CHEMICAL TOOLS

Optogenetics in the hot seat

Optogenetic and thermogenetic tools have been limited to applications for single-state control of cellular processes. A single-component optogenetic tool was found to act as both a temperature sensor and a photoreceptor, enabling multi-state control of developmental signaling.

Maxwell Z. Wilson

Traditionally, 'cellular' (non-neuronal) optogenetic tools can be difficult to work with because they are built from multiple components, require adding endogenous chromophores, or can be inactivated by N- and C-terminal protein fusions, making expert knowledge of each tool a requirement for successful operation. In addition, these tools have largely been limited to integration of a single environmental signal, typically a pulse of blue (~450-nm) or red (~650-nm) light. In this issue of *Nature Chemical Biology*, Benman et al. present the application of a new single-component optogenetic tool, BcLov4, that controls signal transduction pathways and responds to both heat and blue light[1].

In response to blue light, the BcLov4 photoreceptor translocates from the cytoplasm to the cell membrane all by itself. This single-component membrane translocation makes BcLov4 an attractive candidate for expanding optogenetic tool use because of its simplicity. The protein accomplishes this feat by unsheathing a polybasic amphipathic helix that nestles into the inner leaflet of the plasma membrane with high affinity[2].

During their analysis, Benman et al. discovered a curious phenomenon: the BcLov4 receptor responds to heat as well as blue light[1]. To systematically understand the effects of temperature on BcLOV4, the authors made clever use of their 96-well light-delivery device, the Opto-Plate, a custom piece of hardware that allows fully programmable delivery of blue, red and infrared wavelengths[3]. Benman et al. used the infrared LEDs as microcontrollable heaters, showing that they could precisely scan through temperature in addition to blue light intensity. After modeling responses to these inputs, the authors found that the duration in which BcLov4 can be photoconverted is determined by temperature, with higher temperatures reducing the window of time during which photoconversion can occur (Fig. 1a). Moreover, at lower temperatures

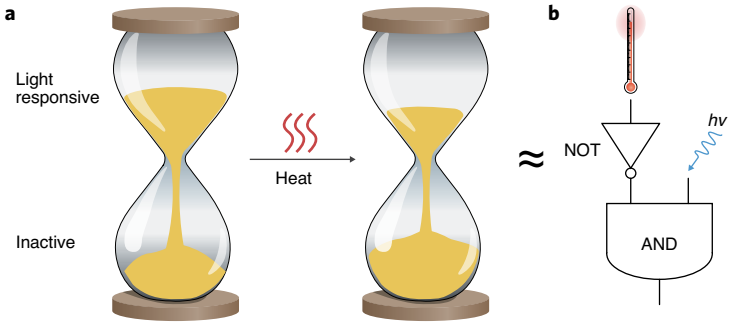

Fig. 1 | A schematic representation of the BcLOV4 protein's integration of both light and heat. **a**, At lower temperatures, BcLOV4 remains photoactivatable, but as temperature is increased and light is applied, the protein transitions into the non-photoswitchable, inactive state at a higher rate. **b**, This integration of light and heat can be used as an AND NOT logic gate where the addition of heat prior to light stimulus causes the protein to not respond. This dual signal integration can be used for multi-state control.

(<30 °C), BcLov4 photoconversion is almost perfectly reversible, allowing the control of cell signaling events in model organisms that grow at room temperature, such as *Drosophila melanogaster* and zebrafish. In addition, this thermo-responsive property enables multi-state control through temperature manipulation, approximating a *light* AND NOT *heat* gate (Fig. 1b). Excitingly, this discovery places BcLov4 in a new class of post-translational 'thermogenetic' tools that are more responsive than the classic heat-shock-responsive elements that require transcription. Although the BcLov4 receptor was previously used to regulate cell migration[4,5], the authors now demonstrate the applicability of their single-domain approach to controlling the Erk and Akt developmental signaling pathways.

The study serves as a powerful reminder that environmental receptors, including optogenetic proteins, have been selected to respond to their environment to benefit the organisms that produce them. In this case, the fungus *Botrytis cinerea* (famous for causing the 'noble rot' of wine grapes), from which BcLov4 is derived, has some apparent need for the temporal modulation of light perception by ambient temperature. Yet the biological function of this multi-state integrator remains unknown.

Indeed, the findings raise many interesting questions. Heat-responsive regulatory proteins typically denature temperature-sensitive regions to expose new binding interfaces that initiate downstream responses. How does BcLov4 sense temperature, and what makes this heat switch almost completely irreversible? Applying analytical techniques, such as hydrogen exchange mass spectrometry or NMR, to BcLOV4 in its active and heat-inactivated states could help unveil how this protein senses heat. Such studies may even lead to potential structural changes that increase or decrease the temperature threshold. Further engineering to make the thermogenetic switch fast, reversible and perhaps even able to drive (rather than inhibit) membrane recruitment would also benefit the study of complex developmental signaling networks.

Future work dissecting the organismal context of this single-domain signal integrator promises insights into the molecular basis of environmental sensing

by fungi and may even lead to methods of controlling *B. cinerea* with brief pulses of heat and light. Thus, studying the mechanisms of this thermo-optogenetic protein promises to reveal how natural environmental sensing regulates cell decisions, enabling the development of advanced tools to dissect how complex signals are decoded in model organisms. ❐

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Competing interests
The author declares no competing interests.

ENZYMOLOGY

A complex struggle for direction

To avoid strife at the interface of basic carbon and nitrogen metabolism, *Bacillus subtilis* has developed a rather combative solution. If needed, its glutamate synthase suppresses conflicting glutamate breakdown by directly binding and immobilizing its metabolic opponent, glutamate dehydrogenase.

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Changes in the environment, such as in nutrient availability, necessitate adaptations of cellular metabolism. The available regulatory modalities are manifold, including the differential transcription of metabolic genes and the post-translational modification or allosteric regulation of already present metabolic enzymes. Allosteric regulation can be mediated by many types of (bio-)molecules, including other enzymes. Here, of special note are 'metabolons', a term coined by Paul Srere for transient complexes of enzymes that form to optimize metabolic throughput[1]. While several such synergistic complexes have been studied functionally and structurally, in this issue Jayaraman et al.[2] describe a metabolic complex in *B. subtilis* that could be regarded as the exact opposite of a classical metabolon. When necessary, six protomers of the heterodimeric glutamate synthase GltAB smother the homohexameric glutamate dehydrogenase (GDH) GudB from all sides, suppressing its activity in a 1.6-megadalton 'counter-enzyme' complex, and thereby switching the metabolic program from glutamate breakdown to its synthesis[2] (Fig. 1).

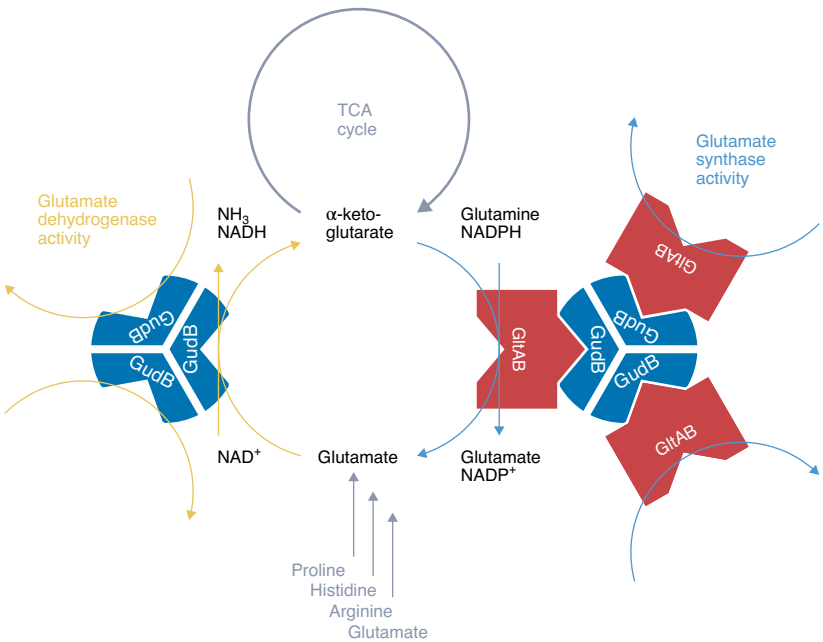

Fig. 1 | The GudB–GltAB counter-enzyme system at the intersection of carbon and nitrogen metabolism. The GudB homo- and the GudB–GltAB heterocomplex are shown schematically in a top view, with the bottom half of the complex omitted. On the left, GudB is working on glutamate breakdown, while on the right, GltAB is working toward glutamate synthesis, overruling GudB activity by blocking its active site.

Glutamate is a key metabolite that is generally maintained at high concentrations and serves as a cellular nitrogen reservoir. When glutamate or other amino acids that can be catabolized via glutamate (such as histidine or arginine) are available as nutrients, GDH enzymes are utilized to access them as carbon and nitrogen sources, via conversion to α-ketoglutarate and ammonia. However, in glutamate-limited conditions, the glutamate level has to be actively maintained via glutamate synthesis, using α-ketoglutarate as a substrate. In this scenario, GDH activity is redundant, if not counterproductive. Dan Tawfik's investigations into the regulatory mechanisms of two *B. subtilis* GDH paralogs, RocG and GudB[3,4], led to the discovery that the latter can be inactivated in a direct interaction with its counter-enzyme GltAB. Together with collaborator James