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Molecular basis of viroid RNA-templated transcription

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Transcription is a fundamental process catalyzed by DNA-dependent RNA polymerases (DdRPs). Interestingly, some DdRPs can use both DNA and RNA as templates for transcription. This RNA-dependent RNA polymerase (RdRP) activity of DdRPs is used by RNA-based pathogens such as viroids and hepatitis delta virus for replication. In addition, RdRP activity of DdRPs widely occurs in various organisms to regulate gene transcription. Despite the importance of this intrinsic RdRP activity of DdRPs, associated factors and mechanisms are in their infancy stage. We employed potato spindle tuber viroid (PSTVd) as a model to study RNA-templated transcription. Here, we present evidence showing that circular PSTVd templates are critical for the synthesis of longer-than-unit-length (-) strand products. Further, we show transcription factor IIS is dispensable for PSTVd replication supporting *de novo* transcription on PSTVd RNA templates. The absence of canonical general transcription factor, TFIIS from PSTVd-templated transcription complex led to the hypothesis that RNA-templated transcription has a distinct organization on the RNA template. To test this hypothesis, we used our well-established *in vitro* transcription (IVT) system and demonstrated that RNA polymerase II (Pol II) accepts minus-strand for transcription. In addition, transcription factor TFIIIA-7ZF is needed to aid Pol II transcription activity. Further analyses of the critical zinc finger domains in TFIIIA-

7ZF revealed that the first three zinc finger domains are pivotal for template binding. Notably, we identified a remodeled Pol II complex for viroid transcription that is missing Rpb4, Rpb5, Rpb6, Rpb7, and Rpb9. General transcription factors for DNA-templated transcription are also absent in the transcription complex on the RNA template. This remodeled Pol II complex still possesses the transcription activity on PSTVd RNA template. Collectively, our data illustrate a distinct organization of Pol II complex on viroid RNA templates, providing new insights into viroid replication, the evolution of transcription machinery, as well as the mechanism of RNA-templated transcription.

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CHAPTER I

INTRODUCTION: VIROID AS AN EXCELLENT MODEL SYSTEM TO STUDY RNA-DEPENDENT RNA POLYMERASE ACTIVITY OF RNA POLYMERASE II

This chapter is a modified version of "Potato spindle tuber viroid RNA-templated transcription: factors and regulation" published in Viruses [1] and has been reproduced here with the permission of the copyright holder. I have significantly contributed to the main concepts explained in this review. Figures 1.3 and 1.5 were based on data obtained by Dr. Ying Wang. This review served as an outline of my Ph.D. project.

1.1 Biology of viroid replication

Viroids are single-stranded circular noncoding RNAs with a genome size of 250-400 nucleotides (nt). Without protein-coding capacity, viroids utilize plant machinery for replication and spread, often leading to disease. The first viroid, potato spindle tuber viroid (PSTVd), was discovered in 1971 [2, 3]. About 40 viroids have been reported since then and are categorized into two families, *Avsunviroidae* and *Pospiviroidae*. Members of the *Avsunviroidae* family replicate in chloroplasts. Some evidence suggested the involvement of plastid-encoded RNA polymerase (PEP) in the replication of viroids in *Avsunviroidae* [4]. However, based on the sensitivity of avocado sunblotch viroid replication to tagetitoxin (an inhibitor of chloroplast RNA polymerase), a nucleus-encoded RNA polymerase (NEP) is considered as the replication enzyme [5]. All other viroids belong to the *Pospiviroidae* family. In general, viroids of the *Pospiviroidae* replicate in the nucleus. They have highly conserved regions in their rod-shaped structure. These

regions include left-terminal, pathogenicity, central, variable, and right-terminal domains [6]. Nuclear-replicating viroids replicate via an asymmetric rolling-circle mechanism (Figure 1.1). First, (+) circular RNA is transcribed into concatemeric linear (-) strand RNA in the nucleoplasm. This intermediate RNA acts as the template for concatemeric linear (+) strand RNA. It is then cleaved into unit-length monomers. Subsequently, these monomers are circularized via end-to-end ligation and transported into neighboring cells.

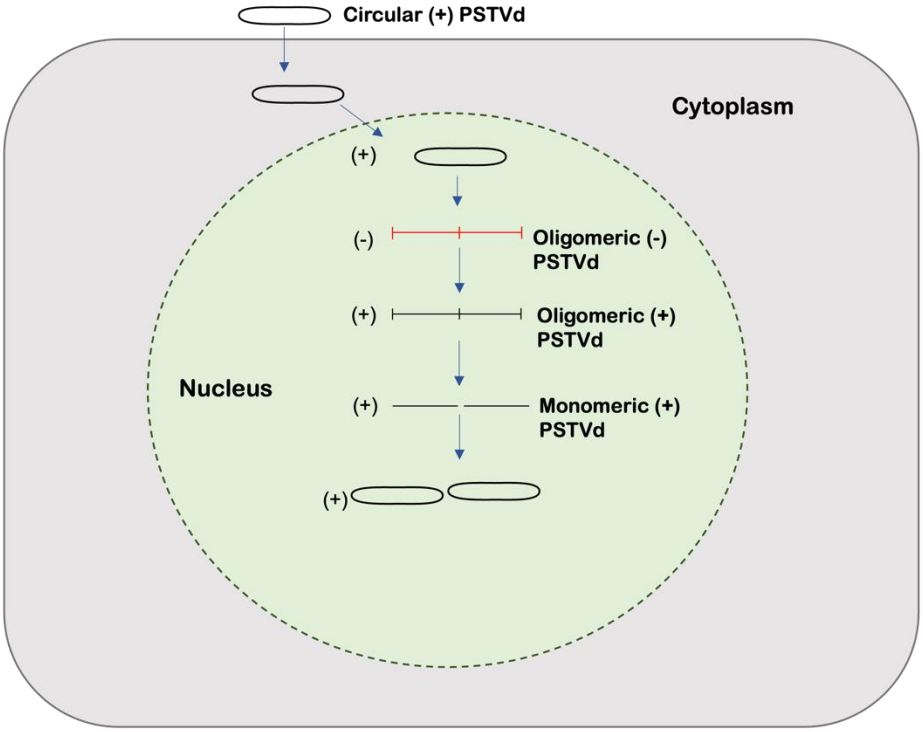


Figure 1.1 Asymmetric rolling circle replication of PSTVd.

The nodes in the (-) and (+) concatemers depict the cleavage sites for generating unit-length products. Red lines depict antisense orientation of PSTVd, while black lines depict sense orientation of PSTVd.

Transcription from circular genome to multimeric (-) intermediates rely on the RNA-dependent RNA polymerase (RdRP) activity of DNA-directed RNA polymerase II (Pol II) [7, 8].

The intrinsic RdRP activity of DNA-dependent RNA polymerases (DdRP) was first reported by Dezélée et al. in 1974 [9]. However, the identification of Pol II as the authentic polymerase for the replication of PSTVd [9], after the assumption regarding the involvement of a tomato RdRP [10], was the first to demonstrate the intrinsic RdRP activity of a DdRP in a natural biological process. This discovery was strongly supported by the evidence that (1) purified Pol II can transcribe PSTVd template and generate full-length (−) strand product and (2) α-amanitin, at a concentration that specifically inhibits Pol II activity, inhibited PSTVd-templated transcription [11]. Following this discovery, similar observations confirmed the involvement of Pol II in the replication of various viroids of the *Pospiviroidae* family based on α-amanitin treatment and/or Pol II binding [12-20]. The evidence showing Pol II binding to circular PSTVd RNA template *in vivo* has also been reported recently [21]. The human hepatitis delta virus (HDV) also utilizes this RdRP activity of DdRPs for propagation [22, 23]. Further, recent studies showed that the RdRP activity of DdRPs is critical for bacterial response to nutrient shortage showing broader biological significance beyond pathogen infection. Under nutrient deficient conditions, 6S RNA in *Escherichia coli* functionally engages in the active site of DdRP and prevents it from transcribing a DNA template. In response to nutrient availability, the DdRP transcribes a few nucleotides from 6S RNA and liberates DdRP from 6S RNA. Mammalian Pol II can use a non-coding RNA, B2 RNA, as a template to extend its short strand by 18 nt. This extension causes destabilization of B2 RNA and represents a novel posttranscriptional mechanism for altering the stability of B2 RNA. Thus, the RdRP activity of DdRPs exerts diverse biological functions, extending from pathogen infection to regulating gene expression across kingdoms.

The high-resolution crystallographic structure of yeast Pol II shows that Pol II utilizes the same active site for interacting with DNA and RNA templates [24]. Thus, the RdRP activity of Pol

II shows that an ancestor of Pol II replicated early RNA genomes. This ancient RdRP activity of Pol II has persisted in modern life in catalyzing the transcription of viroids and HDV.

1.2 Factors involved in PSTVd transcription

Pol II requires various co-factors for successful transcription [22, 23]. However, the factors involved in PSTVd-templated transcription by Pol II are an outstanding question. Loop E motif, located in the central conserved region of PSTVd is implicated in replication and other functions. This motif is similar to the loop E motifs on archaeal and eucaryal 5S rRNAs. With the discovery of loop E RNA motif on PSTVd, 5S rRNA binding proteins such as transcription factor IIIA (TFIIIA) and ribosomal protein L5 (RPL5) are considered to be possible candidates for co-factors for PSTVd-templated transcription. TFIIIA is a single copy gene conserved in eukaryotes [25]. In mammalian cells and yeast, TFIIIA is required for the transcription of 5S rRNA and transports it to the cytoplasm [26].

TFIIIA has nine C2H2 type zinc finger domains (ZFs) [25, 27]. The first plant TFIIIA gene from *Arabidopsis thaliana* was cloned in 2003 [28]. Two later reports showed that the TFIIIA gene in land plants has a conserved alternative splicing pattern [29, 30]. One splicing variant encodes the canonical TFIIIA with nine ZFs (TFIIIA-9ZF), while the other splicing variant, TFIIIA-7ZF, has seven ZFs due to the short 5S rRNA-mimic intron that introduces premature stop codon (PTC) [21]. However, the biogenesis of TFIIIA-7ZF is under debate due to the reports that suggests PTC near the 5' end of *TFIIIA-7ZF* is regulated by the non-sense mediated decay (NMD) pathway [29-31]. A recent study showed that transcripts regulated by NMD usually have a very long 3' untranslated region in plants [32]. This may not be the case for *TFIIIA-7ZF* because it contains a normal open reading frame after PTC. By contrast, the uORF encoding two ZFs may play a regulatory role in translating the downstream ORF (TFIIIA-7ZF),

as exemplified in several recent studies in plants [33-37]. Early work failed to demonstrate the translation of TFIIIA-7ZF [29, 30]. However, with the development of TFIIIA-specific antibodies, immune blotting results from *A. thaliana* and *Nicotiana benthamiana* showed the presence of TFIIIA-7ZF protein in plants [21, 31]. Later, it was concluded that the production of TFIIIA-7ZF is a result of the proteolytic process [31]. This conclusion was based entirely on complementing the potential lethality of a homozygous *A. thaliana* mutant (SALK_008626C) that has a T-DNA insertion in TFIIIA locus. However, the T-DNA insertion is at the end of C-terminal region leaving all the nine ZFs unaffected and the homozygous plant is viable according to Arabidopsis Biological Resource Center (https://www.arabidopsis.org/ servlets/SeedSearcher action=detail&stock_number=SALK_008626c). Therefore, data supports a model where TFIIIA-7ZF is directly translated from *TFIIIA-7ZF*. This position is supported by (1) the molecular weight of the observed TFIIIA-7ZF protein is in line with the predicted ORF [21, 31] and (2) the altered expression of the splicing regulator (RPL5) directly leads to changes in the TFIIIA-7ZF accumulation (see below for more details) [38].

After the discovery of the plant *TFIIIA* gene, Eiras et al. [39] first showed *Arabidopsis* TFIIIA-9ZF can bind to (+) PSTVd *in vitro* . Following this report, Wang et al. [21] further confirmed that both TFIIIA-9ZF and TFIIIA-7ZF can bind to (+) PSTVd strand both *in vivo* and *in vitro.* TFIIIA-9ZF has a higher affinity for (+) PSTVd compared to TFIIIA-7ZF [21, 39]. However, only TFIIIA-7ZF interacts with (-) PSTVd *in vitro* and *in vivo*. The major binding site for TFIIIA-7ZF was mapped to the lower portion of PSTVd left terminal domain [21], coinciding with the loop structures critical for replication *in vivo* [40] and Pol II binding [41], as well as near the transcription initiation site [14]. In contrast, TFIIIA-9ZF binds to the right terminal domain, where RNA motifs critical for trafficking reside [40, 42]. Interestingly, TFIIIA-

9ZF exclusively accumulated in nucleoli in PSTVd-infected leaves, while TFIIIA-7ZF located in both nucleoplasm and nucleoli [21]. The difference in spatial distribution patterns makes TFIIIA-7ZF, but not TFIIIA-9ZF, more likely to function together with Pol II, an enzyme localized in the nucleoplasm. Manipulating the expression of TFIIIA-7ZF affected PSTVd titers in the nucleus, which further supported that TFIIIA-7ZF is the bona fide transcription factor for PSTVd. The most direct evidence was from an *in vitro* transcription assay that resulted in the production of longer-than-unit-length (-) PSTVd. This assay was successful using purified TFIIIA-7ZF, but not purified bovine serum albumin or TFIIIA-9ZF, when added with purified Pol II and circular PSTVd template [21]. Therefore, TFIIIA-7ZF is a dedicated factor critical for Pol II-dependent transcription on PSTVd RNA template to generate longer-than-unit-length products. However, it remains to be determined if TFIIIA-7ZF is also critical for transcription initiation.

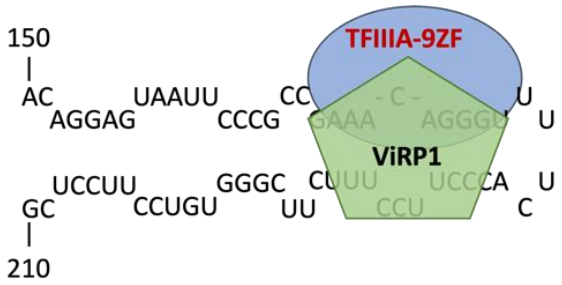


Figure 1.2 Binding sites of TFIIIA-9ZF and VirP1 in the right terminal region of PSTVd genome.

Another remaining question is the functional implication of the interaction between TFIIIA-9ZF and PSTVd. TFIIIA-9ZF is often the dominant form in plants and binds to PSTVd with a high affinity [21, 39]. Due to technical challenges, TFIIIA-9ZF loss-of-function assay was not achieved, although over-expression of TFIIIA-9ZF did not appear to affect PSTVd

replication [21]. It is interesting to note that the TFIIIA-9ZF binding site partially overlaps with that of VirP1 (Figure 1.2), another PSTVd host factor [21, 42, 43]. Studies have shown that VirP1 binds to RY motifs close to PSTVd right terminal domain and regulates PSTVd replication [43, 44]. A recent report showed that VirP1 can facilitate the nuclear import of a viral satellite RNA, which raises a possibility that VirP1 may also regulate the nuclear import of PSTVd [45]. In this regard, it is worth testing whether VirP1 and/or TFIIIA-9ZF regulate PSTVd nuclear import. On the other hand, it is also of interest to understand the critical ZFs in TFIIIA-7ZF for RNA-templated transcription, which will shed light on the molecular mechanism in regulating the RdRP activity of a DdRP and the coordination of multiple ZFs in function.

Transcription is a complex process that requires a group of factors functioning cooperatively with a polymerase. The composition of the required machinery is clear for DNA-dependent transcription through decades of studies [22, 23]. By contrast, the required factors involved in RNA-templated transcription by Pol II or other DdRPs remain elusive, despite that Pol II utilizes the same active site for both RNA and DNA templates [24]. Studies on human hepatitis delta virus (HDV; relying on Pol II for transcription and replication) suggested that a preinitiation complex is composed of multiple general transcription factors, such as TFIIA, TFIIB, TFIID (containing TATA-box binding protein; TBP), TFIIE, TFIIF, TFIIH, and TFIIS [46]. This observation shows that transcription machinery for DNA-templates and RNA-templates share certain similarities. However, a hepatitis delta antigen (HDAg-S) is required in the Pol II elongation process [47], indicating the presence of unique factors for RNA-templated transcription by DdRPs. Based on our unpublished data and numerous studies on TFIIIA-9ZF truncated protein [48-51], TFIIIA-7ZF does not possess 5S rDNA-binding capacity. The RNA-binding capacity allows TFIIIA-7ZF to act as an RNA-templated transcription factor, which is

required for the production of longer-than-unit-length PSTVd by Pol II [21]. Besides TFIIIA-7ZF and the largest and second largest subunits of Pol II, other factors for PSTVd-templated transcription await identification. In an effort to show the in vivo interaction of TFIIIA-7ZF and Pol II using co-immunoprecipitation, we detected the enrichment of TBP in the immunoprecipitated fraction using TFIIIA-7ZF as a bait (Figure 1.3). This result supports the involvement of TBP in the machinery for PSTVd-templated transcription in plants, but future functional analyses are needed. In addition, it will be insightful to understand the protein composition of purified Pol II used for PSTVd-templated *in vitro* transcription.

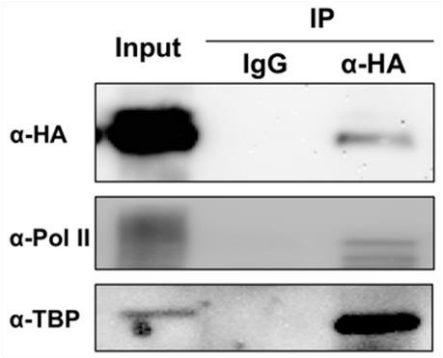


Figure 1.3 Co-immunoprecipitation of TFIIIA-7ZF/Pol II

Overexpressed and HA-tagged TFIIIA-7ZF served as bait and successfully pulled down endogenous Pol II largest subunit and TBP. IgG and α-HA-conjugated resins were used as negative and positive treatments, respectively. The experimental methods were described previously [21]. IP, immunoprecipitated fractions

1.3 RPL5 as a regulator of TFIIIA splicing and PSTVd replication

RPL5 is a well-studied ribosomal protein that directly binds to 5S rRNA to form 5S RNP (ribonucleoprotein particle), which traffics to nucleoli for ribosomal assembly. There is no distinct RNA-binding domain in RPL5, and instead, nearly the whole protein is required for 5S rRNA binding [26]. A recent study found that alternative splicing of TFIIIA is regulated by

RPL5. RPL5 binds to the 5S rRNA-mimic intron in TFIIIA pre-mRNA thus facilitating intron removal [30]. PSTVd directly interacts with RPL5 through PSTVd central conserved region (CCR) [38]. The RPL5-PSTVd interaction is abolished when the loop E motif in CCR is disrupted. Upon PSTVd infection, the expression of RPL5 is induced about 2-fold. However, this induction fails to repress TFIIIA-7ZF accumulation, likely due to reduced protein availability through a direct RPL5-PSTVd interaction in vivo [38]. This observation suggested that PSTVd impairs RPL5 regulation over TFIIIA splicing during infection. Further over-expression of RPL5 in infected plants repressed TFIIIA-7ZF expression and reduced PSTVd titer [38]. Therefore, RPL5 as a negative factor for PSTVd, may be exploited in future breeding efforts. Furthermore, RPL5 and TFIIIA-7ZF constitute an RPL5/TFIIIA-7ZF regulatory cascade critical for PSTVd replication.

1.4 Possible PSTVd RNA conformations during transcription

PSTVd and members of the *Pospiviroidae* family fold into a rod-shaped structure with five domains: the left and right terminal domains; the pathogenicity domain; the central domain; and the variable domain [6]. This structural organization has been supported by various studies using a combination of *in vivo* and *in vitro* approaches [52-54]. Pol II transcription on (+) strand PSTVd RNA initiates at the left terminal domain, according to the mapped initiation site [14] and electron microscopy images [15]. Purified Pol II was also shown to bind with a PSTVd fragment containing the left terminal domain [41]. Therefore, it is intuitive to assume that PSTVd stays in a rod-shaped conformation during transcription and the transcription initiates at the nucleotide 1 in the left terminal loop (loop 1). However, in this view, half of the Pol II enzyme might be outside of the template at the initiation step (Figure 1.4A) and Pol II might be forced to make one or more “U turns” on a rod-shaped template during elongation. Alternatively,

a bifurcated conformation might form when interacting with TFIIIA-7ZF and Pol II during transcription initiation (Figure 1.4B). This bifurcated conformation has been determined by several studies and likely exists in various nuclear-replicating viroids, despite the fact that these viroids have distinct primary sequences [53, 55, 56]. Notably, the wildtype PSTVd fragment binding with Pol II in an in-situ assay [41] has an intrinsic capacity to fold into a bifurcated conformation or a rod-shaped conformation. The bifurcated terminal structure might provide a structural support for Pol II docking at the initiation step and might also favor the activity of Pol II by forming a stem-loop structure [24]. Furthermore, it does not against the recent structural analysis of PSTVd genomic RNA *in vivo* [52], because this alternative structure might transiently exist in only a few molecules that are masked by the dominant rod-shaped conformation. It is important to note that studying PSTVd RNA conformations during transcription processes (i.e., initiation, elongation, etc.) is critical for understanding PSTVd biogenesis and broadly for RNA-templated transcription by DdRPs. Given the lack of conclusive experimental evidence for either of the models, it is worth testing which conformation is involved in transcription initiation in the near future.

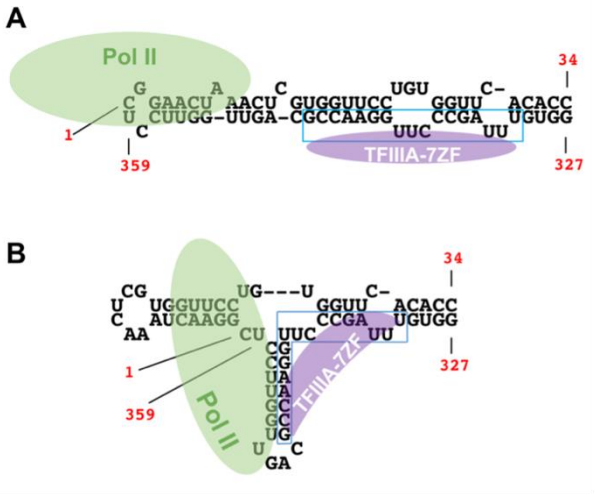


Figure 1.4 Two possible left terminal conformations during replication.

A. Traditional rod-shape left terminal conformation. B. Alternative bifurcated left terminal conformation. The mapped TFIIIA-7ZF binding region is highlighted in a blue box.

PSTVd and 10 other viroids in the genus *Pospiviroid* share conserved CCRs in terms of sequences and structures [54]. Thus, it is reasonable to assume that these viroids all exploit the RPL5/TFIIIA-7ZF regulatory cascade for replication. However, other members in *Pospiviroidae* possess CCRs with various sequences and structures [54]. Whether these viroids also employ the RPL5/TFIIIA-7ZF regulatory cascade for replication remains an outstanding question. In an *in vivo* RNA immunoprecipitation assay, we found that TFIIIA-7ZF and RPL5 cloned from *N. benthamiana* also binds to hop stunt viroid (HSVd) (Figure 1.5), which has a distinct CCR as compared with PSTVd [54, 57]. How RPL5 recognizes various CCRs needs to be determined. In addition, it is also worth testing whether the sequence variations in both RPL5 and TFIIIA-7ZF from various hosts contribute to PSTVd host ranges. Lastly, this system offers a platform to test if the RPL5/TFIIIA-7ZF regulatory cascade is employed for replication by other viroids of the family *Pospiviroidae*.

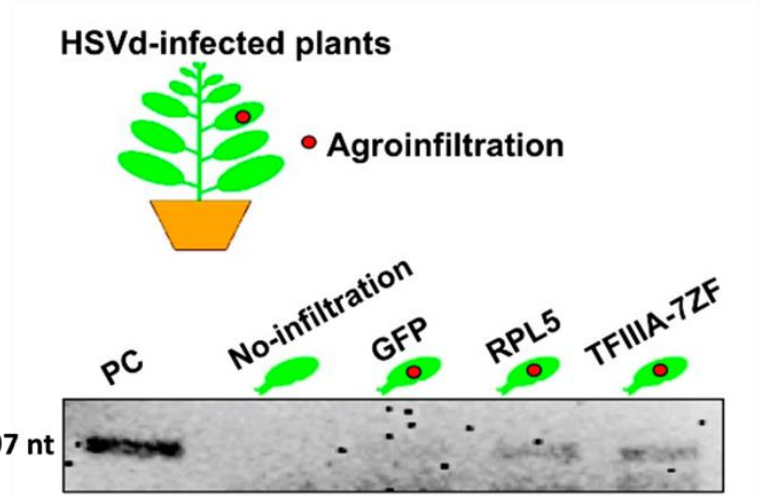


Figure 1.5 RNA-immunoprecipitation supporting that TFIIIA-7ZF and RPL5 bind to HSVd *in vivo*

HA-tagged proteins (GFP, RPL5, and TFIIIA-7ZF) were expressed in HSVd-infected *N. benthamiana* plants via agroinfiltration and served as baits. RPL5 and TFIIIA-7ZF successfully pulled down HSVd RNA. Non-infiltrated and GFP-expressing leaves served as negative controls. The experimental methods were described previously [21]. PC, monomeric HSVd cDNA served as a size marker.

Comparison of TFIIIA-7ZF/TFIIIA-9ZF and RPL5 binding with 5S rRNA and PSTVd generates interesting insights. It is well understood that TFIIIA-9ZF binds to the 5S rRNA region covering helixes I, III, and V as well as loops A, B, and E [58, 59]. By contrast, RPL5 binds to helix II and loop C of 5S rRNA [60]. Albeit PSTVd and 5S rRNA share the recurring loop E motif [61, 62], TFIIIA variants do not bind to PSTVd loop E but recognize loops in two terminal domains [21]. Instead, RPL5 binds to PSTVd loop E [38]. Replacing PSTVd loop E with the sequences of *N. benthamiana* 5S rRNA loop E results in a non-infectious strain (Figure 1.6), despite that both loop E motifs likely share similar 3-dimensional structural arrangements [62]. These observations raise the question regarding what additional mechanisms determine the specificity in protein-RNA interactions other than RNA motifs.

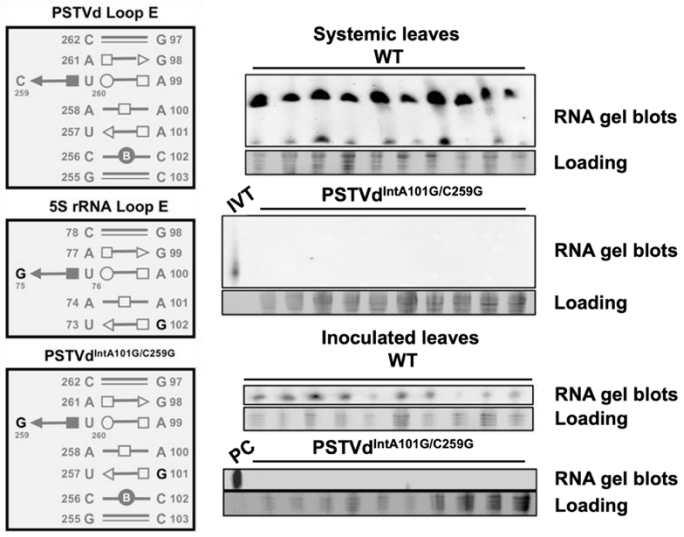


Figure 1.6 Loss of infectivity of PSTVd variant with 5S rRNA loop E sequences.

Left panel displays loop E structural arrangements in wildtype (WT) PSTVd, *N. benthamiana* 5S rRNA, and a loop E swapped PSTVd$^{IntA101G/C259G}$. The right panel shows RNA gel blots detecting the infection of WT PSTVd and PSTVd$^{IntA101G/C259G}$ in local and systemic leaves of *N. benthamiana* plants. Ethidium bromide staining of rRNAs serves as loading control. PC, a verified RNA sample from PSTVd-infected leaves served as a positive control. IVT, the unit-length *in vitro* transcript served as a control. Note: only circular PSTVd (an indicator of replication) is shown in the northern blots for inoculated leaves. The "○, □, △" symbols depict Watson-Crick, Hoogsteen, and sugar edges, respectively. These symbolic annotations of loop E 3-dimensional structures were previously described in detail [61, 63].

1.5 Transcription on (-) strand PSTVd

It is generally accepted that transcription from (+) strand genome to (-) intermediates of nuclear-replicating viroids are catalyzed by Pol II [11, 21]. However, it is less clear whether the transcription from (-) PSTVd intermediates to multimeric (+) PSTVd intermediates is also Pol II-dependent. Studies using different inhibitors led to different conclusions [16]. More importantly, the assays in these studies mixed both (-) and (+) PSTVd that can be templates for each other, generating difficulties in data interpretation. It is notable that both TFIIIA-7ZF and Pol II, but not TFIIIA-9ZF, bind to (-) PSTVd *in vitro* and *in vivo* [21], rendering them possible to function

in the transcription on the (-) PSTVd template. Nevertheless, it awaits further tests for clarification.

1.6 RNA-templated transcription of PSTVd and HDV: A brief comparison

Emerging evidence outlines plant machinery for PSTVd-templated transcription with a composition of Pol II's largest and second largest subunits, TFIIIA-7ZF [11, 21], and potentially TBP. TFIIIA-7ZF is a splicing variant from a single copy gene, TFIIIA. The alternative splicing process of TFIIIA is regulated by RPL5 through binding to the 5S rRNA-mimic intron. RPL5 binding facilitates the intron removal and favors the production of TFIIIA-9ZF [30]. PSTVd modulates its own replication through a direct interaction with RPL5, resulting in impaired RPL5 regulation over TFIIIA splicing and optimized expression of TFIIIA-7ZF (Figure 1.7A) [38]. It is interesting to compare this mechanism with the transcription on the HDV RNA template. HDV-templated transcription relies on the orchestrated activities of Pol II [64] and the virus-encoded HDAg-S [47]. Recently, a study showed the formation of a preinitiation complex on the HDV RNA template [46]. HDV contains only one HDAg gene encodes two proteins (HDAg-S and HDAg-L) with distinct roles. HDAg-L functions in viral assembly [65] while the shorter form HDAg-S is required for replication and transcription of HDV by Pol II [47, 64, 66]. Unlike the alternative splicing mechanism that regulates the expression of two TFIIIA proteins, there is a specific A-to-I RNA editing resulting in a translational read-through. This A-to-I RNA editing is catalyzed by RNA adenosine deaminase (ADAR1). The exact regulatory mechanism for this editing is under debate. It is believed that group 1 HDV viruses, but not other sub-types, can use HDAg-S to influence the RNA editing [67]. Besides, it has also been shown that HDV RNA secondary structures and viral replication may influence this RNA editing (Figure 1.7B).

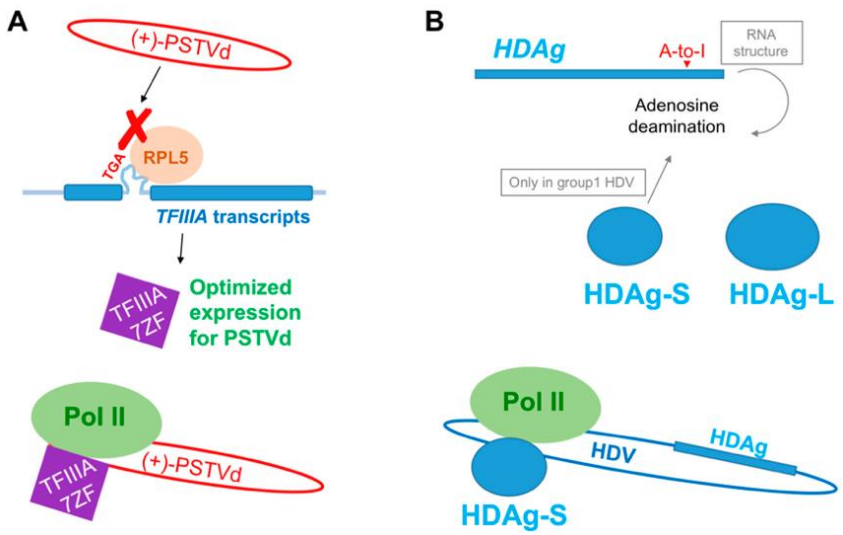


Figure 1.7 Possible regulatory mechanisms for the expression of TFIIIA-7ZF and HDAg-S

A. PSTVd directly binds to RPL5 to regulate alternative splicing of TFIIIA, resulting in optimized TFIIIA-7ZF expression and PSTVd replication; B. The expression of HDAg-S and HDAg-L is regulated through A-to-I RNA editing at a specific site. HDV RNA structure may affect the editing. In addition, HDAg-S itself may also impact the editing efficiency in group 1 HDV.

1.7 Summary

Some DdRPs possess RdRP activity, reflecting their likely evolution from RdRPs that first arose to transcribe RNA templates [24]. Prior to my work, studies have identified TFIIIA-7ZF as a dedicated transcription factor for Pol II-dependent transcription using PSTVd RNA template. The main objective of my research is to probe the machinery for RNA-templated transcription by Pol II and the underlying mechanism. First, we will use a recently developed IVT assay to determine the role of TFIIS in RNA-templated transcription. TFIIS is thought to facilitate Pol II to transcribe RNA templates in a prime-based transcription model. In this model, TFIIS enhances the nicking of the RNA strand and exposes a region of single-stranded RNA that can serve as a template. using the template, Pol II can extend from the nicking site by adding nucleotides. In such scenario, the nick site serves as a “primer” for subsequent transcription extension. However, TFIIS is dispensable for the *de novo* transcription model. TFIIS is a single-

copy gene in *Arabidopsis*. A recent report showed that *Arabidopsis tfiis* null mutant is viable and largely resembles WT plants, except that the seeds show an early germination phenotype. Therefore, *Arabidopsis tfiis* null mutant provides an excellent platform to determine the involvement of TFIIS in RNA-templated transcription. Here we anticipate observing no difference in PSTVd replication between WT and *tfiis* protoplasts, which will support that TFIIS is dispensable for PSTVd RNA-templated transcription. This observation will also favor the *de novo* transcription model. Alternatively, if we observed reduced circular PSTVd production in *tfiis* protoplasts, it will indicate that TFIIS is required for PSTVd replication, which favors the prime-based model.

Biochemically reconstituted systems have been successfully used to characterize the required factors and functional mechanisms underlying DNA-dependent transcription. Our IVT assay generates multimeric products representing the processes occurring in cells. This reconstituted IVT assay allows testing the role of additional factors in RNA-templated replication. Specific binding of Pol II to a DNA promoter requires the coordinated assembly of Pol II and six general transcription factors, little is known regarding how this enzyme recognizes an RNA template. We will investigate the direct interaction of Pol II with PSTVd RNA to define the Pol II subunit(s) and transcription factors involved in the formation of transcription complex on RNA template. For this purpose, we will use RNA affinity chromatography followed by mass spectrometry analysis to identify the components on the RNA template.

CHAPTER II

EVIDENCE SUPPORTING THAT RNA POLYMERASE II CATALYZES *DE NOVO* TRANSCRIPTION USING POTATO SPINDLE TUBER VIROID CIRCULAR RNA TEMPLATE

This chapter is a modified version of "Evidence supporting that RNA Polymerase II catalyzes *de novo* transcription using potato spindle tuber viroid circular RNA templates" published in Viruses [68] and has been reproduced here with the permission of the copyright holder. I carried out experiments for all the data except for Figure 2.7 (done by Dr. Ying Wang).

2.1 Abstract

Transcription is a fundamental process that mediates the interplay between genetic information and phenotype. Emerging evidence indicates that RNA polymerase II (Pol II) can catalyze transcription using both DNA and RNA templates. It is well established that Pol II initiates *de novo* transcription on DNA templates. However, whether Pol II performs *de novo* transcription or relies on primers for initiation (primed transcription) on RNA templates is unclear. Using potato spindle tuber viroid (PSTVd) as a model, we presented evidence showing that circular PSTVd template is critical for the synthesis of longer-than-unit-length (-) strand products, which supports the *de novo* transcription based on the asymmetric rolling circle model of PSTVd replication. We further showed that the crucial factor for primed transcription, transcription factor IIS (TFIIS), is dispensable for PSTVd replication in cells. Together, our data support the *de novo* transcription on PSTVd RNA templates catalyzed by Pol II. This result has

significant implications in understanding the mechanism and machinery underlying Pol II-catalyzed transcription using other RNA templates.

2.2 Introduction

Transcription is a fundamental process that mediates the interplay between genetic information and phenotype and is thus vital for development, responses to environmental cues, and diseases [69-72]. As the first and indispensable step towards protein production, transcription is catalyzed by DNA-dependent RNA polymerases (DdRPs) that use DNA templates to synthesize RNA [73-75]. Since the first eukaryotic RNA polymerase was identified in 1969 [76], the composition of the required machinery is well understood for DNA-dependent transcription through decades of research [23, 74, 77-79]. Each DdRP cooperatively associates with a group of factors to form the transcription machinery. Taking RNA polymerase II (Pol II) as an example, TFIID binds to DNA promoters and recruits TFIIB, thereby constituting a platform to assemble the preinitiation complex (PIC) that includes TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH [80]. Subsequently, TFIIS and other factors together constitute the elongation complex [81]. It is known that DdRPs can use both DNA and RNA templates for transcription [9]. Some RNA-based pathogens use the RNA-dependent RNA polymerase (RdRP) activity of DdRPs for replication, such as viroids and the human hepatitis delta virus (HDV) [82]. Viroids replicating in chloroplasts use the nucleus-encoded RNA polymerase (NEP) [5], while viroids replicating in the nucleus rely on Pol II [11, 13, 17, 20]. Numerous lines of evidence support that Pol II catalyzes transcription/replication using HDV RNA templates [47, 64, 83], but there also exists evidence showing that Pol I and Pol III may participate in HDV replication [84-87]. The RdRP activity of DdRPs widely occurs in various organisms to regulate gene expression as well. For instance, under nutrient-deficient conditions, the bacterial DdRP interacts with the

noncoding 6S RNA of *Escherichia coli* and stops transcribing DNA templates. In response to nutrient availability, the DdRP uses the 6S RNA template to transcribe a short RNA that leads to the de-sequestration of the DdRP. The free DdRP then binds to DNA promoters and initiates the synthesis of mRNAs [88]. Mammalian Pol II binds to a hairpin formed by the noncoding B2 RNA and extends the short strand by using the longer strand of the hairpin sequence as the template for transcription. This extension destabilizes of the B2 RNA, representing a novel posttranscriptional mechanism to regulate RNA stability [89]. Therefore, the RdRP activity of DdRPs is emerging as a critical regulatory mechanism of gene expression across kingdoms and its function in pathogen infections.

DdRPs are known for initiating *de novo* transcription on DNA templates [90]. However, it seems that DdRPs may have different modes when using RNA templates for transcription. Although the bacterial DdRP initiates *de novo* transcription when using the 6S RNA template [88], the evidence clearly shows that Pol II utilizes the fold-back 3'-end in the B2 RNA terminal loop as the primer to extend the B2 RNA [89]. This extension demonstrates that Pol II can perform the so-called primed transcription in eukaryotic cells, further supported by investigating the active Pol II structure on RNA templates [24]. It is also indicated that one general transcription factor, TFIIS, can promote the cleavage of RNA templates to generate an exposed 3'-end as RNA primers for transcription [24]. Whether Pol II exclusively performs primed transcription on RNA templates is a critical question in virology because it directly relates to the replication of HDV and nuclear-replicating viroids. Whether HDV replication/transcription is primed remains to be clarified [91, 92]. The primed transcription model may explain how Pol II catalyzes the synthesis of HDV mRNAs (HDAg), but it is difficult to explain the rolling circle replication if the circular viral genome is cleaved [91, 92]. Based on the primed transcription, it

predicts the existence of the covalently linked templates and products, a chimeric intermediate remains to be identified [91, 92]. Furthermore, the experimental systems supporting the primed transcription on RNA templates (B2 RNA and HDV) only generated very short RNA products [24, 89, 93-95], but the HDV concatemers are much longer in host cells. As for nuclear-replicating viroids, experimental evidence is needed to support the proposed *de novo* replication by Pol II.

We recently established an IVT system for studying the Pol II-catalyzed replication of potato spindle tuber viroid (PSTVd), the type species of nuclear-replicating viroids [110]. We identified the eukaryotic transcription factor TFIIIA-7ZF as a critical factor facilitating Pol II-catalyzed transcription using the (+) strand PSTVd RNA template [21, 38]. We also mapped the TFIIIA-7ZF binding site [21], which is next to the transcription initiation site of (-) strand intermediates [14] and is shown vital for Pol II binding [41] and PSTVd replication [40]. Therefore, this binding site likely acts as an RNA promoter. Furthermore, we observed the longer-than-unit-length products in the *in vitro* transcription assay [21] mimicking the process in cells [96]. Based on these available resources and information, we attempted to understand whether Pol II transcription using circular PSTVd RNA templates is initiated in a primed mode or *de novo* mode. Here, we improved the Pol II purification scheme for the IVT assay and directly verified the importance of circular templates for generating the longer-than-unit-length (-) strand products, a critical intermediate in the rolling circle replication model [97-99]. We further showed that PSTVd replication was not affected in *tfiis* loss-of-function protoplasts, supporting the *de novo* replication of PSTVd by Pol II. Our observations have significant implications for understanding the molecular mechanism of Pol II-catalyzed transcription on RNA templates.

2.3 Materials and methods

2.3.1 Plant growth and protoplast assays

Arabidopsis thaliana wildtype and *tfiis* mutant (SALK_027258C) plants were grown in a growth chamber with an 8/16 h light/dark cycle at 22 ˚C. Both lines were obtained from Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH, USA). Using 4-week-old plants, protoplasts were isolated following an established protocol [100, 101]. Briefly, we included 3% cellulose (Onozuka Yakult Pharmaceutical IND., Tokyo, Japan) and 0.8% macerase (MilliporeSigma, Burlington, MA, USA) in the digestion buffer (0.4 M mannitol, 20 mM KCl, 20 mM MES, 10 mM $CaCl_2$, 0.1% BSA, 5 mM-mercaptoethanol, pH 5.7) for digesting leaf blades with the lower epidermis layer removed by tapes. After 1 h digestion, protoplasts were pelleted by centrifugation at 150x g for 1 min. The pelleted protoplasts were then incubated in the W5 buffer (5 mM MES, 154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, pH 5.7) on ice for 30 min followed by incubation for 10 min with the MMg solution (4 mM MES, 0.4 M mannitol, 15 mM $MgCl_2$, pH 5.7) at room temperature. About 10^5 cells in 200 µL MMg buffer were supplemented with 5 µg $PSTVd^{RZ\text{-}Int}$ RNA [102, 103], then incubated with 200 µL PEG solution (A ~8 mL stock including 40% (w/v) PEG4000, 0.2 M mannitol, 0.1 M $CaCl_2$) for 5 min at room temperature. Finally, protoplasts were briefly washed with the W5 solution and incubated in the WI solution (0.4 M mannitol, 20 mM KCl, 20 mM MES, pH 5.7) for 2 days at room temperature before RNA purification. The protoplast assay was repeated three times.

Genomic DNA was extracted, using a published protocol [104] for genotyping using TF2Sf (5’-cggttgtaaagaggctaaggtgaa-3’) and TF2Sr (5’-caacagaacttccagtggttgtcac-3’) primer pair, and TF2Sr and LBb1 (5’-gcgtggaccgcttgctgcaact-3’) primer pairs to detect wildtype cDNA and the T-DNA insertion, respectively.

2.3.2 Recombinant protein purification

Recombinant TFIIIA-7ZF protein with an intein-chitin binding tag was expressed and purified following the previously described method [21] with few modifications. *E. coli* BL21 (DE3) Rossetta strain (MilliporeSigma, Burlington, MA, USA) were grown to A600 = 0.4–0.6. TFIIIA-7ZF protein was then induced by adding IPTG (final concentration 0.4 mM) and $ZnCl_2$ (final concentration 5 µM) to the culture followed by shaking at 20 ˚C for 16 h. Cells were harvested by centrifugation at 6,000x *g* for 20 min. Harvested cells were resuspended in the lysis buffer (20 mM Tris-HCl pH 8.5, 500 mM NaCl, 1 mM PMSF, 5 M $ZnCl_2$ and 0.1% (v/v) Triton X-100). Cells were lysed by sonicating with the Bioruptor (Diagenode, Denville, NJ, USA) followed by centrifugation at 15,000x *g* for 20 min at 4 ˚C. Supernatant was collected and incubated for 1 h with chitin resin (New England Biolabs, Ipswich, MA, USA) that was pre-equilibrated with the lysis buffer. After incubation, chitin resin was washed with 10 bed volume of lysis buffer to remove non-specifically bound proteins followed by incubation for 18 h at room temperature in the cleavage buffer (10 mM Tris-HCl pH 7.5, 20 mM KCl, 5 µM $ZnCl_2$ and 10 mM DTT) for on-column cleavage to release TFIIIA-7ZF from the intein tag. Fractions containing TFIIIA-7ZF were desalted using Sephadex-G25 (GE Healthcare Life Sciences, Pittsburgh, PA, USA) equilibrated with storage buffer (10 mM Tris-HCl pH 7.5, 20 mM KCl, 5 µM $ZnCl_2$). The desalted protein was concentrated with an Amicon protein concentrator (MilliporeSigma, Burlington, MA, USA). Glycerol was added to the final concentration of 20% (v/v) before storing at -20 ˚C.

2.3.3 Purification of Pol II from wheat germ

Purification of Pol II from wheat germ was carried out following a published protocol [105] with modifications. All operations were performed at 4 ˚C, and all centrifugations were

carried out for 15 min. One hundred grams wheat germ (Bob's Red Mill, Milwaukie, OR, USA) was ground in a Waring Blender with 400 mL of buffer A (50 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, and 75 mM $(NH_4)_2SO_4$). The resulting homogenate was diluted with 100 mL of buffer A and followed by centrifugation at 15,000x *g*. The supernatant was filtered through one layer of Miracloth (MilliporeSigma, Burlington, MA, USA). The resulting crude extract containing Pol II was precipitated by an addition of 0.075 volume of 10% (v/v) Polymin P with rapid stirring. The resulting mixture was subject to centrifugation at 10,000x *g*. The pellet was washed with 200 mL of buffer A. The insoluble fraction, which contains Pol II, was resuspended with buffer B (50 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, and 0.2 M $(NH_4)_2SO_4$). The resulting suspension was centrifuged at 10,000x *g* to remove insoluble materials.

$(NH_4)_2SO_4$ precipitation was carried out by slowly adding 20 g of solid $(NH_4)_2SO_4$ per 100 mL of the above supernatant with stirring. The mixture was centrifuged, and the pellet was dissolved in buffer C (0.05 M Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, 25% ethylene glycol) plus 0.1% Brij 35 (Thermo Fisher Scientific, Waltham, MA, USA) to make final $(NH_4)_2SO_4$ concentration 0.15 M as determined by conductivity reading. The resulting solution was applied to DEAE Sepharose FF (GE Healthcare Life Sciences, Pittsburgh, PA, USA) equilibrated with buffer C plus 0.15 M $(NH_4)_2SO_4$. Then the column was washed with five bed volume buffer C containing 0.15 M $(NH_4)_2SO_4$. Finally, bound Pol II was eluted with buffer C containing 0.25 M $(NH_4)_2SO_4$. Fractions containing Pol II were pooled. The $(NH_4)_2SO_4$ concentration was adjusted to 75 mM by diluting with buffer A without $(NH_4)_2SO_4$. Concentration was determined by measuring conductivity. The resulting solution was applied to SP Sepharose FF (GE Healthcare Life Sciences, Pittsburgh, PA, USA) equilibrated with buffer C

containing 75 mM $(NH_4)_2SO_4$. After washing the column with the same buffer, Pol II was eluted using buffer C containing 0.15 M $(NH_4)_2SO_4$. Eluted fractions containing Pol II were pooled based on the presence of Rpb1 subunit of Pol II, detected by immunoblotting. Ethylene glycol (VWR Chemicals BDH, Radnor, PA, USA) was added to a final concentration of 50% (v/v) before storing at -20 ^{0}C.

2.3.4 Immunoblotting

Purified protein samples together with the BLUItra prestained protein ladder (FroggaBio, Wheatfield, NY, USA) were separated on an SDS-PAGE gel, followed by transferring to an Amersham Protran 0.45 µm NC nitrocellulose membrane (GE Healthcare Lifesciences, Pittsburgh, PA, USA) using the Mini-PROTEAN Tetra Cell (BioRad, Hercules, CA, USA). After 1 h incubation with 1% (w/v) nonfat milk in 1X TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) at room temperature, the 8WG16 monoclonal antibody (Thermo Fisher Scientific, Waltham, MA, USA) at 1:1000 against the largest subunit of Pol II was added and incubated overnight at 4 ˚C. After three washes with 1X TBST (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20 (v/v)), HRP-conjugated secondary antibody against mouse IgG (Millipore Sigma, Burlington, MA, USA) was added at 1:8,000 dilution. The membrane was washed three times with 1X TBST and incubated with HRP substrates (Li-COR Biosciences, Lincoln, NE, USA). The signals were detected with C-DiGit (Li-COR Biosciences, Lincoln, NE, USA).

2.3.5 RNA preparation *in vitro*

PSTVd RNA circularization was performed following a previously described method [106]. Ten µg of linear PSTVd RNA was incubated at 37 ˚C for 2 h in a final volume of 200 µL containing 150 units of calf intestinal alkaline phosphatase (New England Biolabs, Ipswich, MA,

USA), 1X calf intestinal alkaline phosphatase buffer, and 80 units of RNase inhibitor (New England Biolabs, Ipswich, MA, USA). After incubation, the phenol-chloroform-based method was used to remove proteins followed by precipitation using ethanol. RNA pellet was resuspended in 10 µL nuclease-free water and incubated at 37 ˚C for 2 h in a final volume of 100 µL containing 100 units of T4 polynucleotide kinase (New England Biolabs, Ipswich, MA, USA), 80 units of RNase inhibitor (Thermo Fisher Scientific, Waltham, MA, USA), 1X T4 polynucleotide kinase buffer, and 1 mM ATP. Following incubation, proteins were removed using the phenol/chloroform method, followed by precipitation using ethanol. The pellet was dissolved in 20 µL of nuclease-free water and incubated at 37 ˚C for 2 h in a final volume of 200 µL containing 1 mM ATP, 150 units of T4 RNA ligase (New England Biolabs, Ipswich, MA, USA), 80 units of RNase inhibitor, and 1X T4 RNA ligase buffer. Circular PSTVd RNA was separated by a 5% (w/v) polyacrylamide/8 M urea gel and detected by the Gel Doc XR+ gel documentation system (BioRad, Hercules, CA, USA) after ethidium bromide staining before gel excision. The excised gel was incubated with 0.3 M NaCl at 4 ˚C overnight. Gel pieces were removed using Costar Spin-X column (Corning, Corning, NY, USA) by centrifugation. The circular PSTVd RNA in the flow-through fraction was precipitated using ethanol. The resulting pellet containing circular RNA was resuspended in nuclease-free water and stored in -80 ˚C.

To prepare digoxigenin (DIG)-labeled (+) strand and (-) strand probes for RNA gel blots, *Sma*I-linearized pInt$^{95\text{-}94}$(+) and pInt$^{95\text{-}94}$ (-) were used as templates for *in vitro* transcription with T3 MAXIscript (Thermo Fisher Scientific, Waltham, MA, USA). For detecting 5S rRNA, (DIG)-labeled riboprobes were transcribed from a *Nco*I-linearized pGEMT-5SrRNA template [40] using a SP6 MAXIscript kit (Thermo Fisher Scientific, Waltham, MA, USA). To prepare *in vitro* transcripts for the protoplast transfection assay, PSTVd$^{RZ\text{-}Int}$ plasmid [50, 51, 54] was

linearized by *Hin*dIII (New England Biolabs, Ipswich, MA, USA). The PSTVd$^{RZ\text{-}Int}$ construct in pTZ18R was originally described by Hu et al. [51], and the 5'-Hammerhead ribozyme-PSTVd-Paperclip ribozyme-3' cassette was later cloned into pGEM-T [50, 54]. The linearized plasmid was used as the template for *in vitro* transcription using T7 MEGAscript (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's manual. The products were purified using the MEGAclear kit (Thermo Fisher Scientific, Waltham, MA, USA). The products contain four RNA species, including the unit-length (+) PSTVd as shown in Jiang et al. [101]. To prepare the size markers, the PSTVd$^{RZ\text{-}Int}$ plasmids [102, 103] were digested with *Hin*dIII (New England Biolabs, Ipswich, MA, USA) and then were subjected to *in vitro* transcription using T7 MEGAscript (Thermo Fisher Scientific, Waltham, MA, USA).

2.3.6 Pol II-catalyzed *in vitro* transcription

Pol II-catalyzed *in vitro* transcription was carried out using a previously developed protocol [11, 21, 75]. For DNA-dependent RNA transcription, 50 µL reaction contained 50 mM HEPES-KOH pH 7.9, 2 mM $MnCl_2$, 100 mM KCl, 10% (v/v) glycerol, 1 U/µL SuperaseIn RNase inhibitor (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 mM rATP, 0.5 mM rCTP, 0.5 mM rGTP, 0.35 mM rUTP, 0.15 mM DIG-labeled (Enzo Life Sciences, Farmingdale, NY, USA) rUTP, 150 ng DNA template, and 300 ng of partially purified Pol II. For RNA-dependent RNA transcription, 4 µM BSA (New England Biolabs, Ipswich, MA, USA) and TFIIIA-7ZF were treated with 1 unit of Turbo DNase (Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 37 ˚C. Then, 360 ng circular PSTVd, 300 ng partially purified Pol II, pretreated BSA and various amounts of TFIIIA-7ZF were incubated at 28 ˚C for 15 min before adding reaction buffer containing 50 mM HEPES-KOH pH 7.9, 1 mM $MnCl_2$, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$,

10% (v/v) glycerol, 1 unit/µL SuperaseIn RNase inhibitor (Thermo Fisher Scientific,Waltham, MA, USA), 0.5 mM rATP, 0.5 mM rCTP, 0.5 mM rGTP, 0.5 mM rUTP. Transcription reactions were incubated at 28 ˚C for 4 h. The reactions were terminated by 0.8 U/µL proteinase K (New England Biolabs, Ipswich, MA, USA) treatment at 37 ˚C for 15 min, followed by incubation at 95 ˚C for 5 min. The Pol II-catalyzed *in vitro* transcription assay was repeated three times.

2.3.7 RNA purification. RNA gel blots, and dot blots

Transfected protoplasts were subjected to centrifugation at 1,000x *g* for 2 min. The solution was removed, followed by wash with 1X PBS once. The pellet protoplasts were mixed with 100 µL RNAzol RT (Molecular Research Center, Cincinnati, OH, USA) and 35 µL ddH_2O. RNA was purified following the manufacturer's manual. For dot blots, DNA-dependent reactions were treated with Turbo DNase (Thermo Fisher Scientific, Waltham, MA, USA) for 20 min and then subjected to RNA purification by mixing 2 volumes ethanol followed by 14,000x *g* centrifugation at 4 ˚C. The precipitated RNA was dissolved in 10 µL nuclease-fee H_2O. Total RNA from protoplasts or RNA from Pol II-catalyzed *in vitro* transcription were separated on a 5% (w/v) polyacrylamide/8 M urea gel for 1 h at 200 V. Then RNA was transferred to Hybond-XL nylon membrane (GE Healthcare Lifesciences, Pittsburgh, PA, USA) using the Trans-Blot SD semi-dry transfer cell (BioRad, Hercules, CA, USA) followed by UV cross-linking. Membranes were blocked by ULTRAhyb ultrasensitive hybridization buffer (Thermo Fisher Scientific, Waltham, MA, USA) followed by overnight hybridization with (DIG)-labeled riboprobes at 65 ˚C. Following the instructions of the DIG northern starter kit (MilliporeSigma, Burlington, MA, USA), membranes were washed and incubated with antibodies against DIG label. Transcripts were identified using the Immun-Star AP chemiluminescence kit (BioRad, Hercules, CA, USA). Signals were obtained using C-DiGit (Li-COR Biosciences, Lincoln, NE,

USA). Transcription products of DNA-dependent reactions were spotted on a Hybond-XL nylon membrane (GE Healthcare Lifesciences, Pittsburgh, PA, USA) followed by UV cross-linking. Membranes were blocked with ULTRAhyb ultrasensitive hybridization buffer (Thermo Fisher Scientific, Waltham, MA, USA), washed and incubated with the specific antibody against DIG labeling (MilliporeSigma, Burlington, MA, USA). The signals were detected with C-DiGit (Li-COR Biosciences, Lincoln, NE, USA).

2.4 Results

2.4.1 An efficient method to partially purify functional Pol II

We recently established a Pol-II-catalyzed *in vitro* system to study RNA-templated transcription [21]. In this system, we mixed circular PSTVd as the RNA template, recombinant TFIIIA-7ZF protein purified from *E. coli,* and immuno-purified Pol II from plants. To obtain an immune-purified Pol II complex, we agroinfiltrated *Nicotiana benthamiana* plants with a plasmid harboring a FLAG-tag fused cDNA of the second largest subunit of Pol II cloned from *A. thaliana* [107]. However, the Pol II expressing efficiency varied among batches. To circumvent this shortcoming, we revisited a Polymin P (PEI)-based purification method that was developed in the mid-1970s to prepare active Pol II from wheat germs [105]. At low ionic strength, PEI precipitates DNA genomes and many DNA-binding proteins. Following re-suspension, the protein solution sequentially passes through the DEAE Sepharose FF column and the phosphocellulose column. The final elution contains the major components of the Pol II complex. However, the phosphocellulose column has been discontinued. Using the DEAE Sepharose FF column alone resulted in the contamination of strong nuclease activities in the purified Pol II fraction (Figure 2.1A). After some initial trials, we found that the SP-Sepharose column can replace the phosphocellulose column to remove nucleases (Figure 2.1A) and obtain

the relatively pure Pol II complex (Figure 2.1B). Using the specific antibody against the largest subunit of Pol II, we confirmed that the purified fraction contains Pol II (Figure 2.1C).

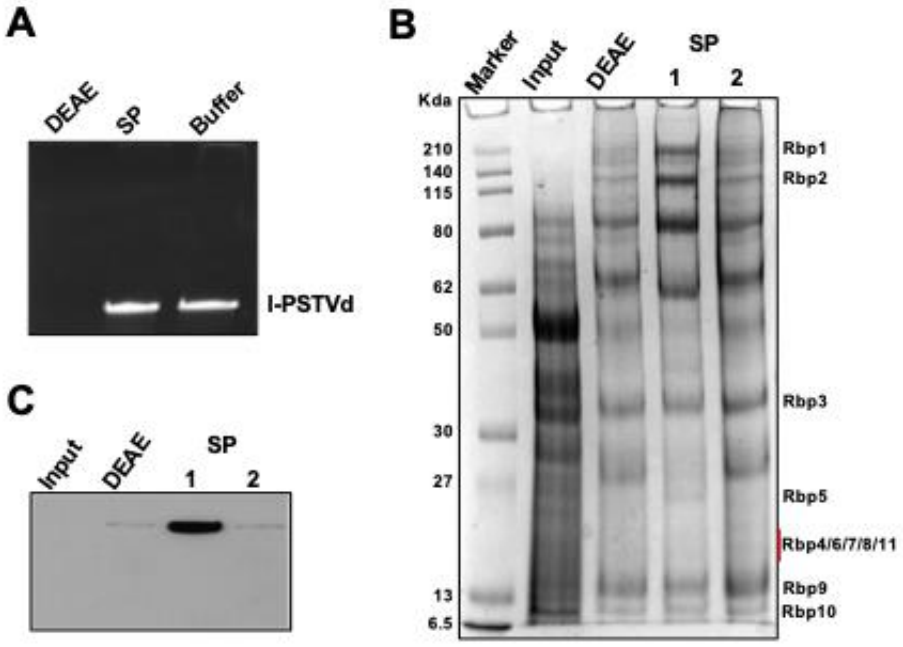


Figure 2.1 Pol II purification.

(A) Eluate from the DEAE Sepharose FF column contains nuclease activity as demonstrated by RNA degradation in lane DEAE. The eluate from DEAE was further purified using the SP Sepharose FF column to remove the nuclease activity, as shown in the SP lane. DEAE, the eluate from the DEAE Sepharose FF column. Buffer, protein storage buffer (0.05 M Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, 0.15 M $(NH_4)_2SO_4$) and 50% ethylene glycol). l-PSTVd, 480 ng linear PSTVd. (B) Purified protein factions were visualized in an SDS-PAGE gel after coomassie blue staining. Protein bands corresponding to the putative Pol II subunits were labeled based on the molecular weight of the corresponding homologs in *Arabidopsis*. See Table 2.1 for details. SP, the elution fractions (1 and 2, elution using 0.15 M and 0.3 M ammonium sulfate, respectively) from the SP Sepharose FF column. (C) Immunoblot detection of the largest subunit of Pol II, Rbp1, using the 8WG16 antibody. Input, raw lysate of wheat germ.

We then tested the activity of purified Pol II for transcription. When we used p35S::RZ-PSTVd plasmid [102] as the template, we could not observe any specific band in RNA gel blots using the PSTVd specific probe (Figure 2.2A). This is expected because the purified polymerase

fraction likely lacks the necessary general transcription factors to initiate DNA-directed transcription from specific promoters [108, 109]. To confirm that the purified Pol II possesses enzymatic activity, we performed dot blots to examine the rNTP incorporation efficiency.

Table 2.1 Pol II subunits

Function	*Saccharomyces cerevisiae* Pol II	*Arabidopsis* Homologs	Mw (kDa)
Catalytic	RPB1	At4g35800	204.62
Assembly	RPB2	At4g21710	135.02
	RPB3	At2g15430	35.46
	RPB11	At3g52090	17.14
	RPB10	At1g11475	8.31
	RPB12	At5g41010	5.9
	RPB6	At5g51940	16.65
	RPB8	At2g04630	16.74
Auxillary	RPB5	At1g54250	16.51
	RPB4	At3g59600	16.6
	RPB7	At3g22320	24.3
	RPB9	At5g09920	15.93

Using the same DNA template, we supplied the rNTPs mixed with (DIG)-labeled rUTP for Pol II to perform transcription. The products were precipitated to remove free DIG-rUTP before dot blots. As shown in Figure 2.2B, dot blots showed that the purified Pol II was active for rNTP incorporation, evidenced by a much stronger signal. The signal in the middle of Figure 2.2B might be attributable to the precipitated Pol II with incorporated (DIG)-labeled rUTP

nucleotides. Nevertheless, the signal in the reaction without DNA templates is much weaker than that from the reaction with the supply of DNA templates.

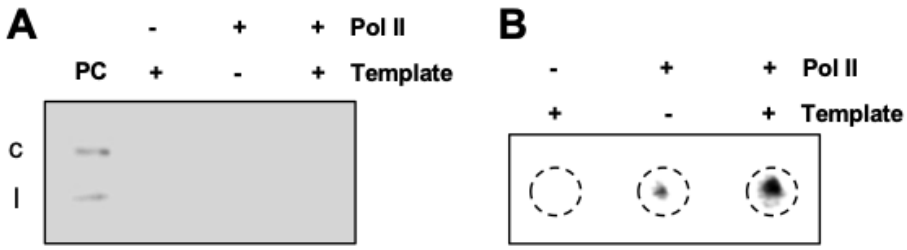


Figure 2.2 The activity of purified Pol II on DNA template.

(A) The purified Pol II cannot generate specific RNA products from the p35S::RZ-PSTVd plasmid. (B) Pol II possesses nucleotide-incorporating activity as shown in a dot blot. PC, the mixture of circular and linear PSTVd RNA served as the positive control. c, circular PSTVd. l, linear PSTVd.

2.4.2 Circular templates are critical for generating longer-than-unit-length intermediates

The discovery of the circular (+) PSTVd [110, 111], concatemeric (-) PSTVd [98], and the double-stranded RNA complex containing the unit-length and the longer-than-unit-length (+) and (-) PSTVd RNAs [112] led to the proposal of an asymmetric rolling circle model of PSTVd replication [97, 99] (Figure 2.3A). It has been assumed that Pol II catalyzes the transcription *de novo* using PSTVd circular RNA templates to generate multimeric (-) PSTVd intermediates. In contrast, if Pol II employs the TFIIS-based primed transcription mechanism for PSTVd, it would generate the chimeric (+) PSTVd and (-) PSTVd fusion intermediates with a length ranging from close to the unit-length to oligomeric PSTVd pending on the nick site (Figure 2.3B). In the primed transcription scenario, a circular RNA template may not be required, since the nicked 3'-end of the linear PSTVd can serve as a primer.

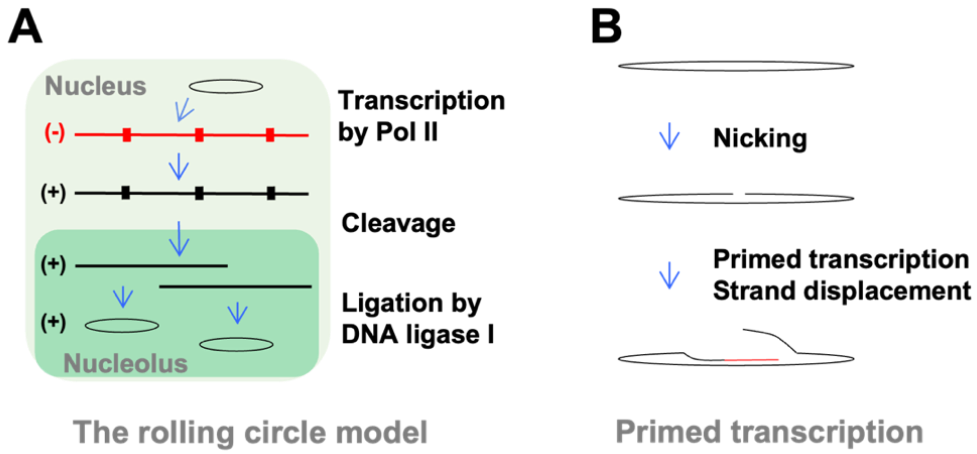


Figure 2.3 PSTVd rolling circle model.

(A) Rolling circle mechanism. (B) A hypothetical primed transcription scheme. The nodes in the (-) and (+) concatemers in (a) depict the cleavage sites for generating unit-length copies. Black lines depict the sense orientation of PSTVd, while red lines the depict antisense orientation of PSTVd.

Using the partially purified Pol II from wheat germ, we performed the *in vitro* transcription assay and tested the activity of the purified Pol II in catalyzing transcription on RNA templates. Half of the reaction mixture was subjected to RNA gel blots to detect products (Figure 2.4A), while the other half was used to detect templates (Figure 2.4B). As shown in Figure 2.4A, the purified Pol II alone exhibits very weak activity in transcribing PSTVd circular RNA templates. Adding 100 ng TFIIIA-7ZF to the *in vitro* transcription significantly enhanced the Pol II activity on the circular RNA template and produced of the longer-than-unit-length (-) strand products. Interestingly, compared with the supplementation of 100 ng TFIIIA-7ZF, adding more TFIIIA-7ZF led to the drastic reduction of circular templates and failure in generating the longer-than-unit-length (-) strand product (Figure 2.4). Occasionally, we observed the circular template remained at a comparable concentration with 100 ng and 160 ng TFIIIA-7ZF in the

reactions but still only detected the longer-than-unit-length (-) strand products in the reaction with 100 ng TFIIIA-7ZF (Figure 2.5).

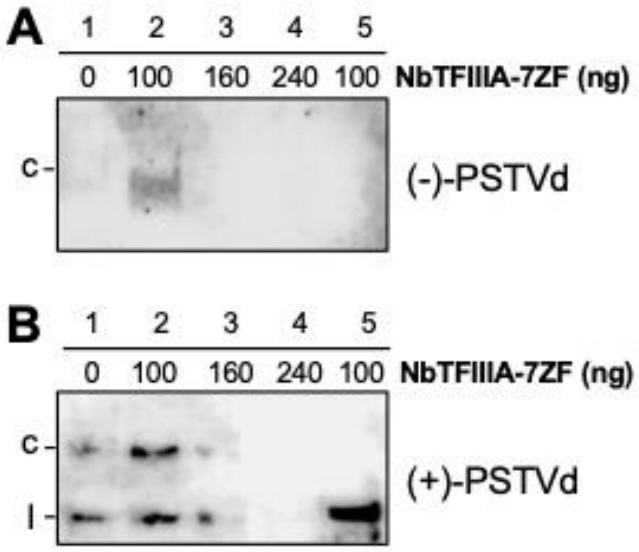


Figure 2.4 Pol II-catalyzed transcription using PSTVd circular RNA templates.

(A) *In vitro* transcription products detected by the (+) PSTVd riboprobe. (B) PSTVd templates detected by the (-) PSTVd riboprobe. Lanes 1-4 were supplied with circular PSTVd as templates, while lane 5 was supplied with linear PSTVd as the template. c, circular PSTVd. l, linear PSTVd.

These observations demonstrated that the ratio of TFIIIA-7ZF and the circular template is critical for generating (-) PSTVd intermediates. Under the condition for efficient synthesis of (-) PSTVd intermediates, the stoichiometric ratio among TFIIIA-7ZF, Pol II, and the PSTVd circular template is estimated to be 2:12:6 based on protein and RNA concentrations. The template degradation is likely attributable to the presence of divalent ions (i.e., Mn^{2+}) in the reaction, because incubating the circular templates with Mn^{2+} alone also resulted in a significant reduction in circular templates (Figure 2.6).

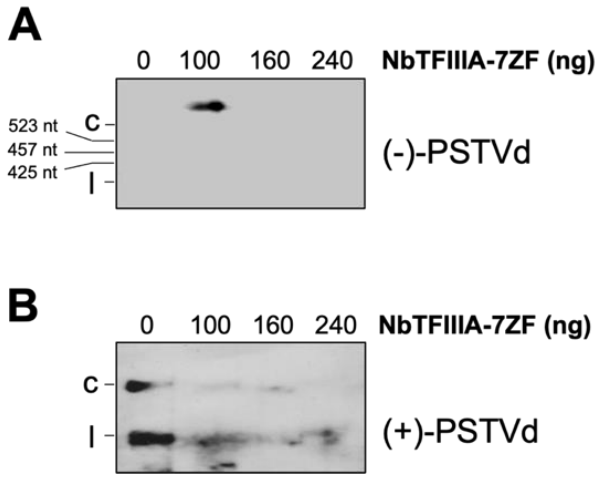


Figure 2.5 Pol II-catalyzed transcription depends on TFIIIA-7ZF to template ratio.

(A) *In vitro* transcription products detected by the (+) PSTVd riboprobe. We used full length RNA with a hammerhead ribozyme, an l-PSTVd, and a paperclip ribozyme as a size marker. The ribozyme activity result in four RNA species with 523 nt, 457 nt,425 nt and 359 nt linear PSTVd. (B) The PSTVd templates detected by the (-) PSTVd riboprobe. C, circular PSTVd. L, linear PSTVd.

Notably, a mixture of 100 ng TFIIIA and 300 ng Pol II, the condition allowing effective transcription on circular RNA templates, failed to generate longer-than-unit-length product using the linear (+) PSTVd template (Figure 2.4). Given the importance of the circular template, the proposed HDV primed transcription [24, 93-95], using the activity of the TFIIS/Pol II complex to nick the circular RNA and free the cleaved 3’ end as the primer, is unlikely to exist in PSTVd replication.

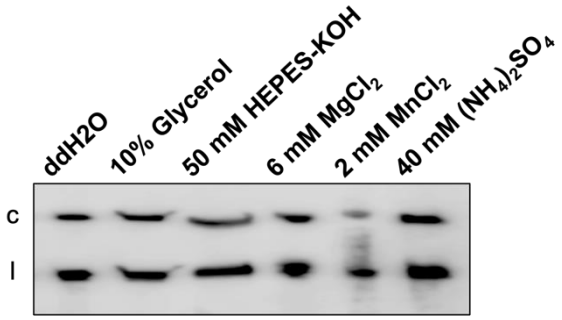


Figure 2.6 Template degradation by reaction buffer.

Reactions containing PSTVd RNA template were incubated with above buffer components for 4 h at 28 ˚C and RNA gel blot was performed to identify remaining RNA. C, circular PSTVd. l, linear PSTVd.

2.4.3 PSTVd replication is independent of TFIIS

Since TFIIS plays a crucial role in the proposed primed transcription model for HDV [24], we decided to test its role in PSTVd replication using the *tfiis* loss-of-function *Arabidopsis* mutant for the protoplast transfection assay. TFIIS is critical for plant growth [113, 114]. Albeit the homozygous *tfiis* loss-of-function *Arabidopsis* mutant is viable [113], loss of TFIIS results in early germination [113] and reduced tolerance to UV radiation [115]. In addition, more than seven hundred genes encoded in the nuclear genome exhibited significant differential expression patterns in the *tfiis* mutant [113]. We chose SALK_027258C as the material because it is a well-established *tfiis* loss-of-function mutant [113]. As shown in Figure 2.7, genomic PCR confirmed that our protoplasts were generated from homozygous *tfiis* loss-of-function plants. Using a previously established protoplast transfection system [101], we transfected wildtype and mutant protoplasts with linear PSTVd RNA inoculum and harvested the total RNA two days post inoculation. As shown in Figure 2.7, we could observe the accumulation of circular PSTVd in both wildtype and *tfiis* protoplasts, which reflects the successful replication of PSTVd based on previous studies [44, 61, 96, 101, 116]. Thus, our observation indicates that PSTVd replication is

unlikely via the TFIIS-based primed transcription mechanism.

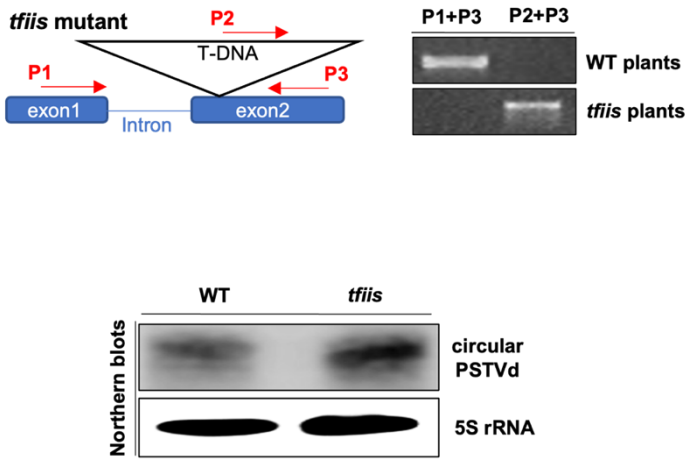


Figure 2.7 PSTVd replication in protoplasts.

Top panel shows the schematic presentation of T-DNA disruption in the TFIIS locus and the genotyping result. The genotyping PCR, using two sets of primers (P1+P3 and P2+P3) confirmed wildtype plants and *tfiis* mutants. The bottom panel shows PSTVd replication in wildtype and *tfiis* protoplasts. The sequence of P2 primer is derived from the inserted T-DNA, while the P1 and P3 primers are derived from the endogenous gene. WT, wildtype.

2.5 Discussion

The RdRP activity of Pol II is critical for gene regulation [88, 89] and pathogen replication (i.e., HDV and nuclear-replicating viroids) [1, 7, 82]. Pol II initiates transcription *de novo* on DNA templates in all eukaryotic cells [90] but utilizes the primed transcription mechanism on B2 noncoding RNA templates in mammalian cells [89]. Structural analysis [24] and some replication assays [24, 93-95] also suggested that Pol II may utilize the primed transcription mechanism for HDV infection, with the aid of TFIIS activity [24]. Whether the primed transcription mechanism is common for Pol II to transcribe RNA templates should be clarified. Nuclear-replicating viroids rely on Pol II activity for replication [11, 13, 17, 20], which has long been assumed to be *de novo* transcription. The discoveries of circular PSTVd [110,

111], longer-than-unit-length (-) PSTVd [98], as well as the double-stranded RNA composed of unit-length and longer-than-unit-length (+) and (-) PSTVd [112] led to the proposal of the rolling circle replication model [98]. Later, some evidence disproved the existence of circular (-) PSTVd and established the asymmetric rolling circle mechanism [99]. The asymmetric rolling circle replication is also common to all nuclear-replicating viroids [7, 8]. Here, we collected experimental evidence directly showing that circular (+) PSTVd, but not the unit-length linear (+) PSTVd, serves as the template for Pol II-catalyzed transcription. The generation of longer-than-unit-length (-) strand intermediates depends on the concentration of TFIIIA-7ZF and circular (+) strand templates. When using the circular (+) PSTVd as the template in our *in vitro* transcription assay, the circular RNA is often degraded to generate unit-length linear (+) PSTVd and smaller fragments (Figure 2.4B). The longer-than-unit-length products could not be detected when the circular templates were completely degraded (Figure 2.4A). In addition, linear (+) PSTVd RNA alone failed to serve as templates for generating longer-than-unit-length product (Figure 2.4), indicating that only the circular (+) PSTVd serves as the template for the synthesis longer-than-unit-length (-) PSTVd intermediates catalyzed by Pol II. Furthermore, we presented direct evidence that TFIIS is not required for PSTVd replication in protoplast cells (Figure 2.7). These observations, together, strongly support that Pol II catalyzes *de novo* transcription using PSTVd circular RNA templates.

It is also noteworthy that our purified Pol II cannot enable promoter-based transcription on DNA for the synthesis of specific products (Figure 2.2A), which is in line with previous reports that the Pol II enzyme requires a cohort of factors to enable regulated transcription [108, 109]. However, this purified Pol II is able to utilize circular PSTVd RNA templates to synthesize the longer-than-unit-length products, with the aid of TFIIIA-7ZF in a dosage-dependent fashion

(Figure 2.4A). This observation further confirmed the importance of TFIIIA-7ZF in RNA-templated transcription catalyzed by Pol II, particularly for the regulation of Pol II processivity. Given that TFIIIA-7ZF is a novel transcription factor for RNA-templated transcription and TFIIS, as a conserved general transcription factor, is dispensable for the PSTVd RNA-templated transcription catalyzed by Pol II, this polymerase should employ novel machinery to catalyze transcription on PSTVd RNA templates. This finding has significant implications in other RNA-templated transcription by DdRPs.

CHAPTER III

A REMODELED RNA POLYMERASE II COMPLEX CATALYZING VIROID RNA-TEMPLATED TRANSCRIPTION

This chapter is a modified version of "A remodeled RNA polymerase II complex catalyzing viroid RNA-templated transcription" published in PLOS Pathogens [117] and has been reproduced here with the permission of the copyright holder. I performed almost all the experiment and prepared samples for nLC-MS/MS. The nLC-MS/MS assay was performed at the IGBB core facility on campus by Drs. Olga Pechanova and Tibor Pechan.

3.1 Abstract

Viroids, a fascinating group of plant pathogens, are subviral agents composed of single-stranded circular noncoding RNAs. It is well-known that nuclear-replicating viroids exploit host DNA-dependent RNA polymerase II (Pol II) activity for transcription from circular RNA genome to minus-strand intermediates, a classic example illustrating the intrinsic RNA-dependent RNA polymerase activity of Pol II. The mechanism for Pol II to accept single-stranded RNAs as templates remains poorly understood. Our previous finding that TFIIS is dispensable for PSTVd replication implies that the Pol II machinery is somehow remodeled to adapt to RNA templates. To test this hypothesis, we reconstituted a robust *in vitro* transcription system and demonstrated that Pol II also accepts minus-strand viroid RNA template to generate plus-strand RNAs. Further, we purified the Pol II complex on RNA templates for nano-liquid chromatography-tandem mass spectrometry analysis and identified a remodeled Pol II missing Rpb4, Rpb5, Rpb6, Rpb7, and

Rpb9, contrasting to the canonical 12-subunit Pol II or the 10-subunit Pol II core on DNA templates. Interestingly, the absence of Rpb9, which is responsible for Pol II fidelity, explains the higher mutation rate of viroids compared to cellular transcripts. This remodeled Pol II is active for transcription with the aid of TFIIIA-7ZF and appears not to require other canonical general transcription factors (such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIS), suggesting a distinct mechanism/machinery for viroid RNA-templated transcription. Transcription elongation factors, such as FACT complex, PAF1 complex, and SPT6, were also absent in the reconstituted transcription complex. Further analyses of the critical zinc finger domains in TFIIIA-7ZF revealed the first three zinc finger domains pivotal for RNA template binding. Collectively, our data illustrated a distinct organization of Pol II complex on viroid RNA templates, providing new insights into viroid replication, the evolution of transcription machinery, and the mechanism of RNA-templated transcription.

3.2 Introduction

Viroids are circular noncoding RNAs that infect crop plants [118, 119]. After five decades of studies, the host machinery for viroid infection has not been fully elucidated [118-120]. There are two viroid families, *Avsunviroidae* and *Popspiviroidae* [121, 122]. Members of *Pospivoirdae* replicate in the nucleus via the rolling circle mechanism (Figure 1.1) and rely on the enzymatic activity of DNA-dependent RNA polymerase II (Pol II) [118-120]. Specifically, ample evidence supports that Pol II activity is critical for the synthesizing of oligomer minus-strand or (-) intermediates using viroid circular genomic RNAs as templates [1, 118]. However, it remains controversial whether Pol II also uses (-) oligomers as templates for transcription [1, 118].

By and large, DNA-dependent RNA polymerases (RNA polymerases or DdRPs) catalyze

transcription using DNA templates, which is a fundamental process of life. A group of general transcription factors facilitates RNA polymerases to achieve highly regulated transcription, from initiation to elongation and termination. Taking Pol II as an example, this 12-subunit complex functions in concert with general transcription factors during transcription initiation around DNA promoter regions [123-126]. In previous reports, a reconstituted system for the DNA promoter-driven transcription requires Pol II and five general transcription factors (TFIIB, TFIID, TFIIE, TFIIF, and TFIIH) [127, 128]. Interestingly, the 10-subunit Pol II core (without Rpb4/Rpb7 heterodimer) is sufficient for transcription elongation [129, 130]. Transcription elongation is also regulated by multiple factors, including TFIIS, TFIIF, PAF1 (RNAPII-associated factor 1) complex (PAF1-C), FACT complex (histone chaperone), SPT6, etc [131-133].

Since 1974, RNA polymerases possess intrinsic RNA-dependent RNA polymerase (RdRp) activity to catalyze RNA polymerization using RNA templates [9]. This intrinsic RdRp activity of RNA polymerases was found in bacteria and mammalian cells, as well as exploited by subviral pathogens (i.e., viroids and human hepatitis delta virus (HDV)) for propagation [82, 89, 134]. Pol II transcription using viroid or HDV RNA templates can yield RNA products over 1,000 nt in cells, comparable to some products from DNA templates. To ensure such efficient transcription, specific factors are needed. HDV encodes an S-HDAg protein to promote Pol II activity on its RNA template [47]. Using potato spindle tuber viroid (PSTVd) as a model, we showed that an RNA-specific transcription factor (TFIIIA-7ZF with seven zinc finger domains) interacts with Pol II and enhances Pol II activity on circular genomic RNA template [21, 68]. However, it remains unclear how S-HDAg or TFIIIA-7ZF functions with Pol II for RNA-templated transcription.

Biochemically reconstituted systems have been successfully used to characterize the

required factors and functional mechanisms underlying DNA-dependent transcription [74]. However, reconstituted transcription systems using RNA templates often exhibited poor activity. For example, all currently available *in vitro* transcription (IVT) systems using HDV templates have the premature termination issue generating products less than 100 nt [91, 92], which may not reflect the transcription process in cells. We recently established an IVT system for PSTVd that can generate longer-than-unit-length products (more than 360 nt) [21, 68], mimicking the replication process in cells [96]. Using this IVT system we presented evidence showing that circular PSTVd templates are critical for the synthesis of longer-than-unit-length (-) strand products in chapter II. Further, we showed transcription factor IIS is dispensable for PSTVd replication supporting *de novo* transcription on PSTVd RNA templates. The absence of canonical general transcription factor, TFIIS from PSTVd-templated transcription complex led to the hypothesis that RNA-templated transcription has a distinct organization on an RNA template.

We tested this hypothesis using our IVT system. First, we confirmed that Pol II and TFIIIA-7ZF function together in transcribing (-) PSTVd oligomers to (+) oligomers. Interestingly, we found that the Pol II complex remaining on (-) PSTVd RNA template has a distinct composition compared with the 12-subunit Pol II or the 10-subunit Pol II core via nano-liquid chromatography-tandem mass spectrometry analysis (nLC-MS/MS). Rpb4, Rpb5, Rpb6, Rpb7, and Rpb9 were absent in the remodeled Pol II. Notably, Rpb9 is responsible for the fidelity of Pol II transcription. Thus, the absence of Rpb9 may explain the much higher mutation rate of viroid RNA-templated transcription catalyzed by Pol II. Several elongation factors for DNA templates, such as the PAF1 complex and SPT6, were also absent in the transcription complex on RNA templates. More importantly, essential general transcription factors (including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIS) were all absent in the transcription

complex on PSTVd RNA template, clearly demonstrating the distinct regulations between DNA-dependent and RNA-templated transcription. This distinct Pol II retains the catalytic activity to generate (+)-PSTVd oligomers with the aid of TFIIIA-7ZF. We further showed that nearly all seven zinc finger domains of TFIIIA-7ZF are critical for function. In particular, the first three zinc fingers are pivotal for binding with RNA templates. This IVT system opens the door to further dissecting the mechanism underlying viroid RNA-templated transcription. Our findings also provide new insights into the organization of Pol II complex on RNA templates, which has profound implications for understanding RNA-templated transcription as well as viroid transcription and its high mutation rates.

3.3 Materials and methods

3.3.1 Molecular constructions

We have previously reported WT TFIIIA-7ZF cloned from *Nicotiana benthamiana* in bacteria expression vector pTXB1 (New England Biolabs, Ipswich, MA) [21]. The TFIIIA- 7ZF mutants were generated via site-directed mutagenesis using the WT TFIIIA-7ZF in pTXB1 as the template (See 3.1 Table for primer information). We have also reported the pInt95-94(-) and pInt95-94(+) constructs for generating PSTVd probes to detect sense and antisense PSTVd RNAs, respectively [21] . PSTVd dimer construct was inherited from late Professor Biao Ding and was illustrated in Figure 3.1. All constructs have been verified by Sanger sequencing.

3.3.2 Protein purification

The protocol for recombinant protein purification was based on our reported protocol [21]. Various recombinant TFIIIA-7ZF proteins with an intein-chitin binding domain (CBD) tag were overexpressed using the *Escherichia coli* BL21(DE3) Rosetta strain (EMD Millipore,

Burlington, MA). About 500 mL of bacterial culture was collected and re-suspended in lysis buffer (20 mM Tris-HCl pH 8.5, 500 mM NaCl, 1 mM PMSF, 5 M $ZnCl_2$ and 0.1% (v/v) Triton X-100). After sonication with Bioruptor (Diagenode, Denville, NJ), samples were subjected to centrifugation at 15,000x *g* for 1 h at 4 ˚C. The cell lysate was collected and incubated for 1 h with 2 mL of 50% slurry of chitin resin (New England Biolabs) before loading onto an empty EconoPac gravity-flow column (Bio-Rad Laboratories, Hercules, CA). After washing, resin was incubated for 18 h at 4 ˚C in a cleavage buffer [20 mM Tris-HCl (pH 8.5), 500 mM NaCl, 50 mM dithiothreitol, and 5 μM $ZnSO_4$]. Fractions containing recombinant TFIIIA-7ZF proteins were dialyzed against 20 mM HEPES, pH 7.5, 200 mM NaCl, 50 μM $ZnSO_4$, and 5 mM DTT. Protein concentrations were estimated by Coomassie Brilliant Blue staining of an SDS-PAGE gel using bovine serum albumin of known concentrations as reference standards.

Table 3.1 Primer sequences for cloning.

Primer name		Sequences
zf1	f	cacttgacgagaaatctcttgcagc
	r	gctgcaagagatttctcgtcaagtg
zf2	f	caacatgactcggaatgtcaatgagatgc
	r	gcatctcattgacattccgagtcatgttg
zf3	f	gcatccaaattaaagaaaaatgaggattctc
	r	gagaatcctcatttttctttaatttggatgc
zf4	f	tgcctcaaggaaaacgtggagagttg
	r	caactctccacgttttccttgaggca
zf5	f	gaatattaagcggaatctccgtacgcatg
	r	catgcgtacggagattccgcttaatattc
zf6	f	atcaaatcttattcagaacgtcaaagctg
	r	cagctttgacgttctgaataagatttgat
zf7	f	cgtgagagatagaaatgaaaagtctggc
	r	gccagacttttcatttctatctctcacg

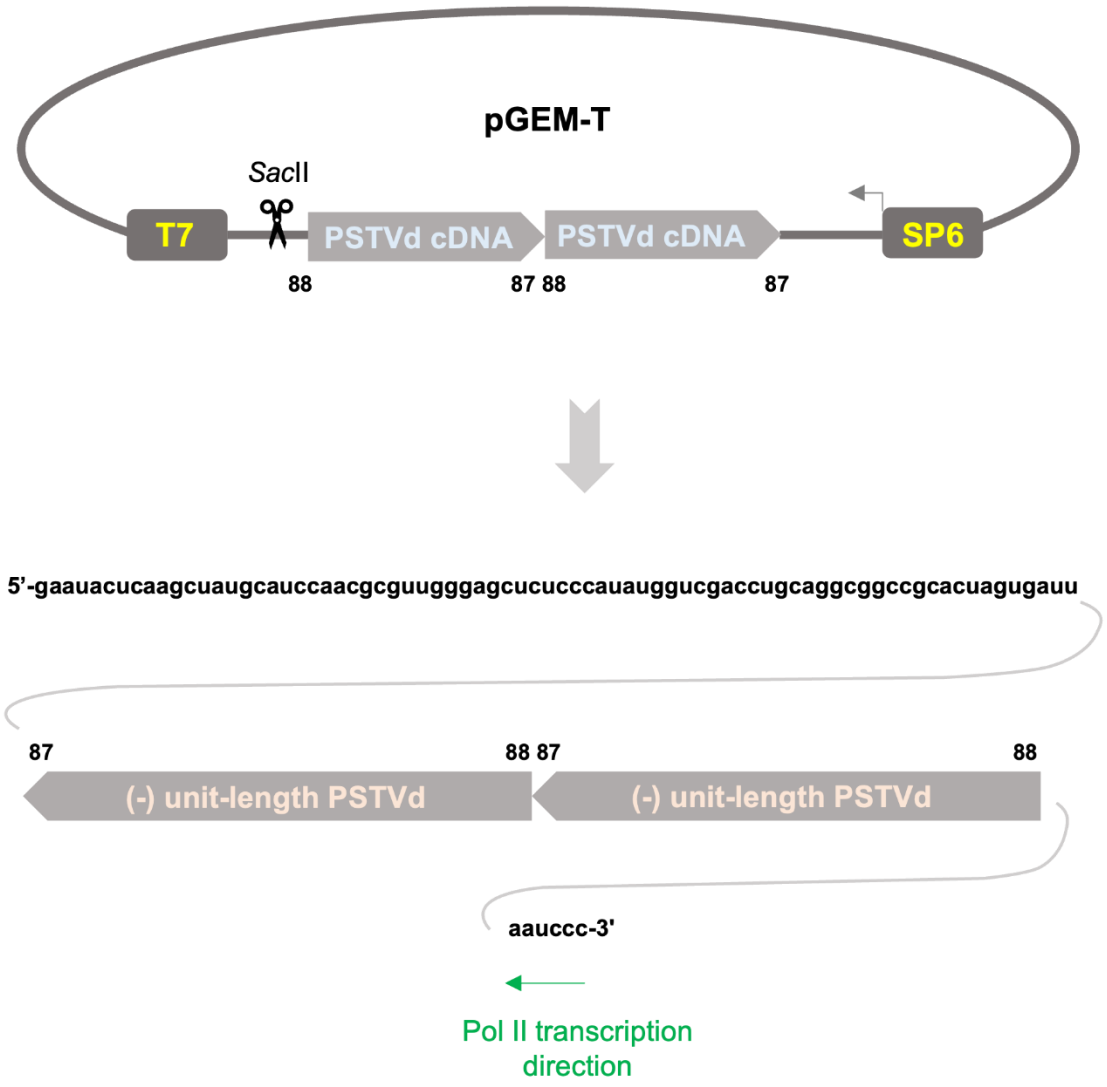


Figure 3.1 Illustration for the construct and the corresponding (-) PSTVd dimer template.

Top panel shows the pGEM-T plasmid containing PSTVd dimer cDNA and *Sac*II restriction site. Plasmid was linearized with *Sac*II and transcribed with SP6 MAXIscript transcription kit (Thermo Fisher Scientific, Waltham, MA, USA). Bottom panel shows the (-) PSTVd dimer containing non-viroid sequences on both ends.

Purification of Pol II from wheat germ was carried out following our published protocol [68]. All operations were performed at 4 ˚C, and all centrifugations were carried out for 15 min. One hundred grams raw wheat germ (Bob's Red Mill, Milwaukie, OR) was ground in a Waring Blender with 400 mL of buffer A [50 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, and 75

mM $(NH_4)_2SO_4$]. The resulting homogenate was diluted with 100 mL of buffer A and followed by centrifugation at 15,000x *g*. The supernatant was filtered through one layer of Miracloth (MilliporeSigma, Burlington, MA). The resulting crude extract containing Pol II was precipitated by an addition of 0.075 volume of 10% (v/v) Polymin P with rapid stirring. The resulting mixture was subject to centrifugation at 10,000x *g*. The pellet was washed with 200 mL of buffer A. The insoluble fraction, which contains Pol II, was resuspended with the buffer B [50 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, and 0.2 M $(NH_4)_2SO_4$]. The resulting suspension was centrifuged at 10,000x *g* to remove insoluble material. $(NH_4)_2SO_4$ precipitation was carried out by slowly adding 20 g of solid $(NH_4)_2SO_4$ per 100 mL of the above supernatant with stirring. The mixture was centrifuged, and the pellet was dissolved in buffer C (0.05 M Tris-HCl pH 7.9, 0.1 mM EDTA, 1 mM DTT, 25% ethylene glycol) plus 0.1% Brij 35 (Thermo Fisher Scientific, Waltham, MA) to make final $(NH_4)_2SO_4$ concentration 0.15 M. The $(NH_4)_2SO_4$ concentration was determined by conductivity. The resulting solution was applied to DEAE Sepharose FF (GE Healthcare Life Sciences, Pittsburgh, PA) equilibrated with buffer C plus 0.15 M $(NH_4)_2SO_4$. The column was then washed with five-bed volume with buffer C containing 0.15 M $(NH_4)_2SO_4$. Finally, bound Pol II was eluted with buffer C containing 0.25 M $(NH_4)_2SO_4$. Fractions containing Pol II were pooled. The $(NH_4)_2SO_4$ concentration was adjusted to 75 mM by diluting with buffer A without $(NH_4)_2SO_4$. The resulting solution was applied to SP Sepharose FF (GE Healthcare Life Sciences) equilibrated with buffer C containing 75 mM $(NH_4)_2SO_4$. After washing the column with the same buffer, Pol II was eluted using buffer C containing 0.15 M $(NH_4)_2SO_4$. Eluted fractions containing Pol II were pooled. Ethylene glycol (VWR Chemicals BDH, Radnor, PA) was added to a final concentration of 50% (v/v) before storing at −20 ^{0}C.

3.3.3 *In* vitro transcription assay

Pol II-catalyzed *in vitro* transcription was carried out based on our recently developed protocol [21, 68]. BSA (New England Biolabs) and TFIIIA-7ZF were treated with 1 unit of Turbo DNase (Thermo Fisher Scientific) for 10 min at 37 ˚C. Then, 0.39 pmol (-) PSTVd dimer, 0.27 pmol partially purified Pol II, pretreated BSA (4 μM final concentration) and various amounts of TFIIIA-7ZF were incubated at 28 ˚C for 15 min. The reaction system was adjusted to contain 50 mM HEPES-KOH pH 7.9, 1 mM $MnCl_2$, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$, 10% (v/v) glycerol, 1 unit/μL SuperaseIn RNase inhibitor (Thermo Fisher Scientific), 0.5 mM rATP, 0.5 mM rCTP, 0.5 mM rGTP, 0.5 mM rUTP. Transcription reactions (50 μL) were incubated at 28 ˚C for 4 h. About 0.8 U/μL proteinase K (New England Biolabs) was applied to terminate the reaction by incubation at 37 ˚C for 15 min, followed by incubation at 95 ˚C for 5 min. The Pol II-catalyzed *in vitro* transcription assay was repeated three times for each TFIIIA-7ZF variant. For the IVT assay in Figure 3.5, TFIIIA-7ZF and partially purified Pol II were subsequentially bound to immobilized desthiobiotinylated RNA templates (see the section below for details). After washing twice, additional RNA templates without desthiobiotinylation were supplied together with NTPs. The reaction was then performed the same as the abovementioned. This assay was repeated twice.

3.3.4 RNA-based affinity purification

Using Pierce RNA 3’ end desthiobiotinylation kit (Thermo Fisher Scientific, Waltham, MA, USA), 50 pmol of PSTVd dimer RNA was labeled following the manufacturer’s instructions. Labeled RNA was purified using MEGAclear kit (Thermo Fisher Scientific, Waltham, MA, USA) and heated at 65 ˚C for 10 min followed by incubation at room temperature for 12 min. Labeled RNA was bound to the 50 μL of streptavidin magnetic beads (Thermo Fisher

Scientific, Waltham, MA, USA). Magnetic beads were collected and washed twice with equal volume of 20 mM Tris-HCl, pH 7.5. Beads were subsequently washed with reaction buffer containing, 50 mM HEPES-KOH pH 8, 2 mM $MnCl_2$, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$, 10% glycerol. DNase-treated 150 pmol of recombinant TFIIIA-7ZF was incubated with RNA-bound beads in a 50 μL reaction at 28 ˚C for 15 min. Then, 27 pmol of partially purified Pol II was added to the reaction and incubated at 28 ˚C for another 15 min. Next, beads were washed twice with wash buffer (20 mM Tris-HCl, pH 7.5, 10 mM NaCl, 0.1% Tween-20) and bound proteins were eluted with 1x SDS-loading buffer by heating 95 ˚C for 5 min.

3.3.5 RNA gel blots

Detailed protocol has been reported previously [38]. Briefly, after electrophoresis in 5% (w/v) polyacrylamide/8 M urea gel for 1 h at 200 V, RNAs were then transferred to Hybond-XL nylon membranes (Amersham Biosciences, Little Chalfont, United Kingdom) by a Bio-Rad semi-dry transfer cassette and were immobilized by a UV-crosslinker (UVP, Upland, CA). The RNAs were then detected by DIG-labeled UTP probes. PSTVd RNAs were prepared as described before [21]. *Sma*I-linearized pInt95-94(-) and pInt95-94(+) were used as templates for generating probes, using the MAXIclear kit (Thermo Fisher Scientific). The DIG-labeled probes were used for detecting PSTVd RNAs. RNA gel blot signals were obtained using ChemiDoc (Bio-Rad Laboratories) and quantified using ImageJ (https://imagej.nih.gov/ij/). The quantified signals with biological replicates were subject to graphing and statistical analysis using the built-in functions of Prism (GraphPad Software, LLC).

3.3.6 Immunoblots

Protein samples were separated on an SDS-PAGE gel and transferred to a nitrocellulose membrane (GE Healthcare Lifesciences) using the Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories). After 1 h incubation with 1% (w/v) nonfat milk in 1X TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) at room temperature, membranes were incubated with primary antibodies overnight at 4 ˚C. After three washes with 1X TBST (50 mM Tris- HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20), HRP-conjugated secondary antibodies were added. Membranes were washed three times with 1X TBST and incubated with HRP substrates (Li-COR Biosciences, Lincoln, NE). The signals were detected with ChemiDoc and quantified using ImageJ. Graphing and statistical analyses were performed using the built-in functions of Prism.

For immunoblotting, polyclonal antibodies against TFIIIA were diluted at 1:2,000 and the monoclonal 8WG16 antibodies (Thermo Fisher Scientific) were diluted at 1:1,000. HRP-conjugated anti-mouse serum (Bio-Rad) was diluted at 1:5,000. HRP-conjugated anti-rabbit serum (Thermo Fisher Scientific) was diluted at 1:3,000. For silver staining, we followed the instructions of the Silver BULLit kit (Amresco, Solon, OH).

3.3.7 Nano-liquid chromatography-tandem mass spectrometry analysis (nLC-MS/MS)

Prior to mass spectrometry, samples were subjected to in-solution digestion. Briefly, reduction treatment (100 mM dithiothreitol and 15 min incubation at 65 ˚C) and alkylation treatment (100 mM iodoacetamide/45 min incubation at room temperature) were followed by 16 h incubation at 37 ˚C with sequencing grade trypsin (Promega, Madison 18 WI). Tryptic peptides were acidified with formic acid, lyophilized, and stored at -80 ˚C. As described previously [135], two micrograms of digested protein were subjected to nLC-MS/MS analysis using the LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) directly linked to the Ultimate

3000 UPLC system (Thermo Fisher Scientific), with the following modification: mass spectra of intact and fragmented peptides were collected in the orbitrap and linear ion trap detector, respectively. All data files were deposited to PRIDE database [136] (accession PXD033736). The. raw mass spectral files were searched using the SEQUEST algorithm of the Proteome Discoverer (PD) software version 2.1 (Thermo Fisher Scientific). Tolerances were set to 10 ppm and 0.8 Da to match precursor and monoisotopic fragment masses, respectively. The *Triticum aestivum* NCBI protein database (as of February 2022, with 122, 221 entries) and its reversed copy served as the target and decoy database, respectively, to allow calculation of False Discovery Rate (FDR). All proteins presented in results were filtered by FDR<1% and identified by minimum of 2 peptides and 2 PSMs (peptide-spectrum matches) in each replicate. The PD result data files showing peptide/protein-ID relevant parameters for each replicate are given in Appendix A.

3.4 Results

Because PSTVd replication from circular genomic RNA to (-) oligomers and then to plus-strand or (+) oligomers is a continuous process, failure to tease apart each step resulted in controversial data, as evidenced by previous reports [137, 138]. To understand whether Pol II can catalyze transcription using (-) viroid oligomers, we established a reconstituted *in vitro* transcription system using partially purified Pol II from wheat germ [11, 68] and the (-) PSTVd dimer as RNA template. The experimental flow is summarized in Figure 3.2. In brief, *in vitro* transcribed (-) PSTVd dimer was mixed with partially purified Pol II with or without the supplement of TFIIIA-7ZF proteins expressed in and purified from bacteria. Noteworthy is that our (-) PSTVd dimer RNA contains non-viroid sequences on both ends, as detailed in Figure 3.1. We will discuss the presence of non-viroid sequences below.

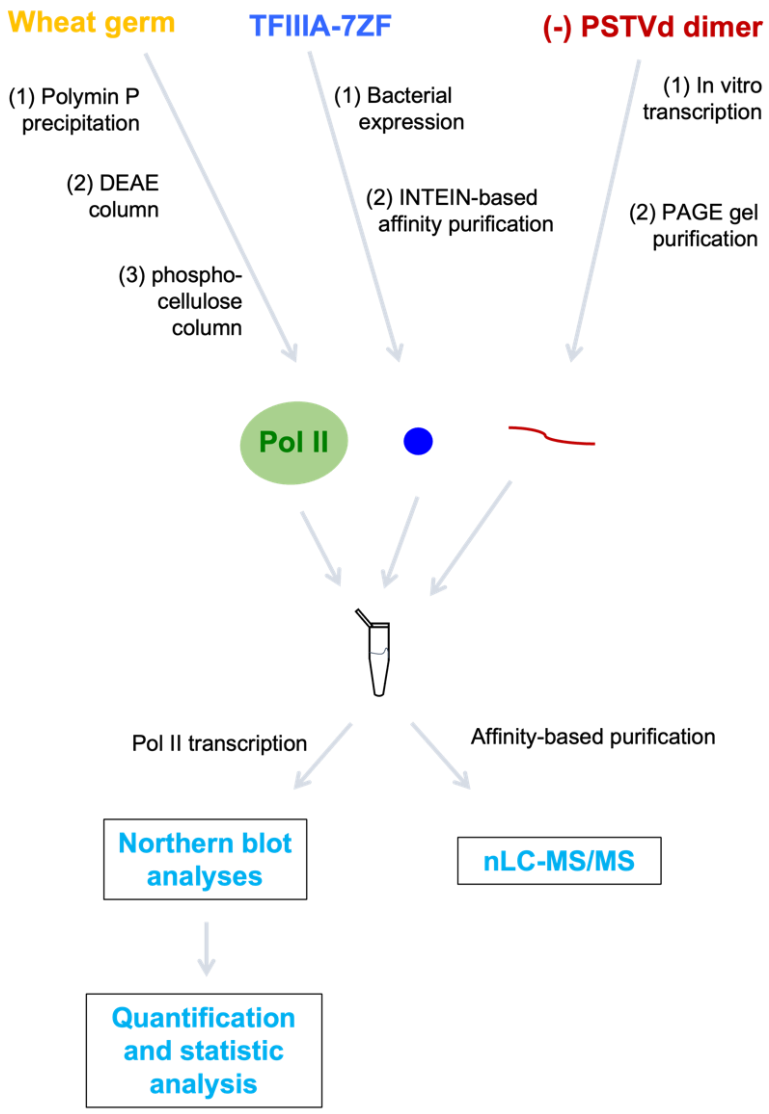


Figure 3.2 Flow chart illustrating the reconstituted *in vitro* transcription system.

We partially purified Pol II from wheat germ and TFIIIA-7ZF proteins overexpressed in *E. coli* were purified to homogeneity. *In vitro* transcription was carried out by mixing TFIIIA-7ZF with (-) PSTVD dimer followed by addition of partially purified Pol II to TFIIIA-7ZF (-) PSTVd RNA protein complex. *In vitro* transcribed products were identified by performing northern blot analyses. Using an RNA-based affinity purification we analyzed the composition of Pol II complex on the RNA template.

Based on our previous report [68] and the rolling circle replication model (Figure 1.1), the linear (+) PSTVd (i.e., the product from IVT assay in this report) cannot serve as a template

for further transcription. Therefore, this IVT assay only focuses on the transcription step from (-) PSTVd dimer to (+) PSTVd dimer. As shown in Figure 3.3, Pol II consistently exhibited a weak activity in transcribing (-) PSTVd template to full-length (+) PSTVd oligomer with a length similar to the template. We then tested the role of the RNA-specific transcription factor (TFIIIA-7ZF) in this transcription reaction by supplying various amounts of the protein. The reactions were subject to RNA gel blotting analyses with sequence-specific riboprobes. Comparing the results of RNA gel blots for (-) PSTVd dimer template and the (+) PSTVd product from the same samples demonstrated the specificity of the riboprobes as well as confirmed the identity of the IVT products (Figure 3.3). The product signals from samples with Pol II but without TFIIIA-7ZF (Pol II only) were quantified and set as 1. Then the product signals from samples with various amounts of TFIIIA-7ZF protein were normalized to the signal from "Pol II only" sample in the same blot. As shown in Figure 3.1, TFIIIA-7ZF can significantly increase the RdRp activity (more than 10-fold) of Pol II on the (-) PSTVd dimer template which is statistically significant (p-value 0.02). Therefore, our results indicate that Pol II can accept (-) PSTVd oligomers as a template for transcription, expanding the known natural RNA templates for DdRPs.

We then analyzed the composition of Pol II complex on the viroid RNA template using RNA-based affinity purification. Briefly, a desthiobiotinylated cytidine (Bis)phosphate was ligated to the 3'end of (-) PSTVd dimer, which was then mounted to magnetic streptavidin beads. After sequential supplying of TFIIIA-7ZF and partially purified Pol II, the magnetic beads were washed before elution. Through silver staining, common patterns and distinct bands can be observed between elution fraction and partially purified Pol II (Figure 3.4), implying that certain factors may be enriched by or removed from RNA templates.

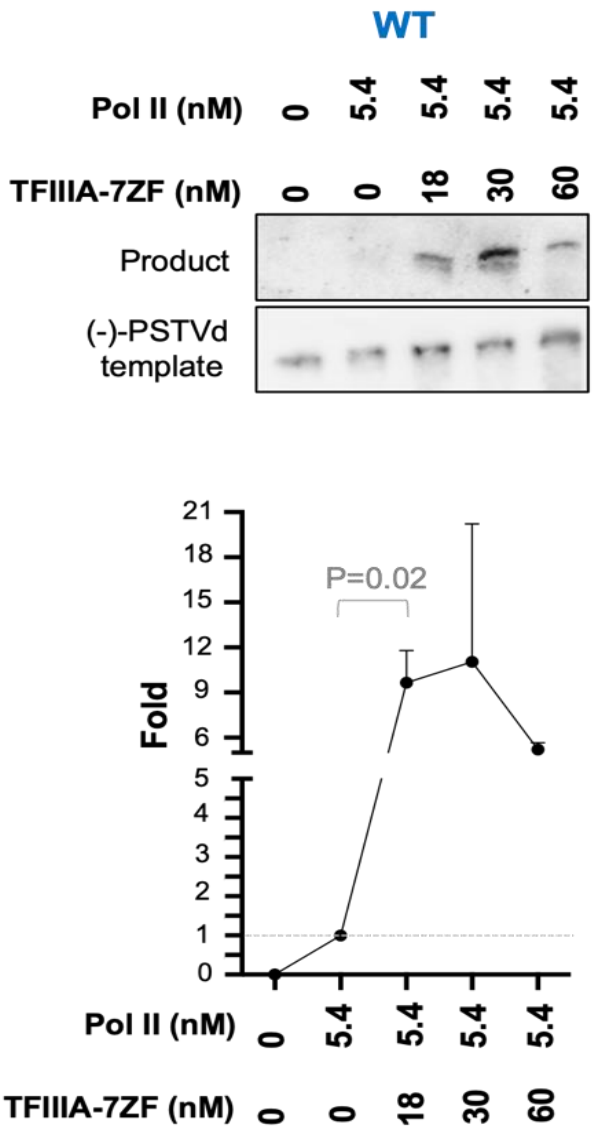


Figure 3.3 Reconstituted *in vitro* transcription system.

Partially purified Pol II, (-) PSTVd dimer RNA (7.8 nM), and various amounts of TFIIIA-7ZF used for reaction. Sequence-specific riboprobes were used to detect (-) PSTVd templates and (+) PSTVd products (at the position close to dimer PSTVd). Quantification of product intensities was performed using ImageJ. The first lane signal was set as 0 and the second lane signal was set as 1. Data from three replicates were used for graphing the fold increases induced by various amount of TFIIIA-7ZF.

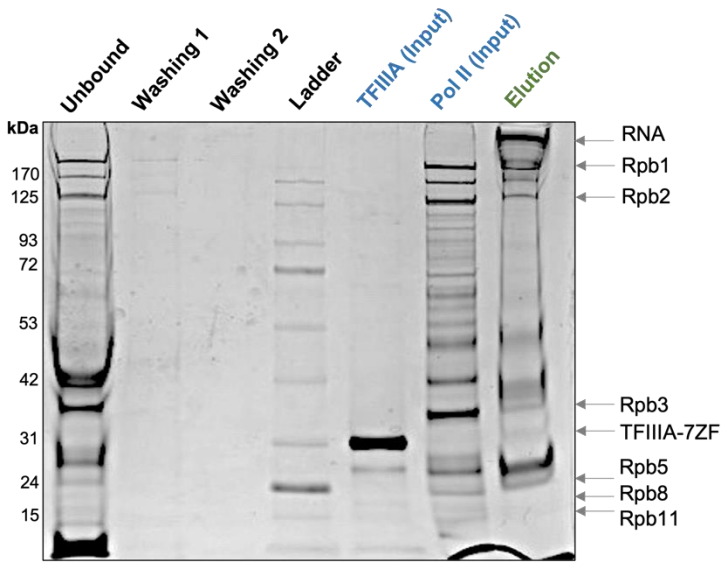


Figure 3.4 Silver staining of RNA-based affinity purification.

Pol II subunits are labeled based on the predicted molecular weight.

We then performed nLC-MS/MS to reveal the protein factors in partially purified Pol II as well as the Pol II complex remaining on the RNA template (proteins identified in each replicate are listed in A1-A8 Datasets). We identified 11 out of 12 Pol II subunits in partially purified Pol II with high confidence (false discovery rate below 0.01, identified by a minimum of 2 peptides, and 2 peptide-spectrum matches) in all three replicates (Figure 3.5, Table 3.2, A1 and A2).

The smallest subunit Rpb12 (~6 kDa) was absent, which is possibly caused by sample loss during the size cut-off enrichment of samples for nLC-MS/MS. This issue has been seen in another study [133]. Interestingly, only six subunits were confidently identified in the Pol II complex remaining on the RNA template with high confidence: Rpb1, Rpb2 and Rpb8 were found in all three replicates while Rpb3, Rpb10, and Rpb11 were found in two out of three replicates (Table 3.2, A1 and A2). Rpb5 can only be detected in one replicate of Pol II remaining

on the RNA template (Table 3.2).

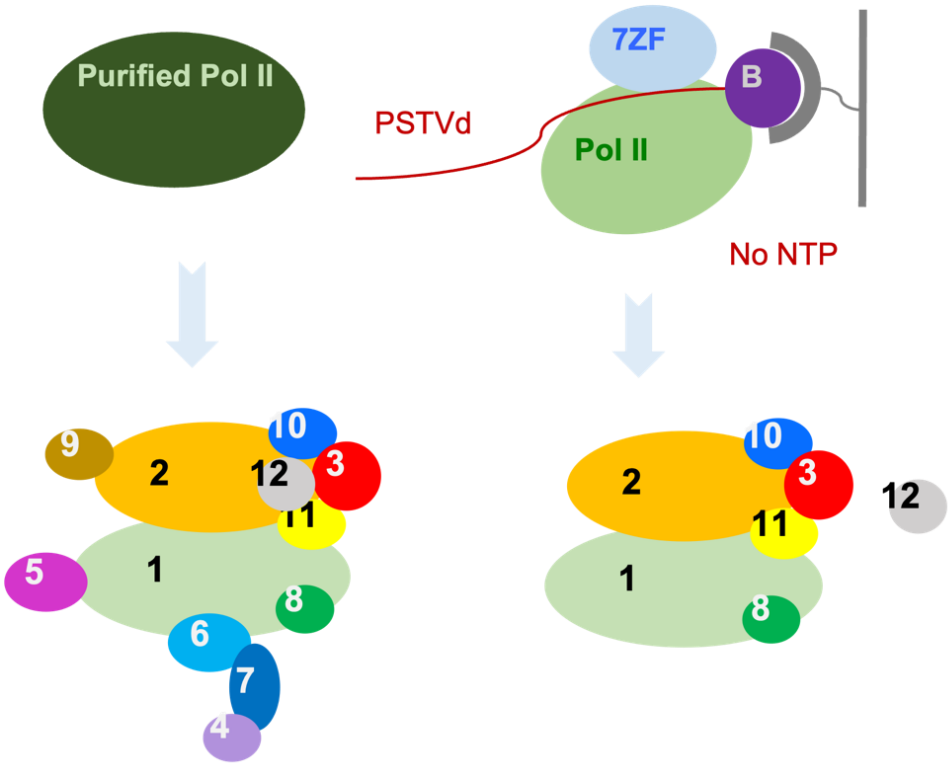


Figure 3.5 Schematic presentation for RNA-based affinity purification followed by nLC-MS/MS identifying protein factors in partially purified Pol II and remodeled Pol II.

Therefore, we consider it is less likely to participate in the Pol II complex on the RNA template. Rpb1 and Rpb2 form the catalytic core of Pol II, while Rpb3, Rpb10, Rpb11 are components of a subassembly group all critical for Pol II assembly [75]. Rpb8 is an auxiliary factor [75] . Given that the Pol II complex remaining on the RNA template has a distinct composition, we termed it remodeled Pol II hereafter. To test whether the remodeled Pol II has any catalytic activity, we repeated the RNA-based affinity purification followed by IVT. As shown in Figure 3.6, this remodeled Pol II indeed possessed transcription activity in generating (+) PSTVd oligomers comparable to partially purified Pol II.

Table 3.2 Peptide counts for each Pol II subunit in all nLC-MS/MS replicates

	Partially purified Pol II			Pol II on RNA template		
	1	2	3	1	2	3
Rpb1	222	166	175	26	36	46
Rpb2	67	56	63	26	22	22
Rpb3	21	16	15	0	9	8
Rpb4	7	5	5	0	0	0
Rpb5	20	16	18	0	2	0
Rpb6	4	4	4	0	0	0
Rpb7	5	6	5	0	0	0
Rpb8	10	8	8	6	4	4
Rpb9	9	6	8	0	0	0
Rpb10	6	4	6	3	0	2
Rpb11	12	7	8	2	0	2
Rpb12	222	166	175	26	36	46

Besides the Pol II complex in the partially purified samples, we also found the presence of several general transcription factors and transcription elongation factors for DNA-dependent transcription. However, they were all absent in the remodeled Pol II in the nLC-MS/MS analysis. For example, we found TFIIF in all three repeats of partially purified Pol II but could not confidently detect it in any of the remodeled Pol II samples (Figure 3.7, Table A1 and A2). In addition, TFIIE was found in two out of three partially purified Pol II repeats but could not be confidently detected in any of the remodeled Pol II samples (Table A1 and A2). Therefore, TFIIE and TFIIF are likely not required for viroid RNA templated transcription.

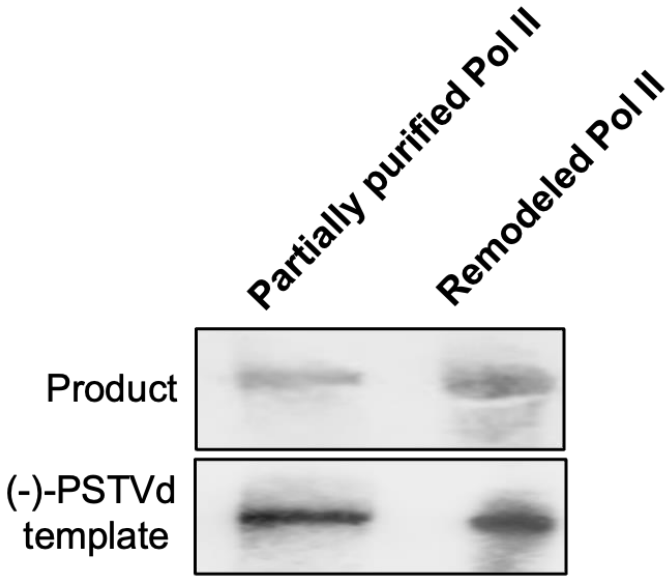


Figure 3.6 IVT assay demonstrating the activity of remodeled Pol II

The first lane contains free RNA as template, while the second lane contains mixed free RNA and desthiobiotinylated RNA as template. For the second lane, labeled RNA was used first to reconstitute the remodeled Pol II, and the free RNA was then supplied together with rNTPs. Reaction condition is described in Methods details.

Noteworthy is that all the rest of the canonical general transcription factors (including TFIIA, TFIIB, TFIID, TFIIH, and TFIIS) were absent in our partially purified or remodeled Pol II, indicating that they are dispensable for (-) PSTVd RNA-templated transcription. SPT6 is a histone chaperone that interacting with Rpb4/Rpb7 heterodimer during transcription elongation on DNA templates [132]. All Rpb4/Rpb7 and SPT6 were absent in the remodeled Pol II sample (Figure 3.7, Table A1 and A2). The FACT complex, including SPT16 and SSRP1-B, is also a histone chaperone assisting Pol II elongation on DNA templates. The FACT complex does not interact with Pol II directly during transcription on chromatin [139], and neither of its components was present in the remodeled Pol II sample (Figure 3.7, Table A1 and A2). The PAF1-C, including PAF1, CTR9, LEO1, and RTF1, regulates transcription-coupled histone

modifications. PAF1-C components extensively interact with Pol II. PAF1-LEO1 is anchored to the external domains of Rpb2. RTF1 is in proximity to PAF1. The trestle helix in CTR9 binds to Rpb5 and the surrounding region, while the tetratricopeptide repeats interact with Pol II around Rpb11 and Rpb8 [132, 140]. Similarly, the PAF1-C was mainly absent in the remodeled sample on the RNA template (except for the significantly reduced CTR9 found in two out of three replicates) (Figure 3.7, Table A1 and A2).

Since TFIIIA-7ZF is critical for Pol II to perform transcription using RNA templates, we attempted to identify the functional domain(s) of TFIIIA-7ZF. TFIIIA-7ZF has seven C2H2-type zinc finger domains. We mutated each zinc finger domain by changing the first histidine in the C2H2 domain to asparagine, which is commonly used to disrupt the local structure of a C2H2 motif [137, 141]. We then used those variants for the IVT assay. As shown in Figure 3.8, all mutants exhibited greatly reduced activity in aiding Pol II transcription on viroid RNA templates. Mutants *zf1*, *zf2*, *zf3*, and *zf6* completely lost the activity, while mutants *zf4*, *zf5*, and *zf7* can increase Pol II activity about two-fold (Figure 3.8), which is a much weaker activity as compared with more than 10-fold increase stimulated by wildtype (WT) TFIIIA-7ZF (Figure 3.3). Despite the low amounts of products generated, products all had a similar length to the template.

Interestingly, *zf1*, *zf2*, and *zf3* exhibited significantly reduced affinity to (-) PSTVd dimer RNA, which led to significantly reduced Pol II bound to the RNA templates. The amount of the remodeled Pol II was also reduced, to a lesser extent, in the presence of *zf4*, *zf5*, or *zf7*, which explains the reduced transcription activity in the corresponding IVT reactions. Interestingly, *zf5* had a similar binding ability to the RNA template resembling WT and *zf6* variant, but the amount of Pol II on the RNA template was significantly decreased in the presence of *zf5* as compared

with the presence of WT or *zf6* (Figure 3.9). This observation suggests that the fifth zinc finger domain is possibly involved in Pol II binding.

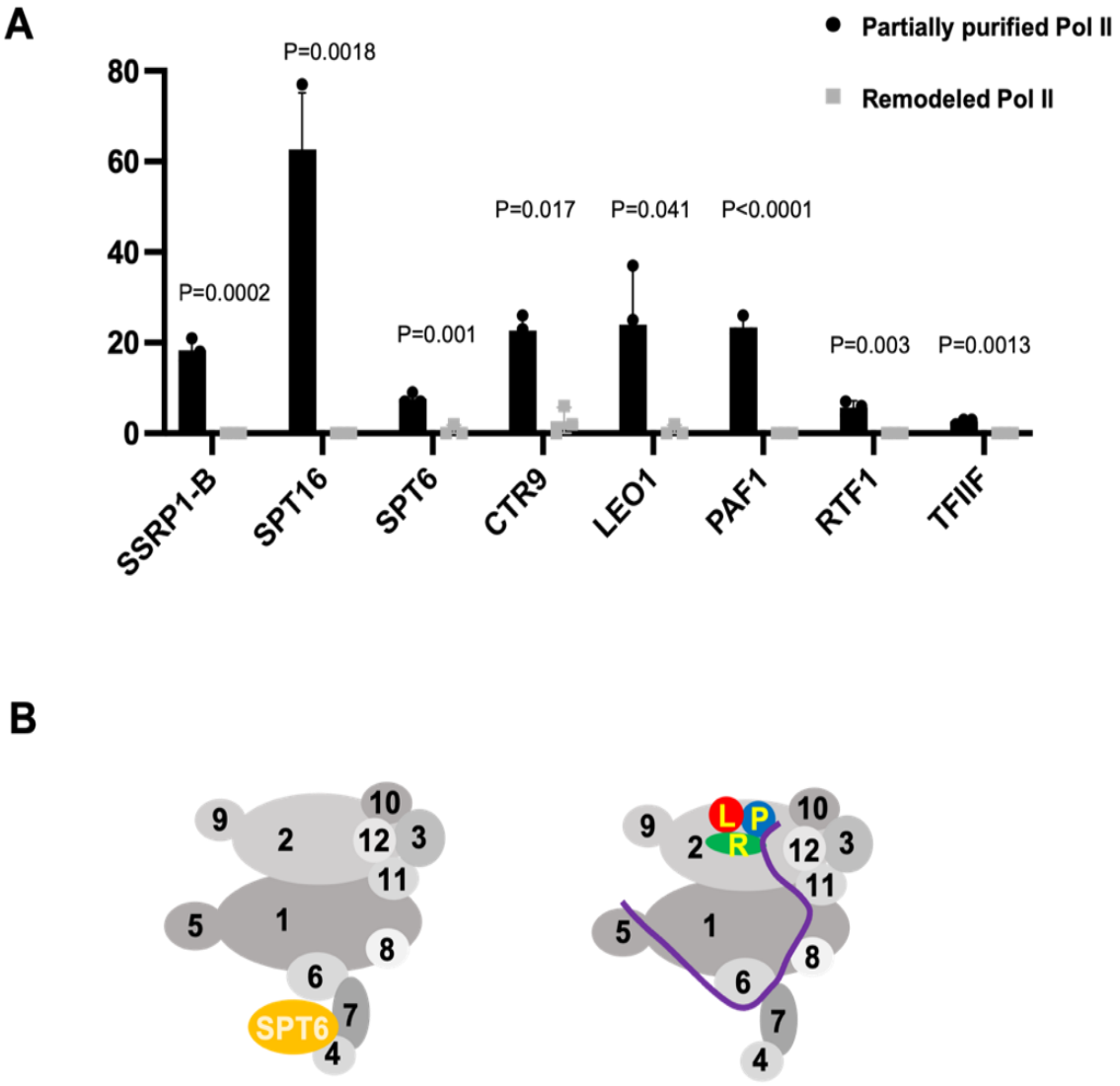


Figure 3.7 Analyses of transcription elongation factors.

(A) Analyses on peptide counts of the FACT complex (SSRP1-B, SPT16), SPT6, PAF1-C, and TFIIF in partially purified Pol II and remodeled Pol II. P values were calculated via two-tailed T-test, a built-in function in Prism. The summarized nLC-MS/MS data are listed in Table A1 and A2. The original data can be found in Tables A3-A8. (B) Schematic presentation of Pol II interactions with SPT6 and PAF1-C during DNA-dependent transcription, based on [16]. P, PAF1. L, LEO1. R, RTF1. Purple line, CTR9.

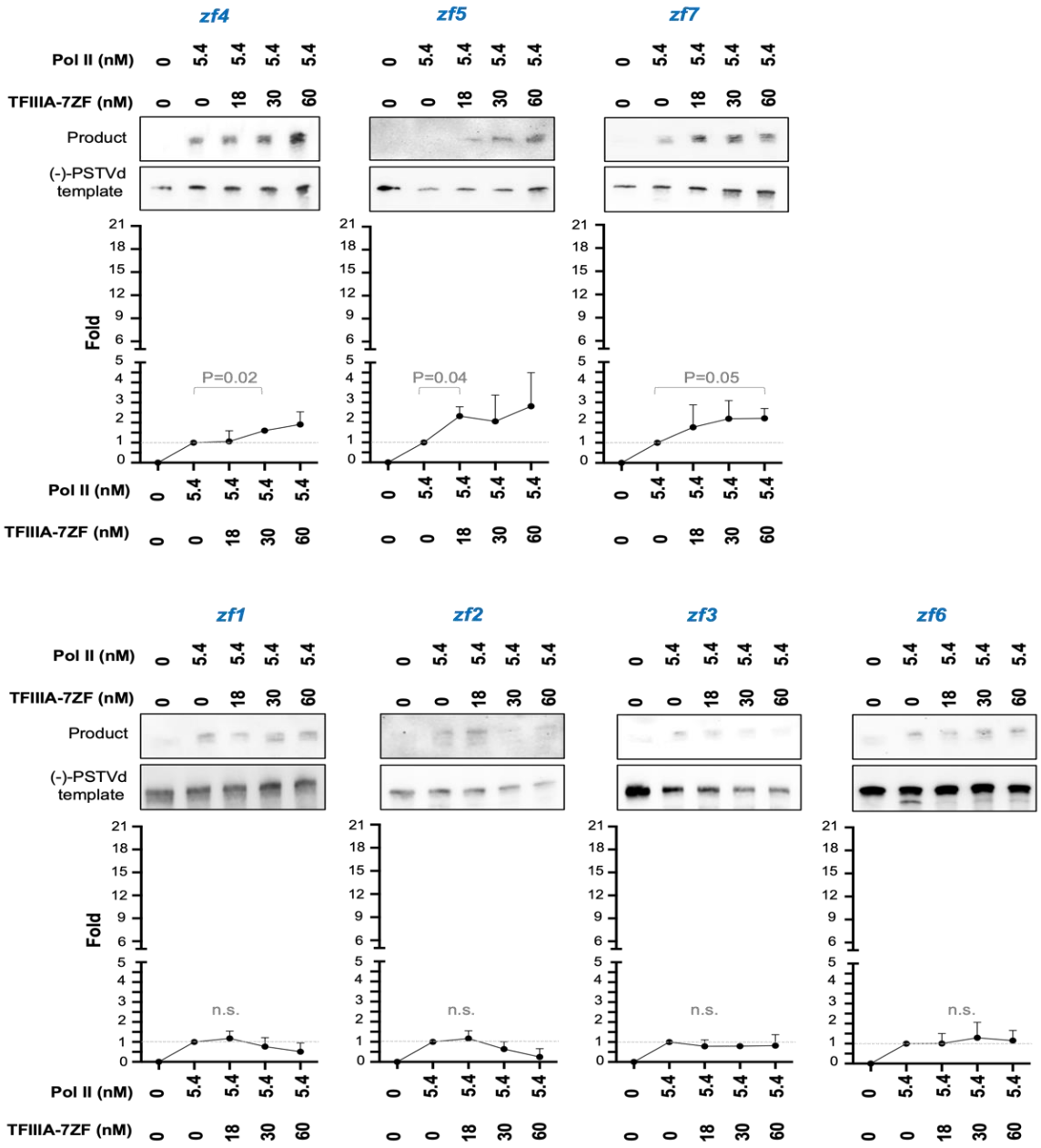
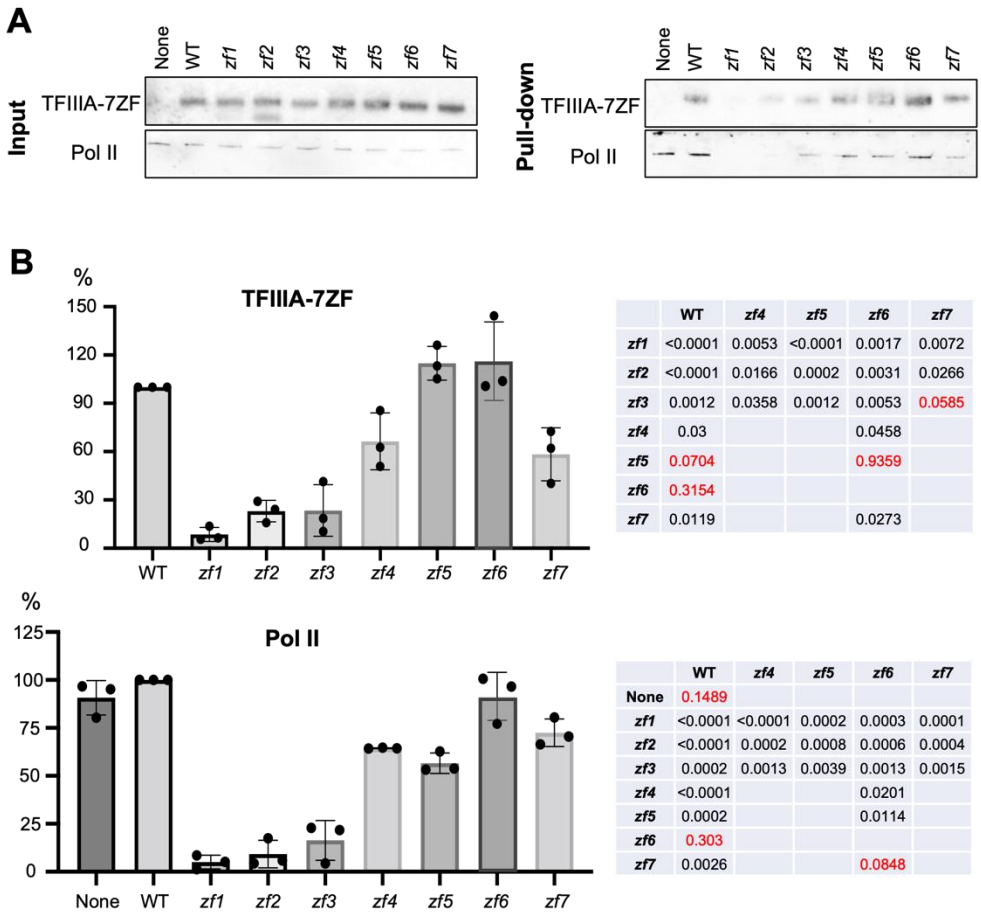


Figure 3.8 Analyses of the role of TFIIIA-7ZF zinc finger domains in aiding Pol II activity on RNA templates

Reconstituted *in vitro* transcription (IVT) system using partially purified Pol II, (-) PSTVd dimer RNA (7.8 nM), and various amounts of TFIIIA- 7ZF mutants. Sequence-specific riboprobes were used to detect (-) PSTVd templates and (+) PSTVd products (at the position close to dimer PSTVd). Fold changes were analyzed as described in Figure 3.3. P values for the most significant fold changes were listed as compared with Pol II only samples. n.s., no significant comparisons were identified.

3.5 Discussion

Using a robust IVT platform, we found that a remodeled Pol II and TFIIIA-7ZF can efficiently utilize (-) PSTVd dimer for RNA-templated transcription. TFIIIA-7ZF significantly enhances Pol II transcription activity on RNA templates. This remodeled Pol II represents a new minimal organization of the functional Pol II complex. In this remodeled Pol II, we observed the catalytic core (Rpb1 and Rpb2), a subassembly group (Rpb3, Rpb10, Rpb11), and an assembly factor Rpb8. Rpb12 was not detected in our samples due to technical shortcomings. Since Rpb12 is a conserved subunit in the subassembly group containing Rpb3, Rpb10, and Rpb11 [75], we speculate that Rpb12 is also present in the remodeled Pol II complex. However, it awaits clarification in the future. Rpb4/Rpb7 heterodimer, Rpb6, Rpb9, and likely Rpb5 were absent in the remodeled Pol II. The Rpb4/Rpb7 heterodimer is not essential for elongation and is not included in the Pol II core [129, 130]. Rpb6 is involved in contact with TFIIS and TFIIH [142, 143], neither of which was present in the transcription complex on the (-) viroid RNA template. This is in line with our recent observation that TFIIS is dispensable for PSTVd replication [68]. Rpb9 is critical for Pol II fidelity by delaying NTP sequestration [144, 145]. Pol II fidelity is also partially regulated by TFIIS [138, 146]. Interesting, both Rpb9 and TFIIS were absent in the remodeled Pol II samples, which could explain the observation that nuclear-replicating viroids have a much higher mutation rate than cellular Pol II transcripts [147]. Rpb5 is proposed to make contacts with DNA promoters and coordinate the opening/closing of the Pol II DNA cleft [148-152], which is not involved in RNA-templated transcription.



	WT	zf4	zf5	zf6	zf7
zf1	<0.0001	0.0053	<0.0001	0.0017	0.0072
zf2	<0.0001	0.0166	0.0002	0.0031	0.0266
zf3	0.0012	0.0358	0.0012	0.0053	0.0585
zf4	0.03			0.0458	
zf5	0.0704			0.9359	
zf6	0.3154				
zf7	0.0119			0.0273	

	WT	zf4	zf5	zf6	zf7
None	0.1489				
zf1	<0.0001	<0.0001	0.0002	0.0003	0.0001
zf2	<0.0001	0.0002	0.0008	0.0006	0.0004
zf3	0.0002	0.0013	0.0039	0.0013	0.0015
zf4	<0.0001			0.0201	
zf5	0.0002			0.0114	
zf6	0.303				
zf7	0.0026			0.0848	

Figure 3.9 Analyses on the binding ability of TFIIIA-7ZF mutants.

WT and mutants TFIIIA-7ZF proteins were used for RNA-based affinity purification in the presence of partially purified Pol II. (A) Immunoblots for input and RNA-bound TFIIIA-7ZF (anti TFIIIA) and the Rpb1 subunit of Pol II (8WG16). None, no TFIIIA-7ZF protein supplied. (B) Quantification of immunoblotting results in (A). Protein signals in Pull-down blots were normalized to the corresponding signals in the Input blots. The normalized signals in WT (TFIIIA and Rpb1) were set as 100. Three replicates were performed for quantification and statistical analyses. Two tailed T-test was used to calculate P values (listed in tables), by using the built-in function in Prism.

It has been proposed that an RNA polymerase may have evolved to transcribe RNA templates first and then transitioned to use DNA templates in modern life forms [75]. Interestingly, Rpb4/Rpb7 heterodimer is present in all archaeal and eukaryotic cells but not in bacteria [126, 153], further suggesting that organization changes occurred in RNA polymerases

during the course of evolution. The remodeled Pol II identified here only retains a minimal set of factors, while most of the missing subunits are absent in bacterial RNA polymerases (i.e., Rpb4, Rpb5, Rpb7, and Rpb9) [154-156]. Our discovery of a remodeled Pol II actively transcribing RNA templates may provide a handle to explore the functional evolution of transcription machinery further.

The high-resolution crystallographic structure of Pol II core-Rpb4/Rpb7-TFIIS showed that Pol II utilizes the same active site for interacting DNA and RNA templates, revealed by using chimeric RNA templates [24]. Later, one study using a chimeric RNA template containing an HDV fragment sequence suggested multiple general transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIS) may potentially be involved in the initiation of RNA-templated transcription [46]. However, neither experimental system could yield long RNA products, suggesting that those transcription complexes might not be optimal for subviral RNA templates. In the transcription complex on PSTVd RNA template, we could not detect the presence of TFIIE or TFIIF, despite that they were both identified confidently in at least two out of three replicates in partially purified Pol II (Table A1 and A2). Notably, other Pol II-associated general transcription factors (TFIIA, TFIIB, TFIID, TFIIH, and TFIIS) were also absent in partially purified Pol II and remodeled Pol II samples. Although we cannot rule out the possibility that a minute amount of general transcription factors were remaining in our samples below the detection capacity of nLC-MS/MS, it is unlikely for them to play a role in a stoichiometric ratio to Pol II resembling the transcription complex on DNA templates. Therefore, our observation argues that those general transcription factors for DNA-dependent transcription are dispensable in the transcription complex on RNA templates, at least for (-) PSTVd RNA templates. This is in drastic contrast to the requirement for the formation of a preinitiation

complex (PIC) on DNA templates [157]. Thus, our results outlined a distinct organization of transcription complex on viroid RNA templates.

All seven zinc finger domains of TFIIIA-7ZF are critical in aiding RNA-templated transcription. The first three zinc finger domains are pivotal for RNA template binding. Interestingly, Pol II exhibited weaker affinity to RNA templates in the presence of either *zf1*, *zf2*, or *zf3* mutant. It is intuitive to speculate that free *zf1*, *zf2*, and *zf3* proteins sequestered Pol II and prevented Pol II from binding to RNA templates, significantly reducing transcription activity. While *zf5* mutant has a similar ability in binding RNA templates as WT and *zf6*, Pol II was decreased considerably on RNA template in the presence of *zf5* compared to WT or *zf6*. Thus, the fifth zinc finger domain is possibly involved in Pol II binding. It is unclear why the *zf6* mutant also greatly diminished Pol II activity in generating the oligomer product as the amount of *zf6* and Pol II remaining on the RNA template resembles that in the reaction with WT TFIIIA-7ZF. Since this assay only tested TFIIIA-7ZF and Pol II binding to the RNA templated before reaction initiates, reasonable speculation is that *zf6* might be critical for transcription initiation or even elongation.

Interestingly, TFIIIA-7ZF aids Pol II activity on circular (+) PSTVd RNA template [21, 68]. The optimized stoichiometric ratios among Pol II, TFIIIA-7ZF, and RNA templates are slightly different between the IVT assays for circular (+) PSTVd [68] and (-) PSTVd dimer templates (reported here). This is likely attributed to the higher amount of circular (+) RNA template used for IVT, which is in agreement with the common observation that circular (+) PSTVd accumulates to a much higher level *in vivo* as compared with the (-) PSTVd oligomers. It may be informative to compare the organizations of the Pol II complex on distinct RNA templates. However, as we reported recently [68], the Pol II/TFIIIA-7ZF complex does not have

any detectable activity to transcribe linear (+) PSTVd RNA template. Therefore, we cannot use the labeled linear (+) PSTVd RNA to purify Pol II for nLC-MS/MS analyses.

Our reconstituted IVT system is robust for exploring the factors and functional mechanism underlying viroid RNA-templated transcription, particularly for studying transcription initiation and elongation. The IVT system reported here paves the way to dissect the machinery and mechanism involved in viroid RNA-templated transcription. One immediate question is whether there is an RNA promoter residing in the (-) PSTVd dimer. Our (-) PSTVd dimer contains a very short non-viroid sequence at its 3' end (6 nt), which falls in the region for the initiation of Pol II transcription. This 6 nt non-viroid sequence appears not to affect Pol II initiation, which is in line with the observation that (-) hop stunt viroid dimer surrounded by non-viroid sequences on both ends remains infectious in plants [158]. Therefore, it is reasonable to state that a certain element(s) within (-) viroid oligomers guides Pol II initiation. Meanwhile, it is interesting that Pol II seems to initiate transcription only from the 3' end of the RNA template, despite an identical viroid sequence in the middle of the oligomer RNA template. We expect our IVT system can help future investigations on these topics.

Of note, the mechanism underlying transcription termination on the viroid RNA template remains unknown [120]. Future investigations using our reconstituted IVT system may help analyze the transcription termination mechanism (regulated or run-off) and provide a handle for experimental tests in plants. In addition, the detailed regulation and mechanism for the remodeled Pol II/TFIIIA-7ZF complex on viroid RNA templates remains to be elucidated, which may be addressed by structural analyses in the future.

Although IVT systems are powerful to dissect the machinery and mechanism underlying the biological processes that are difficult to analyze *in vivo*, they may introduce some variations.

For example, the requirement of general transcription factors for the forming of PIC in reconstituted assays is largely in agreement with data obtained from *in vivo* studies but with minor variations [157]. On the other hand, the FACT complex and PAF1 complex regulate transcription elongation in cells but are dispensable for *in vitro* transcription [129, 130], which may be attributed to the lack of histone binding for *in vitro* DNA templates. Therefore, despite the fact that we did not observe the FACT components and most of the PAF1 complex (except for the significantly reduced CTR9) in our IVT system (Figure 3.7, Table A1 and A2), we cannot rule out the possibility that they might function in the cellular environment. While the finding of a minimal organization of Pol II complex with a reduced set of factors catalyzing (-) PSTVd RNA-templated transcription is novel, developing an *in vivo* experimental system is desired to corroborate this finding. Such investigations must overcome the challenge of distinguishing the remodeled Pol II on viroid RNA from the dominant Pol II complex/subunits in associated with DNA in cells.

CHAPTER IV

EMERGING VALUE OF THE VIROID MODEL IN MOLECULAR BIOLOGY AND BEYOND

This chapter is a modified version of "Emerging value of the viroid model in molecular biology and beyond" published in Virus Research [120] and has been reproduced here with the permission of the copyright holder. I have played a major role in developing the concepts explained in this chapter.

4.1 Abstract

Viroids are single-stranded circular noncoding RNAs that infect plants. Research in the past five decades has deciphered the viroid genome structures, viroid replication cycles, numerous host factors for viroid infection, viroid motifs for intracellular and intercellular trafficking, interactions with host defense machinery, etc. In this chapter, I mainly focus on some significant questions that remain to be tackled, centered around how the RNA polymerase II machinery performs transcription on RNA templates of nuclear-replicating viroids and how viroid RNAs coordinate multiple structural elements for diverse functions. Research on viroids has led to seminal discoveries in biology, and I expect the research directions outlined in this chapter to continue providing essential knowledge inspiring other areas of biology.

4.2 Introduction

The first viroid, potato spindle tuber viroid (PSTVd), was discovered and named in 1971 [2, 3]. Since then, about 40 viroids have been reported and categorized into two families, *Pospiviroidae* and *Avsunviroidae* [121, 122]. All the known viroids are circular noncoding RNAs that infect plants [82, 118, 119]. Members of the family *Pospiviroidae* possess rod-shaped genome structures and replicate in the nucleus. Transcription from their circular genome to multimeric (-) intermediates is known to harness DNA-dependent RNA polymerase II (Pol II) [11, 17]. An RNA-specific transcription factor (TFIIIA-7ZF) is critical for redirecting Pol II to use the RNA genome of PSTVd, a representative species of *Pospiviroidae* [21]. Viroids of the family *Avsunviroidae* all replicate in chloroplasts and adapt a highly branched portion in their RNA genomes [82, 118, 119]. They rely on nuclear-encoded polymerase (NEP) for RNA-templated transcription [5]. Here, I mainly discuss some of the emerging questions in plant-viroid interactions and provide testable hypotheses for future investigations, mainly revolving around the composition and mechanism of Pol II machinery on viroid RNA templates.

4.3 Viroid RNA-templated transcription

Mounting evidence supports that viroids of the family *Pospiviroidae* use host Pol II to catalyze the transcription from circular genome to linear multimeric (-) intermediates [11, 17]. In contrast to Pol I, Pol III and NEP, Pol II has a specialized carboxy-terminal domain (CTD) that supports co-transcriptional regulation [159]. While Pol II-catalyzed cellular mRNA synthesis is proceeding, a 7-methylguanosine cap (m^7G-CAP) is attached to nascent transcripts and introns are removed. This is feasible because the conserved “YSPTSPS” heptapeptide repeats in the Pol II CTD domain can recruit distinct factors based on the different phosphorylation statuses [160, 161]. Viroids clearly do not exploit the whole set of co-transcription machinery for their own

replication, therefore providing opportunities to understand the regulation of transcription on RNA templates with limited co-transcriptional regulation.

4.3.1 Viroid RNAs do not possess a m^7G-CAP

The m^7G-CAP attachment can be a simultaneous process during the transcription of many cellular mRNAs [159]. When nascent transcripts reach a length of ~20–30 nt, these short transcripts become capped near the RNA exit channel of the Pol II complex [162]. The phosphorylation of Ser5 in the heptapeptide repeats is critical to recruiting nuclear capping enzyme and guanine-7-methyltransferase for co-transcriptional capping [163-165]. The capping process coincides with the promoter-proximal pause during the transcription of many metazoan genes [162, 166, 167]. During this transcriptional pause, the nuclear capping enzyme interacts with the elongation factor SPT4/5. Phosphorylation of SPT4/5 releases pausing to resume productive elongation [168-170]. The promoter-proximal pause serves as a checkpoint for coupling the m^7G-CAP addition and elongation as well as allows the preparation of permissive DNA templates. To date, there is no evidence showing that viroid transcripts possess any m^7G-CAP. In addition, transcriptional pausing has not been demonstrated in the RNA-templated transcription either. These observations prompt the question of whether the phosphorylation codes of the Pol II CTD domain are necessary for RNA-templated transcription. Notably, Pol II-dependent RNA-templated transcription of B2 ncRNA and the human hepatitis delta virus (HDV) genomic/antigenomic RNAs do not possess m^7G-CAP either [89, 92]. The HDAg mRNA from HDV with a defined m^7G-CAP accounts for an extremely low percentage of total HDV RNAs [171], but it is unclear whether the m^7G-CAP in HDAg mRNA is added through co-transcriptional activity or other activities such as through cytosolic m^7G-CAP enzyme. Therefore, the replication of viroids and HDV genomes relies on Pol II but has no known co-

transcriptional regulation. Future studies on viroid replication may provide insights into the composition and modus operandi of Pol II complex on RNA templates.

4.3.2 Splicing and nuclear export

Intron splicing can occur co-transcriptionally or post-transcriptionally [159, 172, 173]. The assembly of spliceosomes on nascent transcripts regulates co-transcriptional splicing [174]. Importantly, splicing and nuclear export are coupled via the TREX complex, which is conserved in metazoans and contains both splicing factors and nuclear export factors [175]. Hence, splicing is necessary step for the nuclear export of most cellular mRNAs. Nuclear-replicating viroids do not harbor any intron, despite their dependence on Pol II for replication. Moreover, unlike the cellular mRNAs that are transported to the cytoplasm directly from their synthesis sites in the nucleoplasm, PSTVd appears to be transported into the nucleolus from the production site before nuclear export [176]. Therefore, viroids probably harness another route for nuclear export. Alternatively, viroid nuclear export relies on another pathway yet-to-be elucidated. One hypothesis is that viroids can use the 5S rRNA nuclear export pathway through transcription factor IIIA (the full-length protein with nine zinc fingers; TFIIIA-9ZF) [177]. Evidence showed that TFIIIA-9ZF interacts with PSTVd *in vivo*, but the functional significance of this interaction awaits future investigation [21].

4.3.3 Transcription termination

It is well accepted that cleavage at the poly(A) site (i.e., AAUAAA) leads to the addition of the poly(A) tail to mRNAs and the exonuclease-involved transcription termination [178-180]. However, the transcripts using the AAUAAA signal for termination only account for ~10% in plants [100]. Recently, a specific histone deacetylase (HDA6) has been shown to regulate the

usage of poly(A) sites other than AAUAAA in *Arabidopsis*, possibly through modifying the epigenetic hallmarks on DNA templates surrounding the AAUAAA sites [181]. This observation indicates that plants may regulate transcriptional termination via multiple means. The regulation of Pol II termination on RNA templates has not been fully understood, except for the HDV HDAg gene that contains a functional AAUAAA element [182]. Early studies found that the replication intermediates from circular (+) viroid templates have relatively unique size ranges based on viroid species. For example, PSTVd (-) intermediates can be tetramers [98, 183] or hexamers [184]; citrus exocortis viroid (CEVd) (-) intermediates are roughly 6-8 times monomer in size [185]; and hop stunt viroid (HSVd) (-) intermediates are dominantly dimers or tetramers [112]. While it is intuitive to consider the template run-off theme that Pol II leaves templates after several rounds of transcription on circular RNA templates, other possibilities involving regulatory factors cannot be ruled out. The relatively species-specific consistency in the lengths of (-) intermediates implies the existence of regulation over transcription termination when Pol II uses viroid genomic RNA as templates for transcription. Interestingly, reports showed that HSVd interacts with HDA6 directly and affects its cellular function [186]. Whether HDA6 is involved in regulating transcription termination on RNA templates may be clarified in future investigations.

4.4 Concluding remarks

As a peculiar group of subviral agents, viroids can serve as a productive model to tackle fundamental questions in molecular biology and beyond. PSTVd is the first known natural RNA redirecting a DNA-dependent RNA polymerase (DdRP) for transcription. To date, intrinsic RNA-dependent RNA polymerase (RdRP) activity has been found in many DdRPs [1]. These observations imply a missing link in the molecular evolution of transcription, where RNA

templates emerged first before DNA templates [24]. The viroid model can help dissect the needed factors for redirecting DdRPs to use RNA templates and distinguish the differences in regulating efficient transcription on DNA and RNA templates, which is a critical handle to understand further the evolution and function of the transcription complexes and mechanism. Using PSTVd as a model we present evidence showing that circular PSTVd templates are critical for the synthesis of longer-than-unit-length (-) strand products. Further we show TFIIS is dispensable for PSTVd replication supporting *de novo* transcription on PSTVd RNA templates. using PSTVd (-) dimer we demonstrate that RNA polymerase II (Pol II) accepts minus-strand for transcription and transcription factor TFIIIA-7ZF aids Pol II transcription activity. Analyses of critical zinc finger domains in TFIIIA-7ZF revealed that the first three zinc finger domains are pivotal for template binding. Notably, we identify a reorganized Pol II complex for viroid transcription. Collectively, our data illustrate a distinct organization of Pol II complex on viroid RNA templates, providing new insights into viroid replication, the evolution of transcription machinery, as well as the mechanism of RNA-templated transcription.

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APPENDIX A

PROTEOME DISCOVERER RESULT DATA

Table A.1 Summary results for transcription related factors in three replicates of partially purified Pol II

Accession	Description	Coverage			#Peptides			#PSMs			#Unique peptides		
		1	2	3	1	2	3	1	2	3	1	2	3
XP_044391332.1	DNA-directed RNA polymerase II subunit RPB1-like [Triticum aestivum]	18.3		23.9	29		4	127		4	1		4
XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	52.4	51.4	51.3	96	83	85	406	338	332	2	3	3
XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	52.3	50.7	51.2	97	83	86	408	335	336	3	3	4
XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	76.8	57.4	59.0	67	56	63	286	218	252	67	56	63
XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	73.4	60.7	59.8	21	16	15	97	78	77	3	16	15
XP_044410738.1	DNA-directed RNA polymerase II subunit 4-like [Triticum aestivum]	66.9	61.2	46.0	7	5	5	23	20	17	7	5	5
XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	58.4	54.1	56.9	12	11	11	58	43	41	11	11	10
XP_044350539.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	44.8	31.0	35.7	8	5	7	17	8	11	7	5	6
XP_044455885.1	DNA-directed RNA polymerases II, IV and V subunit 6A-like [Triticum aestivum]	35.2	36.6	35.2	4	4	4	21	26	14	4	4	4
XP_044407805.1	DNA-directed RNA polymerase II subunit RPB7 [Triticum aestivum]	48.0	35.0	35.0	5	6	5	21	19	17	5	6	5
XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	52.0	35.8	35.8	5	4	4	17	15	16	1	1	1
XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	52.0	35.8	35.8	5	4	4	15	18	14	1	1	1
XP_044378512.1	DNA-directed RNA polymerases II, IV and V subunit 9A-like [Triticum aestivum]	19.3	19.3	33.3	2	2	4	6	6	9	1	1	3
XP_044395610.1	DNA-directed RNA polymerases II, IV and V subunit 9B [Triticum aestivum]	86.0	54.4	54.4	7	4	4	34	14	26	6	3	3
XP_044341880.1	DNA-directed RNA polymerase subunit 10-like protein isoform X4 [Triticum aestivum]	77.5	76.1	77.5	6	4	6	24	19	21	1	1	1
XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like (RPB10) [Triticum aestivum]	41.7	40.9	41.7	6	4	6	25	17	23	1	1	1
XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	78.3	60.8	67.5	12	7	8	42	30	35	12	7	8
XP_044351723.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]	8.1	8.7	15.5	7	7	5	15	12	11	7	1	5
XP_044408236.1	DNA-directed RNA polymerase III subunit 2-like [Triticum aestivum]	13.6	9.5	8.0	10	7	5	14	8	7	10	7	5
XP_044332823.1	DNA-directed RNA polymerases I and III subunit rpac1-like [Triticum aestivum]	19.4	16.6	13.5	5	4	3	13	8	6	5	4	3
XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]	6.4	2.9	4.1	5	2	4	8	6	6	5	2	4
XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]	36.9	35.3	33.5	16	21	18	50	52	49	16	21	18
XP_044330076.1	FACT complex subunit SPT16-like [Triticum aestivum]	42.4	36.5	37.5	37	26	28	91	61	71	1	1	2
XP_044454990.1	FACT complex subunit SPT16 [Triticum aestivum]	44.7	38.7	37.5	40	28	29	91	64	71	2	3	3
XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]	6.4	7.7	8.5	7	7	9	13	11	16	7	7	9
XP_044338627.1	protein IWS1 homolog 1-like [Triticum aestivum]	6.4		5.1	4		2	6		3	4		2
XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	37.4	31.5	31.0	26	23	19	43	50	50	1	23	19
XP_044332259.1	protein LEO1 homolog [Triticum aestivum]	34.7	21.0	30.8	19	10	13	41	25	42	2	10	2
XP_044457053.1	protein LEO1 homolog [Triticum aestivum]	34.8		30.9	18		12	37		39	1		1
XP_044338201.1	protein PAF1 homolog [Triticum aestivum]	23.8	22.1	24.0	12	11	11	23	27	31	3	3	3
XP_044429807.1	protein PAF1 homolog [Triticum aestivum]	30.8	21.8	23.9	14	11	11	26	23	32	2	2	2
XP_044387377.1	protein RTF1 homolog [Triticum aestivum]	16.7	11.7	19.8	6	4	7	13	5	13	6	4	7
XP_044343360.1	general transcription factor IIE subunit 1-like [Triticum aestivum]	8.2	10.3		3	4		7	8		3	4	
XP_044394533.1	general transcription factor IIF subunit alpha-like isoform X1 [Triticum aestivum]	6.4	8.8	8.8	2	3	3	7	5	7	2	3	3

Table A.1 (continued)

Accession	Description	Coverage			#Peptides			#PSMs			#Unique peptides		
		1	2	3	1	2	3	1	2	3	1	2	3
XP_044365145.1	polyadenylation factor subunit 2-like [Triticum aestivum]	5.6		5.6	2		2	3		4	2		2
XP_044380741.1	pre-mRNA-splicing factor 38-like [Triticum aestivum]	9.4	12.7	9.4	3	4	3	5	7	5	3	4	3
XP_044323484.1	splicing factor 3B subunit 4-like [Triticum aestivum]	18.5	18.5		4	4		11	6		4	4	
XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]	11.3	12.4	12.6	7	8	7	22	13	15	7	8	7
XP_044421055.1	splicing factor, arginine/serine-rich 19-like [Triticum aestivum]	28.3	28.9	25.4	6	5	5	13	9	7	3	4	3
XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	8.6	3.7	6.4	15	6	9	29	10	15	15	6	9
XP_044348996.1	protein MLN51 homolog [Triticum aestivum]	18.4	11.7	10.7	8	4	5	22	12	11	1	1	1
XP_044417769.1	DExH-box ATP-dependent RNA helicase DExH12-like [Triticum aestivum]	20.5	16.9	17.6	23	20	23	51	37	46	23	20	23

Table A.2 Summary results for transcription related factors in three replicates of Remodeled Pol II

Accession	Description	Coverage			#Peptides			#PSMs			#Unique peptides		
		1	2	3	1	2	3	1	2	3	1	2	3
XP_044391332.1	DNA-directed RNA polymerase II subunit RPB1-like [Triticum aestivum]												
XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]		13.1	17.1		18	23		31	43		1	1
XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	22.6	13.0	17.0	26	18	23	60	30	41	18	1	1
XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	31.4	28.3	25.3	26	22	22	64	51	56	26	22	22
XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]		42.9	42.6		9	8		19	19		9	8
XP_044410738.1	DNA-directed RNA polymerase II subunit 4-like [Triticum aestivum]												
XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]		15.8			2			5			2	
XP_044350539.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]												
XP_044455885.1	DNA-directed RNA polymerases II, IV and V subunit 6A-like [Triticum aestivum]												
XP_044407805.1	DNA-directed RNA polymerase II subunit RPB7 [Triticum aestivum]												
XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	18.9	18.2		3	2		5	3		1	1	
XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	18.9	18.2	14.4	3	2	4	5	4	9	1	1	1
XP_044378512.1	DNA-directed RNA polymerases II, IV and V subunit 9A-like [Triticum aestivum]												
XP_044395610.1	DNA-directed RNA polymerases II, IV and V subunit 9B [Triticum aestivum]												
XP_044341880.1	DNA-directed RNA polymerase subunit 10-like protein isoform X4 [Triticum aestivum]												
XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like (RPB10) [Triticum aestivum]	18.2		18.2	3		2	5		3	3		2
XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	32.5		32.5	2		2	3		4	2		2
XP_044351723.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]												
XP_044408236.1	DNA-directed RNA polymerase III subunit 2-like [Triticum aestivum]												
XP_044332823.1	DNA-directed RNA polymerases I and III subunit rpac1-like [Triticum aestivum]												
XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]		2.2			2			4			2	
XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]												

Table A.2 (continued)

Accession	Description	Coverage			#Peptides			#PSMs			#Unique peptides		
		1	2	3	1	2	3	1	2	3	1	2	3
XP_044330076.1	FACT complex subunit SPT16-like [Triticum aestivum]												
XP_044454990.1	FACT complex subunit SPT16 [Triticum aestivum]												
XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]		1.7			2			3			2	
XP_044338627.1	protein IWS1 homolog 1-like [Triticum aestivum]												
XP_044414262.1	protein CTR9 homolog [Triticum aestivum]		7.8			6			7			6	
XP_044332259.1	protein LEO1 homolog [Triticum aestivum]			4.7			2			2			2
XP_044457053.1	protein LEO1 homolog [Triticum aestivum]												
XP_044338201.1	protein PAF1 homolog [Triticum aestivum]												
XP_044429807.1	protein PAF1 homolog [Triticum aestivum]												
XP_044387377.1	protein RTF1 homolog [Triticum aestivum]												
XP_044343360.1	general transcription factor IIE subunit 1-like [Triticum aestivum]												
XP_044394533.1	general transcription factor IIF subunit alpha-like isoform X1 [Triticum aestivum]												
XP_044365145.1	polyadenylation factor subunit 2-like [Triticum aestivum]												
XP_044380741.1	pre-mRNA-splicing factor 38-like [Triticum aestivum]												
XP_044323484.1	splicing factor 3B subunit 4-like [Triticum aestivum]												
XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]			3.7			2			2			2
XP_044421055.1	splicing factor, arginine/serine-rich 19-like [Triticum aestivum]												
XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]		1.4	1.6		2	2		2	4		2	2
XP_044348996.1	protein MLN51 homolog [Triticum aestivum]												
XP_044417769.1	DExH-box ATP-dependent RNA helicase DExH12-like [Triticum aestivum]												

Table A.3 nLC-MS/MS for partially purified Pol II (replicate 1)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	554.732	52.30193	97	408	3	1	1868	208	6.2	1357.06	97
High	XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	554.296	52.41416	96	406	2	1	1864	207.8	6.32	1349.72	96
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	356.307	76.82228	67	286	67	1	1221	138.2	7.15	953.9	67
High	XP_044327129.1	DNA-directed RNA polymerase II subunit RPB1-like [Triticum aestivum]	0	304.503	30.17751	52	234	1	1	1859	206.3	6.14	742.17	52
High	XP_044391332.1	DNA-directed RNA polymerase II subunit RPB1-like [Triticum aestivum]	0	151.51	18.30454	29	127	1	1	1852	206.5	6.16	390.36	29
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	114.253	51.65049	21	122	10	1	515	56.1	6.11	396.82	21
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	138.36	42.63006	21	116	7	1	692	77	5.52	453.77	21
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	123.695	63.06483	23	109	10	1	509	55.4	6.62	354.14	23
High	XP_044370961.1	vicilin-like seed storage protein At4g36700 [Triticum aestivum]	0	123.964	40.72022	20	109	8	1	722	81.1	5.85	411.55	20

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044377882.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	108.805	50.69034	22	108	9	1	507	55.3	6.96	351.83	22
High	XP_044376355.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	150.72	65.89242	39	97	4	1	818	90.8	5.26	322.64	39
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	123.709	73.4139	21	97	3	1	331	36.7	4.88	342.03	21
High	XP_044362370.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	142.272	61.62362	38	95	3	1	813	90.1	5.29	308.56	38
High	XP_044330076.1	FACT complex subunit SPT16-like [Triticum aestivum]	0	121.127	42.39631	37	91	1	1	1085	121	5.59	256.03	37
High	XP_044454990.1	FACT complex subunit SPT16 [Triticum aestivum]	0	132.465	44.70046	40	91	2	1	1085	121	5.57	258.52	40
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	95.089	57.8125	18	86	18	1	320	34.5	5.5	287.51	18
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	117.079	65.91928	22	85	8	1	446	50.2	4.81	304.25	22
High	XP_044321810.1	FACT complex subunit SPT16-like [Triticum aestivum]	0	117.617	41.06814	37	85	1	1	1086	121	5.63	234.92	37
High	XP_044343678.1	tubulin alpha chain [Triticum aestivum]	0	97.842	67.40576	18	78	2	1	451	49.7	5.02	274.14	18
High	XP_044380751.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	103.332	73.4139	19	77	1	1	331	36.6	4.84	260.74	19
High	XP_044368225.1	tubulin alpha-3 chain [Triticum aestivum]	0	99.901	67.62749	18	77	2	1	451	49.7	5.02	273.5	18
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	84.508	97.34513	11	61	3	1	113	11.5	4.41	233.84	11
High	XP_044423298.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	83.523	25.86818	23	58	6	1	1411	158.7	5.58	195.97	23
High	XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	64.29	58.37321	12	58	11	1	209	24.1	9.44	207.45	12
High	XP_044360175.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	78.326	30.37791	16	56	5	1	688	76.9	6.09	213.59	16
High	XP_044429961.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	83.282	23.68794	22	55	5	1	1410	158.7	5.64	184.24	22
High	XP_044324377.1	60S acidic ribosomal protein P2A [Triticum aestivum]	0	74.731	100	10	54	10	1	113	11.6	4.41	189.6	10
High	XP_044332842.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	82.208	50.10267	23	53	2	1	487	53.4	5.64	180.31	23
High	XP_044417769.1	DExH-box ATP-dependent RNA helicase DExH12-like [Triticum aestivum]	0	81.629	20.54104	23	51	23	1	2181	246.8	6.01	170.23	23
High	XP_044367849.1	63 kDa globulin-like protein [Triticum aestivum]	0	55.522	41.23377	19	50	1	1	616	69.4	7.06	126.99	19
High	XP_044390710.1	tubulin beta-4 chain [Triticum aestivum]	0	84.978	55.95506	18	50	3	1	445	49.9	4.87	173.08	18
High	XP_044365264.1	63 kDa globulin-like protein [Triticum aestivum]	0	58.518	46.2585	21	50	4	1	588	66.3	8.27	127.71	21
High	XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]	0	73.031	36.92078	16	50	16	1	669	74.4	5.8	177.27	16
High	XP_044450611.1	60S acidic ribosomal protein P2B [Triticum aestivum]	0	68.883	97.32143	10	49	2	1	112	11.4	4.34	188.75	10
High	XP_044383134.1	tubulin alpha-2 chain-like [Triticum aestivum]	0	65.079	54.54545	15	49	1	1	451	49.8	4.96	164.85	15
High	XP_044448581.1	importin subunit alpha-1b-like [Triticum aestivum]	0	80.522	51.31086	17	48	14	1	534	58.6	5.25	183.96	17
High	XP_044337674.1	heat shock 70 kDa protein 4-like [Triticum aestivum]	0	72.827	37.34568	18	48	3	1	648	71	5.25	165.81	18
High	XP_044324637.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	75.854	43.73717	21	48	2	1	487	53.4	5.83	161.29	21
High	XP_044421003.1	protein CTR9 homolog [Triticum aestivum]	0	88.583	37.86317	27	47	2	1	1067	122	6	163.2	27
High	XP_044356354.1	importin subunit alpha-1a isoform X2 [Triticum aestivum]	0	78.518	56.48855	19	47	16	1	524	57.3	5.19	165.81	19
High	XP_044438520.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	56.269	44.19643	12	46	3	1	448	47.4	4.75	151.18	12
High	XP_044339487.1	tubulin beta-5 chain [Triticum aestivum]	0	80.3	57.94183	19	45	7	1	447	50.3	4.83	147.68	19
High	XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	0	83.547	37.35955	26	43	1	1	1068	122	6	146.46	26
High	XP_044423543.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	78.4	40.74447	23	43	2	1	994	110.2	5.4	140.03	23

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044367850.1	63 kDa globulin-like protein [Triticum aestivum]	0	49.269	33.55155	15	43	1	1	611	68.9	7.59	111.77	15
High	XP_044339880.1	RNA-binding protein P-like [Triticum aestivum]	0	53.466	29.16667	12	42	1	1	480	49.5	5.12	125.84	12
High	XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	0	50.329	78.33333	12	42	12	1	120	14.1	7.62	113.22	12
High	XP_044348474.1	RNA-binding protein P-like [Triticum aestivum]	0	52.174	31.57895	13	42	1	1	475	48.7	5.27	126.14	13
High	XP_044356450.1	RNA-binding protein P-like [Triticum aestivum]	0	53.295	37.29167	13	41	1	1	480	49.4	5.12	125.85	13
High	XP_044363031.1	heat shock cognate 70 kDa protein-like [Triticum aestivum]	0	62.195	35.92085	17	41	5	1	657	71.8	5.29	141.75	17
High	XP_044332259.1	protein LEO1 homolog [Triticum aestivum]	0	76.026	34.7432	19	41	2	1	662	75.7	4.63	144.86	19
High	XP_044423644.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	51.965	48.307	13	40	3	1	443	46.9	4.82	120.38	13
High	XP_044373111.1	63 kDa globulin-like protein [Triticum aestivum]	0	51.416	32.40132	16	40	5	1	608	68.3	7.72	102.65	16
High	XP_044430028.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	73.928	40.74447	23	39	2	1	994	110.1	5.36	123.89	23
High	XP_044391423.1	elongation factor 2-like [Triticum aestivum]	0	54.51	27.28351	13	38	13	1	843	93.7	6.16	139.23	13
High	XP_044430572.1	60S acidic ribosomal protein P1-like [Triticum aestivum]	0	41.201	67.27273	7	38	7	1	110	11.2	4.59	140.7	7
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	43.262	81.66667	7	38	1	1	120	12.1	4.55	129.48	7
High	XP_044457053.1	protein LEO1 homolog [Triticum aestivum]	0	67.737	34.79576	18	37	1	1	661	75.5	4.59	128.97	18
High	XP_044438842.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	40.985	81.66667	7	37	1	1	120	12.1	4.55	126.08	7
High	XP_044369320.1	heat shock cognate 70 kDa protein 2 [Triticum aestivum]	0	51.569	26.85185	14	36	1	1	648	71.1	5.16	123.07	14
High	XP_044457664.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	44.792	30.72034	13	34	3	1	472	51.7	6.51	102.62	13
High	XP_044373110.1	63 kDa globulin-like protein [Triticum aestivum]	0	42.249	29.50558	13	34	4	1	627	70.6	7.17	94.81	13
High	XP_044395610.1	DNA-directed RNA polymerases II, IV and V subunit 9B [Triticum aestivum]	0	48.676	85.96491	7	34	6	1	114	13	6.64	117.28	7
High	XP_044374291.1	heterogeneous nuclear ribonucleoprotein Q-like isoform X1 [Triticum aestivum]	0	44.714	22.04007	8	32	8	1	549	60.4	5.2	108.86	8
High	XP_044343777.1	protein MLN51 homolog [Triticum aestivum]	0	54.014	35.90504	12	31	12	1	674	72.8	5.53	116.71	12
High	XP_044341061.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	58.042	44.27711	9	31	9	1	332	36.2	6.43	112.2	9
High	XP_044340424.1	protein MLN51 homolog [Triticum aestivum]	0	46.68	27.84993	12	30	5	1	693	75.5	5.34	95.73	12
High	XP_044397879.1	uncharacterized protein LOC123121865 [Triticum aestivum]	0	31.422	7.663551	8	30	2	1	1070	120.7	5.53	97.41	8
High	XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	0	44.11	8.58351	15	29	15	1	2365	275.4	8.78	81.18	15
High	XP_044437934.1	protein PAF1 homolog [Triticum aestivum]	0	39.105	30.64516	14	28	3	1	620	70.7	7.23	92.18	14
High	XP_044346616.1	protein PAF1 homolog [Triticum aestivum]	0	37.142	27.79553	13	27	1	1	626	71.4	7.97	86.55	13
High	XP_044429807.1	protein PAF1 homolog [Triticum aestivum]	0	36.633	30.84416	14	26	2	1	616	70.2	7.05	85.91	14
High	XP_044399391.1	elongation factor 1-alpha isoform X1 [Triticum aestivum]	0	27.147	11.62162	6	26	2	1	740	83.1	8.97	81.08	6
High	XP_044383709.1	heat shock cognate 70 kDa protein 2 [Triticum aestivum]	0	40.01	20.67901	11	26	1	1	648	71.2	5.21	83.87	11
High	XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like [Triticum aestivum]	0	41.923	41.66667	6	25	1	1	132	14.7	8.27	85.82	6
High	XP_044451529.1	embryonic protein DC-8-like [Triticum aestivum]	0	47.139	22.8022	12	25	2	1	728	73.1	8.41	101.53	12
High	XP_044389258.1	heat shock protein 81-3 [Triticum aestivum]	0	36.128	24.42857	13	25	9	1	700	80.4	5.03	85.45	13
High	XP_044371936.1	coatomer subunit alpha-3-like [Triticum aestivum]	0	38.219	15.35304	14	25	8	1	1218	135.7	7.01	64.08	14
High	XP_044418568.1	embryonic protein DC-8-like [Triticum aestivum]	0	51.204	28.60999	13	25	4	1	741	74.2	7.34	97.12	13

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044383314.1	dnaJ protein homolog [Triticum aestivum]	0	31.778	36.10451	9	24	7	1	421	46.8	5.92	84.58	9
High	XP_044341880.1	DNA-directed RNA polymerase subunit 10-like protein isoform X4 [Triticum aestivum]	0	42.424	77.46479	6	24	1	1	71	8.2	5.83	81.61	6
High	XP_044455423.1	probable nucleolar protein 5-2 [Triticum aestivum]	0	43.997	35.71429	11	23	11	1	560	61.7	8.72	75.13	11
High	XP_044330175.1	elongation factor 1-alpha-like [Triticum aestivum]	0	22.817	15.88367	5	23	1	1	447	49.1	9.13	73.68	5
High	XP_044424493.1	60S ribosomal protein L9 [Triticum aestivum]	0	37.34	53.43915	8	23	8	1	189	21.3	9.8	84.32	8
High	XP_044338201.1	protein PAF1 homolog [Triticum aestivum]	0	33.247	23.83901	12	23	3	1	646	73.6	7.71	73.7	12
High	XP_044345306.1	embryonic protein DC-8-like [Triticum aestivum]	0	41.765	17.75312	10	23	2	1	721	72.9	8.48	88.58	10
High	XP_044410738.1	DNA-directed RNA polymerase II subunit 4-like [Triticum aestivum]	0	30.384	66.90647	7	23	7	1	139	15.8	4.92	76.48	7
High	XP_044364944.1	coatomer subunit alpha-3-like [Triticum aestivum]	0	37.24	14.13311	12	23	6	1	1217	136.3	7.12	64.88	12
High	XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]	0	30.315	11.30536	7	22	7	1	858	96.7	5	65.86	7
High	XP_044348996.1	protein MLN51 homolog [Triticum aestivum]	0	32.969	18.4438	8	22	1	1	694	75.4	5.3	68.3	8
High	XP_044389229.1	protein LTV1 homolog [Triticum aestivum]	0	28.745	17.14876	7	22	1	1	484	54	4.73	72.38	7
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	36.339	40.84507	7	22	7	1	284	30.9	9.58	73.47	7
High	XP_044431117.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	30.626	29.57111	9	22	3	1	443	46.9	4.79	62.64	9
High	XP_044455885.1	DNA-directed RNA polymerases II, IV and V subunit 6A-like [Triticum aestivum]	0	16.024	35.21127	4	21	4	1	142	16.2	4.27	71.28	4
High	XP_044407805.1	DNA-directed RNA polymerase II subunit RPB7 [Triticum aestivum]	0	28.863	48.0226	5	21	5	1	177	19.5	6.52	60.33	5
High	XP_044369898.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	28.916	30.54755	7	21	2	1	347	38.6	6.77	72.75	7
High	XP_044369337.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	30.588	52.17391	7	21	7	1	322	33.6	6.15	71.86	7
High	XP_044330857.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	24.991	35.77982	6	20	3	1	218	24	6.79	55.55	6
High	XP_044443808.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	21.293	21.77914	4	20	2	1	326	36.1	4.82	48.05	4
High	XP_044365948.1	glucose and ribitol dehydrogenase homolog [Triticum aestivum]	0	25.986	32.47126	8	20	8	1	348	37.7	9	64.5	8
High	XP_044367851.1	protein CDC73 homolog isoform X1 [Triticum aestivum]	0	25.99	32.01058	8	19	4	1	378	41.9	6.7	52.43	8
High	XP_044390882.1	cupincin-like [Triticum aestivum]	0	17.681	15.0495	7	19	7	1	505	56.5	7.43	53.52	7
High	XP_044375308.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	24.954	30.54755	7	19	2	1	347	38.7	6.77	65.27	7
High	XP_044397238.1	ATP synthase subunit alpha, mitochondrial [Triticum aestivum]	0	21.822	20.82515	8	18	8	1	509	55.3	5.9	52.77	8
High	XP_044419436.1	nucleolar protein 56-like [Triticum aestivum]	0	31.352	23.49727	7	18	7	1	549	61	8.72	64.86	7
High	XP_044365259.1	protein CDC73 homolog [Triticum aestivum]	0	21.291	23.40426	8	17	4	1	376	41.9	6.71	42.68	8
High	XP_044350539.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	24.913	44.7619	8	17	7	1	210	24.8	9.47	56.08	8
High	XP_044355245.1	uncharacterized protein LOC123077097 [Triticum aestivum]	0	18.04	19.07131	7	17	7	1	603	66.8	5.19	49.23	7
High	XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	19.121	52.02703	5	17	1	1	148	16.8	5.87	50.16	5
High	XP_044362453.1	40S ribosomal protein S3a [Triticum aestivum]	0	23.402	37.26236	6	16	6	1	263	29.9	9.79	58.85	6
High	XP_044319569.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	15.886	22.47706	4	15	1	1	218	24	6.54	40.73	4
High	XP_044443577.1	squamous cell carcinoma antigen recognized by T-cells 3-like [Triticum aestivum]	0	20.722	13.23706	7	15	1	1	831	94.1	6.14	45.22	7
High	XP_044362686.1	tubulin alpha-2 chain [Triticum aestivum]	0	21.45	30.95768	8	15	4	1	449	49.8	5.2	45.96	8
High	XP_044425255.1	DNA damage-binding protein 1-like [Triticum aestivum]	0	23.311	12.01835	8	15	8	1	1090	121.8	5.34	40.26	8

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	19.076	52.02703	5	15	1	1	148	16.9	5.87	47.03	5
High	XP_044351723.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]	0	22.414	8.100358	7	15	7	1	1395	155	8.82	44.07	7
High	XP_044430325.1	heterogeneous nuclear ribonucleoprotein A/B-like [Triticum aestivum]	0	28.31	25.55831	6	15	1	1	403	41.4	5.08	56.46	6
High	XP_044408236.1	DNA-directed RNA polymerase III subunit 2-like [Triticum aestivum]	0	30.727	13.56958	10	14	10	1	1157	129.7	8.47	41.82	10
High	XP_044417542.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	25.138	20.6422	7	14	1	1	436	45.7	4.96	48.75	7
High	XP_044343932.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	33.384	66.22517	8	14	1	1	151	16.9	6.09	56.65	8
High	XP_044355041.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	29.603	66.22517	8	14	1	1	151	16.9	6.09	52.15	8
High	XP_044366056.1	coatomer subunit beta'-1-like isoform X1 [Triticum aestivum]	0	23.712	15.63877	8	14	6	1	908	102.4	4.98	48.16	8
High	XP_044383422.1	eukaryotic translation initiation factor 3 subunit B-like [Triticum aestivum]	0	12.248	10.54092	5	14	5	1	721	83.2	5.14	38.77	5
High	XP_044407136.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	22.862	20.45977	7	14	1	1	435	45.7	4.98	42.51	7
High	XP_044419370.1	coatomer subunit beta'-2-like isoform X1 [Triticum aestivum]	0	22.512	12.01264	8	14	6	1	949	107.2	4.94	42.4	8
High	XP_044423311.1	heterogeneous nuclear ribonucleoprotein 1-like isoform X1 [Triticum aestivum]	0	27.272	25.68579	6	14	1	1	401	41.3	5.05	51.86	6
High	XP_044422783.1	squamous cell carcinoma antigen recognized by T-cells 3-like [Triticum aestivum]	0	20.925	12.35012	7	14	1	1	834	94.3	6.14	45.12	7
High	XP_044324396.1	40S ribosomal protein S14 [Triticum aestivum]	0	21.52	33.11258	5	14	5	1	151	16.3	10.56	43.58	5
High	XP_044332823.1	DNA-directed RNA polymerases I and III subunit rpac1-like [Triticum aestivum]	0	15.507	19.43662	5	13	5	1	355	39.5	5.16	33.03	5
High	XP_044421055.1	splicing factor, arginine/serine-rich 19-like [Triticum aestivum]	0	21.682	28.34225	6	13	3	1	374	40.9	4.7	42.1	6
High	XP_044387377.1	protein RTF1 homolog [Triticum aestivum]	0	18.662	16.66667	6	13	6	1	660	73.4	8.72	43.58	6
High	XP_044323959.1	casein kinase II subunit alpha-2-like isoform X2 [Triticum aestivum]	0	20.602	22.06704	7	13	5	1	358	42.3	8.22	34.8	7
High	XP_044325394.1	coatomer subunit gamma-2-like [Triticum aestivum]	0	23.105	19.46007	10	13	10	1	889	98.6	5.14	39.92	10
High	XP_044432754.1	16.9 kDa class I heat shock protein 2-like [Triticum aestivum]	0	22.003	42.40506	5	13	1	1	158	18	5.71	44.4	5
High	XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]	0	17.846	6.372549	7	13	7	1	1632	182.8	5.14	32.37	7
High	XP_044371305.1	40S ribosomal protein Sa-2-like [Triticum aestivum]	0	20.623	18.83117	3	12	3	1	308	33.2	5.02	39.04	3
High	XP_044439510.1	endoplasmin homolog [Triticum aestivum]	0	16.75	7.77512	5	12	5	1	836	96	5.21	36.93	5
High	XP_044437687.1	glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic [Triticum aestivum]	0	23.369	39.76261	7	11	5	1	337	36.5	7.18	38.76	7
High	XP_044323484.1	splicing factor 3B subunit 4-like [Triticum aestivum]	0	21.278	18.47826	4	11	4	1	368	40.3	8.15	36.88	4
High	XP_044377892.1	late embryogenesis abundant protein 1-like [Triticum aestivum]	0	14.536	24.14773	5	11	3	1	352	37.4	6.9	34.77	5
High	XP_044403314.1	glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic-like isoform X1 [Triticum aestivum]	0	21.507	27.37752	5	11	3	1	347	38.2	7.66	35.57	5
High	XP_044433310.1	17.4 kDa class I heat shock protein-like [Triticum aestivum]	0	14.676	43.67089	5	11	1	1	158	17.9	5.49	35.67	5
High	XP_044383398.1	60S ribosomal protein L4-1-like [Triticum aestivum]	0	13.914	23.51485	6	10	6	1	404	44.2	10.6	30.28	6
High	XP_044407747.1	probable helicase MAGATAMA 3 [Triticum aestivum]	0	12.133	13.4058	7	10	7	1	828	91.2	6.11	24.21	7
High	XP_044421771.1	rRNA 2'-O-methyltransferase fibrillarin 1-like [Triticum aestivum]	0	17.062	28.15534	4	10	4	1	309	32.4	10.0	34.12	4
High	XP_044407359.1	luminal-binding protein 2 isoform X2 [Triticum aestivum]	0	18.156	9.774436	5	10	3	1	665	73.2	5.21	32.2	5
High	XP_044362751.1	NHP2-like protein 1 [Triticum aestivum]	0	18.145	48.4375	3	10	3	1	128	13.9	7.12	41.27	3
High	XP_044387740.1	actin-3-like [Triticum aestivum]	0	13.853	19.8939	4	10	4	1	377	41.6	5.49	30.47	4
High	XP_044442770.1	avenin-like b6 [Triticum aestivum]	0	13.084	23.21429	4	10	4	1	280	31.9	7.91	30.67	4

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044375751.1	17.9 kDa class I heat shock protein [Triticum aestivum]	0	13.008	46.49682	6	10	2	1	157	17.5	6.06	34.91	6
High	XP_044414306.1	STOREKEEPER protein-like [Triticum aestivum]	0	17.842	20.81081	5	10	2	1	370	40.2	4.74	27.94	5
High	XP_044352787.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	18.665	53.28947	6	9	2	1	152	16.9	6.81	29.09	6
High	XP_044383044.1	casein kinase II subunit alpha-2-like [Triticum aestivum]	0	8.447	11.02362	3	9	1	1	381	43.9	8.88	16.72	3
High	XP_044345724.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	16.546	16.41791	3	9	3	1	335	36.3	6.52	35.28	3
High	XP_044364863.1	lariat debranching enzyme-like [Triticum aestivum]	0	12.45	18.64754	5	9	5	1	488	55.1	6.13	29.03	5
High	XP_044346113.1	uncharacterized protein LOC123067343 [Triticum aestivum]	0	10.575	4.8	2	9	1	1	500	54.6	9.55	29.76	2
High	XP_044451591.1	ATP synthase subunit beta, mitochondrial-like [Triticum aestivum]	0	18.86	18.73874	6	9	6	1	555	59.3	6.2	31.87	6
High	XP_044366277.1	40S ribosomal protein S20 [Triticum aestivum]	0	10.579	44.09449	5	9	5	1	127	14.1	9.44	18.84	5
High	XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]	0	11.891	6.368821	5	8	5	1	1052	115.8	5.38	22.33	5
High	XP_044323868.1	small nuclear ribonucleoprotein-associated protein B'-like [Triticum aestivum]	0	14.919	15.4717	4	8	4	1	265	27.9	11.3	28.56	4
High	XP_044365658.1	uncharacterized protein LOC123087672 [Triticum aestivum]	0	9.711	14.06728	4	8	1	1	327	36.2	5.15	19.51	4
High	XP_044343298.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	10.318	26.92308	4	8	3	1	182	20.1	9.73	22.92	4
High	XP_044363825.1	eukaryotic translation initiation factor 3 subunit I [Triticum aestivum]	0	9.073	12.88344	3	8	3	1	326	36.1	6.96	21.93	3
High	XP_044432232.1	heat shock protein 81-1 [Triticum aestivum]	0	11.875	9.428571	5	8	1	1	700	80.4	5.06	24.06	5
High	XP_044376616.1	uncharacterized protein LOC123098635 [Triticum aestivum]	0	6.926	14.48763	3	8	3	1	283	32.1	9.77	21.55	3
High	XP_044360348.1	late embryogenesis abundant protein 1-like [Triticum aestivum]	0	16.352	16.80912	4	8	2	1	351	37.3	6.95	23.04	4
High	XP_044382638.1	ribosome biogenesis protein BOP1 homolog isoform X1 [Triticum aestivum]	0	8.437	5.980529	5	8	5	1	719	81.5	5.73	16.66	5
High	XP_044339126.1	nuclear ubiquitous casein and cyclin-dependent kinase substrate 1-like [Triticum aestivum]	0	7.251	15.67398	3	7	1	1	319	34.2	5	20.15	3
High	XP_044407809.1	calnexin homolog [Triticum aestivum]	0	8.516	8.488964	3	7	3	1	589	65.6	4.93	25.65	3
High	XP_044407705.1	small nuclear ribonucleoprotein E-like [Triticum aestivum]	0	10.17	65.90909	4	7	4	1	88	10.3	9.89	21.88	4
High	XP_044451243.1	chaperone protein ClpB1 [Triticum aestivum]	0	16.259	10.23965	6	7	6	1	918	101	6.24	21.75	6
High	XP_044418692.1	eukaryotic initiation factor 4A-like [Triticum aestivum]	0	15.999	13.28502	4	7	4	1	414	46.9	5.58	25.29	4
High	XP_044394533.1	transcription initiation factor IIF subunit alpha-like isoform X1 [Triticum aestivum]	0	10.186	6.430868	2	7	2	1	622	68.7	5.95	25.86	2
High	XP_044343360.1	general transcription factor IIE subunit 1-like [Triticum aestivum]	0	11.773	8.154506	3	7	3	1	466	52.9	5.16	16.9	3
High	XP_044445530.1	40S ribosomal protein S25-2 [Triticum aestivum]	0	14.366	33.33333	4	7	4	1	108	12.1	10.5	20.1	4
High	XP_044391616.1	uncharacterized protein LOC123114265 [Triticum aestivum]	0	8.997	14.28571	4	7	1	1	322	35.8	5.19	17.15	4
High	XP_044348900.1	developmentally-regulated G-protein 2 [Triticum aestivum]	0	18.33	22.30576	5	7	5	1	399	44.5	8.56	22.26	5
High	XP_044457854.1	small nuclear ribonucleoprotein SmD1a-like [Triticum aestivum]	0	15.42	45.61404	3	7	3	1	114	12.7	11.2	26.19	3
High	XP_044383254.1	histone H2A.2.1-like [Triticum aestivum]	0	17.773	39.35484	3	7	2	1	155	16.5	10.5	27.98	3
High	XP_044412707.1	60S ribosomal protein L6-like [Triticum aestivum]	0	6.914	13.69863	3	7	1	1	219	24.5	10.1	15.8	3
High	XP_044372055.1	small nuclear ribonucleoprotein SmD3b-like [Triticum aestivum]	0	10.527	27.40741	4	7	4	1	135	14.5	11.0	22.45	4
High	XP_044410540.1	pre-mRNA-processing protein 40A-like isoform X2 [Triticum aestivum]	0	12.259	6.480558	4	7	4	1	1003	114.2	7.24	20.74	4
High	XP_044423143.1	60S ribosomal protein L22-2-like [Triticum aestivum]	0	5.701	25.19084	3	6	3	1	131	14.5	9.55	16.25	3
High	XP_044347284.1	phytepsin isoform X1 [Triticum aestivum]	0	10.87	14.73477	4	6	4	1	509	54.5	5.52	18.19	4

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044403120.1	60S ribosomal protein L6-like [Triticum aestivum]	0	4.756	15.06849	3	6	1	1	219	24.6	10.1	9.41	3
High	XP_044344314.1	17.5 kDa class II heat shock protein-like [Triticum aestivum]	0	9.227	37.03704	4	6	3	1	162	17.6	5.47	21.82	4
High	XP_044419470.1	heterogeneous nuclear ribonucleoprotein 1-like isoform X1 [Triticum aestivum]	0	12	19.3154	4	6	1	1	409	40.9	6.29	17.32	4
High	XP_044362961.1	17.9 kDa class I heat shock protein-like [Triticum aestivum]	0	6.781	29.74684	4	6	2	1	158	17.5	5.69	18.77	4
High	XP_044449381.1	low-temperature-induced 65 kDa protein-like isoform X1 [Triticum aestivum]	0	6.899	8	2	6	1	1	600	61.3	4.98	21.16	2
High	XP_044397682.1	40S ribosomal protein S17-4-like [Triticum aestivum]	0	12.465	39.16084	4	6	4	1	143	16.5	10.2	18.93	4
High	XP_044378512.1	DNA-directed RNA polymerases II, IV and V subunit 9A-like [Triticum aestivum]	0	7.554	19.29825	2	6	1	1	114	13	6.84	15.08	2
High	XP_044340278.1	RWD domain-containing protein 1-like isoform X1 [Triticum aestivum]	0	6.967	20.63492	4	6	4	1	252	28.5	4.21	16.1	4
High	XP_044351580.1	microfibrillar-associated protein 1-like [Triticum aestivum]	0	11.371	7.256236	2	6	1	1	441	52.1	5.15	26.34	2
High	XP_044425015.1	40S ribosomal protein S27 isoform X1 [Triticum aestivum]	0	11.584	18.51852	2	6	2	1	162	18.2	8.4	22.38	2
High	XP_044355746.1	nuclear ubiquitous casein and cyclin-dependent kinase substrate 1-like [Triticum aestivum]	0	6.994	15.67398	3	6	1	1	319	34.2	5	17.9	3
High	XP_044415288.1	translation initiation factor eIF-2B subunit epsilon-like [Triticum aestivum]	0	16.65	11.12619	5	6	5	1	737	82.2	4.74	20.71	5
High	XP_044339239.1	uncharacterized protein At2g34160-like [Triticum aestivum]	0	4.738	25.16129	2	6	1	1	155	16.2	5.01	13.94	2
High	XP_044413926.1	26S rRNA (cytosine-C(5))-methyltransferase NOP2B-like [Triticum aestivum]	0	9.351	10.34031	4	6	4	1	764	84.5	5.88	18.58	4
High	XP_044338627.1	protein IWS1 homolog 1-like [Triticum aestivum]	0	9.76	6.403013	4	6	4	1	531	59.1	5.34	12.99	4
High	XP_044376983.1	60S ribosomal protein L23-like [Triticum aestivum]	0	9.635	32.85714	3	6	3	1	140	15	10.4	15.2	3
High	XP_044425964.1	histone deacetylase HDT2 [Triticum aestivum]	0	10.509	20.67039	4	6	4	1	358	38.5	5	15.03	4
High	XP_044365009.1	eukaryotic peptide chain release factor subunit 1-3-like [Triticum aestivum]	0	7.944	8.695652	3	5	3	1	437	49	5.6	12.41	3
High	XP_044327283.1	ice nucleation protein-like [Triticum aestivum]	0	6.573	7.973422	2	5	1	1	602	61.5	5.1	18.46	2
High	XP_044414706.1	dehydrin DHN4 [Triticum aestivum]	0	7.086	15.58442	2	5	2	1	231	23.2	9.23	15.67	2
High	XP_044412497.1	heterogeneous nuclear ribonucleoprotein A0-like [Triticum aestivum]	0	12.96	18.84058	4	5	1	1	414	41.3	6.29	14.07	4
High	XP_044379771.1	importin subunit beta-1-like [Triticum aestivum]	0	5.823	4.931193	3	5	3	1	872	95.8	4.74	12.61	3
High	XP_044359714.1	uncharacterized protein LOC123080794 [Triticum aestivum]	0	7.31	24.85207	2	5	2	1	169	17.2	6.33	14.83	2
High	XP_044380741.1	pre-mRNA-splicing factor 38-like [Triticum aestivum]	0	10.645	9.390863	3	5	3	1	394	47	9.04	14.86	3
High	XP_044333162.1	nascent polypeptide-associated complex subunit alpha-like protein 1 [Triticum aestivum]	0	5.648	14.14634	2	5	2	1	205	22.2	4.42	11.21	2
High	XP_044388315.1	40S ribosomal protein S19 [Triticum aestivum]	0	6.366	20	4	5	4	1	155	17.1	9.89	11.44	4
High	XP_044325436.1	nucleolin-like [Triticum aestivum]	0	5.822	7.455013	4	5	4	1	778	87.6	5.69	8.89	4
High	XP_044435946.1	V-type proton ATPase catalytic subunit A [Triticum aestivum]	0	5.882	5.162242	3	5	3	1	678	74.3	5.4	10.84	3
High	XP_044387719.1	probable mediator of RNA polymerase II transcription subunit 26c [Triticum aestivum]	0	13.171	20.93023	3	5	3	1	344	38.1	5.9	18.58	3
High	XP_044323344.1	histone H2A.4 [Triticum aestivum]	0	5.619	28.14815	2	5	1	1	135	14.1	10.0	15.19	2
High	XP_044363227.1	uncharacterized protein LOC123085629 [Triticum aestivum]	0	4.882	6.430868	3	5	3	1	622	67.2	7.11	10.7	3
High	XP_044349510.1	WD-40 repeat-containing protein MSI4-like isoform X2 [Triticum aestivum]	0	6.131	14.06593	3	5	3	1	455	50.3	6.37	12.14	3
High	XP_044406245.1	SNF1-related protein kinase regulatory subunit gamma-like PV42a [Triticum aestivum]	0	6.176	14.15525	3	5	3	1	438	46.5	5.33	12.31	3
High	XP_044375395.1	GPN-loop GTPase QQT2-like isoform X1 [Triticum aestivum]	0	13.472	7.551487	2	5	2	1	437	48.5	4.74	17.61	2

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044404915.1	proliferating cell nuclear antigen-like isoform X1 [Triticum aestivum]	0	5.775	13.33333	2	5	2	1	270	29.7	4.69	14.81	2
High	XP_044391737.1	U2 small nuclear ribonucleoprotein A'-like [Triticum aestivum]	0	7.652	12.98246	3	5	1	1	285	31.9	5.15	13.31	3
High	XP_044404494.1	nucleolar and coiled-body phosphoprotein 1-like [Triticum aestivum]	0	13.128	13.97849	3	5	1	1	372	40.8	4.7	14.74	3
High	XP_044373534.1	dehydrin DHN4-like [Triticum aestivum]	0	10.467	9.767442	2	5	2	1	430	41.2	9.06	20.05	2
High	XP_044324199.1	eukaryotic translation initiation factor 3 subunit H-like isoform X1 [Triticum aestivum]	0	7.803	9.912536	3	4	3	1	343	38.8	4.92	10.58	3
High	XP_044365487.1	protein BCCIP homolog isoform X1 [Triticum aestivum]	0	3.436	5.30303	2	4	2	1	396	44.4	4.89	9.85	2
High	XP_044425441.1	catalase isozyme 1 isoform X1 [Triticum aestivum]	0	4.286	4.878049	2	4	2	1	492	56.4	7.25	9.91	2
High	XP_044339240.1	phospholipase D alpha 1 [Triticum aestivum]	0	11.404	8.251232	4	4	4	1	812	92	5.48	10.87	4
High	XP_044412687.1	NAP1-related protein 2-like [Triticum aestivum]	0	10.118	27.09163	3	4	3	1	251	28.8	4.37	15.54	3
High	XP_044341401.1	aspartate aminotransferase, cytoplasmic-like [Triticum aestivum]	0	4.864	6.373626	2	4	2	1	455	49.6	8.47	9.97	2
High	XP_044326777.1	zinc finger CCCH domain-containing protein 32-like [Triticum aestivum]	0	4.188	4.992867	2	4	2	1	701	78.3	5.81	12.04	2
High	XP_044406649.1	uncharacterized protein LOC123130924 [Triticum aestivum]	0	8.405	21.47239	2	4	2	1	163	17.4	9.31	13.18	2
High	XP_044380766.1	polyadenylate-binding protein 8 [Triticum aestivum]	0	6.16	3.072197	2	4	2	1	651	70.7	7.37	10.29	2
High	XP_044375745.1	17.9 kDa class I heat shock protein-like [Triticum aestivum]	0	4.853	33.12102	3	4	1	1	157	17.5	6.06	13.39	3
High	XP_044451775.1	ubiquitin-40S ribosomal protein S27a-like [Triticum aestivum]	0	5.117	15.2027	3	4	3	1	296	33.6	9.96	8.75	3
High	XP_044407948.1	26S proteasome non-ATPase regulatory subunit 2 homolog A-like [Triticum aestivum]	0	5.38	4.581006	3	4	3	1	895	97.7	5.21	9.83	3
High	XP_044377405.1	histone H2B.11-like [Triticum aestivum]	0	5.506	11.68385	2	4	1	1	291	31.3	9.79	8.32	2
High	XP_044340029.1	coatomer subunit beta-2-like [Triticum aestivum]	0	3.764	2.631579	2	4	2	1	950	105.3	5.72	9.17	2
High	XP_044365544.1	U2 small nuclear ribonucleoprotein A'-like [Triticum aestivum]	0	7.229	12.98246	3	4	1	1	285	31.9	5.15	11.12	3
High	XP_044433605.1	heat shock 70 kDa protein BIP5-like [Triticum aestivum]	0	5.874	5.255255	3	4	1	1	666	73.1	5.29	9.85	3
High	XP_044342538.1	PHD finger protein ALFIN-LIKE 6-like [Triticum aestivum]	0	7.102	11.78707	2	4	2	1	263	29.5	5.49	15.22	2
High	XP_044451126.1	general transcription factor IIF subunit 2-like [Triticum aestivum]	0	6.103	12.1673	2	4	2	1	263	29.6	8.84	14.16	2
High	XP_044381680.1	histone H2A-like [Triticum aestivum]	0	6.49	23.89937	2	4	1	1	159	16.6	10.6	14.14	2
High	XP_044453329.1	23.2 kDa heat shock protein-like [Triticum aestivum]	0	8.211	19.17808	3	4	3	1	219	23.9	5.74	12.71	3
High	XP_044334411.1	coatomer subunit delta-1-like [Triticum aestivum]	0	8.35	13.02682	4	4	4	1	522	57.4	6.01	9.82	4
High	XP_044418242.1	peptidyl-prolyl cis-trans isomerase [Triticum aestivum]	0	6.947	20.46784	3	4	3	1	171	18.4	8.25	9.55	3
High	XP_044371551.1	alpha-amylase/trypsin inhibitor CM3 [Triticum aestivum]	0	7.944	38.69048	4	4	4	1	168	18.2	7.44	9.63	4
High	XP_044455762.1	developmentally-regulated G-protein 3 [Triticum aestivum]	0	8.678	7.608696	2	4	2	1	368	41.1	8.28	14.74	2
High	XP_044398240.1	DNA-directed RNA polymerase I subunit 2-like [Triticum aestivum]	0	6.228	2.104377	2	3	2	1	1188	133	8.53	8.09	2
High	XP_044371307.1	eukaryotic translation initiation factor 3 subunit K-like [Triticum aestivum]	0	3.718	8.849558	2	3	2	1	226	25.9	5.16	7.85	2
High	XP_044404772.1	protein HEADING DATE REPRESSOR 1-like [Triticum aestivum]	0	6.05	19.6347	3	3	3	1	219	24.7	4.69	8.31	3
High	XP_044365145.1	polyadenylation factor subunit 2-like [Triticum aestivum]	0	7.298	5.579399	2	3	2	1	466	48.7	5.26	9.77	2
High	XP_044407707.1	eukaryotic translation initiation factor 3 subunit F-like [Triticum aestivum]	0	4.752	9.931507	2	3	2	1	292	31.8	5.25	7.1	2
High	XP_044411052.1	glycine-rich protein 2-like [Triticum aestivum]	0	8.563	16.73152	2	3	1	1	257	24.1	6.38	9.9	2
High	XP_044418248.1	glycine-rich protein 2-like [Triticum aestivum]	0	8.917	16.07843	2	3	1	1	255	24.1	6.51	10.27	2

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044323100.1	ruvB-like 2 [Triticum aestivum]	0	5.112	4.852321	2	3	2	1	474	51.6	5.63	9.26	2
High	XP_044431145.1	60S ribosomal protein L17-2 isoform X1 [Triticum aestivum]	0.001	2.203	10.27027	2	3	1	1	185	21.3	10.2	4.74	2
High	XP_044344629.1	late embryogenesis abundant protein, group 3 isoform X1 [Triticum aestivum]	0	7.946	9.821429	2	3	2	1	224	23.2	8.84	10.2	2
High	XP_044363492.1	40S ribosomal protein S21 [Triticum aestivum]	0	7.323	39.02439	2	3	2	1	82	9.2	7.34	9.47	2
High	XP_044373379.1	14-3-3-like protein A [Triticum aestivum]	0	4.556	14.12214	2	3	2	1	262	29.4	4.88	7.42	2
High	XP_044381431.1	eukaryotic translation initiation factor 3 subunit C-like [Triticum aestivum]	0	5.208	2.693966	2	3	2	1	928	104.8	5.68	8.02	2
High	XP_044423659.1	something about silencing protein 10-like [Triticum aestivum]	0	4.361	5.648855	2	3	2	1	655	72.8	5.22	8.33	2
High	XP_044423454.1	uncharacterized protein At2g34160-like [Triticum aestivum]	0	6.14	29.54545	2	3	1	1	132	14.6	5.19	9.71	2
High	XP_044348087.1	late embryogenesis abundant protein Lea14-A-like [Triticum aestivum]	0	8.358	26.49007	2	3	2	1	151	16.3	5.02	13.64	2
High	XP_044339931.1	cysteine proteinase inhibitor 12 [Triticum aestivum]	0	6.241	12.7572	2	3	2	1	243	26.6	6.87	11.32	2
High	XP_044382764.1	dnaJ protein homolog 2-like [Triticum aestivum]	0	5.549	9.026128	3	3	1	1	421	46.3	6.55	9.46	3
High	XP_044333576.1	60S ribosomal protein L14-1 [Triticum aestivum]	0	4.648	16.2963	2	3	2	1	135	15.5	10.1	8.39	2
High	XP_044346863.1	uncharacterized protein DDB_G0286299-like [Triticum aestivum]	0	2.328	3.071017	2	3	2	1	521	56.3	5.49	2.16	2
High	XP_044341100.1	DNA-directed RNA polymerase III subunit RPC3-like [Triticum aestivum]	0	4.336	3.984064	2	3	2	1	502	57	7.66	7.56	2
High	XP_044324467.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	5.891	15.87302	2	3	1	1	189	20.6	9.88	10.64	2
High	XP_044391898.1	ruBisCO large subunit-binding protein subunit alpha-like [Triticum aestivum]	0	6.579	7.823129	3	3	3	1	588	61.7	5.39	8.24	3
High	XP_044407019.1	NAD-capped RNA hydrolase DXO1-like isoform X1 [Triticum aestivum]	0	3.123	4.271845	2	3	2	1	515	56.7	5.21	6.53	2
High	XP_044363220.1	U2 small nuclear ribonucleoprotein B"-like [Triticum aestivum]	0	2.664	4.72103	2	3	2	1	233	26.2	9.38	6.88	2
High	XP_044394558.1	probable histone H2A variant 2 [Triticum aestivum]	0	5.196	27.33813	2	3	1	1	139	14.6	10.3	10	2
High	XP_044335377.1	uncharacterized protein LOC123055427 [Triticum aestivum]	0	4.241	16.8	2	3	2	1	375	40.7	4.97	8.68	2
High	XP_044342852.1	microfibrillar-associated protein 1-like [Triticum aestivum]	0	7.448	6.802721	2	3	1	1	441	52.1	5.15	10.71	2
High	XP_044443955.1	nucleolin 1-like isoform X1 [Triticum aestivum]	0	5.871	4.72028	2	3	2	1	572	59.4	5.5	9.81	2
High	XP_044426988.1	late embryogenesis abundant protein 31-like [Triticum aestivum]	0	7.004	15.16245	2	3	2	1	277	28.3	4.35	11.12	2
High	XP_044413205.1	H/ACA ribonucleoprotein complex subunit 3-like protein [Triticum aestivum]	0	3.439	35.9375	2	3	2	1	64	7.5	9.94	7.07	2
High	XP_044415900.1	uncharacterized protein LOC123140679 [Triticum aestivum]	0	3.406	15.75758	2	2	2	1	165	19	5.6	5.36	2
High	XP_044404949.1	SAP-like protein BP-73 [Triticum aestivum]	0	5.573	9.043928	2	2	2	1	387	42.1	5.5	6.94	2
High	XP_044450447.1	glycine-rich protein 2 [Triticum aestivum]	0.002	2.015	20.34632	2	2	2	1	231	21.5	6.1	2.08	2
High	XP_044325775.1	coatomer subunit epsilon-1-like [Triticum aestivum]	0	4.836	12.89199	2	2	2	1	287	31.6	5.45	6.08	2
High	XP_044322718.1	50S ribosomal protein L7/L12-like [Triticum aestivum]	0	4.139	21.60494	2	2	2	1	162	16.7	8.31	5.48	2
High	XP_044330891.1	H/ACA ribonucleoprotein complex subunit 4-like [Triticum aestivum]	0	3.313	7.072368	2	2	2	1	608	66.7	9.2	5.11	2
High	XP_044325823.1	26S proteasome non-ATPase regulatory subunit 1 homolog A-like isoform X1 [Triticum aestivum]	0	6.515	2.999063	2	2	2	1	1067	115.4	5.34	6.21	2
High	XP_044448020.1	WEB family protein At5g16730, chloroplastic-like [Triticum aestivum]	0	5.194	2.261554	2	2	2	1	1017	112.7	4.88	4.74	2
High	XP_044324623.1	trihelix transcription factor ASIL2-like [Triticum aestivum]	0	3.217	5.528846	2	2	2	1	416	44.9	6.61	4.15	2
High	XP_044396807.1	60S ribosomal protein L17-1-like [Triticum aestivum]	0	2.498	8	2	2	1	1	200	23	10.5	2.43	2
High	XP_044323640.1	chaperonin CPN60-1, mitochondrial-like [Triticum aestivum]	0	3.24	3.135889	2	2	2	1	574	61.1	5.55	4.54	2

Table A.3 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044344319.1	17.5 kDa class II heat shock protein-like [Triticum aestivum]	0	2.378	17.90123	2	2	1	1	162	17.7	5.73	5.57	2
High	XP_044374025.1	DNA-directed RNA polymerases I and III subunit RPAC2-like [Triticum aestivum]	0	3.327	19.47368	2	2	2	1	190	20.6	7.11	3.77	2
High	XP_044324182.1	nuclear transport factor 2-like isoform X1 [Triticum aestivum]	0	2.932	5.172414	2	2	2	1	522	55.7	5.34	4.52	2

Table A.4 nLC-MS/MS for partially purified Pol II (replicate 2)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	365.579	51.4485	83	338	3	1	1864	207.8	6.32	935.17	83
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	362.31	50.74946	83	335	3	1	1868	208	6.2	924.15	83
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	244.618	57.41196	56	218	56	1	1221	138.2	7.15	572.52	56
High	XP_044362369.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	120.636	55.74572	37	88	2	1	818	90.8	5.26	256.42	37
High	XP_044362370.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	118.496	56.45756	36	87	1	1	813	90.1	5.29	254.31	36
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	102.834	34.68208	17	97	7	1	692	77	5.52	320.62	17
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	99.276	42.63261	21	91	10	1	509	55.4	6.62	241.05	21
High	XP_044370961.1	vicilin-like seed storage protein At4g36700 [Triticum aestivum]	0	95.024	33.241	17	91	8	1	722	81.1	5.85	288.76	17
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	91.935	53.36323	21	73	6	1	446	50.2	4.81	205.42	21
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	86.354	60.72508	16	78	16	1	331	36.7	4.88	227.26	16
High	XP_044454990.1	FACT complex subunit SPT16 [Triticum aestivum]	0	84.748	38.70968	28	64	3	1	1085	121	5.57	157.42	28
High	XP_044429961.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	79.318	28.51064	23	49	4	1	1410	158.7	5.64	137.62	23
High	XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]	0	79.28	35.27653	21	52	21	1	669	74.4	5.8	161.13	21
High	XP_044377882.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	78.379	50.88757	21	83	10	1	507	55.3	6.96	209.85	21
High	XP_044330076.1	FACT complex subunit SPT16-like [Triticum aestivum]	0	77.803	36.4977	26	61	1	1	1085	121	5.59	145.73	26
High	XP_044423298.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	75.904	25.37208	21	47	2	1	1411	158.7	5.58	130.42	21
High	XP_044390710.1	tubulin beta-4 chain [Triticum aestivum]	0	74.713	48.98876	19	55	3	1	445	49.9	4.87	143.99	19
High	XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	0	74.59	31.46067	23	50	23	1	1068	122	6	154.93	23
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	73.813	47.37864	18	89	9	1	515	56.1	6.11	230.12	18
High	XP_044339487.1	tubulin beta-5 chain [Triticum aestivum]	0	71.758	42.28188	16	52	3	1	447	50.3	4.83	134.45	16
High	XP_044343678.1	tubulin alpha chain [Triticum aestivum]	0	69.01	43.68071	12	62	5	1	451	49.7	5.02	195.76	12
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	67.355	61.875	17	69	17	1	320	34.5	5.5	194.06	17
High	XP_044423543.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	60.483	34.50704	20	44	20	1	994	110.2	5.4	118.2	20
High	XP_044356354.1	importin subunit alpha-1a isoform X2 [Triticum aestivum]	0	58.411	50	16	44	13	1	524	57.3	5.19	127.26	16
High	XP_044417769.1	DExH-box ATP-dependent RNA helicase DExH12-like [Triticum aestivum]	0	56.3	16.87299	20	37	20	1	2181	246.8	6.01	99.4	20
High	XP_044360175.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	54.511	24.7093	12	39	4	1	688	76.9	6.09	125.59	12
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	53.481	100	11	43	4	1	113	11.5	4.41	138.41	11

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044451529.1	embryonic protein DC-8-like [Triticum aestivum]	0	51.024	25.27473	11	34	4	1	728	73.1	8.41	104.79	11
High	XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	49.277	54.06699	11	43	11	1	209	24.1	9.44	123.84	11
High	XP_044363031.1	heat shock cognate 70 kDa protein-like [Triticum aestivum]	0	47.34	32.11568	16	40	3	1	657	71.8	5.29	108.22	16
High	XP_044337674.1	heat shock 70 kDa protein 4-like [Triticum aestivum]	0	47.057	29.93827	16	42	2	1	648	71	5.25	117.58	16
High	XP_044448581.1	importin subunit alpha-1b-like [Triticum aestivum]	0	46.165	36.89139	12	28	9	1	534	58.6	5.25	75.83	12
High	XP_044450611.1	60S acidic ribosomal protein P2B [Triticum aestivum]	0	44.793	95.53571	8	39	1	1	112	11.4	4.34	131.49	8
High	XP_044365264.1	63 kDa globulin-like protein [Triticum aestivum]	0	43.116	30.10204	13	36	4	1	588	66.3	8.27	84.01	13
High	XP_044324637.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	42.835	28.33676	11	24	8	1	487	53.4	5.83	74.22	11
High	XP_044383134.1	tubulin alpha-2 chain-like [Triticum aestivum]	0	40.959	25.27716	8	36	1	1	451	49.8	4.96	109.53	8
High	XP_044367849.1	63 kDa globulin-like protein [Triticum aestivum]	0	40.631	26.13636	12	40	1	1	616	69.4	7.06	94.53	12
High	XP_044324377.1	60S acidic ribosomal protein P2A [Triticum aestivum]	0	40.533	58.40708	7	45	7	1	113	11.6	4.41	126.92	7
High	XP_044332259.1	protein LEO1 homolog [Triticum aestivum]	0	40.059	20.99698	10	25	10	1	662	75.7	4.63	77.44	10
High	XP_044339880.1	RNA-binding protein P-like [Triticum aestivum]	0	38.277	26.45833	12	39	12	1	480	49.5	5.12	108.69	12
High	XP_044417498.1	probable nucleolar protein 5-2 [Triticum aestivum]	0	36.41	27.85714	9	18	9	1	560	61.8	8.81	55.63	9
High	XP_044367850.1	63 kDa globulin-like protein [Triticum aestivum]	0	35.997	23.07692	11	37	2	1	611	68.9	7.59	85.28	11
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	35.043	85	7	31	2	1	120	12.1	4.55	82.76	7
High	XP_044430572.1	60S acidic ribosomal protein P1-like [Triticum aestivum]	0	35.011	67.27273	7	45	7	1	110	11.2	4.59	139.48	7
High	XP_044383709.1	heat shock cognate 70 kDa protein 2 [Triticum aestivum]	0	34.979	21.2963	12	27	2	1	648	71.2	5.21	70.69	12
High	XP_044418568.1	embryonic protein DC-8-like [Triticum aestivum]	0	34.757	20.51282	9	20	2	1	741	74.2	7.34	60.41	9
High	XP_044438520.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	33.527	34.59821	8	29	2	1	448	47.4	4.75	84.18	8
High	XP_044457664.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	33.479	24.78814	10	25	7	1	472	51.7	6.51	66.84	10
High	XP_044419370.1	coatomer subunit beta'-2-like isoform X1 [Triticum aestivum]	0	33.314	16.43836	10	27	8	1	949	107.2	4.94	73.97	10
High	XP_044423644.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	32.828	38.37472	9	27	2	1	443	46.9	4.82	77.59	9
High	XP_044389229.1	protein LTV1 homolog [Triticum aestivum]	0	32.706	20.04132	8	23	2	1	484	54	4.73	72.95	8
High	XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	0	32.1	60.83333	7	30	7	1	120	14.1	7.62	74.77	7
High	XP_044438842.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	31.79	81.66667	6	29	1	1	120	12.1	4.55	80.9	6
High	XP_044431117.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	30.31	34.76298	8	24	3	1	443	46.9	4.79	68.71	8
High	XP_044364944.1	coatomer subunit alpha-3-like [Triticum aestivum]	0	29.888	13.80444	10	22	6	1	1217	136.3	7.12	55.75	10
High	XP_044323130.1	tubulin alpha-1 chain [Triticum aestivum]	0	29.597	23.33333	7	24	1	1	450	49.6	5.1	68.51	7
High	XP_044343777.1	protein MLN51 homolog [Triticum aestivum]	0	29.575	24.48071	9	18	9	1	674	72.8	5.53	49.94	9
High	XP_044371936.1	coatomer subunit alpha-3-like [Triticum aestivum]	0	27.833	12.97209	10	17	6	1	1218	135.7	7.01	40.44	10
High	XP_044374291.1	heterogeneous nuclear ribonucleoprotein Q-like isoform X1 [Triticum aestivum]	0	27.618	19.30783	7	20	7	1	549	60.4	5.2	66.96	7
High	XP_044429807.1	protein PAF1 homolog [Triticum aestivum]	0	27.478	21.75325	11	23	2	1	616	70.2	7.05	60.18	11
High	XP_044407805.1	DNA-directed RNA polymerase II subunit RPB7 [Triticum aestivum]	0	27.466	35.02825	6	19	6	1	177	19.5	6.52	51.91	6
High	XP_044430325.1	heterogeneous nuclear ribonucleoprotein A/B-like [Triticum aestivum]	0	27.264	26.30273	7	12	2	1	403	41.4	5.08	36.55	7
High	XP_044422439.1	protein PAF1 homolog [Triticum aestivum]	0	26.215	23.13916	11	27	1	1	618	70.5	7.43	72.83	11

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044383314.1	dnaJ protein homolog [Triticum aestivum]	0	25.94	34.20428	9	18	7	1	421	46.8	5.92	48.02	9
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	25.894	36.97183	8	22	8	1	284	30.9	9.58	50.74	8
High	XP_044363815.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	25.478	48.13665	8	27	2	1	322	33.6	6.25	72.91	8
High	XP_044397879.1	uncharacterized protein LOC123121865 [Triticum aestivum]	0	24.88	8.878505	7	18	1	1	1070	120.7	5.53	56.57	7
High	XP_044346607.1	60S ribosomal protein L9 [Triticum aestivum]	0	24.857	38.09524	6	18	6	1	189	21.3	9.77	52.26	6
High	XP_044369898.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	24.742	35.73487	7	18	7	1	347	38.6	6.77	55.34	7
High	XP_044365854.1	elongation factor 2 [Triticum aestivum]	0	24.691	22.3013	10	21	10	1	843	93.7	6.16	52.61	10
High	XP_044373111.1	63 kDa globulin-like protein [Triticum aestivum]	0	23.871	17.76316	7	23	1	1	608	68.3	7.72	49.38	7
High	XP_044423311.1	heterogeneous nuclear ribonucleoprotein 1-like isoform X1 [Triticum aestivum]	0	23.781	26.43392	6	11	1	1	401	41.3	5.05	32.72	6
High	XP_044374587.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	23.115	36.64596	7	25	1	1	322	33.6	6.25	66.28	7
High	XP_044338201.1	protein PAF1 homolog [Triticum aestivum]	0	23.043	22.13622	11	27	3	1	646	73.6	7.71	72.26	11
High	XP_044322364.1	nucleolar protein 56-like [Triticum aestivum]	0	21.427	19.62963	7	13	7	1	540	60.1	8.73	33.28	7
High	XP_044397238.1	ATP synthase subunit alpha, mitochondrial [Triticum aestivum]	0	21.307	17.2888	6	12	6	1	509	55.3	5.9	30.85	6
High	XP_044330175.1	elongation factor 1-alpha-like [Triticum aestivum]	0	21.137	16.10738	5	19	1	1	447	49.1	9.13	50.81	5
High	XP_044373110.1	63 kDa globulin-like protein [Triticum aestivum]	0	20.894	10.36683	5	18	1	1	627	70.6	7.17	55.67	5
High	XP_044437687.1	glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic [Triticum aestivum]	0	20.487	27.59644	6	12	4	1	337	36.5	7.18	32.44	6
High	XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]	0	20.47	12.35431	8	13	8	1	858	96.7	5	35.47	8
High	XP_044341061.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	20.432	26.20482	5	16	5	1	332	36.2	6.43	58.58	5
High	XP_044341880.1	DNA-directed RNA polymerase subunit 10-like protein isoform X4 [Triticum aestivum]	0	19.811	76.05634	4	19	1	1	71	8.2	5.83	54.87	4
High	XP_044367853.1	protein CDC73 homolog isoform X2 [Triticum aestivum]	0	19.373	28.38196	6	15	3	1	377	41.9	6.77	28.36	6
High	XP_044356966.1	protein MLN51 homolog [Triticum aestivum]	0	18.891	13.11239	6	17	3	1	694	75.4	5.38	33.07	6
High	XP_044410738.1	DNA-directed RNA polymerase II subunit 4-like [Triticum aestivum]	0	18.197	61.15108	5	20	5	1	139	15.8	4.92	54.74	5
High	XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like [Triticum aestivum]	0	18.194	40.90909	4	17	1	1	132	14.7	8.27	47.41	4
High	XP_044362453.1	40S ribosomal protein S3a [Triticum aestivum]	0	18.144	33.46008	6	15	6	1	263	29.9	9.79	41.54	6
High	XP_044366056.1	coatomer subunit beta'-1-like isoform X1 [Triticum aestivum]	0	17.993	10.35242	5	10	3	1	908	102.4	4.98	27.55	5
High	XP_044381819.1	heat shock protein 81-3 [Triticum aestivum]	0	17.44	10.71429	6	15	2	1	700	80.4	5.03	38.57	6
High	XP_044336485.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	17.383	52.98013	7	13	1	1	151	16.8	5.72	36.2	7
High	XP_044343932.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	17.216	44.37086	6	12	1	1	151	16.9	6.09	35.36	6
High	XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]	0	17.162	7.659314	7	11	7	1	1632	182.8	5.14	22.72	7
High	XP_044425255.1	DNA damage-binding protein 1-like [Triticum aestivum]	0	17.121	11.74312	7	12	7	1	1090	121.8	5.34	17.21	7
High	XP_044399391.1	elongation factor 1-alpha isoform X1 [Triticum aestivum]	0	17.037	9.72973	5	17	1	1	740	83.1	8.97	48.33	5
High	XP_044355245.1	uncharacterized protein LOC123077097 [Triticum aestivum]	0	16.981	18.40796	7	14	7	1	603	66.8	5.19	38.04	7
High	XP_044362751.1	NHP2-like protein 1 [Triticum aestivum]	0	16.502	48.4375	3	9	3	1	128	13.9	7.12	22	3
High	XP_044412707.1	60S ribosomal protein L6-like [Triticum aestivum]	0	16.499	35.15982	8	15	8	1	219	24.5	10.15	35.63	8
High	XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	0	16.31	3.678647	6	10	6	1	2365	275.4	8.78	24.99	6

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044351723.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]	0	15.797	8.74552	7	12	1	1	1395	155	8.82	29.02	7
High	XP_044395610.1	DNA-directed RNA polymerases II, IV and V subunit 9B [Triticum aestivum]	0	15.58	54.38596	4	14	3	1	114	13	6.64	31.42	4
High	XP_044432754.1	16.9 kDa class I heat shock protein 2-like [Triticum aestivum]	0	15.36	28.48101	4	9	1	1	158	18	5.71	30.63	4
High	XP_044365948.1	glucose and ribitol dehydrogenase homolog [Triticum aestivum]	0	15.204	20.11494	5	11	5	1	348	37.7	9	29.75	5
High	XP_044455885.1	DNA-directed RNA polymerases II, IV and V subunit 6A-like [Triticum aestivum]	0	15.004	36.61972	4	26	4	1	142	16.2	4.27	67.87	4
High	XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	14.943	35.81081	4	18	1	1	148	16.9	5.87	57.95	4
High	XP_044342950.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]	0	14.904	8.74552	7	11	1	1	1395	155	8.82	24.51	7
High	XP_044451591.1	ATP synthase subunit beta, mitochondrial-like [Triticum aestivum]	0	14.862	10.27027	4	7	4	1	555	59.3	6.2	14.46	4
High	XP_044330857.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	14.445	35.3211	5	13	3	1	218	24	6.79	22.19	5
High	XP_044432232.1	heat shock protein 81-1 [Triticum aestivum]	0	14.37	10.28571	6	15	1	1	700	80.4	5.06	36.27	6
High	XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	14.258	35.81081	4	15	1	1	148	16.8	5.87	45.74	4
High	XP_044414877.1	protein CDC73 homolog [Triticum aestivum]	0	14.255	20.99738	5	11	2	1	381	42.3	6.71	23.65	5
High	XP_044442770.1	avenin-like b6 [Triticum aestivum]	0	14.217	27.14286	5	13	5	1	280	31.9	7.91	23.4	5
High	XP_044348996.1	protein MLN51 homolog [Triticum aestivum]	0	13.718	11.67147	4	12	1	1	694	75.4	5.3	18.76	4
High	XP_044403314.1	glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic-like isoform X1 [Triticum aestivum]	0	13.431	17.86744	4	8	2	1	347	38.2	7.66	24.85	4
High	XP_044421055.1	splicing factor, arginine/serine-rich 19-like [Triticum aestivum]	0	13.227	28.87701	5	9	4	1	374	40.9	4.7	31.43	5
High	XP_044433310.1	17.4 kDa class I heat shock protein-like [Triticum aestivum]	0	12.976	30.37975	4	8	1	1	158	17.9	5.49	21.68	4
High	XP_044439510.1	endoplasmin homolog [Triticum aestivum]	0	12.926	9.808612	6	9	6	1	836	96	5.21	22.46	6
High	XP_044345724.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	12.886	16.41791	3	6	3	1	335	36.3	6.52	19.46	3
High	XP_044323484.1	splicing factor 3B subunit 4-like [Triticum aestivum]	0	12.689	18.47826	4	6	4	1	368	40.3	8.15	18.77	4
High	XP_044344314.1	17.5 kDa class II heat shock protein-like [Triticum aestivum]	0	12.632	37.03704	4	12	4	1	162	17.6	5.47	34.74	4
High	XP_044371305.1	40S ribosomal protein Sa-2-like [Triticum aestivum]	0	12.392	18.18182	3	10	3	1	308	33.2	5.02	23.01	3
High	XP_044418242.1	peptidyl-prolyl cis-trans isomerase [Triticum aestivum]	0	12.304	32.16374	4	15	4	1	171	18.4	8.25	38.89	4
High	XP_044424477.1	40S ribosomal protein S14-like [Triticum aestivum]	0	11.8	16.32653	4	9	4	1	196	20.8	11.03	22.72	4
High	XP_044347284.1	phytepsin isoform X1 [Triticum aestivum]	0	11.796	15.52063	5	7	5	1	509	54.5	5.52	17.87	5
High	XP_044408236.1	DNA-directed RNA polymerase III subunit 2-like [Triticum aestivum]	0	11.587	9.507347	7	8	7	1	1157	129.7	8.47	11.49	7
High	XP_044375395.1	GPN-loop GTPase QQT2-like isoform X1 [Triticum aestivum]	0	11.362	10.52632	3	8	3	1	437	48.5	4.74	17.47	3
High	XP_044407359.1	luminal-binding protein 2 isoform X2 [Triticum aestivum]	0	11.314	8.120301	5	9	4	1	665	73.2	5.21	21.88	5
High	XP_044340919.1	WD-40 repeat-containing protein MSI4-like isoform X2 [Triticum aestivum]	0	11.209	11.20879	3	5	3	1	455	50.3	6.37	11.68	3
High	XP_044332823.1	DNA-directed RNA polymerases I and III subunit rpac1-like [Triticum aestivum]	0	11.043	16.61972	4	8	4	1	355	39.5	5.16	14.32	4
High	XP_044456782.1	40S ribosomal protein S15 [Triticum aestivum]	0	10.992	26.62338	2	8	2	1	154	17.4	10.27	23.41	2
High	XP_044387377.1	protein RTF1 homolog [Triticum aestivum]	0	10.856	11.66667	4	5	4	1	660	73.4	8.72	14.87	4
High	XP_044325394.1	coatomer subunit gamma-2-like [Triticum aestivum]	0	10.834	10.57368	5	8	4	1	889	98.6	5.14	17.02	5
High	XP_044352787.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	10.717	40.13158	5	8	1	1	152	16.9	6.81	21.47	5
High	XP_044380741.1	pre-mRNA-splicing factor 38-like [Triticum aestivum]	0	10.652	12.69036	4	7	4	1	394	47	9.04	19.38	4

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044350539.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	10.618	30.95238	5	8	5	1	210	24.8	9.47	12.94	5
High	XP_044348900.1	developmentally-regulated G-protein 2 [Triticum aestivum]	0	10.573	15.78947	4	7	4	1	399	44.5	8.56	20.98	4
High	XP_044445530.1	40S ribosomal protein S25-2 [Triticum aestivum]	0	10.57	30.55556	4	7	4	1	108	12.1	10.56	19.46	4
High	XP_044399791.1	40S ribosomal protein S2-3-like [Triticum aestivum]	0	10.518	5.734767	2	8	2	1	279	30.4	10.17	22.87	2
High	XP_044417542.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	10.26	11.46789	3	9	1	1	436	45.7	4.96	22.79	3
High	XP_044390882.1	cupincin-like [Triticum aestivum]	0	9.921	9.50495	4	8	2	1	505	56.5	7.43	19.79	4
High	XP_044335842.1	glycine-rich protein 2 [Triticum aestivum]	0	9.657	22.27074	3	5	1	1	229	21.4	6.1	9.85	3
High	XP_044319569.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	9.457	18.34862	3	8	1	1	218	24	6.54	15.58	3
High	XP_044362686.1	tubulin alpha-2 chain [Triticum aestivum]	0	9.43	9.576837	3	9	1	1	449	49.8	5.2	22.78	3
High	XP_044343298.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	9.291	28.57143	4	9	3	1	182	20.1	9.73	24.88	4
High	XP_044391737.1	U2 small nuclear ribonucleoprotein A'-like [Triticum aestivum]	0	9.127	15.08772	3	6	3	1	285	31.9	5.15	16.69	3
High	XP_044407136.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	9.101	11.26437	3	8	1	1	435	45.7	4.98	16.98	3
High	XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]	0	9.033	2.946768	2	6	2	1	1052	115.8	5.38	18.23	2
High	XP_044343360.1	general transcription factor IIE subunit 1-like [Triticum aestivum]	0	8.889	10.30043	4	8	4	1	466	52.9	5.16	7.44	4
High	XP_044364918.1	actin-1-like [Triticum aestivum]	0	8.632	13.52785	3	5	3	1	377	41.8	5.58	10.79	3
High	XP_044422783.1	squamous cell carcinoma antigen recognized by T-cells 3-like [Triticum aestivum]	0	8.286	3.597122	3	7	3	1	834	94.3	6.14	14.39	3
High	XP_044333684.1	nucleolin-like [Triticum aestivum]	0	8.249	7.593308	3	5	2	1	777	87.4	5.57	14.46	3
High	XP_044398966.1	dnaJ protein homolog 2-like [Triticum aestivum]	0	8.228	11.40143	3	4	1	1	421	46.3	6.55	11.83	3
High	XP_044438002.1	V-type proton ATPase subunit B 1-like [Triticum aestivum]	0	8.126	13.13485	5	6	5	1	571	62.9	5.54	11.91	5
High	XP_044366277.1	40S ribosomal protein S20 [Triticum aestivum]	0	7.887	25.98425	3	6	3	1	127	14.1	9.44	12.77	3
High	XP_044457854.1	small nuclear ribonucleoprotein SmD1a-like [Triticum aestivum]	0	7.867	28.94737	2	6	1	1	114	12.7	11.22	20.08	2
High	XP_044339126.1	nuclear ubiquitous casein and cyclin-dependent kinase substrate 1-like [Triticum aestivum]	0	7.832	18.18182	3	9	3	1	319	34.2	5	17.2	3
High	XP_044450447.1	glycine-rich protein 2 [Triticum aestivum]	0	7.808	23.37662	3	4	1	1	231	21.5	6.1	6.46	3
High	XP_044415288.1	translation initiation factor eIF-2B subunit epsilon-like [Triticum aestivum]	0	7.693	4.070556	2	5	2	1	737	82.2	4.74	14.08	2
High	XP_044410540.1	pre-mRNA-processing protein 40A-like isoform X2 [Triticum aestivum]	0	7.677	4.685942	3	6	3	1	1003	114.2	7.24	8.85	3
High	XP_044323957.1	casein kinase II subunit alpha-2-like isoform X1 [Triticum aestivum]	0	7.302	10.52632	3	6	3	1	361	41.9	8.57	14.95	3
High	XP_044409014.1	glycine-rich protein 2-like [Triticum aestivum]	0	7.173	12.5	2	4	2	1	232	21.7	6.1	8.28	2
High	XP_044377405.1	histone H2B.11-like [Triticum aestivum]	0	7.16	11.68385	3	7	2	1	291	31.3	9.79	14.66	3
High	XP_044421771.1	rRNA 2'-O-methyltransferase fibrillarin 1-like [Triticum aestivum]	0	7.135	10.35599	3	6	3	1	309	32.4	10.08	15.38	3
High	XP_044412687.1	NAP1-related protein 2-like [Triticum aestivum]	0	7.13	14.34263	2	3	2	1	251	28.8	4.37	10.69	2
High	XP_044407747.1	probable helicase MAGATAMA 3 [Triticum aestivum]	0	7.082	6.15942	4	7	4	1	828	91.2	6.11	13.21	4
High	XP_044379771.1	importin subunit beta-1-like [Triticum aestivum]	0	7.03	5.619266	3	4	3	1	872	95.8	4.74	12.38	3
High	XP_044363027.1	heat shock cognate 70 kDa protein-like [Triticum aestivum]	0	7.015	4.251701	3	7	1	1	588	64.7	7.96	16.99	3
High	XP_044412497.1	heterogeneous nuclear ribonucleoprotein A0-like [Triticum aestivum]	0	6.865	12.56039	3	4	1	1	414	41.3	6.29	7.52	3
High	XP_044419470.1	heterogeneous nuclear ribonucleoprotein 1-like isoform X1 [Triticum aestivum]	0	6.811	12.95844	3	3	1	1	409	40.9	6.29	7.44	3

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044321538.1	heat shock protein 83 [Triticum aestivum]	0	6.798	6.882022	4	8	1	1	712	81.2	5.05	19.03	4
High	XP_044414706.1	dehydrin DHN4 [Triticum aestivum]	0	6.689	15.58442	2	7	2	1	231	23.2	9.23	14.2	2
High	XP_044394558.1	probable histone H2A variant 2 [Triticum aestivum]	0	6.622	27.33813	2	3	1	1	139	14.6	10.32	9.22	2
High	XP_044399554.1	cupincin-like [Triticum aestivum]	0	6.509	6.504065	3	4	1	1	492	55.1	7.15	4.52	3
High	XP_044402921.1	heterogeneous nuclear ribonucleoprotein A0-like [Triticum aestivum]	0	6.377	12.83293	3	3	1	1	413	41.3	6.29	7.46	3
High	XP_044426597.1	protein SEEDLING PLASTID DEVELOPMENT 1-like [Triticum aestivum]	0	6.281	6.906475	3	6	3	1	695	75.6	5.71	14.42	3
High	XP_044381680.1	histone H2A-like [Triticum aestivum]	0	6.248	23.89937	2	3	1	1	159	16.6	10.64	9	2
High	XP_044451243.1	chaperone protein ClpB1 [Triticum aestivum]	0	6.245	4.139434	3	4	3	1	918	101	6.24	12.39	3
High	XP_044362953.1	17.4 kDa class I heat shock protein [Triticum aestivum]	0	6.218	13.92405	2	6	1	1	158	17.5	6.06	14.65	2
High	XP_044346113.1	uncharacterized protein LOC123067343 [Triticum aestivum]	0	6.12	7	3	10	2	1	500	54.6	9.55	16.99	3
High	XP_044339931.1	cysteine proteinase inhibitor 12 [Triticum aestivum]	0	6.037	12.7572	2	3	2	1	243	26.6	6.87	8.18	2
High	XP_044404772.1	protein HEADING DATE REPRESSOR 1-like [Triticum aestivum]	0	5.877	15.52511	2	5	2	1	219	24.7	4.69	13.68	2
High	XP_044390792.1	40S ribosomal protein S12-like [Triticum aestivum]	0	5.873	18.75	2	3	2	1	144	15.3	5.74	7.37	2
High	XP_044346863.1	uncharacterized protein DDB_G0286299-like [Triticum aestivum]	0	5.86	6.142035	2	3	1	1	521	56.3	5.49	9.05	2
High	XP_044376616.1	uncharacterized protein LOC123098635 [Triticum aestivum]	0	5.779	14.48763	3	5	3	1	283	32.1	9.77	7.27	3
High	XP_044365658.1	uncharacterized protein LOC123087672 [Triticum aestivum]	0	5.731	7.64526	2	4	2	1	327	36.2	5.15	10.29	2
High	XP_044343945.1	60S ribosomal protein L4-1-like [Triticum aestivum]	0	5.689	11.38614	3	5	3	1	404	44.3	10.61	11.83	3
High	XP_044323344.1	histone H2A.4 [Triticum aestivum]	0	5.647	28.14815	2	4	1	1	135	14.1	10.05	11.13	2
High	XP_044325436.1	nucleolin-like [Triticum aestivum]	0	5.622	3.984576	2	3	1	1	778	87.6	5.69	7.46	2
High	XP_044361617.1	uncharacterized protein LOC123083734 [Triticum aestivum]	0	5.599	15.84158	2	3	2	1	202	22.2	5.16	7.35	2
High	XP_044451441.1	late embryogenesis abundant protein, group 3-like [Triticum aestivum]	0	5.496	21.36364	2	4	2	1	220	22.7	9.01	9.93	2
High	XP_044435946.1	V-type proton ATPase catalytic subunit A [Triticum aestivum]	0	5.477	3.982301	2	3	2	1	678	74.3	5.4	8.2	2
High	XP_044382638.1	ribosome biogenesis protein BOP1 homolog isoform X1 [Triticum aestivum]	0	5.419	5.702364	3	4	3	1	719	81.5	5.73	8.15	3
High	XP_044364554.1	eukaryotic translation initiation factor 3 subunit B-like [Triticum aestivum]	0	5.414	6.796117	3	5	3	1	721	83.4	5.12	9.69	3
High	XP_044374025.1	DNA-directed RNA polymerases I and III subunit RPAC2-like [Triticum aestivum]	0	5.342	23.68421	3	4	3	1	190	20.6	7.11	8.91	3
High	XP_044380766.1	polyadenylate-binding protein 8 [Triticum aestivum]	0	5.243	8.294931	3	5	3	1	651	70.7	7.37	11.19	3
High	XP_044324467.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	5.181	15.87302	2	6	1	1	189	20.6	9.88	20.24	2
High	XP_044388315.1	40S ribosomal protein S19 [Triticum aestivum]	0	5.153	14.19355	3	6	3	1	155	17.1	9.89	12.79	3
High	XP_044371551.1	alpha-amylase/trypsin inhibitor CM3 [Triticum aestivum]	0	5.122	19.04762	2	4	2	1	168	18.2	7.44	7.3	2
High	XP_044394533.1	transcription initiation factor IIF subunit alpha-like isoform X1 [Triticum aestivum]	0	5.108	8.842444	3	5	3	1	622	68.7	5.95	12.13	3
High	XP_044407354.1	catalase isozyme 2-like [Triticum aestivum]	0	5.002	5.465587	2	4	2	1	494	56.9	7.06	7.28	2
High	XP_044431145.1	60S ribosomal protein L17-2 isoform X1 [Triticum aestivum]	0	4.882	10.27027	2	6	2	1	185	21.3	10.24	11.45	2
High	XP_044338442.1	uncharacterized protein DDB_G0286299-like [Triticum aestivum]	0	4.809	6.225681	2	2	1	1	514	55.7	5.62	5.59	2
High	XP_044364863.1	lariat debranching enzyme-like [Triticum aestivum]	0	4.688	9.631148	3	3	3	1	488	55.1	6.13	4.71	3
High	XP_044324199.1	eukaryotic translation initiation factor 3 subunit H-like isoform X1 [Triticum aestivum]	0	4.57	7.28863	2	2	2	1	343	38.8	4.92	5.05	2

Table A.4 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044362808.1	coatomer subunit gamma-1 [Triticum aestivum]	0	4.563	3.125	2	4	1	1	896	99.6	5.2	9.2	2
High	XP_044404142.1	26S rRNA (cytosine-C(5))-methyltransferase NOP2B-like [Triticum aestivum]	0	4.559	3.104787	2	5	2	1	773	85.4	5.39	11.81	2
High	XP_044375497.1	40S ribosomal protein S7 [Triticum aestivum]	0	4.451	13.02083	2	3	2	1	192	22.2	9.79	5.97	2
High	XP_044378512.1	DNA-directed RNA polymerases II, IV and V subunit 9A-like [Triticum aestivum]	0	4.38	19.29825	2	6	1	1	114	13	6.84	13.82	2
High	XP_044322198.1	60S ribosomal protein L22-2-like [Triticum aestivum]	0	4.362	16.15385	2	4	1	1	130	14.5	9.55	9.68	2
High	XP_044404494.1	nucleolar and coiled-body phosphoprotein 1-like [Triticum aestivum]	0	4.338	11.55914	2	2	1	1	372	40.8	4.7	4.83	2
High	XP_044407705.1	small nuclear ribonucleoprotein E-like [Triticum aestivum]	0	4.315	30.68182	2	2	2	1	88	10.3	9.89	4.71	2
High	XP_044416379.1	SNF1-related protein kinase regulatory subunit gamma-like PV42a [Triticum aestivum]	0	4.207	7.272727	3	4	3	1	440	46.8	5.6	2.2	3
High	XP_044346726.1	ubiquitin-40S ribosomal protein S27a-like [Triticum aestivum]	0	4.092	14.28571	3	4	3	1	231	26.2	9.66	5.93	3
High	XP_044359714.1	uncharacterized protein LOC123080794 [Triticum aestivum]	0	4.048	24.85207	2	3	2	1	169	17.2	6.33	9	2
High	XP_044348807.1	ras-related protein RIC1 isoform X1 [Triticum aestivum]	0	4.042	12.21719	2	3	2	1	221	24.3	5.74	6.66	2
High	XP_044406649.1	uncharacterized protein LOC123130924 [Triticum aestivum]	0	3.918	15.95092	2	4	2	1	163	17.4	9.31	6.37	2
High	XP_044360276.1	17.9 kDa class I heat shock protein-like [Triticum aestivum]	0	3.813	14.19355	2	3	2	1	155	17.3	6.6	7.16	2
High	XP_044334998.1	zinc finger CCCH domain-containing protein 32-like [Triticum aestivum]	0	3.782	3.708987	2	2	2	1	701	78.2	5.55	3.72	2
High	XP_044399570.1	40S ribosomal protein S18-like [Triticum aestivum]	0	3.775	13.81579	2	3	2	1	152	17.7	10.74	7.87	2
High	XP_044344786.1	oil body-associated protein 2A-like [Triticum aestivum]	0	3.741	10.08065	2	3	2	1	248	27.7	7.69	5.57	2
High	XP_044343562.1	protein H2A.6-like [Triticum aestivum]	0	3.642	19.48718	2	3	1	1	195	20.4	10.14	8.39	2
High	XP_044425964.1	histone deacetylase HDT2 [Triticum aestivum]	0	3.515	14.24581	2	4	2	1	358	38.5	5	9.95	2
High	XP_044362961.1	17.9 kDa class I heat shock protein-like [Triticum aestivum]	0	3.496	16.4557	2	5	2	1	158	17.5	5.69	10.86	2
High	XP_044423143.1	60S ribosomal protein L22-2-like [Triticum aestivum]	0	3.37	16.03053	2	3	1	1	131	14.5	9.55	5.6	2
High	XP_044318289.1	universal stress protein PHOS34 [Triticum aestivum]	0	3.271	18.07229	2	3	2	1	166	17.9	6.19	7.28	2
High	XP_044403247.1	small nuclear ribonucleoprotein SmD1a-like [Triticum aestivum]	0	3.251	28.94737	2	4	1	1	114	12.7	11.22	11.42	2
High	XP_044415364.1	60S ribosomal protein L23 [Triticum aestivum]	0	3.207	17.14286	3	4	3	1	140	15	10.48	4.92	3
High	XP_044361663.1	uncharacterized protein LOC123083827 [Triticum aestivum]	0	3.16	11.42857	2	4	2	1	140	15.2	6.79	4.83	2
High	XP_044377892.1	late embryogenesis abundant protein 1-like [Triticum aestivum]	0	3.057	11.93182	2	2	2	1	352	37.4	6.9	2.67	2
High	XP_044337279.1	putative H/ACA ribonucleoprotein complex subunit 1-like protein 1 [Triticum aestivum]	0	2.54	19.89796	2	2	2	1	196	20.3	11.43	4.78	2
High	XP_044323640.1	chaperonin CPN60-1, mitochondrial-like [Triticum aestivum]	0	2.518	3.484321	2	3	2	1	574	61.1	5.55	7.06	2
High	XP_044333048.1	eukaryotic peptide chain release factor subunit 1-2-like [Triticum aestivum]	0	2.502	3.837472	2	3	2	1	443	49.4	5.3	6.06	2
High	XP_044323100.1	ruvB-like 2 [Triticum aestivum]	0	2.481	4.64135	2	3	2	1	474	51.6	5.63	4.24	2
High	XP_044415108.1	40S ribosomal protein S30 [Triticum aestivum]	0	2.125	17.74194	2	2	2	1	62	6.9	12.03	2.37	2

Table A.5 nLC-MS/MS for partially purified Pol II (replicate 3)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	391.432	51.17773	86	336	4	1	1868	208	6.2	788.99	86
High	XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	386.411	51.28755	85	332	3	1	1864	207.8	6.32	781.3	85
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	263.055	59.04996	63	252	63	1	1221	138.2	7.15	559.97	63
High	XP_044362369.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	105.197	57.82396	33	82	2	1	818	90.8	5.26	201.43	33
High	XP_044362370.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	102.645	55.96556	32	80	1	1	813	90.1	5.29	193.62	32
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	98.049	36.12717	20	86	7	1	692	77	5.52	245.73	20
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	97.33	55.59921	21	97	8	1	509	55.4	6.62	207.78	21
High	XP_044377882.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	92.048	52.66272	23	96	10	1	507	55.3	6.96	201.97	23
High	XP_044370961.1	vicilin-like seed storage protein At4g36700 [Triticum aestivum]	0	90.08	34.62604	19	87	7	1	722	81.1	5.85	249.41	19
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	90.014	53.36323	19	81	6	1	446	50.2	4.81	200.9	19
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	87.753	53.00971	19	103	9	1	515	56.1	6.11	242.84	19
High	XP_044454990.1	FACT complex subunit SPT16 [Triticum aestivum]	0	82.229	37.51152	29	71	3	1	1085	121	5.57	130.03	29
High	XP_044330076.1	FACT complex subunit SPT16-like [Triticum aestivum]	0	80.594	37.51152	28	71	2	1	1085	121	5.59	128.23	28
High	XP_044339487.1	tubulin beta-5 chain [Triticum aestivum]	0	73.268	44.74273	16	58	4	1	447	50.3	4.83	140.03	16
High	XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]	0	72.538	33.48281	18	49	18	1	669	74.4	5.8	134.21	18
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	72.254	59.81873	15	77	15	1	331	36.7	4.88	191.38	15
High	XP_044390710.1	tubulin beta-4 chain [Triticum aestivum]	0	72.129	48.98876	17	55	3	1	445	49.9	4.87	134.41	17
High	XP_044429961.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	72.063	21.56028	19	51	3	1	1410	158.7	5.64	125.45	19
High	XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	0	71.046	30.99251	19	50	19	1	1068	122	6	113.95	19
High	XP_044417769.1	DExH-box ATP-dependent RNA helicase DExH12-like [Triticum aestivum]	0	69.436	17.56075	23	46	23	1	2181	246.8	6.01	112.26	23
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	69.308	51.5625	13	54	13	1	320	34.5	5.5	142.2	13
High	XP_044423298.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X3 [Triticum aestivum]	0	66.981	21.47413	18	51	2	1	1411	158.7	5.58	117.05	18
High	XP_044356354.1	importin subunit alpha-1a isoform X2 [Triticum aestivum]	0	63.892	46.18321	16	49	13	1	524	57.3	5.19	136.96	16
High	XP_044343678.1	tubulin alpha chain [Triticum aestivum]	0	61.285	39.24612	12	53	5	1	451	49.7	5.02	139.04	12
High	XP_044423543.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	56.488	32.69618	19	32	19	1	994	110.2	5.4	75.61	19
High	XP_044365264.1	63 kDa globulin-like protein [Triticum aestivum]	0	56.253	38.77551	17	56	5	1	588	66.3	8.27	97.05	17
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	55.224	100	11	46	4	1	113	11.5	4.41	124.12	11
High	XP_044324637.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	51.293	34.08624	14	34	10	1	487	53.4	5.83	87.4	14
High	XP_044360175.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	50.766	30.08721	15	36	4	1	688	76.9	6.09	107.19	15
High	XP_044448581.1	importin subunit alpha-1b-like [Triticum aestivum]	0	49.921	39.70037	14	35	11	1	534	58.6	5.25	85.29	14
High	XP_044367850.1	63 kDa globulin-like protein [Triticum aestivum]	0	47.217	34.53355	16	50	4	1	611	68.9	7.59	84.43	16
High	XP_044339880.1	RNA-binding protein P-like [Triticum aestivum]	0	47.149	29.79167	15	40	2	1	480	49.5	5.12	92.75	15
High	XP_044332259.1	protein LEO1 homolog [Triticum aestivum]	0	47.056	30.81571	13	42	2	1	662	75.7	4.63	104.69	13
High	XP_044450611.1	60S acidic ribosomal protein P2B [Triticum aestivum]	0	46.75	97.32143	9	39	2	1	112	11.4	4.34	108.42	9
High	XP_044348474.1	RNA-binding protein P-like [Triticum aestivum]	0	46.605	36	15	40	2	1	475	48.7	5.27	89.43	15

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	45.095	56.9378	11	41	10	1	209	24.1	9.44	103.16	11
High	XP_044373111.1	63 kDa globulin-like protein [Triticum aestivum]	0	43.709	26.80921	13	41	4	1	608	68.3	7.72	76.07	13
High	XP_044457053.1	protein LEO1 homolog [Triticum aestivum]	0	43.245	30.86233	12	39	1	1	661	75.5	4.59	96.43	12
High	XP_044365854.1	elongation factor 2 [Triticum aestivum]	0	40.879	19.45433	10	27	10	1	843	93.7	6.16	63.04	10
High	XP_044324377.1	60S acidic ribosomal protein P2A [Triticum aestivum]	0	39.578	58.40708	7	43	7	1	113	11.6	4.41	114.85	7
High	XP_044451529.1	embryonic protein DC-8-like [Triticum aestivum]	0	39.178	22.8022	11	24	2	1	728	73.1	8.41	70.63	11
High	XP_044343777.1	protein MLN51 homolog [Triticum aestivum]	0	38.468	32.78932	12	26	12	1	674	72.8	5.53	74.8	12
High	XP_044423644.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	37.722	33.63431	11	27	2	1	443	46.9	4.82	71.63	11
High	XP_044438842.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	37.597	81.66667	6	38	1	1	120	12.1	4.55	100.94	6
High	XP_044337674.1	heat shock 70 kDa protein 4-like [Triticum aestivum]	0	37.244	22.53086	12	35	3	1	648	71	5.25	90.31	12
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	37.006	81.66667	6	35	1	1	120	12.1	4.55	96.08	6
High	XP_044457664.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	36.655	25.63559	10	27	6	1	472	51.7	6.51	59.69	10
High	XP_044455423.1	probable nucleolar protein 5-2 [Triticum aestivum]	0	36.057	31.25	10	22	1	1	560	61.7	8.72	51.37	10
High	XP_044345306.1	embryonic protein DC-8-like [Triticum aestivum]	0	36.021	19.97226	11	23	2	1	721	72.9	8.48	63.53	11
High	XP_044417498.1	probable nucleolar protein 5-2 [Triticum aestivum]	0	35.481	31.25	10	23	1	1	560	61.8	8.81	50.04	10
High	XP_044383134.1	tubulin alpha-2 chain-like [Triticum aestivum]	0	35.062	20.84257	8	33	1	1	451	49.8	4.96	79.59	8
High	XP_044438520.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	35.056	35.49107	10	32	3	1	448	47.4	4.75	82.56	10
High	XP_044430572.1	60S acidic ribosomal protein P1-like [Triticum aestivum]	0	34.567	67.27273	7	45	7	1	110	11.2	4.59	119.93	7
High	XP_044429807.1	protein PAF1 homolog [Triticum aestivum]	0	34.102	23.86364	11	32	2	1	616	70.2	7.05	72.58	11
High	XP_044395610.1	DNA-directed RNA polymerases II, IV and V subunit 9B [Triticum aestivum]	0	30.874	54.38596	4	26	3	1	114	13	6.64	75.86	4
High	XP_044363031.1	heat shock cognate 70 kDa protein-like [Triticum aestivum]	0	30.769	19.78691	11	31	2	1	657	71.8	5.29	81.21	11
High	XP_044368141.1	coatomer subunit alpha-3 [Triticum aestivum]	0	30.389	13.80444	10	19	5	1	1217	136.3	7.02	40.74	10
High	XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	0	30.177	67.5	8	35	8	1	120	14.1	7.62	74.14	8
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	29.97	47.53521	10	28	10	1	284	30.9	9.58	68.32	10
High	XP_044383314.1	dnaJ protein homolog [Triticum aestivum]	0	29.463	22.80285	7	21	7	1	421	46.8	5.92	49.09	7
High	XP_044338201.1	protein PAF1 homolog [Triticum aestivum]	0	29.192	23.99381	11	31	3	1	646	73.6	7.71	76.45	11
High	XP_044341880.1	DNA-directed RNA polymerase subunit 10-like protein isoform X4 [Triticum aestivum]	0	28.785	77.46479	6	21	1	1	71	8.2	5.83	49.24	6
High	XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like [Triticum aestivum]	0	28.433	41.66667	6	23	1	1	132	14.7	8.27	53.48	6
High	XP_044422439.1	protein PAF1 homolog [Triticum aestivum]	0	28.111	26.69903	12	29	2	1	618	70.5	7.43	69.75	12
High	XP_044419370.1	coatomer subunit beta'-2-like isoform X1 [Triticum aestivum]	0	28.086	15.48999	9	21	8	1	949	107.2	4.94	48.22	9
High	XP_044418568.1	embryonic protein DC-8-like [Triticum aestivum]	0	28.031	23.07692	10	16	2	1	741	74.2	7.34	43.51	10
High	XP_044389229.1	protein LTV1 homolog [Triticum aestivum]	0	27.314	21.07438	7	15	7	1	484	54	4.73	46.46	7
High	XP_044366633.1	60S ribosomal protein L9-like [Triticum aestivum]	0	26.736	41.26984	6	21	6	1	189	21.3	9.83	53.5	6
High	XP_044431117.1	plant UBX domain-containing protein 4-like [Triticum aestivum]	0	26.251	23.92777	9	18	2	1	443	46.9	4.79	42.47	9
High	XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]	0	26.16	12.58741	7	15	7	1	858	96.7	5	30.26	7
High	XP_044341061.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	26.118	40.96386	7	19	7	1	332	36.2	6.43	56.04	7

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044373110.1	63 kDa globulin-like protein [Triticum aestivum]	0	25.063	15.62998	7	23	3	1	627	70.6	7.17	47.46	7
High	XP_044430325.1	heterogeneous nuclear ribonucleoprotein A/B-like [Triticum aestivum]	0	24.663	30.02481	8	14	8	1	403	41.4	5.08	33.51	8
High	XP_044323130.1	tubulin alpha-1 chain [Triticum aestivum]	0	24.568	14.88889	6	22	1	1	450	49.6	5.1	49.75	6
High	XP_044407805.1	DNA-directed RNA polymerase II subunit RPB7 [Triticum aestivum]	0	24.246	35.02825	5	17	5	1	177	19.5	6.52	40.02	5
High	XP_044387377.1	protein RTF1 homolog [Triticum aestivum]	0	24.153	19.84848	7	13	7	1	660	73.4	8.72	30.3	7
High	XP_044363379.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	23.706	34.29395	8	20	1	1	347	38.6	6.73	47.99	8
High	XP_044371936.1	coatomer subunit alpha-3-like [Triticum aestivum]	0	23.324	10.01642	8	13	3	1	1218	135.7	7.01	25.79	8
High	XP_044325394.1	coatomer subunit gamma-2-like [Triticum aestivum]	0	23.307	14.06074	8	16	6	1	889	98.6	5.14	31.7	8
High	XP_044363815.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	22.957	54.34783	8	21	2	1	322	33.6	6.25	48.92	8
High	XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]	0	22.815	8.517157	9	16	9	1	1632	182.8	5.14	26.69	9
High	XP_044381819.1	heat shock protein 81-3 [Triticum aestivum]	0	22.546	14.57143	9	18	2	1	700	80.4	5.03	42.15	9
High	XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	0	21.963	6.384778	9	15	9	1	2365	275.4	8.78	38.23	9
High	XP_044369898.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	21.832	34.29395	8	20	1	1	347	38.6	6.77	42.96	8
High	XP_044374291.1	heterogeneous nuclear ribonucleoprotein Q-like isoform X1 [Triticum aestivum]	0	21.422	13.47905	6	20	6	1	549	60.4	5.2	61.68	6
High	XP_044426735.1	heat shock protein 81-1 [Triticum aestivum]	0	21.1	13	8	16	1	1	700	80.4	5.06	38.25	8
High	XP_044366056.1	coatomer subunit beta'-1-like isoform X1 [Triticum aestivum]	0	19.891	15.63877	8	13	7	1	908	102.4	4.98	21.03	8
High	XP_044352297.1	general transcription factor IIE subunit 1-like [Triticum aestivum]	0	19.413	15.45064	5	11	5	1	466	52.9	5.16	23.47	5
High	XP_044374587.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	18.886	42.85714	7	18	1	1	322	33.6	6.25	42.65	7
High	XP_044375308.1	ricin B-like lectin R40C1 [Triticum aestivum]	0	18.564	28.81844	7	16	1	1	347	38.7	6.77	36.19	7
High	XP_044330175.1	elongation factor 1-alpha-like [Triticum aestivum]	0	18.35	15.88367	5	18	1	1	447	49.1	9.13	41.01	5
High	XP_044349953.1	DNA damage-binding protein 1 [Triticum aestivum]	0	17.713	11.37615	7	11	7	1	1090	121.9	5.36	17.86	7
High	XP_044399391.1	elongation factor 1-alpha isoform X1 [Triticum aestivum]	0	17.463	9.594595	5	15	1	1	740	83.1	8.97	39.55	5
High	XP_044343932.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	16.923	41.0596	5	11	1	1	151	16.9	6.09	21.47	5
High	XP_044336485.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	16.776	49.66887	6	11	1	1	151	16.8	5.72	17.58	6
High	XP_044414877.1	protein CDC73 homolog [Triticum aestivum]	0	16.569	29.13386	8	13	3	1	381	42.3	6.71	22.02	8
High	XP_044367853.1	protein CDC73 homolog isoform X2 [Triticum aestivum]	0	16.417	34.48276	9	13	4	1	377	41.9	6.77	20.45	9
High	XP_044455885.1	DNA-directed RNA polymerases II, IV and V subunit 6A-like [Triticum aestivum]	0	16.387	35.21127	4	14	4	1	142	16.2	4.27	29.42	4
High	XP_044421055.1	splicing factor, arginine/serine-rich 19-like [Triticum aestivum]	0	16.337	25.40107	5	7	3	1	374	40.9	4.7	17.21	5
High	XP_044356966.1	protein MLN51 homolog [Triticum aestivum]	0	16.311	10.66282	6	11	2	1	694	75.4	5.38	25.25	6
High	XP_044355245.1	uncharacterized protein LOC123077097 [Triticum aestivum]	0	15.833	17.91045	5	10	5	1	603	66.8	5.19	26	5
High	XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	15.143	35.81081	4	14	1	1	148	16.9	5.87	35.04	4
High	XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	15.032	35.81081	4	16	1	1	148	16.8	5.87	41.49	4
High	XP_044371305.1	40S ribosomal protein Sa-2-like [Triticum aestivum]	0	14.999	25.64935	4	11	4	1	308	33.2	5.02	20.9	4
High	XP_044350539.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	14.826	35.71429	7	11	6	1	210	24.8	9.47	20.35	7
High	XP_044348996.1	protein MLN51 homolog [Triticum aestivum]	0	14.75	10.66282	5	11	1	1	694	75.4	5.3	22.62	5
High	XP_044365948.1	glucose and ribitol dehydrogenase homolog [Triticum aestivum]	0	13.989	20.11494	5	12	5	1	348	37.7	9	28.37	5

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044422687.1	glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic isoform X1 [Triticum aestivum]	0	13.928	15.84699	4	10	2	1	366	39.9	7.55	29.96	4
High	XP_044410738.1	DNA-directed RNA polymerase II subunit 4-like [Triticum aestivum]	0	13.209	46.04317	5	17	5	1	139	15.8	4.92	27.16	5
High	XP_044415288.1	translation initiation factor eIF-2B subunit epsilon-like [Triticum aestivum]	0	13.123	8.005427	4	7	4	1	737	82.2	4.74	20.45	4
High	XP_044370943.1	40S ribosomal protein S3a [Triticum aestivum]	0	12.655	23.57414	4	11	1	1	263	30	9.79	30.14	4
High	XP_044330857.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	12.572	36.23853	5	13	2	1	218	24	6.79	26.69	5
High	XP_044375395.1	GPN-loop GTPase QQT2-like isoform X1 [Triticum aestivum]	0	12.342	10.98398	3	8	3	1	437	48.5	4.74	19.62	3
High	XP_044362453.1	40S ribosomal protein S3a [Triticum aestivum]	0	12.267	23.57414	4	12	1	1	263	29.9	9.79	33.32	4
High	XP_044451591.1	ATP synthase subunit beta, mitochondrial-like [Triticum aestivum]	0	11.988	13.33333	5	5	5	1	555	59.3	6.2	12.04	5
High	XP_044391737.1	U2 small nuclear ribonucleoprotein A'-like [Triticum aestivum]	0	11.736	23.50877	5	11	5	1	285	31.9	5.15	20.32	5
High	XP_044403314.1	glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic-like isoform X1 [Triticum aestivum]	0	11.716	17.86744	4	8	2	1	347	38.2	7.66	21.74	4
High	XP_044442770.1	avenin-like b6 [Triticum aestivum]	0	11.237	23.21429	4	8	4	1	280	31.9	7.91	19.14	4
High	XP_044408236.1	DNA-directed RNA polymerase III subunit 2-like [Triticum aestivum]	0	11.127	8.038029	5	7	5	1	1157	129.7	8.47	13.68	5
High	XP_044456782.1	40S ribosomal protein S15 [Triticum aestivum]	0	11.026	26.62338	2	7	2	1	154	17.4	10.2	15.83	2
High	XP_044397238.1	ATP synthase subunit alpha, mitochondrial [Triticum aestivum]	0	10.922	13.35953	5	9	5	1	509	55.3	5.9	19.42	5
High	XP_044346113.1	uncharacterized protein LOC123067343 [Triticum aestivum]	0	10.838	7	3	11	2	1	500	54.6	9.55	19.82	3
High	XP_044424477.1	40S ribosomal protein S14-like [Triticum aestivum]	0	10.765	14.79592	4	8	4	1	196	20.8	11.0	13.01	4
High	XP_044407359.1	luminal-binding protein 2 isoform X2 [Triticum aestivum]	0	10.724	8.120301	5	8	3	1	665	73.2	5.21	15.88	5
High	XP_044404494.1	nucleolar and coiled-body phosphoprotein 1-like [Triticum aestivum]	0	10.647	20.16129	4	6	2	1	372	40.8	4.7	13.06	4
High	XP_044348900.1	developmentally-regulated G-protein 2 [Triticum aestivum]	0	10.396	15.78947	4	5	4	1	399	44.5	8.56	10.35	4
High	XP_044419436.1	nucleolar protein 56-like [Triticum aestivum]	0	10.391	11.11111	4	5	4	1	549	61	8.72	8.32	4
High	XP_044451243.1	chaperone protein ClpB1 [Triticum aestivum]	0	10.168	5.119826	4	6	4	1	918	101	6.24	14.27	4
High	XP_044362751.1	NHP2-like protein 1 [Triticum aestivum]	0	9.976	48.4375	2	7	2	1	128	13.9	7.12	11.79	2
High	XP_044362808.1	coatomer subunit gamma-1 [Triticum aestivum]	0	9.733	5.915179	4	6	2	1	896	99.6	5.2	12.43	4
High	XP_044418242.1	peptidyl-prolyl cis-trans isomerase [Triticum aestivum]	0	9.539	23.39181	4	9	4	1	171	18.4	8.25	14.87	4
High	XP_044323957.1	casein kinase II subunit alpha-2-like isoform X1 [Triticum aestivum]	0	9.477	11.08033	4	8	4	1	361	41.9	8.57	18.54	4
High	XP_044417542.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	9.298	12.61468	3	6	1	1	436	45.7	4.96	15.84	3
High	XP_044342950.1	DNA-directed RNA polymerase III subunit 1-like [Triticum aestivum]	0	9.243	4.874552	4	7	4	1	1395	155	8.82	13.33	4
High	XP_044450447.1	glycine-rich protein 2 [Triticum aestivum]	0	9.047	12.55411	2	4	2	1	231	21.5	6.1	14.3	2
High	XP_044378512.1	DNA-directed RNA polymerases II, IV and V subunit 9A-like [Triticum aestivum]	0	8.849	33.33333	4	9	3	1	114	13	6.84	15.6	4
High	XP_044352787.1	16.9 kDa class I heat shock protein 1-like [Triticum aestivum]	0	8.816	36.84211	4	6	1	1	152	16.9	6.81	7.81	4
High	XP_044432754.1	16.9 kDa class I heat shock protein 2-like [Triticum aestivum]	0	8.65	28.48101	3	6	1	1	158	18	5.71	12.4	3
High	XP_044417838.1	pre-mRNA-processing protein 40A-like isoform X3 [Triticum aestivum]	0	8.514	4.137235	3	5	1	1	991	113.3	6.95	10.9	3
High	XP_044319569.1	1-Cys peroxiredoxin PER1 [Triticum aestivum]	0	8.501	24.31193	4	7	1	1	218	24	6.54	16.76	4
High	XP_044415795.1	zinc finger CCCH domain-containing protein 19-like [Triticum aestivum]	0	8.393	5.792683	3	6	3	1	656	72.7	5.49	3.58	3
High	XP_044412707.1	60S ribosomal protein L6-like [Triticum aestivum]	0	8.348	23.28767	4	6	4	1	219	24.5	10.1	11.42	4
High	XP_044421771.1	rRNA 2'-O-methyltransferase fibrillarin 1-like [Triticum aestivum]	0	8.321	10.35599	3	7	3	1	309	32.4	10.0	15.29	3

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044410540.1	pre-mRNA-processing protein 40A-like isoform X2 [Triticum aestivum]	0	8.292	4.087737	3	6	1	1	1003	114.2	7.24	14.64	3
High	XP_044366277.1	40S ribosomal protein S20 [Triticum aestivum]	0	8.182	22.04724	4	7	4	1	127	14.1	9.44	18.38	4
High	XP_044422783.1	squamous cell carcinoma antigen recognized by T-cells 3-like [Triticum aestivum]	0	8.149	5.035971	3	6	3	1	834	94.3	6.14	13.59	3
High	XP_044333684.1	nucleolin-like [Triticum aestivum]	0	8.146	7.593308	3	7	2	1	777	87.4	5.57	17.37	3
High	XP_044364918.1	actin-1-like [Triticum aestivum]	0	8.017	15.64987	3	6	3	1	377	41.8	5.58	13.41	3
High	XP_044390882.1	cupincin-like [Triticum aestivum]	0	7.959	7.722772	3	6	3	1	505	56.5	7.43	14.49	3
High	XP_044433310.1	17.4 kDa class I heat shock protein-like [Triticum aestivum]	0	7.958	30.37975	3	5	1	1	158	17.9	5.49	4.6	3
High	XP_044445530.1	40S ribosomal protein S25-2 [Triticum aestivum]	0	7.926	21.2963	3	8	3	1	108	12.1	10.5	21.26	3
High	XP_044346863.1	uncharacterized protein DDB_G0286299-like [Triticum aestivum]	0	7.885	10.36468	3	4	3	1	521	56.3	5.49	9.31	3
High	XP_044344314.1	17.5 kDa class II heat shock protein-like [Triticum aestivum]	0	7.795	37.03704	4	6	4	1	162	17.6	5.47	15.8	4
High	XP_044380741.1	pre-mRNA-splicing factor 38-like [Triticum aestivum]	0	7.744	9.390863	3	5	3	1	394	47	9.04	11.07	3
High	XP_044407136.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	7.584	12.41379	3	5	1	1	435	45.7	4.98	9.85	3
High	XP_044371551.1	alpha-amylase/trypsin inhibitor CM3 [Triticum aestivum]	0	7.547	30.95238	3	8	3	1	168	18.2	7.44	14.49	3
High	XP_044439510.1	endoplasmin homolog [Triticum aestivum]	0	7.545	5.263158	3	7	3	1	836	96	5.21	16.48	3
High	XP_044410325.1	FAM10 family protein At4g22670-like [Triticum aestivum]	0	7.349	11.38716	3	5	1	1	483	50.6	5.11	9.85	3
High	XP_044368583.1	PHD finger protein ALFIN-LIKE 8-like [Triticum aestivum]	0	7.32	16.92913	2	4	2	1	254	28.5	6.06	7.05	2
High	XP_044343562.1	protein H2A.6-like [Triticum aestivum]	0	7.263	31.28205	3	6	1	1	195	20.4	10.1	15.59	3
High	XP_044399570.1	40S ribosomal protein S18-like [Triticum aestivum]	0	7.071	13.81579	2	4	2	1	152	17.7	10.7	9.62	2
High	XP_044389137.1	histone H2A [Triticum aestivum]	0	7.067	38.36478	3	5	1	1	159	16.6	10.6	10.28	3
High	XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]	0	6.963	4.087452	4	6	4	1	1052	115.8	5.38	10.74	4
High	XP_044332823.1	DNA-directed RNA polymerases I and III subunit rpac1-like [Triticum aestivum]	0	6.837	13.52113	3	6	3	1	355	39.5	5.16	9.68	3
High	XP_044344629.1	late embryogenesis abundant protein, group 3 isoform X1 [Triticum aestivum]	0	6.736	9.821429	2	5	2	1	224	23.2	8.84	15.55	2
High	XP_044394533.1	transcription initiation factor IIF subunit alpha-like isoform X1 [Triticum aestivum]	0	6.689	8.842444	3	7	3	1	622	68.7	5.95	16.56	3
High	XP_044365145.1	polyadenylation factor subunit 2-like [Triticum aestivum]	0	6.642	5.579399	2	4	2	1	466	48.7	5.26	5.84	2
High	XP_044360348.1	late embryogenesis abundant protein 1-like [Triticum aestivum]	0	6.642	14.52991	3	5	3	1	351	37.3	6.95	6.63	3
High	XP_044363825.1	eukaryotic translation initiation factor 3 subunit I [Triticum aestivum]	0	6.575	12.26994	3	5	3	1	326	36.1	6.96	8.18	3
High	XP_044391112.1	40S ribosomal protein S2-3-like [Triticum aestivum]	0	6.558	10.58394	2	6	2	1	274	29.9	10.1	16.57	2
High	XP_044343945.1	60S ribosomal protein L4-1-like [Triticum aestivum]	0	6.485	11.38614	3	6	3	1	404	44.3	10.6	8.05	3
High	XP_044457854.1	small nuclear ribonucleoprotein SmD1a-like [Triticum aestivum]	0	6.466	28.94737	2	6	2	1	114	12.7	11.2	16.41	2
High	XP_044345724.1	guanine nucleotide-binding protein subunit beta-like protein A [Triticum aestivum]	0	6.416	12.23881	2	3	2	1	335	36.3	6.52	5.49	2
High	XP_044343298.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	6.303	21.97802	3	5	2	1	182	20.1	9.73	14.08	3
High	XP_044407705.1	small nuclear ribonucleoprotein E-like [Triticum aestivum]	0	6.254	53.40909	3	4	3	1	88	10.3	9.89	6.19	3
High	XP_044364863.1	lariat debranching enzyme-like [Triticum aestivum]	0	6.215	8.606557	2	6	2	1	488	55.1	6.13	8.63	2
High	XP_044325436.1	nucleolin-like [Triticum aestivum]	0	6.051	3.984576	2	5	1	1	778	87.6	5.69	12.83	2
High	XP_044388315.1	40S ribosomal protein S19 [Triticum aestivum]	0	6.042	23.87097	4	4	4	1	155	17.1	9.89	6.25	4
High	XP_044334998.1	zinc finger CCCH domain-containing protein 32-like [Triticum aestivum]	0	5.863	7.275321	3	4	3	1	701	78.2	5.55	9.66	3

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044397682.1	40S ribosomal protein S17-4-like [Triticum aestivum]	0	5.845	37.06294	3	5	1	1	143	16.5	10.2	6.39	3
High	XP_044423659.1	something about silencing protein 10-like [Triticum aestivum]	0	5.824	7.175573	2	2	2	1	655	72.8	5.22	5.09	2
High	XP_044390792.1	40S ribosomal protein S12-like [Triticum aestivum]	0	5.691	18.75	2	3	2	1	144	15.3	5.74	4.87	2
High	XP_044414706.1	dehydrin DHN4 [Triticum aestivum]	0	5.657	15.58442	2	6	2	1	231	23.2	9.23	18.48	2
High	XP_044449381.1	low-temperature-induced 65 kDa protein-like isoform X1 [Triticum aestivum]	0	5.655	8	2	2	2	1	600	61.3	4.98	3.09	2
High	XP_044390303.1	translation initiation factor eIF-2B subunit delta-like [Triticum aestivum]	0	5.552	7.830343	2	3	2	1	613	66.4	8.72	6.43	2
High	XP_044425964.1	histone deacetylase HDT2 [Triticum aestivum]	0	5.545	18.15642	3	5	3	1	358	38.5	5	7.99	3
High	XP_044426597.1	protein SEEDLING PLASTID DEVELOPMENT 1-like [Triticum aestivum]	0	5.488	6.906475	3	5	3	1	695	75.6	5.71	11.81	3
High	XP_044396038.1	probable mediator of RNA polymerase II transcription subunit 26c [Triticum aestivum]	0	5.402	14.66276	2	2	2	1	341	37.9	5.92	5.13	2
High	XP_044323868.1	small nuclear ribonucleoprotein-associated protein B'-like [Triticum aestivum]	0	5.395	7.924528	2	3	2	1	265	27.9	11.3	6.86	2
High	XP_044339931.1	cysteine proteinase inhibitor 12 [Triticum aestivum]	0	5.394	12.7572	2	3	2	1	243	26.6	6.87	5.56	2
High	XP_044448668.1	60S ribosomal protein L10-2 [Triticum aestivum]	0	5.193	13.24201	3	5	3	1	219	24.7	10.4	7.92	3
High	XP_044324467.1	40S ribosomal protein S10-1-like [Triticum aestivum]	0	5.172	15.87302	2	9	1	1	189	20.6	9.88	27.63	2
High	XP_044325471.1	40S ribosomal protein S17-4-like [Triticum aestivum]	0	5.042	41.95804	3	5	1	1	143	16.4	10.2	4.15	3
High	XP_044338399.1	ruvB-like protein 1 isoform X1 [Triticum aestivum]	0	5.01	5.645161	2	3	2	1	496	54.2	6.01	5.02	2
High	XP_044338627.1	protein IWS1 homolog 1-like [Triticum aestivum]	0	4.901	5.084746	2	3	2	1	531	59.1	5.34	4.53	2
High	XP_044339126.1	nuclear ubiquitous casein and cyclin-dependent kinase substrate 1-like [Triticum aestivum]	0	4.633	18.18182	3	4	3	1	319	34.2	5	8.74	3
High	XP_044404772.1	protein HEADING DATE REPRESSOR 1-like [Triticum aestivum]	0	4.613	15.52511	2	3	2	1	219	24.7	4.69	7	2
High	XP_044372277.1	oleosin 18 kDa-like [Triticum aestivum]	0	4.603	23.71134	2	3	2	1	194	19.3	9.69	5.3	2
High	XP_044370535.1	small heat shock protein, chloroplastic-like [Triticum aestivum]	0	4.548	17.69547	3	3	3	1	243	26.9	8.12	2.81	3
High	XP_044407707.1	eukaryotic translation initiation factor 3 subunit F-like [Triticum aestivum]	0	4.385	9.931507	2	3	2	1	292	31.8	5.25	7.73	2
High	XP_044365009.1	eukaryotic peptide chain release factor subunit 1-3-like [Triticum aestivum]	0	4.382	4.805492	2	4	1	1	437	49	5.6	8.72	2
High	XP_044347284.1	phytepsin isoform X1 [Triticum aestivum]	0	4.275	6.483301	2	4	2	1	509	54.5	5.52	10.35	2
High	XP_044348807.1	ras-related protein RIC1 isoform X1 [Triticum aestivum]	0	4.218	20.36199	3	4	3	1	221	24.3	5.74	6.13	3
High	XP_044375497.1	40S ribosomal protein S7 [Triticum aestivum]	0	4.194	19.27083	2	2	2	1	192	22.2	9.79	4.59	2
High	XP_044372055.1	small nuclear ribonucleoprotein SmD3b-like [Triticum aestivum]	0	4.161	9.62963	2	3	2	1	135	14.5	11.0	5.87	2
High	XP_044404707.1	eukaryotic translation initiation factor 3 subunit G-like [Triticum aestivum]	0	4.152	11.41869	2	2	2	1	289	31.4	7.03	3.04	2
High	XP_044377405.1	histone H2B.11-like [Triticum aestivum]	0	4.14	11.34021	2	4	1	1	291	31.3	9.79	6.88	2
High	XP_044365658.1	uncharacterized protein LOC123087672 [Triticum aestivum]	0	3.972	7.033639	2	2	2	1	327	36.2	5.15	4.35	2
High	XP_044380766.1	polyadenylate-binding protein 8 [Triticum aestivum]	0	3.948	4.608295	2	3	2	1	651	70.7	7.37	4.54	2
High	XP_044323344.1	histone H2A.4 [Triticum aestivum]	0	3.92	28.14815	2	4	1	1	135	14.1	10.0	9.36	2
High	XP_044407019.1	NAD-capped RNA hydrolase DXO1-like isoform X1 [Triticum aestivum]	0	3.841	4.854369	2	3	2	1	515	56.7	5.21	2.23	2
High	XP_044323040.1	eukaryotic peptide chain release factor subunit 1-3-like [Triticum aestivum]	0	3.835	5.491991	2	3	1	1	437	49.1	5.52	4.34	2
High	XP_044425468.1	HMG1/2-like protein [Triticum aestivum]	0	3.8	18.63354	2	5	2	1	161	17.2	6.92	13.73	2
High	XP_044362953.1	17.4 kDa class I heat shock protein [Triticum aestivum]	0	3.779	13.92405	2	2	1	1	158	17.5	6.06	4.14	2

Table A.5 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044394558.1	probable histone H2A variant 2 [Triticum aestivum]	0	3.726	27.33813	2	3	1	1	139	14.6	10.3	7.51	2
High	XP_044431145.1	60S ribosomal protein L17-2 isoform X1 [Triticum aestivum]	0	3.632	10.27027	2	4	2	1	185	21.3	10.2	6.19	2
High	XP_044403524.1	H/ACA ribonucleoprotein complex subunit 3-like protein [Triticum aestivum]	0	3.587	43.75	2	2	2	1	64	7.5	9.79	4.35	2
High	XP_044318289.1	universal stress protein PHOS34 [Triticum aestivum]	0	3.48	18.07229	2	4	2	1	166	17.9	6.19	8.98	2
High	XP_044374025.1	DNA-directed RNA polymerases I and III subunit RPAC2-like [Triticum aestivum]	0	3.384	14.21053	2	4	2	1	190	20.6	7.11	6.2	2
High	XP_044346726.1	ubiquitin-40S ribosomal protein S27a-like [Triticum aestivum]	0	3.351	7.792208	2	3	2	1	231	26.2	9.66	1.6	2
High	XP_044361617.1	uncharacterized protein LOC123083734 [Triticum aestivum]	0	3.234	12.37624	2	4	2	1	202	22.2	5.16	8.56	2
High	XP_044438002.1	V-type proton ATPase subunit B 1-like [Triticum aestivum]	0	3.089	3.85289	2	2	2	1	571	62.9	5.54	3.75	2
High	XP_044364554.1	eukaryotic translation initiation factor 3 subunit B-like [Triticum aestivum]	0	2.932	4.160888	2	3	2	1	721	83.4	5.12	0	2
High	XP_044340029.1	coatomer subunit beta-2-like [Triticum aestivum]	0	2.747	3.578947	2	2	2	1	950	105.3	5.72	0	2
High	XP_044433605.1	heat shock 70 kDa protein BIP5-like [Triticum aestivum]	0	2.71	3.603604	2	2	1	1	666	73.1	5.29	3.5	2
High	XP_044346700.1	eukaryotic translation initiation factor 3 subunit A-like [Triticum aestivum]	0	2.336	2.470356	2	2	2	1	1012	117.1	9.33	1.62	2

Table A.6 nLC-MS/MS for remodeled Pol II (replicate 1)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	57.64	22.59101	26	60	18	1	1868	208	6.2	199.44	26
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	54.084	31.44963	26	64	26	1	1221	138.2	7.15	205.03	26
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	33.605	56.49547	12	36	12	1	331	36.7	4.88	150.49	12
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	29.042	35.42601	13	28	13	1	446	50.2	4.81	104.24	13
High	XP_044391333.1	DNA-directed RNA polymerase II subunit RPB1-like [Triticum aestivum]	0	24.002	8.516336	10	28	2	1	1867	207.7	6.25	78.28	10
High	TFIIIA-7ZF;	Nicotiana benthamiana	0	21.548	42.85714	9	19	9	1	287	33	8.07	60.8	9
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	20.743	79.64602	5	23	2	1	113	11.5	4.41	97.86	5
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	19.85	15.17341	6	19	2	1	692	77	5.52	78.91	6
High	XP_044370961.1	vicilin-like seed storage protein At4g36700 [Triticum aestivum]	0	18.088	15.09695	6	15	2	1	722	81.1	5.85	56.7	6
High	XP_044365264.1	63 kDa globulin-like protein [Triticum aestivum]	0	17.468	20.40816	8	29	4	1	588	66.3	8.27	85.19	8
High	XP_044450611.1	60S acidic ribosomal protein P2B [Triticum aestivum]	0	16.416	78.57143	4	18	1	1	112	11.4	4.34	74.45	4
High	XP_044360175.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	14.66	11.48256	5	15	1	1	688	76.9	6.09	55.25	5
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	13.438	20.62868	7	18	3	1	509	55.4	6.62	61.99	7
High	XP_044416593.1	tubulin alpha-2 chain isoform X2 [Triticum aestivum]	0	12.35	21.98732	6	13	5	1	473	52.2	7.39	51.54	6
High	XP_044373110.1	63 kDa globulin-like protein [Triticum aestivum]	0	11.933	11.80223	6	23	3	1	627	70.6	7.17	64.81	6
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	10.669	16.8932	6	15	2	1	515	56.1	6.11	48.05	6

Table A.6 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044373111.1	63 kDa globulin-like protein [Triticum aestivum]	0	10.37	13.65132	6	23	2	1	608	68.3	7.72	66.04	6
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	10.358	18.4375	3	10	3	1	320	34.5	5.5	38.48	3
High	XP_044324377.1	60S acidic ribosomal protein P2A [Triticum aestivum]	0	7.528	38.0531	3	7	3	1	113	11.6	4.41	24.61	3
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	6.118	13.38028	3	8	3	1	284	30.9	9.58	25.7	3
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	5.529	24.16667	2	7	2	1	120	12.1	4.55	24.22	2
High	XP_044392326.1	FACT complex subunit SSRP1-B-like isoform X1 [Triticum aestivum]	0	5.215	5.231689	2	4	2	1	669	74.4	5.8	12.27	2
High	XP_044324637.1	peptidyl-prolyl cis-trans isomerase FKBP53-like [Triticum aestivum]	0	4.842	6.776181	3	5	3	1	487	53.4	5.83	15.71	3
High	XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	4.819	18.91892	3	5	1	1	148	16.8	5.87	19.35	3
High	XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	4.545	18.91892	3	5	1	1	148	16.9	5.87	19.92	3
High	XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like [Triticum aestivum]	0	4.046	18.18182	3	5	3	1	132	14.7	8.27	13.7	3
High	XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	0	3.352	32.5	2	3	2	1	120	14.1	7.62	9.01	2
High	XP_044423543.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	3.075	2.615694	2	3	2	1	994	110.2	5.4	8.62	2
High	XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	0	2.673	2.247191	2	2	2	1	1068	122	6	5.62	2
High	XP_044364068.1	heterogeneous nuclear ribonucleoprotein Q-like isoform X1 [Triticum aestivum]	0	2.561	6	2	3	2	1	550	60.3	5.2	9.55	2
High	XP_044362686.1	tubulin alpha-2 chain [Triticum aestivum]	0	2.283	7.7951	2	3	1	1	449	49.8	5.2	9.85	2
High	XP_044340687.1	elongation factor 2-like [Triticum aestivum]	0	1.722	4.270463	2	3	2	1	843	93.7	6.07	10.7	2

Table A.7 nLC-MS/MS for remodeled Pol II (replicate 2)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	44.842	28.25553	22	51	22	1	1221	138.2	7.15	157.01	22
High	TFIIIA-7ZF;	Nicotiana benthamiana	0	44.128	58.88502	13	47	13	1	287	33	8.07	168.69	13
High	XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	33.22	13.09013	18	31	1	1	1864	207.8	6.32	100.39	18
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	32.149	12.95503	18	30	1	1	1868	208	6.2	92.39	18
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	29.267	30.26906	11	29	5	1	446	50.2	4.81	104.78	11
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	22.225	42.9003	9	19	9	1	331	36.7	4.88	76.12	9
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	18.83	60.17699	5	24	5	1	113	11.5	4.41	97.59	5
High	XP_044339487.1	tubulin beta-5 chain [Triticum aestivum]	0	18.419	23.04251	8	18	2	1	447	50.3	4.83	58.87	8
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	13.216	16.89587	6	18	2	1	509	55.4	6.62	56.95	6
High	XP_044343678.1	tubulin alpha chain [Triticum aestivum]	0	13.089	25.94235	9	16	7	1	451	49.7	5.02	49.74	9
High	XP_044367849.1	63 kDa globulin-like protein [Triticum aestivum]	0	13.02	17.85714	8	24	8	1	616	69.4	7.06	62	8

Table A.7 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	12.872	27.1875	5	18	5	1	320	34.5	5.5	67.51	5
High	XP_044370961.1	vicilin-like seed storage protein At4g36700 [Triticum aestivum]	0	12.692	8.587258	5	15	1	1	722	81.1	5.85	50.4	5
High	XP_044362369.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	11.927	15.03667	8	10	8	1	818	90.8	5.26	31.46	8
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	10.994	7.65896	5	15	1	1	692	77	5.52	47.45	5
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	9.866	13.59223	5	15	1	1	515	56.1	6.11	44.95	5
High	XP_044324377.1	60S acidic ribosomal protein P2A [Triticum aestivum]	0	9.02	38.0531	3	6	3	1	113	11.6	4.41	24.08	3
High	XP_044414262.1	protein CTR9 homolog [Triticum aestivum]	0	7.815	7.771536	6	7	6	1	1068	122	6	20.7	6
High	XP_044356354.1	importin subunit alpha-1a isoform X2 [Triticum aestivum]	0	6.729	12.40458	4	7	2	1	524	57.3	5.19	20.4	4
High	XP_044451529.1	embryonic protein DC-8-like [Triticum aestivum]	0	6.657	6.730769	3	4	3	1	728	73.1	8.41	14.79	3
High	XP_044432754.1	16.9 kDa class I heat shock protein 2-like [Triticum aestivum]	0	6.337	37.34177	3	6	3	1	158	18	5.71	23.32	3
High	XP_044448581.1	importin subunit alpha-1b-like [Triticum aestivum]	0	5.881	10.48689	3	5	1	1	534	58.6	5.25	15.24	3
High	XP_044429959.1	protein SHORT ROOT IN SALT MEDIUM 1-like isoform X1 [Triticum aestivum]	0	5.365	3.198887	3	5	3	1	1438	161.4	5.67	17.15	3
High	XP_044430028.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	4.85	5.130785	4	6	4	1	994	110.1	5.36	18.17	4
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	4.369	24.16667	2	4	2	1	120	12.1	4.55	16.17	2
High	XP_044340687.1	elongation factor 2-like [Triticum aestivum]	0	4.32	4.270463	2	2	2	1	843	93.7	6.07	7.89	2
High	XP_044322202.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	4.126	18.24324	2	4	1	1	148	16.9	5.87	14.03	2
High	XP_044450962.1	transcription elongation factor SPT6 homolog isoform X1 [Triticum aestivum]	0	3.99	1.654412	2	3	2	1	1632	182.8	5.14	10.24	2
High	XP_044424477.1	40S ribosomal protein S14-like [Triticum aestivum]	0	3.906	12.2449	2	4	2	1	196	20.8	11.0	12.15	2
High	XP_044324940.1	DNA-directed RNA polymerases II and IV subunit 5A-like [Triticum aestivum]	0	3.818	15.78947	2	5	2	1	209	24.1	9.44	15.89	2
High	XP_044330432.1	DNA-directed RNA polymerases II, IV and V subunit 8B-like [Triticum aestivum]	0	3.756	18.24324	2	3	1	1	148	16.8	5.87	9.77	2
High	XP_044346607.1	60S ribosomal protein L9 [Triticum aestivum]	0	3.636	16.93122	2	3	2	1	189	21.3	9.77	11.13	2
High	XP_044439510.1	endoplasmin homolog [Triticum aestivum]	0	3.603	3.4689	2	3	2	1	836	96	5.21	10.14	2
High	XP_044437687.1	glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic [Triticum aestivum]	0	3.391	10.38576	2	5	2	1	337	36.5	7.18	13.64	2
High	XP_044339880.1	RNA-binding protein P-like [Triticum aestivum]	0	3.269	5	2	4	2	1	480	49.5	5.12	10.83	2
High	XP_044399391.1	elongation factor 1-alpha isoform X1 [Triticum aestivum]	0	3.247	5.810811	3	5	3	1	740	83.1	8.97	14	3
High	XP_044346616.1	protein PAF1 homolog [Triticum aestivum]	0	3.097	4.313099	2	3	2	1	626	71.4	7.97	10.54	2
High	XP_044436933.1	putative transcription elongation factor SPT5 homolog 1 [Triticum aestivum]	0	2.981	2.186312	2	4	2	1	1052	115.8	5.38	10.77	2
High	XP_044362686.1	tubulin alpha-2 chain [Triticum aestivum]	0	2.941	9.35412	3	3	1	1	449	49.8	5.2	9.05	3
High	XP_044381819.1	heat shock protein 81-3 [Triticum aestivum]	0	2.715	3	2	2	2	1	700	80.4	5.03	6.18	2
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	2.692	7.394366	2	5	2	1	284	30.9	9.58	14.17	2
High	XP_044372277.1	oleosin 18 kDa-like [Triticum aestivum]	0	2.426	23.71134	2	2	2	1	194	19.3	9.69	6.4	2
High	XP_044373534.1	dehydrin DHN4-like [Triticum aestivum]	0	2.267	9.767442	2	3	2	1	430	41.2	9.06	7.9	2
High	XP_044442770.1	avenin-like b6 [Triticum aestivum]	0	2.14	10.35714	2	2	2	1	280	31.9	7.91	5.34	2
High	XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	0.004	1.722	1.353066	2	2	2	1	2365	275.4	8.78	4.74	2

Table A.8 nLC-MS/MS for remodeled Pol II (replicate 3)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides Sequest HT
High	XP_044398982.1	DNA-directed RNA polymerase II subunit RPB2 [Triticum aestivum]	0	48.142	25.30713	22	56	22	1	1221	138.2	7.15	170.84	22
High	XP_044422531.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	47.978	17.11373	23	43	1	1	1864	207.8	6.32	129	23
High	XP_044444971.1	DNA-directed RNA polymerase II subunit RPB1-like isoform X2 [Triticum aestivum]	0	46.001	16.97002	23	41	1	1	1868	208	6.2	117.38	23
High	XP_044336839.1	60S acidic ribosomal protein P2B-like [Triticum aestivum]	0	26.966	79.64602	6	29	6	1	113	11.5	4.41	112.55	6
High	XP_044388213.1	DNA-directed RNA polymerases II, IV and V subunit 3-like [Triticum aestivum]	0	26.387	42.59819	8	19	8	1	331	36.7	4.88	68.28	8
High	TFIIIA-7ZF;	Nicotiana benthamiana	0	25.4	41.11498	8	18	8	1	287	33	8.07	62.86	8
High	XP_044443638.1	60S acidic ribosomal protein P0 [Triticum aestivum]	0	23.924	18.4375	4	17	4	1	320	34.5	5.5	75.68	4
High	XP_044424578.1	tubulin beta-3 chain-like [Triticum aestivum]	0	18.341	21.30045	8	18	3	1	446	50.2	4.81	63.37	8
High	XP_044360175.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	15.072	12.5	7	16	2	1	688	76.9	6.09	50.73	7
High	XP_044376297.1	vicilin-like seed storage protein At2g18540 [Triticum aestivum]	0	13.854	9.248555	6	15	1	1	692	77	5.52	48.63	6
High	XP_044363443.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	13.692	18.46758	8	17	2	1	509	55.4	6.62	47.93	8
High	XP_044416593.1	tubulin alpha-2 chain isoform X2 [Triticum aestivum]	0	12.886	18.18182	5	12	2	1	473	52.2	7.39	43.81	5
High	XP_044367849.1	63 kDa globulin-like protein [Triticum aestivum]	0	11.504	13.7987	7	19	7	1	616	69.4	7.06	46.08	7
High	XP_044377882.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	10.436	13.41223	6	12	1	1	507	55.3	6.96	32.76	6
High	XP_044339487.1	tubulin beta-5 chain [Triticum aestivum]	0	9.227	16.77852	6	9	1	1	447	50.3	4.83	27.65	6
High	XP_044363815.1	WD repeat-containing protein VIP3-like [Triticum aestivum]	0	9.074	8.695652	2	6	2	1	322	33.6	6.25	17.23	2
High	XP_044323130.1	tubulin alpha-1 chain [Triticum aestivum]	0	8.791	14.44444	4	9	1	1	450	49.6	5.1	29.02	4
High	XP_044369843.1	vicilin-like seed storage protein At2g28490 [Triticum aestivum]	0	8.756	14.17476	5	9	1	1	515	56.1	6.11	24.57	5
High	XP_044339880.1	RNA-binding protein P-like [Triticum aestivum]	0	6.491	4.583333	3	7	3	1	480	49.5	5.12	24.35	3
High	XP_044424046.1	60S acidic ribosomal protein P3-like [Triticum aestivum]	0	6.249	24.16667	2	6	2	1	120	12.1	4.55	24.46	2
High	XP_044381884.1	DNA-directed RNA polymerases II, IV and V subunit 11-like [Triticum aestivum]	0	5.84	32.5	2	4	2	1	120	14.1	7.62	10.53	2
High	XP_044362369.1	cell division control protein 48 homolog E-like [Triticum aestivum]	0	5.794	9.290954	6	7	6	1	818	90.8	5.26	15.23	6
High	XP_044430139.1	40S ribosomal protein S3-3 [Triticum aestivum]	0	5.571	11.61972	3	5	3	1	284	30.9	9.58	13.78	3
High	XP_044451529.1	embryonic protein DC-8-like [Triticum aestivum]	0	5.538	5.082418	2	4	2	1	728	73.1	8.41	12.81	2
High	XP_044430572.1	60S acidic ribosomal protein P1-like [Triticum aestivum]	0	4.92	45.45455	2	4	2	1	110	11.2	4.59	13.05	2
High	XP_044445530.1	40S ribosomal protein S25-2 [Triticum aestivum]	0	4.492	25	2	4	2	1	108	12.1	10.56	11.88	2
High	XP_044346607.1	60S ribosomal protein L9 [Triticum aestivum]	0	4.179	13.75661	2	3	2	1	189	21.3	9.77	9.44	2
High	XP_044439510.1	endoplasmin homolog [Triticum aestivum]	0	4.173	3.708134	2	4	2	1	836	96	5.21	12.25	2

Table A.8 (continued)

Protein FDR Confidence	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequest HT	# Peptides SequestHT
High	XP_044340687.1	elongation factor 2-like [Triticum aestivum]	0	3.91	4.270463	2	3	2	1	843	93.7	6.07	11.08	2
High	XP_044423543.1	110 kDa U5 small nuclear ribonucleoprotein component CLO-like [Triticum aestivum]	0	3.795	3.219316	2	4	2	1	994	110.2	5.4	10.98	2
High	XP_044405773.1	splicing factor 3B subunit 2-like [Triticum aestivum]	0	3.735	3.729604	2	2	2	1	858	96.7	5	5.41	2
High	XP_044399391.1	elongation factor 1-alpha isoform X1 [Triticum aestivum]	0	3.676	2.567568	2	4	2	1	740	83.1	8.97	10.88	2
High	XP_044356354.1	importin subunit alpha-1a isoform X2 [Triticum aestivum]	0	3.654	6.679389	2	4	2	1	524	57.3	5.19	7.83	2
High	XP_044431686.1	pre-mRNA-processing-splicing factor 8A-like [Triticum aestivum]	0	3.277	1.649049	2	4	2	1	2365	275.4	8.78	11.86	2
High	XP_044332259.1	protein LEO1 homolog [Triticum aestivum]	0	2.228	4.682779	2	2	2	1	662	75.7	4.63	5.27	2
High	XP_044387598.1	DNA-directed RNA polymerases I, II, and III subunit RPABC5-like [Triticum aestivum]	0	1.85	18.18182	2	3	2	1	132	14.7	8.27	2.9	2