In addition, we are using the frog system to study size changes that occur during early development by preparing extracts from embryos at different stages. The fertilized egg is 1 mm in diameter in *X. laevis*, and undergoes rapid cleavages without growth so that the first ~4000 cells of the embryo are generated with minimal transcription or translation. I believe *Xenopus* will allow us to crack fundamental questions of how organelle size is determined.

What are the limitations to the system? Some of the limitations are technical. Egg and embryo extract approaches are not for the faint-hearted. Months can go by when the system doesn't work quite right and this can be incredibly frustrating. Also, there is the inevitable question: "But is this how it works in real cells?" As we identify organelle scaling factors in *Xenopus*, the plan is to test whether these mechanisms also operate in mammalian somatic systems. Many human diseases are associated with defects in cellular scaling. For example, the nucleus is enlarged and misshapen in many cancer cells. With some luck, the discoveries we make in *Xenopus* could lead to a greater understanding of diseases such as cancer. Every system has its advantages and disadvantages, and I am a strong proponent of the view that basic research as an open-minded exploration of the unknown in any system — even goo extracted from frog eggs — can be the key that leads to profound discoveries.

Who are your heroes? I am very proud of my scientific lineage, from Marc Kirschner and Tim Mitchison to Andrew Murray, Tony Hyman and many of my contemporaries who trained in their laboratories. But my biggest heroes are my colleagues. I am impressed on a daily basis by people in my lab and in my department, at my University, and in the broader cell biology community. I rely on my fellow faculty at Berkeley for inspiration and support, and all kinds of practical help, and they have never let me down. I was fortunate to start my lab at around the same time as my neighbors, Matt Welch and Karsten Weis, and we have always been close friends. It is the collective efforts of students and postdocs in my group, and our collaborators, that lead to the fascinating discoveries and the progress that we've made.

As an avid cyclist, I also have bicycle heroes. My favorite professional cyclist right now is Fabian Cancellara, who just won the Tour of Flanders and Paris-Roubaix. My own private bicycle hero is my husband Steve Hill, who inspires me to travel and have a life outside of the lab.

Any advice for young cell biologists? You'd think after running a lab for over 10 years that I might have it all worked out, but I still struggle getting papers published and grants funded, and teaching effectively. It has helped me a lot to talk with my peers about how they approach these challenges, and throughout my career the best advice has come from my contemporaries, rather than from more senior colleagues. Maybe it is not advice so much as just talking about difficult decisions or shared anxieties. It is low budget therapy that only costs as much as lunch or a couple of beers.

Although a career in science always seems to be a struggle, I find it pays to be nice. Remember that life continues to be like second grade, with some kids unwilling to share their toys and deliberately picking on one another. Instead of embracing the model in which no one takes you seriously if you aren't a hyper-competitive a**hole, I find the scientific world is a much sweeter place if you are generous and friendly. Believe it or not, scientific rigor does not mandate nasty comments in reviews of grants and manuscripts. A big benefit of open interaction is that it inspires collaboration, which helps overcome limitations, whether it simply involves a reagent, an approach, or even a way of thinking that does not come naturally to you, but enhances the impact of what can be learned.

And this is the key to thinking big. Fantasize about what you want to discover, and the different methods you could apply (or develop!) to get there, and who you could talk to and collaborate with to make it happen. No matter what hell you have to go through to get your project funded and published, it will be rewarding nevertheless.

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Quick guide

Phytochromes

Peter H. Quail

What are the phytochromes? The phytochromes (phys) are a superfamily of sensory photoreceptors. They were discovered in plants over half a century ago, but the era of whole-genome sequencing revealed that phy-related sequences are also widely dispersed across the microbial world (mainly cyanobacteria and eubacteria, as well as some filamentous fungi). The canonical phy molecule is a soluble dimer of chromoprotein subunits, each of which consists of a polypeptide with a single, covalently-linked bilin (tetrapyrrole) chromophore, which is responsible for light perception (Figure 1A). Each polypeptide folds into two major domains: an amino-terminal sensory domain which cradles the chromophore, and a carboxy-terminal 'output' or 'regulatory' domain. Each domain in turn contains subdomains in various combinations, identified by sequence similarity to several known structural and/or functional domains (Figure 1A). The core sensory domain typically consists of a PAS, GAF and PHY subdomain, with an additional variable amino-terminal extension in certain phys. The carboxy-terminal domain typically contains a subdomain related to those found in two-component histidine kinases (HKRD), with two additional upstream PAS domains in the plant phys. Recent crystallographic and solution NMR studies of the sensory domain of bacterial phys have begun to provide beautiful images of the three-dimensional structure of the photoreceptor molecule.

What are the biological functions of the phys? In the most general sense, the function of the phys is to monitor information from the environment in the form of light signals and direct responses in the organism appropriate to the prevailing conditions. More specifically, the phys constantly monitor multiple physical parameters of the impinging light signals (including presence/absence, color (wavelength), intensity

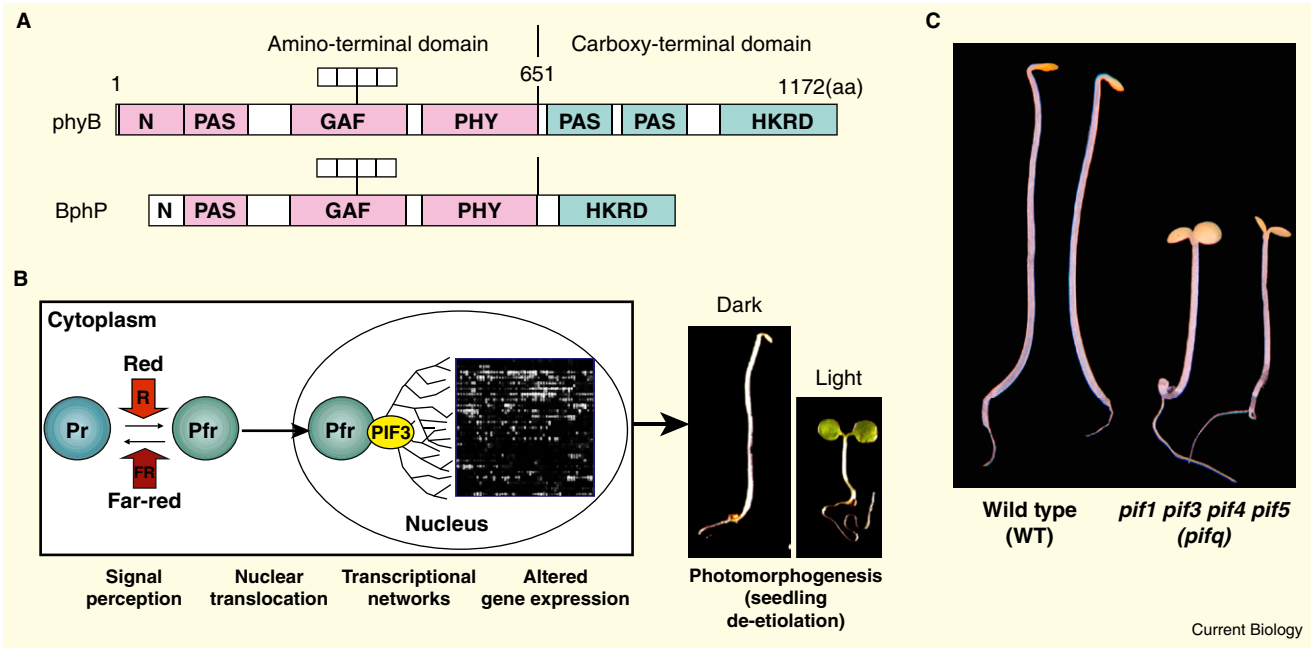

Figure 1. Phytochrome (phy) domain structure and intracellular signaling pathway.

(A) phy domain structure. Higher plant phy polypeptides, represented by phyB (top), contain two major structural domains (amino- and carboxy-terminal) and several subdomains (N (non-conserved amino-terminal extension), PAS (PER, ARNT, SIM), GAF (cGMP-specific phosphodiesterase, adenylate cyclase, FhlA), PHY (phytochrome) and HKRD (His-kinase related domain)) within each. Small partitioned box above GAF represents the chromophore. Microbial phys, represented by bacteriophytochrome (BphP), have a similar overall architecture but lack the twin caboxy-terminal PAS subdomains. (B) Plant phy photoperception and signal transduction in regulating seedling photomorphogenesis. phy molecules switch reversibly between their Pr and Pfr conformers upon sequential absorption of red (R) and (FR) photons. Pfr formation (signal perception) triggers rapid translocation into the nucleus where the activated molecule interacts with bHLH-class, phy-interacting transcription factors (PIFs), such as PIF3, initiating alterations in gene expression, via a transcriptional network, that culminates in the switch from skotomorphogenesis to photomorphogenesis (seedling de-etiolation). About 10% (2,400) of the genes in the *Arabidopsis* genome display altered expression within 24 h of first exposure to R light, whereas 1% (250 genes) change expression within 1 h of initial exposure ('early-response' genes). (C) The dark side: multiple PIF bHLH factors repress photomorphogenesis in darkness. A quadruple *pif1 pif3 pif4 pif5* (*pifq*) mutant of *Arabidopsis* displays a constitutively photomorphogenic (*cop*)-like phenotype in completely dark-grown seedlings. (Figure coutesy of B. Al-Sady, P. Leivar, E. Monte and J. Tepperman.)

(fluence rate) and diurnal duration (photoperiodicity)) and transduce this information via intracellular signaling pathways that elicit molecular and cellular responses specific to the organism and developmental state. In plants, the phys regulate multiple facets of growth, development and reproduction throughout the life cycle, including seed germination, the switch from heterotrophic to photoautotrophic seedling development (termed de-etiolation), juvenile vegetative development and architecture (through a process called neighbor-detection/shade-avoidance), flowering and senescence. A wealth of information has been gathered over the years on the montage of underlying cellular, subcellular, biochemical and molecular processes that drive these overt morphogenic responses, including stimulation or inhibition of cell expansion rates, switching of cell fate, induction of organelle biogenesis, alterations in metabolic pathways, modulation of hormone activities, regulation of the circadian clock and global changes in gene expression. Less is known about the functions of the phys in microorganisms but the data indicate adaptive functions, not only in photosynthetic bacteria, but also potentially in heterotrophic bacteria.

What is the mechanism of photosensory perception? The sensory function of the phy molecule resides in its capacity to switch reversibly (within milliseconds) between two stable conformers, called Pr (for red light (R)-absorbing) and Pfr (for far-red light (FR)-absorbing), upon sequential photoexcitation by R and FR light (Figure 1B). The Pr form is biologically inactive (at least in plants), whereas the Pfr form is biologically active. With a few bacterial exceptions, the molecule is synthesized in the inactive Pr form, so that initial light-induced Pfr formation is the signal that the host organism has been exposed to light for the first time, as occurs for young seedlings emerging from subterranean darkness after germination. In fully light-exposed organisms, under sustained irradiation conditions, a dynamic photoequilibrium is established between the Pr and Pfr forms, with the proportion of molecules in each form at any instant being determined by the relative levels (photon fluxes) of R and FR wavelengths in the incoming light signal. The phys thus provide a primitive form of color vision to the host organism, especially in the region of the spectrum containing photosynthetically active radiation. A variety of spectroscopic data, including recent NMR-based solution-structural analysis, have shown that photon absorption by the chromophore triggers rapid isomerization-driven ring rotation in the tetrapyrrole, and that this induces conformational changes

in the surrounding protein that presumably lead to the reversible switching in biological activity of the photoreceptor.

What is the nature of the intracellular signal transduction pathway? The phy molecule is continuously synthesized and accumulates in the cytoplasm in the inactive Pr form until it is exposed to light for the first time. In higher plants, initial photoinduced conversion to the Pfr form triggers rapid (within minutes) translocation into the nucleus where the activated photoreceptor initiates a cascade of changes in gene expression that are also detectable within minutes (Figure 1B). In microbes, the pathway is less well-defined but appears likely to involve a two-component signaling mechanism.

What is the nature of the phy-regulated transcriptional network in higher plants? About 10% or so of the genes in the genome display phy-regulated changes in expression during the seedling de-etiolation transition triggered by initial exposure of post-germinative seedlings to light (Figure 1B). Unsurprisingly, the predicted or established functions of the large majority of these genes correlate strongly with the various morphogenic changes observed during deetiolation; such genes include numerous photosynthetic genes related to the biogenesis of active chloroplasts, various auxin-, gibberellin-, cytokinin- and ethylene hormone pathway-related genes potentially mediating growth responses, and metabolic genes reflecting the transition from heterotrophic to autotrophic growth. In addition, oscillations in the expression of core circadian clock genes are triggered through the phy system by this initial exposure of seedlings to light, and repetitive diurnal exposure of plants to light at the dawn, dark-to-light transition provides a signal through the phy system that maintains the phase of the clock in sync with the ambient day–night cycle. The loci that respond most rapidly to the initial light signal ('early-response' genes) are enriched in genes encoding transcription factors, suggesting a role as direct regulators of downstream genes in the phy-directed transcriptional network.

What is the mechanism of transcriptional regulation by the photoactivated phy molecule? The phys (primarily phyA and phyB) interact, specifically in the Pfr form, with a subset of constitutively nuclear, basic helix-loop-helix (bHLH) transcription factors termed **p**hytochrome-**i**nteracting **f**actors (PIFs), immediately upon light-induced phy translocation into the nucleus. This interaction triggers rapid phosphorylation and subsequent degradation of the PIF protein via the ubiquitin proteasome system. The data suggest, therefore, that the phys regulate at least some target genes by directly modulating the abundance of transcription factors that control the transcriptional activity of those genes. This model is supported by the findings that a quadruple mutant (called *pifq*), null for four PIF family members (PIF1, 3, 4 and 5), largely phenocopies, when grown in darkness, the morphogenic development of wild-type seedlings grown in the light (Figure 1B,C), and that the global gene expression pattern of this dark-grown mutant also strongly phenocopies the pattern normally induced by light in the wild-type plant. These data also indicate that the PIF proteins function to repress photomorphogenic development in darkness and that phy photoactivation reverses this repression through induced PIF proteolysis.

What is the biochemical mechanism of signal transfer from the activated phy photoreceptor molecule to its immediate signaling partners? The most prominent current proposed mechanism of phy signaling is that the photoreceptor molecule is a light-regulated protein kinase that transphosphorylates physically interacting transduction-chain partners upon photoactivation. There is strong evidence for this mechanism in the case of the few prokaryotic phys that have been examined. In these cases, the histidine kinase activity predicted from the conserved HKRD domain in these molecules (Figure 1A) has been verified biochemically and shown to be light regulated. In canonical plant phys, however, the issue remains controversial. The first confounding factor is that these phys generally lack the phospho-acceptor histidine residue conserved in the two-component prokaryotic kinases, and no biochemically detectable His-kinase activity has been reported. The plant phys thus appear to have lost this catalytic activity during evolution. This conclusion has led to the alternative proposal that the plant phys have acquired serine/threonine kinase activity, more typical of eukaryotes, during evolution. The rapid *in vivo* phosphorylation of the PIF proteins triggered by light-induced binding of the phy molecule to the bHLH protein in the nucleus, mentioned above, is consistent with this possibility. Similarly, *in vitro* kinase assays of purified plant phys have detected the presence of serine/threonine kinase activity, potentially attributable to the phy molecule itself. However, several caveats remain. Firstly, the plant phy sequences lack the canonical signature motifs found in the majority of eukaryotic serine/threonine kinases, invoking the need to propose that they are atypical kinases. Secondly, the potential for the presence of contaminating kinase activity in the *in vitro* assays has not been rigorously eliminated. Thirdly, and most importantly, evidence that mutagenesis of the plant-phy kinase-like sequence motif results in loss of the protein kinase activity detected either *in vitro*, or by PIF phosphorylation *in vivo*, has not been forthcoming to date. In fact, there is evidence that deletion of the entire carboxy-terminal domain (containing the HKRD subdomain; Figure 1A) does not reduce phy activity *in vivo*. It must be concluded, therefore, that there is currently no unequivocal evidence that the plant phys possess intrinsic, autonomous protein kinase activity. Other alternatives, such as phy-mediated recruitment of third-party kinases to target proteins, remain viable.

What is on the horizon? Rapid strides are being made in defining the structural features of the phy molecule through crystallographic and solution NMR studies on the sensory domain of bacterial phys. Expansion of this work to include the entire molecule and to solve the structure of a plant phy can be expected to provide invaluable insight into the mechanism of phy signaling. The availability

of increasingly economical new-generation high-throughput DNA-sequencing technologies can be expected to permit genome-wide definition of the primary phy-regulated transcriptional network through the use of ChIP-seq and RNA-seq procedures. Proteomic approaches, such as mass spectrometric analysis, may provide an avenue for unravelling the current enigma of the capacity of the phy molecule to induce phosphorylation of signaling partners *in vivo*, through direct interaction, in the absence of apparent evidence of autonomous protein kinase activity intrinsic to the photoreceptor molecule itself.

Where can I find out more?

Bae, G., and Choi, G. (2008). Decoding of light signals by plant phytochromes and their interacting proteins. Annu. Rev. Plant Biol. *59*, 281–311.

Briggs, W.R., and Spudich, J.L. (2005). Handbook of Photosensory Receptors (Weinheim: Wiley).

Castillon, A., Shen, H., and Huq, E. (2007). Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks. Trends Plant Sci. *12*, 514–521.

Essen, L.-O., Mailliet, J., and Hughes, J. (2008). The structure of a complete phytochrome sensory module in the Pr ground state. Proc. Natl. Acad. Sci. USA *105*, 14709–14714.

Fankhauser, C., and Chen, M. (2008). Transposing phytochrome into the nucleus. Trends Plant Sci. *13*, 596–601.

Franklin, K., and Quail, P.H. (2010). Phytochrome functions in *Arabidopsis* development. J. Exp. Bot. *61*, 11–24.

Leivar, P., Monte, E., Oka, Y., Liu, T., Carle, C., Huq, E. and Quail, P.H. (2008). Multiple phytochrome-interacting bHLH transcription factors repress premature photomorphogenesis during early seedling development in darkness. Curr. Biol. *18*, 1815–1823.

Leivar, P., Tepperman, J.M., Monte, E., Calderon, R.H., Liu, T.I., and Quail, P.H. (2009). Definition of early transcriptional circuitry involved in light-induced reversal of PIF (phytochrome-interacting bHLH factor)-imposed repression of photomorphogenesis in young Arabidopsis seedlings. Plant Cell *21*, 3535–3553.

Quail, P.H. (2007). Phytochrome Interacting Factors. In Light and Plant Development. G.C. Whitelam and K.J. Halliday, eds. (Blackwell), pp. 81–105.

Rockwell, R.C., Su, Y.-S., and Lagarias, J.C. (2006). Phytochrome structure and signaling mechanisms. Annu. Rev. Plant Biol. *57*, 837–858.

Schäfer, E., and Nagy, F. (2006). Photomorphogenesis in Plants and Bacteria (Doedrecht: Springer).

Ulijasz, A.T., Cornilescu, G., Cornilescu, C.C., Zhang, J., Rivera, M., Markley, J.L. and Vierstra, R.D. (2010). Structural basis for the photoconversion of a phytochrome to the activated Pfr form. Nature *463*, 250–254.

Whitelam, G.C., and Halliday, K.J. (2007). Light and Plant Development (Blackwell).

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Lethal intergroup aggression leads to territorial expansion in wild chimpanzees

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Chimpanzees make lethal coalitionary attacks on members of other groups [1]. This behavior generates considerable attention because it resembles lethal intergroup raiding in humans [2]. Similarities are nevertheless difficult to evaluate because the function of lethal intergroup aggression by chimpanzees remains unclear. One prominent hypothesis suggests that chimpanzees attack neighbors to expand their territories and to gain access to more food [2]. Two cases apparently support this hypothesis, but neither furnishes definitive evidence. Chimpanzees in the Kasekela community at Gombe National Park took over the territory of the neighboring Kahama community after a series of lethal attacks [3]. Understanding these events is complicated because the Kahama community had recently formed by fissioning from the Kasekela group and members of both communities had been provisioned with food. In a second example from the Mahale Mountains, the M group chimpanzees acquired part of the territory of the adjacent K group after all of the adult males in the latter disappeared [4]. Although fatal attacks were suspected from observations of intergroup aggression, they were not witnessed, and as a consequence, this case also fails to furnish conclusive evidence. Here we present data collected over 10 years from an unusually large chimpanzee community at Ngogo, Kibale National Park, Uganda. During this time, we observed the Ngogo chimpanzees kill or fatally wound 18 individuals from other groups; we inferred three additional cases of lethal intergroup aggression based on circumstantial evidence (see Supplemental Information). Most victims were caught in the same region and likely belonged to the same neighboring group. A causal link between lethal intergroup aggression and territorial expansion can be made now that the Ngogo chimpanzees use the area once occupied by some of their victims.

From 1999 to 2008, the Ngogo chimpanzees utilized a territory of 28.76 km^2 (Figure 1A). During this period, they occasionally made forays into the territories of their neighbors on boundary patrols (Figure 1A). Patrols involve considerable travel, but little feeding or socializing; patrollers are unusually silent and move in single file line, while attending to signs of other chimpanzees [1]. Seventeen of the 18 observed fatal attacks were made by coalitions of Ngogo males on patrol (Supplemental Information). Thirteen of the 21 cases of lethal intergroup aggression (61.9%) occurred northeast of the Ngogo territory in a circumscribed region that corresponded to an area of heavy patrol activity (Figure 1B). Four victims were adult males, while 9 others were immatures. All 13 chimpanzees were unhabituated to human presence, and as a result, we do not know the exact size of their community. If its size is similar to those of chimpanzee communities studied elsewhere (X = 46.6, SD = 18.7, n = 8 communities [5]), the 13 fatalities represent a mortality rate of 2,790 per 100,000 individuals per year. Alternatively, a rate of 867 per 100,000 individuals per year results if one assumes the northeast community is as large as Ngogo's (150 individuals). These values are extremely high, exceeding median rates of mortality due to intergroup violence reported for humans in agricultural and hunter–gatherer populations by factors of 1.5–5 and 5–17, respectively [6]. They are also 23–75 times higher than the median rate suffered by individuals in nine well-studied chimpanzee communities [6].

Recent observations of the Ngogo chimpanzees reveal that they have expanded their territory considerably to the northeast into the area previously occupied by their neighbors (Figure 1B). Large, mixed-sex parties of Ngogo chimpanzees started to use this area regularly in June 2009, spending 43 of 132 observation days (32.6%) in the newly acquired territory over the next 5 months. They traveled, fed, and socialized in this region in ways