

Supplementary information S1 (box) | Evolution of the E2F family

Proteins related to the E2Fs are conserved across various species of plants and animals. The efforts to elucidate E2F function in mammals have traditionally been complicated by the existence of a large number of family members with extensive functional overlap. RB-E2F studies in the elegant model organism *Drosophila melanogaster*, which has one activator (dE2F1) and one repressor E2F (dE2F2), have yielded many essential insights into the role of E2F proteins in cell proliferation, differentiation and apoptosis¹. In *Caenorhabditis elegans*, three E2F-like proteins have been described including EFL-1, EFL-2 and F49E12.6. It is well established that EFL-1, DPL-1 (DP) and LIN-35 (RB) are all components of the SynMuv B pathway that inhibits ectopic vulval development cell non-autonomously²⁻⁴. Consistent with emerging functions for E2Fs in mammals that are unrelated to cell proliferation, *efl-1* and *dpl-1* have recently been shown to be essential for fertility in *C. elegans* by regulating the expression of developmental rather than cell cycle-related programs independent of *lin-35*^{5,6}. EFL-1 is most closely related to the mammalian repressor E2F4, but less is known about EFL-2 and F49E12.6 since their knockdown through RNAi did not result in notable phenotypes in the worm⁷⁻⁹. In the model plant system *Arabidopsis thaliana*, two activators and 4 repressors have been identified¹⁰. Like mammalian E2F activators, AtE2Fa-b interacts with AtDP (DP) to bind DNA and activate E2F-responsive genes. Studies have shown that AtE2Fa expression is at a maximum late in G₁, acts to induce S phase and endoreplication (see Glossary) and that AtE2Fb plays a similar role¹¹⁻¹³. Interestingly, AtE2Fc has a truncated C-terminal transactivation domain and has been shown *in vitro* to down-regulate the expression of E2F targets such as *AtCDC6* (*Cdc6*)^{14,15}. AtE2Fa-c are all capable of binding the plant RBR (Rb) protein. In contrast, the “atypical” AtE2Fd-f are considered the plant orthologues of mammalian E2F7 and E2F8 and participate in the regulation of plant cell growth and endoreplication¹⁶⁻¹⁸.

References

1. van den Heuvel, S. & Dyson, N. J. Conserved functions of the pRB and E2F families. *Nat Rev Mol Cell Biol* 9, 713-24 (2008).
2. Ceol, C. J. and Horvits, H. R. *dpl-1* DP and *efl-1* E2F act with *lin-35* Rb to antagonize Ras signaling in *C. elegans* vulval development. *Mol Cell* 7, 461-73 (2001).

3. Myers, T. R. and Greenwald, I. lin-35 Rb acts in the major hypodermis to oppose ras-mediated vulval induction in *C. elegans*. *Dev Cell* 8, 117-23 (2005).
4. Lu, X. & Horvitz, H. R. lin-35 and lin-53, two genes that antagonize a *C. elegans* Ras pathway, encode proteins similar to Rb and its binding protein RbAp48. *Cell* 95, 981-91 (1998).
5. Chi, W. & Reinke, V. Promotion of oogenesis and embryogenesis in the *C. elegans* gonad by EFL-1/DPL-1 (E2F) does not require LIN-35 (pRB). *Development* 133, 3147-57 (2006).
6. Chi, W. & Reinke, V. DPL-1 (DP) acts in the germ line to coordinate ovulation and fertilization in *C. elegans*. *Mech Dev* 126, 406-16 (2009).
7. Kamath, R. S. et al. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421, 231-7 (2003).
8. Rual, J. F. et al. Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Res* 14, 2162-8 (2004).
9. Sönnichsen, B. et al. Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature* 434, 462-9 (2005).
10. Mariconti, L. et al. The E2F family of transcription factors from *Arabidopsis thaliana*. Novel and conserved components of the retinoblastoma/E2F pathway in plants. *J Biol Chem* 277, 9911-9 (2002).
11. Rossignol, P. et al. AtE2F-a and AtDP-a, members of the E2F family of transcription factors, induce *Arabidopsis* leaf cells to re-enter S phase. *Mol Genet Genomics* 266, 995-1003 (2002).
12. De Veylder, L. et al. Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa-DPa transcription factor. *EMBO J* 21, 1360-8 (2002).
13. Sozzani, R. et al. Interplay between *Arabidopsis* activating factors E2Fb and E2Fa in cell cycle progression and development. *Plant Physiol* 140, 1355-66 (2006).
14. Kosugi, S. & Ohashi, Y. Interaction of the *Arabidopsis* E2F and DP proteins confers their concomitant nuclear translocation and transactivation. *Plant Physiol* 128, 833-43 (2002).
15. del Pozo, J. C., Boniotii, M. B. & Gutierrez, C. *Arabidopsis* E2Fc functions in cell division and is degraded by the ubiquitin-SCF(AtSKP2) pathway in response to light. *Plant Cell* 14, 3057-71 (2002).
16. Lammens, T., Li, J., Leone, G., & De Veylder, L. Atypical E2Fs: new players in the E2F transcription factor family. *Trends Cell Biol* 3, 111-8 (2009).
17. Lammens, T. et al. Atypical E2F activity restrains APC/CCCS52A2 function obligatory for endocycle onset. *Proc Natl Acad Sci U S A* 105, 14721-6 (2008).
18. Ramirez-Parra, E. et al. Role of an atypical E2F transcription factor in the control of *Arabidopsis* cell growth and differentiation. *Plant Cell* 16, 2350-63 (2004).