Published in final edited form as:

Nat Chem Biol. 2017 September ; 13(9): 1058–1059. doi:10.1038/nchembio.2464.

Let there be light on kinetochores

Ana C. Figueiredo^{1,2} and Helder Maiato^{1,2,3,4,*}

¹Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

²Instituto de Investigação e Inovação em Saúde – i3S, Universidade do Porto, Porto, Portugal

³Department of Biomedicine, Faculdade de Medicina, Universidade do Porto, Porto, Portugal

⁴Whitman Center, Marine Biological Laboratory, Woods Hole, MA, USA

Abstract

Kinetochores form the critical interface with spindle microtubules that account 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.

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 mitosis¹. These proteins include the cytoplasmic dynein and the kinesin-7/CENP-E motors required for the congression of peripheral chromosomes to the spindle equator², as well as the spindle assembly checkpoint (SAC) proteins Mad1 and Mad2 that monitor kinetochore-microtubule attachments and regulate anaphase onset³. In this issue of *Nature Chemical Biology*, Zhang *et al.*⁴ have developed a new set of optogenetic tools that allowed spatial and temporal control of motor protein function and SAC signaling at kinetochores using light.

Kinetochore function has been studied using classical genetics, RNAi, chemical inhibition and micromanipulation techniques, such as laser microsurgery. Although instrumental for 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 ground-breaking technology that combines genetic and optical methods to activate/inactivate specific molecular events in living cells/tissues at high spatial and temporal resolution. Originally developed to control neuronal activity and brain function in freely moving animals⁵, optogenetic tools have been successfully adapted to induce protein-protein interactions at the kinetochore of living cells⁶. Accordingly, a photocaged chemical dimerizer genetically engineered to localize to kinetochores by an anchoring protein was shown to selectively recruit tagged cytosolic proteins to individual kinetochores upon uncaging the chemical dimerizer with light. However, this proof-of-concept dimerizer showed limitations related with low light sensitivity and slow reversal.

*Correspondence: maiato@i3s.up.pt.

Competing financial interests

The authors declare no competing financial interests.

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/blue-light (inducing dimerization) or UV-light-inducible cleavage (reversing dimerization). The authors have replaced the previously developed photocaged module by a red-shifted coumarin-based cage, allowing uncaging with significantly less light and longer wavelengths, thus reducing phototoxicity. Spatiotemporal control of dimerization reversal was achieved using a chemical dimerizer containing a photocleavable linker between the two ligand modules (eDHFR and Halo tags, respectively).

CENP-E-mediated congression relies on the ability of this kinetochore motor, but not on microtubule-plus-end-directed motors located on chromosomes arms (known as chromokinesins), to bias the movement of polar chromosomes towards the spindle equator⁷. Zhang *et al.*⁴ have now tested whether this directional transport of chromosomes towards 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 (Fig. 1a). 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 towards the spindle equator (Fig. 1b). This experiment elegantly substantiates the view of CENP-E as the critical kinetochore motor that accounts for the congression of polar chromosomes towards the spindle equator⁷, and is fully consistent with recent findings suggesting that CENP-E is guided towards the equator by a microtubule code⁸.

Whether CENP-E motor activity is further 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 function⁷ 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 shed light on 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 to maintain chromosome positioning at the spindle equator, CENP-E enhances the stability of 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. 1c). Thus, mitotic progression could now be regulated by light, opening the way for future applications, such as cancer therapeutics.

Overall, the work of Zhang and colleagues demonstrate the versatility and potential of optogenetics to tackle important biological problems that might extend way beyond the study of kinetochore function. The development of dimerizers with different chemical properties (e.g. alternative photocages with different wavelengths) or ligands (e.g. 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.

References

1. Cheeseman IM. Cold Spring Harbor perspectives in biology. 2014; 6
2. Maiato H, Gomes AM, Sousa F, Barisic M. Biology. 2017; 6
3. Joglekar AP. Biology. 2016; 5
4. Zhang H, et al. Nat Chem Biol. 2017
5. Deisseroth K. Nat Methods. 2011; 8:26–29. [PubMed: 21191368]
6. Ballister ER, Aonbangkhen C, Mayo AM, Lampson MA, Chenoweth DM. Nat Commun. 2014; 5
7. Barisic M, Aguiar P, Geley S, Maiato H. Nature cell biology. 2014; 16:1249–1256. [PubMed: 25383660]
8. Barisic M, et al. Science (New York, N.Y.). 2015; 348:799–803.
9. Gudimchuk N, et al. Nature cell biology. 2013; 15:1079–1088. [PubMed: 23955301]
10. Putkey FR, et al. Developmental cell. 2002; 3:351–365. [PubMed: 12361599]

Figure 1. Optogenetic control of kinetochore function.

(a) Kinesin-7/CENP-E inhibition with 20 nM GSK923295 in human HeLa cells showing the typical clustering of few chromosomes at the spindle poles (image credits: Elsa Logarinho/CID Lab). Scale = 5 μ m. Kinetochore motor function on chromosomes located on a single pole (dashed area) could now be specifically controlled with light. (b) Recruitment of the motor domain of conventional Kinesin-1 (left) or of Kinesin-7/CENP-E (right) to polar chromosomes using a light-inducible chemical dimerizer in CENP-E inhibited/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 towards the spindle equator, along detyrosinated microtubules (MTs). (c) Mad1 recruitment from cytosol to metaphase kinetochores after addition of a photocleavable dimerizer results in metaphase arrest (top). Light-induced cleavage of the chemical dimerizer caused the release of Mad1 from kinetochores and anaphase onset (bottom). *Escherichia coli* dihydrofolate reductase (eDHFR), trimetoprim (TMP).