Introns play an essential role in splicing-dependent formation of the exon junction complex

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Pre-mRNA splicing specifically deposits the exon junction complex (EJC) onto spliced mRNA, which is important for downstream events. Here, we show that EJC components are primarily recruited to the spliceosome by association with the intron via the intron-binding protein, IBP160. This initial association of EJC components occurs in the absence of the final EJC-binding site on the exon. RNA interference [RNAi] knockdown of IBP160 arrested EJC association with cytoplasmic RNAs following nonsense-mediated decay. We propose that the intron has a crucial role in the early steps of EJC formation and is indispensable for the subsequent formation of a functional EJC.

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The removal of introns from pre-mRNAs is conducted by a large ribonucleoprotein complex called the spliceosome. During the splicing reaction, it has been recognized that the spliceosome deposits a set of protein factors on the nascent mRNA that shape subsequent events in gene expression [Maniatis and Reed 2002; Maquat 2004]. The exon junction complex [EJC] is a multiprotein complex that is deposited 20–24 nucleotides [nt] upstream of an exon–exon junction upon completion of the splicing reaction [Le Hir et al. 2000; Maquat 2004; Tange et al. 2004]. To date, 16 proteins including transiently interacting factors have been identified as EJC components [Tange et al. 2005]. The tetrameric core of this complex is composed of eIF4AIII, Y14, Magoh, and MNL51; the EJC is anchored to the upstream exon of the mature mRNA by eIF4AIII [Ballut et al. 2005; Tange et al. 2005]. The EJC is a multifunctional complex involved in mRNA export, nonsense-mediated mRNA decay [NMD], translation, and mRNA localization in metazoons [Maquat 2004; Tange et al. 2004]. Among the EJC components, REF, Y14, and/or Magoh have been observed to bind to the mRNA export receptor TAP [Kataoka et al. 2001; Le Hir et al. 2001], whereas RNPS1 and Y14 interact with hUpf3, a factor required for NMD [Kim et al. 2001, Lykke-Andersen et al. 2001]. The EJC is only assembled onto mature mRNA that has been produced as a result of pre-mRNA splicing; however, the pathway of assembly remains to be described in molecular detail. Proteomic analyses have revealed that the majority of EJC components are recruited to the spliceosome prior to the second catalytic step of the splicing reaction [Jurica et al. 2002, Makarov et al. 2002, Reichert et al. 2002, Merz et al. 2007]. Immunoprecipitation experiments conducted in a simple in vitro splicing system indicated stepwise binding of successive EJC components to the mRNA [Kataoka and Dreyfuss 2004]. The composition of the complex that interacts with the sequence upstream of the exon junction, which corresponds to the EJC-binding site, is dynamically altered during the splicing reaction [Reichert et al. 2002]. However, the spliceosomal protein(s) responsible for recruiting EJC components other than SRm160 remain unidentified. We recently demonstrated that IBP160, a helicase-like spliceosomal protein, specifically binds −40 nt upstream of the intron branch site in the spliceosomal C1 complex. IBP160 binding is not sequence dependent, but is position dependent [Hirose et al. 2006]. IBP160 is critical for assembly of the intron-encoded box C/D snoRNA. However, it also binds introns that do not encode a snoRNA, suggesting that the function of IBP160 during pre-mRNA processing is more general than snoRNA biogenesis.

In this paper, we provide evidence that IBP160 is responsible for the initial binding of EJC components to the spliceosome by linking them to the intron in the C1 complex. The association of EJC components with the spliceosome can occur in the absence of the final EJC-binding region in the upstream exon. For two noncoding RNAs [ncRNAs], depletion of IBP160 by RNA interference [RNAi] reduced EJC deposition and resulted in NMD arrest, which is dependent on the integrity of the EJC. These data suggest that a significant role of the intron and the intron-binding protein IBP160 is the initial binding of EJC components to the spliceosome, which is a prerequisite for subsequent assembly of the functional EJC.

Results and Discussion

We recently reported that SRm160, an EJC component, is associated with intron-bound IBP160 in the C1 complex [Hirose et al. 2006]. This association raises the interesting possibility that the function of IBP160 may be correlated with that of SRm160. Site-specific UV cross-linking enabled us to visualize intron-bound IBP160 in the C1 complex [Fig. 1A] This technique was applied here to examine whether intron-bound IBP160 associates with EJC components other than SRm160. For these experiments, Adenovirus [Adv] pre-mRNA was prepared as a substrate for an in vitro splicing assay. A GG32P-labeled substrate was introduced to arrest the second catalytic step of splicing, resulting in the capture of the C1 complex. Next, the mutated substrate pre-mRNA was specifically 32P-labeled at the −40 nt position relative to the branch site. The 32P-label was then transferred by UV
cross-linking to the protein bound to this specific site, IBP160. Thus, we could easily distinguish intron-bound IBP160 from unbound IBP160 or other proteins. Extensive RNase treatment after UV cross-linking releases 32P-IBP160, but maintains its interactions with associated proteins. In this assay, therefore, the association of EJC components with IBP160 can be assessed simply by examining whether the cross-linked 32P-labeled IBP160 coimmunoprecipitates with a given EJC component. Figure 1B shows that antibodies against SRm160, as well as two other EJC components, eIF4AIII or RNPS1, coimmunoprecipitated the cross-linked IBP160. Further immunoprecipitation experiments using nuclear extracts from HEK293 cells, each expressing a different Flag-tagged EJC component (see Supplementary Fig. 1A), revealed that all the EJC components efficiently coimmunoprecipitated the cross-linked IBP160 (Fig. 1C). These data indicate that the majority of EJC components indirectly bind the intron by interacting with IBP160 in the C1 complex. Similar results were obtained with a β-globin pre-mRNA with a GG 3′ splice site mutation (Supplementary Fig. 1B). To examine whether the cross-linked (32P-labeled) IBP160 is disrupted into smaller subcomplexes upon RNase A/T1 treatment after UV cross-linking, our immunoprecipitations did not merely precipitate the entire spliceosome. This hypothesis was confirmed by our experiment showing that U2, U5, and U6 snRNAs could no longer be detected in the RNase A/T1-treated nuclear extracts that were used for immunoprecipitation (data not shown). Furthermore, size fractionation by gel filtration chromatography of the subcomplex containing cross-linked IBP160 reproducibly indicated the presence of two populations of IBP160 subcomplexes with peaks at 620 kDa and 340 kDa (Fig. 1D). These data support the above results, and indicate that the spliceosomes are disrupted and divided into smaller subcomplexes, and that a subcomplex containing the cross-linked IBP160 transiently associates with EJC components. The other components of the IBP160 subcomplex remain to be identified. The previous proteome analysis suggested that IBP160 is closely linked to the PRP19-associated complex (NTC) (Makarov et al. 2002, Makarova et al. 2004). We also observed that Flag-hPRP19 coprecipitates with the cross-linked IBP160 (data not shown), suggesting that the IBP160 subcomplex may overlap at least in part with the NTC. These results suggest that the intron-bound IBP160 may play an instructive role in the initial association of EJC components with the spliceosome. Alternatively, the recruited components may already be assembled into the intact EJC on the upstream exon EJC-binding site in the C1 complex and secondarily associate with IBP160.

To examine the importance of the upstream exon in the association of EJC components with the C1 complex, two modified Adv-GG substrates with short upstream exons [17 nt: 17GG; 38 nt: 38GG] were employed for in vitro splicing (Fig. 2A). A previous study showed that shortening the upstream exon to 17 nt, but not 38 nt, results in defective EJC formation on the spliced mRNA because of the lack of an EJC-binding site (Le Hir et al. 2001). Consistent with previous reports, we confirmed by RNase H protection and immunoprecipitation that correct EJC assembly took place in our in vitro splicing system (Supplementary Fig. 3). If the EJC were fully as-
siRNAs against hUPF1, eIF4AIII, or IBP160 were intro-
ducing the ability of EJC assembly during in vitro splicing could not be as-
dicated that depletion of IBP160 from nuclear extracts down IBP160 in HeLa cells. Since our recent results in-
triguing possibility that the intron bound to the
with intron-bound IBP160. These observations raise the
(Shibuya et al. 2006), were found to associate with in-
retain the ability to associate with the spliceosome
lost the ability to induce NMD or EJC formation but
more, two mutants of Flag-eIF4AIII (mutations in motif
reached the same conclusion (Merz et al. 2007). Further-
the upstream exon that eventually serves for the assem-
sembled and anchored to the upstream exon in the C1
complex, the association of EJC components with the
spliceosome would be abolished only with the 17GG
construct. We found that the splicing of both 17GG and
38GG proceeded efficiently to the C1 complex (Fig. 2B).
Immunoprecipitation of cross-linked IBP160 with each
Flag-tagged EJC component clearly showed that shorten-
ing the upstream exon to 17 nt did not affect the asso-
ciation of EJC components with IBP160 in the C1 com-
plex [Fig. 2C], indicating that the initial association of
EJC components with the spliceosome does not require
the upstream exon that eventually serves for the assem-
bly of the intact EJC. A recent proteomic analysis
reached the same conclusion [Merz et al. 2007]. Fur-
thermore, two mutants of Flag-eIF4AIII (mutations in motif
Ia or motif VI) [see Supplementary Fig. 1B], which have lost
the ability to induce NMD or EJC formation but retain
the ability to associate with the spliceosome
(Shibuya et al. 2006), were found to associate with in-
tron-bound IBP160 [Fig. 1E]. This supports our hypo-
thesis that the initial binding of EJC components to the
spliceosome is tightly linked to their ability to interact
with intron-bound IBP160. These observations raise the
intriguing possibility that the intron bound to the
IBP160 subcomplex is required for subsequent EJC
assembly.
To demonstrate the significance of IBP160 in EJC func-
tion, we used small interfering RNA [siRNA] to knock
down IBP160 in HeLa cells. Since our recent results in-
dicated that depletion of IBP160 from nuclear extracts destabilizes in vitro spliced exons [Hirose et al. 2006],
EJC assembly during in vitro splicing could not be as-
essed in extracts from IBP160-depleted cells. Instead,
we investigated the effect of IBP160 depletion by exam-
ing the ability of ΔIBP160 cells to induce NMD.
siRNAs against hUPF1, eIF4AIII, or IBP160 were intro-
duced into HeLa cells and were found to efficiently de-
plete each protein [Fig. 3A]. Subsequent quantitative
RT–PCR measurement of the levels of two endogenous
NMD target ncRNAs [Gas5 and UHG] [Tycowski et al.
1996; Smith and Steitz 1998] indicated that both the
Gas5 and UHG ncRNAs were markedly stabilized in
ΔIBP160 (350%–700%) and ΔeIF4AIII cells (300%–400%)
while the levels of two control mRNAs [β-actin and
GAPDH] were unaltered [Fig. 3B]. These data confirm
that these ncRNAs are genuine NMD targets, and that
NMD arrest was induced by defective EJC formation.
Importantly, knockdown of IBP160 increased the levels
of the two ncRNAs (250%–350%) as much as those in
ΔeIF4AIII cells, while the levels of the control mRNAs
were almost unaltered [Fig. 3B]. The increase in the le-
vels of the ncRNAs was observed in the cytoplasmic frac-
tion [Fig. 3B], indicating that the increase is not caused
by defects in nuclear export. Recently, it has been re-
ported that a subset of shuttling SR proteins [e.g., 9G8
and SRp20] is able to recruit TAP onto the mature
mRNA and to enhance mRNA export [Huang et al.
2003]. It is possible that these mRNA export adaptors
might compensate for any defects in EJC function during
nuclear export. IBP160 is a general intron-binding pro-
tein, but was previously shown not to be essential for
splicing in vitro [Hirose et al. 2006]. Northern blot anal-
ysis also showed that aberrant accumulation of unspliced
RNA species of all four transcripts were not observed in
ΔIBP160 cells [Fig. 3C]. Taken together, these data
strongly suggest that EJC assembly failed in the absence
of IBP160, leading to NMD arrest in the ΔIBP160 cells.
To demonstrate the role of IBP160 more directly, the
levels of EJC-bound cellular RNAs in control and
ΔIBP160 cells were compared. EJC-associated cellular
RNAs were obtained by coimmunoprecipitating the cy-
toplasmic fraction of siRNA-treated HeLa cells experi-
ments with øeIF4AIII or øY14 antibodies. Since EJC is
obliterated by the translating ribosome upon mRNA ex-
port to the cytoplasm [Dostie and Dreyfuss 2002], it is
difficult to obtain substantial amounts of EJC-associated
cytoplasmic mRNAs. We chose UHG and Gas5 ncRNAs
to capture the cytoplasmic ribonucleoprotein complexes
associated with EJC, because we expected that EJC
would remain associated with these ncRNAs, which
possess translatable small ORFs close to their 5′ termini
[see Supplementary Fig. 4]. Coimmunoprecipitation of
Gas5 and UHG ncRNAs with eIF4AIII and Y14 revealed
that the EJC association was remarkably reduced in
ΔIBP160 cells, while that of PABP1 remained relatively
constant [Fig. 3D]. These data additionally suggest that
the intron-mediated primary association of EJC with the
spliceosome is a prerequisite for EJC assembly onto the
ligated exon.
Our immunoprecipitation experiments indicated that
IBP160 remains associated with the lariat intron but may
dissociate prior to debranching of the lariat structure,
since lariats, but not linearlized intron fragments, were
detected in the IBP160 precipitates [Supplementary Fig.
5A]. In contrast, association of SRm160 with the lariat
intron was poorly detected. Instead, it was found to sta-
ble associate with the ligated exon [Supplementary Fig.
5B]. These data suggest that remodeling of the IBP160
subcomplex takes place upon exon ligation, resulting in
the production of distinct post-splicing complexes con-
taining the ligated exon and the lariat intron [Fig. 4]. It
has been reported that hPRP22 plays a critical role in the

Figure 2. Association of EJC components with the C1 complex in
the absence of the final EJC-binding site in the upstream exon. [A]
The two shortened pre-mRNA splicing substrates are shown sche-
masically. The lengths of the upstream exons are shown on the left.
The labeled nucleotide and the 3′ splice site mutation are shown as
in Figure 1A. [B] In vitro splicing of 17GG and 38GG pre-mRNAs.
The identities of the RNA species are shown on the right. [C] Co-
immunoprecipitation of IBP160 cross-linked either to 38GG or to
17GG, indicated as 38 or 17 above each lane, with the øFlag anti-
body as in Figure 1C.
We observed that Flag-hPRP22 efficiently coprecipitated the cross-linked IBP160 (Supplementary Fig. 6). This interaction requires the intact RS domain of hPRP22, which was previously reported to be necessary for association with the spliceosome (Ohno and Shimura 1996). This result suggests that hPRP22 is another component of the IBP160 subcomplex that may be involved in remodeling of the IBP160 subcomplex for the release of ligated exons. It would be intriguing to try similar coimmunoprecipitation experiments using cross-linked IBP160 with EJC components in the presence of previously reported dominant-negative hPRP22 mutants (Ohno and Shimura 1996). We reported previously that the functional EJC was deposited by the U12-type (minor) spliceosome (Hirose et al. 2004). Our immunoprecipitation of RNA species produced by in vitro splicing of P120 pre-mRNA containing a U12-type intron shows that IBP160 is associated with the lariat U12-type intron (Supplementary Fig. 5C). This suggests that the role played by IBP160 in EJC formation is a common feature of splicing by both the U2- and U12-type spliceosomes. In summary, we have demonstrated an interaction of EJC components with the intron-bound spliceosomal subcomplex containing IBP160 in the C1 stage prior to exon ligation. This initial binding of EJC components to the intron is likely to be essential for the assembly of the functional EJC that induces NMD (Fig. 4). Our data provide a novel mechanistic insight into the role of introns during mRNA biogenesis.

**Materials and methods**

**Plasmid construction**
The Adv, β-globin, Gas5, and P120 splicing substrates have been described previously (Hirose et al. 2004, 2006). The 38GG and 17GG Adv mutants were constructed by PCR cloning.
Site-specific labeling of RNA

Site-specific labeling of splicing substrates has been described previously (Hirose et al. 2006). In brief, for labeling at -40 nt relative to the branch site of the Adv intron or β-globin intron, PCR was used to obtain the template DNA for either the upstream RNA or the downstream RNA, relative to the site to be labeled. These PCR fragments were used as templates for in vitro transcription with T7 RNA polymerase. For the upstream RNA, transcription was performed in the presence of an RNA cap analog [Invitrogen]. For the downstream RNA, GMP was added into the transcription reaction. The 5' terminus of the purified downstream RNA was dephosphorylated with CIP, followed by 32P-labeling with T4 polynucleotide kinase. The capped upstream RNA (10 pmol) was ligated to the 5'-3' labeled downstream RNA (15 pmol) with T4 DNA ligase (50 U; Roche) in the presence of a bridging DNA oligonucleotide (30 mer, 15 pmol) for 3 h at 25°C. The ligated RNA was separated from the unligated RNAs and purified by PAGE.

Preparation of nuclear extracts, in vitro splicing, and UV cross-linking

Nuclear extracts from HeLa cells were prepared by standard procedures (Dignam et al. 1983). Nuclear extracts containing Flag-tagged proteins were prepared from HEK293 cells transfected with a pcDNA3-Flag plasmid as described (Hirose et al. 2004). The expression of each Flag-tagged protein was confirmed by the appearance of a single band in Western blots using α-Flag antibody [M2; Sigma]. In vitro splicing reactions and UV cross-linking were carried out as described previously (Hirose et al. 2006). In brief, pre-mRNAs with site-specific 32P labeling (100 fmol) were incubated in 20 μL of reaction mixtures containing 2.4 mM MgCl2, 0.5 mM ATP, 20 mM creatine phosphate, 2% polyvinyl alcohol, and 12 μL of HEK293 nuclear extract (6 μL) were mixed. In vitro splicing of the upstream RNA, transcription was performed in the presence of an RNA cap analog [Invitrogen]. For the downstream RNA, GMP was added into the transcription reaction. The 5' terminus of the purified downstream RNA was dephosphorylated with CIP, followed by 32P-labeling with T4 polynucleotide kinase. The capped upstream RNA (10 pmol) was ligated to the 5'-3' labeled downstream RNA (15 pmol) with T4 DNA ligase (50 U; Roche) in the presence of a bridging DNA oligonucleotide (30 mer, 15 pmol) for 3 h at 25°C. The ligated RNA was separated from the unligated RNAs and purified by PAGE.

Antibodies and immunoprecipitation

Antibodies were purchased or provided as follows: anti-Flag M2 and anti-PARP1 from Sigma, anti-SRm160 from B. Blencowe, anti-eIF4AIII from G. Dreyfuss, and anti-RPS1 from A. Mayeda. Anti-IBP160 was obtained as antiseraum against the synthetic peptide as described (Hirose et al. 2006). For communoprecipitation experiments, the anti-SRm160 monoclonal antibodies were bound to protein A-Sepharose (PAS) beads via rabbit anti-mouse IgG + IgM (Pierce). All other antibodies were bound directly to Dynabeads protein G (Invitrogen). For immunoprecipitation, the in vitro splicing reaction was fivefold larger [100 μL]. The reaction mixture was diluted 10-fold with NET2 buffer immediately after RNA treatment and mixed with the antibody-Dynabeads conjugates for 3 h at 4°C. The beads were washed four times with NET2 buffer using an automatic magnetic bead washer [Thermo]. Bound proteins were eluted by directly adding SDS loading buffer to the beads. For immunoprecipitation of the EJC-associated cellular RNAs, the cytoplasmic fraction of HeLa cells (∼2 × 10^6 cells) was prepared as described (Dostie and Dreyfuss 2002) and mixed with the antibody-Dynabeads conjugates for 3 h at 4°C. The beads were washed four times with HNT buffer [Dostie and Dreyfuss 2002] using an automatic magnetic bead washer [Thermo]. Precipitated RNAs were recovered using Sepasol-RNAI (Nacalai).

RNAi, Northern blot, and quantitative RT–PCR

HeLa cells were grown to 95% confluency in six-well tissue culture dishes. The siRNAs for IBP160 (Hirose et al. 2006), hUPF1 (Mendell et al. 2002), or eIF4AIII [Shibuya et al. 2004] were synthesized by Sigma Genosys, Inc. The control siRNA was purchased from Ambion. The siRNA duplex [50 nM] was administered to the HeLa cells using Lipofectamine 2000 reagent according to the manufacturer’s instructions [Invitrogen]. After 48 h (68 h for the ΔIBP160 cells), total RNA and cytoplasmic RNA were isolated using TRizol reagent or a PARIS kit, respectively. Quantitative RT–PCR and Northern blotting were carried out as described previously [Sasaki et al. 2007].

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Wilm, M., and Luhrmann, R. 2002. Small nuclear ribonucleoprotein remodeling during catalytic activation of the spliceosome. *Science* **298**: 2205–2208.

Makarova, O.V., Makarov, E.M., Urlaub, H., Will, C.L., Gentzel, M., Wilm, M., and Luhrmann, R. 2004. A subset of human 35S U5 proteins, including Prp19, function prior to catalytic step 1 of splicing. *EMBO J.* **23**: 2381–2391.

Maniatis, T. and Reed, R. 2002. An extensive network of coupling among gene expression machines. *Nature* **416**: 499–506.

Maquat, L.E. 2004. Nonsense-mediated mRNA decay: Splicing, translation and mRNP dynamics. *Nat. Rev. Mol. Cell Biol.* **5**: 89–99.

Mendell, J.T., ap Rhys, C.M., and Dietz, H.C. 2002. Separable roles for rent1/hUpf1 in altered splicing and decay of nonsense transcripts. *Science* **298**: 419–422.

Merz, C., Urlaub, H., Will, C.L., and Luhrmann, R. 2007. Protein composition of human mRNPs spliced in vitro and differential requirements for mRNP protein recruitment. *RNA* **13**: 116–128.

Nojima, T., Hirose, T., Kimura, H., and Hagiwara, M. 2007. The interaction between cap-binding complex and RNA export factor is required for intronless mRNA export. *J. Biol. Chem.* **282**: 15645–15651.

Ohno, M. and Shimura, M. 1996. A human RNA helicase-like protein, HRH1, facilitates nuclear export of spliced mRNA by releasing the RNA from the spliceosome. *Genes & Dev.* **10**: 997–1007.

Reichert, V.L., Le Hir, H., Jurica, M.S., and Moore, M.J. 2002. 5' Exon interactions within the human spliceosome establish a framework for exon junction complex structure and assembly. *Genes & Dev.* **16**: 2778–2791.

Sasaki, Y.T.F., Sano, M., Kin, T., Asai, K., and Hirose, T. 2007. Identification and characterization of human non-coding RNAs with tissue-specific expression. *Biochem. Biophys. Res. Commun.* **357**: 991–996.

Shibuya, T., Tange, T.O., Sonenberg, N., and Moore, M.J. 2004. elf4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat. Struct. Mol. Biol.* **11**: 346–351.

Shibuya, T., Tange, T.O., Stroupe, M.E., and Moore, M.J. 2006. Mutational analysis of human elf4AIII identifies regions necessary for exon junction complex formation and nonsense-mediated mRNA decay. *RNA* **12**: 360–374.

Smith, C.M. and Steitz, J.A. 1998. Classification of gas5 as a multi-small-nucleolar-RNA (snoRNA) host gene and a member of the 5'-terminal oligopyrimidine gene family reveals common features of snoRNA host genes. *Mol. Cell. Biol.* **18**: 6897–6909.

Tange, T.O., Nott, A., and Moore, M.J. 2004. The ever-increasing complexities of the exon junction complex. *Curr. Opin. Cell Biol.* **16**: 279–284.

Tange, T.O., Shibuya, T., Jurica, S., and Moore, M.J. 2005. Biochemical analysis of the EJC reveals two new factors and a stable tetrameric protein core. *RNA* **11**: 1869–1883.

Tarn, W.Y. and Steitz, J.A. 1996. A novel spliceosome containing U11, U12, and U5 snRNPs excises a minor class (AT–AC) intron in vitro. *Cell* **84**: 801–811.

Tycowski, K.T., Shu, M.-D., and Steitz, J.A. 1996. A mammalian gene with introns instead of exons generating stable RNA products. *Nature* **379**: 464–466.
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