Chapter 10

Regulation of Alternative Splicing by Signal Transduction Pathways

Kristen W. Lynch*

Abstract

Alternative splicing is now recognized as a ubiquitous mechanism for controlling gene expression in a tissue-specific manner. A growing body of work from the past few years has begun to also highlight the existence of networks of signal-responsive alternative splicing in a variety of cell types. While the mechanisms by which signal transduction pathways influence the splicing machinery are relatively poorly understood, a few themes have begun to emerge for how extracellular stimuli can be communicated to specific RNA-binding proteins that control splice site selection by the spliceosome. This chapter describes our current understanding of signal-induced alternative splicing with an emphasis on these emerging themes and the likely directions for future research.

Introduction

To maintain viability, most, if not all, cells within an organism must be capable of responding to a changing environment. For example, neuronal and muscle cells must respond to activation to promote behaviors and movement; cells in the liver, kidney and intestines must regulate their metabolic pathways in response to changing nutrient and hormonal environments and lymphoid cells must respond to any immune challenge to prevent or control infection. Such flexibility requires that individual cells have the ability to change function rapidly and precisely in response to a given stimulus. In general, cellular responsiveness is accomplished through the activity of signal transduction cascades that transmit signals from the cell surface to the relevant cellular machinery, often involving alterations in the protein composition of the cells.

Changes in protein expression occur through many different mechanisms and much work has focused on signal-induced regulation of transcription and translation. However, in the past few years there has been a growing recognition of the importance of signal-induced changes in alternative splicing as a mechanism for mediating biologically relevant cellular responses. Thus, the interface of the splicing and signaling fields is an emerging area of study. This chapter focuses on this interface, with a particular emphasis on the mechanisms by which signal transduction pathways affect the activity of splicing regulatory proteins. While much remains to be discovered about this process, the recent elucidation of a few pathways and growing information on several others, has begun to provide clear paradigms for how signaling pathways can impinge upon the splicing machinery and the biologic implications of such regulation. These mechanistic paradigms, described below, are grouped into categories for clarity, but it should be noted that in many cases one signal-induced perturbation will trigger another (e.g., phosphorylation and localization), so the groupings below are somewhat arbitrary and should not be viewed as "either-or", but rather as allied mechanisms that can act together to ensure a particular functional effect on alternative splicing.

*Kristen W. Lynch—Department of Biochemistry, UT Southwestern Medical Center, Dallas, Texas 75390-9038, USA. Email: kristen.lynch@utsouthwestern.edu

Alternative Splicing in the Postgenomic Era, edited by Benjamin J. Blencowe and Brenton R. Graveley.

Molecular "Hubs" Help Link the Extracellular World to the Splicing Machinery

To a first approximation, signaling cascades are thought of primarily as cytoplasmic pathways that respond directly to changing environments and stimuli at the cell surface. In contrast to this cytoplasmic activity, pre-mRNA splicing occurs in the nucleus, well separated from cell surface receptors. However, many members of the SR and hnRNP splicing regulatory protein families (refer to chapter by Lin and Fu, and Martinez-Contreras et al) shuttle between the nucleus and cytoplasm, as do other splicing factors, proteins that modify the splicing machinery and signaling proteins.[1-3] Therefore, it is not surprising that a wide variety of interactions have been described between components of the RNA processing machinery and traditional signaling molecules, many of which are discussed below. In particular, a few proteins have gained attention as potential docking "hubs" that integrate and transmit molecular information between a variety of signaling pathways and the RNA processing machinery. These molecular hubs include hnRNP K and Sam68.

HnRNP K contains three KH-type RNA binding domains and typically binds to C-rich sequences in RNA (for a review see ref. 4). Interspersed between the KH domains are proline-rich regions and sites of tyrosine phosphorylation which, respectively, bind SH3 and SH2 domains-protein-protein interaction motifs that are ubiquitous amongst signaling molecules. Not surprisingly, hnRNP K has been shown to bind to a wide variety of signaling proteins including Vav, Src-family kinases and various PKC isoforms. In addition, the Src-kinases, PKCs, Erk1/2 and JNK can all directly phosphorylate specific residues within hnRNP K and thereby regulate the various protein-protein and protein-RNA interactions involving this hnRNP protein.[4] The majority of studies related to the function of hnRNP K focus on its role in controlling mRNA stability and translation in the cytoplasm. However, this protein is present in nuclear extract preparations and has been found to interact with other hnRNPs and SR proteins that are involved in the regulation of splicing.[5,6] HnRNP K has also been shown to bind splicing enhancers and silencers within regulated pre-mRNAs.[7] While a conclusive mechanistic link has not yet been made between signaling pathways and regulated splicing via hnRNP K, it is likely that such a link exists. Indeed, phorbol esters, cytokines and hormones can all induce phosphorylation of hnRNP K and alter its ability to interact with RNAs[8,9] and at least one of the RNAs to which hnRNP K binds is *CD45*, which undergoes stimulus-regulated alternative splicing (A.A. Melton, J. Jackson and K.W.L. in preparation; see below).

Sam68 is not a classical hnRNP protein, but rather a member of the STAR (Signal-Transduction and RNA) family of proteins which contain a single RNA binding KH domain as well as multiple potential binding sites for SH2, SH3 and WW domains.[10] Sam68 was first identified as a protein that is tyrosine phosphorylated by Src during mitosis and as a protein that promotes cell cycle progression.[11-13] Further studies have demonstrated that Sam68 is also a target of serine/threonine phosphorylation, methylation and acetylation.[10] Within the cytoplasm, Sam68 is thought to function as an adaptor protein that nucleates signaling complexes proximal to several cell surface receptors. Sam68 is tyrosine phosphorylated in an inducible manner upon activation of the T-cell receptor, insulin receptor, or by stimulation of cells with leptin, leading to increased association with molecules such as PI3K, JAKs, Ras-GAP, Grb-2 and PLC-1 and the activation of downstream effector pathways.[10,14]

Sam68 also interacts with a variety of splicing factors, including several hnRNPs and other STAR proteins, as well as with proteins involved in transcription.[10,14,15] Within the nucleus, the adaptor function of Sam68 likely contributes to its ability to promote signal-induced splicing, as indicated by recent studies linking Sam68 to the signal-responsive inclusion of the *CD44* variable exon 5.[15-17] *CD44* encodes a cell surface glycoprotein that is involved in cell migration, invasion and proliferation.[18] The extracellular domain of CD44 is encoded, in part, by ten variable exons that are inducibly included upon antigen stimulation of T-cells through a pathway involving the activation of the MAP kinase pathway (Ras-Raf-MEK-Erk).[19,20] Work from several groups studying the induced inclusion of *CD44* variable exon 5 (*CD44v5*) has led to the formulation of a model in which, upon stimulation, Sam68 binds to an ESE within *CD44v5* together with the SR-related

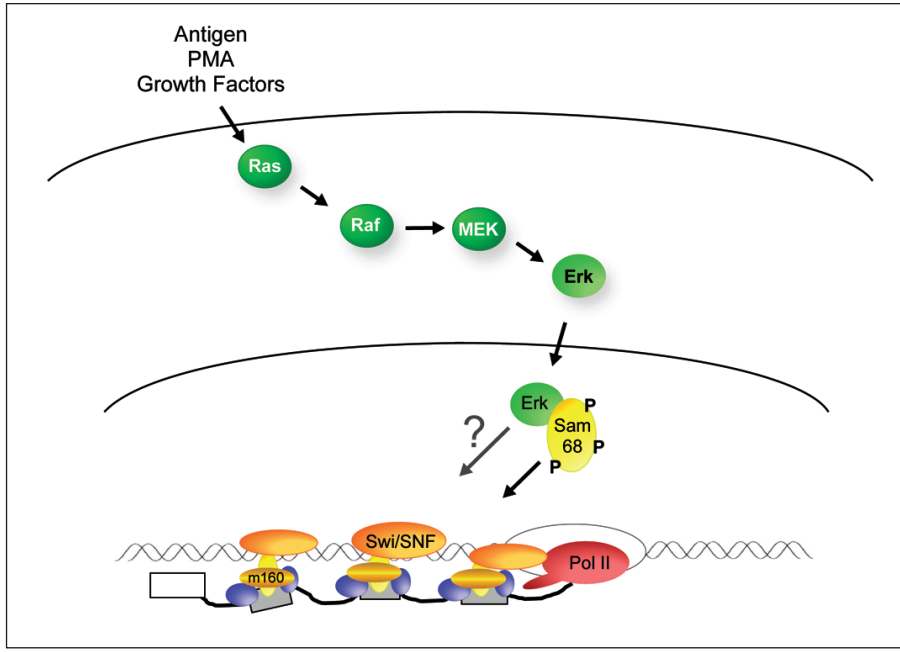

Figure 1. Model of the activity of the adaptor proteins Sam68 in the signal-induced regulation of *CD44*. Activation of the Ras-Raf-MEK-Erk pathway by various stimuli leads to phosphorylation of Sam68 by Erk. This phosphorylation of Sam68 and/or other Erk-dependent modifications (indicated by "?") leads to Sam68 binding to *CD44* variable exons (grey boxes) along with SRm160 ("m160") which promotes assembly of spliceosomal components (blue ovals) on these exons. Sam68 also interacts with the Swi/SNF chromosome remodeling complex which causes slowing of RNA polymerase II (Pol II) elongation, thus further promoting use of weak exons.

protein SRm160 (see Fig. 1).[16,17] Sam68 then also interacts with the Brm subunit of the Swi/SNF chromatin remodeling complex, thus stalling RNA polymerase II and promoting the inclusion of weak exons (see ref. 15 and discussion of transcription-coupled splicing below, and chapter by Kornblihtt). How activation of the MAP kinase pathway induces this Sam68-dependent regulation is not yet fully understood. Erk is known to phosphorylate Sam68, however mutation of the putative phosphorylation sites on Sam68 only marginally decreases its ability to enhance *CD44v5* inclusion,[16] suggesting that there must be other molecular links between Ras activation and the Sam68/SRm160/Brm complex. Finally, the Sam68-related proteins SLM-1 and SLM-2 have been shown to have activities similar to that of Sam68, both in terms of protein-protein interactions and CD44 splicing. This suggests that many or all members of the STAR family may serve as molecular links to alter splicing in response to extracellular stimuli.[10,21]

Posttranslational Modifications of Splicing Machinery

Signaling molecules can directly interact with and influence many other components of the splicing machinery. As discussed in the chapters by Lin and Fu, and Martinez-Contreras et al, the regulation of splicing is often achieved by the action of SR and hnRNP proteins. These proteins bind to sequences within and flanking alternative exons (i.e., ESEs, ESSs, ISEs, ISSs) and promote or inhibit spliceosome assembly at the nearby splice sites (refer to chapter by Chasin). It follows then that changing the activity of these splicing factors by posttranslational modifications is likely a major mechanism for altering splicing pathways.

The activity of the SR family of splicing factors is strongly influenced by the phosphorylation state of these proteins, which cycles during the splicing reaction and in response to a variety of stimuli and cell cycle conditions.[22-28] At least four different kinase families have been shown to phosphorylate SR proteins. The most specific of these is the SRPK family, which includes two closely related SR protein kinases, SRPK1 and SRPK2. These proteins bind to a unique "docking site" within SR proteins that both confers substrate specificity and restricts the catalytic activity to the N-terminal half of the RS domain, thereby resulting in a partially or hypophosphorylated protein.[29] The Clk family of dual-specificity kinases also phosphorylate members of the SR protein family, but with significantly reduced substrate specificity compared to the SRPKs.[29,30] Importantly, in contrast to the limited range of SRPK phosphorylation sites on SR proteins, the Clk family of kinases are able to phosphorylate the entire RS domain to yield a hyperphosphorylated form of SR proteins.[29,31] Thus, the SRPK and Clk families of kinases have differential effects on SR protein function (see below). Finally, both Topoisomerase I and Akt have also been shown to phosphorylate SR proteins. These enzymes phosphorylate overlapping sites that are likely to be distinct from the optimal phosphorylation sites of the SRPKs and Clks.[32-35] The activity of all of these SR kinases is presumably countered by phosphatases, with at least PP1 and PP2A having been shown to function on SR proteins and/or be required for splicing.[36-38]

HnRNP proteins, as well as other non-SR splicing factors, can also be modified by phosphorylation, methylation, SUMOylation and acetylation, although the enzymes responsible for such alterations have only been described for the first two of these modifications.[39-42] PKA, Casein Kinase II and Mnk1/2 have been shown to phosphorylate hnRNP I/PTB, hnRNP C and hnRNP A1, respectively,[43-46] while the PRMT family of methyltransferases modify many of the RGG box-containing hnRNPs.[42] While much remains to be learned with respect to the mechanisms by which these posttranslational modifications of SR, hnRNP and other splicing proteins change in response to extracellular stimuli and influence specific alternative splicing patterns, many groups have now correlated changes in the phosphorylation of SR and hnRNP proteins with the signal-induced regulation of several alternative splicing events.

A well described system in which phosphorylation of an SR protein mediates signal-induced changes in splicing is the insulin-induced inclusion of the variable βII exon within the *PKCβ* gene.[47] This induced change in the alternative splicing of *PKCβ* results in the expression of a PKCβ isozyme that is necessary for glucose uptake and is thus a critical aspect of the cellular response to insulin.[47,48] Inclusion of the βII exon is dependent on the activity of SRp40, an SR family protein, which binds to an intronic sequence downstream of the regulated exon.[34,49] Upon insulin treatment the PI-3 kinase (PI3K) pathway activates Akt which in turn phosphorylates SRp40 on a specific serine residue (Ser86) (see Fig. 2). Blocking of PI3K, Akt, SRp40, Ser86, or the binding site for SRp40 within the *PKCβ* gene all abolish the ability of insulin treatment to induce PKCβII expression.[34,49,50] However, it has yet to be determined how phosphorylation of SRp40 leads to increased exon inclusion; namely, whether phosphorylation of Ser86 increases the association of SRp40 with the *PKCβ* pre-mRNA, or rather increases the ability of SRp40 to activate exon inclusion via interactions with other splicing factors once it is bound to the pre-mRNA.

A second system in which phosphorylation of SR proteins is linked to changes in splicing is in the growth factor-induced alternative splicing of the *fibronectin* EDA exon.[51] In this case, phosphorylation of the SR proteins SF2/ASF and 9G8, again through the PI3K/Akt pathway, induces inclusion of EDA (see Fig. 2).[35] Importantly, phosphorylation of SF2/ASF or 9G8 by the Clk or SRPK family members have opposite effects on EDA splicing, thus providing evidence that the various SR kinases target different residues within SR proteins to achieve distinct functional consequences.[35] A second interesting aspect of the effect of Akt on SR proteins noted in this study is that this phosphorylation not only alters the activity of SR proteins in mediating splicing, but also influences the activity of SR proteins in translation.[35] This dual effect of Akt-dependent phosphorylation implies that alternative splicing is only one of several possible steps in RNA metabolism that can be affected by signal-induced phosphorylation of SR proteins.

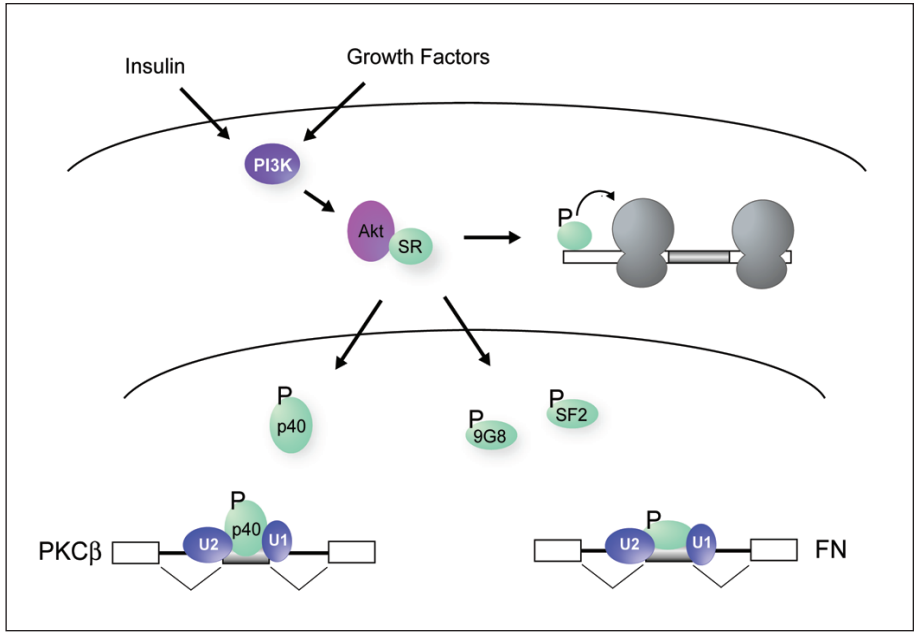

Figure 2. Model for signal-induced regulation of alternative splicing via direct phosphorylation of SR proteins. Activation of PI3K-Akt pathway by insulin or growth factors results respectively in phosphorylation of at least SRp40 or SF2/ASF and 9G8. Phosphorylation of these SR proteins is necessary for their ability to promote inclusion of weak exons in the *PKCβ* and *fibronectin* (FN) genes respectively. In addition, growth factor-dependent phosphorylation of SF2/ASF and 9G8 has been link to an increase in SR-stimulated translation of cytoplasmic mRNA. Grey shapes represent ribosomes, U1/U2 represent spliceosomal components.

Signal-Induced Changes in Localization of Splicing Factors

While posttranslational modifications may directly alter the activity of a splicing factor, the phosphorylation state of SR and hnRNP proteins can also influence their subcellular localization (see also chapters by Lin and Fu, and Martinez-Contreras et al). Since changes in the availability of splicing regulatory proteins can dramatically influence splicing patterns of specific genes, changing the localization of an SR or hnRNP protein is another potential means for achieving signal-induced alterations in splicing. Many, if not all, splicing factors localize at least to some extent in sub-nuclear foci known as "speckles". These speckles are thought to function as storage sites for proteins not actively engaged with pre-mRNA,[52,53] although speckles may be important to facilitate efficient splicing in cells.[54-56] Under normal growth conditions, splicing factors traffic between the speckles and nascent transcripts in a phosphorylation-dependent manner.[27,53] Furthermore, recent studies have shown that particular SR proteins are only recruited from the speckles to a nascent transcript when they are specifically engaged in the splicing of that transcript.[57] Interestingly, differential phosphorylation of SR proteins by overexpression of some kinases has been shown to influence their localization to speckles.[29,58,59] However, a change in speckle association is unlikely to explain all of the signal-induced changes in SR protein function since phosphorylation of SF2/ASF by Akt does not cause an apparent alteration in speckle pattern, yet can promote growth-factor induced inclusion of the *fibronectin* EDA exon as described above.[35]

A second mechanism by which phosphorylation can alter cellular localization is by altering the affinity of a protein for a nuclear transport factor. HnRNP A1, a well characterized cargo

of the nucleocytoplasmic transport protein Transportin, has a serine-rich region (referred to as the F-peptide) immediately neighboring its nuclear localization signal (NLS). Extensive studies by Caceres and colleagues have demonstrated that a signaling cascade involving the kinases p38 and MNK1/2 phosphorylates hnRNP A1 within the F-peptide in response to osmotic stress (see Fig. 3).[45,60,61] This phosphorylation prevents binding of hnRNP A1 to Transportin and results in retention of hnRNP A1 in the cytoplasm, where it ultimately localizes to stress granules.[45,60] The resulting decrease in nuclear concentration of hnRNP A1 reduces its ability to compete with the SR protein SF2/ASF in 5' splice site selection,[62] thereby leading to the predicted shift towards use of proximal 5' splice sites in *E1A* transcripts from a transfected reporter pre-mRNA.[61] Interestingly, the majority of the confirmed or predicted substrates for Transportin (aka Karyopherin β2) are RNA binding proteins and a recent determination of the structure of Transportin in complex with the NLS of hnRNP A1 demonstrates that an overall basic character in the vicinity of the NLS is an important determinant for the binding of cargo to Transportin.[63,64] This analysis of the general binding requirements of Transportin, together with studies demonstrating a phosphorylation-dependent increase in the cytoplasmic

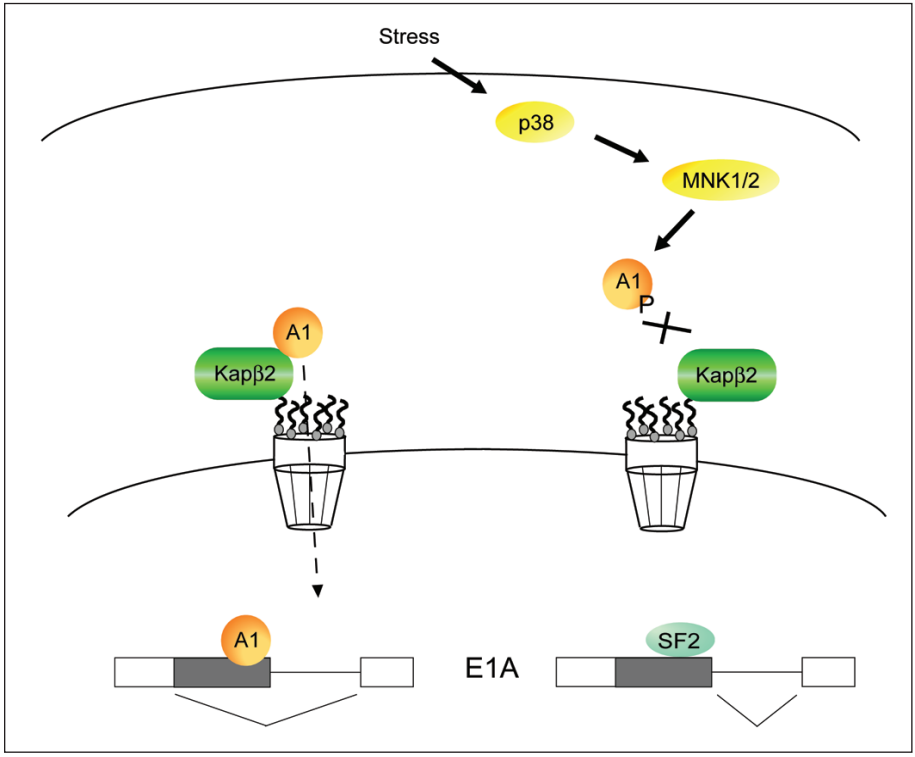

Figure 3. Model for signal-induced regulation of nuclear import. Under normal conditions Transportin (or Karyopherinβ2, Kapβ2) mediates nuclear import of hnRNP A1, which promotes use of distal 5' splice sites in the *E1A* pre-mRNA. Upon activation of the p38 stress response pathway, MNK1/2 phosphorylates hnRNP A1, thereby inhibiting binding of A1 to Transportin and preventing nuclear import of A1. Inhibition of nuclear import of A1 results in a reduction in the nuclear concentration of this proteins and allows for competing proteins, such as SF2/ASF, to preferentially bind target genes. In the case of the *E1A* pre-mRNA this results in increased utilization of proximal 5' splice sites.

localization of a few other RNA binding proteins,[46] suggests that regulation of nucleocytoplasmic transport may be a common mechanism for changing the nuclear concentration of RNA binding proteins, thus resulting in altered splicing patterns in response to extracellular cues (Fig. 3).

Other Mechanisms: Altered Protein-Protein Interactions and Protein Expression

As discussed above, both the change in nucleocytoplasmic localization of splicing proteins and the dispersion of speckles are due to a widespread disruption of protein-protein interactions via increased phosphorylation. However, posttranslational modifications can also cause specific changes in protein-protein interactions, such as those described above for the adaptor proteins hnRNP K and Sam68. While there is little direct confirmation of specific signal-induced alterations of protein-protein interactions leading to changes in splicing regulation, some recent data suggest evidence for such mechanisms. For instance, in the Sam68-dependent regulation of *CD44*, described above, signal-induced modifications to Sam68, SRm160 and Brm might influence the ability of these proteins to complex with one another. Analysis of the alternative splicing of another gene regulated in response to T-cell activation, namely *CD45*, also suggests a role of signal-regulated protein-protein interactions.

The *CD45* gene encodes a transmembrane protein tyrosine phosphatase that is involved in the regulation of signal transduction pathways in lymphocytes. In T-cells, three variable exons within *CD45* are skipped upon antigen stimulation. Recent work has shown that this signal-induced exon repression is due to the recruitment of the splicing factor PSF to an ESS within the *CD45* variable exons (Melton A, Jackson J and Lynch KW., in preparation). Interestingly, there is no difference in the nuclear concentration of PSF between resting and activated cells, nor any detectable change in the posttranslational modification of this protein. However, PSF only binds to the *CD45* ESS in response to cellular activation. PSF is known to interact with a wide spectrum of splicing factors, transcription factors and nuclear matrix proteins.[65-68] Moreover, PSF interacts with activated PKC isoforms within the cell nucleus,[69] and specific epitopes within PSF have been shown to be masked upon changing cellular conditions.[70] Therefore, a reasonable hypothesis for the signal-induced repression of *CD45* exons by PSF is that upon activation of T-cells binding partners of PSF are modified so as to either recruit PSF to the *CD45* pre-mRNA or, alternatively, to release PSF from an otherwise sequestered conformation.

Arguably, the simplest mechanism through which alternative splicing could be regulated in response to environmental cues would be through the increased or decreased expression of critical regulatory factors. Signaling pathways are known to stimulate many ubiquitous transcription factors such as NFκB, NFAT and nuclear receptors, as well as factors involved in mRNA stability/translation and proteosome-mediated degradation.[71-73] These various mechanisms typically induce broad changes in proteome expression. Not surprisingly, many SR proteins and other splicing factors have been found to be differentially expressed in a signal-dependent manner in a variety of cell types.[74-76] However, it remains to be determined whether such changes in the overall expression of splicing factors truly lead to altered splicing patterns, or whether splicing proteins are already in such excess that increased expression does not significantly alter these patterns. One example in which changes in protein expression have been shown to directly influence splicing patterns occurs during the development of erythrocytes. At a specific stage during erythropoesis there is a marked decrease in the expression of the hnRNP A/B proteins. This decrease in hnRNP A/B expression correlates temporally with the increased inclusion of exon 16 in the gene encoding the cytoskeletal protein 4.1R.[77] Since biochemical studies have shown that hnRNP A1 binds to an ESS within the *4.1R* exon 16 and causes exon skipping, the decreased expression of the hnRNP A/B proteins almost certainly is the cause of *4.1R* exon 16 inclusion in mature erythroblasts.[77]

Regulation Via Cross-Talk with Signal-Responsive Changes in Transcription

All of the paradigms for altering splicing regulation described above involve the direct manipulation of the activity or accessibility of a splicing factor. However, in the cell pre-mRNA splicing does not occur in isolation, but rather it is linked temporally, spatially and mechanistically with other mRNA production events. In particular, many recent studies have demonstrated extensive cross-talk between the transcription and splicing machineries (refer to chapter by Kornblihtt). Given the substantial effects of signaling pathways on transcription, it would not be surprising if at least some of the signal-induced regulation of transcription factors have secondary effects on alternative splicing (Fig. 4).

The primary ways in which transcription has been shown to effect splicing are summarized by two models: the "kinetic model" and the "recruitment model" (refer to chapter by Kornblihtt). The mechanism that has gained the most experimental support thus far is the kinetic model, also known colloquially as the "first come, first serve" model.[78,79] This model is based on the premise that, given the length of a typical mammalian intron, the time lag between the transcription of one exon and the transcription of the next exon is often sufficiently long that the first exon can be bound by the spliceosome before the next exon is present. This time lag potentially allows a "weak exon" (i.e., an exon with suboptimal splice sites, the absence of splicing enhancer elements and/or the presence of splicing silencer elements) to be recognized by the spliceosome without having to compete with a subsequent "strong" exon. It follows then that a reduced rate of transcriptional

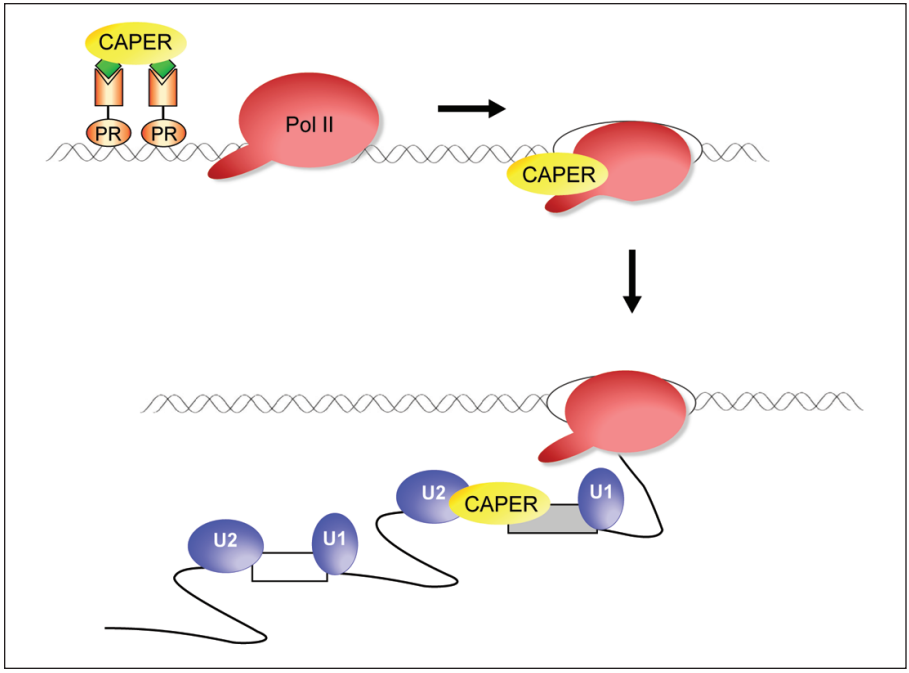

Figure 4. Model for hormone-induced alternative splicing via transcription. Binding of progesterone (green diamond) to the progesterone receptor (PR) recruits the CAPER proteins. CAPER then activates transcription by RNA polymerase II (Pol II) and promotes use of weak exons (grey box) in pre-mRNAs transcribed from the PR-dependent promoter, presumably by binding to 3' splice sites and recruiting spliceosomal components (U1/U2).

elongation favors the recognition and inclusion of weak exons, whereas an increased rate of transcription favors exon skipping.

In its simplest form, the recruitment model proposes that binding of splicing factors to the RNA polymerase II complex increases their local concentration proximal to the nascent transcript, thereby enhancing otherwise weak interactions between the splicing factors and the pre-mRNA.[80,81] However, a further complexity of the recruitment model is that transcription activators or co-activators bound at the promoters may differentially influence recruitment of splicing factors. This promoter specificity was initially suggested by studies of the *fibronectin* gene, in which the SR proteins 9G8 and SF2/ASF enhance the inclusion of the variable EDI exon by binding to an ESE, but this only occurs when the EDI containing gene is transcribed from its endogenous promoter.[82]

Sam68-dependent regulation of *CD44* splicing is one example of signal-induced regulation that relates to the kinetic model of cotranscriptional alternative splicing (see Fig. 1). In addition, the signal-induced transcription factor NFκB has been shown to increase transcription elongation,[83] suggesting that genes transcribed in an NFκB-dependent manner may also undergo signal-regulated alternative splicing through changes in transcriptional kinetics.

Signaling pathways also appear to alter splicing patterns via the recruitment model, as revealed in studies of nuclear hormone-dependent alternative splicing.[84] Work by the Berget and O'Malley groups has demonstrated that both progesterone and estrodiol can cause changes in alternative splicing profiles, but only when pre-mRNA transcription is driven by promoters that are dependent on the corresponding nuclear hormone receptors for activity.[85,86] At least in the case of progesterone-responsive alternative splicing, it was further shown that, in the presence of progesterone, the U2AF^{65}-like co-activators CAPERα and β are recruited to the progesterone receptor where they induce both transcription and alternative splicing.[87] Therefore, as predicted by the recruitment model for transcription-coupled splicing, signal-induced changes in promoter occupancy can directly recruit splicing regulatory proteins that influence splicing of the transcribed pre-mRNA.

Coordinated Regulation

The primary goal of signal transduction pathways within a cell is to evoke a specialized response to any given environmental condition. Often an optimal response requires the coordinated activity of a broad spectrum of genes and proteins. For instance, neuronal depolarization induces ion trafficking across the cell membrane as well as protein and vesicle transport, whereas a T-cell must migrate, proliferate and secrete various proteins in response to antigen stimulation as part of an effective immune response. In order to achieve such a robust and comprehensive response, signaling pathways frequently activate a program of related events rather than just the expression of one individual gene or protein. For instance, the activation of NFκB by antigen stimulation of a T-cell leads to the induction of transcription of multiple genes involved in promoting cell division and inhibiting apoptosis.[71] Similarly, regulation of multiple alternative splicing events by a given extracellular stimulus could amplify potential physiological consequences. Not surprisingly, therefore, even the few examples of signal-induced alternative splicing that have been characterized demonstrate coordinated regulation. Analysis of the *CD45* gene identified a motif within the signal-responsive ESS that is present in other exons which are differentially spliced in response to T-cell activation.[88] Similarly, two regulatory sequences (intronic and exonic) have been identified as a hallmark of exons that are alternatively spliced in response to neuronal depolarization.[89,90] The identification of these signal-responsive regulatory motifs has allowed for the bioinformatic identification of novel examples of signal-induced alternative splicing and strongly suggests that genes which contain such sequences are regulated in a coordinated manner through common mechanisms.[88-91] The recent development of microarrays designed to monitor the levels of alternatively spliced isoforms has further allowed for the systematic identification of genes that undergo alternative splicing in a signal-dependent manner.[102] Subsequent studies of these signal-regulated genes are likely to reveal

additional signal-responsive splicing regulatory sequences and allow for the grouping of genes into families of mechanistically-coordinated alternative splicing events.

Achieving Specificity in Signal-Responsiveness of Alternative Splicing

Despite the importance of coordinating regulation of splicing, one obvious question raised by our understanding of the mechanisms underlying signal-induced alternative splicing discussed above is regarding how specificity is achieved. That is, if Akt can phosphorylate several SR proteins and these proteins are ubiquitous splicing factors, why are the effects of these posttranslational modifications restricted from the splicing of other genes that are not regulated upon activation of Akt? Even in the case of coordinated regulation of a family of genes it is clear that some level of specificity still is at play in determining which splicing events are regulated by a particular cellular stimulus.

While we don't yet have a sufficiently clear understanding of regulated splicing in general and signal-responsive splicing in particular, to completely understand the question of specificity, current data does suggest that specificity may be conferred at the level of signaling pathways, RNA binding and/or differential sensitivity to the activity of individual splicing factors. Within the signaling field, specificity is largely understood to be conferred by location or co-association of proteins.[92] In other words, while a protein such as Sam68 may be capable of interacting with a wide range of proteins, under any given cellular condition Sam68 may only co-associate with a subset of potential partners and thus will only be able to transmit signals to certain downstream effectors. At the level of RNA binding, specificity of RNA-protein interactions is also often conferred by co-association of proteins within enhancer or silencer complexes.[93,94] Therefore, the signal-induced regulation of a particular gene may require the combinatorial effect of multiple transduction pathways, each altering the activity of one component of a larger complex. In such a scenario, a stimulus that only triggered one signaling pathway would not affect a more complex target gene. Alternatively, loss of one protein from a particular regulatory complex may be compensated for by other binding partners. A potentially related aspect of specificity is the recent discovery that a decrease in the expression of even core spliceosomal proteins has differential effects on the splicing of specific transcripts.[95] While some of this differential activity may be due to compensating protein-protein interactions, this phenomenon is primarily understood to be due to differences in the rate-limiting step of splicing for different transcripts. In other words, decreased activity of a splicing factor involved in 3' splice site selection will have the greatest effect on substrates which have weak or variable 3' splice sites.[96] Together, the specificity inherent in signaling and splicing mechanisms likely work in concert to achieve the necessary balance between strength and precision of signal-induced changes in alternative splicing.

Feed-Back and Feed-Forward

Interestingly, many of the genes that have been shown to undergo changes in splicing pattern in response to extracellular stimuli are themselves receptors or other signaling molecules. These include, among others, *CD45, CD44, NMDAR1* and *PKCIIβ*.[47,90,97] Importantly, the differential proteins expressed by all of the above-mentioned genes have been shown to have distinct signaling properties, often affecting the very signaling pathway that leads to their differential splicing pattern.[48,98-100] This strongly suggests that there is a possibility of feedback or feed-forward in which the initial activation of a signaling pathway is either promoted/maintained or turned off via signal-induced alternative splicing.

One example of such feedback is in *CD44* alternative splicing.[99] As mentioned above, activation of the Ras signaling pathway enhances inclusion of ten variable exons that encode part of the extracellular domain of CD44. Specifically, inclusion of variable exon 6 (*CD44v6*) promotes CD44 involvement in a coreceptor complex with hepatocyte growth factor and the tyrosine kinase Met that in turn promotes Ras signaling.[101] Activation of quiescent cells with growth factors leads to an initial burst of MAP kinase activation followed several hours later by a second, prolonged wave of MAP kinase activity. The inclusion of *CD44v6* that occurs in response to the initial burst of Ras

activation is necessary for the subsequent wave of Ras signaling, as demonstrated by the loss of the second pulse of Ras activity when *CD44v6*-containing transcripts are specifically repressed.[99] Therefore, at least with regards to Ras signaling, alternative splicing is an important feedback mechanism to generate the sustained activation phenotype necessary to drive cells forward to proliferation.

Summary

While signal-induced alternative splicing is no doubt a prevalent phenomenon,[90,91,102] we are only just beginning to scratch the surface in terms of identifying such regulated events and in understanding the mechanisms by which they occur. The examples provided in this chapter are in no way meant to be an exhaustive list, but rather are presented as examples of how extracellular stimuli might influence the splicing machinery so as to alter splicing patterns. At present there is still only cursory data in support of many of the proposed mechanisms; however, what is clear is that there are likely numerous pathways by which changes in growth conditions or in the environment can be communicated to the splicing machinery and many critical physiological processes are influenced by signal-induced changes that impact on splicing. Clearly the questions surrounding signal-induced alternative splicing represent an important frontier that warrants major investigation.

References

1. Pinol-Roma S, Dreyfuss G. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 1992; 355(6362):730-732.
2. Caceres JF, Screaton GR, Krainer AR. A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. Genes Dev 1998; 12(1):55-66.
3. Khokhlatchev AV, Canagarajah B, Wilsbacher J et al. Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell 1998; 93(4):605-615.
4. Bomsztyk K, Denisenko O, Ostrowski J. hnRNP K: one protein multiple processes. Bioessays 2004; 26(6):629-638.
5. Kim JH, Hahm B, Kim YK et al. Protein-protein interaction among hnRNPs shuttling between nucleus and cytoplasm. J Mol Biol 2000; 298(3):395-405.
6. Shnyreva M, Schullery DS, Suzuki H et al. Interaction of two multifunctional proteins. Heterogeneous nuclear ribonucleoprotein K and Y-box-binding protein. J Biol Chem 2000; 275(20):15498-15503.
7. Expert-Bezancon A, Le Caer JP, Marie J. Heterogeneous nuclear ribonucleoprotein (hnRNP) K is a component of an intronic splicing enhancer complex that activates the splicing of the alternative exon 6A from chicken beta-tropomyosin pre-mRNA. J Biol Chem 2002; 277(19):16614-16623.
8. Ostrowski J, Kawata Y, Schullery DS et al. Insulin alters heterogeneous nuclear ribonucleoprotein K protein binding to DNA and RNA. Proc Natl Acad Sci USA 2001; 98(16):9044-9049.
9. Ostrowski J, Schullery DS, Denisenko ON et al. Role of tyrosine phosphorylation in the regulation of the interaction of heterogenous nuclear ribonucleoprotein K protein with its protein and RNA partners. J Biol Chem 2000; 275(5):3619-3628.
10. Lukong KE, Richard S. Sam68, the KH domain-containing superSTAR. Biochim Biophys Acta 2003; 1653(2):73-86.
11. Fumagalli S, Totty NF, Hsuan JJ et al. A target for Src in mitosis. Nature 1994; 368(6474):871-874.
12. Taylor SJ, Shalloway D. An RNA-binding protein associated with Src through its SH2 and SH3 domains in mitosis. Nature 1994; 368(6474):867-871.
13. Taylor SJ, Resnick RJ, Shalloway D. Sam68 exerts separable effects on cell cycle progression and apoptosis. BMC Cell Biol 2004; 5:5.
14. Najib S, Martin-Romero C, Gonzalez-Yanes C et al. Role of Sam68 as an adaptor protein in signal transduction. Cell Mol Life Sci 2005; 62(1):36-43.
15. Batsche E, Yaniv M, Muchardt C. The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat Struct Mol Biol 2006; 13(1):22-29.
16. Matter N, Herrlich P, Konig H. Signal-dependent regulation of splicing via phosphorylation of Sam68. Nature 2002; 420(6916):691-695.
17. Cheng C, Sharp PA. Regulation of CD44 Alternative Splicing by SRm160 and Its Potential Role in Tumor Cell Invasion. Mol Cell Biol 2006; 26(1):362-370.
18. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 2003; 4(1):33-45.
19. Konig H, Ponta H, Herrlich P. Coupling of signal transduction to alternative pre-mRNA splicing by a composite splice regulator. EMBO J 1998; 17:2904-2913.

20. Weg-Remers S, Ponta H, Herrlich P et al. Regulation of alternative pre-mRNA splicing by the ERK MAP-kinase pathway. EMBO J 2001; 20(15):4194-4203.
21. Stoss O, Olbrich M, Hartmann AM et al. The STAR/GSG family protein rSLM-2 regulates the selection of alternative splice sites. J Biol Chem 2001; 276(12):8665-8673.
22. Xiao SH, Manley JL. Phosphorylation of the ASF/SF2 RS domain affects both protein-protein and protein-RNA interactions and is necessary for splicing. Genes Dev 1997; 11(3):334-344.
23. Xiao SH, Manley JL. Phosphorylation-dephosphorylation differentially affects activities of splicing factor ASF/SF2. EMBO J 1998; 17(21):6359-6367.
24. Sanford JR, Ellis JD, Cazalla D et al. Reversible phosphorylation differentially affects nuclear and cytoplasmic functions of splicing factor 2/alternative splicing factor. Proc Natl Acad Sci USA 2005; 102(42):15042-15047.
25. Graveley B. Sorting out the complexity of SR protein functions. RNA 2000; 6:1197-1211.
26. Cao W, Jamison SF, Garcia-Blanco MA. Both phosphorylation and dephosphorylation of ASF/SF2 are required for pre-mRNA splicing in vitro. RNA 1997; 3(12):1456-1467.
27. Misteli T, Caceres JF, Clement JQ et al. Serine phosphorylation of SR proteins is required for their recruitment to sites of transcription in vivo. J Cell Biol 1998; 143(2):297-307.
28. Huang Y, Yario TA, Steitz JA. A molecular link between SR protein dephosphorylation and mRNA export. Proc Natl Acad Sci USA 2004; 101(26):9666-9670.
29. Ngo JC, Chakrabarti S, Ding JH et al. Interplay between SRPK and Clk/Sty kinases in phosphorylation of the splicing factor ASF/SF2 is regulated by a docking motif in ASF/SF2. Mol Cell 2005; 20(1):77-89.
30. Colwill K, Feng LL, Yeakley JM et al. SRPK1 and Clk/Sty protein kinases show distinct substrate specificities for serine/arginine-rich splicing factors. J Biol Chem 1996; 271(40):24569-24575.
31. Colwill K, Pawson T, Andrews B et al. The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intracellular distribution. EMBO J 1995; 15:265-275.
32. Labourier E, Rossi F, Gallouzi IE et al. Interaction between the N-terminal domain of human DNA topoisomerase I and the arginine-serine domain of its substrate determines phosphorylation of SF2/ASF splicing factor. Nucleic Acids Res 1998; 26(12):2955-2962.
33. Rossi F, Labourier E, Forne T et al. Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. Nature 1996; 381(6577):80-82.
34. Patel NA, Kaneko S, Apostolatos HS et al. Molecular and genetic studies imply Akt-mediated signaling promotes protein kinase CbetaII alternative splicing via phosphorylation of serine/arginine-rich splicing factor SRp40. J Biol Chem 2005; 280(14):14302-14309.
35. Blaustein M, Pelisch F, Tanos T et al. Concerted regulation of nuclear and cytoplasmic activities of SR proteins by AKT. Nat Struct Mol Biol 2005; 12(12):1037-1044.
36. Misteli T, Spector DL. Serine/threonine phosphatase 1 modulates the subnuclear distribution of pre-mRNA splicing factors. Mol Biol Cell 1996; 7(10):1559-1572.
37. Mermoud JE, Cohen P, Lamond AI. Ser/Thr-specific protein phosphatases are required for both catalytic steps of pre-mRNA splicing. Nucleic Acids Res 1992; 20(20):5263-5269.
38. Shi Y, Reddy B, Manley JL. PP1/PP2A phosphatases are required for the second step of Pre-mRNA splicing and target specific snRNP proteins. Mol Cell 2006; 23(6):819-829.
39. Krecic AM, Swanson MS. hnRNP complexes: composition, structure and function. Curr Opin Cell Biol Jun 1999; 11(3):363-371.
40. Vassileva MT, Matunis MJ. SUMO modification of heterogeneous nuclear ribonucleoproteins. Mol Cell Biol 2004; 24(9):3623-3632.
41. Kim SC, Sprung R, Chen Y et al. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell 2006; 23(4):607-618.
42. Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. Mol Cell 2005; 18(3):263-272.
43. Magistrelli G, Jeannin P, Herbault N et al. A soluble form of CTLA-4 generated by alternative splicing is expressed by nonstimulated human T-cells. Eur J Immunol 1999; 29(11):3596-3602.
44. Mayrand SH, Dwen P, Pederson T. Serine/threonine phosphorylation regulates binding of C hnRNP proteins to pre-mRNA. Proc Natl Acad Sci USA 1993; 90(16):7764-7768.
45. Guil S, Long JC, Caceres JF. hnRNP A1 relocalization to the stress granules reflects a role in the stress response. Mol Cell Biol 2006; 26(15):5744-5758.
46. Xie J, Lee JA, Kress TL et al. Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. Proc Natl Acad Sci USA 2003; 100(15):8776-8781.
47. Chalfant CE, Mischak H, Watson JE et al. Regulation of alternative splicing of protein kinase C beta by insulin. J Biol Chem 1995; 270(22):13326-13332.
48. Cooper DR, Watson JE, Patel N et al. Ectopic expression of protein kinase CbetaII, -delta and -epsilon, but not -betaI or -zeta, provide for insulin stimulation of glucose uptake in NIH-3T3 cells. Arch Biochem Biophys 1999; 372(1):69-79.

49. Patel NA, Chalfant CE, Watson JE et al. Insulin regulates alternative splicing of protein kinase C beta II through a phosphatidylinositol 3-kinase-dependent pathway involving the nuclear serine/arginine-rich splicing factor, SRp40, in skeletal muscle cells. J Biol Chem 2001; 276(25):22648-22654.
50. Patel NA, Apostolatos HS, Mebert K et al. Insulin regulates protein kinase CbetaII alternative splicing in multiple target tissues: development of a hormonally responsive heterologous minigene. Mol Endocrinol 2004; 18(4):899-911.
51. Blaustein M, Pelisch F, Coso OA et al. Mammary epithelial-mesenchymal interaction regulates fibronectin alternative splicing via phosphatidylinositol 3-kinase. J Biol Chem 2004; 279(20):21029-21037.
52. Lamond AI, Spector DL. Nuclear speckles: a model for nuclear organelles. Nat Rev Mol Cell Biol 2003; 4(8):605-612.
53. Misteli T, Caceres JF, Spector DL. The dynamics of a pre-mRNA splicing factor in living cells. Nature 1997; 387(6632):523-527.
54. Moen Jr PT, Johnson CV, Byron M et al. Repositioning of muscle-specific genes relative to the periphery of SC-35 domains during skeletal myogenesis. Mol Biol Cell 2004; 15(1):197-206.
55. Shopland LS, Johnson CV, Byron M et al. Clustering of multiple specific genes and gene-rich R-bands around SC-35 domains: evidence for local euchromatic neighborhoods. J Cell Biol 2003; 162(6):981-990.
56. Shopland LS, Johnson CV, Lawrence JB. Evidence that all SC-35 domains contain mRNAs and that transcripts can be structurally constrained within these domains. J Struct Biol 2002; 140(1-3):131-139.
57. Mabon SA, Misteli T. Differential recruitment of pre-mRNA splicing factors to alternatively spliced transcripts in vivo. PLoS Biol 2005; 3(11):374e.
58. Ding JH, Zhong XY, Hagopian JC et al. Regulated cellular partitioning of SR protein-specific kinases in mammalian cells. Mol Biol Cell 2006; 17(2):876-885.
59. Gui JF, Lane WS, Fu X-D. A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature 1994; 369:678-682.
60. Allemand E, Guil S, Myers M et al. Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. Proc Natl Acad Sci USA 2005; 102(10):3605-3610.
61. van der Houven van Oordt W, Diaz-Meco MT, Lozano J et al. The MKK(3/6)-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. J Cell Biol 2000; 149(2):307-316.
62. Eperon IC, Makarova OV, Mayeda A et al. Selection of alternative 5' splice sites: role of U1 snRNP and models for the antagonistic effects of SF2/ASF and hnRNP A1. Mol Cell Biol 2000; 20(22):8303-8318.
63. Lee BJ, Cansizoglu AE, Suel KE et al. Rules for nuclear localization sequence recognition by karyopherin beta 2. Cell 2006; 126(3):543-558.
64. Chook YM, Blobel G. Karyopherins and nuclear import. Curr Opin Struct Biol 2001; 11(6):703-715.
65. Meissner M, Dechat T, Gerner C et al. Differential nuclear localization and nuclear matrix association of the splicing factors PSF and PTB. J Cell Biochem 2000; 76(4):559-566.
66. Rosonina E, Ip JY, Calarco JA et al. Role for PSF in mediating transcriptional activator-dependent stimulation of pre-mRNA processing in vivo. Mol Cell Biol 2005; 25(15):6734-6746.
67. Emili A, Shales M, McCracken S et al. Splicing and transcription-associated proteins PSF and p54nrb/nonO bind to the RNA polymerase II CTD. RNA 2002; 8(9):1102-1111.
68. Shav-Tal Y, Zipori D. PSF and p54(nrb)/NonO—multi-functional nuclear proteins. FEBS Lett 2002; 531(2):109-114.
69. Rosenberger U, Lehmann I, Weise C et al. Identification of PSF as a protein kinase Calpha-binding protein in the cell nucleus. J Cell Biochem 2002; 86(2):394-402.
70. Shav-Tal Y, Cohen M, Lapter S et al. Nuclear relocalization of the pre-mRNA splicing factor PSF during apoptosis involves hyperphosphorylation, masking of antigenic epitopes and changes in protein interactions. Mol Biol Cell 2001; 12(8):2328-2340.
71. Gerondakis S, Grumont R, Rourke I et al. The regulation and roles of Rel/NF-kappa B transcription factors during lymphocyte activation. Curr Opin Immunol 1998; 10(3):353-359.
72. Guhaniyogi J, Brewer G. Regulation of mRNA stability in mammalian cells. Gene 2001; 265(1-2):11-23.
73. Metivier R, Reid G, Gannon F. Transcription in four dimensions: nuclear receptor-directed initiation of gene expression. EMBO Rep 2006; 7(2):161-167.
74. Schimmer BP, Cordova M, Cheng H et al. Global profiles of gene expression induced by adrenocorticotropin in Y1 mouse adrenal cells. Endocrinology 2006; 147(5):2357-2367.
75. Uematsu F, Takahashi M, Yoshida M et al. Distinct patterns of gene expression in hepatocellular carcinomas and adjacent noncancerous, cirrhotic liver tissues in rats fed a choline-deficient, L-amino acid-defined diet. Cancer Sci 2005; 96(7):414-424.
76. Screaton GR, Caceres JF, Mayeda A et al. Identification and characterization of three members of the human SR family of pre-mRNA splicing factors. EMBO J 1995; 14:4336-4349.

77. Hou VC, Lersch R, Gee SL et al. Decrease in hnRNP A/B expression during erythropoiesis mediates a pre-mRNA splicing switch. EMBO J 2002; 21(22):6195-6204.
78. Kornblihtt AR. Chromatin, transcript elongation and alternative splicing. Nat Struct Mol Biol 2006; 13(1):5-7.
79. Kornblihtt AR, de la Mata M, Fededa JP et al. Multiple links between transcription and splicing. RNA 2004; 10(10):1489-1498.
80. Bentley DL. Rules of engagement: cotranscriptional recruitment of pre-mRNA processing factors. Curr Opin Cell Biol 2005; 17(3):251-256.
81. Maniatis T, Reed R. An extensive network of coupling among gene expression machines. Nature 2002; 416(6880):499-506.
82. Cramer P, Caceres JF, Cazalla D et al. Coupling of transcription with alternative splicing: RNA pol II promoters modulate SF2/ASF and 9G8 effects on an exonic splicing enhancer. Mol Cell 1999; 4(2):251-258.
83. West MJ, Lowe AD, Karn J. Activation of human immunodeficiency virus transcription in T-cells revisited: NF-kappaB p65 stimulates transcriptional elongation. J Virol 2001; 75(18):8524-8537.
84. Lonard DM, O'Malley BW. Expanding functional diversity of the coactivators. Trends Biochem Sci 2005; 30(3):126-132.
85. Auboeuf D, Honig A, Berget SM et al. Coordinate regulation of transcription and splicing by steroid receptor coregulators. Science 2002; 298(5592):416-419.
86. Auboeuf D, Dowhan DH, Kang YK et al. Differential recruitment of nuclear receptor coactivators may determine alternative RNA splice site choice in target genes. Proc Natl Acad Sci USA 2004; 101(8):2270-2274.
87. Dowhan DH, Hong EP, Auboeuf D et al. Steroid hormone receptor coactivation and alternative RNA splicing by U2AF65-related proteins CAPERalpha and CAPERbeta. Mol Cell 2005; 17(3):429-439.
88. Rothrock C, Cannon B, Hahm B et al. A conserved signal-responsive sequence mediates activation-induced alternative splicing of CD45. Mol Cell 2003; 12(5):1317-1324.
89. Xie J, Black DL. A CaMK IV responsive RNA element mediates depolarization-induced alternative splicing of ion channels. Nature 2001; 410(6831):936-939.
90. Lee JA, Xing Y, Nguyen D et al. Depolarization and CaM Kinase IV modulate NMDA receptor splicing through two essential RNA elements. PLoS Biol 2007; 5(2):e40.
91. An P, Grabowski PJ. Exon silencing by UAGG motifs in response to neuronal excitation. PLoS Biol 2007; 5(2):e36.
92. Ptashne M, Gann A. Signal transduction. Imposing specificity on kinases. Science 2003; 299(5609):1025-1027.
93. Lynch KW, Maniatis T. Assembly of specific SR protein complexes on distinct regulatory elements of the Drosophila doublesex splicing enhancer. Genes Dev 1996; 10:2089-2101.
94. Markovtsov V, Nikolic JM, Goldman JA et al. Cooperative assembly of an hnRNP complex induced by a tissue-specific homolog of polypyrimidine tract binding protein. Mol Cell Biol 2000; 20(20):7463-7479.
95. Park JW, Parisky K, Celotto AM et al. Identification of alternative splicing regulators by RNA interference in Drosophila. Proc Natl Acad Sci USA 2004; 101(45):15974-15979.
96. Konarska MM, Query CC. Insights into the mechanisms of splicing: more lessons from the ribosome. Genes Dev 2005; 19(19):2255-2260.
97. Lynch KW. Consequences of regulated pre-mRNA splicing in the immune system. Nat Rev Immunol 2004; 4(12):931-940.
98. Hermiston ML, Xu Z, Majeti R et al. Reciprocal regulation of lymphocyte activation by tyrosine kinases and phosphatases. J Clin Invest 2002; 109(1):9-14.
99. Cheng C, Yaffe MB, Sharp PA. A positive feedback loop couples Ras activation and CD44 alternative splicing. Genes Dev 2006; 20(13):1715-1720.
100. Carroll RC, Zukin RS. NMDA-receptor trafficking and targeting: implications for synaptic transmission and plasticity. Trends Neurosci 2002; 25(11):571-577.
101. Orian-Rousseau V, Chen L, Sleeman JP et al. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev 2002; 16(23):3074-3086.
102. Ip JY, Tong A, Pan Q et al. Global analysis of alternative splicing during T-cell activation. RNA 2007; 13:563-572.