

Summary of: Three routes to modulate the pore size of the MscL channelnanovalve.pdf

**** Key findings and quantitative results:**

1. ****CB Disassociation****: Disassociation of the CB is not required for normal gating and conductance of the MscL channel.
2. ****TM2/CB Linker Deletions****: - $\Delta 110-112$: Single channel current decreased to 73.1 ± 1.2 pA, significantly lower than WT (84.7 ± 1.7 pA). - $\Delta 110-115$: Further reduced current to 49.1 ± 5.0 pA, with most openings below 30 pA.
3. ****CB Stability****: - CB remains intact upon gating, suggesting it does not dissociate during normal gating.
4. ****Conductance Modulation****: - $\Delta 110-115$ mutant has significantly less sensitive to membrane tension.
5. ****Engineering a Nanovalve****: - G22C/ $\Delta 110-115$ mutant shows spontaneous openings upon MTSET+ treatment, similar to G22C alone.
6. ****Calcein Efflux Assay****: - G22C/ $\Delta 110-115$ MscL has reduced calcein efflux compared to G22C MscL, consistent with pore size reduction.
7. ****Crosslinking Effects****: - $\Delta 110-112$ mutant current decreased to 49.1 ± 1.7 pA, significantly lower than WT. - $\Delta 110-115$ mutant current decreased to 43.1 ± 3.9 pA, further reducing conductance.
8. ****ZnCl₂ Treatment****: - ZnCl₂ treatment reversibly increases conductance from 49.1 ± 1.7 pA to 74.9 ± 1.7 pA for A110H and 80.2 ± 3.6 pA for A112H.
9. ****Pressure Thresholds****: - Pressure thresholds for gating differ between WT and mutants, demonstrating CB's role in gating.
10. ****Mechanosensitivity****: - Pressure thresholds for WT, $\Delta 110-112$, $\Delta 110-115$, and A110H/A112H mutants are 1.5 ± 0.1 , 1.7 ± 0.1 , 1.8 ± 0.1 , and 1.9 ± 0.2 , respectively.
11. ****Calcein Efflux Assay****: - Calcein efflux from vesicles reconstituted with $\Delta 110-115$ MscL is significantly reduced compared to WT.
12. ****Patch Clamp Recordings****: - Pressure thresholds for gating differ between WT and mutants, demonstrating CB's role in gating.
13. ****Crosslinking****: - $\Delta 110-112$ mutant shows disulfide bridging between subunits, forming a ladder of monomer through pentamer.
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