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CHAPTER 49

MOLECULAR ANALYSIS OF THE SUPRAMOLECULAR USHER PROTEIN COMPLEX IN THE RETINA

Harmonin as the key protein of the Usher Syndrome

Jan Reiners and Uwe Wolfrum*

1. Introduction

Human Usher syndrome (USH) is the most common form of deaf-blindness and also the most frequent case of recessive *retinitispigmentosa.* According to the degree of the clinical symptoms, three different types of the Usher syndrome are distinguished: USH1, USH2 and USH3 (Davenport and Omenn, 1977). USH is genetically heterogeneous with eleven chromosomal loci, which can be assigned to the three USH types (USH1A-G, USH2A-C, USH3A) (Petit, 2001). Out of these, USHl is the most severe form, characterized by profound congenital deafness, constant vestibular dysfunction and prepubertal-onset *retinitispigmentosa.* USH2 patients show a milder congenital deafness, a slightly later onset of *retinitis pigmentosa* and no vestibular dysfunction. The rarest Usher type 3 shows a late onset of *retinitis pigmentosa* and a progressing hearing loss. So far the different USH subtypes have been grouped into one disease basically on the same phenotype of the patients, although the clinical symptoms of the individual differ noticeably. The protein harmonin, responsible for USH1C, is of special interest, since it contains three PDZ domains, known for protein-protein interactions. We have gathered evidence that the different USH proteins are molecularly linked essentially via the scaffold protein harmonin. Harmonin interacts hereby not only with USHl proteins, but also with USH2 proteins. Thus, this is the first evidence for a molecular linkage between USHl and USH2, beyond the shared phenotype.

*Jan Reiners and Uwe Wolfrum, Instiutut fur Zoologie, Universitat Mainz, 55099 Mainz, Germany; E-mail: wolfrum@uni-mainz.de.

2. HARMONIN INTERACTS WITH ALL USHl PROTEINS

All USH proteins belong into different protein classes. Among the USHl proteins there are myosin VIIa (USH1B), two cadherins (cadherin 23/USH1D and protocadherin 15/USH1F) and with harmonin (USH1C) and SANS (USH1G) two scaffold proteins. Among these proteins harmonin is of special interest since it contains three PDZ domains. Recent results showed that all known USHl proteins interact with harmonin via its PDZ domains (Boeda et al., 2002; Siemens et al., 2002; Weil et al., 2003; Adato et al., 2004; Reiners et al., submitted). Thereby, the USH-cadherins cadherin 23 and protocadherin 15 interact with the PDZ2 domain of harmonin. Myosin VIIa and SANS on the other hand bind to harmonin's PDZ1 and PDZ 1/PDZ3 respectively.

In addition, USHl proteins also exhibit homomeric interactions. Harmonin has been shown to initiate homomeric interactions via PDZl and the C-terminal of the major harmonin isoform a1 which provides the basis for polymeric protein chains (Siemens et al., 2002; Adato et al., 2004; Reiners et al., unpublished). Homodimers were also demonstrated for the USH1 proteins, myosin VIIa and SANS (Inoue and Ikebe, 2003; Adato et al., 2004). Moreover, dimerization is commonly found in cadherins and seems likely for the USHl cadherins, cadherin 23 and protocadherin 15 (Bolz et al., 2002). Chains of harmonin may connect these dimers and integrate them into a protein network. In summary, the harmonin scaffold may integrate USHl proteins and their dimers into USHl networks and complexes.

3. HARMONIN INTERACTS WITH ALL USH2 PROTEINS

To date three USH2 genes have been identified. The first isolated USH2 gene was the most common form of the Usher syndrome, USH2A. It encodes for Usherin previously depicted as an extracellular matrix protein (Eudy et al., 1998). Recently, a splice variant of Usherin has been described which contains a transmembrane domain and a cytoplasmatic part including a PDZ binding motif (Van Wijk et al., 2004). At nearly the same time Weston et al. (2004) identified the gene defective in USH2C patients. The isoform causing USH2C is called "very large G-protein coupled receptor 1 b "(VLGR1b), a member of the GPCR-superfamily. Its cytoplasmatic C-terminal tail contains a PDZ binding motif, as well. The affected gene in patients of USH2B was suggested to be the sodium bicarbonate transporter NBC3 (Bok et al., 2003). This prediction was based on following observations: the gene encoding for NBC3 is located in the human USH2B locus and mice lacking the murine ortholog of NBC3 show the USH phenotype. In previous studies, this co-transporter was localized in the kidney, where it interacts with the PDZ-protein NHERF-l via its C-terminal (Pushkin et al., 1999; Pushkin et al., 2003). To summarize, although the USH2 proteins are members of very distinct families of transmembrane proteins, they have promising PDZ binding motifs of the class I (Nourry et al., 2003) at their C-terminus in common.

The interaction of these three proteins with the PDZ1domain of harmonin was demonstrated in GST-pull down assays and using the yeast two-hybrid system (Reiners et al. in prep.). While the USHl proteins rather function as cell adhesion and scaffold proteins, the USH2 proteins seem to be large "functional proteins", in a physiological point of view for the cell. These functional proteins may be positioned and anchored via harmonin in the USHl protein network to form a supramolecular Usher protein complex.

4. FURTHER INTERACTING PARTNERS OF HARMONIN

Harmonin is expressed in many tissues and has been shown to interact with further proteins, which are not directly related to the Usher syndrome. The protein MCC2 was reported to interact with the PDZ1 domain of harmonin (Ishikawa et al., 2001). In pancreatic cells, the protein HARP, a protein with a large homology to SANS, binds to harmonin (Gonez et al., 2001; Johnston et al., 2004).

Recently, we identified further interacting partners of harmonin by yeast two-hybrid screening of a retinal cDNA-library: one of these proteins is the actin-binding protein filamin A. In the cellar environment, filamin A forms homodimers, stabilizes three-dimensional branching of actin filaments and links membrane proteins to the actin cytoskeleton (Gorlin et al., 1990; van der Flier and Sonnenberg, 2001). The interaction of filamin A with harmonin was confirmed by GST-pull down-assays and immuno-precipitations (Reiners et al., in prep.). Like actin-associated motor myosin VIIa (USH1B), filamin A provides a connection between the Usher protein complex and the actin cytoskeleton.

5. SUPRAMOLECULAR USHER PROTEIN COMPLEXES IN THE RETINA

To understand the cellular function of the USH proteins and their complexes respectively, it was essential to determine their subcellular localization. For this purpose, specific antibodies against the different interaction partners were generated and used for subcellular localization in the mammalian retina (Reiners et al., 2003; Wolfrum and Reiners, 2004; Reiners et al., submitted; Reiners et al., in prep.). Immunocytochemical analyses revealed that the partner molecules are localized in several distinct compartments of retinal photoreceptor cells. However, the co-localization of all USH proteins and the other complex partners – a necessary prerequisite for the assembly of a supramolecular complex – was determined in the outer plexiform layer. In this retinal layer the synaptic terminals of photoreceptor cells are sited. Together with our binding studies, this indicates that the identified Usher protein complex partners assemble in the photoreceptor cell synapses.

In the photoreceptor synaptic terminals, USH complexes may play fundamental roles in the structural and functional integrity of this synaptic junction. The scaffold protein harmonin bridges the activity of integral membrane USH2 proteins with the actin cytoskeleton (including filamin A and myosin VIIa) and the cell-cell adhesion sites generated by cadherin 23 and protocadherin 15. It is very likely that there are even more "functional" proteins, which are integrated into this USH complex. This hypothesis is outlined in figure 49.1. Defects of any of the USH-complex partners should result in synaptic dysfunction which in turn may cause *retinitis pigmentosa*, the clinical phenotype in the retina of USH patients.

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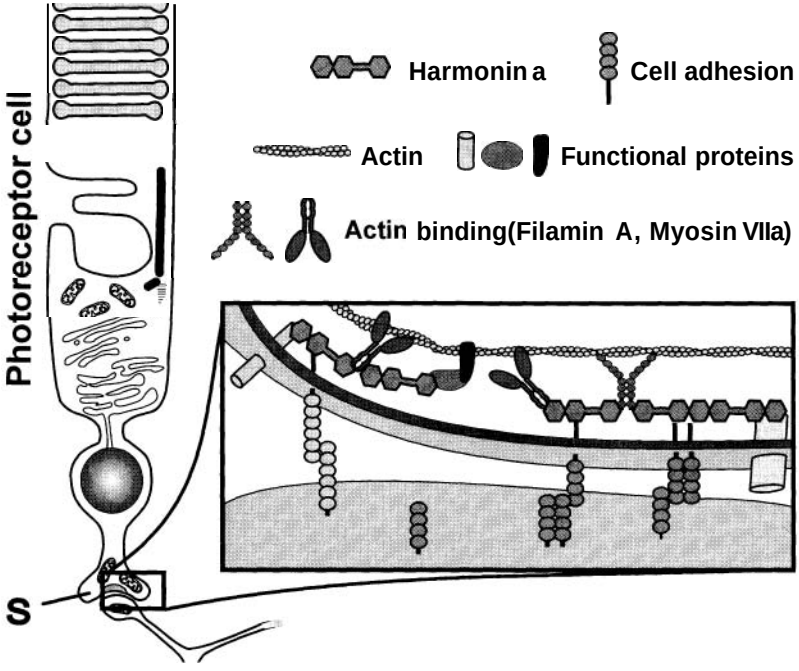

Figure 49.1. Scheme of the supramolecular Usher protein complex at photoreceptor synapse (S). Harmonin (USHIC) may act as the scaffold between the USH-cadherins (cadherin 23 1 USH1D, protocadherin 15 / USH1F) and the actin binding proteins (myosin VIIa / USH1B, filamin A). In this fundament further "functional" proteins (e.g. NBC3 / USH2B) may be anchored and positioned.

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