# The otoferlin interactome in neurosensory hair cells: Significance for synaptic vesicle release and trans-Golgi network (Review)

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**Abstract.** Sound perception in terrestrial vertebrates relies on a structure in the inner ear consisting of the utriculus, sacculus and lagena. In mammals, the lagena has developed into the cochlea where mechanotransduction at ciliated cells leads to ion influx via regulated ion channels. To maintain proper $Ca^{2+}$ concentration many cellular systems use a variety of functional proteins; the neurosensory systems use calcium-sensors like hippocalcin, visinin or recoverin. In cochlear hair cells the 230 kDa protein otoferlin has been suggested to play this role. While several observations support this hypothesis additional data argue for a more expanded functional profile of otoferlin. Evidence for otoferlin's multiple roles and newer results on otoferlin's interacting partners are presented and the existence of a protein complex as a functional unit ('interactome') in the cochlea and further tissues is suggested.

**Contents**



## 1. Introduction

Two neurosensory functions aid in the orientation of mobile terrestrial animals and allow perception over larger distances: the visual and auditory senses. Humans register sound waves in the range of 20-20,000 Hz, while other vertebrates show capacities below or above the human limits. The impressive sensitivity of hearing is derived from the transformation of mechanical signals to ion flux and depolarization resulting in electric signals transmitted to the brain via the eighth nerve. This complex process requires a highly specialized structure and the mammalian cochlea is the result of evolution of a refined instrument for sound perception (1). One key element of the intricate cochlear organ is a set of ciliated cells (inner and outer hair cells) where mechanical movement of the cilia opens or closes cation channels (2). Since the cilia reach into a space filled with endolymph rich with potassium, channel opening leads to potassium influx and depolarization of hair cells. This event, in turn, opens calcium channels, thus leading to further depolarization by allowing calcium influx, which triggers synaptic vesicle exocytosis and neurotransmitter release (3,4). To distinguish temporally between sounds these steps must occur quickly; thus, to facilitate high rates of sustained synaptic transmission inner hair cells contain specialised structures, the so-called synaptic ribbons. These are capable of tethering numerous synaptic vesicles at release sites (5) for a speedy, coordinated process. Moreover, calcium-activated potassium channels control a rapid potassium flux required for improved perception of high frequencies (6). This short description underlines the significance of cations and their channels in the hearing process.

## 2. Calcium sensors

In the many specialised cell types of the body numerous proteins functioning as calcium sensors have been reported (7-12). In neurons of the brain and in neurosensory systems proteins like hippocalcin (13) or visinin and recoverin (14) play a crucial role as key mediators of many cellular functions including synaptic plasticity or regulation of ion channels. In the ciliary membranes, the sites of odorant transduction, for example, the guanylyl cyclase activating protein 1 (GCAP1), responds to the free $Ca^{2+}$ by inhibiting or stimulating further proteins, some of those also linked to phototransduction. This demonstrates that a single $Ca^{2+}$ sensor, working in two opposite modes of signalling, can serve two sensory processes (olfactory and visual) (15).

Acoustic perception is a highly complex sense and one key component, the cochlea of the inner ear, consist of many specialised cell types. Therefore, not surprisingly, more than

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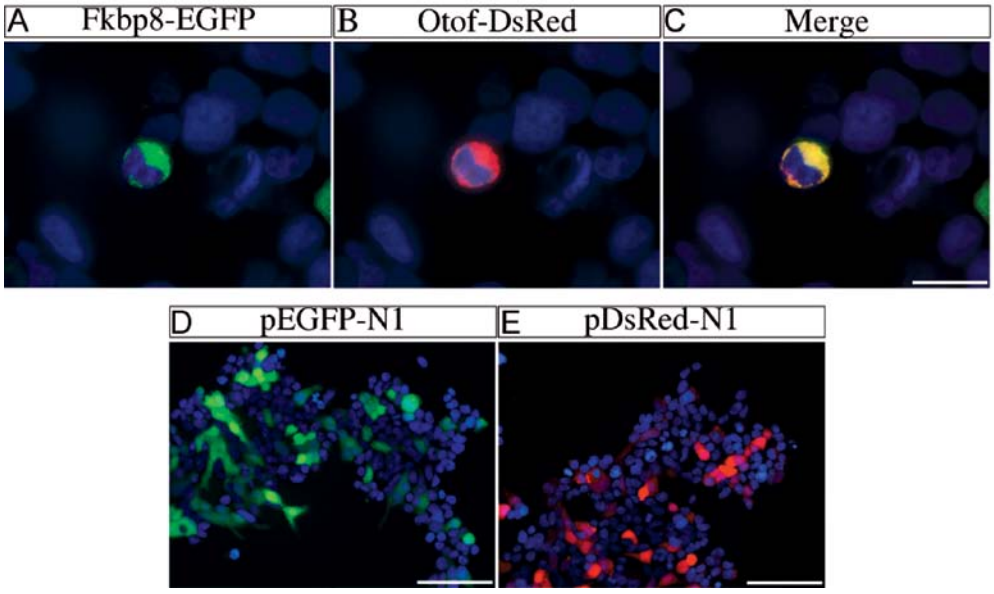


Figure 1. Co-localization of Fkbp8 and otoferlin in HEK293 cells. HEK293 cells were simultaneously transfected with (A) Fkbp8-pEGFP and (B) otoferlin-pDsRed. (C) The resulting mixed colour (yellow) signal of co-localization was observed upon merging of A and B photographs. (D and E) Transfection with empty pEGFP-N1 and pDsRed-N1 leads to a ubiquitous distribution of fluorescent signals in cells. Cell nuclei were counterstained with DAPI. Scale bars, 20 $\mu$m.

200 genes are expected to participate in its structural build-up and function (data from http://hereditaryhearingloss.org/). A number of proteins coded by these genes cooperate in a temporal and spacial setting and elucidation of such interacting complexes, called 'interactomes', has moved into the focus of recent research. For one syndromic hearing defect (Usher syndrome type I) the cooperation of myosin VIIa, harmonin, sans, protocadherin 15 and cadherin 23 was shown to be essentail for the organisation of sensory hair cells (16). In the non-syndromic hearing impairment DFNA4, where the primary target of mutation was found to be MYH14 (17,18), actin filaments interact with 2E4-Kaptin and in stereocilia myosin XVa together with whirlin control the development of these cells (19,20).

### 3. Otoferlin as a calcium sensor

In the auditory system the neurons were reported to contain their own calcium sensors, synaptotagmin I/II (21), that mediate neurotransmitter release in a complex termed SNARE (22). In the inner ear, cochlear and vestibular hair cells may have their sensor protein: otoferlin. This suggestion was based on several observations. (i) Mature hair cells do not show significant amounts of synaptotagmin (23); (ii) synaptotagmin has two $Ca^{2+}$ binding domains (C2A, C2B) and otoferlin displays six C2 domains (C2A-C2F) (24,25). Though the amino acid alignment indicates lack of complete conservation within the OTOF C2 motifs, dysferlin, while also binding $Ca^{2+}$, deviates from the classic C2 core motif as well (26); (iii) otoferlin-deficient mice are deaf but show functional mechanoelectrical transduction. Their hair cells do not release neurotransmitters in response to calcium influx (27); (iv) in hair cells, replenishment of synaptic vesicles was demonstrated to be otoferlin-dependent (28); and (v) five of the six C2 domains of otoferlin stimulate membrane fusion in a calcium-dependent fashion (29).

Table I. Potential interacting partners of otoferlin identified by yeast-2-hybrid system.

| Protein | Cochlea cDNA |
|---|---|
| Fkbp8 | + |
| cAMP | + |
| Col2a1 | + |
| RhoGTPase3 | + |
| MAP kinase2 | + |
| Clusterin | + |
| Pleiotrophin | + |
| Rps21 | + |
| Igfbp2 | + |
| ALG-2 | + |

Expression of this set of candidates was confirmed by RT-PCR in cochlear RNA extracts (33).

### 4. Otoferlin's interactome

In addition to otoferlin's contribution to calcium-dependent exocytosis via 'ribbon synapses', as discussed above, several new data suggest otoferlin's participation in the endosomal trans-Golgi network. While these observations do not exclude otoferlin as a calcium sensor involved in membrane fusion reactions, they may extend its action potential to vesicle recycling/membrane trafficking. One first indication of such additional functions was the demonstration of its expression in a widened pattern, including the brain (25), the vestibular system, neurons and nerve fibers. Also, apart from its presence in cochlear inner hair cells, otoferlin was found to be present in mature outer hair cells responsible for low-frequency

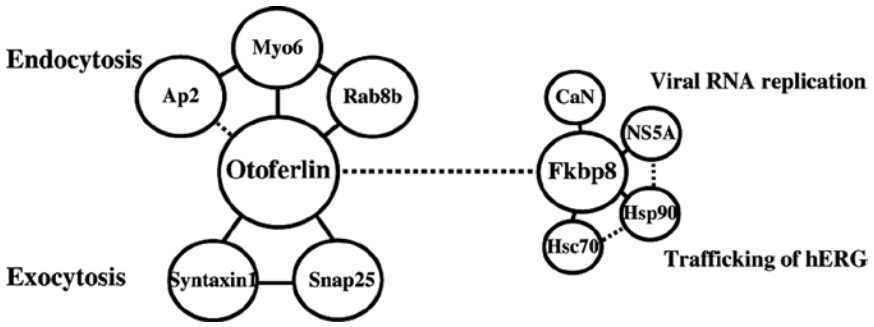


Figure 2. Schematic diagram of otoferlin interactom. Schematic interactom of otoferlin comprises verified (solid line) and hypothetical (broken line) binding partners involved in exocytosis via ribbon synapses (Syntaxin 1, Snap25) and endosomal trans-Golgi trafficking (Myo6, Rab8b, Ap2). The diagram includes Fkbp8, a further potential interactor suggested by yeast-2-hybrid screening, and a set of known binding proteins of Fkbp8 [calcineurin (CaN), HCV nonstructural protein 5A (NS5A), Hsp90 and Hsc70].

processing (30). Its subcellular distribution beyond regions of synaptic vesicle fusion and its association with Golgi markers suggested a more ubiquitous role. Protein-protein association studies demonstrated that otoferlin can not only interact with syntaxin 1 and Snap25, both ribbon synaptic components (27) but with Rab8 and Myo6, two proteins involved in the trans-Golgi network and in cargo sorting (31,32).

Screening for otoferlin's partners using the yeast-2-hybrid system suggested further candidates (33) (Table I). Fkbp8 is a multifunctional protein shown to be involved in patterning of neuronal tissues (34). Since it displays a transmembrane domain it is expected to be localized at such. In fact, it was shown to be associated with the endoplasmic reticulum and the mitochondria (35,36). Additionally, Fkbp8 is an interacting partner of two other membrane-anchored proteins: Bcl-2 and Bcl-xL, which are mitochondria-bound anti-apoptotic molecules (36,37). Fkbp8 is required to antagonize Sonic Hedgehog signaling during formation of the central nervous system and together with Hsp73 and Hsp90 regulates trafficking of hERG potassium channels in the heart (34,38). Moreover, it interacts with the NS5A protein of the hepatitis C virus and recruits Hsp90 to form a complex participating in viral RNA replication (39). Fkbp8 has presently been investigated in more detail and was co-localized with otoferlin in HEK cells (Fig. 1) but not in cochlear hair cells, the site of otoferlin's presence. Fkbp8 displays an interesting expression pattern during cochlear maturation in rodents, i.e. from the time of pre-hearing onset (P5, postnatal day 5) its low level expression increases and peaks at P10 and decreases again after hearing onset (>P15). At the same time, its expression pattern is broadened: in addition to spiral ganglia it appears within cells of the stria vascularis (unpublished data). While these findings assign some role to Fkbp8 in the maturation of rodent hearing, it appears not to be a definite partner in the cochlear otoferlin interactome.

Recent data suggest a further component of this interactome. This candidate, Ap2, is a member of the clathrin-dependent endocytosis complex and is itself a heterotetramere which is called adaptor protein complex 2 (40). Ap2 was demonstrated to interact with Myo6 in intestinal epithelia (41) and otoferlin to bind Myo6 in cochlea (31) suggesting otoferlin's possible complex formation with Ap2 via Myo6. This model was most recently supported by co-localization studies in rodent cochlea. Also, both genes are activated within the same time frame in the rodent brain (42). These data additionally support the idea of otoferlin contributing to endocytosis in auditory hair cells.

In a new model of otoferlin impairment, only one amino acid in a C2 domain was mutated. In these mice, the overall otoferlin structure does not differ significantly from the wild-type protein and the structure of the synapses remains the same. Interaction with $Ca^{2+}$ and synaptic vesicles are expected not to be affected (28). Thus, the authors' explanation aims at a disturbed protein-protein interaction in otoferlin's interactome.

In summary, all these findings suggest that otoferlin executes its function in possibly variable cooperation complexes depending on whether action at ribbon synapses, endocytic or secretory trafficking is required (Fig. 2). Synaptic transmission involving otoferlin as a calcium-sensor has been reported (29) but for neurotransmitter release in hair cells it may not be entirely necessary: hypothyroid rats that lack otoferlin in their IHCs show IHC exocytosis (43). Whether this suggests that an otoferlin interactome may be substituted by a comparable protein complex without otoferlin remains to be elucidated in further investigations. Such studies should show whether a different, yet uncharacterized protein replaces otoferlin's function or whether the otoferlin interactome may only be required for a defined time span during cochlear maturation. Despite such open questions it is clear that otoferlin plays a crucial role in the inner ear's function with mutations resulting in auditory neuropathy (44).

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