The connection between splicing and cancer

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Accepted 17 May 2006
Journal of Cell Science 119, 2635-2641 Published by The Company of Biologists 2006
doi:10.1242/jcs.03053

Summary

Alternative splicing is a crucial mechanism for generating protein diversity. Different splice variants of a given protein can display different and even antagonistic biological functions. Therefore, appropriate control of their synthesis is required to assure the complex orchestration of cellular processes within multicellular organisms. Mutations in cis-acting splicing elements or changes in the activity of regulatory proteins that compromise the accuracy of either constitutive or alternative splicing could have a profound impact on human pathogenesis, in particular in tumor development and progression. Mutations in splicing elements, for example, have been found in genes such as *LKB1*, *KIT*, *CDH17*, *KLF6* and *BRCA1*, and changes in trans-acting regulators can affect the expression of genes such as *Ron*, *RAC1* and *CD44*.

Key words: Splicing, Alternative splicing, Cancer, Signaling

Introduction

The response of cells to environmental signals, as well as their differentiation, death or malignant transformation, involves changes in gene expression. For many years, the modulation of gene expression was thought to be restricted to, or at least dominated by, the control of transcription. According to the Jacob-Monod-Lwoff paradigm, established in bacteria and logically extended to eukaryotes, what made a cell a cell was the combinatorial turning on and off of genes. The discovery of introns and the process of splicing, which eliminates them from the precursor mRNA, introduced an exciting but also perturbing factor in our simplified conceptions of the flow of gene expression in eukaryotes. Introns must be excised precisely to generate bona fide mRNA molecules; otherwise, translational frame shifts would be introduced. Introns are much longer than exons and most of their nucleotide sequence seems to be irrelevant except for the 'donor' and 'acceptor' consensus sequences located at their extremities, and the branching sequence that precedes the acceptor sequence. The spliceosome, a sophisticated nuclear machine comprising five types of small nuclear ribonucleoprotein (snRNP) and hundreds of auxiliary proteins, takes care of intron excision and exon joining repeatedly at each and every one of the seven splicing events that occur on average per human pre-mRNA molecule (Lander et al., 2001).

'What is all this for?', a teleologist might ask. We know now that the high adaptive value of introns resides in the fact that they permitted exon shuffling in the past and alternative splicing in the present (Sharp, 1994). However, the apparent 'irrationality of introns' (I. Eperon, http://www.eurasnet.info/ian_eperon.shtml) was questioned early on following the discovery that mutations that affect splicing, quantitatively or qualitatively, are a widespread source of hereditary diseases. No matter how absurd or energy consuming a biological process appears to be, if its disruption or perturbation causes disease, it must be important, and its conservation in evolution is paramount. The links between splicing and human disease have been extensively reviewed lately (Caceres and Kornblihtt, 2002; Cartegni et al., 2002; Faustino and Cooper, 2003; Pagani and Baralle, 2004). The specific association between splicing and cancer has received less attention (Brinkman, 2004; Venables, 2006), perhaps because the field is still emerging. Here we examine this connection, focusing not only on mutations in cis-acting splicing sequences that are associated with cancer but also on the variations in normal splicing processes and the signals that may affect them in cancer cells.

The basic language of splicing

Splice sites, branch sites, splicing enhancers and splicing silencers

Splicing occurs when a group of proteins and RNPs recognize specific RNA sequences conserved at the boundaries of introns (Fig. 1A). The classical spliceosome, which acts on >99% of introns from genes transcribed by RNA polymerase II (Pol II), recognizes a 5′ donor splice site beginning with a GU dinucleotide and a 3′ acceptor splice site ending with an AG dinucleotide (the GU-AG rule). A second type of spliceosome acts on a minor class of Pol-II-transcribed introns obeying the AU-AC rule (Tarn and Steitz, 1996). In the major class of introns, the 5′ splice site, the branch site in between and the 3′ splice site are recognized by U1 snRNP, U2 snRNP and the auxiliary factor U2AF, respectively. The splicing reaction is completed with the participation of the U4, U6 and U5 snRNPs (Sharp, 1994). Every internal exon is flanked by upstream and downstream splice sites. The situation is different for the terminal exons: the upstream limit of the first exon is the Cap site or transcription initiation site, whereas the downstream limit of the last exon is the polyadenylation site (Fig. 1B). These particular features are important considerations for alternative splicing (see below).

When the sequence of a splice site deviates from the consensus shown in Fig. 1A, the site can still be used, although less efficiently, depending on the number of base changes. These weak sites (Fig. 1C) have less affinity for their spliceosomal protein or RNP partners and are the main cause of alternative splicing. A second class of cis-acting sequence

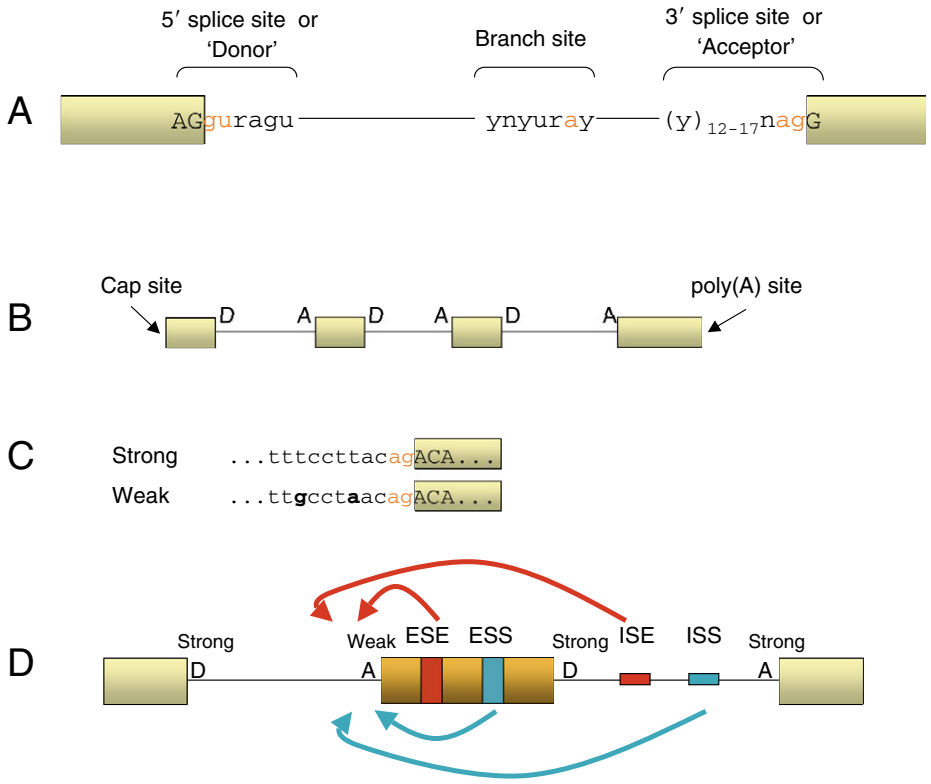

Fig. 1. Cis-acting sequences that control splicing. (A) Consensus sequences for the 5′ splice site (donor), branch site and 3′ splice site (acceptor). (B) Arrangement of donor (D) and acceptor (A) sites in the architecture of a typical eukaryotic gene. Whereas internal exons are limited by acceptor and donor sites, the first exon is limited by the Cap site and a donor, and the last exon is limited by an acceptor and the poly(A) site. (C) Examples of sequences for strong and weak acceptor sites. Red indicates the 3′ acceptor splice site; bold indicates deviations from the consensus sequence. (D) Schematic roles of exonic splicing enhancers (ESE), exonic splicing silencers (ESS), intronic splicing enhancers (ISE) and intronic splicing silencers (ISS) on the recognition of a weak acceptor by the splicing machinery; red indicates enhancing, blue indicates silencing. In A-D, exon sequences are boxed.

can influence the recognition and use of weak sites by the splicing apparatus: the enhancers and silencers of splicing. These are short (~10 nucleotides) conserved sequences located in exons or introns, either isolated or in clusters, that stimulate or inhibit the use of weak splice sites (Fig. 1D). An exonic sequence is defined as an exonic splicing enhancer (ESE) if its mutation reduces inclusion of the corresponding exon into the mature mRNA. Conversely, mutation of an exonic splicing silencer (ESS) increases inclusion of the exon. In most cases, the mechanisms of action of splicing enhancers and silencers involve the specific binding of regulatory proteins such as SR proteins (serine/arginine-rich proteins) or heterogeneous nuclear (hn)RNPs (Dreyfuss et al., 1993; Graveley, 2000; Manley and Tacke, 1996). Certain silencers, instead of binding regulatory proteins, form a particular pre-mRNA secondary structure that hinders the recognition of a neighboring splicing enhancer by SR proteins (Buratti et al., 2004).

Approximately 15% of mutations that cause genetic disease affect pre-mRNA splicing. Some disrupt or create splice sites. If a canonical splice site is completely disrupted, a cryptic splice site nearby is used instead, leading to aberrantly spliced mRNA molecules and failure to produce a functional protein. If the disruption is partial, the cryptic and mutated sites compete, leading to a mixed population of aberrant and normal mRNA molecules, with a reduction in normal protein levels. Creation of a new splice site has similar consequences: the new (aberrant) and the old site compete and there is a concomitant reduction in normal protein levels.

Recently, the role of mutations that create or abolish splicing enhancers and silencers in disease has been examined (Cartegni et al., 2002; Pagani and Baralle, 2004). This is particularly important when one studies single base changes in exonic sequences. Traditionally, these base changes were assumed to produce nonsense, missense or silent substitutions that could only affect the quality of the encoded protein. However, we now know that in many cases they disrupt or create functional ESEs or ESSs, and provoke changes in the levels of inclusion of the exons to which they map.

Alternative splicing

When two or more splice sites compete, alternative splicing generates mRNA variants that yield different polypeptides from a single gene (reviewed by Black, 2003). Alternative splicing is more a rule than an exception: it affects an estimated 60% of human genes (Lander et al., 2001). Its regulation not only depends on the interaction of SR and hnRNP proteins with splicing enhancers and silencers, but is also coupled to Pol II transcription, as in the case of other pre-mRNA processing reactions (reviewed by Bentley, 2005; Kornblihtt et al., 2004; Maniatis and Reed, 2002; Proudfoot et al., 2002).

Fig. 2A illustrates the different modes of alternative splicing, which we distinguish from alternative transcriptional initiation (Fig. 2B). Alternative transcriptional initiation generates mRNA diversity but is not alternative splicing because there are no competing splice sites.

Cancer and mutations that affect splicing

Many examples of cancer-associated alterations in splicing are attributable to mutations that create or disrupt splice sites or splicing enhancers and silencers. However, in only a few cases has a cause-effect relationship been proved. Below we discuss some recent examples, which are summarized in Table 1.

LKB1

LKB1 is a tumor suppressor gene that encodes a serine/threonine protein kinase involved in the control of several

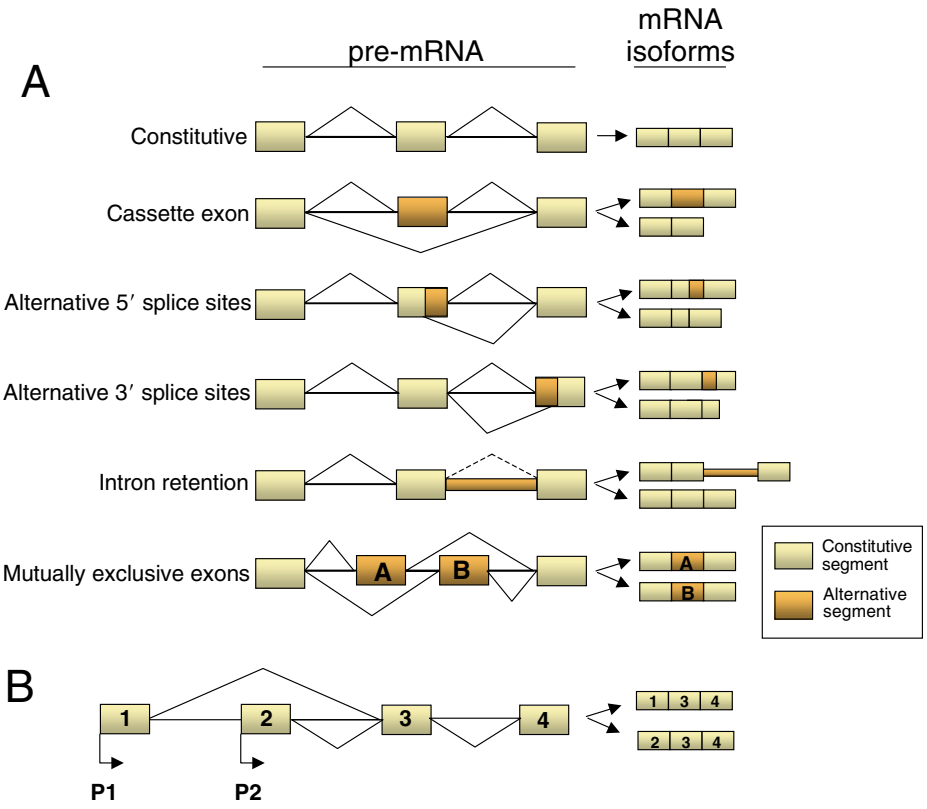

Fig. 2. (A) Different modes of alternative splicing. (B) Alternative promoters provoke mRNA diversity but do not necessarily imply alternative splicing. There are two alternative promoters, P1 and P2. If promoter P2 is used, exons 2, 3 and 4 are constitutively included. If promoter P1 is used, exon 2 simply does not exist for the splicing machinery because, being a 5′ terminal exon of the P1 transcription unit, it lacks an upstream 3′ splice site. Therefore, the splicing machinery has no option but to join exon 1 directly to exon 3, generating a 1-3-4 mRNA.

cellular processes, including cell-cycle arrest, p53-mediated apoptosis, Ras-induced transformation, cell polarity. Its second intron belongs to the minor spliceosome class that obeys the AU-AC rule. Mutations of *LKB1* that lead to reduced levels of the protein are found in patients with Peutz-Jeghers Syndrome (PJS), an autosomal dominant disorder associated with gastrointestinal polyposis and an increased cancer risk. A group of PJS patients carry a mutation that affects the 5′ splice site of intron 2 (IVS2+1A>G). Interestingly, the mutated 5′ splice site is still used but not in conjunction with its normal 3′ splice site. Instead, cryptic, non-canonical 3′ splice sites adjacent to the normal one are used, which leads to frameshifting and consequently to the appearance of premature termination codons in the aberrantly spliced transcripts (Hastings et al., 2005). The mutation thus affects the fidelity of splicing. It also provides a tool to dissect the differences between minor and major spliceosome requirements.

KIT

Another interesting example involves the oncogene *KIT*. The encoded protein is a member of the type III receptor tyrosine kinase family whose constitutive activation is associated with gastrointestinal stromal tumors. Chen et al. found patients with deletions of an intron-exon segment encompassing the 3′ splice

Table 1. Examples of mutations in splicing cis-acting sequences that are associated with malignant disease

Gene (function)	Disease	Mutated sequence	Molecular consequences	Ref.
LKB1 (tumor suppressor)	Peutz-Jeghers Syndrome (increased cancer risk)	IVS2+1A>G	Disruption of minor spliceosome; mutated 5′ splice site is still used, together with cryptic, non-canonical 3′ splice sites; lower protein levels	Hasting et al., 2005
KIT (oncogene)	Gastrointestinal stromal tumor	Deletion of 30 or 34 nucleotides including IVS10 3′ splice site	Aberrant splicing; constitutive protein activation	Chen et al., 2005
CDH17 (LI-cadherin)	Hepatocellular carcinoma	IVS6+35A>G	Generation of an ISS(?); exon 7 skipping	Wang et al., 2005
CDH17 (LI-cadherin)	Hepatocellular carcinoma	E6 codon 651	Generation of a ESS(?); exon 7 skipping	Wang et al., 2005
KLF6 (tumor suppressor)	Prostate cancer	IVS1-27G>A (‘*IVSΔA* allele’)	Generation of binding site (ISE) for SRp40; novel splicing variants act as dominant negatives	Narla et al., 2005
HAS1 (hyaluronan synthase)	Multiple myeloma	E3 C7760T	Exon 4 skipping	Adamia et al., 2005
BRCA1 (tumor suppressor)	Breast and ovarian cancer	E18 G5199T (E1694X)	ESE disruption; exon 18 skipping	Mazoyer et al., 1998

site of intron 10; these deletions concomitantly create an intra-exonic 3′ splice site within exon 11 (Chen et al., 2005). The resulting polypeptides remain in-frame but lack an internal stretch that is crucial for auto-inhibition of the kinase. Structural studies of the mutated kinase revealed a conformation consistent with constitutive activation.

CDH17

Liver intestine cadherin (LI-cadherin) is a cell-cell adhesion protein present in the plasma membrane. Its gene, *CDH17*, has been reported to be overexpressed in hepatocellular carcinomas, as well as in gastric and pancreatic cancer. Similarly, splicing variants lacking exon 7 are strongly associated with poor prognosis and a high incidence of tumor recurrence. Exon 7 skipping seems to be caused by two types of point mutation: disruption of the branch site of intron 6 and a base change at position 651 of exon 6 (Wang et al., 2005). The latter might affect exon 7 inclusion by generating an ESS or disrupting an ESE located in exon 6. Why the lack of the protein segment encoded by exon 7 provokes effects similar to those resulting from overexpression of the full-length *CDH17* remains to be determined.

KLF6

A case in which the molecular defect is better defined is a point mutation in intron 1 of the *KLF6* gene that generates a binding site for the SR protein SRp40. KLF6 is a Kruppel-like Zn-finger transcription factor that functions as a tumor suppressor and is somatically inactivated in prostate cancer (Narla et al., 2005). The new site for SRp40 apparently works as an atypical intronic splicing enhancer (ISE), provoking the use of three cryptic splice sites in exon 2. The proteins predicted to arise from the aberrant mRNAs lack parts of the activation and/or DNA-binding domain and presumably act as dominant-negative mutants.

BRCA1

Germline mutations in the *BRCA1* gene are well-known markers of predisposition to breast and ovarian cancers. An inherited point mutation in exon 18, classifiable as a nonsense mutation, was the first shown to affect splicing: it disrupts an ESE and provokes exon 18 skipping (Mazoyer et al., 1998). Since then, a panoply of mutations that presumably affect splicing enhancers and silencers have been described in *BRCA1*. A recent in silico approach using the program ESEfinder (Cartegni et al., 2003) identified 23 highly conserved ESEs in the 22 exons of the *BRCA1* gene. About 60% of these ESEs are predicted to be affected by sequence variants reported in the Breast Cancer Information Core (Pettigrew et al., 2005). These findings stress how crucial it is when one attempts to interpret the molecular basis of genetic disease to define whether base changes in coding regions act at the translational level (generating amino acid changes, stop codons, or silent mutations) or affect splicing.

Cancer-associated alterations of splicing patterns

The splicing patterns of several genes have been reported to be altered in cancers, including those encoding the prolactin receptor, Ron, Rac1, fibronectin, fibroblast growth factor receptor, CD44, MDM2 and IIp45. The fact that no mutations have been observed in cis-acting splicing elements within these genes suggests that the alterations could be due to changes in trans-acting splicing regulators. Furthermore, certain alternatively spliced isoforms of proteins such as Ron and Rac1 can accumulate in tumors, and overexpression of the tumor-associated isoforms is sufficient to transform cells in culture (Singh et al., 2004; Zhou et al., 2003). Below, we discuss a few examples that affect cell growth, adhesion, migration, invasion and apoptosis, as well as the connection between signaling pathways and splicing regulation.

Ron

Ron is a heterodimeric protein formed by α and β subunits, both derived from the proteolytic cleavage of a common precursor. It is the tyrosine kinase receptor for macrophage-stimulating protein, and binding to this ligand triggers a signaling cascade that regulates a variety of cellular activities, such as cell growth, motility and invasion of extracellular matrices. These occur during epithelial-to-mesenchymal transition (EMT), a process that is essential for embryonic development and also participates in tumor progression in epithelial tissues (Radisky, 2005; Thiery and Sleeman, 2006). An alternatively spliced isoform termed ΔRon was identified in human gastric carcinoma cells and induces an invasive phenotype in transfected cells (Collesi et al., 1996). Δ*Ron* mRNA originates by skipping of exon 11, which leads to the deletion of a 49 amino acid stretch in the β chain of the resulting polypeptide. This deletion abolishes the proteolytical cleavage and renders the protein constitutively active.

Ghigna et al. not only confirmed that *Ron* splicing is altered in breast and colon cancers but also shed light on the molecular mechanism that regulates it (Ghigna et al., 2005). They identified two regulatory elements within exon 12 that control the level of inclusion of exon 11. The binding of the SR protein SF2/ASF to one of these stimulates skipping of exon 11, increasing the level of the ΔRon isoform. More excitingly, not only does overexpression of SF2/ASF lead to morphological and molecular changes characteristic of an EMT, but knockdown of SF2/ASF by RNA interference (RNAi) reduces the levels of ΔRon and concomitantly decreases cell motility. Similarly, RNAi directed against ΔRon reduces cell motility and partially reverses the morphological changes induced by overexpression of SF2/ASF. Thus, SF2/ASF could regulate malignant transformation of certain epithelial tumors by inducing a ΔRon-dependent EMT.

Other trans-acting regulators could act in the same way and we can therefore speculate that variations in expression and/or activity of splicing factors could lead to changes in the splicing patterns of certain mRNAs whose protein products are involved in different stages of tumor progression. The obvious question is which signaling molecules control SR protein activity and what events lead to their deregulation.

Rac

Matrix metalloproteases (MMPs) are upregulated in nearly all cancers (Egeblad and Werb, 2002). Their activity modifies cell-cell and cell-substratum adhesion, promoting tumor cell proliferation, invasion, angiogenesis and metastasis. In addition, they influence genomic surveillance, causing genomic instability (Radisky and Bissell, 2006). MMPs have also been implicated in splicing regulation. Radisky et al. demonstrated that synthesis of Rac1b, an alternatively spliced

isoform of the Rho-family GTPase Rac1, is induced by treatment of normal mammary epithelial cells with MMP-3 and that Rac1b activity is required for MMP-3-triggered EMT in these cells (Radisky et al., 2005).

Rac1b is generated by inclusion of a 57-nucleotide cassette exon. It accumulates in colorectal and breast tumors, and shows transforming properties when overexpressed in cultured cells. Unlike the ΔRon example, the factors that regulate *RAC1* splicing have not yet been characterized. However, preliminary evidence indicates that changes in SR protein expression modulate *RAC1* splicing patterns (F. Pelisch, D. Radisky and A.S., unpublished results). Moreover, MMP-3-dependent Rac1b synthesis triggers an increase in cellular reactive oxygen species (ROS), leading to genomic instability (Radisky et al., 2005), which can drive tumorigenesis. Li and Manley have linked genomic instability to SR protein function (Li and Manley, 2005). They showed that, when cultured cells are depleted of SF2/ASF, nascent pre-mRNA remains hybridized to the template DNA strand, leading to increased damage to the displaced complementary DNA strand. They propose that coupling between transcription and pre-mRNA processing therefore not only assures efficient production of mature mRNAs but also protects chromosomes from potentially deleterious DNA damage. Further investigations will reveal whether the expression and/or activity of certain splicing factors is involved in the genomic instability observed upon Rac1b production.

p53

The delicate balance between cell proliferation, differentiation and death maintains tissue homeostasis within multicellular organisms. Deregulation of any of these processes can lead to tumorigenesis. Inactivation of the *p53* tumor suppressor gene is a very frequent event in human cancer (Oren, 2003). p53 is a crucial protein involved in cell-cycle control, apoptosis and maintenance of genetic stability. It was thought to exist as a single isoform. However, multiple isoforms generated through the use of two different promoters and alternative splicing have recently been discovered, making p53 similar to its relatives p63 and p73 (Bourdon et al., 2005; Prives and Manfredi, 2005; Rohaly et al., 2005). Bourdon et al. showed that these isoforms are expressed in a tissue-dependent manner and that their expression pattern is altered in human breast tumors (Bourdon et al., 2005). The regulators of promoter selection and alternative splicing of p53 still needs to be elucidated.

The p53 regulators MDM2 (and its human analog HDM2) and MDMX (and its human analog HDMX) also undergo alternative splicing. Binding of MDM2 to p53 inhibits its transcriptional function and also facilitates its degradation by the proteasome. MDMX (also named MDM4) heterodimerizes with MDM2, affecting MDM2 activity. More than 40 *MDM2*/*HDM2* transcripts, including alternatively as well as aberrantly spliced forms, have been identified both in tumors and normal tissue. Some of these variants encode proteins that possess transforming properties in vitro and in vivo (Bartel et al., 2002; Lukas et al., 2001). Genomic mutations that can account for the observed usage of cryptic splice sites and the generation of aberrantly spliced isoforms have not been found (Lukas et al., 2001). Moreover, an aberrantly spliced and tumor-specific *HDMX* isoform has been recently described. This transcript, isolated from a thyroid tumor cell line, encodes a protein named HDMX211, which enhances MDM2 protein levels and counteracts its p53-degrading function (Giglio et al., 2005).

Signal transduction, splicing and cancer

Ras pathways

Activation of a pathway involving Ras, PI 3-kinase (PI 3-kinase) and AKT (the Ras/PI 3-kinase/AKT pathway) has been associated with multiple human cancers (Bos, 1989; Scheid and Woodgett, 2001; Vivanco and Sawyers, 2002). This pathway is counterbalanced by the tumor suppressor phosphatase PTEN, which in turn is often inactivated in cancer (Di Cristofano and Pandolfi, 2000). The Ras/PI 3-kinase/AKT pathway leads to changes in activity of SR proteins, in particular SF2/ASF and 9G8. Consequently, it regulates alternative splicing of fibronectin transcripts. Furthermore, AKT can phosphorylate SF2/ASF and 9G8 in vitro (Blaustein et al., 2004; Blaustein et al., 2005). Activation of this pathway by insulin regulates the activity of another SR protein, SRp40, stimulating the inclusion of an alternative exon in protein kinase C (PKC)βII pre-mRNA (Patel et al., 2001; Patel et al., 2005). It is tempting to speculate that deregulation of the Ras/PI 3-kinase/AKT pathway by activating mutations in its components or by PTEN inactivation would have dramatic consequences for the splicing pattern of any of the pre-mRNAs regulated by these splicing factors.

Several reports implicate other Ras-dependent pathways in the regulation of alternative splicing of the genes encoding agrin, CD44 and CD45 (Konig et al., 1998; Lynch and Weiss, 2000; Smith et al., 1997; Weg-Remers et al., 2001; Weg-Remers et al., 2002). *CD44* is one of the most studied alternatively spliced genes in cancer because inclusion of variable exons correlates with tumor development and metastasis (Brinkman, 2004; Stickeler et al., 1999; Venables, 2004). In T-lymphoma cells, phorbol-ester-dependent activation of extracellular-signal-regulated kinase (ERK) leads to phosphorylation of the nuclear RNA-binding protein Sam68, which regulates alternative splicing of variable exon 5 in CD44 (Matter et al., 2002). Cheng and Sharp recently identified SRm160 as another Ras-regulated splicing co-activator responsible for inclusion of this exon in CD44 (Cheng and Sharp, 2006). Furthermore, they demonstrated that silencing of this factor decreases cell invasiveness, providing another link between regulation of alternative splicing and tumorigenesis.

Several questions remain to be answered. First, whether changes in the splicing patterns of different genes involved in neoplastic transformation result from the deregulation of a certain set of splicing factors. Second, whether a given extracellular signal can affect different alternative splicing events that cooperatively participate in tumor progression.

Signaling to the splicing machinery

It is becoming increasingly clear that alternative splicing is regulated by extracellular signals through the activation of complex networks of transduction pathways (Lynch, 2004; Pelisch et al., 2005; Shin and Manley, 2004). However, data addressing how these extracellular signals impinge upon splicing factor activity are scarce (Allemand et al., 2005; Blaustein et al., 2005; Matter et al., 2002; Patel et al., 2005; van der Houven van Oordt et al., 2000; Xie et al., 2003). Serine phosphorylation of the arginine- and serine-rich (RS) domain

is an important modulator of SR protein activity and localization. Protein kinases that phosphorylate SR proteins and their antagonistic hnRNP proteins could play a crucial role in linking extracellular cues to regulation of alternative splicing. A recent study (Ngo et al., 2005) supports a model in which SF2/ASF is first phosphorylated in the cytoplasm by SR-protein-specific kinase 1 (SRPK1) on only a few serine residues within the RS domain. This hypophosphorylated SF2/ASF is imported into the nucleus and stored in nuclear speckles. Release of SF2/ASF from these storage sites and its recruitment to active sites of transcription requires a second round of phosphorylation, which is carried out by CDC-like kinase 1 (CLK). Dephosphorylation of SF2/ASF is required for its activity during mRNA splicing, and so it is conceivable that it must undergo dephosphorylation by as-yet-unknown phosphatases (Huang et al., 2004).

Although SRPK and CLK have been identified as SR protein kinases, signaling pathways that involve these kinases have not been identified. As already mentioned, AKT can also phosphorylate SR proteins, in particular SF2/ASF, 9G8 and SRp40. ERK phosphorylates the RNA-binding protein Sam68, which is involved in the regulation of CD44 alternative splicing (Matter et al., 2002). ERK-dependent activation of Sam68 triggers the formation of a macromolecular complex that contains Sam68, Pol II and Brm (a component of the SWI/SNF chromatin-remodeling complex). This interacts with the nascent transcript, stalling Pol II (Batsche et al., 2006). Such pausing could favor inclusion of the variable exons within the mature mRNA, which would be consistent with the proposed kinetic coupling between transcription and splicing (Kornblihtt, 2006).

The balance of different kinase activities could modulate the function of splicing regulatory proteins and consequently modify splicing patterns. But there are still more questions than answers. What is the SR protein specificity, if any, of all these kinases? Is there a precise order in which each phosphorylation takes place? Which residues are involved in each case? Does each kinase affect different SR protein properties (e.g. RNA binding, protein-protein interaction, or protein localization) or functions (e.g. splicing, surveillance, or translation) (Huang and Steitz, 2005)?

Conclusion and perspectives

Cancer is a multistep process that involves severe changes in gene expression. The more we learn about regulatory pathways that are disturbed during tumorigenesis, the more we realize about the complexity of tissue homeostasis.

Mutations that alter cis-acting splicing elements can modify mRNA quality and therefore protein function. Activation of signaling pathways that can affect the activity of splicing regulatory factors or modify the balance between them can also change the proportions of mRNA splicing isoforms. Both can lead to the deregulation of crucial cellular processes such as adhesion, proliferation, differentiation, death, motility and invasion (Fig. 3), all of which contribute to cancer.

Although still in development, splice-isoform-sensitive microarrays will undoubtedly contribute towards the analysis of changes in splicing patterns associated with cancer (Li et al., 2006; Relogio et al., 2005). Establishing which genes actively participate in different steps of tumor initiation and progression, or whether deregulation of their splicing is a consequence of this, will require both high-throughput techniques and reductionist approaches in culture and animal models.

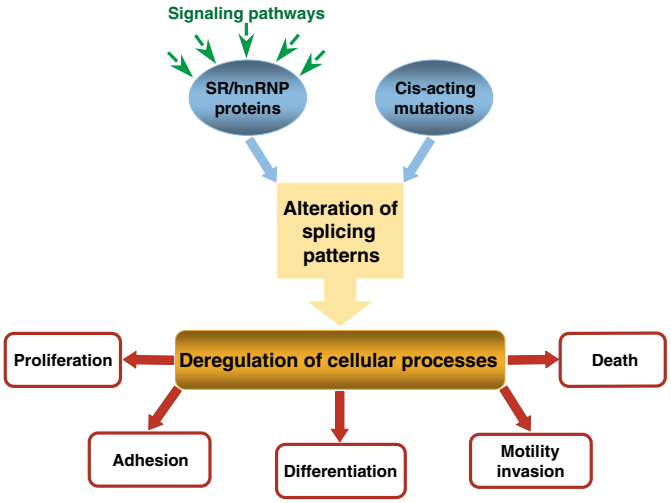

Fig. 3. Causes and consequences of splicing pattern alterations. For simplicity, only SR and hnRNP proteins are shown. However, other factors not belonging to any of these families could also be targets of these signaling pathways (e.g. Sam68).

We thank F. Pelisch and M. Blaustein for suggestions and critical reading of the manuscript, and D. Radisky for helpful discussion. We apologize to colleagues whose interesting work could not be cited owing to space limitations. This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the University of Buenos Aires, the Fundación Antorchas, the Agencia Nacional de Promoción de Ciencia y Tecnología of Argentina, and the European Union Network of Excellence on Alternative Splicing (EURASNET). A.S. and A.R.K. are investigators of the CONICET. A.R.K. is an International Research Scholar of the Howard Hughes Medical Institute.

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