might also cause cancer. It is intriguing that the gene for human *bmf* is located on chromosome 15q14, the site of a candidate tumor suppressor gene lost in many metastatic, but not primary, carcinomas (*25*). Anoikis has been implicated as a barrier against metastatic tumor growth (*26*), raising the possibility that metastatic tumors harboring 15q14 mutations might have abnormalities in the expression or function of Bmf.

References and Notes

1. D. C. S. Huang, A. Strasser, *Cell* **103**, 839 (2000).
2. B. Conradt, H. R. Horvitz, *Cell* **93**, 519 (1998).
3. L. O'Connor *et al.*, *EMBO J.* **17**, 384 (1998).
4. P. Bouillet *et al.*, *Science* **286**, 1735 (1999).
5. X.-M. Yin *et al.*, *Nature* **400**, 886 (1999).
6. B. Conradt, H. R. Horvitz, *Cell* **98**, 317 (1999).
7. E. Oda *et al.*, *Science* **288**, 1053 (2000).
8. K. Nakano, K. H. Vousden, *Mol. Cell* **7**, 683 (2001).
9. J. Yu, L. Zhang, P. M. Hwang, K. W. Kinzler, B. Vogelstein, *Mol. Cell* **7**, 673 (2001).
10. J. Zha, H. Harada, E. Yang, J. Jockel, S. J. Korsmeyer, *Cell* **87**, 619 (1996).
11. H. Puthalakath, D. C. S. Huang, L. A. O'Reilly, S. M. King, A. Strasser, *Mol. Cell* **3**, 287 (1999).
12. The cDNA libraries from day 17 mouse embryos or from mouse embryos from embryonic day 9 to day 1 postpartum were prepared in pAD-GAL4-2.1 (HybriZAP-2.1 kit, Stratagene). The bait vector was made by cloning mouse *mcl*-1 lacking the sequences encoding its hydrophobic COOH-terminus into pGBT-9 (Clontech). Yeast transformation and plasmid rescue were performed as previously described (*11*). Of the 7×10^5 clones screened, one positive clone was obtained. Interaction between Mcl-1 and the novel protein was confirmed by β-galactosidase staining (*11*). Sequence analysis revealed that the clone was a partial one lacking the 5′ end. This partial clone was used as the probe to isolate full-length clones by screening a cDNA library derived from the $p53^{-/-}$ KO52DA20 thymoma cell line. Human *bmf* was isolated by screening a human, activated T cell cDNA library using mouse *bmf* as probe. To screen for Bmf-interacting proteins, mouse *bmf* was subcloned into a pGBT-9 derivative harboring the gene for chloramphenicol acetyltransferase as the selection marker. Out of 5×10^6 clones screened, 60 positive clones were initially selected.
13. A. Krogh, M. Brown, I. S. Mian, K. Sjolander, D. Haussler, *J. Mol. Biol.* **235**, 1501 (1994).
14. H. Puthalakath *et al.*, unpublished observations.
15. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
16. X.-M. Yin, Z. N. Oltvai, S. J. Korsmeyer, *Nature* **369**, 321 (1994).
17. Supplementary material is available on *Science* Online at www.sciencemag.org/cgi/content/full/293/5536/1829/DC1
18. Rat monoclonal antibodies (mAbs) to dynein light chains or Bmf were generated as described (*27*). Antibodies were purified either on a protein-G column (Amersham Pharmacia) or on a Sepharose column conjugated with MAR 18.5 antibody (monoclonal mouse secondary antibody to rat Igκ). Monoclonal antibody 11F7 (rat γ2a/κ) recognizes mouse and human DLC1 and DLC2, whereas 10D6 (rat μ/κ) detects mouse and human DLC1 but not DLC2 [Web fig. 2 (*17*)]. Monoclonal antibodies 9G10 and 12E10 (both rat γ2a/κ) detect endogenous mouse and human Bmf by Western blotting and immunoprecipitation. To generate polyclonal antibodies against Bmf, New Zealand White rabbits were immunized with recombinant mouse Bmf. Sera were purified over a Sepharose column conjugated with recombinant mouse Bmf protein.
19. S. Naisbitt *et al.*, *J. Neurosci.* **20**, 4524 (2000).
20. K. W. Lo, S. Naisbitt, J. S. Fan, M. Sheng, M. Zhang, *J. Biol. Chem.* **276**, 14059 (2001).
21. S. E. Benashski, A. Harrison, R. S. Patel-King, S. M. King, *J. Biol. Chem.* **272**, 20929 (1997).
22. For subcellular fractionation, MCF-7 cells (5×10^6) were lysed in 500 μl of extraction buffer containing 1% Triton X-100 (*28*). Cell debris and nuclei were removed by centrifugation at 2000*g*. The supernatant was centrifuged for 60 min at 4°C at 140,000*g* to obtain the filamentous actin–enriched P1 pellet fraction. The remaining supernatant was incubated for 13 min at 37°C with 2 mM paclitaxel and 5 U apyrase (Sigma). This mixture was then loaded on top of a 0.5-ml cushion of 7.5% sucrose (made in the extraction buffer) and centrifuged at 140,000*g* for 30 min at 30°C. Sedimented material was saved as the dynein-enriched P2 fraction and the supernatant was saved as the S fraction. Fractionation of cell extracts on sucrose gradients was done as described (*11*). Heavier fractions contain cytoskeletal proteins; lighter fractions contain soluble and intracellular membrane-associated proteins.
23. S. M. Frisch, E. Ruoslahti, *Curr. Opin. Cell Biol.* **9**, 701 (1997).
24. A. Strasser, A. W. Harris, M. L. Bath, S. Cory, *Nature* **348**, 331 (1990).
25. W. Wick *et al.*, *Oncogene* **12**, 973 (1996).
26. E. Ruoslahti, J. C. Reed, *Cell* **77**, 477 (1994).
27. L. A. O'Reilly *et al.*, *BioTechniques* **25**, 824 (1998).
28. B. M. Paschal, H. S. Shpetner, R. B. Vallee, *Methods Enzymol.* **196**, 181 (1991).
29. E. M. Espreafico *et al.*, *J. Cell Biol.* **119**, 1541 (1992).
30. R. E. Cheney, *Methods Enzymol.* **298**, 3 (1998).

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Role of the Nonsense-Mediated Decay Factor hUpf3 in the Splicing-Dependent Exon-Exon Junction Complex

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Nonsense-mediated messenger RNA (mRNA) decay, or NMD, is a critical process of selective degradation of mRNAs that contain premature stop codons. NMD depends on both pre-mRNA splicing and translation, and it requires recognition of the position of stop codons relative to exon-exon junctions. A key factor in NMD is hUpf3, a mostly nuclear protein that shuttles between the nucleus and cytoplasm and interacts specifically with spliced mRNAs. We found that hUpf3 interacts with Y14, a component of post-splicing mRNA-protein (mRNP) complexes, and that hUpf3 is enriched in Y14-containing mRNP complexes. The mRNA export factors Aly/REF and TAP are also associated with nuclear hUpf3, indicating that hUpf3 is in mRNP complexes that are poised for nuclear export. Like Y14 and Aly/REF, hUpf3 binds to spliced mRNAs specifically (~20 nucleotides) upstream of exon-exon junctions. The splicing-dependent binding of hUpf3 to mRNAs before export, as part of the complex that assembles near exon-exon junctions, allows it to serve as a link between splicing and NMD in the cytoplasm.

Eukaryotic cells have a conserved surveillance mechanism that serves to ensure that only correctly processed mRNAs will be translated to produce proteins (*1–4*). An important example of this is NMD, which selectively degrades mRNAs that contain premature termination codons, thus avoiding the production of potentially deleterious COOH-terminal–truncated proteins. For NMD, cells must have the capacity to distinguish premature stop codons from legitimate, wild-type stop codons. In mammalian cells, the legitimate stop codon is almost always found on the last exon in the mRNA. If translation terminates more than ~50 to 55 nucleotides (nt) upstream of the last exon-exon junction, the mRNA is subject to rapid decay. This suggests that a mechanism must exist to define exon-exon junctions on mRNAs in the cytoplasm. Such a mechanism would be established by splicing in the nucleus and would persist on the mRNA in the cytoplasm at least through the first round of translation, because translation is required to trigger NMD. Although nucleus-associated NMD has also been suggested (*5–9*), it is likely that mRNA degradation takes

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place mostly in the cytoplasm (*10, 11*).

Recently, splicing has been shown to alter the composition of mRNP complexes (*12–14*). Several proteins have been found to bind to mRNAs produced by splicing on spliced mRNAs, including RNPS1 (*15*), SRm160 (*13, 16*), DEK (*17*), Aly/REF (*18*), and Y14 (*14*). Moreover, these proteins bind at a specific position (24 or 20 nt) upstream of exon-exon junctions (*19, 20*). This splicing-dependent exon-exon junction complex is dynamic in vivo (*19*). Of the proteins identified so far, only the binding of Y14 persists on the mRNAs in the cytoplasm at the same position. Thus, Y14 has the necessary attributes to serve as the mark that indicates premature termination codons, to communicate this information to the cytoplasm, and thereby to trigger NMD. However, it is not known whether Y14 has a direct role in NMD.

The factors that execute the NMD response were first identified in *Saccharomyces cerevisiae* and were named Upf1p, Upf2p/Nmd2p, and Upf3p (*21–23*). Upf orthologs have been found in divergent organisms (*24–29*). Two closely related human Upf3 genes (*hUpf3a* and *hUpf3b*) have been identified, each of which produces several variants via alternative splicing (*28, 29*). Tethering human Upf3 to the β-globin mRNA induces the destruction of the mRNA when bound downstream of a termination codon (*28*). The Upf proteins interact with each other; Upf2 interacts with both Upf1 and Upf3 (*29, 30*) and with translation release factors (*31, 32*), suggesting that a NMD trigger complex can form at the time of translation termination. Although hUpf1, hUpf2, and hUpf3 can exist as a complex, they show different subcellular localization patterns (*28, 29*). Whereas hUpf1 is distributed throughout the cytoplasm, hUpf2 is concentrated in the perinuclear area. hUpf3, by contrast, is predominantly nuclear and shuttles between the nucleus and cytoplasm. This finding suggests that hUpf3 associates first with mRNA while in the nucleus, and that hUpf2 joins the complex upon export. Later, at the time of translation termination, hUpf1 binds to hUpf2, establishing the surveillance complex that mediates the decapping of the mRNA (*28*). Notably, hUpf3 proteins are preferentially associated with spliced mRNAs in vivo, although where and when this binding occurs is not known (*28*). These observations raised the possibility that hUpf3 binds to mRNA near exon-exon junctions and thereby participates in communicating the necessary positional information that links the NMD response to splicing.

Because Y14 binds preferentially to mRNAs produced by splicing, immunoprecipitations with antibodies to Y14 can be used to isolate post-splicing mRNP complexes (*14*). Post-splicing mRNP complexes share some components with general heterogeneous nuclear RNP (hnRNP) complexes but represent a different population. We first examined whether human Upf3 proteins are present in Y14 complexes. To do so, we transiently expressed hUpf3b with a Flag tag at its NH_2-terminus in HeLa cells (*33*). The nucleoplasmic fraction of transfected cells was prepared and subjected to immunoprecipitations followed by Western blotting (*34*) (Fig. 1A). Flag-hUpf3b was coimmunoprecipitated with 4C4, a monoclonal antibody (mAb) to Y14; this result indicates that hUpf3b is associated with Y14 complexes in vivo. A similar experiment showed that Flag-hUpf3a is also associated with Y14 (Fig. 1B). General hnRNP complexes can be efficiently isolated by immunoprecipitation using hnRNP C protein mAb 4F4 (*35*). Because hnRNP proteins bind to nascent transcripts (*36*), most of the RNP complexes that are isolated from the nucleoplasm by 4F4 immunoprecipitation contain pre-mRNAs and splicing intermediates. The hUpf3a protein is more enriched in mRNP complexes than in hnRNP complexes (Fig. 1B). As noted previously, hnRNP A1 is more abundant in general hnRNP complexes (*14*). The hUpf3a protein appears to be more enriched than Aly/REF in mRNP complexes, which suggests that hUpf3a joins mRNP complexes at a later stage. Similar results were obtained with hUpf3b (*37*).

The association of hUpf3 proteins (such

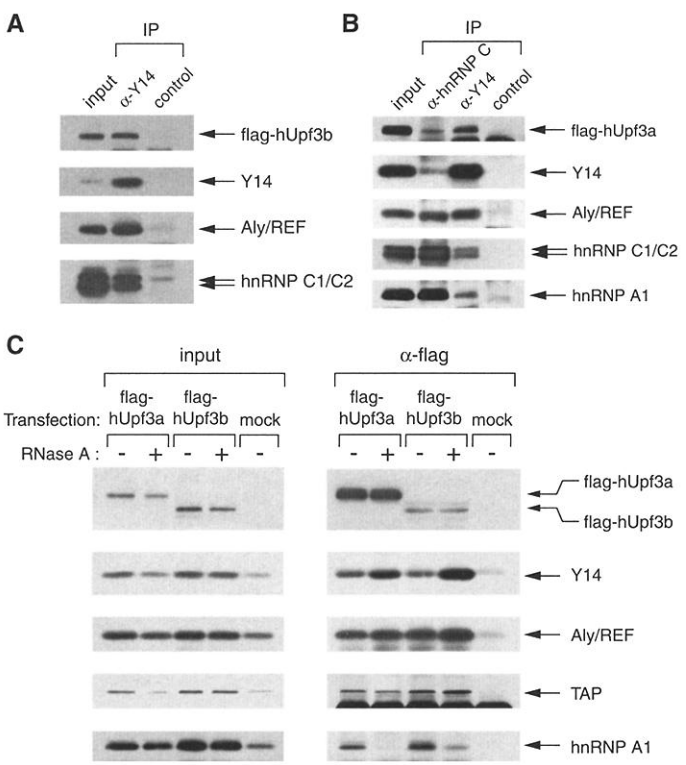

Fig. 1. The hUpf3 proteins are associated with Y14-containing mRNP complexes. **(A)** Immunoprecipitations (IP) were performed using the nucleoplasmic fraction of HeLa cells 24 hours after transfection of Flag-hUpf3b. Y14 mAb 4C4 and nonimmune control antibody (SP2/0) were used for immunoprecipitation. Coimmunoprecipitated proteins were visualized by Western blotting using polyclonal rabbit antibody to Flag, Y14 mAb 1F12, Aly/REF mAb 11G5, and hnRNP C mAb 4F4; 1% of the total was loaded in the input lane. **(B)** Immunoprecipitations and Western blotting were carried out using 293T cells transfected with Flag-hUpf3a; hnRNP C mAb 4F4 was used for immunoprecipitations. Western blotting was done using polyclonal rabbit antibody to Flag, Y14 mAb 1F12, Aly/REF mAb 11G5, hnRNP C mAb 4F4, and hnRNP A1 mAb 4B10; 2% of the total was loaded in the input lane. **(C)** Immunoprecipitations were performed using nucleoplasmic fractions of 293T cells 24 hours after transfection of either Flag-Upf3a or Flag-Upf3b. For mock transfection, the same amount (10 μg per plate) of Flag-pcDNA3 was used. For RNase A treatment, RNase A was added to the nucleoplasmic fraction to a final concentration of 10 μg/ml and preincubated on ice for 15 min before immunoprecipitation Flag mAb M2 was used for immunoprecipitations. Coimmunoprecipitated proteins were visualized by Western blotting using polyclonal rabbit antibody to Flag, Y14 mAb 1F12, Aly/REF mAb 11G5, TAP mAb 53H8, and hnRNP A1 mAb 4B10; 2% of the total was loaded in the input panels.

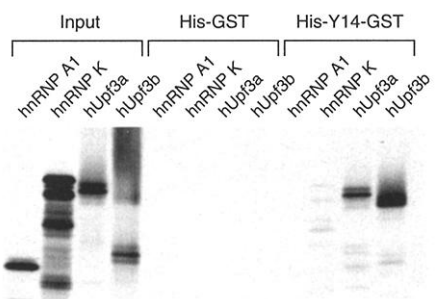

Fig. 2. The hUpf3 proteins interact with Y14 in vitro. Myc-hnRNP A1, myc-hnRNP K, Flag-hUpf3a, and Flag-hUpf3b proteins were translated and labeled with [^{35}S]methionine in vitro and used in a binding assay with 4 μg of recombinant His-GST or His-Y14-GST; 10% of input was loaded on the left side.

as Flag-hUpf3a or Flag-hUpf3b produced by transfection) with mRNP complexes was further examined by immunoprecipitations using Flag mAB M2 followed by Western blotting with Y14 mAb, Aly mAb, TAP mAb, and hnRNP A1 mAb (Fig. 1C). This experiment confirmed that hUpf3 proteins are present in complexes that contain Y14. In addition, we found that hUpf3 proteins are coimmunoprecipitated with the mRNA export factors Aly/REF and TAP, indicating that hUpf3 proteins are part of mRNP complexes that are poised for nuclear export. Moreover, these interactions are not abolished by ribonuclease (RNase) treatment, which suggests that the association of hUpf3 with Y14, Aly/REF, and TAP is mediated by protein-protein interactions (Fig. 1C). In contrast, the coimmunoprecipitation of hnRNP A1 with these proteins is sensitive to RNase treatment, indicating that this interaction is bridged by RNA.

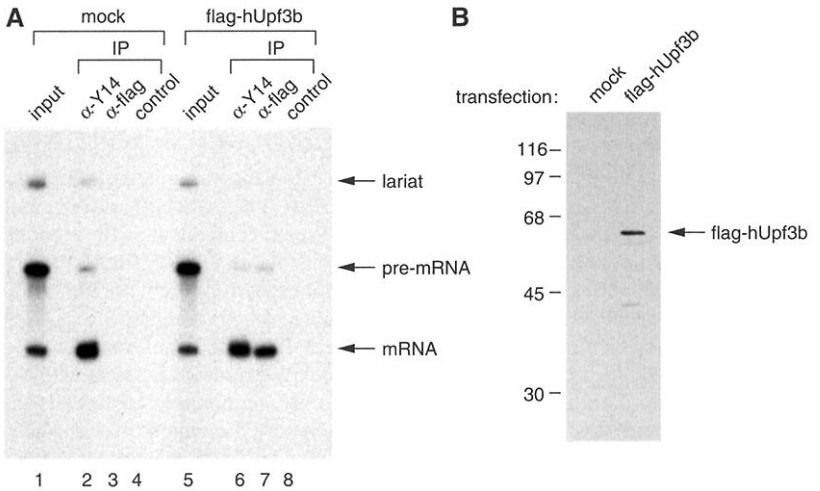

Fig. 3. hUpf3b is associated preferentially with spliced mRNA in vitro. **(A)** Immunoprecipitations were carried out after in vitro splicing of ^{32}P-labeled Ad2 pre-mRNA. For inclusion of Flag-hUpf3b in the reaction, HeLa nuclear extract was added with total cell extract from 293T cells that had been transfected with control plasmid (Flag-pcDNA3, lanes 1 to 4) or Flag-hUpf3b (lanes 5 to 8). Flag mAb M2, Y14 mAb 4C4, and control antibody (SP2/0) were used for immunoprecipitations. **(B)** Western blotting of the extracts from the cells transfected with control plasmid (mock) or Flag-hUpf3b (Flag-hUpf3b). The Flag-hUpf3b protein was detected using polyclonal rabbit antibody to Flag.

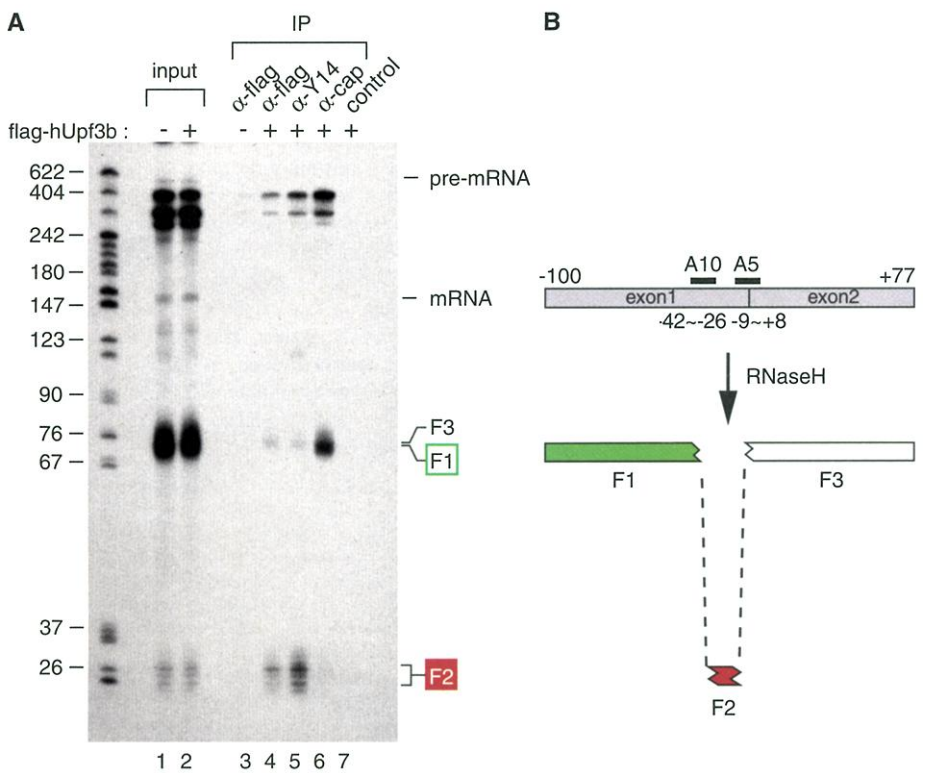

Fig. 4. hUpf3b is located immediately upstream of exon-exon junctions. **(A)** ^{32}P-labeled Ad2 pre-mRNA was incubated under in vitro splicing conditions as in Fig. 3 and subjected to RNase H digestion followed by immunoprecipitations and RNA analysis. Lanes 1 and 2 display the fragmentation pattern of RNAs after splicing and RNase H digestion. Lanes 3 to 7 show the fragments that were immunoprecipitated using the indicated antibodies. 293T cells had been transfected with control plasmid (Flag-pcDNA3, lanes 1 and 3) or Flag-hUpf3b (lanes 2 and 4 to 7). Flag mAb, Y14 mAb 4C4, cap mAb H20, and control nonimmune antibody (SP2/0) were used for immunoprecipitations. **(B)** Positions of the oligonucleotides and the RNA fragments from the spliced Ad2 mRNA are depicted. The red box indicates the fragment bound to hUpf3b and Y14 (F2). The green box indicates the 5′-most fragment (F1).

Because hUpf3 proteins were coimmunoprecipitated with Y14 in an RNase-resistant manner, we investigated whether hUpf3 proteins can bind to Y14. hUpf3 proteins were produced by transcription and translation in vitro and used in binding assays with recombinant Y14 fused to glutathione *S*-transferase (GST) (*38*) (Fig. 2). Two abundant nuclear RNA-binding proteins were used as controls—hnRNP A1, which contains two RBDs and an RGG box (*39*), and hnRNP K, a KH domain–containing protein (*40, 41*)—and neither showed detectable binding. We conclude that both hUpf3a and hUpf3b bind specifically to Y14, although we cannot ascertain that the binding to Y14 is direct because of the presence of other proteins in the reticulocyte lysate. It is nevertheless likely that Y14 is involved in the binding of these NMD factors (hUpf3) to spliced mRNAs.

To determine whether hUpf3 proteins stably interact with RNAs under splicing conditions, we spliced adenovirus MLP ΔIVS (Ad2) pre-mRNA (*14*) in nuclear extract (*42*). In a recent study, Lykke-Andersen *et al.* (*28*) reported that antibodies to hUpf3 could not immunoprecipitate RNA from in vitro splicing reactions, which suggests that hUpf3 proteins may become associated with mRNA at a late post-splicing stage, which does not occur efficiently in vitro. To address the same issue (and because recombinant hUpf3 could not be efficiently produced in bacteria), we produced Flag-hUpf3b protein by transient transfection (Fig. 3B) and added lysates from the transfected cells to the nuclear extract in which the splicing reaction was carried out. We then made use of Flag mAb M2, which is highly efficient in immunoprecipitation. The Flag mAb preferentially immunoprecipitated spliced mRNA (Fig. 3A, lane 7), displaying a similar pattern to that obtained with Y14 mAb 4C4 (lane 6). In contrast, when lysates from mock-transfected cells (transfected with a plasmid lacking hUpf3b cDNA) were added (Fig. 3, lanes 1 to 4), Flag mAb did not immunoprecipitate any RNA (lane 3), indicating that hUpf3b is responsible for the pre-

cipitation of spliced mRNA in this system. Similar results were observed for another splicing substrate, chicken δ-crystallin (CDC) pre-mRNA (*14, 37*).

This system made it possible to map the binding site of hUpf3 on mRNAs (*43*). After incubation of ^{32}P-labeled pre-mRNAs under splicing conditions, antisense deoxyoligonucleotides complementary to specific mRNA sequences were added together with RNase H in order to cleave the mRNA in a sequence-specific manner. We then performed immunoprecipitations with Flag mAb M2, Y14 mAb 4C4, cap mAb H20 (to define the 5′ fragment) (*44*), or control nonimmune antibodies (SP2/0). The total RNA fragments and those that coimmunoprecipitated with each of these antibodies were extracted and resolved by polyacrylamide gel electrophoresis. If Flag-hUpf3b preferentially binds to a certain region, Flag mAb should immunoprecipitate the fragment that contains it (*19*). Cleavage of the Ad2 mRNA with two oligonucleotides, A10 and A5, generates three major fragments (Fig. 4B). The results of in vitro splicing of the Ad2 pre-mRNA followed by RNase H digestion and immunoprecipitation are shown in Fig. 4A. The Flag and Y14 mAbs immunoprecipitated specifically the small fragments designated as F2 (~ –30 to –5 relative to the exon-exon junction) (Fig. 4A, lanes 4 and 5, respectively). In contrast, cap mAb immunoprecipitated the 5′ fragment, confirming the identification of these fragments (Fig. 4A, lane 6). Experiments with the CDC mRNA produced a similar result (*37*), indicating that the position-specific binding of hUpf3b immediately upstream of the exon-exon junction is a general phenomenon.

From these experiments, we conclude that hUpf3 is a bona fide component of the nuclear mRNP complex that is produced by splicing, and that hUpf3 binds specifically upstream of exon-exon junctions. Moreover, hUpf3 interacts with Y14 via protein-protein interaction and thus participates with Y14 in the formation of the complex that assembles –20 nt relative to exon-exon junctions. This splicing-dependent exon-exon junction complex appears to be multifunctional (Fig. 5). One of its functions is to facilitate the export of spliced mRNAs by recruiting proteins, such as Aly/REF and TAP, that can promote mRNA export (*18, 45*). Another likely function of this complex is in NMD. Our findings here suggest that the recruitment of hUpf3 to mRNA is dependent on splicing and occurs in the nucleus where most of hUpf3 is found. Together with other proteins that assemble on the spliced mRNA in the same position, including Y14 and Aly/REF, hUpf3 likely travels with the mRNA to the cytoplasm. Indeed, Upf3p has been detected on polysomes (*46*), and it can form a complex with the cytoplasmic protein hUpf1 (*28*). Our results provide strong evidence that hUpf3 proteins are part of the imprint or mark that delivers the positional information of the excised introns from the nucleus to the cytoplasm. Thus, the splicing-dependent position-specific binding of hUpf3 to mRNAs likely provides the long-sought link between splicing and the NMD pathway.

It can be envisioned that once the mRNA engages the protein synthesis machinery, the leading translating ribosome (or an associated activity) displaces the Y14-hUpf3 complexes from the mRNA. For a normal wild-type mRNA, all the Y14-hUpf3 complexes may be displaced from the mRNA during the first round of translation because the legitimate stop codon is usually found on the last exon. However, if a nonsense mutation occurs more than 50 to 55 nt upstream of the last exon-exon junc-

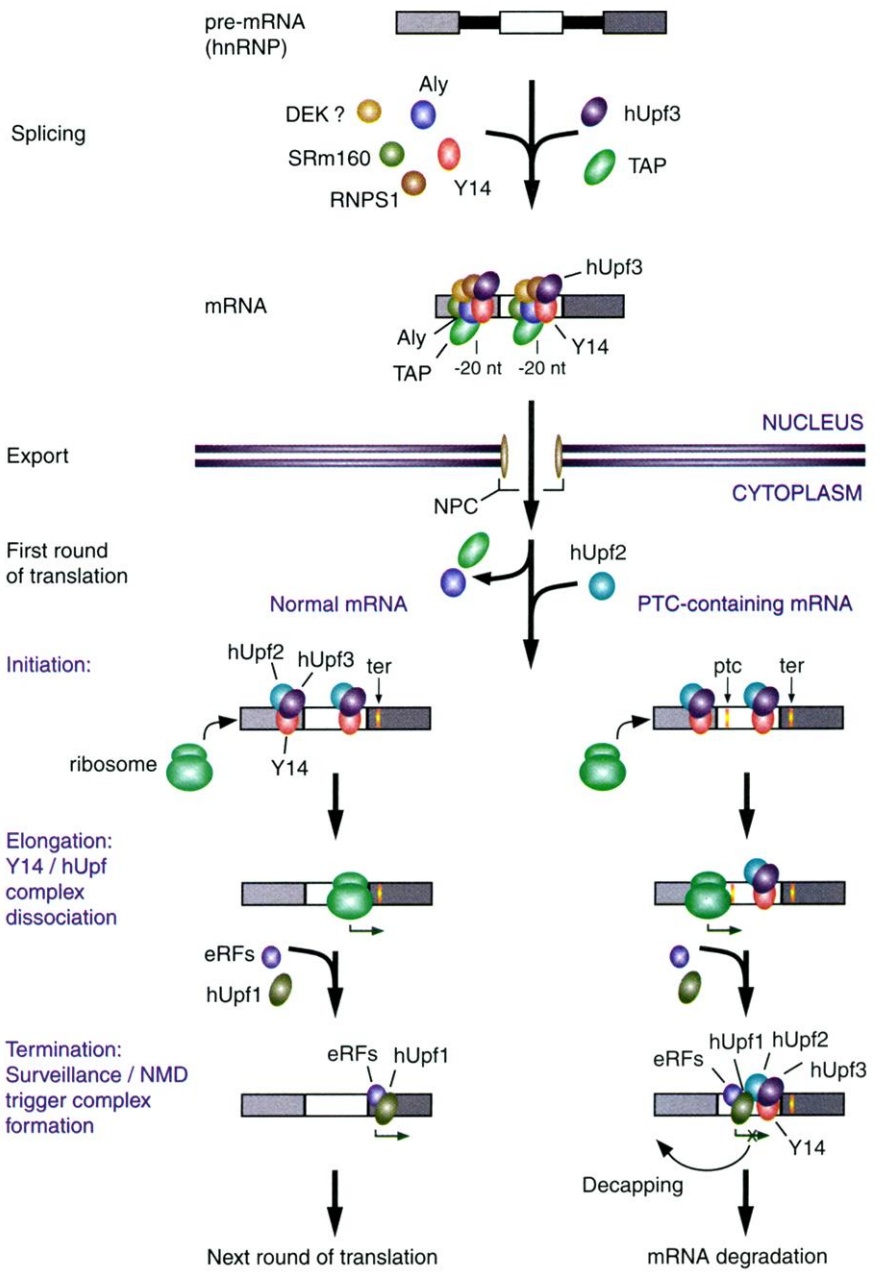

Fig. 5. A model depicting our view of the role of the exon-exon junction complex in providing a functional link among pre-mRNA splicing, nuclear export, and NMD of mRNA. The exon-exon junction complex comprises proteins that bind mRNAs that are produced by splicing in the nucleus. Several of the components of the complex remain associated with newly exported mRNAs (in the same position, 20 nt 5′ to exon-exon junctions) in the cytoplasm. The pre-mRNAs are also with hnRNP proteins, which are omitted here for simplicity. See text for details. Abbreviations: PTC, premature termination codon/nonsense codon; NPC, nuclear pore complex; RF, translation release factor.

tion, the ribosomes will dissociate from the mRNA before they reach the legitimate termination codon, and thus they will fail to remove the remaining Y14-hUpf3 complex. This remaining complex, because it likely contains hUpf3, could recruit hUpf2 and hUpf1, probably together with components of the termination complex (eRF1 and eRF3), and trigger degradation of this mRNA. There may be additional functions for the exon-exon junction in the cytoplasm, such as influencing the efficiency of translation [which for some mRNAs depends on the splicing pattern of the mRNA (*47*)] and the localization of mRNAs. Determining the complete composition and functions of individual components of this complex will shed further light on post-splicing gene regulation.

References and Notes

1. M. W. Hentze, A. E. Kulozik, *Cell* **96**, 307 (1999), and references therein.
2. A. B. Shyu, M. F. Wilkinson, *Cell* **102**, 135 (2000), and references therein.
3. L. E. Maquat, G. G. Carmichael, *Cell* **104**, 173 (2001), and references therein.
4. J. Lykke-Andersen, *Curr. Biol.* **11**, R88 (2001), and references therein.
5. H. C. Dietz, R. J. Kendzior Jr., *Nature Genet.* **8**, 183 (1994).
6. S. Aoufouchi, J. Yelamos, C. Milstein, *Cell* **85**, 415 (1996).
7. S. Li, M. F. Wilkinson, *Immunity* **8**, 135 (1998).
8. S. Brogna, *RNA* **5**, 562 (1999).
9. A. Gersappe, D. J. Pintel, *Mol. Cell. Biol.* **19**, 1640 (1999).
10. R. Thermann *et al.*, *EMBO J.* **17**, 3484 (1998).
11. P. M. Moriarty, C. C. Reddy, L. E. Maquat, *Mol. Cell. Biol.* **18**, 2932 (1998).
12. M. J. Luo, R. Reed, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14937 (1999).
13. H. Le Hir, M. J. Moore, L. E. Maquat, *Genes Dev.* **14**, 1098 (2000).
14. N. Kataoka *et al.*, *Mol. Cell* **6**, 673 (2000).
15. A. Mayeda *et al.*, *EMBO J.* **18**, 4560 (1999).
16. B. J. Blencowe, R. Issner, J. A. Nickerson, P. A. Sharp, *Genes Dev.* **12**, 996 (1998).
17. T. McGarvey *et al.*, *J. Cell Biol.* **150**, 309 (2000).
18. Z. Zhou *et al.*, *Nature* **407**, 401 (2000).
19. V. N. Kim *et al.*, *EMBO J.* **20**, 2062 (2001).
20. H. Le Hir, E. Izaurralde, L. E. Maquat, M. J. Moore, *EMBO J.* **19**, 6860 (2000).
21. P. Leeds, S. W. Peltz, A. Jacobson, M. R. Culbertson, *Genes Dev.* **5**, 2303 (1991).
22. Y. Cui, K. W. Hagan, S. Zhang, S. W. Peltz, *Genes Dev.* **9**, 423 (1995).
23. F. He, A. Jacobson, *Genes Dev.* **9**, 437 (1995).
24. J. T. Mendell, S. M. Medghalchi, R. G. Lake, E. N. Noensie, H. C. Dietz, *Mol. Cell. Biol.* **20**, 8944 (2000).
25. R. Pulak, P. Anderson, *Genes Dev.* **7**, 1885 (1993).
26. H. A. Perlick, S. M. Medghalchi, F. A. Spencer, R. J. Kendzior Jr., H. C. Dietz, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10928 (1996).
27. S. E. Applequist, M. Selg, C. Raman, H. M. Jack, *Nucleic Acids Res.* **25**, 814 (1997).
28. J. Lykke-Andersen, M. D. Shu, J. A. Steitz, *Cell* **103**, 1121 (2000).
29. G. Serin, A. Gersappe, J. D. Black, R. Aronoff, L. E. Maquat, *Mol. Cell. Biol.* **21**, 209 (2001).
30. F. He, A. H. Brown, A. Jacobson, *RNA* **2**, 153 (1996).
31. K. Czaplinski *et al.*, *Genes Dev.* **12**, 1665 (1998).
32. W. Wang, K. Czaplinski, Y. Rao, S. W. Peltz, *EMBO J.* **20**, 880 (2001).
33. The cDNAs encoding hUpf3a and hUpf3b were isolated from 293T cells by reverse transcription polymerase chain reaction (RT-PCR) using the primers that were designed according to the previously reported sequences AF318575 and AF318576 (*29*). Primers for hUpf3a are 5′-CGAGGATCCATGCTGTCGGCCCTAGAAGTGCAGTTCCAC-3′ (sense; underlined nucleotides represent the Bam HI site) and 5′-CGTACTCGAGTCACTCTGCCTCTTCCCTCTTCTCAGGACC-3′ (antisense; underlined nucleotides indicate the Xho I site). Primers for hUpf3b are 5′-CGAGGATCCATGAAGGAAGAGAAGGAGCACAGGCC-3′ (sense; underlined nucleotides represent the Bam HI site) and 5′-CGTACTCGAGTTATCACTCCTCTCCTCCTTCTTTTCTATGGC-3′ (antisense; underlined nucleotides indicate the Xho I site). The PCR products were digested with Bam HI and Xho I and inserted into Flag-pcDNA3, which contains a single Flag epitope between the Hind III and Bam HI sites. The resulting *hUpf3a* gene lacks 12 nucleotides that encode amino acids 255 to 258 (numbering based on AF318575). The *hUpf3b* gene that we isolated was identical to AY013251, which lacks 39 nucleotides of exon 8 from AF318576.
34. Subcellular fractionations of HeLa and 293T cells and immunoprecipitations were done in RSB-100 buffer [10 mM tris-HCl (pH 7.5), 100 mM NaCl, 2.5 mM $MgCl_2$, 35 μg/ml digitonin, and one tablet of protease inhibitor cocktail (Roche) per 50 ml of buffer]. Y14 mAbs 4C4 and 1F12 were used for immunoprecipitation and Western blotting, respectively (*14*). Aly/REF mAb 11G5 was described in (*19*). Flag mAb M2, conjugated on agarose beads, and polyclonal rabbit antibody to Flag were purchased from Sigma and used for immunoprecipitation and Western blotting, respectively. For RNase A treatment, RNase A (5.9 mg/ml, U.S. Biochemical) was added to a final concentration of 10 μg/ml and preincubated on ice for 15 min before immunoprecipitation.
35. Y. D. Choi, G. Dreyfuss, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7471 (1984).
36. E. L. Matunis, M. J. Matunis, G. Dreyfuss, *J. Cell Biol.* **121**, 219 (1993).
37. V. N. Kim, N. Kataoka, G. Dreyfuss, data not shown.
38. hUpf3a and hUpf3b proteins as well as hnRNP A1 and hnRNP K proteins were translated in vitro using reticulocyte lysate (Promega) from plasmids Flag-hUpf3a, Flag-hUpf3b, myc-A1 (*49*), and myc-K (*50*); 4 μg of GST-fusion proteins were used for each binding. Binding buffer consisted of 10 mM tris-HCl (pH 7.5), 200 mM NaCl, 10% glycerol, 0.1% Triton X-100, RNase A (2 μg/ml), and 1 tablet of protease inhibitor cocktail (Roche) per 50 ml of buffer. Washing was done in the same buffer without RNase A.
39. G. Dreyfuss, M. J. Matunis, S. Pinol-Roma, C. G. Burd, *Annu. Rev. Biochem.* **62**, 289 (1993).
40. H. Siomi, M. J. Matunis, W. M. Michael, G. Dreyfuss, *Nucleic Acids Res.* **21**, 1193 (1993).
41. C. G. Burd, G. Dreyfuss, *Science* **265**, 615 (1994).
42. For preparation of templates for in vitro transcription, pCDC (previously referred to as pSP14-15) and pAd2 were linearized with Sma I and Xba I, respectively (*51*). The in vitro splicing reaction with HeLa and 293T cell extracts was performed as described (*51*, *52*) with the following modifications: 293T cells were transfected with either Flag-hUpf3b or Flag-pCDNA3. After 24 hours, whole-cell extract was prepared from transfected 293T cells in buffer D [10 mM Hepes-KOH (pH 7.9), 100 mM KCl, 0.2 mM EDTA] by sonication followed by centrifugation. Each 10 μl of splicing reaction contained 1 μl of HeLa nuclear extract and 5 μl of 293T whole-cell extract.
43. A 40-μl portion of the splicing reaction mixture was treated with 1 unit of RNase H (Promega) and 5 to 7 μM of oligonucleotides in a total reaction volume of 50 μl for 20 min (see figure legends) at 30°C, as described (*19*).
44. P. Bochnig, R. Reuter, P. Bringmann, R. Lührmann, *Eur. J. Biochem.* **168**, 461 (1987).
45. J. P. Rodrigues *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1030 (2001).
46. A. L. Atkin *et al.*, *J. Biol. Chem.* **272**, 22163 (1997).
47. K. Matsumoto, K. M. Wassarman, A. P. Wolffe, *EMBO J.* **17**, 2107 (1998).
48. M. C. Siomi *et al.*, *J. Cell Biol.* **138**, 1181 (1997).
49. W. M. Michael, M. Choi, G. Dreyfuss, *Cell* **83**, 415 (1995).
50. W. M. Michael, P. S. Eder, G. Dreyfuss, *EMBO J.* **16**, 3587 (1997).
51. L. Pellizzoni, N. Kataoka, B. Charroux, G. Dreyfuss, *Cell* **95**, 615 (1998).
52. M. Ohno, Y. Shimura, *Genes Dev.* **10**, 997 (1996).

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Communication of the Position of Exon-Exon Junctions to the mRNA Surveillance Machinery by the Protein RNPS1

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In mammalian cells, splice junctions play a dual role in mRNA quality control: They mediate selective nuclear export of mature mRNA and they serve as a mark for mRNA surveillance, which subjects aberrant mRNAs with premature termination codons to nonsense-mediated decay (NMD). Here, we demonstrate that the protein RNPS1, a component of the postsplicing complex that is deposited 5′ to exon-exon junctions, interacts with the evolutionarily conserved human Upf complex, a central component of NMD. Significantly, RNPS1 triggers NMD when tethered to the 3′ untranslated region of β-globin mRNA, demonstrating its role as a subunit of the postsplicing complex directly involved in mRNA surveillance.

The maturation of mRNA in eukaryotes involves nuclear processing before cytoplasmic translation. The quality of the mRNA is assessed at different stages. For example, export of mRNA requires successful pre-mRNA splicing, and mRNAs