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Learning to breathe: developmental phase transitions in oxygen status

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Abstract (100 words)

Plants are developmentally disposed to considerable changes in oxygen availability, yet our understanding of the importance of hypoxia is almost entirely limited to stress biology. Differential patterns of the abundance of oxygen, nitric oxide ($^{\cdot}$NO) and reactive oxygen species (ROS), and redox potential occur in organs and meristems, and examples are emerging in the literature of mechanistic relationships of these to development. Here, we describe the convergence of these cues in meristematic and reproductive tissues, and discuss the evidence for regulated hypoxic niches, within which oxygen-, ROS-, $^{\cdot}$NO- and redox-dependent signalling curate developmental transitions in plants.

The nature of developmental hypoxia and metabolism

Molecular oxygen is essential for efficient production of ATP through oxidative phosphorylation, serving as the terminal electron acceptor for the mitochondrial electron transport chain. Oxygen and reduction-oxidation (redox) biochemistry pervades cellular metabolism and signalling in plants, as in all aerobic life forms. Yet even in optimal growth conditions, various higher plant tissues such as seeds, tubers and buds reside in a state of low oxygen status [1-3]. Internal oxygen concentrations in such organs range from 1 to 50 μM, compared with an air-saturated concentration of *ca*. 260 μM (*cf*. 21 kPa O_2 partial pressure at standard atmosphere and pressure), and this is reflected in the spatial patterns of metabolic control, energy status and gene expression, particularly anaerobic glycolysis [1, 4, 5]. Despite the fundamental metabolic importance of oxygen, our knowledge of oxygen as a curator of growth, differentiation and reproduction in plants is only beginning to emerge. Increasing evidence points to the presence of regulated hypoxic niches during plant development.

Until recently, oxygen signalling in plants was defined by the consequences of oxygen metabolism, such as changes in energy status, production of reactive oxygen and nitrogen species (ROS, RNS), or the accompanying dynamics of the redox network. By contrast, the basic mammalian hypoxia (low-oxygen) signalling and transduction pathways were defined over 20 years ago [6]. It is now widely accepted that local tissue hypoxia plays a central role in mammalian embryogenesis [7] and constitutes a key regulatory feature of adult stem cell niches [8]. The prevailing model applied to mammalian tissues and stem cells is that low oxygen provides a protective environment, conducive to quiescence, low ROS, and a relatively reduced redox state, all of which promote genome stability [9]. Regulated ROS synthesis in mammalian stem cells is central to the transition to proliferation and differentiation.

Parallel research programs in 2011 provided a step change in our understanding of oxygen signalling in plants, defining an oxygen-dependent N-end rule of proteolysis (discussed further below) [10, 11; see **Box 1**]. Nevertheless, research on N-end rule signalling in plants to date has been largely undertaken in the context of stress, particularly waterlogging and flooding [12, 13]. Thus the current state of the art of developmental oxygen signalling in plants is constrained by the ability to relate stress signalling via the N-end rule to the developmental understanding via redox and energy signalling (see Outstanding Questions). We discuss the roles of hypoxia in plant development and the nexus between oxygen, ROS, nitric oxide ($^{\cdot}$NO) and redox cues. We consider the differential patterns of these cues within organs and meristems, and the evidence suggesting that hypoxic niches are central to meristem function and differentiation in plants. In this context we highlight particular examples from the recent literature on seeds, seedlings and anthers that illustrate functional roles for oxygen status in developmental transitions, in partnership with ROS, RNS and redox status.

Gradients in oxygen, ROS, $^{\cdot}$NO and redox potential in organs and meristems

During evolution, the formation of niches and gradients in oxygen and redox status were important forces shaping multicellular life [14]. Cell identity within multicellular organisms became a critical factor in determining sensitivity to cellular cues including ROS and RNS such as $^{\cdot}$NO. The presence of pockets of cells with a low oxygen status is a prominent feature of many developing, reproductive and quiescent plant tissues (**Fig. 1**). These areas can form when oxygen diffusion fails to keep pace with aerobic respiration or when the oxygen supply is occluded by cell wall modifications, such as the deposition of callose. Within hypoxic

niches, ROS appear to function alongside $^{\cdot}$NO, phytoglobins and plant hormones to regulate developmental events such as growth, flowering and wood formation [15].

Hypoxia may be defined as a condition in which the cellular availability of oxygen is insufficient to support oxidative phosphorylation at full capacity. Glycolytic activity is increased to supply ATP in cells experiencing low oxygen availability and fermentation is induced to recycle pyridine nucleotides, in a response known as the Pasteur Effect. Hypoxia is characterised by specific transcriptional programs that are induced and maintained in response to perception of reduced oxygen availability [12, 13]. Oxygen-limited metabolism triggers the expression of specific set of hypoxia-related genes, such as those encoding sucrose synthase and alcohol dehydrogenase, and leads to remobilisation of carbohydrates to meet the increased glycolytic demand. These conserved transcriptional and metabolic responses are seen across life forms [16]. Survival and release from hypoxia is developmentally programmed to enable effective phase transition from quiescence to active metabolism. By contrast, survival through stress-induced hypoxia thereafter is much less certain. For example, an auxin-induced oxidative state defines the root stem cell niche without risk of programmed cell death [17], while hypoxia resulting from abiotic stress sees a persistent increase in ROS production that is frequently associated with impaired cell function and death [18]. The parallel with mammalian stem cells is tempting to consider [19], where glycolysis predominates and ROS homeostasis defines the balance of quiescence, proliferation and differentiation. Mitochondria in mammalian stem cells appear to fulfil different roles in maintaining cell integrity [20]. It is interesting to consider how such findings may translate to plant development (see Outstanding Questions).

The patterns shown in **Box 2**, particularly tissue oxygen status, may be organ- and species-specific. In the root, oxygen profiles may be influenced by the cortical gas space, surface area to volume ratio, depth below the soil surface and experimental system, such as embedding within versus above agar, and the presence of light. The presence of surface water films and root hairs will likely reduce radial oxygen diffusion into the root, reinforcing the polar oxygen gradient. Species differences will also be significant [21]; for example, maize roots have significant amounts of cortical gas space, whereas pea and *Arabidopsis* roots have little. Nevertheless, current data point to a convergence of polar and radial oxygen gradients to a hypoxic condition in the cells of quiescent centre (QC) and stem cells of roots. Mugnai *et al*. [22] demonstrated considerable induction of alcohol dehydrogenase and pyruvate decarboxylase activities in whole *Arabidopsis* roots only when the meristem was exposed to hypoxia, and that respiratory demand was greatest at the proximal region of the meristem. It should be noted however, that there is no obvious signature of hypoxia in the transcripts enriched in the QC of *Arabidopsis* roots, with exception that one of the hypoxia-inducible Group VII ethylene response factors (ERFVIIs), discussed below was enriched in the QC [23]. Patterns in ROS and $^{\cdot}$NO in the root apical meristem appear to be highly specific to developmental state, as is also the case in a typical seed (**Fig. 2**). The known functions of ROS and $^{\cdot}$NO in roots and seeds are discussed in subsequent sections. Meanwhile, the state in the shoot apical meristem is less clear, confounded by technical challenges identifying the meristem proper and combining this with available resolution of technologies (see Outstanding Questions) [24]. Hence, while current evidence suggest gradients in tissue oxygen status converge to a hypoxic state in the vital tissues such as the QC and stem cells of roots, more mechanistic evidence is required from other organs and in a range of conditions. Nevertheless, these features point to a potentially important role for oxygen-, ROS- and $^{\cdot}$NO-dependent signalling during plant development.

The N-end rule of proteolysis in a developmental context

Responses to hypoxia in animals are mediated by the hypoxia-inducible factor (HIf1α) transcription factor; oxygen-dependent modification of HIF1α by prolyl hydroxylases initiates its degradation via the proteasome, whilst decreased oxygen levels lead to its accumulation and a concomitant induction of the hypoxic transcriptome [6]. A functionally analogous, but qualitatively different, protein degradation-based mechanism for sensing oxygen also exists in plants, where ERFVIIs act as 'master regulators' of hypoxia responsive gene expression [13, 25]. Under normoxic conditions, ERFVIIs are degraded in an oxygen- and ·NO-dependent manner via the N-end rule pathway of targeted proteolysis (see **Box 1**), whilst a small stable subpopulation localises to the plasma membrane [10, 11, 26, 27]. Under hypoxia, ERFVIIs localise to nucleus, where newly synthesised ERFVIIs also accumulate, to activate gene expression. These nuclear ERFVIIs are then rapidly destroyed upon re-oxygenation, which quickly dampens the hypoxic transcriptional response, providing the cell with a sensitive mechanism for directly adjusting transcription relative to oxygen availability. ERFVIIs regulate the expression of over half of the 'core 49' hypoxia induced genes that are activated and preferentially translated across cell types when oxygen is depleted [16, 26, 28, 29]. These include genes associated with glycolysis and ethanol fermentation, various transcription factors, and genes coding for proteins of unknown function that likely contribute to cellular homeostasis under oxygen deficiency.

There is mounting evidence that oxygen- and ·NO-dependent ERFVII regulation by the N-end rule pathway is important for coordinating responses during developmentally-imposed hypoxia and the transition to oxygen-replete conditions, in addition to stress. The examples of seed dormancy, germination and photomorphogenesis are described in subsequent sections

and **Figure 2**. In addition, it has previously been shown that loss of function mutants for several enzymatic components for N-end rule pathway display aberrant phenotypes relating to leaf and shoot development and the timing of leaf senescence [30, 31]. This finding could implicate roles for oxygen and $^{\cdot}$NO in the control of development and senescence processes. However, the oxygen/$^{\cdot}$NO-dependent branch of the N-end rule pathway only provides these enzymes with a subset of their substrates, and the relevant targets need to be identified in order to establish a firm link to regulation by oxygen and/or $^{\cdot}$NO levels. It is also interesting to consider that under hypoxia, several genes are induced that attenuate ERFVII activity, providing feedback mechanisms to fine tune the response [32, 33]. This includes the plant cysteine oxidases, which are critical for oxygen-dependent ERFVII destruction (**Box 1**), and the trihelix transcription factor HYPOXIA RESPONSIVE ATTENUATOR 1 (HRA1), which negatively regulates the activity of the ERFVII RAP2.12 through direct protein-protein binding [33]. Giuntoli *et al*. [33] demonstrated through histochemical staining that HRA1 is expressed in young growing leaves of the rosette and meristematic regions under non-stressed conditions, and the authors speculated that it may play a role in counterbalancing the extent of the hypoxic transcriptional response in developmental contexts where oxygen availability is reduced. Further analyses are required to confirm such a role for HRA1.

Sources and roles for reactive oxygen and nitrogen species in development

Cellular energy metabolism employs reductive anabolic reactions to store energy, and oxidative catabolic reactions to release energy. While oxygenic photosynthesis and respiration operate four-electron exchange mechanisms between oxygen and water, without release of partially reduced intermediates, many enzymes catalyse partial oxygen reduction producing superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Consequently, ROS levels are intrinsically linked to oxygen availability, and therefore constitute important components of

oxygen and hypoxia signalling. These and other redox signals have become integrated in every aspect of plant biology and are crucial regulators of pre- and post-translational gene expression, cell division and expansion, and cell defence, morphology, and fate [34]. Within this context, cellular antioxidants not only determine the extent of ROS accumulation in the different compartments of the plant cell but they also can act as signal transmitters. The intracellular compartments that are major ROS producers show substantial plasticity in organelle shape, with extensions such as stromules, peroxules and matrixules playing crucial roles in inter-organelle communication [35]. For example, ROS accumulation triggers direct stromule-nucleus communication that facilitates direct transfer of oxidants and proteins [36]. The sensitivity of different tissues and organs to ROS accumulation, and to oxidation, is regulated to a large extent by the abundance and intracellular distribution of low molecular weight antioxidants such as glutathione and ascorbate [34]. Antioxidant enzymes and redox-sensitive proteins also calibrate tissue sensitivity to redox signalling appropriate to the conditions.

The major sites of intracellular ROS production in plants are the chloroplasts, mitochondria and peroxisomes [37]. Direct electron transfer to oxygen occurs during photosynthesis and respiration leading to $O_2^{\cdot -}$ production [38]. $O_2^{\cdot -}$ is converted to H_2O_2 by superoxide dismutase (SOD) [39]. In peroxisomes, ROS are produced by a number of different oxidases including glycolate oxidase and xanthine oxidase and through β-oxidation of fatty acids. In addition, ROS are produced in the apoplast by different enzymes including: the plasma membrane-bound NADPH-oxidases (RBOH); class III secretory plant peroxidases; amine oxidases such as polyamines oxidases (PAO); germin-like oxalate oxidases, and; quinone reductases [40]. Of these, RBOH-mediated ROS production has been linked to signal transduction pathways that mediate plant cell growth and development [41]. For example, tip growth in pollen tubes

and root hairs is regulated by ROS-mediated cell wall loosening and stiffening [42]. PAO has also been associated with pollen tube extension by promoting Ca^{2+} influx followed by RBOH activation [42, 43]. Apoplastic H_2O_2 also regulates cell division and expansion during leaf development, where a MYB-like transcription factor KUA1 represses peroxidase expression during cell expansion [44].

ROS production and redox homeostasis are considered to play key roles in root [45] and shoot [24] meristem development. A mechanistic relationship between ROS localisation and cell identity in the root was determined by Tsukagoshi *et al*. (see **Box 2**) [46]. There, the UPBEAT1 transcription factor, expressed in the extension and differentiation zones, represses peroxidase activity, moderating the balance of H_2O_2 and $O_2^{\cdot-}$ in the differentiation and meristem zones, independent of the auxin gradient [46]. $^{\cdot}NO$ also appears to be required to maintain root meristem cell identity, as dependent on the auxin gradient [47], and two recent studies pointed to the importance of mitochondrial ROS homeostasis in cell-specific signalling, determining the identity of the root distal stem cells [48], and the maintenance of the shoot apical meristem [49]. These conclusions are in line with the general consensus that redox regulation is involved in multiple processes related to self-renewal and differentiation. Nevertheless, caution must be used when interpreting some of these approaches [50]. There also remains debate on the oxidation state and ROS synthesis in the cells of the root QC. In maize, current data show the QC cells are maintained in a highly oxidised state, and where oxidation of the core redox buffers ascorbate and glutathione is functionally related to the polar auxin gradient, interacting with hormonal and transcriptional controls [17, 51-53]. More recently in *Arabidopsis*, the redox potential in the medial plane of the root was shown to be most reduced in the area of QC and stem cells [54]. These data are in line with the enrichment of genes encoding enzymes leading to or requiring glutathione in the QC of

Arabidopsis [23]. There is a need to resolve the basis of these differences, whether genetic, physiological or due to the experimental system. Moreover, no signal study to date has investigated each of the oxygen-dependent cues in one system.

Mitochondrial plasticity in relation to oxygen availability

It is implicit that considerable adjustment of mitochondrial metabolism is required to ensure that energy metabolism is sustained under hypoxia. Respiratory electron transport generates ROS as an inevitable consequence of oxidative phosphorylation, $^{\cdot}$NO through participation in Hb-$^{\cdot}$NO cyclic respiration (discussed further below), and regenerates pyridine nucleotides to enable continued cytosolic and organelle functions. The importance of mitochondrial ROS homeostasis in the identity and fate of the root- [48] and shoot- apical meristem [49] was introduced above. Accumulating evidence suggests that the availability of oxygen and the requirements of oxidative phosphorylation can alter the composition, numbers and structure of mitochondria. Mitochondrial biogenesis and interdependence with chloroplast during seed germination is illustrated in **Figure 2**. Rice seedlings germinated under anaerobic conditions initially develop a normal mitochondrial structure, but later the mitochondria showed degraded cristae with vesicles [55]. Even within 48h of anoxia, mitochondria had reduced protein levels of tricarboxylic acid cycle components and cytochrome-containing complexes of the respiratory chain, resulting in repressed respiratory functionality [56]. In other tissues, oxygen deprivation can lead to the generation of giant mitochondria, as in *Arabidopsis* leaves [57] and tobacco cells [58]. However, the response of mitochondrial structure to hypoxia may depend on whether cells are in a quiescent or metabolically active state, or whether the experimental context is stress-acclimation or developmental (see Outstanding Questions).

The glutathione redox potentials of root mitochondria have been estimated using ro-GFP. Such measurements showed that root mitochondria were substantially more reduced (*ca*. -360 mV) than the surrounding cytosol (*ca*. -320 mV) of the same tissues [59, 60]. Moreover, mitochondria were found to be much more able to buffer changes in redox state than the cytosol [59]. This is consistent with the observation that mitochondria accumulate more glutathione than any other compartment of plant cells [61]. In contrast to the other cell types in the maize root, the QC cells were found to have little or no glutathione, as discussed above [53]. The mitochondria within this oxidising environment look structurally similar to those in the cells surrounding the QC [62]. However, compared to mitochondria in the adjacent, rapidly dividing cells, the QC mitochondria have much lower tricarboxylic acid cycle enzyme activities, with a much reduced capacity to generate ATP and NADH [62]. A similar situation has been described for potato tuber mitochondria, which reside in very low oxygen environments [63]. Nevertheless, it is not known whether quiescent cells of meristems, including shoot meristems, are specifically hypoxic, and hence whether these features are a consequence of low oxygen or low metabolic requirements for quiescence (see Outstanding Questions).

Phytoglobins and the haemoglobin-nitric oxide cycle under hypoxia

Phytoglobins are also important in the survival in hypoxic conditions that arise during development, and are central to cell fate decisions during embryogenesis, as well as during seed germination, xylem formation, and lateral and adventitious root development [64, 65]. *HAEMOGLOBIN* (*Hb)1* is a core hypoxia-responsive gene, which is induced by hypoxia alongside $^{\cdot}$NO accumulation [66]. Heterologous expression of *Vitreoscilla Hb* in several plant species led to improved energy status and enhanced growth [67]. The overexpression of *Hb1*

in *Arabidopsis* led to enhanced shoot development [68], and to earlier bolting [69], while silencing of *Hb1* and *Hb2* proved to be lethal [70].

The Hb-·NO cycle has been suggested to relieve mitochondrial transport chain inhibition by ·NO under hypoxia [71]. In the process of Hb-·NO cyclic respiration or nitrate-·NO respiration, nitrate is first reduced to nitrite by nitrate reductase. Nitrite is then transported from the cytosol to the mitochondria, where it is reduced to ·NO, via the mitochondrial electron transport chain. ·NO then diffuses from the mitochondrial matrix to the cytosol, where it is oxidised by Hb [66]. To complete the cycle MetHb is regenerated by a MetHb reductase [72]. In this way, ·NO accumulation in developmentally hypoxic tissues may be controlled by the non-symbiotic Hbs in an NADH-coupled reaction, while facilitating respiration and ATP production.

Hypoxia and re-oxygenation during plant development

Regulated hypoxia and re-oxygenation have recently been shown to play a critical role in non-stress-associated plant development. Here we highlight particular examples as case studies: seed germination and bud burst, photomorphogenesis and anther development, to illustrate roles for oxygen availability, and related ROS/ RNS levels, in the control of these processes.

Seed germination and bud burst

Seeds and latent buds are spatially complex organs, which transit from quiescence to extension and synthetic growth over a period of hours to days [2, 73]. Prior to germination or bud burst, the organ is hypoxic, <50 μM [O_2], heterotrophic and desiccated, often <0.3 g H_2O.g DW^{-1} (*cf.* up to 260 μM [O_2], 3-12 g H_2O.g DW^{-1}) [74]. Imbibition sees a rapid relief

from desiccation, and gradual relaxation of hypoxia, accompanied by spatiotemporal bursts of ROS and $^{\cdot}NO$ (**Fig. 2**). The biogenesis of mitochondria and chloroplast appears to be partially interdependent, with chloroplast metabolism being initially photoheterotrophic, relying on mitochondria to re-oxidise pyridine nucleotides and to sustain the cytosolic and plastid adenylate pools (described in **Fig. 2**) [4]. In the seed, hydration [75] and local oxidation [76, 77] occurs initially within the embryonic axis and peripheral tissues, with synthesis of ROS, principally $O_2^{\cdot-}$ and H_2O_2, driven by apoplastic peroxidases and NADPH oxidases. During imbibition, ROS appear to function in cell wall elasticity ($O_2^{\cdot-}$, $^{\cdot}OH$) and cross-linking (H_2O_2), to enable extension growth of the radicle. Genetic analysis of the NADPH/ NADP-thioredoxin reductase/ thioredoxin system also indicates a role for redox regulation of hydrolytic proteins during imbibition and radicle extension, a feature that has been exploited in preventing precocious germination [78]. The rise in internal oxygen is augmented by the restriction of oxidative phosphorylation by partially-nitrite-dependent $^{\cdot}NO$ synthesis, which may inhibit complex IV, enabling photosynthetic oxygen to accumulate [79]. $^{\cdot}NO$ synthesis is prominent in the peripheral tissues of the seed during imbibition [80], associated with an increase in *S*-nitrosothiols in the embryo [81]. In the bud, hydration appears to be facilitated by $O_2^{\cdot-}$-mediated development of protoxylem [2], and degradation of callose occlusions of the plasmodesmata [81]. However, no spatial resolution of RNS in the bursting bud is yet known.

Mechanistic relationships between oxygen- and RNS-dependent signalling and germination have recently emerged, notably the role of $^{\cdot}NO$ in attenuating abscisic acid (ABA)-dependent repression of germination. The ERFVII transcription factors are positive regulators of the *ABA INSENSITIVE 5* (*ABI5*) transcription factor, which acts downstream of ABA to repress germination [27]. The enhanced degradation of ERFVIIs during germination, as $^{\cdot}NO$ and

oxygen levels rise, attenuates the action of ABI5. Oxygen and ·NO appear both to be required for the destabilisation of the ERFVIIs by the N-end rule pathway [27], while ·NO/ RNS appear to function to further attenuate ABI5 signalling without direct dependence on oxygen via two further mechanisms. Firstly, ·NO promotes the degradation of ABI5 during germination by the *S*-nitrosylation of cysteine-153 [82]. Secondly, tyrosine nitration acts upstream of ABI5 by inactivating the PYR/PYL/RCAR receptor [83], leading to the dephosphorylation of the SUCROSE NONFERMENTING-RELATED KINASE 2 (SnRK2), and thus preventing the activity of this positive regulator of ABI5. Hence by several modes, RNS-dependent modifications enable germination by attenuating ABA-dependent repression. At present, the only direct link to oxygen signalling is via the proteolysis of ERFVIIs, however further dissection of these interactions are required.

Anther development

Recent studies have shown that reproductive cell differentiation from pluripotent precursor cells is controlled by hypoxia in developing maize anthers. In contrast to animals, which sequester germ line cells during embryogenesis, the somatic-to-germinal switch in plants is regulated post-embryonically in response to endogenous and environmental cues. Maize anthers develop in tightly encased tassels that undergo short-term transient hypoxia (*ca*. 1.2-1.4 kPa pO_2, 15-30 μM [O_2]) due to diffusion limitation and constraint by non-photosynthetic, rapidly growing leaves with a high metabolic demand [84]. Reduced oxygen availability in the anther lobe triggers the activity of the glutaredoxin MALE STERILE CONVERTED ANTHER 1 (MSCA1) in the central multipotent somatic cells, specifying them as germ initial (archesporial) cells that then enlarge and secrete MULTIPLE ARCHESPORIAL CELLS 1 (MAC1) protein, which represses proliferation and directs the development of surrounding supportive tissues [84, 85]. Analysis of microdissected

archesporial cells revealed gene expression patterns biased towards reduced ROS accumulation, enhanced reductive capacity, and alternative metabolism, indicating that these cells bypass the electron transport chain to limit potentially harmful ROS production and accommodate hypoxia [86]. Intriguingly, artificial manipulation of redox status in developing anthers (using hypoxia or hyperoxia treatments) revealed that every cell has the capacity to develop as a germ cell, suggesting that the natural hypoxic gradient that forms during the early development of this tissue is required for normal spatiotemporal reproductive cell differentiation [84]. Genetic studies in other species also highlight ROS management as an important component of fertility in plants. For example, the *Arabidopsis* glutaredoxin ROXY regulates floral organ and germline development [87], whilst mutants in the rice glutaredoxin MICROSPORELESS1 are male sterile similarly to maize *mcsa1* mutants [88]. Thus, redox status and hypoxia may play a conserved role in the regulation of meiotic fate acquisition.

Photomorphogenesis

Following germination, newly emerged seedlings growing in the dark adopt a skotomorphogenic developmental program, characterised by a rapidly elongating hypocotyl, yellow folded cotyledons and an apical hook [89]. Once exposed to light, photomorphogenesis is induced, where cotyledons expand, hypocotyl growth ceases and mature chloroplasts develop. This growth transition coincides with the initiation of photosynthesis and a congruent production of ROS, which is potentially damaging to the plant. Long-term growth in the dark exacerbates photo-oxidative damage upon light perception, due to accumulation of the chlorophyll precursor protochlorophyllide [90]. Recent work has shown that environmental hypoxia (which frequently occurs in soils) acts as a positive developmental cue for facilitating seedling survival during de-etiolation, particularly following extended darkness [91]. Under low oxygen conditions, stable ERFVIIs

repress several photomorphogenic traits, restrict chlorophyll biosynthesis and limit protochlorophyllide abundance, which increases the capacity for seedling survival through limiting ROS production upon exposure to light. Accordingly, it was shown that *Arabidopsis* seedlings grown under hypoxia survived much longer periods of skotomorphogenesis than those grown in normoxia [91]. Following emergence, seedlings are typically exposed to atmospheric oxygen levels, and endogenous $^{\cdot}$NO production also increases [92], which collectively would induce ERFVII destabilisation to relieve their repressive function and facilitate the light-induced transition to photomorphogenesis. Thus, hypoxia facilitates seedling survival by coordinating photomorphogenesis.

Concluding statements

Recent insights from root apical meristems, seeds, seedlings and anthers point to a mechanistic function for hypoxic niches and re-oxygenation events during plant development, where the roles of ROS, $^{\cdot}$NO and redox-signalling become paramount in determining the balance of quiescence, proliferation and differentiation. Our summary of these concepts is presented in the diagram in **Figure 1B**. Importantly, it is clear that these cues rarely act in isolation. The combination of more deliberate attention with the use of more sensitive cellular technologies will improve our understanding of how these cues cooperate to effect developmental programming, and at the interface with environmental perception.

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Text boxes and Figure legends

Figure 1. Demonstrated and hypothetical gradients in tissue oxygen and redox status in plant developmental phase transitions. **A.** Axillary bud burst and anther meiosis are developmentally augmented by oxygenation and hypoxia respectively [2, 84]. Cell identity and fate, and organ polarity of the root apical meristem (RAM) are governed by differential patterns in ROS, ·NO and gradients in oxygen status and redox potential (see **Box 2**). We hypothesise these cues are functionally relevant in the shoot apical meristem (SAM). Climacteric-type fruit ripen with a rapid burst of respiration, resulting in hypoxia-driven transcription [93]. Germination and the skoto-photomorphogenic transition are detailed in **Figure 2**. **B.** Accumulating evidence has highlighted the key functions of ROS and ·NO in defining the balance of plant cell proliferation and differentiation. In addition, recent evidence suggests hypoxia plays an important role in the maintenance of quiescence in plants, as it does in animals, by constraining oxidative metabolism and stabilising transcription factors [9, 12, 25, 74]. In the accompanying model we illustrate how tissue oxygen status might influence the balance between quiescence, proliferation and differentiation via regulated stabilisation/ destabilisation of N-end rule transcription factors, and influencing the cellular redox poise, and specifically through the differential generation of ROS species and ·NO. We consider that mitochondria and plasma membrane-bound NADPH-oxidases (RBOH), together with peroxidases (POX) are particularly important in regulating specific ROS expression and the cellular redox poise in this context. Rights for photographic images were purchased from www.shutterstock.com.

Figure 2. Typical spatiotemporal profiles of internal oxygen [O_2], ROS and ·NO during seed imbibition and germination, and biogenesis of plastids and mitochondria during

imbibition through to de-etiolation. Quiescent seeds are hypoxic, and plastids and mitochondria are prototypical, with poorly developed inner membranes [94-96]. During imbibition, hypoxia is gradually relieved, while ROS play a role in radicle extension, $^{\cdot}$NO plays a role in activating hydrolytic activities in the endosperm. Plastids differentiate to etioplast, characterised by a prolamellar body (PLB) and prothylakoid membranes [Pth; 96]. Mitochondria rapidly develop inner membranes (IMM) and cristae, protein import capacity and subsequently a functional electron transport chain [ETC; 94, 95]. Upon exposure to light, plastids have primordial thylakoid membranes (Th) and grana (Gr), and functional photosynthetic apparatus, which is co-dependent on mitochondria [photoheterotrophic; 4]. Here, chloroplast provide oxygen and reducing power (NAD(P)H), which augments oxidative phosphorylation in the mitochondria via external NAD(P)H dehydrogenase (Ext NDH), ETC and ATP synthase (ATPase), which enables recycling of NAD(P)H and P_i for continued photosynthesis [4, 97]. Nitrate-depended $^{\cdot}$NO serves to partially inhibit oxidative phosphorylation, augmented the increase in internal [O_2] [4, 79]. The progressive switch from Ext NDH to the tricarboxylic acid cycle (TCA) is hypothetical. Absence of arrows between fully functional chloroplasts and mitochondria does not imply absence of relationships.

Box 1. The N-end rule pathway

The eukaryotic N-end rule pathway of proteolysis is a highly conserved branch of the ubiquitin proteasome system that targets proteins for degradation based on their N-terminus [27, 98]. Substrates of the pathway undergo a number of regulated N-terminal processing events to produce an ‘N-degron’ prior to ubiquitination and destruction. There are two known divisions of the pathway: the Ac/N-end rule targets proteins that have been N-terminally acetylated, whilst the Arg/N-end rule degrades proteins bearing specific unmodified (but post-transcriptionally exposed) hydrophobic or basic N-terminal amino acids [27, 99].

Primary residues of the Arg/N-end rule are directly recognised by specific E3 ligases (Arg/N-recognins), whereas secondary and tertiary residues (including Nt-Cys) must first undergo chemical modification followed by N-terminal arginylation before they are turned over. The Cysteine-branch of the Arg/N-end rule regulates oxygen and $^{\cdot}$NO perception and transduction, through controlling the stability of proteins initiating with the residues Met-Cys [10, 11, 27, 100]. In mammals this includes several RGS proteins, which monitor oxygen availability to coordinate angiogenesis [100]. In plants, the group VII ERF transcription factors (ERFVIIs) – of which there are five in *Arabidopsis* - have a Met-Cys- N-terminus, embedded in a longer consensus sequence [13]. ERFVIIs are processed via the N-end rule pathway as such (**Fig. I**):

(i) Cytoplasmic methionine amino peptidases (MetAPs) cleave Nt-Met.

(ii) Exposed tertiary Nt-Cys is oxidised to Cys-sulfenic or Cys-sulfonic acid in an oxygen- and NO-dependent manner. In plants this oxidation is catalysed by plant cysteine oxidases (PCOs), which use oxygen as a co-substrate [32]; functionally homologous enzymes in the animal Arg/N-end rule are yet to be identified.

(iii) Oxidised Nt-Cys functions as a secondary residue of the pathway and likely targeted by Arginyl t-RNA transferase (ATE), which conjugates an arginine molecule to produce Nt-Arg-Cys.

(iv) Nt-Arg, a primary destabilising residue, can be recognised by the Arg/N-recognin PROTEOLYSIS6 (PRT6), which leads to degradation by the 26S proteasome.

It is through this regulated, condition-dependent control of their stability that the ERFVIIs function as homeostatic sensors of oxygen and $^{\cdot}$NO availability [10, 11, 27].

(FIGURE IN BOX)

Figure I. Schematic diagram of the major steps in the oxygen/ ·NO branch of the N-end rule pathway of targeted proteolyis, as described in accompanying text.

Box 2. Differential localisation of ROS and ·NO in root tissues with respect to oxygen and redox status.

It is worthwhile considering the tissue patterning of the various oxygen-related cues in meristematic tissues. The root apical meristem is a convenient developmental model, for its relative polar and radial simplicity [51]. Even more-so in the context of oxygen and ROS metabolism, due to the lack of light. Oxgyen enters the root by inward radial diffusion from the rhizosphere or cotical gas space diffusion from shoot system [101]. Armstrong and colleagues [101] measured and modelled polar and radial patterns of oxygen concentration in maize roots. In **Figure II**, two stylised profiles are shown, representing the modelled (upper) and measured (lower) transect through the proximal meristem [101]. Assuming these are reflective of the range, we see the steep radial gradient towards a minimum of <10% air-saturated [O_2], *i.e.* <25 μM [O_2] or 2 kPa O_2 partial pressure in the vascular tissue. Although not shown here, data from Armstrong *et al.*, [101] clearly demonstrate a strong polar gradient also, whereby more proximal tissues are more oxygenated.

Studies of ROS and ·NO localisation have demonstrated rather discrete and differential patterns. Hydrogen peroxide (H_2O_2) is concentrated towards the extension and differentiation zones, particularly the epidermis and vascular tissues, as well as the columella and lateral root cap [46, 102]. By contrast, superoxide ($O_2^{\cdot-}$) is predominantly localised to the vascular and dermal tissues of the proximal meristem and elongation zone [46, 102]. Although not shown here, both H_2O_2 and $O_2^{\cdot-}$ were previously found to be more concentrated in the quiescent centre cells (QC) than the proximal meristem [53]. Meanwhile, ·NO localisation is concentrated towards the cortical and endodermal stem cells [47]. A recent study of redox

729 status demonstrated a relatively reduced cellular environment in the proximal meristem and
730 columella, including the QC cells [54]. Although only polar data were presented [54], authors
731 indicated there was no evidence of a radial gradient.
732 We consider variables affecting these findings, such as genetic and experimental conditions
733 in the main text. To date, no single study has examined these data in one system.
734 Nevertheless, taken together these data illustrate the importance of both polar and radial
735 gradients in oxygen status and of tissue-specific localisation of ROS and $^{\cdot}$NO, and potentially
736 redox in the root apical meristem.
737
738 (FIGURE IN BOX)
739 **Figure II.** Differential tissue distributions of oxygen, ROS, $^{\cdot}$NO and redox potential in a
740 stylised root. Two alternative profiles of an oxygen transect through the proximal meristem
741 (dashed line) are presented; the upper (yellow) line is the modelled profile, the lower (orange)
742 is the measured profile, both interpreted from [101]. The redox profile through a longitudinal
743 plane is interpreted from [54], H_2O_2 (purple) and $O_2^{\cdot-}$ (blue) localisation from [46, 102] and
744 $^{\cdot}$NO localisation (green) from [47].
745