DRMY1 promotes robust morphogenesis by sustaining translation of a hormone signaling protein

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SUMMARY

Robustness is the invariant development of phenotype despite environmental changes and genetic perturbations. In the Arabidopsis flower bud, four sepals initiate at robust positions and times and grow to equal size to enclose and protect the inner floral organs. We previously characterized the mutant development related myb-like1 (drmy1), where 3-5 sepals initiate at irregular positions and variable times and grow to different sizes, compromising their protective function. The molecular mechanism underlying this loss of robustness was unclear. Here, we show that drmy1 has reduced TARGET OF RAPAMYCIN (TOR) activity, ribosomal content, and translation. Translation reduction decreases the protein level of ARABIDOPSIS RESPONSE REGULATOR7 (ARR7), a rapidly synthesized and degraded cytokinin signaling inhibitor. The resultant upregulation of cytokinin signaling disrupts the robust positioning of auxin signaling, causing variable sepal initiation. Our work shows that the homeostasis of translation, a ubiquitous cellular process, is crucial for the robust spatiotemporal patterning of organogenesis.

Keywords: Robustness, TOR, translation, cytokinin, auxin, ARR, ribosomopathy, Arabidopsis, sepal, morphogenesis

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INTRODUCTION

Robustness, or canalization, is the invariant, reproducible development of phenotype, unchanged by environmental fluctuations, genetic perturbations, or gene expression noise\textsuperscript{1-4}. Commonly, within an individual, a given number of organs develop at well-defined positions to a robust final size and shape, which is crucial for fitness under stabilizing selection\textsuperscript{2}. For example, transplanted eyes, limbs, and kidneys in mammals grow to a mature size similar to their donor, irrespective of the mature size of the same type of organ in the recipient\textsuperscript{5-7}. The pairs of wings and halteres in Drosophila develop to robust, precisely coordinated final size and shape, which are required for flight\textsuperscript{8-11}. The characteristic cruciform flower in Brassicaceae consists of four petals\textsuperscript{12}, a trait that can contribute to pollinator attraction\textsuperscript{13}. The robust positioning of leaves around the shoot apical meristem in plants, or phyllotaxis, ensures optimal light capture\textsuperscript{14-16}. While these examples of developmental robustness have been documented for a long time, the underlying molecular mechanisms have just begun to be unveiled.

Earlier studies looking for genes involved in maintaining robustness have found HEAT SHOCK PROTEIN 90 (HSP90). Mutations of HSP90 cause a diverse array of phenotypic changes in plants, fruit fly, zebrafish, worm, and humans\textsuperscript{4,17,18}. Notably, the display and severity of these changes vary between individuals and even between different parts of the same individual, indicating that developmental robustness is disrupted\textsuperscript{17,18}. HSP90 encodes a protein chaperone which has clients from nearly all developmental and signaling pathways\textsuperscript{4}. HSP90, therefore, is a hub gene that affects numerous other genes within the gene network\textsuperscript{2}. Disruption of such a hub gene would therefore trigger many defects in numerous developmental processes. Similarly, genes involved in central cellular processes such as chromatin remodelling\textsuperscript{19-21}, transcription\textsuperscript{19,20}, translation\textsuperscript{22,23}, and protein degradation\textsuperscript{24,25} are also hub genes, and they have been found to be important for developmental robustness in various systems including fungi, animals, and plants. How these broadly acting hub genes contribute to the robustness of tissue-specific developmental phenotypes is still largely unclear.

We have developed the Arabidopsis sepal as a system to elucidate the mechanisms maintaining robustness in organ size and shape\textsuperscript{26-28}. Sepals are the outermost floral organs whose function is to enclose buds and protect the developing inner organs, i.e. petals, stamens, and carpels, before the flower blooms. To fulfill this protective function, each flower robustly develops four sepals of equal length, allowing them to close at the top (Figure 1A, middle left); these four sepals are of equal width and positioned 90° from each other, leaving no gap on the sides (Figure 1A, top left). This robustness in sepal size and shape stems from the robust initiation of the four sepal primordia from the floral meristem with precisely coordinated spatiotemporal patterns\textsuperscript{26} (Figure 1A, bottom left). The initiated sepal primordia attain robust final size and shape by spatiotemporal averaging of cellular growth variability during sepal elongation, and synchronous progression of a whole-flower growth termination signal from tip to base\textsuperscript{27}. In addition, noise in gene expression must be kept low to ensure sepal size robustness\textsuperscript{29}. We previously characterized a mutant in DEVELOPMENT RELATED MYB-LIKE 1 (DRMY1) that
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devlops flowers where the inner organs are exposed due to gaps between sepal's. The gaps are caused by variability in sepal development. Specifically, some sepal's are shorter than others, leaving gaps on the top (Figure 1A, middle right); the arrangement of sepal's around the flower deviate from the canonical form such that parts of the flower are not covered by a sepal, leaving gaps on the side (Figure 1A, top right). This variability in the size, number, and position of the mature sepal originates from the earliest stages of floral development where the initiation of sepal primordia is variable in spatiotemporal patterns (Figure 1A, bottom right). Variability in sepal initiation, in turn, is driven by the loss of robust patterning of auxin and cytokinin, two plant hormones critical for organ initiation and morphogenesis, in the floral meristem before sepal initiation. However, the molecular mechanism through which DRMY1 maintains robust hormone patterning is still unknown.

In this study, we eludicate a mechanism through which DRMY1 maintains robust hormone patterning and thus robust sepal initiation. Specifically, we find that DRMY1 maintains proper activity of TARGET OF RAPAMYCIN (TOR), a crucial regulator of ribosome biogenesis and protein translation, and thereby sustains translation in vivo. When DRMY1 is mutated, the protein level of ARABIDOPSIS RESPONSE REGULATOR7 (ARR7), a rapidly synthesized and degraded cytokinin signaling inhibitor, is reduced in the floral meristem. Consequently, cytokinin signaling uniformly increases in the meristem periphery, causing variability in auxin patterning and sepal initiation. We further propose that the increase in cytokinin signaling may be a survival mechanism to alleviate the translation rate reduction when ribosomal content is limited. In summary, our work shows that the hub processes of TOR signaling and translation, which occur in every cell, have very specific roles in robust organ primordium initiation by sustaining the rapid synthesis of a hormone signaling protein.

RESULTS

The drmy1 mutant has reduced TOR activity, ribosome content, and translation rate

DRMY1 encodes a MYB/SANT domain protein which may exert transcriptional regulation. To look for differentially expressed genes in drmy1 which may be candidates underlying variable sepal initiation, we performed RNA-sequencing (RNA-seq) in drmy1 and wild type (WT) of apetala1 (ap1) cauliflower (cal) AP1-GR background. The ap1 cal AP1-GR inflorescence produces numerous tightly packed ball-shaped meristems, which, upon induction of AP1-GR with dexamethasone, synchronously initiate sepal primordia, allowing us to collect large quantities of floral meristems with sepal primordia initiating (Stage 3) (Figure S1A). We crossed drmy1 into ap1 cal AP1-GR and performed RNA-seq on induced inflorescences of WT and drmy1 in this background. We detected transcripts from a total of 21,496 genes, of which 1,042 (4.8%) were differentially expressed in drmy1 (Figure S1B; Supplemental Dataset 1). We found that the 443 genes downregulated at the transcript level in drmy1 were most enriched in the gene ontology (GO) term “Translation”, a fundamental and ubiquitous cellular process that
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converts genetic information from transcript to protein. Within this term, genes encoding ribosomal components were most downregulated (Figure S1C). The 443 downregulated genes were also enriched in several other GO terms related to ribosome biogenesis and assembly (Figure 1B). We therefore hypothesized that ribosome abundance and translation rate are lower in \textit{drmy1}, potentially altering the accumulation of proteins critical to developmental robustness.

To determine whether and how ribosome abundance and translation are affected in \textit{drmy1}, we performed polysome profiling in induced inflorescences of WT and \textit{drmy1} in \textit{ap1 cal AP1-GR} background. Compared to WT, polysomal peaks are drastically reduced in \textit{drmy1} (Figure 1D; Supplemental Dataset 2). To see whether this reduction in ribosomal content affected translation rate (\textit{de novo} protein synthesis) \textit{in vivo}, we performed puromycin labeling. Samples were incubated with puromycin, an amino acid-tRNA analog that is incorporated into nascent polypeptide chains and can be detected using an anti-puromycin antibody to infer global translation rate\textsuperscript{38,39}. In both young seedlings and induced \textit{ap1 cal AP1-GR} inflorescences, we found that the puromycin level detected in \textit{drmy1} mutant samples was much reduced compared to WT (Figure 1E), indicating translation rate is reduced. We hypothesized that a reduction in protein translation should likely result in a decrease in protein level. For this, we looked at a ubiquitously expressed membrane marker \textit{UBQ10::mCherry-RCI2A}, and found that it had a small (~25\%) but significant decrease in fluorescence intensity in the inflorescence meristem and young floral buds of \textit{drmy1} compared with WT. We also measured its fluorescence intensity in the ribosomal mutant \textit{ul4y (rpl4d)}\textsuperscript{40} and we found that the decrease in fluorescence intensity in \textit{drmy1} is even greater than in \textit{ul4y} (Figure S1G-J). These results validate our proteomics findings, and show that ribosome content and translation are indeed reduced in the \textit{drmy1} mutant.

To test how the global repression of translation in \textit{drmy1} impacts its proteome, we extracted total soluble protein from induced inflorescences of WT and \textit{drmy1} in \textit{ap1 cal AP1-GR} background and performed mass spectrometry. We identified a total of 5,077 proteins, of which 548 (10.8\%) were differentially accumulated in \textit{drmy1} (Figure S1B; Supplemental Dataset 1). These differentially accumulated proteins were enriched in GO terms related to translation and ribosomal assembly (Figure 1C). Despite the overall reduction in ribosomes (Figure 1D), relative to other proteins, ribosomal components are more abundant in \textit{drmy1} (Figure S1D; Supplemental Dataset 1). This is not true for all proteins involved in translation; poly-A binding proteins and tRNA synthetases, for example, are relatively less abundant in \textit{drmy1} than in WT. Moreover, the 26S proteasome responsible for targeted protein degradation is much more abundant in \textit{drmy1} than in WT (Supplemental Dataset 1). In concert, these results demonstrate that the machinery responsible for maintaining protein homeostasis is substantially dysregulated in \textit{drmy1}.

A key signaling pathway that regulates protein homeostasis is TARGET OF RAPAMYCIN (TOR)\textsuperscript{41,42}. TOR is a hub that integrates information from light, sugars, nutrient availability, etc., to promote growth-related processes, including ribosome biogenesis and translation, and to repress catabolic processes, including protein degradation by autophagy and the proteasome\textsuperscript{33,34,43-45}. We therefore hypothesize that the decrease in ribosomal content and protein
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Translation in *drmy1* may reflect altered TOR signaling. To test for signatures of transcriptomic changes that have been well defined in seedlings under TOR inhibition\textsuperscript{34,46,47}, we performed RNA-seq on seedlings of WT, *drmy1*, WT treated with AZD-8055 (a potent TOR inhibitor), and mock-treated WT (Supplemental Dataset 3). We found that the *drmy1* mutation causes transcriptomic changes similar to TOR inhibition (Figure 1F). A significant portion of genes differentially expressed under TOR inhibition vs. mock were also differentially expressed in *drmy1* vs. WT (466/2044 = 22.8%; hypergeometric test, p = 4.7x10\textsuperscript{-108}). Not only were these 466 genes differentially expressed in both situations, but also most of them were coherently downregulated or upregulated (439/466 = 94.2%, Chi-square test, p < 2.2x10\textsuperscript{-16}; Figure S1E). Genes coherently downregulated in both situations were enriched in GO terms related to translation and ribosome biogenesis, and, most strikingly, a quarter of them were under the GO term “translation” (Figure 1F, S1F). These similar transcriptomic changes support our hypothesis that TOR activity is reduced in *drmy1*. To further test this hypothesis, we measured TOR activity in WT and *drmy1* by assaying the phosphorylation of its direct substrate, RIBOSOMAL PROTEIN eS6 KINASE (S6K)\textsuperscript{5,48,49}. While the total protein level of S6K did not change in *drmy1*, we found that S6K phosphorylation decreased by half, demonstrating reduced TOR activity (Figure 1G, 1H). Overall, these results are consistent with the idea that *drmy1* has reduced TOR activity—a main pathway controlling ribosome biogenesis and translation—which causes reduced ribosomal content and translation rate.

**Defects in TOR activity, ribosome integrity, and translation disrupt robust sepal initiation**

We next asked whether defects in TOR activity, ribosome, or translation have any effects on robust sepal initiation like the *drmy1* mutation does (Figure 2A vs 2B; also see Zhu et al.\textsuperscript{26}). In a WT bud, initiation is robust in that four sepal primordia of similar size form evenly spaced around the periphery of the floral meristem (Figure 2A, 2H). Angles between them vary little, i.e., they are all at around 90° angles from each other (Figure 2I, 2J). By contrast, in *drmy1* buds, three to five sepal primordia initiate and grow to different sizes (Figure 2B, 2H; also see Zhu et al.\textsuperscript{26}). The *drmy1* sepal primordia are generally unevenly spaced, and angles between them have a high coefficient of variation (CV) (Figure 2I, 2J). To determine whether defects in ribosomes can cause the same sepal initiation defects, we imaged three ribosomal mutants, *ul4z* (*rpl4a*), *ul4y*, and *ul18z* (*rpl5a*)\textsuperscript{40}, each mutated in a gene encoding a ribosomal component that is also downregulated in *drmy1* at the transcript level (Figure S1C). The *ul4z* mutant bud shows reduced size of the inner sepal primordia relative to the outer sepal primordia (Figure 2C), and slightly more variable spatial distribution of sepal primordia (Figure 2J), although it always develops four sepal primordia (Figure 2H). This is a weaker phenotype than *drmy1* but has similar characteristics. The *ul4y* and *ul18z* mutants show great variability in the number and position of sepal primordia (Figure 2D, 2E, 2H, 2J), more similar to *drmy1*. We also crossed these ribosomal mutants with *drmy1* to study sepal variability in the double mutants (Figure S2A-H). In *drmy1 ul4z*, *drmy1 ul4y*, and *drmy1 ul18z/+*, on average, sepal initiation was as variable as in the *drmy1* single mutant (Figure S2I, S1J). However, there were buds with no outgrowth in the adaxial or lateral regions of the bud periphery (Figure S2B, S2E, S2G), buds with six sepal primordia (Figure S2C, S2F, S2H), and...
buds with two outer sepal primordia (Figure S2D, S2H), which were not seen in the single mutants. Note that we were unable to characterize the homozygous drmy1 ul18z double mutant because they were embryo-lethal (Figure S2K), further supporting the idea that ribosomal mutations enhance the phenotypic defects in drmy1.

We then imaged mutants with reduced TOR activity to determine whether sepal initiation is also less robust. lst8-1-1 is a T-DNA insertional mutant of the TOR complex component LST8-1 and is weakly hypomorphic in TOR activity. We found that lst8-1-1 shows variable sepal initiation in a small proportion of buds (4/41, 9.8%) (Figure 2F, 2H, 2J). The spaghetti1 mutant defective in TOR complex 1 (TORC1) assembly showed a level of variability comparable to the drmy1 mutant and the ribosomal mutants ul4y and ul18z (Figure 2G, 2H, 2J). Mutants with more severe disruption of TOR activity are embryo lethal and could not be analyzed. These results show that reduction in TOR activity can cause variability in sepal initiation, similar to drmy1.

To corroborate these findings, we directly inhibited translation by in vitro culture of dissected WT inflorescences on 2 µM cycloheximide (CHX, a chemical inhibitor of translation) for 9-10 days. This is a low concentration that does not completely block translation, as inflorescences were still alive after 10 days in this condition. Compared with mock, CHX-treated inflorescences develop buds that have 2 to 6 sepal primordia of variable sizes that are unevenly spaced around the bud periphery (Figure 2K, 2M, 2N). These phenotypes are stronger than drmy1. Similarly, we directly inhibited TOR activity by continuous bi-daily application of 2 nmol Torin2 to the growing shoot apex for 15 days, and we observed variable sepal initiation (Figure 2L, 2M, 2N). Overall, data reported above show that inhibition of TOR activity and translation can disrupt the robustness of sepal initiation, in terms of sepal primordium number, position, and size.

We previously showed that the drmy1 mutant bud develops sepals of different sizes because some sepal primordia initiate much later than others. The late-initiating primordia remain smaller throughout development. They end up as smaller sepals relative to those that initiated earlier, leaving gaps that expose the developing inner floral organs. We asked whether TOR or ribosomal defects disrupt the relative timing of sepal initiation just as the drmy1 mutation does. We live imaged WT and ul4y every six hours during sepal initiation and quantified the amount of time the bud takes to initiate the inner and lateral sepals after it initiates the outer sepal. In WT, after the initiation of the outer sepal, most buds initiate the inner sepal within 6 hours and the lateral sepals within 12 hours (Figure 3A, 3C; also see Zhu et al.). In ul4y, the initiation times of the inner and lateral sepals are more variable and delayed relative to the outer sepal, with 7.5% of the buds initiating the inner sepal 18 hours after the outer sepal, and 32.5% of the buds initiating the lateral sepals 18 hours after the outer sepal (Figure 3B, 3C). Similarly, we compared the relative timing of sepal initiation in Torin2 vs mock-treated WT buds. While in most mock-treated buds, the inner and lateral sepals initiate within 12 hours after the outer sepal (Figure 3D, 3F), in Torin2-treated buds they are much more variable and delayed (Figure 3E, 3F), to a similar extent as previously observed in drmy1. In some cases, the inner and lateral sepals initiate more than
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30 hours after the outer sepal. These results show that TOR and ribosomal defects can disrupt the precisely orchestrated initiation timing of sepal primordia.

Does the variability in initiation timing cause variable sizes and gaps in mature sepalas, as in drmy1 (Figure S3A,B,G,H; also see Zhu et al. 26)? We imaged the mature sepalas of the ribosomal mutants ul4z, ul4y, ul18z, as well as the TOR component mutant lst8-1-1. Surprisingly, unlike drmy1, the sepalas in ul4z, ul4y, ul18z enclose the inner floral organs perfectly, leaving no gaps, regardless of sepal number (Figure S3C-E). Small gaps still exist in buds of lst8-1-1, although sepal size differences appear greatly reduced (Figure S3F). Further dissection shows that in these mutants, sepalas within the same flower are of similar sizes, although sepalas from different flowers can be of vastly different sizes, most conspicuously for lst8-1-1 (Figure S3I-N). This is unlike drmy1, where sepal size variability is equally high comparing sepalas within the same flower or from different flowers (Figure S3H, S3M-N). Upon closer examination, while sepalas initiating late in drmy1 buds remain small, continuously leaving a gap in the sepal whorl (Figure S3O-P), those in ul4y were able to catch up with the other sepalas and close the gap (Figure S3Q). Our results are consistent with the hypothesis that there exists a size-coordinating mechanism independent of TOR or ribosome function that allows sepalas within the same bud to reach the same mature length, and that this mechanism is disrupted in drmy1. Such a mechanism requires further investigation in future studies.

Inhibition of TOR activity and translation increase cytokinin signaling and disrupts the robust spatial pattern of auxin and cytokinin signaling

Auxin and cytokinin are two important plant hormones critical to many aspects of plant development30–32, and there is accumulating evidence that they act synergistically in the shoot apical meristem to promote lateral organ initiation16,53,54. We previously showed that, in a WT floral meristem prior to sepal initiation, auxin and cytokinin signaling are concentrated at the four incipient primordia, which is required for robust sepal initiation from these regions (Figure 4A, S4A; Zhu et al. 26). In the drmy1 mutant, cytokinin signaling becomes stronger and diffuse around the bud periphery (Figure 4A-B). Auxin signaling also becomes more diffuse, forming irregular auxin maxima that are less focused than those in WT, except at the incipient outer sepal where it remains robust (Figure 4A, S4B; Zhu et al. 26). These changes in hormone signaling correlate with variable sepal initiation (Figure S4B)26. We wondered whether ribosomal mutations have similar effects on auxin and cytokinin signaling. To this end, we imaged the auxin signaling reporter DR5::3xVENUS-N7 and the cytokinin signaling reporter TCS::GFP in floral meristems of the ribosomal mutant ul4y. Both reporters lose their robust spatial pattern except in the incipient outer sepal (Figure 4A, S4C). The hormone signaling patterns were quantified by circular histogram analysis (see Methods for details). For each of DR5 and TCS, WT buds showed four clear peaks ~90 degrees apart from each other, with very little signal in between, whereas in drmy1 and ul4y, peaks were barely seen except at the incipient outer sepal (at 45 degrees), and there was greater noise and variation all around the bud (Figure 4C-D). Diffuse bands of auxin signaling that typically occurs in the adaxial or lateral periphery of drmy1 and ul4y buds (Figure S4B and S4C, brackets).
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can later resolve into several distinct auxin maxima of various intensity and at various positions, correlated with the initiation of sepal primordia of various sizes at these same positions (Figure S4B and S4C, red arrowheads).

We also tested whether drug treatments that inhibit TOR activity or translation can disrupt the robust hormone patterning. Buds treated in vitro with the translation inhibitor CHX (2 µM) for 3 days showed a 50% increase in cytokinin signaling, and both auxin and cytokinin signaling became diffuse around the bud periphery (Figure 4E-H). By day 6, cytokinin signaling was still diffuse all around, and increased to more than two-fold relative to mock (Figure 4I, 4J, 4L). Auxin signaling formed maxima of variable number at variable positions (Figure 4I arrowheads, 4K), correlated with the variable initiation of sepal outgrowth at these positions (Figure S4D-E). Similar changes occurred in buds treated in vitro with the TOR inhibitor AZD-8055 (2 µM) for 6 days (Figure 4I-L). For both CHX and AZD-8055, the disruptions of hormone signaling are similar to drmy1. In vivo treatment using another TOR inhibitor Torin2 for 15 days increased cytokinin signaling by 70%, although it did not make auxin and cytokinin signaling more diffuse (Figure S4F-I). Overall, these results show that defects in TOR activity and translation increase cytokinin signaling, and disrupt the precise spatial patterning of cytokinin and auxin signaling required for robust sepal initiation.

An increase in cytokinin signaling is necessary and sufficient for variable auxin signaling and sepal initiation under translation inhibition

Auxin is a critical hormone in organogenesis. As shown above, variable patterning of auxin signaling correlates with variable sepal initiation during inhibition of TOR activity and translation. We wondered what caused auxin to lose its robust patterning under such conditions. It was previously reported that the ribosomal mutants ul4y, ul18z, and el24y have reduced protein levels of AUXIN RESPONSE FACTOR (ARF) 3, 5, and 7, key transcription factors that mediate the auxin signaling response. The transcripts of these ARFs contain upstream open reading frames (uORFs), requiring translation reinitiation to translate their main open reading frames, a process defective in ul4y, ul18z, and el24y. We therefore hypothesized that drmy1 loses robust auxin signaling pattern because of reduced translation of uORF-containing transcripts, including those of certain ARFs. To begin, we utilized our transcriptomics and proteomics data, and considered that the protein-transcript ratio of a gene should reflect both its level of translation and stability. Therefore, following our hypothesis, genes containing uORFs should, in general, have a lower protein-transcript ratio in drmy1 than in WT. We calculated the difference of this ratio between drmy1 and WT for all 5,086 gene-protein pairs in our inflorescence dataset, and compared the ratio against the number of uORFs in each transcript (Figure S5A; uORF data from von Arnim et al.). We found a small but significant decrease in the protein-transcript ratio in drmy1 for the 724 genes containing at least 2 uORFs in their transcripts, supporting the hypothesis that drmy1 has reduced translation reinitiation for uORF-containing transcripts, just like the ribosomal mutants ul4y, ul18z, and el24y. Then, we examined whether the translation reinitiation of uORF-containing ARFs are indeed reduced in the drmy1...
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We selected ARF3/ETTIN, ARF5/MONOPTEROS, and ARF6, which have 2, 6, and 6 uORFs respectively, and as controls, ARF8 and ARF10 which do not contain uORFs. None of these ARFs were differentially expressed in drmy1 at the transcript level, except ARF10 which was slightly upregulated (Figure S5B). We utilized promoter-fluorescent protein fusion reporters (Figure S5C) which have the same uORFs in the promoter region as the corresponding ARF genes if the genes have them. These reporters reflect transcriptional and uORF-mediated translational regulation. pARF3::N3xGFP, pARF5::ER-EYFP-HDEL, and pARF6::N3xGFP contain uORFs and thus, following our hypothesis, are expected to drastically decrease in fluorescence intensity in drmy1 compared to WT. pARF8::N3xGFP and pARF10::N3xGFP do not have uORFs and are thus expected to have comparable or higher fluorescence intensity in drmy1. Surprisingly, we saw no correlation between the presence of uORFs and decrease in fluorescent intensity in drmy1 (Figure S5C). While it might arise from additional layers of regulation on these ARFs, this result suggests that the decrease in translation reinitiation of uORF-containing ARFs is not the main factor explaining the loss of robust auxin signaling pattern in drmy1.

It was previously reported that external application of cytokinin increases auxin biosynthesis in actively growing tissue including the shoot apex, young leaves, and roots, and cytokinin application also changes the expression and polarity of PIN-FORMED (PIN) polar auxin transport carriers. We previously noticed that external application of 6-benzylaminopurine (BAP), a synthetic cytokinin, induced additional convergence points of PIN1 and increased variability in auxin signaling, causing variability in sepal initiation (Zhu et al., in this reference see Fig. 4e, Extended Data Fig. 7e and 7f). Here, we confirmed this observation by circular histogram analysis (Figure 5A-D). While the mock-treated WT buds showed four clear peaks of DR5 signal with very little signal in between (Figure 5A-B), those treated with 5 µM BAP showed a less robust spatial pattern, with less distinguishable peaks and larger variation all around the bud (Figure 5C-D). Thus, excessive cytokinin is sufficient for the variable spatial pattern of auxin signaling.

We then wondered whether an increase in cytokinin signaling (Figure 4) is the cause of variable pattern of auxin signaling under translation-limited conditions such as drmy1. To test this hypothesis, we crossed drmy1 containing the DR5 reporter with a triple mutant of ARABIDOPSIS RESPONSE REGULATOR (ARR) 1, 10, and 12, the three most highly expressed B-type ARRs in our RNA-seq (Supplementary Dataset 1) which are crucial for the activation of cytokinin-responsive genes. While buds of arr1,10,12 did not show apparent phenotypic differences from WT, the quadruple mutant drmy1 arr1 arr10,12 largely rescued the drmy1 phenotype, with much less variability in sepal number and position (Figure 5E-G). While mature buds of drmy1 have sepals of variable sizes, leaving gaps and exposing the inner floral organs (Figure S6D vs. S6A), those of drmy1 arr1 arr10,12 have sepals of robust sizes that are able to close (Figure S6E). Likewise, mutation in a cytokinin receptor WOODEN LEG (WOL)/ARABIDOPSIS HISTIDINE KINASE 4 (AHK4) showed a similar rescue of the drmy1 sepal phenotype (Figure 5E-G, S6F).

While the auxin signaling reporter DR5 was diffuse and variable in drmy1 except in the incipient outer sepal (Figure 5H-I), in drmy1 arr1 arr10,12, it was focused in all the four incipient sepals that
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were robustly positioned, although the signal intensity in the incipient outer sepal was much higher than others (Figure 5J-K). These results indicate that cytokinin signaling is required for the variability in auxin signaling pattern and sepal initiation in *drmy1*.

Furthermore, we wanted to test whether cytokinin signaling is required for variability in more general conditions where translation is inhibited. The translation inhibitor CHX disrupted robustness in auxin signaling and sepal initiation in WT (Figure 2K, 4E, 4I), and we tested whether these effects are still present in *arr1,10,12* and *wol* mutants. We found that, unlike WT, sepal initiation remained mostly robust in *arr1,10,12* and *wol* after ten days of 2 µM CHX treatment (Figure 5L-N). While DR5 in WT became diffuse and occurred in variable positions after three days of CHX treatment (Figure 5O-P, arrow), DR5 in *arr1,10,12* remained robust and concentrated at the four incipient sepal primordia (Figure 5Q-R). These results suggest that elevated cytokinin signaling level is the primary cause for variability in auxin patterning under translation-inhibited conditions. Thus, in WT, maintaining a low level and focused cytokinin signaling is crucial for robust auxin patterning and sepal initiation.

Upregulation of cytokinin signaling is required to sustain translation and fitness in *drmy1*

Why does the plant upregulate cytokinin signaling at the cost of robust morphogenesis under translation-inhibited conditions? Early studies revealed that cytokinin signaling can stimulate translation\(^{67-71}\), by increasing transcription or protein abundance of ribosomal components or biogenesis factors\(^{72-74}\) and modification of initiation and elongation factors\(^{75}\). We therefore hypothesized that an increase in cytokinin signaling under translation-inhibited conditions such as in *drmy1* serves to sustain a survivable rate of translation in a feedback loop.

We first validated that, under our growth conditions, an increase in cytokinin signaling (*arr1,10,12*; Figure 6A; also see Karunadasa et al.\(^{67}\)) in 14-day-old seedlings. We then tested whether cytokinin signaling is required to sustain translation, especially in *drmy1* (Figure 6B-C). The cytokinin receptor single mutant *wol* does not differ from WT in translation rate 8 days after germination but shows a reduced translation rate at day 14. Conversely, at day 8, *drmy1* seedlings showed a drastically reduced translation rate compared to WT, but by day 14, translation rate in *drmy1* increased and matched WT. In the *drmy1* *wol* double mutant, however, translation rate was unable to recover at day 14 and remained lower than either single mutant. Our data suggests that cytokinin signaling is required to sustain translation in *drmy1*, despite reduced ribosomal content (Figure 1D).

We then examined whether removal of cytokinin signaling and consequent failure to sustain translation in *drmy1* affects plant vitality and reproduction. As expected, at day 14, the *drmy1* *wol* double mutant plants were extremely small, with tiny and chlorotic cotyledons and true leaves (Figure 6D). These plants typically produced tiny rosettes and short inflorescences with a few chlorotic buds that develop into small, short siliques (Figure 6E, S6F). Similarly, the *drmy1* *arr1,10,12* quadruple mutant plants are slightly chlorotic and accumulate anthocyanins in the rosette leaves (Figure 6F). They produced a tiny inflorescence composed of very few buds (Figure
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6F, S6E) and, in the end, siliques in which all seeds had aborted (Figure 6G). Overall, these results show that the growth defects in drmy1 are exaggerated by downregulation of cytokinin signaling. While it is possible that drmy1 can be particularly sensitive to other defects caused by cytokinin downregulation, our results are consistent with the hypothesis that the upregulation of protein synthesis by increased cytokinin signaling promotes the survival of mutants with reduced ribosomal content such as drmy1.

Translation inhibition decreases the level of ARR7, a cytokinin signaling inhibitor protein

What causes cytokinin signaling to increase in plants with reduced TOR activity and translation (Figure 4)? It was previously known that cis-type cytokinins can be synthesized from tRNAs by the tRNA isopentenyltransferases (IPTs), IPT2 and IPT9. We hypothesized that the decrease in translation rate may increase the availability of tRNAs as substrates for cytokinin biosynthesis, increasing the level of cytokinins. To test this idea, we extracted cytokinins from induced inflorescences of WT and drmy1 in ap1 cal AP1-GR background (Figure S1A). We measured the level of three cytokinin bases, trans-Zeatin (tZ), cis-Zeatin (cZ), and isopentenyladenine (iP), and their corresponding nucleosides (tZR, cZR, and iPR), using liquid chromatography-mass spectrometry. Surprisingly, we found no significant difference in their levels between WT and drmy1, and notably, the amount of cis-Zeatin was barely detectable in all samples (Figure S7A). This suggests that the increase in cis-type cytokinin synthesis is not the mechanism underlying the increase in cytokinin signaling under our translation-inhibited conditions.

We then considered the effects that a decrease in translation rate might have on the protein components of the cytokinin signaling pathway. A-type ARR proteins are inhibitors of cytokinin signaling. They are rapidly induced upon cytokinin application and serve to dampen cytokinin response in the tissue. These proteins also have a fast turnover rate, being rapidly depleted upon blocking translation with a half-life ranging from 60 to 180 min. The rapid synthesis and degradation of these proteins may be crucial for maintaining homeostasis of cytokinin signaling during developmental processes. We therefore hypothesized that, during sepal initiation, translation defects in drmy1 cause reduced synthesis of A-type ARR proteins, decreasing them to a level insufficient to repress cytokinin signaling (Figure 7A).

We set out to test whether the level of A-type ARR proteins are reduced in drmy1. We were unable to detect fluorescence in the inflorescence of a published GFP-tagged A-type ARR line under the endogenous promoter (pARR4::ARR4-GFP). We reasoned that this was because A-type ARRs have low protein levels in the inflorescence (none was detected in our proteomics dataset) and fast turnover rates. We therefore employed LlamaTagging, a recently developed method to visualize the abundance of nuclear-localized proteins with short half-lives. Rapidly degraded proteins cannot be visualized through fusion with standard fluorescent proteins, because fluorescent proteins take time to fold and mature before they fluoresce, and the protein of interest is degraded before the maturation of the fluorescent protein. On the other hand, the
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LlamaTag folds immediately. A LlamaTag with a high affinity for GFP can be encoded as a translational fusion with a nuclear-localized protein of interest. In this case, soon after translation, the fusion immediately binds GFP in the cytosol and translocates the GFP with the protein to the nucleus. Thus, increased GFP fluorescence in the nucleus indicates higher abundance of the protein of interest. We decided to focus on ARR7, the A-type ARR that is most highly expressed in our RNA-seq dataset and not differentially expressed in drmy1 (Figure S7B; Supplemental Dataset 1). We designed a construct with ARR7 fused with GFP-specific LlamaTag by a short linker, driven by the ARR7 native promoter (pARR7::ARR7-linker-llama-ARR7ter; ARR7-llama for short). This construct was co-transformed with cytosol-localized GFP containing a nuclear exclusion signal (pUBQ10::sfGFP-nes-UBQ3ter, GFP-nes for short; Figure 7C). When ARR7-llama is produced in the cytosol, LlamaTag binds to cytosolic GFP. ARR7 then localizes to the nucleus, guided by its C-terminal nuclear localization signal, dragging GFP into the nucleus. Thus, when ARR7-llama is present at a low level, GFP is predominantly cytosol-localized; at an intermediate level, GFP may be nearly equally localized in the cytosol and nucleus; at a high level, most GFP are transported by ARR7-llama into the nucleus, and thus GFP signal is mainly seen in the nucleus (Figure 7D).

As a proof of concept, we treated this reporter in WT background with 200 µM BAP. We found that GFP signal became more nuclear-localized within 5 hours of the treatment (Figure S7B), agreeing with an increased expression and stability of A-type ARR proteins upon cytokinin application as previously reported.

We then compared the localization of GFP signal in floral meristems of WT and drmy1 before sepal initiation. Following our hypothesis, if the increase in cytokinin signaling in drmy1 is caused by reduced translation of A-type ARR proteins, there should be a decrease in ARR7 level, and thus an increase in cytosolic GFP signal, in drmy1 vs WT. On the other hand, if the increase in cytokinin signaling in drmy1 is caused by any other mechanism, we should see an increase in ARR7 level and nuclear GFP signal because cytokinin signaling increases the gene expression and protein stability of A-type ARRs. We found that WT buds had slightly more nuclear-localized GFP signal than cytosol-localized GFP signal, with brighter spots corresponding to the nucleus surrounded by darker grooves in between corresponding to the cytosol (Figure 7E-F). In contrast, in the periphery of drmy1 buds, GFP signal localizes more to the cytosol than to the nucleus, with darker spots surrounded by brighter grooves (Figure 7E-F). More nuclear GFP was present near the center of drmy1 buds. This result indicates nuclear ARR7 protein concentration is reduced in the drmy1 mutant, particularly in the zone where sepal initiate. To see whether this conclusion holds in other translation-inhibited conditions, we treated WT plants carrying the ARR7-llama and GFP-nes reporters with the translation inhibitor CHX and the TOR inhibitor AZD-8055. 2 µM CHX treatment for 24 hours drastically reduced the nuclear localization of the GFP signal and increased its cytosolic localization (Figure 7G). 2 µM AZD-8055 treatment for 72 hours had a milder but similar effect (Figure 7H). These treatments did not affect the localization of the GFP signal in plants without ARR7-llama (Figure S7C-D). These results show that conditions that decrease translation rate generally decrease the nuclear level of ARR7 protein. Further, these
results are consistent with our hypothesis that disruption of protein translation upregulates cytokinin signaling in the floral meristem due to depletion of A-type ARR proteins. This suggests that the rapid synthesis of A-type ARR proteins is crucial for maintaining homeostasis of cytokinin signaling during sepal initiation.

Given that the level of nuclear ARR7 was reduced in drmy1, we next asked whether increasing the level of ARR7 would restore robustness in drmy1. We found that while WT buds carrying the ARR7-llama and GFP-nes constructs do not phenotypically differ from WT without these constructs (Figure 7I-J, S7E-F), drmy1 plants with these constructs show a partial restoration of sepal initiation robustness, particularly in terms of sepal primordium position (Figure 7K-N, S7G-H). In stage 9-12 buds, some buds have robustly sized sepals that are able to close properly, while others still have variably sized sepals that leave gaps just like in drmy1 (Figure S7H). Thus, increasing ARR7 level by adding an extra functional transgene of ARR7-llama to the genome can partially restore robustness in both sepal initiation and mature sepal size in drmy1. This indicates that the decrease in ARR7 protein level in drmy1 is at least partially responsible for its variability in these aspects.

We also considered other protein components of auxin and cytokinin signaling that are being rapidly synthesized and degraded during developmental processes, and therefore, may be depleted under translation defects. AUXIN/INDOLE-3-ACETIC ACID INDUCIBLE (Aux/IAA) proteins are auxin signaling inhibitors that are rapidly induced by auxin\(^90,91\). In the presence of auxin, they are rapidly degraded, mediated by the ubiquitin E3 ligase SKP1, CUL1, F-BOX PROTEIN (SCF) complex involving TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB)\(^92-95\). Degradation is dependent on the Short Linear Motif (SLiM) degron contained within Domain II (DII)\(^94-96\). We hypothesized that the level of DII-containing proteins including Aux/IAAs would be drastically decreased in drmy1 because they are unable to be rapidly synthesized to keep up with their degradation. To test this, we used the R2D2 reporter\(^97\), which contains a DII fused with 3xVENUS (pUS7Y::DII-n3xVENUS), and as a control, a mutated non-degraded DII fused with tdTomato (pUS7Y::mDII-ntdTomato). We compared this reporter in drmy1 vs. WT. The ratio of VENUS to tdTomato was not reduced in drmy1, but instead slightly but significantly elevated (Figure S7I-J). In addition, drmy1 has stochastic patches of DII-VENUS degradation, consistent with its often mislocalized auxin maxima (Figure 4A, S4B), unlike WT which had four patches of degradation corresponding to the four incipient sepal primordia where auxin maxima robustly form (Figure 4A, S4A). Overall, these results suggest that the level of DII-containing Aux/IAA proteins is not reduced in drmy1, despite the high requirement for synthesis due to their rapid turnover. They also indicate that not all proteins that are rapidly synthesized and depleted in response to hormone signaling are equally affected by translation inhibition, which may result in different changes in hormone signaling output under such condition.

**DISCUSSION**
Robustness, the strikingly reproducible development of phenotype, has fascinated biologists for decades\(^2\). The Arabidopsis flower robustly develops four sepals of equal size. This stems from the robust initiation of four sepal primordia from the floral meristem, which is in turn dictated by the robust patterning of auxin and cytokinin controlled by DRMY1\(^26\). Here we elucidated how DRMY1 controls robust hormone patterning and thus robust sepal initiation. We show that DRMY1 sustains TOR activity, ribosomal content, and translation. We further show that inhibition of TOR activity or translation is sufficient to cause variability in the timing, position, and number of sepal primordia, mimicking the \textit{drmy1} phenotype. Our findings are in concert with previous studies that have shown robustness is often maintained by genes involved in central cellular processes\(^2\). In our case, the rate of translation in wild type maintains a proper level of ARR7, which needs to be rapidly synthesized to dampen cytokinin signaling. ARR7 in turn ensures a normal level and spatial pattern of cytokinin signaling and indirectly auxin signaling, and thus robust sepal initiation (Figure 7O, top). In the \textit{drmy1} mutant, the reduced TOR activity, ribosomal content, and translation rate causes inability to rapidly synthesize ARR7, which are short-lived proteins that are easily depleted\(^93\). Consequently, cytokinin signaling is elevated, disrupting the robust spatial distribution of both cytokinin and auxin, leading to variable sepal initiation (Figure 7O, bottom). Blocking cytokinin signaling in \textit{drmy1} is sufficient to restore robust initiation of four sepal primordia, but has severe consequences on the overall fitness of the plant. Our results reveal how defects in hub cellular processes such as TOR signaling and translation can have very tissue-specific phenotypic effects.

It was discovered long ago that extrinsic cytokinin application to plant tissue or cell-free extracts can promote mRNA translation\(^68–71\). Recent studies further confirmed that the up-regulation of translation by cytokinin is at least in part mediated by the cytokinin signaling pathway\(^67,75\). Here, we show that cytokinin signaling in floral buds is upregulated in translation-inhibited conditions, such as \textit{drmy1}, AZD-8055 treatment, or CHX treatment (Figure 4; also see Zhu et al.\(^26\)), through reduced level of ARR7 (Figure 7). The enhanced cytokinin signaling maintains translation rate at a level necessary for the survival and reproduction of the plant (Figure 6). We propose that there is a homeostasis mechanism where plants leverage increased cytokinin signaling to rescue the translation rate reduction caused by deficient TOR activity and ribosomal content (Figure 7O, bottom). It remains to be tested how widely this mechanism is applicable to other mutants with ribosomal defects, or whether parallel mechanisms operate in other species across kingdoms.

While translation-inhibited plants likely upregulate cytokinin signaling to maintain protein synthesis, this upregulation negatively affects developmental robustness. We have previously shown that exogenous cytokinin application to the WT floral meristem increases variability in PIN1 convergence and auxin signaling patterns, and consequently, in sepal initiation. Mutation of a cytokinin signaling inhibitor \textit{AHP6} causes similar variability in sepal initiation. These effects are more pronounced in the \textit{drmy1} mutant, which by itself has increased and diffuse cytokinin signaling\(^26\). Here, we provide additional evidence that increased and diffuse cytokinin signaling is necessary for such variability. While \textit{drmy1} and CHX-treated WT floral meristems are variable in
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auxin signaling pattern and sepal initiation (Figure 2, 4), mutations in wol and arr1,10,12, which
decreases cytokinin signaling, largely restore robustness (Figure 5). Robustness is also restored
in the mature sepals of drmy1 wol and drmy1 arr1,10,12, enabling sepal closure (Figure S6).
Similar effects in restoring robustness are seen when an extra functional transgene of ARR7
(pARR7::ARR7-llama) is introduced to the drmy1 mutant (Figure 7, S7). Our results suggest that
cytokinin upregulation is necessary and sufficient for variability in auxin patterning and sepal
initiation, indicating that the cytokinin signaling changes are primary defects in drmy1, and the
auxin signaling changes are secondary. Our results suggest a mechanism different from that
previously reported in ul4y, ul18z, and el24y, where ribosomal mutations affect auxin signaling
through reduced translation reinitiation of uORF-containing mRNAs, including those of AUXIN
RESPONSE FACTOR (ARF) 3, 5, and 7. While we found that uORF-containing mRNAs
generally have reduced protein-transcript ratio in drmy1 suggestive of reduced translation, we did
not see a consensus reduction in the level of uORF-containing promoter reporters of ARFs (Figure
S5). This suggests that the variable auxin signaling pattern in drmy1 is unlikely to result from
changes in uORF-mediated translational regulation of ARFs. Overall, our results suggest that
homeostasis in cytokinin signaling is crucial for maintaining robust patterns of auxin signaling and
robust morphogenesis in the floral meristem.

Mutations affecting ribosome biogenesis or translation have long attracted interest due to
the surprisingly tissue-specific phenotypes they cause\(^\text{98}\). In humans, these mutations have been
associated with diseases collectively known as ribosomopathies, where patients show various
abnormalities in blood, skeleton, hair, teeth, and pancreas, as well as intellectual disability and
increased risk of cancer\(^\text{99–104}\). Ribosomal protein mutants have been characterized in numerous
other species with similarly diverse impacts. They display a range of specific phenotypic changes,
such as altered pigmentation and skeletal structure in mouse\(^\text{105–107}\) and zebrafish\(^\text{108}\), shorter
bristles and notched wing margins in fruit fly\(^\text{109}\), abnormal gonad development in worm\(^\text{110}\), and
pointed leaves and abnormal vascular patterning in Arabidopsis\(^\text{57,58,111–113}\). Here, we show that
the Arabidopsis mutant drmy1 has reduced TOR activity, ribosomal content, and translation rate,
causing variable sepal initiation which phenocopies the ribosomal mutants ul4y and ul18z and the
TORC1 assembly mutant spaghetti-1 (Figure 2, 3). We therefore propose that drmy1 is an
Arabidopsis ribosomopathy mutant like those previously characterized\(^\text{112}\).

Several mechanisms have been proposed to explain why ribosomopathies do not usually
cause a general reduction in growth, but rather affect development in tissue-specific ways. These
include extra-ribosomal functions of certain ribosomal proteins\(^\text{114–118}\), altered translation behavior
of ribosomal variants on certain mRNAs\(^\text{119}\), different competitiveness of mRNAs for scarce
ribosomes\(^\text{57–59,120–123}\), and high translation rate requirement for certain proteins\(^\text{124,125}\). For example,
neurotransmitter release in animals relies on constant synthesis of the synaptic vesicle protein
Syt1\(^\text{126}\). A Drosophila Minute mutant, uS15/+ , shows reduced synthesis of Syt1, which in turn
reduces ecysdysone secretion in 5-HT neurons, causing delayed larval-to-pupal transition\(^\text{124}\).
Similarly, the human apoptosis inhibitor Mcl-1 has a half-life of ~30 min and thus requires a high
translation rate to maintain its proper level. Under translation inhibition, Mcl-1 is rapidly degraded,
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causing apoptosis\textsuperscript{125}. Here, we show that the level of ARR7, another rapidly synthesized and degraded protein\textsuperscript{83}, is drastically reduced under translation inhibition, which underlies the upregulation of cytokinin signaling and loss of robustness in auxin signaling and morphogenesis (Figure 7). This mechanism parallels those previously found in animal systems\textsuperscript{124,125}, and highlights how defects in translation, which occurs in every cell, can have tissue-specific effects on how cells robustly arrange into organs. Outside the floral meristem, the \textit{drmy1} mutant shows other phenotypic changes such as enlarged shoot apical meristem, reduced apical dominance, phyllotaxy defects, and reduced root system, all of which are related to altered cytokinin/auxin signaling activity\textsuperscript{26}.

In addition, we note that not all proteins that are rapidly synthesized and depleted in response to hormone signaling are equally affected under broad translation inhibition. Our data suggest that DII-containing proteins, including auxin signaling inhibitors Aux/IAAs, are not present at a lower level in \textit{drmy1} (Figure S7H-I). Aux/IAAs are degraded by the proteasome in an auxin-dependent manner\textsuperscript{96}, while A-type ARRs can also be degraded by selective autophagy when phosphorylated, in addition to proteasomal degradation\textsuperscript{84}. In addition, the transcription of Aux/IAA genes can be induced under translation inhibition, possibly as a feedback mechanism to maintain Aux/IAA protein homeostasis\textsuperscript{90}. Therefore, distinct turnover mechanisms and/or transcriptional regulation may shape distinct effects of translational inhibition on the level of these hormone signaling proteins.

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AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

The authors declare no competing interests.
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FIGURE TITLES AND LEGENDS

Figure 1. *drmy1* has reduced ribosome abundance, translation rate, and TOR activity.

(A) Top row, stage 12 buds of WT (left) and *drmy1* (right) viewed from the top. Arrowheads point to sepals. Note that the *drmy1* bud has 5 sepals of unequal size and unevenly spaced, exposing the stamens and carpels. Middle row, stage 12 buds of WT (left) and *drmy1* (right) viewed from the side. Asterisk shows the gap between sepals with petals and carpels exposed. Bottom row, stage 5 buds of WT (left) and *drmy1* (right) containing 35S::mCitrine::RCl2A (plasma membrane marker). Arrowheads point to sepal primordia. Note that the *drmy1* bud has 5 sepal primordia of different sizes. Scale bars are 0.5 mm for stage 12 bud images and 25 µm for stage 5 bud images.

(B-C) Gene ontology (GO) enrichment of downregulated genes (B) and differentially accumulated proteins (C) in *drmy1* compared to WT, in the *ap1 cal AP1-GR* background. Shown are the top 8 GO terms and their enrichment p-values. A complete list can be found in Supplemental Dataset 1. Arrowheads highlight terms related to ribosome biogenesis or translation.

(D) Ribosome profiles of WT (blue) and *drmy1* (red) in the *ap1 cal AP1-GR* background, representative of 3 biological replicates each. Polysomal peaks are highlighted. All replicates can be found in Supplemental Dataset 2.

(E) Puromycin labeling of WT vs *drmy1*. Left, puromycin labeling in WT and *drmy1* seedlings. From left to right: WT pre-treated with CHX, two biological replicates of WT pre-treated with mock, and two biological replicates of *drmy1* pre-treated with mock. All groups were then treated with puromycin. For seedlings to match in size, WT seedlings were 8 days old and *drmy1* seedlings were 10 days old. Right, puromycin labeling in WT and *drmy1* inflorescences of induced *ap1 cal AP1-GR* background. From left to right: WT co-treated with puromycin and CHX, three biological replicates of WT treated with puromycin, and three biological replicates of *drmy1* treated with puromycin. In both experiments, RuBisCO large subunit on Ponceau S-stained membrane is shown as a loading control (bottom).

(F) Coherent alteration of gene expression by *drmy1* and AZD-8055 TOR inhibitor treatment. Shown here is a scatterplot of RNA log 2 fold change in *drmy1* vs WT (x-axis), and WT+AZD vs WT+Mock (y-axis), in 7-day-old seedlings. Genes are color-coded based on the following categories: genes in “Structural constituents of the ribosome” (GO:0003735) and its offspring terms (magenta); all other genes in “Translation” (GO:0006412) and its offspring terms (orange); all other genes (gray). Blue line shows a linear regression of all points ($R^2 = 0.1446$, $p < 2.2 \times 10^{-16}$). Note that the axes were trimmed to (-3,3) for ease of display.

(G-H) Phosphorylation of the direct TOR substrate, S6K-pT449, in WT and *drmy1*. Representative images are shown in (G). Top, Western blot against S6K-pT449. Middle, Western blot against total S6K protein. Bottom, Ponceau S staining as a loading control. (H) Quantification of the intensity of S6K-pT449 over Ponceau normalized by WT, in three experiments, shows that TOR activity decreased by half in *drmy1*. (mean ± SD; *, $p<0.05$).
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**Figure 2.** Defects in TOR activity, ribosome integrity, and translation cause variable sepal initiation.

(A-G) Representative images of stage 5 buds in WT (A), *drmy1* (B), *ul4z* (C), *ul4y* (D), *ul18z* (E), *lst8-1-1* (F), and *spaghetti-1* (G). Tissue morphology is visualized by either propidium iodide (a cell wall-staining dye) or a plasma membrane marker. Arrowheads indicate sepal primordia that are variable in number, position, and size. Note that *ul4z* flowers always develop four sepal primordia, although of different sizes; *lst8-1-1* occasionally (4/41, 9.8%) develops buds with more than four sepal primordia. Scale bars, 25 µm.

(H) Quantification of sepal primordium number, comparing *ul4z* (*n* = 52 buds), *ul4y* (*n* = 53 buds), *ul18z* (*n* = 52 buds), *lst8-1-1* (*n* = 41 buds), and *spaghetti-1* (*n* = 84 buds) with WT (*n* = 51 buds). Asterisks indicate statistically significant (p < 0.05) differences from WT in Fisher’s contingency table tests.

(I) Illustration of robust versus variable positioning of sepal primordia. Primordia are considered robustly positioned if they are evenly distributed around the edge of the bud. Within each bud, angles between adjacent primordia with respect to the center of the bud are measured, and coefficient of variation (CV) is calculated. A bud with robustly positioned primordia would have similar angular values and a low CV value. A bud with variably positioned primordia would have very different angular values and a high CV value.

(J) Quantification of variability in primordium positioning (CV) in the same buds as in (H), following illustration in (I). Asterisks indicate statistically significant (p < 0.05) differences from WT in Wilcoxon’s rank sum tests.

(K) Representative images of buds from *in vitro*-cultured WT inflorescences treated with mock or 2 µM CHX for 9 days (see Material and Methods). Arrowheads indicate sepal primordia that are variable in number, position, and size. Scale bars, 25 µm.

(L) Representative images of buds from WT plants treated with mock or 2 nmol Torin2 for 15 days (see Material and Methods). Arrowheads indicate sepal primordia that are variable in number, position, and size. Scale bars, 25 µm.

(M-N) Quantification of variability in primordium number (M) and positional variability (N) similar to (H,J), comparing CHX-treated (*n* = 31 buds), CHX-mock (*n* = 42 buds), Torin2-treated (*n* = 51 buds) and Torin2-mock buds (*n* = 56 buds).
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**Figure 3. TOR and ribosomal defects cause variability in the timing of sepal initiation.**

(A-C) 6h-interval live imaging of the sepal initiation process in WT (A) and *ul4y* (B), which is quantified in (C). n = 48 buds for WT; n = 40 buds for *ul4y.*

(D-F) 6h-interval live imaging of the sepal initiation process in buds from WT plants treated with mock or 2 nmol Torin2 bi-daily for 15 days, which is quantified in (F). n = 31 buds for mock; n = 15 buds for Torin2.

In (A,B,D,E), top rows show the 35S::mCitrine-RCI2A membrane marker, and bottom rows show Gaussian curvature heatmaps calculated from the same image stacks. Asterisks indicate sepal initiation events, defined as a dark red band (primordium with positive curvature) separated from the floral meristem by a dark blue band (boundary with negative curvature) in the Gaussian curvature heatmap. Scale bars, 25 µm.

In (C,F), the amount of time between outer and inner sepal initiation (left) and between outer and lateral sepal initiation (right) were calculated for each bud, and summarized over all the buds. Asterisks indicate statistically significant (p < 0.05) differences in the distribution of relative initiation timing in Fisher’s contingency table tests.
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**Figure 4. Defects in TOR activity, ribosome integrity, and translation cause variability in auxin and cytokinin signaling.**

(A-D) The ribosomal mutant *ul4y* loses robustness in auxin and cytokinin signaling. (A) Representative images of late stage 2 buds of WT, *drmy1*, and *ul4y*, showing the auxin signaling reporter *DR5::3xVENUS-N7* in yellow, the cytokinin signaling reporter *TCS::GFP* in cyan, and both merged with Chlorophyll (in WT) or *UBQ10::mCherry-RCI2A* (in *drmy1* and *ul4y*) in magenta. (B) Quantification of TCS intensity (integrated density divided by area) from maximum intensity projection images, normalized to mean of WT. Shown are mean ± SD. Asterisks show statistically significant differences from WT in two-tailed Student’s t-tests (*drmy1*, *p* = 2.1x10^{-6}; *ul4y*, *p* = 3.4x10^{-5}). (C) Circular histogram of DR5 distribution around the bud. Each bud was divided into 360 sectors of 1° each. Within each sector, DR5 signal measured in pixel intensity units (0-255 range) was summed. This sum was plotted along the x-axis starting from the sector at 1:30 position (between the incipient outer sepal and incipient lateral sepal on the right) going counterclockwise. I.e. in WT, the outer sepal is near 45°, the inner sepal near 225°, and the lateral sepals near 45° and 135° (vertical dotted lines). The mean was plotted as a solid line, and mean ± SD was plotted as a shaded area. (D) Circular histogram of TCS distribution around the bud. Sample size for (A-D): WT, *n* = 12 buds; *drmy1*, *n* = 15 buds; *ul4y*, *n* = 10 buds.

(E-H) 3 days of translation inhibition causes increased and diffuse cytokinin signaling, and diffuse auxin signaling. (E) Representative images of late stage 2 buds from dissected and cultured WT inflorescences treated with mock or 2 µM CHX for 3 days. Shown are *DR5::3xVENUS-N7* in yellow, *TCS::GFP* in cyan, and both merged with Chlorophyll in magenta. (F) Quantification of TCS intensity from maximum intensity projection images, normalized to mean of WT mock day 3. Shown are mean ± SD. Asterisk shows statistically significant difference in a two-tailed Student’s t-test (*p* = 2.0x10^{-4}). (G) Circular histogram of DR5 distribution around the bud. (H) Circular histogram of TCS distribution around the bud. Sample size for (E-H): WT mock day 3, *n* = 10 buds; WT CHX day 3, *n* = 12 buds.

(I-L) 6 days of TOR or translation inhibition causes increased and diffuse cytokinin signaling, and randomly positioned auxin signaling maxima. (I) Representative images of late stage 2 buds from dissected and cultured WT inflorescences treated with mock, 2 µM CHX, or 2 µM AZD for 6 days. Shown are *DR5::3xVENUS-N7* in yellow, *TCS::GFP* in cyan, and both merged with Chlorophyll in magenta. Arrowheads point to randomly positioned auxin maxima in buds of the CHX or AZD group. (J) Quantification of TCS intensity from maximum intensity projection images, normalized to mean of WT mock day 6. Shown are mean ± SD. Asterisks show statistically significant differences from mock in two-tailed Student’s t-tests (CHX, *p* = 1.0x10^{-3}; AZD, *p* = 1.2x10^{-4}). (K) Circular histogram of DR5 distribution around the bud. (L) Circular histogram of TCS distribution around the bud. Sample size for (I-L): WT mock day 6, *n* = 12 buds; WT CHX day 6, *n* = 11 buds; WT AZD day 6, *n* = 10 buds. Scale bars in (A,E,I) represent 25 µm.
Figure 5. Cytokinin signaling is required for variability in auxin signaling and sepal initiation under translation inhibition.

(A-D) Cytokinin treatment makes auxin signaling diffuse. Shown are late stage 2 WT buds under mock (A,B) or 5 µM cytokinin (BAP) treatment (C,D) for 4 days. (A,C) Representative images of the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with Chlorophyll in magenta. (B,D) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). Arrows point to DR5 signal in variable positions. Sample size: WT Mock n = 10, WT BAP n = 10. Also see Zhu et al. (2020), Extended Data Figure 7e.

(E-G) Cytokinin signaling is required for variable sepal initiation in drmy1. (E) Representative images of stage 5 buds in WT, drmy1, arr1,10,12, drmy1 arr1,10,12, wol, and drmy1 wol. Arrowheads indicate initiated sepal primordia that are variable in number, position, and size. (F,G) Quantification of sepal primordium number (F) and positional variability (G), comparing Mock and CHX withi each genotype. Sample size: WT Mock n = 29, WT CHX n = 19, arr1,10,12 CHX n = 19, wol Mock n = 15, wol CHX n = 19. Asterisks indicate statistically significant (p < 0.05) differences in Fisher’s contingency table tests (F) and Wilcoxon’s rank sum tests (G) respectively. ns indicates no significant difference.

(H-K) Cytokinin signaling is required for variable patterning of auxin signaling in drmy1. Shown are late stage 2 buds of WT vs drmy1 (H,I), and arr1,10,12 vs drmy1 arr1,10,12 (J,K). (H,J) Representative images of the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with propidium iodide in magenta. Arrows point to diffuse DR5 signal in variable positions in the drmy1 bud. Arrowheads show four robust DR5 maxima in drmy1 arr1,10,12. (I,K) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). For ease of visualization, circular histograms of drmy1 and drmy1 arr1,10,12 between 90 and 360 degrees are enlarged and shown as insets (y-axis range 0-0.4). Sample size: WT n = 19, drmy1 n = 16, arr1,10,12 n = 13, drmy1 arr1,10,12 n = 9.

(L-N) Cytokinin signaling is required for variable sepal initiation under translation inhibition. (L) Representative images of stage 6 buds in WT, arr1,10,12, and wol, treated with Mock or 2 µM CHX for 10 days. Arrowheads indicate variable initiation of sepal primordia. (M,N) Quantification of sepal primordium number (M) and positional variability (N), comparing Mock and CHX within each genotype. Sample size: WT Mock n = 29, WT CHX n = 19, arr1,10,12 Mock n = 18, arr1,10,12 CHX n = 19, wol Mock n = 15, wol CHX n = 19. Asterisks indicate statistically significant (p < 0.05) differences in Fisher’s contingency table tests (M) and Wilcoxon’s rank sum tests (N) respectively. ns indicates no significant difference.

(O-R) Cytokinin signaling is required for diffuse auxin signaling under translation inhibition. Shown are late stage 2 buds of WT (O,P) and arr1,10,12 (Q,R), treated with Mock or 2 µM CHX for 3 days. (O,Q) Representative images of the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with Chlorophyll in magenta. Arrows point to diffuse DR5 signal in variable positions in CHX-treated WT. Arrowheads show four robust DR5 maxima in CHX-treated arr1,10,12. (P,R) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). Sample size: WT Mock n = 17, WT CHX n = 18, arr1,10,12 Mock n = 7, arr1,10,12 CHX n = 7. Scale bars in (A,C,E,H,J,L,O,Q) represent 25 µm.
Translation and developmental robustness

**Figure 6. Upregulation of cytokinin signaling is required to maintain translation and fitness in drmy1.**

(A) Puromycin labeling of WT seedlings with 4 h CHX pre-treatment (control), and three biological replicates each of WT and arr1 35S::ARR1 seedlings with 4 h mock pre-treatment. All seedlings are 14 days old. RuBisCO large subunit in Ponceau S-stained membrane is shown as a loading control. Also see Karunadasa et al. (2020).

(B,C) Puromycin labeling of WT seedlings with 4 h CHX pre-treatment (control), and two biological replicates of WT, drmy1, wol, and drmy1 wol seedlings with 4 h mock pre-treatment. Seedlings are 8 days old in (B) and 14 days old in (C). RuBisCO large subunit in Ponceau S-stained membrane is shown as a loading control.

(D) Representative 14 days old seedling images of WT, drmy1, wol, and drmy1 wol used in (C). Notice that drmy1 wol is very small and pale. Scale bars, 5 mm.

(E) Representative aerial part images of 42 days old plants of WT, drmy1, wol, and drmy1 wol. Inset shows enlarged drmy1 wol plant; notice that it has a tiny rosette and inflorescence. Scale bars, 5 cm. See also Supplemental Figure 6F.

(F) Representative aerial part images of 74 days old plants of WT, drmy1, arr1,10,12, and drmy1 arr1,10,12. Inset shows enlarged drmy1 arr1,10,12 plant; notice its pale leaves accumulating anthocyanin, and short inflorescence. Scale bars, 5 cm. See also Supplemental Figure 6E.

(G) Dissected siliques of arr1,10,12 (left) and drmy1 arr1,10,12 (right) showing developing seeds. Notice that while arr1,10,12 occasionally have aborted seeds, all seeds in the drmy1 arr1,10,12 silique were aborted. Scale bars, 0.2 mm.
Figure 7. A-type ARR protein levels are sensitive to TOR and translation inhibition.

(A) The hypothesis. A-type ARR proteins are rapidly synthesized and degraded to dampen cytokinin signaling. Translation inhibition causes inability to rapidly synthesize these proteins in response to cytokinin signaling, resulting in an upregulation of cytokinin signaling.

(B) Expression of A-type ARR genes in WT vs drmy1 inflorescences (ap1 cal AP1-GR) measured in RNA-seq. Shown are the five A-type ARR genes with the highest expression, ranked by mean expression level in WT. Asterisk indicates statistically significant difference, while ns means no significant difference. P-values: ARR7, p = 0.807; ARR4, p = 0.611; ARR15, p = 0.532; ARR8, p = 0.0115; ARR9, p = 0.0416.

(C) A GFP-channel image of a stage 2 bud of GFP-nes (pUBQ10::sfGFP-nes-UBQ3ter). For this panel and (E-H), each image was brightness to reveal GFP distribution patterns. A square region taken from the image containing 5-10 cells is enlarged and shown on the top right. Within the enlargement, GFP intensity was quantified along the dotted line and plotted on the bottom right. X-axis, pixels (range 0-238). Y-axis, GFP intensity in gray value (smoothened by taking the average intensity of 11-pixel neighborhoods; range 90-210). Scale bars, 25 µm.

(D) Illustration of the Llama Tag system used in this study. Plants were co-transformed with ARR7-llama (pARR7::ARR7-linker-llama-ARR7ter) and GFP-nes (pUBQ10::sfGFP-nes-UBQ3ter). Without ARR7-llama, the GFP is localized in the cytosol. ARR7-llama is produced in the cytosol and translocates into the nucleus. When this happens, the Llama Tag capable of binding GFP drags GFP into the nucleus (note that from our observation it is excluded from the nucleolus). Therefore, at low ARR7-llama levels, GFP signal is mainly seen in the cytosol. At intermediate levels, the GFP is at comparable intensities between the cytosol and the nucleus, and no clear pattern can be seen. At high ARR7-llama levels, GFP is mainly seen in the nucleus.

(E,F) GFP channel images of stage 2 buds from two independent transgenic lines of ARR7-llama GFP-nes, 7-4 (E) and 7-6 (F), in WT (top) vs drmy1 (bottom). Images are representative of n = 17 (line 7-4, WT), n = 40 (line 7-4, drmy1), n = 9 (line 7-6, WT), and n = 6 (line 7-6, drmy1) buds.

(G) GFP channel images of WT ARR7-llama GFP-nes buds treated with mock (top) or 2 µM CHX (bottom) for 24 hours. The mock image is representative of n = 20 buds (12 from line 7-4, 5 from line 7-6, and 3 from line 7-12). The CHX image is representative of n = 19 buds (11 from line 7-4, 5 from line 7-6, and 3 from line 7-12).

(H) GFP channel images of WT ARR7-llama GFP-nes buds treated with mock (top) or 2 µM AZD-8055 (bottom) for 72 hours. The mock image is representative of n = 13 buds (8 from line 7-4 and 5 from line 7-6). The AZD-8055 image is representative of n = 11 buds (8 from line 7-4 and 3 from line 7-6).

(I-L) ARR7-llama partially restores robustness in drmy1 sepal primordia. (I-L) Representative stage 5 or 6 buds from WT (l), WT with the ARR7-llama and GFP-nes constructs (J), drmy1 (K), and drmy1 with these constructs (L). (M) Quantification of sepal primordium number. ns indicates no significance difference in a Fisher’s exact test (WT vs ARR7-llama, p = 1; drmy1 vs drmy1 ARR7-llama, p = 0.44). (N) Quantification of variability in sepal primordium position. Asterisk indicates statistically significant difference (p = 5.7x10^{-6}), while ns indicates no statistically significant difference (p = 0.91). Data for WT and drmy1 were reused from Figure 2H, 2J. Data for ARR7-llama GFP-nes and drmy1 ARR7-llama GFP-nes were pooled from line 7-4 and 7-6.
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Sample size: WT n = 51, ARR7-llama GFP-nes n = 16, drmy1 n = 67, drmy1 ARR7-llama GFP-nes n = 20. Scale bars, 25 µm.

(O) Working model. In WT, DRMY1 maintains TOR activity and translation, which sustains the rapid translation of A-type ARRs in response to cytokinin signaling. This suppresses excessive cytokinin signaling, allowing auxin and cytokinin signaling to interact and form robust spatial patterns. Robust patterning of auxin and cytokinin signaling gives rise to robustly numbered, positioned, and sized sepal primordia. In drmy1, A-type ARR protein levels are reduced due to insufficient TOR activity, ribosome content, and translation rate. Cytokinin signaling is upregulated, which rescues the translation rate reduction in a homeostatic mechanism. This upregulation of cytokinin signaling disrupts the robust spatial pattern of both cytokinin and auxin signaling, which in turn causes variable sepal initiation.
MATERIALS AND METHODS

Plant material

Most Arabidopsis plants were in Col-0 background (WT). ap1 cal 35S::AP1-GR was in Ler background. drmy1 (Col-0) was backcrossed to Ler twice and then crossed with ap1 cal 35S::AP1-GR to obtain drmy1 ap1 cal 35S::AP1-GR. R2D2 was originally in Col-Utrecht background and was backcrossed twice into WT (Col-0) and drmy1 (Col-0). The following mutants and reporters were previously described: drmy1-126, wol-1127, spaghetti-1 (trp5-1)128, ap1 cal 35S::AP1-GR (Ler)35,6 arr1-1 35S::ARR167, DR5::3xVENUS-N7129, TCS::GFP130, pARF5::ER-EYFP-HDEL131, pUS7Y-mDII-NdTomato-pUS7Y-DII-N3xVENUS (R2D2)97, 35S::mCirtine-RC12A26, UBQ10::mCherry-RC12A26. The following mutants and reporter lines were obtained from Arabidopsis Biological Resource Center (ABRC): ul14z (SALK_130595), ul14y (SALK_029203), ul18z (SALK_089798), arr1-3 arr10-5 arr12-132 (CS39992), lst8-1-1 (SALK_002459), pARF3::N3xGFP133 (CS67072), pARF6::N3xGFP133 (CS67078), pARF8::N3xGFP133 (CS67082), pARF10::N3xGFP133 (CS67086).

Llama-tagged ARR7 construct

For the LlamaTag system, we first generated plasmid pVV13 containing linker llama. We amplified the Llama Tag (from a plasmid containing vhhGFP4134) and added a linker sequence of tccggagcactgctcgctgctgctgcggcgccactagt at its 5’ end by two rounds of overlap PCRs. Primers for the first round were oVV64 and oVV53, and primers for the second round were oVV35 and oVV53. After the second round, we A-tailed the PCR product according to the Promega manufacturer’s protocol. A-tailed product was ligated to the pGEMTeasy vector according to the Promega ligation protocol, to create the plasmid pVV13.

To make pARR7::ARR7-llama, a genomic fragment of pARR7::ARR7 minus the stop codon and terminator was amplified from the Arabidopsis (Col-0) genome using the primers oSK197 and oSK198. The linker-llama fragment was PCR-amplified from pVV13 using the primers oSK199 and oSK200. The ARR7 stop codon, 3' UTR, and terminator was amplified from the Arabidopsis (Col-0) genome using the primers oSK201 and oSK202. pMLBART backbone was digested with NotI, and all fragments were assembled into pMLBART using NEBuilder according to the manufacturer’s protocol.

To make pUBQ10::sfGFP-NES:UBQ3ter, sfGFP sequence was amplified from the 35S-sfGFP-nosT plasmid135 (Addgene # 80129) using primers UsfGM-F1 and UsfGnes-R1. The UBQ10 promoter was amplified from the UPG plasmid136 (Addgene # 161003) using primers OutALFd and UsfGM-R1. The UBQ3 terminator was amplified from the UPG plasmid136 (Addgene # 161003) using primers UsfGnes-F1 and OutALRb. Primer overhangs spanning the junction between sfGFP and the UBQ3 terminator contain the sequence of the mouse PKIα NES. pCambia1300 backbone was digested with BamHI and Kpnl, and all fragments were Gibson-assembled into the backbone. Sequences of primers, pARR7::ARR7-llama, and pUBQ10::sfGFP-NES:UBQ3ter can be found in Supplemental Dataset 5.

Col-0 plants were co-transformed with pARR7::ARR7-llama and pUBQ10::sfGFP-NES:UBQ3ter, and selected with Basta (for pARR7::ARR7-llama) + Hygromycin (for
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pUBQ10::sfGFP-NES:UBQ3ter). Surviving T1 plants were screened for clear nuclear signal in the inflorescence, and 5 independent T1 plants were selected and crossed into drmy1. F2 plants from each line were again selected with Basta + Hygromycin and genotyped. One line showed cosegregation with the DRMY1 locus. Two lines showed severe silencing in the F2 and could not be used. Two lines (7-4 and 7-6), though with minor silencing in F2, were used for imaging and image analysis. F3 plants of 7-4 and 7-6 had severe silencing, and therefore only F2 were imaged.

Plant growth conditions

For most experiments, seeds were sown in wetted Lamber Mix LM-111 soil and stratified at 4°C for 3-5 days. For experiments including drmy1 wol and drmy1 arr1,10,12, all seeds were sown onto ½ MS plates with 0.05% (w/v) MES, 1% (w/v) sucrose, 1.2% (w/v) agar, pH 5.7, and stratified at 4°C for a week. They were grown for 7-10 days before being transplanted to soil (for imaging of inflorescence or aerial part of the plant) or left on the plates until desired time of the experiment (for seedling imaging or puromycin labeling).

Most plants were grown under 16 h – 8 h light-dark cycles (fluorescent light, ~100 µmol m⁻¹ s⁻¹) at 22°C in a Percival walk-in growth chamber. We found that the drmy1 phenotype is more pronounced in this condition than under continuous light. The ap1 cal 35S::AP1-GR and drmy1 ap1 cal 35S::AP1-GR plants were grown in soil under continuous light at 16°C to prevent premature floral induction.

Flower staging

Flower buds were staged as previously described. Briefly, stage 1 is when the floral meristem emerges, but not yet separated, from the inflorescence meristem. Stage 2 is when the floral meristem separates from the inflorescence meristem but with no floral organs initiated. Stage 3 is when sepal primordia initiate. Stage 4 is when sepal primordia bend to cover part of the floral meristem. Stage 5 is when stamen primordia initiate. Stage 6 is when sepal primordia completely cover the floral meristem.

RNA-seq data collection and analysis

For RNA-seq in the inflorescence, bolting ap1 cal 35S::AP1-GR and drmy1 ap1 cal 35S::AP1-GR plants were induced daily with an aqueous solution containing 10 µM dexamethasone (Sigma-Aldrich), 0.01% (v/v) ethanol, and 0.015% (v/v) Silwet L-77 (Rosecare.com). When sepals initiated from the floral meristems, usually on the fourth day after three daily inductions, three inflorescence samples per genotype (including inflorescence meristems and buds under stage 6) were collected and immediately put into liquid nitrogen. RNA extraction, library preparation, RNA-seq, and data analysis for inflorescence samples were done as previously described with a few changes. After read mapping, genes with at least two raw reads in at least two biological replicates in either WT or drmy1 were kept for downstream analysis. For differentially expressed genes, we set a log2 fold change threshold of ±1 and a BH-adjusted p-value threshold of 0.05. For GO term enrichment, gene-GO mapping data was obtained from TAIR (https://www.arabidopsis.org/download_files/GO_and_PO_Annotations/Gene_Ontology_Annotations/ATH_GO_GOSLIM.txt)
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The R package “topGO”\textsuperscript{137} (version 2.38.1) was used for the enrichment, with statistic “fisher”, algorithm “weight01”, annotation function “annFUN.gene2GO”, and minimum node size 10. The results were ranked by their p-value, and the first 8 terms were plotted.

For RNA-seq in seedlings, WT and \textit{drmy1} seedlings were grown to quiescence (7 days) in ½ MS liquid media as previously described\textsuperscript{34}. After 7 days, the media was replaced with ½ MS liquid media containing 15 mM glucose and incubated for 24 hours to activate TOR. Seedlings were then incubated with or without AZD-8055 in addition to 15 mM glucose in ½ MS liquid media for 2 hours before collecting tissue. RNA was extracted from 100 mg pooled seedlings using the Spectrum Plant Total RNA Kit (Sigma). This RNA was used as a template for RNA-Seq library synthesis and sequencing, which was performed by Novogene. RNA-seq data for AZD-8055 treated WT and \textit{drmy1} seedlings were preprocessed with fastp (v. 0.22.0) using default parameters. Preprocessed reads were then mapped to the TAIR10 reference genome using STAR (v. 2.7.10z_alpha_220314). Following alignment, BAM output files from STAR were used to generate feature counts for transcripts using subread-featureCounts (v. 2.0.3) and the Araport11 transcriptome. TPMs were generated using TPMCalculator (v. 0.0.3). Differential expression analysis was performed using feature count data and DESeq2 (v. 1.36.0).

A list of genes with uORFs based on gene models of the TAIR10 Arabidopsis genome assembly were downloaded from von Arnim et al.\textsuperscript{62}. For each gene, within each genotype, protein-transcript ratio was calculated as the ratio between mean protein abundance and mean transcript TPM across all bio-reps in our proteomics and RNA-seq datasets, respectively. This was log2-transformed, and the difference between \textit{drmy1} and WT was calculated. This was used as an indicator of translation rate difference between \textit{drmy1} and WT, although we acknowledge that other factors such as protein stability may affect this number. This was plotted against the number of uORFs in each gene model (0, 1, or ≥ 2).

Proteomics

Five induced inflorescence samples of WT and \textit{drmy1} in \textit{ap1 cal AP1-GR} background were collected as described above. Samples were ground in liquid nitrogen. Total soluble proteins were extracted in ice-cold extraction buffer (50 mM PBS-HCl (pH 8.0) buffer with 150 mM NaCl, 2% NP-40, 1 mM PMSF, 1x Roche cOmplete protease inhibitor cocktail (Sigma 11697498001), and 1x Halt TM Phosphatase inhibitor cocktail (ThermoFisher 78420)) and filtered through Pierce™ Micro-Spin Columns (30 μm pore size; Thermo Scientific 89879). Extracts were RuBisCO-depleted using Seppro Bubisco Kit (Sigma SEP070-1KT), concentrated, denatured, reduced, cysteine blocked, trypsin-digested, and TMT 10-plex labeled. Then, mass spectrometry was done using an UltiMate 3000 RSCLnano / Orbitrap Fusion system (Thermo Scientific). Raw data was searched against the NCBI protein database using PD 2.3 (Thermo Scientific) with Sequest HT searching engine. Precursor-based protein identification and relative quantification was done using the standard processing workflow in PD 2.3, with an additional node of Minora Feature Detector. Proteins with at least 2 supporting peptides were kept for downstream analysis. For each protein, data was fit with an ANOVA model and a p-value was calculated. Proteins with a p-value < 0.05 were considered differentially accumulated in \textit{drmy1}. GO term enrichment was done as above, using genes corresponding to the differentially accumulated proteins.
Polysome extraction and profiling

Three induced inflorescence samples of WT and *drmy1* in *ap1 cal AP1-GR* background were collected as described above, and polysomes were extracted as previously described. Briefly, samples were ground in liquid nitrogen, mixed with an extraction buffer (0.2 M Tris pH 9.0, 0.2 M KCl, 0.025 M EGTA, 0.035 M MgCl$_2$, 1% (w/v) Brij-35, 1% (v/v) Triton X-100, 1% (v/v) Igepal CA-630, 1% (v/v) Tween-20, 1% (w/v) Sodium deoxycholate, 1% (v/v) Polyoxyethylene 10 tridecyl ether, 5 mM Dithiothreitol, 1 mM Phenylmethylsulfonyl fluoride, 100 µg/ml cycloheximide, 100 µg/ml chloramphenicol, 40 U/ml RNasin, 0.5 mg/ml Heparin), and let sit on ice for 10 min. Samples were centrifuged at 4°C 4,000 g for 5 min, supernatant was transferred to a new tube, centrifuged at 4°C 16,000 g for 15 min, and supernatant was filtered through Miracloth.

Polysome extracts were profiled as previously described. Briefly, samples were loaded onto 15%-45% sucrose density gradients and centrifuged at 4°C 38,000 rpm in a SW41 rotor. Separated samples were fractionated at a rate of 0.375 mL/min in an Isco fractionation system, and absorbance at 254 nm was recorded.

Puromycin labeling

Puromycin labeling was done as previously described, with slight modifications. In seedlings, when comparing WT and *drmy1*, in order to control for plant size, WT seedlings were grown for 8 days and *drmy1* seedlings were grown for 10 days (Figure 1E). When comparing WT, *drmy1*, *wol*, and *drmy1 wol*, we were unable to control for plant size because *drmy1 wol* seedlings were too small. We therefore controlled for plant age, and seedlings were grown to specified age (8 days for Figure 6B and 14 days for Figure 6C). Seedlings were harvested from plates and incubated with an incubation buffer (½ MS, 0.05% (w/v) MES, 1% (w/v) sucrose, 0.1% (v/v) Tween-20, 0.1% (v/v) DMSO, 1x Gamborg vitamin mix, pH 5.7), with or without 50 µM CHX, for 4 hours in an illuminated growth chamber. Then, the buffer was replaced with a fresh incubation buffer (which is same as above, but contains 50 µM puromycin (GoldBio P-600-100)), and incubation continued for another 45 min.

In inflorescences of WT and *drmy1* in *ap1 cal AP1-GR* background, inflorescences were DEX-induced as described above. Inflorescence samples were collected and put in an incubation buffer (½ MS, 1% (w/v) sucrose, 0.02% (v/v) Silwet L-77, 0.1% (v/v) DMSO, 50 µM puromycin, 1x Gamborg vitamin mix, pH 5.7), with or without 100 µM CHX. Samples were vacuum infiltrated for 15 minutes and then put on a rocking shaker in an illuminated growth chamber for 45 minutes.

In both cases, at the end of the incubation, samples were washed three times with water, blotted dry, weighed, and frozen in liquid nitrogen. Soluble proteins were extracted as described above. Puromycin incorporated into the proteins were detected in a Western blot using a mouse-origin anti-puromycin monoclonal antibody (12D10, Sigma MABE343, lot # 3484967) and a goat-anti-mouse HRP-conjugated secondary antibody (Abcam ab6789, lot # 3436981). RuBisCO large subunit in Ponceau S-stained membrane was used as a loading control.

TOR activity assay
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WT and drmy1 seedlings were grown in a six-well plate containing ½ MS liquid media. After seven days, the media were replaced with half-strength MS liquid media plus 15 mM glucose and incubated for 24 hours. At least 120 quiescent seedlings per sample were collected and frozen in liquid nitrogen. Protein was then extracted from the plant tissue in 100 mM MOPS (pH 7.6), 100 mM NaCl, 5% SDS, 0.5% b-mercaptoethanol, 10% glycerin, 2 mM PMSF, and 1x PhosSTOP phosphatase inhibitor (Sigma). S6K-pT449 was detected by Western blot using a phosphospecific antibody (Abcam ab207399) and an HRP-conjugated goat anti-rabbit IgG secondary antibody (Jackson Immuno Research 111-035-003). Total S6K was detected using a custom monoclonal antibody described by Busche et al. Total protein visualized in Ponceau S-stained membrane was used as a loading control.

Confocal microscopy

Confocal imaging of reporter lines in the inflorescence were done as previously described. Briefly, main inflorescences (not side branches) were cut and dissected with a Dumont tweezer (Electron Microscopy Sciences, style 5, no. 72701-D) to remove buds older than stage 9 or 10. The inflorescences were then inserted upright into a small petri dish (VWR, 60 x 15 mm) containing inflorescence culture medium (1/2 MS, 1% (w/v) sucrose, 1x Gamborg vitamin mixture, 0.1% (v/v) plant preservative mixture (Plant Cell Technology) 1% (w/v) agarose, pH 5.8), leaving most of the stem inside the medium and the buds outside. They were then further dissected to reveal stage 6 and younger buds, immersed with water, and imaged under a Zeiss710 upright confocal microscope with a 20x Plan-Apochromat water-dipping lens (1.0 NA). For live imaging experiments, inflorescence samples were put in a continuous-light growth chamber between time points. To prevent bacterial growth, samples were transferred onto fresh media every 2 to 3 days, and for live imaging experiments lasting longer than 6 days, once in the middle, plants were incubated with an aqueous solution of 100 µg/ml Carbenicillin (GoldBio, C-103-5, lot # 0129.091814A) for 30 minutes.

To visualize tissue morphology of inflorescence samples without a reporter, samples were stained for 5 minutes with an aqueous solution of 0.1 mg/ml propidium iodide (PI) and 0.1% (v/v) Tween-20, washed three times with water, and imaged. The following laser and wavelength were used in confocal imaging. Chlorophyll, excitation 488 nm, emission 647-721 nm. PI, excitation 514 nm, emission 566-659 nm. mCherry, excitation 594 nm, emission 600-659 nm. tdTomato, excitation 561 nm, emission 566-595 nm. For EYFP/VENUS/mCitrine, in 35S::mCitrine-RCI2A, excitation 514 nm, emission 519-580 nm; in DR5::3xVENUS-N7, excitation 514 nm, emission 519-569 nm; in pARF5::ER-EYFP-HDEL, excitation 514 nm, emission 519-550 nm; in R2D2, excitation 488 nm, emission 493-551 nm. For GFP/sfGFP, in pARR7::ARR7-llama UBQ10::sfGFP-NES, excitation 488 nm, emission 493-569 nm; in pARF3::N3xGFP, pARF6::N3xGFP, pARF8::N3xGFP, and pARF10::N3xGFP, excitation 488 nm, emission 493-564 nm; in TCS::GFP, excitation 488 nm, emission 493-513 nm.

Visualization of tissue morphology

For single-channel image stacks intended for the visualization of tissue morphology (35S::mCitrine-RCI2A or PI), stacks were 3D-rendered using the ZEN confocal software.
(Processing -> 3D). Parameters were set to best visualize tissue morphology, typically, minimum 5-10, ramp 60-80, maximum 100. Buds were rotated to desired orientation, and screenshots were taken using the "Create Image" button. For fluorophores that are dimmer, less sharp, or have a noisy background (UBQ10::mCherry-RCI2A or Chlorophyll), stacks were converted from LSM to TIF using ImageJ, loaded into MorphoGraphX, and screenshots were taken using the built-in screenshot function in MorphoGraphX.

To aid visualizing tissue morphology and determine the timing of sepal initiation, each stack was fitted with a surface, and a Gaussian curvature heatmap was calculated from the surface (see below). We consider a sepal primordium as initiated when we see a dark red band of positive Gaussian curvature (primordium) separated from the center of the floral meristem by a dark blue band of negative Gaussian curvature (boundary).

Gaussian curvature heatmaps were calculated as previously described, with slight modifications. Briefly, stacks underwent the following processes in MorphoGraphX: Gaussian blur (3 times; X/Y/Z sigma = 1 µm for the first 2 times, and 2 µm for the third time), edge detection (threshold = 2000-8000 depending on the brightness of the stack, multiplier = 2.0, adapt factor = 0.3, fill value = 30000), marching cube surface (cube size = 8 µm, threshold = 20000), subdivide mesh, smooth mesh (passes = 5), subdivide mesh, smooth mesh (passes = 5), project mesh curvature (type = Gaussian, neighborhood = 10 µm, autoscale = no, min curv = -0.0015, max curv = 0.0015). For ease of visualization, the lookup table “jet” was applied to the mesh.

Quantification of sepal initiation robustness

For sepal primordium number, screenshots were taken of stage 3-6 buds of indicated genotypes, in either ZEN or MorphoGraphX. The number of sepal primordia initiated were counted from these screenshots.

For variability in sepal primordium positioning, within each bud, an angular distance was measured between each pair of adjacent sepal primordia (with vertex at the center of the bud), using ImageJ. Note that the last pair was not measured – the angular distance was calculated as the sum of all other angular distances subtracted from 360°. A CV value (standard deviation divided by mean) was calculated from all the measured or calculated angular distances. Buds with sepal primordia evenly distributed around the bud periphery should have a small CV value, i.e. all angles are around 90° for four-sepal buds (or 72° for five-sepal buds, etc.). Buds whose sepal primordia distributed variably or randomly around the bud periphery will have widely varying angular distances between adjacent sepal primordia, and thus large CV values.

Relative sepal initiation timing was quantified as previously described. Briefly, dissected inflorescence samples were live-imaged every 6 hours. A Gaussian curvature heatmap was generated for each sample at each time point and was used to determine the time point at which a sepal primordium initiates. A sepal primordium is considered initiated at time point Tn if it is absent at time point T(n-1) but becomes present at time point Tn. Within the same bud, we counted the number of time points between outer and inner sepal initiation, and between outer and lateral sepal initiation, and multiplied them by 6 hours to get the relative initiation timing of these sepals.
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Quantification of fluorescent reporters

For TCS::GFP, pARF3::N3xGFP, pARF5::ER-YFP-HDEL, pARF6::N3xGFP, pARF8::N3xGFP, pARF10::N3xGFP, pUS7Y::mDII-NtdTomato, pUS7Y::DII-N3xVENUS, and UBQ10::mCherry-RCI2A, total signal (integrated density) was quantified from maximum intensity projection images using ImageJ\textsuperscript{141,142}. Fluorescence intensity was measured in pixel intensity units (0-255 range). Signal intensity was calculated as total signal divided by area.

For both TCS::GFP and DR5:3xVENUS-N7, circular histogram analysis was done as previously described\textsuperscript{26}. Briefly, individual buds were cropped out of image stacks, channels were split using FIJI and saved in TIF format, and TIF stacks were imported into MorphoGraphX. Signal from outside the buds (e.g. inflorescence meristem, parts of other buds within the same image) was manually removed using the Voxel Edit function. Buds were positioned so that the incipient sepal primordia are in the XY plane: the incipient outer sepal is at 45°, the incipient inner sepal and the inflorescence meristem are at 225°, and the incipient lateral sepals are at 135° and 315°, respectively. Fluorescence intensity was measured in pixel intensity units (0-255 range). A circular histogram of bin width 1° centered around the Z axis was exported for each replicate expressing DR5 and/or TCS. Multiple circular histograms of the same reporter and genotype were pooled and mean ± SD were plotted.

For GFP signal in plants carrying pUBQ10::sfGFP-nes-UBQ3ter and pARR7::ARR7-linker-llama-ARR7ter reporters, screenshots were taken in MorphoGraphX as described above. Screenshots were subtracted of a background determined using blank regions with no tissue, and brightened to the same level to reveal differences in GFP distribution patterns. A square region containing 5-10 cells were taken from each screenshot, and GFP intensity (in gray value ranging from 0 to 255) along a straight line of 239 pixels in length was quantified using ImageJ\textsuperscript{141,142} (Analyze -> Plot profile). For ease of visualization, the curves were smoothed by taking the average of the gray value of 11 neighboring pixels (including itself) as the value of each pixel.

In vitro drug treatments on inflorescence samples

For cycloheximide (CHX) treatment, a stock solution of 10 mM CHX was made from powder (Sigma C1988) in pH 4.0 water. The stock solution was filter-sterilized and stored in -20°C and replaced every six months to prevent degradation. The stock solution was added to an autoclaved and cooled (but not solidified) inflorescence culture medium (see above) to a final concentration of 2 µM. The medium was not pH-buffered. The medium was made at the beginning of each experiment and was stored at 4°C. After each imaging session (at day 3 and day 6), new medium was taken out of 4°C, warmed to room temperature, and inflorescence samples were transferred onto the new medium.

For AZD-8055 treatment, a stock solution of 16 mM AZD-8055 was prepared from powder (Cayman Chemical 16978) in DMSO within days of use, and stored in -80°C. The stock was serial-diluted with water to 2 mM, and added to autoclaved and cooled (but not solidified) inflorescence culture medium (see above) to a final concentration of 2 µM. For the mock medium, DMSO was added to the inflorescence culture medium to a final concentration of 0.0125% (v/v).

For 6-benzylaminopurine (BAP) treatment, a stock solution of 50 mM BAP was prepared from powder (Alfa Aesar A14678) in DMSO, and stored in -80°C. The stock was added to an
autoclaved and cooled (but not solidified) inflorescence culture medium (see above) to a final concentration of 5 µM. For the mock medium, DMSO was added to the inflorescence culture medium to a final concentration of 0.01% (v/v).

Inflorescences were dissected and inserted into regular inflorescence culture medium without drugs, and pre-treatment image stacks were captured. Then, they were transferred into specified treatment or mock media, and imaged at the specified time points.

**In vivo Torin2 treatment**

Starting at 14 days after germination, twice each day for 15 days, 2 nmol of Torin2 (Cayman Chemical 14185) in 20 µl of aqueous solution containing 0.5% DMSO and 0.5% Tween-20 was applied to the center of the rosette using a pipette. For mock, 20 µl aqueous solution containing 0.5% DMSO and 0.5% Tween-20 was applied. At the end of the 15-day treatment period, inflorescences were dissected and put in the inflorescence culture medium for imaging.

To prevent Torin2 degradation, throughout the duration of this experiment, the Torin2 stock solution in DMSO was kept in -80°C and replaced each week, and the treatment and mock solutions were kept in 4°C and replaced each day.

**Imaging of whole plant, whole inflorescence, silique, and mature sepals**

For whole-plant imaging, aerial parts of the plants were removed from the pots, flattened, put on a dark cloth, and imaged with a cell phone (iPhone 12, iOS 16.2).

For whole-inflorescence imaging, inflorescences consisting of open flowers and unopened buds were removed from the plant and held with forceps. Images were taken under a Zeiss Stemi 2000-C Stereo Microscope with a cell phone (iPhone 12, iOS 16.2).

For silique imaging, siliques on inflorescences sufficiently distant from the shoot apex that were developed and started to ripen were picked with forceps, opened with a razor blade, and imaged under a Zeiss Stemi 2000-C Stereo Microscope with a cell phone (iPhone 12, iOS 16.2).

Mature sepal imaging was done as previously described. Briefly, mature sepals from stage 15 flowers (10th to 25th flower on the inflorescence) were dissected and sandwiched between two slides to flatten. Images were taken using a Canon Powershot A640 camera attached to a Zeiss Stemi 2000-C Stereo Microscope. Minor damages were manually fixed, and undesired objects such as pollen grains were manually removed from these images. Sepals with major damages were discarded. Then, a contour was extracted from each sepal using custom python scripts. This gave us measurements such as length, width, area, etc. of each sepal. To measure between-flower variability of length, within each genotype and for each of outer, inner, and lateral positions, a CV (standard deviation divided by mean) of all sepals was calculated (for example, a CV of length of all outer sepals in WT). To determine statistical significance, genotypes were compared pairwise using permutation tests. To measure within-flower variability of length, a CV was calculated for all sepals within each flower (for example, a CV of length of outer, inner, and two lateral sepals in WT bud #10). For accurate calculation of CV, flowers with length data of at least four sepals were included in the analysis. To determine statistical significance, genotypes were compared pairwise using Wilcoxon rank sum tests.
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Cytokinin extraction and measurement

Cytokinin extraction was based on a previously published protocol\textsuperscript{144} with modifications. Briefly, five inflorescence samples of induced \textit{ap1 cal 35S::AP1-GR}, and six inflorescence samples of induced \textit{drmy1 ap1 cal 35S::AP1-GR} were collected as described above. Samples were ground in liquid nitrogen and twice extracted in methanol : water : formic acid (15:4:1). 200 pg of BAP per sample was added as an internal control. Extracts were centrifuged at 14,650 rpm in -4°C for 30 min, and supernatant was evaporated of methanol and reconstituted in 1% (v/v) acetic acid. Samples were passed through an Oasis MCX SPE column (Waters 186000252), washed with 1% acetic acid, washed with methanol, and eluted with 0.35 M ammonia in 70% methanol. Eluents were evaporated to complete dryness, reconstituted in 5% acetonitrile, and sent for LC-MS.

LC-MS was done as previously described\textsuperscript{145}, with modifications. Briefly, 1 µl of each sample was injected into a Thermo Fisher Scientific Vanquish Horizon UHPLC System coupled with a Thermo Q Exactive HF hybrid quadrupole-orbitrap high-resolution mass spectrometer equipped with a HESI ion source. Samples were separated on a C18 ODS column (AQUITY UPLC BEH C18, 1.7 µm, 2.1 × 100 mm, Waters), at a flow rate of 0.3 ml/min, with linear gradients of solvent A (0.1% formic acid) and solvent B (0.1% formic acid in methanol) according to the following profile: 0 min, 99.0% A + 1.0% B; 4.0 min, 55.0% A + 45.0% B; 7 min, 30.0% A + 70.0% B; and then with isocratic conditions: 8 min, 1.0% A + 99.0% B; 12 min, 99.0% A + 1.0% B.

Cytokinins were detected using the positive ion mode. For tZ, tZR, iP, iPR, and the internal control BAP, peaks were identified from an external standard mix composed of 0.1 µg/ml each of BAP (Alfa Aesar A14678), tZ (Sigma Z0876), tZR (Sigma Z3541), iP (Cayman Chemical 17906), and iPR (Cayman chemical 20522) in 5% acetonitrile. For cZ and cZR, peaks were identified based on previously reported precursor m/z and retention time\textsuperscript{146}. Using Xcalibur (Thermo Scientific), peak area was quantified for each cytokinin in each sample, normalized against the peak area of BAP (internal control) and sample fresh weight, and then normalized against the average abundance of tZ in WT samples.

Software

Image processing was done in ImageJ (version 2.9.0/1.53t, build a33148d777)\textsuperscript{141,142} and MorphoGraphX (version 2.0, revision 1-294, CUDA version 11.40)\textsuperscript{143}. Data processing was done in RStudio (R version 4.0.5 “Shake and Throw” (2021-03-31))\textsuperscript{147}. Graphs were made using the package ggplot2 (version 3.3.3)\textsuperscript{148}. Fisher’s contingency table tests were done using the function fisher.test in R. Wilcoxon rank sum tests were done using the function wilcox.test in R. Hypergeometric tests were done using the function phyper in R. Data fitting with ANOVA was done using the function aov in R. Figures were assembled in Adobe Illustrator (version 25.4.1). An RGB color profile “Image P3” was used for all the figures.
SUPPLEMENTAL INFORMATION TITLES AND LEGENDS

Supplemental Figure 1. Evidence that the drmy1 mutant has ribosomal and translation defects, associated with Figure 1.

(A) The drmy1 phenotype is reproduced in the ap1 cal AP1-GR system (Ler background). Shown are representative buds of ap1 cal AP1-GR (top row) and drmy1 ap1 cal AP1-GR (bottom row) at day 0 (before DEX induction), day 3 (after 3 DEX inductions, when tissue is collected for RNA, protein, or cytokinin extraction), and day 5 (after 5 DEX inductions). Arrowheads show sepal primordia that are of variable number, position, and sizes. Asterisks indicate periphery of the floral meristem that has limited or no sepal outgrowth in drmy1 ap1 cal AP1-GR compared with ap1 cal AP1-GR. Scale bars, 25 µm.

(B) Summary of the inflorescence RNA-seq and proteomics datasets. Shown are numbers of genes in each category. Down, downregulated in drmy1; NS, not significantly changed between drmy1 and WT; Up, upregulated in drmy1; NA, not available. Note that in the combined dataset (gene-protein pairs), different genes encoding for the same protein were separately counted, so were different proteins encoded by the same gene. See also Supplemental Dataset 1.

(C) Violin and box plots of log2 fold change in RNA between drmy1 and WT in induced ap1 cal AP1-GR inflorescence, for genes encoding ribosomal components (“Structural constituents of the ribosome” GO:0003735, and its offspring terms) and all other genes involved in translation (“Translation” GO:0006412, and its offspring terms). The following genes are labeled on the graph: UL4Z (AT3G09630), log2FC = -0.492; UL4Y (AT5G02870), log2FC = -0.509; UL18Z (AT3G25520), log2FC = -0.459. Note that the x-axis was trimmed to (-2,2) for ease of display.

(D) Violin and box plots of log2 fold change in protein level between drmy1 and WT in induced ap1 cal AP1-GR inflorescence, for genes in the same categories as in (C). The following genes are labeled on the graph: UL4Z (AT3G09630), log2FC = 0.352; UL4Y (AT5G02870), log2FC = 0.509; UL18Z (AT3G25520), log2FC = 0.742.

(E) Coherent regulation of gene expression by drmy1 and AZD-8055. Shown is a contingency table of genes downregulated (Down), not significantly changed (NS), and upregulated (Up) in drmy1 vs WT (columns), and in AZD-8055-treated WT vs mock-treated WT (rows). Bold font shows the number of genes in each category, and gray font shows the expected number of genes if there were no correlation between two conditions (calculated as row margin x column margin / total number of genes). Categories where the number of genes is above expectation are highlighted blue, and categories where the number of genes is below expectation are highlighted red. Chi-square test p < 2.2x10^-16.

(F) Gene ontology enrichment of genes coherently downregulated by both drmy1 and AZD-8055. Shown are the top 8 terms and their enrichment p-values. A complete list can be found in Supplementary Dataset 3.

(G-J) Fluorescence of a constitutively expressed marker supports the hypothesis that drmy1 has reduced translation rate. (G-I) are representative confocal images of UBQ10::mCherry-RCI2A in dissected inflorescences of WT (G), drmy1 (H), and ul4y (I). Numbers show how the signal is divided based on the stage of floral meristem when quantified (IM+1, inflorescence meristem plus stage 1; 2, stage 2; 3, stage 3). Scale bars, 25µm. (J) shows quantification of signal intensity (i.e.
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integrated density divided by area) in all images divided as in (G-I). Mean ± SD are shown. Data was fit using a two-way ANOVA model with genotype and stage as two additive factors. Asterisks show statistically significant pairwise contrasts between WT and *drmy1* \( p < 2 \times 10^{-16} \) and between WT and *ul4y* \( p = 2.1 \times 10^{-15} \). Sample sizes: WT IM+1, n = 30; *drmy1* IM+1, n = 22; *ul4y* IM+1, n = 18; WT stage 2, n = 99; *drmy1* stage 2, n = 100; *ul4y* stage 2, n = 52; WT stage 3, n = 39; *drmy1* stage 3, n = 27; *ul4y* stage 3, n = 26.
Supplemental Figure 2. Ribosomal mutations enhance the *drmy1* phenotype, associated with Figure 2.

(A-H) Examples of stage 5 buds from *drmy1* (A), *drmy1 ul4z* (B-D), *drmy1 ul4y* (E-F), and *drmy1 ul18z/+* (G-H). In (B,E,G) sepal primordia within each bud have bigger size differences than typical *drmy1* single mutant buds; asterisks show giant outer sepal primordia and brackets show bud peripheral regions with little or no primordium outgrowth. In (C,F,H), arrowheads show 6 sepal primordia within each bud, which does not occur in *drmy1*. In (D,H), asterisks show the presence of two outer sepal primordia within a bud, instead of one in *drmy1*. Scale bars, 25µm.

(I-J) Quantification of sepal primordium number (I) and positional variability (J), comparing each of *drmy1 ul4z* (n = 60), *drmy1 ul4y* (n = 61), and *drmy1 ul18z/+* (n = 69) with *drmy1* (n = 67). “ns” indicates no significant difference in Fisher’s contingency table tests (I) and Wilcoxon’s rank sum tests (J) respectively. Data of *drmy1* is reproduced from the same dataset presented in Figure 2.

(K) Dissected young silique of *drmy1 ul18z/+* mother plant. Arrowheads point to aborted ovules. Scale bar, 200 µm.
Supplemental Figure 3. Sepal primordia in ribosome and TOR mutants catch up in growth to form uniformly sized mature sepals within the bud, associated with Figure 3.

(A-F) Representative inflorescences images (left) of WT (A), drmy1 (B), ul4z (C), ul4y (D), ul18z (E), and lst8-1-1 (F), with boxed regions enlarged (right). Blue arrowheads show sepals of regular length, and red arrowheads show sepals shorter than others. Note that sepals in drmy1 were unable to close due to unequal lengths, while sepals in ul4z, ul4y, and ul18z, and close like in WT. Sepals in lst8-1-1 were unable to close although there is no apparent variation in length. Scale bars, 0.5 mm.

(G-L) Dissected sepals from a representative bud of WT (G), drmy1 (H), ul4z (I), ul4y (J), ul18z (K), and two buds of lst8-1-1 (L). Note that sepals in the drmy1 bud are of different sizes. Sepals within each bud of ul4z, ul4y, ul18z, and lst8-1-1 are of similar sizes, although there can be variation between different buds of the same genotype. O, outer sepal. I, inner sepal. L, lateral sepal. Scale bars, 200 µm.

(M) Quantification of between-flower variability of sepal length. Length was measured from all imaged outer sepals of each genotype, and coefficient of variation (CV) was calculated. A two-sided permutation test (100,000 permutations) for CV difference not equating to zero was done for each pair of genotypes, and results were represented by letters (left). Similar analysis was done for the inner sepal (middle), and the lateral sepal (right). Sample size: Outer sepal, WT n = 35, drmy1 n = 43, ul4z n = 37, ul4y n = 42, ul18z n = 39, lst8-1-1 n = 43. Inner sepal, WT n = 34, drmy1 n = 46, ul4z n = 38, ul4y n = 44, ul18z n = 37, lst8-1-1 n = 44. Lateral sepal, WT n = 65, drmy1 n = 84, ul4z n = 81, ul4y n = 89, ul18z n = 76, lst8-1-1 n = 82.

(N) Quantification of within-flower variability of length. Flowers with length data from at least four sepals were analyzed. A CV of length from all sepals within each flower was calculated, and mean ± SD was plotted, grouped by genotype. A Wilcoxon rank sum test was done for each pair of genotypes, and results were represented by letters. Sample size: WT n = 31 buds, drmy1 n = 38 buds, ul4z n = 33 buds, ul4y n = 36 buds, ul18z n = 32 buds, lst8-1-1 n = 39 buds.

(O-Q) Live imaging of sepal development from stage 3 to 6 in a bud each of WT, drmy1, and ul4y, showing chlorophyll or propidium iodide channel, and Gaussian curvature calculated from the surface. Note that both drmy1 and ul4y have inner sepals that initiate late (day 2, asterisk). The drmy1 inner sepal develops slowly, and leaves the bud open at day 3 (red arrowhead). The ul4y inner sepal catches up with the rest of the sepals and closes the bud (blue arrowhead). Scale bars, 25 µm.
Supplemental Figure 4. Inhibition of TOR activity and translation causes auxin maxima formation at variable positions, correlated with variable positions of sepal primordia, associated with Figure 4.

(A-E) Variable patterning of auxin signaling in drmy1, ul4y, and CHX-treated WT buds corresponds to variable sepal initiation. Shown are a representative bud each of the labeled genotype or treatment live-imaged over three or four days. In all but the last time point, in the top row is DR5::3xVENUS-N7 (yellow), in the middle row is a composite of DR5::3xVENUS-N7 (yellow) and Chlorophyll (magenta), and in the bottom row is Gaussian curvature calculated from a surface extracted from the Chlorophyll channel. In the last time point, propidium iodide is shown on the top, and Gaussian curvature calculated from a surface extracted from the propidium iodide channel is shown on the bottom. (A) In WT, four robustly positioned auxin maxima at day 1 correlates with four robustly positioned sepal primordia at day 4 (blue arrowheads). (B) In drmy1, at day 1 there are three robustly positioned auxin maxima (blue arrowheads). At day 2, a diffuse band of auxin signaling occurs in the adaxial periphery of the bud, joining with one of the lateral auxin maxima (red bracket). At day 3, this diffuse band splits into three auxin maxima (red arrowheads), making a total of 5. The maxima correlate with the five sepal primordia at day 4, three at robust positions (blue arrowheads) and two at irregular positions (red arrowheads). (C) In ul4y, at day 1 there are two auxin maxima at robust positions (blue arrowheads), one at robust lateral position but much weaker (red arrowhead), and a band of weak auxin signaling in the adaxial periphery of the bud (red bracket). At day 2, the weak auxin maxima at lateral position got stronger, and the weak band split into two auxin maxima on the adaxial side (red arrowheads). These five auxin maxima correspond to the five sepal primordia at day 3, three in robust positions (blue arrowheads) and two in irregular positions (red arrowheads). (D) In the WT bud treated with Mock, four robust auxin maxima at day 6 of the treatment corresponds to four robust sepal primordia seen at day 9 (blue arrowheads). (E) In the WT bud treated with CHX, at day 6 there are three stronger auxin maxima (blue arrowheads) and two weaker ones (red arrowheads), corresponding to three bigger primordium outgrowth regions (blue arrowheads) and two smaller ones (red arrowheads) at day 9. For ease of display, the DR5 channel in CHX-treated WT was brightened three times relative to mock. Scale bars, 25 µm.

(F-I) TOR inhibition using Torin2 causes increased cytokinin signaling, and occasional variability in both auxin and cytokinin signaling. (F) Representative images of late stage 2 buds from WT plants treated with mock or 2 nmol Torin2 for 15 days. Shown are DR5::3xVENUS-N7 in yellow, TCS::GFP in cyan, and both merged with propidium iodide in magenta. Note that 3/16 (19%) buds had variable number and position of DR5 and TCS maxima, and 13/16 (81%) had robust DR5 and TCS maxima, although TCS intensity is higher in both cases compared with mock-treated buds. Scale bars, 25 µm. (G) Quantification of TCS intensity from maximum intensity projection images, normalized to the mean of WT mock. Shown are mean ± SD. Asterisk shows statistical significance in a two-tailed Student’s t-test compared with WT mock (p = 1.2x10^{-4}). (H) Circular histograms of DR5 distribution around the bud (mean ± SD). (I) Circular histograms of TCS distribution around the bud (mean ± SD). For calculation of circular histograms, please see Figure 4 legends and Materials and Methods. Sample size: WT mock, n = 11 buds; WT Torin2, n = 16 buds.
Supplemental Figure 5. Translation of uORF-containing ARFs is not universally downregulated in drmy1, associated with Figure 5.

(A) drmy1 has a lower protein-transcript ratio than WT for genes with at least 2 uORFs. 5,086 transcript-protein pairs in our dataset were grouped according to the maximum number of uORFs in all transcript isoforms (0, n = 3,485; 1, n = 874; ≥ 2, n = 724)\(^2\). For each pair, protein-transcript ratio was calculated, log-transformed, and the difference between drmy1 and WT was plotted. A negative value means this gene has less protein per transcript in drmy1 than WT, and could indicate reduced translation or protein stability. Medians for each group: 0 uORF, -0.00367; 1 uORF, -0.00808; ≥ 2 uORFs, -0.0243. Asterisk show statistically significant difference from Group 0 in a Wilcoxon rank sum test (p = 3.167x10\(^{-4}\)), while ns means no significant difference from Group 0 (p = 0.167).

(B-D) There is no universal decrease in the expression of uORF-containing ARF reporters. (B) Transcript level of three activator ARFs (ARF5, ARF6, ARF8) and two repressor ARFs (ARF3, ARF10) in RNA-seq (n = 3 per genotype). Note that ARF3, ARF5, and ARF6 contain uORFs before the main ORF, and ARF8 and ARF10 do not. Asterisks show statistically significant differences between WT and drmy1, and ns means no significance difference. p values: ARF3, p = 0.583; ARF5, p = 0.497; ARF6, p = 0.603; ARF8, p = 0.058; ARF10 p = 0.019. (C) Transcriptional reporters for these ARFs (pARF3::n3xGFP, pARF5::erYFP, pARF6::n3xGFP, pARF8::n3xGFP and pARF10::n3xGFP) were imaged in WT and drmy1, and representative late stage 2 buds were shown (cyan, GFP or YFP; magenta, propidium iodide). Note that the pARF3, pARF5, and pARF6 reporters contain the same uORFs as the genes, reflecting a combination of transcriptional and uORF regulations. Scale bars, 20 µm. (D) Quantification of GFP intensity. Sample size: pARF3 WT, n = 22; pARF3 drmy1, n = 25; pARF5 WT, n = 22; pARF5 drmy1, n = 22; pARF6 WT, n = 19; pARF6 drmy1, n = 28; pARF8 WT, n = 25; pARF8 drmy1, n = 31; pARF10 WT, n = 20; pARF10 drmy1, n = 29. Asterisks show statistically significant differences between WT and drmy1 in Wilcoxon rank sum tests, and ns means no significance difference. p values: pARF3, p = 0.3797; pARF5, p = 6.22x10\(^{-5}\); pARF6, p = 2.868x10\(^{-1}\); pARF8, p = 0.5127; pARF10 p = 7.073x10\(^{-1}\).
Supplemental Figure 6. Cytokinin signaling causes variability in mature sepal number and size in *drmy1*, associated with Figure 5.

Shown are top-view inflorescence images of WT (A), *arr1,10,12* (B), *wol* (C), *drmy1* (D), *drmy1 arr1,10,12* (E), and *drmy1 wol* (F), with boxed areas of individual buds enlarged and shown on the right. In the enlarged views, blue arrowheads point to sepals of regular size, and red arrowheads point to sepals that are much smaller. Scale bars, 0.5 mm.
Supplemental Figure 7. ARR7-llama partially restores robustness in mature sepal number and size, associated with Figure 7.

(A) Cytokinin abundance does not significantly change in drmy1. Shown is mean ± SD of levels of trans-zeatin (tZ), cis-Zeatin (cZ), N⁶-(Δ²-Isopentenyl)adenine (iP), trans-Zeatin riboside (tZR), cis-Zeatin riboside (cZR), and N⁶-(Δ²-Isopentenyl)adenosine (iPR) quantified by LC-MS in induced WT and drmy1 inflorescences of ap1 cal AP1-GR background. Levels are normalized to the mean tZ level in WT. Sample size: n = 5 for WT; n = 6 for drmy1. ns means no significant difference between WT and drmy1 in a two-sided Wilcoxon rank sum test. P-values: tZ, p = 0.2468; cZ, p = 0.7922; iP, p = 0.2468; tZR, p = 0.1775; cZR, p = 0.6623; iPR, p = 0.6623.

(B) The ARR7-llama reporter responds to externally applied cytokinin. Shown are GFP channel images of stage 2 buds from ARR7-llama GFP-nes line 7-4 in WT, before (top) or after (bottom) 5 hours of 200 µM BAP treatment. Images are representative of n = 6 buds from line 7-4 and n = 3 buds from line 7-6.

(C) CHX treatment does not change the localization of GFP-nes. Shown are GFP channel images of stage 2 buds from GFP-nes in WT, treated with mock (top) or 2 µM CHX (bottom) for 24 hours. Images are representative of n = 10 buds (mock) and n = 9 buds (CHX).

(D) AZD-8055 treatment does not change the localization of GFP-nes. Shown are GFP channel images of stage 2 buds from GFP-nes in WT, treated with mock (top) or 2 µM AZD-8055 (bottom) for 72 hours. Images are representative of n = 10 buds (mock) and n = 11 buds (AZD-8055). For (B-D), each image was brightened to reveal patterns of GFP distribution. A square region taken from the image containing 5-10 cells is enlarged and shown on the top right. Within the enlargement, GFP intensity was quantified along the dotted line and plotted on the bottom right. X-axis, pixels (range 0-238). Y-axis, GFP intensity in gray value (smoothened by taking the average intensity of 11-pixel neighborhoods; range 90-175). Scale bars, 25 µm.

(E-H) The ARR7-llama construct partially rescues the mature sepal variability in drmy1. Shown are inflorescence images of WT (E), ARR7-llama GFP-nes (F), drmy1 (G), and drmy1 ARR7-llama GFP-nes (H). The boxed regions were enlarged and shown on the right of each panel. Note that while drmy1 buds have normal-sized (blue arrowheads) and smaller (red arrowheads) sepals, some buds in drmy1 ARR7-llama GFP-nes have robustly sized sepals (H, middle) while others still show variability (H, right). Scale bars, 0.5 mm.

(I-J) drmy1 has decreased and disrupted pattern of DII degradation. (I) Representative late stage 2 buds of WT and drmy1 showing DII-n3xVenus (cyan), mDII-ntdTomato (magenta), and merge. For ease of display, the VENUS channel was brightened 3 times relative to the tdTomato channel. Scale bars, 25 µm. (J) Quantification of VENUS/tdTomato ratio using ImageJ. Note that a universal background of 6 gray value per pixel (determined in blank regions without tissue) were subtracted from each image before quantification. Sample size: WT, n = 8 buds; drmy1, n = 19 buds. Asterisk shows statistically significant difference in a Wilcoxon rank sum test (p = 0.01335).
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Supplemental Dataset 1. Inflorescence RNA-seq and proteomics.
Supplemental Dataset 2. Unprocessed ribosome profiles.
Supplemental Dataset 3. Seedling RNA-seq.
Supplemental Dataset 4. Data used in graphs.
Supplemental Dataset 5. DNA sequences.
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Figure 1. *drmy1* has reduced ribosome abundance, translation rate, and TOR activity.

(A) Top row, stage 12 buds of WT (left) and *drmy1* (right) viewed from the top. Arrowheads point to sepals. Note that the *drmy1* bud has 5 sepals of unequal size and unevenly spaced, exposing the stamens and carpels. Middle row, stage 12 buds of WT (left) and *drmy1* (right) viewed from the side. Asterisk shows the gap between sepals with petals and carpels exposed. Bottom row, stage 5 buds of WT (left) and *drmy1* (right) containing 35S::mCitrine-RC12A (plasma membrane marker). Arrowheads point to sepal primordia. Note that the *drmy1* bud has 5 sepal primordia of different sizes. Scale bars are 0.5 mm for stage 12 bud images and 25 µm for stage 5 bud images.

(B-C) Gene ontology (GO) enrichment of downregulated genes (B) and differentially accumulated proteins (C) in *drmy1* compared to WT, in the *ap1 cal AP1-GR* background. Shown are the top 8 GO terms and their enrichment p-values. A complete list can be found in Supplemental Dataset 1. Arrowheads highlight terms related to ribosome biogenesis or translation.

(D) Ribosome profiles of WT (blue) and *drmy1* (red) in the *ap1 cal AP1-GR* background, representative of 3 biological replicates each. Polysomal peaks are highlighted. All replicates can be found in Supplemental Dataset 2.

(E) Puromycin labeling of WT vs *drmy1*. Left, puromycin labeling in WT and *drmy1* seedlings. From left to right: WT pre-treated with CHX, two biological replicates of WT pre-treated with mock, and two biological replicates of *drmy1* pre-treated with mock. All groups were then treated with puromycin. For seedlings to match in size, WT seedlings were 8 days old and *drmy1* seedlings were 10 days old. Right, puromycin labeling in WT and *drmy1* inflorescences of induced *ap1 cal*
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22 *AP1-GR* background. From left to right: WT co-treated with puromycin and CHX, three biological replicates of WT treated with puromycin, and three biological replicates of *drmy1* treated with puromycin. In both experiments, RuBisCO large subunit on Ponceau S-stained membrane is shown as a loading control (bottom).

(F) Coherent alteration of gene expression by *drmy1* and AZD-8055 TOR inhibitor treatment. Shown here is a scatterplot of RNA log 2 fold change in *drmy1* vs WT (x-axis), and WT+AZD vs WT+Mock (y-axis), in 7-day-old seedlings. Genes are color-coded based on the following categories: genes in “Structural constituents of the ribosome” (GO:0003735) and its offspring terms (magenta); all other genes in “Translation” (GO:0006412) and its offspring terms (orange); all other genes (gray). Blue line shows a linear regression of all points ($R^2 = 0.1446$, $p < 2.2 \times 10^{-16}$). Note that the axes were trimmed to (-3,3) for ease of display.

(G-H) Phosphorylation of the direct TOR substrate, S6K-pT449, in WT and *drmy1*. Representative images are shown in (G). Top, Western blot against S6K-pT449. Middle, Western blot against total S6K protein. Bottom, Ponceau S staining as a loading control. (H) Quantification of the intensity of S6K-pT449 over Ponceau normalized by WT, in three experiments, shows that TOR activity decreased by half in *drmy1*. (mean ± SD; *, p<0.05).
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Figure 2. Defects in TOR activity, ribosome integrity, and translation cause variable sepal initiation.

(A-G) Representative images of stage 5 buds in WT (A), *drmy1* (B), *ul4z* (C), *ul4y* (D), *ul18z* (E), *lst8-1-1* (F), and *spaghetti-1* (G). Tissue morphology is visualized by either propidium iodide (a cell wall-staining dye) or a plasma membrane marker. Arrowheads indicate sepal primordia that are variable in number, position, and size. Note that *ul4z* flowers always develop four sepal primordia, although of different sizes; *lst8-1-1* occasionally (4/41, 9.8%) develops buds with more than four sepal primordia. Scale bars, 25 µm.

(H) Quantification of sepal primordium number, comparing *drmy1* (n = 67 buds), *ul4z* (n = 52 buds), *ul4y* (n = 53 buds), *ul18z* (n = 52 buds), *lst8-1-1* (n = 41 buds), and *spaghetti-1* (n = 84 buds) with WT (n = 51 buds). Asterisks indicate statistically significant (p < 0.05) differences from WT in Fisher’s contingency table tests.

(I) Illustration of robust versus variable positioning of sepal primordia. Primordia are considered robustly positioned if they are evenly distributed around the edge of the bud. Within each bud, angles between adjacent primordia with respect to the center of the bud are measured, and coefficient of variation (CV) is calculated. A bud with robustly positioned primordia would have
similar angular values and a low CV value. A bud with variably positioned primordia would have very different angular values and a high CV value.

(J) Quantification of variability in primordium positioning (CV) in the same buds as in (H), following illustration in (I). Asterisks indicate statistically significant (p < 0.05) differences from WT in Wilcoxon’s rank sum tests.

(K) Representative images of buds from in vitro-cultured WT inflorescences treated with mock or 2 µM CHX for 9-10 days (see Material and Methods). Arrowheads indicate sepal primordia that are variable in number, position, and size. Scale bars, 25 µm.

(L) Representative images of buds from WT plants treated with mock or 2 nmol Torin2 for 15 days (see Material and Methods). Arrowheads indicate sepal primordia that are variable in number, position, and size. Scale bars, 25 µm.

(M-N) Quantification of variability in primordium number (M) and positional variability (N) similar to (H,J), comparing CHX-treated (n = 31 buds), CHX-mock (n = 42 buds), Torin2-treated (n = 51 buds) and Torin2-mock buds (n = 56 buds).
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Figure 3. TOR and ribosomal defects cause variability in the timing of sepal initiation.

(A-C) 6h-interval live imaging of the sepal initiation process in WT (A) and ul4y (B), which is quantified in (C). n = 48 buds for WT; n = 40 buds for ul4y.

(D-F) 6h-interval live imaging of the sepal initiation process in buds from WT plants treated with mock or 2 nmol Torin2 bi-daily for 15 days, which is quantified in (F). n = 31 buds for mock; n = 15 buds for Torin2.

In (A,B,D,E), top rows show the 35S::mCitrine-RCI2A membrane marker, and bottom rows show Gaussian curvature heatmaps calculated from the same image stacks. Asterisks indicate sepal initiation events, defined as a dark red band (primordium with positive curvature) separated from the floral meristem by a dark blue band (boundary with negative curvature) in the Gaussian curvature heatmap. Scale bars, 25 µm.

In (C,F), the amount of time between outer and inner sepal initiation (left) and between outer and lateral sepal initiation (right) were calculated for each bud, and summarized over all the buds.
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Asterisks indicate statistically significant (p < 0.05) differences in the distribution of relative initiation timing in Fisher’s contingency table tests.
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Figure 4. Defects in TOR activity, ribosome integrity, and translation cause variability in auxin and cytokinin signaling.

(A-D) The ribosomal mutant ul4y loses robustness in auxin and cytokinin signaling. (A) Representative images of late stage 2 buds of WT, drmy1, and ul4y, showing the auxin signaling reporter DR5::3xVENUS-N7 in yellow, the cytokinin signaling reporter TCS::GFP in cyan, and both merged with Chlorophyll (in WT) or UBQ10::mCherry-RCI2A (in drmy1 and ul4y) in magenta. (B) Quantification of TCS intensity (integrated density divided by area) from maximum intensity projection images, normalized to mean of WT. Shown are mean ± SD. Asterisks show statistically significant differences from WT in two-tailed Student’s t-tests (drmy1, p = 2.1x10^-6; ul4y, p = 3.4x10^-5). (C) Circular histogram of DR5 distribution around the bud. Each bud was divided into 360 sectors of 1° each. Within each sector, DR5 signal measured in pixel intensity units (0-255 range) was summed. This sum was plotted along the x-axis starting from the sector at 1:30 position (between the incipient outer sepal and incipient lateral sepal on the right) going...
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counterclockwise. I.e. in WT, the outer sepal is near 45°, the inner sepal near 225°, and the lateral
sepals near 45° and 135° (vertical dotted lines). The mean was plotted as a solid line, and mean
± SD was plotted as a shaded area. (D) Circular histogram of TCS distribution around the bud.
Sample size for (A-D): WT, n = 12 buds; 

(E-H) 3 days of translation inhibition causes increased and diffuse cytokinin signaling, and diffuse
auxin signaling. (E) Representative images of late stage 2 buds from dissected and cultured WT
inflorescences treated with mock or 2 µM CHX for 3 days. Shown are DR5::3xVENUS-N7 in
yellow, TCS::GFP in cyan, and both merged with Chlorophyll in magenta. (F) Quantification of
TCS intensity from maximum intensity projection images, normalized to mean of WT mock day 3.
Shown are mean ± SD. Asterisk shows statistically significant difference in a two-tailed Student’s
t-test (p = 2.0x10⁻⁴). (G) Circular histogram of DR5 distribution around the bud. (H) Circular
histogram of TCS distribution around the bud. Sample size for (E-H): WT mock day 3, n = 10 buds;
WT CHX day 3, n = 12 buds.

(I-L) 6 days of TOR or translation inhibition causes increased and diffuse cytokinin signaling, and
randomly positioned auxin signaling maxima. (I) Representative images of late stage 2 buds from
dissected and cultured WT inflorescences treated with mock, 2 µM CHX, or 2 µM AZD for 6 days.
Shown are DR5::3xVENUS-N7 in yellow, TCS::GFP in cyan, and both merged with Chlorophyll
in magenta. Arrowheads point to randomly positioned auxin maxima in buds of the CHX or AZD
group. (J) Quantification of TCS intensity from maximum intensity projection images, normalized
to mean of WT mock day 6. Shown are mean ± SD. Asterisks show statistically significant
differences from mock in two-tailed Student’s t-tests (CHX, p = 1.0x10⁻³; AZD, p = 1.2x10⁻⁴). (K)
Circular histogram of DR5 distribution around the bud. (L) Circular histogram of TCS distribution
around the bud. Sample size for (I-L): WT mock day 6, n = 12 buds; WT CHX day 6, n = 11 buds;
WT AZD day 6, n = 10 buds. Scale bars in (A,E,I) represent 25 µm.
Figure 5. Cytokinin signaling is required for variability in auxin signaling and sepal initiation under translation inhibition. 

(A-D) Cytokinin treatment makes auxin signaling diffuse. Shown are late stage 2 WT buds under mock (A,B) or 5 µM cytokinin (BAP) treatment (C,D) for 4 days. (A,C) Representative images of
the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with Chlorophyll in magenta. (B,D) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). Arrows point to DR5 signal in variable positions. Sample size: WT Mock n = 10, WT BAP n = 10. Also see Zhu et al. (2020), Extended Data Figure 7e.

(E-G) Cytokinin signaling is required for variable sepal initiation in drmy1. (E) Representative images of stage 5 buds in WT, drmy1, arr1,10,12, drmy1 arr1,10,12, wol, and drmy1 wol. Arrowheads indicate initiated sepall primordia that are variable in number, position, and size. (F,G) Quantification of sepal primordium number (F) and positional variability (G), comparing WT (n = 58) with drmy1 (n = 31), arr1,10,12 (n = 24) with drmy1 arr1,10,12 (n = 20), and wol (n = 36) with drmy1 wol (n = 39). Asterisks indicate statistically significant (p < 0.05) differences in Fisher’s contingency table tests (F) and Wilcoxon’s rank sum tests (G) respectively. ns indicates no significant difference.

(H-K) Cytokinin signaling is required for variable patterning of auxin signaling in drmy1. Shown are late stage 2 buds of WT vs drmy1 (H,I), and arr1,10,12 vs drmy1 arr1,10,12 (J,K). (H,J) Representative images of the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with propidium iodide in magenta. Arrows point to diffuse DR5 signal in variable positions in the drmy1 bud. Arrowheads show four robust DR5 maxima in drmy1 arr1,10,12. (I,K) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). For ease of visualization, circular histograms of drmy1 and drmy1 arr1,10,12 between 90 and 360 degrees are enlarged and shown as insets (y-axis range 0-0.4). Sample size: WT n = 19, drmy1 n = 16, arr1,10,12 n = 13, drmy1 arr1,10,12 n = 9.

(L-N) Cytokinin signaling is required for variable sepal initiation under translation inhibition. (L) Representative images of stage 6 buds in WT, arr1,10,12, and wol, treated with Mock or 2 µM CHX for 10 days. Arrowheads indicate variable initiation of sepal primordia. (M,N) Quantification of sepal primordium number (M) and positional variability (N), comparing Mock and CHX within each genotype. Sample size: WT Mock n = 29, WT CHX n = 19, arr1,10,12 Mock n = 18, arr1,10,12 CHX n = 19, wol Mock n = 15, wol CHX n = 19. Asterisks indicate statistically significant (p < 0.05) differences in Fisher’s contingency table tests (M) and Wilcoxon’s rank sum tests (N) respectively. ns indicates no significant difference.

(O-R) Cytokinin signaling is required for diffuse auxin signaling under translation inhibition. Shown are late stage 2 buds of WT (O,P) and arr1,10,12 (Q,R), treated with Mock or 2 µM CHX for 3 days. (O,Q) Representative images of the auxin signaling reporter DR5::3xVENUS-N7 in yellow, and DR5 merged with Chlorophyll in magenta. Arrows point to diffuse DR5 signal in variable positions in CHX-treated WT. Arrowheads show four robust DR5 maxima in CHX-treated arr1,10,12. (P,R) Circular histograms of the DR5::3xVENUS-N7 signal, showing mean (solid line) and mean ± SD (shaded area). Sample size: WT Mock n = 17, WT CHX n = 18, arr1,10,12 Mock n = 7, arr1,10,12 CHX n = 7. Scale bars in (A,C,E,H,J,L,O,Q) represent 25 µm.
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Figure 6. Upregulation of cytokinin signaling is required to maintain translation and fitness in *drmy1*.

(A) Puromycin labeling of WT seedlings with 4 h CHX pre-treatment (control), and three biological replicates each of WT and *arr1 35S::ARR1* seedlings with 4 h mock pre-treatment. All seedlings are 14 days old. RuBisCO large subunit in Ponceau S-stained membrane is shown as a loading control. Also see Karunadasa et al. (2020).

(B,C) Puromycin labeling of WT seedlings with 4 h CHX pre-treatment (control), and two biological replicates of WT, *drmy1*, *wol*, and *drmy1 wol* seedlings with 4 h mock pre-treatment. Seedlings are 8 days old in (B) and 14 days old in (C). RuBisCO large subunit in Ponceau S-stained membrane is shown as a loading control.

(D) Representative 14 days old seedling images of WT, *drmy1*, *wol*, and *drmy1 wol* used in (C). Notice that *drmy1 wol* is very small and pale. Scale bars, 5 mm.

(E) Representative aerial part images of 42 days old plants of WT, *drmy1*, *wol*, and *drmy1 wol*. Inset shows enlarged *drmy1 wol* plant; notice that it has a tiny rosette and inflorescence. Scale bars, 5 cm. See also Supplemental Figure 6F.

(F) Representative aerial part images of 74 days old plants of WT, *drmy1*, *arr1,10,12*, and *drmy1 arr1,10,12*. Inset shows enlarged *drmy1 arr1,10,12* plant; notice its pale leaves accumulating anthocyanin, and short inflorescence. Scale bars, 5 cm. See also Supplemental Figure 6E.

(G) Dissected siliques of *arr1,10,12* (left) and *drmy1 arr1,10,12* (right) showing developing seeds. Notice that while *arr1,10,12* occasionally have aborted seeds, all seeds in the *drmy1 arr1,10,12* silique were aborted. Scale bars, 0.2 mm.
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**Figure 7.** A-type ARR protein levels are sensitive to TOR and translation inhibition.

**(A)** The hypothesis. A-type ARRs are rapidly synthesized and degraded to dampen cytokinin signaling. Translation inhibition causes inability to rapidly synthesize these proteins in response to cytokinin signaling, resulting in an upregulation of cytokinin signaling.

**(B)** Expression of A-type ARR genes in WT vs *dmmy1* inflorescences (*ap1 cal AP1-GR*) measured in RNA-seq. Shown are the five A-type ARR genes with the highest expression, ranked by mean expression level in WT. Asterisk indicates statistically significant difference, while *ns* means no
significant difference. P-values: ARR7, p = 0.807; ARR4, p = 0.611; ARR15, p = 0.532; ARR8, 
= 0.0115; ARR9, p = 0.0416.

(C) A GFP-channel image of a stage 2 bud of GFP-nes (pUBQ10::sfGFP-nes-UBQ3ter). For this 
panel and (E-H), each image was brightened to reveal GFP distribution patterns. A square region 
taken from the image containing 5-10 cells is enlarged and shown on the top right. Within the 
elongation, GFP intensity was quantified along the dotted line and plotted on the bottom right. 
X-axis, pixels (range 0-238). Y-axis, GFP intensity in gray value (smoothened by taking the 
average intensity of 11-pixel neighborhoods; range 90-210). Scale bars, 25 µm.

Illustration of the Llama Tag system used in this study. Plants were co-transformed with ARR7-
llama (pARR7::ARR7-linker-llama-ARR7ter) and GFP-nes (pUBQ10::sfGFP-nes-UBQ3ter). 
Without ARR7-llama, the GFP is localized in the cytosol. ARR7-llama is produced in the cytosol 
and translocates into the nucleus. When this happens, the Llama Tag capable of binding GFP 
drags GFP into the nucleus (note that from our observation it is excluded from the nucleolus). 
Therefore, at low ARR7-llama levels, GFP signal is mainly seen in the cytosol. At intermediate 
ARR7-llama levels, GFP is at comparable intensities between the cytosol and the nucleus, and 
no clear pattern can be seen. At high ARR7-llama levels, GFP is mainly seen in the nucleus.

(E,F) GFP channel images of stage 2 buds from two independent transgenic lines of ARR7-llama 
GFP-nes, 7-4 (E) and 7-6 (F), in WT (top) vs drmy1 (bottom). Images are representative of n = 
17 (line 7-4, WT), n = 40 (line 7-4, drmy1), n = 9 (line 7-6, WT), and n = 6 (line 7-6, drmy1) buds. 

(G) GFP channel images of WT ARR7-llama GFP-nes buds treated with mock (top) or 2 µM CHX 
(bottom) for 24 hours. The mock image is representative of n = 20 buds (12 from line 7-4, 5 from 
line 7-6, and 3 from line 7-12). The CHX image is representative of n = 19 buds (11 from line 7-4, 
5 from line 7-6, and 3 from line 7-12).

(H) GFP channel images of WT ARR7-llama GFP-nes buds treated with mock (top) or 2 µM AZD-
8055 (bottom) for 72 hours. The mock image is representative of n = 13 buds (8 from line 7-4 and 
5 from line 7-6). The AZD-8055 image is representative of n = 11 buds (8 from line 7-4 and 3 from 
line 7-6).

(I-L) ARR7-llama partially restores robustness in drmy1 sepal primordia. (I-L) Representative 
stage 5 or 6 buds from WT (I), WT with the ARR7-llama and GFP-nes constructs (J), drmy1 (K), 
and drmy1 with these constructs (L). (M) Quantification of sepal primordium number. ns indicates 
no significant difference in a Fisher's exact test (WT vs ARR7-llama, p = 1; drmy1 vs drmy1 
ARR7-llama, p = 0.44). (N) Quantification of variability in sepal primordium position. Asterisk 
indicates statistically significant difference (p = 5.7x10⁻⁶), while ns indicates no statistically 
significant difference (p = 0.91). Data for WT and drmy1 were reused from Figure 2H, 2J. Data 
for ARR7-llama GFP-nes and drmy1 ARR7-llama GFP-nes were pooled from line 7-4 and 7-6. 
Sample size: WT n = 51, ARR7-llama GFP-nes n = 16, drmy1 n = 67, drmy1 ARR7-llama GFP-
nes n = 20. Scale bars, 25 µm.

(O) Working model. In WT, DRMY1 maintains TOR activity and translation, which sustains the 
rapid translation of A-type ARRs in response to cytokinin signaling. This suppresses excessive 
cytokinin signaling, allowing auxin and cytokinin signaling to interact and form robust spatial 
patterns. Robust patterning of auxin and cytokinin signaling gives rise to robustly numbered, 
positioned, and sized sepal primordia. In drmy1, A-type ARR protein levels are reduced due to
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insufficient TOR activity, ribosome content, and translation rate. Cytokinin signaling is upregulated, which rescues the translation rate reduction in a homeostatic mechanism. This upregulation of cytokinin signaling disrupts the robust spatial pattern of both cytokinin and auxin signaling, which in turn causes variable sepal initiation.