Cell. Mol. Life Sci.
DOI 10.1007/s00018-016-2257-3

Cellular and Molecular Life Sciences

REVIEW

The role of post-translational modifications in hearing and deafness

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Received: 9 March 2016 / Revised: 21 April 2016 / Accepted: 26 April 2016
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Abstract Post-translational modifications (PTMs) are key molecular events that modify proteins after their synthesis and modulate their ultimate functional properties by affecting their stability, localisation, interaction potential or activity. These chemical changes expand the size of the proteome adding diversity to the molecular pathways governing the biological outcome of cells. PTMs are, thus, crucial in regulating a variety of cellular processes such as apoptosis, proliferation and differentiation and have been shown to be instrumental during embryonic development. In addition, alterations in protein PTMs have been implicated in the pathogenesis of many human diseases, including deafness. In this review, we summarize the recent progress made in understanding the roles of PTMs during cochlear development, with particular emphasis on the enzymes driving protein phosphorylation, acetylation, methylation, glycosylation, ubiquitination and SUMOylation. We also discuss how these enzymes may contribute to hearing impairment and deafness.

Keywords Inner ear · Cochlea · Post-translational modifications · Development · Ototoxicity

Abbreviations

ABR	Auditory brainstem response
ADP	Adenosine diphosphate
Ac-CoA	Acetyl-coenzyme A
ALG10B	Alpha-1,2-glucosyltransferase
ARHL	Age-related hearing loss
Atoh1	Protein atonal homolog 1
ATP	Adénosine triphosphate
Aos1/Uba2	Ubiquitin-like 1-activating enzyme E1B
CBP	CREB-binding protein
CREB	cAMP-responsive element binding protein
Cx26	Connexin-26
DFNB61	Autosomal recessive deafness-61
DNA	Deoxyribonucleic acid
DUBs	Deubiquitinating enzymes
E1	Ubiquitin activating enzyme
E2	Ubiquitin conjugating enzyme
E3	Ubiquitin ligase
ERK1/2	Extracellular signal-regulated kinases 1/2
ERM	Ezrin, radixin and moesin proteins
Fbx2	F-Box protein 2 (also called Fbs1 or OCP1)
FGF	Fibroblast growth factor
GCN5	General control nonderepressible
GJP2	Gap junction protein type 2
GPI	Glycosylphosphatidylinositol
GTP	Guanosine triphosphate
H3K9	Histone H3 lysine 9
HCs	Hair cells
IDH2	Isocitrate dehydrogenase
IHCs	Inner hair cells
JAB1	Jun activation domain-binding protein 1
JAMMs	JAB1/MPN/MOV34 metalloenzymes
JNK	c-Jun N-terminal kinase
KATs	Lysine acetyl-transferases
KDACi	Lysine deacetylases inhibitors
KDACs	Lysine deacetylases
KDMs	Histones lysine demethylation enzymes

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Published online: 04 May 2016

KDM4B	Lysine (K)-specific demethylase 4B (also called jumonjiD2)
LKB1	Liver serine/threonine kinase B1
MAP3K1	Mitogen-activated protein kinase kinase kinase 1
MAP3K4	Mitogen-activated protein kinase kinase kinase 4 (also called MEKK4)
MAP3K7	Mitogen-activated protein kinase kinase kinase 7 (also called Tak1)
MAPK	Mitogen-activated protein kinase
MOV34	Proteasome 26S subunit, non-ATPase 7 (PSMD7)
MPN	MPR1/PAD1 N-terminal
MPR1	sigMa 1278b gene for L-proline-analog resistance
mRNA	Messenger ribonucleic acid
MYST	Named for the founding members MOZ, YBF2, SAS2 and TIP60
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NEDD8	Neural precursor cell expressed, developmentally down-regulated 8
NF-κB	Nuclear factor-kappa B
OCP1	Organ of corti protein 1 (also called Fbs1 or Fbx2)
OHCs	Outer hair cells
OTUs	Ovarian tumor proteases
PAD1	Protein-arginine deiminase 1
PCAF	p300/CBP-associated factor
PCP	Planar cell polarity
Pk	Prickle1
PKMT	Protein lysine methyl transferases
PRMT	Protein arginine methyl transferases
PTK7	Protein tyrosine kinase 7
PTMs	Post-translational modifications
RhoA Ras	Homolog gene family, member A
ROCK1/2	Rho-associated protein kinase 1/2
SAH	*S*-Adenosyl homocysteine
SAM	*S*-Adenosyl methionine
SCs	Supporting cells
SCF	SKP1-cullin-F-box
Scr	Proto-oncogene tyrosine-protein kinase
SGN	Spiral ganglion neurons
SLC26A4	Solute carrier family 26 (anion exchanger), member 4
SLC26A5	Solute carrier family 26 (anion exchanger), member 4
SMAD	Homologs of the Drosophila protein, mothers against decapentaplegic (MAD)
Smurf1/2	SMAD specific E3 ubiquitin protein ligase 1/2
SUMO	Small Ubiquitin-like MOdifier
UBC9	UBiquitin-conjugating 9
UCHs	Ubiquitin C-terminal hydrolases
Usp53	Ubiquitin-specific protease 53
USPs	Ubiquitin-specific proteases
Wnt	Wingless-related integration site

Introduction

Proteins can be regulated after translation by the reversible or irreversible addition of functional groups (e.g., phosphorylation, acetylation, and methylation), peptides (e.g., ubiquitination, SUMOylation) or other complex molecules (e.g., glycosylation). These post-translational modifications (PTMs) diversify and extend protein function beyond what is dictated by gene transcripts by altering the structure of proteins. Through changes in protein conformation, PTMs have been shown to modulate the stability, localisation, interacting partners or activity of their substrates. The precise effect of PTMs depends on the nature of the covalent modification, the identity of the substrate and the residue that is specifically targeted by the chemical reaction. Hence, PTMs are crucial for regulating the functions of many eukaryotic proteins and play pivotal roles in a highly diverse range of cellular functions. It is now well accepted that these modifications are required for a tight regulation of cell proliferation, differentiation and apoptosis and are instrumental for tissue development and homeostasis [1–3]. In addition, dysregulation of PTMs contributes to many pathologies, including neurodegenerative diseases and deafness [4, 5].

The organ of audition, called the cochlea, is a coiled structure located in the ventral part of the inner ear. It houses mechanosensory hair cells (HCs), which detect sound through the deflection of an apical hair bundle, consisting of approximately 100 stereocilia, and convert it into electric signals. The cochlea also comprises the supporting cells (SCs) that provide mechanical and physiological support to the neighboring HCs. The sensory HCs are innervated by the peripheral processes of the spiral ganglion neurons (SGNs), constituting the first relay to the auditory cortex.

PTMs are tissue and cell specific and a better understanding of these modifications during cochlear development will help to identify potential new therapeutic strategies. However, the identification of specific PTMs relies on mass spectrometry and proteomic analyses that are extremely difficult to conduct on the developing cochlea because of the limited amount of material one can recover from this small tissue. Nevertheless, the role of different enzymes responsible for transferring or removing

PTMs has been studied during inner ear development by genetic invalidation, through the use of pharmacological inhibitors or by ex vivo knock down experiments.

Overview of different PTMs encountered in the cochlea

Over the past years, we have accumulated a growing knowledge of the pleiotropic roles of the enzymes that affect protein function via covalent modifications. In this review, we highlight the main enzymes driving PTMs that have been shown to play a role during the development of the auditory portion of the inner ear. We have focused on those mediating phosphorylation, acetylation, methylation, glycosylation, ubiquitination and SUMOylation (Table 1).

Phosphorylation—the most common PTM—consists of the transfer of a phosphoryl residue (PO_3^{2-}) from the universal phosphoryl donor ATP to the side chain of three amino acids, i.e, serine, threonine and tyrosine. The enzymes that perform phosphorylation are named kinases and can be classified according to their substrate(s), whereas dephosphorylation is executed by phosphatases that are classified based on the structure of their catalytic domain [6–8]. Phosphorylation is a key step in the activation of many signaling cascades because this reversible chemical modification of proteins allows transient functional changes, enabling the cells to respond rapidly to environmental cues. Hence, protein phosphorylation plays a crucial role in conveying molecular signals that will dictate a switch in cellular activity and is instrumental in the development and homeostasis of many tissues, including the inner ear.

Table 1 Different PTMs and their functions

PTM type	General functions
Chemical group	
Phosphorylation ($-PO_3^{-2}$)	Enzymatic activation/inactivation
	Modulation of molecular interactions
	Signaling
Acetylation ($-COCH_3$)	Regulation of gene expression (histones)
	Protein stability and localisation
Methylation ($-CH_3$)	Regulation of gene expression (histones or DNA)
	Protein–protein and protein-DNA interactions
	Protein activation
Protein/peptides	
Ubiquitination	Protein activity, localisation and degradation
SUMOylation	Protein activity
Complex molecules	
Glycosylation	Protein folding and stability
	Cell–cell adhesion

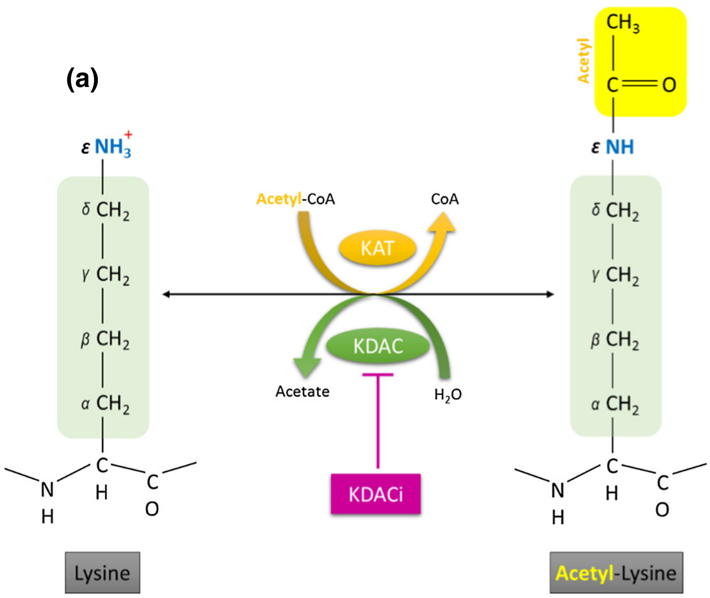
Only PTMs that have been shown to play a role during cochlear development or that are involved in hearing impairment are shown

Among the numerous intracellular cascades involving protein phosphorylation, mitogen-activated protein kinase (MAPK) pathways play an important role in the development and function of the mouse inner ear. The mammalian MAPK family includes the extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 MAPK. These large families of serine/threonine kinases exist in three tiers of protein kinases that are successively activated by phosphorylation: MAPK kinase kinase (MAP3K) activates MAPKK (or MAP2K), which then activates MAPK, which itself phosphorylates a large array of substrates.

Acetylation, consisting of the transfer of an acetyl group ($COCH_3$) to nitrogen, is a very common protein modification. The acetyl group may be transferred from acetyl-coenzyme A (Ac-CoA) to the α-amino group of the first amino acid residue (*N*-alpha acetylation), but this modification occurs mainly during protein translation and is rarely post-translational. In contrast, *N*-epsilon acetylation is among the most common PTM and consists of the addition of an acetyl group from Ac-CoA to the terminal ε-amino group of a lysine residue (Fig. 1a). At a mechanistic level, lysine acetylation neutralizes the positive charge but also increases the hydrophobicity and size of its lateral chain. By inducing conformational changes, reversible lysine acetylation can affect protein activity, localisation, and specific interaction as well as stability, thereby controlling a variety of cellular processes such as apoptosis, proliferation and differentiation [9–14].

For many years, the study of acetylation was mainly restricted to histones and their role in modulating chromatin structure and gene transcription. More recently, other substrates have been shown to be targeted and regulated by acetylation, among which are a large number of transcription factors. This discovery reinforced the importance of PTMs in controlling transcriptional events and cell function. Today, numerous nuclear, cytoplasmic or mitochondrial proteins have been described as acetylated, and the acetylome is thought to comprise almost 2000 proteins [15], which is close to the size of the phosphoproteome. The enzymes responsible for this reversible modification are known as lysine acetyl-transferases (KATs) and lysine deacetylases (KDACs) [16]. At present, over 20 human KATs have been identified. Among them, three main families have been described based on their sequence and structural similarity. These are the CBP/p300, the GCN5/PCAF and the MYST families (Fig. 1b). Even though different KAT families can target the same protein, they acetylate different lysine sites [17]. KDACs are classified

(a)



(b)

(De)-acetylation enzymes	Main families
KATs	CBP/p300
	GCN5/PCAF
	MYST
KDACs	I (KDAC1,2,3 and 8)
	IIA (KDAC4,5,7 and 9) & IIB (KDAC6 and 10)
	III or sirtuins (SIRT1-7)
	IV (KDAC11)

Fig. 1 **a** Reaction of lysine acetylation by lysine acetyl-tranferase enzymes (KAT) and deacetylation (KDAC) on proteins. **b** The main KATs and KDACs families are summarized in this table. These enzymes are classified regarding their sequence and structural similarities. *KDACi* KDAC inhibitor, *CBP* CREB-binding protein, *p300* E1A-associated 300 kDa protein, *GCN5* general control of amino acid synthesis protein 5-like 2, *PCAF* P300/CBP-associated factor

in four classes, from I to IV, based on their function and DNA sequence similarity [18, 19]. All the KDACs contain zinc and are known as "Zn-dependent lysine deacetylases", except the class III encompassing NAD^+-dependent enzymes known as sirtuins [20].

The addition of methyl groups to specific nucleotide residues on proteins, mostly on histones, is a major epigenetic controller of gene expression. This common modification on nuclear proteins takes place on arginine and lysine residues, causing changes in protein–protein interactions, protein activation and chromatin compaction [21–23]. Lysine residues can undergo up to three methylations, while arginine residues typically undergo mono or dimethylation (Fig. 2a). Each of these specific methylations is used as a distinct recognition element for the transcriptional machinery. The selective addition of methyl groups to specific sites of histones is controlled by the action of protein lysine or arginine methyl transferases, PKMT and PRMTs, respectively. The addition of methyl groups needs *S*-adenosyl methionine (SAM) as the methyl donor, which is transformed into *S*-adenosyl homocysteine (SAH). This reaction is reversible, and demethylation is carried out by histones lysine demethylation enzymes (KDMs), classified from KDM1 to KDM6 (Fig. 2b).

It is well established that many proteins do not fold correctly, are mistrafficked or are less stable unless they are glycosylated. Glycosylation refers to the enzymatic process anchoring sugar moieties to proteins, lipids, or other organic molecules. The majority of proteins synthesized in the rough endoplasmic reticulum undergo glycosylation. The process produces glycans, fundamental cellular biopolymers, of which there are five main classes according to their glycosidic linkage: *N*-, *O*- and *C*-linked glycans, phospho-glycans and glycosylphosphatidylinositol (GPI)-glycans. The addition or removal of sugar moieties onto the amide group of asparagine (*N*-linked) or the hydroxyl group of serine or threonine (*O*-linked) gives rise to characteristic branched or chain structures [24]. Each type of sugar structure plays specific roles in human biology [25]. For example, *N*-glycosylation is associated with protein folding and stability, protein–protein complex formation, protease resistance, inter- and intra-cellular transport, and cell–cell recognition and signaling. *O*-Linked glycosylation impacts upon antibacterial immune defence, inflammatory response, and ABO blood type determination. Glycans can serve structural and functional roles in membranes and secreted molecules, and can also play a role in cell–cell adhesion via sugar-binding proteins called lectins [26, 27]. Given the wide range of biological actions dependent upon protein glycosylation, it is not surprising that over 100 inherited human glycosylation disorders have been identified in the last 20 years [28–30]. Mutations affecting glycan synthesis or structure give rise to a broad phenotypic spectrum and most of the disorders are characterized by deficient embryonic or early postnatal development of multiple organs [31]. Interestingly, mutations in genes encoding various glycosylated proteins have been associated with syndromic and non-syndromic forms of deafness in humans.

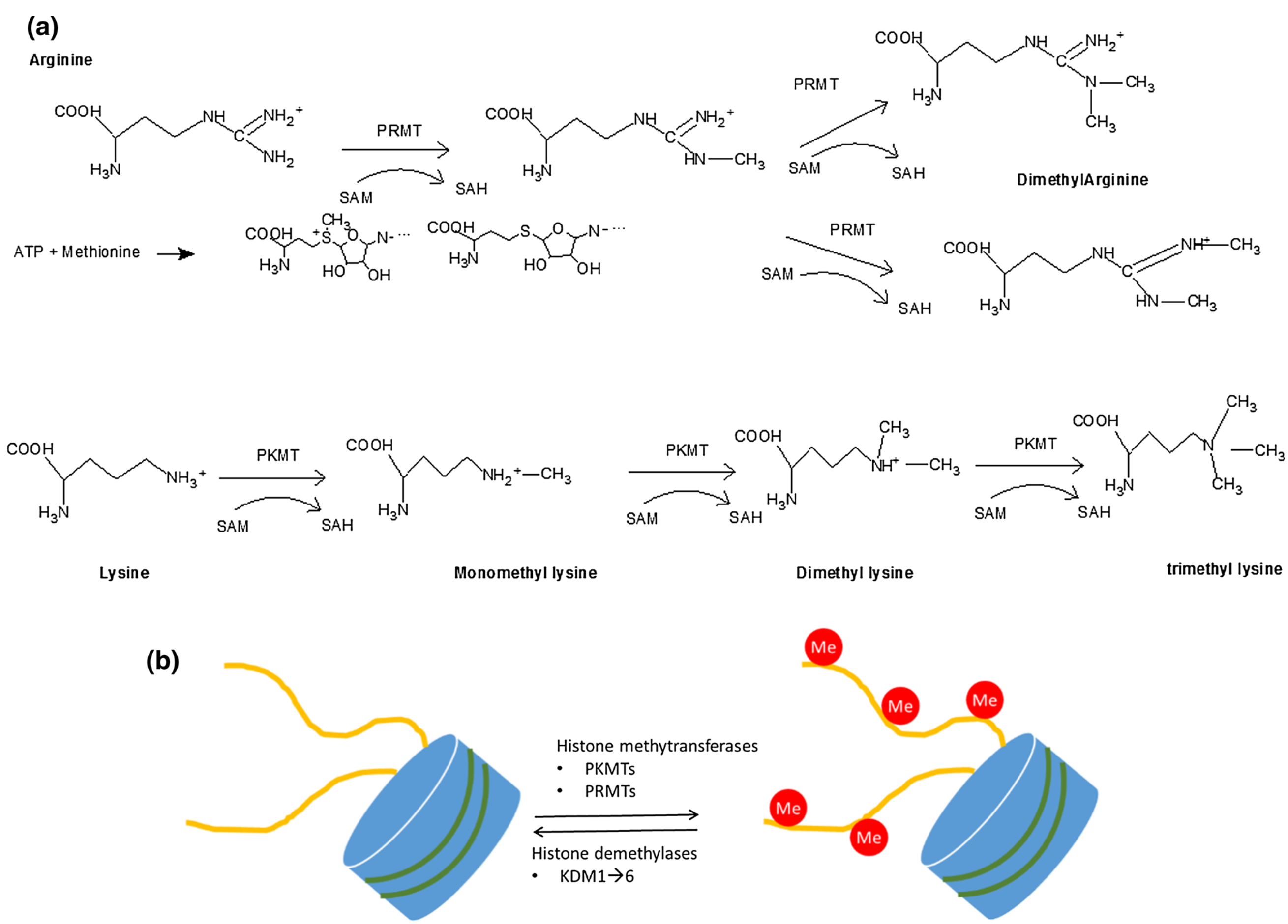


Fig. 2 **a** Methylation of proteins by arginine-specific (PRMT) or by lysine-specific (PKMTs) methyltransferases. Lysine methylation can undergo up to three methylations, while arginine undergo mono or dimethylation. **b** Reaction of (de-)methylation on histones. Demethylation is carried out by histones lysine demethylation enzymes, classified from KDM1 to KDM6. *SAM S*-adenosyl methionine, *SAH S*-adenosyl-homocysteine, *ATP* adenosine triphosphate

Ubiquitination involves the covalent attachment of ubiquitin to a target protein, and is carried out by three enzymes: ubiquitin activating enzyme, E1, ubiquitin conjugating enzyme, E2, and ubiquitin ligase, E3. Ubiquitin is first activated by E1, through an ATP-dependent step forming a thiol ester bond between the C terminus of ubiquitin and the active cysteine of the E1. The ubiquitin molecule is then transferred to the active site cysteine of the second enzyme of the complex, E2, before reaching the final enzyme, E3, which binds the target substrate and labels it with ubiquitin. Ubiquitination affects proteins in many ways: it can act as a signal for their degradation via the proteasome, change their cellular location, or modify their activity [32–36]. Protein ubiquitination is reversed by deubiquitinating enzymes (DUBs), which are classified in five families: ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), Josephins, and JAB1/MPN/MOV34 metalloenzymes (JAMMs) [37] (Fig. 3). Regulation of protein turnover is essential for cellular homeostasis in all tissues, including the inner ear [38–40]; therefore the mutation of any enzyme involved in this process will affect inner ear development and hearing.

Aside from ubiquitin, the best-studied ubiquitin-like protein is Small Ubiquitin-like MOdifier (SUMO), which conjugates to lysine residues within its substrates. In contrast to ubiquitin, SUMO is not used to tag proteins for degradation, but to modify their function. SUMOylation is catalyzed by an enzymatic cascade that closely resembles that of ubiquitination. It requires the hetero-dimeric E1 Aos1/Uba2, the E2 enzyme Ubc9 and an E3 SUMO-ligase. SUMOylation is involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle [41–44]. The effects of SUMOylation depend largely on the function of the targeted protein.

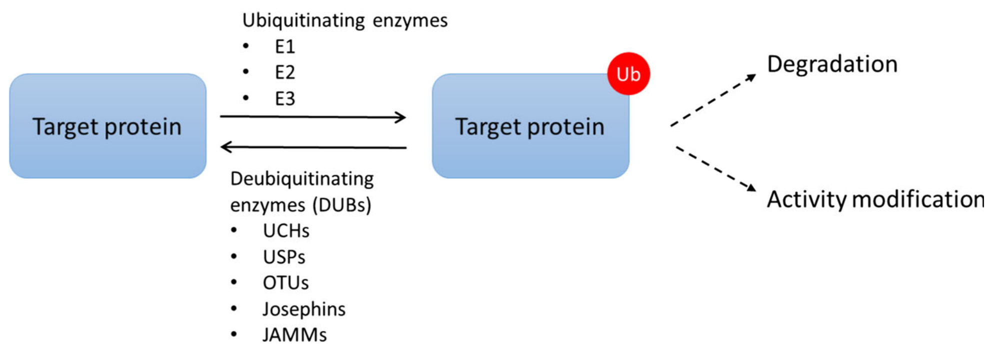


Fig. 3 Reaction of ubiquitination and deubiquitination of proteins. *UCHs* ubiquitin C-terminal hydrolases, *USPs* ubiquitin-specific proteases, *OTUs* ovarian tumor proteases and *JAMMs* JAB1/MPN/MOV34 metalloenzymes

Roles of PTMs during cochlear development

Cochlear development involves an exquisite coordination of multiple processes, beginning with the specification of the otic placode, a thickened portion of cranial ectoderm adjacent to the hindbrain (Fig. 4). The invagination of this otic placode, starting at E8.5 in mice, first forms the otic cup, which then closes off to form a vesicle known as the otocyst. Later, cell proliferation and specification of progenitors gives rise to the neural, sensory and non-sensory cells that form the mature inner ear. Proliferation of the sensory progenitors occurs between E12.5 and E14.5, whereas the differentiation of the sensory epithelium into HCs and SCs takes place from E14.5 in a basal-to-apical gradient [45–47]. Two types of HCs have been described: the inner hair cells (IHCs), which are the true transducers of sound, and the outer hair cells (OHCs), which play a role in the amplification and modulation of the sound. The mature sensory epithelium is a mosaic of a single row of IHCs and three rows of OHCs, and several types of interdigitating non-sensory supporting cells. The apical hair bundles of the HCs are uniformly oriented along the medio-lateral axis of the cochlea—a patterning event that is regulated by the planar cell polarity (PCP) pathway. In parallel to the development of the sensory epithelium, epithelial neuroblasts delaminate from the otic epithelium at E9.5 to form the neurons of the cochleo-vestibular ganglion, which innervate the HCs [48]. All these developmental steps rely on specific transcriptional programs implemented by the occurrence of appropriate PTMs.

Early cochlear development (E8.5–E12.5)

Epigenetic regulation of gene expression by histone methylation is crucial for inner ear formation. During chick otic placode invagination, KDM4B (also known as jumonjiD2), acts to remove of H3K9me2/3 (di- and tri-) methylation marks at regulatory regions of the Dlx3 locus, controlling morphological changes in the otic epithelium [49]. KDM4B loss of function results in a reduction in the expression domain of several inner ear markers (Soho1, Pax2, Sox10 and Dlx3), and otic vesicle deformities. As well as methylation, acetylation is also necessary for the correct formation of the otic vesicle. Knockdown of KDAC1 in zebrafish embryos results in decreased expression of members of the FGF family, inducing smaller otic vesicles and subsequently resulting in a malformed inner ear [50]. Phosphorylation of Rho-associated kinase 1 and 2 (ROCK1/2) is also implicated in the early stages of the chicken otic development (E8.5). ROCK1 and 2 bind to, and are activated by, GTP-bound Rho GTPases (Rho A, B or C). The ROCK pathway is crucial when the superficially located otic placode is transformed into an otic vesicle following the invagination of the epithelium [51]. The restricted localisation of RhoA to the apical junctions of the epithelial cells results in a localised, apical activation of ROCK1/2, which, by acting on actomyosin contractility, leads to an apically polarized constriction of the cells and invagination of the otic placode. SUMOylation is also very important for early steps of inner ear development [52, 53]. Indeed, SUMOylation of SoxE proteins (i.e., Sox8, Sox9 and Sox10), which in turn regulates the expression of Pax8, a key specifier of the mouse otic placode [54], is necessary for the proper formation of otic vesicle.

Late cochlear development (E13.5–P0)

Later during development, numerous pathways involving phosphorylation have been shown to play major roles in the cochlea. MAP3K1 has been shown to be necessary for HC development and survival in mice (starting around E16.5), by regulating some key cochlear morphogenesis genes through p38 phosphorylation [55, 56]. MAP3K4 (or MEKK4) is also important for cochlear cell differentiation through phosphorylation of ERK1/2 [57]. Indeed, loss of MAP3K4 activity leads to an aberrant differentiation of HCs and SCs and a misregulation of cell patterning in the auditory epithelium. Consequently, mice lacking MAP3K4 signaling exhibit elevated auditory thresholds in auditory brainstem response (ABR) recordings following sound stimulation. Recently, phosphorylation and activation of Tak1 (also known as MAP3K7) has also been shown to be involved in

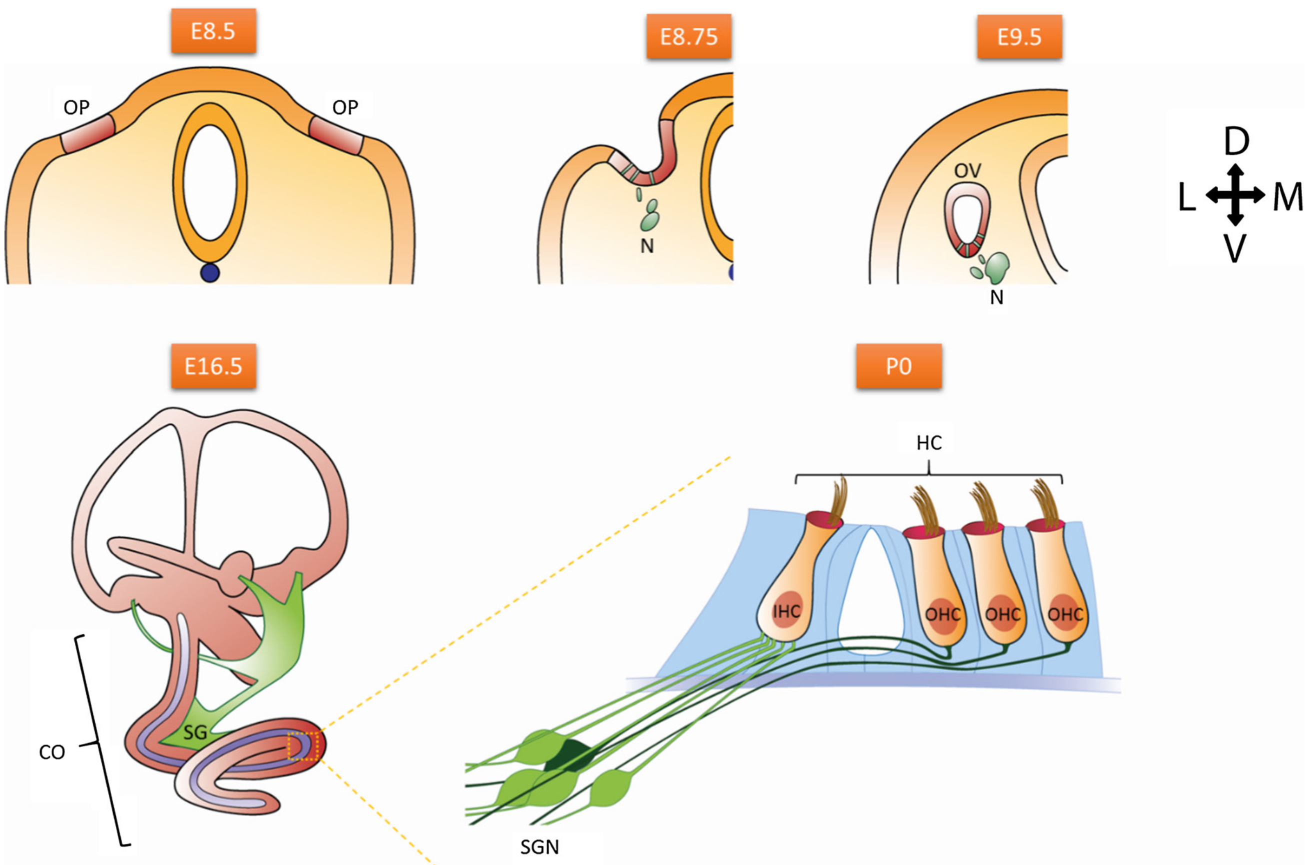


Fig. 4 Schematic illustration of inner ear development. The cochlea derives from the otic placode (OP), which is a thickened portion of cranial ectoderm. This region invaginates around E8.5–E8.75 to form the otic vesicle (OV). Neuroblasts (N) start to delaminate at E8.75 to give rise to the future spiral ganglion (SG), as shown at the stage E16.5. At this point, the coiled-shaped structure of the cochlea (CO) is easily recognized. Different types of cells are found by looking closer at the cochlear sensory epithelium at P0. Supporting cells are shown in *blue*. Hair cells (HC) can be separated into inner hair cells (IHC) and outer hair cells (OHC). Note the distinctive hair bundle, essential for the mechanotransduction of the sound, at the apical surface of HC. These cells are innervated by the spiral ganglion neurons (SGN). *OP* otic placode, *OV* otic vesicle, *N* delaminating neuroblasts, *CO* cochlea, *SG* spiral ganglion, *SGN* spiral ganglion neurons, *HC* hair cells, *IHC* inner hair cells, *OHC* outer hair cells

cochlear development. More specifically, it has been involved in the specification of the tonotopic axis—the spatial arrangement of different frequency components of sound signals—in the chicken cochlea [58]. The ROCK pathway is another intracellular signaling pathway involving phosphorylation that contributes to late cochlear development. In a recent study, it has been demonstrated that PTK7 (a transmembranous tyrosine kinase receptor lacking kinase activity), in association with Src (a non-receptor tyrosine kinase protein), regulates phosphorylation of ROCK2 at cell–cell contacts. Junctional ROCK2 spatially organizes the actomyosin cytoskeleton to regulate PCP in the mouse auditory sensory epithelium [59]. Recently, phosphorylation of ezrin/radixin/moesin (ERM) proteins by liver serine/threonine kinase B1 (LKB1) has been shown to be required for the normal development of cochlear stereocilia and thus for auditory function in mice. LKB1 knockout mice have malformed stereocilia bundles, progressive OHCs death, and impaired hearing with increased ABR thresholds [60].

The smad ubiquitination regulatory factors, Smurf1 and Smurf2, are E3 ubiquitin ligases that play key roles in regulating signaling, cell polarity and motility [61] by targeting proteins for localized degradation. In the cochlea, smurfs have been shown to play a role in PCP, acting on non-canonical Wnt pathway through the degradation of Prickle1 (Pk), a core PCP component. This ubiquitin-mediated degradation alters the asymmetric distribution of Pk and therefore disrupts the correct position of the hair cell's stereocilia [40].

Another important PTM for HC differentiation is acetylation. Indeed, it has been recently shown that dynamic changes in histone acetylation are indispensable for Atoh1 expression—a transcription factor that is both necessary and sufficient for HC differentiation [62].

Roles of PTMs in the mature cochlea and their impact after noise or ototoxic exposure

A better understanding of the effects of PTMs during and after cochlear injury will help to identify potential new therapeutic strategies to restore audition. Among PTMs, phosphorylation plays an important role following noise or ototoxic drug exposure [63, 64]. Noise trauma leads to phosphorylation, and thus activation of ERK, JNK or p38 in cochlear HCs, SCs and SGNs [65]. The activation of these pathways is time-dependent and varies with injury type (i.e., temporary or permanent). An early increase in pERK, as a protective response, occurs within 3–6 h following noise-induced trauma, through the activation and phosphorylation of cAMP-responsive element binding protein (CREB), which initiates the transcription of survival-promoting genes. The use of conditional knockout mice recently confirmed the role of ERK in regulating HC survival by decreasing their susceptibility to noise [66]. Mice deficient for ERK2 display normal hearing, but are more vulnerable to noise as compared to WT littermates. Activation of the ERK signaling pathway also plays an important role in HC regeneration in the neonatal avian utricle following gentamicin-induced damage [67]. Here, pERK induces the phosphorylation of pRb, which forces utricular SCs to re-enter the cell cycle. These new SCs differentiate into HCs and therefore participate to HC regeneration. Contrastingly, the activation of JNK and p38 kinases by noise or ototoxic exposure activates mediators of apoptosis in the cochlea, such as c-Jun, activating transcription factor 2 (ATF2), [E twenty six (Ets)-like transcription factor 1] (Elk1) and tumor protein 53 (p53) [68–70]. Accordingly, intratympanic administration of compounds that inhibit pJNK activity has been shown to protect from hearing loss after noise exposure or ototoxic insult in both mice and zebrafish [68, 71–73]. Therefore, JNK inhibitors are currently under investigation for the treatment of acute sensorineural hearing loss. Similarly, p38 MAPK specific inhibitors also protect against hearing loss by preventing noise- or gentamicin-induced HC death in mice [74, 75].

The ROCK pathway is another intracellular signaling pathway involving phosphorylation that contributes to auditory function in the mature cochlea [59, 60, 76]. The ROCK proteins phosphorylate a vast array of downstream targets that contribute to the stabilization of F-actin, phosphorylation of myosin light chains and coupling of actin–myosin filaments to the plasma membrane, leading to increased acto-myosin force generation and cell contractility. ROCK2 is also implicated in the mouse cochlea in response to noise exposure. Traumatic noise has been shown to decrease the activity of RhoA in OHCs, resulting in cell death and hearing loss [77]. Accordingly, the levels of cochlear ROCK2 and the phosphorylation of ERM are also decreased following intense sound exposure [76]. The fact that down-regulation of ROCK2 or ERM proteins worsens the noise-induced loss of OHCs suggests that ROCK2-mediated modulation of ERM phosphorylation and OHCs cytoskeleton could be targeted to protect from noise-induced hearing loss.

Dysregulation of acetylation has been implicated in various human diseases, and thus KDACs are attractive therapeutic targets for numerous diseases, including deafness [78–81]. Indeed, aminoglycoside antibiotics—the most common ototoxic drugs—decrease histone acetylation before inducing loss of HCs in organotypic cultures of organ of Corti [79]. Furthermore, aminoglycoside-induced ototoxicity can be reduced both in vitro and in vivo by a treatment with KDACi through NF-κB activation and increased expression of pro-survival genes [80, 81]. Modifications of histone acetylation have also been reported in the elderly mouse cochlea, suggesting that epigenetic changes may participate in the process of presbycusis [23]. Sirtuins have been reported to play a role in hearing and age-related hearing loss (ARHL) [82–85], and treatment with resveratrol—an activator of Sirt1-induces protective effects on hearing in aged mice [82]. Furthermore, in response to calorie restriction, expression of Sirt3, which directly deacetylates and activates mitochondrial isocitrate dehydrogenase 2 (Idh2), is increased, leading to increased NADPH levels that protect cochlear HCs from oxidative stress-induced cell death [84, 85].

The mechanical functionality of OHCs, controlled by the motor protein prestin, is regulated at least in part by histone acetylation/deacetylation. Cultured organs of Corti treated with butyric acid, a broad spectrum KDACi [86], show an increased expression of prestin mRNA [87]. Whether this increased expression is triggered by histone acetylation at the level of prestin gene promoter still needs to be elucidated. Acetylation is also involved in the inherent capacity of avian HCs to regenerate after cochlear injury, a capacity that is lost in mammals. Treatment with KDACi decreases supporting cell proliferation in cultures of avian regenerating utricles after ototoxic insult, suggesting that histone deacetylation is a positive regulator of regenerative proliferation [88]. However, inhibiting histone deacetylation does not affect avian HC differentiation. It would be of great interest to have a more complete knowledge of the changes in gene expression that occur during regeneration and their regulatory mechanisms in order to identify new avenues to induce human HC regeneration.

Histone methylation is another important PTM that is involved after cochlear injury. H3K9 methylation governs the susceptibility of HCs to injury. H3K9me2, known to be critical for gene silencing, rapidly increases following aminoglycoside-induced HC damage and precedes the death of the HCs [89]. Yu and colleagues have demonstrated that blocking this increased level of H3K9me2, by using specific inhibitors of histone methyltransferases G9a/GLP, suppresses the onset of apoptosis and prevents the subsequent HC death. This study highlights a novel role for histone methylation in otoprotection, which is of potential therapeutic value for sensorineural hearing loss management.

Ubiquitination also plays an essential role for the normal function of the cochlea and after noise trauma. For example, a point mutation affecting the putative catalytic domain of ubiquitin-specific protease 53 (Usp53) leads to the degeneration of OHCs and rapid progressive hearing loss in KO mice. It also increases the susceptibility to noise-induced hearing loss in heterozygous mice [39].

Implications of PTMs in human hereditary deafness

Approximately 50 % of prelingual deafness are hereditary and can be divided into syndromic or non-syndromic hearing loss. Among them, a variety of PTMs are sites of deafness-causing mutations. Pendred syndrome, characterized by a familial goiter and congenital hearing loss [90], is associated with mutations of the *SLC26A4* gene, which encodes for the anion exchanger pendrin. Most of the mutations characterized in humans result in the absence of pendrin glycosylation, preventing its targeting to the plasma membrane, and therefore its $Cl^{-}/HCO3^{-}$ exchange activity [91–94]. Another important role of glycosylation in syndromic neurosensory hearing loss has been identified in Usher syndrome type 3 [95]. Here, one of the most frequent mutations, missense N48K, results in deficient *N*-glycosylation of clarin-1—a protein that is essential for F-actin

Table 2 Major roles of different PTMs in cochlear development and function, otoprotection and involving mouse or human deafness and presbycusis

Player	Context	Associated role/disease	References
Developement			
KDM4B	Histone methylation	Inner ear invagination	Uribe et al. [49]
KDAC1	Histone deavetylation	Inner ear invagination	He et al. [50]
ROCK2	ROCK-pERM pathway	Remodeling af actin cytoskeleton and PCP	Han et al. [76]
SoxE	SUMOylation	Specification of the otic placode	Taylor and Labonne [52]
MAP3K1	MAPK/ERK pathway	Cochlear hair cell development and survival	Yousaf et al. [55], Parker et al. [56]
MAP3K4	MAPK/ERK pathway	Cochlear hair cell development	Haque et al. [57]
MAP3K7 (Tak1)	MAPK/ERK pathway	Tonotopy in avian cochlea	Mann et al. [58]
Atoh1	Histone acetylation	Hair cell differentiation	Stojanova et al. [62]
LKB1	ROCK-pERM pathway	Development and maintenance of stereocilia	Men et al. [60]
Cochlear function and otoprotection			
ERK2	MAPK/ERK pathway	Hair cell survival in NIHL	Kurioka et al. [66]
JKN and p38	MAPK/ERK pathway	Otoprotection	Eshraghi et al. [71], Tabuchi et al. [74]
ROCK2	ROCK-pERM pathway	Protection from NIHL	Han et al. [76]
Prestin	Histone (de-)acetylation	Mechanical functionality of OHC	Gross et al. [87]
H3K9me2	Aminoglycosides injury	Otoprotection of hair cells	Yu et al. [89]
Smurf1/2	Ubiquitination	Cell polarity and PCP	Kazmierczak et al. [39]
Usp53	Deubiquitination	Survival of OHC and susceptibility to NIHL	Narimatsu et al. [40]
Mouse/human deafness or presbycusis			
Sirt1	Hypoxia-induced stress	Protection in ARHL	Wang et al. [83]
Sirt3	Oxidative stress	Protection in ARHL	Someya et al. [84], Han and Someya [85]
SLC26A4 (Pendrin)	Pendrin glycosylation	Pendred syndrome (hearing loss)	Azroyan et al. [92]
CLRN1	*N*-Glycosylation	Usher syndrome type III (deafness)	Kremer et al. [97], Gopal et al [98]
SLC26A5 (prestin)	Prestin glycosylation	Non-syndromic hearing loss (DFNB61)	Rajagopalan et al. [100]
Alg10B	*N*-Linked glycosylation pathway	Non-syndromic deafness	Probst et al. [102]
Connexin-26	Fbx2-mediated ubiquitination	Deafness	Nelson et al. [38], Henzl et al. [104]

ARHL age-related hearing loss, *NIHL* noise-induced hearing loss, *PCP* planar cell polarity, *OHC* outer hair cells organization. This mutated clarin-1 no longer localizes to the sub-plasma membrane micro-domain of HC stereocilia, which results in the disorganization of the actin cytoskeleton [96–98]. Glycosylation is also important for the regulation of SLC26A5 (prestin) activity in OHCs, by controlling its self-association and cellular trafficking to the cell membrane [99, 100]. Mutations of *SLC26A5* gene are potentially associated to DFNB61, an autosomal recessive non-syndromic hearing loss [101]. A point mutation in the alpha-1,2-glucosyltransferase (Alg10B) gene, coding for a protein involved in the asparagine *N*-linked glycosylation pathway, causes non-syndromic deafness in mice [102]. Mice carrying this mutation show a number of cochlear abnormalities, with numerous OHCs missing and an atypical expression of prestin in many of the remaining OHCs.

Mutations in the connexin-26 (Cx26)—also called Gap junction protein type 2 (GJP2)—gene account for nearly 50 % of all non-syndromic hearing loss. More than 50 mutations have been reported to be associated with autosomal recessive (DFNB1) and autosomal dominant (DFNA3) forms of hearing impairment. Among those mutations, some are sites of PTMs [103]. For example, Cx26 is acetylated in its N-terminal domain (Lys15) as well as in cytoplasmic loop (Lys102) and both lysine residues are mutated in non-syndromic recessive deafness. However, each mutation alters the sequence of Cx26, which can have its own effects in addition to eliminating the PTM. Interestingly, Cx26 is a potential target for Fbx2 (also called Fbs1 or OCP1), a subunit of the SKP1-cullin-F-box (SCF) E3 ubiquitin ligase complex [38], and it has been shown that cochlear degeneration occurs in mice lacking Fbx2. Cx26 and Fbx2 have been shown to co-precipitate in vitro, and increased levels of Cx26 have been found in Fbx2 KO mice. It is thus tempting to suggest that Cx26 is a substrate for Fbx2-mediated ubiquitination and degradation, and deafness observed in Fbx2 KO mice is caused by a failure of Cx26 regulation. However, in vivo interactions between Fbx2 and Cx26 remain controversial [38, 104].

Conclusion

PTMs are crucial regulators of cell biology by playing key roles in processes such as the rapid on and off switching of signaling networks or the regulation of enzymatic activities. PTMs such as acetylation, methylation, glycosylation, ubiquitination and SUMOylation of specific sites in proteins have key roles in the regulation of both cochlear development and function (Table 2). Therefore, their mutation or knock down can contribute to deafness. Research into the role of PTMs in the cochlea is far from complete, with a number of PTMs as yet unstudied. Processes such as neddylation, in which the ubiquitin-like protein NEDD8 is conjugated to its target proteins, or ADP-ribosylation, in which one or more ADP-ribose moieties are added to a target molecule, have been implicated in developmental signaling and in the pathogeny of many diseases [105–109]. Whether these modifications play a role during cochlear development and hearing remain to be demonstrated.

Improving our knowledge of the biological effects of PTMs may open promising avenues towards preventing and treating hearing impairment and deafness. Furthermore, a better understanding of how PTMs affect gene regulatory networks during ototoxic injury will also offer a new and exciting field in therapeutics. The identification of new candidates regulating inner ear organization by PTM would be of great interest in developing new strategies for the regeneration of the auditory portion of the inner ear following its injury from trauma or disease.

Acknowledgments BM is Research Director from the Belgian National Funds for Scientific Research (FNRS). This work was supported by Grants from the FSR-FNRS, the Fonds Léon Fredericq, the Fondation Médicale Reine Elisabeth, and the Belgian Science Policy (IAP-VII network P7/07). We thank Bernard Minguet for his assistance with Fig. 4.

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