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Atypical E2Fs: new players in the E2F transcription factor family

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Abstract

As major regulators of the cell cycle, apoptosis and differentiation, E2F transcription factors have been studied extensively in a broad range of organisms. The recent identification of atypical E2F family members further expands our structural, functional and molecular view of the cellular E2F activity. Unlike other family members, atypical E2Fs have a duplicated DNA-binding domain and control gene expression without heterodimerization with DP proteins. More recently, knock-out strategies in plants and mammals have pinpointed that atypical E2Fs play a critical role in plant cell size control, endocycle regulation, proliferation, and apoptotic response upon DNA stress. Here, we review the emerging biochemical and genetic data on these unique E2F members and discuss their roles in plant and animal development. Their position at the crossroads between proliferation and DNA stress response marks these novel E2F proteins as interesting study objects in the field of tumor biology.

The E2F family of transcription factors

Transcription, representing a first step in gene expression, is tightly regulated by transcription factors (TFs), their co-factors, RNA polymerases, and chromatin modifying proteins [1]. E2F represents an important class of TFs whose family members hold an evolutionarily conserved DNA-binding domain. Typically, E2F proteins associate with a dimerization partner (DP) protein to form a heterodimeric complex that binds to the promoter of a multitude of target genes [2]. Over the past decade, a central role in development has been revealed for these E2F/DP TFs [3]. In particular, it is now clear that the E2F/DP pathway is crucial for the regulation of DNA replication, apoptosis and cellular differentiation [4], whereas deregulation of its activity has a high impact on health and disease [5–7].

E2F TFs have been identified in higher eukaryotes, such as mammals [8–10], worm [11], frog [12] and fly [13,14], as well as in plants [15–17]. More recently, through the survey of genome data, a novel evolutionarily conserved branch of E2F-related TFs has been discovered, first in plants [18–20] and subsequently in mammals [21–26]. Due to their peculiar structural properties, they were designated atypical E2F proteins, and consequentially were found to be mechanistically and functionally distinct from their typical relatives. Recent studies have

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revealed that these atypical E2F proteins are involved in post-mitotic development [27–29], embryogenesis [30], DNA stress response [30,31] and possibly carcinogenesis [32]. In the light of these emerging data, here we review the current knowledge of the atypical E2F TFs in mammals and plants and highlight some unresolved issues.

Identification of atypical *E2F* genes

Six classical E2F proteins have been described in mammals (E2F1 to E2F6) [33] and three in *Arabidopsis thaliana* (E2Fa to E2Fc) [34] that all possess one N-terminally located DNA-binding domain (DBD) immediately followed by a dimerization domain, allowing interaction with a DP partner protein (Figure 1). This dimerization is a prerequisite for high-affinity, sequence-specific binding of the E2F proteins to the *cis*-acting elements of target genes (Box 1) [2,19]. With the completion of nuclear genome sequencing in the model plant *Arabidopsis* [35], it became evident that plants hold a novel class of E2F TFs. To determine all *Arabidopsis* E2F genes, its genome was scrutinized for genes containing motif(s) homologous to the E2F DNA-binding domain [20]. Surprisingly, three genes were detected that were related to E2Fs, but obviously differed structurally [18–20]. Because of their phylogenetic positions between E2F and DP, they were either designated DP-E2F-Like (DEL1 to DEL3), or E2Fe/E2L3, E2Fd/E2L1 and E2Ff/E2L2 (the E2F/DEL nomenclature will be used in this review). By a similar approach, analogous genes were identified in the genomes of human and mouse and were designated *E2f7* and *E2f8*, [21–26]. Noteworthy, *E2f7* was also detected independently in a screen for human E2F1-regulated genes [22]. Additionally, with the OrthoMCL tool and database (www.orthomcl.org), orthologous atypical E2F proteins were discovered in many organisms, including *Caenorhabditis elegans* (nematode worm) and *Oryza sativa* (rice). Rather unexpectedly, no atypical E2F family members have been reported for *Drosophila melanogaster* (fruit fly).

Box 1

Typical E2Fs and RB transcriptional regulation

The RB/E2F/DP pathway is conserved in mammals and plants. Mammals possess three RB family members (pRB, p107, and p130) and six classical E2Fs (E2F1 to E2F6) [33]. The model plant species *Arabidopsis* holds one RB-related (RBR1) protein and three typical E2Fs (E2Fa to E2Fc) [58]. According to whether E2Fs act positively or negatively on gene transcription, they are grouped into transcriptional activators (E2F1 to E2F3 in mammals and E2Fa and E2Fb in plants) or suppressors (E2F4, E2F5 and E2F6 in mammals and E2Fc in plants) [19,33]. Typical E2F family members regulate the expression of a diverse set of genes, among which the DNA replication machinery genes have been studied most extensively [33,34]. In addition, E2F TFs have been implicated in the control of the expression of genes involved in proliferation, apoptosis, DNA repair, and differentiation [33]. The RB proteins counteract the activity of E2F TFs through masking the transactivation domain of E2F proteins and recruiting chromatin-modifying proteins [59]. Hyperphosphorylation of RB by cyclin-dependent kinases causes its dissociation from the E2F factors, thereby liberating their transactivation domain and allowing transcription of E2F target genes [60].

Molecular features of atypical E2F proteins

The rather low overall sequence similarity (~20% on amino acid level) between atypical and typical E2F proteins implies an important structural difference between these two classes. The most peculiar characteristic of atypical E2F members is the duplication of the DBD (Figure 1a) that comprises DNA-binding and dimerization residues. Typical E2F family members

possess only one such domain, whereas the second domain that is required for DNA binding is obtained through dimerization with the E2F-binding partner DP. Consistently with the presence of two DBDs, atypical E2Fs recognize and bind consensus E2F-binding sites of target genes in a DP-independent fashion, as shown by gel retardation assays. Moreover, their binding of the target DNA is completely abolished by mutation of either of the two DBDs [18, 22–26]. Likely, the duplicated DBD structurally mimics the DNA-binding interface of E2F-DP heterodimers. Indeed, three-dimensional models of the atypical E2F7/E2F8, based on the solved E2F4-DP2 structure, reveal a structure compatible with DNA binding (Figure 1b) [23, 26] with DBD1 and DBD2 adopting the functions of the E2F and DP, respectively. Currently, it is still unclear whether atypical E2Fs arose from the gene duplication of classical E2Fs or the latter lost a second DBD during evolution.

Curiously, the duplicated DNA-binding domain of atypical E2F proteins also contains the heterodimerization residues (Figure 1a), previously shown to make contact with the DP protein in the E2F/DP heterodimer [36]. However, DP does not interact with atypical E2Fs as illustrated by the observation that the mainly cytoplasmatic DP-green fluorescent protein (GFP) signal remains cytoplasmatic upon co-expression of *E2f7*, whereas co-expression of *E2f1* results in a nuclear DP-GFP signal [23]. It turned out that candidates for interactions with this dimerization motif are the atypical E2F members themselves. The possibility of such interactions was demonstrated by co-immunoprecipitation of epitope-tagged versions of E2F7 and E2F8 produced in human cells [22,26,30]. Detailed homo- and heterodimerization studies indicated that the dimerization of E2F7/E2F8 depends solely on the integrity of the dimerization residues within the DBD [31]. Interestingly, E2F7 and E2F8 have preferential dimerization states with E2F7 homodimers formed preferentially over E2F7/E2F8 heterodimers and E2F8 homodimers being the least preferred form [30]. In agreement with these biochemical data, *E2f7*^{+/-};*E2f8*^{-/-} knockout mice (in which E2F7 homodimers are able to form) were normal, whereas *E2f7*^{-/-};*E2f8*^{+/-} mice (only E2F8 homodimers can form) had postnatal developmental defects [30]. The dimerization issue has not been conclusively addressed in plants.

While atypical E2F proteins contain an extra DBD, they do not possess several features found in most typical E2Fs. First, in contrast to E2F activator proteins (Box 1), they lack a transactivation domain (Figure 1a). Knockout of atypical *E2F* genes in plants and mammals results in the upregulation of defined subsets of E2F-regulated genes, suggesting that atypical E2F proteins operate as transcriptional repressors [27,28,30,31]. Moreover, in competition assays, E2F7/E2F8 and E2F/DELS can counteract the activating E2Fs, which is consistent with their role as transcriptional inhibitors [18,19,21–26]. Notably, the negative effect of atypical E2F proteins requires the integrity of the DBD, including their dimerization residues [23,26, 31].

Compared to the typical E2Fs, the classical retinoblastoma (RB)-binding domain is also absent in the atypical E2Fs (Figure 1a). RB and its family members inhibit E2F activity and trigger silencing of gene expression through their simultaneous association with E2Fs and chromatin-modifying enzymes (Box 1). In other words, atypical E2F proteins likely exert their repressive function in an RB-independent manner. Similarly to the classical E2F6 repressor, they might operate through recruitment of chromatin-modifying repressor complexes [37], but competition with activating E2F proteins for the same E2F binding site could also be involved.

Information concerning domains/motifs other than those mentioned above is scarce. Studies with GFP reporter constructs have demonstrated the nuclear localization of E2F7/E2F8 and E2F/DEL proteins [18,21,26]. Consistent with this observation, all atypical E2F proteins contain a putative nuclear localization signal (NLS). Whereas typical E2F proteins possess a single NLS sequence in their N-terminal region, atypical E2Fs hold a bipartite NLS in their C-

terminal tail [19,23,25,33]. Yet, the functional significance of this difference remains unknown. Additionally, *in silico* analysis of E2F7 and E2F8 identified motifs that are known to occur in proteins susceptible to ubiquitin-mediated degradation [25, T.L., J.L., G.L., and L.D.V., unpublished observations], which might provide a potential explanation for the observed instability of these atypical E2F members [25].

Transcriptional regulation of atypical *E2F* expression

The expression of E2F activators is the highest at the G1-to-S transition, whereas that of typical E2F repressors remains unchanged throughout the entire cell cycle [19,38]. In contrast to typical E2F repressors, the expression of atypical E2F members is cell cycle regulated. Transcription of *E2f7/E2f8* is induced at the G1-to-S transition and mRNA levels peak during S-to-G2 [21–26]. In accordance with these observations and the role of E2F7/E2F8 as repressors of gene expression, *E2f1*, a target of E2F7/E2F8, has a complementary expression profile that is disturbed upon double ablation of *E2f7* and *E2f8*, particularly during the S-to-G2 phase of the cell cycle [30,31]. In plants, the expression profile of atypical *E2F* genes is slightly different: they all reach the highest level at the G2-M boundary, but *E2Fe/DEL1* and *E2Ff/DEL3* display an additionally peak at G1-S [19,29]. The transcript levels of the *E2Fe/DEL1* target gene, *CELL CYCLE SWITCH52 (CCS52A2)*, has a complementary profile, as anticipated from the role of E2Fe/DEL1 as repressors of gene transcription during G2-M [29].

Limited information is available on the tissue-specific expression of these atypical *E2F* genes. *E2f7* and *E2f8* share a very similar expression pattern: both are highly expressed in the fetus as well as in the placenta at the mid-gestation stage of mouse embryo development [30, J.L. and G.L., unpublished observations]. In adults, they are highly expressed in skin and thymus, moderately in spleen, and less abundantly in intestine and testis. No, or very low, expression occurs in brain, muscle and stomach [21,26]. Thus, in mammals, atypical *E2f* transcripts are particularly present in tissues with proliferative potential. In plants, *E2F/DEL* transcripts are detected at high levels in young, growing tissue, such as young leaves and immature flower buds, whereas they are low in mature tissues, such as adult leaves [18]. Thus, similar as the mammalian atypical *E2f* genes, *E2F/DEL* transcript levels are also positively correlated with the proliferation state of cells and tissues. However, *E2Ff/DEL3* appears to be an exception. Analysis of the pollen transcriptome has indicated high levels of *E2Ff/DEL3* in mature pollen [39]. The vegetative pollen nucleus is thought to be arrested in G1, and thus high *E2Ff/DEL3* levels in pollen might repress transcription of S-phase genes and be (at least partially) responsible for blocking the nucleus in G1. Unfortunately, no clear pollen phenotype could be detected in *E2Ff/DEL3* knock-down plants [27].

Interestingly, the atypical *E2F* genes themselves are E2F targets [22]. High expression levels of *E2f7* and *E2f8* were observed in HeLaS3 cells that have a deregulated RB-E2F pathway [25]. Similarly, plants overproducing the E2Fa-DPa factor accumulate high levels of the *E2Ff/DEL3* transcript [40]. In agreement with these observations, analysis of the mammalian and *Arabidopsis* atypical *E2F* genes has identified consensus E2F-binding sequences on their promoters and chromatin immunoprecipitation assays have provided evidence for a direct association of E2F1/3/4/7 to the *E2f7/E2f8* promoters [22,25]. These findings reinforce the notion that the E2F network exists as a complex map of cross-talking pathways [2,33,34,38].

Biological functions of atypical E2F proteins

Recent insights into the *in vivo* functions of atypical E2F proteins have emerged from the analysis of knockout mice and mutant plants [27–30]. These preliminary data have indicated

that these atypical E2Fs fulfill critical functions during development, controlling cell size, endocycle, proliferation and DNA damage response.

Atypical E2F proteins and cell size control

The study of E2Ff/DEL3 in *Arabidopsis* provided the first biological evidence for an atypical E2F protein function [27]: depletion or overexpression of *E2Ff/DEL3* resulted in a significantly increased or decreased root and hypocotyl size, respectively. These differences are caused by changes in cell size, but not in cellular proliferation, because the size of the meristems, the plant proliferating centers, was not affected [27]. In plants, root and hypocotyl growth relies extensively on cell expansion, a process that starts after cells stop proliferating and initiate their differentiation program. This cell expansion demands the remodeling of preexisting cell walls [41–43]. One important step in this mechanism is the loosening of the cell walls, a process carried out by the expansin protein family. Interestingly, *E2Ff/DEL3* was shown to bind directly and modulate expression of several expansin members [27] (Figure 2). Together, these data indicate an important role for *E2Ff/DEL3* in restricting cell expansion through transcriptional repression of genes involved in cell wall biosynthesis. In mammals, no function in cell size control has been assigned to any atypical E2Fs, probably because in animals growth occurs commonly by division, and not often by cell expansion [44].

Atypical E2F proteins and cell cycle control

Similar to what was observed for *E2Ff/DEL3*, overexpression of *E2Fe/DEL1* also has a negative impact on plant size [28,29], but the mechanism underlying this phenotype is completely different. In *E2Fe/DEL1* transgenic plants, the cell size decreases not because of a repression of cell wall-modifying genes, but due to a reduced DNA ploidy content [28]. In dicotyledonous plants (flowering plants with two embryonic seed leaves), exit from the mitotic cell cycle is often accompanied by the onset of an alternative cell cycle, named endoreduplication, in which DNA replication is followed by incomplete mitosis (Box 2). This DNA amplification is thought to support cellular growth. Mitotic cell cycle progression and endoreduplication are linked events. Premature or delayed onset of endoreduplication results in an increased or decreased DNA ploidy level, respectively [45–47], and accordingly, larger or smaller cells (Box 2). Recently, the altered cell size and associated changes in DNA ploidy levels in *E2Fe/DEL1* knockout plants have been proven to result from a premature onset of the endoreduplication program [29]. More specifically, *E2Fe/DEL1* was found to control the expression of the Anaphase-Promoting Complex/Cyclosome (APC/C) activator gene *CCS52A2*, which is homologous to the mammalian *Cdh1* gene [29] (Figure 2). APC/C is an E3-ubiquitin ligase that marks proteins for degradation by the 26S proteasome [48]. *CCS52A2* has been implicated in the control of endocycle onset in *Arabidopsis*, probably through degradation of mitotic cyclins [49,50]. Thus, by regulating the *CCS52A2* expression, E2Fe/DEL1 probably determines the time point at which cells switch from mitosis to endoreduplication and, consequently, controls cell size [29]. The distinct ways in which E2Fe/DEL1 and E2Ff/DEL3 govern cell size reinforce the idea that in plants cell expansion is regulated by different systems, roughly divided into ploidy-dependent (via E2Fe/DEL1) and ploidy-independent (via E2Ff/DEL3) growth control. It would be interesting to study the functional relationship between E2Fe/DEL1 and E2Ff/DEL3 to obtain a deeper insight into the coordination and crosstalk between the different levels of cell size regulation in plants.

Box 2

Roles of atypical E2Fs in the endoreduplication process

During development, cells exit the cell cycle and start to differentiate to become specialized in a specific function. Exit from the mitotic cell cycle is sometimes accompanied by the onset of an alternative cycle, called endoreduplication. During endoreduplication, DNA

replication is followed by incomplete mitosis, allowing cells to increase their DNA content dramatically in a discrete two-fold way [61,62] (figure in this box). Endoreduplicating cells were first observed in plants, but, since then, also frequently described in metazoans as well. Whereas in *Arabidopsis* endoreduplicating cells are found at the whole plant level, in mammals they seem to be confined to certain organs, such as placenta, liver and during megakaryocytic development [51,52,63]. The physiological role of endoreduplication is poorly understood, and much speculated. The nuclear-endoplasmatic theory postulates that endoreduplication serves as a base for cell size, at least in plants [64]. Intriguingly, a function for endoreduplication has been postulated in DNA stress tolerance [61,65]. Since endoreduplicating cells possess more gene copies, they would be better protected from deleterious mutations. Research of the past 20 years has implicated many genes in the control of the endoreduplication initiation and progression [58,61,62]. Importantly, recent reports have demonstrated that the *Arabidopsis thaliana* atypical E2Fe/DEL1 acts as a specific inhibitor of endoreduplication (28,29) (see fig in this box). By contrast, the mammalian atypical E2F7 and E2F8 serves as safe-guards, preventing mitotic progression in endoreduplicating cells (see fig in this box).

In mammals, the best example of developmentally programmed endocycle is the placental trophoblast giant cells [51] that amplify their genomes and attain a >1000C polytene configuration through successive rounds of DNA replication in the absence of karyokinesis or cytokinesis [52]. Interestingly, similarly to E2Fe/DEL1, the mammalian atypical E2F7 and E2F8 also play a critical role in endocycle control. In mice, loss of *E2f7* and *E2f8* results in endocycle defects in the trophoblast giant cells. These mutant giant cells strikingly segregate their chromosomes and complete karyokinesis in the absence of cytokinesis [J.L. and G.L., unpublished observations]. Although the underlying mechanism of this aberrant mitosis remains unknown, atypical E2Fs are clearly involved in the endocycle control, with the plant E2Fe/DEL1 protein controlling the timing of endocycle onset and mammalian E2F7/E2F8 probably governing the maintenance of endocycle progression (Box 2).

Atypical *E2F* genes are also expressed in cell types that do not endoreduplicate, indicating they might have other biological functions beyond the endocycle control. Indeed, data do suggest that atypical *E2Fs* play a role in general cell cycle control, particularly in proliferation. For example, loss of *E2f7* and *E2f8* function causes ectopic DNA replication in spongiotrophoblasts as well as in giant cells [J.L. and G.L., unpublished observations]. Consistent with this cellular phenotype, at the molecular level, global gene profiling and chromatin immunoprecipitation-polymerase chain reaction (ChIP-PCR) analyses revealed that a majority of direct target genes

of E2F7 and E2F8 are functionally involved in cell cycle control, particularly in DNA replication during the G1-to-S transition [J.L. and G.L., unpublished observations]. Conversely, early studies have demonstrated that overexpression of *E2f7/E2f8* in cell cultures repressed cell proliferation [21–26] and decreased the colony-forming potential of HeLa cells [22].

Atypical E2F proteins and DNA damage response

The recent generation of *E2F7/E2f8* double knockout mice has provided insight into the role of atypical E2Fs in cell survival because these embryos exhibited widespread apoptosis [30]. Furthermore, upon treatment of wild-type cells with DNA-damaging agents, E2F7 and E2F8 protein levels increased [31]. The most interesting observation, however, is that these high levels coincided with an augmented E2F7/E2F8 occupancy onto the *E2f1* promoter [31] (Figure 2). In contrast, absence or low levels of E2F7 or E2F8 increases the *E2f1* expression and renders cells more susceptible to DNA damage. *E2f1* appears to be a physiological target for stress-induced apoptosis, because such apoptosis could be rescued by co-depletion of *E2f1* *in vitro* as well as *in vivo* [30,31]. Thus, both E2F7 and E2F8 seem to act as critical regulators of E2F1 activity that had been proposed previously to determine the outcome of DNA damage. Low levels of E2F1 have been suggested to be necessary to recruit repair complexes, whereas high levels of E2F1 would activate transcription of genes that drive cells into the apoptotic program [53–55]. Importantly, as described above, *E2f7* and *E2f8* themselves are transcriptional targets of E2F1, illustrating the existence of a negative feedback loop. The activation of *E2f7* and *E2f8* by E2F1 at the G1-to-S transition, followed by the repression of *E2f1* by E2F7 and E2F8 during S-G2 might limit the window of E2F1 activity during the cell cycle. In the *E2f7E2f8* double mutant, persistent activity of E2F1 during G2 might account for the increased sensitivity to cell death [30,56]. Taken together, these data indicate that E2F7/E2F8 are an important arm of the E2F transcriptional network, which is responsible for fine-tuning E2F1 activity upon DNA damage and, consequently, involved in regulating cell viability. However, the control of DNA damage response by atypical E2F proteins could be more complex than anticipated. For example, in addition to the direct repression of *E2f1* expression, E2F7/E2F8 could also compete with E2F1 binding to the genes involved in the early apoptotic response and, thus, balance their activity. Future research will need to confirm or exclude this possibility.

In contrast to mammals, cell survival does not seem to be affected by the knockout of individual atypical *E2F* genes in *Arabidopsis* [27–29], probably because the stringency and checkpoint control mechanisms that exist between animals and plants differ. Since plant cells are fixed within tissues due to their rigid cell walls, there is no risk of metastasis of damaged cells. Rather than being eliminated by cell death, damaged cells are pushed into a differentiation program that precludes cells with mutated DNA from becoming part of the gametophytic cells [57]. Moreover, thanks to their plasticity, plants grow directly by induction of new meristems, escaping from the DNA stress sources [58]. Together, these mechanisms might exclude the need for a sensitive apoptotic program. Nevertheless, the generation of doubly and triply mutated *E2F/DEL* cohorts will be crucial for understanding the role of atypical E2F proteins in DNA damage response in plants.

Concluding remarks

Atypical E2F proteins represent an evolutionarily conserved branch of the E2F TFs family, in which the presence of a duplicated DBD provides these new E2F players the unique ability to bind DNA in a DP-independent manner. Interestingly, in most organisms with E2F proteins, at least one DP and one atypical E2F are recognized. This evolutionary coexistence possibly reinforces the strong communication and interdependence of E2F family members, which is indispensable for correct development.

Recently, a preliminary clinical study has associated low *E2f7* levels in ovarian tumor tissues with low patient survival and potential development of resistance to anti-cancer drugs [32]. Similarly, Kaplan-meier survival curves for *E2f7* (Rembrandt-glioma database see: <http://caintegrator-info.nci.nih.gov/rembrandt>) indicate that low *E2f7* levels also correlate with poor survival in glioma patients. Interestingly, *E2f7* is located at chromosome 12q21, a region of which its deletion is associated with poor prognosis for pancreatic cancer [22]. In sharp contrast to *E2f7*, high levels of *E2f8* predict poor survival in glioma patients (Rembrandt-glioma database see: <http://caintegrator-info.nci.nih.gov/rembrandt>). Thus, although *E2f7* and *E2f8* were shown to act synergistically [30,31], clinical observations argue that, at least in carcinogenesis, *E2f8* might have a different role than *E2f7* has [32]. Of course, further studies with large sets of patients will be essential to confirm the predictive values of *E2f7* and *E2f8*. To date, no cancer research has been specifically performed on these newly identified E2F factors, however, given their critical roles in controlling proliferation, proper mitotic entry and DNA damage-induced apoptosis, it is reasonable to imagine that they might function as putative tumor suppressors in the tumor setting. In this regard, using mouse models to examine the potential tumor suppressor function of these atypical E2F factors would be very informative. No tumor phenotype was observed in *E2f7* or *E2f8* single knockout mice [J.L. and G.L., unpublished observations], which is likely due to the functional compensation between these two E2F factors. The *E2f7* and *E2f8* conventional double knockout animals die *in utero*, which unfortunately excludes the possibility to investigate their potential tumor repressor activity in the late stage of development. To overcome this early lethality, future experiments employing conditional knockout strategies should be considered. With no doubt, a lot of questions remain to be unraveled to understand these most recently identified but least understood atypical E2F members (Box 3). Answering these questions will not only provide a much deeper insight into the atypical E2Fs, but also will contribute greatly to the understanding of the whole family of E2F TFs.

Box 3

Outstanding questions

The five most important research questions that need to be addressed in order to understand atypical E2Fs function are:

1. By which mechanism do atypical E2F proteins suppress gene transcription? Is simple competition with classical E2F proteins or active repression involved? In the latter case, which are the co-repressors?
2. Which are the target genes of the different atypical E2Fs? Could this information explain the observed phenotypes of knockouts?
3. What are the essential domains present in atypical E2F proteins and how do they contribute to their activities?
4. How are the activities of atypical E2F proteins controlled at the posttranscriptional level?
5. Do atypical *E2F* genes behave as tumor suppressor genes and how do they exert this function?

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Figure 1. Schematic representation of the E2F proteins and their three-dimensional structures
(a) The E2F protein family and DP proteins, and their structural organization and interaction regions with DNA and binding partners. The most peculiar differences are shown between classical E2Fs, DP proteins and atypical E2Fs. To indicate that the DBD1 of the atypical E2F resembles most that of the classical E2F, they have been given the same color (blue) as well as for DBD2 and that of the DP (red). In this general schematic representation, no difference in length, domain positions and other functional domains is indicated. **(b)** Structure of the E2F4/DP2 heterodimer with E2F4 (blue) and DP2 (red) bound to an E2F DNA consensus sequence (Protein Data Bank code 1CF7). Structural models based on homology modeling with the solved E2F4/DP2 structure depicting the interaction between DBD1 (blue) and DBD2 (red) of E2F7 or E2Fe/DEL1. The putative DBDs of the E2F7 and E2Fe/DEL1 were aligned with the E2F4 and DP2 DBDs using the ClustalW program. Modeling requests were submitted to the SWISS-MODEL protein modeling server with the previously solved E2F4/DP2 crystal structure 1CF7 as the template. DBD, DNA-binding domain; DD, dimerization domain

Figure 2.

Overview of the atypical E2F functions. Dashed lines mark biological links that are not fully supported yet by experimental data. Arrows indicate positive effects; bars represent repressive paths. Until now, no functional data for E2Fd/DEL2 has been reported. The observed cell elongation and upregulated expression of expansin genes in *E2Ff/DEL3* knock-down plants positions this atypical E2F in the control of cell wall loosening. *E2Fe/DEL1* levels have been reported to control the onset of endoreduplication through the transcriptional control of *CCS52A2*, an APC/C activator gene related to the mammalian *CDH1* gene. Both *CCS52A2* and *CDH1* have previously been implicated in the regulation of endoreduplication. *E2F7/E2F8* have been observed to bind the *CDH1* promoter; whether or not this has a functional consequence remains to be determined. Additional *E2F7/E2F8* target genes include *E2F1*, minichromosome maintenance (*MCM*) genes and cell division cycle 6 (*CDC6*). Whereas the transcriptional upregulation of *E2F1* contributes to the observed apoptosis phenotype in *E2f7/E2f8* double knockout embryos, the *MCM* and *CDC6* targets reveal a role for atypical E2Fs in controlling cell proliferation. Importantly, the atypical *E2F7/E2F8* themselves are E2F1 targets, generating a negative feedback loop. Whether this also is the case for the plant E2F/DEL proteins remains to be demonstrated.

Table 1

The mammalian and *Arabidopsis* atypical E2F proteins

Symbol	Species	Accession	Amino acids	NLS	Destruction motif
E2F7	Human	NM_203394	910/713	Yes	KEN box ^b
E2F8	Human	NM_024680	867	Yes	KEN box ^b
E2F7	Mouse	NM_178609	905	Yes	KEN box ^b
E2F8	Mouse	NM_001013368	860	Yes	KEN box ^b
E2Fe/DEL1 ^a	<i>Arabidopsis thaliana</i>	A3G48160	403	Yes	No
E2Fd/DEL2 ^a	<i>Arabidopsis thaliana</i>	A5G14960	359	Yes	No
E2Ff/DEL3 ^a	<i>Arabidopsis thaliana</i>	A3G01330	354	Yes	No

^aThese proteins have been assigned several other names: DEL1=E2Fe=E2L3; DEL2=E2Fd=E2L1 and DEL3=E2Ff=E2L2.

^bThe KEN box motif is known as a recognition sequence for binding of adaptor proteins of the APC/C, which marks these proteins for ubiquitin-mediated degradation by the 26S proteasome.