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The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of *Xenopus* oocytes

Christof G.Körner, Michael Wormington[1], Martina Muckenthaler[2], Sandra Schneider[3], Eva Dehlin[4] and Elmar Wahle[4,5]

Institut für Biochemie and [3]Institut für Genetik, Universität Giessen, Heinrich-Buff-Ring 58, 35392 Giessen, Germany, [1]Department of Biology, University of Virginia, Charlottesville, VA 22903-2477, USA and [2]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

[4]Present address: Universität Halle-Wittenberg, Institut für Biochemie, 06099 Halle, Germany

[5]Corresponding author
e-mail: ewahle@biochemtech.uni-halle.de

C.G.Körner and M.Wormington contributed equally to this work

Exonucleolytic degradation of the poly(A) tail is often the first step in the decay of eukaryotic mRNAs and is also used to silence certain maternal mRNAs translationally during oocyte maturation and early embryonic development. We previously described the purification of a poly(A)-specific 3′-exoribonuclease (deadenylating nuclease, DAN) from mammalian tissue. Here, the isolation and functional characterization of cDNA clones encoding human DAN is reported. Recombinant DAN overexpressed in *Escherichia coli* has properties similar to those of the authentic protein. The amino acid sequence of DAN shows homology to the RNase D family of 3′-exonucleases. DAN appears to be localized in both the nucleus and the cytoplasm. It is not stably associated with polysomes or ribosomal subunits. *Xenopus* oocytes contain nuclear and cytoplasmic DAN isoforms, both of which are closely related to the human DAN. Anti-DAN antibody microinjected into oocytes inhibits default deadenylation during progesterone-induced maturation. Ectopic expression of human DAN in enucleated oocytes rescues maturation-specific deadenylation, indicating that amphibian and mammalian DANs are functionally equivalent.

Keywords: deadenylation/3′-exoribonuclease/mRNA stability/oocyte maturation/poly(A) tails

Introduction

The cellular concentrations of specific mRNAs are controlled by the rates of both synthesis and degradation. mRNAs are not degraded by random nucleolytic events but by defined pathways and at rates that are specific for different RNAs (Beelman and Parker, 1995; Ross, 1995, 1996; Caponigro and Parker, 1996a; Jacobson and Peltz, 1996).

Two degradation pathways have been described in which the poly(A) tail plays a prominent role. In *Escherichia coli*, poly(A) tails are added to mRNAs and to mRNA fragments generated by the endonuclease RNase E. The poly(A) tail appears to serve as an unstructured entry site for the 'degradosome', a complex containing, among other proteins, the processive 3′-exonuclease polynucleotide phosphorylase. Polynucleotide phosphorylase digests the poly(A) tail and then advances into the coding sequence. An RNA-dependent ATPase, also a component of the degradosome, helps the exonuclease overcome inhibitory structures in the mRNA body (Cohen, 1995; O'Hara *et al*., 1995; Xu and Cohen, 1995; Py *et al*., 1996). A similar mechanism operates in chloroplasts (Lisitsky *et al*., 1997). Recently, it has been reported that RNase E can also degrade poly(A) tails (Huang *et al*., 1998).

In eukaryotes, exonucleolytic shortening of the poly(A) tail also initiates the decay of many, but not all, mRNAs (Wilson and Treisman, 1988; Shyu *et al*., 1991; Muhlrad and Parker, 1992; Decker and Parker, 1993; Dellavalle *et al*., 1994). However, in contrast to the bacterial process, the nuclease responsible for deadenylation does not appear to advance rapidly into the coding sequence. Instead, the second step in the major pathway of mRNA degradation, at least in *Saccharomyces cerevisiae*, is the removal of the 5′ cap by a specific pyrophosphatase (Beelman *et al*., 1996; LaGrandeur and Parker, 1998). Decapping, which does not take place until the poly(A) tail has been shortened to 10–15 nucleotides, exposes the mRNA to 5′-exonucleases, the most important of which is encoded by the *XRN1* gene (Muhlrad *et al*., 1994; Caponigro and Parker, 1996a). A second general pathway of mRNA decay involves 3′–5′ degradation of the mRNA body after deadenylation (Jacobs Anderson and Parker, 1998).

While observation of the initial deadenylation process in mammalian cells is straightforward, the subsequent steps are so rapid that a lack of detectable intermediates has precluded a complete analysis of the degradation pathway. However, indirect evidence favors decapping as the second step (Couttet *et al*., 1997). A mouse homolog of the XRN1 exonuclease has also been described (Bashkirov *et al*., 1997).

In general, stable mRNAs are deadenylated slowly whereas unstable mRNAs are deadenylated rapidly. Rapid decay depends on destabilizing elements within either the 3′-untranslated region (3′-UTR) or the coding sequences. Mutations in these elements usually result in both mRNA stabilization and a corresponding decrease in the rate of deadenylation (Muhlrad and Parker, 1992; Decker and Parker, 1993; Lagnado *et al*., 1994; Schiavi *et al*., 1994; Zubiaga *et al*., 1995; Xu *et al*., 1997). Conversely, inactivation of a stabilizing element in the α-globin mRNA leads to both destabilization and accelerated deadenylation (Morales *et al*., 1997). These data strongly suggest that the rate of mRNA decay can be controlled by the rate of poly(A) tail shortening. However, depending on the particular mRNA, subsequent steps can also be rate-limiting (Caponigro and Parker, 1996a,b). The so-called AU-rich

destabilizing elements also promote the degradation of RNAs that are not polyadenylated (Fan *et al*., 1997).

In addition to its role in mRNA decay, deadenylation is an evolutionarily conserved mechanism to silence maternal mRNAs translationally during oocyte maturation and early embryogenesis in diverse species (Richter, 1996). Translational inactivation by deadenylation may be explained at least in part by the fact that the poly(A) tail plays an important role in the initiation of translation (Sachs *et al*., 1997). In contrast to the situation in somatic cells, deadenylation during the meiotic maturation of both *Xenopus* and mouse oocytes does not require specific *cis*-sequences but is the default pathway for mRNAs that are not protected from deadenylation by active extension of their poly(A) tails (Fox and Wickens, 1990; Varnum and Wormington, 1990; Huarte *et al*., 1992). Deadenylation of maternal mRNAs is not restricted to the default reaction in mature oocytes. The 3′-UTRs of certain *Xenopus* mRNAs that are polyadenylated during maturation contain additional elements that promote their deadenylation after fertilization, thereby restricting their translation to mature oocytes (Bouvet *et al*., 1994; Legagneux *et al*., 1995). Sequence-specific deadenylation has also been defined as a crucial determinant of embryonic patterning in *Drosophila* (Wreden *et al*., 1997). In all of these developmental situations, deadenylation does not destabilize mRNAs immediately, but appears to be a prerequisite for their subsequent degradation at later stages (Audic *et al*., 1997). This uncoupling of deadenylation from mRNA turnover differs from both *S.cerevisiae* and metazoan somatic cells in which poly(A) removal rapidly triggers mRNA decay.

In no case have the enzymes responsible for poly(A) degradation been identified with certainty. In yeast, genetic evidence shows that deadenylation depends, directly or indirectly, on the poly(A)-binding protein, PAB I (Sachs and Davis, 1989; Caponigro and Parker, 1995). A PAB I-dependent poly(A) nuclease (PAN) has been purified (Lowell *et al*., 1992; Sachs and Deardorff, 1992), but PAN mutants have only mild defects in deadenylation, so the existence of additional poly(A)-degrading enzymes seems certain (Boeck *et al*., 1996; Brown *et al*., 1996). In oocytes, PAB I inhibits deadenylation (Wormington *et al*., 1996), and the same may be true in somatic cells (Bernstein *et al*., 1989).

We recently described the purification of a candidate enzyme for deadenylation in mammalian cells (Körner and Wahle, 1997). The deadenylating nuclease (DAN) is a poly(A)-specific 3′-exoribonuclease. In the presence of PAB I, this enzyme produces a pattern of deadenylation intermediates resembling that observed *in vivo*. We now report the cloning of a human cDNA encoding DAN. With respect to catalytic properties and primary structure, the enzyme is related to the DAN that catalyzes default deadenylation during the meiotic maturation of *Xenopus* oocytes.

Results

Cloning of cDNAs for DAN

Sequencing of peptides derived from purified bovine DAN (Körner and Wahle, 1997) resulted in three unique amino acid sequences, one mixture of two sequences and one minor, ambiguous sequence. Database searches identified a family of human expressed sequence tags (ESTs) that

M E I I R S N F K S N L H K V Y Q A I E E A D F F A I
D G E F S G I S D G P S V S A L T N G F D T P E E R
Y Q K L K K H S M D F L L F Q F G L C T F K Y D Y T
D S K Y I T **K S F N F Y V F P K P F N R S S P**
D V K F V C Q S S S I D F L A S Q G F D F N K V F
R N G I P Y L N Q E E E R Q L R E Q Y D E K R S Q A
N G A G A L S Y V S P N T S K C P V T I P E D Q K K
F I D Q V V E K I E D L L Q S E E N K N L D L E P C T
G F Q R K L I Y Q T L S W K Y P K G I H V E T L E T
E K K E R Y I V I S K V D E E E R K R R E Q Q K H A
K E Q E E L N D A V G F S R V I H A I A N S G K L
V I G H N M L L D V M H T V H Q F Y C P L P A D L S
E F K E M T T C V F P R L L D T K L M A S T Q P F K
D I I N N T S L A E L E K R L K E T P F N P P K V E S
A E G F P S Y D T A S E Q L H E A G Y D A Y I T G L
C F I S M A N Y L G S F L S P P K I H V S A R S K L I
E P F F N **K L F L M R V M D** I P Y L N L E G P D L
Q P K R D H V L H V T F P K E W K T S D L Y Q L F S
A F G N I Q I S W I D D T S A F V S L S Q P E Q V K I
A V N T S **K Y A E S Y** R I Q T Y A E Y M **G** R K Q
E E K Q I K R K W T E D S W K E A D S K R L N P Q
C I P Y T L Q N H Y Y R N N S F T A P S T V G K R N
L S P S Q E E A G L E D G V S G E I S D T E L E Q T
D S C A E P L S E G R K K A K K L K R M K K E L S P
A G S I S K N S P A T L F E V P D T W

Fig. 1. Amino acid sequence of human DAN. The sequence was derived from the cDNA. Peptide sequences corresponding to those obtained from purified bovine DAN after Lys C digestion (see Material and methods) are in bold. In the last peptide, three amino acids were different between the human cDNA and the bovine peptide sequence. Peptides corresponding to those obtained from the *Xenopus* p62 DAN isoform after trypsin digestion are underlined. The *Xenopus* peptides had the following sequences: 1, (I/L)F(I/L)MR; 2, Y(I/L)V (I/L)SK; 3, (I/L)MASVPFK; and 4, (I/L)QTYAEY(I/L)EK.

contained several of these peptide sequences. Complete sequencing of the longest EST clone revealed an open reading frame (ORF) coding for a protein of 639 amino acids with a molecular mass of 73.5 kDa (Figure 1; DDBJ/EMBL/GenBank accession No. AJ005698), in agreement with the apparent molecular mass of purified DAN. The sequence contained all unambiguous DAN peptide sequences. The 57 nucleotides preceding the first AUG codon did not contain an in-frame stop codon. Attempts to amplify additional upstream sequence from a cDNA library were not successful. N-terminal amino acid sequence could also not be obtained. Thus, there is no firm evidence that the first AUG in the clone is in fact the true start codon. However, the sequence surrounding this codon (AGAAUGG) corresponds to the 'Kozak rules' (Kozak, 1991), and experiments presented below suggest that the amino acid sequence as shown in Figure 1 is complete. The DAN-coding region is followed by 0.7 kb of 3′-UTR and a poly(A) tail preceded by the rare polyadenylation signal AUUAAA.

A 3′-exoribonuclease with catalytic properties similar to those of mammalian DAN has also been purified from

Xenopus oocytes (P.R.Copeland and M.Wormington, unpublished). Two polypeptides of 62 and 74 kDa co-purified with the activity. Several tryptic peptide sequences were obtained from the p62 isoform, and very good matches to these are present in the human DAN sequence (Figure 1).

A Northern blot of poly(A)$^+$ RNA from HeLa cells showed a single 3.1 kb band hybridizing to a DAN probe (data not shown), in good agreement with the size of the cDNA clone. The databases contain numerous DAN ESTs derived from a wide variety of tissues, consistent with ubiquitous expression of the gene. Widespread expression was confirmed by a multi-tissue Northern blot (K.Buiting and B.Horsthemke, personal communication).

Expression of DAN in E.coli

DAN was expressed in *E.coli* as a fusion protein with an N-terminal Met-Ala-His$_6$ tag. Purification on an Ni^{2+}-NTA column, followed by anion exchange chromatography, resulted in a nearly homogeneous preparation of a protein of the expected molecular mass, which had poly(A)-specific 3′-exonuclease activity (Figure 2; see below). These results confirm that the cDNA clone encodes DAN.

Rabbit antibodies raised against a C-terminal DAN fragment expressed in *E.coli* precipitated DAN activity from partially purified fractions (data not shown). They also recognized recombinant DAN as well as bovine and human DAN in Western blots (Figures 2 and 3). Recombinant DAN appeared slightly larger than the enzyme present in an SDS lysate of HeLa cells, presumably due to the presence of the His tag. This is consistent with the first ATG in the cDNA sequence being used as the initiation codon *in vivo*.

Properties of recombinant DAN

Trichloroacetic acid (TCA) precipitation assays with homogeneously labeled poly(A) were used for an initial characterization of the enzyme. Like bovine DAN (Körner and Wahle, 1997), recombinant DAN was active depending on phosphate charge neutralization under two different reaction conditions. In the absence of salt, activity was absolutely dependent on the presence of spermidine, with an optimum of 1 mM. Under these conditions, the specific activity of recombinant DAN (79 000 U/mg) was not significantly different from that of purified bovine DAN (110 000 U/mg). With spermidine, salt was inhibitory at all concentrations. In the absence of spermidine, DAN activity was salt dependent, with an optimum of 150 mM potassium acetate. This is again similar to bovine DAN (Körner and Wahle, 1997). However, the activity of the recombinant protein under these conditions (960 U/mg) was lower than that of DAN purified from calf thymus (8070 U/mg). Possibly, the activity is influenced either by a post-translational modification or by another protein contaminating the bovine enzyme preparation.

Recombinant DAN was also assayed with a capped, polyadenylated RNA as a substrate, and products were analyzed by denaturing gel electrophoresis. In the presence of salt, DAN degraded the poly(A) tail rapidly; completely deadenylated RNA accumulated transiently (Figure 4A, lane 3, and B) and was degraded upon further incubation. A fraction of the substrate RNA was always resistant to degradation, possibly due to an unknown modification of the 3′ end, e.g. phosphorylation (Körner and Wahle, 1997). When the assay was carried out under the same conditions

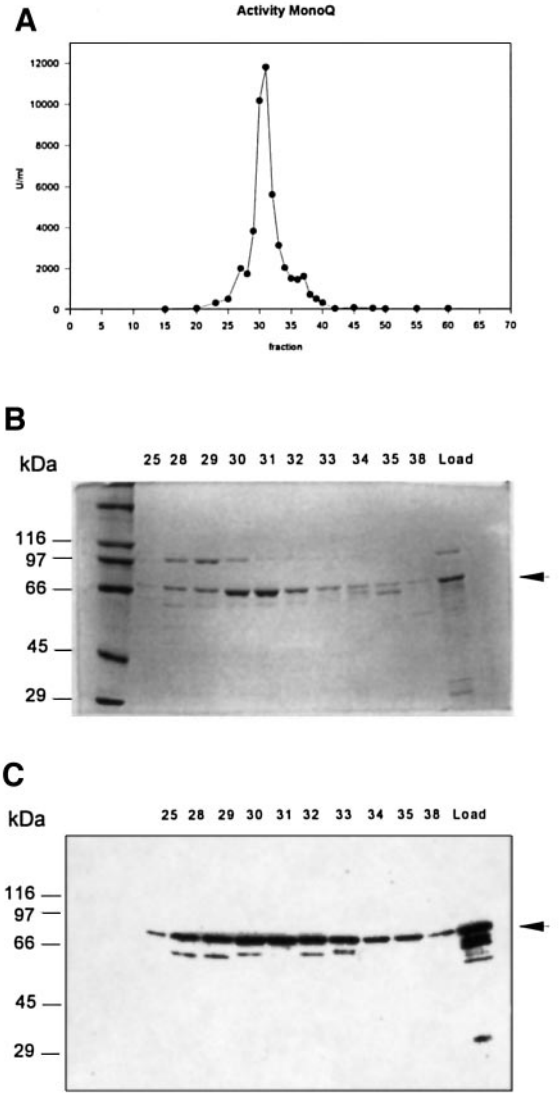

Fig. 2. Expression of recombinant human DAN. (**A**) Activity profile of the final MonoQ column. DAN activity was monitored by a TCA precipitation assay in the presence of spermidine. (**B**) SDS–polyacrylamide gel of MonoQ fractions. Aliquots (10 µl) of the fractions indicated at the top were separated on an 8% gel. (**C**) Western blot analysis of an SDS–polyacrylamide gel corresponding to (B). A 5 µl aliquot of the MonoQ fractions was applied. DAN was detected with a rabbit serum directed against the C-terminal 176 amino acids of human DAN. The molecular mass of marker proteins (in kDa) is indicated on the left. The 74 kDa band co-migrating with DAN activity is indicated by an arrowhead on the right. Load (5 µl of the Ni^{2+}-NTA eluate).

but in the presence of PAB I, the nuclease activity was partially inhibited, and phased poly(A) shortening was observed. Prominent deadenylation intermediates differed in length by ~30 nucleotides, obviously due to protection of the poly(A) tail by PAB I (Figure 4, lanes 7–11). Again, deadenylated RNA was generated but degraded upon further incubation. In the absence of salt and spermidine, DAN was activated weakly by PAB I (data not shown). These results are very similar to those described previously for the authentic protein (Körner and Wahle, 1997). Together, they show that recombinant DAN is a 3′-exonuclease with a preference for a poly(A) substrate.

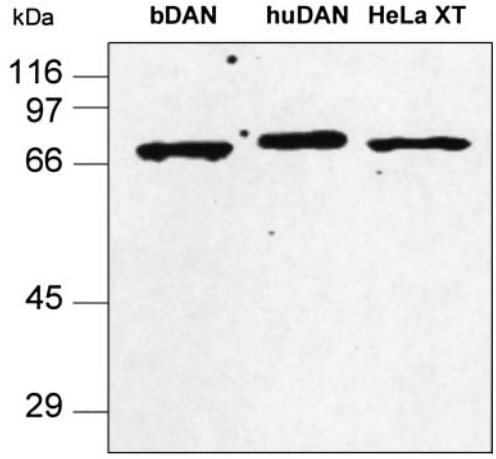

Fig. 3. Western blot analysis of DAN. Purified bovine DAN (bDAN) (Körner and Wahle, 1997), recombinant human DAN (huDAN, fraction 31 of the column shown in Figure 2) and an SDS lysate of HeLa cells were probed with affinity-purified anti-DAN antibodies. The molecular mass of marker proteins (in kDa) is indicated on the left.

Localization of DAN in mammalian cells

Since deadenylation initiates the decay of mature mRNAs, DAN, if it is involved in this process, should be localized in the cytoplasm. Affinity-purified antibodies were used to investigate the localization of DAN in HeLa cells. Diffuse staining was observed mostly in the cytoplasm, but some nuclear staining was detectable. Within the nucleus, nucleoli sometimes were labeled more strongly than the nucleoplasm (Figure 5). Control experiments with pre-immune serum showed very weak labeling, mostly in the nucleus (data not shown). In nuclear and cytoplasmic fractions of HeLa cells, similar amounts of DAN were detectable by Western blots (data not shown).

In Western blots of total cell lysates, the antibodies used usually recognized a single 74 kDa polypeptide (Figure 3). However, since a second, weaker band was detected erratically in some blots, additional experiments were performed to confirm the localization. A construct in which the green fluorescent protein (GFP) was fused to the N-terminus of full-length DAN was expressed in Cos-1 cells by transient transfection. Labeling of cells was heterogeneous: while approximately two-thirds showed a clear nuclear signal, one-third displayed cytoplasmic fluorescence, either exclusively or in addition to the nuclear signal (Figure 5). Based on these data and those described below, we tentatively conclude that DAN is localized in both the nucleus and the cytoplasm.

A possible association of DAN with mRNA or ribosomes during translation was investigated by the analysis of polysomes on sucrose gradients, followed by Western blotting. DAN was found exclusively in the upper fractions of such gradients (Figure 6). A stable association with polysomes or ribosomal subunits was not detectable. As a control, PAB I was detected easily in the polysome fractions (Figure 6) and shifted to the top of the gradient when polysomes were dissociated by EDTA (data not shown).

Human and Xenopus oocyte DANs are functionally related

His-tagged, full-length DAN was used to generate an additional rabbit polyclonal antibody. After affinity purification, this antibody recognized both the p62 and p74 polypeptides that co-purify with *Xenopus* DAN activity through poly(A)–Sepharose chromatography (P.Copeland and M.Wormington, unpublished data; Figure 7A, lane 6).

The default deadenylation of maternal mRNAs in *Xenopus* oocytes is triggered by progesterone-induced meiotic maturation. Poly(A) removal is not observed before dissolution of the oocyte nucleus, and enucleated oocytes

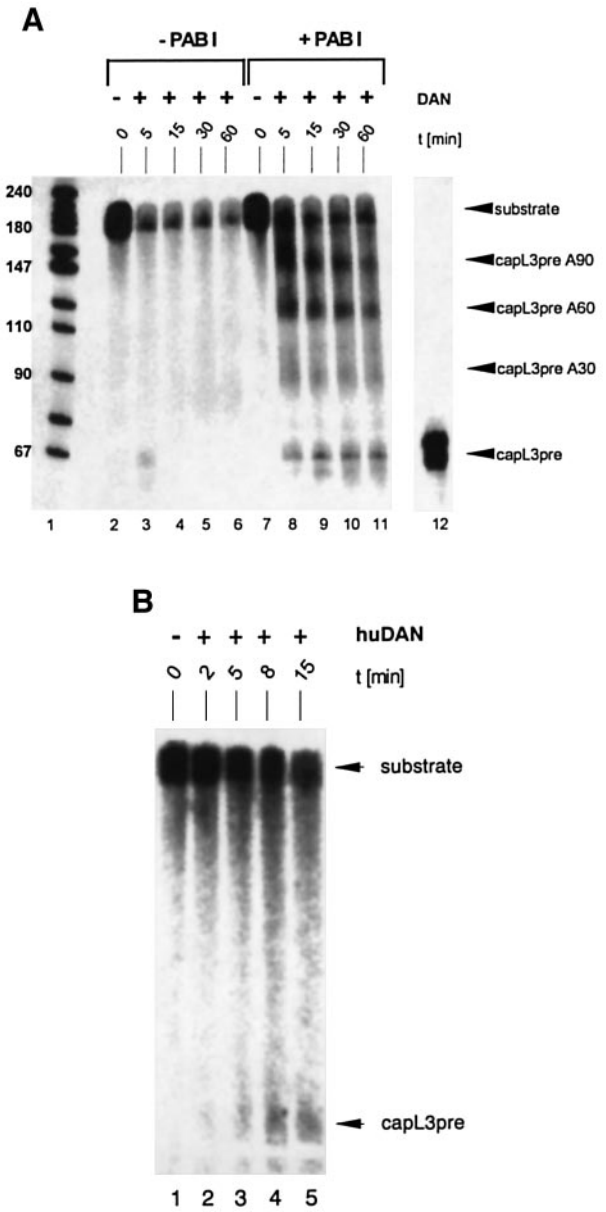

Fig. 4. mRNA deadenylation by recombinant human DAN. (**A**) Two reaction mixtures were assembled, each containing 30 fmol of capped and polyadenylated (A_{115}) L3pre RNA in 50 µl of reaction buffer plus 120 mM KAc. One mixture received, in addition, 126 fmol of PAB I. Both mixtures were incubated at 37°C with 90 fmol of human DAN. Aliquots were taken at the times indicated and analyzed on a denaturing 8% polyacrylamide gel. Sizes (in nucleotides) of DNA size standards (lane 1) are indicated on the left. Substrate and decay products are indicated on the right. Lane 12, non-polyadenylated capped L3pre RNA. (**B**) A reaction mixture was assembled containing 20 fmol of capped and polyadenylated (A_{150}) L3pre RNA in 50 µl of reaction buffer containing 120 mM Kac. The mixture was incubated at 37°C with 20 fmol of human DAN. Aliquots were taken at the times indicated and analyzed as in (A).

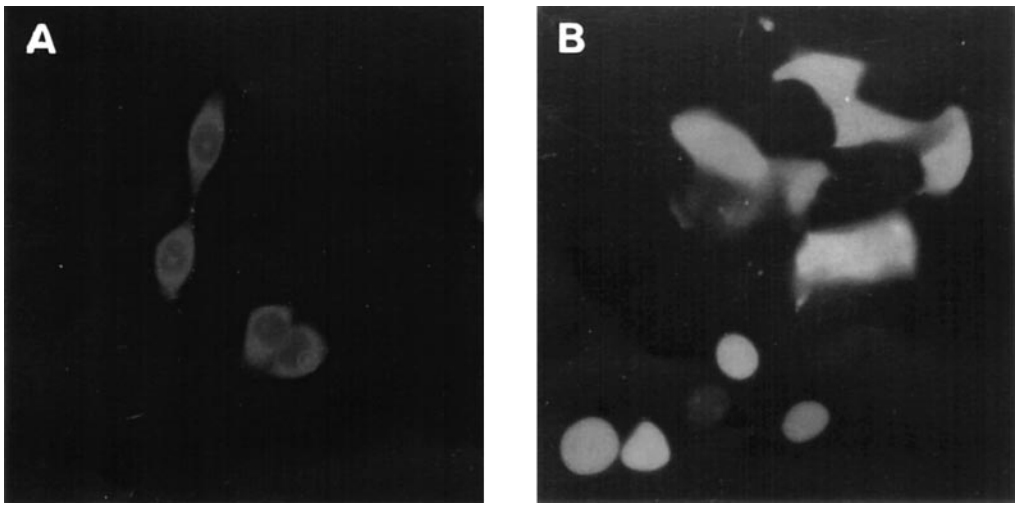

Fig. 5. Intracellular localization of DAN. (**A**) Immunofluorescence detection of DAN. Affinity-purified antibody directed against the C-terminus was used. The same cells were also stained with 4′,6-diamidino-2-phenylindole (DAPI) for identification of the nuclei (not shown). (**B**) Localization of the GFP–DAN fusion. The lower left corner contains cells with nuclear staining, whereas the upper right corner contains cells with cytoplasmic staining. Nuclei and cytoplasm were identified by phase-contrast microscopy (not shown).

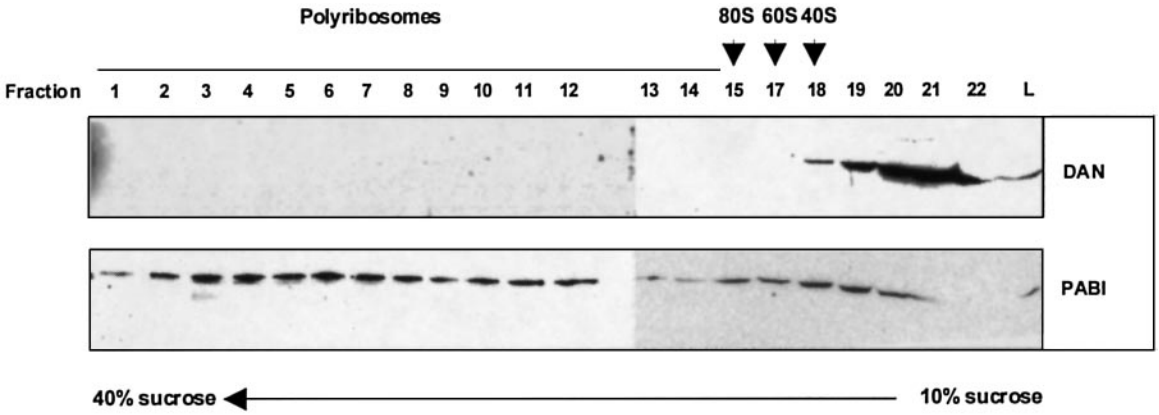

Fig. 6. Sedimentation profile of human DAN in a cytoplasmic extract. A HeLa cell cytoplasmic extract was sedimented through a 10–40% sucrose gradient (see Materials and methods). The sedimentation of DAN and PAB I was monitored by Western blot analysis. DAN was detected with a rabbit serum directed against the entire protein (205). PAB I was detected with a monoclonal antibody (10E10). The positions of the 40S and 60S subunits, the 80S monosomes, as well as the polyribosomes are indicated. L, load (HeLa cell cytoplasmic extract).

lack default DAN activity *in vivo*. Thus, default deadenylation is prevented in stage VI oocytes by the sequestration of one or more DAN components within the nucleus and is initiated upon their release into the cytoplasm at maturation (Fox and Wickens, 1990; Varnum *et al*., 1992). The predominant protein detected by the DAN antibody in both oocyte and egg extracts was p74 (Figure 7A, lanes 2 and 3). A second protein of 62 kDa probably corresponds to the p62 species of purified *Xenopus* DAN. The two proteins exhibited distinct nuclear–cytoplasmic distributions in stage VI oocytes; p62 was cytoplasmic, whereas p74 was exclusively nuclear (Figure 7A, compare lanes 3, 4 and 5). The nuclear localization of p74 is consistent with the idea that the default deadenylase activity is sequestered in the nucleus. As cytoplasmic extracts lack default deadenylation activity *in vitro*, the functionality of p62 in stage VI oocytes and its relationship to p74 remain to be determined. The p74 present in oocyte and egg extracts migrates as a protein doublet, the relative intensities of which are altered as a consequence of maturation (Figure 7A, compare lanes 2 and 3). Similar changes in electrophoretic mobility due to altered phosphorylation after oocyte maturation have been reported for other proteins involved in poly(A) metabolism (Hake and Richter, 1994; Ballantyne *et al*., 1995). Additional immunoblot analyses indicated that both p74 and p62 accumulate throughout oogenesis and persist after fertilization (not shown).

When a synthetic mRNA encoding human DAN with an N-terminal myc 9E10 epitope tag was injected into stage VI oocytes, myc-DAN was distributed equally between the nucleus and cytoplasm, as indicated by immunoblots (Figure 7B). This is consistent with the intracellular distribution seen in mammalian cells (see above).

Having established that p62 and p74 are related to the human DAN, we determined if affinity-purified anti-DAN antibody can inhibit the default deadenylation of endogenous mRNAs in mature oocytes *in vivo* and deplete *Xenopus* DAN activity *in vitro*. The progesterone-induced maturation of stage VI oocytes triggers the deadenylation of endogenous ribosomal protein L5 and S22 mRNAs. The cytoplasmic injection of anti-DAN antibody prevented this reaction (Figure 8A). Injection of pre-immune IgG was not inhibitory (Figure 8A, lanes 5 and 6). The anti-DAN antibody also depleted partially purified *Xenopus* DAN

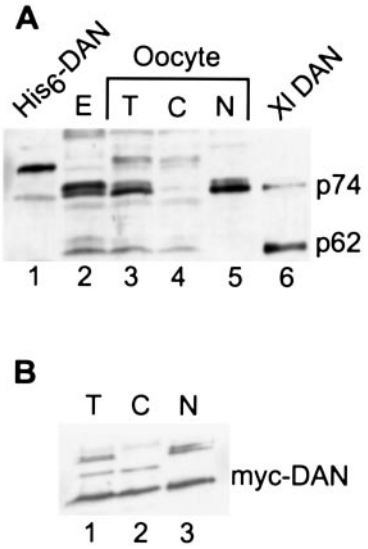

Fig. 7. Immunoblot analysis of DAN in *Xenopus* oocytes. (**A**) Immunoblot analysis of endogenous DAN. Lane 1 contained 10 ng of purified human His_6-DAN derived from the pET30 construct (see Materials and methods). Lanes 2–5 each contained one oocyte-equivalent of protein isolated from unfertilized egg (E), stage VI oocyte (T), oocyte cytoplasm (C) and oocyte nucleus (N). Lane 6, 15 ng of poly(A)–Sepharose-purified *Xenopus* DAN. (**B**) Immunoblot analysis of ectopically expressed human myc-DAN in *Xenopus* oocytes. Each lane contained one oocyte-equivalent of total (T), cytoplasmic (C) or nuclear (N) protein.

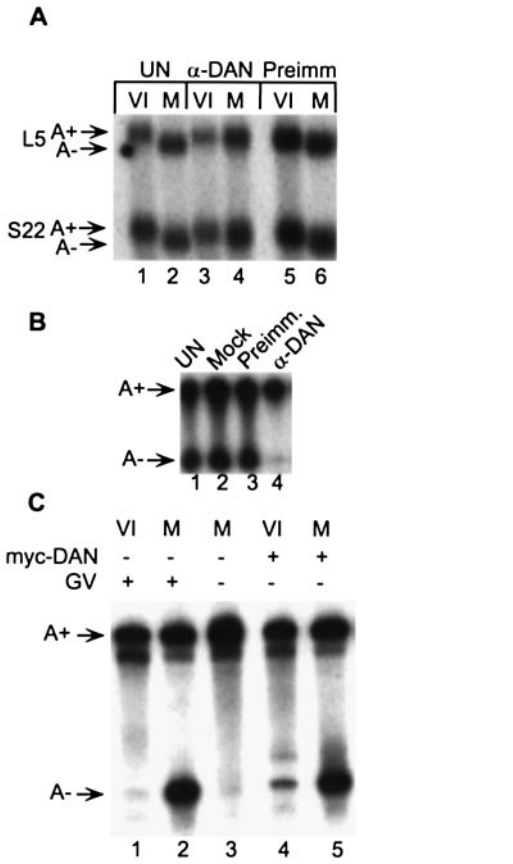

Fig. 8. DAN-dependent mRNA deadenylation in *Xenopus* oocytes. (**A**) Deadenylation of endogenous rp-S22 and L5 mRNAs. Endogenous RNAs were analyzed by Northern blotting in stage VI (VI) and progesterone-matured (M) oocytes. Arrows denote positions of polyadenylated and deadenylated transcripts. Each lane contained total RNA extracted from two oocytes. Stage VI oocytes were either uninjected (lanes 1 and 2) or cytoplasmically injected with 100 ng of affinity-purified anti-DAN IgG (lanes 3 and 4) or pre-immune IgG (lanes 5 and 6) and incubated in either the absence (VI) or presence (M) of progesterone. (**B**) Deadenylation *in vitro*. Poly(A)–Sepharose-purified *Xenopus* DAN (lane 1) was either mock-depleted with protein A–Sepharose (lane 2), pre-immune serum pre-bound to protein A–Sepharose (lane 3), or depleted with anti-DAN serum pre-bound to protein A–Sepharose (lane 4), prior to the addition of radiolabeled deadenylation substrate RNA. Reaction products were separated by 6% polyacrylamide–7 M urea gel electrophoresis. (**C**) Complementation of enucleated oocytes by DAN mRNA. Stage VI oocytes were injected cytoplasmically with radiolabeled G52 deadenylation substrate RNA. Control (GV+) and enucleated (GV–) oocytes were incubated in the absence (VI) or presence (M) of progesterone. A subset of enucleated oocytes were injected cytoplasmically with 20 ng of synthetic myc-DAN RNA (+) prior to progesterone treatment. RNA was extracted and electrophoresed on a 6% polyacrylamide–7 M urea gel.

in vitro (Figure 8B). Immunoblot analysis confirmed that both p62 and p72 were depleted quantitatively (not shown). Based on these results, we conclude that *Xenopus* DAN catalyzes default deadenylation during oocyte maturation.

We then determined if the human DAN can substitute for the *Xenopus* nuclease and catalyze default deadenylation during oocyte maturation *in vivo*, thereby establishing their functional equivalence. A synthetic mRNA encoding human myc-DAN was injected into enucleated oocytes that had been injected previously with a radiolabeled deadenylation substrate, G52 RNA. The oocytes were then incubated in either the presence or absence of progesterone, and deadenylation of the G52 substrate RNA was assayed. In agreement with earlier studies (Varnum *et al*., 1992), enucleation eliminated maturation-specific deadenylation (Figure 8C, compare lanes 2 and 3). However, injection of the synthetic myc-DAN mRNA efficiently rescued deadenylation in enucleated oocytes upon progesterone treatment (Figure 8C, lane 5). A small amount of deadenylation was observed in myc-DAN-injected enucleated oocytes even without progesterone treatment (Figure 8C, lane 4). This precocious poly(A) removal most likely reflects the absence of the nucleus and thus the lack of sequestration of the ectopically expressed DAN. However, deadenylation was stimulated 10-fold by progesterone treatment (Figure 8C, compare lanes 4 and 5). This raises the interesting possibility that, although cytoplasmic myc-DAN is present in the RNA-injected oocytes, post-translational modifications, such as phosphorylation, that occur in response to maturation may be required for its maximal activity.

Discussion

We have cloned a cDNA for a mammalian deadenylating nuclease, DAN. The identity of the clone was confirmed by the expression of catalytically active protein in *E.coli* and in *Xenopus* oocytes. Database searches revealed homologs of DAN in diverse organisms, including plants. Surprisingly, no yeast gene with obvious similarity to DAN was detected in these searches. Mian and colleagues recently have defined the RNase D family of 3′-exonucleases, including both DNases and RNases (Mian, 1997; Moser *et al*., 1997). One member of this family, not proven to be an exonuclease so far and included based solely on sequence criteria, is the potential *Caenorhabditis elegans* homolog of DAN. In all RNase D family members, the amino acids known to be essential for catalytic activity are arranged in three clusters

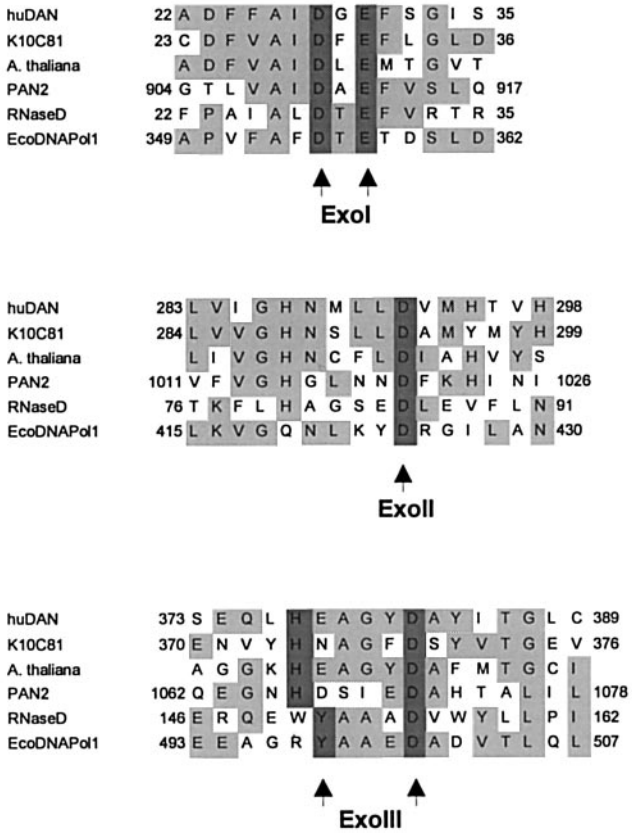

Fig. 9. DAN is a member of the RNase D family. Aligment of human DAN with members of the RNase D family (Moser *et al*., 1997). huDAN, human DAN; K10C81, sequence from the *C.elegans* genome project; *Arabidopsis thaliana*, sequence from the *Arabidopsis* genome project (DDBJ/EMBL/GenBank accession No. AC002304); PAN2, *S.cerevisiae* poly(A) nuclease subunit 2; EcoDNAPol1, *E.coli* DNA polymerase I; RNaseD, *E.coli* RNase D. Conserved amino acids of the ExoI, ExoII and ExoIII motifs characteristic for this class of exonucleases are marked by arrowheads and boxed with dark shading. Other conserved amino are boxed in gray. Numbers at the margins refer to the amino acids at the boundaries of these domains in the respective proteins. The *Arabidopsis* sequence appears to contain introns, therefore the sequences shown here cannot be numbered with confidence. This may also be the reason why it was not included in the aligment of Moser *et al*. (1997). Accession numbers can be found in Moser *et al*. (1997).

initially defined by Bernad *et al*. (1989). Their functions are known mostly from studies of the exonuclease domain of *E.coli* DNA polymerase I (Joyce and Steitz, 1994). Three acidic side chains, two in the ExoI motif and one in the ExoIII motif, directly bind the two Mg^{2+} ions essential for catalysis. An additional acidic residue, in the ExoII motif, binds one of the metal ions through bridging water molecules (Joyce and Steitz, 1994). All of these amino acids are highly conserved within the family and are also found in DAN (Figure 9). In the 3′-exonuclease of DNA polymerase I, a tyrosine residue in the ExoIII motif orients the attacking water molecule (Joyce and Steitz, 1994). This tyrosine is not conserved in some RNase D family members. Among the nucleases not sharing the tyrosine, most have a histidine at the preceding position, which may substitute for the tyrosine in binding the water molecule (Moser *et al*., 1997). Replacement of this histidine by alanine in DNA polymerase III of *Bacillus subtilis* led to inactivation of the exonuclease (Barnes *et al*., 1995). The histidine is also found in DAN (Figure 9).

The yeast poly(A) nuclease, PAN, shares with DAN the specificity for poly(A) substrates, even when PAN's co-factor PAB I is replaced by the non-specific activator spermidine (Lowell *et al*., 1992). A subunit of PAN, the 127 kDa protein PAN2p, also belongs to the RNase D family (Moser *et al*., 1997). Thus, PAN and DAN are likely to share not only their preferred substrate but also their catalytic mechanism. However, PAN is no more similar to DAN than several other ORFs from the yeast genome. Thus, no conclusions can be drawn with respect to similar biological roles of PAN and DAN. As the RNase D family is composed of diverse exonucleases, it also remains to be demonstrated experimentally that those enzymes with stronger sequence similarity to DAN are in fact poly(A)-specific 3′-exoribonucleases.

Several lines of evidence strongly suggest that one isoform of *Xenopus* DAN catalyzes default deadenylation in mature oocytes. (i) A poly(A)-specific 3′-exoribonuclease with strong sequence similarity to human DAN has been purified from oocyte extracts, and antibodies raised against the recombinant human enzyme recognize the two polypeptides which co-purify with this activity. (ii) The default nuclease activity is sequestered in the nucleus of immature oocytes and thereby segregated away from its cytoplasmic mRNA substrates until it is released at germinal vesicle breakdown during maturation (Varnum *et al*., 1992). In agreement with this, Western blots show that the major p74 isoform of DAN is exclusively nuclear prior to maturation. (iii) Injection of anti-DAN antibody inhibits deadenylation in mature oocytes *in vivo*. (iv) Finally, human DAN can restore default deadenylation upon progesterone treatment of enucleated oocytes.

While the data presented here are consistent with previous proposals that sequestration of the deadenylation activity in the nucleus is involved in its regulation, three observations suggest that sequestration of the nuclease itself may not be a sufficient explanation. First, stage VI oocytes, which do not deadenylate their mRNAs, nevertheless contain a putative cytoplasmic isoform of DAN, p62. Secondly, injection of mRNA encoding human DAN into enucleated oocytes leads to accumulation of the enzyme in the cytoplasm. However, deadenylation activity is feeble unless maturation is induced by progesterone. Thirdly, progesterone-induced oocyte maturation is accompanied by a change in electrophoretic mobility of p74 DAN consistent with its possible phosphorylation. The nature, effect and regulation of the modification remain to be established. It will also be interesting to see whether a similar modification can account for the difference in activity between the recombinant DAN and the cognate enzyme purified from mammalian cells.

The default deadenylation reaction in mature oocytes differs from poly(A) removal in embryos and somatic cells as it does not require specific sequences in the substrate RNA. Moreover, it differs from deadenylation in somatic cells in that it does not lead to immediate degradation of the deadenylated RNA. It remains to be determined whether DAN is also involved in other deadenylation reactions such as the sequence-dependent deadenylation observed in *Xenopus* embryos and in somatic cells.

The substrate specificity of DAN remains to be clarified. The enzyme purified from calf thymus prefers poly(A) over other homopolymers and, with polyadenylated RNA as a substrate, an accumulation of

deadenylated RNA is readily observed. However, further incubation leads to complete degradation of the RNA body (Körner and Wahle, 1997). With recombinant protein, production of deadenylated RNA is visible but is also transient. Bacterially expressed DAN has not yet been assayed with homopolymer substrates. However, in several types of assays, we have seen differences between the mammalian and recombinant DANs (C.G.Körner and E.Wahle, unpublished data; see Results). The question of substrate specificity thus has to be investigated more thoroughly once recombinant DAN has been purified from a eukaryotic source. Whereas the deadenylated RNA is degraded slowly by DAN *in vitro*, it is stable in oocytes, including those ectopically expressing human DAN. Thus, other factors appear to affect the substrate specificity of the nuclease. The simplest possibility would be a protection of the mRNA body by RNA-binding proteins. In fact we have found that a relatively non-specific hnRNP protein can stabilize the deadenylated RNA in *in vitro* assays with purified DAN (C.G.Körner and E.Wahle, unpublished data).

DAN is not stably associated with polysomes in either somatic cells (this study) or *Xenopus* oocytes (Varnum and Wormington, 1990). The relationship between translation and mRNA decay is not clear (Jacobson and Peltz, 1996). Muckenthaler *et al*. (1997) have reported that a dissociation of mRNAs from ribosomes induced by various means leads to enhanced deadenylation. Thus, while deadenylation is the proximal cause of translational silencing in oocytes and embryos, it can be a consequence of translational inactivation in somatic cells. It is possible that DAN is responsible for this reaction and that the translational machinery protects mRNA in some way from the attack of this nuclease.

Materials and methods

Identification of human DAN cDNA clones

The purification of bovine DAN has been described previously (Körner and Wahle, 1997). Several active side fractions of three Superdex columns were pooled and applied to a phenyl-Superose column as described. DAN was purified further on a 100 µl Smart MonoQ (PC 1.6/5) column (Pharmacia). The two peak fractions were applied to a preparative SDS–polyacrylamide gel. *In situ* digestion by protease LysC, peptide separation and sequencing were performed by Toplab GmbH, Munich, Germany. The following sequences were obtained: (1) KSFNFYVFPK; (2) KPFNRSSPD(V/K)K; (3) KYAESYWIQTYADYVG; and (4) a 1:1 mixture of KEQEELNDA and KLFLMRVMD. Trypsin digestion of purified *Xenopus* p62 DAN, peptide separation and sequencing were performed by the Keck Biomedical Mass Spectrometry Laboratory at the University of Virginia Health Sciences Center (P.Copeland and M.Wormington, unpublished). EST database searches were performed with the advanced BLAST programs provided by the NCBI. I.M.A.G.E. consortium clone ID 301901, identified through peptide 3, and I.M.A.G.E. consortium clone ID 645295, identified through peptides 1 and 2, were obtained from the Ressourcenzentrum im Deutschen Genomprojekt, Berlin, Germany, and from the UK HGMP Resource Centre, Hinxton, Cambridge, UK, respectively (Lennon *et al*., 1996). Both were sequenced completely. Clone 301901 coded for the C-terminal 176 amino acids of DAN and the entire 3′-UTR. Clone 645295 contained the complete human DAN ORF, the 5′-UTR and part of the 3′-UTR. Sequencing was performed on a ABI373A sequencer with the ABI dye terminator cycle sequencing kit. The combined sequence of the two cDNAs has been deposited in the DDBJ/EMBL/GenBank database (AJ005698).

Plasmids

For generation of plasmid pGMMCS, the T7 expression vector pGM10 (Martin and Keller, 1996), containing the PAB II cDNA sequence (Nemeth *et al*., 1995) with an N-terminal Met-Ala-His_6 tag was digested with *Xho*I and *Bam*HI, and the fragment containing the 3′ portion of the PAB II insert was replaced with an *Xho*I–*Bam*HI fragment of the multiple cloning site of pBluescript KS (+/–). The resulting plasmid contains a T7 promoter-controlled coding sequence starting with Met-Ala-His_6, followed by the 5′ end of the PAB II-coding sequence and a multicloning site. An *Nde*I site located at the junction between the His_6 tag and the PAB II sequence can be used, in conjunction with the multicloning site, to replace the remaining PAB II sequence by any coding sequence desired.

For plasmid pGMMCS301901, the cDNA sequence encoding the C-terminal 176 amino acids of huDAN was amplified from clone 301901 by PCR with primers *Nde*I 301901, CCATATCCATATGCTTTTCA-GTGCCTTTGGTAAC, and *Xho*I 301901, AGTACTCGAGTTAC-AATGTGTCAGG. After digestion with *Nde*I and *Xho*I, the cDNA fragment was inserted into pGMMCS digested with *Nde*I and *Xho*I. The cDNA sequence was confirmed.

To produce plasmid pGMMCS645295, the coding sequence of huDAN was amplified from clone 645295 by PCR with the primers *Nde*I 645295, AGTGTCGCATATGGAGATAATCAGGAGCA, and *Xho*I 645295, AGTACTCAGACAGCGGTTTGCTGCCCTCA. The product was subcloned into the vector pCR2.1 with the TA-cloning kit (Invitrogen). After digestion with *Nde*I and *Xho*I, the cDNA fragment was inserted into pGMMCS digested with *Nde*I and *Xho*I. Most of the PCR-generated sequence was replaced with the *Dra*III–*Bst*EII fragment from the original clone, and the sequence of the remaining PCR-generated portion of the clone was confirmed. An independent His_6-DAN clone was generated as follows. The plasmid 645295 was digested with *Hha*I, blunt-ended with T4 DNA polymerase and digested with *Xho*I. The fragment containing the entire DAN-coding region preceded by 13 amino acids derived from the 5′-UTR (RARVGPRFRPLCR) was isolated and cloned into the *Eco*RV and *Xho*I sites of pET30a (Novagen).

For myc-DAN, complementary oligonucleotides encoding the myc 9E10 epitope (Evan *et al*., 1985) were annealed and cloned into the *Eco*RI and *Bgl*II sites of SP64TEN (Klein and Melton, 1994) to generate SP64-myc. The entire DAN-coding region was amplified from the plasmid 645295 using the primers DAN5, CAGATCTGCAGAATGGAGAT, and DAN3, GCCCTCAGATCTTGGTTACCATGT. The PCR product was digested with *Bgl*II and ligated to *Bgl*II-digested SP64-myc to generate myc-DAN. DNA was linearized with *Sal*I to generate a transcription template for SP6 RNA polymerase that encodes full-length DAN with the myc 9E10 epitope at its N-terminus.

To generate pEGFP-DAN, a synthetic double-stranded oligonucleotide encoding the hemagglutinin (HA) tag (Wilson *et al*., 1984) preceded by a *Kpn*I site was cloned into the *Nde*I site of pGMMCS645295, and the orientation was confirmed by sequencing. The DAN-coding sequence including the HA tag was cut out as a *Kpn*I–*Xho*I fragment and ligated into the corresponding sites of pEGFP-N1 (Clontech). The resulting plasmid expresses DAN fused to the C-terminus of EGFP.

Expression and purification of recombinant human DAN

DAN was expressed as a fusion with an N-terminal His tag as follows: pGMMCS645295 was transformed into *E.coli* BL21 (pLysS). Cells were grown at 33°C in LB medium supplemented with 100 µg/ml carbenicillin, induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and harvested 1 h later. Cells were resuspended in buffer A [50 mM Tris, pH 7.9, 300 mM KCl, 0.1 mM MgAc, 1 mM β-mercaptoethanol, 0.4 µg/ml leupeptin, 0.7 µg/ml pepstatin, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)] and sonicated. The lysate was centrifuged and the supernatant incubated with 2 ml of Ni^{2+}-NTA resin for 2 h at 4°C. The resin was packed into a column and washed with 25 ml of buffer A and then with 20 ml of buffer B [buffer A plus 10% (v/v) glycerol, 0.02 (v/v) NP-40, without magnesium, pH 6.3]. The protein was eluted with 5 ml of buffer B containing 500 mM imidazole. After dialysis against buffer C [50 mM Tris, pH 7.9, 20 mM KCl, 10% (v/v) glycerol, 0.02% (v/v) NP-40, 1 mM EDTA, 0.5 mM PMSF], the sample was applied to a 1 ml MonoQ column (Pharmacia) equilibrated with buffer C containing 50 mM KCl. The column was washed with five bed volumes of this buffer and developed with a gradient (40 bed volumes) from 50 to 500 mM KCl at 1 ml/min. DAN activity eluted in a sharp peak at ~190 mM KCl.

Antibodies

Escherichia coli BL21 (pLysS) was transformed with pGMMCS301901. Synthesis of the C-terminal DAN fragment was induced by IPTG in a 2.5 l culture volume, cells were harvested after 5 h and resuspended in buffer A (100 mM NaH_2PO_4, 10 mM Tris, pH 7.9, 8 M urea). They were lysed by sonication and the lysate was centrifuged and incubated with 3 ml of Ni-NTA resin for 2 h at room temperature. The resin was packed into a column and washed with 70 ml of buffer A, pH 6.3. After further washing with 15 ml of buffer B [300 mM NaCl, 10% (v/v) glycerol, 50 mM Tris pH 7.9, 0.01 (v/v) NP-40, 8 M urea], the protein was renatured on the column with

two 60 ml gradients at 45 ml/h with buffer B from 8 to 4 M urea (at room temperature) and from 4 to 0 M urea (at 4°C). The protein was eluted with buffer B + 500 mM imidazole, dialyzed against buffer B without urea and used for the production of polyclonal rabbit antibodies by Eurogentec, Belgium. Antibodies were affinity purified as follows. The C-terminal DAN fragment (2.2 mg) was coupled to a 1 ml NHS-activated HiTrap® column (Pharmacia), and antibodies were purified from 3 ml of serum according to the manufacturer's instructions. They were supplemented with 300 μg/ml bovine serum albumin (BSA) and 0.02% (w/v) NaN_3, and the buffer was exchanged against phosphate-buffered saline (PBS) by centricon (Amicon) concentration.

Full-length His_6-DAN was expressed from the pET30a construct and purified through Ni^{2+}-NTA chromatography as described, except that all buffers included 6 M urea. The protein was used by Covance Research Products, USA, to generate the rabbit polyclonal anti-DAN antiserum, 205. Antibodies were affinity purified using SDS–gel-purified His_6-DAN immobilized on a Hybond C membrane (Harlow and Lane, 1988). IgG was purified from pre-immune serum by affinity chromatography on protein A–Sepharose (Harlow and Lane, 1988). Affinity-purified anti-DAN and pre-immune IgGs were adjusted to 2.5 mg/ml in 88 mM NaCl prior to microinjection into oocytes.

The monoclonal antibody 10E10, directed against PAB I, was a gift from Matthias Görlach, Jena (Görlach *et al*., 1994).

Immunodepletions

The construction of pG52 and its use as a template to synthesize a capped deadenylation substrate RNA have been described (Varnum and Wormington, 1990). *Xenopus* DAN was purified from *Xenopus* ovaries by sequential chromatography of S100 extracts on DEAE–Sephacel, heparin–Sepharose and poly(A)–Sepharose columns (P.R.Copeland and M.Wormington, unpublished). For immunodepletions, 15 ng of poly(A)–Sepharose-purified *Xenopus* DAN was incubated for 30 min at 4°C with either 2.5 mg of protein A–Sepharose beads alone (mock depletion) or protein A–Sepharose beads pre-sorbed to either pre-immune or anti-DAN 205 IgG. Beads were removed by centrifugation and the supernatants assayed for deadenylation activity using ^{32}P-labeled G52 RNA (Wormington *et al*., 1996). Reaction products were electrophoresed on a 6% polyacrylamide–7 M urea gel and visualized using a Molecular Dynamics PhosphorImager.

Cell culture and microscopy

HeLa and Cos-1 cells were grown on coverslips in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Immunofluorescence experiments with HeLa cells were performed as described (Spector and Smith, 1986; Eggert *et al*., 1997). Cells were fixed with 100% ethanol for 10 min on ice. Affinity-purified antibody against the C-terminus of DAN was the primary antibody, the secondary antibody was tetramethylrhodamine isothiocyanate (TRITC)-conjugated swine anti-rabbit IgG (Dako, Denmark). Cells were stained with DAPI (Sigma) at 5 μg/ml in PBS for 1 min. Cos-1 cells were transfected with 2 μg of pEGFP-DAN per 60 mm dish with Lipofectin (Gibco-BRL) according to the manufacturer's instructions. Cells were washed twice with PBS and examined in a Zeiss Axiophot microscope.

Polysome gradients

A published procedure (Rogers and Munro, 1987) was used with slight modifications. HeLa cells (~5×10^7 cells in monolayer culture) were treated with 10 μg/ml cycloheximide for 10 min before harvesting and lysed for 10 min on ice in 0.5 ml of buffer containing 10 mM HEPES pH 7.2, 0.15 M KCl, 10 mM $MgCl_2$, 20 mM dithiothreitol (DTT), 0.5% NP-40, 150 μg/ml cycloheximide and 100 U/ml Rnasin (Promega). The nuclei and mitochondria were pelleted for 10 min in a refrigerated microcentrifuge. The supernatant was adjusted to 0.25 M KCl, layered onto a 11 ml 10–40% sucrose gradient in 20 mM HEPES, pH 7.2, 0.25 M KCl, 10 mM $MgCl_2$, 20 mM DTT, 150 μg/ml cycloheximide in a 14×95 mm centrifuge tube and centrifuged for 135 min at 32 000 r.p.m. in a Beckman SW41 Ti rotor at 4°C. The gradient was separated into 22 fractions, and the polyribosome profile was monitored by absorption at 254 nm. Proteins were TCA precipitated and analyzed by Western blotting.

Oocyte manipulation, microinjection, RNA and protein isolation

Defolliculated stage VI oocytes were isolated from collagenase-dissociated ovarian fragments and maintained at 18°C in modified Barth's saline (MBS). For immunoneutralization assays, stage VI oocytes were injected cytoplasmically with 100 ng of either affinity-purified anti-DAN 205 antibody or pre-immune IgG as a control. Oocytes were then incubated for an additional 12 h in either the presence or absence of 10 μg/ml of progesterone. Maturation was ascertained by the appearance of a white spot at the animal pole indicative of germinal vesicle breakdown. Total RNA was extracted from oocytes and analyzed by Northern blotting using probes corresponding to ribosomal proteins L5 and S22 (Wormington *et al*., 1996). RNAs were visualized by phosphoimagery.

Oocytes were enucleated as described (Varnum *et al*., 1992). Briefly, oocytes were first injected cytoplasmically with 5 fmol of ^{32}P-labeled G52 RNA, incubated for 60 min in MBS and transferred to 0.5× MBS for 5 min. A small incision was made with Dumont #5 forceps at the top of the animal pole, and the nucleus was extruded gently by squeezing the oocyte. The enucleated oocytes were placed in healing solution for 20 min and then transferred to full-strength MBS. Only oocytes whose nuclei were removed intact and whose enucleation wounds were healed completely within 1 h were used for subsequent manipulation. Enucleated oocytes and control stage VI oocytes were then injected cytoplasmically with 20 ng of synthetic myc-DAN RNA and incubated for 6 h in MBS and for an additional 12 h in either the presence or absence of 10 μg/ml of progesterone. In some cases, the maturation of progesterone-treated enucleated oocytes was confirmed by incubation in the presence of 0.5 μg/ml of the calcium ionophore, A23187. This resulted in the rapid contraction of the pigmented region of the cortex, indicative of the activation of mature oocytes. Total RNA was extracted, electrophoresed on a 6% polyacrylamide–7 M urea gel and visualized by phosphoimagery.

To ascertain the nuclear–cytoplasmic distribution of endogenous DAN and ectopically expressed myc-DAN, stage VI oocytes were injected cytoplasmically with 20 ng of synthetic RNA and incubated for 24 h in MBS. Total non-yolk, nuclear and cytoplasmic protein fractions were isolated from RNA-injected and control, uninjected, stage VI and progesterone-matured oocytes as described (Wormington, 1989), separated by SDS–PAGE (12.5% polyacrylamide) and analyzed by immunoblot analysis. To detect endogenous *Xenopus* DAN, anti-DAN antiserum 205 was used at a 1:1000 dilution. To detect myc-DAN, mAb 9E10 (Berkeley Antibody Co.) was used at a 1:5000 dilution. Immunoreactive proteins were visualized using appropriate alkaline phosphatase-conjugated secondary antibodies in conjunction with the Promega Western Blot AP detection system according to the manufacturer's protocol.

Other methods

DAN assays were performed and substrates prepared as described (Körner and Wahle, 1997). PAB I was the preparation described in Körner and Wahle (1997). SDS–polyacrylamide gels were run according to Laemmli (1970). Western blots were done by the semi-dry procedure (Kyse-Andersen, 1984). They were blocked in TNT buffer [20 mm Tris–HCl, pH 7.5, 150 mM NaCl, 0.05% (v/v) Tween-20] containing 5% non-fat dry milk. The same buffer was also used for incubation with antisera and washing. Blots were incubated with diluted antibodies for 2–3 h at room temperature and washed. Proteins were detected by peroxidase-conjugated swine anti-rabbit immunoglobulins (DAKO, Glostrup, Denmark) and chemiluminescence staining (SuperSignal Kit, Pierce).

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Note added in proof

The human gene for DAN has been mapped to chromosome 16 (K.Buiting, C.Körner, B.Ulrich, E.Wahle and B.Horsthemke, manuscript submitted for publication). During the preparation of the manuscript it was noticed that the abbreviation DAN has been used previously for a gene encoding a transcription factor and potential tumor suppressor [Ozaki *et al*. (1997) The genomic analysis of human DAN gene. *DNA Cell Biol*., **16**, 1031–1039, and references therein]. Therefore, the HUGO Nomenclature Committee has assigned the name ‘poly(A)-specific ribonuclease (PARN)’ to the gene/protein described here. We will use this name in future communications.