Published in final edited form as:

Curr Opin Chem Biol. 2016 October ; 34: 62–71. doi:10.1016/j.cbpa.2016.06.009.

Go in! Go out! Inducible control of nuclear localization

Barbara Di Ventura[1] and **Brian Kuhlman**[2,3]

[1]Center for Quantitative Analysis of Molecular and Cellular Biosystems (BioQuant), University of Heidelberg, Heidelberg, Germany

[2]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, North Carolina, USA

[3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina, USA

Abstract

Cells have evolved a variety of mechanisms to regulate the enormous complexity of processes taking place inside them. One mechanism consists in tightly controlling the localization of macromolecules, keeping them away from their place of action until needed. Since a large fraction of the cellular response to external stimuli is mediated by gene expression, it is not surprising that transcriptional regulators are often subject to stimulus-induced nuclear import or export. Here we review recent methods in chemical biology and optogenetics for controlling the nuclear localization of proteins of interest inside living cells. These methods allow researchers to regulate protein activity with exquisite spatiotemporal control, and open up new possibilities for studying the roles of proteins in a broad array of cellular processes and biological functions.

Introduction

Many cellular processes are naturally regulated via control of nuclear import and export [1]. For instance, gene transcription is often repressed by sequestering transcription factors in the cytosol [2]. To enter and exit the nucleus, macromolecules dynamically interact with nuclear transport receptors which facilitate passage through nuclear pore complexes [3,4]. The interaction between cargo molecules and the transport receptors is frequently mediated by short linear motifs on the cargo called nuclear localization signals (NLS) or nuclear export signals (NES) [3,5]. The discovery of these interactions and the control mechanisms that regulate them is enabling new technologies which allow cell biologists to manipulate when and where proteins enter the nucleus. These inducible systems offer a powerful alternative to traditional gene knock-out or RNA knock-down approaches for studying dynamic processes.

Correspondence to: barbara.diventura@bioquant.uni-heidelberg.de, bkuhlman@email.unc.edu.

In this review, we briefly describe the protein interactions that mediate nucleocytoplasmic transport and provide examples of how these interactions are regulated in natural systems. We then present the methods that have been devised for inducible control of nuclear localization. These approaches fall into two broad categories: control via small molecules and control via light-mediated protein switches (Figure 1). Some of these methods lead to non-reversible nuclear accumulation/depletion, while some of the light-based techniques are compatible with repeated cycles of import and/or export. Reversible control is particularly promising for studying the influence of protein dynamics on cellular decisions. We conclude with a perspective on the most important challenges that need to be overcome to permit the widespread usage of these tools in research labs.

Nucleocytoplasmic transport

Transport in and out of the nucleus occurs through the nuclear pore complex (NPC) [4,6]. Nuclear pore proteins with arrays of phenylalanine-glycine repeats create a permeability barrier in the NPC that restricts the flow of proteins through the pore [7,8]. Smaller proteins (< 40 kDa) can diffuse through the barrier, while larger proteins require the assistance of nuclear transport receptors [9]. Karyopherins are the primary transport receptors used in the cell and can act as importins, helping proteins get into the nucleus, or exportins, helping proteins leave the nucleus [3]. Karyopherins generally bind to specific linear motifs on their transport cargoes.

Importin-α is a karyopherin that binds to nuclear localization signals (NLSs) on cargoes and interacts with Importin-β to direct proteins to the nucleus. The sequence and structural determinants of binding between Importin-α and NLS motifs have been extensively studied [10], and it has been shown that the affinity of Importin-α for a particular NLS influences transport efficiencies [11]. NLSs are often short sequences of positively charged lysines and arginines. CRM1 (chromosome region maintenance 1, exportin1) is the major nuclear export receptor and interacts with nuclear export signals (NESs) that are rich in hydrophobic amino acids [12]. Loading and unloading of cargo from the transport receptors is controlled by the GTPase Ran [13]. For a more extensive explanation of how nucleocytoplasmic transport functions we refer readers to other reviews focused on this topic [10,14,15].

Controlling nuclear localization as a strategy to control protein activity

Most cellular processes need to occur at specific times and locations within a cell for accurate and specific cellular function. Therefore, the appropriate proteins need to be activated in the right place, at the right time. This can be accomplished by always maintaining the proteins in the correct location, but keeping them inactive until needed [16,17]. Alternatively, activated proteins can be recruited to the correct location when needed [18,19]. Both strategies allow for rapid control as protein activation via post-translational modification and recruitment can occur much more quickly than transcription and translation. Gene expression is a prominent example of a process regulated this way [20,21]. Transcription factors and transcriptional co-regulators often are denied access to the nucleus where they would find their interacting partners (DNA, co-factors, etc.), remaining cytoplasmic until the proper stimuli trigger their nuclear accumulation. Nuclear accumulation can also be transient, to allow for adaptation and termination of cellular

response. Transient control is achieved by quickly shifting from rapid nuclear import to rapid export.

For example, Signal transducer and activator of transcription 1 (STAT1) is a transcription factor that is kept in a latent, monomeric form in the cytoplasm in the absence of activating signals (cytokines, hormones and growth factors). Upon signal transduction cascade initiation, STAT1 is phosphorylated at the plasma membrane by activated kinases (e.g. members of the Janus kinase (JAK) family) on a conserved tyrosine at its C- terminus and dimerizes [22–25]. Dimer formation induces a conformational change that presents a non-classical NLS to Importin-α5 leading to transport of phosphorylated, dimeric STAT1 into the nucleus [26,27]. Once inside the nucleus, phosphorylated, dimeric STAT1 binds to its cognate DNA responsive elements. DNA binding conceals STAT1 NES and prevents its dephosphorylation [28], thus nuclear accumulation is further enhanced through repression of export.

NF-κB is another example of a transcription factor kept in a latent form in the cytoplasm by interaction with its inhibitor IκB. The NF-κB/IκB complex constantly shuttles between the nucleus and the cytoplasm, but, since the NES on IκB is stronger than the NLS on NF-κB (partly concealed in the complex [29,30]), the resulting localization is heavily shifted towards the cytoplasm [31]. Upon arrival of the stimulus onto the cells, IκB gets phosphorylated, which leads to its ubiquitination and proteasomal degradation [32]. Once its inhibitor is removed, NF-κB can be imported in the nucleus via its fully exposed NLS.

Transcription can also be initiated by exporting repressors out of the nucleus, and terminated by re-importing the repressors in the nucleus [33]. For instance, class II histone deacetylases HDAC4 and −5 bind to the transcription factor MEF2 turning it into a repressor [34–37]. During myogenesis, this repression has to be released to allow transcription of muscle genes. This is achieved through export of HDAC4 and −5 from the nucleus. The mechanism involves phosphorylation of HDAC5 by calcium-calmodulin-dependent protein kinase (CaMK) on two serines (at positions 259 and 498) which triggers binding to the chaperone 14-3-3 and exposure of the otherwise cryptic NES on HDAC5 [38]. Binding to 14-3-3 may additionally block the NLS on HDAC5, thus having two synergistic effects to localize the protein to the cytosol [38]. In order for HDAC5 to reenter the nucleus dephosphorylation at the two serines is required, likely involving protein phosphatase function in this process.

Importantly, in the last years it is becoming evident that the dynamics of nuclear accumulation play a critical role in determining the cellular response to specific stimuli [39–42]. In order to unravel the importance of these dynamics on determining cellular output, methods to reversibly, quickly and precisely control the nuclear localization of proteins of interest are needed.

Methods to control nuclear localization

Chemical Control

Discovered in a screen for antifungal antibiotics, leptomycin B (LMB) is a branched-chain fatty acid that binds covalently to cysteine 528 on the surface of CRM1 and inhibits binding

to NES motifs [43]. Low nanomolar concentrations of LMB are sufficient to induce nuclear accumulation of proteins dependent on CRM1 for export, and similar molecules are currently being tested for anti-cancer activity [44]. However, LMB is a blunt tool for studying the function of individual proteins as it blocks the export of a large set of proteins, and long incubation times can lead to the induction of apoptosis or cell cycle arrest.

To control the transport of individual proteins, strategies have been developed that involve genetically modifying the protein of interest. Upon ligand binding, class-1 nuclear receptors, such as the estrogen receptor (ER), dissociate from heat shock proteins and translocate to the nucleus. Taking advantage of this natural mechanism, researchers have fused nuclear receptors to proteins of interest (POIs) in order to create ligand-dependent import [45]. In some cases, this has required engineering NES and/or NLS in the fusion protein to tune the export and import properties of the switch [46,47]. A fusion between ER and Cre recombinase is frequently used for inducing site-specific recombination with the ER ligand, tamoxifen [48].

Several groups have demonstrated that rapamycin-induced dimerization of the FK506 binding protein (FKBP) and the FKBP12-rapamycin-binding (FRB) domain of mTOR can be used to control the nuclear import or export of POIs (Figure 1) [49–53]. With this strategy one half of the dimer system is fused to the POI and the other half is fused to a NLS, NES, or to a protein that is known to undergo robust localization to the nucleus or cytosol. In *Saccharomyces cerevisiae* the ribosomal protein Rpl13a has proven effective at directing nuclear export as it is abundantly expressed and rapidly transported to the cytoplasm after being assembled into ribosomes in the nucleus [51]. Following exposure to rapamycin, the target is transported to the cytosol within 15 minutes. Similarly, nuclear import via rapamycin-induced recruitment of a FKBP-NLS chimera has been shown to occur in 10–15 minutes in yeast [50]. These approaches have been used to study a variety of processes including transcription [54], spindle positioning [55], and establishment of cell polarity [56]. One challenge that can arise when using rapamycin is that will also bind and inhibit endogenous mTOR, which can lead to unwanted toxicity. In yeast, this problem can be circumvented by using rapamycin-resistant strains that contain a mutated *TOR1* and deleted *FPR1*, a yeast homolog to FKBP12 [50,51]. Alternatively, rapamycin analogs (rapalogs) have been developed that only bind to mutated forms of FRB [57]. It has been shown that by using multiple rapalogs and engineered proteins simultaneously it is possible to direct a POI to the nucleus with one rapalog, and to the cytosol with a separate rapalog [58]. For further details on chemical dimerization systems we refer readers to other reviews on this topic [59–61].

Light-inducible control

Irreversible control—Light-inducible control of nuclear transport has gained significant interest lately because it offers some advantages when compared to chemical induction. Using lasers, light can be applied with very tight spatial resolution in living cells and animals, and it can be rapidly turned on and off. In an early example of light-mediated control, an NLS peptide was synthesized with a critical lysine modified with a photocleavable group sensitive to UV light [62]. The modified NLS was conjugated to

BSA*in vitro* and then microinjected into cells. Only after UV stimulation could the NLS bind to importins, and recruit BSA to the nucleus. More recently, to avoid the need for microinjection, a genetically encoded system was developed for expression of caged lysines and used to activate a NLS fused to a transcription factor (Figure 1). This approach is not reversible and requires special expression systems for the caged lysine [63].

Another UV light-based approach consists in using the *Arabidopsis thaliana* UVR8 photoreceptor which binds to the E3 ubiquitin ligase COP1 after irradiation with UV-B light (280–315 nm) [64,65]. The basic idea behind this approach is very similar to the one that characterizes rapamycin-based methods: one of the two components should reside inside the nucleus the whole time, while the other component fused to the POI should be mostly cytoplasmic but still shuttling inside the nucleus where it can find its binding partner, in this case after illumination with UV-B light. When testing accumulation of GFP fused to UVR8, Crefcoeur and colleagues observe slow kinetics of nuclear accumulation [66]. Kinetics might get slower for a POI larger than GFP [67–69]. This system is irreversible, therefore it is mostly suited for applications where the protein to be light-controlled has an all-or-none function (e.g. Cre recombinase). In this case, import kinetics are likely less critical.

Reversible control

Methods based on the red/far-red responsive PhyB-PIF system: The plant phytochrome B (PhyB) exists in three conformations: 1) the apo-form not bound to the chromophore phycocyanobilin (PCB); 2) the chromophore-bound, red light absorbing Pr conformation, which is biologically inactive; and 3) the chromophore-bound, infrared absorbing Pfr conformation, which is active [70]. PhyB in the Pfr form interacts with phytochrome interacting factors (PIFs) and the interaction is released by infrared illumination, which brings PhyB back to its red-absorbing, non-PIF binding Pr state [71]. This light-dependent complex formation has been successfully used in the engineering of optogenetic control of protein-protein interactions [72–76]. More recently the system has been adapted to specifically control nuclear translocation. There are two slightly different versions of PhyB-PIF-mediated nuclear translocation.

In one version, developed by Yang and co-workers for usage in budding yeast, PhyB is either anchored to the nucleus by means of a fusion to the histone H2B or kept nuclear by means of fusion to a NLS [77]. The POI is fused to a fragment of PIF6 (aa 1–100) and is equally distributed between cytoplasm and nucleus. Nuclear localization is likely due to the presence of a weak NLS on PIF. Once red light is shone on the cells, PhyB and PIF interact, thereby causing the retention of the POI in the nucleus. This method has been shown to work with fluorescent proteins of various sizes (e.g. mCitrine, GFP-GFP or Venus-Venus-Venus) and with the biologically relevant protein cyclin Clb2. Using their optogenetic system, the authors investigated interesting aspects of Clb2 biology, such as the time during the cell cycle at which it is needed for nuclear fission. Importantly, this tool is not restricted to nuclear localization, as by simply selecting another type of anchor the POI can be recruited to various cellular addresses, including small organelles such as the spindle pole body, peroxisomes and the nucleolus. Indeed, by choosing the plasma membrane as anchor,

the POI can be depleted from the nucleus, effectively producing an optogenetic version of techniques based on chemically induced dimerization.

In another variation developed by Beyer and co-workers for use in mammalian cells, the POI is fused to PhyB and can contain a NES to allow return of the fusion protein to the cytoplasm after nuclear localization (without additional NES the fusion protein is trapped in the nucleus and the tool is, thus, irreversible [78]). The PIF used here is the full length PIF3 from *Arabidopsis thaliana*. The authors take advantage of the biology of the system, whereby PhyB gets translocated in the nucleus by virtue of its interaction with PIF3 under red light [78] (Figure 1). This system has been used to control gene expression in mammalian cells and zebrafish using a synthetic transcription factor based on the TetR binding domain and the VP16 transactivation domain.

Both approaches based on the PhyB-PIF system share many properties. First of all, there is no light absorption (and therefore complex formation) until the PCB chromophore is added, which provides a tight off state for the start of experiments. When addition of the chromophore is not a problem –e.g. in cell culture or at the early stages of development in zebrafish larvae (when done by injection) – these methods are quite advantageous. However, having to externally add the chromophore mitigates other advantages of red light, i.e. its deeper penetration into tissue, making the use of the PhyB-PIF system in animals for instance more challenging. While production of PCB by Chinese hamster ovary cells has been established by introducing two enzymes that convert heme into PCB [79], the question remains whether adopting the same strategy in animals would be possible given that heme is a very important cofactor in many reactions, among which cellular respiration.

The interaction between PhyB and PIF can be quickly disrupted at any time point by shining infrared light onto the cells, meaning that the tools have a high temporal resolution, only limited by nucleocytoplasmic transport itself. Reversibility is, though, obtained at the cost of equipping the microscope with a filter set or laser for infrared light. Finally, these methods are based on two components and, therefore, the concentration of each one needs to be carefully titrated to achieve quantitative control of protein nuclear accumulation kinetics.

Methods based on the blue light responsive LOV2 domain of *Avena sativa*: Light Oxygen Voltage (LOV) domains are protein domains that sense changes in oxygen, redox potential and light in cells [80,81]. The second LOV domain of *Avena sativa* phototrophin 1 (*As LOV2*) has been extensively used for creating optogenetic switches because it is small, binds a chromophore that is abundant in nature (Flavin Mononucelotide, FMN), and undergoes a well characterized structural transition with blue light stimulation that can be harnessed to regulate protein-protein interactions and protein function (see Box 1 for more details).

Recently, methods to control nuclear protein import and export with the *As LOV2* domain have been developed [82,83,86,87] (Figure 1). These methods all control the accessibility of engineered NLSs and NESs by embedding them in the Jα helix of *As LOV2* so that they are more exposed when the Jα helix undocks from the rest of the protein in the lit state (Box 1 and Figure 2a). Interestingly, the tools for import (LINuS and LANS), as well as those for

export (LEXY and LINX), were independently developed in two laboratories, providing evidence that this approach is robust, can be used on different proteins, in several cell lines and animals, and for different biological applications.

One useful feature of these systems is that they are single-component (Figure 2 b–d). The POI is fused to the modified *As LOV2* (which is as small as GFP) bearing either a caged NLS (LINuS and LANS) or NES (LEXY and LINX), and is expressed in cells. In the presence of blue light, the exposed NLS/NES interacts with the endogenous import/export machinery triggering accumulation of the fusion protein in the nucleus/cytoplasm (Figure 2). These methods have been used to control entry into mitosis [87], gene expression from synthetic [82,83,86,87] or natural transcription factors such as p53 [86] and an E3 ubiquitin ligase that modifies histone H2B in yeast [82]. In the latter study, rapid removal of the E3 ligase from the nucleus allowed the investigators to monitor the *in vivo* stability of the monoubiquitylated H2B. Surprisingly, the modification was removed within two minutes, highlighting the dynamic nature of epigenetic modifications and demonstrating the usefulness of light-activated tools for studying dynamic processes.

Methods based on the LOV2 domain suffer from the problem of “leakiness”, i.e. activation of the system prior to illumination. This is due to the fact that the LOV2 domain always exists in its two conformations and light only shifts the equilibrium towards the lit state [94]. This means that, even in the dark, there is always some spontaneous uncaging of the NLS/NES with consequential import/export of the fusion protein. To compensate for this, Niopek and colleagues, and Yumerefendi and colleagues added a constitutively exposed countersignal –that is, a NES for their import tools and a NLS for their export tools –to ensure that the fusion protein rapidly returns to the appropriate compartment in the dark state. Notably, such additional sequences might not be necessary when the POI already contains them. Interestingly, these tools can also be used in combination with other optogenetic switches; for instance, by using the LYNX switch in tandem with a light-induced dimer that recruited the LYNX-POI fusion to the mitochondria in the light, the authors further reduced nuclear levels in the lit state [82]. In another example, the tools for nuclear import and export were combined to control with the same light pulse the movement of two fluorescent proteins in opposing directions [86]. One attractive feature of *As LOV2*-based systems is that the FMN chromophore is endogenously made by all cell types, readily allowing experiments in animals [96]. The shallow tissue penetration of blue light can be bypassed for example by using upconversion nanoparticles (UCNPs) which convert near-infrared light into visible light [97–100].

Opto-LMB: Niopek and colleagues also created a different version of their export LEXY tool which effectively corresponds to a light-inducible LMB treatment (the authors call it Opto-LMB). By fusing the engineered *As LOV2*-NES domain to histone H2B, their anchored a light-inducible CRM1 “sponge” into the nucleus. When cells are illuminated with blue light, the NES gets exposed binding the endogenous export receptors, making them unavailable to bind to and export other cargos [86]. Albeit being less tight than LMB itself, Opto-LMB is genetically encoded and fully reversible, thus problems arising from LMB toxicity can be avoided. Moreover, as NESs of different strengths can be caged within

the LOV2 domain, this tool can be tuned to block the export of a subset of endogenous targets.

Conclusion and outlook

Since many biological processes are controlled by the timely translocation of proteins in or out of the nucleus, being able to induce such translocation with an external trigger is very important for cell biological studies. Nowadays, this is possible with the tools presented here, among which cell biologists can find the one most suited for the specific application (Table 1). Still, in most cases, optimization of the selected switch is required to obtain optimal results with the POI in any given cellular system (see for instance [102]).

While many studies are conducted with overexpressed proteins (i.e. transient transfection of plasmid-bore engineered construct in cells), modern genome-engineering techniques such as CRISPR/Cas [103] will allow for more studies with protein expressed from their endogenous locus. In this case, single-component tools are advantageous requiring only appending the photoswitch to either terminus of the POI. Notably, Yumerefendi and colleagues demonstrated that LANS functioned when integrated in the genome of *C. elegans* [83].

Finally, a challenge for the future will be to find ways to directly control the localization of unmodified, endogenous proteins. We speculate that combining the switches presented here with engineered peptides or proteins [104] that bind with high specificity and affinity their targets is a promising strategy in this direction.

Acknowledgments

We thank Robert Grosse for critical reading of the manuscript. This work was supported by the BMBF 031L0079 grant (BDV) and NIH DA036877 grant (BK).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Weis K. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. Cell. 2003; 112:441–451. [PubMed: 12600309]
2. Komeili A, O'Shea EK. Nuclear transport and transcription. Curr Opin Cell Biol. 2000; 12:355–360. [PubMed: 10801461]
3. Chook YM, Suel KE. Nuclear import by karyopherin-betas: recognition and inhibition. Biochim Biophys Acta. 2011; 1813:1593–1606. [PubMed: 21029754]
4. Gorlich D, Kriwacki RW. Editorial Overview: Functional and Mechanistic Landscape of the Nuclear Pore Complex. J Mol Biol. 2016
5. Gorlich D, Henklein P, Laskey RA, Hartmann E. A 41 amino acid motif in Importin-αlpha confers binding to importin-beta and hence transit into the nucleus. EMBO J. 1996; 15:1810–1817. [PubMed: 8617226]
6. Knockenhauer KE, Schwartz TU. The Nuclear Pore Complex as a Flexible and Dynamic Gate. Cell. 2016; 164:1162–1171. [PubMed: 26967283]

7. Schmidt HB, Gorlich D. Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear porelike permselectivity. Elife. 2015:4.
8. Terry LJ, Wente SR. Flexible gates: dynamic topologies and functions for FG nucleoporins in nucleocytoplasmic transport. Eukaryot Cell. 2009; 8:1814–1827. [PubMed: 19801417]
9. Mohr D, Frey S, Fischer T, Guttler T, Gorlich D. Characterisation of the passive permeability barrier of nuclear pore complexes. EMBO J. 2009; 28:2541–2553. [PubMed: 19680228]
10. Christie M, Chang CW, Rona G, Smith KM, Stewart AG, Takeda AA, Fontes MR, Stewart M, Vertessy BG, Forwood JK, et al. Structural Biology and Regulation of Protein Import into the Nucleus. J Mol Biol. 2015
11. Hodel AE, Harreman MT, Pulliam KF, Harben ME, Holmes JS, Hodel MR, Berland KM, Corbett AH. Nuclear localization signal receptor affinity correlates with in vivo localization in Saccharomyces cerevisiae. J Biol Chem. 2006; 281:23545–23556. [PubMed: 16785238]
12. Hutten S, Kehlenbach RH. CRM1-mediated nuclear export: to the pore and beyond. Trends Cell Biol. 2007; 17:193–201. [PubMed: 17317185]
13. Matsuura Y. Mechanistic Insights from Structural Analyses of Ran-GTPase-Driven Nuclear Export of Proteins and RNAs. J Mol Biol. 2015
14. Tran EJ, King MC, Corbett AH. Macromolecular transport between the nucleus and the cytoplasm: Advances in mechanism and emerging links to disease. Biochim Biophys Acta. 2014; 1843:2784–2795. [PubMed: 25116306]
15. Mor A, White MA, Fontoura BM. Nuclear trafficking in health and disease. Curr Opin Cell Biol. 2014; 28:28–35. [PubMed: 24530809]
16. Gadaleta RM, Magnani L. Nuclear receptors and chromatin: an inducible couple. J Mol Endocrinol. 2014; 52:R137–R149. [PubMed: 24363438]
17. Latz E, Verma A, Visintin A, Gong M, Sirois CM, Klein DC, Monks BG, McKnight CJ, Lamphier MS, Duprex WP, et al. Ligand-induced conformational changes allosterically activate Toll-like receptor 9. Nat Immunol. 2007; 8:772–779. [PubMed: 17572678]
18. Michaelson D, Abidi W, Guardavaccaro D, Zhou M, Ahearn I, Pagano M, Philips MR. Rac1 accumulates in the nucleus during the G2 phase of the cell cycle and promotes cell division. J Cell Biol. 2008; 181:485–496. [PubMed: 18443222]
19. Wiatrowski HA, Carlson M. Yap1 accumulates in the nucleus in response to carbon stress in Saccharomyces cerevisiae. Eukaryot Cell. 2003; 2:19–26. [PubMed: 12582119]
20. Fujihara SM, Nadler SG. Modulation of nuclear protein import: a novel means of regulating gene expression. Biochem Pharmacol. 1998; 56:157–161. [PubMed: 9698068]
21. Suzuki H, Tashiro S, Hira S, Sun J, Yamazaki C, Zenke Y, Ikeda-Saito M, Yoshida M, Igarashi K. Heme regulates gene expression by triggering Crm1-dependent nuclear export of Bach1. EMBO J. 2004; 23:2544–2553. [PubMed: 15175654]
22. Fu XY. A transcription factor with SH2 and SH3 domains is directly activated by an interferon alpha-induced cytoplasmic protein tyrosine kinase(s). Cell. 1992; 70:323–335. [PubMed: 1638633]
23. Gutch MJ, Daly C, Reich NC. Tyrosine phosphorylation is required for activation of an alpha interferon-stimulated transcription factor. Proc Natl Acad Sci U S A. 1992; 89:11411–11415. [PubMed: 1280824]
24. Schindler C, Shuai K, Prezioso VR, Darnell JE Jr. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science. 1992; 257:809–813. [PubMed: 1496401]
25. Shuai K, Stark GR, Kerr IM, Darnell JE Jr. A single phosphotyrosine residue of Stat91 required for gene activation by interferon-gamma. Science. 1993; 261:1744–1746. [PubMed: 7690989]
26. Sekimoto T, Imamoto N, Nakajima K, Hirano T, Yoneda Y. Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. EMBO J. 1997; 16:7067–7077. [PubMed: 9384585]
27. Wenta N, Strauss H, Meyer S, Vinkemeier U. Tyrosine phosphorylation regulates the partitioning of STAT1 between different dimer conformations. Proc Natl Acad Sci U S A. 2008; 105:9238–9243. [PubMed: 18591661]

28. Meyer T, Marg A, Lemke P, Wiesner B, Vinkemeier U. DNA binding controls inactivation and nuclear accumulation of the transcription factor Stat1. Genes Dev. 2003; 17:1992–2005. [PubMed: 12923054]
29. Huxford T, Huang DB, Malek S, Ghosh G. The crystal structure of the IkappaBalpha/NF-kappaB complex reveals mechanisms of NF-kappaB inactivation. Cell. 1998; 95:759–770. [PubMed: 9865694]
30. Jacobs MD, Harrison SC. Structure of an IkappaBalpha/NF-kappaB complex. Cell. 1998; 95:749–758. [PubMed: 9865693]
31. Huang TT, Kudo N, Yoshida M, Miyamoto S. A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes. Proc Natl Acad Sci U S A. 2000; 97:1014–1019. [PubMed: 10655476]
32. Chen Z, Hagler J, Palombella VJ, Melandri F, Scherer D, Ballard D, Maniatis T. Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. Genes Dev. 1995; 9:1586–1597. [PubMed: 7628694]
33. Le Gallic L, Sgouras D, Beal G Jr, Mavrothalassitis G. Transcriptional repressor ERF is a Ras/mitogen-activated protein kinase target that regulates cellular proliferation. Mol Cell Biol. 1999; 19:4121–4133. [PubMed: 10330152]
34. Miska EA, Karlsson C, Langley E, Nielsen SJ, Pines J, Kouzarides T. HDAC4 deacetylase associates with and represses the MEF2 transcription factor. EMBO J. 1999; 18:5099–5107. [PubMed: 10487761]
35. Wang AH, Bertos NR, Vezmar M, Pelletier N, Crosato M, Heng HH, Th'ng J, Han J, Yang XJ. HDAC4, a human histone deacetylase related to yeast HDA1, is a transcriptional corepressor. Mol Cell Biol. 1999; 19:7816–7827. [PubMed: 10523670]
36. Lemercier C, Verdel A, Galloo B, Curtet S, Brocard MP, Khochbin S. mHDA1/HDAC5 histone deacetylase interacts with and represses MEF2A transcriptional activity. J Biol Chem. 2000; 275:15594–15599. [PubMed: 10748098]
37. Lu J, McKinsey TA, Nicol RL, Olson EN. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. Proc Natl Acad Sci U S A. 2000; 97:4070–4075. [PubMed: 10737771]
38. McKinsey TA, Zhang CL, Olson EN. Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. Mol Cell Biol. 2001; 21:6312–6321. [PubMed: 11509672]
39. Nelson DE, Ihekwaba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, et al. Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science. 2004; 306:704–708. [PubMed: 15499023]
40. Hao N, O'Shea EK. Signal-dependent dynamics of transcription factor translocation controls gene expression. Nat Struct Mol Biol. 2012; 19:31–39.
41. Purvis JE, Karhohs KW, Mock C, Batchelor E, Loewer A, Lahav G. p53 dynamics control cell fate. Science. 2012; 336:1440–1444. [PubMed: 22700930]
42. Hansen AS, O'Shea EK. Encoding four gene expression programs in the activation dynamics of a single transcription factor. Curr Biol. 2016; 26:R269–R271. [PubMed: 27046808] •• Using a small molecule, different dynamics of the yeast transcription factor Msn2 are generated and shown to activate different promoters.
43. Kudo N, Matsumori N, Taoka H, Fujiwara D, Schreiner EP, Wolff B, Yoshida M, Horinouchi S. Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. Proc Natl Acad Sci U S A. 1999; 96:9112–9117. [PubMed: 10430904]
44. Dickmanns A, Monecke T, Ficner R. Structural Basis of Targeting the Exportin CRM1 in Cancer. Cells. 2015; 4:538–568. [PubMed: 26402707]
45. Eilers M, Picard D, Yamamoto KR, Bishop JM. Chimaeras of myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells. Nature. 1989; 340:66–68. [PubMed: 2662015]
46. Kakar M, Davis JR, Kern SE, Lim CS. Optimizing the protein switch: altering nuclear import and export signals, and ligand binding domain. J Control Release. 2007; 120:220–232. [PubMed: 17574289]

47. Davis JR, Mossalam M, Lim CS. Controlled access of p53 to the nucleus regulates its proteasomal degradation by MDM2. Mol Pharm. 2013; 10:1340–1349. [PubMed: 23398638]
48. Feil R, Brocard J, Mascrez B, LeMeur M, Metzger D, Chambon P. Ligand-activated site-specific recombination in mice. Proc Natl Acad Sci U S A. 1996; 93:10887–10890. [PubMed: 8855277]
49. Klemm JD, Beals CR, Crabtree GR. Rapid targeting of nuclear proteins to the cytoplasm. Curr Biol. 1997; 7:638–644. [PubMed: 9285717]
50. Geda P, Patury S, Ma J, Bharucha N, Dobry CJ, Lawson SK, Gestwicki JE, Kumar A. A small molecule-directed approach to control protein localization and function. Yeast. 2008; 25:577–594. [PubMed: 18668531]
51. Haruki H, Nishikawa J, Laemmli UK. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. Mol Cell. 2008; 31:925–932. [PubMed: 18922474]
52. Xu T, Johnson CA, Gestwicki JE, Kumar A. Conditionally controlling nuclear trafficking in yeast by chemical-induced protein dimerization. Nat Protoc. 2010; 5:1831–1843. [PubMed: 21030958]
53. Ding L, Laor D, Weisman R, Forsburg SL. Rapid regulation of nuclear proteins by rapamycin-induced translocation in fission yeast. Yeast. 2014; 31:253–264. [PubMed: 24733494]
54. Crickard JB, Fu J, Reese JC. Biochemical Analysis of Yeast Suppressor of Ty 4/5 (Spt4/5) Reveals the importance of Nucleic Acid Interactions in the Prevention of RNA Polymerase II Arrest. J Biol Chem. 2016
55. Schweiggert J, Stevermann L, Panigada D, Kammerer D, Liakopoulos D. Regulation of a Spindle Positioning Factor at Kinetochores by SUMO-Targeted Ubiquitin Ligases. Dev Cell. 2016; 36:415–427. [PubMed: 26906737]
56. Wu CF, Chiou JG, Minakova M, Woods B, Tsygankov D, Zyla TR, Savage NS, Elston TC, Lew DJ. Role of competition between polarity sites in establishing a unique front. Elife. 2015:4.
57. Liberles SD, Diver ST, Austin DJ, Schreiber SL. Inducible gene expression and protein translocation using nontoxic ligands identified by a mammalian three-hybrid screen. Proc Natl Acad Sci U S A. 1997; 94:7825–7830. [PubMed: 9223271]
58. Bayle JH, Grimley JS, Stankunas K, Gestwicki JE, Wandless TJ, Crabtree GR. Rapamycin analogs with differential binding specificity permit orthogonal control of protein activity. Chem Biol. 2006; 13:99–107. [PubMed: 16426976]
59. Putyrski M, Schultz C. Protein translocation as a tool: The current rapamycin story. FEBS Lett. 2012; 586:2097–2105. [PubMed: 22584056]
60. Corson TW, Aberle N, Crews CM. Design and Applications of Bifunctional Small Molecules: Why Two Heads Are Better Than One. ACS Chem Biol. 2008; 3:677–692. [PubMed: 19112665]
61. Pollock R, Clackson T. Dimerizer-regulated gene expression. Curr Opin Biotechnol. 2002; 13:459–467. [PubMed: 12459338]
62. Watai Y, Sase I, Shiono H, Nakano Y. Regulation of nuclear import by light-induced activation of caged nuclear localization signal in living cells. FEBS Lett. 2001; 488:39–44. [PubMed: 11163792]
63. Engelke H, Chou C, Uprety R, Jess P, Deiters A. Control of protein function through optochemical translocation. ACS Synth Biol. 2014; 3:731–736. [PubMed: 24933258] • Genetically encoded modified lysines are used to generate a conditional NLS on a protein of interest that gets recognized by importins only after UV irradiation.
64. Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schafer E, Nagy F, Jenkins GI, et al. Perception of UV-B by the Arabidopsis UVR8 protein. Science. 2011; 332:103–106. [PubMed: 21454788]
65. Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M, Albert A, Cloix C, Jenkins GI, Oakeley EJ, et al. Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J. 2009; 28:591–601. [PubMed: 19165148]
66. Crefcoeur RP, Yin R, Ulm R, Halazonetis TD. Ultraviolet-B-mediated induction of protein-protein interactions in mammalian cells. Nat Commun. 2013; 4:1779. [PubMed: 23653191] • A method to control the import of a protein of interest based on the UVR8 photoreceptor is presented.
67. Lowe AR, Siegel JJ, Kalab P, Siu M, Weis K, Liphardt JT. Selectivity mechanism of the nuclear pore complex characterized by single cargo tracking. Nature. 2010; 467:600–603. [PubMed: 20811366]

68. Lyman SK, Guan T, Bednenko J, Wodrich H, Gerace L. Influence of cargo size on Ran and energy requirements for nuclear protein import. J Cell Biol. 2002; 159:55–67. [PubMed: 12370244]
69. Ribbeck K, Gorlich D. The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. EMBO J. 2002; 21:2664–2671. [PubMed: 12032079]
70. Rockwell NC, Su YS, Lagarias JC. Phytochrome structure and signaling mechanisms. Annu Rev Plant Biol. 2006; 57:837–858. [PubMed: 16669784]
71. Ni M, Tepperman JM, Quail PH. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature. 1999; 400:781–784. [PubMed: 10466729]
72. Toettcher JE, Weiner OD, Lim WA. Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. Cell. 2013; 155:1422–1434. [PubMed: 24315106] •• Using the PhyB/PIF system, different dynamics of Ras/Erk activation are generated and shown to lead to different cellular responses.
73. Toettcher JE, Gong D, Lim WA, Weiner OD. Light-based feedback for controlling intracellular signaling dynamics. Nat Methods. 2011; 8:837–839. [PubMed: 21909100]
74. Toettcher JE, Gong D, Lim WA, Weiner OD. Light control of plasma membrane recruitment using the Phy-PIF system. Methods Enzymol. 2011; 497:409–423. [PubMed: 21601096]
75. Levskaya A, Weiner OD, Lim WA, Voigt CA. Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature. 2009; 461:997–1001. [PubMed: 19749742]
76. Leung DW, Otomo C, Chory J, Rosen MK. Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway. Proc Natl Acad Sci U S A. 2008; 105:12797–12802. [PubMed: 18728185]
77. Yang X, Jost AP, Weiner OD, Tang C. A light-inducible organelle-targeting system for dynamically activating and inactivating signaling in budding yeast. Mol Biol Cell. 2013; 24:2419–2430. [PubMed: 23761071] •• Using the PhyB/PIF system, a palette of anchors is generated to reversibily target a protein of interest to almost any organelle in budding yeast. Very interesting biological studies are conducted to understand better the timing of activation and the requirements in terms of subcellular localization for the mitotic cyclin Clb2.
78. Beyer HM, Juillot S, Herbst K, Samodelov SL, Muller K, Schamel WW, Romer W, Schafer E, Nagy F, Strahle U, et al. Red Light-Regulated Reversible Nuclear Localization of Proteins in Mammalian Cells and Zebrafish. ACS Synth Biol. 2015; 4:951–958. [PubMed: 25803699] •• A method to reversibly control the nuclear accumulation of a protein of interest in mammalian cells based on the PhyB/PIF is presented. Very tight and efficient control of gene expression with red light in mammalian cells and zebrafish is demonstrated.
79. Muller K, Engesser R, Timmer J, Nagy F, Zurbriggen MD, Weber W. Synthesis of phycocyanobilin in mammalian cells. Chem Commun (Camb). 2013; 49:8970–8972. [PubMed: 23963496]
80. Briggs WR, Christie JM, Salomon M. Phototropins: a new family of flavin-binding blue light receptors in plants. Antioxid Redox Signal. 2001; 3:775–788. [PubMed: 11761327]
81. Kottke T, Hegemann P, Dick B, Heberle J. The photochemistry of the light-, oxygen-, and voltage-sensitive domains in the algal blue light receptor phot. Biopolymers. 2006; 82:373–378. [PubMed: 16552739]
82. Yumerefendi H, Lerner AM, Zimmerman SP, Hahn K, Bear JE, Strahl BD, Kuhlman B. Light-induced nuclear export reveals rapid dynamics of epigenetic modifications. Nat Chem Biol. 2016 •• Paper introducing LINX, an *As LOV2*-based method to reversibly control cytoplasmic accumulation of a protein of interest in mammalian cells and yeast. The tool is used to study the dynamics of epigenetic modifications in yeast.
83. Yumerefendi H, Dickinson DJ, Wang H, Zimmerman SP, Bear JE, Goldstein B, Hahn K, Kuhlman B. Control of Protein Activity and Cell Fate Specification via Light-Mediated Nuclear Translocation. PLoS One. 2015; 10:e0128443. [PubMed: 26083500] •• Paper introducing LANS, an *As LOV2*-based method to reversibly control nuclear accumulation of a protein of interest in mammalian cells and *C. elegans*. This work shows that genomic incorporation of LANS can be used to control an endogenously expressed protein in worms.
84. Yi JJ, Wang H, Vilela M, Danuser G, Hahn KM. Manipulation of endogenous kinase activity in living cells using photoswitchable inhibitory peptides. ACS Synth Biol. 2014; 3:788–795. [PubMed: 24905630]

85. Strickland D, Lin Y, Wagner E, Hope CM, Zayner J, Antoniou C, Sosnick TR, Weiss EL, Glotzer M. TULIPs: tunable, light-controlled interacting protein tags for cell biology. Nat Methods. 2012; 9:379–384. [PubMed: 22388287]

86. Niopek D, Wehler P, Roensch J, Eils R, Di Ventura B. Optogenetic control of nuclear protein export. Nat Commun. 2016; 7:10624. [PubMed: 26853913] •• Paper introducing LEXY, an *As LOV2*-based method to reversibly control cytoplasmic accumulation of a protein of interest in mammalian cells. Here an optogenetic version of LMB is presented.

87. Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, Di Ventura B. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. Nat Commun. 2014; 5:4404. [PubMed: 25019686] •• Paper introducing LINuS, an *As LOV2*-based method to reversibly control nuclear accumulation of a protein of interest in mammalian cells and yeast.

88. Mart RJ, Meah D, Allemann RK. Photocontrolled Exposure of Pro-apoptotic Peptide Sequences in LOV Proteins Modulates Bcl-2 Family Interactions. Chembiochem. 2016; 17:698–701. [PubMed: 26493687]

89. Lungu OI, Hallett RA, Choi EJ, Aiken MJ, Hahn KM, Kuhlman B. Designing photoswitchable peptides using the AsLOV2 domain. Chem Biol. 2012; 19:507–517. [PubMed: 22520757]

90. Guntas G, Hallett RA, Zimmerman SP, Williams T, Yumerefendi H, Bear JE, Kuhlman B. Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins. Proc Natl Acad Sci U S A. 2015; 112:112–117. [PubMed: 25535392]

91. Crosson S, Moffat K. Structure of a flavin-binding plant photoreceptor domain: insights into light-mediated signal transduction. Proc Natl Acad Sci U S A. 2001; 98:2995–3000. [PubMed: 11248020]

92. Harper SM, Neil LC, Gardner KH. Structural basis of a phototropin light switch. Science. 2003; 301:1541–1544. [PubMed: 12970567]

93. Zoltowski BD, Vaccaro B, Crane BR. Mechanism-based tuning of a LOV domain photoreceptor. Nat Chem Biol. 2009; 5:827–834. [PubMed: 19718042]

94. Yao X, Rosen MK, Gardner KH. Estimation of the available free energy in a LOV2-J alpha photoswitch. Nat Chem Biol. 2008; 4:491–497. [PubMed: 18604202]

95. Strickland D, Yao X, Gawlak G, Rosen MK, Gardner KH, Sosnick TR. Rationally improving LOV domain-based photoswitches. Nat Methods. 2010; 7:623–626. [PubMed: 20562867]

96. Hayashi-Takagi A, Yagishita S, Nakamura M, Shirai F, Wu YI, Loshbaugh AL, Kuhlman B, Hahn KM, Kasai H. Labelling and optical erasure of synaptic memory traces in the motor cortex. Nature. 2015; 525:333–338. [PubMed: 26352471] • An example of how light-activated control of intracellular signaling can be used to control and study complicated biological processes in living animals.

97. Shen J, Zhao L, Han G. Lanthanide-doped upconverting luminescent nanoparticle platforms for optical imaging-guided drug delivery and therapy. Adv Drug Deliv Rev. 2013; 65:744–755. [PubMed: 22626980]

98. He L, Zhang Y, Ma G, Tan P, Li Z, Zang S, Wu X, Jing J, Fang S, Zhou L, et al. Near-infrared photoactivatable control of Ca(2+) signaling and optogenetic immunomodulation. Elife. 2015:4. •• Paper introducing Opto-CRAC, an AsLOV2-based tool to control calcium oscillations in non-excitable cells including T lymphocytes, macrophages and dendritic cells. Here the usage of upconversion nanoparticles to convert near-infrared light to visible light is shown.

99. Chen G, Qiu H, Prasad PN, Chen X. Upconversion nanoparticles: design, nanochemistry, and applications in theranostics. Chem Rev. 2014; 114:5161–5214. [PubMed: 24605868]

100. Cheng L, Yang K, Shao M, Lu X, Liu Z. In vivo pharmacokinetics, long-term biodistribution and toxicology study of functionalized upconversion nanoparticles in mice. Nanomedicine (Lond). 2011; 6:1327–1340. [PubMed: 21834646]

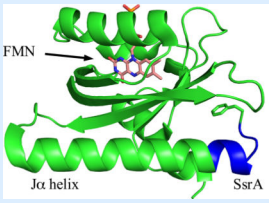
101. Juillot S, Beyer HM, Madl J, Weber W, Zurbriggen MD, Romer W. Signalling to the nucleus under the control of light and small molecules. Mol Biosyst. 2016; 12:345–349. [PubMed: 26672971]

102. Wehler P, Niopek D, Eils R, Di Ventura B. Optogenetic control of nuclear protein import in living cells using light-inducible nuclear localization signals (LINuS). Current Protocols in Chemical Biology. 2016; 8:1–15. [PubMed: 26995352]

103. Puchta H. Genome engineering using CRISPR/Cas: getting more versatile and more precise at the same time. Genome Biol. 2016; 17:51. [PubMed: 26987411]

104. Reverdatto S, Burz DS, Shekhtman A. Peptide aptamers: development and applications. Curr Top Med Chem. 2015; 15:1082–1101. [PubMed: 25866267]

Box 1




```
                                521                 546                 Reference
AsLOV2          RDAAEREGVMLIKKTAENIDEAAKELPDANLRPEDLWANHSKVVL
LANS            RDAAEREGVMLIKKTAENIDMAAKRVKLD---------------- [67]
LINuS           RDAAEREGVMLIKKTAENIAAAKRVKLD---------------- [71]
biLINuS10       RDAAEREGVMLIKKTAENIDEAAKRLPDANLAAAAAAAAKKKKLD [71]
LEXY            RDAAEREGVMLIKKTAENIDELLKELADLNLD------------ [70]
LINX            RDAAEREGVMLIKKTAENIDEAAKELAGLDL------------- [66]
LOV-PKI         RDAAEREGVMLIKKTAENIDEGRTGRRNAI--------------- [68]
LOV-ipaA        RDAAEREGVMLIKKTANNIIKAAKDVTTSLSKVLKNIN------- [73]
TULIP           RDAAEREGVMLAKKTAENIDKAVDTWV----------------- [69]
LOV-BID1        RDAAEDIGVNIARHLAQVGDSIDRSIPDANLRPEDLWAN------ [72]
iLID(LOV-SsrA) HGAAEREAVMLIKKTAFQIAEAANDENYF---------------- [74]
```

Using the AsLOV2 domain to regulate peptide-protein interactions. Several studies have demonstrated that the LOV2 domain from *Avena sativa* phototropin 1 (*As LOV2*) can be used to effectively cage peptides so that they have reduced affinity for their binding partners in the dark, but binding is restored in the light [82–90]. This change in binding affinity can be used to regulate a variety of cellular processes including nuclear import and export. *As LOV2* binds a flavin mononucleotide (FMN) in the center of the protein. Upon irradiation with blue light, a metastable covalent bond is formed between a cysteine in the core of the protein and a carbon atom of the FMN [91]. NMR studies indicate that this leads to large conformational changes in the protein, including undocking and unfolding of the C-terminal helix of the protein (the Jα helix) [92]. When the light is turned off, the metastable bond breaks within seconds to hours, depending on the LOV domain ortholog (for *As LOV2* the reversion time is ~30 sec.) [93], and the the Jα helix refolds. It is important to point out that the changes to the Jα helix between the lit and dark state are not an all or none process, but rather there is always an equilibrium between the docked/folded state and the undocked/unfolded state. For wild type *As LOV2* the docked/undocked ratio is 98.4/1.6 In the dark and 9/91 in the lit state [94]. The sequence alignment shows the various positions at which investigators have placed peptides of interest (underlined residues) in the *As LOV2* to achieve light sensitivity. In order to achieve caging in the dark it is important to have at least a few critical residues from the peptide embedded in the last helical turn of the Jα helix. In creating the chimeric sequences, it is also important to conserve the hydrophobicity of the Jα residues that are packed against the core of the domain. The crystal structure shown here is of the *As LOV2* domain with the SsrA peptide embedded in the Jα helix [90]. This construct also includes an engineered phenylalanine at the end of the Jα helix that packs back against the domain and further holds the dark state closed. Other Jα helix mutations have also been discovered that stabilize the closed state of the protein, and can be used to improve switching [95].

Highlights

- Protein localization regulates function
- Gene expression is controlled by nuclear localization of transcriptional regulators
- The basic signals dictating nuclear import and export have been deciphered
- Small molecules can be used to control nuclear transport of engineered proteins
- Light-activatable proteins can provide reversible control of nuclear localization

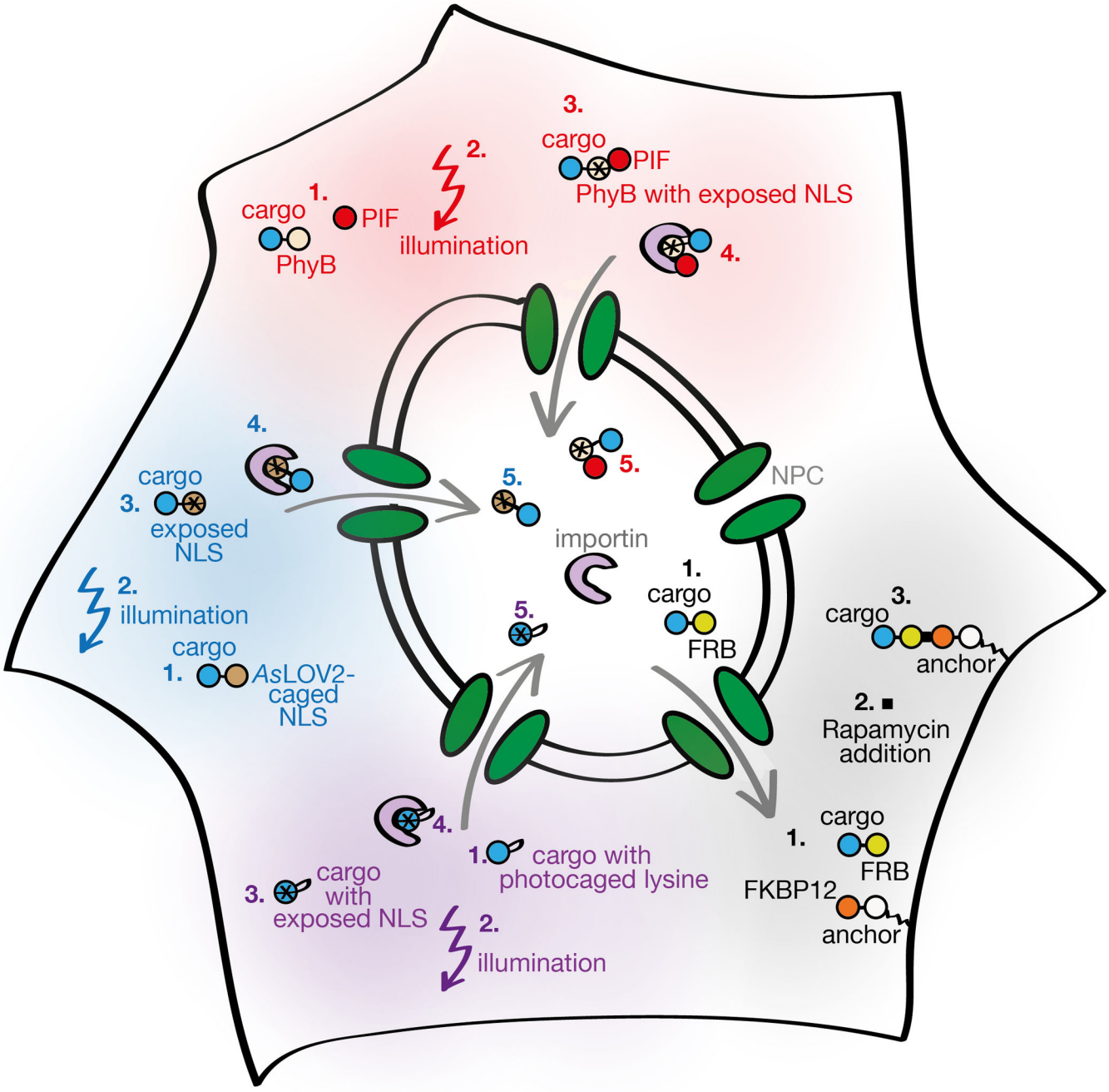


Figure 1.

Overview of methods for external control of nuclear protein localization. For simplicity, only some of the methods are depicted here. Different color zones correspond to different methods. The selected colors roughly indicate the wavelengths used for illumination: UV light for uncaging lysines, blue light to activate the LOV domain and red/infrared light to activate/de-activate the PhyB/PIF system. Numbers indicate temporally sequential events. In the case of the lysine caged with a photo-removable protective group, the cargo is depicted with a small black tongue, to indicate the presence of the unnatural amino acid. NCP:

nuclear pore complex; FRB: FKBP and rapamycin binding domain; *As LOV2: Avena sativa* LOV2 domain from phototrophin 1; PhyB: phytochrome B; PIF: phytochrome interacting factor.

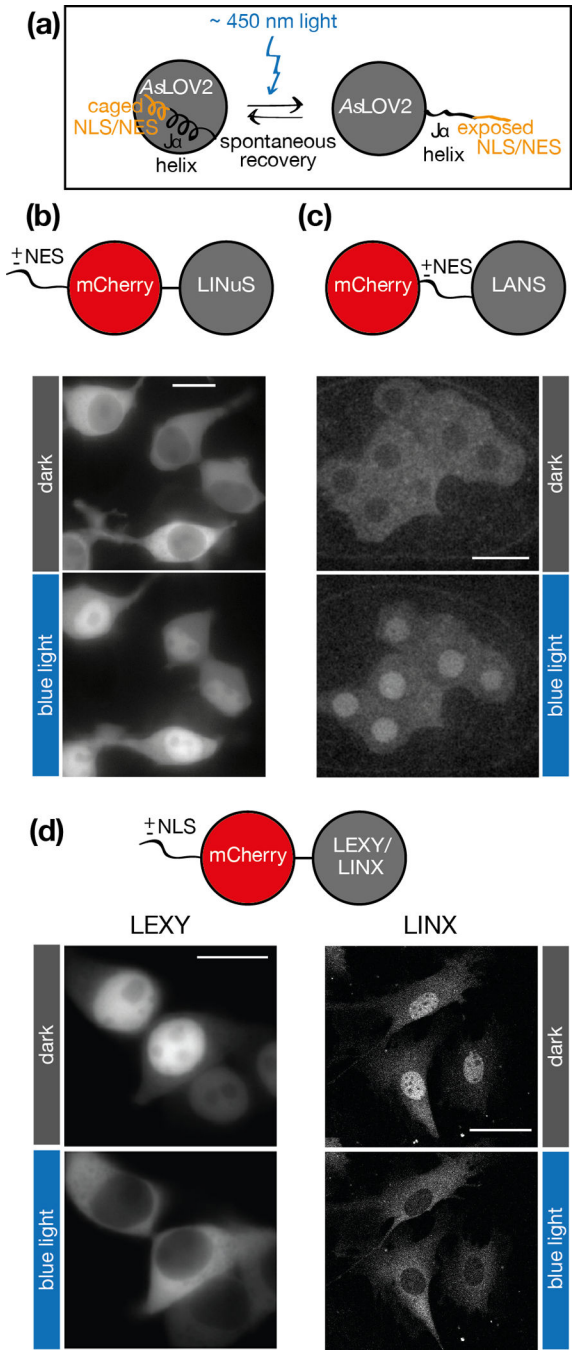


Figure 2.

As LOV2-based methods for controlling import and export. (a) Schematic drawing showing the conformational change of *As LOV2* upon light absorption leading to Jα helix undocking and unfolding and consequential exposure of NLS/NES motifs. This mechanism is common to LINuS, LANS, LEXY and LINX. (b–d) Upper panel: schematic drawing of the construct. Thin black line, flexible linker. (b) Representative images of HEK 293T cells expressing NES-mCherry-LINuS before and after blue light illumination. Scale bar, 15 μm. LINuS here is the biLINuS22 variant. NES, PKIt NES. (c) Representative images of a *C. elegans* embryo

before and after blue light illumination. Scale bar, 10 μm. LANS here is the variant LANS4. (d) Representative images of HEK 293T cells (left) and mouse fibroblasts (IA32) cells (right) transfected with the indicated construct before and after blue illumination. Scale bar, 20 μm (left) and 50 μm (right).

Table 1

Methods for Inducible Control of Nuclear Localization

	System/ Molecule	Engineered Function	Critical Features	Selected References
Chemical Induction	Leptomycin B (LMB)	Broad inhibitor of nuclear export	Covalent inhibitor of CRM1 Non-reversible	[36]
	Fusions with nuclear receptors	Ligand binding induces nuclear import	Estrogen, Glucocorticoid, and Progesterone receptors have been used Commonly used to control Cre recombinase One-component system	[39] [40]
	Rapamycin-dependent dimerization	Protein of interest recruited to cytosolic or nuclear proteins via dimerization	Common technique in yeast (“anchor away”) Requires rapamycin-resistant yeast strain Non-reversible inhibition in <15 minutes Two-component system	[49–53]
Light Induction	Photocaged lysine in a NLS	Inducible nuclear import of fusion proteins	Non-reversible / UV-light sensitive Requires cells engineered for use with non-natural amino acids One-component system	[62,63]
	PhyB/PIF dimerization	Inducible nuclear import or export of fusion proteins	Reversible: 650 nm -> PhyB/PIF complex forms, 750 nm -> PhyB/PIF complex dissociates Very tight spatio-temporal control Chromophore must be supplied in non-plant systems Two-component system	[77,78,101]
	Caging of NLS with *As*LOV2	Inducible nuclear import of fusion proteins	Reversible: 450 nm -> nucleus, dark -> cytoplasm Chromophore abundant in most systems One-component system	[83,87]
	Caging of NES with *As*LOV2	Inducible nuclear export of fusion proteins	Reversible: 450 nm -> cytoplasm, dark -> nucleus Chromophore abundant in most systems One-component system	[82,86]
	UVR8 dimerization with COP1/NLS	Inducible nuclear import of fusion proteins.	UV-B light sensitive Slower than other systems (responds in minutes to hours); Two-component system	[66]