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**Alternative splicing: An important mechanism in stem cell biology**

Chen K *et al.* **Alternative splicing in stem cells**

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**Abstract**

Alternative splicing (AS) is an essential mechanism in post-transcriptional regulation and leads to protein diversity. It has been shown that AS is prevalent in metazoan genomes and the splicing pattern is dynamically regulated in different tissues and cell types including embryonic stem cells. These observations suggest that AS may play critical roles in stem cell biology. Since embryonic stem cells and induced pluripotent stem cells have the ability to give rise to all types of cells and tissues, they hold the promise of future cell-based therapy. A lot of efforts have been devoted to understanding the mechanisms underlying stem cell self-renewal and differentiation. However, most of the studies focused on the expression of a core set of transcription factors and regulatory RNAs. The role of AS in stem cell differentiation was not clear. Recent advancements of high-throughput technologies allow profiling dynamic splicing patterns and cis-motifs that are responsible for AS at a genome-wide scale and provide novel insights in a number of studies. In this review we discussed some recent findings involving AS and stem cells. An emerging picture from these findings is that AS is integrated in the transcriptional and post-transcriptional networks and together they control the pluripotency maintenance and differentiation of stem cells.

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**Key words:** Alternative splicing; Stem cell; Pluripotency; Differentiation; Splicing factor

**Core tip:** Alternative splicing (AS) produces multiple transcript isoforms from a single gene, and the regulation of cell-type-specific splicing pattern is crucial for the properties and functions of cells including pluripotent stem cells. A better understanding of the role of AS in stem cell pluripotency maintenance and differentiation will offer potential new approaches for enhancing the production of induced pluripotent stem cells and/or better controlling cell differentiation for research or therapeutic purpose. In this brief review, we provide a timely update of recent studies related to stem cell regulation and splicing at a genome-wide scale.

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**INTRODUCTION**

The splicing of messenger RNA precursors, namely the precise removal of introns and the joining of exons, is a crucial yet highly dynamic and flexible process in the synthesis of mature eukaryotic mRNAs. Alternative splicing (AS)-the inclusion of different exons in mature mRNA by selecting different splice sites in pre-mRNA, can result in different transcript isoforms from a single gene and give rise to a much larger number of proteins compared to the number of genes encoded in metazoan genomes[1–3]. AS regulation plays important roles in almost every aspect of eukaryotic biological processes, including cell growth, death, pluripotency maintenance, differentiation, development, circadian rhythms, response to external changes and diseases etc[4,5]. Recent advancement of high-throughput RNA sequencing technology revealed greater number of multi-exon genes can produce alternatively spliced transcripts than previously thought[6,7]. In human, more than 90% of genes were estimated undergo AS in different tissues and/or cell types. Comparing to other RNA processing mechanisms such as alternative transcription initiation, RNA editing and alternative poly(A) site selection, AS is the most prominent one in generating mRNA complexity. In addition, AS events can introduce premature termination codons (PTCs) in mature mRNAs, triggering mRNA degradation by the process of nonsense-mediated mRNA decay (NMD)[8,9]. AS events can also cause mRNA UTR (un-translated region) variation, which affects mRNA translation efficiency, stability and localization[10–12].

Splicing of pre-mRNA involves the formation of active splicing complexes on pre-mRNAs via a step-wise assembly process. The basal splicing machinery (spliceosome) comprises of five small nuclear ribonucleoprotein particles (snRNPs) such as U1, U2, U4/U6 and U5 in the case of the major spliceosome, and U11, U12, U4atac/U6atac and U5 in the case of the minor spliceosome. AS is primarily regulated by [approximately](http://www.iciba.com/approximately) 200 RNA-binding proteins (splicing factors) together with basal splicesome through direct recognition of short sequence motifs near exon/intron boundaries[13]. Depending on the pattern of exon inclusion/skipping, AS events can be categorized into at least six major types including cassette exon skipping, mutually exclusive exons, alternative 5’ splice site selection, alternative 3’ splice site selection, alternative retained intron and tandem cassette (Figure 1). There are more complex patterns but their numbers are much less than these major types, therefore most analyses of AS events are focusing on these six types, particularly cassette exon skipping which represents the majority of AS events.

The knowledge of the crosstalk between splicing and other layers of gene regulatory network is fundamentally important for understanding biological processes such as cell differentiation, development and pluripotency maintenances. In this review, we will highlight recent progress related to these themes, with an emphasis on studies involving both AS and stem cell research, to provide timely insight into AS regulation and its important roles in cell fate determination. The general principle of splicing regulation have been covered in detail in a number of excellent reviews, readers who are interested in the mechanisms of splicing regulation can refer to these reviews[9–11,14–21].

**GENOME-WIDE METHODS APPLIED IN AS RESEARCH**

Our understanding and knowledge about AS has increased rapidly during last decade, thanks to the advancement of several high-throughput technologies. To better understand AS regulation, it is necessary to be familiar with the basic principles of these technologies. Here, we summarized some of the technologies that were applied to study AS at a genome-wide scale.

The first genome-scale AS study was carried out using microarray platform. Traditional microarrays have been designed to measure the total level of expression of a gene, without the discrimination of its different isoforms[22–24]. For probing AS events, several splicing-sensitive microarray platforms have been developed[3,25,26]. Although vary from one another, these splicing-sensitive microarrays all utilize short oligonucleotide probes that designed across exon-exon junctions. cDNA samples were derived from mRNA and hybridized to the probes (Figure 2A). The signal intensity of these junction probes can then be used to infer exon inclusion ratio by sophisticated algorithms[27–37]. These microarrays have been applied in a number of studies to generate genome-scale profiling of AS and provided quantitative measurements of AS at different time points of development, across tissues, and upon perturbation of interesting splicing factors[28,31,32,34,35,37]. From these pioneer studies, genome-level regulatory mechanisms of AS were better understood, largely transformed our view about AS in every aspects including their evolution, dynamic regulation and their organization in global transcription networks[2,19].

Recently, RNA sequencing technology has been evolving rapidly, and has become the method of choice for genome-wide AS analysis. In RNA sequencing method, cDNA fragments derived from poly(A) selected RNA population are sequenced from the ends and generate a large number of short sequence tags (reads). These reads can then be mapped (aligned) back to the reference transcriptome and splice-mapped reads can reveal the exon-exon junctions (Figure 2B)[38,39].

Compared to microarray, RNA sequencing (RNA-Seq) does not rely on probes pre-designed across exon-exon junctions based on prior knowledge about the transcriptome under study, thus novel exons and splice junctions can be detected in an unbiased manner. RNA sequencing also has other advantages such as no cross-hybridization issues, higher sensitivity and broader dynamic range[40–46]. As the technology keeps improving and cost continues to decline, longer read length and deeper sequencing coverage can lead to more accurate AS detection at a reasonable price.

High-throughput RT-PCR has also been developed and used for monitoring AS changes[47–49]. Although the number of AS events monitored is limited by priori knowledge from reference AS database, in theory, it has the advantage of avoiding biases towards the highly expressed genes and can quantify AS of medium- and extremely low-expressed genes[50]. There is also a very good correlation between percent-spliced-in (PSI, the percent of transcripts that include a specific AS exon; Figure 2) values obtained with RNA-Seq data and the PCR-based method for events in which RNA-Seq data had enough coverage to produce confident PSI estimates, suggesting that PCR-based method is consistent with RNA-Seq and these two methods can complement each other[48,51].

Methods for directly mapping RNA-binding protein (RNP) and mRNA interaction transcriptome-wide *in vivo* have also been developed, complementing AS events profiling to decipher the regulatory network of splicing by RNP. To identify binding targets, specific RNP together with its associated RNP complex is immunoprecipitated from cell lysate, bound RNA transcripts are then purified and subjected to high-throughput sequencing[52–54]. After mapping the reads back to the reference genome sequence, potential binding locations of RNPs can then be inferred by computer algorithms. RNP complexes can be immunoprecipitated under native condition; however, this can increase the risk of losing low-affinity yet specific *in vivo* bindings or obtaining artificial bindings following cell extraction[55]. A cross-linking step is usually performed to circumvent these problems. Several methods have been developed in this area. CLIP-Seq (cross-linking immunoprecipitation and high-through sequencing, also named HITS-CLIP) method uses UV light to crosslink proteins with RNAs[56]. In PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking Immunoprecipitation), photoreactive ribonucleotide analogs are used to treat cell and are incorporated into RNAs before UV treatment[57]. And iCLIP (individual-nucleotide resolution Cross-Linking and ImmunoPrecipitation) employs a self-circularization strategy to achieve individual-nucleotide resolution[58]. RNP mapping combined with AS profiling can be used for constructing “RNA maps” which correlate binding site positions with splicing regulatory differences upon perturbation of specific splicing factors.

**AS IN STEM CELLS**

Advanced technologies have been adopted to profile AS in stem cells recently. Extensive AS patterns were observed in stem cells and their contribution to pluripotency maintenance and differentiation has been noted.

***Pervasive splicing in embryonic stem cells***

Embryonic stem cells (ESCs) are pluripotent cells which can self-renew and has the ability to differentiate into all three germ layers[59,60]. As ESCs can generate most if not all of the cell types of a human body, they serve as an excellent model for studying early embryonic development. ESC is also a valuable source for producing differentiated cells for potential cell therapeutic purposes[61,62]. Thus, intensive efforts have been devoted to the stem cell gene expression profiling, and genes related with pluripoentcy were uncovered[63,64]. However, only recent advances in next generation sequencing technology made it possible to profile the AS pattern of a given cell/tissue at a global scale. A number of genome-wide studies showed specific transcriptome changes during the differentiation of ESCs into different lineages[65–70].

In 2005, Pritsker *et al*[65] started using EST collections derived from stem cells to identify splice variants in ESCs and HSCs, represents one of the first AS analyses in stem cells on a genome-wide scale. AS was detected in > 1000 genes. Although the technology is out-dated nowadays, it showed AS generates large diversity in stem cell molecular repertoire.

Following studies using advanced technologies further confirmed the pervasive AS in ESC. A study by Wu *et al*[66] adopted three types of RNA sequencing technologies and profiled the transcriptome changes during the differentiation of hESCs into the neural lineage. The authors combined Illumina single and paired-end reads (sequence reads from both ends of cDNA fragments; 35 bp reads) and longer Roche 454 FLX and Titanium sequencing reads (250-450 bp reads) to discern transcript structure and analyze transcriptome complexity. Transcriptome profiles of cells in ESC stage, N1 (early neural initiation) stage, N2 (neural progenitor) stage, and N3 (early glial-like) stage were reconstructed from mapped sequencing reads. Utilizing the unique spliced junction reads detected from each gene across all four stages, the authors then calculated a “junction complexity index” and found that splicing isoform diversity is highest in undifferentiated hESCs and decreases upon differentiation, a phenomenon they named “isoform specialization”. Observation like this can only be achieved with genome-scale study, demonstrating the power and potential of RNA sequencing in AS researches. In 2010, Revil *et al*[71] applied splicing-sensitive exon microarray technology to profile alternative isoform expression in embryonic day 8.5, 9.5 and 11.5 embryos and placenta. Although the profiling was not performed using pure ESCs, their analysis results revealed frequent AS during embryonic development stages. Intriguingly, a number of RNA binding proteins, including putative splicing factors, are differentially expressed and spliced across development stages, suggesting these RNA binding proteins may be involved in regulating tissue and temporal variations in isoform expression.

***During reprogramming, the AS profile of iPSCs is reversed to an ESC like state***

It is well known that when somatic cells are reprogrammed to pluripotent stem cells the transcription of most genes reversed to ESCs like state. An interesting question is that whether this is also true for AS?

Several recent studies answered this question by profiling both iPSC and ESCs AS pattern in a genome-scale. Ohta *et al*[72] combined RNA-seq and high-throughput absolute qRT-PCR to analyze splicing pattern changes during the reprogramming process. Indeed, the somatic cell splicing profiles revert to pluripotent-like state during reprogramming. In addition, to determine whether alterations in splicing patterns are specific for pluripotent stem cells, the authors identified 27 genes which undergo alterations during reprogramming process, and profiled splicing pattern of these genes across multiple tissues by qRT-PCR. Interestingly, the splicing patterns in iPSCs were most similar to testes compared to other tissues, suggesting an intriguing hypothesis that pluripotent stem cell regulate AS using the same mechanism as the testes does. Other work also showed that splicing pattern is similar between iPSCs and ESCs[48,51]. These observations raised the possibility that manipulating specific splicing regulators can potentially fine tune the reprogramming process.

**AS INFULENCES PLURIPOTENCY**

In addition to investigating AS patterns during ESCs differentiation, efforts have also been made to determine the functional impact of AS in ESCs[65]. In the study of Pritsker *et al*[65], not only splicing complexity in ESCs was observed, they also found that AS can modify multiple components of signaling pathways which are important for stem cell function. The distribution of splice variants across different classes of genes indicated that tissue-specific genes have higher tendency to undergo AS than ubiquitously expressed genes. Comparison between all orthologous genes which undergoes ASin human and mouse transcriptome showed that the patterns of AS are only weakly conserved, supporting that AS patterns evolve fast[73,74]. Because multiple genes in stem cells undergo AS and that these genes are enriched in regulatory proteins, stem cell molecular networks are highly dependent on AS.

Salomonis *et al*[67] investigated the roles of AS and alternative promoter selection (APS) in differentiating mouse embryonic stem cells using Affymetrix exon-exon junction microarray. Among approximately 7500 genes and 40000 putative exon-exon junctions represented on the microarray, the authors identified 170 unique alternative exons (AEs) corresponding to 144 genes. Sixty-seven percent of these AEs were predicted to alter protein sequence and domain composition. Pathway analysis of these genes showed enrichment in genes associated with Wnt and TGF-beta receptor signaling pathway, actin cytoskeleton, lipid transport, muscle contraction, mRNA metabolism and embryonic development. Most of these 170 AEs were conserved between mouse and human, suggesting their functional importance. In order to examine the functional impact of AS, two genes Serca2 and Tcf3 that showed large difference in expression level of alternative isoforms were selected for examination. Gene Serca2 is a Ca2+ pump that hydrolyzes ATP during the translocation of calcium from the cytosol to sarco/endoplasmic reticulum[75]. PCR experiment confirmed that one of its isoform (Serca2b) with an additional 44 amino acids and a longer 3’UTR region, was expressed in both ESC and embryoid bodies (EBs), whereas another isoform with a shorter alternative 3’UTR (Serca2a) was mainly expressed in EBs. Interestingly, the 3’UTR of Serca2b was predicted to be targeted by many microRNAs (miR-200b, miR-214, *etc.*) but not Serca2a in both mouse and human. This is consistent with the observation that SERCA2b mRNA is more degraded than SERCA2a in an experiment *in vitro*[76], indicating 3’UTR of SERCA2b can inhibit protein expression. Using a library of miRNA mimics, the authors further confirmed that miR-200b and miR-214 among other miRNAs were indeed targeting SERCA2b. Bioinformatics analysis showed that miR-214 and miR-200b binding sites were enriched for inhibitor genes of cardiac differentiation, indicating they play functional roles in cardiac development by repressing cardiac inhibitor gene expression. In addition, previous studies showed that miR-200b and Serca2a are both highly induced upon cardiac differentiation[75,77,78]. Taken together, this study suggests that Serca2 can avoid direct repression by miRNAs through selectively expressing one of its isofroms (Serca2a) which has no miRNA target sites in differentiated ESCs. This observation implicates the ability of AS to regulate protein expression, without affecting gene or miRNA transcription. The other gene studied is Tcf3 (TCF7L1 in human) a Wnt signaling transcription factor and a repressor for ESC self-renewal. Tcf3 inhibits ESC self-renewal through repressing Nanog and Oct4 transcription[79,80]. The authors identified a longer isoform of Tcf3 [Tcf3(l)] which is enriched in ESCs but down-regulated upon differentiation. Tcf3(l) includes a 42-bp cassette exon which encodes an additional 14 amino acids overlapping with the Groucho binding domain[81,82]. This domain is necessary for Tcf3 to repress Nanog expression[79]. Tcf3(l) is up-regulated in ESCs compared to EBs, while a shorter isoform of Tcf3, Tcf3(s), is expressed at a constant level. Selective knockdown (KD) of Tcf3(l) in ESCs revealed several distinct targets of transcriptional repression compared to Tcf3(s). For example, knocking down any Tcf3 isoform increased Nanog expression whereas Oct4 was up-regulated only when knocking down Tcf3(l). Knocking down either or both Tcf3 isofroms can lead to delayed differentiation in ESCs. Interestingly, Tcf3(l) knockdown and Tcf3(s) knockdown inhibit distinct differentiation pathways, raising the intriguing hypothesis that isoform-specific regulation of Tcf3 targets affects distinct lineage commitment decisions. In short, these examples demonstrated that, specific AS events can modulate transcriptional networks involved in pluripotency maintenance *vs* differentiation.

**AS IS INTEGRATED IN THE CORE REGULATORY CIRCUIT OF ESCs**

During last decade, a core set of transcription factors (TFs) including OCT4 (POU5F1), NANOG and SOX2 among others which control the pluripotency of ESCs has been uncovered[83]. Together with specific microRNAs and long non-coding RNAs, these TFs control the expression of gene cohorts required for establishment and maintenance of embryonic stem cells pluripotency[84–86].

The AS of the core TFs can directly influence pluripotency control. One classic example is the pluripotency gene *OCT4*. The *OCT4* gene was identified encoding three isoforms which were named *OCT4A*, *OCT4B* and *OCT4B1*[87]. Two of its isoforms *OCT4A* and *OCT4B* were shown encoding different binding domains which resulted in different target genes. While *OCT4A* can regulate genes that are responsible for stemness[83,88], *OCT4B* does not has the ability to maintain ESC self-renewal and it regulates genes that are responsive to cell stress.

Not only the AS of core TFs can affect pluripotency control, AS of several other genes is also linked to stem cell self-renewal and lineage specification[89–93]. Genes that have ESC-specific isoforms are particularly intriguing. A study conducted by Gabut *et al*[94] used microarray profiling to compare patterns of AS in undifferentiated and differentiated hESCs and identified an evolutionarily conserved ESC specific AS event of gene FOXP1 (Forkhead box transcription factor 1). Experimental validation showed that inclusion of FOXP1 exon 18b is specific to self-renewing, pluripotent hESCs, thus this transcript isoform was named “FOXP1-ES”. The inclusion of exon 18b within FOXP1-ES changes the DNA-binding specificity of FOXP1, causes FOXP1-ES to regulate distinct programs of gene expression in hESCs. The knockdown of FOXP1-ES results in a significant decrease in the expression of the pluripotency genes OCT4, NANOG, NR5A2, GDF3 and TDGF1 and an increase in expression of differentiation-associated genes including GAS1, HESX1, SFRP4. Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) was performed to identify genes that directly regulated by FOXP1-ES and FOXP1 in hESC. The FOXP1-ES binding target genes significantly overlap with the set of genes that are dependent on FOXP1-ES expression in hESCs and a set of genes that are regulated by OCT4 in hESCs. Over-expression of Foxp1-ES in mESC also promotes mESC self-renewal and pluripotency. Collectively, this study provided evidence that an AS switch regulating the FOXP1-ES isoform is integrated into the core circuit of transcriptional regulatory network required for ESC pluripotency and iPSC reprogramming.

**SPLICING FACTORS INVOLVED IN THE MAINTENANCE OF PLURIPOTENCY**

Compared to transcription factors, little is known about splicing factors that may contribute to stem-cell self-renew and lineage specification. Technology advancement allows the identification of functional RNA cis-elements related to AS and splicing factors in stem cells recently.

In 2007, Yeo *et al*[68] studied the AS events in hESCs and neural progenitors using exon array combined with sophisticated algorithms to identify exons undergoing AS. The analysis showed that RBFOX binding motif GCAUG was enriched proximal to a set of exons that are alternatively spliced in hESCs, suggesting RBFOX splicing factors may play a critical role in hESC. Following this study, the same group constructed an RNA map for RNA binding protein RBFOX2 to identify functional RNA elements in the human genome in hESCs[91]. RBFOX2 is expressed abundantly in hESC cell lines, whereas RBFOX1 is not. Using CLIP-seq technology, thousands of RBFOX2 RNA targets were uncovered, representing approximately 7% of human genes in hESC. Many RBFOX2 targets are themselves splicing factors, suggesting that RBFOX2 might act as an upstream regulator of many splicing factors. Interestingly, RBFOX2 pre-mRNA is also the target of itself, supporting the autoregulation of RBFOX2. It is possible that AS of RBFOX2 pre-mRNA may result in distinct proteins that can target different pre-mRNAs. RBFOX2 depletion in hESCs led to rapid cell death, indicating that RBFOX2 is important in maintaining hESCs viability. However, RBFOX2 depletion in neural progenitor cells or primary human fetal neural stem cells did not cause cell death, suggesting RBFOX2 has different set of targets in different cell types.

Several recent studies have also used CLIP-seq to map binding sites of specific splicing factors. Combined with AS profiling, these studies revealed several splicing factors that are potentially related with pluripotency maintenance. The work of Han *et al*[51] demonstrated a systematic strategy to study specific splicing factors function. They combined RNA-seq, CLIP-seq datasets and “splicing code” analysis (a computational method which predicts cis-elements that promote or repress specific splicing events) to identify splicing regulators that are differentially expressed between stem cells and differentiated cells and control cell-specific AS. Particularly, MBNL1 and MBNL2 were found to have the lowest relative expression levels in stem cells (ES and iPS cells) compared with differentiated cells in both human and mouse, suggesting these proteins carry out their function through repressing ES cell specific AS events. The authors tested the hypothesis using FOXP1 transcription factor. As mentioned previously, isoform FOXP1-ES contains an ESC specific exon which allows FOXP1 to bind and activate genes (OCT4 and NANOG, *etc.*) required for pluripotency. Supporting the hypothesis, the experiments showed that the FOXP1-ES specific exon was retained in differentiated cells in which MBNL1 and MBNL2 were knocked down, whereas, over-expression of MBNL1 and MBNL2 in ES cells promoted differentiated-cell-like splicing patterns. Furthermore, MBNL knockdown enhanced the efficiency of reprogramming from fibroblasts into iPSCs about two fold. Taken together, the study revealed MBNL protein expressions play a functional role in differentiation by promoting FOXP1-ES specific exon skipping, and knocking down them can facilitate reprogramming somatic cell into iPSCs.

Another study by Venables *et al*[48] found that splicing factor MBNL1 and RBFOX2 cooperate together to control plupotency in stem cell. The authors adopted high-throughput RT-PCR technology to monitor splicing changes of more than 3000 AS events annotated in the RefSeq database during the induction of fibroblasts into iPSCs and their subsequent re-differentiation. Comparing the AS profiles in fibroblasts and iPSCs and fibroblasts re-differentiated from iPSCs, the authors observed that AS changes are reversible during these processes. Their finding uncovered a program of concerted AS changes involved in late mesoderm differentiation. The authors selected 47 AS regions (in different genes) whose splicing profiles showed an equivalent near-perfect anti-correlation in reversible stem cell induction and redifferentiation, and used these AS regions to investigate splicing mechanism involved in stemness and maintenance of pluripotency. To identify the splicing factors involved in pluripotency and reprogramming, the authors knocked down 81 potential splicing factors in various cell lines and monitored these 47 AS regions using RT-PCR. The differences of PSI values (between iPSCs and original fibroblasts used for inducing pluripotent cells) of these 47 AS events were then compared with the differences of PSI values before and after 81 splicing factors were knocked down individually during reprogramming fibroblasts into iPSCs. They found that MBNL1 knockdown correlated most strongly with the splicing profile change of the induction of pluripotency. Splicing factor RBFOX2 knockdown showed the second highest correlation with the induction of pluripotency. By knocking down both MBNL1 and RBFOX2 in fibroblast, significant correlation between splicing changes the induction of pluripotency was observed and the correlation is even higher than knocking down MBNL1 and RBFOX2 individually, suggesting MBNL1 and RBFOX2 cooperate to establish splicing program involved in stem cell differentiation.

Ohta *et al*[72] used siRNA screen in pluripotent stem cells to identify RNA-binding proteins that are involved in the reprogramming process by enhancing stem cell specific AS. After a screen of 92 RNA-binding proteins, 9 RNA-binding protein-coding genes that affected the splicing patterns effectively were examined to access the impact on somatic cell reprogramming using shRNA knockdown. The down-regulation of U2af1 and Srsf3 was found to suppress both the efficiency of AP-positive (Alkaline phosphatase-positive) colony formation and ESC marker gene expression.

AS can also affect RNA stability through nonsense mediated decay (NMD). Most recently, Jangi *et al*[95] performed a genome-wide analysis of RBFOX2 activity in mESCs by mapping RBFOX2 binding sites to transcriptomic changes upon the loss of RBFOX2. Using iCLIP and RNA-seq technologies, the authors identified more than 200 AS-NMD (AS-coupled nonsense mediated decay) splicing events that are mediated by RBFOX2 in mESCs. These events showed minimal splicing changes but appreciable changes in gene expression upon RBFOX2 knockdown due to the degradation of the NMD-inducing isoform. About 70 of these AS-NMD events are within genes encoding RNA binding proteins. Many of these RBPs are also auto-regulated. A large fraction of bound but apparently unregulated events likely generate NMD isoforms. This led to the hypothesis that RBFOX2 can control gene expression level by regulating AS-NMD. The authors further demonstrated that Rbfox2 determines a threshold for the ratio of NMD to non-NMD isoforms for several of these RBPs. These findings uncovered an unexpectedly broad multilayered regulatory network controlled by RBFOX2, and established a model for how autoregulatory splicing networks are tuned.

**CONCLUSION**

Induced pluripotent stem cell holds the promise of future cell-based therapy. A thorough understanding of mechanisms underlying stem cell pluripotency and differentiation is critical for harnessing the cell reprogramming process. In this review, we have summarized recent progress in the field of AS and its role in stem cell pluripotency maintenance and differentiation. It was found that AS is pervasive in stem cell, and reprogramming reverts the splicing pattern to an ESC like state. AS has fundamental impact on stem cell differentiation by regulating different isoforms of the core pluripotency transcription factors. AS of genes other than the core factors is also linked to stem cell self-renewal and lineage specification. Additionally, Splicing factors can regulate pluripotency by affecting stem cell specific AS. In summary, these findings showed a picture in which AS is integrated in the transcriptional and post-transcriptional networks and the crosstalks between AS and other layers of gene regulatory network have fundamental effect on stem cell pluripotency maintenance and differentiation. These findings can lead to novel approaches for improving iPSCs derivation and a better control of cell differentiation.

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**Figure 1 Major alternative splicing events in metazoan transcriptome.** Major types of alternative splicing events are shown. Brown boxes indicate constitutive exons, while boxes in other colors indicate alternative spliced exons.



**Figure 2 Methods to profile alternative splicing events.** Diagram depicted methods used for profiling alternative splicing events. Cassette exon AS event is used as illustration for simplicity. “E1” and “E2” are constitutively spliced exons, “A” is an alternative exon. Probes designed for specific regions are noted with a “Pr-“ prefix. (A) Splicing sensitive microarray for AS profiling. Adopted from[2]. (B) RNA sequencing for AS profiling. PSI (percent splice in value) #E1A, #E1E2, #AE2 are number of reads mapped to the spliced junctions.