Chapter 21
Role of Alternative Splicing of the Serotonin Receptor 2C in the Prader–Willi Syndrome

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21.1 Editing

The 5-HT_{2C} receptor pre-messenger ribonucleic acid (pre-mRNA) undergoes both editing and alternative pre-mRNA splicing. Both events occur in the cell nucleous and are summarized below. RNA editing is the chemical modification of RNA bases. Common editing events include 2′-*O*-methylation, the addition of a methyl group on the ribose; conversion of cytidine to uridine; and conversion from adenine to inosine. The modification of the bases is catalyzed by deaminating enzymes that hydrolyze specific amino groups of the bases. The cytidine-to-uridine editing is catalyzed by cytidine deaminase, and the adenine-to-inosine editing is catalyzed by adenosine deaminase acting on RNAs (ADARs) (reviewed in Jepson and Reenan 2008 and Mehler and Mattick 2007). Editing of RNA has multiple effects on the resulting RNAs. It can lead to alteration of coding capacity, altered microRNA (miRNA) or small inhibitory RNA (siRNA) target populations, heterochromatin formation, nuclear sequestration, cytoplasmic sequestration, inhibition of miRNA and siRNA processing, and altered alternative splicing patterns (Nishikura 2006). The 5-HT_{2C} receptor pre-mRNA undergoes adenine-to-inosine editing on at least five editing sites. The combination of these sites could generate 32 isoforms theoretically, but not all the predicted mRNA forms have been identified (reviewed in Werry et al. 2008). As the translational machinery interprets an inosine as a guanosine the adenine-to-guanine editing changes the protein sequence that is encoded by the edited pre-mRNA. Due to the degeneracy of the genetic code, some of the edited mRNAs encode the same protein, which reduced the number to proteins generated by editing to 24.

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21.2 Pre-mRNA Splicing

21.2.1 General Mechanisms

In addition to editing, the 5-HT_{2C} receptor pre-mRNA undergoes alternative splicing. Alternative splicing affects an estimated 95% of human intron containing genes and is one of the most important mechanisms to increase the use of information encoded in eukaryotic genomes (Pan et al. 2008; Wang et al. 2008). The mechanism of splicing catalysis has been studied in considerable detail (Jurica and Moore 2003; Wahl et al. 2009). Critical for the catalysis are transient interactions between the pre-mRNA and five small nuclear ribonucleoprotein (snRNPs) (reviewed in Biamonti and Caceres 2009; Stark and Luhrmann 2006). The interaction is based on imperfect base complementarity between the snRNPs and the pre-mRNA (Sharp 1994).

In contrast to the constitutive splicing mechanism, it is not fully understood how splice sites, especially the alternative ones, are selected. Currently, it is not possible to accurately identify alternative spliced exons from genomic DNA sequences. The problem is that splice sites exhibit a large degree of sequence variations and only the four GU-AG nucleotides flanking the intron are conserved (Stamm et al. 2006; Thanaraj and Clark 2001). The splicing machinery needs therefore additional signals that define an exon (Robberson et al. 1990). This signal is provided by transient complexes of splicing regulatory proteins and pre-mRNA. Once these complexes have formed, they interact with components of the core spliceosome, which allows the correct identification of splice sites.

Serine-arginine-rich proteins (SR-proteins) and heterogenous ribonuclear-proteins (hnRNPs) are the major classes of proteins identified in complexes forming on pre-mRNA. These proteins bind to short degenerate sequences on the pre-mRNA. The degeneracy of the sequences allows the coding requirements of the pre-mRNA to be independent from splicing requirements. Depending on which proteins they bind, sequence elements on the pre-mRNA can act as either enhancers or silencers, which either promote or antagonize exon usage. Splicing regulatory proteins generally possess RNA-binding and protein-interaction domains that allow weak, transient binding between protein and pre-mRNA as well as among proteins that assemble on the pre-mRNA. The combination of these multiple weak interactions ultimately leads to the accurate recognition of exons by the spliceosome (Smith and Valcarcel 2000; Maniatis and Tasic 2002; Hertel 2008).

21.2.2 Control of Alternative Splicing

The formation of the exon-recognition complexes is subject to numerous controls. The first level of control is the variation of regulatory factor concentrations that often differ between cell types (Hanamura et al. 1998). In addition to this regulation,

the activity of splicing regulatory proteins is regulated by post-translational modifications, especially reversible phosphorylation. Phosphorylation influences the binding affinity between splicing regulatory proteins and can therefore control the formation of exon-recognition complexes. It is not fully understood what controls the phosphorylation of splicing factors, but numerous studies indicate that well-established signaling routes, such as mitogen activated protein (MAP)-kinase, Ca^{2+}-dependent kinase, and cyclic adenosine monophosphate (cAMP)-dependent pathways are involved. This model explains why numerous cellular stimuli, such as receptor activation or membrane depolarization can influence splice site selection. This paradigm also implies that tissue- and cell-specific differences in alternative splicing could be due to different signals that cells receive (reviewed in Stamm 2008; Shin and Manley 2004; Stamm 2002).

21.3 Gene Structure and Processing of the 5-HT_{2C} Pre-mRNA

21.3.1 Gene Structure

The 5-HT_{2C} receptor pre-mRNA is composed of at least seven exons (Fig. 21.1a). The entire human 5-HT_{2C}R gene from exon I to exon VI spans around 326 kilobase (kb) of DNA. Current databases annotate six exons and five introns. Recently, a human 5-HT_{2C} receptor mRNA was identified that contains a novel 91 nucleotide long alternatively spliced exon in the 5′ untranslated region (UTR) between exon II and III (accession numbers M81778, DR003480). In addition, in mouse, a not previously annotated exon is located between Exon II and III of the mouse 5-HT_{2C}R gene (mRNA accession number BC098327). This exon is unrelated to the human one. All introns of the serotonin receptor are larger than the mammalian average of 3365 nt (Thanaraj and Stamm 2003). The largest one is intron IV that spans about 117 kb. The 5′ UTR of the gene comprises exons I and II and a part of exon III. The coding region of the 5-HT_{2C}R cDNA spans from part of exon III to exon VI (Fig. 21.1b).

In contrast to many G-protein-coupled receptors that do not contain introns in their coding regions, the coding sequence of the human 5-HT_{2C}R gene is interrupted by three introns. The long 3′ UTR of the receptor is generated by exon VI. Key features of the gene structure, such as the positions of the intron–exon junctions as well as the promoter regions have been conserved between rodents and humans. This suggests that similar *cis*- and *trans*-acting elements regulate gene expression in both species. In its 5′ UTR, the 5-HT_{2C} receptor gene hosts at least one snoRNA (HBI-36) between exons II and III and two putative miRNAs hsa-mir-1264 and hsa-mir-1298. However, the function of these RNAs remains to be determined. Theoretically, the three alternative exons can be combined to generate nine mRNA isoforms. Combining these nine mRNA isoforms with 32 variants generated by pre-mRNA editing predicts that the 5-HT_{2C}R gene can generate 288 mRNA isoforms. It is not clear whether all of these isoforms are actually generated. Detailed RT-PCR studies produced evidence for [AU1]

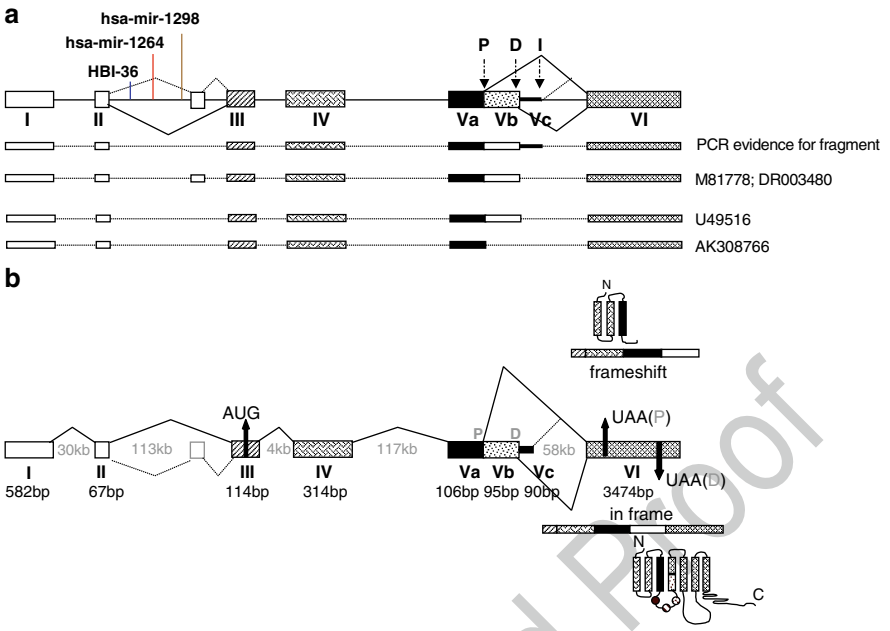

Fig. 21.1 Gene structure of serotonin receptors. (**a**) Overview of pre-mRNAs generated from the serotonin receptor. Exons are indicated by boxes or a thick line and roman numerals. Introns are indicated as lines. Exons that contribute to the open reading frame are shaded. Splicing patterns are indicated by lines. Annotated and predicted RNA isoforms are indicated underneath the gene structure. Exon V undergoes alternative splicing. The splice sites I to III are indicated. The proximal, distal, and intronic splice sites I (P, D, and I) are indicated. (**b**) Protein isoforms and splicing events. The structure of the human 5-HT_{2C}R receptor is indicated as in panel (**a**), and the intron and exon lengths are indicted. P and D indicate the location of proximal and distal splice sites, respectively. An arrow pointing towards AUG indicates the start codon of the longest open reading frame. UAA(P) and UAA(D) are the stop codons resulting from usage of the proximal and distal splice site. Exons are indicated as boxes with roman numerals. The mRNA isoforms and the resulting proteins are schematically shown. The shading reflects contribution of the different exons to the protein composition. Exon Vb encodes the second intracellular loop, and due to RNA editing, three amino acids, which are indicated as dots, are variable

fragments of most of the isoforms (Burns et al. 1997; Niswender et al. 1999; Wang et al. 2000; Fitzgerald et al. 1999; Hackler et al. 2006). As the expression of these isoforms changes due to environmental stress and is altered in disease processes (Englander et al. 2005), their regulation has been studied in detail.

21.3.2 Alternative Pre-mRNA Processing of Exon Vb

Exon V of the 5-HT_{2C} pre-mRNA has three alternative 5′ splice sites, proximal P, distal D, and intronic I. Different authors use different nomenclatures for these

sites, for example, splice site I, II, and III. Their location is shown in Fig. 21.1a. The usage of these sites define exon Vb and Vc (Fig. 21.1a). There is RT-PCR evidence for the usage of the intronic site I (also named *donor site III*), but a full-length mRNA containing exon Vc has not been described. However, the RT-PCR data suggest that RNAs with this exon exist (Flomen et al. 2004). Alternative usage of exon Vb is documented in mRNA databases. Exon Vb is of special importance for the regulation of the 5-HT_{2C} pre-mRNA, since it is located in the coding region of the protein and is targeted by both RNA editing and alternative splicing. Exon Vb encodes the part of the protein that composes its second intracellular loop. This loop couples to the G protein and is therefore essential for signaling. The exon is 95 nucleotides long and, thus, can not accommodate an integer number of the three-nucleotide long codons. Therefore, skipping of this exon causes a frameshift and leads to the generation of a truncated receptor mRNA. It is not clear whether this mRNA is translated into a nonfunctional receptor that lacks the G-protein-coupling ability or undergoes nonsense-mediated RNA decay. Nonsense mediated decay is a posttranscriptional surveillance mechanism that can degrade mRNA with premature stop codons (Neu-Yilik and Kulozik 2008). Since there is no published evidence for the expression of short 5-HT_{2C} mRNA forms, it is likely that skipping of exon Vb leads to the degradation of the resulting mRNA. Exon Vb is localized in a predicted extended secondary structure that harbors editing sites described above (Fig. 21.2). In addition to the five edited adenine residues in exon Vb, a sixth site in exon Vc has been described (Flomen et al. 2004). Editing of these nucleotides changes the encoded protein in the second intracellular loop that is involved in receptor signaling.

In addition to influencing the encoded protein, editing influences the splicing of exon Vb when tested in cell culture based assays (Flomen et al. 2004; Kishore and Stamm 2006a). The distal splice site (TAGgtaaat) deviates on two positions from the consensus, "optimal" 5′ splice site (AAGgtaagt). The splice site is not used when analyzed in reporter gene assays. In these types of assays, a fragment of the gene is transfected into cells and the splicing pattern is analyzed by subsequent RT-PCR (reviewed in Tang et al. 2005; Stoss et al. 1999). These analyses showed that when the splice site is mutated into the consensus sequence, the exon is included into the mRNA (Kishore and Stamm 2006a). However, even after this splice site is mutated into a perfect mammalian consensus, exon Vb is still predominantly skipped. This suggested the existence of a splicing silencer element in the exon. Such a silencing element was bioinformatically predicted in the exon (Kishore and Stamm 2006a). This splicing silencing element partially overlaps with the adenine editing sites. Their conversion from adenine to inosine in the editing process weakens the splicing silencer, and as a result, exon Vb is now included (Flomen et al. 2004; Kishore and Stamm 2006a). These experiments were performed in transfected cell lines that have a different set of splicing regulatory proteins than differentiated neurons that express the 5-HT_{2C} receptor under physiological conditions. When the effect of editing of exon Vb was studied in knock-in mouse models no effect on

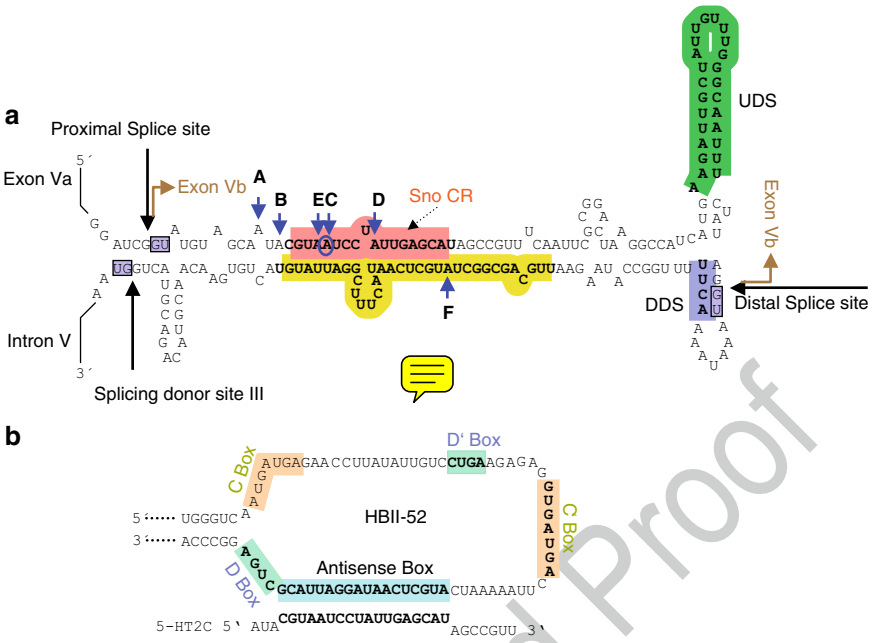

Fig. 21.2 Predicted RNA structure of exon Vb and complementarity to the snoRNA HBII-52. (**a**) RNA structure: The sequence of the serotonin receptor 5-HT$_{2C}$ exon V is indicated. *Arrows* point to the five nucleotides that are edited from A to I (A–E editing sites). Structural elements are indicated by shading: UDS upstream distal splice site, DDS downstream distal splice site, *sno-CR* snoRNA complementarity region. The base at editing site C fulfills the requirements to be 2′-*O*-methylated (circle) by the snoRNA. Exon Vb is indicated by *arrows*; the GU nucleotides of the proximal, distal, and donor site III are boxed. (**b**) Complementarity between exon Vb and the snoRNA HBII-52. The snoRNA is shown in 3′–5′ orientation to illustrate the base pairing with the sno-CR of the serotonin receptor. The structural elements of the snoRNA, the C, C′ box, D, D′ box, and antisense box are indicated

exon inclusion was found. In these mice, the wild-type exon Vb was substituted with an exon that had adenine-to-guanine mutations at the five editing sites (Kawahara et al. 2008). This suggests the presence of an activity in neurons that promotes exon Vb inclusion and can overwrite the influence of a splicing silencer.

Exon Vb harbors an 18 nt sequence that exhibits full complementarity to the antisense box of a small nucleolar RNA (snoRNA), HBII-52. The official name of this snoRNA is SNORD115, but the historical name HBII-52 for "second human brain library, clone number 52," is widely used in the literature. The snoRNA HBII-52 is expressed only in neurons (Cavaille et al. 2000), and its coexpression with 5 HT$_{2C}$ splicing reporter constructs promotes exon Vb inclusion in cell culture based assays (Kishore and Stamm 2006a). This finding suggested that the snoRNA HBII-52 is involved in splice site selection of the 5-HT$_{2C}$ receptor pre-mRNA.

21.4 Small Nucleolar Rnas

21.4.1 Traditional View of Snornas

Small nucleolar RNAs are small, noncoding RNAs. Based on their sequence, they can be subdivided into C/D and H/ACA snoRNAs. C/D box snoRNAs have C and D boxes as characteristic sequence elements that help form the snoRNA particle, or snoRNP. Small nucleolar RNAs reside in introns from which they are released during pre-mRNA processing of the hosting gene. During the splicing reaction, [AU2] the intron is released as a lariat structure that contains a 2′ to 5′ phosphodiester bond at the adenosine branch point. The lariat is opened by a debranching enzyme, and the intron is typically rapidly removed by nuclease action. If the intron contains snoRNAs, proteins associate with the snoRNA sequences and prevent their further degradation. As a result, the snoRNA that "resides" in an intron is released as a snoRNP.

A major function attributed to C/D box snoRNAs is their guiding of 2′-*O*-methylation in ribosomal, transfer, and snRNAs. The guiding activity of the snoRNAs is achieved by the formation of a specific RNA:RNA duplex between the snoRNA and its target. Most snoRNAs contain two regions to interact with other RNAs, the antisense boxes. Each antisense box exhibits sequence complementarity to its target and forms a short, transient double strand with it. On the target RNA, the nucleotide base pairing with the snoRNA nucleotide positioned five nucleotides downstream of the snoRNA D box is methylated on the 2′-*O*-hydroxyl group (reviewed in Matera et al. 2007). Several snoRNAs show complementarity towards pre-rRNA, but the rRNA is not 2′-*O*-methylated at the predicted positions (Steitz and Tycowski 1995). Recently, numerous C/D box snoRNAs were discovered that show no sequence complementarity to other RNAs, suggesting that C/D box snoRNAs might have function other than 2′-*O*-methylation (Filipowicz and Pogacic 2002). Furthermore, bioinformatics analysis of high-throughput sequencing data provided evidence for shorter forms of snoRNAs, suggesting that snoRNAs could be precursors for miRNA-like nuclear RNAs (Taft et al. 2009; Scott et al. 2009).

21.4.2 Small Nucleolar Rnas Missing in the Prader–Willi Syndrome

HBII-52 is a neuron-specific C/D box snoRNA (Cavaille et al. 2000). The snoRNA resides in the SNURF-SNRPN locus (Fig. 21.3a). Loss of expression from this locus is the most likely cause for Prader–Willi syndrome (PWS) (Butler et al. 2006).

In contrast to most other C/D box snoRNAs, HBII-52 contains only a single antisense box. This antisense box exhibits sequence complementarity to exon Vb of the 5-HT_{2C} receptor and is phylogenetically highly conserved (Nahkuri et al. 2008). In most species that express HBII-52, clusters of this snoRNA are expressed.

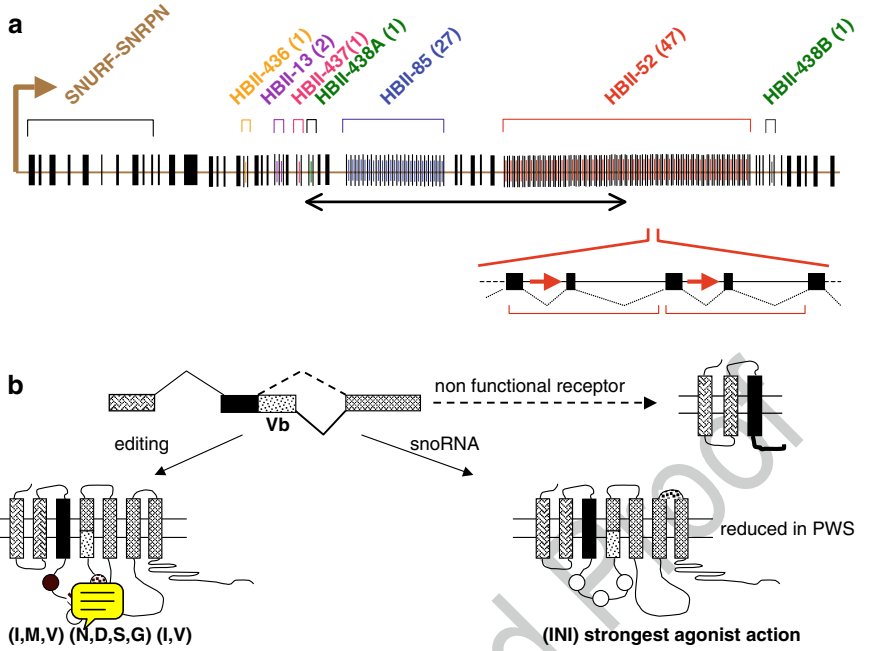

Fig. 21.3 5-HT$_{2C}$ receptor and HBII-52 snoRNA. (**a**) Small nucleolar RNAs are generated from the SNURF-SNRPN locus. The *SNURF-SNRPN* gene, located in the Prader–Willi critical region is schematically shown. Exons are shown as boxes, introns as horizontal lines. The bracket labeled SNURF-SNRPN indicates the protein-coding part of the pre-mRNA. Small nucleolar RNAs are located between noncoding exons and are shown as shorter and lighter vertical lines. Their names are indicated on top of the gene structure. A magnification of two snoRNAs (arrows) from the HBII-52 cluster is shown as an enlargement. A thick line with double *arrows* shows the microdeletion that causes PWS in one patient (Sahoo et al. 2008). (**b**) Model for HBII-52 action on the serotonin receptor pre-mRNA. Exon Vb is alternatively spliced. Skipping of the exon leads to a premature stop codon and is predicted to generate a nonfunctional receptor. However, it is not clear whether this nonfunctional protein is formed, since the mRNA is predicted to undergo nonsense mediated decay. Editing of the pre-mRNA promotes exon Vb inclusion but changes the amino acid composition in the second intracellular loop that couples to the G protein. The three positions that are changed by editing are indicated with circles, and the possible amino acids are shown below. The snoRNA promotes inclusion of the exon without editing and leads to a receptor that has the amino acids INI (isoleucine, asparagine, isoleucine) at the positions. This receptor has the strongest agonist response, and its expression is reduced in people with Prader–Willi syndrome

For example, humans posses 47 HBII-52 copies flanked by noncoding exons. The exon–snoRNA–exon structure is arranged in tandem (Fig. 21.3a). Each of the copies exhibits sequence complementarity to exonVb of the Serotonin receptor $_{2C}$ mRNA. The corresponding mouse MBII-52 snoRNAs are expressed throughout the mouse brain. They are most abundant in hippocampus, but absent in choroid plexus and some thalamic nuclei (Rogelj et al. 2003). The expression of MBII-52 is upregulated during early memory consolidation in the hippocampus (Rogelj et al. 2003). This indicates that snoRNAs could convey a “memory signal.”

ExonVb of the serotonin receptor is expressed throughout the brain, but it is mostly absent in the choriod plexus. In contrast, the snoRNA MBII-52 is expressed throughout the brain, but is absent in the choroid plexus, indicating a correlation between HBII-52 expression and exon Vb usage. We therefore analyzed the influence of HBII-52 on 5-HT_{2C}R pre-mRNA processing. In these experiments, the snoRNA and a 5-HT_{2C} receptor splicing reporter were transiently expressed in cell lines. This allowed to analyze the influence of HBII-52 on exon Vb inclusion of reporter. We found that HBII-52 promotes usage of exon Vb. In addition, a transient binding of the snoRNA to the 5 HT_{2C} receptor RNA could be detected by UV cross-link followed by RT-PCR (Kishore and Stamm 2006a). These experiments suggested that the snoRNA HBII-52 can promote inclusion of exon Vb and could be a factor that "overwrites" the splicing silencing element in this exon.

21.4.3 Mechanism of Snorna Acting on the Serotonin Receptor

The mechanism used by snoRNPs to change splice site selection is not obvious, as snoRNPs are mainly located in the nucleolus and splicing takes place in the nucleoplasma. However, snoRNAs are generated in the nucleoplasma during the splicing reaction and intron release. They can therefore contact pre-mRNA during their generation. In addition, snoRNAs share some proteins with the splicing machinery. For example, the 15.5 K protein that was originally identified as part of a the C/D box snoRNP complex where it binds to a conserved kink turn binds also to a similar structure in the U4 snRNA where it interacts with the splicing factor hPrp31 (Liu et al. 2007). This raises the possibility that the 15.5 K protein bound to snoRNPs interferes with the U4/U6 rearrangement during the splicing reaction by interacting with hPrp31.

Insight into the mechanism came from experiments that analyzed the RNAs from a single HBII-52 expression unit by RNase protection analysis, which directly quantifies the expressed RNAs. The data indicated that the HBII-52 expression unit generates several RNAs. Mutation studies showed that these shorter RNAs are only made when their precursor snoRNA contains intact C and D boxes. This indicates that they are most likely generated by further processing of the snoRNA and were therefore termed *psnoRNAs* (for processed snoRNAs) (Kishore et al. 2010). The main product of the 48 HBII-52 expressing units that are missing in PWS is therefore not a C/D box snoRNA but a psnoRNA that lacks several nucleotides at the ends. This shorter version lacks the stem of the snoRNA that is crucial for the assembly of a functional snoRNPs but still contains the antisense box needed for targeting to pre-mRNA. In addition to this form, three other shorter RNAs (60 to 37 nt) could be detected. The psnoRNAs were present in the nucleoplasma, where they could interact with pre-mRNA. The analysis of the protein composition showed that the RNAs associate with hnRNPs commonly implicated in splice site regulation but not with the known structural C/D box snoRNA proteins or the 2′-*O*-methylase (Kishore et al. 2010). This strongly

suggest that HBII-52 has a role different from C/D box snoRNAs that function in 2′-*O*-methylation of RNA.

As the major HBII-52 psnoRNA form still contains the antisense box that targets the serotonin receptor exon Vb sequence, it is likely that this RNA form brings processing factors to this exon, similar to a bifunctional oligonucleotide. Studies of miRNAs, Dscam selector RNA or U1 snRNAs showed that RNA:RNA interactions can tolerate multiple mismatches towards their targets. This indicates that HBII-52 could also regulate other splicing events.

A bioinformatic analysis predicted about 220 alternative exons that have evolutionary conserved sites that exhibit limited complementarity to the antisense box. Five of these exons were regulated by HBII-52 expression (Kishore et al. 2010). In each of the identified exons there were three mismatches between the 18 nt antisense element and the target RNA, which is reminiscent of U1, where the majority of 5′ splice sites has a mismatch in two of the nine possible bases.

Together, these data indicate that HBII-52 derived RNAs promote exon Vb inclusion either by recruiting other pre-mRNA processing factors to 5-HT_{2C} pre-mRNA or by forming novel small RNAs that interfere with the splicing process.

21.5 Prader–Willi Syndrome

21.5.1 Organization of Sno[illegible] in the Prader–Willi Critical Region

The HBII-52 snoRNA resides in the SNURF-SNRPN locus (for small RNP in neurons [SmN] upstream reading frame). The SNURF-SNRPN locus whose loss of expression causes PWS spans more than 460 kb and contains at least 148 exons (Runte et al. 2001) (Fig. 21.3a). The locus is maternally imprinted, meaning that only the allele from the father is expressed. The loss of expression from this paternal allele, most frequently through genomic deletions causes PWS. The SNURF-SNRPN locus has a complex architecture. Ten exons in the 5′ part of the gene are transcribed into a bicistronic mRNA that encodes the SNURF (SmN upstream reading frame) and the SmN (small RNP in Neurons) protein. Adjacent to the SNURF-SNRPN gene is a bipartite imprinting center (IC) that silences most maternal genes of the PWS critical region. The large 3′ UTR region of the SNURF-SNRPN gene harbors clusters of the C/D box snoRNAs HBII-85 and HBII-52 that are present in at least 24 and 47 copies, respectively. In addition, the region harbors single copies of other C/D box snoRNAs: HBII-13, HBII-436, HBII-437 HBII-438A, and HBII-438B. The snoRNAs are flanked by noncoding exons and show a large degree of conservation between mammalian species. Their flanking, noncoding exons are only poorly conserved, suggesting that the snoRNAs are important, not the flanking exons (Fig. 21.3a). The snoRNAs in this locus show a tissue-specific expression. Expression of HBII-52 could be detected only in brain, whereas other snoRNAs are

expressed also in nonbrain tissues (reviewed in Kishore and Stamm 2006b). A link between PWS and snoRNAs was supported by the recent finding that a microdeletion containing only snoRNAs, including all the HBII-85 and most of the HBII-52 cluster leads to the PWS phenotype (Sahoo et al. 2008) (Fig. 21.3a). The accumulated data strongly suggest that the loss of snoRNA expression from the SNURF-SNRNP region plays a decisive role in PWS.

21.5.2 *Features of Prader–Willi Syndrome*

Prader–Willi syndrome is a congenital disease with an incidence of about 1 in 8,000 to 20,000 live births. Prader–Willi syndrome is the most common genetic cause of marked obesity in humans. The excess weight causes type II diabetes as a major complication. This makes PWS the most frequent genetic cause for type II diabetes (Butler et al. 2006). Early PWS is characterized by a failure to thrive, feeding difficulties and hypogonadism. Later, the patients are characterized by short stature and develop mild to moderate mental retardation, behavioral problems and hyperphagia that leads to severe obesity. Children with PWS show low levels of growth hormone, IGF-I, and insulin as well as elevated levels of ghrelin (Eiholzer et al. 1998a, b; Cummings et al. 2002) and often exhibit central adrenal insufficiency (de Lind van Wijngaarden et al. 2008). Subsequently, growth hormone substitution was approved for treatment of children with PWS (Carrel et al. 2006). The growth hormone substitution represents to date the only pharmaceutical therapy and is successful in weight management.

[AU3]

21.5.3 *Serotonin Receptor in Patients with Prader–Willi Syndrome*

HBII-52 snoRNA expression has not been detected in people with Prader–Willi syndrome (Kishore and Stamm 2006a; Cavaille et al. 2000). This raises the question whether these patients also exhibit an imbalance in the serotonin mRNA isoforms. Therefore, brain samples from patients with Prader–Willi syndrome were analyzed by RT-PCR. By using primers that specifically recognize the five editing sites, the 5-HT_{2C} mRNA isoforms could be compared between Prader–Willi patients and age-matched controls. The experiments indicated a significantly reduced editing in three of the four tested sites when the same brain regions are compared. This suggests a reduced expression of the nonedited pre-mRNA in Prader–Willi patients. However, due to the intrinsic problems with human tissues, the protein composition could not be analyzed. These data support a model where HBII-52 promotes exon inclusion of the nonedited exon Vb (Fig. 21.3b).

Exon Vb encodes the second intracellular loop of the receptor that couples to G proteins. Editing changes the amino acids in this loop and alters the receptor

properties. The nonedited version of the receptor shows the highest efficacy towards serotonin. Changing the amino acids through editing generates multiple receptor isoforms with 10- to 100-fold lower efficacy (Wang et al. 2000). These conclusions have been derived from studies performed cell culture. The studies have been recently recapitulated in a knock-in mouse model (Kawahara et al. 2008). Mouse lines were engineered where the wild-type 5-HT_{2C} receptor allele was exchanged with an allele generating only the fully edited receptor version. In the mutant allele all five adenosine residues were replaced by guanine residues, which are similar to the inosine residues generated by editing. The mice harboring the fully edited VGV allele of serotonin receptor 2C showed growth retardation, an increased energy expenditure, and a constitutively activated sympathetic nervous system, as well as hyperphagia (Kawahara et al. 2008). These mice did not express any nonedited INI allele. These findings were confirmed by a second, similar mouse model (Morabito et al. 2010). Together, these data indicate the importance of the physiological balance of 5-HT_{2C} splice variants. Some aspects of the phenotype, such as hyperphagia and growth retardation, correlate with the PWS phenotype (Morabito et al. 2010).

21.5.4 Is There a Link Between HBII-52 Expression and Hunger Control?

A molecular link between a defect in the 5-HT_{2C} production and PWS is an attractive hypothesis, as the 5-HT_{2C} receptor plays a crucial role in hunger control and satiety, which is the major problem in PWS. Since HBII-52 promotes the generation of the most active receptor, it acts like a "genetic agonist" of the serotonin receptor. The administration of selective 5-HT_{2C}R agonists, such as d-fenfluramine has a strong appetite-suppressing effect (Vickers et al. 2001). Underlining the importance of the 5-HT_{2C} receptor for hunger control, the mouse knockout of 5-HT_{2C}R is hyperphagic and develops obesity. Expression of the 5-HT_{2C}R in the arcuate nucleus, a major hunger control center reverses the hyperphagic phenotype (Xu et al. 2008). As mentioned above, when a mutant of the receptor that represents the fully edited 5-HT_{2C}R is expressed in knockout mice, the resulting mice remain hyperphagic (Kawahara et al. 2008; Morabito et al. 2010). Collectively, the data strongly support a model where the loss of HBII-52 causes a loss of the mRNA isoform that encodes the most active form of the receptor, which is necessary for proper hunger control.

21.6 Conclusion

Together, the data indicate that the snoRNA HBII-52 contributes to alternative splicing regulation of the 5-HT_{2C} receptor. The exact molecular mechanism of the regulation is currently unveiled. The HBII-52 snoRNA is processed into smaller

snoRNA fragments (Kishore et al. 2010) that most likely directly influence the 5-HT_{2C} receptor pre-mRNA. Finally, it is likely HBII-52 controls other pre-mRNAs, and it is therefore expected that the 5-HT_{2C} receptor mRNA is not the only deregulated RNA in PWS. However, since mouse models of the 5-HT_{2C} receptor mutants recapitulate some aspects of PWS, we expect that the dysregulation of the receptor plays a decisive role in this disease.

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[AU7]