REVIEW

Nuclear post-transcriptional control of gene expression

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ABSTRACT

The mammalian nucleus has considerable control over nascent transcripts. The basic mechanisms of post-transcriptional processing are well understood and recently some of the principles underlying the regulation of nuclear processing events have been elucidated. Here we review the recent progress in identification of signalling pathways that modulate the action of key RNA-binding proteins which regulate splicing, and the mechanisms of action of the C-terminal domain of RNA polymerase II that co-ordinate transcription with nuclear mRNA processing events.

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INTRODUCTION

The human genome contains about 40 000 genes (International Human Genome Sequencing Consortium 2001, Venter *et al*. 2001). This is a relatively small number of genes considering the complexity of humans: for example, *Saccharomyces cerevisiae* and *Caenorhabditis elegan*s genomes have 6000 (Goffeau *et al*. 1996) and 19 000 (*C. elegans* Sequencing Consortium 1998) genes respectively. The substantially increased human biological repertoire is provided by interactions between genes (epistasis), regulation of transcriptional programmes and control of post-transcriptional processing. This review will consider post-transcriptional events in the nucleus. Once a pre-messenger RNA (pre-mRNA) has been produced by transcription, substantial changes must occur before the mRNA is ready for export from the nucleus to the translation machinery. In addition, the kinetics of post-transcriptional processing must be co-ordinated with transcription in order to ensure efficient gene expression. Furthermore, variation in post-transcriptional processing provides substantial mRNA and protein diversity, with multiple isoforms generated from single genes. For example, the single human insulin-like growth factor-I (IGF-I) gene has three alternatively spliced isoforms (Jansen *et al*. 1983, Rotwein 1986, Chew *et al*. 1995), and since there are two promoters each expressing alternative signal peptides (Tobin *et al*. 1990), six peptide variants are made. Of the post-transcriptional events, splicing and polyadenylation are the major processes generating diversity, with alternative splicing being quantitatively more important than alternative polyadenylation (Claverie 2001). Regulation of mRNA stability and export contributes to the expression levels of a gene (Staton *et al*. 2000), but these processes have less influence on mRNA and protein heterogeneity. RNA editing is another nuclear process that generates protein diversity and this has been comprehensively reviewed elsewhere (Smith *et al*. 1997*a*, Holland *et al*. 1999). This review focuses on the regulation of pre-mRNA splicing and polyadenylation.

SPLICING

As organisms become more complex, the proportion of genes containing introns rises. Although fewer than 5% of *S. cerevisiae* genes contain introns, about 26% of expressed transcripts are derived from these genes (Ares *et al*. 1999). Thus, the presence of splicing confers an expression advantage. In humans, most genes contain introns. Furthermore, up to 60% of genes are alternatively spliced (International Human Genome Sequencing Consortium 2001, Kan *et al*. 2001). This generates substantial protein diversity (Black 2000).

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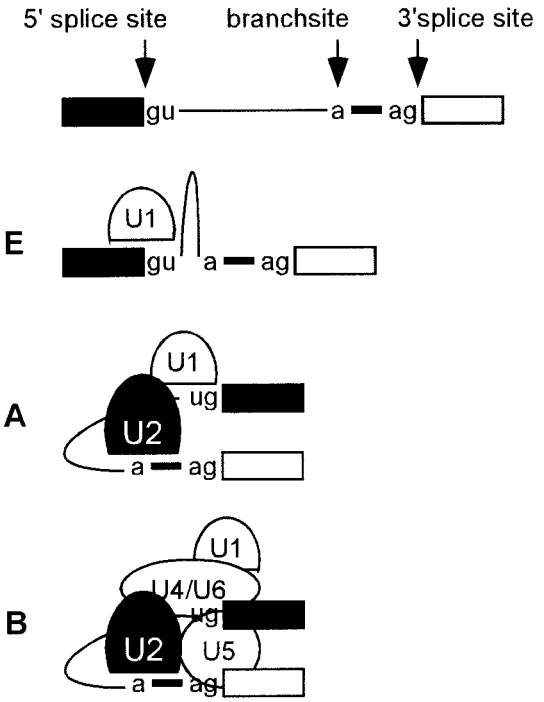

FIGURE 1. Basic mechanisms of splice site selection and spliceosome assembly. The 5′ and 3′ splice sites and the branchsite are indicted by arrows and the exons are shown as boxes. The intron is shown as a line. The bold line in the intron, between the branchsite and the 3′ splice site, represents the polypyrimidine tract. The stepwise assembly of the spliceosome complexes, early (E), and subsequent (A and B), is shown. Note that only the U1, U2 and U4/U6.U5 snRNPs are depicted and the many non-snRNP proteins are omitted for clarity. g, guanine; u, uracil; a, adenine.

The basic mechanisms of intron removal are well understood (Staley & Guthrie 1998, Reed & Oalandjian 2000). The intron is cleaved from the exons at the 5′ and 3′ ends, called the splice sites. The 5′ splice site consists of a short intronic sequence that loosely fits a consensus of GURAGU (G, guanine; U, uracil; A, adenine; and R, purine). The 3′ splice site consists of three elements: a branchsite (consensus YNYURAY, where Y is a pyrimidine); a stretch of pyrimidines (the polypyrimidine tract); and, finally the sequence CAG or UAG. These sequence elements must be recognised by the spliceosome, a multi-unit complex of proteins and RNA. The RNA components are small nuclear RNAs, U1, U2, U4, U5 and U6, assembled into ribonucleoprotein particles (snRNPs). Initially, the 5′ splice site of major introns is bound by U1 snRNP via Watson–Crick base-pairing (Fig. 1). A stepwise assembly of the spliceosome then occurs around the splice sites. The polypyrimidine tract of the 3′ splice site is bound by U2 snRNP and the U4/U6.U5 snRNPs are recruited. Correct selection of the splice sites is vital for gene expression, and regulating the use of different possible splice sites is fundamental to alternative splicing. Although necessary for splicing, the interaction between the U1 snRNP and the 5′ splice site is insufficient to account for the fidelity and flexibility of splicing because many sequences that match the 5′ splice site consensus are present in introns and exons and are bound by U1 snRNP, but are never used (Eperon *et al*. 1993, Sun & Chasin 2000). The main method of selecting the correct splice sites involves the co-ordinate recognition of nearby 3′ and 5′ splice sites, usually across an exon (called exon definition). The problem arises in explaining why other splice site-like sequences are not used for splicing even if they bind U1 and lie close to the sites that are used. This problem is usually explained by the surrounding sequence context (i.e. regulatory elements), which modulates the recruitment of the spliceosome.

Major advances in understanding regulated alternative splicing have come with the identification of some sequence elements involved in promoting exon selection (enhancers) or repressing splicing (silencers). The factors functioning through several regulatory sequences have been isolated. Many important non-snRNP proteins are involved in spliceosome assembly and function. A number of these also regulate splice site selection, in particular, a family of splicing factors characterised by RNA-recognition motifs and domains containing serine and arginine (SR) repeats, the SR proteins (Graveley 2000). SR proteins are required for general or constitutive splicing and are crucial mediators of regulated alternative splicing. Thus, members of the SR protein family bind and function at vertebrate exonic enhancers (Lavigueur *et al*. 1993, Sun *et al*. 1993, Ramchatesingh *et al*. 1995, Gontarek & Derse 1996, Du *et al*. 1997, Selvakumar & Helfman 1999). Consensus exonic sequence motifs for several SR proteins have been derived experimentally (Liu *et al*. 1998, 2000, Schaal & Maniatis 1999) and are useful in predicting function (Liu *et al*. 2001).

Hormonal activation of signalling pathways can lead to modulation of the action of splicing factors and subsequent alteration in splice site choice. Several endocrine genes are subject to hormonally regulated alternative splicing (Chew 1997, Webster & Huang 1999) (Table 1), although these have only been partially characterised. A well-studied model is the splicing of three alternative exons of the fibronectin pre-mRNA (Magnuson *et al*. 1991, Inoue *et al*. 1999), where regulation reflects a

TABLE 1. Catalogue of hormonally regulated alternative splicing events

	Stimulus	Reference
Alternatively spliced mRNA		
Insulin receptor	Dexamethasone	Kosaki & Webster (1993), Norgren *et al*. (1993, 1994*a*)
	Glucose	Norgren *et al*. (1994*a*), Huang *et al*. (1996)
	Insulin	Huang *et al*. (1994, 1996), Norgren *et al*. (1994*b*), Sell *et al*. (1994), Wiersma *et al*. (1997)
Cal/CGRP	Dexamethasone	Cote & Gagel (1986)
PKC beta	Insulin	Chalfant *et al*. (1995)
IGF-I	Growth hormone	Chew *et al*. (1995), Lin *et al*. (1998), Lowe *et al*. (1988)
FGF-R	Cytokines	Zhao *et al*. (1994)
TNFα	2-Aminopurine	Jarrous *et al*. (1996)
PTP1B	PDGF, EGF, bFGF	Shifrin & Neel (1993)
TNFβ, β-globin	src	Neel *et al*. (1995)
Hac1	UPR	Cox & Walter (1996)
hPMCA2	Calcium	Zacharias & Strehler (1996)
CD44	Phytohaemagglutinin	Screaton *et al*. (1995)
	TPA, PDGF, IGF-I	Fichter *et al*. (1997)
	Via hnRNP A1	Matter *et al*. (2000)
CD45	Phytohaemagglutinin	Screaton *et al*. (1995)
	Concanavalin A	Konig *et al*. (1998)
	PKC and ras	Lynch & Weiss (2000)
Fibronectin EIIIB (rat)	Insulin, via HRS	Du *et al*. (1997)
Fibronectin ED (human)	TGFβ1, vitD, RA	Magnuson *et al*. (1991), Inoue *et al*. (1999)
Kv3.1 channel	bFGF/depolarisation	Liu & Kaczmarek (1998)
Agrin	NGF	Smith *et al*. (1997*b*)
MHC-B	NGF	Itoh & Adelstein (1995)
SRp20	Serum/cell cycle	Jumaa *et al*. (1997)
BK channel	Hypophysectomy/ACTH	Xie & McCobb (1998)
NR1	Alcohol	Hardy *et al*. (1999), Winkler *et al*. (1999)
TRbeta	T3	Williams (2000)

bFGF, basic fibroblast growth factor; Cal/CGRP, calcitonin/calcitonin gene-related peptide; EGF, epidermal growth factor; FGF-R, fibroblast growth factor receptor; hPMCA2, human plasma membrane Ca-ATPase; Kv3.1, potassium voltage-gated channel; MHC-B, myosin heavy chain II-B; NGF, nerve growth factor; NR1, N-methyl-D-aspartate receptor subunit 1; PDGF, platelet-derived growth factor; PTP1B, phosphotyrosine-1B; TGF, tumour growth factor; TNF, tumour necrosis factor; TPA, 12-tetradecanoate 13-acetate; TRbeta, thyroid hormone receptor-β; UPR, unfolded protein response.

balance of splicing factors binding to several enhancers and silencers (Lavigueur *et al*. 1993, Caputi *et al*. 1994, Huh & Hynes 1994, Staffa *et al*. 1997). A hormonal stimulus, insulin, changes splicing in rat fibronectin exon EIIIB and is associated with increased levels of the rat SR protein, SRp40 (Du *et al*. 1997). This is not an isolated example, and there are several alternative splicing systems where SR proteins regulate exon selection. Alternative splicing in the mouse SRp20 pre-mRNA changes in response to serum stimulation or withdrawal. This change in splicing involves the SR proteins SF2/ASF (splicing factor-2/alternative splicing factor) and SRp20 (Jumaa & Nielsen 1997, Jumaa *et al*. 1997). In a different system, alternative splicing of CD44 and CD45 pre-mRNAs occurs in response to cytokine-induced T-cell differentiation (Screaton *et al*. 1995), via protein kinase C (PKC) and Ras pathways (Konig *et al*. 1998, Lynch & Weiss 2000). SF2/ASF and other SR proteins alter CD44 and CD45 splicing (Lemaire *et al*. 1999, ten Dam *et al*. 2000, Wang *et al*. 2001). Our recent data indicate the presence of an exonic splicing enhancer in a regulated exon of the human IGF-I gene and efficient splicing to this exon requires both the enhancer and the SR protein SF2/ASF (P J Smith & S L Chew, unpublished observations). These data are consistent with the well-documented splicing of growth hormone pre-mRNA. The removal of the last intron in this transcript is dependent on an exonic enhancer element and the SR protein, SF2/ASF (Sun *et al*. 1993). More recently, several SF2/ASF functional sites have been mapped in the downstream exon (Dirksen *et al*. 2000) and the actions of SF2/ASF at the enhancer is synergistic with another SR protein family member, 9G8, when the concentrations of SF2/ASF are low (Li *et al*. 2000). Thus, local concentrations and ratios of SR proteins are important in determining enhancement of splicing in alternative splicing systems.

Although more enhancers have been studied and characterised experimentally, recent evidence shows

that the predominant mechanism of splicing may involve silencers (Fairbrother & Chasin 2001). This may be to ensure that multiple illegitimate splice sites and false exons contained within large introns are repressed and do not disrupt the reading frame. However, predicting silencer function is not possible at present, and the few silencers characterised have a diverse set of sequences (see references in Chew *et al*. 2000). Several protein factors have been identified that function at silencers, and these include members of the hnRNP (heterogeneous nuclear RNA particle) family. Recently, hnRNP A1 has been shown to regulate exon silencing in CD44 pre-mRNA and to be a target of oncogenic signalling pathways (Matter *et al*. 2000). Alternative splicing of the STREX exon in the BK channel pre-mRNA in the adrenal medulla or neuronal tissue is regulated by hypophysectomy, adrenocorticotrophin therapy or neuronal depolarisation (Xie & McCobb 1998). The calmodulin kinase IV pathway has recently been shown to repress splicing of the STREX exon through an element within a 54-nucleotide intronic region of the STREX 3′ splice site (Xie & Black 2001). A splicing factor called polypyrimidine tract binding protein (PTB) regulates alternative splicing of several genes by blocking the binding of factors such as $U2AF^{65}$ to the polypyrimidine tract. However, calmodulin kinase IV activation does not alter the phosphorylation of PTB, so the splicing factors through which this pathway influences the silencer element are presently unknown.

The ratio of the SR protein SF2/ASF to hnRNP A1 determines splice site selection in several genes (Graveley 2000) (Fig. 2) and this is a mode by which external signals may alter splicing patterns. For example, a stress-induced p38 MAP-kinase signalling pathway induces hnRNP A1 phosphorylation, changes its localisation, and this switches alternative splicing (van der Houven van Oordt *et al*. 2000). The activity of SR proteins may be regulated by several mechanisms (Manley & Tacke 1996, Graveley 2000), including phosphorylation by kinases (Misteli 1999, Murray 1999), cellular localisation (Misteli *et al*. 1998) and varying tissue concentrations (Hanamura *et al*. 1998).

There are therefore two potential mechanisms for regulating alternative splicing by signalling pathways in differentiated tissues: (i) via a change in the ratio of ubiquitous splicing factors such as SF2/ASF, hnRNP A1; or (ii) through the use of splicing factors that are specific to a particular tissue or signalling pathway. These mechanisms are not mutually exclusive. Tissue-specific splicing factors are described, particularly in the context of neural and testes alternative splicing, and are closely related to general splicing factors (Venables *et al*. 1999, Markovtsov *et al*. 2000). To date, there is no evidence of a splicing factor specific to a signalling pathway.

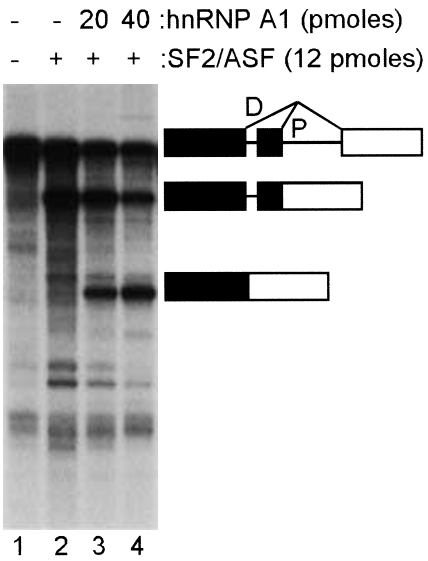

FIGURE 2. Action of SF2/ASF and hnRNP A1 in the selection of alternative splice sites. Splicing assays were performed in S100 cytosolic Hela cell extracts using a β-globin pre-mRNA, 5′D16 (Reed & Maniatis 1986). This pre-mRNA has duplicated 5′ splice sites (Krainer *et al*. 1990): P, a proximal 5′ splice site and D, a distal 5′ splice site, giving alternative splicing patterns indicated by the sloping lines. Recombinant SF2/ASF (S L Chew) and hnRNP A1 (kindly made by L Manche, Cold Spring Harbour Laboratory) were added in amounts indicated above the lanes. No mRNA products were seen in the absence of SF2/ASF (lane 1). An appropriate increase in the proportion of D to P splice site usage occurred with an increase in hnRNP A1 to SF2/ASF ratio (lanes 2–4).

POLYADENYLATION/CLEAVAGE

The components of the polyadenylation machinery have been isolated and characterised (Barabino & Keller 1999). Two multi-protein complexes are involved: cleavage and polyadenylation specificity factor (CPSF) binds the AAUAAA motif, while cleavage stimulation factor (CstF) binds the downstream GU-rich region. CstF consists of three subunits of 77, 64 and 50 kDa (CstF-77, CstF-64 and CstF-50). CPSF, CstF, two cleavage factors ($CF\ I_m$ and $CF\ II_m$) and poly(A) polymerase cleave the pre-mRNA, and then CPSF and poly(A) polymerase add the poly(A) tail of between 20–200 (A) nucleotides. The efficiency of polyadenylation and length of the tail may be regulated by the

action of poly(A) binding factor II. Several patterns of regulation are possible. Firstly, alternative polyadenylation/cleavage signals may be used. This changes the length of the 3′ untranslated region included in the mRNA. Second, the length of the poly(A) tail can vary. Third, the same polyadenylation signal is used, but the site of cleavage changes, as in the thyroglobulin pre-mRNA (Pauws *et al*. 2001).

Hormonal stimuli regulate polyadenylation and cleavage site selection and the length of the polyadenylation tail (Santra & Carter 1999). A good example of regulation of polyadenylation site usage is the action of follicle-stimulating hormone (FSH) during spermatogenesis. FSH stimulation promotes usage of an upstream polyadenylation/cleavage site in the cAMP-responsive element modulator-tau (CREM-τ) pre-mRNA, resulting in the exclusion of an instability element and an increase in CREM-τ levels (Foulkes *et al*. 1993). A comprehensive review of alternative poly(A) site selection has been published (Edwalds-Gilbert *et al*. 1997). An example of the regulation of poly(A) tail length is the effect of bromocriptine on the rat prolactin pre-mRNA (Carter *et al*. 1993).

The mechanisms by which external signals regulate polyadenylation or cleavage are not clear. More is understood about how cellular differentiation, growth control and DNA repair processes interact with the polyadenylation/cleavage machinery. Progress has been made in the context of B-lymphocyte differentiation, where the level of CstF-64 regulates polyadenylation site selection. The binding of CstF-64 and alternative polyadenylation/cleavage site selection can be blocked by hnRNP F (Veraldi *et al*. 2001). A link between cellular growth control and polyadenylation and cleavage is indicated by the modulation of the phosphorylation status and function of poly(A) polymerase by cyclin-dependent kinases (Colgan *et al*. 1998). Cyclin B(1) binds poly(A) polymerase directly (Bond *et al*. 2000). The polyadenylation/cleavage machinery is also regulated by DNA repair and tumour suppression mechanisms. Thus, the breast cancer gene product BRCA1 interacts with a BRCA1-associated RING domain protein (BARD1). DNA damage inhibits polyadenylation via the formation of a complex between BARD1/BRCA1 and CstF-50 (Kleiman & Manley 2001).

CO-ORDINATION

There is now substantial detail about the coupling of transcription to splicing and polyadenylation. The C-terminal domain (CTD) of RNA polymerase II (pol II) directs splicing and polyadenylation factors to the pre-mRNA (McCracken *et al*. 1997, Hirose & Manley 1998, 2000, Misteli & Spector 1999). This co-ordination between transcription and splicing also influences alternative splicing. Thus, the nature of the transcriptional promoter and complex influences splice site selection in the fibronectin pre-mRNA, via recruitment of SR proteins SF2/ASF and 9G8 (Cramer *et al*. 1999). A change in the conformation of the CTD of pol II may be the mode by which nuclear hormones and their receptors influence alternative splicing (Fig. 3). SR proteins and exonic splicing enhancers also function in both steps of the splicing reaction (Chew *et al*. 1999), perhaps to ensure co-ordination of the different steps of splicing during up-regulation of gene expression. The co-ordination between splicing and polyadenylation is well illustrated in the calcitonin/CGRP

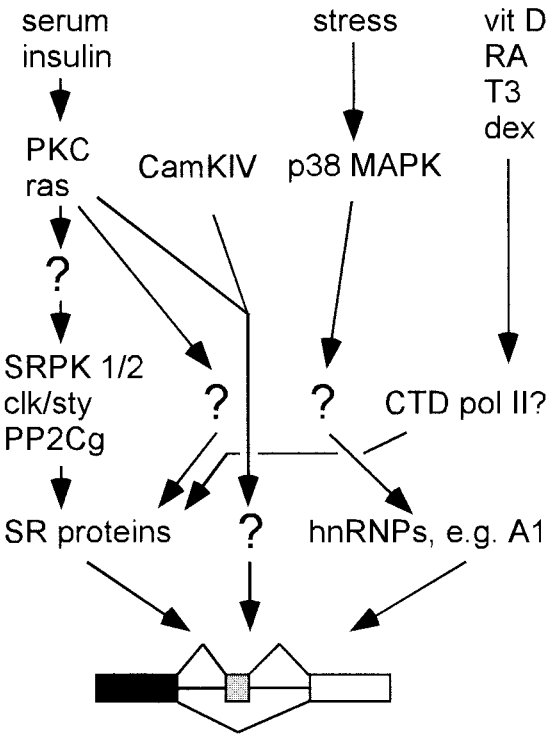

FIGURE 3. Schematic showing possible pathways involved in the regulation of splicing. The primary signals include insulin, serum stimulation, stress responses or nuclear hormones (vit D, vitamin D; RA, retinoic acid; T3, thyroid hormones; dex, dexamethasone). The areas of uncertainty are shown by question marks. For example, it is not clear if PKC and ras pathways function via SR protein kinases (SRPK) or through an unknown set of intermediates. Other abbreviations: PP2Cg, protein phosphatase 2 gamma; clk/sty, clk/sty kinase; CamKIV, calmodulin kinase IV.

pre-mRNA, where polyadenylation and splicing factors interact to regulate the use of alternative terminal exons (Lou & Gagel 1999).

DISRUPTION

Disruption of existing splice sites or introduction of new splice sites via DNA sequence mutations may result in incorrect pre-mRNA splicing leading to genetic disease. One of the earliest understandings of a mechanism of genetic disease was in the splicing defect of a thalassaemic globin gene (Treisman *et al*. 1982). It is now clear that DNA mutations resulting in abnormal splicing cause a substantial proportion of genetic disease (Krawczak *et al*. 1992). The commonest functional consequence of genetic mutations in many familial endocrine diseases is aberrant splicing; e.g. in *CYP21B* (Speiser *et al*. 1992, Kapelari *et al*. 1999), *NF1* (Ars *et al*. 2000) and *MENIN* genes (Mutch *et al*. 1999). DNA mutations affecting splicing may be classified into those that disrupt the splice sites themselves (Krawczak *et al*. 1992), or those that change non-splice site sequences (Valentine 1998). In the former class, mutations at 5′ splice sites may cause activation of nearby cryptic 5′ splice sites, or skipping of the entire adjacent upstream exon (Robberson *et al*. 1990). In the latter class, mutations of non-splice site sequences may disrupt regulatory elements for nearby splice sites (Liu *et al*. 2001). Another mode of disrupting normal splicing is through mutations that activate the splicing of a false exon which is normally never expressed, and several examples occur in cystic fibrosis (Friedman *et al*. 1999). In an endocrine example, we showed a point mutation in a false exon was necessary and sufficient for splicing of the false exon, thus disrupting the growth hormone receptor mRNA and causing Laron syndrome (Metherell *et al*. 2001).

SUMMARY

Recent insights have added greatly to our understanding of the mechanisms governing the regulation of post-transcriptional mRNA nuclear processing. SR proteins and hnRNPs are important regulators of pre-mRNA splicing and bind pre-mRNA at key regulatory elements. Signalling pathways alter splicing and the properties of such RNA-binding proteins. The details of the intermediates between the signalling pathways and splicing protein factors are still unclear (Fig. 3). There may be a specific set of proteins that directly couple signalling cascades and RNA-binding proteins. Alternatively, the action of signalling on RNA-binding protein function may be indirect, perhaps via effects on the cell cycle, as several kinases and phosphatases associated with modulating phosphorylation status of splicing proteins are also cell cycle-regulated factors (Burns & Gould 1999). The action of some of the steroid hormones on alternative splicing may be indirect, through changes in the structure of the transcriptional complex and the configuration of the CTD of pol II. Thus, there is still much work required to fully elucidate the molecular mechanisms and importance of pre-mRNA splicing in regulating gene expression. Insight into these mechanisms will have an impact on our understanding of certain genetic endocrine diseases and perhaps in development of novel therapies for the future.

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