Published in final edited form as:

Wiley Interdiscip Rev RNA. 2018 May ; 9(3): e1470. doi:10.1002/wrna.1470.

Intron Retention in Viruses and Cellular Genes: Detention, Border Controls and Passports

David Rekosh and Marie-Louise Hammarskjöld

The Myles H. Thaler Center for AIDS and Human Retrovirus Research and the Department of Microbiology, Immunology and Cancer Biology, University of Virginia, Charlottesville VA, 22908The Department of Microbiology, University of Venda, Thohoyandou, 0950 South Africa

Abstract

Intron retention (IR), where one or more introns remain in the RNA after splicing, was long thought to be rare in mammalian cells, albeit common in plants and some viruses. Largely due to the development of better methods for RNA analysis, it has now been recognized that intron retention is much more common than previously thought and that this mechanism is likely to play an important role in mammalian gene regulation. To date, most publications and reviews about intron retention have described the resulting mRNAs as “dead end” products, with no direct consequence for the proteome. However, there are also many reports of mRNAs with retained introns giving rise to alternative protein isoforms. Although this was originally revealed in viral systems, there are now numerous examples of *bona fide* cellular proteins that are translated from mRNAs with retained introns. These new isoforms have sometimes been shown to have important regulatory functions. In this review, we will highlight recent developments in this area and the research on viruses that led the way to the realization of the many ways in which mRNAs with retained introns can be regulated.

Graphical Abstract

Correspondence to: Marie-Louise Hammarskjöld.

David Rekosh, dr4u@virginia.edu, ORCID ID #: 0000-0003-3662-4176, no COI

Marie-Louise Hammarskjöld*, mh7g@virginia.edu, ORCID ID #: 0000-0002-9710-8960, no COI

Introduction

It has now been 40 years since mRNA splicing was first reported (Berget, Moore, & Sharp, 1977; Chow, Gelinas, Broker, & Roberts, 1977). The initial publications described the remarkable discovery that Adenovirus mRNAs in infected human host cells mapped to separate, discontinuous parts of the viral genome, signifying that they originated from an initial pre-mRNA where some parts were “spliced” out and the remaining parts were joined together by a then unknown process. Initially, this mechanism was considered a viral “phenomenon”, but it soon became clear that this was a process that is extensively used by the “host” cell as well (Breathnach, Mandel, & Chambon, 1977; Tilghman et al., 1978).

To define which segments were left in the mRNA and which segments were “thrown away”, the terms “exons” and “introns” were coined (Gilbert, 1978). In subsequent years, it became clear that splicing is a much more complicated process than originally thought, with mechanisms that allow different segments to be discarded in different mRNAs from the same gene (for early reviews see (Black, 2003; Breitbart, Andreadis, & Nadal, 1987)). We now know that this process, known as “alternative” splicing, is extensively used in higher eukaryotic systems and that it contributes greatly to proteome diversity and “plasticity. Many different types of alternative splicing have been described, including exon “skipping”, different use of nearby 5′ and 3′ splice sites, “alternative” use of exons (cassette exons) and intron “retention” (IR). mRNAs with retained introns, the subject of this review, retain one or more complete introns in the final mRNA product (IR-mRNAs).

Even though alternative splicing was shown to be frequently used by viruses many years ago, it remained controversial for a long time how common alternative splicing was in the genes of their host cells. With the completion of the human genome sequence and many other genome projects, and the many novel methods to analyze RNA (most importantly RNASeq), it is now widely accepted that 90% or more of human genes are alternatively spliced in a dynamic fashion and that most genes can give rise to multiple mRNA isoforms (Braunschweig et al., 2014). To date, exon skipping has been recognized as the most common form of alternative splicing in humans and other mammals (Keren, Lev-Maor, & Ast, 2010), whereas intron retention has been reported to be the dominant form in plants (Ner-Gaon et al., 2004). Intron retention was until recently overlooked and considered rather rare in mammals (Modrek, Resch, Grasso, & Lee, 2001), but was early on recognized as an important and common mechanism of alternative splicing in many viruses (Gama-Carvalho, Condado, & Carmo-Fonseca, 2003; Kienzle et al., 1999; Phelan, Dunlop, & Clements, 1996; Sedlackova et al., 2008), with many examples of introns serving as protein coding exons, blurring the distinction between introns and exons. This has been especially well studied in retroviruses (M.-L. Hammarskjöld, 1997) (see below). Yet, IR in “host” cell genes was not the subject of many studies until just a few years ago.

It has now become clear that IR is common also in most mammalian cells, with a current estimate of at least 30% of human genes using this mechanism (Boutz, Bhutkar, & Sharp, 2015; Braunschweig et al., 2014). The realization of “widespread” IR in many cells, which often seems to change in development, differentiation and during stress conditions, has resulted in many publications in the last few years (Edwards et al., 2016; Memon et al.,

2016; Pimentel et al., 2016; Yap, Lim, Khandelia, Friedman, & Makeyev, 2012). Estimates of intron retention are likely to increase even further as “long read” sequencing (using PacBio, Oxford Nanopore and other new technologies) and single cell sequencing become more widespread. It is also now recognized that IR is often changed in many types of cancer and other diseases (Dvinge & Bradley, 2015).

Despite the increased recognition of IR as a common form of alternative splicing, it often continues to be described as a negative “feed-back” mechanism that has very minor effects on proteome diversity (Green et al., 2003). This is largely due to the fact that IR-mRNAs are subject to restrictions at multiple levels. Firstly, it is clear that IR-mRNAs are often subject to nuclear retention or detention (Boutz et al., 2015; M.-L. Hammarskjold, 2001). This is likely to reflect the fact that mRNA export is generally coupled to the completion of splicing with removal of all introns (Chang & Sharp, 1989; Legrain & Rosbash, 1989). In addition, if IR-mRNAs are exported to the cytoplasm they would be expected to frequently be subject to Nonsense Mediated Decay (NMD) (Ishigaki, Li, Serin, & Maquat, 2001; Kurosaki & Maquat, 2016; Maquat & Carmichael, 2001). NMD is an RNA surveillance mechanism that serves to degrade mRNAs that contain premature termination codons (PTCs) and other aberrant mRNAs. This would serve to limit the translation of many of the IR-mRNAs. However, recent studies have suggested that NMD is regulated and may be less active in some cell types, such as neuronal cells, as well as during “stress” conditions (see below) (Hug, Longman, & Caceres, 2016; Karam, Wengrod, Gardner, & Wilkinson, 2013; Nasif, Contu, & Muhlemann, 2017). This could mean that mRNAs which would normally be degraded could be translated into stable proteins in these situations.

How IR is regulated and what determines the fate of mRNAs with retained introns remains largely unexplored. However, in the last few years it has become clear that IR is dynamic in space and time and may play important roles in processes such as development, differentiation and aging. Intron retention is often tissue specific and recent publications suggest that it may be particularly prevalent in neuronal cells, where it may ultimately play a role in synaptic plasticity (Bell et al., 2008; Buckley et al., 2011; Mauger, Lemoine, & Scheiffele, 2016; Yap et al., 2012).

In this review, we summarize what is known about intron retention and the regulation and fate of mRNAs with retained introns, starting with the very well studied and established role of this in retroviral systems and ending with the more controversial and less well studied roles of intron retention in mammalian systems.

RETAINED INTRONS IN RETROVIRUSES

A mechanism for export and expression of mRNA with retained introns was first discovered in human immunodeficiency virus (HIV)

As in the case of many other molecular mechanisms that have been discovered in mammalian cells, intron retention and regulation of mRNAs with retained introns were initially recognized in analysis of viral interactions with host cells. When molecular studies of HIV and its replication became prevalent in the mid 1980’s, it was quickly demonstrated that transcription from the integrated HIV genome produces one primary full length

transcript that gives rise to a large number of alternatively spliced mRNAs, compared to the two or three subgenomic mRNAs that are generated from simpler retroviruses (Purcell & Martin, 1993; Schwartz, Felber, Benko, Fenyo, & Pavlakis, 1990). The HIV alternatively spliced mRNAs encode accessory and regulatory proteins, in addition to the structural proteins (Gag, Pol and Env) that are common to all retroviruses. One of these regulatory proteins, Rev, is involved in export and expression of many of the HIV mRNA species (Fernandes, Booth, & Frankel, 2016; Pollard & Malim, 1998). In 1989, several groups, including ours, demonstrated that Rev works *in trans* on the Rev Response Element (RRE), a highly structured *cis*-acting element present in some HIV mRNAs (see Figure 1) (Hadzopoulou-Cladaras et al., 1989; M. L. Hammarskjold et al., 1989; M.H. Malim, Hauber, Le, Maizel, & Cullen, 1989). If the RRE is mutated or deleted or if Rev is not present, these mRNAs, which all retain one or two introns, are not exported from the nucleus of the host cell. The mRNAs whose export is dependent on Rev include the “unspliced” HIV genomic RNA, which retains two introns. This RNA also serves as the mRNA for the Gag and Pol proteins that are encoded within the first intron. Removal of this intron from the full length mRNA gives rise to a class of incompletely spliced mRNAs that also encode proteins within a retained intron.

The Rev/RRE mechanism generated a lot of interest among RNA biologists from the very beginning, since Rev was the first example of a protein necessary for export of mRNAs in human cells. It was also shown that Rev and the RRE were absolutely essential for HIV replication and no HIV variant has ever been isolated, where this is not the case. Additional studies demonstrated that Rev is not a *bona fide* mRNA export receptor, but that it functions as an “adaptor” through recruitment of a host cell protein, Crm1 (also known as Exportin 1 or Xpo1)(Fornerod, Ohno, Yoshida, & Mattaj, 1997). At about the same time, Crm1 was also shown to be involved in nucleo-cytoplasmic export of many proteins (Fornerod et al., 1997; Fukuda et al., 1997; Neville, Stutz, Lee, Davis, & Rosbash, 1997; Ossareh-Nazari, Bachelier, & Dargemont, 1997), as well as U snRNAs (Fornerod et al., 1997). Before export, RanGTP is also recruited to the Crm1 complexes (Figure 2) (Askjaer, Jensen, Nilsson, Englmeier, & Kjems, 1998; Kuersten, Ohno, & Mattaj, 2001).

The specific domain in Rev that recruits Crm1 was originally called the “activation” domain (Hope, Bond, McDonald, Klein, & Parslow, 1991; M. H. Malim, Bohnlein, Hauber, & Cullen, 1989), but was later recognized as a “nuclear export signal” (NES) (Fischer, Huber, Boelens, Mattaj, & Luhrmann, 1995). In fact, Rev was one of the first proteins that was identified to have an NES. Like many cellular proteins with nuclear export signals, Rev also has a nuclear localization signal (NLS) and shuttles between the nucleus and the cytoplasm even when it is not bound to RRE-RNA (Kalland, Szilvay, Brokstad, Saetrevik, & Haukenes, 1994; Meyer & Malim, 1994). However, recent studies indicate that Crm1 is recruited in a different way when Rev is bound to RRE-RNA, potentially for the purpose of differential regulation of protein and mRNA-export (Booth, Cheng, & Frankel, 2014; Sherer et al., 2011).

It has remained unclear to what extent Crm1 plays a role in export of cellular mRNAs. However, Crm1 is clearly involved in the export of eIF4E-dependent mRNAs (Culjkovic, Topisirovic, Skrabanek, Ruiz-Gutierrez, & Borden, 2006; Siddiqui & Borden, 2012) and

also in mRNA export regulated by HuR/ELAV proteins (Fries et al., 2007; Kimura, Hashimoto, Nagase, & Fujisawa, 2004; Schutz et al., 2006). While we can only speculate how HIV developed the Rev/RRE mechanism for export of mRNA with retained introns, it seems probable that it may have been originally exapted from a host cell mechanism by an ancient “complex” retrovirus. The viral adoption of a *cis*-acting RNA element, as well as a gene encoding the transacting protein that acts on this element would have allowed for co-evolution of these factors. This would not have been possible if only the *cis*-acting sequence had been exapted (see the section on CTEs below). This makes it difficult to trace a potential host cell origin, since both the RNA element and the protein acting on it could have changed substantially.

Another reason to postulate a host cell origin for Rev/RRE regulation is the fact that many retroviruses have now been shown to utilize similar mechanisms that can be cross-substituted, even though export is mediated by proteins and RNA elements that do not share much sequence similarity with Rev and the RRE, beyond the presence of an RNA binding domain (which often is also an NLS) and an NES in the transacting protein. These complex retroviruses include all of the animal lentiviruses, as well as Human T-Cell Leukemia Viruses (HTLV-1 and 2) (Hidaka, Inoue, Yoshida, & Seiki, 1988; Seiki, Inoue, Hidaka, & Yoshida, 1988) Jaagsiekte Sheep Retrovirus (JSRV) (Hofacre, Nitta, & Fan, 2009; Nitta, Hofacre, Hull, & Fan, 2009), and Mouse Mammary Tumor Virus (MMTV) (Mertz, Simper, Lozano, Payne, & Dudley, 2005). In fact, for many years the last two viruses were thought to be simpler retrovirus. Intriguingly, the “youngest” family of Human Endogenous Retroviruses (HERV-K) that exists in many near-full length copies in the human genome, also uses a Crm1 binding protein known as Rec to bind to a *cis*-acting element in its genome RNA (the RcRe) to achieve export and expression of HERV mRNA with retained introns (Magin, Lower, & Lower, 1999; Magin-Lachmann et al., 2001). Curiously, HIV Rev can function in conjunction with the RcRe element (Bogerd, Wiegand, Yang, & Cullen, 2000) and the HTLV-I Rev equivalent, Rex, can function with the RRE to promote RNA export (Hanly et al., 1989; Lewis, Williams, Rekosh, & Hammarskjold, 1990), despite lack of any obvious sequence similarities. In contrast, Rec does not functionally interact with the RRE.

The discovery of the Rev/RRE mechanism originally led several groups to suggest that this served as a means for HIV to regulate its replication into an early and late phase (Cullen & Greene, 1989). It was postulated that since Rev is encoded by a fully spliced message that would be expressed “early”, export and expression of the mRNAs for the structural proteins would only be possible after Rev built up to sufficient levels in the infected cell, since these mRNAs are all dependent on Rev for export.

Whereas a slight delay in structural protein production may be a consequence of this mechanism, we favored an alternative explanation for the necessity for Rev/RRE regulation. We postulated that host cell restrictions would normally prevent the export of mRNA with retained introns and that Rev/RRE enabled the virus to bypass these restrictions (M.-L. Hammarskjold, 2001; M. L. Hammarskjold, Li, Rekosh, & Prasad, 1994; X. B. Lu, Heimer, Rekosh, & Hammarskjold, 1990). Rev and the RRE in HIV and similar mechanisms in other complex retroviruses would thus provide the IR-mRNAs with a “passport” to allow them to travel to the cytoplasm and also successfully avoid NMD, resulting in productive translation.

This hypothesis was quickly supported by studies from the Sharp and Rosbash laboratories that demonstrated that cellular mRNAs with retained introns were restricted at the level of nucleo-cytoplasmic export, both in mammalian and yeast systems (Chang & Sharp, 1989; Legrain & Rosbash, 1989). However, this hypothesis did not explain how the simpler retroviruses that do not encode any regulatory proteins would be able to circumvent these restrictions, since the full length genomic RNA of every retrovirus always contains at least one intron. The solution to this apparent enigma came with our discovery of a *cis*-acting RNA element in the simpler retrovirus Mason-Pfizer Monkey Virus (MPMV) (Figure 1) (Bray et al., 1994). This element interacts directly with a host cell protein that serves as an mRNA export receptor (Gruter et al., 1998).

The identification of the MPMV constitutive transport element and how this led to the discovery of Nxf1, a conserved cellular mRNA export receptor

In the early 1990's, a serendipitous finding led to the discovery of the first Constitutive Transport Element (CTE) (Bray et al., 1994). Previous to its discovery, all mammalian expression vectors that produced the HIV GagPol and Env proteins from DNA transfected into the nucleus required Rev and the RRE, when Env was expressed from an mRNA with a retained intron (Rekosh, Nygren, Flodby, Hammarskjöld, & Wigzell, 1988; Smith, Cho, Hammarskjöld, & Rekosh, 1990). However, it was surprisingly revealed that when a fragment of the Mason Pfizer Monkey Virus genome was used to provide a poly A site and transcription termination signals, Rev was no longer required for Env protein expression. Further experiments showed that the reason for this was the presence of a small MPMV sequence just upstream of the polyA signal. When this sequence was transplanted to an HIV GagPol expression vector with a retained intron, it provided Rev/RRE independent expression as well. Finally, it was shown that this small element could be used to replace both Rev and the RRE to promote HIV replication and that deletion of it from the MPMV genome, led to retention of the full length MPMV genomic RNA in the nucleus (see Figure 2) (R.K. Ernst, Bray, Rekosh, & Hammarskjöld, 1997). Since the element functioned constitutively in host cells without a viral protein, it was given the name CTE. Probing of the secondary structure of CTE RNA demonstrated that the element forms a single stem-loop, with two inner single stranded loops that have almost identical nucleotide sequence and a small single stranded top loop (see Figure 1) (R. K. Ernst, Bray, Rekosh, & Hammarskjöld, 1997). Single nucleotide mutations in and around the inner loops were shown to abolish CTE function.

These results strongly suggested that the inner loop regions of the CTE interacted with a host cell protein directly involved in mRNA export. Attempts to find this protein led to the identification of Nuclear Export Factor 1 (Nxf1) (originally named Tap) (Gruter et al., 1998), which is now believed to be a major mRNA export receptor in most metazoans (Herold, Klymenko, & Izaurralde, 2001). The Nxf1 protein is highly conserved in evolution and there is significant sequence homology between Nxf proteins in species as divergent as worms, flies, fish and mammals (Herold et al., 2000). In addition, Mex67, an mRNA export receptor in yeast, shows some homology to Nxf1 (Katahira et al., 1999). Curiously, no Nxf protein has been identified in plants, and relatively little is known to date about plant mRNA export (Gaouar & Germain, 2013).

Further studies of Nxf1 and its role in mRNA export demonstrated that Nxf1 functions in conjunction with small proteins, now known as Nxt (previously also known as p15) (Guzik et al., 2001). Two different Nxt genes (1 and 2) have been identified in mammals and they have both been shown to promote Nxf1 function (Braun, Herold, Rode, Conti, & Izaurralde, 2001; Herold et al., 2001; Ossareh-Nazari et al., 2000). It has also been shown that Nxf1 is only one member of a related gene family. Thus there are several other NXF genes, especially in higher mammals (NXF2 to NXF5 in humans) (Herold et al., 2000; Sasaki et al., 2005). With the exception of Nxf2, none of the other human proteins have been demonstrated to have a role in mRNA nucleo-cytoplasmic export, but several studies suggest that they may be involved in cytoplasmic RNA trafficking (Katahira et al., 2008; Takano, Miki, Katahira, & Yoneda, 2007; Tretyakova et al., 2005). Interestingly, whereas the NXF1 gene is present on somatic chromosomes and the Nxf1 protein appears to be expressed in most cells, expression of the other genes (that are all present on the X-chromosome) appear to be largely limited to brain and testis. Their exact function, and whether they have any role in expression of cellular mRNAs with retained introns, remains to be determined.

Early mutational and other analysis suggested that an Nxf1/Nxt heterodimer interacts with the MPMV CTE in each of the two inner loop regions (R.K. Ernst et al., 1997; R. K. Ernst et al., 1997; Kang, Bogerd, Yang, & Cullen, 1999). Several years ago, a crystal structure of a partial CTE-Nxf1/Nxt1 complex, containing one inner loop, was published (Teplova, Wohlbold, Khin, Izaurralde, & Patel, 2011), supporting that the CTE functions by direct binding of a Nxf1/Nxt heterodimer, and that this allows export of the resulting mRNP complex (see Figure 2). However, in the case of completely spliced cellular mRNAs, Nxf1 seems to be recruited through “adapter” proteins rather than through direct sequence specific RNA binding (for a recent review see (Bjork & Wieslander, 2017)). Even though it remains somewhat controversial exactly how Nxf1 is recruited to fully spliced mRNAs, it is believed that proteins involved in splicing recruit Nxf1, once splicing is completed.

Subsequent to the discovery of the MPMV CTE, it has been revealed that many simpler retroviruses use CTE-like elements to recruit Nxf1 and Nxt. These include several other MPMV related betaretroviruses (Zolotukhin, Valentin, Pavlakis, & Felber, 1994), and also alpharetroviruses such as Rous Sarcoma Virus (Ogert & Beemon, 1995; Paca, Ogert, Hibbert, Izaurralde, & Beemon, 2000) and the gammaretrovirus, Mouse Moloney Leukemia virus (Pessel-Vivares, Ferrer, Laine, & Mougél, 2014; Sakuma et al., 2014). The elements in the alpha and gamma retroviruses display no obvious sequence similarity to the MPMV CTE so it is not clear how these elements recruit Nxf1. Finally, it has also been reported that several other “nuclear” viruses, such as Influenza viruses (Satterly et al., 2007) and Herpesviruses (Johnson & Sandri-Goldin, 2009) also use Nxf1 in expression of their mRNAs, although the specific mechanisms remain less defined.

How simpler retroviruses evolved to use *cis*-acting RNA elements to recruit Nxf1/Nxt remained an enigma for a long time. It was proposed that the MPMV CTE mimicked a cellular protein, thus “tricking” Nxf1 to directly recognize unspliced or partially spliced mRNA in a way that would not normally occur in the host cell. However, this theory was refuted when it was revealed that CTEs are also used by the host cell and that they provide a

mechanism for export and expression of host cell mRNAs with retained introns (Li et al., 2006).

RETAINED INTRONS IN CELLULAR GENES

The NXF1 gene contains a conserved CTE in an intron that is subject to intron retention

More than 10 years after the discovery of the MPMV CTE, we discovered that the NXF1 gene itself contains an element with a high degree of structural similarity and primary sequence homology to the CTE (see Figure 1) (Li et al., 2006). However, the element is smaller than the MPMV CTE and contains only one binding site for Nxf1, compared to the two binding loops that are present in the MPMV CTE. The CTE from the NXF1 gene can substitute for the MPMV CTE to provide Rev/RRE independent HIV expression, but requires a duplication of the element for efficient function in this context. The element is present in intron 10 of the NXF1 gene of many species (see below) and an alternative NXF1 mRNA isoform retains this intron (see Figure 2). In human cells, the IR-mRNA can be translated into a 356 amino acid truncated “small” Nxf1 protein, due to a stop codon in the retained intron (Li et al., 2006). Whereas this IR-mRNA can be readily detected in most tissues, it is not clear if the protein is produced in all of these. However, the protein has been demonstrated in brain, spleen and liver in rodent studies (Li et al., 2016). Intriguingly, the small Nxf1 protein is highly expressed in rat hippocampal neurons, as well as some cortical neurons and shows partial co-localization with Staufen proteins in neuronal granules in mouse N2A neuroblastoma cells. Although the exact role of the small Nxf1 protein remains unexplored, it can serve as an alternative Nxf1 co-factor to replace Nxt in export and expression of CTE-containing RNA, likely by forming heterodimers with the full length Nxf1 protein. Similarly to the large Nxf1 protein, sNxf1 also associates with polyribosomes, implying a potential role in translational regulation.

Mutations in the NXF1 CTE previously shown to abolish MPMV CTE function, resulted in loss of export of IR-mRNA both in HIV reporter assays and NXF1 mini-constructs containing intron 10 (Li et al., 2006). Also, unpublished data from our lab show that CRISPR/Cas9 mediated deletions in the NXF1 CTE in 293T cells does not change the levels of NXF1 mRNA that retains the intron, but prevents the export of this RNA from the nucleus, indicating that a functional CTE is required for export.

CTEs with high sequence similarity are present in the corresponding intron in the NXF1 gene in most mammalian species and are also conserved in many species of teleost fish (including Zebrafish and Latimeria) (Wang, Rekosh, & Hammarskjold, 2015). In fact, there are only a few nucleotide differences between the fish CTEs and the human CTE, making this an ultraconserved intron sequence element. Additionally, the Zebrafish CTE functions efficiently in mammalian cells with either Zebrafish Nxf1/Nxt or the mammalian versions of these proteins. These results strongly suggest that CTE function, in conjunction with Nxf1/Nxt, is an evolutionarily conserved eukaryotic mechanism for regulated export and expression of mRNA with retained introns that was exapted from the host genome by an “ancient” retrovirus. Even though it remains possible that this is only a feed-back mechanism to specifically affect NXF1 gene expression, it seems likely that other RNA

elements, which interact directly with Nxf1 to function like CTEs may be used to regulate other eukaryotic genes.

Widespread intron retention has now been reported in many organisms and tissues

As described above, for many years, intron retention was mainly reported in viral systems and was thought to be rare in mammals and other organisms (with the exception of plants). However, the frequent use of this mechanism by viruses as an essential part of replication in their respective host cells was an early indication that intron retention might also be a common cellular mechanism. Before the development of RNA “deep” sequencing technologies, which enabled an unbiased view, annotation of intron retention was rarely seen in databases and publications. To a large extent this was due to the fact that most scientists were not looking for introns in their mRNA preparations and, even when they were detected, they were often thought to represent pre-mRNAs rather than final mRNA products. Thus findings of IR were often de-emphasized in publications. In fact, even when isoforms with retained introns were reported and deposited in NCBI and other databases, they were often removed by the curators, since they were flagged as NMD targets and predicted to be degraded if they reached the cytoplasm.

It has become clear in recent years that mRNA splicing is mostly co-transcriptional and that it occurs before poly A addition (Bentley, 2014; Beyer, Bouton, & Miller, 1981; Merkhofer, Hu, & Johnson, 2014; Osheim, Miller, & Beyer, 1985). Thus the fear that observed intron retention is really contamination with pre-mRNA species, especially if the RNA is poly A selected, is likely to be largely unfounded. These realizations, as well as the more unbiased analysis of massive amounts of whole transcriptome data from many organisms and tissues, have now led to many reports of intron retention in the recent literature (Edwards et al., 2016; Memon et al., 2016; Pimentel et al., 2016; Yap et al., 2012). In addition, long read sequencing using for example PacBio Iso-seq and Oxford Nanopore technologies can now be used to directly and accurately assess intron retention, even in the case of long introns. In a very recent study, using Iso-seq on RNA isolated from human cerebellum, IR was identified in 3,590 of the 11,466 genes covered (31,3%) with many genes having evidence of multiple retained introns (S. J. Zhang et al., 2017). The majority of these events are currently not annotated in either RefSeq and GENCODE. Since these results were obtained from only one specific organ, at a single point in time, it can be surmised that IR will be observed in many other genes in other organs and conditions. These studies all contribute to the fact that intron retention is rapidly becoming acknowledged as a much more common form of alternative splicing than previously appreciated. Because of this growing recognition there have been several recent reviews on this general subject (Hube & Francastel, 2015; Jacob & Smith, 2017; Wong et al., 2017; Wong et al., 2013).

One of the first transcriptome studies reporting wide-spread intron retention was published by the Blencowe group. They examined evidence for intron retention in polyA+ Illumina RNASeq data from over 40 human and mouse tissues (Braunschweig et al., 2014). The study also examined the extent to which IR events are conserved between the equivalent organs (whole brain, cerebellum, heart, muscle, liver, kidney, and testis) from up to 11 vertebrate species spanning 440 million years of evolution. The reads in these data sets were aligned to

a comprehensive set of reference sequences comprising exon-intron junctions, intron midpoint sequences, and exon-exon junctions resulting from intron removal. The degree of IR was then represented using the metric percent intron retention (PIR), which is a measure of the percentage of transcripts in which a given intron is retained. Additional steps were also taken to allow distinction between true intron retention and alternative transcript start and stop sites, alternative 5' and 3' splice-site selection, and/or overlap with transcripts from other genes, including RNA transcribed from the opposite strand. Overall, this study found the highest level of IR in neural and immune cell types, whereas IR was detected less often in ES and muscle cells. The results also indicated that introns detected as retained in poly(A)⁺ RNA were associated with a set of features that distinguished them from general introns (including elevated G/C content, short length and relatively weak 5' and 3' splice sites). Taken together, the data suggest that retained introns comprise three distinct types with different evolutionary origins (Type A, B and C), where Type A are ancestral introns flanked by constitutive exons, Type B are introns that arose by “intronization” of ancestral exonic sequence and Type C are located adjacent to one or more alternative exons that may or may not be conserved between species. The data also suggested that Type A is the most frequent, whereas type B represents the rarest form of IR. Based on this, it was concluded that retained introns comprise a heterogeneous group with different types that harbor distinct features, likely reflecting different evolutionary origins and functional properties.

There are obvious exceptions to these general IR features. For example, as mentioned above, the retained intron 10 in the NXF1 gene that harbors the CTE represents one of the longest introns in NXF1 (almost 4 kB in zebrafish and almost 2 kB in most mammalian species) (Li et al., 2006; Wang et al., 2015). Even though the Blencowe study found many examples of IR, it is likely that many were missed, since the analysis was based on short Illumina reads. It is likely that Iso-seq and similar techniques will discover many more IR events in various tissues and allow a clearer and more complete picture of IR as more and more data are reported. This kind of analysis will also more accurately reveal tissue specific aspects of IR, i.e. how IR varies from tissue to tissue in individual genes.

The general importance of IR in neural cell types and the important roles that this may have in the normal function of hippocampal neurons in the brain have been highlighted in studies from the Eberwine laboratory. This group has showed intron retention in a number of mRNAs expressed in such cells in the mouse and they have also showed that many of these mRNAs are exported to the cytoplasm. In many of these mRNAs, mouse ID repeat elements, a class of mouse SINE retrotransposons, are used for export (Buckley, Khaladkar, Kim, & Eberwine, 2014; Buckley et al., 2011). This group has also reported that many of these mRNAs eventually undergo splicing in the cytoplasm of neuronal cells. However, this remains controversial, due to the lack of evidence for a machinery for mRNA splicing in this compartment.

Several other recent studies have also demonstrated regulated intron retention in many genes. Specifically, IR has been shown to be dynamic in hematopoietic systems. Thus intron retention has been reported to serve important roles in granulocyte (Wong et al., 2013) and erythroid differentiation (Boutz et al., 2015; Braunschweig et al., 2014; Edwards et al., 2016; Pimentel et al., 2016). Additionally, a database maintained at the Barres laboratory

web site (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html) shows intron retention in many genes in normal mouse brain, with significant differences in different cells (Y. Zhang et al., 2014).

In spite of the many recent studies suggesting the importance of regulated intron retention, the factors that determine this remain poorly understood and the fates of the resulting mRNAs remain controversial. This is in part due to the fact that most genome wide studies have generally focused on reporting the IR-mRNAs in total mRNA, without exploring whether they are exported or associated with polyribosomes. In many cases,, it has been generally assumed that the IR-mRNAs would not be exported to the cytoplasm or that they would be subject to NMD if they were. Thus it has been argued that IR-mRNAs do not substantially add to proteome diversity (Ge & Porse, 2014; Wong et al., 2013; Yap et al., 2012). This mechanism for down-regulation of gene expression has become known as RUST (Regulated Unproductive Splicing and Translation) (Green et al., 2003). However, in recent years, it has become clear that NMD is also a complex mechanism that can be down-regulated under certain conditions such as stress and or in development and differentiation of certain cells (see below) (Hug et al., 2016; Karam et al., 2013; Nasif et al., 2017). This indicates a more complicated picture and the RUST theory has to be reconciled with the fact that many NMD IR-mRNA candidates have been reported to give rise to alternative protein isoforms. The small Nxf1 protein isoform is only one such example (Li et al., 2016; Li et al., 2006).

Intron retention and expression of novel protein isoforms

In spite of the prevailing dogma that proteins are only rarely expressed from IR-mRNAs, there are many examples in the literature of novel isoforms expressed from mRNAs with retained introns. We have already discussed the many viral proteins that are expressed from IR-mRNA and the small Nxf1 protein that is expressed from the NXF1 mRNA that retains intron 10 (Li et al., 2006), but there are multiple additional examples. For example, it was demonstrated that Robo3, which serves as an axonal guidance receptor in mouse development, exists in 2 different isoforms. One of these isoforms retains an intron. Translation gives rise to a truncated protein that opposes the action of the protein that is translated from the isoform that lacks this intron (Chen, Gore, Long, Ma, & Tessier-Lavigne, 2008).

Novel protein isoforms resulting from intron retention have also been reported for many other genes, including ERBB2 (Herstatin), ID3, PASD1, CEACAM6, Kallikrein genes, TRP-2, ANIA-6 (CCNL1) and PRX (Berke et al., 2001; Doherty, Bond, Jardim, Adelman, & Clinton, 1999; Dytrych, Sherman, Gillespie, & Brophy, 1998; S. T. Forrest, Barringhaus, Perlegas, Hammarskjöld, & McNamara, 2004; Kurio et al., 2008; Liggins, Brown, Asker, Pulford, & Banham, 2004; Lupetti et al., 1998; Michael et al., 2005). A very recent publication adds the EIF2B5 gene', which serves as an important "master" regulator of translation', to this list (Brady et al.) (see below). A recent review provides a table with many other examples (Wong, Au, Ritchie, & Rasko, 2016).

The factors that act on these mRNAs to overcome cellular restrictions have generally not been explored, but it is possible that some of the mRNAs encoding these isoforms will turn

out to contain *cis*-acting elements akin to the CTE. In fact, published and unpublished data from our laboratory indicate that several mammalian genes contain elements that work as CTEs and that at least some of these functionally interact with Nxf1/Nxt (Bor et al., 2006).

“Detained” versus “retained” introns

Widespread IR in total polyA+ RNA from mouse embryonic stem cells and has also been reported in a study from the Sharp laboratory (Boutz et al., 2015). Many of these events were also observed in human cell lines, as well as in adult mouse liver. In their publication, the authors reported that many of these IR-mRNAs were not exported to the cytoplasm, leading them to coin the term “detained” introns, as opposed to “retained” introns, which are introns retained in mRNA that can be exported. However, the mechanisms that detain these mRNAs in the nucleus are still poorly understood. The authors also suggested that intron detention could provide an additional gene regulation mechanism, since the detained mRNAs would be protected from NMD, which only occurs in the cytoplasm. If detention were coupled to delayed splicing, which occurs at a later time point in response to some kind of “trigger” signal, the resulting RNAs could then be exported and translated, without the need for new transcription, permitting a more rapid response to changing demands in the cell (Figure 3).

A very recent study by Naro et al. reported an orchestrated IR program in meiotic mammalian spermatocytes (40.9 % of observed regulated splicing events) that appears to be akin to the “detained” intron regulation described by the Sharp lab (Naro et al., 2017; Naro & Sette, 2017). The study showed that regulated IR in many genes generated long-lived intron-retaining mRNAs that could be later translated in post-meiotic germ cells, in which transcription is shut off. Many of the genes with IR had strong relevance for gamete function. The IR-RNAs were shown to initially localize to the nucleus, but seemed to be recruited to polyribosomes days after synthesis. Although the authors did not formally prove that the mRNAs that were translated in the cells were directly derived from the IR-mRNA, they favored the idea that delayed splicing, followed by nucleocytoplasmic export, could explain this. The notion that stored IR transcripts can be spliced later in a transcription independent manner, allowing delayed translation in response to physiological or developmental signals, is also supported by a recent study of IR in mouse neuronal cells (Mauger et al., 2016), as well as data from a study of spermatogenesis in the fern *Marsilea vestita* (Boothby, Zipper, van der Weele, & Wolniak, 2013).

It is also quite possible that some mRNAs with “detained introns” could possess functional CTE-like elements that would enable their export and translation without splicing of the detained intron, when the proper transacting proteins that interact functionally with these elements are present. This could permit the coordinated export of several different mRNAs and would make them akin to mRNA with “retained introns”. In this context it is interesting to note that the Sharp laboratory study placed NXF1 intron 10 in their list of “detained” introns. As mentioned above, we know that a truncated small Nxf1 protein can be stably translated from the mRNA that retains intron 10, at least in some tissues (Li et al., 2016). However, although the mRNA can be readily observed in most tissues and cell lines, the protein is not always detected. Thus it is possible that the intron 10 containing Nxf1 mRNA

is detained in the nucleus in some situations, but that it can be exported to the cytoplasm and translated in response to specific signals. This could provide another mechanism for coordinated gene expression of proteins from mRNAs with retained introns

Intron retention in cancer and other diseases

In addition to IR in normal tissues, it has become clear that IR is increased and altered in many disease states. This seems to be especially prevalent in cancers, where an increase in IR has been observed in many different forms of cancer, compared to normal cells from the same tissues (Dvinge & Bradley, 2015; Goodison, Yoshida, Churchman, & Tarin, 1998). Although the true significance of this and how it may contribute to progression of disease remains to be elucidated, this clearly affects gene regulation in the cancer cells and may lead to expression of new protein isoforms. In the study mentioned above that described a new protein isoform of EIF2B5, exon-level analyses demonstrated > 1,000 mRNAs as alternatively spliced during hypoxia in head and neck cancer cells, including many mRNAs with retained introns (Brady et al., 2017). Most notably, the authors found a unique retained intron in EIF2B5. It was shown that the mRNA that retained this intron was expressed in solid tumors in a stage-dependent manner and that the IR created a premature termination codon (PTC). Nevertheless, the authors found that a 65kDa truncated protein isoform was translated from the IR mRNA in these cells. This short isoform was shown to oppose the action of the full-length protein to inhibit global translation. Furthermore, they reported that expression of the truncated protein resulted in increased survival of head and neck cancer cells under hypoxia, providing evidence that the alternate isoform enables cells to adapt to conditions of low oxygen. Another example of a protein expressed from an IR mRNA is Cyclin D1B (Comstock et al., 2009; F. Lu, Gladden, & Diehl, 2003; Solomon et al., 2003). This isoform produces a truncated form of Cyclin D1 due a PTC in the retained intron. This form of the protein is upregulated in certain cancers and has been reported to have increased transforming activity. Upregulation of IR has also been observed in many other diseases, such as autoimmune diseases and neurological diseases such as Amyotrophic Lateral Sclerosis (ALS) (Lin et al., 1998). In the case of these diseases, it remains less clear if new protein isoforms are expressed and if so, how these relate to the phenotype of the disease.

Novel protein isoforms resulting from intron retention can have beneficial roles in disease

In some instances, a novel protein isoform expressed from an IR-mRNA can have a beneficial effect. One example of this is IR in the ID3 gene that seems to play an important role in proper “repair” after vascular injury. The Id3 protein that is translated from a fully spliced mRNA promotes smooth muscle cell proliferation in response to damage to the neointima layer of a vessel. The Id3a protein that is expressed transiently from an ID3 mRNA with a retained intron during the “repair” process has an opposing function to Id3. Thus expression of Id3a appears to represent a feed-back loop that acts to prevent excess smooth muscle cell proliferation after vessel injury (S. Forrest & McNamara, 2004). Another example similar to this is the IR that has been observed in the Calcineurin gene. IR in this gene results in a new protein isoform that improves cardiac function after myocardial infarction without inducing cardiac hypertrophy (Felkin et al., 2011). Taken together, studies such as these suggest that IR and protein expression from the resulting mRNAs may

represent an important mechanism in the regulation of “stress” and injury responses. This is also supported by studies in plants that show that IR appears to be involved in regulation of processes such as early flowering and responses to temperature changes (Filichkin & Mockler, 2012; Jiao & Meyerowitz, 2010; Kwon, Park, Kim, Baldwin, & Park, 2014; Ner-Gaon et al., 2004; Remy et al., 2014; Vitulo et al., 2014). The expression of proteins from IR mRNAs in these situations may be facilitated by the reported down-regulation of NMD during hypoxia and other stress conditions (Gardner, 2010; Karam et al., 2013).

CONCLUSION: INTRON RETENTION IS A FUNDAMENTALLY DIFFERENT TYPE OF ALTERNATIVE SPLICING THAT CAN BE REGULATED AT MANY LEVELS

Even though IR and the fate of mRNAs that retain introns remain incompletely understood, we know now that these mRNAs can be regulated at many levels in the cell. It was shown many years ago that mRNAs with retained introns appear to be restricted at the level of nucleo-cytoplasmic export, but the factors that mediate retention still remain largely unknown. While we have learned that this restriction can be overcome by *cis*-acting elements in the RNA (for example RREs and CTEs) and the transacting factors that bind to them, it is not clear how this provides the IR- mRNAs with a “passport” that allows their transit out of the nucleus. However, it is generally hypothesized that the nuclear pore serves as a “border control” in this process. Specifically, the Tpr protein that makes up the basket on the nuclear side of the pore, has been proposed to play a specific role in proof-reading of mRNA and it has been demonstrated that perturbation of Tpr function by limited knockdown with siRNA leads to increased export of some mRNAs with retained introns (Coyle, Bor, Rekosh, & Hammarskjöld, 2011).

As described above, several studies have reported that some of the mRNAs with IR never leave the nucleus and are eventually degraded by nuclear exosomes. However, it is not clear that these detained mRNAs are prevented from exiting the nucleus, without splicing of the introns, in all tissues and under all conditions. It is possible that many of these isoforms can undergo nucleo-cytoplasmic export and even get translated in certain tissues or during development or in certain conditions of stress. IR would ensure that these mRNAs would only be exported and translated if the right *cis*-acting elements and transacting factors are present. This may provide a very dynamic way to regulate the synthesis of the resulting proteins as needed. This could thus be a very important facet of gene regulation that has remained almost completely unexplored to date. If this is really true and how many genes are regulated in this way remains to be determined, but it is an intriguing possibility. New technologies, such as single cell RNA seq (Saliba, Westermann, Gorski, & Vogel, 2014) and long-read RNA sequencing (Byrne et al., 2017; Kuo et al., 2017; S. J. Zhang et al., 2017) now make it much easier to investigate exactly how dynamic IR is and if IR-mRNAs reach the cytoplasm and if they are present on polyribosomes. Once this is established, it can be determined if and when these mRNAs give rise to novel protein isoforms. The transacting factors that act on mRNAs that contain CTE-like export elements can also be identified in cross-linking and other experiments.

NMD is clearly an important mechanism for regulation of IR-mRNA expression in the cytoplasm, since many of these mRNAs contain PTCs. This mechanism would be expected to especially limit expression if the mRNA does not have CTE-like elements that appear to circumvent NMD by an unknown mechanism. However, as mentioned above, several recent studies have shown that NMD is also subject to regulation. Recently, it was shown that NMD is repressed during neural development by the expression of a specific miRNA, miR-128 (Bruno et al., 2011). This leads to upregulation of many NMD targets that encode proteins that are of importance for differentiation and neuron function. It is likely that this would also lead to increased expression from cytoplasmic mRNAs with retained introns in neuronal cells. As mentioned above, it has also been reported that NMD is repressed by a variety of stress conditions, such as hypoxia, and nutrient deprivation (Gardner, 2010; Hug et al., 2016; Karam et al., 2013). There could thus be many situations that would allow stabilization and translation of IR-mRNAs in a regulated and concerted fashion.

In summary, yet another discovery that was initially thought to represent a solely viral phenomenon, may be just as important as a mechanism for the host cell. Further research will be necessary to pinpoint how this unique form of alternative splicing is used for gene regulation and how and when IR is used in coordinated post-transcriptional regulation.

Acknowledgments

Concepts developed by the authors and publications from the authors' laboratories were supported by the following grants from the National Institutes of Health: R01GM087651; R01GM110009; R01206275 Additional funding and partial salary support for DR and MLH was provided by the Myles H. Thaler and Charles H. Ross Jr. professorships at the University of Virginia. The authors are also adjunct professors in the Department of Microbiology at the University of Venda, Thohoyandou, South Africa.

References

- Askjaer P, Jensen TH, Nilsson J, Englmeier L, Kjems J. The specificity of the CRM1-Rev nuclear export signal interaction is mediated by RanGTP. *J Biol Chem.* 1998; 273(50):33414–33422. [PubMed: 9837918]
- Bell TJ, Miyashiro KY, Sul JY, McCullough R, Buckley PT, Jochems J, ... Eberwine J. Cytoplasmic BK(Ca) channel intron-containing mRNAs contribute to the intrinsic excitability of hippocampal neurons. *Proc Natl Acad Sci U S A.* 2008; 105(6):1901–1906. [PubMed: 18250327]
- Bentley DL. Coupling mRNA processing with transcription in time and space. *Nat Rev Genet.* 2014; 15(3):163–175. DOI: 10.1038/nrg3662 [PubMed: 24514444]
- Berget SM, Moore C, Sharp PA. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. *Proc Natl Acad Sci U S A.* 1977; 74(8):3171–3175. [PubMed: 269380]
- Berke JD, Sgambato V, Zhu PP, Lavoie B, Vincent M, Krause M, Hyman SE. Dopamine and glutamate induce distinct striatal splice forms of Ania-6, an RNA polymerase II-associated cyclin. *Neuron.* 2001; 32(2):277–287. S0896-6273(01)00465-2 [pii]. [PubMed: 11683997]
- Beyer AL, Bouton AH, Miller OL Jr. Correlation of hnRNP structure and nascent transcript cleavage. *Cell.* 1981; 26(2 Pt 2):155–165. [PubMed: 6174239]
- Bjork P, Wieslander L. Integration of mRNP formation and export. *Cell Mol Life Sci.* 2017; 74(16): 2875–2897. DOI: 10.1007/s00018-017-2503-3 [PubMed: 28314893]
- Black DL. Mechanisms of alternative pre-messenger RNA splicing. *Annu Rev Biochem.* 2003; 72:291–336. DOI: 10.1146/annurev.biochem.72.121801.161720 [PubMed: 12626338]
- Bogerd HP, Wiegand HL, Yang J, Cullen BR. Mutational definition of functional domains within the Rev homolog encoded by human endogenous retrovirus K. *J Virol.* 2000; 74(20):9353–9361. [PubMed: 11000203]

- Booth DS, Cheng Y, Frankel AD. The export receptor Crm1 forms a dimer to promote nuclear export of HIV RNA. *Elife*. 2014; 3doi: 10.7554/eLife.04121
- Boothby TC, Zipper RS, van der Weele CM, Wolniak SM. Removal of retained introns regulates translation in the rapidly developing gametophyte of *Marsilea vestita*. *Dev Cell*. 2013; 24(5):517–529. DOI: 10.1016/j.devcel.2013.01.015 [PubMed: 23434411]
- Bor Y, Swartz J, Morrison A, Rekosh D, Lodomery M, Hammarskjöld ML. The Wilms' tumor 1 (WT1) gene (+KTS isoform) functions with a CTE to enhance translation from an unspliced RNA with a retained intron. *Genes Dev*. 2006; 20(12):1597–1608. [PubMed: 16738405]
- Boutz PL, Bhutkar A, Sharp PA. Detained introns are a novel, widespread class of post-transcriptionally spliced introns. *Genes Dev*. 2015; 29(1):63–80. DOI: 10.1101/gad.247361.114 [PubMed: 25561496]
- Brady LK, Wang H, Radens CM, Bi Y, Radovich M, Maity A, ... Koumenis C. Transcriptome analysis of hypoxic cancer cells uncovers intron retention in EIF2B5 as a mechanism to inhibit translation. *PLoS Biol*. 2017; 15(9):e2002623.doi: 10.1371/journal.pbio.2002623 [PubMed: 28961236]
- Braun IC, Herold A, Rode M, Conti E, Izaurralde E. Overexpression of TAP/p15 heterodimers bypasses nuclear retention and stimulates nuclear mRNA export. *J Biol Chem*. 2001; 276(23):20536–20543. [PubMed: 11259411]
- Braunschweig U, Barbosa-Morais NL, Pan Q, Nachman EN, Alipanahi B, Gonatopoulos-Pournatzis T, ... Blencowe BJ. Widespread intron retention in mammals functionally tunes transcriptomes. *Genome Res*. 2014; 24(11):1774–1786. DOI: 10.1101/gr.177790.114 [PubMed: 25258385]
- Bray M, Prasad S, Dubay JW, Hunter E, Jeang KT, Rekosh D, Hammarskjöld ML. A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev-independent. *Proc Natl Acad Sci U S A*. 1994; 91(4):1256–1260. [PubMed: 8108397]
- Breathnach R, Mandel JL, Chambon P. Ovalbumin gene is split in chicken DNA. *Nature*. 1977; 270(5635):314–319. [PubMed: 593351]
- Breitbart RE, Andreadis A, Nadal GB. Alternative splicing: a ubiquitous mechanism for the generation of multiple protein isoforms from single genes. *Annu Rev Biochem*. 1987; 56:467–495. [PubMed: 3304142]
- Bruno IG, Karam R, Huang L, Bhardwaj A, Lou CH, Shum EY, ... Wilkinson MF. Identification of a microRNA that activates gene expression by repressing nonsense-mediated RNA decay. *Mol Cell*. 2011; 42(4):500–510. DOI: 10.1016/j.molcel.2011.04.018 [PubMed: 21596314]
- Buckley PT, Khaladkar M, Kim J, Eberwine J. Cytoplasmic intron retention, function, splicing, and the sentinel RNA hypothesis. *Wiley Interdiscip Rev RNA*. 2014; 5(2):223–230. DOI: 10.1002/wrna.1203 [PubMed: 24190870]
- Buckley PT, Lee MT, Sul JY, Miyashiro KY, Bell TJ, Fisher SA, ... Eberwine J. Cytoplasmic intron sequence-retaining transcripts can be dendritically targeted via ID element retrotransposons. *Neuron*. 2011; 69(5):877–884. DOI: 10.1016/j.neuron.2011.02.028 [PubMed: 21382548]
- Byrne A, Beaudin AE, Olsen HE, Jain M, Cole C, Palmer T, ... Vollmers C. Nanopore long-read RNAseq reveals widespread transcriptional variation among the surface receptors of individual B cells. *Nat Commun*. 2017; 8:16027.doi: 10.1038/ncomms16027 [PubMed: 28722025]
- Chang DD, Sharp PA. Regulation by HIV Rev depends upon recognition of splice sites. *Cell*. 1989; 59(5):789–795. [PubMed: 2686839]
- Chen Z, Gore BB, Long H, Ma L, Tessier-Lavigne M. Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. *Neuron*. 2008; 58(3):325–332. DOI: 10.1016/j.neuron.2008.02.016 [PubMed: 18466743]
- Chow LT, Gelinis RE, Broker TR, Roberts RJ. An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. *Cell*. 1977; 12(1):1–8. [PubMed: 902310]
- Comstock CE, Augello MA, Benito RP, Karch J, Tran TH, Utama FE, ... Knudsen KE. Cyclin D1 splice variants: polymorphism, risk, and isoform-specific regulation in prostate cancer. *Clin Cancer Res*. 2009; 15(17):5338–5349. DOI: 10.1158/1078-0432.CCR-08-2865 [PubMed: 19706803]
- Coyle JH, Bor YC, Rekosh D, Hammarskjöld ML. The Tpr protein regulates export of mRNAs with retained introns that traffic through the Nxf1 pathway. *RNA*. 2011; 17(7):1344–1356. rna.2616111 [pii]. DOI: 10.1261/rna.2616111 [PubMed: 21613532]

- Culjkovic B, Topisirovic I, Skrabanek L, Ruiz-Gutierrez M, Borden KL. eIF4E is a central node of an RNA regulon that governs cellular proliferation. *J Cell Biol.* 2006; 175(3):415–426. jcb. 200607020 [pii]. DOI: 10.1083/jcb.200607020 [PubMed: 17074885]
- Cullen BR, Greene WC. Regulatory Pathways Governing HIV-1 Replication. *Cell.* 1989; 58:423–426. [PubMed: 2569361]
- Doherty JK, Bond C, Jardim A, Adelman JP, Clinton GM. The HER-2/neu receptor tyrosine kinase gene encodes a secreted autoinhibitor. *Proc Natl Acad Sci U S A.* 1999; 96(19):10869–10874. [PubMed: 10485918]
- Dvinge H, Bradley RK. Widespread intron retention diversifies most cancer transcriptomes. *Genome Med.* 2015; 7(1):45.doi: 10.1186/s13073-015-0168-9 [PubMed: 26113877]
- Dytrych L, Sherman DL, Gillespie CS, Brophy PJ. Two PDZ domain proteins encoded by the murine periaxin gene are the result of alternative intron retention and are differentially targeted in Schwann cells. *J Biol Chem.* 1998; 273(10):5794–5800. [PubMed: 9488714]
- Edwards CR, Ritchie W, Wong JJ, Schmitz U, Middleton R, An X, ... Blobel GA. A dynamic intron retention program in the mammalian megakaryocyte and erythrocyte lineages. *Blood.* 2016; doi: 10.1182/blood-2016-01-692764
- Ernst RK, Bray M, Rekosh D, Hammarskjöld ML. A structured retroviral RNA element that mediates nucleocytoplasmic export of intron-containing RNA. *Mol Cell Biol.* 1997; 17:135–144. [PubMed: 8972193]
- Ernst RK, Bray M, Rekosh D, Hammarskjöld ML. Secondary structure and mutational analysis of the Mason-Pfizer monkey virus RNA constitutive transport element. *RNA.* 1997; 3(2):210–222. [PubMed: 9042947]
- Felkin LE, Narita T, Germack R, Shintani Y, Takahashi K, Sarathchandra P, ... Lara-Pezzi E. Calcineurin splicing variant calcineurin Abeta1 improves cardiac function after myocardial infarction without inducing hypertrophy. *Circulation.* 2011; 123(24):2838–2847. DOI: 10.1161/CIRCULATIONAHA.110.012211 [PubMed: 21632490]
- Fernandes JD, Booth DS, Frankel AD. A structurally plastic ribonucleoprotein complex mediates post-transcriptional gene regulation in HIV-1. *Wiley Interdiscip Rev RNA.* 2016; doi: 10.1002/wrna.1342
- Filichkin SA, Mockler TC. Unproductive alternative splicing and nonsense mRNAs: a widespread phenomenon among plant circadian clock genes. *Biol Direct.* 2012; 7:20.doi: 10.1186/1745-6150-7-20 [PubMed: 22747664]
- Fischer U, Huber J, Boelens WC, Mattaj IW, Luhrmann R. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell.* 1995; 82(3):475–483. [PubMed: 7543368]
- Fornerod M, Ohno M, Yoshida M, Mattaj IW. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell.* 1997; 90(6):1051–1060. [PubMed: 9323133]
- Forrest S, McNamara C. Id family of transcription factors and vascular lesion formation. *Arterioscler Thromb Vasc Biol.* 2004; 24(11):2014–2020. [PubMed: 15345510]
- Forrest ST, Barringhaus KG, Perlegas D, Hammarskjöld ML, McNamara CA. Intron retention generates a novel Id3 isoform that inhibits vascular lesion formation. *J Biol Chem.* 2004; 279(31):32897–32903. [PubMed: 15159391]
- Fries B, Heukeshoven J, Hauber I, Gruttner C, Stocking C, Kehlenbach RH, ... Chemnitz J. Analysis of nucleocytoplasmic trafficking of the HuR ligand APRIL and its influence on CD83 expression. *J Biol Chem.* 2007; 282(7):4504–4515. [PubMed: 17178712]
- Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, Yanagida M, Nishida E. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature.* 1997; 390(6657):308–311. DOI: 10.1038/36894 [PubMed: 9384386]
- Gama-Carvalho M, Condado I, Carmo-Fonseca M. Regulation of adenovirus alternative RNA splicing correlates with a reorganization of splicing factors in the nucleus. *Exp Cell Res.* 2003; 289(1):77–85. [PubMed: 12941606]
- Gauvar O, Germain H. mRNA export: threading the needle. *Front Plant Sci.* 2013; 4:59.doi: 10.3389/fpls.2013.00059 [PubMed: 23526740]

- Gardner LB. Nonsense-mediated RNA decay regulation by cellular stress: implications for tumorigenesis. *Mol Cancer Res.* 2010; 8(3):295–308. DOI: 10.1158/1541-7786.MCR-09-0502 [PubMed: 20179151]
- Ge Y, Porse BT. The functional consequences of intron retention: alternative splicing coupled to NMD as a regulator of gene expression. *Bioessays.* 2014; 36(3):236–243. DOI: 10.1002/bies.201300156 [PubMed: 24352796]
- Gilbert W. Why genes in pieces? *Nature.* 1978; 271(5645):501. [PubMed: 622185]
- Goodison S, Yoshida K, Churchman M, Tarin D. Multiple intron retention occurs in tumor cell CD44 mRNA processing. *Am J Pathol.* 1998; 153(4):1221–1228. [PubMed: 9777953]
- Green RE, Lewis BP, Hillman RT, Blanchette M, Lareau LF, Garnett AT, ... Brenner SE. Widespread predicted nonsense-mediated mRNA decay of alternatively-spliced transcripts of human normal and disease genes. *Bioinformatics.* 2003; 19(Suppl 1):i118–121. [PubMed: 12855447]
- Gruter P, Taberero C, von KC, Schmitt C, Saavedra C, Bachi A, ... Izaurralde E. TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol Cell.* 1998; 1(5):649–659. [PubMed: 9660949]
- Guzik BW, Levesque L, Prasad S, Bor YC, Black BE, Paschal BM, ... Hammarskjöld ML. NXT1 (p15) is a crucial cellular cofactor in TAP-dependent export of intron-containing RNA in mammalian cells. *Mol Cell Biol.* 2001; 21(7):2545–2554. [PubMed: 11259602]
- Hadzopoulou-Cladaras M, Felber BK, Cladaras C, Athanassopoulos A, Tse A, Pavlakis GN. The rev (trs/art) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a cis-acting sequence in the env region. *J Virol.* 1989; 63(3):1265–1274. [PubMed: 2783738]
- Hammarskjöld ML. Regulation of retroviral RNA export. *Sem in Cell and Dev Bio.* 1997; 8:83–90.
- Hammarskjöld ML. Constitutive transport element-mediated nuclear export. *Curr Top Microbiol Immunol.* 2001; 259:77–93. [PubMed: 11417128]
- Hammarskjöld ML, Heimer J, Hammarskjöld B, Sangwan I, Albert L, Rekosh D. Regulation of human immunodeficiency virus env expression by the rev gene product. *J Virol.* 1989; 63(5):1959–1966. [PubMed: 2704072]
- Hammarskjöld ML, Li H, Rekosh D, Prasad S. Human immunodeficiency virus env expression becomes Rev-independent if the env region is not defined as an intron. *J Virol.* 1994; 68(2):951–958. [PubMed: 8289397]
- Hanly SM, Rimsky LT, Malim MH, Kim JH, Hauber J, Duc Dodon M, ... Greene WC. Comparative analysis of the HTLV-I Rex and HIV-1 Rev trans-regulatory proteins and their RNA response elements. *Genes Dev.* 1989; 3(10):1534–1544. [PubMed: 2482226]
- Herold A, Klymenko T, Izaurralde E. NXF1/p15 heterodimers are essential for mRNA nuclear export in *Drosophila*. *RNA.* 2001; 7(12):1768–1780. [PubMed: 11780633]
- Herold A, Suyama M, Rodrigues JP, Braun IC, Kutay U, Carmo-Fonseca M, ... Izaurralde E. TAP (NXF1) belongs to a multigene family of putative RNA export factors with a conserved modular architecture. *Mol Cell Biol.* 2000; 20(23):8996–9008. [PubMed: 11073998]
- Hidaka M, Inoue J, Yoshida M, Seiki M. Post-transcriptional regulator (rex) of HTLV-1 initiates expression of viral structural proteins but suppresses expression of regulatory proteins. *EMBO J.* 1988; 7(2):519–523. [PubMed: 2835230]
- Hofacre A, Nitta T, Fan H. Jaagsiekte sheep retrovirus encodes a regulatory factor, Rej, required for synthesis of Gag protein. *J Virol.* 2009; 83(23):12483–12498. JVI.01747-08 [pii]. DOI: 10.1128/JVI.01747-08 [PubMed: 19776124]
- Hope TJ, Bond BL, McDonald D, Klein NP, Parslow TG. Effector domains of human immunodeficiency virus type 1 Rev and human T-cell leukemia virus type I Rex are functionally interchangeable and share an essential peptide motif. *J Virol.* 1991; 65(11):6001–6007. [PubMed: 1920623]
- Hube F, Francastel C. Mammalian introns: when the junk generates molecular diversity. *Int J Mol Sci.* 2015; 16(3):4429–4452. DOI: 10.3390/ijms16034429 [PubMed: 25710723]
- Hug N, Longman D, Caceres JF. Mechanism and regulation of the nonsense-mediated decay pathway. *Nucleic Acids Res.* 2016; 44(4):1483–1495. DOI: 10.1093/nar/gkw010 [PubMed: 26773057]

- Ishigaki Y, Li X, Serin G, Maquat LE. Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20. *Cell*. 2001; 106(5):607–617. [PubMed: 11551508]
- Jacob AG, Smith CW. Intron retention as a component of regulated gene expression programs. *Hum Genet*. 2017; doi: 10.1007/s00439-017-1791-x
- Jiao Y, Meyerowitz EM. Cell-type specific analysis of translating RNAs in developing flowers reveals new levels of control. *Mol Syst Biol*. 2010; 6:419.doi: 10.1038/msb.2010.76 [PubMed: 20924354]
- Johnson LA, Sandri-Goldin RM. Efficient nuclear export of herpes simplex virus 1 transcripts requires both RNA binding by ICP27 and ICP27 interaction with TAP/NXF1. *J Virol*. 2009; 83(3):1184–1192. DOI: 10.1128/JVI.02010-08 [PubMed: 19019956]
- Kalland KH, Szilvay AM, Brokstad KA, Saetrevik W, Haukenes G. The human immunodeficiency virus type 1 Rev protein shuttles between the cytoplasm and nuclear compartments. *Mol Cell Biol*. 1994; 14(11):7436–7444. [PubMed: 7935458]
- Kang Y, Bogerd HP, Yang J, Cullen BR. Analysis of the RNA binding specificity of the human tap protein, a constitutive transport element-specific nuclear RNA export factor. *Virology*. 1999; 262(1):200–209. [PubMed: 10489353]
- Karam R, Wengrod J, Gardner LB, Wilkinson MF. Regulation of nonsense-mediated mRNA decay: implications for physiology and disease. *Biochim Biophys Acta*. 2013; 1829(6–7):624–633. DOI: 10.1016/j.bbarm.2013.03.002 [PubMed: 23500037]
- Katahira J, Miki T, Takano K, Maruhashi M, Uchikawa M, Tachibana T, Yoneda Y. Nuclear RNA export factor 7 is localized in processing bodies and neuronal RNA granules through interactions with shuttling hnRNPs. *Nucleic Acids Res*. 2008; 36(2):616–628. DOI: 10.1093/nar/gkm556 [PubMed: 18063567]
- Katahira J, Strasser K, Podtelejnikov A, Mann M, Jung JU, Hurt E. The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. *EMBO J*. 1999; 18(9):2593–2609. [PubMed: 10228171]
- Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. *Nat Rev Genet*. 2010; 11(5):345–355. DOI: 10.1038/nrg2776 [PubMed: 20376054]
- Kienzle N, Young DB, Liaskou D, Buck M, Greco S, Sculley TB. Intron retention may regulate expression of Epstein-Barr virus nuclear antigen 3 family genes. *J Virol*. 1999; 73(2):1195–1204. [PubMed: 9882321]
- Kimura T, Hashimoto I, Nagase T, Fujisawa J. CRM1-dependent, but not ARE-mediated, nuclear export of IFN- α 1 mRNA. *J Cell Sci*. 2004; 117(Pt 11):2259–2270. DOI: 10.1242/jcs.01076 [PubMed: 15126627]
- Kuersten S, Ohno M, Mattaj IW. Nucleocytoplasmic transport: Ran, beta and beyond. *Trends in cell biology*. 2001; 11(12):497–503. [PubMed: 11719056]
- Kuo RI, Tseng E, Eory L, Paton IR, Archibald AL, Burt DW. Normalized long read RNA sequencing in chicken reveals transcriptome complexity similar to human. *BMC Genomics*. 2017; 18(1):323.doi: 10.1186/s12864-017-3691-9 [PubMed: 28438136]
- Kurio H, Murayama E, Kaneko T, Shibata Y, Inai T, Iida H. Intron retention generates a novel isoform of CEACAM6 that may act as an adhesion molecule in the ectoplasmic specialization structures between spermatids and sertoli cells in rat testis. *Biol Reprod*. 2008; 79(6):1062–1073. *biolreprod*. 108.069872 [pii]. DOI: 10.1095/biolreprod.108.069872 [PubMed: 18685128]
- Kurosaki T, Maquat LE. Nonsense-mediated mRNA decay in humans at a glance. *J Cell Sci*. 2016; 129(3):461–467. DOI: 10.1242/jcs.181008 [PubMed: 26787741]
- Kwon YJ, Park MJ, Kim SG, Baldwin IT, Park CM. Alternative splicing and nonsense-mediated decay of circadian clock genes under environmental stress conditions in *Arabidopsis*. *BMC Plant Biol*. 2014; 14:136.doi: 10.1186/1471-2229-14-136 [PubMed: 24885185]
- Legrain P, Rosbash M. Some cis- and trans-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell*. 1989; 57(4):573–583. [PubMed: 2655924]
- Lewis N, Williams J, Rekosh D, Hammarskjöld ML. Identification of a cis-acting element in human immunodeficiency virus type 2 (HIV-2) that is responsive to the HIV-1 rev and human T-cell leukemia virus types I and II rex proteins. *J Virol*. 1990; 64(4):1690–1697. [PubMed: 2157051]

- Li Y, Bor YC, Fitzgerald MP, Lee KS, Rekosh D, Hammarskjöld ML. An NXF1 mRNA with a retained intron is expressed in hippocampal and neocortical neurons and is translated into a protein that functions as an Nxf1 cofactor. *Mol Biol Cell*. 2016; 27(24):3903–3912. DOI: 10.1091/mbc.E16-07-0515 [PubMed: 27708137]
- Li Y, Bor YC, Misawa Y, Xue Y, Rekosh D, Hammarskjöld ML. An intron with a constitutive transport element is retained in a Tap messenger RNA. *Nature*. 2006; 443(7108):234–237. [PubMed: 16971948]
- Liggins AP, Brown PJ, Asker K, Pulford K, Banham AH. A novel diffuse large B-cell lymphoma-associated cancer testis antigen encoding a PAS domain protein. *Br J Cancer*. 2004; 91(1):141–149. 6601875 [pii]. DOI: 10.1038/sj.bjc.6601875 [PubMed: 15162151]
- Lin CL, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, Clawson L, Rothstein JD. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron*. 1998; 20(3):589–602. [PubMed: 9539131]
- Lu F, Gladden AB, Diehl JA. An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. *Cancer Res*. 2003; 63(21):7056–7061. [PubMed: 14612495]
- Lu XB, Heimer J, Rekosh D, Hammarskjöld ML. U1 small nuclear RNA plays a direct role in the formation of a rev-regulated human immunodeficiency virus env mRNA that remains unspliced. *Proc Natl Acad Sci U S A*. 1990; 87(19):7598–7602. [PubMed: 2217190]
- Lupetti R, Pisarra P, Verrecchia A, Farina C, Nicolini G, Anichini A, ... Traversari C. Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. *J Exp Med*. 1998; 188(6):1005–1016. [PubMed: 9743519]
- Magin C, Lower R, Lower J. cORF and RcRE, the Rev/Rex and RRE/RxRE homologues of the human endogenous retrovirus family HTDV/HERV-K. *J Virol*. 1999; 73(11):9496–9507. [PubMed: 10516058]
- Magin-Lachmann C, Hahn S, Strobel H, Held U, Lower J, Lower R. Rec (formerly Corf) function requires interaction with a complex, folded RNA structure within its responsive element rather than binding to a discrete specific binding site. *J Virol*. 2001; 75(21):10359–10371. DOI: 10.1128/JVI.75.21.10359-10371.2001 [PubMed: 11581404]
- Malim MH, Bohnlein S, Hauber J, Cullen BR. Functional dissection of the HIV-1 Rev trans-activator--derivation of a trans-dominant repressor of Rev function. *Cell*. 1989; 58(1):205–214. [PubMed: 2752419]
- Malim MH, Hauber J, Le SV, Maizel JV, Cullen BR. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature*. 1989; 338:254–257. [PubMed: 2784194]
- Maquat LE, Carmichael GG. Quality control of mRNA function. *Cell*. 2001; 104(2):173–176. [PubMed: 11207359]
- Mauger O, Lemoine F, Scheiffele P. Targeted Intron Retention and Excision for Rapid Gene Regulation in Response to Neuronal Activity. *Neuron*. 2016; 92(6):1266–1278. DOI: 10.1016/j.neuron.2016.11.032 [PubMed: 28009274]
- Memon D, Dawson K, Smowton CS, Xing W, Dive C, Miller CJ. Hypoxia-driven splicing into noncoding isoforms regulates the DNA damage response. *NPJ Genom Med*. 2016; 1:16020.doi: 10.1038/npjgenmed.2016.20 [PubMed: 28480052]
- Merkhofer EC, Hu P, Johnson TL. Introduction to cotranscriptional RNA splicing. *Methods in molecular biology*. 2014; 1126:83–96. DOI: 10.1007/978-1-62703-980-2_6 [PubMed: 24549657]
- Mertz JA, Simper MS, Lozano MM, Payne SM, Dudley JP. Mouse mammary tumor virus encodes a self-regulatory RNA export protein and is a complex retrovirus. *J Virol*. 2005; 79(23):14737–14747. 79/23/14737 [pii]. DOI: 10.1128/JVI.79.23.14737-14747.2005 [PubMed: 16282474]
- Meyer BE, Malim MH. The HIV-1 Rev trans-activator shuttles between the nucleus and the cytoplasm. *Genes and Devel*. 1994; 8:1538–1547. [PubMed: 7958838]
- Michael IP, Kurlender L, Memari N, Yousef GM, Du D, Grass L, ... Diamandis EP. Intron retention: a common splicing event within the human kallikrein gene family. *Clin Chem*. 2005; 51(3):506–515. clinchem.2004.042341 [pii]. DOI: 10.1373/clinchem.2004.042341 [PubMed: 15650036]

- Modrek B, Resch A, Grasso C, Lee C. Genome-wide detection of alternative splicing in expressed sequences of human genes. *Nucleic Acids Res.* 2001; 29(13):2850–2859. [PubMed: 11433032]
- Naro C, Jolly A, Di Persio S, Bielli P, Setterblad N, Alberdi AJ, ... Sette C. An Orchestrated Intron Retention Program in Meiosis Controls Timely Usage of Transcripts during Germ Cell Differentiation. *Dev Cell.* 2017; 41(1):82–93. e84. DOI: 10.1016/j.devcel.2017.03.003 [PubMed: 28366282]
- Naro C, Sette C. Timely-regulated intron retention as device to fine-tune protein expression. *Cell Cycle.* 2017; 16(14):1321–1322. DOI: 10.1080/15384101.2017.1337983 [PubMed: 28594251]
- Nasif S, Contu L, Muhlemann O. Beyond quality control: The role of nonsense-mediated mRNA decay (NMD) in regulating gene expression. *Semin Cell Dev Biol.* 2017; doi: 10.1016/j.semedb.2017.08.053
- Ner-Gaon H, Halachmi R, Savaldi-Goldstein S, Rubin E, Ophir R, Fluhr R. Intron retention is a major phenomenon in alternative splicing in Arabidopsis. *Plant J.* 2004; 39(6):877–885. [pii]. DOI: 10.1111/j.1365-3113X.2004.02172.xTPJ2172 [PubMed: 15341630]
- Neville M, Stutz F, Lee L, Davis LI, Rosbash M. The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr Biol.* 1997; 7(10):767–775. [PubMed: 9368759]
- Nitta T, Hofacre A, Hull S, Fan H. Identification and mutational analysis of a *Rej* response element in Jaagsiekte sheep retrovirus RNA. *J Virol.* 2009; 83(23):12499–12511. JVI.01754-08 [pii]. DOI: 10.1128/JVI.01754-08 [PubMed: 19776134]
- Ogert, RA., Beemon, KL. Identification of a constitutive transport element in the 3' untranslated region of the Rous Sarcoma virus RNA. Paper presented at the Retroviruses; Cold Spring Harbor. 1995.
- Osheim YN, Miller OL Jr, Beyer AL. RNP particles at splice junction sequences on *Drosophila* chorion transcripts. *Cell.* 1985; 43(1):143–151. [PubMed: 3935315]
- Ossareh-Nazari B, Bachelier F, Dargemont C. Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science.* 1997; 278(5335):141–144. [PubMed: 9311922]
- Ossareh-Nazari B, Maison C, Black BE, Levesque L, Paschal BM, Dargemont C. RanGTP-binding protein NXT1 facilitates nuclear export of different classes of RNA in vitro. *Mol Cell Biol.* 2000; 20(13):4562–4571. [PubMed: 10848583]
- Paca RE, Ogert RA, Hibbert CS, Izaurralde E, Beemon KL. Rous sarcoma virus DR posttranscriptional elements use a novel RNA export pathway. *J Virol.* 2000; 74(20):9507–9514. [PubMed: 11000220]
- Pessel-Vivares L, Ferrer M, Laine S, Mougél M. MLV requires Tap/NXF1-dependent pathway to export its unspliced RNA to the cytoplasm and to express both spliced and unspliced RNAs. *Retrovirology.* 2014; 11:21.doi: 10.1186/1742-4690-11-21 [PubMed: 24597485]
- Phelan A, Dunlop J, Clements JB. Herpes simplex virus type 1 protein IE63 affects the nuclear export of virus intron-containing transcripts. *J Virol.* 1996; 70(8):5255–5265. [PubMed: 8764035]
- Pimentel H, Parra M, Gee SL, Mohandas N, Pachter L, Conboy JG. A dynamic intron retention program enriched in RNA processing genes regulates gene expression during terminal erythropoiesis. *Nucleic Acids Res.* 2016; 44(2):838–851. DOI: 10.1093/nar/gkv1168 [PubMed: 26531823]
- Pollard VW, Malim MH. The HIV-1 Rev protein. *Annu Rev Microbiol.* 1998; 52:491–532. [PubMed: 9891806]
- Purcell DF, Martin MA. Alternative splicing of human immunodeficiency virus type 1 mRNA modulates viral protein expression, replication, and infectivity. *J Virol.* 1993; 67(11):6365–6378. [PubMed: 8411338]
- Rekosh D, Nygren A, Flodby P, Hammarskjöld ML, Wigzell H. Coexpression of human immunodeficiency virus envelope proteins and tat from a single simian virus 40 late replacement vector. *Proc Natl Acad Sci U S A.* 1988; 85(2):334–338. [PubMed: 2829181]
- Remy E, Cabrito TR, Batista RA, Hussein MA, Teixeira MC, Athanasiadis A, ... Duque P. Intron retention in the 5' UTR of the novel ZIF2 transporter enhances translation to promote zinc tolerance in arabidopsis. *PLoS Genet.* 2014; 10(5):e1004375. PGENETICS-D-13-02254 [pii]. doi: 10.1371/journal.pgen.1004375 [PubMed: 24832541]

- Sakuma T, Davila JI, Malcolm JA, Kocher JP, Tonne JM, Ikeda Y. Murine leukemia virus uses NXF1 for nuclear export of spliced and unspliced viral transcripts. *J Virol*. 2014; 88(8):4069–4082. DOI: 10.1128/JVI.03584-13 [PubMed: 24478440]
- Saliba AE, Westermann AJ, Gorski SA, Vogel J. Single-cell RNA-seq: advances and future challenges. *Nucleic Acids Res*. 2014; 42(14):8845–8860. DOI: 10.1093/nar/gku555 [PubMed: 25053837]
- Sasaki M, Takeda E, Takano K, Yomogida K, Katahira J, Yoneda Y. Molecular cloning and functional characterization of mouse Nxf family gene products. *Genomics*. 2005; 85(5):641–653. [PubMed: 15820316]
- Satterly N, Tsai PL, van Deursen J, Nussenzweig DR, Wang Y, Faria PA, ... Fontoura BM. Influenza virus targets the mRNA export machinery and the nuclear pore complex. *Proc Natl Acad Sci U S A*. 2007; 104(6):1853–1858. DOI: 10.1073/pnas.0610977104 [PubMed: 17267598]
- Schutz S, Chemnitz J, Spillner C, Frohme M, Hauber J, Kehlenbach RH. Stimulated expression of mRNAs in activated T cells depends on a functional CRM1 nuclear export pathway. *J Mol Biol*. 2006; 358(4):997–1009. S0022-2836(06)00227-0 [pii]. DOI: 10.1016/j.jmb.2006.02.041 [PubMed: 16580684]
- Schwartz S, Felber BK, Benko DM, Fenyo EM, Pavlakis GN. Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. *J Virol*. 1990; 64(6):2519–2529. [PubMed: 2335812]
- Sedlackova L, Perkins KD, Lengyel J, Strain AK, van Santen VL, Rice SA. Herpes simplex virus type 1 ICP27 regulates expression of a variant, secreted form of glycoprotein C by an intron retention mechanism. *J Virol*. 2008; 82(15):7443–7455. DOI: 10.1128/JVI.00388-08 [PubMed: 18495765]
- Seiki M, Inoue J, Hidaka M, Yoshida M. Two cis-acting elements responsible for posttranscriptional trans-regulation of gene expression of human T-cell leukemia virus type I. *Proc Natl Acad Sci U S A*. 1988; 85(19):7124–7128. [PubMed: 3174625]
- Sherer NM, Swanson CM, Hue S, Roberts RG, Bergeron JR, Malim MH. Evolution of a species-specific determinant within human CRM1 that regulates the post-transcriptional phases of HIV-1 replication. *PLoS Pathog*. 2011; 7(11):e1002395.doi: 10.1371/journal.ppat.1002395 [PubMed: 22114565]
- Siddiqui N, Borden KL. mRNA export and cancer. *Wiley Interdiscip Rev RNA*. 2012; 3(1):13–25. DOI: 10.1002/wrna.101 [PubMed: 21796793]
- Smith AJ, Cho MI, Hammarckjöld ML, Rekosh D. Human immunodeficiency virus type 1 Pr55gag and Pr160gag-pol expressed from a simian virus 40 late replacement vector are efficiently processed and assembled into viruslike particles. *J Virol*. 1990; 64(6):2743–2750. [PubMed: 1692347]
- Solomon DA, Wang Y, Fox SR, Lambeck TC, Giesting S, Lan Z, ... Knudsen ES. Cyclin D1 splice variants. Differential effects on localization, RB phosphorylation, and cellular transformation. *J Biol Chem*. 2003; 278(32):30339–30347. DOI: 10.1074/jbc.M303969200 [PubMed: 12746453]
- Takano K, Miki T, Katahira J, Yoneda Y. NXF2 is involved in cytoplasmic mRNA dynamics through interactions with motor proteins. *Nucleic Acids Res*. 2007; 35(8):2513–2521. DOI: 10.1093/nar/gkm125 [PubMed: 17403691]
- Teplova M, Wohlbold L, Khin NW, Izaurralde E, Patel DJ. Structure-function studies of nucleocytoplasmic transport of retroviral genomic RNA by mRNA export factor TAP. *Nat Struct Mol Biol*. 2011; 18(9):990–998. DOI: 10.1038/nsmb.2094 [PubMed: 21822283]
- Tilghman SM, Tiemeier DC, Seidman JG, Peterlin BM, Sullivan M, Maizel JV, Leder P. Intervening sequence of DNA identified in the structural portion of a mouse beta-globin gene. *Proc Natl Acad Sci U S A*. 1978; 75(2):725–729. [PubMed: 273235]
- Tretyakova I, Zolotukhin AS, Tan W, Bear J, Propst F, Ruthel G, Felber BK. Nuclear export factor family protein participates in cytoplasmic mRNA trafficking. *J Biol Chem*. 2005; 280(36):31981–31990. [PubMed: 16014633]
- Vitolo N, Forcato C, Carpinelli EC, Telatin A, Campagna D, D'Angelo M, ... Valle G. A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. *BMC Plant Biol*. 2014; 14:99. 1471-2229-14-99 [pii]. doi: 10.1186/1471-2229-14-99 [PubMed: 24739459]

- Wang B, Rekosh D, Hammarskjold ML. Evolutionary conservation of a molecular machinery for export and expression of mRNAs with retained introns. *RNA*. 2015; 21(3):426–437. DOI: 10.1261/rna.048520.114 [PubMed: 25605961]
- Wong JJ, Au AY, Ritchie W, Rasko JE. Intron retention in mRNA: No longer nonsense: Known and putative roles of intron retention in normal and disease biology. *Bioessays*. 2016; 38(1):41–49. DOI: 10.1002/bies.201500117 [PubMed: 26612485]
- Wong JJ, Gao D, Nguyen TV, Kwok CT, van Geldermalsen M, Middleton R, ... Rasko JEJ. Intron retention is regulated by altered MeCP2-mediated splicing factor recruitment. *Nat Commun*. 2017; 8:15134.doi: 10.1038/ncomms15134 [PubMed: 28480880]
- Wong JJ, Ritchie W, Ebner OA, Selbach M, Wong JW, Huang Y, ... Rasko JE. Orchestrated intron retention regulates normal granulocyte differentiation. *Cell*. 2013; 154(3):583–595. S0092-8674(13)00834-9 [pii]. DOI: 10.1016/j.cell.2013.06.052 [PubMed: 23911323]
- Yap K, Lim ZQ, Khandelia P, Friedman B, Makeyev EV. Coordinated regulation of neuronal mRNA steady-state levels through developmentally controlled intron retention. *Genes Dev*. 2012; 26(11):1209–1223. 26/11/1209 [pii]. DOI: 10.1101/gad.188037.112 [PubMed: 22661231]
- Zhang SJ, Wang C, Yan S, Fu A, Luan X, Li Y, ... Li CY. Isoform Evolution in Primates through Independent Combination of Alternative RNA Processing Events. *Mol Biol Evol*. 2017; 34(10):2453–2468. DOI: 10.1093/molbev/msx212 [PubMed: 28957512]
- Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keefe S, ... Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014; 34(36):11929–11947. DOI: 10.1523/JNEUROSCI.1860-14.2014 [PubMed: 25186741]
- Zolotukhin AS, Valentin A, Pavlakis GN, Felber BK. Continuous propagation of RRE(–) and Rev(–)RRE(–) human immunodeficiency virus type 1 molecular clones containing a cis-acting element of simian retrovirus type 1 in human peripheral blood lymphocytes. *J Virol*. 1994; 68(12):7944–7952. [PubMed: 7966585]

FIGURE 2.

Three Mechanisms that Promote the Export of mRNA with Retained Introns.

(A) Transcription from the HIV provirus produces multiple mRNAs that retain introns and encode different HIV proteins. Their nucleocytoplasmic export is mediated by Rev binding to the RRE, multimerizing and forming a complex with Crm1 and Ran-GTP which targets them to the nuclear pore for export and subsequent translation in the cytoplasm.

(B) Transcription from the MPMV provirus generates an unspliced mRNA that encodes the MPMV Gag and GagProPol proteins within an intron. Export to the cytoplasm is achieved by the binding of Nxf1/Nxt1 to the CTE. In addition to export, Nxf1 is required for efficient translation. (C) The NXF1 gene expresses an alternatively spliced mRNA isoform that retains intron 10, but has spliced out all of the other introns. Similarly to the unspliced MPMV mRNA, it is exported to the cytoplasm by the interaction of a CTE within intron 10 and Nxf1/Nxt1. In the cytoplasm this mRNA is translated into a truncated Nxf1 (sNxf1) due to a UGA stop codon within the intron. The sNxf1 protein can replace Nxt1 as a cofactor in Nxf1 mediated export.

FIGURE 3.

The Fate of Detained and Retained Introns. Detained introns never reach the cytoplasm. mRNAs that contain them may be slowly spliced or spliced in response to a specific signal allowing them to exit the nucleus as a fully spliced mRNA. If they are not spliced they are eventually degraded. On the other hand, retained introns can be exported to the cytoplasm through the interaction of an RNA sequence, the constitutive transport element (CTE), or a CTE-like element, with cellular export proteins. In the case of the CTE, the cellular export factor is Nxf1. Other, as yet unidentified cellular proteins, may play similar roles through interaction with other CTE-like elements. This export may be regulated by differential expression of the export proteins in space and time. Once in the cytoplasm, mRNAs with retained introns can have several fates as indicated.