Center for Lung Biology Pulmonary NewsLetter

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What's New in Pulmonary Science?

Dr. Wito Richter joined the Department of Biochemistry & Molecular Biology at USA last year, and is our most recent newcomer to the Center for Lung Biology. Wito has a long-standing interest in the structure and function of Type 4 cAMP-phosphodiesterases (PDE4s), a large group of isoenzymes that hydrolyze and inactivate the second messenger cAMP. His interest in cAMP started during his graduate studies at Leipzig University, Germany, and continued through subsequent postdocal and faculty positions at Stanford University and the University of California San Francisco.

The PDE4 family comprises four genes, PDE4A-D, and each is expressed as multiple protein variants. Through the characterization of genetic models of PDE4 subtype ablation and the identification of macromolecular signaling complexes that tether specific PDE4 variants to beta-adrenergic receptors, the ryanodine receptor, L-type calcium channels, phospholamban, or the scaffold protein Shank2, Wito's work has contributed to the idea that individual PDE4 isoforms serve unique, and non-overlapping physiological and pathophysiological roles in the body. Wito is particularly interested in exploiting this insight for the development of novel therapeutics that target individual PDE4 subtypes or PDE4 signaling complexes and are expected to exhibit a much improved safety profile compared to the non-selective PDE4 inhibitors available to date. Along this line, a main focus of the lab is the idea to target compartmentalized pools of Type 4 PDEs (PDE4s) as a therapeutic approach for Cystic Fibrosis (CF).

CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) that impair the expression and/or function of this epithelial anion channel. Deletion of phenylalanine 508 (ΔF508) is the most common mutation found in CF patients and triggers misfolding and premature degradation of the translated protein. As a result, the number of channels inserted into the epithelial membranes is insufficient to support normal ion transport, which triggers pathologies in multiple organs including the lungs, pancreas, intestines and liver. At present, inflammatory lung disease is the main contributor to morbidity and mortality in CF patients. Here, CFTR insufficiency in airway gland and epithelial cells triggers a vicious cycle of airway obstruction with dehydrated and sticky mucus, chronic bacterial infections and hyperinflammation, which eventually results in tissue destruction and loss of respiratory function (see Pierre Kadeba's excellent perspective in the Nov 2014 CLB Newsletter). Wito pursues the idea that inhibition of PDE4, via the resulting increase in cAMP/PKA signaling, can serve to both activate CFTR, thus alleviating the underlying cause of CF, and limit mucus expression and hyperinflammation in the lungs, thus ameliorating its main symptoms.

What's New in Research Training?

Our Lung Biology track in the PhD program was initiated in 2002, followed by award of the predoctoral T32 on "Cell Signaling and Lung Pathobiology" in 2004. We thought it would be good to highlight a few of our former trainees who began training during that time. Where are they now?

Natavia Middleton (Gillespie, T32 trainee; PhD 2008): Natavia moved directly into academia. She is an Assistant Professor in Biology at the Technical College of the Lowcountry in Beaufort, SC. She has been key to development of a new agriscience certificate program there funded by NSF and is currently Interim Dean of the College of Arts and Sciences.

Nutan Prasain (Stevens; PhD 2008): After postdoctoral training at Indiana University, Nutain recently was appointed as an Assistant Research Professor in the Herman B. Wells Center for Pediatric Research at Indiana University, Indianapolis, IN. His work focuses on stem cell and progenitor cell biology.

Kathy Bonness (Honkanen, T32 trainee; PhD 2009): After postdoctoral training at UT Southwestern, Kathy moved to industry at Indica Labs with a focus on biomedical imaging. She is now a consultant for mentoring and career development in Portland, OR.

Christina McManus (Stevens, T32 trainee; PhD 2010): Christina is currently an Assistant Professor of Physiology at the Alabama College of Osteopathic Medicine in Dothan, AL.

Salina Gairhe (McMurtry; PhD 2010): Salina completed a first postdoctoral experience at the University of South Alabama. She currently is a postdoctoral fellow in the intramural research program at NIH in Bethesda, MD.

We will introduce other former trainees in future issues of the newsletter.

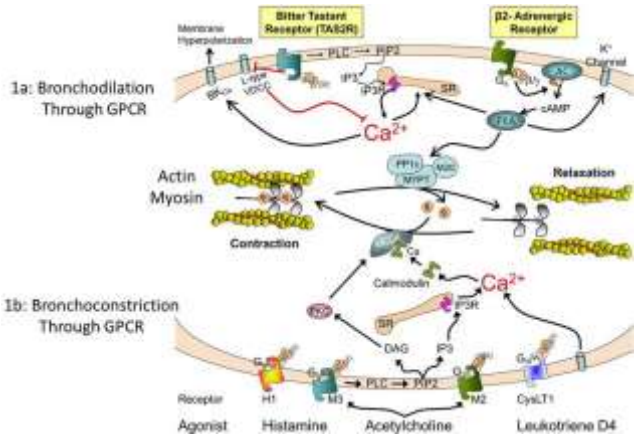
We have included two recent Center for Lung Biology "Did you know…" articles in this issue of the newsletter. This series, authored by our predoctoral trainees focus on historical perspectives for influential discoveries that led to the modern understanding of pulmonary and critical care medicine. The first article, authored by Jared McClendon asked "Did you know …there are taste buds in the respiratory tract?". Next, the article authored by Ningyong Xu, asked "Did you know…that the protein conducting a fly's visual phototransduction has the same origin as those regulating human lung permeability?".

Did You Know…

…there are taste buds in the respiratory tract?

There are taste receptors in the airways, and moreover that they may be future targets for treating obstructive airway diseases? Most are familiar with epithelial sensory receptors, first identified in 2000, on the tongue and palate as they allow us to differentiate bitter tastants [1]. However, bitter taste receptors, or TAS2Rs, are also surprisingly located in several extraoral locations, including pulmonary and vascular tissues. For example, in 2003, TAS2Rs were discovered in the nasal cavity where they regulate respiratory rate and promote sneezing [1]. In addition, in 2009, TAS2Rs on ciliated epithelial cells in the nasal cavity were shown to increase beat frequency [2]. Perhaps most intriguing, airway smooth muscle (ASM) expression of TAS2Rs was observed to regulate bronchial tone [3].

TAS2Rs are G protein-coupled receptors (GPCRs). A variety of other GPCRs on ASM regulate bronchial tone and serve as therapeutic targets for obstructive airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) [4]. These standard-of-care therapies (β-agonists) signal through the β2 adrenergic receptor, a GPCR, and increase the second messenger cAMP leading to relaxation of ASM and bronchodilation (Figure 1A). In contrast, contractile agonists such as leukotriene-D4, acetylcholine and histamine signal through other GPCRs and increase intracellular calcium leading to contraction of ASM and bronchoconstriction (Figure 1B).



Signaling cascades of TAS2Rs and GPCRs in ASM cells to regulate bronchodilation and bronchoconstriction. Adapted from [3, 9].Figure 1A. GPCR signaling leading to ASM relaxation and bronchodilation. In ASM, activation of TAS2Rs and Ggust in localized regions of the cell may lead to increased intracellular calcium, activation of membrane channels including large conductance calcium dependent potassium channels (BKCa) leading to membrane hyperpolarization, and potent ASM relaxation (black lines). Also, activated TAS2Rs may directly inhibit L-type voltage dependent calcium channels (VDCC) and blunt agonist induced calcium influx, leading to ASM relaxation (red lines). Activation of β2-Adrenergic receptors elicit airway smooth relaxation by Gs coupled activation of adenylate cyclase (AC), production of cyclic AMP (cAMP) and activation of protein kinase A (PKA), which phosphorylates multiple substrates to decrease intracellular cell calcium concentration. Decreasing calcium reduces activation of myosin light chain kinase (MLCK) thus favoring myosin light chain dephosphorylation by myosin phosphatase (complex of PP1c, MYPT and M20) and relaxation of ASM.Figure 1B. GPCR signaling leading to ASM contraction and bronchoconstriction. Bronchoconstrictor agonists activate multiple GPCRs and elicit ASM contraction through activation of multiple downstream signaling pathways that ultimately increase intracellular calcium. Intracellular calcium interacts with calmodulin to activate myosin light chain kinase (MLCK). Increasing calcium increases activation of MLCK thus favoring myosin light chain phosphorylation and contraction of ASM.

On oral sensory epithelia, bitter compounds activate TAS2Rs and lead to increased intracellular calcium, TRP channel activation, membrane depolarization, and neurotransmitter release. Since increased intracellular calcium and membrane depolarization on ASM promote bronchoconstriction, the discovery of TAS2Rs on ASM led to the hypothesis that TAS2Rs would mediate increased intracellular calcium on ASM and subsequent bronchoconstriction. However, a series of experiments by Stephen Liggett and colleagues revealed that agonist-induced activation of bitter taste receptors on ASM cells caused localized calcium-dependent signaling leading to membrane hyperpolarization, and marked airway smooth muscle relaxation (Figure 1A) [5]. These results are now under debate as data from other groups shows activation of TAS2Rs does not produce a localized calcium increase. The suggested alternative is that TAS2R activation prevents agonist-induced contraction by limiting calcium influx through L-type voltage dependent calcium channels, thereby reducing calcium sensitivity leading to ASM relaxation (Figure 1A) [6, 7].

Regardless of the specific mechanism, delivery of bitter tastants proved more effective at reversing acute airway bronchoconstriction than β-agonists in experimental asthma [3, 8]. Thus, if issues regarding selectivity, toxicity, distribution, and palatability of bitter tastants can be solved, future drug therapy to control obstructive airway diseases may leave more than a bitter taste behind [9].

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Did You Know…

…that the protein conducting a fly's visual phototransduction has the same origin as those regulating human lung permeability?

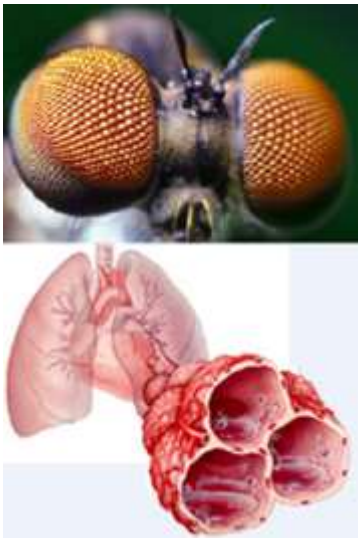

Figure 1. A. Drosophilaeye photoreceptor cells contain TRP channels, while the human TRPC homologs are involved in lung permeability B (figure adapted from[4,5]).

From *Drosophila* to humans, the transient receptor potential (TRP) family of proteins is evolutionarily conserved [1, 2] (Figure 1). Two TRP proteins of the canonical subfamily, TRPC1 and TRPC4, are molecular candidates of store-operated Ca^{2+} (SOC) entry channel subunits involved in increasing human lung permeability.

In 1969, Cosens and Manning discovered the *Drosophila trp* gene by identifying an eye mutation in flies with visual deficiencies [3]. While the normal photoreceptor cells had a sustained membrane depolarization, the mutant cells only had a transient depolarization in response to continuous light. This phenomenon of transient membrane depolarization led to the origin of the name "*trp*" (transient receptor potential). Twenty years later, Montell proposed that "*trp* is the structural gene for the light-sensitive channels" [6]. Around the same time, Putney hypothesized capacitative Ca^{2+} entry [7] to describe agonist-induced endoplasmic reticulum Ca^{2+} release followed by Ca^{2+} influx through plasma membrane channels. Now, this mechanism is widely referred to as SOC entry [8]. In 1993, Minke linked TRP channel and SOC entry together and hypothesized that the *Drosophila* TRP channel may actually be the molecular basis of the mammalian SOC channels [9, 10].

The molecular basis of the SOC channels is complex and involves different proteins including TRP channel proteins in different animal and tissue types. In 1995, a human homolog of the *Drosophila* TRP channel, TRPC1 was identified [1]. While the physiological role of TRPC proteins is still unclear, a pathophysiological effect has been identified. Knockdown of TRPC1 reduced thapsigargin-induced SOC entry and calcium current in human pulmonary artery endothelial cells [11]. Overexpression of TRPC1 increased thrombin-induced SOC entry and lung permeability in both human pulmonary artery and microvascular endothelium [12]. In addition to TRPC1, TRPC4 was found to have a similar role. At the beginning of the 21st century, TRPC4 knockout mice were generated [13, 14]. This type of mouse has lower lung microvascular permeability after thrombin exposure when compared to wild type animals, indicating that TRPC4 also contributes to the increase in lung permeability [14]. From *Drosophila* visual phototransduction to human lung permeability, the *trp* family of genes is conserved during evolution [12, 14].

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