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Meeting Report, Session 2: "Connexin Binding Proteins"

Session Chair and Summary Author: Matthias M. Falk

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Although in electron-microscopic images gap junctions (GJs) appear not to be decorated substantially with proteins on their cytoplasmic surface, biochemical analyses have revealed quite a number of proteins that interact directly with the cytoplasmic C-terminus (CT), especially of connexin 43 (Cx43). In general, Cx43 interacting proteins fall into three categories: regulatory proteins, such as ZO-1 that control the assembly, function, and degradation of GJ channels; multiple kinases that phosphorylate distinct Cx43-CT serine and tyrosine residues; and structural interactions with microtubules and actin filaments. In the second session of this meeting ("Connexin Binding Proteins") the potential function of known Cx-interacting proteins was evaluated, and several novel Cx-binding proteins were reported.

A number of presentations addressed the function of zonula occludens 1 (**ZO-1**) a MAGUK (Membrane Associated Guanylate Kinase) scaffold protein family member that binds to the C-termini of at least 7 different connexins (Cx31.9, Cx36, Cx43, Cx45, Cx46, Cx47, and Cx50), however, the functional relevance for ZO-1 at GJs has remained largely elusive. Andrew Hunter, Robert Gourdie and colleagues (Medical University of South Carolina, Charleston, SC, USA) obtained considerable evidence that ZO-1 controls

channel accretion and possibly plaque fusion and thereby might regulate GJ size and distribution. Xinbo Li, Cristina Ciolafan, James Nagy and coworkers (University of Manitoba, Winnipeg, Manitoba, Canada) obtained evidence that astrocyte (Cx30, Cx43), oligodendrocyte (Cx32, Cx47) and retinal (Cx36) connexins interact with ZO-1 and the Y-box transcription factor 3 (**MsY3**), the mouse ortholog of **ZONAB**. ZONAB was previously reported to interact with ZO-1 in MDCK cells and to regulate expression of the proto-oncogene **Erb2** in these cells. Thus, signaling through ErbB2 may in part be regulated by interactions between connexins, ZO-1 and the transcription factor ZONAB/MsY3. Vincent Chen, James Nagy, and coworkers also obtained evidence that **plectin**, a widely expressed intermediate filament binding protein implicated in epidermolysis bullbosa simplex muscular dystrophy interacts with ZO-1 at Cx43 based GJs. Thus, GJs, ZO-1 and plectin may function together to regulate cytoskeleton remodeling, cell proliferation, and differentiation.

Two new scaffolding proteins were reported to interact with connexins. Bonnie Warn-Cramer and colleagues (University of Hawaii, Honolulu, HI, USA) identified **14-3-3 theta**, a small acidic protein that interacts directly with the Cx43-CT, specifically with Ser373. 14-3-3 binds to over 200 different proteins and it's known functions include the induction of conformational changes, masking of specific molecular sites, and serving as a molecular scaffold. 14-3-3's binding to the Cx43-CT was dependent on phosphorylation of Ser373. The authors identified the Ser/Thr kinase Akt as the likely kinase responsible for Ser373 phosphorylation. Based on these and additional observations, 14-3-3 theta is believed to bind in an Akt mediated phosphorylation to Cx43 and to induce conformational changes or alter Cx43's association with other

proteins. Heather Duffy and colleagues (Columbia University and Albert Einstein College of Medicine, Yeshiva University, New York, NY, USA) reported that another PDZ-containing scaffolding protein, human disc large (**hDlg**) interacts with Cx32 in murine liver and that the two proteins colocalize in the membranes of hepatocytes. hDlg acts as a tumor suppressor protein via its interaction with Ademateous Polyposis Coli (APC), and loss of the hDlg interaction with APC is tumorigenic in multiple tissues. In Cx32 null mice, hDlg looses its localization at hepatocyte membranes and instead localizes within the nucleus. Thus, loss of Cx32 mis-localizes hDlg and suggests a possible mechanism for the hepato-tumorigenesis observed in Cx32 null mice.

Another cancer related protein, the CCN (Cyr61, CTGF, NOV) gene family member **CCN3** was reported by Wun-Chey Sin and Christian Naus (University of British Columbia, Vancouver, BC, Canada) to interact directly with the Cx43-CT. Based on the known implication of CCN family members in tumorigenesis and metastasis, and the potential role of Cx43 in regulating cell growth via association of its cytoplasmic tail with signaling molecules the authors are investigating a potential role of the CCN3/Cx43 interaction in breast tumorigenesis.

Finally, a number of proteins were described that interact with connexins and regulate their trafficking, and degradation. Francisco del Castillo, Christine Petit and colleagues (Institut Pasteur, Paris, France) reported the identification of a novel protein, **consortin**, that is involved in the intracellular sorting of Cx26 and Cx30. Consortin co-localizes with Golgi complex markers, and expression of a mutant consortin resulted in an intracellular accumulation of connexins. Elissavet Kardami and coworkers (University of Manitoba, Winnipeg, Manitoba, Canada) reported a direct interaction of the Cx43-CT

with the Damaged DNA-Binding Protein 1 (**DDB1**). DDB1 interacted preferentially with phosphorylated Cx43. DDB1 can participate in DNA repair in the cell nucleus, however, it can also recruit and target proteins for proteolytic degradation in response to DNA damage. In view of the described properties of DDB1 their results suggest a potential involvement of DDB1 in regulating the subcellular distribution of Cx43, its recruitment to the plasma membrane and regulating its degradation. Michelle Piehl, Matthias Falk and colleagues (Lehigh University, Bethlehem, PA, USA) reported a specific interaction of the Cx43-CT with the alternative adaptor protein disabled 2 (**Dab2**), the coat protein **clathrin**, **myosin-VI**, **actin filaments**, and the large GTPase **dynamin** that are involved in the internalization, inward movement and degradation of GJs, a process that involves the formation of double-membrane vesicles known as annular GJs.

As evident from this meeting, the number of connexin interacting proteins is rapidly increasing. Known functions of these proteins suggest a tight regulation of GJ assembly, cell-to-cell communication, and channel degradation, as well as a critical role of plasma membrane-localized Cx43 in other cell signaling functions. The fast pace of current GJ research suggests that many known and new functions of connexins will be deciphered in the near future.