mammalian visual cortex) (Sanes and Zipursky 2010). The field needs to know whether there are other cells that encode global motion in the protocerebrum and if so, how they accomplish that task. Also, the behavioral impact of the motion-opponent circuit discovered by Mauss et al. still needs fleshing out. In theory, investigating visually guided behaviors in flies (Maisak et al., 2013) could be used to implicate this circuit in separating self-motion versus object motion as the fly moves through space.

The implications of the findings in Mauss et al. extend beyond the parallels to the mammalian direction-selective circuit because they also raise several new ideas about how the brain can use conflicting signals to disambiguate sensory scenes. Not only can this idea be applied to other types of visual signals, but it may also provide insight into the general purpose of having conflicting sensory inputs converge in the same targets. Across multiple sensory modalities, such convergent computations may result in the refinement of percepts of the external world, depending on the specificity with which they are organized (Sosulski et al., 2011). In the meantime, the new results of Mauss et al. indicate that the brain is highly selective in how it organizes the flow and transformation of directional visual information, and they imply that such stringency may be essential for accurate decoding of feature-rich visual scenes.

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Optical Control of Microtubule Dynamics in Time and Space

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Small molecule inhibitors of microtubule dynamics are widely used as cell biology research tools and clinically as cancer chemotherapeutics. By slight modification to the chemical structure of a known microtubule inhibitor, combretastatin A-4, Borowiak et al. develop a photoswitchable derivative that can be turned "on" and "off" with low-intensity light to spatially and temporally control microtubule dynamics.

Microtubules are abundant, dynamic intracellular polymers that perform key functions in important processes such as mitosis, intracellular transport, and migration. Microtubule-directed small molecules, which inhibit microtubule dynamics, are widely used and valuable tools in cell biology as well as successful chemotherapeutics clinically, most likely a result of their ability to perturb mitosis (Dumontet and Jordan, 2010). However, due to the abundance of tubulin and the importance of microtubules not only during mitosis but also in interphase, treatment with microtubule-directed drugs often leads to systemic side-effects, such as peripheral neuropathies (Carlson and Ocean, 2011). The ability to spatially and temporally control the activity of such drugs could provide a significant advancement in the tolerability, increasing their overall clinical value. In this issue of *Cell*, Borowiak et al. develop a group of photoswitchable microtubule inhibitors, referred to as Photostatins (PSTs), which can be turned "on" and "off" with UV or visible light, respectively (Borowiak et al., 2015). Thus, their work establishes a novel tool for optically controlling microtubule dynamics in space and time.

Borowiak et al. designed PSTs as analogs of combretastatin A-4, part of a group of small molecules that bind to the colchicine-binding domain at the interdimer interface between α- and β-tubulin, which have been developed particularly for their vascular-disrupting properties at the tumor tissue level (Dumontet and Jordan, 2010). Critically, different combretastatin A-4 isomers have variable

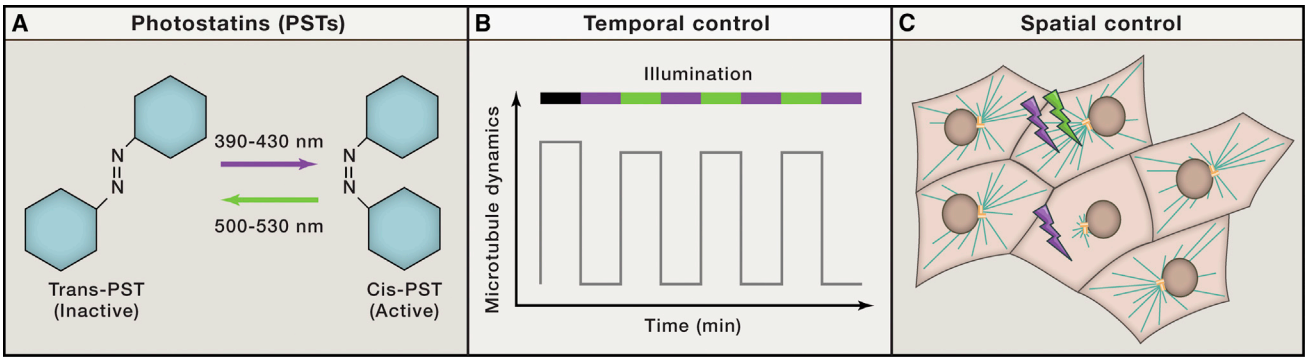

Figure 1. Photostatin Activity Can Be Turned "On" and "Off" with Low-Intensity Light to Temporally and Spatially Control Microtubule Dynamics

(A) In this issue, Borowiak et al. develop and characterize Photostatins (PSTs), small molecule microtubule inhibitors that disassemble microtubules. PST bioactivity can be turned "on" and "off" with UV (390–430 nm) and green (500–530 nm) light, respectively.

(B) Temporal control of microtubule dynamics using PSTs. In the dark (black), normal microtubule dynamics are observed in the presence of the inactive *trans*-PSTs. When exposed to UV light (purple), *trans*-PSTs isomerize to the active *cis*-PSTs and rapidly attenuate microtubule dynamics. Dynamics are then rescued by exposure to green light, converting back to inactive *trans*-PSTs. PST activity is reversible and stable across many on-off cycles.

(C) Single-cell spatial control of microtubule dynamics using PSTs. Microtubule (green lines) dynamics are perturbed in individual cells illuminated with UV light (purple). UV pulse immediately followed by a rescuing green pulse results in normal dynamics. Cells adjacent to those illuminated with either UV or a combination of UV/green are unaffected.

cytotoxic potencies (Tron et al., 2006). Taking advantage of this feature, Borowiak et al. replaced the C=C double bond with an isosteric N=N double bond, resulting in a molecule that can be rapidly photoswitched between the *trans* (inactive) and *cis* (active) isomers on a timescale of seconds using UV (380–420 nm) and green (500–530 nm) light, respectively (Figure 1A). Photoisomerization is reversible and stable over many activation-inactivation cycles. Additionally, the active *cis*-PSTs spontaneously isomerize to *trans*-PSTs with a halftime of 0.75–120 min in the absence of UV illumination (dark regime), ensuring that the concentration of active *cis* isomers diminishes away from the illumination region without the need for inactivation by green light.

The development of the PSTs provides a significant technological advance in the ability to spatially and temporally control microtubule assembly dynamics as well as the cytotoxic effects of microtubule-targeting small molecules. Borowiak et al. demonstrate the efficacy of PSTs as microtubule inhibitors in vitro, in single cells, and in vivo. Consistent with other agents binding to the colchicine domain on tubulin (Dumontet and Jordan, 2010; Stanton et al., 2011), PSTs result in G_2/M phase arrest and eventual cell death in a concentration dependent manner, inhibit the assembly of microtubule polymer in vitro, disrupt the cellular microtubule array, and lead to a loss of single microtubule assembly dynamics. Additionally, PST-1 competes with colchicine for binding to tubulin. Most importantly, Borowiak et al. show that each of the documented effects of PSTs can be controlled by the nature of illumination, exhibiting more than 100-fold less potency when in a dark state, compared to pulsed illumination with UV light.

To characterize the effects of PSTs on single microtubule dynamics Borowiak et al. imaged the end-binding protein EB3, which recognizes the dynamic microtubule plus-end with rapid binding/unbinding kinetics (Maurer et al., 2012), forming a comet-like signal as the microtubule grows. When illuminated with pulsed UV light (405 nm), EB3 comet number, lifetime, speed, and distance traveled were rapidly reduced. But unlike other microtubule inhibitors, these dynamics can subsequently be recovered by illumination with pulsed green light (514 nm), returning close to the control value (Figure 1B). This inhibition and recovery of individual microtubule dynamics continued through multiple UV/green light activation/inactivation cycles, where switching between illuminations was performed in 2 min phases, thus demonstrating the novel ability of the PSTs to be temporally controlled in a reversible manner within live cells.

In addition to temporal control, Borowiak et al. also show that bioactivity of PSTs can be spatially controlled in vivo, with precision on the order of a single cell (Figure 1C). Using the *C. elegans* embryo model system, single cells were targeted with millisecond pulses of either 405 nm light alone or a combination of 405 nm and 514 nm light, in which a 514 nm rescue pulse was applied immediately after the activating 405 nm pulse. Blastomeres were identified and observed using a mCherry-tagged marker for the cell membrane and histone H2B. Illumination was applied when chromosomes aligned at the mid-plane during entry to metaphase. Cells targeted with the 405 nm light were blocked in metaphase, while those targeted with 405 + 514 nm light proceeded through division normally. Further, untargeted neighboring cells exhibited normal mitosis and continued as in a normal embryo in both cases. Thus, the effects of PSTs can be reversibly and optically controlled inside living organisms with single-cell spatial precision.

The discovery and characterization of PSTs is an exciting advancement in the field of small molecule inhibitors and the field of cell biology research. This technology has potential to have an immediate impact as a research tool to control microtubule dynamics with single cell precision on the order of seconds without the

use of complicated microfluidic devices. Normal progression through mitosis requires multiple steps, including spindle assembly, proper kinetochore connections, and chromosome segregation, each relying on different aspects of microtubule dynamics (Inoué and Salmon, 1995; London and Biggins, 2014). It would be interesting to see the effects of activating PSTs at different points in the cell cycle. For example, since PSTs induce microtubule disassembly, activation during metaphase could induce depolymerization, thus exerting excess disassembly-coupled pulling forces on sister chromatids, which could be used to spatially-temporally induce a premature anaphase-like state for a subset of kinetochores.

While the cell biological utility is clear, it remains to be demonstrated that PSTs, or further derivatives, can succeed clinically, with a number of issues that need to be addressed. First, the observed effects of active *cis*-PSTs require concentrations in the micromolar range (typically 0.5–10 μM), whereas combretastatin A-4, as well as more clinically relevant drugs, induce similar effects at nanomolar concentrations (Jordan and Wilson, 2004). Further, clinically relevant drug concentrations are estimated to be in the nanomolar range after infusion (Zasadil et al., 2014). Thus, despite having a greater than 100-fold higher potency than the inactive *trans* isomer, the *cis*-PST affinity may be too low to be practical in the clinic. Further, the need for UV light in photoisomerization may limit the clinical applicability as shorter wavelength light has relatively poor tissue penetration and may require an implantable light emitting device depending on the tumor location. Additionally, prolonged exposure to UV light, as necessary for maintaining the *cis* isomer due to the fast spontaneous inactivation, could result in additional cytotoxic effects. While more work will be necessary for development as a clinical therapy, the work by Borowiak et al. opens the door for future development of optically controlled microtubule inhibitors. It will be interesting to see how it develops in time, and what exciting discoveries will be made using this new tool.

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