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Heidi Szemenyei, *et al.*

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Materials and Methods
SOM Text
Figs. S1 to S9
References

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TOPLESS Mediates Auxin-Dependent Transcriptional Repression During *Arabidopsis* Embryogenesis

Heidi Szemenyei,[1,2] Mike Hannon,[1,2] Jeff A. Long[1]*

The transcriptional response to auxin is critical for root and vascular development during *Arabidopsis* embryogenesis. Auxin induces the degradation of AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) transcriptional repressors, freeing their binding partners, the AUXIN RESPONSE FACTOR (ARF) proteins, which can activate transcription of auxin response genes. We show that TOPLESS (TPL) can physically interact with IAA12/BODENLOS (IAA12/BDL) through an ETHYLENE RESPONSE FACTOR (ERF)–associated amphiphilic repression (EAR) motif. TPL can repress transcription in vivo and is required for IAA12/BDL repressive activity. In addition, *tpl-1* can suppress the patterning defects of the *bdl-1* mutant. Direct interaction between TPL and ARF5/MONOPTEROS, which is regulated by IAA12/BDL, results in a loss-of-function *arf5/mp* phenotype. These observations show that TPL is a transcriptional co-repressor and further our understanding of how auxin regulates transcription during plant development.

The phytohormone auxin has been implicated in many aspects of plant biology, including pattern formation during embryogenesis, lateral organ development, and cell expansion (*1*). Auxin mediates these activities through a signaling cascade involving AUXIN RESPONSE FACTORS (ARFs), which constitute a family of transcription factors that activate or repress expression of auxin response genes (*2*), and the AUX/IAA family of transcriptional repressors (*3*). AUX/IAAs negatively regulate auxin-mediated transcription by binding ARFs through conserved domains III and IV found in both types of proteins (*4*, *5*).

Domain II of AUX/IAAs interacts with the auxin receptor TRANSPORT INHIBITOR RESISTANT (TIR1), part of a Skp1/cullin/F-box protein (SCF) complex (*6*–*8*). Auxin stabilizes this interaction, leading to the degradation of AUX/IAAs and allowing ARFs to activate transcription. Mutations in AUX/IAAs that disrupt the interaction with the TIR1 protein family abolish their auxin-induced degradation (*8*). For example, *bodenlos-1* (*bdl-1*) contains a stabilizing mutation (P74S, substitution of proline 74 with serine) in *IAA12* (*9*). IAA12/BDL physically interacts with AUXIN RESPONSE FACTOR5/MONOPTEROS (ARF5/MP) and represses its activity (*9*). Consistent with this, both *bdl-1* mutants and loss-of-function *ARF5/MP* alleles display reduced vasculature and form a "basal peg" instead of a root and hypocotyl (the seedling stem) (*10*–*12*).

Although no prior connection has been made to auxin signaling, the most severe *topless-1* (*tpl-1*) phenotype, a homeotic transformation of the apical pole (the shoot) into a second basal pole (the root), suggests that it plays a role in this pathway (*13*). *tpl-1* is temperature sensitive and displays a range of phenotypes, all disrupting the patterning of the apical half of the embryo. The *tpl-1* mutation is a histidine substitution at asparagine 176 (N176H), and this allele acts as a dominant negative for the TOPLESS RELATED (TPR) family (*14*). On the basis of its domain structure and genetic interactions with a histone deacetylase and a histone acetyl transferase, TPL has been proposed to be a transcriptional co-repressor (*14*).

Transcriptional co-repressors do not bind DNA directly, but are recruited to DNA through interactions with DNA-binding transcription factors (*15*). Determining a co-repressor's binding partners can provide insight into its biological relevance. Therefore, we performed yeast two-hybrid screens to find protein interactors using full-length TPL or the N terminus of TPL (Fig. 1A). Multiple AUX/IAAs, including IAA12/BDL, were among the positive clones in both screens (table S2). IAA12/BDL contains the four conserved domains found in most AUX/IAAs (domains I to IV) (Fig. 1A). Through truncation analyses, we determined that domain I of IAA12/BDL is necessary and sufficient to interact with the C-terminal to lissencephaly homology (CTLH) domain of TPL (*16*) (Fig. 1, B and C).

Domain I of the AUX/IAAs contains an ERF-associated amphiphilic repression (EAR) motif (*17*), which is known to be involved in transcriptional repression (*18*). To determine if this motif is essential for the interaction with TPL, we tested an IAA12/BDL construct in which three leucines at the core of the EAR domain were changed to alanines (IAA12/BDL mEAR) (Fig. 1A). These changes to the EAR domain severely weakened the interaction between TPL and IAA12/BDL both in yeast two-hybrid assays (Fig. 1C) and assays in which glutathione *S*-transferase (GST)–tagged in vitro–translated products were used (Fig. 1D).

We also tested the interaction between IAA12/BDL and TPL using plant lysates. We found that column-bound GST-IAA12/BDL and GST-N-TPL could affinity purify TPL fused to a 6× hemagglutinin tag (TPL-HA) from plant extracts (Fig. 1E), whereas purified GST or beads alone could not, further indicating that TPL interacts with IAA12/BDL. These results also demonstrate that TPL can homodimerize, a characteristic shared with other transcriptional co-repressors (*19*). GST-N-TPL N176H could also pull down BDL P74S–green fluorescent protein (GFP) from plant lysates (Fig. 1F). Mutations in the EAR domain of GST-IAA12/BDL disrupted the interaction with TPL (Fig. 1E). These interactions were also observed in a tobacco transient expression system (*20*) by means of bimolecular fluorescence complementation (BiFC) (*21*) (fig. S2).

To investigate the biological significance of these physical interactions, we analyzed *tpl-1bdl-1* double mutants. *bdl-1* seedlings form a basal peg (Fig. 2C), lacking hypocotyl and root structures of wild-type seedlings (Fig. 2A), whereas *tpl-1* roots appear normal (Fig. 2B). *tpl-1bdl-1* seedlings formed hypocotyls and roots (Fig. 2D and table S3), indicating that *tpl-1* can suppress the basal patterning defects seen in *bdl-1*. *bdl-1* mutants also display a reduction in cotyledon (seed leaf) vasculature development (Fig. 2G). Wild-type and *tpl-1* cotyledons develop a primary midvein with loops of lateral veins along the margins (Fig. 2, E and F). In *bdl-1*, these veins are either absent or severely reduced (n = 70) (Fig. 2G) (*10*). Vasculature formation was

[1]Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. [2]Division of Biological Sciences, Section of Cell and Developmental Biology, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.

*To whom correspondence should be addressed. E-mail: long@salk.edu

restored in the cotyledons of *tpl-1bdl-1* seedlings, although lateral veins often did not form loops ($n = 50$) (Fig. 2H). Therefore, *tpl-1* can suppress defects in both the apical and basal halves of *bdl-1* seedlings.

We then examined these genetic interactions during embryogenesis. In wild-type embryos, the hypophysis (the uppermost cell of the extra-embryonic suspensor) divides asymmetrically, resulting in a lens-shaped cell (Fig. 2I) that will form the quiescent center of the root meristem. These cells express the synthetic auxin response reporter $DR5_{rev}$::*GFP* (Fig. 2M) (*4*, *22*), which is correlated with the accumulation of high auxin concentrations and is dependent on MP activity (*22*, *23*). *tpl-1* embryos (Fig. 2J) also generate a lens-shaped cell and display a wild-type $DR5_{rev}$::*GFP* expression pattern (Fig. 2N). *bdl-1* embryos, in which the hypophysis divides abnormally, lack the lens-shaped cell (Fig. 2K), and $DR5_{rev}$::*GFP* expression is not detectable in hypophyseal cell derivatives ($n = 50$) (Fig. 2O) (*10*, *23*). In *tpl-1bdl-1* embryos, the lens-shaped cell is restored (Fig. 2L), and $DR5_{rev}$::*GFP* expression is again detected in the developing embryonic root ($n = 55$) (Fig. 2P). This rescue, combined with the interaction of TPL and the EAR domain of IAA12/BDL, suggests that TPL is involved in IAA12/BDL-mediated transcriptional repression. Therefore, we hypothesized that in the *tpl-1* background, IAA12/BDL cannot fully repress transcription of its target genes.

To test this hypothesis, we used a UAS/GAL4-based transcriptional repression assay in planta (Fig. 3A) (*24*). Plants expressing TPL-GAL4 and IAA12/BDL P74S-GAL4 fusions displayed a decrease in β-glucoronidase (GUS) activity in a wild-type background compared to the control line (Fig. 3B) (74.6 ± 1.2% and 76.7 ± 6.9% of the control, respectively). Their representative staining patterns are shown in Fig. 3, C to F. The ability of IAA12/BDL P74S-GAL4 to repress transcription was diminished in the *tpl-1* background (Fig. 3, B and E). These data show that TPL can repress transcription and suggest the role of TPL as a transcriptional co-repressor in AUX/IAA-regulated transcriptional repression.

Single and multiple loss-of-function *aux/iaa* mutants are reported to have subtle or no discernible phenotypes (*25*). However, we hypothesized that further loss of AUX/IAA function might enhance the *tpl-1* phenotype. Therefore, we examined the effect of a transferred DNA (T-DNA) insertion allele (Salk_138684) (*26*) of *IAA12/BDL* on *tpl-1*. This allele of *IAA12/BDL* has no obvious phenotype, whereas *tpl-1* exhibits a range of phenotypes when grown at 21°C (*13*). A higher frequency of severe seedling phenotypes was seen in the double-mutant background versus *tpl-1* alone when grown at 21°C (table S1), suggesting that decreased AUX/IAA function contributes to the *tpl-1* phenotype.

AUX/IAAs are proposed to bind ARFs and inhibit transcription of ARF target genes. Because IAA12/BDL binds ARF5/MP and also interacts with TPL, we reasoned that these three proteins act in a complex. We examined this possibility using BiFC (*21*) in a tobacco transient expression system (*20*). Although we observed no direct interaction between either TPL or TPL N176H and ARF5/MP (Fig. 4A and fig. S2I), interaction was observed upon coexpression of stabilized IAA12/BDL (Fig. 4, B and C). This suggests that these proteins can exist in a complex and that IAA12/BDL represses ARF5/MP by recruiting

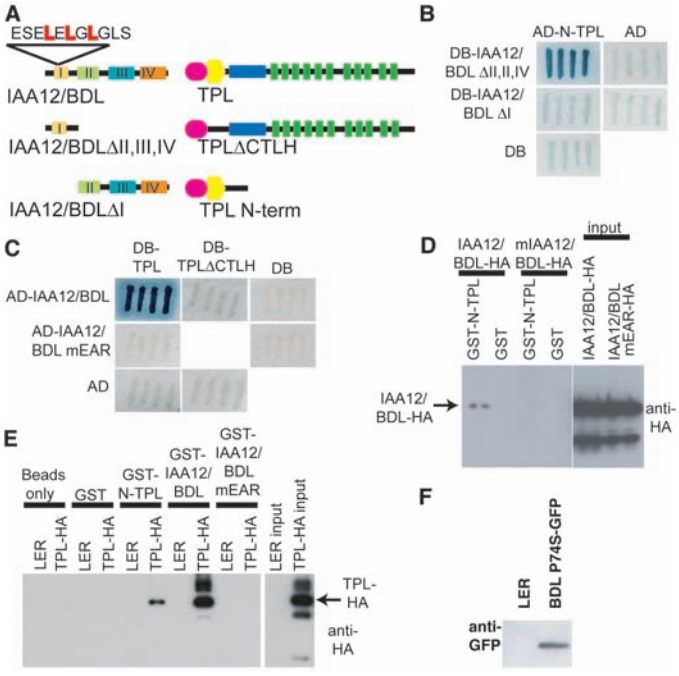

Fig. 1. Interactions between TPL and IAA12/BDL. (**A**) Diagrams of TPL and IAA12/BDL constructs for interaction studies. Core residues of the EAR domain of IAA12/BDL are indicated in red. Pink ellipses represent LisH domains, yellow hexagons represent CTLH domains, blue boxes represent proline-rich regions, and green boxes represent WD40 repeats of TPL. (**B**) TPL interacts with domain I of IAA12/BDL in yeast. Interaction does not occur when domain I is truncated. (**C**) TPL and IAA12/BDL interact much less strongly when the EAR domain is mutated (IAA12/BDL mEAR) compared to the wild type. IAA12/BDL does not interact with TPL without the CTLH domain. AD, activation domain; DB, DNA binding domain. (**D**) The GST-N-TPL fusion protein can interact with in vitro–translated IAA12-HA, but does not interact strongly with IAA12/BDL mEAR. (**E**) Interaction results of extracts from nontransgenic or transgenic plants expressing TPL-HA with either beads alone or bound GST fusion proteins. LER indicates wild-type Landsberg *erecta* extracts. (**F**) GST-N-TPL N176H pulldown assay from nontransgenic or transgenic plants expressing BDL P74S-GFP. (See supplementary fig. 1, A and B, for loading controls.)

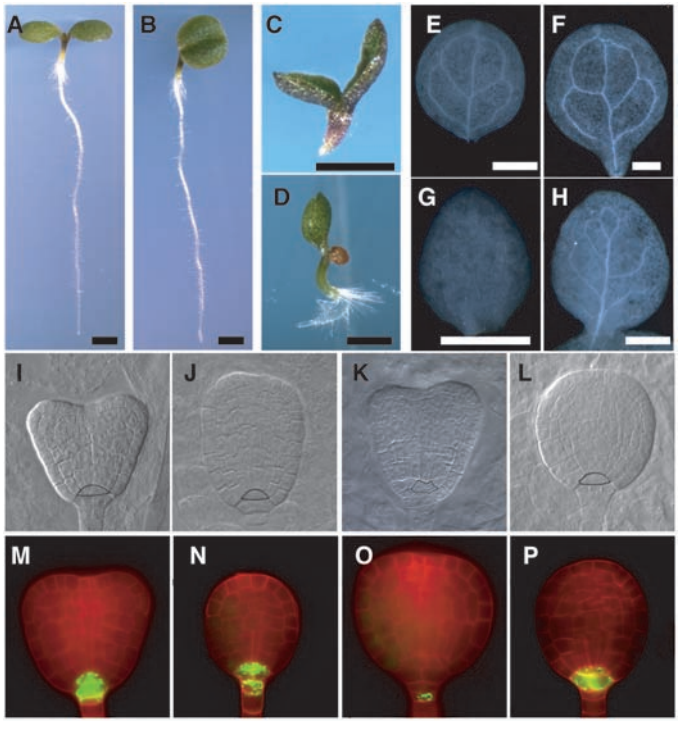

Fig. 2. *tpl-1* suppresses *bdl-1*. (**A** to **D**) Seedlings, 4 days postgermination (dpg). Wild type (A), *tpl-1* (B), *bdl-1* (C), and *bdl-1tpl-1* (D). (**E** to **H**) Cleared cotyledons (4 dpg). Wild type (E), *tpl-1* (F), *bdl-1* (G), and *bdl-1tpl-1* (H). (**I** to **L**) Transition-stage embryos. Wild type (I), *tpl-1* (J), *bdl-1* (K), and *bdl-1tpl-1* (M). Lens-shaped cell and derivatives are outlined. (**M** to **P**) $DR5_{rev}$::*GFP*-expressing embryos. Wild type (M), *tpl-1* (N), *bdl-1* (O), and *bdl-1tpl-1* (P). Scale bars: (A to D) 0.5 mm; (E to H) 1 mm.

TPL. It also shows that TPL N176H does not disrupt this complex.

To determine if the EAR domain of IAA12/BDL allows IAA12/BDL to bridge TPL and ARF5/MP, we generated a chimeric protein containing domains III and IV of IAA12/BDL fused to the C terminus of TPL. This fusion protein should enable TPL to directly interact with ARF5/MP without requiring the EAR motif and should be unaffected by auxin due to the absence of domain II. First, we confirmed this interaction in a yeast two-hybrid assay (fig. S3), and then expressed this construct in plants under the control of the IAA12/BDL promoter. In 15 independent transgenic lines, we observed phenotypes similar to those of *bdl-1* and *arf5/mp* loss-of-function mutants (Fig. 4, D to F).

In light of these results, we propose a model in which TPL is a transcriptional co-repressor involved in the repression of auxin response genes through its physical interaction with the EAR domain of IAA12/BDL (Fig. 4G), and that this process is defective in *tpl-1*, resulting in the derepression of auxin response genes (Fig. 4H). Our results are also consistent with a report that application of a histone deacetylase inhibitor can suppress the lateral root defects seen in the stabilized *iaa14/solitary-root* mutant (*27*).

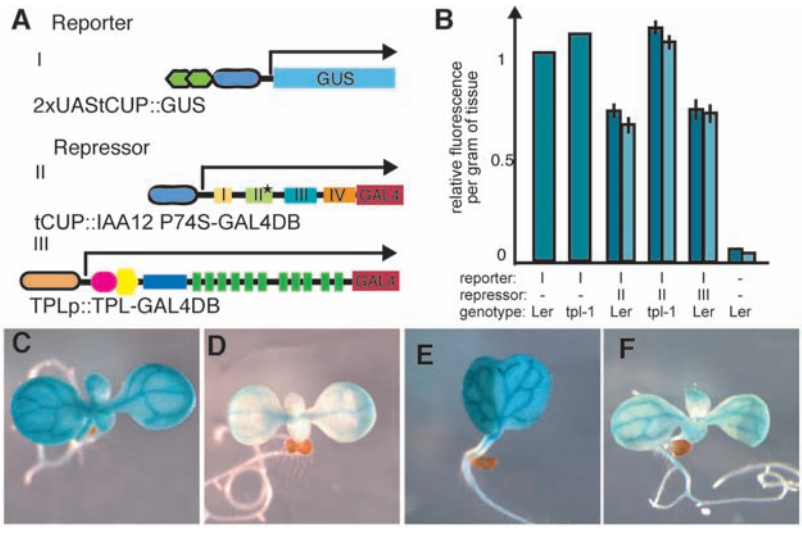

Fig. 3. TPL can repress transcription and affects the ability of IAA12/BDL to repress. (**A**) Diagram of constructs analyzed in repressor assay. (**B**) β-Glucuronidase (GUS) activity measured by MUG assay (4-methylumbelleferyl glucuronide breakdown into 4-methylumbilliferone) of two independent transgenic repressor lines (dark blue/light blue). Experiment was done in duplicate. Repressor activity is relative to MUG activity of the reporter in wild-type Ler, where error bars show standard error of the mean. (**C** to **F**) GUS staining patterns in plants expressing reporter only (C), tCUPp::BDL P74S-GAL4DB and reporter in a wild-type background (D), tCUPp::BDL P74S-GAL4DB and reporter in the *tpl-1* background (E), and TPLp::TPL-GAL4DB and reporter in a wild-type Ler background (F).

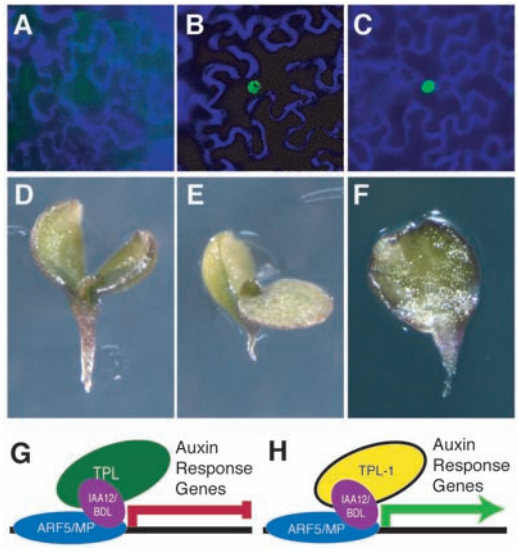

Fig. 4. TPL functions to repress auxin response through its physical interaction with IAA12/BDL. (**A** to **C**) Bimolecular fluorescence complementation studies in tobacco. Tobacco transformed with TPL-SPYCE and ARF5/MP-SPYNE (A); BDL P74S, TPL-SPYCE, and ARF5/MP-SPYNE (B); or BDL P74S, TPL N176H-SPYCE, and ARF5/MP-SPYNE (C). (**D** to **F**) Phenotypes of transgenic plants expressing the TPL-IAA12/BDL III/IV fusion construct. Seedling lacking root (D); seedling lacking root and hypocotyl (E); seedling consisting of single cotyledon with no hypocotyl or root (F). (**G**) Model of TPL-mediated transcriptional repression of auxin response genes. (**H**) Schematic of derepression of auxin response genes in the *tpl-1* context.

The coupling of TPL to ARFs through AUX/IAAs provides the plant an elegant mechanism to control ARF transcriptional activity in an auxin-dependent manner. It will be key to determine if all AUX/IAA proteins use TPL in this manner. It will also be of interest to determine if other EAR domain–containing transcription factors use TPL to facilitate their transcriptional repression activity in *Arabidopsis* as well as in other plant species.

References and Notes

1. P. J. Davies, in *Plant Hormones: Biosynthesis, Signal Transduction, Action!*, P. J. Davies, Ed. (Kluwer, Dordrecht, Netherlands, 2004), pp. 1–15.
2. T. Ulmasov, G. Hagen, T. J. Guilfoyle, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5844 (1999).
3. S. B. Tiwari, X. J. Wang, G. Hagen, T. J. Guilfoyle, *Plant Cell* **13**, 2809 (2001).
4. T. Ulmasov, J. Murfett, G. Hagen, T. J. Guilfoyle, *Plant Cell* **9**, 1963 (1997).
5. J. Kim, K. Harter, A. Theologis, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11786 (1997).
6. S. Kepinski, O. Leyser, *Nature* **435**, 446 (2005).
7. N. Dharmasiri, S. Dharmasiri, M. Estelle, *Nature* **435**, 441 (2005).
8. W. M. Gray, S. Kepinski, D. Rouse, O. Leyser, M. Estelle, *Nature* **414**, 271 (2001).
9. T. Hamann, E. Benkova, I. Baurle, M. Kientz, G. Jurgens, *Genes Dev.* **16**, 1610 (2002).
10. T. Hamann, U. Mayer, G. Jurgens, *Development* **126**, 1387 (1999).
11. C. S. Hardtke, T. Berleth, *EMBO J.* **17**, 1405 (1998).
12. T. Berleth, G. Jürgens, *Development* **118**, 575 (1993).
13. J. A. Long, S. Woody, S. Poethig, E. M. Meyerowitz, M. K. Barton, *Development* **129**, 2797 (2002).
14. J. A. Long, C. Ohno, Z. R. Smith, E. M. Meyerowitz, *Science* **312**, 1520 (2006).
15. Z. Paroush *et al.*, *Cell* **79**, 805 (1994).
16. R. D. Emes, C. P. Ponting, *Hum. Mol. Genet.* **10**, 2813 (2001).
17. S. B. Tiwari, G. Hagen, T. J. Guilfoyle, *Plant Cell* **16**, 533 (2004).
18. M. Ohta, K. Matsui, K. Hiratsu, H. Shinshi, M. Ohme-Takagi, *Plant Cell* **13**, 1959 (2001).
19. M. Pinto, C. G. Lobe, *J. Biol. Chem.* **271**, 33026 (1996).
20. O. Voinnet, S. Rivas, P. Mestre, D. Baulcombe, *Plant J.* **33**, 949 (2003).
21. M. Walter *et al.*, *Plant J.* **40**, 428 (2004).
22. J. Friml *et al.*, *Nature* **426**, 147 (2003).
23. D. Weijers *et al.*, *Dev. Cell* **10**, 265 (2006).
24. K. Wu, L. Tian, J. Hollingworth, D. C. Brown, B. Miki, *Plant Physiol.* **128**, 30 (2002).
25. P. J. Overvoorde *et al.*, *Plant Cell* **17**, 3282 (2005).
26. J. M. Alonso *et al.*, *Science* **301**, 653 (2003).
27. H. Fukaki, N. Taniguchi, M. Tasaka, *Plant J.* **48**, 380 (2006).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S3
Tables S1 to S3
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