Characterization of FEN-1 from *Xenopus laevis*

cDNA CLONING AND ROLE IN DNA METABOLISM*

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Deoxyribonucleases have important functions in DNA replication, repair, and various styles of genetic recombination (1). At the replication fork, for example, the 3′ → 5′ exonuclease activity of DNA polymerase removes misincorporated nucleotides before they can be extended (2). In base excision repair, incision by an endonuclease near the damaged site initiates the removal of that damage through the action of an exonuclease (3). In addition, most models of homologous recombination invoke exonucleolytic resection to produce a recombinagenic single strand prior to interaction of homologous sequences (4, 5).

The nuclease that is now commonly designated FEN-1 was initially isolated and partially characterized in 1969 by Lindahl et al. (6), who called it DNase IV. They found that it had 5′ → 3′ exonuclease activity that was capable of removing thymine dimers, as well as the standard nucleotides, from double-stranded DNA (7, 8). Several groups working on *in vitro* DNA replication rediscovered this enzyme based on its role in the removal of RNA primers during lagging strand synthesis (9–11). When Waga et al. (12, 13) reconstituted an SV40-based replication system entirely with purified proteins, one of the required components was maturation factor-1, which is identical to FEN-1. The name FEN-1 was coined by Harrington and Lieber (14), who purified it as an activity capable of cleaving branched substrates, a property also described by Murante et al. (15). The acronym is now understood to denote both the novel flap endonuclease activity and the five-prime exonuclease activity.

FEN-1 genes have also been characterized in fungi, where the consequences of loss-of-function mutations have been investigated. In the fission yeast *Schizosaccharomyces pombe*, the FEN-1 homologue is the product of the rad2 gene, which was identified because mutants exhibit mild sensitivity to ultraviolet irradiation (16). In *Saccharomyces cerevisiae*, the corresponding gene is *RAD27* (also called *RTH1* and *ERC11*) (17–19). Knockout mutants in *S. cerevisiae* are viable, but are compromised for growth at high temperature. At 30 °C, this strain shows elevated rates of spontaneous mutation, mitotic recombination, and chromosome loss (17–20), which is similar to mutants with defects in other aspects of DNA replication. Furthermore, it exhibits synthetic lethality when combined with otherwise viable mutations in genes for two other replication factors, Dna2 helicase (21) and Ctf4 accessory factor.1 Apparentley, there is a backup function for the primary role of FEN-1 in replication, but it operates at reduced efficiency. This backup might be provided by related nucleases, like Rad2 (16, 22, 23) or Exo1 (24, 25), although double mutants with rad27 are viable.

In addition to a role in replication, FEN-1 is apparently required for some types of DNA repair. Eliminating *RAD27* function in yeast renders the cells sensitive to DNA-methylating agents, like methyl methanesulfonate (17–19). This suggests a role for FEN-1 in base excision repair, a notion that is supported by biochemical findings with reconstituted systems (26–29). As an alternative to β-elimination after base removal, FEN-1 can act in conjunction with displacement synthesis by DNA polymerase to excise several nucleotides, including the abasic residue. Participation in the repair of UV damage is somewhat controversial (17–19); but in both *S. pombe* (16) and *S. cerevisiae* (17), the reported increase in UV sensitivity is quite modest, and FEN-1 is not required in the major nucleotide excision repair pathway. Remarkably, FEN-1 mutants are further disabled in combination with mutations in the double strand break repair pathway (17–20). It may be that lesions accumulated in the absence of FEN-1 are normally repaired by a *RAD52*-dependent mechanism.

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1 T. Formosa, personal communication.
When assayed directly on oligonucleotide substrates, FEN-1 shows greater exonuclease activity at nicks in double-stranded DNA than on simple exposed ends (30, 31). It can remove both ribo- and deoxyribonucleotides from the 5′-end at the nick (32). On a double-stranded substrate with a 5′-ending single-stranded branch, FEN-1 cleaves at the base of the branch; it shows no activity on 3′-ending branches (14, 15). Inhibition by oligonucleotides annealed to the distal end of the branch implies that the enzyme loads on the free 5′ end and travels to the branch point before cleavage (14, 30). These activities are consistent with functions in the trimming of the 5′-ends of Okazaki fragments during replication and in the removal of damaged sites during repair. Since FEN-1 is active both at nicks and at branch sites, it could function either prior to or following DNA polymerase or after some displacement synthesis.

Because of our long-term interest in processes of DNA metabolism in Xenopus laevis oocytes, eggs, and embryos (33), we undertook to isolate the Xenopus homologue of FEN-1 (xFEN-1). A PCR^2-based strategy using known sequences of the fungal and mammalian enzymes proved successful. In addition, we have tested possible roles for xFEN-1 using defined DNA substrates in Xenopus oocyte extracts. In a parallel study, Kim et al. (29) also isolated xFEN-1 and demonstrated its capabilities in base excision repair.

EXPERIMENTAL PROCEDURES

PCR Primers and Conditions—Three degenerate primers based on conserved sequences of the mammalian and fungal FEN-1 homologues were used to isolate internal fragments of xFEN-1 cDNA by RT-PCR with oocyte RNA as template (34). First strand synthesis was performed with primer F1-5′-(5′-ATRCAANRRTCACAAAGTGYTC-3′) and avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). Amplification was carried out with F1-5 in combination with either F2-1′-(5′-CAYTNTAGGGNATGGNTATATG-3′) or F3-1′-(5′-TAYCCTNTGTGGYAGCAGTTCAG-3′) with Taq DNA polymerase for 40 cycles of 30 s at 95 °C, 30 s at 52 °C, and 1 min at 72 °C. Upon gel electrophoresis, bands of the expected sizes were obtained with both primer pairs. The DNAs were purified and cloned into pBluescript SK + (Stratagene), and the sequences of several clones were determined using vector primers and the ABI373 automated sequencing system.

Additional xFEN-1 cDNA sequences were also obtained by RT-PCR. For this purpose, 3′-end cDNA 3′-5′-AACCGGGCTCGAGTTCCTTATGTGTATCG-3′ and 5′-end (Amersham Pharmacia Biotech) and hybridized with the probe in 0.5 M NaPO₄, 7% SDS, and 1% bovine serum albumin at 65 °C overnight (37).

For amplification of cDNA 3′-5′-AAAATGGGAATTCACGGTTTGGC-3′ (Stratagene), and the sequences of several clones were determined with Taq polymerase. The resulting products were labeled by random priming and used to screen plaque lifts from a randomly labeled, linear pBR322 DNA as a substrate (41). After incubation for 3 ha that room temperature, the DNA was extracted and fractionated by electrophoresis on a 1% agarose gel and visualized by autoradiography of the dried gel.

Flap Endonuclease Assay—The flap substrate was the complex of three oligonucleotides described by Harrington and Lieber (14). The protruding single-stranded tail was labeled by treating the appropriate oligonucleotide with [γ-32P]ATP and polynucleotide kinase before preparing the complex. Activity was measured in a 15-μl reaction containing 25 μg of flap substrate in 50 mM Tris (pH 8.0), 10 mM MnCl₂, and 100 μg/ml bovine serum albumin with the indicated amount of crude extract or purified protein. When crude fractions were assayed, 10 μg of sonicated salmon sperm DNA was added to divert competing nucleases. After incubation for 30 min at 30 °C, reactions were terminated with 15 μl of 95% formamide, 10 mM EDTA, 1 mM MgCl₂, and 1 mg/ml xylene cyanol and heated to 95 °C for 5 min. Products were separated by electrophoresis on a 12% polyacrylamide gel containing 7 M urea and visualized by autoradiography of the dried gel.

Oocyte Extracts and Assays—Nuclear (germinal vesicle (GV)) extracts of Xenopus oocytes were prepared in I buffer (20 mM Tris (pH 7.5), 75 mM KCl, 7 mM MgCl₂, 0.1 mM EDTA, and 2 mM dithiothreitol) as described previously (39, 40). Both manual and bulk extracts have been used with comparable results. DNA synthesis was assayed by incubating the GV extract with single-stranded M13mp19 DNA and the four dNTPs, including [α-32P]dCTP (40). After incubation for 3 h at room temperature, the DNA was extracted and fractionated by electrophoresis on a 1% agarose gel and visualized by autoradiography of the dried gel. Enonuclease activity was assayed in the GV extracts using randomly labeled, linear pBR322 DNA as a substrate (41). After incubation for various times, aliquots were removed, mixed with 5 μg of sonicated salmon sperm DNA, and precipitated with 1 x HCl and 100 mM sodium pyrophosphate. Acid-soluble radioactivity was measured by scintillation counting.

Gel Filtration Chromatography—A 7-ml column of Superdex-200 (Amersham Pharmacia Biotech) was equilibrated with 1 buffer plus 10% glycerol and protease inhibitors (41) and with the KCl concentration was increased to 100 mM. GV extract (0.1 ml in the same buffer was applied to the column; some samples were incubated under DNA synthesis conditions, with M13 DNA and dNTPs as above, for 20 min prior to chromatography. The column was calibrated with molecular mass markers supplied by Amersham Pharmacia Biotech. In all cases, 0.5-ml fractions were collected at a flow rate of 0.1 ml/min. Column fractions were analyzed by Western blotting, as described above, with antisera...
The Xenopus FEN-1 sequences are both 382 amino acids in length, the same size as the S. cerevisiae and S. pombe (see Fig. 1 for locations of the primers.) RT-PCR was performed on total Xenopus oocyte RNA, and fragments of the expected sizes were readily obtained. Cloning and sequence analysis confirmed the identity of several PCR products. Based on these sequences, additional RT-PCR amplifications were done with primers designed to recover the 5' and 3'-ends of the cDNA. The PCR products were also used as probes to screen an embryo head cDNA library (36). Full-length cDNAs were recovered and sequenced.

The predicted sequences of the xFEN-1 proteins are given in Fig. 1. X. laevis has a pseudotetraploid genome (42), and as is typical for genes isolated from this organism, we recovered two classes of cDNAs. These differ from each other in amino acid sequence by 4.5% and in DNA sequence by 9% in the coding region and more extensively in the 5'- and 3'-untranslated regions. xFEN-1a was represented more abundantly than xFEN-1b both in the oocyte PCR products and in the clones from the embryo library, but clearly both were expressed as RNA in these stages.

The Xenopus FEN-1 sequences are both 382 amino acids in length, the same size as the S. cerevisiae and very similar to the others. xFEN-1a and xFEN-1b show 80% identity and 92% similarity to the human and mouse proteins. Comparison with the fungal sequences showed 55% identity and 72% similarity. Conserved residues include those identified as being important for substrate binding and catalysis in human FEN-1 (43), which are also retained in the larger 5' → 3' exonuclease family (44). The Xenopus sequences are clearly members of the highly conserved FEN-1 group.

Expression of xFEN-1 in Bacteria—To produce large quantities of the recombinant, we cloned xFEN-1a and xFEN-1b cDNAs into bacterial expression vectors. High levels of expression were obtained, particularly of an N-terminally His-tagged version of xFEN-1a in the pET16b vector (Fig. 2). Unfortunately, essentially all of the overexpressed protein was insoluble. It was readily purified on an Ni2+ nitrilotriacetic acid column after solubilization from inclusion bodies in 6 M urea, but attempts to obtain folded soluble enzyme, by dilution or by dialysis into non-denaturing media, were not very successful.

Surprisingly, an allelic variant of xFEN-1a, called xFEN-1a', showed much better solubility (Fig. 2). The most abundant alleles of xFEN-1a and xFEN-1b have valine at position 33, whereas in xFEN-1a', like the fungal and mammalian sequences, this amino acid is isoleucine (Fig. 1). The reasons for the favorable solubility properties of xFEN-1a' remain obscure, but they are manifested only in the context of the pRSETc vector and the BL21 pLysS host, among the combinations we have tested. Using conventional chromatography steps, we purified overexpressed xFEN-1a' close to homogeneity (data not shown).

Flap Endonuclease Assay—We constructed the same substrate used by Harrington and Lieber (14) to test the debranching activity of xFEN-1 (Fig. 3A). It is composed of three separate oligonucleotides, and the 5'-end of the single-stranded branch was labeled with 32P using polynucleotide kinase.

We found that, by using a buffer containing Mn2+ instead of Mg2+ and by including high concentrations of linear competitor DNA, we could reliably assay flap endonuclease activity in crude extracts of bacteria. Although E. coli DNA polymerase I has been shown to possess debranching activity (45), none was detected in extracts from cells carrying the vector alone (Fig. 3B). Cells expressing xFEN-1a, xFEN-1a', or xFEN-1b repro-
ducibly gave cleavage at the base of the flap, releasing oligo-
nucleotides of the expected size (Fig. 3B). Despite their relative
insolubility, xFEN-1a and xFEN-1b appear to be as active as
xFEN-1a'. Placing a His10 tag on the N terminus of any of the
xFEN-1 proteins abolished its activity (Fig. 3B), so this modi-
fication was abandoned.

Complementation of the Yeast rad27 Mutation—A more
stringent test of the activity of the xFEN-1 proteins was con-
ducted using a yeast strain deleted for its own FEN-1 homo-
logue, RAD27. This strain grows reasonably well at room tem-
perature, but not at 36 °C (17–19). Each of the xFEN-1
homologues was placed under the control of the yeast Gal1
promoter in the plasmid pYES2. Introduction of these con-
structs into the rad27 mutant restored its ability to grow at
36 °C (Fig. 4). The rad27 mutant is also hypersensitive to
DNA-methylating agents; like growth at high temperature, the
ability to grow in the presence of 0.01% methyl methanesul-
fonate was restored by expression of the xFEN-1 proteins (Fig.
4). Complementation of the yeast mutation demonstrated that
all three of the Xenopus homologues are capable of performing
at least some of the normal biological functions of the enzyme.
Similarly, expression of human FEN-1 was shown to com-
plement some phenotypes of an S. pombe rad27 mutant (16).

The rescue illustrated in Fig. 4 was performed on media
containing glucose as a carbon source. Under these conditions,
expression from the Gal promoter is minimal, but evidently
some level of the protein is produced. Growth on galactose, to
induce overproduction of the xFEN-1 proteins, was lethal at all
temperatures. We tentatively conclude that too much of the
Xenopus enzymes was deleterious. A comparable experiment to
overproduce yeast FEN-1 was not performed.

Western Blots with Anti-xFEN-1 Antiserum—His-tagged
xFEN-1a was purified in its denatured form from overexpress-
ing bacteria. After solubilization in urea and enrichment on an
Ni2+-nitrilotriacetic acid column, it was subjected to electro-
phoresis on an SDS-polyacrylamide gel. The prominent 43-kDa
band was cut out and used to immunize a rabbit. The resulting
antiserum was highly specific for xFEN-1. In Western blots
with proteins from bacteria, no bands were detected from cells
carrying the vector alone (Fig. 5A). In samples from cells ex-
pressing His-tagged xFEN-1a, a single band at the expected
molecular mass appeared. The same antiserum detected, with
essentially the same efficiency, all versions of xFEN-1, includ-
ing xFEN-1a without the His tag (Fig. 5A).

In a protein extract from full-grown (stage VI) Xenopus
oocytes, a single strong band at the expected size of 43 kDa was
detected (Fig. 5B), along with two minor bands at 47 and 23
kDa. The identities of the minor bands are not known. When
the oocytes were manually dissected into nuclear and cytoplas-
mic fractions, the 43- and 47-kDa bands proved to be essen-
tially entirely nuclear, whereas the 23-kDa band was largely
cytoplasmic. Nuclear localization is expected for xFEN-1, given
its functions in DNA metabolism.
The appearance of xFEN-1 protein during oocyte development was also examined by Western blot analysis (Fig. 5C). In stage 1, the earliest stage accessible in a mature ovary (46), a very low level of the 23-kDa cross-reacting species was seen, with gradually increasing amounts in sequentially larger stages. xFEN-1 was detected as a very faint band in stage II oocytes, and the amount increased dramatically from stages III to V. This pattern of accumulation is essentially the same as that measured for two other features of oocyte DNA metabolism: homologous recombination capability and total nuclear exonuclease activity (41).  

**Inhibition of xFEN-1 Activity—**One of our goals was to determine what role xFEN-1 plays in various aspects of DNA metabolism that are manifested in *Xenopus* oocytes. We tested the anti-xFEN-1 antiserum for inhibitory activity so that it could be used to determine what processes require the enzyme. As a first step, we showed that flap endonuclease activity is readily assayed in GV extracts from stage VI oocytes. As with the bacterial extracts, we performed the assays in Mn²⁺-containing buffer that minimized confounding activities of other nucleases. The products of treatment of the flap substrate were very similar to those produced by the bacterially expressed enzyme (Fig. 6, compare lanes 1, 2, and 5).

Two protocols were used to demonstrate that the antiserum effectively inhibits this reaction. In one approach, xFEN-1 was immunodepleted from the GV extract by precipitation with *Staphylococcus aureus*; and in the other, the antiserum was simply incubated with the extract prior to and during the reaction. In both cases, the activity was essentially completely inhibited (Fig. 6, lanes 4 and 7). In parallel samples, preimmune serum had no inhibitory activity (Fig. 6, lanes 3 and 6). Evidently at least some of the anti-xFEN-1 antibodies in the antiserum bind the enzyme in such a manner as to block its function.

**xFEN-1 in DNA Synthesis—**A role for xFEN-1 in particular aspects of DNA metabolism in oocytes was tested using the inhibitory antiserum. The mammalian homologues of the enzyme have been identified repeatedly as components of the DNA replication apparatus. Although *Xenopus* oocytes and oocyte extracts are not capable of replication, they will synthesize a second strand on a single-stranded circular DNA template (40, 47, 48). The process is presumably analogous to lagging strand synthesis during chromosomal replication and/or to mechanisms of DNA repair.

Addition of single-stranded M13 DNA to a GV extract led to efficient double-stranded DNA synthesis (Fig. 7). Supercoiled, nicked circular, and multimeric forms were recovered. Co-incubation with the anti-xFEN-1 antiserum blocked this synthesis. The effect was to inhibit total synthesis, but no change in product distribution or accumulation of specific intermediates was noted. Again, preimmune serum had no effect on DNA synthesis at comparable concentrations. It was not possible to perform immunodepletion experiments in this assay since the addition of *S. aureus*, even without antiserum, was sufficient to inhibit DNA synthesis in the extracts. In parallel experiments
with ammonium sulfate-concentrated antiserum, we found that DNA synthesis and flap endonuclease activities were inhibited at essentially the same input of antibodies (data not shown).

Because FEN-1 functions primarily, if not exclusively, in the maturation of Okazaki fragments during lagging strand synthesis, it was surprising to find that the antiserum blocked overall dNTP incorporation so effectively. This observation suggested that xFEN-1 might be part of a complex that includes other replication components that function together. To explore this possibility, GV extracts were subjected to gel filtration chromatography, and proteins were located by Western blot analysis.

As shown in Fig. 8B, in the extract alone, xFEN-1 eluted as a monomer, coincident with ovalbumin, which is essentially the same size. Under the same conditions, PCNA, another required replication protein, fractionated at the position expected for its size as a homotrimer (~90 kDa). When DNA and dNTPs were added, both xFEN-1 and PCNA shifted to positions indicative of larger sizes (Fig. 8A). Some xFEN-1 eluted with DNA at the exclusion volume of the column (fractions 10 and 11), but the majority was in a species that was centered on fraction 13. The center of the PCNA distribution was also located at this position, suggesting that, under conditions that support active DNA synthesis, xFEN-1 and PCNA become incorporated into a complex, which may also contain other proteins required for lagging strand production. An interaction between FEN-1 and PCNA in mammalian systems has been reported (49, 50). We note in passing that the 47-kDa cross-reacting polypeptide behaves like part of a very large complex, eluting near the excluded volume of the column both in simple extracts and under DNA synthesis conditions.

xFEN-1 in Recombination—Any linear DNA injected into Xenopus oocyte nuclei or incubated in GV extracts is subject to 5′→3′ degradation that leaves single-stranded 3′-tails, thereby preparing substrates for homologous recombination (33, 39, 51). Total exonuclease activity in the extracts is conveniently measured by the release of acid-soluble products from a random-primed, 32P-labeled substrate (41). The kinetics of exonuclease digestion were monitored in the presence and absence of anti-xFEN-1 antiserum (Fig. 9). No interference with this activity was detected, even at concentrations sufficient to block flap endonuclease and DNA synthesis essentially completely. Thus, xFEN-1 is not responsible for the major exonuclease activity in GV extracts.

It proved to be impossible, however, to use the antiserum to test a role for xFEN-1 in the recombination process more directly. Addition even of preimmune serum blocked this reaction completely. The multistep process of recombination is extremely sensitive to dilution (41) and evidently to the addition of complex reagents.

**DISCUSSION**

FEN-1 is a remarkable enzyme that plays important roles in DNA replication and in base excision repair in eukaryotic or-
organisms (52). In its purified form, its principal activity is that of a flap endonuclease (i.e., a debranching enzyme) with specificity for single-stranded branches having an exposed 5′-end. It also shows 5′ → 3′ exonuclease activity at nicks and, to a limited extent, at double-stranded ends. Its role is thought to be in the removal of unwanted nucleotides as gap filling is completed, either in the joining of Okazaki fragments during DNA replication or in the removal of damaged segments during DNA repair.

In cells, the function of FEN-1 is almost certainly coordinated with other components of the machinery of DNA metabolism through protein-protein interactions. The key to the assembly of complexes appropriate to the specific needs of the cell may be PCNA, the so-called sliding clamp (13). This protein exists as a homotrimer of a 29-kDa subunit, which can bind DNA through a central hole (53). PCNA has been shown to interact directly with a number of replication and repair proteins, including FEN-1 (29, 49, 50), DNA polymerase δ (54), replication factor C (55–57), DNA ligase I (58), a DNA methyltransferase (59), and XPG (60). Similar amino acid sequences in these various proteins are responsible for binding to PCNA and Ctf4, an accessory factor for DNA polymerase α/primase.

Using an antiserum against the X. laevis homologue of FEN-1, we have provided additional evidence for its incorporation into a multiprotein complex. In oocyte nuclear extracts, the formation of such a complex is dependent on conditions that support DNA synthesis. In the absence of added template and precursors, xFEN-1 behaves as a monomer in gel filtration chromatography. Upon initiation of DNA synthesis, xFEN-1 is recruited into a larger complex that apparently also contains PCNA. Addition of anti-xFEN-1 antiserum to extracts that would otherwise synthesize DNA completely blocks this reaction. Either assembly of the appropriate complex requires the presence of xFEN-1 and sequestration of that component prevents assembly, or the multiple activities of an assembled complex are inhibited by antibody binding to one component.

This result is different from what is observed if FEN-1 is simply omitted from a DNA replication reaction. In that case, synthesis proceeds, but lagging strands of the product are not covalently joined (12). This suggests that, at least with purified proteins, the necessary assemblies can form in the absence of FEN-1. The Xenopus oocyte may be exceptional with regard to the timing of interaction of these factors for two reasons. First, the large stockpiles of replication and repair components are stored for use during embryogenesis, but are inactive in the oocyte (63). Addition of DNA may simply attract the various proteins or may induce chemical modifications (e.g., phosphorylation or dephosphorylation) that encourage the necessary interactions. Second, the oocytes are arrested at prophase of the first meiotic nuclear division. Conceivably, the induction of complex formation upon DNA addition reflects normal cell cycle-dependent regulation of replication activities.

Given the need for assembling FEN-1 into multicomponent complexes, it is perhaps surprising that the expression of the Xenopus proteins in yeast was able to provide phenotypic complementation of both temperature sensitivity (presumably a replication defect) and sensitivity to methyl methanesulfonate (a repair defect). In an in vitro study, human PCNA was shown to stimulate the activity of yeast FEN-1, but the opposite combination (yeast PCNA with human FEN-1) gave no evidence of interaction (50). It should be noted that the amino acid sequence motif identified in FEN-1 and other proteins that interact with PCNA (61, 62) is conserved in the Xenopus and yeast proteins.

We also investigated the role of xFEN-1 in the single strand annealing mechanism of homologous recombination that is prominent in Xenopus oocytes and nuclear extracts. Although it was not possible to examine recombination reactions directly due to nonspecific inhibition by serum components, we showed that xFEN-1 was not responsible for the major exonuclease activity in the extracts. Since recombination is completely dependent on 5′ → 3′ resection of substrate ends, both in vivo and in vitro (33), some other enzyme must be the major contributor to this process. It remains possible that xFEN-1 participates in a later stage of recombination, perhaps in the processing of annealed intermediates or in the trimming of nicked molecules prior to sealing by DNA ligase (33).

The amino acid sequences of xFEN-1 presented no surprises. The Xenopus protein is highly homologous to its mammalian counterparts and retains sequences that are most highly conserved in the fungal homologues. As is frequently true for X. laevis, two sequence variants of xFEN-1 were recovered, reflecting a relatively recent interspecies hybridization that combined two complete functional genomes (42). Surprisingly, an allelic variant of one of these sequences was the only one to exhibit good solubility when expressed in E. coli. The structural consequences of substituting Ile for Val at position 33 are expected to be subtle, but may have a significant effect on the rate of folding to the native conformation.

During oocyte development, xFEN-1 begins to appear in detectable amounts in mid-sized, vitellogenic oocytes and continues to accumulate into late stages. This pattern is typical of components that are stored in oocytes for use in embryogenesis. In particular, both total exonuclease activity and the capability to support homologous recombination accumulate with essentially the same kinetics (41). After fertilization, cell divisions occur very rapidly in Xenopus embryos, approximately once every 0.5 h (64). Replication and segregation of the genome to daughter cells are two of the principal metabolic activities during these stages. Since it is important that the integrity of the genome be maintained, activities required to replicate DNA and to repair any damage that may be incurred must be present in sufficient quantities in the oocyte to support these processes until more leisurely cell cycles take over at mid-blastula.

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