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A suppressor of a yeast splicing mutation (*prp8-1*) encodes a putative ATP-dependent RNA helicase

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FIVE small nuclear RNAs (snRNAs) are required for nuclear pre-messenger RNA splicing: U1, U2, U4, U5 and U6[1,2]. The yeast U1 and U2 snRNAs base-pair to the 5′ splice site and branch-point sequences of introns respectively[1]. The role of the U5 and U4/U6 small nuclear ribonucleoprotein particles (snRNPs) in splicing is not clear, though a catalytic role for the U6 snRNA has been proposed[3]. Less is known about yeast splicing factors, but the availability of genetic techniques in *Saccharomyces cerevisiae* has led to the identification of mutants deficient in nuclear pre-mRNA splicing (*prp2-prp27*)[4,5]. Several *PRP* genes have now been cloned and their protein products characterized. The PRP8 protein is a component of the U5 snRNP and associates with the U4/U6 snRNAs/snRNP to form a multi-snRNP particle believed to be important for spliceosome assembly[6]. We have isolated extragenic suppressors of the *prp8-1* mutation of *S. cerevisiae* and present here the preliminary characterization of one of these suppressors, *spp81*. The predicted amino-acid sequence of the SPP81 protein shows extensive similarity to a recently identified family of proteins thought to possess ATP-dependent RNA helicase activity. The possible role of this putative helicase in nuclear pre-mRNA splicing is discussed.

To identify genes encoding splicing factors that interact with the PRP8 protein, we isolated extragenic suppressors of a *prp8-1* mutation. We sought suppressors that can not only suppress a *prp8-1* mutation but also render the growth of the cells cold-sensitive, as this would greatly aid the analysis of the mutants. Eleven such mutants were isolated which define three complementation groups termed *spp81*, *spp82* and *spp83* (Table 1). Tetrad analysis showed that the cold-sensitive phenotype and the ability to suppress a *prp8-1* mutation were genetically linked in each case and that the cold-sensitive mutations were unlinked to the *prp8-1* mutation and segregated as single recessive nuclear mutations (data not shown). Nine (at least seven of which were independent isolates) of the eleven mutations defined the *SPP81* gene. The ability of the *spp81-3* mutation to suppress the splicing defect of a *prp8-1* temperature-sensitive mutant was confirmed at the molecular level by examining the amount of precursor RNA accumulation at various temperatures. Compared with that observed for the *prp8-1* mutant SPJ8.31, the strain DJY65 (*prp8-1 spp81-3*) failed to accumulate significant amounts of precursor RNA at 34 °C (Fig. 1).

In the process of determining whether the suppressor mutations were linked to the *PRP8* gene, plasmids carrying the wild-type *PRP8* gene and flanking DNA were introduced into *spp81* mutants. The *spp81-3* mutants grew very slowly when transformed with plasmid p8000 (2μ *URA3 PRP8*) compared with those transformed with the control plasmid, *YEP24* (2μ *URA3*). With *spp81-2* mutants the growth defect was more pronounced (no transformants could be obtained), whereas wild-type yeast, *spp82* mutants and *spp83* mutants transformed with plasmid p8000 did not give this phenotype (Table 1). The effect was specific to the *PRP8* gene and was not observed with 2μ plasmids carrying the wild-type *PRP2, PRP4* or *PRP11* genes. Transformation of both *spp81-2* and *spp81-3* mutants with either high (p8000) or low (p8500, *CEN URA3 PRP8*) copy-number plasmids carrying the wild-type *PRP8* gene

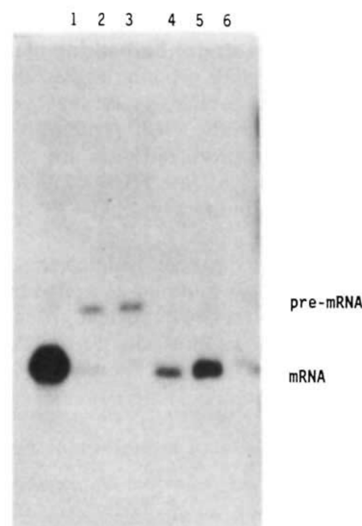

FIG. 1 Northern blot analysis of the *prp8-1* suppressor mutant DJY65 (*MATa his3 leu2 ura3-52 prp8-1 spp81-3*). The yeast actin gene, which contains one intron[21], was used as a probe to demonstrate suppression of the *prp8-1* temperature-sensitive mutation by the *spp81-3* mutation. Lanes 1-3, SPJ8.31 (*prp8-1*) 23 °C, 34 °C and 36 °C; lanes 4-6, DJY65 (*prp8-1 spp81-3*) 23 °C, 34 °C and 36 °C.
METHODS. The actin-encoding plasmid pYA301 (ref. 21) was labelled by the random-priming method[22]. RNA was prepared from cultures which had been grown at 30 °C before shifting them to either 23 °C, 34 °C, or 36 °C for 2 h as described by Jackson *et al.*[7]. Total RNA was extracted as described by Hopper *et al.*[23] and equivalent amounts of total RNA were run on a 1.2% agarose gel. Northern-blotting prehybridization, hybridization and washing conditions were as described by Jackson *et al.*[7].

TABLE 1 Effect of extra copies of the *PRP8* gene on *spp81* mutants

Relevant genotype	No. of isolates	*CEN PRP8* p8500	2μ *PRP8* p8000
PRP8 spp81-2	9	+/−	−
PRP8 spp82-1	1	+	+
PRP8 spp83-1	1	+	+

Cultures of strain SPJ8.31 (*MATa ura3-52 his3 leu2 prp8-1*) were mutagenized with ultraviolet light to a level at which roughly 90% of cells were killed. Mutagenized cells were divided into aliquots and grown in the dark at 30 °C for 2 days. Aliquots of these pools were spread on YPD plates[27] and incubated at 34 °C. Putative cold-sensitive suppressors were identified by screening for weak or no growth at 18 °C and 15 °C on YPD plates. Putative suppressor mutations were then crossed to the wild-type strain KY118 (*MATα ura3-52 trp1-289 lys2-801*^{am} *adel-101*^{oc} *his3Δ200*)[28] and then crossed into the parental strain SPJ8.31 as described by Sherman *et al.*[27]. Complementation analysis was performed as described by Sherman *et al.*[27]. To test for interactions with the wild-type *PRP8* gene yeast strains were transformed with *PRP8*-containing plasmids as described by Ito *et al.*[29] and plated out at 30 °C. +, growth; +/−, poor growth; −, no growth.

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revealed that the growth defect was related to the copy number of the *PRP8* gene. Yeast strains harbouring plasmids p8000 or p8500 overproduce the PRP8 protein roughly fourfold and two-fold, respectively[7]. These results suggest that the product of the *SPP81* gene interacts with the PRP8 protein. It is possible that the excess PRP8 protein produced does not assemble into the U5 snRNP and this pool of free PRP8 protein can effectively sequester a limiting amount of mutant SPP81 protein into a functionally inactive complex.

A suppressor (*SRN1*) has been isolated that suppresses the defects of several of the *prp* mutants, as well as that of the *rnal-1* mutation which is not defective in nuclear pre-mRNA splicing[8]. The molecular basis of suppression by the dominant *SRN1* suppressor is unclear, but in view of the nonspecific nature of its suppression, it is probable that the *SRN1* gene product suppresses the defects indirectly. We therefore determined whether the *spp81* suppressors were more specific than the *SRN1* suppressor. The strain DJY63 (*spp81-3*) was crossed to strains harbouring an *rna1-1, prp2-1, prp3-1, prp4-1* or *prp11-1* mutation, and tetrads from each cross were analysed. In no case did *spp81-3* suppress the temperature-sensitive defects of these *prp* and *rna1* mutations (data not shown). These results suggest that the *spp81* mutations are unlikely to be alleles of the *SRN1* gene and imply that the suppression is more specific than that observed with the *SRN1* mutation.

```
   1  CTCGAGAAAAAAAATATAAAAGAGATGGAGGAACGGGAAAAAGTTAGTTGTGGTGATAGGTGGCAA
  67  GTGGTATTCCGTAAGAACAACAAGAAAAGCATTTCATATTATGGCTGAACTGAGCGAACAAGTGCA
                                                   M  A  E  L  S  E  Q  V  Q
 133  AAATTTAAGCATCAACGACAACAACGAGAATGGTTATGTTCCTCCTCACTTAAGAGGAAAACCAAG
        N  L  S  I  N  D  N  N  E  N  G  Y  V  P  P  H  L  R  G  K  P  R
 199  AAGTGCCAGAAATAACAGTAGCAACTACAATAACAACAACGGCGGCTACAACGGTGGCCGTGGCGG
        S  A  R  N  N  S  S  N  Y  N  N  N  N  G  G  Y  N  G  G  R  G  G
 265  TGGCAGCTTCTTTAGCAACAACCGTCGTGGTGGTTACGGCAACGGTGGTTTCTTCGGTGGAAACAA
        G  S  F  F  S  N  N  R  R  G  G  Y  G  N  G  G  F  F  G  G  N  N
 331  CGGTGGCAGCAGATCTAACGGCCGTTCTGGTGGTAGATGGATCGATGGCAAACATGTCCCAGCTCC
        G  G  S  R  S  N  G  R  S  G  G  R  W  I  D  G  K  H  V  P  A  P
 397  AAGAAACGAAAAGGCCGAGATCGCCATATTTGGTGTCCCCGAGGATCCAAATTTCCAATCTTCTGG
        R  N  E  K  A  E  I  A  I  F  G  V  P  E  D  P  N  F  Q  S  S  G
 463  TATTAACTTCGATAACTACGATGATATTCCAGTGGACGCCTCTGGTAAGGATGTTCCTGAACCAAT
        I  N  F  D  N  Y  D  D  I  P  V  D  A  S  G  K  D  V  P  E  P  I
 529  CACAGAATTTACCTCACCTCCATTGGACGGATTGTTATTGGAAAACATCAAATTGGCCCGTTTCAC
        T  E  F  T  S  P  P  L  D  G  L  L  L  E  N  I  K  L  A  R  F  T
 595  CAAGCCAACACCTGTGCAAAAATACTCCGTCCCTATCGTTGCCAACGGCAGAGATTTGATGGCCTG
        K  P  T  P  V  Q  K  Y  S  V  P  I  V  A  N  G  R  D  L  M  A  C
 661  TGCGCAGACCGGTTCTGGTAAGACTGGTGGGTTTTTATTCCCAGTGTTGTCCGAATCATTTAAGAC
        A  Q  T  G  S  G  K  T  G  G  F  L  F  P  V  L  S  E  S  F  K  T
 727  TGGACCATCTCCTCAACCAGAGTCTCAAGGCTCCTTTTACCAAAGAAAGGCCTACCCAACTGCTGT
        G  P  S  P  Q  P  E  S  Q  G  S  F  Y  Q  R  K  A  Y  P  T  A  V
 793  CATTATGGCTCCAACTAGAGAGTTGGCCACCCAAATTTTCGATGAAGCCAAGAAATTTACTTATAG
        I  M  A  P  T  R  E  L  A  T  Q  I  F  D  E  A  K  K  F  T  Y  R
 859  ATCCTGGGTCAAGGCCTGCGTCGTCTACGGTGGTTCTCCAATTGGTAACCAACTAAGAGAAATTGA
        S  W  V  K  A  C  V  V  Y  G  G  S  P  I  G  N  Q  L  R  E  I  E
 925  ACGTGGTTGCGATCTTTTAGTCGCTACTCCAGGTCGTTTGAATGACTTGTTGGAACGTGGTAAAAT
        R  G  C  D  L  L  V  A  T  P  G  R  L  N  D  L  L  E  R  G  K  I
 991  TTCTTTGGCCAACGTCAAGTATTTGGTTCTAGATGAAGCTGATAGAATGTTGGATATGGGTTTCGA
        S  L  A  N  V  K  Y  L  V  L  D  E  A  D  R  M  L  D  M  G  F  E
1057  ACCTCAAATTAGACATATTGTCGAAGACTGTGATATGACTCCTGTTGGTGAAAGACAAACTCTGAT
        P  Q  I  R  H  I  V  E  D  C  D  M  T  P  V  G  E  R  Q  T  L  M
1123  GTTCTCAGCTACTTTTCCCGCTGATATCCAACATTTGGCCCGTGATTTCTTAAGTGACTACATCTT
        F  S  A  T  F  P  A  D  I  Q  H  L  A  R  D  F  L  S  D  Y  I  F
1189  TTTGTCTGTTGGTAGAGTCGGTTCTACTTCAGAAAACATTACTCAAAAAGTCTTATACGTTGAAAA
        L  S  V  G  R  V  G  S  T  S  E  N  I  T  Q  K  V  L  Y  V  E  N
1255  TCAAGATAAGAAGTCAGCCTTATTGGATCTATTGTCTGCATCCACTGACGGTTTGACTTTGATCTT
        Q  D  K  K  S  A  L  L  D  L  L  S  A  S  T  D  G  L  T  L  I  F
1321  TGTCGAAACTAAGAGAATGGCAGATCAATTGACCGATTTCCTGATCATGCAAAACTTTAGAGCTAC
        V  E  T  K  R  M  A  D  Q  L  T  D  F  L  I  M  Q  N  F  R  A  T
1387  CGCCATTCATGGTGACCGTACCCAATCTGAGAGAGAACGTGCCTTGGCCGCCTTCAGATCTGGTGC
        A  I  H  G  D  R  T  Q  S  E  R  E  R  A  L  A  A  F  R  S  G  A
1453  CGCTACTTTATTGGTTGCCACAGCTGTCGCAGCTAGAGGTCTAGATATTCCAAACGTCACCCACGT
        A  T  L  L  V  A  T  A  V  A  A  R  G  L  D  I  P  N  V  T  H  V
1519  TATCAACTACGATTTACCAAGTGATGTCGATGATTACGTCCATAGAATTGGTAGAACTGGTCGTGC
        I  N  Y  D  L  P  S  D  V  D  D  Y  V  H  R  I  G  R  T  G  R  A
1585  CGGTAACACCGGTCTTGCCACTGCCTTTTTCAACAGTGAAAACAGTAACATTGTTAAAGGTTTGCA
        G  N  T  G  L  A  T  A  F  F  N  S  E  N  S  N  I  V  K  G  L  H
1651  TGAAATTTTGACTGAAGCTAACCAAGAAGTCCCATCATTCTTGAAGGACGCTATGATGAGTGCTCC
        E  I  L  T  E  A  N  Q  E  V  P  S  F  L  K  D  A  M  M  S  A  P
1717  AGGTAGCAGAAGCAACAGCCGTAGAGGCGGTTTCGGTCGCAACAACAACAGAGATTACCGTAAGGC
        G  S  R  S  N  S  R  R  G  G  F  G  R  N  N  N  R  D  Y  R  K  A
1783  CGGAGGCGCTAGCGCAGGCGGCTGGGGCTCTTCAAGAAGCAGAGATAACTCTTTCAGAGGCGGTAG
        G  G  A  S  A  G  G  W  G  S  S  R  S  R  D  N  S  F  R  G  G  S
1849  TGGCTGGGGTAGCGATTCCAAGTCTTCTGGCTGGGGTAACAGCGGTGGTTCAAACAACTCTTCTTG
        G  W  G  S  D  S  K  S  S  G  W  G  N  S  G  G  S  N  N  S  S  W
1915  GTGGTGATTTCAGACAAACTAGGGTGAGGATTCTTCGTTTTCTGCTCTAGCATGTCTTATATTTTC
        W
1981  TTTCTCCAATCACTTTTTTTATCCAAGTCCTGAAAATACTGTGTTGTTTGTTTCTCTCTAGCCGTT
2047  GATTGGCCCCATATTTTTTTTATTTTCTCACTGGGAGCCACTAATCTGTTACTTTTTTTCCACTCT
2113  TATTTTTTATCCATCGTTTTTTTGTTTTTTTTTTCTTTCATGGTTCTAATATTATTTTACAATTGT
2179  ACTATAAAATGCCTTTGCTAATATTAACTAAAAAACTAAAGATTTCACTATGATTATAAGCAATTA
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FIG. 2 Nucleotide sequence of the *SPP81/DED1* gene and the predicted amino-acid sequence of the SPP81/DED1 protein. The first 444 nucleotides of the sequence shown are identical to the sequence previously determined by Struhl[9] except for GT instead of TG at positions 216–217. The one long open reading frame continues that noted by Struhl[9] and its identity as *SPP81/DED1* is supported by the transcript mapping data reported by Struhl[9]. No consensus splice site signals were found within or near this open reading frame, suggesting that it comprises the complete coding region, as supported also by the size of the protein identified on western blots (see text). This sequence plus an additional 612 nucleotides (to the *Sal*I site at the 3′ end) have been deposited with the EMBL, Genbank and DDBJ nucleotide sequence data bases.

METHODS. The complete sequence of both strands of the 2.8-kb *Xho*I–*Sal*I fragment was determined using Sequenase (USB) after generating a set of nested deletions in M13 vectors using a modification[24] of the method of Henikoff[25].

Genetic analysis of the *spp81-2* and *spp81-3* mutations showed that they are tightly linked to the *HIS3* gene on chromosome XV. Two genes of unknown function, *PET56* and *DED1*, have been isolated previously by virtue of their proximity to *HIS3*[9]. To determine whether the cold-sensitive defect of *spp81* mutants could be complemented by DNA from the *HIS3* region, strains DJY57 (*spp81-2 ura3-52*) and DJY63 (*spp81-3 ura3-52*) were transformed with plasmids pYDJ35 (*CEN URA3*) and pYDJ36 (2μ *URA3*) which carry to *HIS3*, *DED*1 and *PET56* genes (the 6.1-kb *Eco*RI–*Sal*I fragment from YIP1 (refs 10, 11)). Both plasmids were capable of complementing the cold-sensitive growth defect of *spp81* mutants. Subcloning experiments showed that the 2.8-kb *Xho*I–*Sal*I fragment from YIP1 (ref. 10) could also complement the mutations in both high and low copy-number plasmids (data not shown). As this fragment encodes *DED1*, an essential gene of previously unknown function[9], the results suggested that the *spp81* mutations might be alleles of *DED1*. To test this, a DNA fragment containing *DED1* was examined for its ability to gene-convert the *spp81* locus in an *spp81-3* mutant, and thereby allow the strain to grow at low temperature. The 2.8-kb *Xho*I–*Sal*I fragment was gel-purified and transformed with pFL38 (*CEN URA3*) into the cold-sensitive strain DJY63 (*spp81-3*). The resulting transformants were analysed for their ability to grow at the nonpermissive temperature. Of the 32 Ura$^+$ transformants screened, four had acquired the ability to grow at 18 °C by gene-conversion, thus demonstrating that the *spp81* mutations are indeed alleles of *DED1*.

The complete nucleotide sequence of the *DED1* gene has been determined (Fig. 2) and indicates that this gene encodes a protein of approximate relative molecular mass 65,000 (65K). Antibodies were raised against the product of a *trpE*::*DED1* fusion gene containing the C-terminal 492 amino acids of *DED1* and affinity-purified against the product of a *lacZ*::*DED1* fusion gene containing the same *DED1* fragment. On western blots of total yeast proteins, the antibodies revealed a single band of ~70K; this band was several-fold more intense in strains carrying a high copy-number *DED1* plasmid (data not shown). The predicted DED1 protein sequence showed no similarity to keratins or other intermediate-filament proteins beyond the small region near the N terminus as previously noted by Struhl[9]. But the predicted DED1 amino-acid sequence showed strong similarity to a recently identified family of proteins which are thought to possess ATP-dependent RNA helicase activity (Fig. 3)[12–15]. The DED1 protein is particularly similar to the mouse PL10 protein[16] (62% identical and 78% similar over the highly conserved region). Over the entire length of the proteins, the DED1 protein shows 49% identity, and 69% similarity to the PL10 protein (Fig. 3). These figures are significantly higher than those for other members of this family[16], raising the possibility that the DED1 protein is the yeast homologue of the PL10 protein.

The identification of a putative RNA helicase associated with RNA splicing is of considerable interest as the requirement for RNA helicase activity seems to be a general feature of RNA splicing mechanisms. For example, a putative RNA helicase has been identified that is involved in the splicing of some group I and II mitochondrial introns[17]. The ATP-dependence of the

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splicing reaction and the occurrence of intermolecular base-pairing between several RNA molecules during the reaction suggests several possible roles for RNA helicases. For example, both the U1 and U2 snRNAs bind to pre-mRNA by base-pairing interactions, at the 5′ splice site and the branch point respectively[2]. Also, the U4 and U6 snRNAs are associated in the U4/U6 snRNP by 24 intermolecular base-pairs. During the splicing reaction these RNA molecules have to be unwound, either wholly or partially[2,18,19]. Other possible roles for an RNA helicase in splicing exist; for example, the removal of secondary structure from introns may permit them to adopt a more favourable conformation for splicing and the binding of splicing factors. It is possible that splicing requires several different RNA helicases with different specificities. Indeed, the products of the *PRP5*[20] and *PRP28* genes are putative RNA helicases (C. Guthrie and E. Strauss personal communication), and a gene encoding a protein similar (and functionally related) to the SPP81/DED1 protein has recently been identified[30]. In view of the known interaction between the PRP8 protein, the U5 snRNP and the U4/U6 snRNP, a role for the SPP81/DED1 protein in facilitating the unwinding of U4 from U6 is an attractive idea.

These results suggest that the PRP8 protein interacts with an ATP-dependent RNA helicase. So far we have no information about where in the splicing reaction the SPP81/DED1 protein is required, nor indeed whether it is normally involved in the splicing mechanism; it is possible that only the mutant form of the SPP81/DED1 protein functions in splicing. We are currently investigating the role of the SPP81/DED1 protein in nuclear pre-mRNA splicing, in particular its interaction with the PRP8 protein and other spliceosome components. □

```
SPP81/DED1  MAELSEQVQ----------NLSINDNNENG-------YVPPHLRGKPRSA---RNNMSNY
PL10        MSHVAEEDELGLDQQLAGLDLTSRDSQSGGSTASKGRYIPPHLRNREAAKAFYDKDGSRW
            *....*. .            .*. .*....*       *.*****..  .       .. *..

SPP81/DED1  NNNNGGYNG------GRGGGSFFSNNRRGGYGNGGFFG----------GNNGGSRSNGR-
PL10        SKDKDAYSSFGSRSDTRAKSSFFSD--RGGSGSRGRFDERGRSDYESVGSRGGRSGFGKF
            ......*..      .*. .****.  *** *. * *.          *..**... *.

SPP81/DED1  ---SGGRWIDGKHV-----PAPRNEKAEIAIFGVPEDPNFQSSGINFDNYDDIPVDASGK
PL10        ERGGNSRWCDKADEDDWSKPLPPSERLEQELFSGGN------TGINFEKYDDIPVEATGN
               ...** *   .      * *..*. * ..*.   .       .****..******.*.*.

SPP81/DED1  DVPEPITEFTSPPLDGLLLENIKLARFTKPTPVQKYSVPIVANGRDLMACAQTGSGKTGG
PL10        NCPPHIESFSDVEMGEIIMGNIELTRYTRPTPVQKHAIPIIKEKRDLMACAQTGSGKTAA
            . * .*..*..  .......**.*.*.*.******...**. . **************..
Consensus                                                     AQ G GKT

SPP81/DED1  FLFPVLSESFKTGPS---PQPESQGSFYQRKAYPTAVIMAPTRELATQIFDEAKKFTYRS
PL10        FLLPILSQIYTDGPGEALRAMKENGKYGRRKQYPISLVLAPTRELAVQIYEEARKFSYRS
            **.*.**. ...**.   .. ...*.. .**.**.....*******.**..**.**.***
Consensus                                            PTRELA Q

SPP81/DED1  WVKACVVYGGSPIGNQLREIERGCDLLVATPGRLNDLLERGKISLANVKYLVLDEADRML
PL10        RVRPCVVYGGADIGQQIRDLERGCHLLVATPGRLVDMMERGKIGLDFCKYLVLDEADRML
            .*..******. **.*.*..****.********* *..*****.*.  ************
Consensus                                                      LDEAD

SPP81/DED1  DMGFEPQIRHIVEDCDMTPVGERQTLMFSATFPADIQHLARDFLSDYIFLSVGRVGSTSE
PL10        DMGFEPQIRRIVEQDTMPPKGVRHTMMFSATFPKEIQMLARDFLDEYIFLAVGRVGSTSE
            *********.***. .*.* * *.*.******* .** ******..****.*********
Consensus                                   SAT

SPP81/DED1  NITQKVLYVENQDKKSALLDLLSAST-DGLTLIFVETKRMADQLTDFLIMQNFRATAIHG
PL10        NITQKVVWVEEADKRSFLLDLLNATGKDSLILVFVETKKGADSLEDFLYHEGYACTSIHG
            ******..**..**.* *****.*.. *.*.*.*****. ** *.***   ...  *.***

SPP81/DED1  DRTQSERERALAAFRSGAATLLVATAVAARGLDIPNVTHVINYALPSDVDDYVHRIGRTG
PL10        DRSQRDREEALHQFRSGKSPILVATAVAARGLDISNVKHVINFDLPSDIEEYVHRIGRTG
            **.*..** ** .****  ...*************.**.****..****...*********
Consensus                                                     Y HRIGR

SPP81/DED1  RAGNTGLATAFFNSENSNIVKGLHEILTEANQEVPSFLKDAMMSAP---GSRSNSRR---
PL10        RVGNLGLATSFFNERNINITKDLLDLLVEAKQEVPSWLENMAFEHHYKGGSRGRSKSRFS
            *.** ****.***. * **.*.*  ..*.**.*****.*..  .. .    ***..*..

SPP81/DED1  GGFGRNNNRDYRKAGGASAGGWGSSRSRDNSFRGGS-----GWGSDS-----KSSGWGNS
PL10        GGFG---ARDYRQSSGASSSSFSSGRASNSRSGGGSHGSSRGFGGGSYGGFYNSDGYGGN
            ****   .****...***.....*.*.....  ***      *.*..*     .*.*.*..

SPP81/DED1  GGSNNSSWW
PL10        YSSQGVDWWGN
             .*.. .**
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FIG. 3 Alignment of the predicted SPP81/DED1 amino-acid sequence with that of the mouse protein PL10. Alignment was created using the Clustal program[26] and the facilities of the SERC computing Laboratory, Daresbury, Cheshire, UK. *, Amino acids identical in both proteins; •, positions in which both proteins have similar amino acids. The consensus line shows the sequences conserved between these and other DEAD box proteins[13].

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The *Xenopus* localized messenger RNA An3 may encode an ATP-dependent RNA helicase

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THE maternal messenger RNA An3 was originally identified localized to the animal hemisphere of *Xenopus laevis* oocytes, eggs and early embryos[1,2]. *Xenopus* embryos depend on mRNA and protein present in the egg before fertilization (maternal molecules) to provide the information needed for early development. Localization of maternal mRNA gives cells derived from different regions of the egg distinctive capacities for protein synthesis. We show here that An3 mRNA encodes a protein with 74% identity to a protein encoded by the testes-specific mRNA PL10 found in mouse[3], which is proposed to have RNA helicase activity. Because the gene encoding An3 mRNA is reactivated after gastrulation and remains active throughout embryogenesis[1,2], we have examined its distribution in embryonic and adult tissues. Unlike PL10 mRNA, which is primarily restricted to the testes, An3 mRNA is broadly distributed in later development.

An3 was predicted to be represented by two mRNAs of approximately 5.1 and 3.5 kilobases (kb). The 2.4-kb An3 com-

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