriophage λ expression vector Uni-ZapXR (Stratagene). Two libraries of 0.8 × 10⁶ clones (Xlalp1) and 3.7 × 10⁶ clones (Xlalp2) were constructed and each was amplified to give 10⁸ p.f.u./ml. For screening purposes equal volumes from each library were used.

Isolation of mRNP particles, proteins and peptides
Poly(A)+ RNP particles were isolated from clarified homogenates of the presence of [α-³²P]CTP (400 Ci/mmol, Amersham). Hybridization of the probe to transfers was carried out at 65°C in a solution containing 1% dried skimmed milk, 1× SSC and 2% SDS. The transfers were then washed in decreasing salt concentrations to a final step of 0.1× SSC at 80°C, dried and exposed in contact with X-ray film (Agfa).

Immunoblotting
Antisera were raised in rabbits against an electrophoretically-purified protein of 54 kDa isolated from oocyte mRNP particles. IgG fractions were obtained by binding to, and elution from, protein A–Sepharose (Pharmacia). Affinity-purified antibodies were prepared by binding IgG to nitrocellulose membrane saturated with extracts of bacterially-expressed pGEX 4T/Xp54 fusion proteins. Samples equivalent to two oocytes or embryos, or to 10² cells or 10 µg of fusion protein, were separated by SDS–PAGE, transferred to nitrocellulose and reacted with anti-p54 at a dilution of 1/3000. Detection was with peroxidase-conjugated anti-rabbit IgG (Sigma) reacted with diaminobenzidine/hydrogen peroxide.

Glycerol gradients
Samples containing ~150 µg of poly(A)+ mRNP were loaded on to gradients of 10–25% glycerol made up in gradient buffer: 100 mM KCl; 2 mM MgCl₂; 1 mM DTT; 10 mM Tris–HCl, pH 7.5. Centrifugation was at 18 000 r.p.m. for 14 h at 0°C, using a Beckman SW28 rotor.

Density gradients
Gradients of 20–60% Nycozen in the buffer: 2 mM MgCl₂; 1 mM EDTA; 20 mM Tris–HCl, pH 7.5; were prepared according to the manufacturer’s specifications (Nyegaard & Co., Oslo, Norway). Samples containing ~150 µg of poly(A)+ mRNP in 0.5 ml buffer were loaded on to 10 ml gradients, which were centrifuged at 36 000 r.p.m. for 18 h at 0°C using a Beckman SW55T rotor. Twenty-one 0.5 ml fractions were collected for analysis. Densities were determined by optical refractometry using the equation: density ρ (g/cm³) = 3.242η – 3.323 where η is the refractive index.

Riboprobe synthesis and helicase assay
The vector pGEM-T (Promega) was used to synthesize complementary RNA transcripts from the T7 and an SP6 promoters. In order to generate transcripts of suitable lengths part of the polylinker region was deleted by cutting at the Nsi I and Pst I restriction sites, which were then ligated. T7 strand: the plasmid was linearized at the Spe I site, generating a 60 base transcript; 5’-GGGGCGAATTGG-GCCCGACGTCGCA TGCTCCCGGCCGCCA TGGCCGCGG-

Phospholabelling
To test for phosphorylation by the mRNP-associated protein kinase activity (9), poly(A)+ mRNP were adjusted to 20 mM Tris–HCl, pH 7.5 and 2 mM MgCl₂, as above. [γ-³²P]ATP (2 µl) (Amersham International, 3000 Ci/mmole) was added to each sample. Reactions proceeded for 30 min at 37°C, then samples were digested with RNase and analysed by SDS–PAGE and autoradiography.
the Apal site, the 3’ overhang of which was blunt-ended with T4 DNA polymerase, generating a 74 base transcript: 5′-GAAATCTCAAGCTATGCAGCCCCTCCATATGTAATATCCCCTC-
CGCCGATGGCGGCGAGCATGCGACGTGGC-3′. The complementary region is underlined (46 bases with a 67% GC content). The T7 transcript was labelled with [α-32P]CTP (Amersham International at 400 Ci/mmol).

To generate the double-stranded probe: 1 μg of the labelled strand, specific activity 2.5 μCi/μg RNA, was mixed with 2 μg of cold strand, in a volume of 100 μl of DEPC-treated dH2O. The mixture was denatured at 95°C for 5 min, then adjusted to 200 mM NaCl: 2 mM EDTA; 10 mM Tris–HCl; pH 7.5, and incubated at 60°C for 1 h for hybridization.

Double-stranded probes were also made (as detailed above) using sense and antisense transcripts from a subclone representing the 3’ end of an oocyte-specific β-tubulin gene. The annealed strands should generate complex structures containing regions of intrastand duplex within the 280 nucleotide length of complementary sequence (23). These structures also present 5’ single-stranded ends.

Any detectable RNA helicase activity unwinds and separates the strands, of which only one is radioactively labelled. Hence the activity is measured as a mobility shift towards the single-stranded form. As a control, the probe was denatured in 1 M glyoxal + 50% DMSO (dimethylsulfoxide) in 10 mM sodium phosphate buffer and placed at 50°C for 1 h. Helicase assays were performed in 50 μl reactions in helicase buffer: 20 mM Tris–HCl, pH 7.5; 80 mM KCl; 1 mM MgCl2; 1.5 mM DTT; with ATP (at 0–1 mM). Double-stranded probe (0.1 μCi per reaction) was included and protein samples (ranging from 2 to 500 ng protein) were tested. Reactions proceeded at 20°C for 20 min, after which 10 μl of gel loading buffer was added (50% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol, in DEPC-treated dH2O) before loading the reactions on an acrylamide gel. The gel contained 6% acrylamide (sequencing grade) in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA) and 10% glycerol. The gel was run for ~2 h at 200 V, fixed for 20 min in 10% acetic acid, dried and set up for autoradiography.

RESULTS

Characterization of a 54 kDa protein from mRNP particles

Poly(A)7 (polyadenylated) mRNP particles from previtellogenic oocytes are isolated by oligo(dT)-cellulose affinity chromatography and separated by SDS–PAGE (Fig. 1A). Most abundant are the ‘masking’ proteins FRGY2a/b, also known as pp60/56 (29), which belong to the family of Y-box transcription factors (10–12). The masking proteins can be efficiently photocrosslinked to a wide range of mRNA sequences (25,28,30). However, other mRNP proteins remain unidentified; particularly the group of abundant proteins in the 50–54 kDa range.

On photocrosslinking [α-32P]ATP to mRNP particles in solution, only two from the complete set of proteins are substantially covalently labelled. Autoradiography of SDS–PAGE gels shows labelled proteins of apparent mass of 68 and 54 kDa (Fig. 1B). For comparison, parallel treatment of mRNP particles with [γ-32P]ATP shows labelling of pp60/56 (FRGY2a/b) by the mRNP-associated protein kinase (CK2) activity. The ATP-binding proteins are distinct from the phospholabelled proteins in not being efficiently photocrosslinked to the RNA. As can be seen in Figure 1B, the amount of crosslinked ATP does not increase in the 54 and 68 kDa bands after RNase treatment, whereas the amount of label (resulting from phosphorylation) resolved in the 56 and 60 kDa bands increases substantially after RNase treatment. It is not known if the phosphorylated material running below the 56 and 60 kDa bands is the 54 kDa ATP-binding protein: this protein is not phosphorylated in vivo, at least in immature oocytes (9), although it does contain potential phosphorylation sites (see Discussion). The slow migrating bands seen after RNase treatment of phosphorylated mRNP probably represent crosslinked FRGY2a/b dimers and multimers (25).

Steps were taken to isolate the 54 kDa ATP-binding protein. By heating suspensions of mRNP particles to 80°C followed by chilling and centrifugation, the protein components are separated into two classes (Fig. 1A): FRGY2a/b remain soluble whereas the other proteins are precipitated (26). The most abundant proteins present in the precipitates are in the size range of 52–54 kDa and include the 54 kDa ATP-binding protein. Digestion of the precipitates with cyanogen bromide yields a series of polypeptides, six of which, in the size range of 5–23 kDa were partially sequenced (Fig. 1C). Four of the peptides could be aligned with the human RNA helicase, p54 (16), and two, (M)GWEKPSPIQ and (M)RQEHRNRFVH, provided a perfect match.

Cloning Xp54 cDNA from a cDNA library using PCR

PCR primers were designed around two of the sequenced peptides: (M)GWEKPSPIQ and (M)RQEHRNRFVH. Based on the alignment with human p54, the peptide sequence MGIFEMGWE was selected to design the forward primer: (5′-ATGGGNAT/C/A)TTTYGARATGGNTGGA-3′, and the peptide sequence HAKMRQEH to was selected to design the reverse primer:
Figure 2. Alignment of amino acid sequences of five members of the subfamily of DEAD-box RNA helicases containing Xp54. Alignments were made using the PILEUP algorithm (Genetics Computer Group, Inc., Version 8.0) and displayed using Seqvu (The Garvan Institute of Medical Research, Version 1.0.1). Comparison is made between proteins from two vertebrates (Xp54 and human p54), Drosophila (ME31B) and two yeasts (Ste13 and DHH1). References are given in the text and the EMBL accession numbers are, in order: X92421; Z11685; M59926; D29795; X66057. Identical sequences are boxed.

(5′-RTGYTCYTGC(G/T)CATYTTCACRTG(A/G/T)AT-3′).

The template used for PCR amplification was double-stranded cDNA derived from stage I oocyte polysomal mRNA. A predicted 0.8 kb product was generated from 25 cycles, and ligated into the pGEM-T vector (Promega) for propagation. A radiolabelled probe was made from the 0.8 kb insert and was used to screen the cDNA library, constructed from stage I polysomal mRNA, for a full-length clone.

Analysis of Xp54 protein structure and homologies

A cDNA clone containing an insert of 2.5 kb was selected from the cDNA library and sequenced (EMBL accession number X92421). The length of the sequenced insert is 2491 bp, containing an open reading frame of 1445 bp extending from positions 204 to 1649. The proposed initiation codon occurs in a sequence context conforming to the eukaryotic consensus and to start sites for other proteins expressed in Xenopus oocytes. A polyadenylation site (AUUAAA) occurs 34 bp upstream from the 3′-terminal poly(A). The 3′ UTR contains AU-rich stretches and three AUUUA motifs which could represent instability elements (31). The predicted molecular mass of the encoded protein is 54.1 kDa, matching the size of the ATP-crosslinked protein already described (Fig. 1B). This protein is referred to as Xp54 (Xenopus p54).

An alignment with the DEAD-box RNA helicase family (not shown) confirms that Xp54 is a member of a subfamily that includes human and mouse p54, Drosophila ME31B, S.pombe Ste13 and S.cerevisiae DHH1. The extent of conservation from yeast to man is on the whole impressive, and in particular, Xenopus Xp54 is 94% identical to human p54 at the amino-acid level. Among the five members of this subfamily, the overall amino acid sequence identity is 54%, and the overall level of similarity is at least 76% when conservative substitutions are taken into account. Figure 2 shows the alignment of Xp54 with its putative homologues.

Whereas all DEAD-box proteins share certain conserved motifs, there is considerable divergence between them in other regions of the proteins, particularly at the N- and C-terminals (14,15). Auxiliary domains, additional to the conserved RNA helicase core, are thought to confer functional specificity to the many members of this family, for example by targeting specific RNA sequences with RNA-binding domains. Although no additional RNA-binding motifs are obvious in human p54 and Xp54, there is present an N-terminus which is rich in uncharged polar residues and may well interact with other macromolecules. It is interesting to note that a similar extension, rich in polar residues, is located at the C-terminals of the yeast proteins (Fig. 2).

Expression of Xp54 mRNA is developmentally regulated

The expression pattern of Xp54 mRNA was examined through oogenesis, early development and in adult tissues (Fig. 3). Hybridization of radiolabelled antisense riboprobes to transfers of poly(A)+ RNA from ovary identifies a transcript of 2.6 kb, but no transcript larger than this, even on high level loading of the gel (Fig. 3A). This observation compares well with the cloned cDNA, which is 2491 bp long and was derived from previtellogenic mRNA. Although a minor signal at 1.5 kb is also apparent, this would barely contain the coding region alone (1445 bp) and its identity remains unknown.

On examining total RNA isolated from different stages of development, a peak of expression of the 2.6 kb transcript is found...
the equivalent human and mouse tissues (16–18). Although a description of a 6.7 kb transcript encoding the homologue p54 in such as ribosomal proteins (Fig. 3C), can be detected in all tissues. In comparison, transcripts encoding general growth components, any Xp54 transcripts in adult tissues (not shown) other than ovary. Embryogenesis may reflect the relatively small contribution to the maximal production of stored mRNP particles: lower levels of Xp54 transcripts in small oocytes corresponds to the period of increase in oocyte volume from stage I to VI. (C) The contrasting pattern of expression through oogenesis of mRNA of 0.5 kb encoding ribosomal protein L22 (EMBL accession number: X94243). The same samples were used but hybridized with an antisense riboprobe to the L22 clone.

at stages I–II (22) of oogenesis (Fig. 3B). A substantial decrease in hybridization signal occurs from oocyte stage III and into early embryogenesis (not shown). The high level of expression of Xp54 transcripts in small oocytes corresponds to the period of maximal production of stored mRNP particles: lower levels of transcript throughout the remainder of oogenesis and into embryogenesis may reflect the relatively small contribution to the net pool of mRNP at these times. Furthermore, we failed to detect any Xp54 transcripts in adult tissues (not shown) other than ovary. In comparison, transcripts encoding general growth components, such as ribosomal proteins (Fig. 3C), can be detected in all tissues and stages of development tested. The failure to detect Xp54 transcripts in a range of somatic tissues is strange in view of the description of a 6.7 kb transcript encoding the homologue p54 in the equivalent human and mouse tissues (16–18). Although a band at ~7 kb was present after hybridization of RNA extracted from embryos and somatic tissues, it was removed after stringent washing of the transfers (not shown). Taken together, these observations lead us to believe that the Xp54 transcripts represent expression of an oocyte-specific gene.

Expression of fusion proteins and their immunoreactivity

GST (glutathione S-transferase) fusion proteins were prepared by subcloning Xp54 into pGEX-4T (Pharmacia) vectors. A polyclonal antiserum, raised against a band-excised mRNP protein of 54 kDa, recognized three out of four Xp54-GST fusion proteins (Fig. 4A). Specific reactions were obtained with fusions containing the middle portion and the carboxyl end of Xp54: PvuII–PvuII; DraI–DraI; XhoI–XhoI. The antiserum did not recognize the PvuII–XhoI fusion protein which encodes 14.3 kDa of Xp54 near the amino end, including the ‘A’ motif involved in ATP-binding. This antiserum is referred to as anti-p54 and was affinity-purified by binding an IgG fraction to nitrocellulose saturated with bacterial extract containing the PvuII–PvuII fusion protein.

Developmental expression of Xp54 protein

Anti-p54 was used to track expression of the Xp54 protein through oogenesis and early embryogenesis (Fig. 4B). The level of Xp54, on a per oocyte/embryo basis, is at a maximum at Dumont stage I and remains fairly constant through to the end of oogenesis and, after fertilization, up to blastula. From blastula, through gastrula and neurula, there is a substantial decline in the level of Xp54. This pattern of expression is similar to that of the Y-box mRNA-packaging proteins, pp60/56 (32), and therefore correlates well with the relative abundance of maternal mRNP particles. However, one difference is that whereas pp60/56 are not detected much after blastula (stage 8), low levels of the immunostained 54 kDa band remain detectable through to at least the free-feeding tadpole (embryonic stage 42).

Although all evidence indicates that the 2.6 kb transcript which encodes Xenopus p54 is exclusively maternal, immunoblotting with anti-p54 shows that a 54 kDa protein is expressed in Xenopus culture cell lines (XTC, from metamorphosing tadpoles; XP, from adult kidney), indicating that a somatic transcript exists to direct the synthesis of a very similar protein. A 54 kDa protein is also detected with anti-p54 in rat ovary extracts (Fig. 4B) and it is reasonable to suppose that similar proteins are expressed across vertebrate species.
Immunostaining of oocytes and culture cells with anti-p54

That Xp54 is a major cytoplasmic protein of small oocytes is seen directly by immunostaining ovarian sections with anti-p54. The cellular distribution of Xp54, then, appears to be similar to that of pp60/56, except for the more intense staining of nucleoli with anti-p54.

Immunostaining of XTC cells with anti-p54 also shows a predominantly particulate cytoplasmic reaction (Fig. 5B). Optical sections, made by confocal laser microscopy, indicate that smaller amounts of immunoreactive material are located in the nucleus. This image is very similar to that shown previously using polyclonal antibodies to human p54 (anti-rck/p54) with Raji cells (17).

Sedimentation properties of Xp54

In order to determine to what extent Xp54 is associated with particles sedimenting at a rate expected of mRNPs, clarified homogenates from previtellogenic oocytes were centrifuged through 15–40% glycerol gradients. It has been shown previously that mRNP particles sediment at 30–100S (5,9). On analysing gradient fractions by SDS–PAGE and immunoblotting, it is seen that a 54 kDa protein, which reacts specifically with anti-p54, mostly sediments between 42S and 80S marker peaks (Fig. 6A). The observed distribution is similar to that of the mRNP packaging proteins pp60/56 using anti-FRGY2 (not shown). The coincidence of pp56/60 and Xp54 suggests that both packaging proteins and RNA helicase are bound to the same population of mRNA molecules. From the presence of some Xp54 near the top of the gradients, it is possible that in early oocytes excess RNA helicase occurs in RNA-free protein complexes, as has been described for pp60/56 and other mRNP proteins (9,33).

Analysis of mRNP density fractions

Previous work with Nycodenz (34) and CsCl gradients (J.S. and M.L., unpublished), has shown a uniform density of mRNP particles, in which all classes of mRNA peak in the same gradient fractions. (Classes of mRNA include those that are translationally-repressed in immature oocytes, such as c-mos and cyclin B1, and those that are translated, such as ribosomal proteins and nucleolin.) The advantage of Nycodenz gradients is that the ionic conditions are not extreme and no prior fixation of RNA/protein complexes is required. On centrifuging poly(A)+ mRNP on 20–60% Nycodenz gradients, the mRNP proteins peak in a fraction corresponding to a buoyant density of 1.21 g/cm³ (Fig. 6B). Since both Xp54 and pp60/56, detected by immunoblotting with anti-p54 (Fig. 6B) and anti-FRGY2 (not shown), occur in this same density fractions, it is again suggested that both packaging proteins and RNA helicase are components of the same mRNP particles.

Separation of helicase activity from other mRNP components

On binding poly(A)+ mRNP to oligo(dT)-cellulose, a subset of proteins, including pp56/60, remains bound to the resin at high salt concentration (2 M NaCl). These proteins are eluted under conditions which release the poly(A)+ RNA (no salt or 60% formamide; 5,25,28). However, before eluting the salt-stable mRNP, individual proteins can be washed off the column by increasing salt concentration (Fig. 7A). At 0.4 M NaCl, a 54 kDa protein is eluted, whereas at 0.6 and 1.0 M NaCl, a much wider range of mRNP proteins elute (Fig. 7B). Selected fractions were immunoblotted with anti-p54 and anti-FRGY2. The immunoblots confirm that most of pp60/56 is only released in the final no salt elutions, although small amounts can be detected from the 0.6 M eluate onwards, but not in the 0.4 M eluate (not shown). In contrast, Xp54 is enriched in the 0.4 M eluate, but is also present in higher salt elutions up to 1.5 M NaCl (Fig. 7C). Thus Xp54 appears to be bound to the mRNP through charge interactions;
Figure 7. Elution of Xp54 from mRNP bound to oligo(dT) cellulose. (A and B) Proteins (Coomassie stained after SDS-PAGE) associated with poly(A)+ RNA are selectively released from the column by changing the salt concentration of the eluent. After extensive washing with buffer containing 0.2 M NaCl, the immunoblot (C) shows that Xp54 elutes mostly between the 0.4 and 1.0 M NaCl steps. Only the 0.4 M fraction is substantially free of other proteins.

DISCUSSION

The RNA helicase Xp54 is an abundant and integral component of stored (non-translating) mRNP in Xenopus oocytes. Xp54 was first identified through peptide sequencing and ATP crosslinking, and then cloned by PCR using degenerate primers. A partially purified fraction containing Xp54 was shown to possess an ATP-dependent RNA helicase activity. Xp54 belongs to the family of DEAD-box proteins, and includes the conserved motifs that are the hallmark of that family. More specifically, Xp54 belongs to a subfamily that includes human p54, to which it is 94% identical, mouse p54, Drosophila ME31B, and S.pombe Ste13 and S.cerevisiae DHH1. Information in the literature describing these putative homologues can now be related to Xp54.

The first member of this subfamily to be cloned was Drosophila ME31B, a maternally expressed gene (19). It is expressed in germ-line cells, including the 15 nurse cells and oocytes, but not the surrounding somatic cells. ME31B mRNA is detectable only in the early embryo, 0–2 h after fertilization. The S.pombe gene STE13 was cloned by functional complementation of a sterility mutant (20). Prior information indicated that STE13 is essential for nitrogen-starvation-induced G1 arrest, leading to the initiation of sexual development (35). The yeast protein is therefore required for the progression of meiosis. Yeast mutants were successfully complemented with a cDNA construct encoding Drosophila ME31B, but not by one encoding Vasa, a well-characterized maternally-expressed RNA helicase, consistent with ME31B being the functional homologue of Ste13. Maekawa et al. (20) suggested that Ste13 may have a role in the translational control of meiotic mRNAs.

Human p54 was first described by Dan and Yunis (16). These authors were investigating the chromosomal translocation t(11;14)(q23;q32) found in the cell line RC-K8 derived from a diffuse, large B-cell lymphoma. A putative RNA helicase, p54, 75% identical to Drosophila ME31B, was found at this break-
The expression of its mRNA was detected at high levels, in a variety of tissues, in the form of a 6.7 kb transcript. More recently, Akao et al. (17) have used an anti-p54 antiserum to detect the 54 kDa protein in different tissues. Significantly, they detect moderate amounts of p54 in the neuroblastoma cell line IMR-32 and glioblastoma cell line T98G, and higher levels in the rhabdomyosarcoma cell line RMS-YM and lung cancer cell lines LU99A and LC39B. These cell lines are derived from tissues in which the same authors do not detect p54, leading to the suggestion that p54 is a candidate proto-oncogene.

As in the case of Drosophila ME31B (19), we were unable to detect Xp54 mRNA in adult tissues. Highest levels of Xp54 mRNA are seen in early oogenesis, when the demand for mRNP proteins is highest. However, Xp54 protein is detected on Western blots at least until the free-feeding tadpole stage, and is detected in the tadpole cell line XTC and the adult kidney cell line XP. It is therefore likely that the cDNA described in this paper is a germ cell-specific transcript, and that a somatic transcript directs the synthesis of a similar protein. The presence of other germ-cell specific transcripts in Xenopus oocytes is well documented (32).

What, then, is the specific function of Xp54? The Xenopus oocyte can be considered to be in a mode of suspended cell proliferation. Until oocyte maturation, the oocyte is stalled in the first meiotic prophase. During this time, the lampbrush chromosomes are highly active in transcribing genes whose products will drive early development. For example, c-mos is transcribed from early oogenesis onwards, but its mRNA is not translated until oocyte maturation (36). Another example is c-myc mRNA which accumulates from early oogenesis and is stored for translation during early embryonic development (37,38). Two possibilities arise. The first is that Xp54, as an integral component of stored mRNP, is required for the efficient translational recruitment of stored mRNAs. More specifically, Xp54 might unwind duplex structures in the 5′ UTR during translation initiation (mimicking the role of eIF4A), or may even unwind duplexes in the coding region and 3′ UTR that might impede elongation. The key property would be the ability to enhance translation at a time when large quantities of product are required. Akao et al. (17) have detected p54 on the rough ER, consistent with a role in translation and our own immunostaining studies on XTC cells lead to a similar conclusion. An alignment of 45 DEAD- and DEXH-box RNA helicases representing all known subfamilies (not shown), suggests that the Xp54 subfamily is more closely related to eIF4A than to any other helicase, again suggesting that Xp54 has a role in translation initiation.

An alternative function might relate to the formation of stored mRNP particles, a process which appears to be initiated in the nucleus (13). In this scenario, Xp54 would unwind RNA sequences to facilitate protein binding, notably of the mRNA-packaging Y-box proteins, FRGY2a/b, which show a marked preference for single-stranded RNA (25). Whereas immunostaining of cell sections (Fig. 5) indicates that Xp54 can be detected in the nucleus, an exclusively nuclear function would not explain the high levels of Xp54 which are maintained in cytoplasmic mRNP. Like the Y-box proteins, which package the mRNA, Xp54 levels decline on progression from rapid embryonic cell cleavage to mid-blastoula, when stored maternal mRNP particles are mostly used up and zygotic transcription takes over (4).

In considering what might regulate the onset of Xp54 helicase activity, two aspects are worth mentioning. (i) It has been shown previously that mRNP particles in oocytes carry an associated casein kinase II (CK2) activity (9) and that phosphorylation of FRGY2a/b stabilizes its binding to mRNA (28,30). Xp54 also has multiple potential CK2 phosphorylation sites: four, out of a total of five, being located near the C-terminus. However, it is significant that Xp54, in contrast with FRGY2a/b, is inefficiently phosphorylated both in vitro and in vivo in immature oocytes (9). Perhaps the turnover of phosphates in Xp54 is much slower, or else the CK2 sites are blocked during the assembly of mRNP particles, only becoming accessible for phosphorylation at some later stage. Modification at these sites, whether by phosphorylation or dephosphorylation, might act as a trigger for helicase activation at an appropriate stage of development. (ii) Full helicase activity in vivo may require the assistance of an additional protein factor, much in the same way that eIF4A requires eIF4B (39). Such a cofactor might only become available at certain times of development, and/or might only bind the mRNP complex in response to an appropriate change in the phosphorylation status of Xp54. It is possible that a cofactor is present in the column fraction used in this report to demonstrate helicase activity, since Xp54 was not purified.

The combination of Xp54, Y-box proteins and CK2 with maternal mRNA should provide a useful model to explore the functioning of stored mRNP particles. Findings would be relevant, not only to germ cells (32) and early development, but also to proliferating somatic cells in which high levels of Y-box proteins are detected (40) and to somatic cells in which an association of Y-box proteins with stored mRNA has been demonstrated (41).

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