Motional dynamics of single Patched1 molecules in cilia are controlled by Hedgehog and cholesterol

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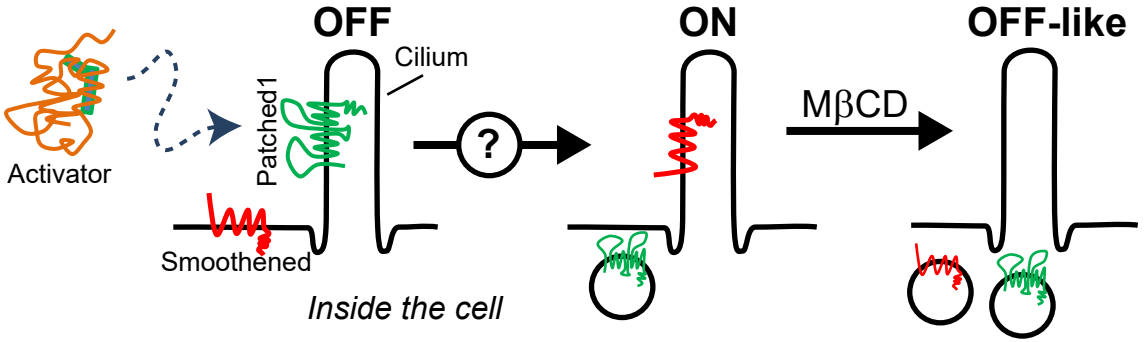
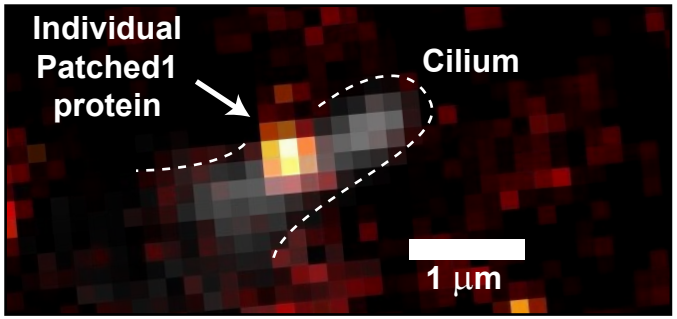
Proceedings of the National Academy of Sciences, Feb 2019, https://doi.org/10.1073/pnas.1816747116

Our study reveals that Hedgehog signaling leads to changes in the motion behavior of the signal-transducing proteins, Patched1 and Smoothened. By genetically encoding a label, then tracking and analyzing the motion of individual proteins on the surface of live cells, we observed significant changes in the restriction of diffusion.

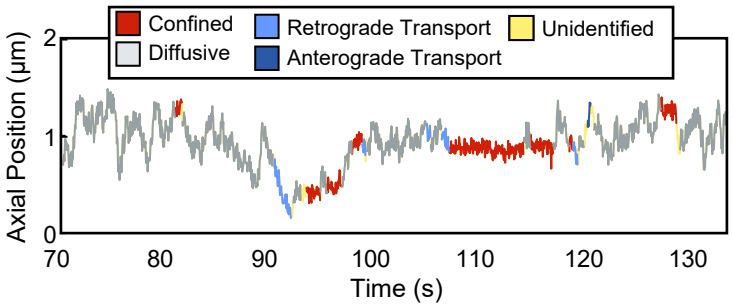
Previously, it was shown that Patched1 represses Smoothened activity; upon pathway activation, the two proteins switch locations in a small, micrometer-scale organelle called the primary cilium. The precise mechanism of the interaction is still uncertain, but the changes we observed occur prior to this population inversion, constituting one of the earliest measurable steps of Hedgehog-signal transduction.



Additionally, by exposing cells to MβCD, a chemical that depletes cholesterol from cells, the motion behavior of both proteins becomes similar to their natural OFF states (in agreement with previous studies showing Smoothened's translocation could be affected with MβCD and by cholesterol). Together, these results implicate cholesterol, or a related molecule, as an intermediate signal carrier between the two proteins.



Single Patched1 trajectory analysis



Aggregated trajectory-data analysis