Experimental procedures

Signalling to the nucleus under the control of light and small molecules

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Plasmids

Table 1. Plasmids used in this study. Abbreviations: pA, polyadenylation signal; PhyB, phytochrome B; PIF3, phytochrome interacting factor 3; P_{SV40}, simian virus 40 promoter; TEV, tobacco etch virus.

Plasmid	Description	Reference
1858 pGAD24-PIF3	Plasmid encoding full-length phytochrome interacting factor 3 (PIF3). Kindly provided by Andreas Hiltbrunner.	Unpublished
pGEMHE-XfA4-mEGFP	Vector for in vitro transcription and expression in Xenopus oocytes containing the coding sequence of monomerizing enhanced GFP (mEGFP A206K). Kindly provided by Maximilian Ulbrich.	Ulbrich *et al.* 2010[1]
pMZ333	P_{SV40} driven mammalian expression vector derived from *XbaI*/*Not*I digested pSAM200[2].	Unpublished
pMZ354	Plasmid encoding full-length PIF3, derived from plasmid 1858 pGAD24-PIF3.	Unpublished
pSJ032	**P_{SV40}::TEV(Full-length)-pA**. Full-length TEV protease was PCR amplified from pRK793 (Addgene #8827) with oligos oSJ046 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCGAATTGCGGCCGCAGGAGGCGCCACCATGGGTCATCATCATCATCATCATCATGGAG-3') and oSJ047 (5'-TCTGGATCGAAGCTTGGGCTGCAGGTCGACTCTAGATTACTAGCGACGGCGACGACGATTCATG-3') for Gibson cloning into pMZ333 digested with *NotI/XbaI*.	This work
pSJ033	**P_{SV40}::split-TEV(C-term)-pA**. The insert was amplified from FRB-NtSNIPER-pMA-T[3] with oligos oSJ050 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCGAATTGCGGCCGCAGGAGGCGCCACCATGGGT	Gray *et al.* 2010[3]

	TATCCGTATGAC-3') and oSJ051 (5'-TCTGGATCGAAGCTTGGGCTGCAGGTCGACTCTAGATTACTAGACCATGAACACTTTATGGCCACCC-3') for Gibson cloning into pMZ333 digested with *NotI/XbaI*.	
pSJ034	**P_{SV40}::split-TEV(N-term)-pA**. The insert was amplified from FKBP-CtSNIPER-pMA-T[3] with oligos oSJ048 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCGAATTGCGGCCGCAGGAGGCGCCACCATGGGTTATCCGTATGAC-3') and oSJ049 (5'-TCTGGATCGAAGCTTGGGCTGCAGGTCGACTCTAGATTACTACGTTTGGAAGTTCGTGGTAACCAGAC-3') for Gibson cloning into pMZ333 digested with *NotI/XbaI*.	Gray *et al.* 2010[3]
pSJ067	**P_{SV40}::PIF3(1-100)-NLS-mEGFP-pA**. PIF3(1-100) was PCR amplified from pMZ354 with oligos oSJ052 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGG-3') and oSJ133 (5'-GCTGTACGCGGAACCAGCACTACCAGCACTACCAGCACTATCCACCTTCCGCTTTTTCTTGGGGAAATCAGAGCAATATCCATCAAGGGAGG-3'), and mEGFP was amplified from pGEMHE with oligos oSJ078 (5'-GATAGTGCTGGTAGTGCTGGTAGTGCTGGTTCCGCGTACAGCATGGTGAGCAAGGGCGAGGAGC-3') and oSJ079 (5'-TCTGGATCGAAGCTTGGGCTGCAGGTCGACTCTAGATTACTACTTGTACAGCTCGTCCATGCCGAG-3') for Gibson cloning into pMZ333 digested with *NotI/XbaI***.**	This work
pSJ068	**P_{SV40}::PIF3(1-100)-NLS-mEGFP-TEVcs-CAAX-pA**. The insert was amplified from pSJ067 with oligos oSJ052 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGG-3') and oSJ053 (5'-ACCACCACCGCCAGACTGAAAATACAGGTTTTCAGAACCACCACCTCCCTTGTACAGCTCGTCCATGCCGAG-3'), and further amplified with oligos oSJ052 (5'-GTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGG-3') and oSJ108 (5'-TCTGGATCGAAGCTTGGGCTGCAGGTCGACTCTAGATTACTACATAATTACACACTTTGTCTTTGACTTCTTTTTCTTCTTTTTACCACCACCACCGCCAGACTGAAAATAC-3') for Gibson cloning into pMZ333 digested with *NotI/XbaI*.	This work
pMZ701	**P_{SV40}::PhyB(1-908)-mCherry-pA**.	Beyer *et al.* 2015[4]

Cell culture and transfection

Human epitheloid cervix carcinoma HeLa cells (ATCC CCL-2) were cultured at 5% CO_2 in DMEM (Life Technologies) containing 4.5 g/l glucose supplemented with 10% fetal calf serum (FCS, Life Technologies) and 4 mM L-Glutamine (Life Technologies). Cells were transfected using polyethyleneimine (PEI, linear, MW: 25 kDa, Polyscience) as described elsewhere[5]. The medium was exchanged 5 hours post-transfection and cells were imaged 30 hours post-transfection.

Light-mediated membrane recruitment

24 hours post-transfection, the cell culture medium was replaced by medium containing 15 µM PCB with or without additional 10 nM rapamycin[6]. After addition of PCB, all steps were carried out under green safe-light (520 nm). Cells were subsequently incubated for 1 hour in darkness in order to allow PhyB holoprotein assembly and were then illuminated with red or far-red light (660 nm or 740 nm, respectively) for 1 hour at a photon flux density of 20 µmol m^{-2} s^{-1}. Illumination was performed with

LED panels emitting at 660 nm or 740 nm light (Roithner, cat. No. LED660N-03, LED740_01AU, respectively) with an electronic controller for adjusting light intensity.

Confocal fluorescence microscopy

For confocal fluorescence imaging, illuminated cells, which were grown on glass coverslips were washed with ice-cold PBS containing Ca^{2+}-/ Mg^{2+}-ions (Life Technologies) and subsequently fixed with 4 % paraformaldehyde (PFA, Roth) at 4 °C for 10 minutes, followed by further fixation for 10 minutes at room temperature. Coverslips were embedded in Mowiol 4-88 (Roth) containing 15 mg/ml 1,4-diazabicyclo[2.2.2]octane (DABCO, Roth) and mounted onto glass microscope slides. Cells were imaged with a confocal fluorescence microscope (Nikon Eclipse Ti-E with A1R confocal laser scanner, 60x oil objective, NA=1.49). mCherry and mEGFP were visualized using excitation lasers of 561 nm and 488 nm, respectively. Image acquisition and analysis were performed with NIS-Elements C (Nikon Instruments, version 4.10.04).

REFERENCES OF EXPERIMENTAL PROCEDURES

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