Netherlands Organisation for Scientific Research
Earth and Life Sciences

Open Programme
Proposal form

The maximum length of a proposal is 11 pages.

1a. Details of proposal

Title: How mushroom forming fungi see the light
Area: O Geo and Biosphere (X) from Molecule to Organism
Summary (scientific summary in English, max. 250 words):

Many organisms depend on, or react to, light. For instance, light is an energy source, directs growth and induces developmental processes. Much is unknown about the role of light and the underlying molecular processes in fungi in general and in mushroom forming fungi in particular. Recently, we demonstrated that mushrooms are formed in a ring in a colony of *Schizophyllum commune* when exposed to 200 lux white light, while mushrooms are formed all over the mycelium when exposed to 1200 lux. We also showed that red light sensitizes the mycelium of *S. commune* for blue light, which is known to induce mushroom formation in this model fungus. In this project, the mechanisms underlying light perception will be studied in *S. commune* as well as heterogeneity in light receptiveness in zones and individual hyphae within the colony. We will identify the different light receptors, study their interactions and their effect on gene expression. Light receptiveness will be studied by exposure of selected parts or individual hyphae whilst monitoring consequences in gene expression and formation of mushrooms. These studies should also reveal how many hyphae should be exposed to induce mushroom formation. This project will make use of innovative technologies including single cell transcriptomics. Recently, we were the first to perform single cell transcriptomics in a microbe. The results of this research will unravel the complexity of a fungal colony and will improve our understanding of the formation of mushrooms in nature and in commercial growth facilities.

1b. Details of applicant

Name: Dr. L.G. Lugones
Gender: (X) Male O Female
Date of birth: 25-11-1962
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1d. Renewed application? O Yes (X) No
In case of a renewed application please indicate the file number of the previous application and summarize the main changes

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1e. Applying for: (X) PhD student O Post Doc O Ship time

1f. Composition of the research group

Name and title	Specialization	Institution	Involvement
Prof. Dr. H.A.B. Wösten	Molecular & Cellular Biology	Utrecht University	Thesis supervisor
Dr. L.G. Lugones	Molecular & Cellular Biology	Utrecht University	Daily supervisor
Dr. W.R. Teertstra	Molecular Biology	Utrecht University	Advisor

2. Summary for the general public

Hoe paddenstoelvormende schimmels het licht zien
Bijna alle organismen zijn afhankelijk van licht. Zo is het een energiebron, geeft het richting aan groei, en initieert het ontwikkelingsprocessen. Ook paddenstoelvorming in *Schizophyllum commune* wordt geïnitieerd door licht In dit project zal de functie van de voorspelde lichtreceptoren worden bepaald in dit modelsysteem. Voorts zal het verschil in lichtgevoeligheid in verschillende zones en hyfen van de kolonie worden bestudeerd. De resultaten zijn van belang om inzicht te krijgen in de complexiteit van kolonies van schimmels alsmede voor ons begrip hoe paddenstoelen in de natuur en tijdens commerciële teelt worden gevormd.

3. Top 5 publications of the applicant and research group related to the proposed research

Relevant Publications 2010-present

1. Ohm RA, de Jong JF, **Lugones LG**, Aerts A, Kothe E, Stajich JE, de Vries RP, Record E, Levasseur A, Baker SE, Bartholomew KA, Coutinho PM, Erdmann S, Fowler TJ, Gathman AC, Lombard V, Henrissat B, Knabe N, Kües U, Lilly WW, Lindquist E, Lucas S, Magnuson JK, Piumi F, Raudaskoski M, Salamov A, Schmutz J, Schwarze FW, vanKuyk PA, Horton JS, Grigoriev IV, **Wösten HAB**. 2010. Genome sequence of the model mushroom *Schizophyllum commune*. *Nature Biotechnology* 28:957-963 **(IF 23,3)**
2. van Peer AF, Wang F, van Driel KG, de Jong JF, van Donselaar EG, Müller WH, Boekhout T, **Lugones LG, Wösten HAB.** 2010. The septal pore cap is an organelle that functions in vegetative growth and mushroom formation of the wood-rot fungus *Schizophyllum commune*. *Environmental Microbiology* 12:833-844 **(IF 5.8)**
3. Ohm RA, de Jong JF, de Bekker C, **Wösten HAB, Lugones LG**. 2011. Transcription factor genes of *Schizophyllum commune* involved in regulation of mushroom formation. *Molecular Microbiology* 81:1433-1445 **(IF 5.0)**
4. de Bekker C, Bruning O, Jonker MJ, Breit TM, **Wösten HAB** 2011. Single cell transcriptomics of neighboring hyphae of Aspergillus niger. Genome Biology 12: R71 (**IF 9.0**)
5. Ohm RA, Aerts D, **Wösten HAB, Lugones LG**. 2012. The blue light receptor complex WC-1/2 of *Schizophyllum commune* is involved in mushroom formation and protection against phototoxicity. Environmental Microbiology **(IF 5.8)**

4. Description of the proposed research

Introduction and Aims

Light is (in)directly responsible for the existence of most organisms. For instance, light can be used as an energy source, is involved in formation of vitamins, gives direction to growth, regulates developmental processes, or gives rise to behavioural and phototactic responses. Photoreceptor proteins are found in all three Domains of life (Heintzen, 2012). They detect light of a certain wavelength of the electromagnetic spectrum. To this end, they may bind light-sensitive molecules called chromophores (Van der Horst & Hellingerwerf, 2003). The role of light and the underlying mechanisms have not been studied thoroughly in fungi when compared to animals and plants. For instance, the function of only two components of the blue light response of the mushroom model *Schizophyllum commune* has been studied (Ohm et al., 2012). Homology searches predict that *S. commune* has three other genes that are involved in the blue light response, as well as a red light receptor.

The aims of this project are:

- To identify proteins of *S. commune* that are involved in red and blue light detection and to establish their role in vegetative growth and mushroom formation.
- To identify interactions between the components of *S. commune* that are involved in detection of blue and red light.
- To study differences in light receptiveness of zones and hyphae within a mycelium and to determine the minimum light responsive unit in the mycelium.

Background

Light plays a key role in the lifecycle of many fungi. It is known to regulate the circadian rhythm, induce the formation of reproductive structures and to induce pigmentation (Purschwitz et al., 2006). The molecular mechanisms controlling the physiological responses of fungi to light have been studied most extensively in *Neurospora crassa* and *Aspergillus nidulans* (Heintzen, 2012). The blue light response in *N. crassa* is mediated by WC-1, WC-2, VIVID and the cryptochrome *nc*CRY-1. The WC-1 protein heterodimerizes with WC-2, forming the White Collar Complex (WCC). This complex controls the circadian rhythm, induces phototropism and influences carotenogenesis (Heintzen, 2012 , Rodriguez-Romaro et al., 2010). The VIVID protein in *N. crassa* detects changes in light intensity and modifies the blue light response of the WCC. The precise function of *nc*CRY-1 in *N. crassa* is not yet known. A Δ*cry-1* strain retains a circadian rhythm albeit out of phase (Heintzen, 2012). *N. crassa* also contains two phytochromes (PHY-1 and PHY-2). However, inactivation of one or both encoding genes did not result in an explicit phenotype. The phytochromes may modify WCC activity as described in *A. nidulans.* Inactivation of the green light opsin receptor NOP-1 also did not result in a phenotype in *N. crassa* (Heintzen, 2012). The function of a putative opsin in the genome of *A. nidulans* is also not known (Bayram et al., 2010). However, the molecular mechanisms underlying the blue and red light responses are relatively well understood in this fungus. *A. nidulans* contains orthologues of the *N. crassa* blue light receptor *nc*CRY-1 and the WCC proteins. LreA and LreB heterodimerize to form the WCC complex of *A. nidulans* and interact with the phytochrome FphA and the Velvet protein (VeA, see below). Inactivation of *lreA* or *lreB* results in increased conidiation irrespective of the presence of light (Purschwitz et al., 2008). This indicates that LreA and LreB suppress formation of asexual structures. Furthermore, the formation of the sexual reproductive structures called cleistothecia was strongly reduced in these knock-out strains when exposed to white light. When *lreA* and *lreB* are inactivated in conjunction with *fphA*, this effect is suppressed. Altogether, it was concluded that LreA and LreB induce the formation of sexual structures and repress the formation of asexual reproductive structures. LreA and LreB are in turn repressed by FphA in the presence of white light, explaining why *A. nidulans* normally fruits in the dark and forms asexual conidia under white light (Purschwitz et al., 2008). The VeA protein is also a positive regulator of sexual development, while simultaneously suppressing asexual development (Kim et al., 2002). It is repressed by the blue light receptor CryA (Bayram et al., 2008a) interacts with the WCC complex and forms a complex with VelB (Bayram et al., 2008b, Bok & Keller, 2004) and LaeA (Sarikaya Bayram et al., 2010). Clearly, the regulation of VeA and its partners is complex. We briefly summarize the roles of VeA and its partners and refer to Krijgsheld et al. (2012) for details. Like the ΔveA strain, the ΔvelB strain does not show light-dependent development. The ΔvelB strain is unable to form cleistothecia in the light and in the dark. However, the effect on asexual sporulation is not as strong as in the ΔveA strain (Bayram et al., 2008b). LaeA is a negative regulator of sexual development when *A. nidulans* is grown in the light and has a positive effect on asexual development (Sarikaya Bayram et al., 2010). The ΔlaeA strain produces five times less conidia in the light when compared to the wild-type. The absolute number of conidia of the ΔlaeA strain is similar in the light and in the dark. An opposite effect is observed for cleistothecia production. Fruiting body formation is markedly increased in the ΔlaeA strain when grown in the light. As a consequence, the number of cleistothecia in the mutant strain is similar in the light and the dark.

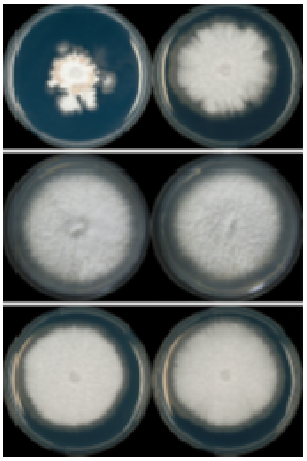

Figure 1: *The morphological phenotypes of S. commune WC-1 and WC-2 mutants in full spectrum light (left) and darkness (right). From top to bottom: The wild type H4-8, the Δwc-1/ Δwc-1 mutant and the Δwc-2/ Δwc-2 mutant. (Ohm et al., 2012)*

Both *N. crassa* and *A. nidulans* are used as model organisms of ascomycetes. The gray shag *Coprinopsis cinerea* and the split gill *Schizophyllum commune* serve as model organisms for mushroom forming basidiomycetes. Both *C. cinerea* and *S. commune* form a vegetative monokaryotic mycelium (i.e. one nucleus per compartment) after germination of a basidiospore. Compatible monokaryotic strains can fuse to form a fertile dikaryon (i.e. two nuclei per compartment). Blue light initiates fruiting body formation in *C. cinerea*. Three genes have been shown to be involved in photomorphogenesis in *C. cinerea*. The *C. cinerea* genes *dst1* and *wc-2* are orthologues of *wc-1* and *wc-2* of *N. crassa*, while *dst2* encodes a novel photomorphogenic protein (Terashima et al., 2005 , Kuratani et al., 2010; Nakazawa et al., 2011). When one of these genes is inactivated, fruiting is impaired and a so-called dark stipe phenotype is observed..Fruiting body formation is initiated but is not completed. Meiosis in the basidia is halted at prophase I, the stipe only slightly elongates and the pileus does not expand and open. *C. cinerea* has no homologue for VIVID but has homologues for VeA, VelB and LaeA (protein ID 1596, 1332 and 4204, Bayram & Braus, 2011), a putative phytochrome (protein ID 2967) and a cryptochrome-like photolyase (protein ID 14649). The role of these proteins is not yet known.

Blue light is also known to induce mushroom formation in the fungus *S. commune*.

We have recently shown that red light primes sensitivity for blue light in this fungus. Orthologues of *wc-1, wc-2* (Ohm et al., 2012) and *dst2* (Protein ID 69239) have been found in *S. commune.* The role of the latter gene is not yet known. However, inactivation of *wc-1* and *wc-2* resulted in a blind phenotype. The resulting dikaryons behave like monokaryons, unable to form mushrooms in white or blue light (Ohm et al. 2012; Figure 1). Compared with the wild type, 183 and 244 genes were more than twofold up- and downregulated in the Δwc-2Δwc-2 dikaryon respectively. Hydrophobin genes are over-represented in the down-regulated genes. Moreover, expression of the transcription factor genes *c2h2* and *hom1*, that are known to be involved in fruiting body development, was more than twofold decreased in the Δwc-2Δwc-2 dikaryon (Ohm et al., 2010). The WC-1 and WC-2 proteins contain two and one protein binding Per-Arnt-Sim (PAS) domain respectively. One of the PAS domains in WC-1 is a modified version, called a Light-Oxygen-Voltage (LOV) domain. This domain binds flavin adenine dinucleotide (FAD), which is a chromophore that detects blue light. The WC-2 protein has a predicted zinc-finger domain of the GATA-type. These data suggest that WC-1 is the light receptor, whereas WC-2 has a role as a transcriptional regulator. The *S. commune dst2* homologue has two FAD-binding domains, one of which works in conjuction with a Berberine and berberine like domain to form a FAD/FMN-containing dehydrogenase domain. The fact that the *S. commune* can bind FAD suggests that the encoding protein is also a blue light receptor protein like WC-1. The genome of *S. commune* does not contain homologues for VIVID or green light receptor genes but it does contain genes encoding a putative cryptochrome (Cry1; protein ID 49182), a phytochrome (Phy1; protein ID 76719)(Ohm et al., 2010) and homologues of VeA, VelB and LaeA (protein ID 112941, 255145 and 14559 respectively). The fact that *cry1* and *phy1* are down-regulated in the Δwc-2Δwc-2 dikaryon suggests a role in the photobiology of *S. commune* (Ohm et al., 2012). The putative cryptochrome of *S. commune* has a FAD-binding domain, as well as a light harvesting cofactor binding domain (Figure 2). The putative phytochrome consists of two parts. Its N-terminal part contains a PAS domain, a GAF domain and a phytochrome domain (Figure 2). The phytochrome domain has a pocket where the red/far-red light detecting chromophore, a billin, is stored. The C-terminal part of the putative phytochrome can be described as the effector part. It contains a histidine-kinase A domain, a histidine-kinase like ATPase domain and a response regulator receiver (REC) domain.

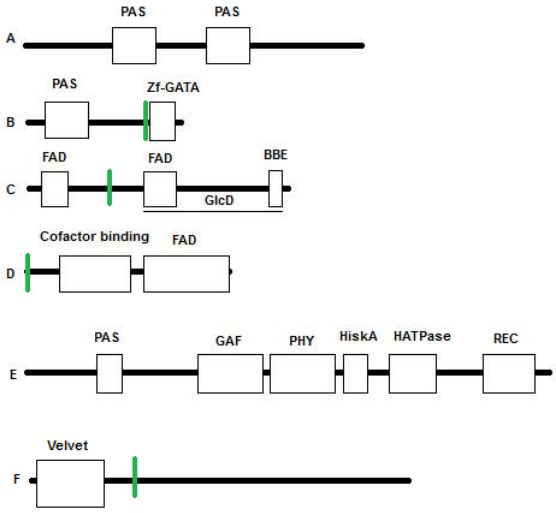

Figure 2: *Domains in proteins shown or predicted to be involved in the light response of S. commune* ***A)*** *WC-1.* ***B)*** *WC-2.* ***C)*** *the dst2 homologue.* ***D)*** *Cry1.* ***E)*** *Phy1* ***F)*** *the VeA homologue.* ***Green:*** *The locations of the Nuclear Localization Sequence for the different proteins. Domains were identified using the Conserved Domain Database (Marchler-Bauer et al., 2011)*

In nature, *S. commune* colonizes wood. Most hyphae grow within the substrate and are not exposed to light. This raises the question whether substrate hyphae are as light sensitive as hyphae that are exposed to the air. We have indications that there is heterogeneity in light sensitivity between different zones of the *S. commune* colony. Mushrooms are formed in a ring when dark-grown colonies of *S. commune* are transferred to light with an intensity of 200 lux. In contrast, the whole colony becomes covered with mushrooms when exposed to 2000 lux. Apart from heterogeneity in light receptiveness there may also be heterogeneity between hyphae within a particular zone. The phenomenon of heterogeneity between neighbouring hyphae has recently been shown in *Aspergillus niger* (Vinck et al., 2011; de Bekker et al., 2011) and we also have indications for heterogeneous expression of the hydrophobin gene sc3 and ribosomal RNA in *S. commune* (Teertstra et al., 2004). It has also not been established whether light induction of a single hypha is sufficient for initiation of mushroom formation or that a cluster of hyphae has to be activated. Moreover, we aim to address whether induction can be transferred to other parts of the colony.

Work plan

1. *Identification of light receptors of S. commune and their role in vegetative growth and mushroom formation.*

The role of *wc-1 and wc-2* in mushroom formation of *S. commune* has been described (Ohm et al., 2012). We will address the function of the predicted blue light receptors *cry1* (protein ID 49182) and *dst2* (protein ID 69239), as well as that of the predicted red light receptor *phyI* (protein ID 76719, Ohm et al., 2010), and the velvet-like protein (protein ID 112914). The genes will be inactivated using the *ku80* deletion strain (de Jong et al., 2010). After reintroduction of an intact *ku80* gene (de Jong et al., 2010), the impact of the deletion on radial growth, biomass formation and fruiting body formation will be assessed in both mono- and dikaryons. This will be done by growing the deletion strains in the dark, in white light (day-light spectrum, 1200 lux), blue light (495nm, 1200 lux), red light (650nm, 1200 lux), and green light (545nm, 1200 lux). Similarly, we will assess the phenotypes of double, triple and quadruple deletions strains. Multiple knockout strains can be easily obtained by crossing. RNAseq will be used to determine the expression profiles of selected mutant strains (i.e. those strains in which mushroom formation is affected).

2. *Transcriptional network of S. commune*

Genes *c2h2* and *hom1* encode transcriptional regulators that are involved in mushroom formation and that are more than 2 fold down-regulated in the Δ*wc-2*Δ*wc-2* strain. This shows that light-regulated development links to the interactive network of transcriptional regulators that are involved in mushroom formation. This network is currently being modelled (In collaboration with T. Gehrmann and Prof. M. Reinders, Delft University of Technology) based on transcriptome data of transcription factor deletion strains. Approaches are followed as described (Yeang et al., 2004; Tu et al., 2006; Ourfali et al., 2007; Kanabar et al., 2009) with specific attention to the network-free approach (Peleg et al., 2010) that does not presume prior knowledge of interactions. The interactive network of transcriptional regulators will be extended by using the expression data obtained in this project. This will be done by T. Gehrmann and Prof. M. Reinders).

3. *Sensitizing of blue light response by red light*

We have shown that red light sensitises dikaryons for blue light. As a result, fruiting bodies are produced one day earlier when compared to dark-grown colonies that have been transferred to blue light. We will assess whether sensitizing by red light occurs via transcriptional and /or post-translational control. Quantitative PCR of RNA of the photoreceptor genes and the gene encoding the VeA-like protein will be performed in mono- and dikaryons of the wild-type and of single, double, triple and quadruple photoreceptor knock-out strains grown in the dark and in white, blue, green and red light (see above).

Physical interactions will be studied between proteins with a phenotype when their gene is inactivated. WC-2, Dst2, Cry1 and the VeA orthologue have a clear nuclear localization sequence, while WC-1 and Phy1 do not. If fusion proteins of the latter proteins with dTomato end up in the nucleus, as observed by fluorescence microscopy, they probably are imported as a complex. By expressing the fusion proteins in the Δ*wc-2* and Δ*veA* strains, it can be assessed whether WC-1, Dst2, Cry1 and Phy1 are imported by WC-2 or Vea. Interactions of these proteins will be further studied by Fluorescence Resonance Energy Transfer (FRET). It will be assessed whether WC-1 and WC-2 physically interact and whether such an interaction takes place between the WCC complex, and Dst2, Cry1, PhyI and Vea (assuming inactivation of their genes results in a phenotype). To this end, fusion proteins with dTomato and GFP (van der Kroft et al., 2008) will be constructed. The reporters GFP and dTomato have been shown to function in *S. commune* (Vinck, 2007; Ohm et al., 2012). If FRET experiments fail, a Yeast II hybrid approach will be used to determine protein-protein interactions.

4. *Heterogeneity of light sensitivity of hyphae*

QPCR will be performed on RNA isolated from 5 different concentric zones of mono- and dikaryotic colonies that have been grown for 4 days in the dark followed by 1 day exposure to 200 lux and 1200 lux white light to assess expression of *wc-1, wc-2, phy1, cry1, dst-2* and *veA,* and the transcription factor genes *c2h2* and *hom1* that are known to be upregulated by light (Ohm, 2010). In the case of the dikaryon, expression will be related to the zone(s) that produce mushrooms. If zonal differences are found, we will use RNAseq to determine the expression profiles of the concentric zones. Clearly, expression profiles of zones will increase the resolution of the interaction network (see **2**) when compared to RNA of the whole colony because RNA of zones that are not reactive to light will level out effects of light in responsive zones. These data will therefore be included in the regulatory network as described in **2**.

Micro-dissection will be used to dissect 50 individual hyphae from zones of the colony that express the photoreceptors. Protocols for isolation of RNA from single hyphae have been developed in our lab (de Bekker et al., 2011). The RNA will be used for QPCR to assess expression of *wc-1, wc-2, phy1, cry1, dst-2* and *veA,* and the transcription factor genes *c2h2* and *hom1*. In the case differences are significant we will perform RNAseq to assess heterogeneity of the transcriptome in 6 different individual hyphae (note that the restricted number of hyphae is due to the high costs of these experiments [see section 8]; when prizes drop we will increase the number of hyphae).

5. Minimal light conditions that impact growth and development
Exposition of a complete colony in a Petri dish to full spectrum light or blue light of 200 lux will induce mushroom formation in a ring between the centre and the periphery of the colony, while mushrooms are formed all over the colony when exposed to 1200 lux. We will reduce the surface area of the colony that is exposed to 200 lux or 1200 lux by using masks. These masks will contain concentric rings or one or more holes of a particular diameter. The diameter of the holes will be reduced until mushroom formation is no longer induced. It may be that exposure of a single hypha to light is sufficient to induce (local) development. We have access to a Chameleon tuneable laser that can be used for two-photon excitation of photoreceptors. The two-photon strategy enables us to excite the photoreceptors in a single hypha or a cluster of hyphae. Location of mushroom formation will be determined after varying exposure times and light intensity.

Contingency strategy
Inactivation of *phy1, cry1, dst2* and *vea* may not result in a phenotype. If this would be the case, we will include studies to assess the role of transcription factor genes that are affected in the Δ*wc-2*Δ*wc-2* strain but that are not yet studied in our lab (see Ohm et al., 2012). Single cell expression analysis and single cell light activation is challenging but we have the expertise and the equipment to make these experiments work.

Innovative aspects
The following aspects of this proposal are innovative:
- Components of the light mediated processes have been identified in mushroom forming fungi. However, functional analysis has not yet been performed on all genes predicted to be involved in light biology. Moreover, the interaction between these proteins is not yet known. This project will identify the proteins that play a role in light mediated processes in *S. commune*, as well as their interactions.
- The transcriptional data obtained in this project will be used to extend the regulatory network involved in mushroom formation. Such a network is unique for mushroom forming fungi and even for fungi in general.
- So far, studies have been performed by exposing whole colonies to the light. Our proposed studies to expose particular zones or even individual hyphae is therefore unique.
- Single cell expression analysis using qPCR or using arrays has only been reported ones in fungi (i.e. in *A. niger* by our group; de Bekker et al. 2011). These methods are crucial to unravel the complexity in filamentous fungi.
- *S. commune* is a model for the formation of mushrooms. The results obtained in this project will be instrumental to understand how other mushroom forming fungi react to light both in nature and in commercial growth facilities.

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5. Timetable of the project

In the first year genes predicted to be involved in the photobiology of *S. commune* will be inactivated and their phenotype will be assessed. Moreover, qPCR will be performed of selected genes using RNA isolated from 5 concentric zones of light exposed colonies. In the second year whole genome expression analysis will be performed of selected deletion strains that are affected in their photobiology as well as of concentric zones of light exposed colonies. The latter will only be performed if qPCR performed in the first year show zonal differences in gene expression. Moreover, qPCR will be performed to assess expression of genes involved in the photobiology in deletion strains affected in light response. In addition, Nuclear import studies and FRET experiments will be performed in the second year. The latter experiment will also be performed in year 3. QPCR and RNAseq of individual hyphae, yeast two hybrid (if necessary), as well as experiments addressing minimal light conditions for zones will be performed in year three. These experiments except for QPCR will also be performed in year 4. This will be combined with experiments addressing minimal conditions of hyphal exposure and with writing of the Thesis. *The transcriptional network experiments will be performed by T. Gehrmann and Prof. M. Reinders (TUD).

	Year 1		Year 2		Year 3		Year 4	
1. Identification of the components of light biology								
a. Gene inactivation & phenotype analysis								
b. RNAseq								
2. Transcriptional network*								
3. Sensitizing of blue light response by red light								
a. qPCR								
b. Nuclear localization								
c. Fret								
d. Yeast two hybrid**								
4. Zonal heterogeneity of light receptiveness								
a. qPCR								
b. RNAseq								
c. Transcriptional network*								
4. Hyphal heterogeneity of light receptiveness								
a. qPCR								
b. RNAseq								
5. Minimal light conditions								
a. Zonal exposure								
b. Hyphal exposure								
6. Writing Thesis								

6. Scientific embedding of the proposed research

Affiliation with national and international research programmes, national and international collaborations

The Microbiology group of Utrecht University is part of the Institute of Biodynamics and Biocomplexity of the Department of Biology. It closely collaborates with research groups within the Department of Biology, and groups within the Science and Medical Faculty of the University of Utrecht. Moreover, the Microbiology group has a strong link to the Fungal Biodiversity Centre CBS of the Royal Dutch Academy of Sciences that is located on the university campus in Utrecht. At the national level, the Microbiology group participates in the research institute Kluyver Centre for Genomics of Industrial Fermentation. Within this institute the group collaborates with the Universities of Leiden, Delft and Groningen. Moreover, we actively collaborate with the Universities of Wageningen and Eindhoven. At the international level we participate in the European network Eurofung, which consists of industry and research groups from universities and institutes. Moreover, our group was part of the annotation consortia of the genomes of *A. niger, Ustilago maydis*, *S. commune* and *A. bisporus.*

7. Societal significance

The research described in this proposal impacts our understanding of mushroom formation in nature and in commercial growth facilities. The cultivation of mushrooms is an important global industry and the Netherlands represents an important part of it. The Netherlands produces approximately 250000 tonnes of white button mushrooms (*Agaricus bisporus*) annually, which represents 12% of the total global production representing a market of approximately 300 million euros. Mushrooms are not only used for consumption, they are also known for the production of pharmaceuticals, enzymes and flavours. The full potential of mushroom farming has yet to be unlocked. Production of commercial species could be improved based on knowledge of the role of light in growth and development and the underlying mechanisms. Moreover, the results of this project may explain in part why many species cannot yet be cultured commercially.

8. Budget

		Year 1	Year 2	Year 3	Year 4
Personnel (mm)		12	12	12	12
Research costs (k€)					
	Equipment				
	Consumables*	8	20	8	8
	Fieldwork/Travel*		2	2	2

* The sums requested for consumables and fieldwork/travel expenses combined should not exceed 50,000 euro for the entire grant period.

Specification of the requested funds:

Equipment: ----

Consumables: The budget will be used for chemicals, enzymes for molecular biology and especially for sequencing of RNA.

Fieldwork/Travel: Funds for international conferences such as the European Conference of Fungal Genetics and the Fungal Genetics Conference at Asilomar (CA, USA).

9. Financial assistance from other sources

Not applicable

10. Statements by the applicant

N/A	I endorse and follow the Code Openness Animal Experiments (if applicable)
YES	I endorse and follow the Code Biosecurity (if applicable)
YES	I have completed this form truthfully

Name: Dr Luis Lugones
Place: Utrecht
Date: December 1, 2012

Please submit the application to NWO in electronic form (pdf format is required!) using the Iris system, which can be accessed via the NWO website (www.iris.nwo.nl). The application must be submitted from the account of the main applicant. For any technical questions regarding submission, please contact the IRIS helpdesk (iris@nwo.nl).