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Publication Date

2018

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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Identifying Protein Interaction between TCP Transcription Factors and Aux/IAAs and ARFs

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Xiang Wang

Committee in charge:

      Professor Mark Estelle, Chair
      Professor Alisa Huffaker
      Professor Martin F. Yanofsky

2018

The Thesis of Xiang Wang is approved and it is acceptable in quality and form

for publication microfilm and electronically:

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Chair

University of California, San Diego

2018

DEDICATION

First of all, this is dedicated to my mother, Qiuyue Jia, and father, Xudong Wang for always being my strongest support in spite of the distance. You teach me how go through all the ups and downs and always encourage me and love me.

I would also like to dedicate this to my girlfriend, Jingyi for you company, love, and all the great food you cooked.

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ABSTRACT OF THE THESIS

Identifying Protein Interaction between TCP Transcription Factors and Aux/IAAs and ARFs

by

Xiang Wang

Master of Science in Biology

University of California San Diego, 2018

Professor Mark Estelle, Chair

In *Arabidopsis thaliana*, the plant hormone auxin plays critical roles at almost all developmental stages by regulating expression of various auxin response genes. It has been known that auxin controls transcription of auxin-response genes through a core $SCF^{TIR1/AFB}$-Aux/IAA-ARF nuclear signaling module. However, little is known about whether other transcription factors also act in modulating auxin responsive gene expression through direct interactions with these core auxin-signaling components. Our previous work showed that a group of bHLH transcription factors named TEOSINTE BRANCHED1, CYCLODEA, PROLIFERATING CELL FACTORS (TCPs) play an important role in auxin/temperature-

regulated cell growth in Arabidopsis seedlings. In this thesis, we checked whether TCPs function in controlling auxin signaling through direct interaction with Aux/IAAs and ARFs, key transcription factors in auxin regulated gene expression. Using yeast two-hybrid assays, we identified interactions between several TCPs and Aux/IAAs and ARFs. We then confirmed some of these interactions with pull-down experiments. Further, we located the domains that mediate the interactions between TCPs and Aux/IAAs and those between TCPs and ARFs using yeast two-hybrid assays with truncated forms of these TFs. In conclusion, our work has identified interactions between TCPs and key TFs that mediate auxin-regulated transcription, and thus provides novel insights into the molecular mechanisms understanding the auxin signaling and crosstalk between auxin signaling and other signaling pathways.

Introduction

Plant hormones are organic molecules synthesized from different metabolic pathways. Plant growth is affected by both external stimuli, including temperature, water, and internal hormone levels [1]. Since the initial discovery of the plant hormone auxin in 1913, several more have been found in the past several decades. Nine main plant hormones have been extensively studied: abscisic acid (ABA), indole-3-acetic acid (IAA or auxin), brassinosteroids (BRs), cytokinin, gibberellic acids (GA), ethylene, jasmonic acid (JA), strigolactones and salicylic acid (SA) [2].

Auxin has a versatile role in plant development. It regulates almost every aspect of plant growth from embryogenesis to root/shoot elongation, as well as later stages such as flowering or fruit maturation [2-4]. Early studies elucidated that auxin can promote hypocotyl growth and lateral root formation while inhibiting primary root elongation [5-7]. However, the molecular mechanism by which a simple molecule could result in a variety of processes was not clear.

Recent studies in *Arabidopsis thaliana* and other plant species have revealed that auxin is perceived through a co-receptor comprised of an F-box protein of the TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX PROTEINS (TIR1/AFBs) family and a transcriptional repressor of the Auxin/INDOLE ACETIC ACID (Aux/IAA) family [8]. TIR1 and the related AFBs function as auxin receptors and are subunits of SKP1–Cul1–F-box (SCF)-type E3 ligase [9, 10]. AUXIN RESPONSIVE FACTORS (ARFs) bind to Auxin-Responsive Elements (AuxREs) in the promoters of auxin response genes. When auxin level is low, Aux/IAAs accumulate in plant cells and interact with both ARFs and a group of transcription corepressors, including TOPLESS (TPL) and TOPLESS RELATEDs (TPRs), which recruit histone deacetylases (HDACs). The resulting complex represses transcriptional activity of ARFs

1

by reducing histone acetylation levels in the chromatin regions harboring AuxREs and thus cause formation of a tighter chromatin structure that prevents expression of auxin response genes [11-13]. When auxin accumulates at high levels, it binds to the pocket of TIR1/AFBs and promotes the interaction between TIR/AFBs and Aux/IAAs which leads to ubiquitination of Aux/IAAs by the SCF[TIR1/AFB] E3 ligase and degradation by the 26S proteasome [14]. Degradation of Aux/IAAs will result in disassembly of the repressor complex and allow ARFs to activate auxin response genes [15, 16].

In *Arabidopsis*, there are 6 members in the TIR1/AFB family, 29 members in the Aux/IAA family, and 23 members in the ARF family [11, 17]. A typical Aux/IAA protein contains three conserved domains, called domain I, domain II and Phox and Bem 1 (PB1) domain, respectively [18]. Domain I functions as a repression domain and mediates the interaction with TPL and TPRs. Domain II contains a degron and is responsible for the interaction with TIR1/AFBs [18-20]. Mutations in the degron may impair the interaction between Aux/IAAs and TIR1/AFBs, thereby stabilizing Aux/IAAs. Plants harboring these mutations are therefore gain-of-function Aux/IAA mutants and are resistant to auxin [14, 21]. The C-terminal PB1 domain of Aux/IAAs and ARFs are homologous and mediate the interactions between Aux/IAAs and those between Aux/IAAs and ARFs [22]. In addition to the PB1 domain, most ARFs have a conserved N-terminal DNA-binding domain that binds to AuxREs and a middle domain (MD) that confers transcription activation or repression activity.

Expression of proteins in each family and their interaction combinations are highly diverse and tissue specific, which may be a reason why auxin has a vital role in regulating a variety of cellular processes [11, 23, 24]. In addition, accumulating evidence shows that other

transcription factors, e.g., PIF4 and BES1, are also involved in regulating expression of auxin response genes through direct interactions with ARFs [16, 25].

Recent studies have shown that several TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR1 (TCP) transcription factors play a role in auxin-regulated plant growth[26]. TCP transcription factors are named from the species in which they were characterized, TEOSINTE BRANCHED1 in maize (Zea mays), CYCLOIDEA in snapdragon (Antirrhinum majus), and PCF (proliferating cell nuclear antigen factor) in rice (Oryza sativa). In Arabidopsis, there are in total 24 TCPs [27], which are classified into two groups based on sequence similarity of a conserved basic helix–loop–helix (bHLH) motif harbored in each TCP. The bHLH motif facilities DNA binding and interactions between individual TCP proteins [27, 28]. TCP4, a Class II TCP, has been shown to promote Arabidopsis hypocotyl growth by activating expression of *YUCCA5*, an auxin biosynthesis gene [26]. Overexpression of a microRNA-resistant form of TCP4 results in significant hypocotyl elongation [26]. Further, it has been shown that TCP4 directly binds to the promoter of YUC5 and regulates its transcription [26]. On the contrary, TCP15, which is a class I TCP, represses transcription of key auxin biosynthesis genes *YUCCA1*, *YUCCA4*, and *YUCCA6* [29]. According to those studies, Class I and Class II TCPs antagonistically regulate auxin biosynthesis.

Although class I TCPs, e.g. TCP15, act as repressors of auxin biosynthesis, our preliminary data indicated that in response to exogenous auxin, hypocotyl elongation is significantly compromised in the *tcp* triple loss-of-function mutant *tcp8/14/15*. We also found that the expression level of some auxin responsive genes, SAUR15, IAA5, IAA19, were also downregulated. These results suggest that class I TCPs may be positive regulators of auxin

signaling in hypocotyls. Furthermore, by comparing IAA7 (one of Arabidopsis Aux/IAAs) Chromatin Immunoprecipitation Sequencing (ChIP-Seq) data and published TCPs DNA Affinity Purification Sequencing (DAP-Seq) data[30] reveal that these two types of transcription factors bind to the same regions in the promoters of many auxin response genes (two examples are shown in Figure 1). These data suggest that Aux/IAAs and TCPs may act in the same complexes to regulate auxin-responsive gene expression. One possibility is that TCPs directly interact with Aux/IAAs and/or ARFs. To test this hypothesis, we conducted Yeast two-hybrid assays and identified interactions between TCPs with Aux/IAAs and ARFs. We then confirmed the interactions using *in vitro* pull-down assays. Finally, we mapped domains of each type of these transcription factors that mediate these interactions using yeast-two-hybrid assays and truncated versions of TCPs, Aux/IAAs and ARFs.

Materials and Methods

Yeast two-hybrid assay

For yeast two-hybrid assays, full-length cDNAs of Arabidopsis *TCP3, 4, 7, 8, 9, 14, 15, 20, 22, 23*, as well as truncated versions of *TCP15* were cloned into pENTR-smaI vector and then into *pGILDA* vector (Clontech) and *pB42AD* plasmid (Clontech) using GateWay cloning (Intitrogen). Full-length cDNAs of *Aux/IAA1, 3, 7, 14, 17, 19*, as well as truncated versions of *Aux/IAA7, 17* were cloned into *pENTR-smaI* vector and then into pGILDA vector (Clontech) and *pB42AD* plasmid (Clontech) using GateWay cloning. Full-length cDNAs of *ARF6, 8*, as well as their middle domain and PB1 domain were cloned into *pENTR-smaI* vector and then into *pGILDA* vector (Clontech) and *pB42AD* plasmid (Clontech) using GateWay cloning. Cloned *pGild*a and *pB42AD* constructs were co-transformed into yeast strain EGY48 (Clontech). Transformants were selected on SD supplemented with –Ura/–His/–Trp drop-out solution (BD Biosciences) plus glucose medium. To test the interaction between TCPs with Aux/IAAs or ARFs, four largest yeast colonies were plated on SD-galactose/raffinose inducing medium containing –Ura/ –His/–Trp drop-out supplement containing 20 mg/ml X-Gal (5- bromo-4-chloro-indolyl-b-D-galactopyranoside).

In vitro pull-down experiments

To express proteins for *in vitro* pull-down assays, full-length cDNAs of Arabidopsis *Aux/IAA7, 17* were cloned into *pENTR/D-Topo* (Life Technologies, K240020SP) cloning vector and then into *pDEST15* with N-terminus GST-tag (Life Technologies) using GateWay recombination (Life Technologies, 11803-012). Full-length cDNAs of Arabidopsis *TCP3*, and *15* were cloned into pENTR-smaI cloning vector and then into pDEST17 with N-terminus 6XHis-tag (Life Technologies) using GateWay recombination (Life Technologies, 11803-012). Fusion

proteins were expressed in E. coli BL21-AI cells. Successful transformants with highest protein expression levels were inoculated in 5ml antibiotic-LB medium and cultured overnight at 37 °C and then transferred into 200ml antibiotic-LB medium. The culture was then induced for protein expression by adding L-Arabinose to a 0.2% final concentration and cultured for 4 hours at 37 °C. After protein induction, the cells were collected by centrifuge (5000rmp, 20mins at 4°C). The cell pellet was re-suspended in 24ml STE buffer (10 mM Tris pH 8.0, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA) and Lysozyme (100ug/ml). DTT (1 M, 120ul), PMSF (100 mM, 240ul), and Triton X-100 (100%, 360ul) were added to the mixture and shaken at 4°C for 30 mins. Supernatant was then collected by centrifuging the mixture at 4°C for 30 mins. For GST-tagged proteins, the supernatant was mixed with pre-washed GST beads and shaken at 4°C for 2 hours. Bead-protein complex was collected by brief centrifugation and washed with STE buffer 6 times. The purified GST-fusion proteins were then re-suspended in 1ml STE buffer and stored at -80°C. For His-tagged proteins, the supernatant was mixed with 600ul of Ni-NTA agarose (Qiagen, 30210) and applied into affinity purification column. The bead-protein complex was then washed with 1ml washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH8.0) and eluted with 2ml elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH8.0). Equal amount of GST-tagged proteins were mixed with His-TCP3, 15 and incubated at 4 C for 4 h followed by washing and protein blots to detect the eluted His-TCP3, 15 levels. Anti-His (Sigma-Aldrich, H1029) at 1:3,000 dilution and anti-GST (Sigma-Aldrich, G7781-.2ML) at 1:5,000 dilution were used for the western blots.

Results

Full-length TCPs interact with Aux/IAAs and ARF6 in Yeast two-hybrid assays

In order to study the protein interactions at a relatively larger scale, the Matchmaker LexA-based two-hybrid system was used. The proteins of interest are fused to either the DNA-binding domain of the LexA protein or a weak transcription activation domain derived from *Escherichia coli* transcription activation domain. If there is an interaction between the protein fused to the DNA-binding domain and the protein fused to the activation domain, it will create a transcription factor that can bind to the LexA promoter region of the lacZ reporter gene and activate its transcription. When lacZ transcription is activated, the yeast can produce an enzyme called ß-galactosidase. ß-galactosidase will then react with the X-gal in the media, in which a blue color then indicates a protein-protein interaction. *pGilda* and *pB42AD* vectors were used to generate DNA-binding domain fused proteins and activation domain fused proteins.

Both Class I TCP7, 15, and Class II TCP 3 showed strong interaction with Aux/IAA7 and 17 (Fig. 2A, C & G). TCP15 also showed interaction with Aux/IAA1 and 14 (Fig. 2A). In addition, TCP3, 7, 8, and 22 showed interaction with ARF6 (Fig. 2C-E & G). TCP8 and 23 did not interact with any of the selected Aux/IAAs (Fig. 2D & F). ARF6 and ARF8 on DNA-binding (BD) construct showed self-activation while other constructs showed no self-activation (Fig. 2H & I)

TCP3 shows interaction with both Aux/IAA7 and 17 in in-vitro pull down assay

In order to confirm the interactions seen in our yeast two-hybrid assays, an alternative *in vitro* pull down assay was conducted. Proteins of interest were fused to GST-tag and HIS-tag. Fusion proteins were mixed and passed through a column that will catch the GST-tagged protein. The proteins trapped on the column were then be washed for detecting using western blot. If the

GST-tagged protein indeed interacts with the HIS-tagged protein, the HIS-tagged protein will be detected using anti-HIS antibody in western blot. *pDEST15* and *pDEST17* were used to generate GST-tagged protein and HIS-tagged protein, separately.

We showed that TCP3 can be pulled down by both Aux/IAA 7 and 17. The input control showed that a similar amount of His-tagged TCP3 were incubated with equal amount of Aux/IAA7 and 17 (Fig. 3A). The gel showed that the protein expression level of TCP3 was higher than that of TCP15 in *Escherichia coli* after 0.2% arabinose induction (Fig. 3B).

TCP15 N-terminal domain mediates interaction with axr2 and Aux/IAA17

Proteins often interact through specific domains. For example, TIR/AFBs interact with Aux/IAAs through the DII domain, and Aux/IAAs interact with ARFs through the PB1 domain. In order to study what domains of TCP15 and Aux/IAA7, 17 mediate their interaction, different truncated versions of each protein were introduced into *pGilda* and *pB42AD* vectors for yeast two hybrid assays.

TCP15 has 4 annotated domains—2 poly-serine domains, TCP domain, and a glycine-rich domain. C1 version contains the N-terminus poly-ser domain and TCP domain; C2 version contains only the TCP domain; C3 version contains the middle poly-ser domain and gly-rich domain (Fig. 4A). The interaction between TCP15 with axr2 and Aux/IAA17 is mediated through the C1 version, which contains both a poly-ser domain and TCP domain, but not the C2 version containing solely TCP domain (Fig. 4B). The C3 version does not mediate the interaction either (Fig. 4B).

Aux/IAA7 N-terminal domain as well as PB1 domain mediate interaction with TCP15

Truncated versions of Aux/IAA7 were generated based on known domains. Aux/IAA7 has 3 annotated domains—repression domain, DII domain, and PB1 domain. The C1 version contains the N-terminal repression domain; the C2 version contains both N-terminal repression domain and DII domain; C3 version contains only the DII domain; C4 version contains PB1 domain (Fig. 5A). TCP15 showed stronger interaction with the C2 version that contains both the repression domain and DII domain than with the C1 version containing the repression domain only (Fig. 5B). The C4 version that contains the PB1 domain also interacted with TCP15 (Fig. 5B).

ARF6 and ARF8 middle domain mediates interaction with TCPs

Since it was shown that TCPs also interacted with ARF6 and ARF8, truncated versions of each protein were also made. Fragments that contain the middle domain and C-terminal PB1 domain were generated. The middle domain of ARF6 and 8 showed interaction with both Class I TCP14, 15, and Class II TCP3. There was no interaction with the C-terminal PB1 domain (Fig. 6A-C). Since the full-length ARF6 and ARF8 had potent self-activation, we also checked whether the middle domain of these proteins had self-activation or not. We showed that there was no self-activation of ARF6 and 8 middle-domain constructs (Fig. 1I). Overall, these results show some evidence for the direct interaction between TCPs with Aux/IAAs and ARFs, and also the domains mediate their interactions.

Discussion

Aux/IAA transcription repressors interact with ARF transcription factors to regulate auxin response genes and plant growth. Aux/IAAs and ARFs are also known to interact with co-repressors or other transcription factors, e.g., TOPLESS, PIF4, to co-regulate auxin response [12, 25]. In recent studies, TCP transcription factors were shown to be involved in antagonistically regulating auxin biosynthesis [26, 29]. Our previous experiments also showed that upon exogenous auxin treatment, the hypocotyl elongation was significantly influenced in a *tcp8/14/15* loss of function triple mutant.

Here, we show that in Y2H assays both Class I and Class II TCPs can interact with Aux/IAAs and ARFs. We noted that most TCPs tested in our experiment showed very strong interactions with Aux/IAA7, 14, and 17 but weak or no interaction with other tested Aux/IAAs. A possible reason is that TCPs may interact with a specific group of Aux/IAAs as Aux/IAA7, 14, and 17 are the closest homologs on the phylogenic tree [23]. Another possibility is that the other Aux/IAAs were poorly expressed in yeast. A western blot to ensure those proteins are truly expressed will be needed in the future.

Based on our Y2H assay results, we further conducted *in vitro* pull down assay of TCP3 and TCP15. We found that TCP3 can be pulled down by both Aux/IAA7 and 17; however, we were not able to detect TCP15. We checked the protein expression level and noticed that TCP15 was poorly expressed in *E.coli*. Although we tried to increase the amount of input TCP15, we were still not able to pull it down with either Aux/IAA7 or 17. Alternative experiments such as Bimolecular Fluorescence Complementation (BiFC) may be used to validate the unsuccessful TCP15 pull down. Co-immunoprecipitation assay will also be needed to confirm that TCPs are in the same complex with Aux/IAAs or ARFs in vivo.

It has been shown that the TCP domain is known to facilitate DNA binding and interactions between individual TCP proteins because of the b-HLH motif [27]. It is also known that the C-terminal PB1 domain of Aux/IAAs and ARFs mediates the interactions between Aux/IAAs to form oligomers and also those between Aux/IAAs and ARFs [22]. Most ARFs also can dimerize through a conserved N-terminal DNA-binding domain. In this thesis, we showed that the TCP15 N-terminus (Amino acid 1-111) contains a poly-serine and TCP domain that mediates interaction with axr2 and Aux/IAA17. Therefore, we hypothesized that it may also facilitate interaction with other proteins. However, the TCP domain itself did not interact with Aux/IAAs. Since we did not have a TCP15 fragment of the N-terminus (Amino acid 1-48) containing the poly-serine domain, we are not sure whether TCP domain requires the poly-serine to interact with Aux/IAA7, and 17 or whether the N-terminal region with the poly-serine domain itself is sufficient for the interaction. Further yeast two-hybrid assays of N-terminal version with the poly-serine domain alone are needed to test the hypothesis. In terms of Aux/IAA7 fragments, we noted that TCP15 showed stronger interaction with the N-terminus (Amino acid 1-123) of Aux/IAA7 that contains the DI domain and DII domain. The PB1 domain also contributes some interaction with TCP15 but to a lesser extent than DI and DII combined. For ARF6 and ARF8 fragments, we found that the middle domain ARF6 and 8 interacted with TCP3, 14, and 15 but not C-terminal that contains PB1 domain. Fragments of the N-terminal DNA-binding domain will be needed to study whether TCPs can interact with ARFs through this domain in addition to the middle domain.

By combining our auxin response experiment with protein interaction screening, we confirmed that TCPs directly interact with Aux/IAAs and ARFs to regulate auxin responsive gene expression. Interestingly, TCPs interact with Aux/IAAs and ARFs through non-canonical

domains, which suggests that TCPs can form a complex with both Aux/IAAs and ARFs. We also know that Class I TCPs such as TCP15 can repress the auxin biosynthesis genes, e.g. YUCCA1, 4, and 6. Therefore, we propose a model in which Class I TCPs repress auxin biosynthesis while interacting with Aux/IAAs and ARFs to promote the auxin signaling (Figure. 7). In conclusion, our work has identified novel interactions between TCPs and key transcription factors and repressors that mediate auxin-induced gene regulation while providing further insights into the molecular mechanisms of auxin signaling.

Figures
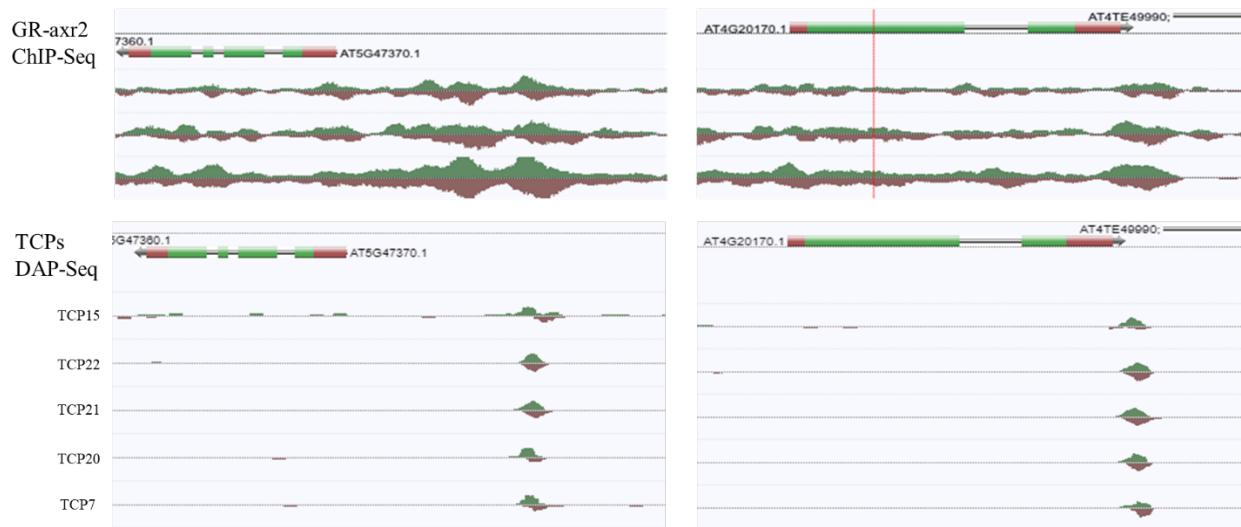
Figure 1. Binding site comparison between GR-axr2 ChIP-Seq and TCP DAP-Seq data. There are overlapping peaks between axr-2 ChIP-Seq and multiple TCPs DAP-Seq data of two auxin response genes. axr-2 is the gain-of-function version of Aux/IAA7. N=3 for GR-axr2 ChIP-Seq experiment.

Figure 2. Yeast two-hybrid assay of full-length TCPs, Aux/IAAs, and ARF6. TCP3, 7, 8, 14, 15, 22, and 23 show interaction with Aux/IAAs or ARF6. A-G) Negative controls for both BD and AD vectors. H and I) axr2 is the gain-of-function version of Aux/IAA7. BD means DNA binding domain (Bait). AD means activation domain (Prey).

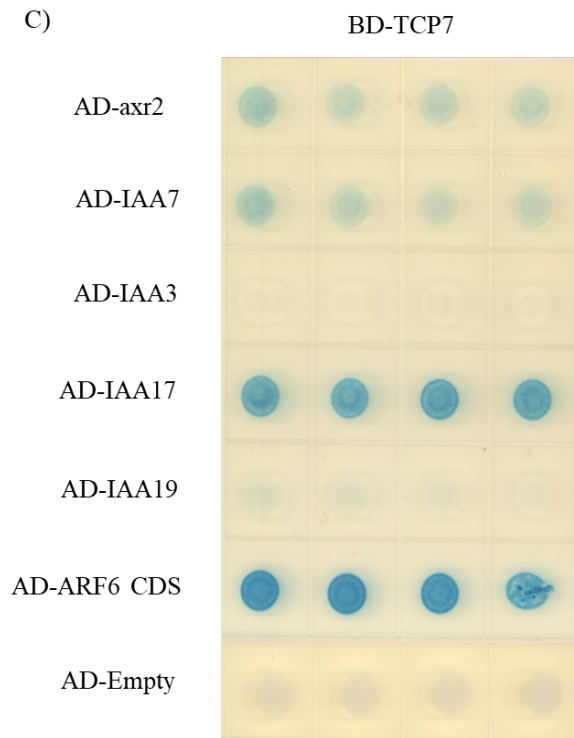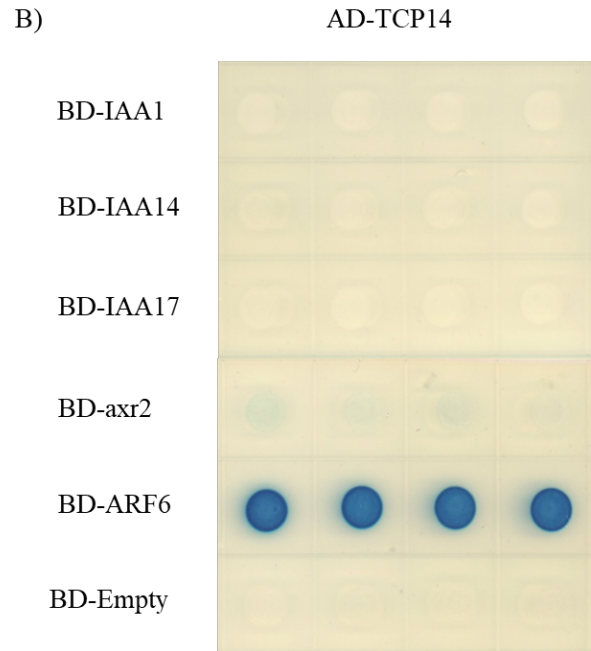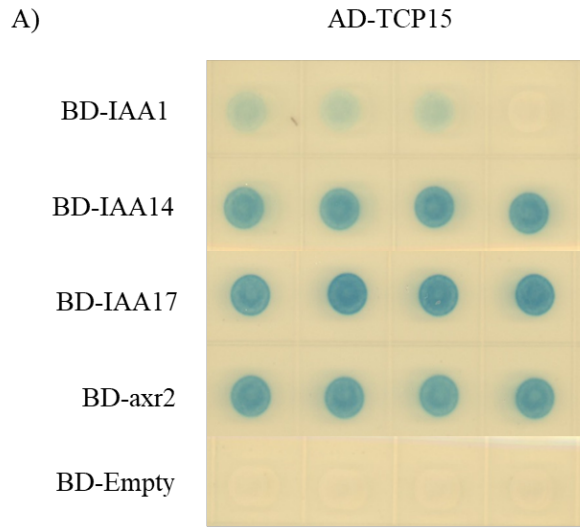A) AD-TCP15

BD-IAA1

BD-IAA14

BD-IAA17

BD-axr2

BD-Empty

B) AD-TCP14

BD-IAA1

BD-IAA14

BD-IAA17

BD-axr2

BD-ARF6

BD-Empty

C) BD-TCP7

AD-axr2

AD-IAA7

AD-IAA3

AD-IAA17

AD-IAA19

AD-ARF6 CDS

AD-Empty

D) BD-TCP8

AD-axr2

AD-IAA7

AD-IAA3

AD-IAA17

AD-IAA19

AD-ARF6 CDS

AD-Empty

E)

BD-TCP22

F)

BD-TCP23

Figure 2, Continued.

G)

BD-TCP3

| | AD-IAA3 |
| AD-IAA7 |
| AD-IAA17 |
| AD-IAA19 |
| AD-ARF6 CDS |
| AD-Empty |

H)

BD-Empty

| AD-ARF6 |
| AD-IAA7 |
| AD-IAA3 |
| AD-IAA17 |
| AD-IAA19 |

I)

AD-Empty

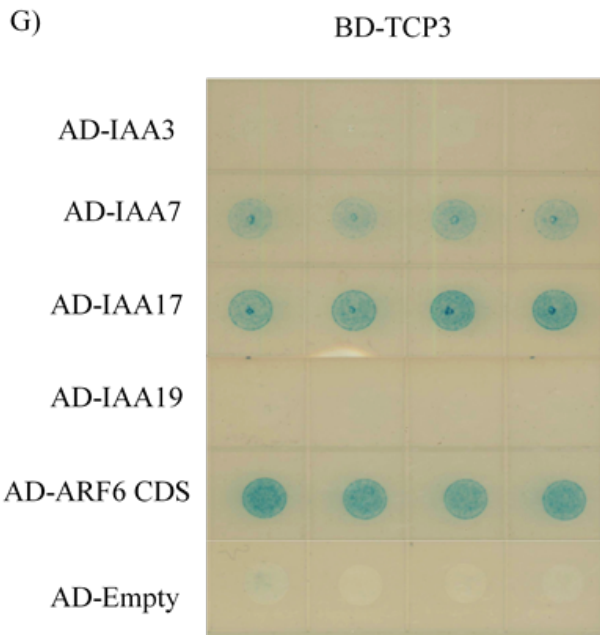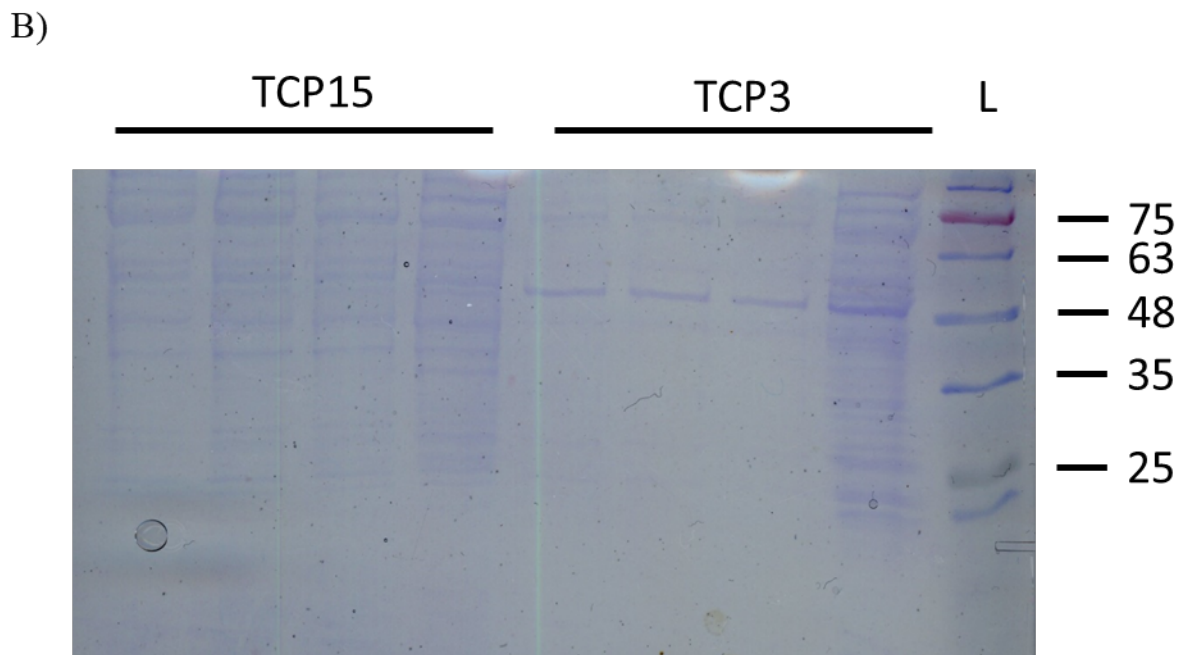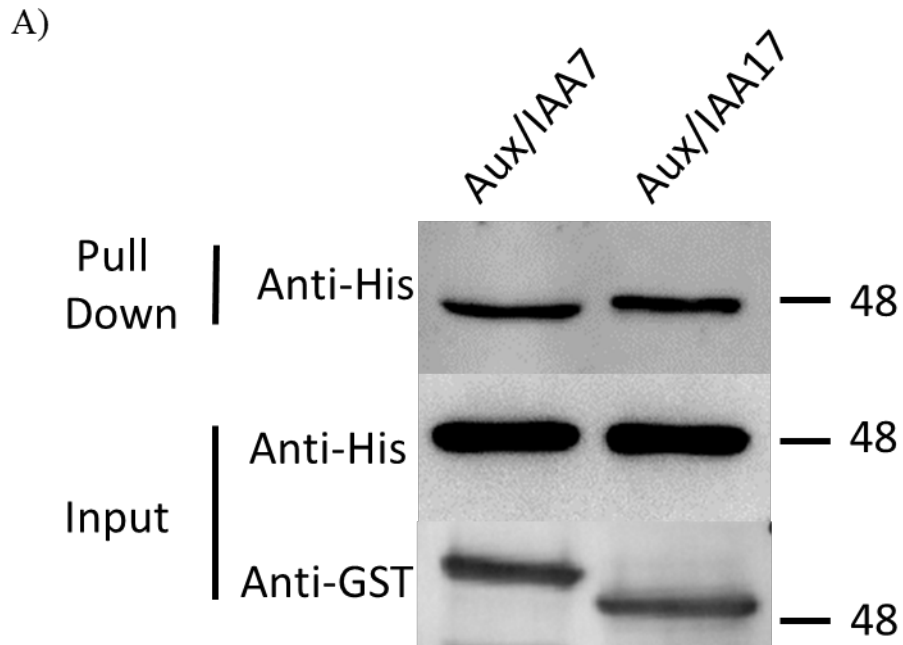| BD-ARF6 |
| BD-ARF6 MID |
| BD-ARF8CDS |
| BD-ARF8 MID |
| BD-IAA14 |
| BD-IAA17 |
| BD-axr2 |
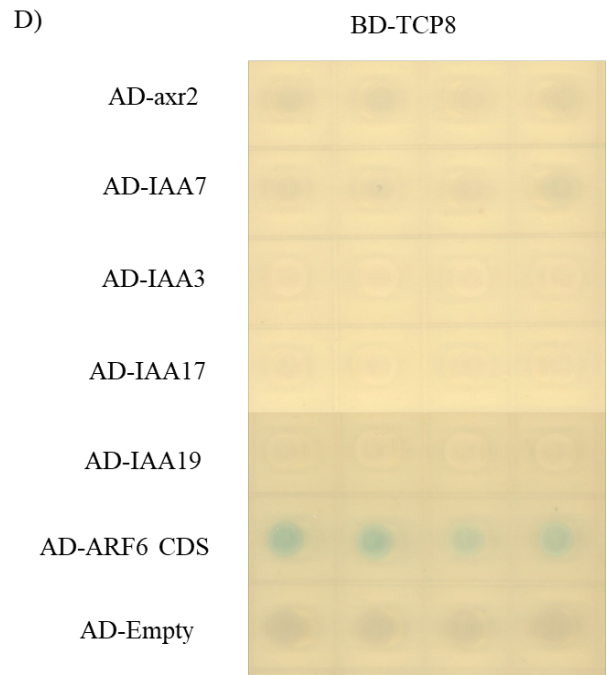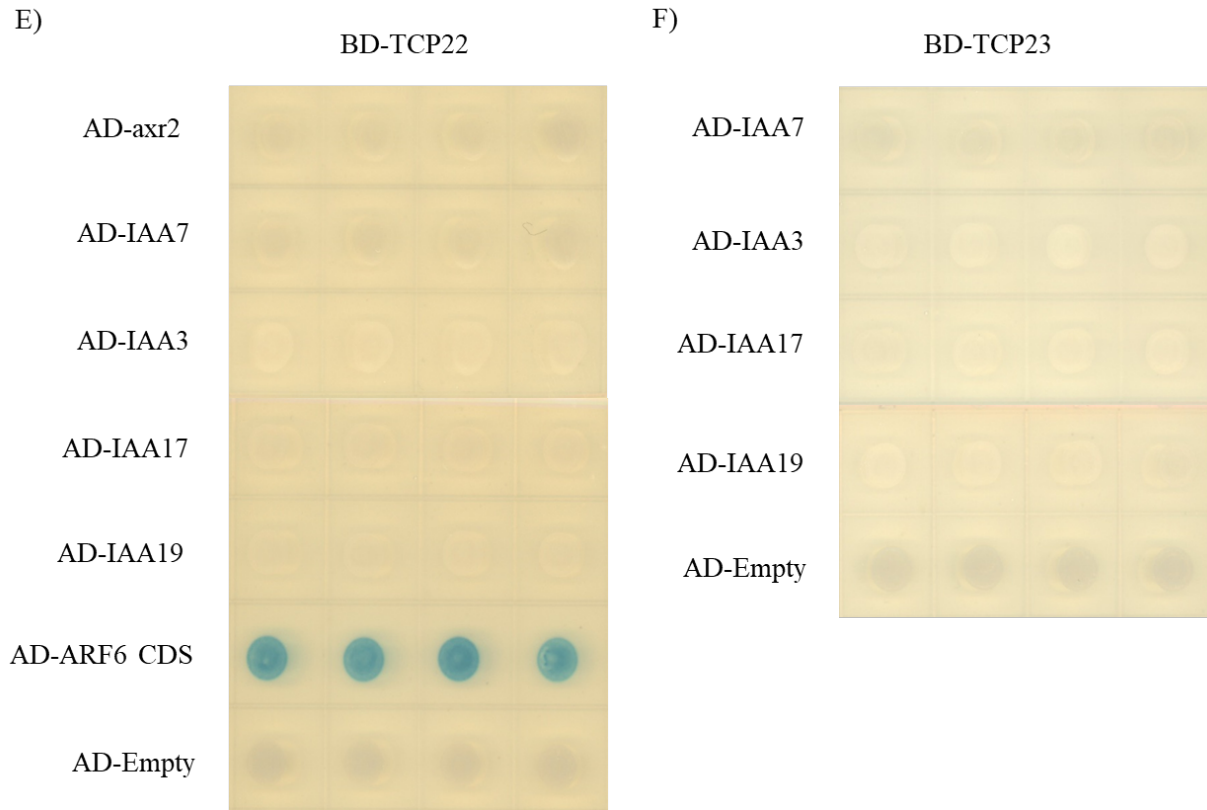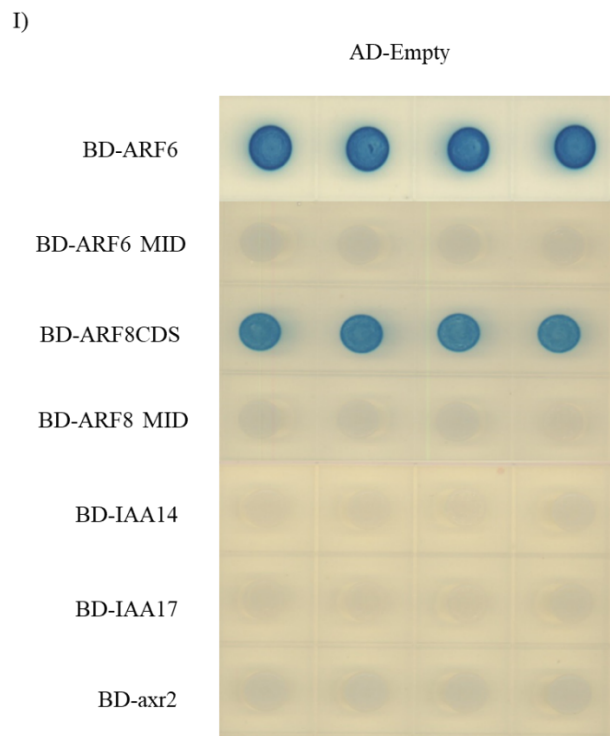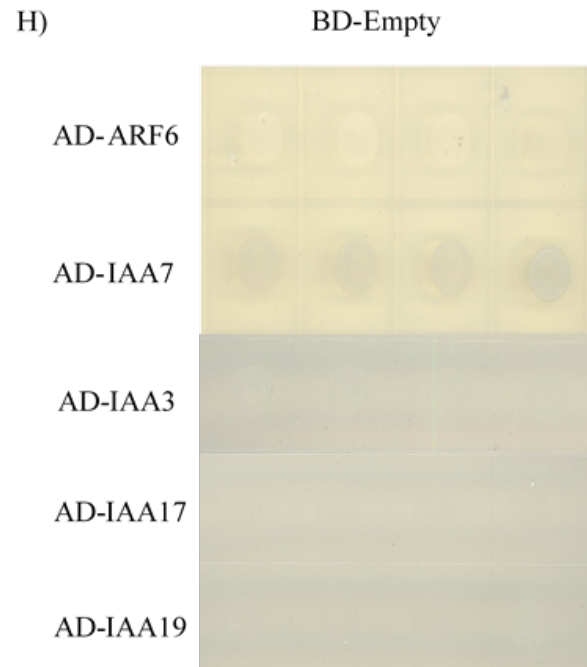
Figure 3. *in vitro* pull-down assay of His-tagged TCP3 with GST-tagged Aux/IAA7, 17. TCP3 interact with both Aux/IAA7 and 17. A) Elution of purified His-TCP3, and His-TCP15 stained with Coomassie Blue. B) L is the protein ladder.
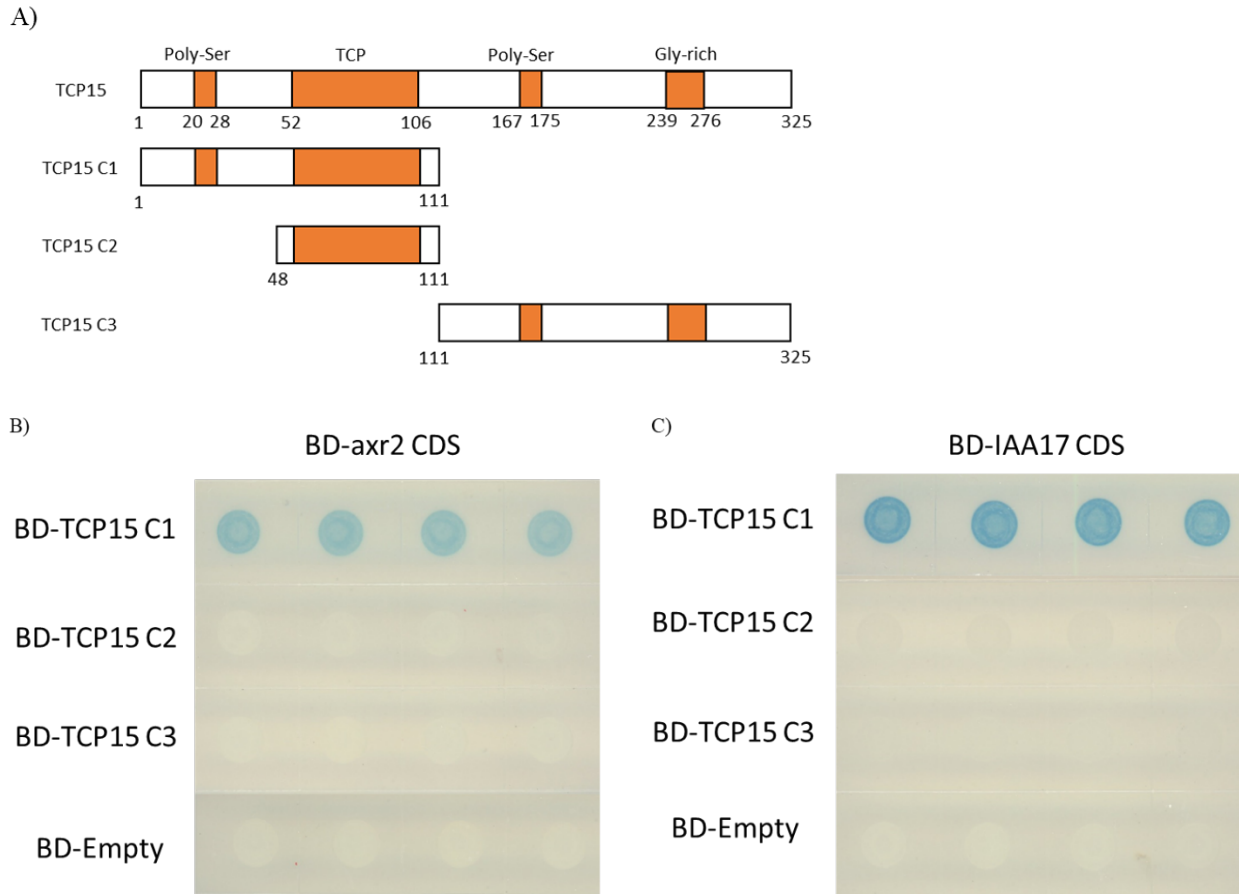
Figure 4. Yeast two-hybrid assay of truncated versions of TCP15 with full-length axr2 and Aux/IAA17. Diagram of annotated full-length and truncated versions TCP15. A) TCP15-C1 interacts with both axr2 and Aux/IAA17. B and C) axr2 is the gain-of-function version of Aux/IAA7. BD means DNA binding domain (Bait). AD means activation domain (Prey).
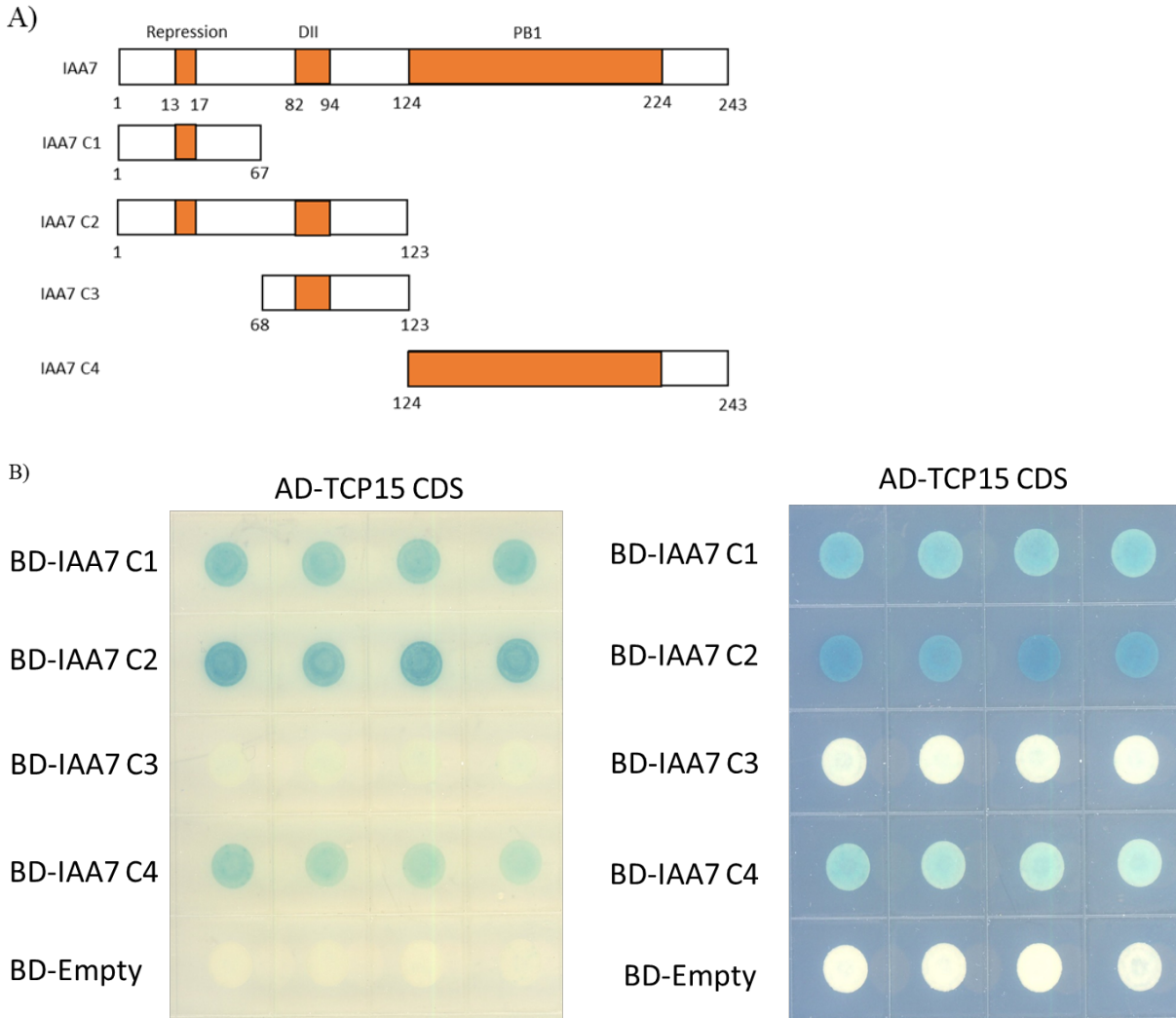
Figure 5. Yeast two-hybrid assay of truncated versions of Aux/IAA7 with full-length TCP15. Diagram of annotated full-length and truncated versions Aux/IAA7. A) Aux/IAA-C1,C2, and C4 but not C3 interact with TCP15. B) axr2 is the gain-of-function version of Aux/IAA7. BD means DNA binding domain (Bait). AD means activation domain (Prey).
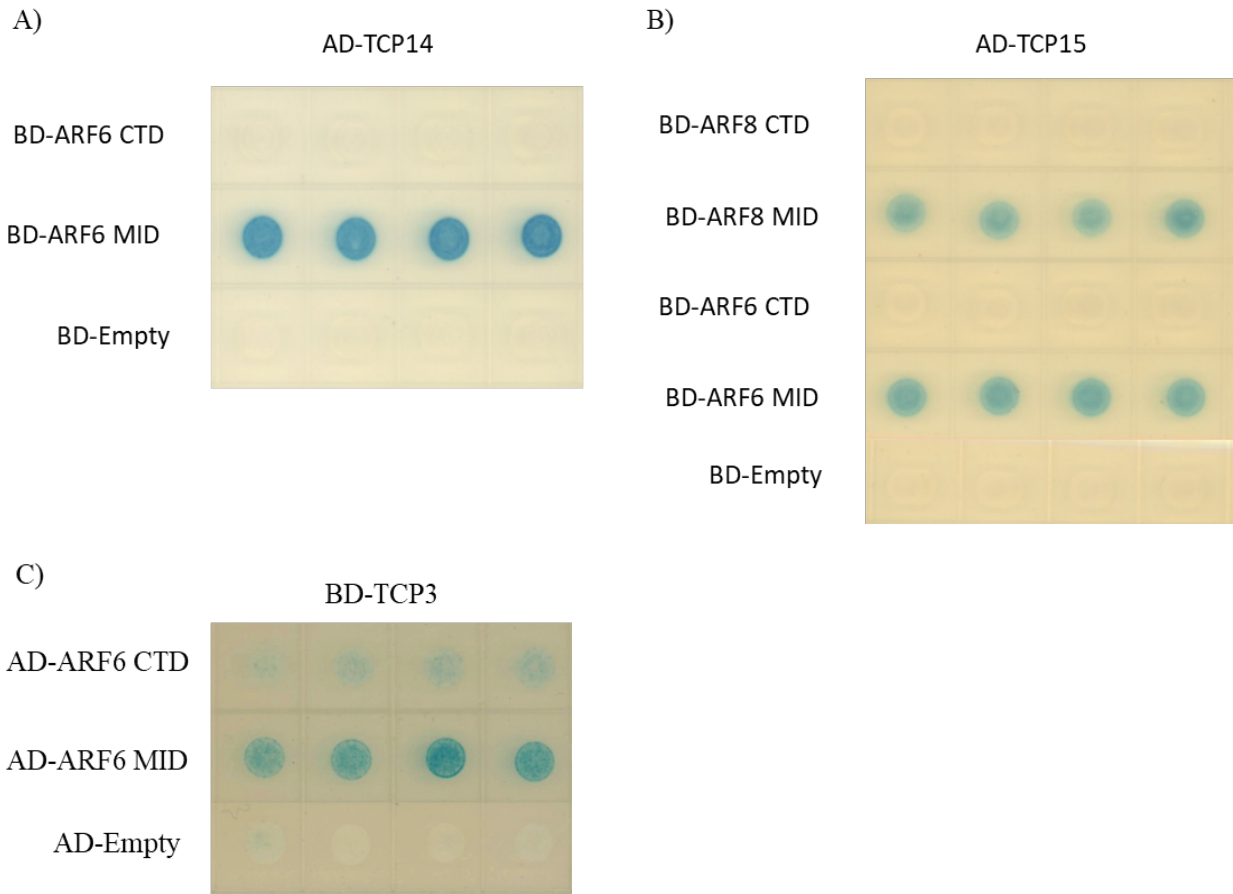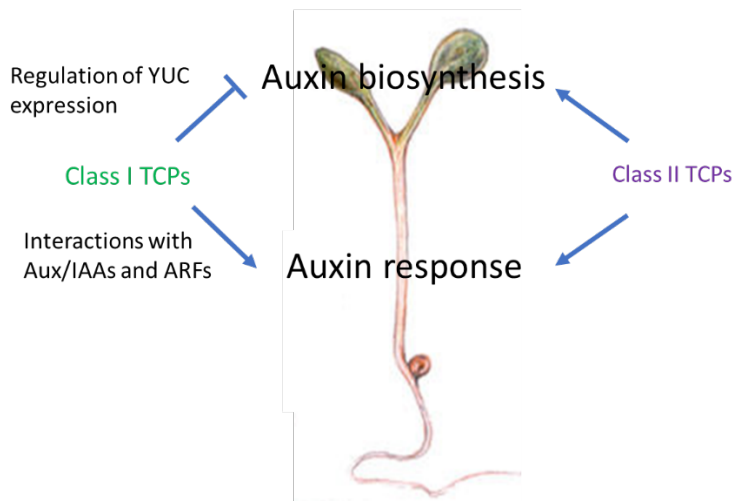
Figure 6. Yeast two-hybrid assay of truncated versions of ARF6 and 8 with full-length TCPs. TCP3, 14, and 15 interact with the middle domain of ARF6 and ARF8. A, B, and C) CTD means C-terminal domain containing PB1 motif. MID means middle domain. BD means DNA binding domain (Bait). AD means activation domain (Prey).
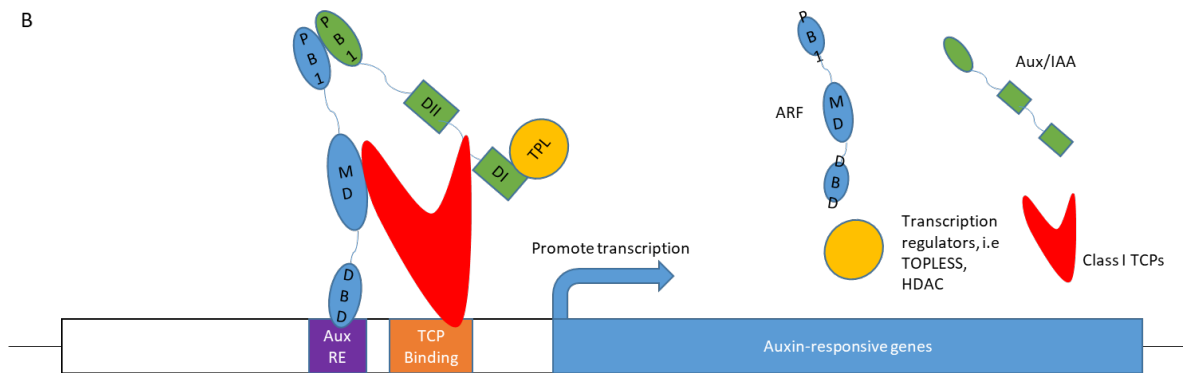
Figure 7. Possible working model for the interactions between TCPs with Aux/IAAs and ARFs in regulating auxin responsive genes. Both Class I and Class II TCPs can promote auxin response; Class I TCPs repress and Class II TCPs promote auxin biosynthesis. A) TCPs interact with Aux/IAAs DI/DII domain while interacting with ARFs middle domain (MD), thus forming a complex. TCPs also bind to the DNA together with ARFs to promote transcription of auxin responsive genes. B)

Table 1. List of Plasmids/Vectors used in this thesis.

Vector Name	Description/Purpose	Selection Marker	Tag
pENTR-smaI	To insert the genes of interest into the entry clone	Kanamycin resistant	N/A
pGilda	Contains the DNA-binding domain of LexA protein driven by GAL1 promoter	Ampicillin resistant Growth on media that lacks tryptophan	LexA-tag
pB42AD	Contains the Activation domain derived from E.coli driven by GAL1 promoter	Ampicillin resistant Growth on media that lacks histidine	HA-tag
pDEST15	Contains a N-terminus GST-tag	Ampicillin resistant	GST-tag
pDEST17	Contains a N-terminus 6xHis-tag	Ampicillin resistant	His-tag (6x)

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