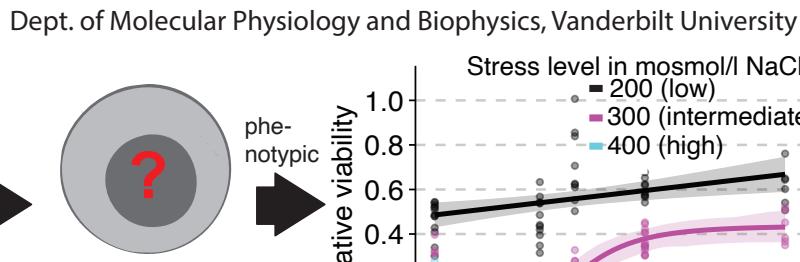
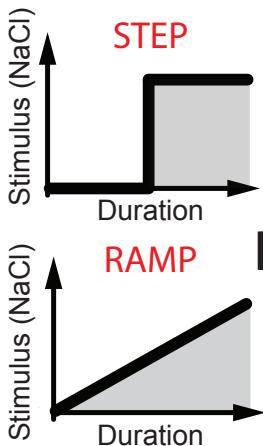


Human cells are protected from apoptosis during gradual stress increase

by accumulating proline

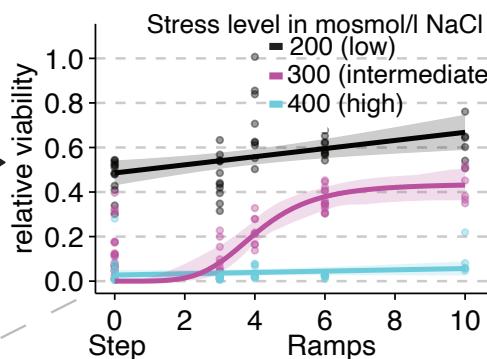
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How does a single cell respond to stress applied by a Step or by a Ramp?

molecular

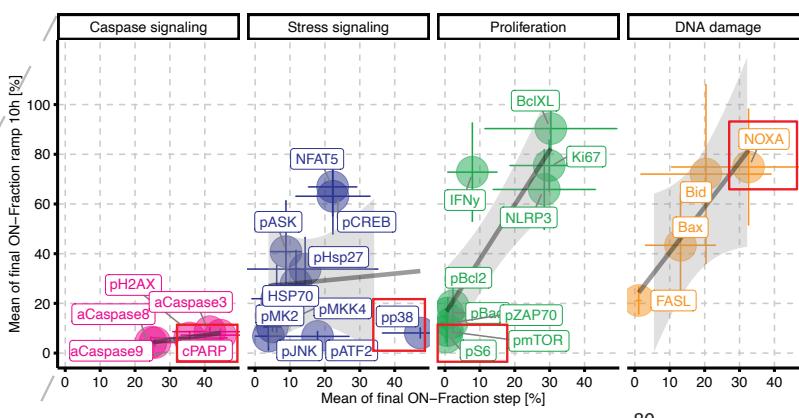


- ★ Increasing stress by a >4h Ramp improves viability of human cells relative to applying the same stress by a Step.
- ★ The effect on viability by the Ramp is strongest at an intermediate stress level.
- ★ The effect is switch-like.

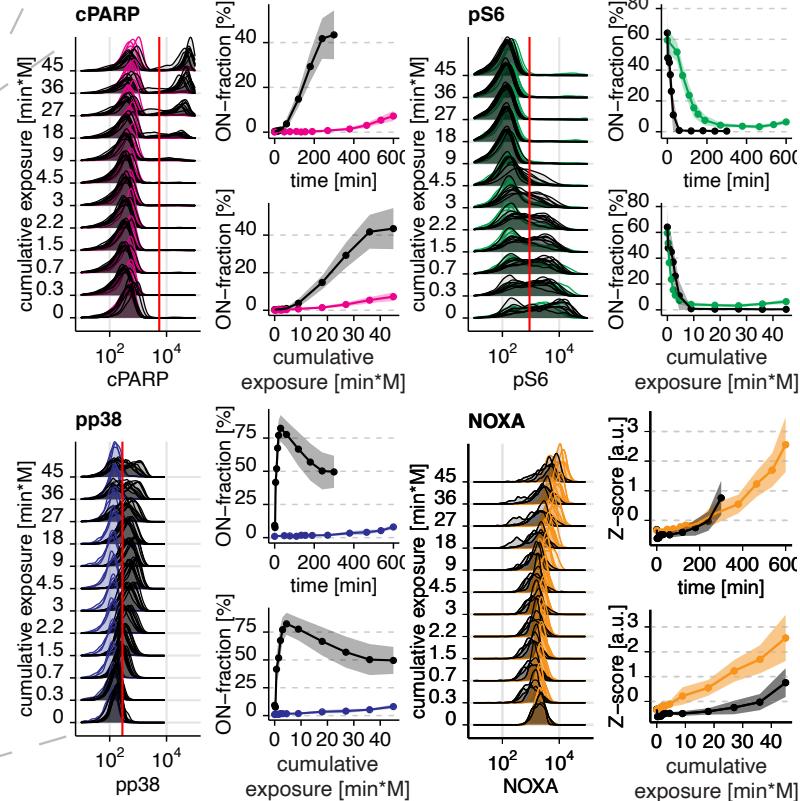
Response of cellular processes in human cells to stress applied by a Step or by a Ramp

- ★ 27 cellular markers of cell state were categorized in 4 groups and measured over time by flow cytometry using specific monoclonal antibodies.
- ★ Mean of the ON-fraction after the time course of exposing human cells to 300 mosmol/l NaCl (intermediate stress level).

- ★ Single-cell distributions over the cumulative exposure (time * concentration at t).
- ★ Black (●) indicates response to step, Color (● ● ● ○ ○ ○) indicates the response to 10h Ramp input to 300 mosmol/l NaCl.
- ★ ON-fraction is obtained by quantifying the percentage of cells that are positive for each marker (above red line) for 3-10 independent experiments.
- ★ Z-score is obtained by subtracting the mean fluorescence from all events of all conditions from the fluorescence of every single cell and dividing by the stand. deviation.

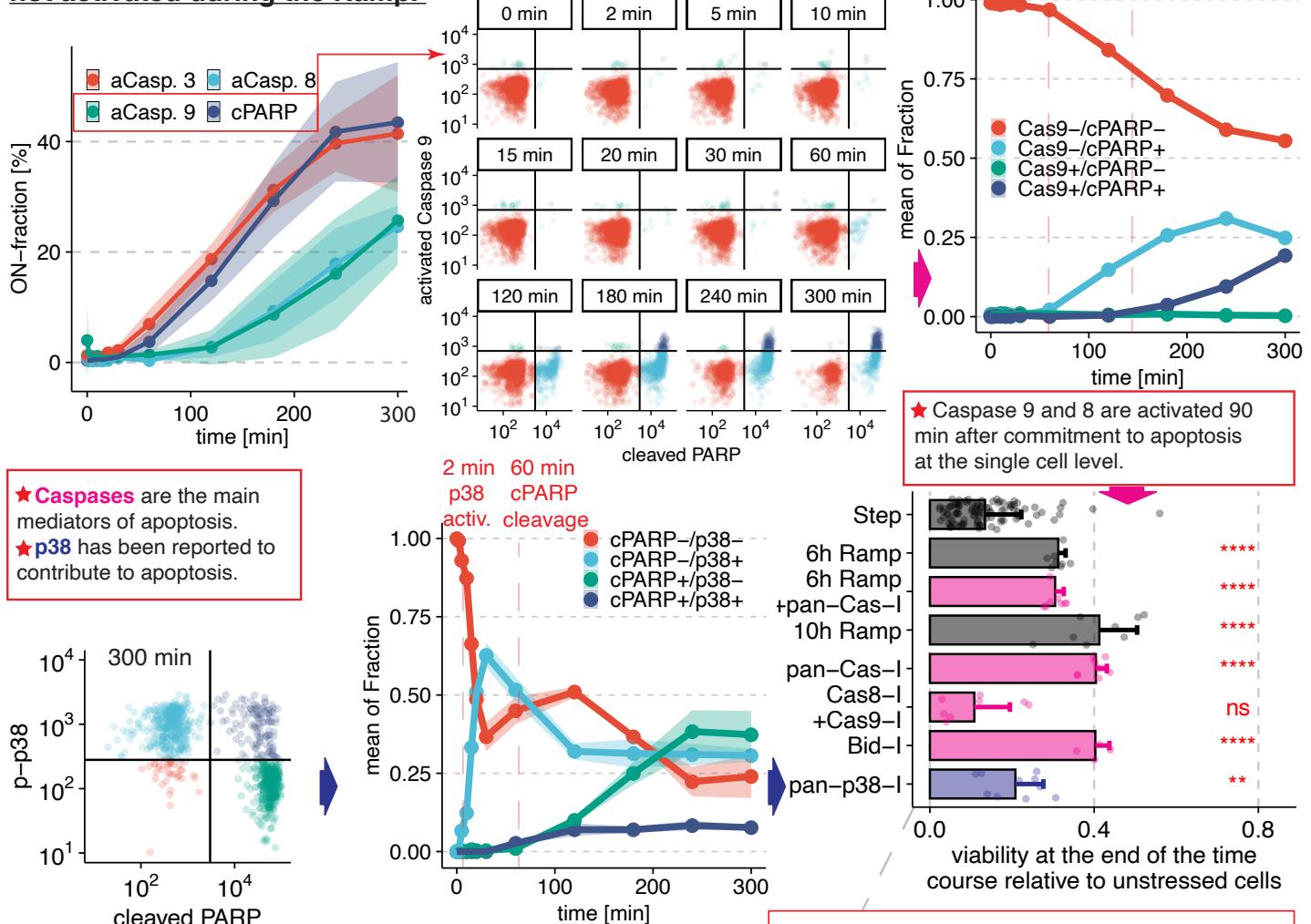


- ★ Markers for apoptosis are differentially regulated between ramp and step exposure to stress.
- ★ Caspases are activated in step, but not in ramp.
- ★ Markers of stress signaling are rather activated in step than in ramp.
- ★ Markers for proliferation, inflammation and DNA damage are activated in both Step and Ramp.



- ★ cleaved PARP, indicating commitment to apoptosis, is strongly induced in cells exposed to a Step of NaCl, but barely induced in cells exposed to a Ramp input to the same stress level.
- ★ phosphorylated p38, evolutionarily conserved stress responsive MAPK, gets transiently activated during the Step exposure to NaCl and adapts, but does not get activated during the Ramp input.
- ★ phosphorylated S6, indicating active translation and proliferation, is reduced dependent on the cumulative exposure, but independent of the input of NaCl.
- ★ NOXA, a protein expressed during DNA damage, is regulated independently of the input profile of the stress.

The mechanisms that kill cells during the Step, but are not activated during the Ramp.



The mechanism that protects cells during the Ramp.

