KINETOCHORE REGULATION

Let there be light

Kinetochores form the critical interface with spindle microtubules that accounts for chromosome movement and segregation fidelity during mitosis. Spatial and temporal control of motor protein and checkpoint signaling at kinetochores is now possible with a new set of optogenetic tools.

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The kinetochore is a hub for more than 100 proteins that assemble at the centromere of each chromatid to provide a physical link between DNA and spindle microtubules during mitosis[1]. These proteins include the cytoplasmic dynein and the kinesin-7 centromere protein E (CENP-E) motors, required for the congression of peripheral chromosomes to the spindle equator[2], as well as the spindle assembly checkpoint (SAC) proteins Mad1 and Mad2 that monitor kinetochore–microtubule attachments and regulate anaphase onset[3]. In this issue, Zhang *et al.*[4] have developed a new set of optogenetic tools that allow spatial and temporal control of motor protein function and SAC signaling at kinetochores using light.

Kinetochore function has been studied using classical genetics, RNA interference (RNAi), chemical inhibition and micromanipulation techniques such as laser microsurgery. Although instrumental in our current understanding of several kinetochore proteins, these approaches lack either temporal or spatial control over protein localization and function. Optogenetics has emerged as a groundbreaking technology that combines genetic and optical methods to activate and/or inactivate specific molecular events in living cells and tissues at high spatial and temporal resolution. Originally developed to control neuronal activity and brain function in freely moving animals[5], optogenetic tools have been successfully adapted to induce protein–protein interactions at the kinetochores of living cells[6]. A photocaged chemical dimerizer selectively recruited tagged cytosolic proteins to individual kinetochores upon uncaging with light. However, this system was limited by low light sensitivity and slow reversibility.

In the present work, Zhang *et al.*[4] developed two new chemical dimerizers that allow fast spatial and temporal control of protein dimerization either by uncaging with UV or blue light (inducing dimerization) or by UV-light-inducible cleavage (reversing dimerization). The authors replaced the previously developed photocaged module with a red-shifted coumarin-based cage, enabling uncaging with substantially less light and longer wavelengths, thus reducing phototoxicity. They also developed a chemical dimerizer containing a photocleavable linker, which allows dimerization reversal with high spatiotemporal resolution.

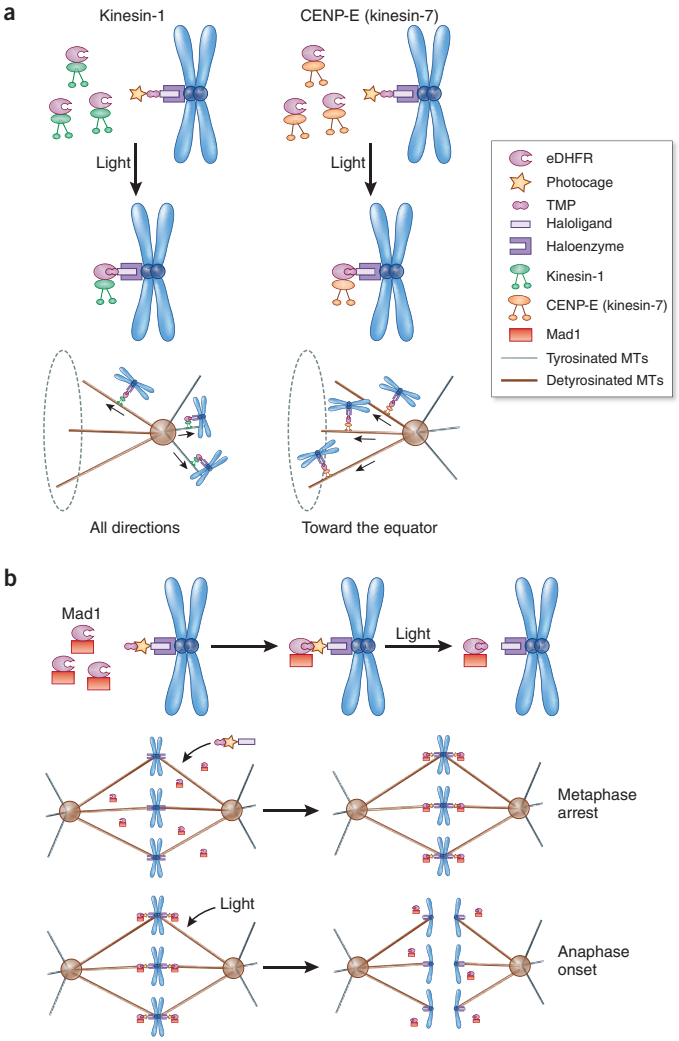

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Figure 1 | Optogenetic control of kinetochore function. (**a**) Recruitment of the motor domain of conventional kinesin-1 (left) or of CENP-E (kinesin-7, right) to polar chromosomes using a light-inducible chemical dimerizer in CENP-E inhibited or depleted cells. Recruitment of kinesin-1 results in the transport of polar chromosomes in all directions, whereas recruitment of CENP-E results in the preferential transport toward the spindle equator, along detyrosinated microtubules (MTs). (**b**) Mad1 recruitment from the cytosol to metaphase kinetochores after addition of a photocleavable dimerizer results in metaphase arrest (top). Light-induced cleavage of the chemical dimerizer causes the release of Mad1 from kinetochores and anaphase onset (bottom). eDHFR, *Escherichia coli* dihydrofolate reductase; TMP, trimethoprim.

CENP-E-mediated congression relies on the ability of this kinetochore motor, but not microtubule plus-end-directed motors located on chromosomes arms (known as chromokinesins), to bias the movement of polar chromosomes toward the spindle equator[7]. Zhang *et al.*[4] have now tested whether this directional transport of chromosomes toward the spindle equator is a unique capacity of CENP-E motors at kinetochores. First, the authors used RNAi or chemical inhibition of CENP-E in human cells to obtain a subset of chromosomes clustered around the spindle poles. With their new light-inducible chemical dimerizer, they specifically recruited either the motor domain of CENP-E or that of conventional kinesin-1 to kinetochores on polar chromosomes and found that only CENP-E preferentially transported chromosomes toward the spindle equator (**Fig. 1a**). This experiment elegantly substantiates the view of CENP-E as the critical kinetochore motor in the congression of polar chromosomes toward the spindle equator[7] and is fully consistent with recent findings suggesting that CENP-E is guided toward the equator by a microtubule code[8].

In addition, whether CENP-E motor activity is required to maintain chromosomes at the equator after completion of alignment has been controversial. The observation that more than 80% of the chromosomes align and maintain alignment after perturbation of CENP-E function[7] indicates that CENP-E is dispensable after chromosome bi-orientation. However, *in vitro* and *in vivo* studies of CENP-E function have suggested a role in the stabilization of end-on kinetochore–microtubule attachments[9,10]. To resolve this controversy, Zhang *et al.*[4] used their new light-inducible chemical dimerizer to artificially recruit kinesin-1 to kinetochores. This caused aligned chromosomes to move away from the metaphase plate, a response that was exacerbated after CENP-E inhibition. These observations suggest that, although dispensable for maintaining chromosome positioning at the spindle equator, CENP-E ensures the formation of functional end-on kinetochore–microtubule attachments on bi-oriented chromosomes, independently of its motor activity.

Finally, Zhang *et al.*[4] used their photocleavable dimerizer to manipulate the kinetochore localization of the SAC protein Mad1. Addition of this dimerizer to mitotic cells in metaphase forced the recruitment of Mad1 to attached kinetochores, re-activating the signaling cascade that prevents sister chromatid separation and entry into anaphase. After light-induced cleavage of the dimerizer, Mad1 disappeared from kinetochores and cells promptly entered anaphase (**Fig. 1b**). Thus, mitotic progression can now be regulated by light, opening the way for future applications such as cancer therapeutics.

Overall, the work of Zhang *et al.*[4] demonstrate the versatility and the potential of optogenetics to tackle important biological problems that may extend way beyond the study of kinetochore function and cell division. The ability to control other molecular motors (for example, cytoplasmic dynein) and other catalytic activities, such as kinases and phosphatases, at kinetochores or other biologically relevant structures represent obvious challenges for the future. An important aspect that must be taken into account relates to the residence time of the docking protein used for optogenetic manipulation, to prevent fast exchange with the soluble endogenous protein pool. This must be articulated with appropriate controls for functional integrity of the manipulated structure after induced dimerization. The development of dimerizers with different chemical properties (for example, alternative photocages with various wavelengths) or ligands (for example, SNAP-tag), combined with CRISPR–Cas9 technology, will certainly broaden the optogenetics toolbox in the near future, allowing the simultaneous manipulation of more than one protein with spatiotemporal precision.

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Competing financial interests

The authors declare no competing financial interests.