Development of bioluminescent probes to analyze the role of NADPH oxidase 1 in circadian clock synchronization

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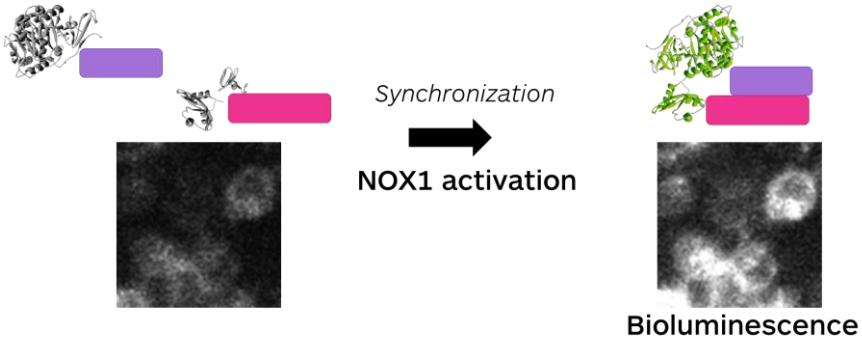
Cellular circadian clocks adjust their circadian phases to external cues through a process called synchronization. We have previously demonstrated that clocks synchronize to cellular stressors such as reactive oxygen species (ROS) and ultraviolet radiation (UV).[1,2] However, the role of physiological ROS signaling in this synchronization process remains elusive.

In this study, we investigated the role of NADPH oxidase (NOX), a primary source of intracellular ROS, in clock synchronization. NOX is an enzyme family that catalyzes the production of cellular $O_2^{\cdot-}$ and H_2O_2, believed to play a unique role in cellular ROS signaling.[3] We observed that the administration of low levels of H_2O_2 enhances clock oscillation. Moreover, we found that genetical knock-down of a NOX family member, NOX1, disrupted clock synchronization. We found that synchronization stimulation increased cellular H_2O_2 levels, while NOX1 suppression decreased them, suggesting that ROS produced by NOX1 mediates cellular synchronization.

To track NOX1 activity in real-time, we developed a split-luciferase complementation probe that detects temporal changes in NOX1 activity. We found that NOX1 activity increased upon synchronization stimulation concomitantly with cellular ROS level increase. Moreover, we discovered that NOX1 activity oscillates in a circadian manner, suggesting that the circadian clock system regulates NOX1 activity.

In conclusion, we propose a model where the circadian clock controls NOX1 activity, which generates ROS signals that, in turn, maintain the circadian clock synchronization.



References: 1) Kawamura, G. et al, *Commun. Biol.* **2018**, 1 (1), 204. 2) Tamaru, T. et al, *PLOS ONE* **2013**, 8 (12), 1–16. 3) Sies, H.; Jones, D. *Nat Rev Mol Cell Biol* **2020**, 21 (7), 363–383.