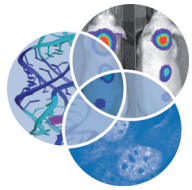
Advanced Review



Regulatory effects of cotranscriptional RNA structure formation and transitions

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RNAs, which play significant roles in many fundamental biological processes of life, fold into sophisticated and precise structures. RNA folding is a dynamic and intricate process, which conformation transition of coding and noncoding RNAs form the primary elements of genetic regulation. The cellular environment contains various intrinsic and extrinsic factors that potentially affect RNA folding *in vivo*, and experimental and theoretical evidence increasingly indicates that the highly flexible features of the RNA structure are affected by these factors, which include the flanking sequence context, physiochemical conditions, *cis* RNA–RNA interactions, and RNA interactions with other molecules. Furthermore, distinct RNA structures have been identified that govern almost all steps of biological processes in cells, including transcriptional activation and termination, transcriptional mutagenesis, 5′-capping, splicing, 3′-polyadenylation, mRNA export and localization, and translation. Here, we briefly summarize the dynamic and complex features of RNA folding along with a wide variety of intrinsic and extrinsic factors that affect RNA folding. We then provide several examples to elaborate RNA structure-mediated regulation at the transcriptional and posttranscriptional levels. Finally, we illustrate the regulatory roles of RNA structure and discuss advances pertaining to RNA structure in plants.

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INTRODUCTION

RNAs play myriad crucial roles in virtually all cellular processes.[1] DNA generally forms a predictable double helix, while RNA consists of a single strand that folds into elaborate bulges, loops, hairpins, pseudo-knots, hammerheads, and other three-dimensional conformations.[2,3] RNA folding is an indispensable process underlying RNA function, and it delineates the process by which an RNA molecule transitions from an unfolded/disordered state to the native/functional conformation.[4,5] The desire to know the diverse functions of RNA has driven extensive research to uncover details of its folding. Some of this research has revealed that two major problems occur during the process of RNA folding.[4,6] First, RNA molecules tend to be misfolded, thereby often becoming trapped in inactive conformations. Second, compared with intermediate structures, the native RNA conformation may not be thermodynamically advantageous, thus requiring a specific protein for stabilization of the tertiary structure.

RNA structures are frequently dynamic and RNAs can undergo distinct conformational transitions; these RNA dynamics serve as a regulatory mechanism and a source of functionality.[7–9] In addition, accumulating evidence suggests that RNA structure begins forming as the RNA is being transcribed. The process of cotranscriptional RNA structural formation is pivotal to determining the functional RNA structures, which can be influenced by a wide range of intrinsic and extrinsic factors.[10] In the following

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sections, we briefly summarize the dynamic properties of RNA structures, and the cellular components that have been exhibited to affect cotranscriptional folding based primarily on experimental evidence *in vivo* supplemented by some theoretical evidence. We then review the various mechanisms that direct RNA folding to achieve a broad spectrum of functions.

HIERARCHY AND DYNAMICS OF RNA CONFORMATION

In general, RNA molecules often do not fold into a single structure because of the intrinsic degeneracies of the canonical base-paring and stacking; they instead form an ensemble or statistical distribution of some interconverting conformations. The population of each configuration depends on its free energy, while the transition rate between conformations depends upon the free-energy barrier of separation.[7,9] Conformational transitions occur when cellular cues, such as protein binding, covalent modification of nucleotides, changes in salt concentrations, and fluctuations in temperature, disrupt the free-energy landscape, which results in a redistribution of distinct conformations. The RNA free-energy landscape is intensely hierarchical and naturally organized into local energetic minima containing secondary and tertiary structures.[6,11] Recently, Mustoe et al.[12] further classified RNA dynamic properties into four different modes that represent transitions between basins on a hierarchical free-energy landscape, including larger-scale secondary-structure transitions, base pair dynamics and tertiary dynamics, stacking dynamics, and other 'jittering' motions.

Mounting experimental and some theoretical evidence exist for the diversity and dynamics of RNA conformation. One example is the well-illustrated dynamics of RNA tetraloops, which are involved in various biochemical processes, including the nucleation in RNA folding and the formation of tertiary stacking.[13] DePaul et al.[13] illustrated the equilibrium conformational dynamics within an RNA tetraloop motif from massively parallel molecular dynamics. First, they described a highly dynamic structure consisting of 15 conformational microstates, the transitions among different conformations ranging from nanosecond to microsecond timescales and six indispensable loop conformations being dominant. Second, they found that the abundant structures of GNRA tetraloops (N represents any ribonucleotide and R is ribonucleotide possess a purine base) involve tertiary contact formation, during which the tetraloops probably adopt nonnative conformations.[13] A riboswitch is another extensively studied example that exhibits RNA structural diversity and dynamics. Riboswitches act as RNA sensors that regulate the transcript in which they reside in *cis* via binding a small molecule ligand.[14,15] Many riboswitches have been studied, including adenine, purine, lysine, thiamine pyrophosphate (TPP), *S*-adenosylmethionine (SAM), and Flavin mononucleotide (FMN).[14,16] By binding a ligand, numerous factors can interact with riboswitches as they are regulating the process of transcription or translation in cells. Timing and cooperation between the binding and association among these factors, the movement of transcriptional complex, and RNA folding are essential to the regulatory outcome.[16] One well-illustrated study is the SAM riboswitch. The different classes of SAM riboswitches can be distinguished by the presence of SAM or *S*-adenosylmethione (SAH), although SAM is highly similar to SAH in structure. The difference is pivotal to preventing the accumulation of toxic SAH and converting SAH into SAM, and the diversity of SAM riboswitches also delineates the possibility of various RNA structural solutions against a massively parallel biochemical challenge.[8,17] In plants, some studies on RNA folding were performed in *Arabidopsis*, using nuclease-based double-strand (ds) RNA-seq and single-strand (ss) -RNA-seq techniques. The complementary libraries showed that RNA folding is a dynamic process because few sequences were present solely in the dsRNA or ssRNA library.[18–20]

FACTORS FOR COTRANSCRIPTIONAL RNA FOLDING

The process of cotranscriptional RNA folding is pivotal for determining the native, functional RNA structures *in vivo*.[21] Although currently available methods for predicting RNA secondary structure have been greatly improved compared to previous approaches, many challenges still remain because the majority of these studies were conducted *in vitro*.[6,12] Consequently, the understanding of RNA folding pathways is still in its infancy because a wide range of intrinsic and extrinsic factors can influence the dynamic characteristics and complexity *in vivo* (e.g., the formation of hairpins and pseudoknots; Figure 1). Several implemented experiments *in vitro* have confirmed that the speed of transcription can influence the cotranscriptional RNA folding, including the folding rate and the transient and final

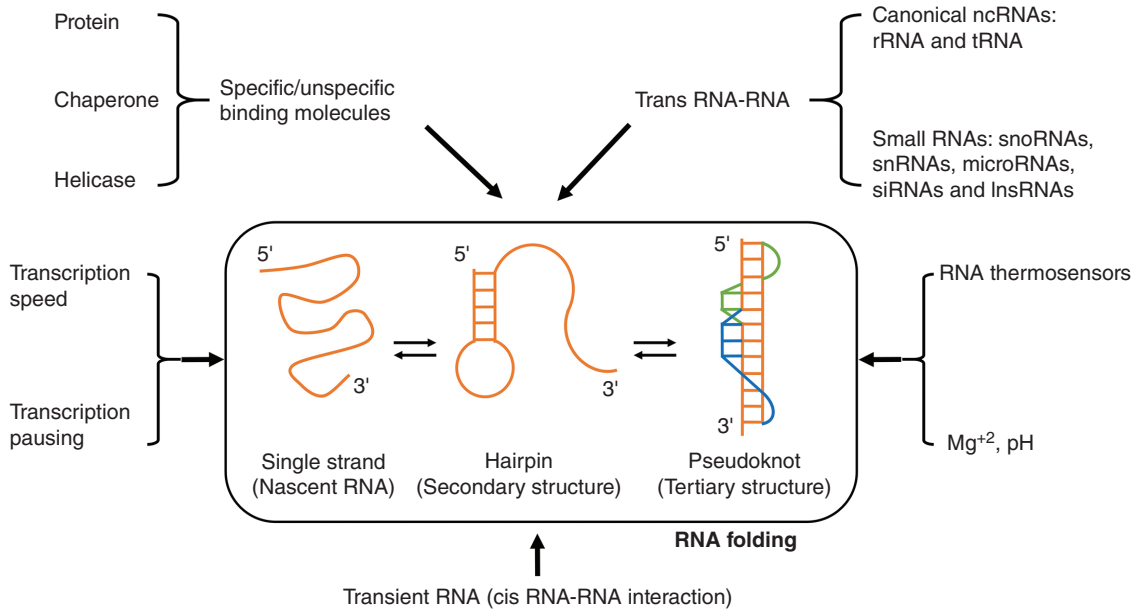

FIGURE 1 | Factors for RNA structural formation and transition *in vivo*.

structures.[22,23] For example, during the process of transcription by phage T7 or *Escherichia coli* polymerase, sequential folding is only twice as fast than refolding, and the yielded native RNA is undifferentiated.[22] Nevertheless, a larger part of circularly permuted variants are folded correctly early during transcription than during the process of refolding. In addition, the speed of transcription is not constant, and thus transcriptional pausing can occur and affect cotranscriptional folding.[24,25] In a previous study, transcriptional pausing played crucial roles in the folding and conformational rearrangement of the *btuB* riboswitch during transcription via *E. coli* RNA polymerase.[26] Transcriptional pausing can prevent the formation of alternate structures at regulatory sites, and it plays a chaperone role that couples the folding of a coenzyme B12 binding aptamer domain and an expression platform.[26,27]

During the process of cotranscriptional RNA folding, structures that have already formed can unpair and produce transient structures. These transient structures, known as *cis* RNA–RNA, can dramatically affect the cotranscriptional folding pathways and the resulting native RNA structures.[9,10,28] Many transient structures exist for only a specific period of time, but they can direct the formation of desired structural conformations through one or more folding pathways.[10] Recently, Zhu et al.[28] performed a computational study in regard to six RNA families with known transient and alternative structures. They found that many transient structures have been evolutionarily conserved to an extent similar to the final structures. They also found that evolutionarily associated sequences have similar transient structural features during the predicted cotranscriptional RNA folding pathways.

In vivo, an RNA transcript can interact with various other molecules that present in the cellular environment. This interaction can affect the cotranscriptional RNA folding pathways and the resulting RNA structures. Examples of such interactions include proteins that specifically bind to RNA to assist RNA folding [29,30], and riboswitches that interact with RNA and affect RNA structural formation.[16,31] In addition, RNA chaperone and helicase can nonspecifically bind to RNA to aid RNA folding by refolding misfolded structures,[32–35] and some distinct intermolecular RNA transcripts can either stabilize or destabilize existing RNA structures, which affects the cotranscriptional folding pathways and the resulting RNA conformations.[4,5,9,10]

Thermal triggers and physiochemical conditions *in vivo* also have significant effects on RNA folding pathways; for example, RNA thermosensors (RNATs) can regulate gene expression during cold and shock response in response to fluctuations in temperature, magnesium ion (Mg^{2+}) concentration, and pH.[36–39] These RNATs possess a RNase E cleavage site, an enzyme found in *E. coli* and plenty of other organisms, in the 5′-UTR of the target gene. The cleavage site is sequestered in a stem-loop at low temperatures, thereby gene expression is not being blocked. Whereas, the stem-loop unfolds at high temperatures, permitting mRNA degradation and

turning off expression of genes.[39] The stability of a compact RNA structure is exquisitely sensitive to the types and concentrations of ion that are present *in vivo*, especially Mg^{2+}.[38] For example, the expression of Mg^{2+} transporters (MgtA) is controlled by their 5′-UTR of stem-loop structures in *Salmonella enterica*.[37] For pH-mediated regulation of RNA folding, a pH-responsive RNA element in *E. coli* in the 5′-UTR of the *alx* gene regulates its translation through a pH-dependent manner.[36,40] At a high pH, the translationally active RNA structure that possesses two well-defined transcriptional pausing sites is formed during transcription, thereby regulating gene expression.[36,40]

FUNCTIONS OF RNA CONFORMATIONAL TRANSITIONS

RNA folding is fundamental and key in gene regulation, acting as a binary switch that is stimulated by specific cellular cues.[4] In living cells, transcription and processing are generally simultaneous rather than sequential, which means processing is cotranscriptional instead of posttranscriptional.[31] Evidence has revealed that cotranscription can boost the accuracy and efficiency of pre-mRNA maturation and allow some new interactions, which have regulatory implications.[10,29] Aside from pre-mRNA processing and transcription being simultaneous, they are interdependent, or mechanistically coupled to each other in time and space.[10,41] In the following, we discuss the importance and regulatory effects of the transition of RNA conformations in transcriptional and posttranscriptional regulation.

Transcription Activation and Termination, and Mutagenesis

Changes in many RNA structures regulate gene expression at the transcriptional level, resulting in either transcription termination or activation. Structural changes of some riboswitches upon ligand binding can regulate RNA transcription.[16,42] In the adenine riboswitch cotranscriptional folding mechanism, the aptamer domain is formed prior to the expression domain by RNA polymerase, and the nascent aptamer domain is able to response to cellular cues and potentially bind to a cognate ligand before the transcription and folding of the expression domain.[16,31] The binding of an adenine riboswitch ligand to an aptamer domain enables formation of an intrinsic terminator RNA hairpin stem structure, which inhibits RNA polymerase extension.[43,44] An anti-terminator stem-loop of expression platform can be formed when the aptamer domain unbounded with ligand, thus causing the poly-U stretch to be sequestered and mRNA synthesis to proceed successfully (Figure 2(a)).[16,45] Differ from the transcriptional regulatory mechanism by riboswitches, other types of RNA conformation transition can regulate gene expression in response to even more complex molecules. For instance, in the human mitochondrial transcription machinery, the primer 3′ end is explicitly identified by transcription termination at conserved sequence block II (CSB II) located in the mitochondrial DNA control region, thereby the formation of guanine (G)-quadruplex structures in nascent RNA depends upon transcription of CSB II results in site-specific termination event.[46–48]

Accurate DNA replication and DNA repair are indispensable for maintaining genomic stability, and many studies have found that DNA damage is prone to occur at genomic loci with higher transcriptional activity.[21] Generally, transcription is mutagenic and DNA damage is partly due to the R-loop formed through the binding of the nascent RNA with its DNA template exposing the DNA nontemplate strand to mutagens and to primers that induce unscheduled error-prone DNA synthesis.[21,49] A recent study revealed that nascent RNA folding can mitigate transcription-associated mutagenesis (Figure 2(b)).[50] The authors illustrated that heightening RNA folding and reducing R-loop formation by synonymous changes in a reporter gene can lower the mutation rate immensely. Over-expression of the gene RNase H1 can degrade the RNA in a DNA-RNA hybrid and decrease the effect, implying that it is R-loop dependent.[50]

Regulation of 5′ Capping and 3′ Polyadenylation

The 5′ capping is the first step for the maturation of a pre-mRNA, and it involves attachment of a 7-methylguanosine cap by a 5′–5′ linkage. This step is crucial for mRNA stability and efficient translation initiation.[51,52] An example is viral RNA-specific cap methylation, in which the 5′ end of the flavivirus plus-sense RNA genome consists of a type 1 cap (m7GpppAmG) and a conserved stem-loop structure. In a West Nile virus model, N-7 cap methylation requires specific nucleotides at the second and third positions and a 5′ stem-loop structure.[51] The nonstructural protein 5 (NS5) derived from four serocomplexes of flaviviruses can specifically methylate the N-7 cap via recognition of the flavivirus RNA 5′ terminus.[51] Comprehensive experiments revealed that different RNA elements are essential to the

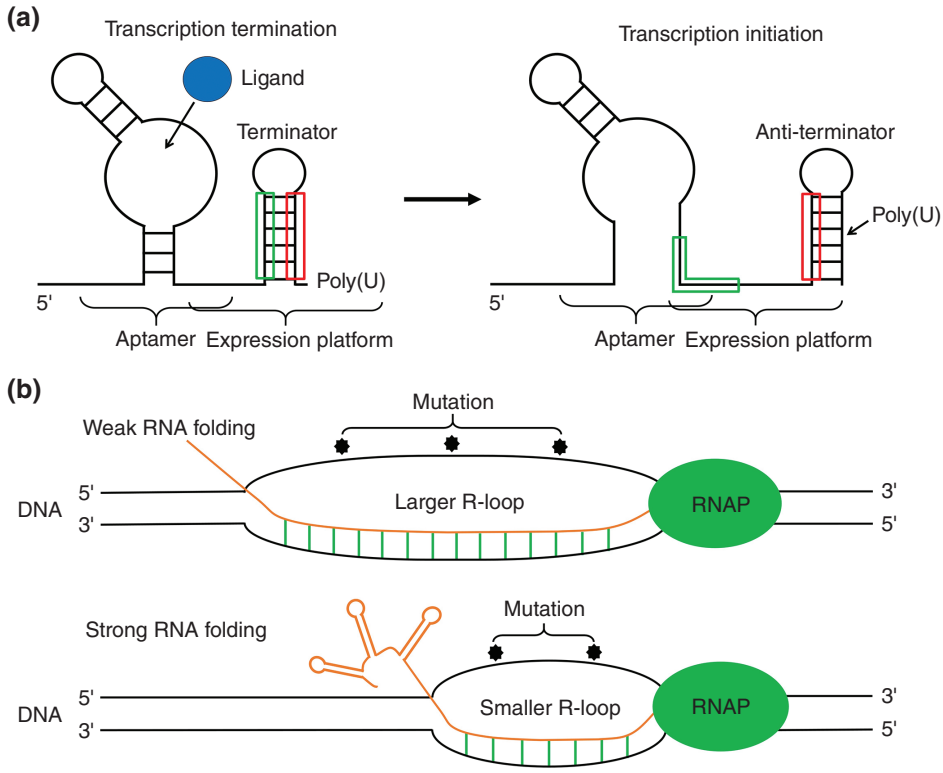

FIGURE 2 | Regulatory effect for cotranscriptional processes of RNA structures formation. (a) The cotranscriptional RNA folding pathways is modulated by adenine riboswitch. Transcription termination resulting from ligand binding to the aptamer domain of the adenine riboswitch and subsequent stabilization of a terminator stem-loop and exposure of a poly-(U) stretch. (b) The cotranscriptional mutagenesis is modulated by RNA folding and R-loop formation. With weak RNA folding, an R-loop accumulated resulting in the exposure time of the naked nontemplate DNA and error-prone DNA synthesis increased, further leading to a higher mutation rate. By comparison, the R-loop is dissolved when RNA folding stronger, resulting in the exposure time of the naked nontemplate DNA and error-prone DNA synthesis is reduced and subsequent a lower mutation rate.

methylations at guanine N-7 of the cap and ribose 2′-OH of the first transcribed nucleotide.[52] The RNA-capping process occurs as the sense strand RNA is being synthesized during the initiation of RNA synthesis, and it requires three enzymatic activities (Figure 3(a)). Another example is the *THZ1* roles for Cdk7 (a subunit of TFIIH phosphorylates RNA polymerase II) in cotranscriptional RNA *5′* capping. *THZ1* is a covalent Cdk7 inhibitor and it can affects Cdk7 during cotranscriptional *5′* capping and pausing.[53] *THZ1* blocks essentially all Pol II subunit CTD phosphorylation instead of influence initiation, and the guanylylation of nascent RNAs is modulated by its length, and modulated by a THZ1-sensitive factor that exist in the nuclear extract.[53]

It is essential that the 3′ end of virtually all eukaryotic RNA polymerase II transcripts is being processed and forming mature polyadenylated mRNA. The polyadenylation signal is located approximately 10–30 nucleotides upstream of the poly(A) addition site in most mRNAs; the poly(A) tails are indispensable structural and functional elements of eukaryotic mRNAs, and they are also crucial for the regulation of mRNA stability.[54,55] In the HIV-1 genome, the HIV-1 poly(A) site can fold into a stable stem-loop structure, which is highly conserved among different simian and human immunodeficiency viruses.[56] It has been demonstrated that the polyadenylation can be suppressed by stable RNA structure encompassing the poly(A) signal. For example, destabilization of the wild-type HIV-1 poly(A) hairpin did not influence polyadenylation efficiency, but stabilization of the hairpin structure strongly repressed HIV-1 polyadenylation.[56–58] Recently, Geisberg et al.[59,60] found that RNA structures involving the poly(A) tail and other 3′ sequences are primary determinants for mRNA isoform stability in yeast (Figure 3(b)). The authors also discovered that distinct 3′ mRNA isoforms derived from the same gene produced different stabilities, and the half-life variability is intensively prevalent across mRNA isoforms from the same gene.[60]

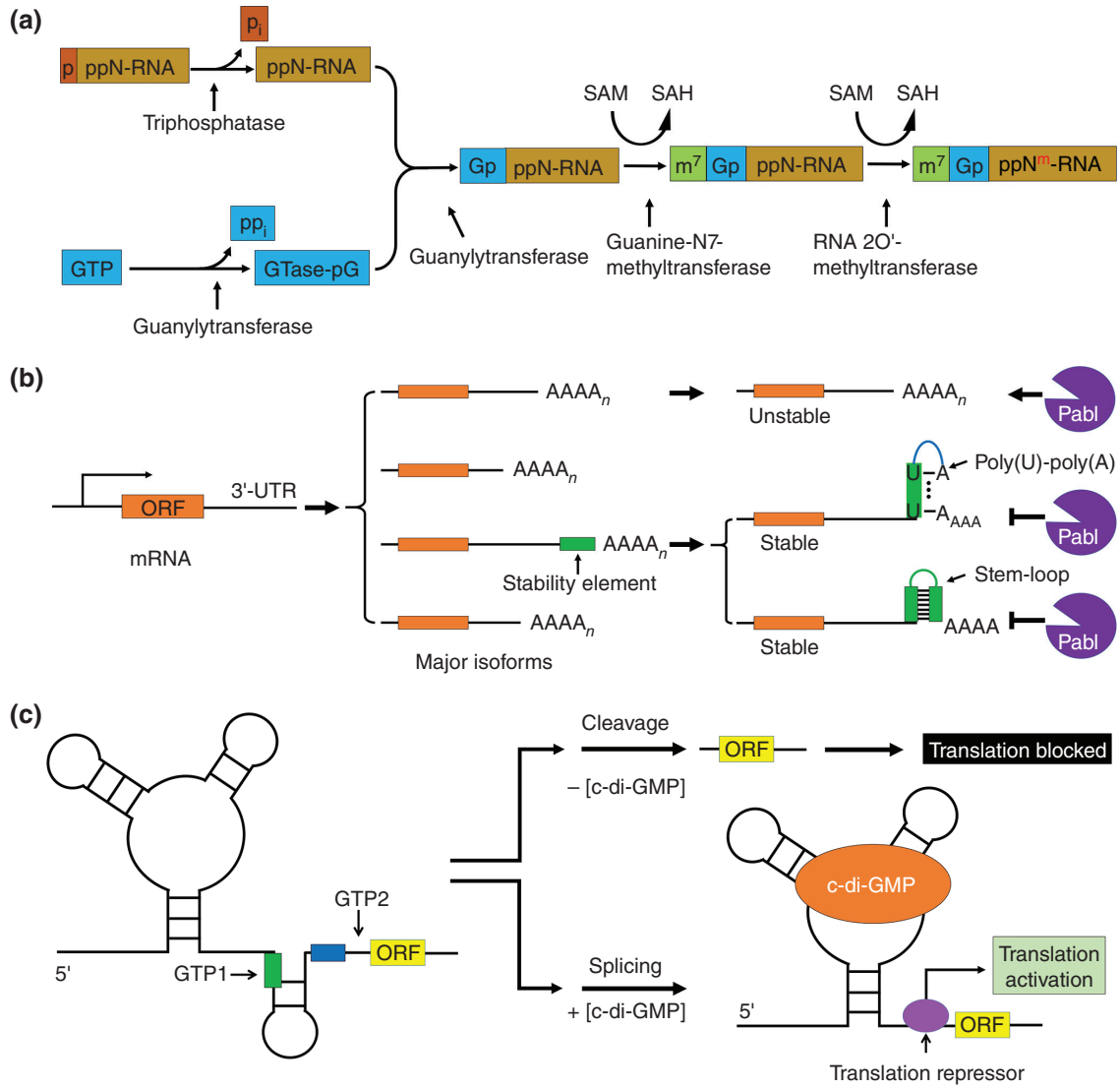

FIGURE 3 | The regulation for mRNA transcriptional and posttranscriptional processing of cotranscriptional RNA structures formation. (a) The cotranscriptional process of 5′-capping of flavivirus RNA. The type 1 cap is formed sense strand RNA through four sequential enzyme activities: triphosphatase activity releases the terminal phosphate from the 5′-triphosphate end of sense strand RNA; a guanosine monophosphate (GMP) is transferred to the 5′ end of dephosphorylated RNA via guanylytransferase activity; subsequently, the capped RNA is methylated first at the N7 position of the guanine cap and then at the ribose 2′-O position of the first RNA. The MTase domain of Guanine-N7-methyltransferase carries out both methylations using *S*-adenosyl-L-methionine (SAM) as a methyl donor and SAM is converted to *S*-adenosyl-L-homosysteine (SAH). (b) The cotranscriptional process of 3′ polyadenylation of RNA involving in mRNA stability in yeast. In this model, four isoforms were demonstrated and one isoform is readily degraded by the exosome complex (Pab1, purple) due to the absence of stability element. In contrast, another isoform possesses a longer half-life due to the presence of a stability element, which the presence of either a polyU-poly(A) tail or a stem-loop near the 3′ terminus blocks exosome-dependent degradation. (c) The intron's self-excision of the ribozyme riboswitch. In the absence of c-di-GMP, the intron take advantage of GTP2 cleavage site, thus yielding truncated RNAs that are not expressed. Binding to c-di-GMP to the riboswitch in the presence of GTP promotes cleavage site 1 (GTP1) and self-excision of the group I self-splicing intron, leading to mRNA is efficiently translated.

Through detection of the half-life of many distinct mRNA isoforms from a single gene, numerous RNA sequence elements associated with the stabilization and destabilization of individual isoform could be identified.[60]

Regulation of Splicing

Accurate and highly efficient recognition of sites for pre-mRNA splicing is important for appropriate transcript expression, and the usage of splice site can be modulated by RNA structures.[61] Many studies

have shown that two basic classes of RNA structures can directly regulate splicing, that is, RNA structures can inhibit splicing and RNA structures can aid splicing.[1,15,61,62] With regard to the riboswitch-mediated regulatory mechanism for splicing, the most widespread riboswitch class in eukaryotic cells is associated with the coenzyme TPP and is typically located in intronic regions of genes. Cheah et al.[15] characterized three TPP riboswitches in a filamentous fungus, and showed that one activates gene expression and the other two suppress gene expression by controlling mRNA alternative splicing. Furthermore, distinct secondary structure between ligand-bound and unbound forms of the pre-mRNA sequester, expose, or relocate splice sites, resulting in alternatively spliced mRNAs.[15] Another type of riboswitch is cyclic di-guanosyl-5′-monophosphate (c-di-GMP), which is sometimes located adjacent to group I self-splicing introns.[63] The binding of c-di-GMP to its aptamer triggers changes in folding that allow GTP to attack a 5′ splice site of the intron, leading to self-excision of the intron, which connects two distantly located halves of the ribosome-binding site (RBS) to produce a translatable mRNA (Figure 3(c)).[64]

Aside from the effects that predominantly involving RNA structure as a physical, but fixed modifier of splicing signals, RNA structures also play a more dynamic role in splicing regulation. Raker et al. described built-in intronic elements for modulating mutually exclusive splicing of the 14-3-3ξ pre-mRNA that were specific and not widely conserved at the secondary structure level among *Drosophilids*. Intriguingly, by activation of the proximal and variable exon outside of the loop and repression of the exon within the loop, along with the competition of RNA pairing, these inter-intronic RNAs synergistically ensured the exclusive selection of one of multiple exons.[1,62]

Regulation of Export and Localization

The export and localization of mRNA within cells are two preliminary steps that are extremely important in the control of gene expression.[21,65] Many mRNAs that encoding secreted proteins contain sequences in the 5′ end of the coding region, which influence RNA localization by the mRNA region that encodes the signal peptide. The mRNA region that encodes the signal peptide also possess pivotal information at the nucleotide level for mRNA localization.[66] The 5′-UTRs and the first 30 coding nucleotides of these mRNAs are crucial for mRNA export.[66] The parallel analysis of RNA structure (PARS) study in yeast revealed that these regions in mRNAs that encoding secreted proteins are less structured on average compared with the same regions in other mRNAs.[67] Studies have shown that RNA structures are pivotal to mRNA export. For instance, misfolded RNA that undergoes incorrect processing in nucleus is prevented from being exported from the nucleus to the cytoplasm, while native RNA that is correctly folded can be exported successfully (Figure 4(a)).[34,35]

In the neurite localization signal mechanism, previous studies indicated that control of RNA localization is via cis-acting RNA elements known as zip-codes, which are primarily found within the 3′-UTRs of mRNAs.[68–70] Subramanian et al.[69] discovered that large amounts of dendritic mRNAs contain a G-quadruplex consensus in their 3′-UTR region. The G-quadruplex structure is a common neurite localization signal and indispensable for efficient and fast localization of mRNAs in cortical neurites.[69] In addition, stem-loop RNA structures are key in regulating their localization in cells, such as the *Drosophila melanogaster fs (1) K10* signal forms a stem loop with two dsRNA helices employing a specific A′-form conformation possessing wide major grooves reminiscent of those in B-form DNA.[65] Another example is a stem-loop in the *oskar* 3′-UTR, which stimulates *oskar* mRNA transfer to the developing oocyte and shows similar functional features with the *fs(1) K10* oocyte localization signal.

Regulation of Translation

Evidence is accumulating that RNA folding can regulate the initiation, elongation, and termination of translation via sequestering or exposing RBS, by influencing the ribosomal RNA structure, or by using other patterns.[66,71] In human myeloid cells, a protein-dependent RNA structure transition in the 3′-UTR region of *VEGFA* mRNA has been investigated, and it regulate translation of *VEGFA* mRNA respond to proteins correlated with two distinct stress stimuli (Figure 4(b)).[27] As shown in Figure 4 (b), interferon-γ (IFN-γ) becomes an activated inhibitor of translation (GAIT) complex by binding a GAIT structural element, and it can facilitate the formation of a translation-silencing (TS) conformer. Under hypoxia stress, the heterogeneous nuclear ribonucleoprotein L (hnRNPL) eliminates the GAIT silencing by occluding the GAIT element and allowing a secondary structural RNA switch to a translation-permissive (TP) conformer.[27] Furthermore, a mechanism of translational regulation on the ribosome has been identified; that is, ribosomes can

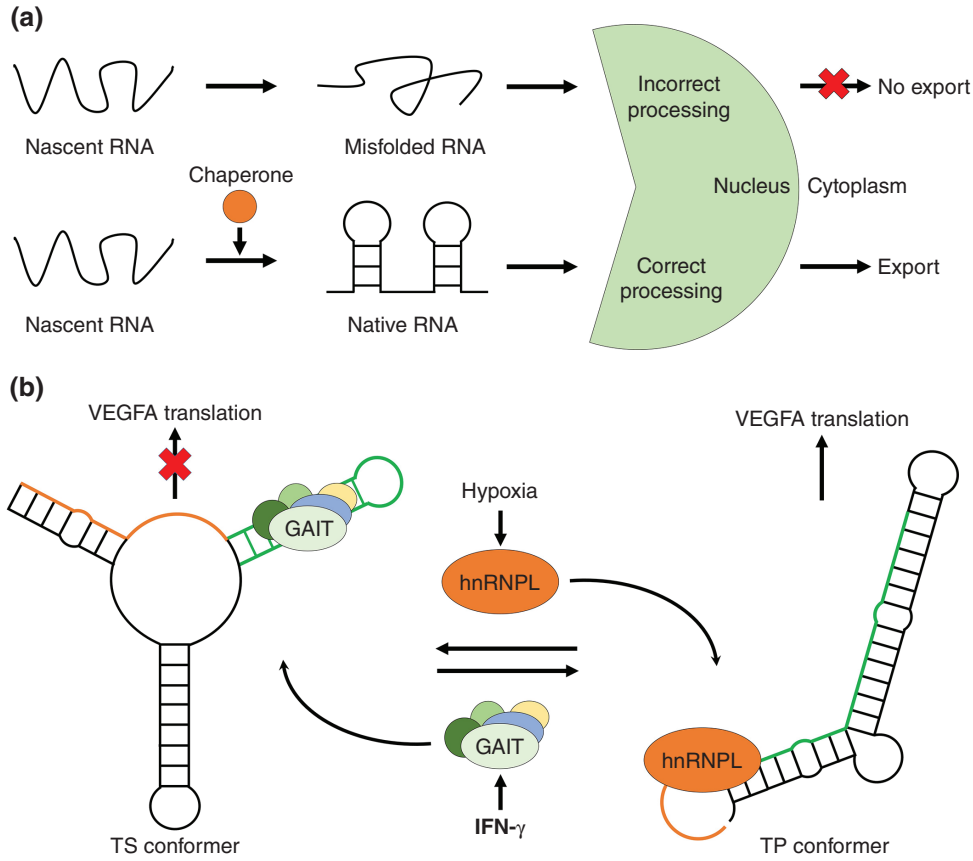

FIGURE 4 | Regulatory effect for posttranscriptional processes of RNA structural transition. (a) The mechanism of RNA being exported from nucleus to cytoplasm depend on whether RNA folded correct or not. Misfolded RNAs are unsuccessfully exported, while native RNAs can be exported from nucleus to cytoplasm through correct processing. (b) The protein-dependent RNA structure transition in the 3′-UTR of *VEGFA* mRNA regulates its translation. Strong and weak RNase cleavage sites are marked by yellow and green circles, respectively. Mutually conformation transition can be occurred between the translation-permissive (TP) conformer and translation-silencing (TS) conformer. *VEGFA* expression was blocked by interferon-γ (IFN-γ) binding to the IFN-γ-activated inhibitor of translation (GAIT) complex to form TS conformer, while hypoxic stress that leads to hnRNPL binding and causes a switch to a TP conformer.

be targeted by a protein that specifically recognizes an alternative rRNA secondary structure, and translation is blocked by a Z-DNA-binding domain attaching to rRNA structures.[72]

Significantly, a plethora of studies demonstrated that RNA structure transitions occurring in the 5′-UTR region, such as hairpin or stem-loop and G-quadruplex structure, has an immense impact on translation.[73–75] For example, the R-Lc mRNA from maize that encodes a transcriptional activator, it is poorly translated on account of a 5′ proximal stem-loop structure that blocks primary initiation of translation, an upstream open reading frame (uORF) that represses reinitiation, and an AU-rich intercistronic that suppresses reinitiation at the coding ORF.[73]

THE IMPACT OF RNA STRUCTURE FORMATION IN PLANTS

Although the understanding of transcriptional and posttranscriptional regulation through RNA structure is not as advanced in plants as in other organisms (bacteria, viruses, and mammals), recent progress in functional characterization of RNA structures has shed new light on the emerging roles of formed RNA structures in plants. In the next few sections, we summarize the roles of RNA structure in various plant regulatory processes.

Regulation by Riboswitches

Previous studies have shown that riboswitches regulate the levels of mRNAs through their conformation

transitions. The TPP riboswitch is one of the most beautiful-studied examples of gene expression control by RNA structure in plants.[76,77] The TPP riboswitch was first discovered in bacteria, and it can be readily distinguished by homology throughout all plant species; it is found in the 3′-UTR of the thiamineC (*THIC*) gene that is essential for thiamine biosynthesis.[76,77] The *THIC* TPP riboswitch regulates the formation of transcripts with alternative 3′-UTR lengths, which affects mRNA accumulation.[77] Meanwhile, riboswitch-mediated regulation of alternative 3′ end processing is crucial for *THIC* expression that depends upon TPP feedback modulation, suggesting that the splicing and alternative 3′ end processing of mRNAs is controlled by metabolite dependent alteration of RNA folding.[76,77] The results highlight the importance of metabolite sensing by riboswitches in plants, and further decipher the significance of alternative 3′ end processing as a mechanism of gene regulation in eukaryotes.

Structure-Encoded Functions of ncRNAs

An increasing number of studies have shown that many noncoding RNAs (ncRNAs) play critical roles in mRNA translation by affecting RNA structure transition in mammals and plants.[30,72,78] ncRNAs comprise a diverse group of transcripts, including not only 'housekeeping' ncRNAs [ribosomal RNAs (rRNA), transfer RNAs (tRNA)], but also 'regulatory' ncRNAs [small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), small-interfering RNAs (siRNAs)]. Earlier attention was given to small regulatory RNAs, such as miRNAs and siRNAs, because they play tremendous important roles in transcriptional and posttranscriptional regulations in plants.[79] Considering the several excellent review articles on this topic,[79,80] we do not go into too much detail here.

Recently, long noncoding RNAs (lncRNAs) that contain >200 nt but lack protein coding potential have been extensively studied.[81] lncRNAs are processed by splicing or nonsplicing or by polyadenylation or nonpolyadenylation, and these processes can occur in the nucleus or cytoplasm.[81] It is well established that interaction between lncRNA and mRNA is involved in the process of cotranscriptional RNA structure formation in bacteria, fungi, and mammals.[10,82] The siRNAs and miRNAs are evolutionarily high conserved and widely involved in posttranscriptional gene expression through canonical base pairing with their targets, while lncRNAs are poorly conserved and they regulate gene expression by diverse mechanisms that are not yet fully understood.[81] LncRNAs are larger and thus possess complex folded structures. Their complicated structures enable lncRNAs to bind DNA, RNA, protein molecules, and/or their combinations in the nucleus, and thus they have multiple regulatory capacities.[81,83] Furthermore, folded structures of lncRNAs appear to be conserved, such as the 'double stem-and-loop' structure for PRC2 binding.[84]

Remarkably, lncRNAs are involved in the various processes of RNA structure formation and transitions, which play essential roles in transcriptional and posttranscriptional regulatory mechanisms in plants, such as vernalization, fertility, photomorphogenesis, phosphate homeostasis, protein re-localization, alternative splicing, and modulation of chromatin loop dynamics.[85,86] Vernalization is the best-studied regulatory process in plants that is known to involve lncRNAs, primarily in the regulation of *FLOWERING LOCUS C* (*FLC*).[84] Recent studies have shown that at least two types of lncRNAs are present in the *FLC* locus. One is the long antisense RNA, called *COOLAIR* (Cold induction long antisense intragenic RNA), is transcribed in antisense orientation in relation to *FLC*. *COOLAIR* is an alternatively spliced lncNAT (long noncoding natural antisense transcripts) under the control of a promoter located downstream of *FLC*, and its promoter activity is regulated by an R-loop (a three-stranded nucleic acid structure formed by an RNA-DNA hybrid plus a displaced ssDNA). *FLC* is transcribed in a chromatin gene-loop before vernalization, while the *FLC* gene-loop is disrupted early during vernalization. The disruption is in accordance with *COOLAIR* transcription, and interactions with local chromatin promote transcriptional shutdown of *FLC*.[84] The second lncRNA, named *COLDAIR* (Cold assisted intronic noncoding RNA), is transcribed by Pol II from the first *FLC* intron in the same direction as *FLC* mRNA. *COLDAIR* recruits polycomb repressive complex 2 (PRC2), increasing the level of H3K27me3 at *FLC* chromatin after vernalization, and maintains the stable silenced state of the locus.[84]

New Insights into RNA Structure and Its Regulatory Effects in Plants

Numerous methods have been developed to investigate plant RNA structures because of their tremendous importance in various biological processes.[18,19,87] The first genome-wide RNA structure probing studies were the nuclease-based ssRNA-/dsRNA-seq investigations performed on *Arabidopsis* unopened flower buds.[18,19] Zheng et al.[18]

compared the constrained RNA folding predictions to *in silico* free energy minimization algorithms, and they validated dsRNA-seq as a higher fidelity alternative to *in silico* techniques. They also showed that conservation at structure hotspots was higher than in flanking regions.[18] Subsequently, Li et al.[19] used high-throughput sequencing technologies to directly assay RNA secondary structure in unopened *Arabidopsis* flower buds. They found that RNA folding was significantly and negatively correlated with total transcript abundance, which is often caused by highly structured mRNAs are prefer to be degraded/processed into small RNAs.[19] A chemical-based approach for structure probing (Structure-seq) has recently been performed on the transcriptome of *Arabidopsis* seedlings.[87] Application of this method yielded the first genome-wide RNA structure map *in vivo* at a nucleotide resolution for any organism, and the quantitative structural information across more than 10,000 transcripts. The authors also found patterns of strong and weak secondary structures at sites of alternative polyadenylation, and strong secondary structures at 5′ splice sites that correlated with unspliced events.[87] Furthermore, a recent study using the protein interaction profile sequencing (PIP-seq) approach demonstrated that the patterns of RNA secondary structure and RNA binding proteins (RBPs) binding are negatively correlated throughout nuclear mRNAs, and demarcate sites of alternative splicing and polyadenylation.[88] The authors also uncovered some protein-bound sequence motifs, and identified their structural contexts, cooccurrences in transcripts that encode functionally related proteins, and interactions with speculate RBPs.[88] In summary, these powerful approaches are uncovering the patterns and functionality of RNA structure on a transcriptome-wide scale, providing bright prospects for a better understanding of this fundamental biological feature.

CONCLUSIONS AND PERSPECTIVES

Significant advances have been achieved in elucidating RNA folding pathways, and these studies have greatly enhanced the understanding of the structure formation of RNA interactions in transcription. Nonetheless, because of the highly dynamic and complex features of RNA conformation transitions, the understanding is still in its infancy. This overview shows that RNA cotranscriptional folding processes are influenced by intrinsic and extrinsic features, such as the RNA sequence itself, the speed of transcription, and trans-interaction partners. *In vivo*, these factors interact in the appropriate cellular conditions and ultimately determine the functional state of a transcript being formed. The formation and state of RNA structure play various roles at almost every step of life, and they differ between *in vivo* and *in vitro* conditions. Future efforts will strengthen understanding of RNA folding processes and develop more comprehensive approaches for identifying RNA secondary and tertiary structures. Important questions center on the relationship between cotranscriptional RNA folding and pre-mRNA processing, how transient RNA structures affect cotranscriptional RNA processing, and conversely, how pre-mRNA processing, particularly splicing, provides feedback for RNA folding.

As highlighted herein, experimental evidence has intensely strengthen our understanding of the regulatory functions of RNA structures in bacteria, fungi, and mammals. In contrast, the understanding of the regulatory mechanisms and functions of RNA structure formation and transitions are still poor in plants, hence, future efforts should enhance the understanding in plants. Experimental strategies for preserving and detecting both native protein-RNA interactions and cotranscriptional RNA folding have immense significance. Furthermore, computational methods are imperative in the era of genome-wide probing, and the errors should be minimized during computational prediction of RNA structures. Ultimately, RNA tertiary structure prediction and analysis need be strengthened to improve understanding of the RNA structural motifs identified from atomic-resolution models and the RNA folding principles. Analyses coupled with functional studies and applied to wider range of systems will provide better comparison and more profound understanding of diverse functions of RNA structure in life.

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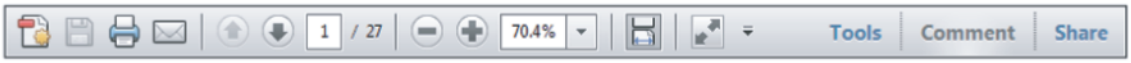

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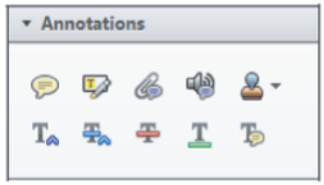

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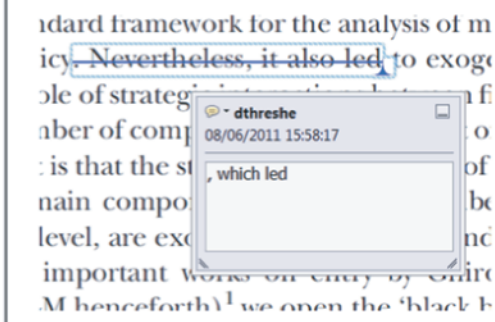

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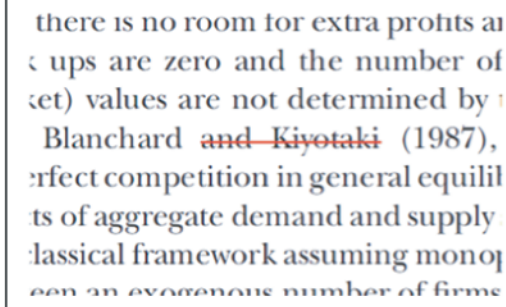

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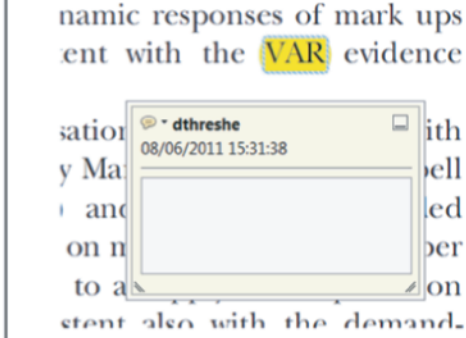

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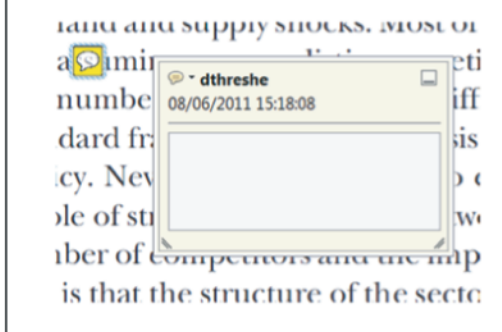

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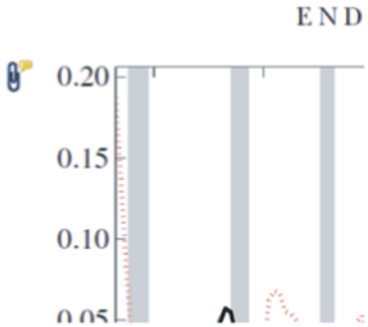

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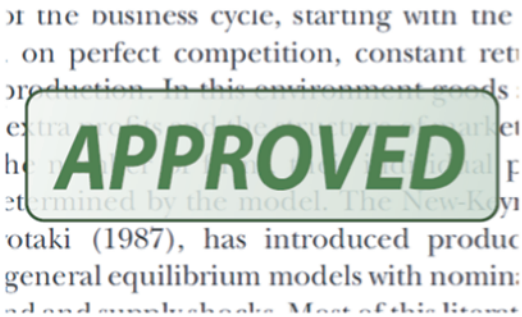

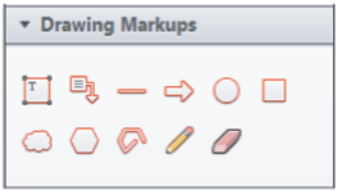

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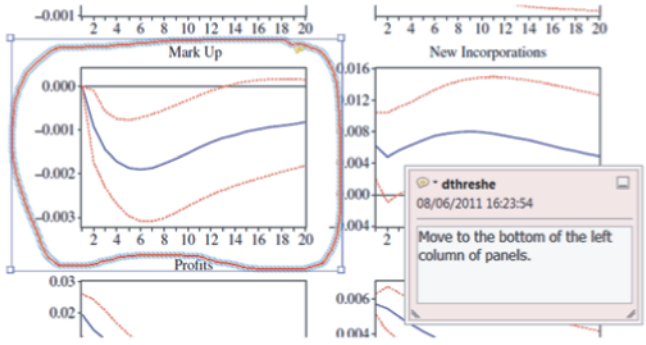

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