Is G1 arrest in plant seeds induced by a p53-related pathway?

Carrie-Ann Whittle, Tannis Beardmore and Mark O. Johnston

In mammals, p53 is crucial for inducing the genes that lead to G1 arrest following DNA damage, enabling DNA repair. However, the possibility that such a system exists in plants has attracted little attention. Even though some plant cDNA sequences with partial homology to p53 have been reported recently, there has been little analysis of how these molecules might relate to DNA damage. The lack of investigation into whether a DNA-damage-induced, p53-mediated G1-arrest pathway might exist in plants is remarkable given that plant DNA, like that of all organisms, is continually under the threat of attack.

Aged seeds are known to contain high levels of DNA damage. The fact that many aged seeds can survive and develop into adult plants suggests that the embryo has an ability to respond to and reduce DNA damage. We believe that there are remarkable parallels between the germination of aged seed (from germination up to the first cell divisions) containing DNA damage and the p53-mediated G1 arrest in mammals. Based on these parallels, we suggest that aged seeds might be a useful model system from which to evaluate the possibility that a p53-related pathway could exist in plants.

p53-Mediated arrest in humans and mice

To understand why p53-mediated G1-arrest might exist in plant seed, it is first necessary to consider the key events enforcing arrest in humans and mice (Fig. 1). Following DNA damage in mammalian cells, p53 levels increase via post-translational modifications [or by modification of its regulator protein, murine or human double minute (MDM2 and HDM2)] and by its translocation from the cytoplasm into the nucleus, each of which circumvents the need for p53 transcription in the presence of a damaged DNA template. Subsequently, p53 might be activated by post-translational modifications (phosphorylation and/or acetylation), thereby enabling the protein to bind DNA and induce transcription of *p21*, a cyclin-dependent-kinase inhibitor[1] (CKI).

p21 is crucial for inducing G1 arrest following DNA damage. First, it binds to complexes containing cyclin and cyclin-dependent kinases (CDKs), thereby inhibiting the modification of the retinoblastoma (Rb) protein and preventing its release from the E2F transcription factors (a step that is necessary for cell-cycle progression). Second, it adheres to the proliferating-cell nuclear antigen (PCNA), a DNA polymerase δ processivity factor, and prevents DNA replication (but does not affect pre-replicative DNA repair[2]). Pre-replicative DNA repair conducted during this enforced G1 arrest might be partially induced by p53 because it has been shown to play a role in base excision repair, nucleotide excision repair, the removal of damage to higher-order DNA structure and the transcriptional activation of other DNA repair genes[1,3–5]. Nevertheless, it is believed that the extension of the G1 arrest by p53 is crucial for enabling both p53-dependent and p53-independent repair to occur, and therefore might be the ultimate determinant of whether a DNA-damaged cell can undergo normal cell division.

Indicators of a p53-related pathway in plant seed

Prolonged G1 arrest in aged seed

If a p53-related DNA-damage-response system exists during seed germination then older seeds containing increased levels of DNA damage[6] would be expected to require a longer time for cell division following imbibition. Evidence indicates that nondormant seeds from older seed lots do have an increased mean germination time relative to their unaged counterparts[7] (Fig. 2a), a characteristic that might primarily exist because of a delay in the onset of DNA replication[8] (i.e. an extension of G1 for those cells in G1 within the dry seed). Possible factors causing this prolonged G1 in older seeds include the prevention of cells from proliferating owing to the effects of a damaged DNA template during early imbibition (thereby impeding effective transcription), the active process of arresting cells in G1, enabling pre-replicative DNA repair, and other unknown causes. Of these, the first possibility seems unlikely because evidence in irradiated and mutagen-treated mammalian cells indicates that the presence of a damaged DNA template does not itself impede the ability of cells to progress through the cell cycle[9]. Given that cells containing DNA damage can divide, it is likely that many aged plant embryonic cells are actively arresting in G1. Nevertheless, because other plant-specific factors, genes or intracellular conditions (e.g. damaged membranes or rRNA) might be causing a prolonged G1 arrest, further molecular evidence is necessary to determine whether a p53-related pathway is responsible.

DNA repair occurs during prolonged G1 in imbibed seed

DNA repair can occur shortly after seed imbibition (for both aged and unaged seeds)[8,10,11] and so the extended time required for aged seeds to germinate might be an active process of prolonging G1 arrest to accommodate the greater levels of pre-replicative repair needed in these embryos. The importance of a prolonged G1 for induction of such repair might be indicated by the artificial enforcement of G1 arrest on aged seeds containing DNA damage by priming (holding seeds at a water potential that prevents germination) or by abscisic acid (ABA) treatment, each of which might extend the time between the start of imbibition and DNA replication (i.e. G1 for many cells)[12]. Such

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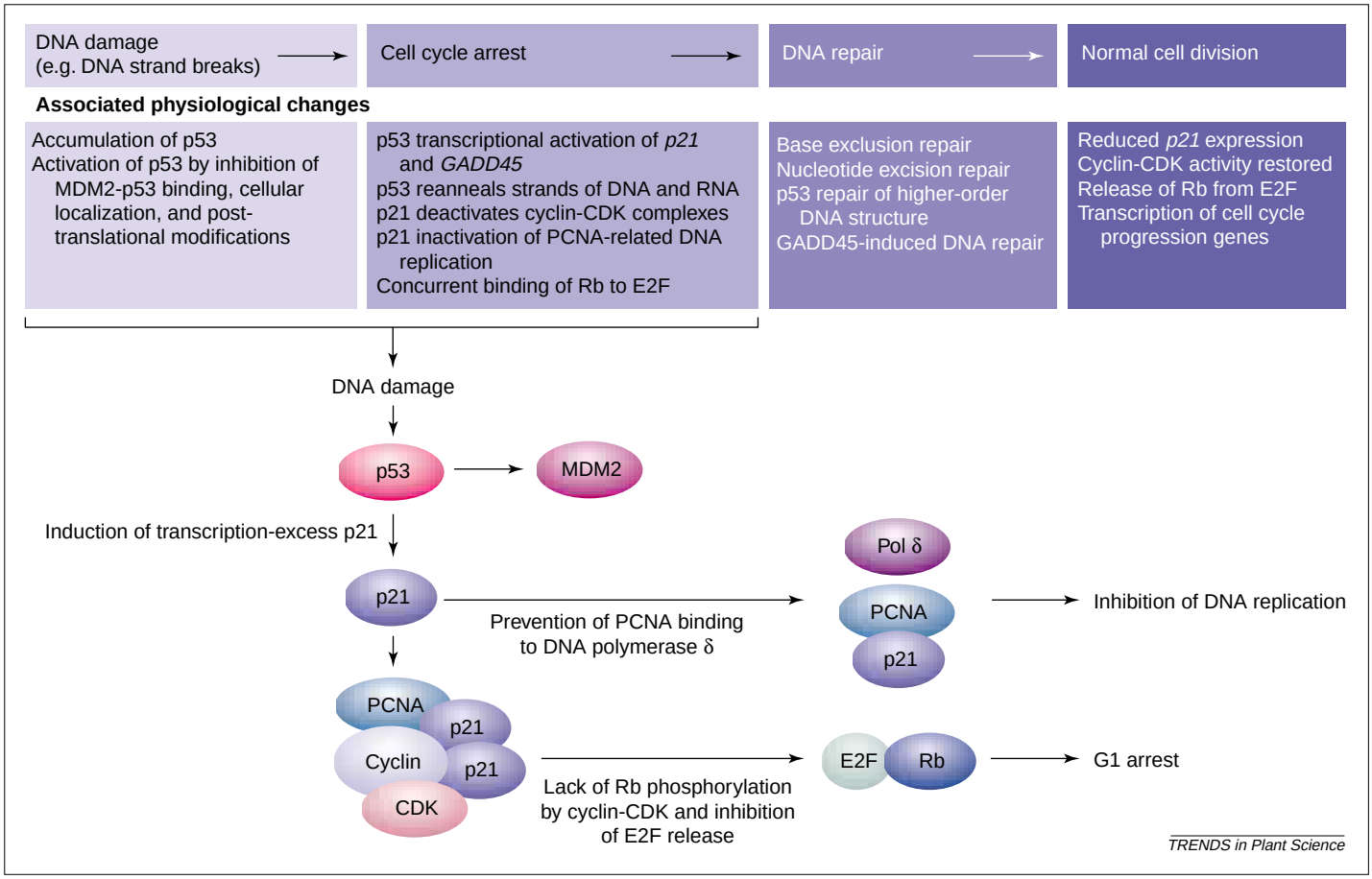

Fig. 1. Pathway leading to p53-induced G1 arrest in humans and mice, and the physiological changes associated with each stage (DNA damage, G1 arrest, DNA repair and cell division). Following DNA damage, p53 induces transcription of *p21*. The p21 protein binds cyclin–cyclin-dependent-kinase (CDK) complexes and proliferating-cell nuclear antigen (PCNA, a trimer), inducing G1 arrest and preventing DNA replication, respectively[1,2]. Abbreviations: E2F, E2F transcription factor; MDM2, murine double minute 2; Pol δ, DNA polymerase δ; Rb, retinoblastoma.

priming and ABA treatment have been shown to reduce the levels of chromosomal aberrations in aged seeds and to enhance the ability to germinate (as indicated by an increase in the proportion of normal germination and a reduction in the mean germination time)[7,12]. In this regard, extension of G1 arrest in aged seeds might be directly related to the level of pre-replicative DNA repair, a feature consistent with the p53-mediated G1 arrest in human and mouse cells. Nevertheless, because ABA treatment and priming might reduce DNA damage by independent pathways, the levels of DNA repair following imbibition among seeds of different ages will need to be determined before the precise relationship between elongation of G1 and the level of DNA repair can be ascertained.

Putative homologues

Although the presence of a prolonged G1 and DNA repair following imbibition of aged seeds might indicate an active process of G1 arrest, the immunodetection of numerous putative cell cycle proteins within plants indicates that a p53-homologous pathway could be responsible for this arrest (Fig. 2b). For example, using a human p53 antibody (Pab-240), a putative p53 homologue has been detected at remarkably high levels in unaged *Zea mays* seeds, with successively lower levels occurring during imbibition and after the start of replicative DNA synthesis[13,14]. This pattern is consistent with the trend observed in mammalian p53.

The tendency of the putative p53 protein to exist before DNA replication suggests that it could be an active enforcer of G1 arrest in both the imbibed embryo and the quiescent seed (suggesting that the protein might be formed during seed maturation). Furthermore, its presence in the quiescent seed suggests that the need for transcription upon imbibition could be circumvented (from a damaged template), a characteristic consistent with the post-translational regulation of p53 inherent in humans and mice. Nevertheless, although the changes in the abundance of this putative p53 protein are consistent with p53-mediated arrest in mammals, the studies published to date only show that a protein region consistent with the human p53 epitope exists in seeds. Further evaluation of the constitution of this protein by protein sequencing, protein–protein interaction studies and the use of antibodies containing other epitope affinities within both aged and unaged seeds will be essential to determine whether a true p53-related homologue exists.

Evidence of a conserved binding site between human p21 and plant PCNA (Ref. 15) suggests that at least some of the downstream components of a p53-induced G1 arrest could be conserved between plants and

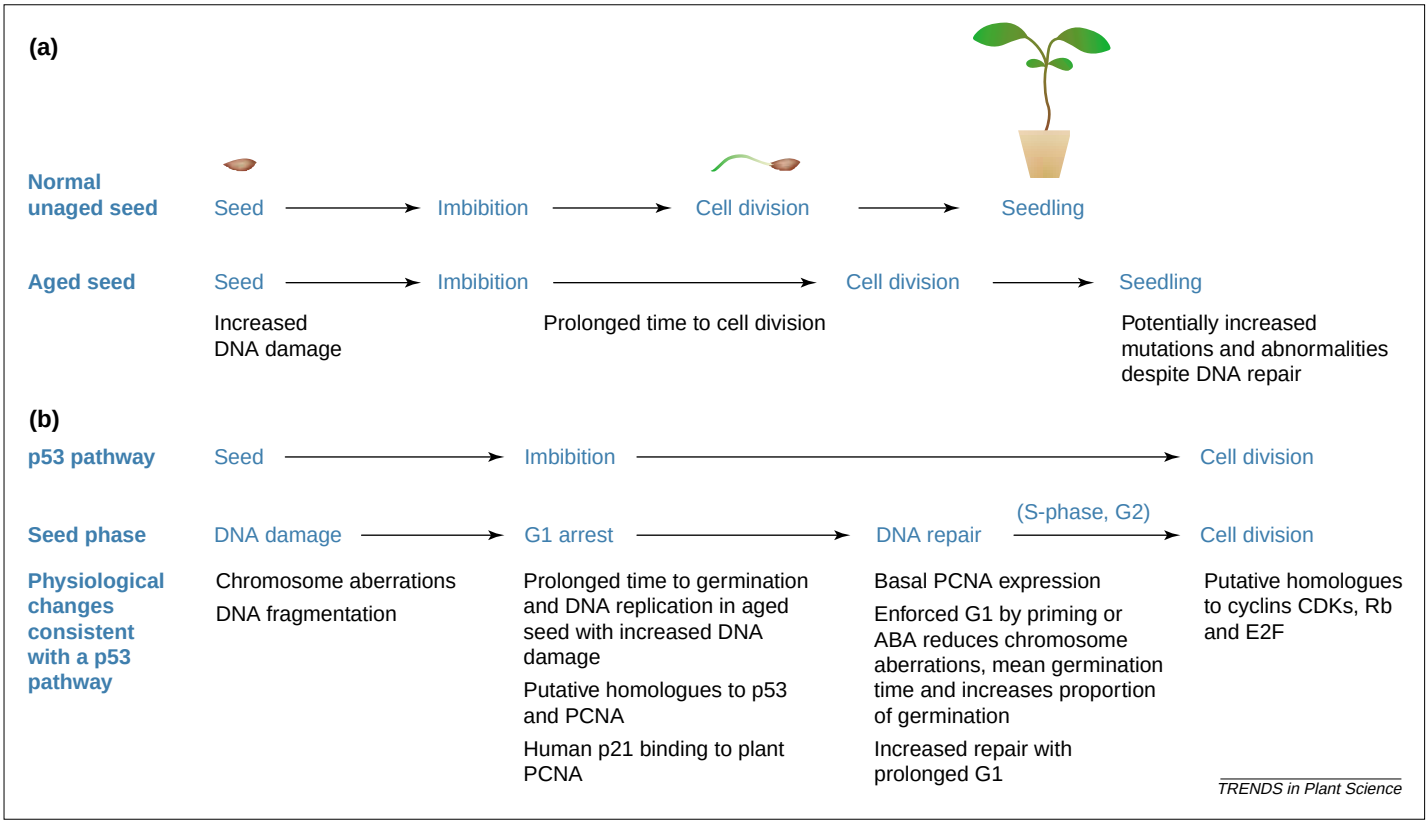

Fig. 2. (a) Steps inherent in cell division of nondormant unaged and aged viable plant seed. Shortly after imbibition (during G1 in most cells), seeds undergo all the processes necessary for cell division, including synthesis of new mRNA, tRNA, rRNA and protein, as well as respiration, mobilization of storage reserves and membrane stabilization. During this early developmental period, DNA repair occurs. For aged seed, with increased levels of DNA damage, the time between imbibition and cell division is prolonged, suggesting that these seeds might have longer to conduct DNA repair. (b) Evidence consistent with the presence of a p53-related pathway in plant seeds during three progressive developmental stages of seed emergence, including seed quiescence, imbibition and cell division, and the parallels with p53-mediated G1 arrest associated with each stage (DNA damage, G1 arrest, DNA repair, cell division). Abbreviations: ABA, abscisic acid; CDK, cyclin-dependent kinase; E2F, E2F transcription factor; PCNA, proliferating-cell nuclear antigen; Rb, retinoblastoma.

mammals (Fig. 2b). Plant PCNA has been shown to function as a cofactor of DNA synthesis in rice (*Oryza sativa*)[16], similar to its function in humans and mice. Thus, it is likely that inhibition of this function would have the same effect in plants as in mammals. This inhibition might be by a p21 homologue; other CKIs have been detected in plants[17]. A putative PCNA homologue (detected using an anti-human antibody) in maize seed indicates that this protein occurs at maximum levels during the first S phase, a feature consistent with PCNA's role in mammalian DNA replication, but occurs at basal levels during the initial hours of imbibition, which is consistent with its role in pre-replicative DNA repair in mammals[18]. In addition to the characterization of these potential homologues, which are important for p53-mediated G1 arrest in seeds, the identification of putative homologues of proteins that are essential for releasing cell cycle arrest [including E2F, Rb, cyclins and CDKs (Refs 14,18,19)] suggests that the complete pathway of DNA damage, p53-induced G1 arrest, DNA repair and the reinitiation of the cell cycle might be inherent in the process of seed germination.

Perspectives and future directions

Determining whether a p53-mediated G1-arrest pathway (or a downstream component of the pathway) is truly inherent in aged seeds will require investigation beyond the use of human probes to include the study of interactions between putative homologues, protein sequencing and a greater focus on the identification of genes. An important component of future analysis will be determining the cell cycle phase distribution (i.e. the relative number of cells in G1 and G2) in aged seeds and during germination. Furthermore, it might be prudent to examine the meristematic regions of the aged seed embryo, because these cells are necessary for normal seedling growth and development (and are thus where DNA damage might have the greatest effects and the need for repair should be highest).

A definitive p53 homologue has not yet been identified in plants even though the entire genome of *Arabidopsis thaliana* has now been sequenced[20], suggesting that, if a p53 plant gene exists, it might share little sequence homology with its human counterpart. Nevertheless, given that plant seeds have such remarkable parallels to the p53-mediated G1-arrest pathway in humans and mice, we believe that the germination of aged seeds might be an especially useful model system to identify possible *p53* RNA and protein homologues, to characterize downstream gene and protein homologues involved in a possible p53-related-pathway, and/or to develop an understanding of possible novel processes underlying DNA damage, G1 arrest and DNA repair in plants.

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Database-assisted promoter analysis

Reinhard Hehl and Edgar Wingender

The analysis of regulatory sequences is greatly facilitated by database-assisted bioinformatic approaches. The TRANSFAC database contains information on transcription factors and their origins, functional properties and sequence-specific binding activities. Software tools enable us to screen the database with a given DNA sequence for interacting transcription factors. If a regulatory function is already attributed to this sequence then the database-assisted identification of binding sites for proteins or protein classes and subsequent experimental verification might establish functionally relevant sites within this sequence. The binding transcription factors and interacting factors might already be present in the database.

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Since the establishment of the central dogma of molecular biology, it has become obvious that the 'transformation' processes of information flow from DNA to RNA to protein are subject to a variety of control mechanisms. The key transmitting step that initiates the information flow, transcription, is mediated in eukaryotes by three different polymerases that transcribe distinct sets of genes. RNA polymerase II transcribes all genes that encode proteins. Many of these genes have a 'core promoter' comprising the initiator site (around +1, the position of the first transcribed nucleotide) and a TATA box at around –30. The transcription apparatus for RNA polymerase II is assembled at the core promoter and, in addition to the enzyme, this machinery includes several general transcription factors. TBP (TATA-binding protein), a subunit of transcription factor IID, is the primary DNA-recognition component for most promoters[1]. The presence of two types of TATA-binding proteins (TBPs) in plants suggests that, although transcription in eukaryotes is highly conserved, fundamental differences might exist[2,3].

The efficiency of the transcription initiation complex formation is largely influenced by the regulatory transcription factors that bind to short sequence elements that activate or repress genes in a manner that is specific for the tissue, the developmental stage or the stress conditions. These regulatory transcription factors interact with the general transcription factors directly or via coactivators[4,5]. To satisfy their specific biological requirements, plants have evolved unique regulatory mechanisms, involving completely new transcription factors that have yet to be found in animals. For example the WRKY ('worky') family of transcription factors, with probably up to 100 members in *Arabidopsis*, regulates the expression of a variety of target genes involved in the response to pathogen infection and other stresses[6]. Another plant-specific family of transcription factors is the Dof proteins, whose actions are related to biological processes unique to plants[7]. Dof proteins might contribute to the expression of genes involved in photosynthesis, in the response to stress and hormone signals, and in carbon metabolism[8]. Other examples of plant-specific factors include the homeodomain-ZIP (HD-ZIP) and GT-box-binding factors[9].

If a particular binding site occurs within a promoter, the relevant transcription factor can bind to this site, assuming that it is present in the nucleus and is in a competent state for binding. Such competent states can involve heterodimer formation or specific post-translational modifications. Transcription factors normally regulate more than one gene. The presence of a particular transcription factor binding site within

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