**WS12.1 Assembly of MUC2 N-terminal with relevance for mucus formation**

H.E. Nilsson1,2, D. Ambort1, M. Bäckström1,3, E. Thomsson1, P. Koeck4, H. Hebert5,6, G.C. Hansson1,2, University of Gothenburg, Medical Biochemistry, Gothenburg, Sweden; 2 Karolinska Institute, Biotechnology and Nutrition, Huddinge, Sweden; 3 University of Gothenburg, Mammalian Protein Expression Core Facility, Gothenburg, Sweden; 4 School of Technology and Health, KTH Royal Institute of Technology, Huddinge, Sweden

MUC2, the main gel forming mucin in intestine, is stored as a densely packed multimonomer in goblet cell secretory granules at high calcium and low pH conditions. This mucin is in contrast to normal small intestine attached to the epithelium in Cystic Fibrosis, something that is likely linked to impaired release and expansion of the packed multimonomer. The MUC2 N-terminal part contains the von Willebrand factor (VWF) domain, which controls the detachment of mucus requires a specific proteolytic cleavage of the packed multimer. The MUC2 N-terminal part contains the von Willebrand factor (VWF) revealed that the D3 domain of MUC2 had a similar subdomain organization, namely, VWD, C8, trypsin-inhibitor-like (TIL) and E in the order, TIL′-VWD1-C8-3-TIL-E-3. Reliable cleavage sites were discovered that trimer that upon gel filtration eluted as a hexamer. The obtained 3D maps revealed a hollow, cage-like structure where six monomers were arranged as a dimer of trimers, confirming that the MUC2 mucin forms branched and non linear structures.

Harriet E. Nilsson and Daniel Ambort have contributed equally.

**WS12.2 Mucins are abnormally concentrated in CF respiratory secretions: role in disease pathogenesis**

A.G. Henderson1, B. Button2, R.C. Boucher1, M. Kesner1, 1 University of North Carolina as Chapel Hill, Cystic Fibrosis and Pulmonary Research Center, Chapel Hill, United States

The pathogenesis of cystic fibrosis lung disease remains unclear. The “2-gel” description of mucus clearance predicts that mucus flow depends on mucin concentration and high concentrations of secreted mucins produce muco-obstructive lung disease. We therefore reiterated the mucin concentration in CF sputum using immunological and biophysical techniques. Mucin concentrations were lower in CF sputum than normal mucin measured using single particle image procession. Sequence comparison to the von Willebrand factor (VWF) revealed that the D3 domain of MUC2 had a similar subdomain organization, namely, VWD, C8, trypsin-inhibitor-like (TIL) and E in the order, TIL′-VWD1-C8-3-TIL-E-3. Reliable cleavage sites were discovered that trimer that upon gel filtration eluted as a hexamer. The obtained 3D maps revealed a hollow, cage-like structure where six monomers were arranged as a dimer of trimers, confirming that the MUC2 mucin forms branched and non linear structures.

Harriet E. Nilsson and Daniel Ambort have contributed equally.

**WS12.3 Detachment of mucin requires a specific proteolytic cleavage in the MUC2 mucin explaining why the cystic fibrosis mucus is attached to the epithelium**

A. Schütte1, A. Ermund1, C. Becker-Pauly2, M.E.V. Johansson1, A.M. Rodriguez-Piñeiro3, F. Bäckhed1, S. Müller1, D. Lottaz2, J.S. Bond1, G.C. Hansson1. 1 University of Gothenburg, Dept. of Medical Biochemistry and Cell Biology, Göteborg, Sweden; 2 Christian Albrechts-University Kiel, Unit for Degradomics of the Protease Web, Institute of Biochemistry, Kiel, Germany; 3 University of Gothenburg, Wallenberg Laboratory for Cardiovascular and Metabolic Research, Dept. of Molecular and Clinical Medicine, Göteborg, Sweden; 4 University of Bern, FACS Lab, Institute of Pathology, Bern, Switzerland; 5 University of Bern, Dept. of Clinical Research, Bern, Switzerland; 6 Penn State University Hershey, Dept. of Biochemistry and Molecular Biology, Hershey, United States

**Objectives:** The mucus of the intestine is a highly organized system which protects the epithelia against microbial challenges from the outside. The mucus layers consist of one major building brick, the MUC2 mucin, which is produced and secreted by goblet cells. This extensively glycosylated molecule forms a loosely structured layer in the ileum which is normally not attached to the epithelium. In contrast, we found recently, that the ileal mucus of Cystic Fibrosis (CF) mice is attached. Here we describe a mechanism which can explain the attachment of the mucus in this disease.

**Methods:** Using an Ussing-type explant system, the attachment of the mucus in the small intestine of CF mice could be reversed by the addition of bicarbonate. Bicarbonate is suggested to be necessary for the normal unfolding of the densely packed mucin.

**Results:** We have now discovered that the zinc-dependent metalloprotease meprin β is responsible for the detachment of the mucins from the epithelium by a cleavage in the MUC2 mucin. Accordingly, the mucus of meprin β-deficient mice was attached and could be released upon addition of the recombinant enzyme. The treatment of CF mucus with the recombinant meprin β alone had no effect. However, the addition of bicarbonate released the CF mucin from its attachment.

**Conclusions:** The findings suggest that unfolding is necessary for exposing the meprin β cleavage sites for releasing the attached CF mucin. Consequently, the unfolding process is impaired in CF. This novel mechanism enables us to shed some light on basic processes in Cystic Fibrosis.

**WS12.4 Planar cell polarity protein network, which controls ciliogenesis and cilia function, is altered in human cystic fibrosis bronchial epithelial cells through response to endoplasmic reticulum stress**

S. Noël1, W. Delbart1, B. Dhogue1, T. Leal1, 1 Université Catholique de Louvain, Louvain Centre for Toxicology and Applied Pharmacology (LEAP), Brussels, Belgium

**Objectives:** Micronuclei clearance (MCC), abnormal in CF lung, is regulated by several mechanisms including epithelial cilia movement. Planar Cell Polarity (PCP) has been described in mammalian lung epithelial cells as a crucial mechanism controlling ciliogenesis and cilia function. Although the majority of studies on cilin CF showed no structural abnormality and normal cilia beat frequency, cilin disorientation was showed to occur secondary to lung inflammation. We hypothesized that CF BEs display abnormal MCC network and that further impaired coordinated cilin function.

**Methods:** We quantified expression of several PCP genes in HBEs and observed influence of CFTR genotype on MCC expression. By Western-Blot and immunofluorescence, we determined which components of PCP network were abnormal in CF cells. We performed pharmacological studies (F508del-CFTR rescue, ER stress inducing) to determine what causes MCC dysfunction in CF-HBEs.

**Results:** We demonstrated that HBEs expressed several PCP genes. Among them, expression of CELS3R (Catherin EGF LAG seven-pass G-type receptor 3) was down-regulated in CF (F508del/F508del) HBEs as compared to non-CF cells. In contrast, Vangl2 (Van Gogh-like 2), FZD3 (Frizzled 3) and PK4 (Prrickle 4) were upregulated in CF cells. Very low levels of immature (unglycosylated, uncleaved) CELS3R protein were found in CF cells. ER stress inducers reduced expression of CELS3R in 16HBE14o− cells line, suggesting that ER stress affects MCC network in HBE cells.

**Conclusion:** Expression and processing of PCP proteins is abnormal in CF-HBEs and may alter coordinated function of cilin within the bronchial epithelium in Cystic Fibrosis.