Light-Induced Mechanical Gain-of-Function in PIEZO Channels via a Chemical Optogenetic Approach

Antoine Le Bris*[1] and Thomas Grutter[2]

[1]Chémo-Biologie Synthétique et Thérapeutique (CBST) UMR7199 – université de Strasbourg, Centre National de la Recherche Scientifique – France

[2]Chémo-Biologie Synthétique et Thérapeutique (CBST) UMR7199 – université de Strasbourg, Centre National de la Recherche Scientifique – France

Résumé

All cells are exposed to mechanical stimuli and respond to them with varying sensitivity. Several proteins can transduce mechanical signals into biological responses, among which the PIEZO1 and PIEZO2 play a prominent role. These proteins form mechano-activated ion channels and are essential in diverse physiological processes, including touch sensation, blood pressure regulation and red blood cell volume control. Dysregulation of PIEZO channels has been associated with various human pathologies. For example, a gain-of-function mutation (R2482H) in PIEZO1 is responsible for xerocytosis, a form of hereditary anemia. As such, PIEZO channels represent promising therapeutic targets.
Currently, most methods to study PIEZO activity rely on mechanical stimulation. Although physiologically relevant, this approach lacks specificity, as it can also activate other mechanosensitive pathways. To overcome this limitation, the laboratory developed a novel chemical optogenetic tool, called mOP1, which allows for rapid and specific activation of PIEZO1 using light. This approach is based on the chemical photoswitch maleimide ethylene azobenzene trimethyl ammonium (MAT), which contains an azobenzene moiety that toggles between *trans* and *cis* configurations under green (525 nm) and UV (365 nm) light, respectively. MAT is covalently attached to a cysteine residue introduced by site-directed mutagenesis (Y2464C) in the PIEZO1 pore domain. In the dark, the MAT adopts the *trans* configuration and the channel remains closed. Upon 365 nm irradiation, MAT switches to *cis* form, inducing a conformational change that rapidly opens the channel, without mechanical stimulation. The goal of this study is to further investigate the biophysical properties of the light-gated ion channels.

The method used in this study is the patch-clamp technique, which is the gold standard for measuring currents in cells. Specifically, I employed the cell-attached configuration, which allows the recording of activity from a small number of channels in a native-like membrane environment. The cells used were HEK293 cells lacking endogenous PIEZO1 activity (HEK P1KO). These cells were transiently transfected with either a plasmid encoding wild-type mouse PIEZO1 (mP1) or a plasmid encoding a cysteine mutant. Prior to recording, cells were incubated with the MAT and extensively washed before patch-clamp recordings. During patch-clamp experiments, negative pressures (suction) were applied through the pipette

*Intervenant

to mechanically stimulate PIEZO1 channels and responses were compared before and after cells were briefly illuminated at either 365 nm or 525 nm to induce photoisomerization of the bound MAT.

Our results show a two-fold increase in current amplitude and slower inactivation kinetics of mechano-induced currents in cells expressing the cysteine mutant when MAT is in the *cis* configuration compared to the *trans* state. These effects are fully reversible and mimic the gain-of-function phenotype of the R2482H mutation linked to xerocytosis. Importantly, these light-dependent effects were not observed in cells expressing wild-type mP1, confirming the specificity of the MAT-mediated modulation.
These findings demonstrate that MAT can induce a reversible gain of function in a mutant PIEZO1, offering a novel and precise way to probe channel activity. This approach enables modeling of PIEZO1 function and dysfunction with high temporal control, paving the way for future *in vivo* studies.

Mots-Clés: PIEZO channels, Chemical optogenetic tool, Patch Clamp, Gain of function