transactions

Biochemical Society Transactions www.biochemsoctrans.org

RNA UK 2008

Independent Meeting held at The Burnside Hotel, Bowness on Windermere, Cumbria, U.K., 18–20 January 2008. Organized and Edited by David Elliot (Newcastle, U.K.), Sarah Newbury (Sussex, U.K.) and Alison Tyson-Capper (Newcastle, U.K.).

Substances that can change alternative splice-site selection

Chiranthani Sumanasekera, David S. Watt and Stefan Stamm[1]

Department of Molecular and Cellular Biochemistry, University of Kentucky, College of Medicine, B283, Biomedical Biological Sciences Research Building, 741 South Limestone, Lexington, KY 40536, U.S.A.

Abstract

Alternative pre-mRNA splicing is an important element in eukaryotic gene expression, as most of the protein-coding genes use this process to generate multiple protein isoforms from a single gene. An increasing number of human diseases are now recognized to be caused by the selection of 'wrong' alternative exons. Research during the last few years identified a number of low-molecular-mass chemical substances that can change alternative exon usage. Most of these substances act by either blocking histone deacetylases or by interfering with the phosphorylation of splicing factors. How the remaining large number of these substances affect splicing is not yet fully understood. The emergence of these low-molecular-mass substances provides not only probes for studying alternative pre-mRNA splicing, but also opens the door to the possible harnessing of these compounds as drugs to control diseases caused by the selection of 'wrong' splice sites.

Importance of alternative splicing

Most of the human protein-coding genes exhibit an exon–intron structure that becomes apparent when their pre-mRNA is processed. During pre-mRNA processing, some sequences are joined and exported into the cytosol as exons (for exported sequences). The introns (intervening sequences) are excised and retained in the nucleus.

In humans, almost all polymerase II transcripts are spliced, and an estimated 74–88% of human genes are alternatively spliced [1,2]. Most of the alternative exons encode portions of proteins, and therefore alternative splicing facilitates the generation of multiple protein isoforms from a single gene. These isoforms often have different biological properties in that they interact with other binding partners and possess different subcellular localizations, catalytic activities or pharmacological properties [3]. Several biological processes are co-ordinated by orchestrated changes in alternative splicing. Well-studied examples of these processes include sex determination in *Drosophila* [4] and certain apoptotic events [5]. The potential of alternative pre-mRNA splicing to increase the number of proteins generated from a single gene demonstrates that alternative pre-mRNA splicing is a central element of eukaryotic gene regulation and explains why it has been selected during evolution.

The recognition of exons in the pre-mRNA is a dynamic process that is coupled with transcription and other pre-mRNA processing steps, such as 5′-end capping, 3′-end processing, polyadenylation and editing. Since the sequences of the splice sites that surround exons are highly degenerate, additional sequences on the pre-mRNA aid in exon recognition. The interplay between these sequences on the pre-mRNA and more than 300 proteins (reviewed in [6]) that have been identified in MS analyses leads ultimately to the recognition of the splice sites by the spliceosome and the joining of exons [7].

The recognition of alternative exons is frequently subjected to regulation. The utilization of an alternative exon depends on the cell type, the developmental stage and/or the reception of cellular signals (reviewed in [8–10]). These changes can occur within 1 h in animal systems [11], and in most systems studied, these changes do not involve *de novo* protein synthesis [8]. Post-translational modifications of splicing factors, such as phosphorylation (reviewed in [12]), glycosylation [13], acetylation [14] or methylation [15], also play key roles in the regulation of splice-site selection.

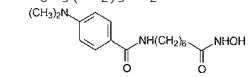
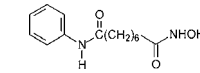
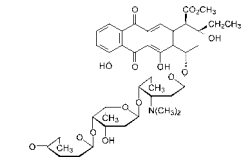
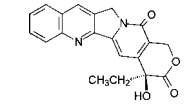
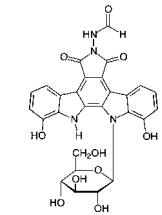
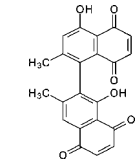
Key words: alternative pre-mRNA splicing, exon, histone deacetylase inhibitor, phosphorylation, serine/arginine-rich protein, splice-site selection.

Abbreviations used: EIPA, 5-(*N*-ethyl)-*N*-isopropylamiloride; FTDP-17, frontotemporal dementia and parkinsonism linked to chromosome 17; HDAC, histone deacetylase; PKA, protein kinase A; PP1, protein phosphatase-1; SAHA, suberoylanilide hydroxamic acid; SMA, spinal muscular atrophy; SMN2, survival of motor neurons 2; SR protein, serine/arginine-rich protein; Topo I, topoisomerase I.

[1]To whom correspondence should be addressed (email stefan@stamms-lab.net).

Table 1 | **Names, putative mechanisms, regulated exons and structures of the low-molecular-mass substances that influence alternative splice-site selection**

Abbreviation: n.d., not defined.

No.	Small molecule name	Mechanism	Regulated exon	Structure	Reference
	HDAC inhibitors				
1	Sodium butyrate	HDAC inhibitor	*SMN2* exon 7	$CH_3(CH_2)_2CO_2Na$	[26]
2	Valproic acid	HDAC inhibitor	*SMN2* exon 7	$(CH_3CH_2CH_2)_2CHCO_2H$	[22]
3	Sodium 4-phenylbutyrate	HDAC inhibitor	*SMN2* exon 7	$C_6H_5(CH_2)_3CO_2Na$	[41]
4	*N*-Hydroxyl-7-[4-(dimethylamino)benzoyl]aminoheptanamide (M344)	HDAC inhibitor	*SMN2* exon 7		[42]
5	SAHA	HDAC inhibitor	*SMN2* exon 7		[43]
	Kinase inhibitors				
6	Aclarubicin	Topo I	*SMN2* exon 7		[44]
7	Camptothecin	Topo I	*CASP-2* exon 9		[45]
8	6-*N*-Formylamino-12,13-dihydro-1,11-dihydroxy-13-(β-D-glucopyranosyl)5*H*-indolo [2,3-*a*]pyrrolo [3,4-*c*]carbazole-5,7(6*H*)-dione (NB-506)	Topo I	*Bcl-X* and *CD44*		[46]
9	Isodiospyrin	Topo I	n.d.		[47]

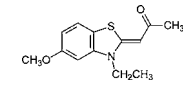
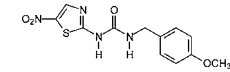
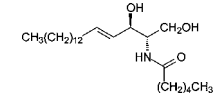
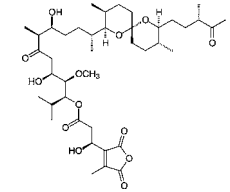
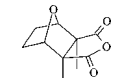
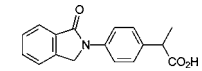
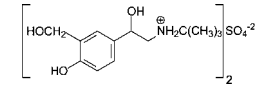
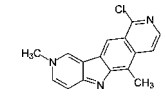
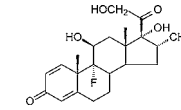
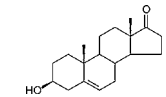
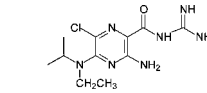
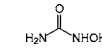
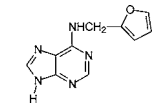
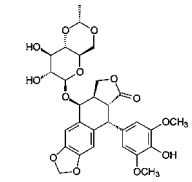
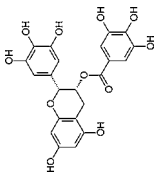
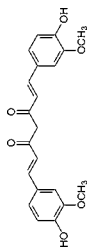
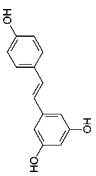
10	(*Z*)-1-(3-Ethyl-5-methoxy-2,3-dihydrobenzothiazol-2-ylidene) propan-2-one (TG003)	CLK kinases	*Clk1/sty* exon 2 and E1A		[29]
11	Lithium chloride	GSK3	*tau* exon 10	LiCl	[21]
12	*N*-(4-Methoxybenzyl)-*N*′-(5-nitro-1,3-thiazol-2-yl)urea (AR-A014418)	GSK3	*tau* exon 10		[21,48]
	Phosphatase inhibitors				
13	Sodium orthovanadate	Non-specific inhibitor	*SMN2* exon 7	Na_3VO_4	[49]
14	*N*-(Hexanoyl)sphingosine (C_6-ceramide)	PP1 regulation	*Bcl-X* and *CASP-9*		[33]
15	Tautomycin	PP1 inhibition	*SMN2* exon 7 and multiple other exons		[32]
16	Cantharidin	PP1 inhibition	*SMN2* exon 7		[32]
	cAMP pathway				
17	*rac*-2-[4-(1-Oxo-2-isoindolinyl)phenyl]propionic acid (indoprofen)	Phosphodiesterase inhibitor?	*SMN2*		[35]
18	2-(t-Butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol sulfate (salbutamol)	Adrenergic antagonist	*SMN2* exon 7		[34]
	Protein–protein interactions				
19	10-Chloro-2,6-dimethyl-2*H*-pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline (IDC16)	SR-protein interaction	HIV-1 mRNA		[38,39]

Table 1 | **(Continued)**

No.	Small molecule name	Mechanism	Regulated exon	Structure	Reference
	Coupling of transcription and splicing				
20	Dexamethasone	Coupling of transcription and splicing	Insulin receptor mRNA		[50]
21	DHEA (dihydroepiandrosterone)	Coupling of transcription and splicing	STREX (stress axis-regulated) exon		[51]
22	Steroid hormones	n.d.	Reporter CD44 mini-gene		[35]
	Ion channels and electrochemical gradients				
23	EIPA	Change in ion gradient	*SMN2*		[52]
24	Glutamate	Change in ion gradient	*Ania-6* mRNA	$C_5H_9NO_4$	[53,54]
	Unknown role				
25	Hydroxyurea	n.d.	*SMN2* exon 7		[55]
26	Ethanol	n.d.	L-type Ca^{2+} channel mRNA	C_2H_5OH	[56]
27	DMSO	Ionic interaction	Multiple		[57]
28	6-Furfuryladenine (kinetin)	n.d.	*IKBKAP* mRNA		[58]
29	Etoposide (VP16)	Topo II inhibition?	*CASP-2* exon 9		[59]

30	EGCG (epigallocatechin gallate)		Down-regulates the expression of hnRNP A2/B1	*SMN2* exon 7, *IKBKAP* mRNA	[60,61]
31	Cucurmin		n.d.	*SMN2* exon 7	[60]
32	Resveratrol		n.d.	*SMN2* exon 7	[60]

The importance of proper splice site recognition is apparent from the growing number of human diseases that are recognized to be caused by the selection of incorrect splice sites [16,17]. These diseases result from either mutations, as in the case of FTDP-17 (frontotemporal dementia and parkinsonism linked to chromosome 17) and Duchenne's muscular dystrophy, or deregulation of the cellular splicing machinery, as exemplified by the numerous changes in alternative splicing seen in cancer [18]. Alternative splicing has therefore rapidly emerged as a new drug target [19], especially since protein isoforms generated by this process can have different pharmacological effects [20]. The unexpected alteration of alternative splice-site selection may also explain the side effects that established drugs have in addition to their principal role.

Given the importance of alternative splicing, it is surprising that no low-molecular-mass compounds have been identified that specifically inhibit certain splicing events or stages in the splicing reaction. The use of RNA-binding molecules as antibiotics, such as gentamicin, chloramphenicol and tetracycline, certainly illustrates that drugs can be targeted against RNA and/or RNA-binding proteins.

The lack of compounds for the activation or inhibition of specific pre-mRNA splicing events is a problem for studying the splicing reaction itself and for the generation of therapeutic approaches to treat diseases caused by missplicing. During the last 5 years, research focused on changing incorrect splice-site selection to the desired outcome led to the identification of several low-molecular-mass components, which are the subject of the present review. So far, the common feature of these compounds is the low degree of specificity with which they affect numerous splicing events.

Several of these components, such as lithium chloride or valproic acid, are in clinical use to treat bipolar disorders or epilepsy respectively. They were only recently shown to influence splice-site selection [21,22]. It is possible that other pharmaceuticals now in clinical use also affect pre-mRNA splicing. Currently, only the use of oligonucleotides allows the unambiguous, specific targeting of a splicing event. This technique is currently being tested for Duchenne's muscular dystrophy and is in the experimental stage for other diseases, such as SMA (spinal muscular atrophy) and thalassemia (reviewed in [23,24]).

Substances regulating splice-site selection

Several screens have been performed to find substances that might change the missplicing that is the cause of SMA or that could be a basis for HIV therapy. In addition, a number of individual investigations discovered several substances capable of changing splice-site selection, which are summarized in Table 1.

HDAC (histone deacetylase) inhibitors

HDAC inhibitors were identified in studies aimed at augmenting the usage of SMN2 (survival of motor neurons 2) exon 7, an outcome that would be a therapeutically valuable approach in the treatment of SMA. These inhibitors include

sodium butyrate, valproic acid, sodium 4-phenylbutyrate, M344 and SAHA (suberoylanilide hydroxamic acid) (Table 1, numbers 1–5). In mouse models of SMA, these compounds increased the life span of the animals (reviewed in [25]). Their mechanism of action, however, is not yet clear. Among possible explanations is the role of HDACs. HDACs regulate the chromatin structure and subsequently gene expression by controlling the acetylation state of histones, which in turn determines histone affinity for DNA. It is possible that application of HDAC inhibitors causes a co-ordinated change in the expression of splicing regulatory factors, which in turn dictates a change in splicing. In support of this hypothesis, a change in SR protein (serine/arginine-rich protein) expression was observed after sodium butyrate application in mice [26]. Another possibility, which has not been systematically investigated, is a direct effect of these substances on the acetylation of splicing-regulatory proteins themselves. Acetylation of SAM68 (68 kDa Src-associated protein in mitosis), for example, regulates its binding to RNA [14], and it is possible that other splicing factors are regulated similarly.

Substances that change the phosphorylation state of splicing-regulatory proteins

As mentioned above, reversible phosphorylation is an important mechanism in controlling the selection of alternative exons. As a consequence, kinase and phosphatase inhibitors also affect alternative splice-site selection. The best understood targets for kinases acting on splicing are the SR proteins, a family of highly conserved pre-mRNA splicing factors. SR proteins can bind to each other and the SR domain serves mainly as a protein–protein interaction domain, allowing different SR proteins to oligomerize. This oligomerization is the basis for the formation of exon enhancer complexes. In addition, the SR domain can bind to RNA. It has been shown in several systems that the phosphorylation state of this domain controls the oligomerization of SR proteins that is the basis for the formation of exon-recognition complexes. The phosphorylation state of the SR domain is controlled by the interplay of multiple kinases and phosphatases, located both in the cytosol and the nucleoplasm [12].

Kinase inhibitors

DNA Topo I (topoisomerase I) effects the phosphorylation of SR proteins [27] and has a dual function in RNA metabolism. It nicks the DNA strand upon transcription, and phosphorylates SR proteins that are known to associate with the nascent pre-mRNA [28]. Numerous antineoplastic drugs that target Topo I were tested for their effect on splice-site selection, and several of them (Table 1, numbers 6–9) changed splice-site selection.

SR proteins are also phosphorylated by a family of nuclear CDC2 (cell division cycle 2 kinase)-related kinases, termed CLK (CDC-like kinase) 1–4. A specific inhibitor against these kinases, TG003 (Table 1, number 10), changes alternative splicing in reporter genes [29] and has been tested as an antiviral agent [30]. GSK3 (glycogen synthase kinase 3) also phosphorylates SR proteins, and its inhibition changes alterative splicing of exon 10 of the *tau* gene [21] (Table 1, numbers 11 and 12). This exon is of medical importance, as mutations causing aberrant usage of the exon leads to frontotemporal dementia (FTDP-17) [31].

Phosphatase inhibitors

PP1 (protein phosphatase-1) binds directly to a phylogenetically conserved motif in the RNA-recognition motif of at least nine different splicing-regulatory proteins [32]. As a consequence, blocking PP1 activity has a strong influence on alternative splicing. The most specific inhibitor for PP1 is tautomycin, which changes alternative splicing in cell culture and mouse models [32]. Similar effects were seen for cantharidin, which inhibits both PP1 and PP2A (protein phosphatase-2A) (Table 1, numbers 13–16). Ceramides activate PP1 by an unknown mechanism that probably involves dissociation of the phosphatase from its inhibitors. C_6-ceramide, shown in Table 1 [14], has been shown to change splice-site selection in some apoptotic genes. The change in alternative splicing of genes involved in apoptosis explains the cytotoxic effect of these drugs that are used in chemotherapy [33].

In vivo, PP1 is blocked by specific protein inhibitors, Inhibitor-1 and DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa), which are activated by phosphorylation by PKA (protein kinase A). Since PKA is activated by a rise in cellular cAMP levels, cAMP increase leads to a decrease in PP1 activity, which has an effect on splice-site selection. This mechanistic link explains the actions of salbutamol [34] (a β_2-adrenergic agonist) and indoprofen [35] (a compound that blocks cyclo-oxygenase and phosphodiesterase) on splicing (Table 1, numbers 17 and 18).

Steroid hormones

The selection of alternative exons is coupled with promoter usage, as the C-terminal domain of polymerase II interacts with components of the splicing machinery. This outcome explains why alternative splicing events can be controlled by steroid hormones when their pre-mRNA transcription is controlled by steroid-responsive promoters [36]. In addition, CAPER [co-activator of AP-1 (activator protein-1) and ER (oestrogen receptor)] proteins have been identified that are activated by steroid hormones and act on both transcription and splicing [37] (Table 1, numbers 20 and 21).

Protein–protein interaction

The interaction between SR proteins can be interrupted by isoquinolines, which target specific members of this class of proteins. These findings can be applied *in vitro* to block HIV replication [38,39] (Table 1, number 19).

Ion channels

Alternative splicing events change in response to neuronal stimulation [11,40]. Several compounds, including valproic acid, glutamate and EIPA [5-(*N*-ethyl)-*N*-isopropylamiloride], affect splicing in cell culture. However, the exact mechanism that links this change in membrane properties to

the splicing machinery remains unclear (Table 1, numbers 23 and 24).

Other components

Still other investigations identified a group of apparently unrelated compounds that affect alternative splicing. The mechanism of action of these compounds remains unclear (Table 1, numbers 25–32).

Future developments

The compounds in Table 1 clearly underscore the dramatic ability of small molecules to affect alternative splicing decisions. Their discovery raises the prospect for finding still other natural, synthetic or semi-synthetic compounds that will directly interact with the spliceosome components or will indirectly alter signal transduction pathways that regulate splice-site selection. Finding substances that possess specificity for a single splicing event or for a certain step in the splicing process represents a future challenge that, once solved, will allow us to understand the fundamental aspects of this intriguing process and to treat human diseases caused by missplicing events.

This work was supported by the EURASNET (European Alternative Splicing Network of Excellence), NIH (National Institutes of Health; P20 RR020171 from the National Center for Research Resources), BMBF (Federal Ministry of Education and Research, Germany), DFG (Deutsche Forschungsgemeinschaft; SFB 473) and the German Cancer Aid.

References

1 Kampa, D., Cheng, J., Kapranov, P., Yamanaka, M., Brubaker, S., Cawley, S., Drenkow, J., Piccolboni, A., Bekiranov, S., Helt, G. et al. (2004) Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22. Genome Res. **14**, 331–342
2 Johnson, J.M., Castle, J., Garrett-Engele, P., Kan, Z., Loerch, P.M., Armour, C.D., Santos, R., Schadt, E.E., Stoughton, R. and Shoemaker, D.D. (2003) Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. Science **302**, 2141–2144
3 Stamm, S., Ben-Ari, S., Rafalska, I., Tang, Y., Zhang, Z., Toiber, D., Thanaraj, T.A. and Soreq, H. (2005) Function of alternative splicing. Gene **344**, 1–20
4 Forch, P. and Valcarcel, J. (2003) Splicing regulation in *Drosophila* sex determination. Prog. Mol. Subcell. Biol. **31**, 127–151
5 Wu, J.Y., Tang, H. and Havlioglu, N. (2003) Alternative pre-mRNA splicing and regulation of programmed cell death. Prog. Mol. Subcell. Biol. **31**, 153–185
6 Jurica, M.S. and Moore, M.J. (2003) Pre-mRNA splicing: awash in a sea of proteins. Mol. Cell **12**, 5–14
7 Smith, C.W. and Valcarcel, J. (2000) Alternative pre-mRNA splicing: the logic of combinatorial control. Trends Biochem. Sci. **25**, 381–388
8 Stamm, S. (2002) Signals and their transduction pathways regulating alternative splicing: a new dimension of the human genome. Hum. Mol. Genet. **11**, 2409–2416
9 Shin, C. and Manley, J.L. (2004) Cell signalling and the control of pre-mRNA splicing. Nat. Rev. Mol. Cell Biol. **5**, 727–738
10 Blaustein, M., Pelisch, F. and Srebrow, A. (2007) Signals, pathways and splicing regulation. Int. J. Biochem. Cell Biol. **39**, 2031–2048
11 Daoud, R., Da Penha Berzaghi, M., Siedler, F., Hubener, M. and Stamm, S. (1999) Activity-dependent regulation of alternative splicing patterns in the rat brain. Eur. J. Neurosci. **11**, 788–802
12 Stamm, S. (2008) Regulation of alternative splicing by reversible phosphorylation. J. Biol. Chem. **283**, 1223–1227
13 Soulard, M., Della Valle, V., Siomi, M.C., Pinol-Roma, S., Codogno, P., Bauvy, C., Bellini, M., Lacroix, J.C., Monod, G., Dreyfuss, G. and Larsen, C.-J. (1993) hnRNP G: sequence and characterization of a glycosylated RNA-binding protein. Nucleic Acids Res. **21**, 4210–4217
14 Babic, I., Jakymiw, A. and Fujita, D.J. (2004) The RNA binding protein Sam68 is acetylated in tumor cell lines, and its acetylation correlates with enhanced RNA binding activity. Oncogene **23**, 3781–3789
15 Rho, J., Choi, S., Jung, C.R. and Im, D.S. (2007) Arginine methylation of Sam68 and SLM proteins negatively regulates their poly(U) RNA binding activity. Arch. Biochem. Biophys. **466**, 49–57
16 Faustino, N.A. and Cooper, T.A. (2003) Pre-mRNA splicing and human disease. Genes Dev. **17**, 419–437
17 Stoilov, P., Meshorer, E., Gencheva, M., Glick, D., Soreq, H. and Stamm, S. (2002) Defects in pre-mRNA processing as causes of and predisposition to diseases. DNA Cell Biol. **21**, 803–818
18 Venables, J.P. (2006) Unbalanced alternative splicing and its significance in cancer. BioEssays **28**, 378–386
19 Hagiwara, M. (2005) Alternative splicing: a new drug target of the post-genome era. Biochim. Biophys. Acta **1754**, 324–331
20 Bracco, L. and Kearsey, J. (2003) The relevance of alternative RNA splicing to pharmacogenomics. Trends Biotechnol. **21**, 346–353
21 Hernandez, F., Perez, M., Lucas, J.J., Mata, A.M., Bhat, R. and Avila, J. (2004) Glycogen synthase kinase-3 plays a crucial role in tau exon 10 splicing and intranuclear distribution of SC35: implications for Alzheimer's disease. J. Biol. Chem. **279**, 3801–3806
22 Brichta, L., Hofmann, Y., Hahnen, E., Siebzehnrubl, F.A., Raschke, H., Blumcke, I., Eyupoglu, I.Y. and Wirth, B. (2003) Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. Hum. Mol. Genet. **12**, 2481–2489
23 Aartsma-Rus, A. and van Ommen, G.J. (2007) Antisense-mediated exon skipping: a versatile tool with therapeutic and research applications. RNA **13**, 1609–1624
24 Eckstein, F. (2007) The versatility of oligonucleotides as potential therapeutics. Expert Opin. Biol. Ther. **7**, 1021–1034
25 Wirth, B., Brichta, L. and Hahnen, E. (2006) Spinal muscular atrophy: from gene to therapy. Semin. Pediatr. Neurol. **13**, 121–131
26 Chang, J.G., Hsieh-Li, H.M., Jong, Y.J., Wang, N.M., Tsai, C.H. and Li, H. (2001) Treatment of spinal muscular atrophy by sodium butyrate. Proc. Natl. Acad. Sci. U.S.A. **98**, 9808–9813
27 Rossi, F., Labourier, E., Forne, T., Divita, G., Derancourt, J., Riou, J.F., Antione, E., Cathela, G., Brunel, C. and Tazi, J. (1996) Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. Nature **381**, 80–82
28 Tazi, J., Rossi, F., Labourier, E., Gallouzi, I., Brunel, C. and Antoine, E. (1997) DNA topoisomerase I: customs officer at the border between DNA and RNA worlds? J. Mol. Med. **75**, 786–800
29 Muraki, M., Ohkawara, B., Hosoya, T., Onogi, H., Koizumi, J., Koizumi, T., Sumi, K., Yomoda, J.I., Murray, M.V., Kimura, H. et al. (2004) Manipulation of alternative splicing by a newly developed inhibitor of Clks. J. Biol. Chem. **279**, 24246–24254
30 Fukuhara, T., Hosoya, T., Shimizu, S., Sumi, K., Oshiro, T., Yoshinaka, Y., Suzuki, M., Yamamoto, N., Herzenberg, L.A. and Hagiwara, M. (2006) Utilization of host SR protein kinases and RNA-splicing machinery during viral replication. Proc. Natl. Acad. Sci. U.S.A. **103**, 11329–11333
31 Gasparini, L., Terni, B. and Spillantini, M.G. (2007) Frontotemporal dementia with tau pathology. Neurodegener. Dis. **4**, 236–253
32 Novoyatleva, T., Heinrich, B., Tang, Y., Benderska, N., Butchbach, M.E., Lorson, C.L., Lorson, M.A., Ben-Dov, C., Fehlbaum, P., Bracco, L. et al. (2008) Protein phosphatase 1 binds to the RNA recognition motif of several splicing factors and regulates alternative pre-mRNA processing. Hum. Mol. Genet. **17**, 52–70
33 Chalfant, C.E., Rathman, K., Pinkerman, R.L., Wood, R.E., Obeid, L.M., Ogretmen, B. and Hannun, Y.A. (2002) *De novo* ceramide regulates the alternative splicing of caspase 9 and Bcl-x in A549 lung adenocarcinoma cells: dependence on protein phosphatase-1. J. Biol. Chem. **277**, 12587–12595
34 Angelozzi, C., Borgo, F., Tiziano, F.D., Martella, A., Neri, G. and Brahe, C. (2007) Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells. J. Med. Genet. **45**, 29–31
35 Lunn, M.R., Root, D.E., Martino, A.M., Flaherty, S.P., Kelley, B.P., Coovert, D.D., Burghes, A.H., Man, N.T., Morris, G.E. and Zhou, J. et al. (2004) Indoprofen upregulates the survival motor neuron protein through a cyclooxygenase-independent mechanism. Chem. Biol. **11**, 1489–1493

36 Auboeuf, D., Honig, A., Berget, S.M. and O'Malley, B.W. (2002) Coordinate regulation of transcription and splicing by steroid receptor coregulators. Science **298**, 416–419
37 Dowhan, D.H., Hong, E.P., Auboeuf, D., Dennis, A.P., Wilson, M.M., Berget, S.M. and O'Malley, B.W. (2005) Steroid hormone receptor coactivation and alternative RNA splicing by U2AF65-related proteins CAPERα and CAPERβ. Mol. Cell **17**, 429–439
38 Soret, J., Bakkour, N., Maire, S., Durand, S., Zekri, L., Gabut, M., Fic, W., Divita, G., Rivalle, C., Dauzonne, D. et al. (2005) Selective modification of alternative splicing by indole derivatives that target SR protein splicing factors. Proc. Natl. Acad. Sci. U.S.A. **102**, 8764–8769
39 Bakkour, N., Lin, Y.L., Maire, S., Ayadi, L., Mahuteau-Betzer, F., Nguyen, C.H., Mettling, C., Portales, P., Grierson, D., Chabot, B. et al. (2007) Small-molecule inhibition of HIV pre-mRNA splicing as a novel antiretroviral therapy to overcome drug resistance. PLoS Pathog. **3**, 1530–1539
40 Xie, J. and Black, D.L. (2001) A CaMK IV responsive RNA element mediates depolarization-induced alternative splicing of ion channels. Nature **410**, 936–939
41 Andreassi, C., Angelozzi, C., Tiziano, F.D., Vitali, T., De Vincenzi, E., Boninsegna, A., Villanova, M., Bertini, E., Pini, A., Neri, G. and Brahe, C. (2004) Phenylbutyrate increases SMN expression *in vitro*: relevance for treatment of spinal muscle atrophy. Eur. J. Hum. Genet. **12**, 59–65
42 Riessland, M., Brichta, L., Hahnen, E. and Wirth, B. (2006) The benzamide M344, a novel histone deacetylase inhibitor, significantly increases SM2 RNA/protein levels in spinal muscular atrophy cells. Hum. Genet. **120**, 101–110
43 Hahnen, E., Eyupoglu, I.Y., Brichta, L., Haastert, K., Trankle, C., Siebzehnrubl, F.A., Riessland, M., Holker, I., Claus, P., Romstock, J. et al. (2006) *In vitro* and *ex vivo* evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy. J. Neurochem. **98**, 193–202
44 Andreassi, C., Jarecki, J., Zhou, J., Coovert, D.D., Monani, U.R., Chen, X., Witney, M., Pollok, B., Zhang, M., Androphy, E. and Burghes, A.H. (2001) Aclarubicin treatment restores SMN levels to cells derived from type I spinal muscular atrophy patients. Hum. Mol. Genet. **10**, 2841–2849
45 Solier, S., Lansiaux, A., Logette, E., Wu, J., Soret, J., Tazi, J., Bailly, C., Desoche, L., Solary, E. and Corcos, L. (2004) Topoisomerase I and II inhibitors control caspase-2 pre-messenger RNA splicing in human cells. Mol. Cancer Res. **2**, 53–61
46 Pilch, B., Allemand, E., Facompre, M., Bailly, C., Riou, J.F., Soret, J. and Tazi, J. (2001) Specific inhibition of serine- and arginine-rich splicing factors phosphorylation, spliceosome assembly, and splicing by the antitumor drug NB-506. Cancer Res. **61**, 6876–6884
47 Ting, C.Y., Hsu, C.T., Hsu, H.T., Su, J.S., Chen, T.Y., Tarn, W.Y., Kuo, Y.H. Whang-Peng, J., Liu, L.F. and Hwang, J. (2003) Isodiospyrin as a novel human DNA topoisomerase I inhibitor. Biochem. Pharmacol. **66**, 1981–1991
48 Bhat, R., Xue, Y., Berg, S., Hellberg, S., Ormo, M., Nilsson, Y., Radesater, A.C., Jerning, E., Markgren, P.O., Borgegard, T. et al. (2003) Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. J. Biol. Chem. **278**, 45937–45945
49 Zhang, M.L., Lorson, C.L., Androphy, E.J. and Zhou, J. (2001) An *in vivo* reporter system for measuring increased inclusion of exon 7 in SMN2 mRNA: potential therapy of SMA. Gene. Ther. **8**, 1532–1538
50 Kosaki, A. and Webster, N.J. (1993) Effect of dexamethasone on the alternative splicing of the insulin receptor mRNA and insulin action in HepG2 hepatoma cells. J. Biol. Chem. **268**, 21990–21996
51 Lai, G.J. and McCobb, D.P. (2002) Opposing actions of adrenal androgens and glucocorticoids on alternative splicing of Slo potassium channels in bovine chromaffin cells. Proc. Natl. Acad. Sci. U.S.A. **99**, 7722–7727
52 Yuo, C.Y., Lin, H.H., Chang, Y.S., Yang, W.K. and Chang, J.G. (2008) 5-(*N*-ethyl-*N*-isopropyl)-amiloride enhances SMN2 exon 7 inclusion and protein expression in spinal muscular atrophy cells. Ann. Neurol. **63**, 26–34
53 Sgambato, V., Minassian, R., Nairn, A.C. and Hyman, S.E. (2003) Regulation of ania-6 splice variants by distinct signaling pathways in striatal neurons. J. Neurochem. **86**, 153–164
54 Meshul, C.K., Bunker, G.L., Mason, J.N., Allen, C. and Janowsky, A. (1996) Effects of subchronic clozapine and haloperidol on striatal glutamatergic synapses. J. Neurochem. **67**, 1965–1973
55 Grzeschik, S.M., Ganta, M., Prior, T.W., Heavlin, W.D. and Wang, C.H. (2005) Hydroxyurea enhances *SMN2* gene expression in spinal muscular atrophy cells. Ann. Neurol. **58**, 194–202
56 Walter, H.J., McMahon, T., Dadgar, J., Wang, D. and Messing, R.O. (2000) Ethanol regulates calcium channel subunits by protein kinase Cδ-dependent and -independent mechanisms. J. Biol. Chem. **275**, 25717–25722
57 Bolduc, L., Labrecque, B., Cordeau, M., Blanchette, M. and Chabot, B. (2001) Dimethyl sulfoxide affects the selection of splice sites. J. Biol. Chem. **276**, 17597–17602
58 Slaugenhaupt, S.A., Mull, J., Leyne, M., Cuajungco, M.P., Gill, S.P., Hims, M.M., Quintero, F., Axelrod, F.B. and Gusella, J.F. (2004) Rescue of a human mRNA splicing defect by the plant cytokinin kinetin. Hum. Mol. Genet. **13**, 429–436
59 Montecucco, A. and Biamonti, G. (2007) Cellular response to etoposide treatment. Cancer Lett. **252**, 9–18
60 Sakla, M.S. and Lorson, C.L. (2008) Induction of full-length survival motor neuron by polyphenol botanical compounds. Hum. Genet. **122**, 635–643
61 Anderson, S.L., Qiu, J. and Rubin, B.Y. (2003) EGCG corrects aberrant splicing of *IKAP* mRNA in cells from patients with familial dysautonomia. Biochem. Biophys. Res. Commun. **310**, 627–633

Received 8 January 2008
doi:10.1042/BST0360483