Retinoblastoma protein goes green: the role of RBR in *Arabidopsis* meiosis

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The EMBO Journal (2011) 30, 631–633. doi:10.1038/emboj.2011.9

In 1986, retinoblastoma was the first identified tumour suppressor gene in animals (Friend *et al.*, 1986). The protein pRB was then recognized as a key negative regulator of cell cycle progression controlling the G1/S transition. Upon phosphorylation by CDK–cyclin complexes, pRB dissociates from bound E2F transcription factors, which in turn are then free to regulate expression of a variety of genes needed for cell cycle progression and differentiation. Later, pRB has been found to play a critical role in chromosome condensation, centromeric function and chromosome stability (reviewed in Sage and Straight (2010)). These findings stress pRB's importance as a node for mediating CDK–cyclin-derived signals to various downstream processes. The mode of operation may be indirect, via the regulation of transcription of E2F-dependent genes, or direct, via binding and sequestration of, e.g., chromatin modifiers like histone methyl transferases or DNA repair-related proteins like BRCA1 (Aprelikova *et al.*, 1999; Sage and Straight, 2010). pRB is conserved and can also be found in higher plants. *Arabidopsis* contains only a single retinoblastoma-related gene (RBR), whereas pRB has two more relatives (p107 and p130) in animals. So far it has not been possible to study plants homozygous for a RBR loss-of-function allele, as the protein is required for both female and male gametophyte development (Ebel *et al.*, 2004). This issue of *The EMBO Journal* features an article by Chen *et al.* (2011) highlighting novel functions of the plant retinoblastoma protein, taking advantage of a genetic rescue strategy to grow plants homozygous for a hypomorphic *rbr* mutant allele. RBR has been studied in plants before concerning its roles in stem cell maintenance, cell differentiation, organ production and gametophyte development (Ebel *et al.*, 2004; Park *et al.*, 2005; Borghi *et al.*, 2010), but the study by Chen and colleagues is the first to unravel a role of pRB in meiosis.

Irrespective of the model system analysed, retinoblastoma proteins have been found to be essential for various cellular processes. Thus, a role in meiosis seemed likely but lacked experimental proof. A recent study in *Arabidopsis* showed that plants homozygous for a certain RBR mutant allele (*rbr-2*) could be rescued by limiting cell proliferation in gametophytes by reducing CDKA;1 activity. A quarter of the progeny from *rbr-2/+; cdka;1/+* self-fertilized plants turned out to be homozygous for *rbr-2*, and survived irrespective of

the presence of the *cdka;1* mutant allele (Chen *et al.*, 2009). In depth analysis of the *rbr-2* mutant plants revealed that wild-type RBR mRNA and protein are present in vegetative cells. In contrast, in meiocytes only a C-terminal truncated RBR protein is present, due to aberrant RBR mRNA splicing in these specialized cells. Accordingly, homozygous *rbr-2* mutant plants displayed only some minor vegetative phenotypic aberrations, but a strong reduction in fertility (1–10% of wild-type plants). This observation indicated a severe meiotic defect and was further analysed in the laboratories of Frédéric Berger and Chris Franklin. The two groups established that meiotic S-phase is not perturbed in *rbr-2* plants and that the first important step for meiotic recombination, the introduction of DNA double-strand breaks (DSBs), occurs normally. Furthermore, the initial processing of meiotic DSBs seems to be unaffected in *rbr-2* meiocytes, as the numbers of foci representing the recombinases DMC1 and RAD51, respectively, are the same as in wild type. Processes downstream of these initial events of homologous recombination are perturbed. During regular meiosis, each chromosome needs to be connected to its homologous partner to exchange genetic information and to provide a physical link for correct chromosome disjunction during the first meiotic division. This is achieved by channelling repair of some of the meiotic DSBs towards the homologous chromosome, thereby connecting parts of the formerly maternal and paternal chromosomes. During late prophase, these connections (a.k.a. crossovers, COs; chiasmata) become visible in meiotic chromosome spreads. In *rbr-2* meiocytes, the formation of these physical links between homologous chromosomes is severely compromised leading to unconnected chromosomes that subsequently segregate at random. Notably, despite the fact that DSBs are made and the DNA repair machinery fails to connect homologous partner chromosomes, no DNA fragmentation was observed. This leads to the assumption that the truncated RBR protein fails to support CO formation but not DNA repair. In line with this idea, proteins correlated with CO promotion, like the MutS homologue MSH4 and the MutL homologue MLH1, appear in strongly reduced foci numbers on chromosomes of *rbr-2* mutants. RBR itself can be detected as numerous distinct foci on meiotic chromosome spreads during meiotic prophase, and its localization depends on meiotic DSB formation. In *rbr-2* buds, the level of full-length RBR mRNA was markedly reduced and no full-length protein could be detected on chromosomes, only the

Figure 1 Multiple functions of RBR during meiosis and gametogenesis. Although not formally shown, RBR may regulate the entry into premeiotic S-phase by regulating E2F-mediated transcription and DNA replication (via interaction with components of the origin of replication complex—ORC), analogously to what has been shown for mitosis (Weinberg, 1995; Ahlander *et al*, 2008). Chen *et al* show that in *Arabidopsis* during meiotic prophase I, fully functional RBR is required for homologous recombination and chromosome condensation. After completion of meiosis, RBR is essential for gametophyte development by inhibiting excessive cell proliferation and regulation of cell differentiation (e.g., via Polycomb Repressive Complex 2—PRC2; Ebel *et al*, 2004; Johnston *et al*, 2008). Additionally, RBR impacts a broad range of developmental processes during vegetative growth (please refer to Park *et al*, 2005; Borghi *et al*, 2010 for further reading).

C-terminal truncated version. Surprisingly, transcription of various meiotic genes, with or without E2F-response elements in their promoters, was reduced. This was unexpected, as pRB is usually known as a transcriptional suppressor by binding and thereby inhibiting E2F-mediated transcription (see Figure 1).

Taken together, the authors speculate that the observed meiotic defects may not be attributed to an unspecific deregulation of meiotic genes, but to a specific, direct impact of the truncated RBR protein on recombination and chromosome structure. Their hypothesis is supported by the fact that the truncated RBR protein lacks the so-called B-box. This highly conserved protein domain has been shown to bind to specific motifs (LxCxE) of pRB interaction partners. As mentioned above, pRB has been found to interact with chromatin

modifiers, DNA repair proteins and condensin complexes (Longworth *et al*, 2008). It may be these factors that are affected in the *rbr-2* mutant background, leading to a certain failure during meiotic progression.

The presented results are of special interest, as they support insights from other organisms that establish pRB as a regulator of diverse cellular events beyond its role as a repressor of E2F-mediated transcription. Furthermore, Chen and colleagues show for the first time that pRB/RBR plays a crucial role during meiosis. Its precise impact on meiosis still has to be established, but the molecular tools for such an analysis are already in place.

Conflict of interest

The authors declare that they have no conflict of interest.

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