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All's Well that Ends Well: Alternative Polyadenylation and its Implications for Stem Cell Biology

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Abstract

Stem cell quiescence, activation, and differentiation are governed by a complex network of molecular pathways. There has been a growing recognition that post-transcriptional modifications, such as alternative polyadenylation (APA) of transcripts, play an important role in regulating gene expression and function. Recent analyses of stem cell populations have suggested that APA controls stem cell fate and behavior. Here, we review recent developments that have shaped our understanding of the control of stem cell behavior by APA and we highlight promising areas for future investigation.

Introduction

The remarkable ability of stem cells to facilitate tissue formation during embryonic development and regeneration in adulthood is contingent upon the regulation and interplay of a plethora of molecular pathways. The advent of microarray and high-throughput sequencing technologies have permitted a global evaluation of these pathways in different cell populations [1–3]. Not only have these platforms created an opportunity to study changes in transcript expression, but they have also revealed a tremendous diversity in transcriptional landscape [4–6]. Of particular interest has been polyadenylation site (PAS) choice because of its potential, by virtue of 3' untranslated region (3' UTR) modifications, both to influence the regulation of the transcript and to result in the production of truncated protein isoforms. In this review, we highlight recent findings that point to APA as a mechanism that influences stem cell behavior.

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APA as a mechanism of transcript regulation

Polyadenylation occurs post-transcriptionally with cleavage at the 3' end of the transcript and the addition of a series of adenosine molecules. In APA, the transcript contains more than one PAS which allows for cleavage at multiple locations and, hence, the production of distinct transcript variants [5]. Numerous methods have been used to detect APA globally, and recent estimates suggest that over 30% and 50% of genes are alternatively polyadenylated in mice and humans, respectively [7,8]. Table 1 provides a comprehensive summary of relevant methods and studies [2,7–36].

Depending upon the position of the alternative PASs, the quantity or the structure of the protein produced can be altered [4,37,38]. If all alternative PASs are located in the 3' UTR (referred to as “UTR-APA” [5] or the “development of tandem UTRs” [28]), the resulting transcripts share an identical protein coding sequence. However, the transcript variants that utilize the more proximal PASs harbor shorter 3' UTRs (Figure 1A). By contrast, if an alternative PAS is located in an internal intron or exon of the coding region (referred to as “coding region APA” [5] or “3' exon switching” [28]), the alternative transcript codes for a truncated protein and alternate 3' UTR [37] (Figure 1B).

Because the coding region is altered in the latter case, those protein isoforms may exhibit functional differences. Interestingly, a recent investigation of tyrosine kinases demonstrated that coding region PAS choice may result in a negative feedback system to fine-tune signaling mediated by the encoded proteins [39]. In particular, APA in the coding region of these receptors may produce soluble isoforms that contain the ligand binding domain but not the transmembrane region or kinase domain. These isoforms thus act as decoy receptors that compete for ligand binding but do not activate signaling [39]. Likewise, a study of a tRNA synthetase showed that APA can create protein isoforms that interfere with the activity of the full-length counterparts [40]. Conversely, CR-APA enhances Cyclin D1 function, as the truncated form is constitutively active [41,42].

Regarding UTR-APA, a global study suggested that transcripts with shorter 3' UTRs tend to be more highly expressed than their longer counterparts [22]. Several mechanisms can explain why changes in 3' UTR length may affect protein abundance. One of the best-characterized processes is that of microRNA (miR)-mediated degradation. In studies of myogenic [43,44], hematopoietic [28], and cancer [45] cells, transcripts bearing shorter 3' UTRs contained fewer miRNA-binding sites, thus allowing these transcripts to evade miRNA-mediated degradation. Transcripts are also subject to length-dependent degradation by the nonsense-mediated decay (NMD) pathway [46,47]. In NMD, Upf1 binds to the 3' UTR in a length-dependent manner, thus eliciting degradation of longer transcripts more rapidly [48].

The 3' UTR contains elements that affect not only transcript degradation but also stability. In a genome-wide computational analysis of sequence and stability data, a number of motifs regulating mRNA stability in the 3' UTR were reported [49]. The truncation of the 3' UTR by APA, and hence the removal of these motifs, could thus affect the steady-state level of the expressed transcripts. Elements in the 3' UTR have also been shown to affect transcript localization [50] and translational efficiency [51], allowing for additional levels of regulation of protein abundance.

APA not only may have an effect on the polyadenylated transcript itself, but also can influence transcription of neighboring genes. In one study of embryogenesis, production of a longer transcript of one gene, *Mest*, inhibited transcription of an overlapping antisense gene, *Copg2* [52]. Intriguingly, because *Mest* is imprinted, transcription of *Copg2* was inhibited only on the allele from which *Mest* was expressed. Thus, *Mest* expression allowed for

mono-allelic expression of Copg2, making it appear as though Copg2 was also imprinted [52].

APA in Stem Cell Biology

A growing body of evidence demonstrates that APA is active in stem cell populations and affects genes that are critical for stem cell function. We will discuss recent findings that relate to adult stem cell populations, as well as embryonic progenitors and induced pluripotent stem (iPS) cells.

Posttranscriptional regulation by APA during development has been reported across multiple species. A recent study of zebrafish development demonstrated that embryos express an abundance of transcripts in which multiple PASs are used [15]. Over a thousand transcripts displayed significant changes in PAS choice during development [15]. Moreover, there was a global trend towards increased usage of distal sites and, hence, an increase in average 3' UTR length [15]. A separate study of zebrafish confirmed this trend, noting that while 3' UTR length decreases in early development during the transition from zygote to blastula, it increases sharply in gastrulation [19]. Similar changes have been observed in mouse [30] and *Drosophila* [53] in which the average 3' UTR length increases in somatic tissues during embryonal and postnatal development [30,53]. Interestingly, analysis of microarray data for a number of cells before, during, and after reprogramming revealed that iPS cells display shortened 3' UTR lengths compared with the somatic cells from which they were derived [29].

APA is important for progenitor function not only during development but also in the adult. In a study of muscle stem cells, or satellite cells, our laboratory found that APA controls expression of a critical myogenic regulator, Pax3, whose transcript contains miR-206 binding sites in the 3' UTR [44]. Curiously, while Pax3 transcript is highly expressed in satellite cells of both limb muscles and the diaphragm, the protein is expressed only in diaphragmatic satellite cells. Because miR-206 is expressed equally in both satellite cell populations, the differential expression of Pax3 protein cannot be explained by differential miR-206 expression. Further investigation revealed that satellite cells in limb muscles use, primarily, the distal Pax3 PASs, thus generating transcripts that contain miR-206 binding sites and allowing for miR-206-mediated suppression of Pax3 protein expression. Satellite cells in the diaphragm, however, primarily use the proximal PAS and thus express Pax3 transcripts with shorter UTRs that are devoid of miR-206 binding sites, allowing these cells to evade miR-206 regulation of Pax3 [44].

A study of muscle differentiation revealed that a critical differentiation regulator, brain-derived neurotrophic factor (BDNF), is regulated by APA [54]. The muscle cell line, C2C12, produces two distinct BDNF transcript variants that differ in 3'UTR length. Production of the shorter form allows BDNF to escape miR-206 mediated repression during differentiation [55]. A recent report of BDNF in neurons also illustrated a role of the 3' UTR in regulating transcript localization and translation. In resting neurons, the long variant is sequestered in ribosome-free ribonucleoprotein particles and hence translationally repressed. During neuronal activation, the long variant is released and robustly translated [56]. Conversely, the short form is consistently translated, allowing for a basal production of the protein [56].

APA is also active in neural stem cells where the RNA-binding protein HuR, which primarily facilitates the initial proliferation stage, is surprisingly also expressed later during neuronal differentiation along with the pro-differentiation proteins HuB, HuC, and HuD. Two recent studies found that the binding of these proteins and HuR itself to the proximal PAS of nascent HuR mRNA induced polyadenylation at a distal site. The longer variant was

less stable, resulting in decreased protein production compared with the shorter form. Thus, the cell could control output of HuR by regulating transcript polyadenylation [57,58]. Although HuR 3' UTR length increased, sequencing analysis of neural stem/progenitor cells and differentiated neurons revealed that global 3' UTR length decreases in differentiation. The mechanisms for this trend remain unknown [17].

Spermatogenesis is also characterized by the production of a multitude of polyadenylation variants of germ cell-specific transcripts [33]. One of these variants is the shortest known variant of the testis-brain RNA-binding protein (TB-RBP), a protein that facilitates intra- and intercellular mRNA transport [59,60]. In the testis, the cleavage stimulation factor-64 (Cstf-64) promotes production of the shortest TB-RBP isoform, which is the most efficiently translated variant [59]. As with neural differentiation, spermatogenesis is also characterized by a global decrease in average 3' UTR length [33].

Within stem cell populations, patterns of expression of factors known to regulate APA further support the idea that changes in 3' UTR length are important in stem cell function. Cleavage and polyadenylation specificity factor-160 (Cpsf-160) as well as Cstf-64 are proteins that bind to PAS sites directly to mediate cleavage and polyadenylation [38,61], and as just noted, Cstf-64 influences APA specificity in the testis. Intriguingly, these factors have been shown to be associated with shorter UTRs. In the mouse, both factors are highly expressed in the male germ cells [62], which display 3' UTR length decreases postnatally [15,30,33,35]. Moreover, they are also upregulated with a corresponding trend towards shorter 3' UTR variants during iPS cell generation [29] and in cancer cells [45]. In contrast, these factors are downregulated during C2C12 differentiation when 3' UTR length increases [30]. Another factor, polypyrimidine tract binding protein (PTB), which has been implicated in alternative splicing and PAS selection [63,64], is also downregulated during myogenic differentiation [65].

Perspectives

A number of questions remain unanswered in the burgeoning field of APA and stem cell regulation. Certainly, further studies of stem cell populations are needed to understand the extent to which APA occurs in different stem cell types and to uncover changes in polyadenylation patterns during quiescence, activation, and differentiation. General patterns that have emerged in the study of pluripotent stem cells include a lengthening of the 3' UTR in embryonic progenitors during development and a decrease in 3' UTR length of somatic cells during reprogramming (Figure 2A). Although there are few large-scale analyses on the 3' UTR length of adult stem cells, the data on proliferating and differentiated somatic cells suggest that adult stem cells will globally express transcripts with shorter 3' UTRs upon exit from quiescence and entry into the cell cycle and will then express longer 3' UTRs upon differentiation (Figure 2A, B).

A number of stem cell populations display molecular and functional heterogeneity, though the full characterization of this phenomenon and its consequences for tissue regeneration and aging have yet to be understood [66]. We postulate that APA-directed 3' UTR length changes may mediate heterogeneity within a population. The global analyses of APA discussed previously have shown that multiple transcript variants can be expressed simultaneously in a cell population. However, this population-based heterogeneity could arise as a result of different patterns at the single cell level with individual cells expressing different isoforms from their neighbors (Figure 3A).

Likewise, changes in 3' UTR length that are observed in populations during state transitions can occur as a result of different patterns at the single cell level. We propose two (non-mutually-exclusive) models by which a stem cell population could exhibit global length

changes during a transition from one state to another (Figure 3B). In this example, we illustrate a hypothetical gene with only two transcript variants, a long form and a short form. In one case (the “conversion” model), a global decrease in UTR length could occur if all cells are capable of expressing both transcripts and the result of the transition is that each cell expresses a higher proportion of the short transcript. In the other case (the “selection” model), in which each cell is capable of expressing only one form, global decrease in UTR length in the population would occur when there is a selection or selective expansion of those cells expressing the short form (Figure 3B). The development of technologies such as single-cell PCR [67–69] and single-cell RNA sequencing [70,71] will surely herald advances in our understanding of APA and stem cell heterogeneity.

Although many studies have addressed the functional consequences of 3' UTR length changes for individual genes, the effects of global changes in 3' UTR length remain elusive. One potential explanation stems from pivotal work in the area of miRNA sponges where it was shown that one could use decoys containing target sites to sequester miRNA molecules and inhibit their activity [72,73]. Interestingly, a number of studies have suggested that the abundance of mRNAs can also dilute the activity of the targeting miRNAs and siRNAs [72,74,75]. Consequently, if multiple transcripts are targeted by the same miRNA, changes in the abundance of any one of those transcripts, which have been termed competing endogenous RNAs (ceRNAs), could influence the abundance of any of the other transcripts [75–80]. We hypothesize that global changes to 3' UTR length could have the same effect. In this model, mRNAs with longer 3' UTRs would act as ceRNAs. If a cell state change were to occur such that 3' UTR lengths were globally decreased, then miRNA availability would be enhanced overall.

Another area for further research is in the regulation of APA. Global studies have suggested that PAS usage varies widely across different tissues with some polyadenylation variants expressed in a tissue-specific manner [24,35]. It is thought that a number of factors involved in polyadenylation and splicing may play a role in specifying the unique polyadenylation signatures of different cells [5,38]. Various techniques have been utilized recently to identify factors that influence polyadenylation on a global level. In one study, high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) was used to identify the RNA binding sites of a neuron-specific splicing factor, Nova. Because this protein was also bound to regions in 3' UTRs, a novel role for this protein in APA was revealed [81]. In another study, Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology was used with RNA sequencing to pinpoint binding motifs for two other regulators of APA, epithelial splicing regulatory protein (Esrp) 1 and Esrp2 [82]. Conversely, in a third study, a reporter for APA and an RNAi-based screen were used to identify a number of factors, including Poly(A)-Binding Protein Nuclear 1 (PABPN1), that regulate APA [83]. Furthermore, recent studies demonstrating that widespread RNA methylation occurs in the 3' UTR have raised the intriguing possibility that RNA methylation can play a role in PAS selection [84,85]. The application of these techniques to stem cell populations will allow for an understanding of how these factors interact to produce the diversity in PAS usage amongst different populations and stem cell states.

Some of these tools have also revealed how changes to 3' UTR processing could induce tissue dysfunction and disease. For example, mutations in PABPN1 cause oculopharyngeal muscular dystrophy [86], and such mutations correlate with global changes in 3' UTR lengths and pervasive gene dysregulation [83]. Moreover, recent investigations have shown that tumor cells exhibit decreased 3' UTR length [12,27,45]. The shorter 3' UTR length of the proto-oncogene IGF2BP1/IMP-1 and consequent enhancement in protein expression suggested a role for APA in cancer progression [45]. These studies may have relevance to the stem cell field to the extent that those global changes are characteristic of cancer stem

cells that may exist in many tumors [87]. A thorough understanding of the extent and patterns of APA in stem cell populations will likely lead to new insights into the regulation of stem cell behavior and heterogeneity as well as tissue maintenance and disease.

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A)

B)

Figure 1.

Major categories of APA. This model refers to a hypothetical gene with three exons and two PASs. A) When both PASs are located in the 3' UTR, then identical proteins are produced. Because the 3' UTR often contains elements regulating transcript stability, degradation, or localization, the quantity of protein produced may be altered depending upon PAS choice. B) When one PAS is located in the coding region, a truncated protein is produced when the proximal PAS is chosen. Ex = exon, PAS = polyadenylation site; thick lines = UTR regions, thin lines = intronic regions.

Figure 2.

3' UTR length changes in stem cell populations and a role for heterogeneity. A) Trends in 3' UTR length in embryonic progenitors (EPs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). During development, embryonic progenitors display a general increase in 3' UTR length. Studies suggest that adult stem cells undergo a decrease in 3' UTR length with proliferation and an increase upon differentiation. The reprogramming of somatic cells to become iPSCs leads to a decrease in 3' UTR length. B) Prediction of global 3' UTR length in adult muscle stem cell lineage progression. In our model, quiescent muscle stem cells (green) are located in close association with the multinucleated muscle fiber. These cells would display a global decrease in 3' UTR length upon exit from quiescence and entry into the cell cycle to generate transit amplifying progenitors and then myoblasts. As they differentiate and, ultimately, fuse to form multinucleated muscle fibers, global 3' UTR length would increase.

Figure 3.

Heterogeneity in 3' UTR length within a cell population for a hypothetical transcript containing two PASs that result in the production of variants with either a long (long variants shown in blue) or short 3' UTR (short variants shown in red). A) In this hypothetical case, sequencing data reveals that the cell population contains a 3:1 ratio of long variants to short variants. In the example of a homogenous population, all cells contain the same 3:1 proportion of transcript variants. In the example of a heterogeneous population, there are two subsets of cells: one that contains only the short variants and the other that contains only the long variants. The proportion of these cells dictates that proportion of short and long variants observed by sequencing of the population. B) Mechanisms of changes in

3' UTR length in cell populations. There may be multiple non-mutually-exclusive scenarios by which a population would exhibit changes in 3' UTR lengths during a transition from one state to another. A theoretical case in which there is global shortening (here represented by a single transcript) in the transition from State A to State B is illustrated for a population of cells undergoing proliferative expansion. In the "Conversion Model", all cells alter their patterns of PAS selection during the transition and the resulting population has a greater proportion of short transcript variants. By contrast, in the "Selection Model", all cells maintain their patterns of PAS usage during the transition, but cells expressing the shorter variant are preferentially selected. Again, the resulting population has a greater proportion of short transcript variants than the starting population.

Table 1**Methods Used to Determine Global Polyadenylation Patterns**

Method	Cell or Tissue Type	Major Findings	Key References
3'-Directed RNA Sequencing			
3'-end Sequencing for Expression Quantification (3-Seq)	Human skin samples	Polyadenylation of human skin samples reveals a lack of systemic changes in 3' end mRNA processing in skin aging.	[9,10]
3'READS	Mouse cell lines (3T3, C2C12) and mouse tissue (whole embryos, brain, testes)	Increased usage of distal PASs during embryonic development and cell differentiation. Suggested a greater importance for the strength of the PAS, rather than the location, for specifying usage.	[37]
3'-Seq	Human cells lines (fibroblast: BJ, mammary epithelial: MCF10A)	E2F transcription factors may contribute to enhanced alternative polyadenylation during cell proliferation.	[11]
Direct RNA Sequencing (DRS)	Yeast (<i>S. cerevisiae</i>), human liver tissue, human brain tissue Major cancers from primary tissue samples (breast, liver, kidney, colon, lung) and cell lines (Hela-S3, K562, MCF7, PC3, HepG2, LNCaP, MCF10A)	Mapping and motif analysis of polyadenylation sites in yeast and human. Negative correlation in expression levels observed between sense and antisense overlapping transcripts. PAS mapping in important cancers and tumor cell lines. Created Expression and Polyadenylation Database (xPAD)	[2,7] [12]
Fluorescence-activated Nuclei Sorting (FANS) and 3'-End-Seq	Intestinal tissue of worm (<i>C. elegans</i>)	Tissue-specific mapping of poly(A) sites in <i>C. elegans</i> revealed widespread APA in the intestine and the existence of U-rich and/or A-rich upstream auxiliary elements in PASs that undergo APA.	[13]
Poly(A)-Position Profiling by Sequencing (3P-Seq)	Worm (<i>C. elegans</i>) Zebrafish tissues (brain, ovaries, testes) from 8 developmental stages	Expanded dataset of <i>C. elegans</i> finding that 30% of mRNA coding for proteins contain multiple PASs. 3' UTR regions appear AU-rich, allowing for genome compaction. Mapping of PAS usage in brain, ovaries, and testes of 8 developmental stages of zebrafish. Of genes that contained multiple PASs, over 1000 display differential usage of the sites through development. 3' UTR length is shortest in the ovaries and greatest in the brain.	[14] [15]
PolyA-Seq	24 tissues in human, rhesus, dog, rat, and mouse	Analysis of 24 tissues in human, rhesus, dog, rat and mouse. PAS usage is more similar across tissues within different species than in different tissues within a species.	[16]
Poly(A) Site Sequencing (PAS-Seq)	HeLa cell line, mouse ES cells, neural stem/progenitor cells, and neurons.	Histone mRNAs show evolutionary conservation of APA and unique patterns for mitochondrial RNA. Lengthening of 3' UTRs in neural stem cell differentiation.	[17]
Sequencing APA Sites (SAP AS)	Human breast cancer cell lines (MCF7 and MB231) and mammary epithelial cell line (MCF10A) Zebrafish at various developmental stages	MCF7 displayed shorter 3' UTRs compared to normal cell lines while MB231 exhibited lengthening of the 3' UTR. Over 4000 genes switch PAS during development with a general decrease in 3' UTR initially in the zygote the blastula transition and an increase in gastrulation.	[18] [19]
RNA Sequencing with Total Transcript Coverage			

Method	Cell or Tissue Type	Major Findings	Key References
3'UTRome	<i>C. elegans</i> at various developmental stages	Many alternative UTR variants are differentially expressed in development and with age, average 3' UTR length decreases.	[20]
Allele-Specific Alternative mRNA Processing (ASARP)	Primary breast cancer tissue and glioblastoma cell line (U87MG)	Mapping of allele-specific expression and alternative polyadenylation patterns from primary cancer tissues and cell lines.	[21]
Relative Expression of Isoforms using Distal PolyA Sites (RUD)	16 human tissues	Analysis of mouse and human transcriptome suggests a correlation between PAS choice and transcriptional activity such that genes are more highly expressed when the proximal PAS is chosen.	[22]
Mixture-of-Isoforms Model (MISO)	Human embryonic kidney cell line (HEK293T)	Suggested that the splicing factor hnRNP H could regulate APA.	[23]
mRNA-SEQ Analysis	Human tissues (adipose, brain, breast, cerebellum, colon, heart, liver, lymph node, skeletal muscle, testes) and cell lines (BT474, HME, MB435, MCF7, T47D)	Patterns of APA and alternative splicing are strongly correlated between tissues suggesting common regulatory mechanisms. Additionally, APA patterns are more strongly correlated between tissues of different individuals than between different tissues.	[24]
Poly(A) Tags (PATs)	<i>Arabidopsis</i> seeds and leaves	70% of <i>Arabidopsis</i> genes encode for multiple PASs with extensive APA in the coding region. Many APA sites correspond to overlapping antisense transcripts.	[25]
Microarray			
Isoform Analysis of Probesets	Lymphoblastoid cell lines	Identification of a SNPs that could alter PAS usage. For example, one SNP creates a functional polyadenylation site that shortens the 3' UTR of <i>Irf5</i> , a gene implicated in systemic lupus erythematosus.	[26]
Probe-Level Analysis of Microarray Data	Mouse leukemia/lymphoma cells (APN, LPC, APC) and B lymphocytes	3' UTR length is decreased on average in tumor samples. Genes that display lengthening show enrichment for cell-cell adhesion and morphology.	[27]
Probe-Level Alternative Transcript Analysis (PLATA)	Primary cells (mouse CD4+ T lymphocytes, human B lymphocytes, human monocytes) and mouse and human tissues (brain, kidney, spleen, heart, skeletal muscle, liver, testes)	Trend towards usage of the proximal PAS in immune cell activation and during proliferation in a broad range of cells and tissues. The data suggests that proximal PAS usage allows cells to circumvent miRNA-mediated regulation of mRNA.	[28]
Relative Usage of Distal Poly(A) Site (RUD)	Mouse iPS cells from B lymphocytes, mouse embryonic fibroblasts, adult neural stem cells. Human iPS cells from neonatal foreskin fibroblasts, fetal lung fibroblasts, and spermatogonial cells. Various mouse embryonic tissues, myogenic cell line (C2C12)	During reprogramming of somatic cells, the 3' UTR length decreases, while the length increases during reprogramming of spermatogonial cells. 3' UTR length increases during mouse embryonic development.	[29] [30]
Expressed Sequence Tag (EST)			
Automated EST Cluster Analysis	Mouse and human samples	Identification of human and murine PASs and widespread APA. Creation of Transcriptome Sailor web tool to visualize ESTs and clustering.	[31]
EST Analysis	Human and mouse samples from dbEST	Identification of PASs across a broad collection of human and mouse samples	[32]

Method	Cell or Tissue Type	Major Findings	Key References
EST Analysis	Mouse testicular cells (spermatagonia, spermatocytes, round spermatids, Sertoli cells)	and evidence for tissue- or disease-specific biases in PAS usage. Creation of ESTparser to visualize PASs and tissue biases. Changes in PAS usage during spermatogenesis with a trend towards truncation of the 3' UTR.	[33]
EST Analysis	Mouse and human samples	54% of human genes and 37% of mouse genes have multiple PASs. Orthologs between the two species display similar polyadenylation patterns.	[8]
Global Study of Poly(A) Site Usage by Gene-based EST Vote (GAUGE)	42 human tissues from polyA_DB	Systemic differences in PAS usage among tissues and identification of potential <i>cis</i> -regulatory elements associated with PASs in the brain. Development of polyA_DB database of mammalian mRNA polyadenylation.	[34,35]
Other			
Digital Gene Expression (DGE) based on Massively Parallel Signature Sequencing (MPSS) and Illumina Sequencing by Synthesis (SBS) and analysis similar to GAUGE	<i>Arabidopsis</i> and rice of various developmental stages and environmental exposures	Approximately 60% of <i>Arabidopsis</i> genes and 47-82% of rice genes contain multiple PASs with 49-66% mapping within the coding region. Genes that show differential PAS usage in different developmental stages make up 10% of the transcriptome.	[36]