



Thought experiment: a hidden signal and an etioreceptor

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Abstract

A pioneer in the study of the effect of light on plant development, Wilhelm Pfeffer (1845–1920), outlined both modes of plant development in darkness and in light—today known as skoto- and photo- morphogenesis, respectively. By exploring the current understanding of these pathways in *Arabidopsis*, we speculate on the possible mechanisms of cell and organ elongation in darkness. We present a thought experiment, which highlights the need for a yet unknown (hidden) external signal, we call aleph (\aleph), and its possible sensor “etioreceptor”. Here we propose for the first time that this system is required for growth and developmental patterning to continue in localized spacetime under minimal growth environments, particularly in the absence of light. If true, this mechanism may have played a fundamental role in organism’s survival during Darwinian (adaptive) evolution (“survival of the fittest”). We present our working model whereby the hidden signal acting through its etioreceptor, increases auxin biosynthesis to facilitate organ expansion, and suggest a function for this system in the auxin biosynthesis pathway.

Keywords Auxin · Darkness · Etiolation · Light · Photomorphogenesis · Spacetime

Abbreviations

ANT	Anthranilate
BBX	B-Box
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
HY5	ELONGATED HYPOCOTYL5
FR	Far-red light
IAM	Indole-3-acetamide
IAN	Indole-3-acetonitrile
IAOx	Indole-3 acetaldoxime
IGP	Indole-3-glycerol phosphate
IGs	Indole glucosinolates
IPyA	Indole-3-pyruvic acid
PAR	Photosynthetically active radiation
R	Red light
SPA	SUPPRESSOR OF PHYA-105
TAM	Tryptamine
Trp	Tryptophan
YUC	YUCCA

Arabidopsis has served as a model plant, helping us unravel intricate mechanisms that regulate plant growth and development. Genomics approaches in *Arabidopsis* led to the identification of the very first single plant gene-based technology, BBX32 (B-Box32), transferred to soybean for improving its field performance and increased grain yield (Khanna et al. 2009; Holtan et al. 2011; Preuss et al. 2012). BBX32 was first identified as a putative negative regulator of photomorphogenesis in a single locus loss-of-function screen with *Arabidopsis* mutants in genes implicated in light signaling (Khanna et al. 2006). It was determined that BBX32 acts antagonistically to ELONGATED HYPOCOTYL5 (HY5) (Holtan et al. 2011). Recent studies have shown that HY5 mediated transcriptional regulation is dependent upon other members of the BBX family, BBX20, BBX21 and BBX22 (Bursch et al. 2020). Accumulation of HY5 protein is directly linked to photomorphogenesis (Osterlund et al. 2000). In darkness, the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1)–SUPPRESSOR OF PHYA-105 (SPA) complex negatively targets positive photomorphogenesis regulators, like HY5 (Galvao and Fankhauser 2015; Podolec and Ulm 2018). Consequently, the *cop1* mutant accumulates positive photomorphogenesis regulators in darkness, it does not need light to trigger photomorphogenesis related changes (Deng et al. 1992). Photomorphogenesis can occur

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artificially when both the signal (light) and the pathway inhibitor (COP1) are absent. Evolutionarily, the presence of this system suggests that development in darkness is primed with anticipation for light to initiate a markedly different growth program. This coordination between dark and light programs continues throughout plant maturity. The success of *Arabidopsis* BBX32 protein in soybean was attributed to coordination of light input to the internal circadian clocks. Ectopic accumulation of BBX32 delayed the expression of morning clock genes resulting in the extension of R3 to R5 developmental stages in soybean with prolonged pod and seed development contributing to increased grain yield (Preuss et al. 2012).

The eminent German botanist, Wilhelm Pfeffer (1845–1920), who died one century ago, was the first to study stem elongation in darkness versus light. In his monumental textbook “Pflanzenphysiologie” (Physiology of Plants), published in two volumes (Pfeffer 1897/1904), Pfeffer depicted two vegetatively grown potato (*Solanum tuberosum*) plants grown either in darkness or in light. The internodes, marked by numbers in Pfeffer’s drawings, displayed large differences with respect to length and diameter/shape, so that he coined the terms “etiolement–development in darkness- versus plant growth in the light (photomorphosis)”. Figure 1a, b shows Pfeffers original “Potato” experiment, supplemented by cross sections of two leaves, taken from *Fagus sylvatica* plants grown either in full sunlight or in deep shade. These pioneering studies document the origin of a research agenda that is today known as “light and plant development,” or the study of photomorphogenesis. Winslow Briggs (1928– 2019) was one of the most prominent and productive researchers “in the wake of Pfeffer”; he not only discovered the blue-light-absorbing phototropins, but also many other features of the “photomorphogenesis-syndrome” outlined for the first time in detail by Pfeffer (1897/1904). Here, we analyze the etiolation response from a hypothetical perspective and explore whether a hidden signal is involved in orchestrating growth and development in the absence of light.

Environmental conditions such as daily dark/light cycles continuously influence plant growth and patterning in the three-dimensions of space. We are accustomed to measuring phenotypic changes at the organ level over periods of time, but the underlying mechanisms occur sequentially at the subcellular level through moments of time in response to the incumbent environmental cues. Other factors shaping plant development in localized space are gravity, temperature, oxygen, carbon dioxide, water, and nutrients. Figure 2 represents this view in Minkowski spacetime (Minkowski 1908), where each frame of three-dimensional space (x, y, z) moves forward in the fourth dimension of time (t). Biological processes occur at each moment (frame of space) and are continuously influenced

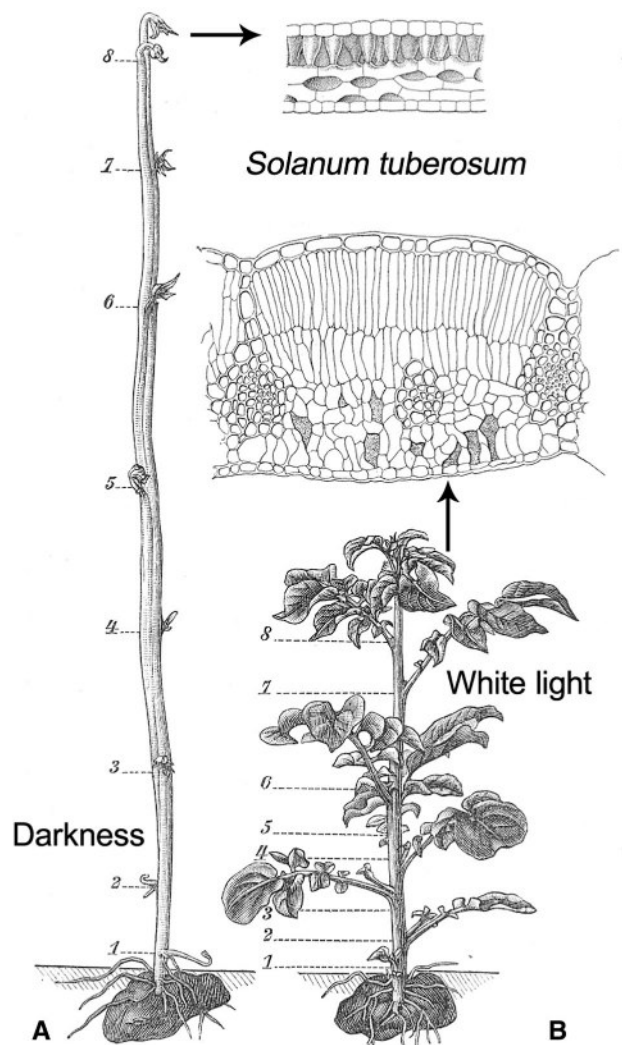


Fig. 1 The effect of light on stem elongation and leaf development. Two Potato (*Solanum tuberosum*) plants, raised from two genetically very similar tubers. In addition, the morphogenetic effects of light on leaf development in *Fagus sylvatica* is depicted (cross-sections shown are of *F. sylvatica*-leaves that were either grown in the dark or in white light, respectively, corresponding to similar leaves shown on potato plants). The cross-sections are original reproductions taken from the classical study of plant morphogenesis. Adapted from Pfeffer (1897/1904)

by their local environmental conditions in spacetime continuum. In this model, seedling growth is viewed in the progression of spacetime. Each moment in time will be influenced by its own environmental conditions to shape the next moment of growth and patterning. We can tell merely by looking at seedlings which ones have experienced growth in darkness and which ones grew in light (Fig. 2). A flash of red light with sufficient intensity at any moment of time will change the developmental direction of etiolated seedlings. Light signaling mechanisms are well understood and we can predict which *Arabidopsis* genes will be activated in the subsequent frames. Similarly,

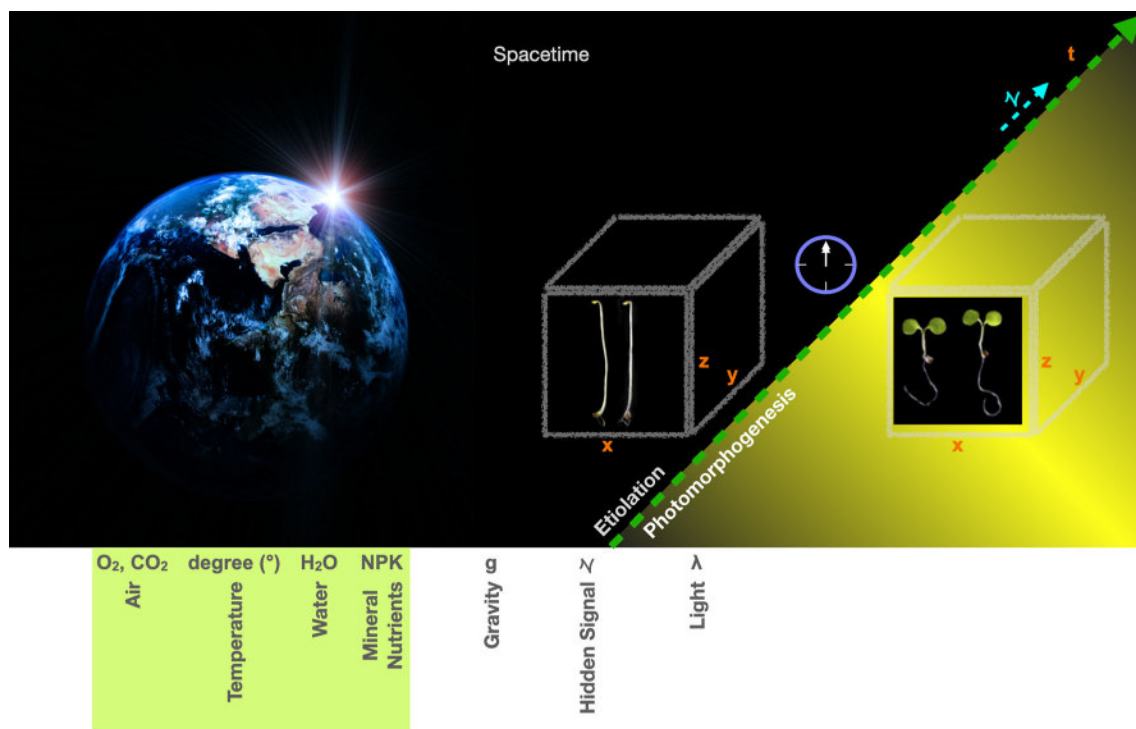


Fig. 2 Representation of plant development in Minkowski spacetime. Earth takes approximately 24 h (1 day) to complete its rotation around the sun from west to east, therefore the sun appears to rise in the east and set in the west, causing diurnal dark/light cycles. Daylight includes direct and indirect radiation from the sun, and it can vary in quantity and quality from dawn to dusk (clock), with a peak at noon from direct sunlight (yellow gradient, lower right corner). Other conditions that support life on earth (particularly plant life) are represented as air (O_2 , CO_2), temperature ($^{\circ}C$), water (H_2O), mineral nutrients (NPK) and gravity (g). Except for any major consequential effects of variation in gravity (altitude/latitude), most of the other conditions can change consequentially (highlighted in green), depending upon the plant's location. Skotomorphogenesis (growth

in darkness, also known as etiolation) and photomorphogenesis (growth in light) are two distinct plant developmental programs that shape seedling morphology within a few days of growth after germination. Insets show 4-day old *Arabidopsis* seedlings grown in continuous darkness (left) or light (right). The three-dimensional frame (x , y , z axes) represents each moment of time throughout developmental patterning, moving forward in the fourth dimension of time (t , green dashed arrow). The proposed hidden signal (λ) is shown as an external force with the possibility that it is a fundamental force underlying the emergence of spacetime (blue dashed arrow). Source, Universe sun rise over planet ID 11,589,967 © Paulpaladin/Dreamstime.com (color figure online)

seedlings growing in light will experience variations in environmental factors, such as temperature, light intensity and quality (R/FR), water and nutrient availability to reprogram their subsequent frames of development into the fourth dimension of time.

We are very familiar with known environmental factors (Table 1) that influence plant growth and development on earth. All of the known factors, except for light, have local origin on earth. Light also exerts local variation (Table 1). It is proposed here that there remains a hidden signal, named Aleph (\aleph) (Khanna and Kutschera 2020), which is not detectable with our current technologies. The need for aleph arises from the dependence of biological systems on variable environmental factors throughout their developmental path along the arrow of time. Here we suggest that a signal is needed for development to proceed in the direction of time. Aleph is postulated to be a universal signal that moves development through the moments of time

(Fig. 2). To simplify, aleph drives growth and development to its fullest potential through time with or without other environmental factors. It can be argued that the evolutionary success of species through drastic environmental pressures requires the existence of aleph to throttle through failed models until an optimized and evolved biological system is developed. In this thought experiment, the idea of aleph is aligned with, but is not dependent upon, the concept that spacetime itself is emergent (Raamsdonk 2010). It is being realized that nearly 96% of the stuff in the universe remains undetected. We are using the term aleph (\aleph) as a placeholder to describe a yet unknown universal signal with an influence on spacetime and consequently on the continuum of growth and development, even in the absence of light.

The need for this hidden signal is further evaluated with our understanding of light and phytohormone auxin

Table 1 Simplified list of proposed and known environmental factors

	Name (symbol) used	Physical nature	Constant or variable	Value or characteristic
<i>(A) Proposed</i>				
Hidden signal	Aleph (\aleph)	Undetected	Constant presence	Predicted similar to light
<i>(B) Known</i>				
Light	Lambda (λ)	Electromagnetic	Variable in quantity, quality, direction and duration	Speed of light (299,792,458.00 m/s)
Gravity	Local gravity (g)	Acceleration	Slight variability (altitude and latitude)	Standard gravity on earth: (9.80665 m/s ²)
Air	Oxygen (O ₂)	Molecular	Variable	78.09% N ₂
	Carbon dioxide (CO ₂)		(altitude and latitude)	20.95% O ₂ 0.93% Ar 0.04% CO ₂
Temperature	Degree (°)	Thermal energy	Variable	Circumstellar Habitable Zone (CHZ)
Water	Water (H ₂ O)	Molecular	Variable	2.5% Fresh Water
				0.001% Atmospheric (of total global water)
Mineral nutrients	Mineral nutrients (NPK)	Elements and compounds	Variable	Macronutrients and micronutrients

List of factors influencing growth and development of plants on earth. The proposed hidden signal, called Aleph (\aleph), is listed as an undetected force predicted to be constantly present with speed similar to that of light (A). Known factors that drive plant growth and development on earth (B). Physical nature of the factors and their behavior (constant or variable) as experienced by organisms is described along with widely accepted values associated with each of the “known” factors. Symbols used here are representative of the factors described. The term Circumstellar Habitable Zone (CHZ) was introduced by astronomer James Kasting in 1993. N (nitrogen), O₂ (oxygen), Ar (argon), CO₂ (carbon dioxide)

signaling pathways in Arabidopsis. In this effort, a potential function for a hypothetical receptor, named Etioreceptor, is presented (Fig. 3). Light is one of the most influential signals to mold plant development. Research with Arabidopsis has led the way in expanding our knowledge of light regulated plant responses. Plants sense different wavelengths of light through specialized photoreceptors; red (R, 600–700 nm) and far-red (FR, 700–750 nm) by phytochromes (phys) (Chen and Chory 2011), UV-B (280–315 nm) by UV Resistance locus 8 (UVR8) (Jenkins 2014), blue (390–500 nm) by cryptochromes (crys) (Chaves et al. 2011), phototropins (phot) (Christie 2007) and Zeitelupe (ztl) family members (Suet-sugu and Wada 2013). Light is essential for the enzymatic reduction of protochlorophyllide in biosynthesis of chlorophylls, but it is not needed in the light-independent biosynthesis of bacteriochlorophylls involved in anoxygenic photosynthesis (Armstrong 1998). As discussed above, early steps of photomorphogenesis can be de-repressed in the absence of light. The developmental program relies upon light for photosynthesis and is designed to grow into the light environment. This is achieved through cell elongation during skotomorphogenesis (etiolation) and during shade avoidance (low R/FR conditions). Auxin

biosynthesis, transport, and sensitivity are increased under low photosynthetically active radiation (PAR) to promote cell elongation (Ma and Li 2019). Auxin biosynthesis occurs de novo using tryptophan (Trp) as a precursor or through a Trp-independent pathway (Zhao 2010), although the evidence for Trp-independent pathway has been challenged (Nonhebel 2015). Tryptophan is an essential amino acid synthesized via the shikimate pathway (Fig. 3), which is active in prokaryotes, fungi, algae, and plants for the production of the three aromatic amino acids. Animals and humans lack the shikimate pathway and rely upon their diet to acquire Trp. In plants, the shikimate pathway is active in the plastid, suggesting its ancestry from a plastid genome (Richards et al. 2006). This ancient pathway is important in plant growth and for the production of a wide range of secondary metabolites for human nutrition and health. In the human brain, it is estimated that 1% of the dietary tryptophan is used to synthesize serotonin (Sandyk 1992; Rambali et al. 2002), a neurotransmitter implicated in numerous psychological processes. Mechanisms regulating the percentage of Trp entering into biosynthesis of these critical regulatory metabolites are not understood.

During skotomorphogenesis, organ expansion is regulated by IAA in combination with other phytohormones.

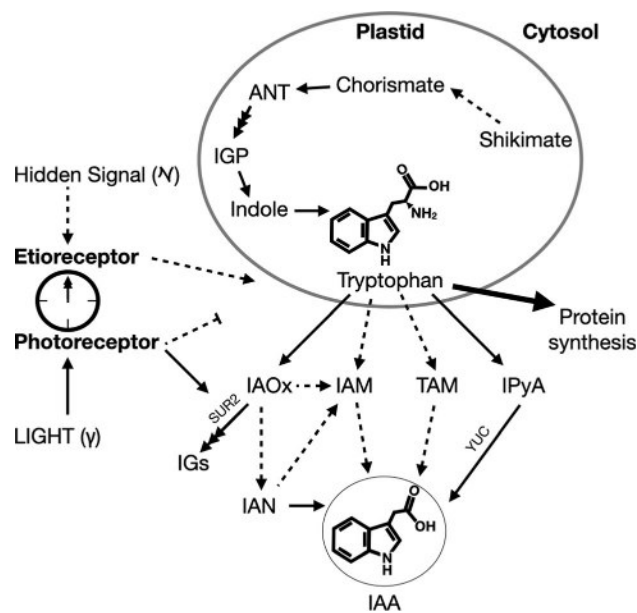


Fig. 3 Auxin biosynthesis pathways. Simplified representation of the tryptophan-dependent indole-3-acetic acid (IAA) biosynthesis pathways (Mikkelsen et al. 2004; Lau et al. 2008; Tao et al. 2008). Some intermediary steps are omitted for simplicity. Shikimate and tryptophan (Trp) biosynthesis is believed to be localized to plastids, whereas auxin biosynthesis is localized in the cytosol (Woodward and Bartel 2005; Tzin and Gallili 2010; Mano and Nemoto 2012). Majority of Trp is used in protein synthesis and as a precursor for synthesis of other compounds. A fraction of the Trp pool enters into the IAA biosynthesis. Pathways with known enzymes and intermediates (solid arrows) and with unknown enzymes and intermediates (dashed arrows) are indicated. Light-activated phyB (photoreceptor) promotes *SUR2* (a suppressor) and represses *TAA1* (an enhancer, not shown), leading to the reduction in IAA levels (Halliday and Martínez-García 2009; Ma and Li 2019). In contrast, the proposed hidden signal (\aleph) perceived by a hypothetical etioreceptor increases the amount of tryptophan entering into auxin biosynthesis pathways, thereby increasing IAA production

IAA causes organ elongation by loosening the outer epidermal wall as summarized in the “cytological model” (Kutschera and Wang 2016; Kutschera and Khanna 2020). The elongation growth requires biochemical processes and demonstrates organ level specificity, in a way similar to the intricate differential processes regulated by light. In this thought experiment, we suggest that an external hidden signal, aleph (\aleph) orchestrates growth processes in the absence of PAR to optimize exposure to light (λ) in the four dimensions, including time. Aleph’s influence may continue in the presence of light on selective growth processes, but it is modulated to express an alternative light-specific developmental program, which dominates for obvious reasons. Light-activated photoreceptor (phyB) suppresses IAA biosynthesis as described (Fig. 3). Here we propose the name “Etioreceptor” for a predicted sensor of the hidden signal, aleph. The predicted etioreceptor is likely to increase IAA biosynthesis. We hypothesize that

etioreceptor is involved in regulating the percentage of Trp entering the IAA biosynthesis pathway (Fig. 3). In this new model, the hidden signal, aleph (\aleph), acts through a specialized etioreceptor to maintain growth and developmental programs in spacetime to facilitate the organism’s survival during Darwinian (adaptive) evolution—in the words of Herbert Spencer the “survival of the fittest”—by attaining desired environmental and nutrient inputs. This process continues throughout the life of the organism in spacetime.

Arabidopsis has proven to be a model system not only for plants, but also in providing valuable insights into biological mechanisms that have expanded our understanding of how signaling pathways work in other biological systems. With reference to Pfeffer’s original discovery of the etiolation response (Fig. 1), here we have presented a thought experiment suggesting there may exist a hidden signal with its own signal perception through an etioreceptor to drive growth in spacetime. We are continuing to explore the nature of the etioreceptor, which is likely to be a known molecule (Khanna, unpublished data) because the *Arabidopsis* genome has been sequenced and major regulatory components in the dark and light signaling pathways are known. *Arabidopsis* will continue to lead this new direction of enquiry in the foreseeable future due to the availability of single gene mutants and the extent of accumulated knowledge of signaling pathways, stay tuned!

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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