Featured Publication Note
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A family of *Salmonella* virulence factors functions as a distinct class of autoregulated E3 ubiquitin ligases

Ubiquitination of proteins is a central eukaryotic regulatory mechanism, which, when defective, can lead to cancer and neurodegenerative disorders. Ubiquitination is a multistep enzymatic process whereby a ubiquitin-activating enzyme (E1) transfers ubiquitin to a family of ubiquitin-conjugating enzymes (E2s), and then ubiquitin-loaded E2s are recruited to their substrates by a family of ubiquitin ligases (E3s). These E3 ligases play a critical role in conferring specificity to the reaction.

Bacterial factors have been identified that exploit these pathways. In this study, researchers investigated a new class of bacterial E3 ligases that do not show any primary amino acid sequence similarity to known eukaryotic proteins. They used a member of this family from *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), called SspH2. The crystal structure of SspH2 was solved, and revealed a 2-domain architecture, a canonical leucine rich repeat (LRR) domain that interacts with a unique E3 ligase C-terminal fold, which they have termed NEL for *Novel E3 Ligase*.

The localization of these different domains of SspH2 when transiently expressed in cultured cells was then examined using immunofluorescence confocal microscopy (see figure). Confocal images were collected at 0.2 µm intervals for 10 µm (HeLa cells) or 25 µm (MDCK cells) on a PerkinElmer Spinning Disc Confocal Microscope using Volocity Acquisition software. The z-stacks were then assembled as a 3D projection using Volocity. The team used the movie making features of Volocity to better illustrate localization (see movie).

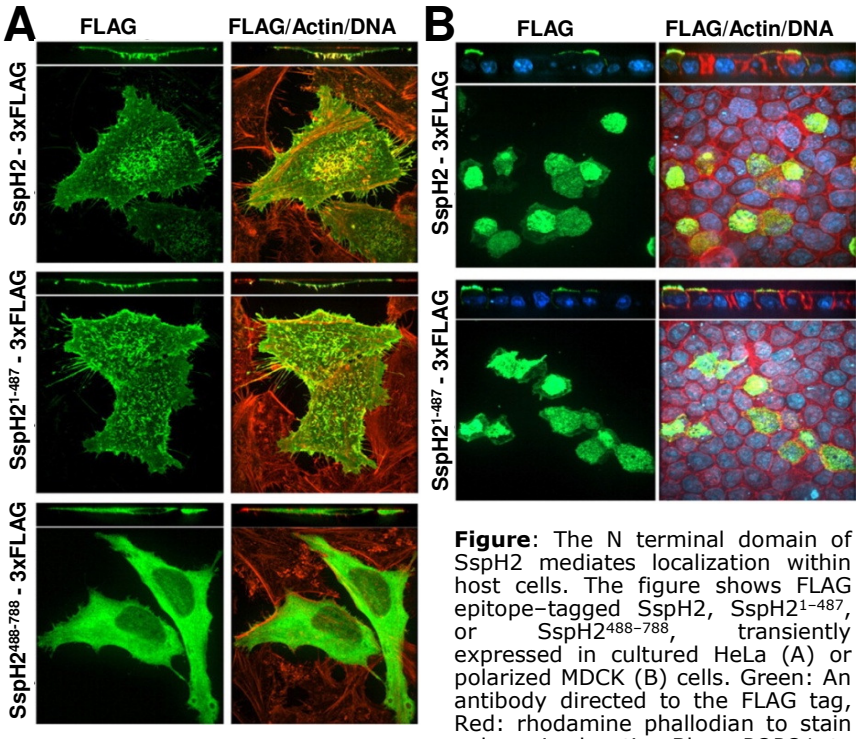

Figure: The N terminal domain of SspH2 mediates localization within host cells. The figure shows FLAG epitope–tagged SspH2, SspH2$^{1–487}$, or SspH2$^{488–788}$, transiently expressed in cultured HeLa (A) or polarized MDCK (B) cells. Green: An antibody directed to the FLAG tag, Red: rhodamine phallodian to stain polymerized actin, Blue: POPO1 to stain DNA.

Part A of the figure shows that a full-length version of SspH2 localizes to the plasma membrane, and is particularly enriched in microvilli. The LRR-containing domain of SspH2 (SspH2$^{1-487}$) had a localization profile virtually indistinguishable from the full-length protein, suggesting that the N-terminal SspH2 region targets the enzymatic activity to the appropriate cellular site. In contrast, the NEL domain (SspH2$^{488-788}$) localizes throughout the cell.

Part B of the figure shows that SspH2 localized almost exclusively in the apical membrane when expressed in polarized epithelial cells, and more specifically, was seen in close association to microvilli (see movie). This unique localization was also dependent on the presence of the N-terminal LRR-containing domain. The results suggest that the SspH2 N-terminal region containing the LRR domain modulates the host interactions of the NEL domain, directing its activity to the apical plasma membrane to engage its putative substrates and the host ubiquitination machinery.

Movie: The movie shows a 3D projection illustrating the localization of wild-type SspH2 transiently expressed in polarized cultured MDCK cells.
http://www.pnas.org/content/vol0/issue2009/images/data/0811058106/DCSupplemental/SM1.mp4.

Cindy M. Quezada, Stuart W. Hicks, Jorge E. Galán and C. Erec Stebbins (2009). A family of *Salmonella* virulence factors functions as a distinct class of autoregulated E3 ubiquitin ligases. PNAS; 106: 4864 - 4869.