RNA splicing, a post-transcriptional process necessary to form a mature mRNA, was discovered in the late 1970s. Two different modes of splicing have been defined, that is, constitutive splicing and alternative splicing. Constitutive splicing is the process of removing introns from the pre-mRNA, and joining the exons together to form a mature mRNA. Alternative splicing, on the other hand, is the process where exons can be included or excluded in different combinations to create a diverse array of mRNA transcripts from a single pre-mRNA and therefore serves as a process to increase the diversity of the transcriptome. The estimated number of alternative splicing events in the human transcriptome has risen sharply over the past decades. In the 1980s, it was thought that about 5% of human genes were subjected to alternative splicing. In 2002, this number had risen to 60%, and now, after implementation of next-generation-sequencing technologies, we know that the vast majority, >95% of mRNAs, are subjected to alternative splicing. Nevertheless, the function of a large fraction of these splice isoforms remains to be elucidated. Furthermore, it is anticipated that in different tissues, or in tissues with different disease states, new isoforms still remain to be identified.

The process of splicing is highly conserved during evolution. Splicing is more prevalent in multicelluar than in unicellular eukaryotes because of the lower number of intron-containing genes in the latter. Later in evolution, alternative splicing becomes more prevalent in vertebrates than in invertebrates. Interestingly, just a single exon-skipping event in the RNA-binding protein (RBP), polypyrimidine tract binding protein 1 has been shown to direct numerous alternative splicing changes between species, indicating that a single splicing event can amplify transcriptome diversity between species. The recent observation that the total number of protein-coding genes does not differ much between species, fueled the hypothesis that alternative splicing largely contributes to organism diversity. And indeed, as we move up the phylogenetic tree, alternative splicing complexity increases, with the highest complexity in primates.

The aim of this review is to give a comprehensive overview of all aspects of constitutive and alternative splicing and their regulators, as well as the importance of these different aspects in human disease, with a focus on the heart. In addition, we discuss possible therapeutic interventions and try to uncover potential future directions of research.
analyses of purified human spliceosomal complexes have indicated that hundreds of proteins can associate with the spliceosome during the splicing process.11 These proteins play critical roles not only in the recognition of the splice sites but also in proper positioning of the pre-mRNA for catalysis. In the mid 1990s, 2 decades after the discovery of splicing, Tarn and Steitz12 identified a second spliceosome. This second spliceosome was called the minor spliceosome and targets a minor class (<1%) of introns (Figure 2). The minor spliceosome is functionally analogous to the major spliceosome but differs in the use of snRNAs (minor snRNAs are U11, U12, U4atac/U6atac, and U5). Because the major spliceosome uses the U2 small nuclear ribonucleoprotein (snRNP) and the minor spliceosome the U12 snRNP, major introns are called U2-type introns and minor introns are U12-type introns. Although the minor spliceosome functions in a similar way as the major spliceosome, there are some distinct differences. First, the sequences of the 5' splice site, 3' splice site, and the branch point sequence differ between the 2 spliceosomes (Figure 2B and 2C).13 Second, splicing conducted by the major spliceosome occurs strictly in the nucleus, whereas splicing by the minor spliceosome occurs mostly in the cytoplasm.14 Third, minor splicing has a considerably slower rate of splicing and can therefore be the rate-limiting step in the maturation of an mRNA.15 Fourth, minor splicing rarely produces alternative isoforms16 and seems mostly used as a mechanism to splicing is active during mitosis and important for cell pro- 

division, the nuclear envelope breaks down, minor splicing is still available. In line with this, it has been shown that 

minor splicing is not a substitute for major splicing, but rather a separate process. Perhaps it serves as a final check of the mRNA in the cytoplasm, and thereby post-transcriptionally controls expression of a specific subset of genes. Even though the minor spliceosome is much less abundant than the major spliceosome,10 its importance is underlined by the fact that mutations in the minor spliceosome snRNAs U4atac lead to severe developmental disorders such as microcephalic osteo-
dysplastic primordial dwarftism type 1.19 Interestingly, it was recently shown that usage of the minor spliceosome is rapidly increased by the activation of cell-stress activated kinase p38 mitogen-activated protein kinase (MAPK) through stabilization of U6atac.17 This resulted in increased expression of hundreds of minor intron-containing genes. Because mRNAs with retained minor introns are often degraded through the nonsense-mediated decay (NMD) pathway (Figure 2A), the activation of p38 MAPK may trigger minor spliceosome usage and thus control gene expression in response to stress.

**Different Types of Alternative Splicing**

Alternative splicing is the process where the spliceosome chooses to include or exclude specific parts of the mRNA. Four modes of alternative splicing are generally observed: (1) exon skipping, (2) mutually exclusive exon usage, (3) alternative splice site selection, and (4) intron retention (Figure 3A). Exon skipping, the most prevalent form of alternative splicing in higher eukaryotes,20 denotes the excision of ≥1 exons and its surrounding introns from a pre-mRNA. Mutually exclusive exon usage represents a form of exon skipping where either one or another exon, but never both, is included in the mature mRNA. Alternative splice site selection relies on the possibility of using different splice sites at the 5' and 3' end of an exon, resulting in longer or shorter exons from the same transcript. The last form is intron retention, a process in which (part of) an intron is retained in the mature mRNA transcript.

These different splicing events can have multiple functional consequences. When the splicing event occurs in the coding region of the mRNA, the most obvious effect is an isoform switch, leading to altered or even opposing functions of the protein. Second, alternative splicing can result in mRNA isoforms with premature stop codons that are degraded through the NMD pathway. Although the NMD pathway was originally identified as an error surveillance pathway, it has become clear that NMD also serves to regulate gene expression. As such, alternative splicing–induced NMD provides a means to control protein expression.21 Finally, alternative splicing events that occur in the 5' or 3' untranslated regions (UTRs) may lead to altered UTRs, which in turn can interfere with mRNA stability, microRNA accessibility, and mRNA localization.

Apart from alternative splicing, there are 2 other processes that can generate multiple mRNA transcripts from a single pre-mRNA or gene: alternative polyadenylation (APA) and alternative first exon usage (or alternative promoter usage; Figure 3B). Eukaryotic pre-mRNAs are processed at their 3'end by cleavage and the addition of a poly(A) tail. Interestingly, most eukaryotic mRNAs have multiple polyadenylation sites, and genomic studies in recent years have indicated that ≈70% of human genes generate alternative mRNA isoforms that differ in length at the 3' end by a process called alternative cleavage and polyadenylation.22 APA
isoforms generally differ in their 3′ UTR, but in about one third of the cases, they also differ in coding sequences.23 Because the 3′ UTR generally harbors functional domains such as microRNA or RBP-binding sites, altered 3′ UTR length by APA may have profound effects on gene expression.24 Alternative first exon usage occurs when the transcriptional machinery starts at a different promoter and leads to different mRNA or protein isoforms at the N terminus. All 3 processes, alternative splicing, APA, and alternative first exon usage, seem to be codependent, but how these processes are functionally linked remains to be elucidated.4,25,26 Nevertheless, APA and alternative first exon usage are, although similar in outcome to alternative splicing, technically not alternative splicing as they are not mediated by the spliceosome.

**Splicing and Noncoding RNAs**

Apart from splicing of protein-coding genes, also long noncoding RNAs (lncRNAs) are subjected to alternatively splicing although to a lesser extent.27 This could be because of the smaller average number of exons in lncRNAs. Interestingly, the production of many noncoding RNAs such as microRNAs and small nucleolar RNAs (snoRNAs) relies on splicing. These noncoding RNAs reside in introns and are spliced out of their host transcript, after which they are processed.28 The expression of these noncoding RNAs is closely linked to the expression of their host mRNAs, and for snoRNAs, it has been suggested that their host mRNAs are merely a byproduct of the snoRNA production process.29 Also, many different noncoding RNAs (including snoRNAs, lncRNAs, and sno-lncRNAs) are being recognized as regulators of alternative splicing. The lncRNA MALAT1, for example, has been shown to regulate the expression level, localization, and activity of SR proteins (serine/arginine-rich proteins).30 It has been suggested that MALAT1, at least in part, works as a molecular sponge for SR proteins. Regulation of MALAT1 thereby indirectly regulates splicing of SR protein targets.

Another example is the formation of circular RNAs. Circular RNAs were long thought to be an accidental byproduct of splicing, but turn out to be highly regulated, suggesting specific functional roles for this new class of RNAs.31 Circular RNAs are synthesized by back-splicing, a form of splicing where a 5′ splice site of an exon is ligated to the 3′ splice site of an upstream exon, thereby creating a circular RNA. Obviously, relatively unknown forms of splicing, like back-splicing, could come into view as new regulators of biological processes.

**Regulation of Splicing**

Alternative splicing is a dynamic and regulated process and can be influenced by an array of variables such as cis-regulatory sequences and trans-acting factors, the transcriptional process, and DNA/RNA methylation. Together, these regulatory RNA features make up the splicing code, a code that determines alternative splicing events or patterns.32 Currently, ≈1500 RBPs have been identified in humans.33 RBPs act as regulators of diverse post-transcriptional processes including constitutive and alternative splicing, mRNA transport and localization, mRNA stability, microRNA inhibition, and mRNA translation. Probably the most well-described RBP families are the SR protein family and the heterogeneous nuclear ribonucleoprotein (hnRNP) protein family. Most
Figure 2. Major and minor splicing. A, Major and minor splicing. The major introns are spliced out, and minor introns are either retained (and the mRNA is most often subsequently degraded) or the minor intron is spliced out, and a mature mRNA is formed. B, The 4 basic splicing signals are the 5′ splice donor site, the 3′ splice acceptor site, the branch point sequence (BPS), and the polypyrimidine tract (PT). Spliceosomal components recognize and bind to these sequences and mediate the splicing reaction. Intronic and exonic splicing enhancers and silencers determine the inclusion rate of exons. The BPS (major, YNYURAY; minor, UCCUUAACU) is located 20 to 50 bp upstream of the 3′ splice site, and the PT (Y10–12) is located in between the BPS and the 3′ splice site (N=any nucleotide, Y=C or U, R=A, or G and S=C or G). C, Minor splicing uses different 5′ and 3′ splice sites and BPS, and lacks the PT. ESE indicates exonic splicing enhancers; ESS, exonic splicing silencers; ISE, intronic splicing silencers; and ISS, intronic splicing silencers.
proteins from these families are ubiquitously expressed and serve critical roles in spliceosomal assembly. SR proteins are characterized by the presence of at least 1 RNA recognition motif and a RS domain. The RNA recognition motif domain is required for RNA-binding, whereas the RS domain functions as a protein interaction domain. Next to the ≈20 described SR proteins, there are many additional RS domain–containing proteins referred to as SR-related proteins.34 The hnRNP protein family is named after its association with hnRNAs, a historical term that is synonymous for pre-mRNA. hnRNP proteins have at least 1 RNA-binding motif, and at least 1 additional functional domain responsible for the regulation of, for example, protein–protein interactions or cellular localization.35 However, because of the similar properties and function of SR and hnRNP proteins, the distinction between the 2 families has blurred over the years. In addition to the ubiquitously expressed RBPs from the SR- and hnRNP protein families, there are numerous tissue-specific or tissue-enriched RBPs. Although most RBPs from the SR- and hnRNP protein families are thought to be redundant, the combination of different

Figure 3. Different types of alternative splicing. A, The different types of splicing are depicted. The process of excising introns and joining exons together is constitutive splicing. Exon skipping is the inclusion or exclusion of one or more exons (known as cassette exons). Cassette exons can be mutually exclusive. Alternative 5’ or 3’ splice site (SS) selection results in shorter or longer exons. Intron retention is the inclusion of (part of) an intron in the mature mRNA. B, Other ways to generate multiple different mRNA transcripts from a single gene are alternative first exon usage and alternative polyadenylation.
RBPs (including tissue-specific RBPs) in the spliceosome determines its specificity in the recognition of alternative exons. The expression of different splice factors has profound effects on the outcome of alternative splicing. There are numerous examples where the balance of different splice factors is regulated in a cell/tissue-specific, developmental, or disease-specific manner. The balance between these splice factors then determines the inclusion or exclusion of specific exons. Perhaps the best example is the antagonistic function of the muscleblind and CUG-BP and ETR-3-like factor (CELF) protein families. During development, muscleblind and CELF protein levels determine the inclusion of fetal exons and the exclusion of adult exons in a set of genes. Besides regulating the expression level of RBPs, their activity can also be controlled (e.g., through phosphorylation), and this also affects pre-mRNA or alternative splicing.

The degree at which an mRNA is alternatively spliced also depends on the usage of strong and weak splice sites. Strong splice sites generally lead to constitutive splicing, as they are always used by the spliceosome. Usage of weak splice sites depends on factors such as splice site sequence, splice site position, and bound splice factors. For example, cis-regulatory sequences known as intronic splicing enhancers, intronic splicing silencers, exonic splicing enhancers, and exonic splicing silencers can be bound by trans-acting RBPs, which in turn recruit spliceosomal components. Based on the sequence and the position of the site in the pre-mRNA, trans-acting RBPs can both enhance or decrease the inclusion of exons. Interestingly, splice sites can be altered by RNA editing, a post-transcriptional process by which cells can make discrete changes to specific nucleotides within an RNA molecule. The proteins responsible for this process are adenosine deaminases that act on RNA (ADARs), and by controlling RNA editing, these proteins are able to regulate splicing indirectly, by affecting splice site sequences.

In addition, the transcription process itself can have a fundamental effect on alternative splicing. Unlike what has been thought for many years, splicing does not occur after transcription, but happens during transcription. As such, the vast majority of human introns are spliced out when transcription is still taking place. The pre-mRNA, therefore, represents a virtual entity. Apart from happening simultaneously, it becomes increasingly clear that also mechanistically, transcription and splicing are linked processes. The 2 mechanisms that underlie the codependence of the processes are recruitment coupling and kinetic coupling. Recruitment coupling is based on the capacity of the transcriptional machinery to recruit RBPs that are also shared by the splicing machinery. For example, it is known that the carboxy-terminal domain of RNA polymerase II (Pol II) recruits serine/arginine-rich splicing factor (SRSF)-3, an SR protein involved in the regulation of alternative splicing of fibronectin. Kinetic coupling relies on the speed at which Pol II transcribes the DNA (elongation rate), thereby influencing the availability of weak and strong splice sites. Although Pol II transcribes DNA, the pre-mRNA becomes available to the spliceosome, even when transcription is not finished. If Pol II-mediated elongation is slow, and a weak splice site becomes available, the splicing machinery will make use of the weak splice site. If, however, elongation is fast and a weak and strong splice sites become available more or less simultaneously, the splicing machinery will favor the strong splice site over the weak splice site. Several examples of this phenomenon exist, but the most compelling evidence is provided by the use of mutant slow Pol II, resulting in a slower elongation rate and altered exon usage of fibronectin.

The fact that Pol II elongation rates influence alternative splicing has led to the hypothesis that epigenetic modifications could also influence alternative splicing because these modifications can influence chromatin structure and therefore the elongation rate of Pol II. Recently, it has also been shown that DNA methylation is enriched in exons when compared with their flanking introns, thereby suggesting that epigenetic modifications play a role in splicing. As it turns out, DNA methylation can be linked to alternative splicing of ≈20% of alternatively spliced exons, both through recruitment coupling and kinetic coupling. With respect to kinetic coupling, Shukla et al demonstrated that methylation of a CCCTC-binding factor (CTCF) site in the human CD45 gene determined the inclusion rate of exon 5. When the CTCF site is not methylated, CTCF can bind and serve as a roadblock for Pol II, effectively slowing down the elongation rate. This, in turn, results in exon inclusion. In contrast, when the CTCF site in exon 5 is methylated, CTCF binding is inhibited, and the exon is excluded, demonstrating that Pol II elongation rates are influenced by DNA methylation status. Nucleosome positioning and histone modifications also affect (alternative) splicing. Nucleosomes are DNA packaging units that consist of histone proteins and a section of DNA. Interestingly, nucleosomes preferentially position at exons, with on average 1 nucleosome per exon. Introns are not devoid of nucleosomes, but the distribution of nucleosomes in introns is far more random. This has led to the idea that nucleosomes aid the splicing machinery to locate exons. The nucleosomes act as a speedbump to slow down elongation, which provides more time for the splicing machinery to recognize the 3′splice site. The fact that alternative exons are more enriched in nucleosomes than constitutive exons points toward a regulatory role for nucleosomes in alternative splicing as well. Like nucleosomes, histone modifications are also enriched on exons. For some histone modifications, this is the result of increased nucleosome density in exons, but even when corrected for nucleosome enrichment, some histone modifications (such as H3K36me3, H3K4me3, and H3K27me2) are still increased, whereas others are reduced (such as H3K9me3). Histone marks can influence alternative splicing both through kinetic and recruitment coupling. The link between DNA methylation and histone modifications is well investigated, and recently Yearim et al revealed that the methylation status of DNA regulates the recruitment of spliceosome components via the chromodomain-containing protein HP1, which was shown to bind directly to H3K9me3, a histone modification that is induced by DNA methylation. Besides the methylation of DNA, RNA methylation (Nd6-methyladenosine [m6A]) has emerged in recent years as an important factor in alternative splicing. In 2015, Liu et al showed that RNA methylation affects RNA secondary structure in such a way that m6A opens up the mRNA for interactions with RBPs.
Splicing Factors in the Developing Heart

Although most of the general mechanisms that control alternative splicing have not been investigated in a cardiac context (ie, minor spliceosome, coupling of transcription and splicing, and DNA methylation), it is becoming evident that alternative splicing plays a pivotal role in development, homeostasis, and disease of the heart.

Using a large-scale screen with splicing microarrays, Kalsotra et al. revealed 63 alternative splicing events in the developing mouse heart. Bioinformatic analysis of the introns flanking these splicing events identified enriched motifs for CELF and muscleblind proteins. Using transgenic and knockout mouse models, Kalsotra et al. subsequently show that CELF and muscleblind proteins determine more than half of the 63 observed developmentally regulated splicing events. During heart development, CELF proteins are downregulated >10-fold, whereas muscleblind proteins are upregulated 4-fold and it appears that the stoichiometric expression of these 2 proteins largely determines the temporal expression of numerous splice isoforms.

RNA-binding motif protein 24 (Rbm24) has recently been shown to be a major regulator of heart and skeletal muscle splicing events. Mice lacking Rbm24 die between E12.5 and E14.5 because of multiple cardiac malformations, including ventricular septum defects, reduced trabeculation and compaction, and dilated atria. Strikingly, sarcomerogenesis was almost completely abolished in knockout embryos. Transcriptome analysis of the hearts of mutant embryos revealed aberrant splicing of 68 Rbm24-dependent genes, of which several are important for cardiac development. Mice with aberrant splicing events had severely reduced trabeculation and a disturbed ratio of triadin isoforms 1 and 2. This is attributed to a dowregulation of triadin and calsequestrin2 and a disturbed ratio of triadin isoforms 1 and 2. Triadin and calsequestrin2 are involved in regulation Ca\textsuperscript{2+} release from the sarcoplasmic reticulum, and loss of SRp38 leads to an increase in Ca\textsuperscript{2+} sparks from the sarcoplasmic reticulum, which indicates a role for SRp38 in regulating Ca\textsuperscript{2+} handling. ASF/SF2 conditional knockout mice die 6 to 8 weeks after birth. Deletion of ASF/SF2 leads to missplicing of several genes, including Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CamKII\delta), cardiac troponin T (cTnT), and LIM-domain binding 3 (LDB3). Interestingly, aberrant alternative splicing of CamKII\delta, cTnT, and LDB3 presented 20 days after birth, even though ASF/SF2 was deleted at the early stages of cardiogenesis. This could mean that ASF/SF2 plays a critical role during the juvenile-to-adult transition period, but is dispensable in earlier stages. Missplicing of CamKII\delta in ASF/SF2 knockout hearts results in disturbed Ca\textsuperscript{2+} handling and severe excitation–contraction coupling defects, which in turn leads to dilated cardiomyopathy (DCM).

SR-rich splicing factor 2 (SRSF2 or SC35) is yet another SR protein that is expressed ubiquitously. Systemic deletion of SC35 in mice results in embryonic lethality, even before the onset of cardiogenesis. Circumventing this problem by generating a heart-specific knockout of SC35 uncovered the role of SC35 in the heart, as these mice developed cardiac hypertrophy and DCM at 5 to 6 weeks of age. Strikingly, the life-span of these mutant mice was not affected. The disease phenotype is associated with a downregulation of ryanodin receptor 2 (Ryr2) in the SC35 knockout hearts. Although an exact mechanism was not provided, the authors speculate that Ryr2 is misspliced and will therefore be decayed through the NMD pathway. In conclusion, ablation of SC35 in the heart shows that proper expression of this splice factor during postnatal heart development is essential to maintain cardiac function.

Little is known about the specific functions for hnRNP proteins in the heart, but conditional deletion of hnRNP U in the mouse heart results in severe DCM and is lethal at 2 weeks after birth. Interestingly, like SRSF1, SRSF2, and SRSF10, hnRNP U is important for proper splicing of Ca\textsuperscript{2+}-handling genes such as CamKII\delta, suggesting that alternative splicing of Ca\textsuperscript{2+}-handling genes is critical in early postnatal heart development.

Alternative Splicing in Disease

Aberrant alternative splicing can both be the cause and the consequence of disease and has been shown to be able to affect disease severity and susceptibility. Mutations in genes that are required for proper function of the spliceosome have been described as a cause for spinal muscular atrophy, retinitis pigmentosa, and Prader–Willi syndrome. Spinal muscular atrophy, for example, is caused by the loss of the survivor of motor neuron-1 (SMN1) gene, which is needed for proper assembly and transport of snRNP. Interestingly, even though SMN1 is ubiquitously expressed, the phenotype is restricted to motor neurons. It is not entirely clear why motor neurons are more sensitive to loss of SMN1-directed snRNP assembly and function, but it is likely that certain splice events are more critical for these cells. In the case of Prader–Willi syndrome, loss of snoRNAs and snoInRNAs encoded in the SNURF/SNRNP locus, cause mis-splicing of the serotonin 2C receptor and other targets, but newer lines of evidence suggest a role for these snoRNAs in RNA editing of the serotonin 2C receptor transcript as well.
Next to mutations in spliceosomal components, mutations in splice sites can have a disturbing effect on splicing. Interestingly, one study revealed that a remarkable amount of ≤15% of the known disease-causing mutations seem to affect splice sites in pre-mRNAs, instead of disrupting the protein coding region of an mRNA. However, this study was conducted in a time that knowledge of the splice code was incomplete, and it therefore disregarded a vast number of mutations that potentially cause splicing abnormalities. Hence, it has been suggested that 15% may be a gross underestimation of the real number, and that the percentage of disease-causing mutations that cause splicing abnormalities, both in splice sites and in splicing-associated genes, is closer to 60%.1-3

Studies on alternative splicing changes in the heart have revealed large differences between development or disease states. Intriguingly, much like reactivation of selected fetal genes, some fetal splice isoforms are also re-expressed in the stressed or diseased heart. For example, splicing of the sarcomeric proteins titin and myomesin is regulated during development, and their fetal isoforms are re-expressed in the stressed heart.4 Apart from the re-expression of fetal isoforms, it is known that alternative splicing is broadly altered in cardiac hypertrophy and disease. Cardiac hypertrophy can be physiological, reversible, and adaptive or progress to pathological hypertrophy with irreversible and maladaptive changes. The precise molecular mechanisms that distinguish the 2 forms are still not entirely understood, but it has become clear that the alternative splicing profiles of the 2 forms are different.5 In hypertrophied and failing mouse hearts, alternative splicing profiles are likewise altered, with the largest differences in failing hearts.6 In humans, Kong et al7 were the first to use a genome-wide approach to study alternative splicing changes in the diseased heart. The splicing profile of diseased and control hearts differed extensively, and subsequently, the authors were able to correctly assign samples to control or disease based solely on the splicing profile. Furthermore, splicing of 4 key sarcomeric genes, troponin T (TNNT)-2, TNNI3, MYH7, and FLNC, were significantly altered in human ischemic cardiomyopathy, DCM, and aortic stenosis. In the pressure-overloaded heart, it even preceded the onset of heart failure (HF). In addition, the ratio of major:minor isoforms of only 3 of these genes, TNNT2, MYH7, and FLNC, was sufficient to correctly assign samples to control or disease with a >98% accuracy, which could be useful as a diagnostic tool.

Although not mediated by the spliceosome, a recent study from our group revealed that 3' end formation of mRNA through APA is also altered in HF. By generating genome-wide polyadenylation maps in the human heart, it was shown that subsets of genes displayed 3' UTR lengthening, whereas others displayed 3' UTR shortening. Interestingly, the genes with altered 3' UTR length were often dysregulated in failing hearts, with an inverse correlation between 3' UTR length and the level of gene expression. This suggests that, in addition to alternative splicing, APA-mediated isoform switches also represent an important layer of gene regulation in HF.8

Splice Factors in the Diseased Heart

We are only beginning to understand the functions of different splice factors, and for many RBPs, their role in the normal and diseased heart has recently been unveiled (Table 1). Remarkably, not many mutations in splicing factors have been described that lead to human cardiac pathologies. To date, only mutations in the splicing factor RNA-binding motif protein 20 (RBM20) have been causally linked to heart disease.6-8 The lack of splice factors in the list of heart disease-causing genes could have multiple explanations. It could be that mutations in splicing factors are severe and embryonically lethal. Several mouse models in which a splicing factor has been knocked out (eg, Rbm24, SC35, SRp20, and SRp38) support this idea.6,7,6,79 Also, before the upcoming of next-generation sequencing, the search for candidate genes used to be hypothesis driven and often did not include splice factors, and consequently, genes encoding splice factors were simply not sequenced in affected patients. It has only recently become possible to look for candidate genes in a broader and often unbiased way, and splicing-related genes can be sequenced on a larger scale.

Nevertheless, in 2009, Brauch et al80 described mutations in RBM20 to be causal for familial DCM. Ever since, mutations in RBM20 have been found in multiple cohorts, being responsible for 3% to 5% of all familial DCM cases.77,78 Subsequently, a molecular mechanism that links RBM20 to alternative splicing of several pivotal cardiac genes including titin was identified by Guo et al.81 Rbm20-deficient rats with a cardiac phenotype that closely resembles the DCM in individuals with RBM20 mutations were analyzed. RNA sequencing of hearts of the Rbm20-deficient rat and a human RBM20 mutation carrier revealed a set of 30 RBM20-dependent alternatively spliced genes that were conserved between rat and human. One of the splicing events that depend on Rbm20 is that of titin’s spring region, and it is thought to be an important determinant of the DCM phenotype.80,81 In mice, the loss of Rbm20 results in a giant isoform of titin (N2BA-G), an increase in titin-based elasticity, and an impaired Frank-Starling mechanism (ie, the ability to increase contractile force with increased sarcomere length).82 Apart from alternative splicing events in titin, it is now known that RBM20 also regulates alternative splicing events in CamKIIδ, Ryr2, and Cacna1c.82 Loss of Rbm20 induces a CamKIIδ switch from CamKIIδ-B and CamKIIδ-C to 2 bigger isoforms (CamKIIδ-A and CamKIIδ-9). This potentially results in dysregulation of the normal function of CamKIIδ and may affect Ca2+-homeostasis and other functions of CamKIIδ. The alternative splicing events in Ryr2 and Cacna1c could likewise impact Ca2+-homeostasis, and together they may contribute to the increased risk of sudden cardiac death in RBM20 mutation carriers. Remarkably, RBM20 expression varies greatly in diseased human hearts, and its expression correlates with splicing of RBM20 target genes.82 This suggests that also in RBM20 mutation negative DCM, RBM20 may play a role in disease progression.

Upregulation of SF3B1, another splice factor, can be sufficient to induce heart disease. SF3B1, an Hif1α-inducible splicing factor, is upregulated in the diseased human and mouse heart and coordinates a shift in ketoheoxokinase
Ketohexokinase is the central fructose-metabolising enzyme and exists in 2 isoforms: ketohexokinase-A and ketohexokinase-C. During hypertrophy and failure, the heart switches toward more glycolysis at the expense of fatty acid metabolism, and the SF3B1-induced shift from ketohexokinase-A to ketohexokinase-C is both necessary and sufficient to enforce fructolysis in the cardiomyocyte. Intriguingly, heart-specific loss of SF3B1 or ketohexokinase prevents the metabolic switch and protects from pathological cardiac growth.

### Table 1. Characteristics and Known Function of Splice Factors in the Heart

<table>
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<tr>
<th>Splice Factor</th>
<th>Expression</th>
<th>Constitutive/Alternative Splicing</th>
<th>Splice Site/Motif</th>
<th>Comments</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Rbm20</td>
<td>Heart and muscle enriched</td>
<td>Alternative splicing</td>
<td>UCUU</td>
<td>Mutations in RBM20 cause DCM in humans Knockout models (mouse/rat) present with DCM</td>
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</tr>
<tr>
<td>Rbm24</td>
<td>Heart and muscle enriched</td>
<td>Alternative splicing</td>
<td>G/A/G/GUG</td>
<td>Mouse knockout is embryonically lethal because of impaired cardiogenesis</td>
<td>56</td>
</tr>
<tr>
<td>Rbfox1</td>
<td>Brain, heart, and skeletal muscle</td>
<td>Alternative splicing</td>
<td>(U)GCAUG</td>
<td>Knockdown in zebrafish results in cardiac and skeletal muscle abnormalities Expression is regulated during heart development Mouse knockout presents with aggravated pressure-overload–induced HF</td>
<td>37, 83, 84</td>
</tr>
<tr>
<td>Rbfox2</td>
<td>Ubiquitous</td>
<td>Alternative splicing</td>
<td>(U)GCAUG</td>
<td>Downregulated in the diseased mouse heart Heart-specific knockout develops DCM and heart failure Knockdown in zebrafish results in cardiac and skeletal muscle abnormalities</td>
<td>84, 85</td>
</tr>
<tr>
<td>SRSF1 (or ASF/SF2)</td>
<td>Ubiquitous</td>
<td>Constitutive and alternative splicing</td>
<td>5' splice site</td>
<td>Heart-specific knockout results in disturbed excitation–contraction coupling and DCM</td>
<td>59</td>
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<tr>
<td>MBNL1</td>
<td>Expressed in many tissues including brain, heart, and skeletal muscle</td>
<td>Alternative splicing</td>
<td>CUG repeats</td>
<td>Crucial for developmentally regulated splice isoforms Knockout presents with DM MBNL is sequestered to CUG repeats in mutant RNAs and fails to properly splice its targets</td>
<td>37, 38, 86</td>
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<td>CELF1 (or CUGBP1) and CELF2</td>
<td>Brain, heart, and skeletal muscle</td>
<td>Alternative splicing</td>
<td>UGU-containing pentamers</td>
<td>Crucial for developmentally regulated splice isoforms (CELF1 and CELF2) CELF1 phosphorylation by PKC is increased in DM and contributes to disease phenotype Transgenic mice present with DM Antagonistic to CELF</td>
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<tr>
<td>SF3B1</td>
<td>Ubiquitous</td>
<td>Alternative and constitutive splicing</td>
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<td>SF3B1 knockdown mouse is protected from pathological cardiac growth Upregulated in the diseased human and mouse heart Coordinates the metabolic switch in hypertrophic/failing heart</td>
<td>89</td>
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<td>SRSF10 (or Srp38)</td>
<td>Ubiquitous</td>
<td>Alternative splicing</td>
<td>GA-rich hexamers</td>
<td>Mouse knockout is embryonically lethal because of impaired cardiogenesis</td>
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<tr>
<td>SRSF2 (or SC35)</td>
<td>Ubiquitous</td>
<td>Constitutive and alternative splicing</td>
<td>Inclusion motif: UCCA/UG Exclusion motif: UGGA/UG</td>
<td>Heart-specific knockout results in cardiac hypertrophy and DCM Interacts with Tbx5 in Holt–Oram syndrome</td>
<td>60, 90, 91</td>
</tr>
<tr>
<td>PTB</td>
<td>Ubiquitous</td>
<td>Alternative splicing</td>
<td>Pyrimidine-rich sequences (eg, UCUUC or CUCUCU)</td>
<td>Knockout model is embryonically lethal before onset of cardiogenesis Potentially antagonistic to RBM24 Downregulated during heart development</td>
<td>56, 92, 93</td>
</tr>
<tr>
<td>Rbm25</td>
<td>Ubiquitous</td>
<td>Alternative splicing</td>
<td>CGGGCA</td>
<td>Upregulated in human heart failure and coordinates splicing of SCN5A</td>
<td>94*</td>
</tr>
<tr>
<td>Luc7l3</td>
<td>Ubiquitous</td>
<td>Alternative splicing</td>
<td>5' splice site</td>
<td>Upregulated in human heart failure, and coordinates splicing of SCN5A</td>
<td>94*</td>
</tr>
<tr>
<td>hnRNP U</td>
<td>Ubiquitous</td>
<td>Constitutive and alternative splicing</td>
<td>Not known</td>
<td>Heart-specific knockout is lethal because of severe DCM</td>
<td>61</td>
</tr>
</tbody>
</table>

ASF indicates alternative splicing factor; CELF, CUG-BP and ETR-3-like factor; DCM, dilated cardiomyopathy; DM, myotonic dystrophy; HF, heart failure; hn-RNP, heterogeneous nuclear-ribonucleoprotein; MBNL, muscleblind; PKC, protein kinase C; PTB, poly pyrimidine tract binding; RBM, RNA-binding motif protein; SF, splicing factor; and SRSF, serine/arginine-rich splicing factor.

*These articles describe the upregulation of 17 splice factors, among which are Rbm25 and Luc7l3, in human heart failure. Only Rbm25 and Luc7l3 were molecularly characterized, but the other 15 splice factors likely play a role in the heart as well.
Members of the FOX-protein family are also dysregulated in heart disease. Rbfox1 expression decreases in failing human and mouse hearts, and the loss of Rbfox1 aggravates pressure-overload–induced HF in mice. Splicing analysis revealed an isoform switch in the myocyte enhancer factor-2 (Mef2) gene family, involving the mutually exclusive exons α1 and α2, which interferes with the transcriptional activity of Mef2. Remarkably, re-expression of Rbfox1 in pressure-overloaded mouse hearts attenuates cardiac hypertrophy and failure.

Expression of Rbfox2 is also decreased in the pressure-overloaded mouse heart, and conditional deletion of Rbfox2 leads to DCM and HF. Splicing analysis of both pressure-overloaded hearts and Rbfox2−/− hearts revealed an enrichment in developmentally regulated splicing events. Strikingly, these splicing events were reversed on loss of Rbfox2, suggesting a role for Rbfox2 in the decompensatory phase during heart disease.

**Mutations in Splice Sites Leading to Human Heart Disease**

There are currently only few examples of mutations in splice sites that directly cause human heart disease (Table 2). One of the first splice site mutations that has been reported to result in heart disease is a mutation in the 5′ splice site of exon 15 of cardiac TNNT2. A G-A transition disrupts the 5′ splice site and leads to truncating mRNA variants. Interestingly, disruption of the splice site leads to not only skipping of exon 15 but also activation of a cryptic splice site in exon 15, resulting in a second aberrant splicing product of TNNT2. Consequently, sarcomeric contractions are impaired and hypertrophic cardiomyopathy ensues. Along the same lines, Bonne et al reported a splice site mutation in myosin-binding protein-C, also encoding a sarcomeric protein, that causes hypertrophic cardiomyopathy. Interestingly, like the TNNT2 mutation, the mutation in myosin-binding protein-C disrupts a splice site and simultaneously activates a cryptic downstream splice site, resulting in aberrant splicing of the myosin-binding protein-C mRNA.

In familial DCM, TTN is the most commonly mutated gene, and in ≥25% of idiopathic familial DCM patients, truncating mutations in TTN are found. Notably, ≥31% of truncating mutations in TTN are splice site mutations.9 These splice site mutations alter full-length TTN and change passive stiffness of the cardiac muscle. Interestingly, TTN isoforms change in heart disease, and aberrant TTN splicing might thus be both a cause and a consequence of heart disease.

In some cases, instead of disrupting a splice site, the mutation gives rise to a new splice site. For example in SCN5A, which encodes the α subunit of the cardiac sodium channel, a 4-bp insertion in exon 27 creates a cryptic splice site, causing a 96-bp deletion that results in the loss of key domains of SCN5A. The mutant channel fails to express any sodium current and leads to Brugada syndrome, a heart disease that is characterized by an abnormal ECG and increased risk of sudden death.

More examples of genes with splice site mutations are listed in Table 2, and it is likely that, with our current understanding of the importance of correct splicing and the use of genome-wide screening methods, many more will follow.

**Myotonic Dystrophy**

Myotonic dystrophy (DM), probably the most well-known splicing-associated disorder, is a neuromuscular disease characterized by muscle wasting, muscle hypercontractility, cardiac conduction defects, and cardiomyopathy. There are 2 types of DM: type 1, which is caused by an expansion of CUG repeats in the 3′UTR of the DMPK gene, and type 2, which is caused by an expansion of CUG repeats in intron 1 in the ZNF9 gene. Healthy individuals have 5 to 40 repeats, whereas DM patients have hundreds to thousands of these repeats. Splicing factors of the muscleblind and CELF family are antagonistically (but separately) dysregulated in DM, and seem to play a crucial role in the development of DM. In this regard, muscleblind proteins were shown to be sequestered to the (C)CUG repeats of the mutant RNA, and aggregated in nuclear foci, which interferes with splicing of muscleblind-targets. In DM type 1 (but not in type 2), CUGBP1 (or CELF) is hyperphosphorylated by protein kinase C, and its activity is increased. In addition, CELF expression is regulated by miR-23a and miR-23b, which are downregulated in DM hearts. The antagonistic functions of muscleblind and CELF are also observed during development, and the additive effect of loss-of-function of muscleblind and gain-of-function of CELF promotes an embryonic-like splicing profile in DM. In mice, both loss-of-function of muscleblind and gain-of-function of CELF are sufficient to induce a DM-like phenotype. Cardiac abnormalities occur in >80% of patients with DM, but the exact molecular mechanisms that underlie these cardiac abnormalities are not completely understood. It probably relates to the disturbed functions of muscleblind and CELF, which are known to regulate splicing of CIC-1, TNNT2, and TNNT3. Apart from splicing disturbances, it is now known that the CUG repeats cause an overall decrease of Mef2 expression. This, in turn, leads to decreased expression of Mef2 target genes and a general reprogramming of the cardiac transcriptome.

Finally, even though it is generally accepted that the CUG repeats are causative for DM1, and it is known that the repeats are necessary and sufficient to induce the disease phenotype, a role for DMPK itself cannot be ruled out, as patients with DM1 have reduced DMPK in the cytoplasm, and mice with loss of DMPK display a mild DM phenotype.

**Therapeutic Potential of Alternative Splicing**

There are various ways in which alternative splicing can be used in the clinic, but some approaches are more promising than others. In a diagnostic setting, alternative splicing profiles or specific splice isoform expression can be used as biomarkers for different human diseases. The combined ratio of major and minor isoforms of only 3 cardiac genes was sufficient to correctly assign samples to either healthy or disease (ischemic cardiomyopathy, DCM, and aortic stenosis) status. Another example is the diagnostic use of EH-myomesin, a re-expressed fetal isoform of myomesin, as a marker for human DCM. Interestingly, EH-myomesin specifically marks DCM,
and not hypertrophic cardiomyopathy or DCM with a left ventricular assist device.\(^{120}\)

With respect to therapy, a promising approach is the use of antisense oligonucleotides (AONs) to redirect splicing. This approach has been tested in a variety of diseases, including Duchenne muscular dystrophy,\(^{121}\) spinal muscular atrophy,\(^{122}\) Hutchinson–Gilford progeria,\(^{123}\) and DM.\(^{124}\) AONs bind to splice sites in a complementary manner, thereby blocking the access of RBPs to their target. In Duchenne muscular dystrophy, AONs are used to induce exon skipping of the exon that contains the pathogenic mutation in the dystrophin gene, partly restoring the functionality of the dystrophin protein. These AONs have already been tested in clinical trials and have provided encouraging results. In these examples, AONs have been used in a loss-of-function fashion, as they block splicing sites (both splicing enhancers and silencers). Conversely, AONs can also be used in a gain-of-function fashion, where they bind to their targets and either mimic the effect of a RBP\(^{125}\) or serve as a binding site for RBPs.\(^{126}\) In this case, the AON comprised a complementary sequence to its target and is coupled to either a synthetic RS domain or a synthetic splice site. Another approach is the use of trans-splicing, a form of splicing common in lower eukaryotes, where 2 different transcripts are spliced together. Trans-splicing relies on the introduction of an exogenous healthy transcript, which subsequently can be spliced together with the mutated pre-mRNA. In short, a wild-type transcript with a complementary sequence to an intron upstream of the mutated exon and a strong splice site at the 3′ end tethers to the mutated pre-mRNA, and the spliceosome then splices the wild-type healthy segment in the mutated mRNA, replacing the mutated exon. This technique is called spliceosome-mediated RNA trans-splicing (SMaRT) and has successfully been used in vitro and in vivo in

| Table 2. Splice Site Mutations That Have Been Shown to Be Causal for Human Heart Disease |
|---------------------------------|---------------------------------|-----------------|---------------------------------|
| **Gene**                        | **Mutation(s) in**              | **Effect of Mutation** | **(Hypothesized) Biological Effect** | **References** |
|---------------------------------|---------------------------------|-----------------|---------------------------------|
| Dilated cardiomyopathy          |                                |                  |                                 |
| TTN                             | Seventeen different variants in 5′ and 3′ splice sites | Skipping of exons leading to truncated TTN | Reduced passive stiffness | 99 |
| Dystrophin                      | Disruption of 5′ splice site exon 1 | Unknown | Complete loss of dystrophin protein expression | 100 |
| Lamin A/C                       | Disruption of splice recognition site exon 2 | Skipping of exon 2 | Unknown | 101 |
| Hypertrophic cardiomyopathy     |                                |                  |                                 |
| TTN                             | 1 variant in a 5′ splice site | Skipping of exons leading to truncated TTN | Reduced passive stiffness | 99 |
| TNN2                            | Disruption of 5′ splice site and activation of cryptic splice site in exon 15 | Skipping of exon 15 Insertion of 13 bp from intron 15 | Altered Ca\(^{2+}\)-dependent binding to tropomyosin | 97 |
| MybpC                           | Disruption of 3′ splice site and activation of cryptic splice site | Out-of-frame with premature stop-codon | Reduced myosin, actin, and titin binding | 98 |
| Arrhythmogenic right ventricular cardiomyopathy |                                |                  |                                 |
| DSG2                            | Disruption of 5′ splice site exon 4 | Exon 4 is skipped | Disturbed desmosomal function | 102 |
| PKP2                            | Disruption of 5′ splice site and activation of cryptic splice site in exon 5 | Exon 5: out-of-frame transcript | Disturbed desmosomal function | 102 |
|                                | Disruption of 5′ splice site exon 7 and 11 Generation of 3′ splice site exon 13 | Skipping of exons 7 and 11 Exon 13: out-of-frame |                                 | |
| JUP                             | Disruption of 3′ splice site and activation of cryptic splice site in exon 4 | Mutant transcript lacks 15 bp of exon 4 | Disturbed desmosomal function | 102 |
| Long-QT syndrome                |                                |                  |                                 |
| Kcnh2 (or hERG)                 | Disruption of 5′ splice site exon 7 | Intron 7 retention or exon 7 skipping | Complete loss of potassium currents from mutated channels | 103–105 |
|                                | Disruption of branch point sequence in intron 9 | Intron 9 retention |                                 | |
|                                | Disruption of 5′ splice site exon 10 | Intron 10 retention |                                 | |
| Kcnq1                           | Disruption of 5′ splice site exon 7 | Skipping of exon 7 | Complete loss of potassium currents from mutated channels | 106 |
| Brugada syndrome                |                                |                  |                                 |
| SCN5A                           | Insertion of TGGG in at intron 27 creating a cryptic splice site | 96-bp deletion in exon 27 | Complete loss of sodium current from mutant channel | 107 |
| Congenital heart disease        |                                |                  |                                 |
| GATA4                           | Splice junction site exon/intron 1 | In silico prediction likely to have altered binding affinity for RBPs SRSF6 and Myf1 | Reduced transcriptional activity of GATA4 | 108 |
| NR2F2                           | Disruption of 5′ splice site exon 3 | In silico prediction likely to skip exon 3 | Reduced transcriptional activity of NR2F2 | 109 |

RBP indicates RNA-binding protein; and SRSF, serine/arginine-rich splicing factor.
Conclusions and Future Directions

The emerging field of RNA biology, especially RNA splicing, has made great leaps forward in the past decade. Much more is known about the regulation of splicing, the splicing code is starting to be unraveled, and the identification of crucial splice factors and their functions, such as RBM20 and SF3B1 in the heart, has led to new insight in disease mechanisms. Still, the understimation of the number of splicing modulators, splicing mutations, and splice isoforms indicates that splicing is disturbed in many more diseases than previously thought, and this might mean that certain diseases should be re-examined for splicing abnormalities. In earlier studies, microarrays were predominantly used to interrogate gene expression and splice isoform differences. However, this technique has serious limitations, as it is restricted by the design of the array probes, and will therefore never capture all known or possible mRNA isoforms. In addition, as it relies on previously annotated genes, novel classes of genes such as lncRNAs have not been analyzed. It is therefore likely that the number of observed alternative splicing events is largely underestimated in these studies. Nowadays, RNA sequencing is the method of choice to study differences in both gene and isoform expression. The advantage of RNA sequencing is that it captures all mRNA isoforms that are present, but the analysis is still hampered by the lack of standardized methods and the incomplete annotation of transcriptomes.

Expression of correct isoforms might prove to be of equal importance for proper cardiac form and function as the total amount of a transcript that is expressed. Analysis of the role of fetal isoforms and correction toward beneficial isoforms might, therefore, be of equal importance for proper heart function as correcting gene expression levels. Furthermore, it is interesting to see that proteins with unrelated biological functions are being identified to also have a role in splicing. For example, the transcription factor Tbx3, classically known for its critical transcriptional role in development and cell fate, was recently identified to have RNA-binding capacity and to coordinate splicing as well. The number of identified RBPs will therefore likely grow in the next years. Another challenge will be to unravel the splicing profiles of different splicing factors and the cooperative role these splicing factors may play. The recent advances in techniques to interrogate protein–RNA interactions, such as HiChIP throughput Sequencing of RNA isolated by Crosslinking Immunoprecipitation (HITS-CLIP; to identify RNA targets of RBPs) and Photo Activatable Ribonucleoside enhanced Crosslinking Immunoprecipitation (PAR-CLIP; to identify binding sites of RBPs), could be used to gain a more complete understanding of RBP target networks. In this regard, many splice factors have overlapping gene targets. For example, CamkIIδ and LDB3 can both be spliced by RBM20 and ASF/SF2, and it will be interesting to see to what extent splicing is coordinated as global splicing profiles or as individual events. Also the intriguing concept of (cardiac) master splicing regulators, that is, splicing factors that regulate entire networks of transcripts, essential for the differentiation, specification, or commitment of a specific cell type or tissue type, will likely gain attention in the next years. The observation that mutations in trans-acting RBPs can cause disease that is restricted to a single tissue or cellular compartment, even when the RBP is ubiquitously expressed (eg, loss of SMN1 in spinal muscular atrophy), argues for the concept of master splicing regulators.

Moreover, some aspects of splicing deserve more attention than they have previously received. For instance, a possible role for the highly evolutionary conserved minor spliceosome in disease, perhaps as a means to control expression of a specific set of genes, remains to be determined. The upregulation of p38 MAPK activity in heart disease suggests that the minor spliceosome is more active in the stressed heart. Therefore, it might be interesting to investigate minor splicing in the context of myocyte proliferation or cardiac regeneration.

In conclusion, although progress has been made in our understanding of alternative splicing, it is clear that this knowledge is still limited. On the bright side, our understanding has already led to promising therapeutic options. Still, the identification of the cardiac splicing code and all the required components of alternative splicing will be crucial for a comprehensive understanding of this dynamic and versatile process in the heart.

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Disclosures

None.

References


van den Hoogenhof et al RNA Splicing in the Heart
The sarcomere.


ial hypertrophic cardiomyopathy. HP, Fiszman M, Komajda M, Schwartz K. Cardiac myosin binding Thierfelder L, Hong K, Guerchicof Am J Physiol Heart Cir

variants in NR2F2 cause congenital heart defects in humans. Am J Hum


Tsugi-Wakisaka K, Ako M, Ishii TM, Ashihara T, Makiyama T, Ohno S, Toyoda F, Dochi K, Matsurah H, Morie H. Identification and func-


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