Polarized condensates confer row identity of hair cell stereocilia

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Sound waves are converted into electric signals by hair bundles (also known as stereocilia) in the cochlear hair cells. Stereocilia, a cluster of actin protrusions at the apical surface of hair cells, are organized into rows of graded height (Figure 1). Mechanical force induced by sound waves leads to stereocilia deflection towards the tallest row and subsequent activation of mechanoelectrical transducer channels at the tips of the shorter rows, thus achieving mechanoelectrical transduction (Gillespie and Muller, 2009). Such a staircase-like architecture is essential for auditory perception as manifested by severe deafness caused by genetic mutations that disrupt stereocilia morphology (Barr-Gillespie, 2015). However, little is known about how this planar asymmetry of stereocilia is achieved.

Several studies have demonstrated that planar cell polarity on the apical surface of hair cells ensures specific localization of the Gpsm2–Gai complex at the tips of row 1 rather than other rows (Tarchini et al., 2013, 2016; Tadenev et al., 2019). There, the Gpsm2–Gai complex interacts with the Whirlin–Myo15–Eps8 module to build a five-component complex, which is believed to greatly elongate row 1 and erect the tallest stereocilia (Tadenev et al., 2019). Nevertheless, it is still puzzling how Gpsm2–Gai promotes stereocilia elongation and defines the row identity of the tallest stereocilia, since the complex is not directly involved in actin dynamic regulation.

In a recently published work (Shi et al., 2022), we characterized the Gpsm2–Whirlin interaction in detail through a combination of biochemical and biophysical approaches. Interestingly, we discovered that Gpsm2 can autonomously form liquid–liquid phase separation (LLPS) in vitro and in living cells. We further showed that the Gpsm2 condensates could coalesce with the Whirlin–Myo15–Eps8 condensates that we described previously (Lin et al., 2021), thus assembling the five-component tip complex density (5×TCD) condensates. The row 1-specific 5×TCD condensates displayed much stronger LLPS ability than Myo15–Eps8 and Whirlin–Myo15–Eps8 did. Consistently, the 5×TCD condensates exhibited more robust actin bundling capability than those without Gpsm2–Gai. The promoted LLPS and actin bundling abilities of the 5×TCD condensates are mainly attributed to Gpsm2 LLPS, as a mutant of Gpsm2 (Gpsm22K5A; all the lysine residues in the ‘poly-K loop’ of Gpsm2 were substituted with alanine) that impaired its LLPS largely weakened the 5×TCD condensate formation and actin bundling. Therefore, we proposed that polarized Gpsm2–Gai promotes the formation of LLPS-mediated 5×TCD condensates. The condensates in turn largely enriched the actin elongation machinery (including but not limited to Eps8 and Myo15) to facilitate robust actin elongation at the tips of row 1, define its unique tallest identity, and instruct differential identity across rows (Figure 1).

Chudley–McCullough syndrome (CMCS) is a rare autosomal recessive neurological disorder characterized by severe to profound sensorineural hearing loss and partial agenesis of the corpus callosum (Mauriac et al., 2017). Mutations in Gpsm2 were found in patients with CMCS. Specifically, a mutation of Gpsm2, p.R318RfsX8, encodes a truncated protein lacking the poly-K loop and the following C-terminal fragment (Mauriac et al., 2017). Given the poly-K loop was essential for LLPS, one would expect that this mutation could interfere with condensate formation. Indeed, both Gpsm22R318RfsX8 itself and the 5×TCD condensates with Gpsm22R318RfsX8 displayed impaired LLPS ability and consequently reduced actin bundling, which may result in abnormal stereocilia morphology. Therefore, our data not only provide critical insights into how Gpsm2–Gai specifies the
tallest stereocilia, but also offer possible mechanistic explanations for the etiology of CMCS-related hearing loss. It is worth noting that future work is definitely needed to elucidate the crucial roles of the TCD condensates in the regulation of stereocilia morphology in both physiological and pathological conditions. It would be attractive to investigate stereocilia development using a genetic mouse model with endogenous Gpsm2 replaced by Gpsm2\textsuperscript{K4A} (LLPS-deficient mutant) or Gpsm2\textsuperscript{*318RfsX8} (CMCS-related mutant).

Our work proves that actin regulatory proteins may form autonomous condensation via LLPS at the tips of stereocilia, creating a compartment for high-efficiency actin dynamics. We reasonably speculate that the analogue actin protrusions (e.g. microvilli and filopodia) may share a similar construction principle (i.e. LLPS-mediated condensation assembly) for dynamic cytoskeletal regulation. Consistently, in microvilli and filopodia, there also exist the electron-dense areas at the distal tips of protrusions, which contain various actin polymerization proteins (e.g. vasodilator-stimulated phosphoprotein and formins) and actin bundling effectors (e.g. fascin, espin, and villin) (Postema et al., 2018; Harker et al., 2019). It would be interesting to test whether these tip complex densities may form via LLPS as well, and if so, whether these tip complex condensates also play essential roles in regulation of cytoskeletal dynamics at the above-mentioned actin protrusions. Taken together, our work would pave the way towards dynamic regulation of actin protrusions in a broad range of cellular processes.

\[This\ \textit{work\ was\ supported\ by\ grants\ from\ the\ National\ Key\ R&D\ Program\ of\ China\ (2018YFA0507900\ to\ J.Z.;\ 2019YFA0508402\ to\ C.W.),\ the\ National\ Science\ Foundation\ of\ China\ (32122036,\ U2032122,\ and\ 31770779\ to\ J.Z.;\ 22122703,\ 91953110,\ and\ 32170767\ to\ C.W.),\ the\ Science\ and\ Technology\ Commission\ of\ Shanghai\ Municipality\ (20511900200),\ the\ Interdisciplinary\ Innovative\ Talent\ Training\ Program\ of\ Shanghai\ Jiao\ Tong\ University\ (to\ J.Z.),\ and\ the\ Scientific\ Research\ Foundation\ for\ Youth\ Scholars\ of\ Shanghai\ Jiao\ Tong\ University\ (AF0890029\ to\ L.L.).}\]

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