Seed Germination

Subjects: Plant Sciences
Contributor: Helen North, Naoto Sano

Mature dry seeds contain thousands of stored mRNAs that have accumulated during seed maturation, and these can serve as templates for protein synthesis during germination.

Keywords: Seed germination ; Long-lived RNA ; longevity

1. Germination Ability of Mature Seeds Using Stored mRNAs

Visible seed germination (also called germination sensu stricto) is often defined as an extension of a part of the embryo (usually the radicle) to penetrate the structures that surround it such as the seed coat[1]. The molecular basis of seed germination has been studied for many years with a landmark discovery made in the 1960s, when de novo protein synthesis was observed in germinating cotton seeds even if transcription was inhibited during imbibition[2][3]. This demonstrated that protein synthesis in the early phase of germination uses pre-existing mRNA templates that are stored in mature dry seeds. Some of these ‘stored mRNAs’ are also referred to as ‘long-lived mRNAs’ because they remain translatable for long periods, even if the seeds are exposed to stressful conditions.

A number of studies have examined whether germination occurs when gene expression is inhibited through seed absorption of exogenously applied drugs. Seed germination was completely blocked in the presence of a cytoplasmic translational inhibitor cycloheximide in lettuce, wheat, Arabidopsis, and rice[4][5][6][7][8], suggesting that *de novo* protein synthesis during imbibition is a prerequisite for germination. RNA polymerase II-mediated transcription can be blocked by α-amanitin and this has been shown to effectively inhibit in vivo transcription during seed imbibition in Arabidopsis and rice, with a significant reduction in UMP incorporation into RNAs synthesized in germinating seeds. Nevertheless, visible germination was observed in the presence of α-amanitin, although germination was retarded[6][9][10]. Rice seeds also showed a similar germination delay with actinomycin D, which intercalates into the DNA template to form a stable complex, thereby inhibiting transcription[7]. These suggest that cytoplasmic translation using stored mRNAs is sufficient for visible seed germination by radicle cell elongation, but that stored mRNAs alone are not enough for normal germination speed. In other words, *de novo* transcription upon imbibition will be required for rapid germination. Nevertheless, lettuce and wheat seeds treated with a transcriptional inhibitor did not show clear germination or embryonic growth[5][11], indicating that germination potential in the presence of inhibitors may differ among plant species or varieties. Additionally, Arabidopsis seed germination was strongly suppressed by a different inhibitor cordycepin (3′-deoxyadenosine)[12], which can be incorporated into RNA and inhibits the elongation of transcripts due to the absence of a hydroxyl group at the 3′ position[13]. Nevertheless, cordycepin may also suppress germination through the inhibition of protein synthesis as it blocks the target of rapamycin (mTOR) signaling pathway at higher concentrations[14]. Furthermore, the latter has been associated with abscisic acid (ABA) signaling and growth processes in seeds and plants[15][16]. Moreover, it cannot be ruled out that uptake efficiency into cells may differ depending on the type of drug. Alternatively, as described below, the accumulation of mRNAs during seed formation can be affected by the environmental conditions in which the mother plant is grown, which could conceivably be responsible for variations observed when the transcription of stored mRNAs is inhibited during germination. It is important to note that while germination *sensu stricto* was not suppressed in seeds treated with a transcription inhibitor, subsequent growth was completely blocked (i.e., cotyledon development in Arabidopsis and radicle elongation in rice)[6][7], indicating that *de novo* transcription upon imbibition is indispensable for normal seedling establishment, which requires cell division and differentiation.

2. Accumulation of Stored mRNAs during Seed Development

Microarray analysis has shown that more than 12,000 and 17,000 different types of stored mRNAs are present in mature dry seeds of Arabidopsis and rice, respectively[17][18]. It is unlikely, however, that all of these stored mRNAs are used in the germination process, as many could be involved in housekeeping activities in cells or persist from seed development processes. Stored mRNAs could be considered as a backup to late seed maturation that take into account the mother

plant's history, adjusting the seed to cope with environmental fluctuations and manage dormancy appropriately, without affecting the ability to germinate. After embryo morphogenesis is completed during seed formation, precocious germination of the embryo can occur if it is removed from immature seed[19][20]. Treating such embryos with a transcriptional inhibitor ascertained when germination-related mRNAs first appear during seed development. In rice, mRNA accumulation was estimated at between 10 to 20 days after flowering (DAF), as actinomycin D inhibited precocious germination of embryos at 10 DAF but not at 20 DAF[21]. RNA-Seq in the same study also detected 529 mRNA candidates unique to germination, which specifically accumulate in embryos at this developmental phase. Similarly, in cotton, germination-specific mRNAs were reported to accumulate between about 30 and 50 DAF[22][23]. Interestingly, young embryos in both rice and cotton exhibited a germination delay compared to mature ones, even without a transcriptional inhibitor. Moreover, during the precocious germination of young embryos, transcription was observed from genes for mRNAs stored for germination (i.e., transcripts of calcium ion and phospholipid signaling-related genes and negative regulators of ABA signaling-related genes increased in young germinating rice embryos[21] as well as inferred increases in transcripts for carboxypeptidase and isocitratase in the cotyledons of young germinating cotton based on enzymatic activities)[22][23]. These imply that some transcription is required in the young embryo during both seed maturation and precocious germination in order to germinate vigorously.

3. Proteins Encoded by Stored mRNAs are Required for Germination

With low transcriptional activities during the early stages of imbibition, protein synthesis must rely on translation from stored mRNAs in seeds. An effective strategy to comprehensively identify the proteins synthesized from stored mRNAs during germination has been proteome analyses combined with the use of a transcriptional inhibitor[6][7][10][24]. These have shown that 12S seed storage proteins and members of the dehydrin family are translated from stored mRNAs in Arabidopsis seeds[6]. Detailed time course proteomics using [^{35}S]-methionine identified similar storage proteins and desiccation tolerance-related proteins as neo-synthesized proteins upon imbibition[25], suggesting that germination begins with a resumption of the seed maturation program through the translation of these mRNAs, and this may be an important checkpoint for seeds to ensure that germination occurs in a favorable environment[26][27]. Recently, proteins translated from stored mRNAs as well as from *de novo* transcribed mRNAs at an initial phase of germination were analyzed in rice. The former proteins were related to glycolysis and translation while the latter were involved in pyruvate metabolism, tricarboxylic acid (TCA) cycle, and momilactone biosynthesis, indicating that upon imbibition, stored mRNAs support the initial production of energy by glycolysis and the activation of translational machinery, whereas de novo transcription accelerates energy production after glycolysis, which enables seeds to germinate vigorously[10]. This seems to be reasonable as ATP production by the TCA cycle in mitochondria may not be fully functional during the initial phase of germination. In higher plants, most ATP is provided by oxidative phosphorylation in mitochondria, but the mitochondria in dry seed cells, termed promitochondria, do not have typical cristae structures and must be repaired and undergo differentiation upon imbibition in order to produce sufficient ATP[28][29]. These developed into more typical mitochondria between 12 to 24 h after imbibition in maize and rice[30][31]. It is also noteworthy that during the seed-to-seedling transition in Arabidopsis, the population of translating mRNAs largely overlapped with genes regulated during hypoxia stress[32], implying the importance of translational control under low-oxygen conditions during germination. Interestingly, mRNAs encoding components of the plastid transcriptional machinery were specifically preserved or even newly synthesized during desiccation and stored in dry seeds and could be immediately available on imbibition[33][34].

4. Selective Translation of Stored mRNAs upon Imbibition

Changes in transcript abundance do not always correlate with observed transitions in protein levels during seed germination[10][25]. One of the mechanisms affecting this is the selective translation of mRNAs involved in germination from over 10,000 types of stored mRNAs. Additionally, the population of transcripts selected for translation has been observed to vary according to the stage within the seed germination program[7][24][25], emphasizing the fact that mRNA translation is both temporal and selective during germination. The latest translatome analyses carried out on Arabidopsis seeds reported that most of the stored mRNAs were detected in the monosome fraction, with translationally quiescent mRNAs associated with single ribosomes, rather than polysomes in which actively translated mRNAs are associated with multiple ribosomes[32][35]. Nevertheless, 17% of monosome specific-stored mRNAs were translationally up-regulated during early seed germination and they encoded proteins involved in processes such as response to water deprivation and cell cycle arrest, which is in agreement with the predicted function of proteins neosynthesized during seed germination[25]. In contrast, non-monosome specific ones (i.e., mRNAs detected from both monosomes and polysomes) were translationally down-regulated during the initial phase of germination[35]. This suggests that the use of stored mRNA for germination involves two step-wise mechanisms: (i) mRNA accumulates as monosomes rather than polysomes during seed formation; and (ii) selective translation of monosomal mRNAs after imbibition. Furthermore, such ribosomal level

regulation could allow seeds to pause translation and stack mRNAs, thereby protecting some specific mRNA populations. How specific mRNAs are targeted to monosome complexes and are specifically translated during imbibition is still unknown, however, the same study found clues that monosome specific-stored mRNAs have features such as shorter transcripts, low GC% in UTRs, weak secondary structure, and a motif (GAAGAAGAA) in 5′UTRs[35].

Selective translation can also result from structural features and regulatory sequences within mRNA[36]. The canonical end modifications of seed stored mRNA remain to be fully characterized. Previous work suggests that both cap-dependent and cap-independent translation can occur from stored mRNA to control seed germination. The cap-independent process occurs through the direct recruitment of ribosomal subunits on specific cis-acting RNA sequences known as internal ribosome entry sites (IRES)[37]. IRES-specific cellular trans-acting factors (ITAF) are important proteins for cap-independent translation initiation. The ErbB3-binding protein 1 (EBP1) is an evolutionarily conserved ITAF found in both animals and plants and previously detected in seeds[38][39]. EBP1 is highly expressed during germination and the protein is over accumulated during seed priming[39]. Furthermore, in maize embryonic axes, some stored mRNAs were efficiently translated via a cap-independent mechanism during germination[40]. As both stored and de novo synthesized mRNAs co-exist during the germination process, it is possible that these different initiation systems allow their selective recruitment during germination. Interestingly, EBP1 is also implicated in stress, and ABA responses[41] and seeds of the Arabidopsis ebp1 mutant were less sensitive to ABA and germinated more rapidly than those of wild type (WT).

References

1. Bewley, J.D.; Seed germination and dormancy. *Plant. Cell.* **1997**, *9*, 1055-1066, 10.1105/tpc.9.7.1055.
2. Dure, L.; Waters, L.; Long-lived messenger RNA: Evidence from cotton seed germination. *Science* **1965**, *147*, 410-412, 10.1126/science.147.3656.410.
3. Waters, L.C.; Dure, L.S. 3rd; Ribonucleic acid synthesis in germinating cotton seeds. *J. Mol. Biol.* **1966**, *19*, 1–27, 10.1016/s0022-2836(66)80046-3.
4. Schultz, C.; Small, J.G.; Inhibition of lettuce seed germination by cycloheximide and chloramphenicol is alleviated by ki netin and oxygen. *Plant. Physiol.* **1991**, *97*, 836-838, 10.1104/pp.97.2.836.
5. Jendrisak, J.; The use of alpha-amanitin to inhibit in vivo RNA synthesis and germination in wheat embryos. *J. Biol Che m.* **1980**, *255*, 8529–8533, .
6. Rajjou, L.; Gallardo, K.; Debeaujon, I.; Vandekerckhove, J.; Job, C.; Job, D.; The effect of alpha-amanitin on the Arabid opsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant. Physi ol.* **2004**, *134*, 1598-1613, 10.1104/pp.103.036293.
7. Sano, N.; Permana, H.; Kumada, R.; Shinozaki, Y.; Tanabata, T.; Yamada, T.; Hirasawa, T.; Kanekatsu, M; Proteomic a nalysis of embryonic proteins synthesized from long-lived mRNAs during germination of rice seeds. *Plant. Cell Physiol.* **2012**, *53*, 687-698, 10.1093/pcp/pcs024.
8. Liu, S.J.; Xu, H.H.; Wang, W.Q.; Li, N.; Wang, W.P.; Lu, Z.; Møller, I.M.; Song, S.Q.; Identification of embryo proteins as sociated with seed germination and seedling establishment in germinating rice seeds. *J. Plant. Physiol.* **2016**, *196*, 79-92, 10.1016/j.jplph.2016.02.021.
9. He, D.; Han, C.; Yao, J.; Shen, S.; Yang, P.; Constructing the metabolic and regulatory pathways in germinating rice se eds through proteomic approach. *Proteomics* **2011**, *11*, 2693-2713, 10.1002/pmic.201000598.
10. Sano, N.; Takebayashi, Y.; To, A.; Mhiri, C.; Rajjou, L.C.; Shotgun proteomic analysis highlights the roles of long-lived m RNAs and de novo transcribed mRNAs in rice seeds upon imbibition. *Plant. Cell Physiol.* **2019**, *60*, 2584-2596, 10.1093/pcp/pcz152.
11. Tao, K.L.; Khan, A.A.; Differential effects of actinomycin d and cordycepin in lettuce seed germination and RNA synthes is. *Plant. Physiol.* **1976**, *58*, 769-772, 10.1104/pp.58.6.769.
12. Bai, B.; Novák, O.; Ljung, K.; Hanson, J.; Bentsink, L.; Combined transcriptome and translatome analyses reveal a role for tryptophan-dependent auxin biosynthesis in the control of DOG1-dependent seed dormancy. *New Phytol.* **2017**, *21 7*, 1077-1085, 10.1111/nph.14885.
13. Holbein, S.; Wengi, A.; Decourty, L.; Freimoser, F.M.; Jacquier, A.; Dichtl, B.; Cordycepin interferes with 3′ end formatio n in yeast independently of its potential to terminate RNA chain elongation. *RNA* **2009**, *15*, 837-849, 10.1261/rna.1458909.
14. Wong, Y.Y.; Moon, A.; Duffin, R.; Barthet-Barateig, A.; Meijer, H.A.; Clemens, M.J.; de Moor, C.H.; Cordycepin inhibits p rotein synthesis and cell adhesion through effects on signal transduction. *J. Biol. Chem.* **2009**, *285*, 2610-21, 10.1074/j

bc.M109.071159.

15. Deprost, D.; Yao, L.; Sormani, R.; Moreau, M.; Leterreux, G.; Nicolaï, M.; Bedu, M.; Robaglia, C.; Meyer, C.; The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Rep.* **2007**, *8*, 864-870, 10.1038/sj.embor.7401043.

16. Salem, M.A.; Li, Y.; Wiszniewski, A.; Giavalisco, P.; Regulatory-associated protein of TOR (RAPTOR) alters the hormonal and metabolic composition of Arabidopsis seeds, controlling seed morphology, viability and germination potential. *Plant. J.* **2017**, *92*, 525-545, 10.1111/tpj.13667.

17. Nakabayashi, K.; Okamoto, M.; Koshiba, T.; Kamiya, Y.; Nambara, E.; Genome-wide profiling of stored mRNA in Arabidopsis thaliana seed germination: epigenetic and genetic regulation of transcription in seed. *Plant. J.* **2005**, *41*, 697-709, 10.1111/j.1365-313x.2005.02337.x.

18. Howell, K.A.; Narsai, R.; Carroll, A.; Ivanova, A.; Lohse, M.; Usadel, B.; Millar, A.H.; Whelan, J.; Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germination process. *Plant. Physiol.* **2008**, *149*, 961-980, 10.1104/pp.108.129874.

19. Ooms, J.; Leon-Kloosterziel, K.M.; Bartels, D.; Koornneef, M.; Karssen, C.M.; Acquisition of desiccation tolerance and longevity in seeds of Arabidopsis thaliana (A comparative study using abscisic acid-insensitive abi3 mutants). *Plant. Physiol.* **1993**, *102*, 1185-1191, 10.1104/pp.102.4.1185.

20. Still, D.W.; Kovach, D.A.; Bradford, K.J.; Development of desiccation tolerance during embryogenesis in rice (Oryza sativa) and wild rice (Zizania palustris) (Dehydrin expression, abscisic acid content, and sucrose accumulation). *Plant. Physiol.* **1994**, *104*, 431-438, 10.1104/pp.104.2.431.

21. Sano, N.; Ono, H.; Murata, K.; Yamada, T.; Hirasawa, T.; Kanekatsu, M.; Accumulation of long-lived mRNAs associated with germination in embryos during seed development of rice. *J. Exp. Bot.* **2015**, *66*, 4035-4046, 10.1093/jxb/erv209.

22. Ihle, J.N.; Dure, L. 3rd.; Synthesis of a protease in germinating cotton cotyledons catalzed by mRNA synthesized during embryogenesis. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 705-710, 10.1016/0006-291x(69)90667-6.

23. Ihle, J.N.; Dure, L.S. 3rd.; The developmental biochemistry of cottonseed embryogenesis and germination. 3. Regulation of the biosynthesis of enzymes utilized in germination. *J. Biol. Chem.* **1972**, *247*, 5048–5055, .

24. De Jiménez, E.S.; Aguilar, R.; Protein synthesis patterns: Relevance of old and new messenger RNA in germinating maize embryos. *Plant. Physiol.* **1984**, *75*, 231-234, 10.1104/pp.75.1.231.

25. Galland, M.; Huguet, R.; Arc, E.; Cueff, G.; Job, D.; Rajjou, L.; Dynamic proteomics emphasizes the importance of selective mRNA translation and protein turnover during Arabidopsis seed germination. *Mol. Cell Proteomics* **2013**, *13*, 252-68, 10.1074/mcp.M113.032227.

26. Galland, M.; Rajjou, L.; Regulation of mRNA translation controls seed germination and is critical for seedling vigor. *Front. Plant. Sci* **2015**, *6*, 284, 10.3389/fpls.2015.00284.

27. Rajjou, L.; Duval, M.; Gallardo, K.; Catusse, J.; Bally, J.; Job, C.; Job, D.; Seed germination and vigor. *Annu. Rev. Plant. Biol.* **2012**, *63*, 507-533, 10.1146/annurev-arplant-042811-105550.

28. Law, S.R.; Narsai, R.; Taylor, N.L.; Delannoy, E.; Carrie, C.; Giraud, E.; Millar, A.H.; Small, I.; Whelan, J.; Nucleotide and RNA metabolism prime translational initiation in the earliest events of mitochondrial biogenesis during Arabidopsis germination. *Plant. Physiol.* **2012**, *158*, 1610-27, 10.1104/pp.111.192351.

29. Law, S.R.; Narsai, R.; Whelan, J.; Mitochondrial biogenesis in plants during seed germination. *Mitochondrion* **2014**, *19*, 214-221, 10.1016/j.mito.2014.04.002.

30. Logan, D.C.; Millar, A.H.; Sweetlove, L.J.; Hill, S.A.; Leaver, C.J.; Mitochondrial biogenesis during germination in maize embryos. *Plant. Physiol.* **2001**, *125*, 662-672, 10.1104/pp.125.2.662.

31. Howell, K.A.; Millar, A.H.; Whelan, J.; Ordered assembly of mitochondria during rice germination begins with pro-mitochondrial structures rich in components of the protein import apparatus. *Plant. Mol. Biol.* **2006**, *60*, 201-223, 10.1007/s11103-005-3688-7.

32. Bai, B.; Peviani, A.; van der Horst, S.; Gamm, M.; Snel, B.; Bentsink, L.; Hanson, J.; Extensive translational regulation during seed germination revealed by polysomal profiling. *New Phytol.* **2016**, *214*, 233-244, 10.1111/nph.14355.

33. Demarsy, E.; Buhr, F.; Lambert, E.; Lerbs-Mache, S.; Characterization of the plastid-specific germination and seedling establishment transcriptional programme. *J. Exp. Bot.* **2011**, *63*, 925-939, 10.1093/jxb/err322.

34. Allorent, G.; Courtois, F.; Chevalier, F.; Lerbs-Mache, S.; Plastid gene expression during chloroplast differentiation and dedifferentiation into non-photosynthetic plastids during seed formation. *Plant. Mol. Biol.* **2013**, *82*, 59-70, 10.1007/s11103-013-0037-0.

35. Bai, B.; van der Horst, S.; Cordewener, J.H.G.; America, T.A.H.P.; Hanson, J.; Bentsink, L.; Seed-stored mRNAs that ar e specifically associated to monosomes are translationally regulated during germination. *Plant. Physiol.* **2020**, *182*, 378 -392, 10.1104/pp.19.00644.

36. Merchante, C.; Stepanova, A.N.; Alonso, J.M.; Translation regulation in plants: An interesting past, an exciting present and a promising future. *Plant. J.* **2017**, *90*, 628-653, 10.1111/tpj.13520.

37. Bonnal, S.; Boutonnet, C.; Prado-Lourenço, L.; Vagner, S.; IRESdb: The internal ribosome entry site database. *Nucleic Acids Res.* **2003**, *31*, 427-428, 10.1093/nar/gkg003.

38. Monie, T.P.; Perrin, A.J.; Birtley, J.R.; Sweeney, T.R.; Karakasiliotis, I.; Chaudhry, Y.; Roberts, L.O.; Matthews, S.; Goodf ellow, I.G.; Curry, S.; et al. Structural insights into the transcriptional and translational roles of Ebp1. *EMBO J.* **2007**, *26*, 3936-3944, 10.1038/sj.emboj.7601817.

39. Catusse, J.; Meinhard, J.; Job, C.; Strub, J.M.; Fischer, U.; Pestsova, E.; Westhoff, P.; Van Dorsselaer, A.; Job, D.; Prot eomics reveals potential biomarkers of seed vigor in sugarbeet. *Proteomics* **2011**, *11*, 1569-1580, 10.1002/pmic.20100 0586.

40. Dinkova, T.D.; Zepeda, H.; Martínez-Salas, E.; Martínez, L.M.; Nieto-Sotelo, J.; de Jiménez, E.S.; Cap-independent tra nslation of maize Hsp101. *Plant. J.* **2005**, *41*, 722-731, 10.1111/j.1365-313x.2005.02333.x.

41. Li, J.; Yu, G.; Sun, X.; Zhang, X.; Liu, J.; Pan, H.; AcEBP1, an ErbB3-Binding Protein (EBP1) from halophyte Atriplex ca nescens, negatively regulates cell growth and stress responses in Arabidopsis. *Plant Sci.* **2016**, *248*, 64-74, 10.1016/j.p lantsci.2016.04.011.
