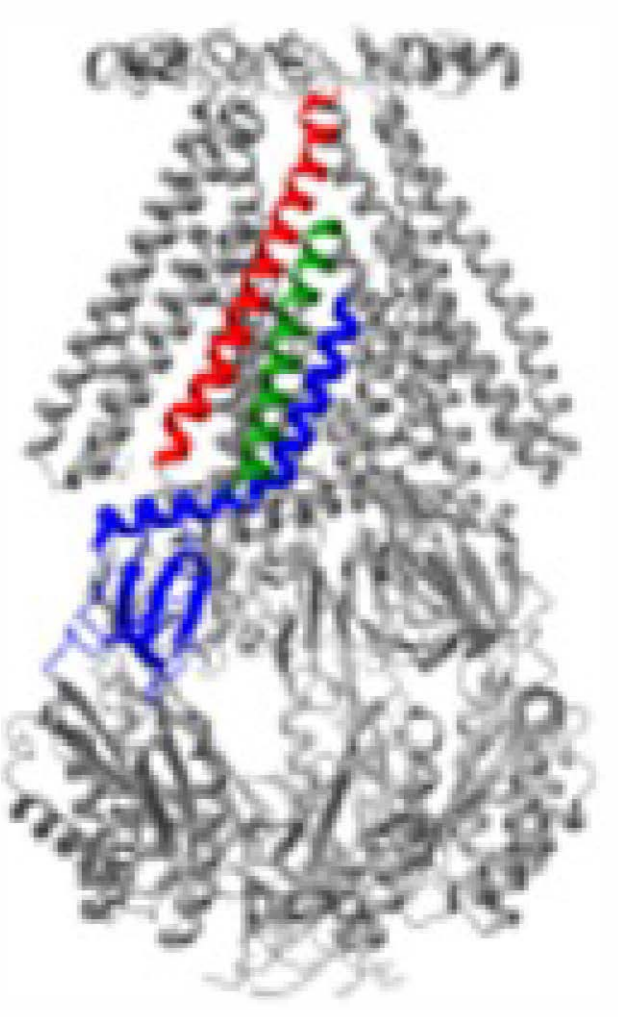


Exploring the effects of various growth conditions on gene expression of mechanosensitive ion channels in *Escherichia coli*

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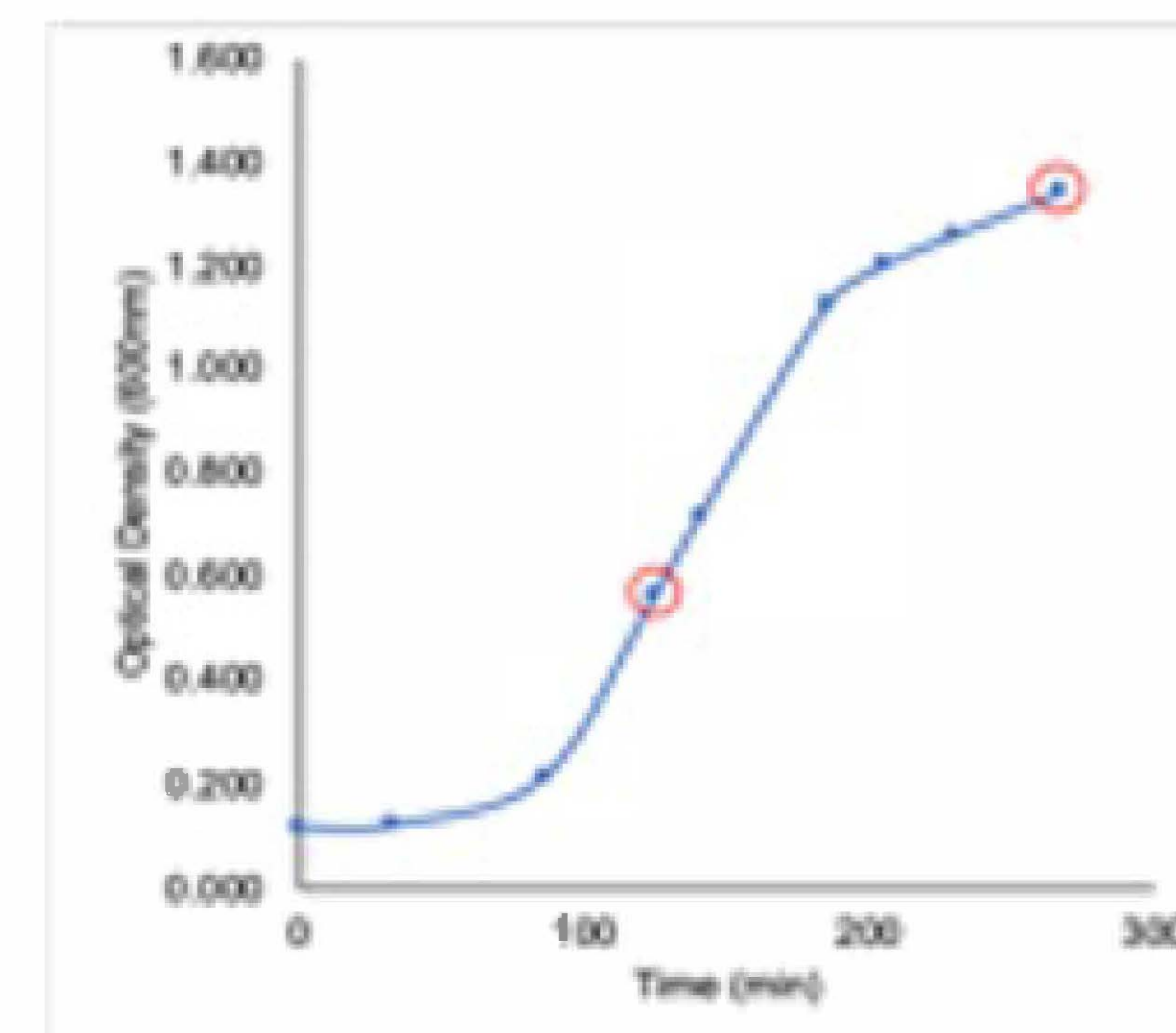
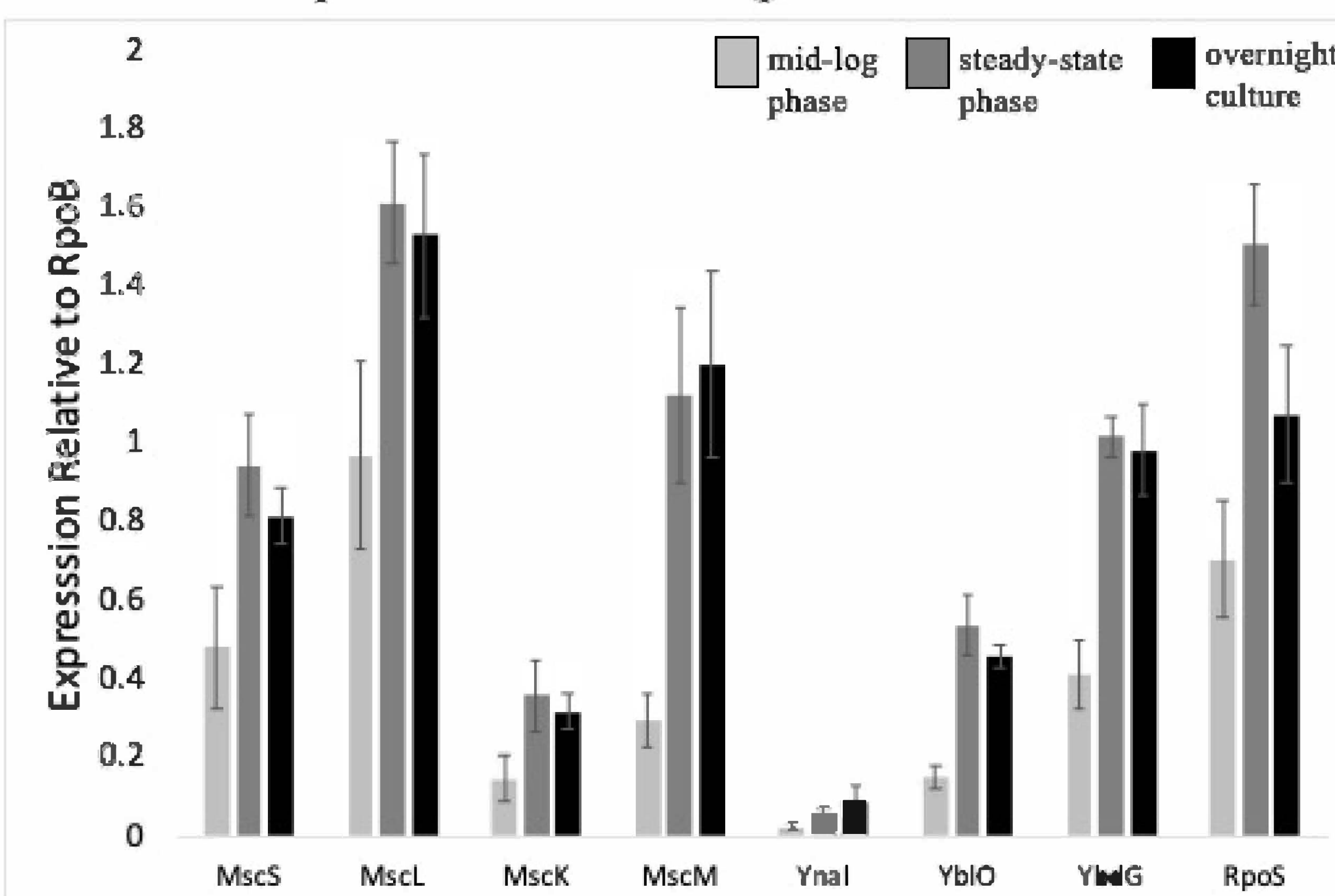


- ❖ Bacterial mechanosensitive ion channels play a key role in the survival response of a cell facing rapidly changing environmental conditions by relieving intracellular pressure and therefore preventing cell lysis
- ❖ The most well studied bacterial mechanosensitive ion channels, from *Escherichia coli* (*E. coli*), are the mechanosensitive channel of large (MscL) and small (MscS) conductance. These channels gate in response to tension within the cell membrane
- ❖ Analysis of the *E. coli* genome identified 5 additional mechanosensitive genes

- ❖ 6 of the 7 predicted MS genes have been shown to gate in response to applied tension
- ❖ MscS, MscK, MscM, and YpiO gate at similar tensions
- ❖ Point mutations in YbdG are tension sensitive YnaI and MscL gate at higher pressures in comparison to the majority of MscS superfamily members
- ❖ Through this study, we reveal each gene's expression as function of a standard ribosomal gene, RpoB, when subjected to varying growth conditions or osmotic downshock

| <i>E. coli</i> Genes | Length of Protein | Function | Gating Threshold (P_x/P_L) |
|----------------------|-------------------|---|--------------------------------|
| MscS | 286 | Opens a 'small' channel in response to applied tension. | 0.63 |
| MscK | 1120 | Opens a 'small' channel, K ⁺ activated channel. | 0.56 |
| MscM (YjeP) | 1107 | Opens a 'mini channel' in response to applied tension. | 0.63 |
| YbdG | 415 | Not detected in standard pressure electrophysiology. It is thought to be involved in recovery from hypo-osmotic up-shock. | ND |
| YnaI | 343 | Opens a 'small' channel at pressure thresholds similar to MscL. | 1.0 |
| YbiO | 741 | Opens a 'small' channel. It is NaCl dependent. | 0.83 |
| MscL | 136 | Opens a 'large' conductance channel in response to applied tension. Directly coupled to the membrane. | 1.0 |
| RpoS | 330 | A sigma factor that is upregulated in response to stress conditions, particularly high salt | N/A |

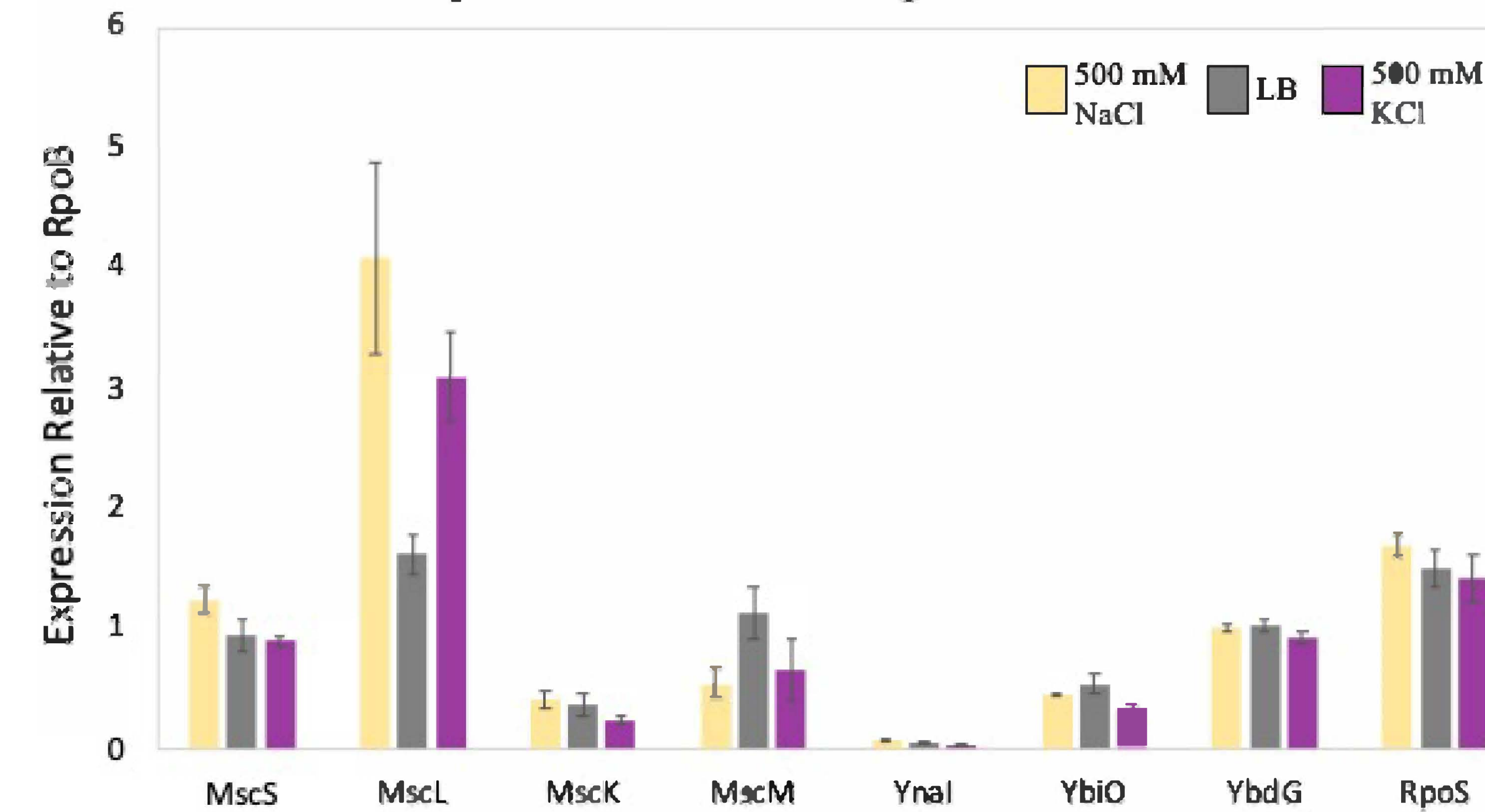
Expression as a function of growth



Representative growth curve, the mid-log and steady state samples are circled in red.

Across all channels, expression level at mid-log phase is roughly half of expression level at steady state or in overnight cultures.

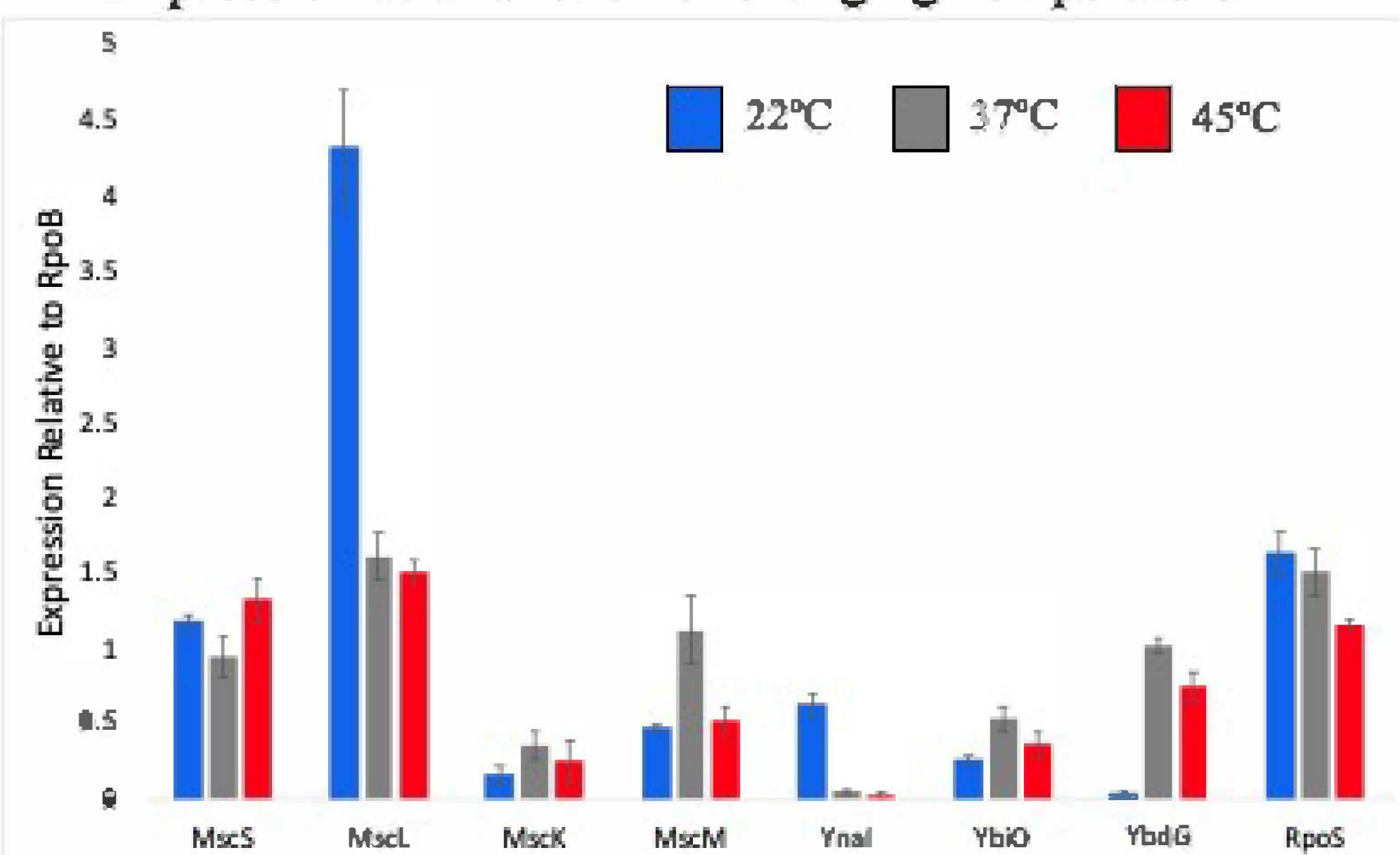
Expression as a function of high salt



With high levels of both NaCl and KCl, we see an upregulation of MscL. We also observe downregulation of MscM and YbiO when grown in high NaCl and KCl conditions.

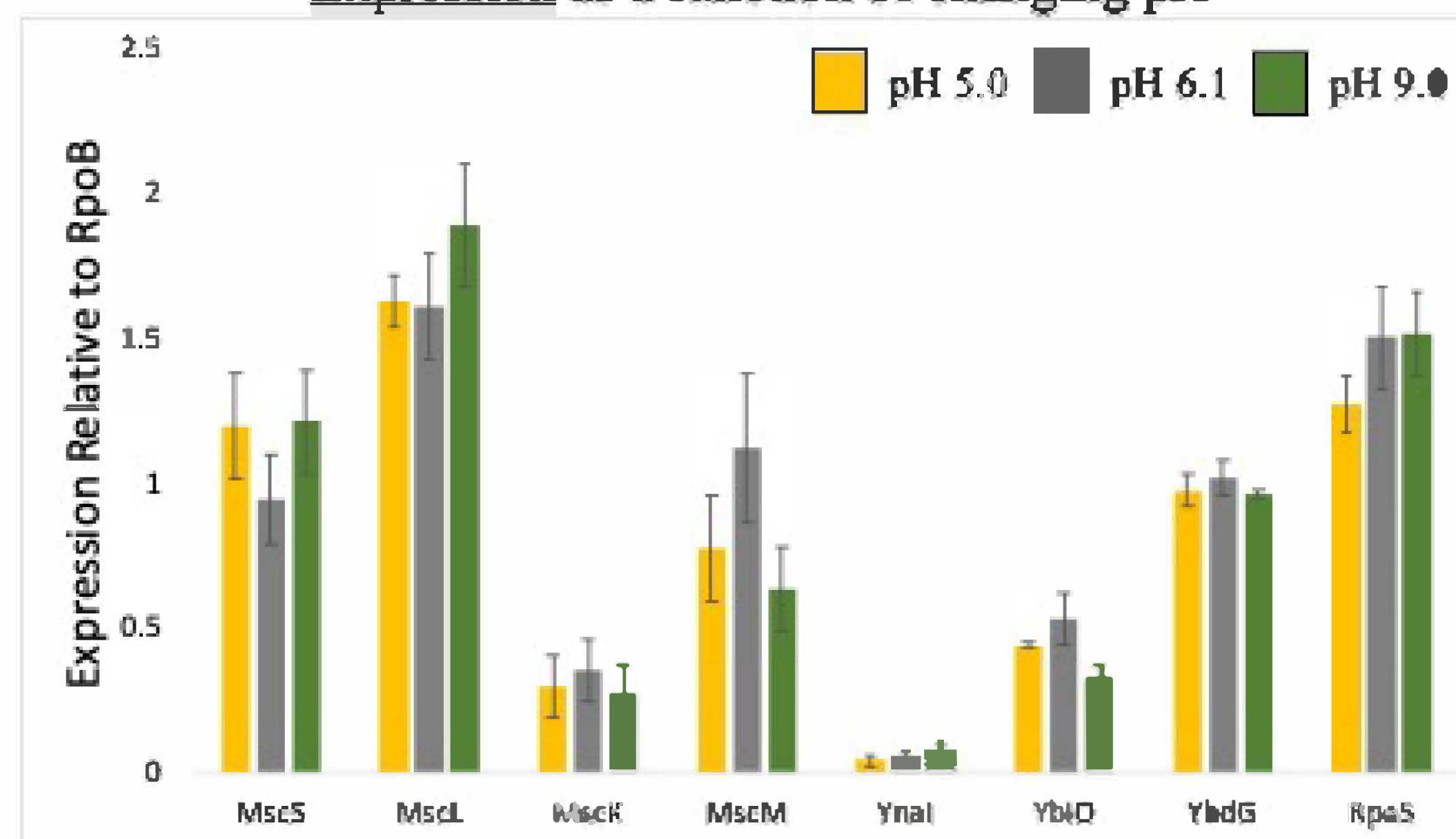
Interestingly, we see downregulation of MscK when subjected to a potassium rich environment.

Expression as a function of changing Temperature



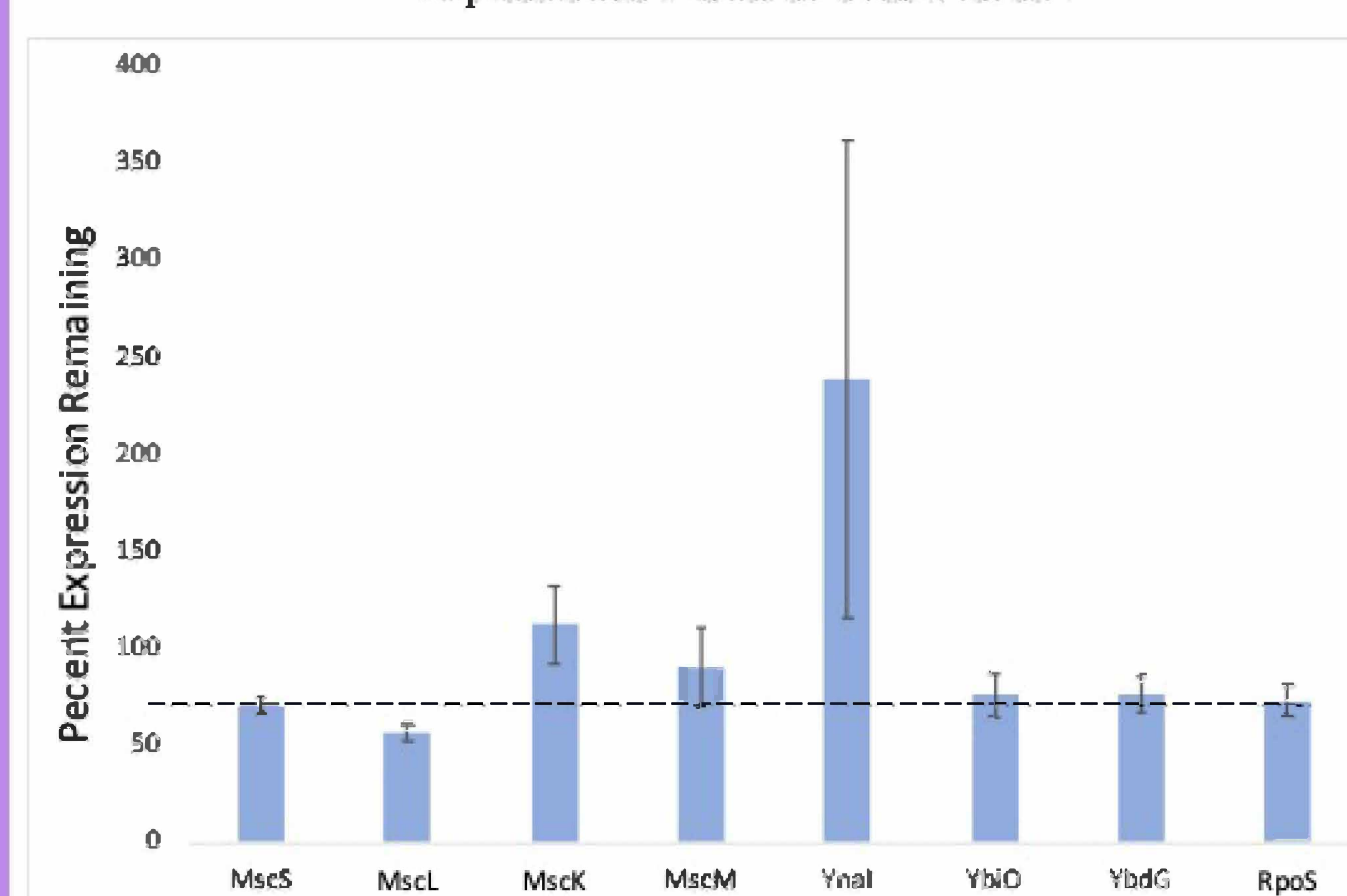
At 22°C, we see upregulation of YnaI and MscL. At 22°C there is less expression of YbdG. At both 22°C and 45°C, we see MscM and YbiO downregulated.

Expression as a function of changing pH



When grown in acidic or basic environments, we observe no notable or significant upregulation/downregulation in expression of any channels, which supports how these channels are only affected by certain conditions.

Expression as a function of downshock



MscL shows the largest decrease in expression within the isotonic solution. MscS, YbiO, and YbdG also show decreases in expression within the isotonic solution, suggesting that these channels play a role in survival from osmotic downshock.

Not all MscS superfamily members play essential roles in survival from osmotic downshock.