Use of Label-free Optical Biosensors to Detect Ion Channel Interactions in Intact Cells

Matthew R. Fleming1, Feng Li3, Alice Gao3, Ravi Marala3, and Leonard K. Kaczmarek1,2
1Dept. of Cellular and Molecular Physiology, 2Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT; 3Corning Life Sciences, Corning, NY

Abstract
Assays examining the interaction between channels and regulatory cytoplasmic proteins generally provide no information on the time course of interactions in living cells. A novel label-free technology (the Corning Epic® system) provides such information using optical sensors embedded in microwells to detect changes in the refractive index at the surface of cells. A redistribution of mass close to the plasma membrane (150 nm from the sensor surface) causes a change in the index of refraction of light impinging on the microwells. We tested the effects of the potassium channel opener bithionol on HEK cells transfected with the Slack potassium channel. Bithionol produces a large persistent decrease in index of refraction that is complete within 5 minutes. The decrease in resonant wavelength is consistent with a decrease in mass close to the plasma membrane. Bithionol treatment of untransfected cells produces only a very small transient decrease in refraction. Activation of protein kinase C, which regulates Slack channels, attenuates the Slack-specific response to bithionol. Thus activation of Slack channels produces a dynamic mass redistribution close to the plasma membrane, potentially representing the dissociation of cytoplasmic proteins from the channel. This method will permit high throughput detection of compounds that modulate channel-protein interactions.

Operating Principle: Cell Based Assays
The Corning Epic® system measures changes in local index of refraction resulting from the ligand-induced dynamic mass redistribution (DMR) within the bottom region (~150 nm) of the cell monolayer. Changes in the index of refraction are manifested by a shift in the resonant wavelength. Mass redistribution occurs as a result of proteins or other large molecules entering or leaving the bottom membrane region of the assay cell. Previous research has shown a DMR occurs upon activation of G-protein coupled receptors (GPCRs), including M1 muscarinic receptors (mACHR) and protease activated receptors (PARs).

Experimental Procedure and Assay Optimization
-HEK-293 is a human carcinoma cell line derived from human embryonic kidney cells
-Cell Culture in Epic Microplates:
1. Generation of stable Slack-expressing HEK-293 cell line
2. Seed transfected and untransfected HEK-293 cells and incubate overnight
3. Incubate and scan plate for 90 minutes
-System Concept
• The Corning Epic system can be utilized to screen for DMR in ion channel overexpressing HEK cells
• Dynamic mass redistribution due to endogenous GPCR activation occurs in both Slack transfected and untransfected HEK cells, but with a different amplitude and timecourse
-Response of endogenous GPCRs in HEK cells
In order to validate our experimental system, carbamylcholine chloride (carbachol), a muscarinic receptor agonist, was applied to HEK cells to activate the cells endogenous M1 mAChRs. SFLLR, an N-terminal TRAF pentapeptide which exhibits against activity against endogenous PAR1 receptors, was also applied. Activation of these G-protein coupled receptors is expected to cause a dynamic mass redistribution.

Conclusions
- The Corning Epic system can be utilized to screen for DMR in ion channel overexpressing HEK cells
- Dynamic mass redistribution due to endogenous GPCR activation occurs in both Slack transfected and untransfected HEK cells, but with a different amplitude and timecourse
- Activation of Slack, a sodium-activated potassium channel, leads to a DMR that may be due to channel-protein interactions.

Future Directions
- Does phosphorylation of Slack through the PKC pathway modulate DMR upon channel stimulation with bithionol?
- Phosphorylate channel with PKC activator TPA prior to bithionol stimulation
- Are any of the proteins known to interact with Slack responsible for the observed DMR?
- RNAi against proteins of interest
- Can we find a Slack specific derivative of bithionol?
- Screen for activity with bithionol derivatives with differing functional groups

References

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