FUS functions in coupling transcription to splicing by mediating an interaction between RNAP II and U1 snRNP

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Pre-mRNA splicing is coupled to transcription by RNA polymerase II (RNAP II). We previously showed that U1 small nuclear ribonucleoprotein (snRNP) associates with RNAP II, and both RNAP II and U1 snRNP are also the most abundant factors associated with the protein fused-in-sarcoma (FUS), which is mutated to cause the neurodegenerative disease amyotrophic lateral sclerosis. Here, we show that an antisense morpholino that base-pairs to the 5' end of U1 snRNA blocks splicing in the coupled system and completely disrupts the association between U1 snRNP and both FUS and RNAP II, but has no effect on the association between FUS and RNAP II. Conversely, we found that U1 snRNP does not interact with RNAP II in FUS knockdown extracts. Moreover, using these extracts, we found that FUS must be present during the transcription reaction in order for splicing to occur. Together, our data lead to a model that FUS functions in coupling transcription to splicing via mediating an interaction between RNAP II and U1 snRNP.

ALS | coupling transcription to splicing | RNA polymerase II | U1 snRNP

It is now well established that the steps in gene expression are extensively coupled to one another, including both physical and functional coupling between RNA polymerase II (RNAP II) transcription and pre-mRNA processing (1–11). Moreover, the majority of nascent transcripts are spliced cotranscriptionally, while the transcripts are still tethered to RNAP II. In vitro systems have been developed for the coupled transcription/splicing (txn/splicing) reaction as well as for cotranscriptional splicing, and these systems have been used to investigate the mechanisms underlying these processes (12–17). In the case of coupled txn/splicing, studies using the in vitro system revealed that transcription potently enhances spliceosome assembly, which in turn leads to a strong enhancement of the splicing reaction (13). Additional studies revealed that the only essential splicing factors that copurify with RNAP II are U1 small nuclear ribonucleoprotein (snRNP) and its associated factors, the serine/arginine-rich (SR) proteins (18–20). This observation is particularly noteworthy because U1 snRNP/SR proteins are the first splicing factors that bind to pre-mRNA during spliceosome assembly (21). Although functional studies indicate that SR proteins play a key role in coupling transcription to splicing (19), the role of U1 snRNP in this coupling event has not been examined. It is also not known how U1 snRNP interacts with RNAP II. U1 snRNA is known to base-pair to the 5' splice site during the earliest steps in spliceosome assembly, and this interaction is essential for splice-site recognition (21). In addition, we and others found that U1 snRNP/SR and RNAP II are among the main factors that associate with the protein fused-in-sarcoma (FUS) (19, 22–27). Understanding the normal roles of FUS and the pathways in which it functions are of great importance because FUS is mutated to cause the fatal motor neuron disease amyotrophic lateral sclerosis (ALS) (28–32). In vivo studies indicate that FUS plays numerous roles in the nucleus, including transcription, splicing, microRNA processing, and DNA repair

(28–32). How FUS is involved in these processes and whether FUS is directly involved have not been established.

Because robust in vitro systems for the coupled txn/splicing reaction are now routinely used (14, 15, 17, 19, 33), we can directly test the roles of U1 snRNP and FUS in this coupled reaction. In recent work, an antisense morpholino oligonucleotide (AMO) that base-pairs to the 5' end of U1 snRNA was used to functionally inactivate U1 snRNP (34, 35). Here, we report that this U1 AMO disrupts the association between U1 snRNP and both FUS and RNAP II, whereas FUS remains associated with RNAP II. However, in FUS knockdown (KD) nuclear extracts, we found that U1 snRNP can no longer interact with RNAP II. Furthermore, our data reveal that FUS must be present during transcription in order for splicing to take place in the coupled txn/splicing system. Together, our data indicate that FUS functions in coupling transcription to splicing via mediating an association between U1 snRNP and RNAP II.

Results and Discussion

U1 AMO Blocks Spliceosome Assembly and Splicing in the Coupled Txn/Splicing System. An extensively characterized U1 AMO (Fig. 1A) is known to inhibit splicing in vivo and also in vitro in uncoupled splicing systems, and to disrupt the essential base-pairing interaction between U1 snRNA and the 5' splice site (34, 35). In our study, we used this U1 AMO to investigate U1 snRNP in the coupled txn/splicing reaction. A size-matched scrambled AMO was used as a negative control. The well-characterized CMV-Ftz DNA template was used to examine the effects of the AMOs on coupled txn/splicing (Fig. S1) (13). This

Significance

The protein fused-in-sarcoma (FUS) is mutated to cause the neurodegenerative disease amyotrophic lateral sclerosis, but its normal cellular role remains to be understood. Previous work showed that FUS associates with both RNA polymerase II (RNAP II) and the essential splicing factor U1 small nuclear ribonucleoprotein (snRNP). Here we were able to directly investigate the functional significance of these interactions using an in vitro system. We show that FUS is essential for the interaction between U1 snRNP and RNAP II and that FUS must be present during the RNAP II transcription reaction in order for splicing to occur. Together, these data indicate that FUS mediates an interaction between RNAP II and U1 snRNP, thereby physically and functionally coupling transcription to splicing.

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Fig. 1. U1 AMO disrupts the interaction between U1 snRNP and RNAP II. (A) Schematic showing the U1 AMO (red line) base paired to U1 snRNA in U1 snRNP. The Sm core and the U1 snRNP-specific proteins (U1-70K, U1A, and U1C) are indicated. (B) Coomassie-stained gel showing the proteins immunoprecipitated by RNAP II (lanes 2 and 4) or control (lanes 1 and 3) antibody after nuclear extract was incubated with the control (lanes 1 and 2) or U1 (lanes 3 and 4) AMO. FUS, the U1 snRNP proteins, and molecular-mass markers (in kDa) are indicated. (C and D) Same as B except proteins (C) or RNA (D) was analyzed by Western blot using the indicated antibodies or on an ethidium bromide-stained gel, respectively. In D, we note that samples 1–6 were prepared side-by-side, but samples 1 and 2 and samples 3–6 were run on two separate gels, as indicated by the white space between the two gels.

analysis revealed that the U1 AMO specifically inhibited splicing, but not transcription, and in a dose-dependent manner (Fig. S1). Moreover, the U1 AMO inhibited spliceosome assembly, and, instead, a faster migrating complex was detected (Fig. S1). Continued incubation did not allow proper spliceosome assembly from this complex (Fig. S1). Furthermore, the U1 AMO blocked both splicing and spliceosome assembly only when the AMO was added to the coupled system before, but not after, transcription (Fig. S2). Finally, when CMV-Ftz was transcribed in the presence of the U1 AMO, followed by dilution into a chase reaction in normal nuclear extract, splicing did not occur (Fig. S2). Together, these data using the U1 AMO indicate that U1 snRNP must be loaded onto pre-mRNA during the transcription reaction in order for efficient spliceosome assembly and splicing to occur. In previous work, we found that SR proteins, which associate with U1 snRNP, also must be recruited to pre-mRNA during the RNAP II transcription reaction in order for splicing to occur (19). In addition, we and others found that U1 snRNP/SR proteins are the only essential splicing factors that associate with RNAP II, and this association occurs even when RNAP II is not present at transcription promoters (18–20). Because U1 snRNP/SR proteins are recruited to pre-mRNA during the earliest steps in the spliceosome assembly pathway

(21), the data lead to a model in which U1 snRNP/SR proteins bound to RNAP II are recruited to promoters, and then, while the nascent pre-mRNA is being synthesized, these splicing factors are recruited to the 5' splice site and adjacent exon to initiate spliceosome assembly. This close physical and functional relationship between RNAP II and U1 snRNP/SR proteins provides a likely explanation for the potent enhancement of spliceosome assembly and splicing observed both in vivo and in vitro when pre-mRNAs are transcribed by RNAP II vs. other polymerases (12–15, 36, 37).

The U1 AMO Disrupts RNAP II Interaction with U1 snRNP, but Not with FUS. To further investigate the significance of the association between U1 snRNP and RNAP II, we next asked whether the U1 AMO affected this interaction. In addition, we investigated the effect of the U1 AMO on the association of FUS with RNAP II, because FUS interacts with both U1 snRNP and RNAP II. Nuclear extracts were treated with the control or U1 AMO, followed by immunoprecipitations (IPs) with a control antibody or an antibody against RNAP II. As expected, analysis of the proteins revealed that both FUS and U1 snRNP components (U1-70K, U1A, and U1C) were specifically coimmunoprecipitated with RNAP II from extracts treated with the control AMO (Fig. 1B, lanes 1 and 2).

Fig. 2. U1 AMO disrupts the interaction between FUS and U1 snRNP. (A) Coomassie-stained gel showing the proteins immunoprecipitated by a control antibody (lanes 1 and 3) or an antibody against U1C (lanes 2 and 4) after nuclear extract was incubated with the control (lanes 1 and 2) or U1 (lanes 3 and 4) AMO. FUS, the U1 snRNP proteins, and molecular-mass markers (in kDa) are shown. (B and C) Same as A, except that proteins were analyzed by Western (B) or RNA was analyzed by ethidium bromide staining (C). snRNAs and 5S rRNA are indicated. (D–F) Same as A–C, except that FUS antibody was used for the IPs.

However, when the same IPs were carried out by using extracts treated with the U1 AMO, the U1 snRNP components were specifically lacking (Fig. 1B, lanes 3 and 4). In contrast, FUS levels were the same in nuclear extract treated with either the control or U1 AMOs (Fig. 1B, lanes 2 and 4). These results, showing loss of U1 snRNP components in the presence of the U1 AMO and no effect on FUS, were also observed on Western blots (Fig. 1C). Moreover, U1 snRNA was present in the RNAP II IP from the control, but not the U1, AMO-treated extract (Fig. 2D). Together, these data indicate that the U1 AMO specifically disrupts the

association between RNAP II and U1 snRNP. In contrast, the interaction between RNAP II and FUS remains intact in the presence of the U1 AMO.

Interaction of FUS with U1 snRNP Is Blocked by the U1 AMO. We next used the AMOs to investigate the interaction between U1 snRNP and FUS. To undertake this investigation, we first used antibodies against U1C to carry out an IP of U1 snRNP, followed by analysis of the proteins on a Coomassie-stained gel (Fig. 2A). This analysis revealed that the U1 snRNP proteins and

Fig. 4. FUS is required during the transcription reaction to promote splicing. (A) CMV-Ftz was incubated under txn/splicing conditions in the control (Cntl; lanes 1 and 2) or FUS (lanes 3 and 4) KD nuclear extracts for 10 and 30 min. Splicing intermediates and products are indicated. (B) CMV-Ftz was incubated under txn/splicing conditions for 10 min to allow transcription in control (lane 1) or FUS (lane 3) KD nuclear extracts followed by addition of α -amanitin. An aliquot of the transcription reaction was then diluted eightfold into fresh nuclear extract and incubated under txn/splicing conditions for an additional 20 min to allow splicing (lanes 2 and 4). (C) Same as B, except that transcription was carried out for 10 min in the normal nuclear extract (lanes 1 and 3) followed by dilution and continued incubation in the control (lane 2) or FUS (lane 4) knockdown nuclear extracts. (D) CMV-Ftz DNA template (lanes 1–4) or naked T7-Ftz pre-mRNA (lanes 5–8) was incubated under identical conditions in control or FUS KD nuclear extract as indicated. Splicing intermediates and products are designated (note that the Ftz-CMV pre-mRNA has a longer first exon owing to the CMV promoter sequence). Ftz-CMV DNA template was incubated for 5 min to allow transcription, followed by continued incubation for 20 min to allow splicing, whereas naked T7-Ftz pre-mRNA was incubated for 0 and 20 min.

1 and 3) and chased in the control or FUS KD nuclear extract. This analysis revealed that the pre-mRNA was efficiently spliced when chased in either the control or FUS KD nuclear extract (Fig. 4C, lanes 2 and 4). Thus, if the pre-mRNA is transcribed in the presence of available FUS, then splicing occurs efficiently in normal nuclear extract. However, if the pre-mRNA is transcribed in the absence of available FUS, then it does not splice in normal nuclear extract. These data indicate that the function of FUS in splicing has to take place during the transcription reaction. This conclusion is further supported by the observation that FUS KD has no significant effect on uncoupled splicing using naked T7-Ftz pre-mRNA, whereas coupled splicing of CMV-Ftz is inhibited in the same FUS KD extract (Fig. 4D).

The data presented here, together with previous observations showing that FUS associates with both RNAP II and U1 snRNP, lead to the model that FUS functions in coupling transcription to splicing via mediating an interaction between RNAP II and U1 snRNP. Considering that transcription-coupled splicing is such a fundamental cellular process, it is possible that its disruption by mutant FUS contributes to the pathogenesis of ALS. In support of this possibility, many of the mutations in FUS that are ALS-causative are located in the nuclear localization signal (NLS),

which leads to mislocalization of FUS to the cytoplasm in ALS patient cells (29, 38, 39). Moreover, we recently obtained evidence that the U1 snRNP core complex (U1 snRNA and the Sm proteins) is comislocalized to the cytoplasm with FUS in ALS patient fibroblasts harboring mutations in the NLS (40). Thus, decreased levels of both FUS and U1 snRNP in the nucleus may lead to the aberrant splicing that has been reported in transfected cells and ALS patient cells expressing FUS with NLS mutations (29, 30, 38, 39). In previous work, FUS was shown to bind to the C-terminal domain of RNAP II and regulate phosphorylation of serine-2 (23, 25, 27). Moreover, when FUS is knocked down, RNAP II accumulates at the start site of transcription, and premature polyadenylation is observed (23, 25). Notably, U1 snRNP has been found to play other important roles in addition to its function in splicing. One of these roles is known as telescripting, in which binding of U1 snRNP to nascent pre-mRNAs is required to prevent premature polyadenylation during transcription (34, 41). The lack of telescripting, which was found to occur on antisense transcripts due to the presence of more polyA sites and fewer 5' splice sites, is thought to be a mechanism for suppressing transcription of antisense RNAs (35). Thus, the observation that premature polyadenylation occurs with FUS

KD (23, 25), together with our observation that FUS mediates an interaction between U1 snRNP and RNAP II, suggests that the RNAP II–FUS–U1 snRNP complex is the entity that functions in telescripting. The data also raise the possibility that defective telescripting may contribute to ALS pathogenesis.

Materials and Methods

Plasmids and Antibodies. The plasmid encoding CMV–Ftz was described (13). The templates for RNAP II transcription were prepared as described (13, 16). Antibodies to U1-70K (9C4.1) were from Millipore; U1C (4H12) was from Sigma; U1A (BJ-7), HA, and tubulin were from Santa Cruz; RNAP II (8WG16) were from Covance; FUS (293A) was from Bethyl; and Smb/B' (Y12), mouse IgG, and rabbit IgG were from Abcam. Mouse IgG, rabbit IgG, and HA were used as negative controls for polyclonal and monoclonal antibodies, respectively. The FUS rabbit polyclonal antibody was described (22).

RNAP II Transcription and Splicing. CMV–Ftz DNA was incubated under *txn*/splicing conditions as described (13, 17). For AMO assays, control or U1 AMOs (34, 35) were added to HeLa cell nuclear extract at a final concentration of 12 μ M. Control or FUS KD nuclear extracts were prepared by using the small-scale nuclear extract procedure (17, 33). For spliceosome assembly, the CMV–Ftz DNA template was incubated under *txn*/splicing conditions for 10 min. G-50 columns were used to remove unincorporated 32 P-UTP. A total of 1 μ L of heparin (6.5 g/L) was added to 10 μ L of G-50 column-purified reactions before loading on 1.2% low-melting-point agarose gels (42). Uncoupled splicing of

naked T7–Ftz pre-mRNA shown in Fig. 4D was carried out in control or FUS KD nuclear extracts side-by-side under identical conditions as used for *txn*/splicing with the CMV–DNA template.

IPs. Antibodies were coupled to protein A Sepharose and then covalently cross-linked to the beads by using dimethylpimelimidate (Sigma). A 500- μ L mixture containing 150 μ L of nuclear extract, 150 μ L of splicing dilution buffer (20 mM Hepes, pH 7.9, 100 mM KCl), 500 μ M ATP, 3.2 mM MgCl₂, and 20 mM creatine phosphate (di-Tris salt) was incubated for 30 min and 30°. After incubation, mixtures were spun at 4° for 5 min at 16,000 $\times$ g. Supernatants were added to 250 mL of IP buffer [1X PBS, 0.1% Triton X-100, 0.2 mM PMSF, protease inhibitor EDTA-free (Roche)], spun at 4 °C for 5 min, and added to 40 mL antibody–cross-linked beads. After rotation overnight at 4 °C, six washes (1.5 mL each) were performed by using wash buffer (1X PBS containing 0.1% Triton X-100, 0.2 mM PMSF, pH 8.0). Proteins were eluted by adding 60 μ L of SDS sample loading buffer, followed by incubation for 20 min at room temperature. DTT was added to a final concentration of 5 mM, and samples were boiled for 10 min and loaded on 4–12% SDS gradient gels. For RNA IPs, total RNA was isolated and analyzed on 6.5% denaturing gels stained with ethidium bromide.

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- Greenleaf AL (1993) Positive patches and negative noodles: Linking RNA processing to transcription? *Trends Biochem Sci* 18(4):117–119.
- Maniatis T, Reed R (2002) An extensive network of coupling among gene expression machines. *Nature* 416(6880):499–506.
- Prasanth KV, Spector DL (2007) Eukaryotic regulatory RNAs: An answer to the 'genome complexity' conundrum. *Genes Dev* 21(1):11–42.
- Burawski S (2009) Progression through the RNA polymerase II CTD cycle. *Mol Cell* 36(4):541–546.
- Margueron R, Reinberg D (2010) Chromatin structure and the inheritance of epigenetic information. *Nat Rev Genet* 11(4):285–296.
- Proudfoot NJ (2011) Ending the message: Poly(A) signals then and now. *Genes Dev* 25(17):1770–1782.
- Hsin JP, Manley JL (2012) The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes Dev* 26(19):2119–2137.
- Gómez Acuña LI, Fiszbein A, Alló M, Schor IE, Kornblihtt AR (2013) Connections between chromatin signatures and splicing. *Wiley Interdiscip Rev RNA* 4(1):77–91.
- Brugiolo M, Herzl L, Neugebauer KM (2013) Counting on co-transcriptional splicing. *F1000Prime Rep* 5:9.
- Müller-McNicoll M, Neugebauer KM (2013) How cells get the message: Dynamic assembly and function of mRNA-protein complexes. *Nat Rev Genet* 14(4):275–287.
- Bentley DL (2014) Coupling mRNA processing with transcription in time and space. *Nat Rev Genet* 15(3):163–175.
- Ghosh S, Garcia-Blanco MA (2000) Coupled in vitro synthesis and splicing of RNA polymerase II transcripts. *RNA* 6(9):1325–1334.
- Das R, et al. (2006) Functional coupling of RNAP II transcription to spliceosome assembly. *Genes Dev* 20(9):1100–1109.
- Natalizio BJ, Garcia-Blanco MA (2005) In vitro coupled transcription splicing. *Methods* 37(4):314–322.
- Hicks MJ, Yang CR, Kotlajich MV, Hertel KJ (2006) Linking splicing to Pol II transcription stabilizes pre-mRNAs and influences splicing patterns. *PLoS Biol* 4(6):e147.
- Yu Y, Das R, Folco EG, Reed R (2010) A model in vitro system for co-transcriptional splicing. *Nucleic Acids Res* 38(21):7570–7578.
- Folco EG, Reed R (2014) In vitro systems for coupling RNAP II transcription to splicing and polyadenylation. *Methods Mol Biol* 1126:169–177.
- Tian H (2001) RNA ligands generated against complex nuclear targets indicate a role for U1 snRNP in co-ordinating transcription and RNA splicing. *FEBS Lett* 509(2):282–286.
- Das R, et al. (2007) SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. *Mol Cell* 26(6):867–881.
- Spiluttini B, et al. (2010) Splicing-independent recruitment of U1 snRNP to a transcription unit in living cells. *J Cell Sci* 123(Pt 12):2085–2093.
- Wahl MC, Will CL, Lührmann R (2009) The spliceosome: Design principles of a dynamic RNP machine. *Cell* 136(4):701–718.
- Yamazaki T, et al. (2012) FUS–SMN protein interactions link the motor neuron diseases ALS and SMA. *Cell Reports* 2(4):799–806.
- Schwartz JC, et al. (2014) FUS is sequestered in nuclear aggregates in ALS patient fibroblasts. *Mol Biol Cell* 25(17):2571–2578.
- Schwartz JC, Wang X, Podell ER, Cech TR (2013) RNA seeds higher-order assembly of FUS protein. *Cell Reports* 5(4):918–925.
- Schwartz JC, et al. (2012) FUS binds the CTD of RNA polymerase II and regulates its phosphorylation at Ser2. *Genes Dev* 26(24):2690–2695.
- Sun S, et al. (2015) ALS-causative mutations in FUS/TLN confer gain and loss of function by altered association with SMN and U1-snRNP. *Nat Commun* 6:6171.
- Kwon I, et al. (2013) Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. *Cell* 155(5):1049–1060.
- Li YR, King OD, Shorter J, Gitler AD (2013) Stress granules as crucibles of ALS pathogenesis. *J Cell Biol* 201(3):361–372.
- Ling SC, Polymenidou M, Cleveland DW (2013) Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis. *Neuron* 79(3):416–438.
- Ramaswami M, Taylor JP, Parker R (2013) Altered ribostasis: RNA-protein granules in degenerative disorders. *Cell* 154(4):727–736.
- Robberecht W, Philips T (2013) The changing scene of amyotrophic lateral sclerosis. *Nat Rev Neurosci* 14(4):248–264.
- Thomas M, Alegre-Abarrategui J, Wade-Martins R (2013) RNA dysfunction and aggregation at the centre of an amyotrophic lateral sclerosis/frontotemporal dementia disease continuum. *Brain* 136(Pt 5):1345–1360.
- Folco EG, Lei H, Hsu JL, Reed R (2012) Small-scale nuclear extracts for functional assays of gene-expression machineries. *J Vis Exp* (64):4140.
- Kaida D, et al. (2010) U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. *Nature* 468(7324):664–668.
- Almada AE, Wu X, Kriz AJ, Burge CB, Sharp PA (2013) Promoter directionality is controlled by U1 snRNP and polyadenylation signals. *Nature* 499(7458):360–363.
- Sisodia SS, Sollner-Webb B, Cleveland DW (1987) Specificity of RNA maturation pathways: RNAs transcribed by RNA polymerase III are not substrates for splicing or polyadenylation. *Mol Cell Biol* 7(10):3602–3612.
- Smale ST, Tjian R (1985) Transcription of herpes simplex virus tk sequences under the control of wild-type and mutant human RNA polymerase I promoters. *Mol Cell Biol* 5(2):352–362.
- Schwartz JC, Cech TR, Parker RR (2015) Biochemical properties and biological functions of FET proteins. *Annu Rev Biochem* 84:355–379.
- Deng H, Gao K, Jankovic J (2014) The role of FUS gene variants in neurodegenerative diseases. *Nat Rev Neurol* 10(6):337–348.
- Yu Y, et al. (2015) U1 snRNP is mislocalized in ALS patient fibroblasts bearing NLS mutations in FUS and is required for motor neuron outgrowth in zebrafish. *Nucleic Acids Res* 43(6):3208–3218.
- Berg MG, et al. (2012) U1 snRNP determines mRNA length and regulates isoform expression. *Cell* 150(1):53–64.
- Das R, Reed R (1999) Resolution of the mammalian E complex and the ATP-dependent spliceosomal complexes on native agarose mini-gels. *RNA* 5(11):1504–1508.