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Structure-based functional analysis of effector protein SifA in living cells reveals motifs important for *Salmonella* intracellular proliferation

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ABSTRACT

The facultative intracellular pathogen *Salmonella enterica* survives and replicates inside the *Salmonella*-containing vacuole (SCV) of mammalian host cells. SifA is a key effector protein translocated by a type III secretion system and involved in formation of *Salmonella*-induced filaments (SIF), extensive tubular endosomal compartments. Recruitment of LAMP1 (lysosomal-associated membrane protein 1)-positive membranes to SIF ensures integrity and dynamics of the membrane network. The binding of SifA to the host protein SKIP (SifA and kinesin interacting protein) was proposed as crucial for this function. Due to structural mimicry SifA has further been proposed to interact with G-proteins. We conducted a mutational study of SifA to identify domains and amino acid residues specifically relevant for intracellular replication and SIF formation. Mutations were designed based on the available structural data of SifA and its interface with SKIP, or modeled for SifA as putative guanine nucleotide exchange factor. We developed a live cell imaging-based approach for volume quantification of the SIF network that allowed determination of subtle changes in SIF network and performed a comprehensive analysis of mutant forms of SifA by this approach. We found that the SifA catalytic loop of WxxxE effectors is as important for SIF formation and intracellular proliferation as the SKIP interaction motif, or the CAAX motif for membrane anchoring of SifA.

1. Introduction

Salmonella enterica serovar Typhimurium is a Gram-negative intracellular pathogen which causes gastroenteritis in humans and a systemic disease in mice that resembles human typhoid fever. *Salmonella* virulence relies on the ability to survive in host cells and proliferate within *Salmonella*-containing vacuole (SCV) (reviewed in LaRock et al., 2015; Liss and Hensel, 2015). The intracellular phase depends on the *Salmonella* pathogenicity island 2 (SPI2)-encoded type III secretion system (T3SS) which is expressed in response to signals in the SCV environment such as low pH and nutritional limitation (Kuhle and Hensel, 2004). The SPI2-T3SS mediates the translocation of at least 30 effector proteins that support bacterial intra-vacuolar survival and replication by altering the membrane and cytoskeleton of the host cells, manipulation of vesicular trafficking, inhibition of cell death pathways and blocking innate and adaptive immunity (LaRock et al., 2015).

A remarkable phenotype induced by intracellular *S. enterica* is the massive remodeling of the host endosomal system with formation of *Salmonella*-induced filaments or SIF as a prominent phenotype (Garcia-del Portillo et al., 1993; Liss and Hensel, 2015). SIF are tubular aggregations of late endosomal/lysosomal vesicles. Presence of lysosomal glycoproteins (lgp) such as lysosome-associated membrane protein 1 (LAMP1) is characteristic for SIF membranes. The *Salmonella* SPI2-T3SS effector protein SifA is crucial for formation of SIF (Stein et al., 1996) and stability of SCV during intracellular replication (Beuzon et al., 2000). Mutant strains lacking SifA are more frequently found in the cytosol of host cells, leading to hyper-replication in epithelial cells or reduced survival in macrophages. In addition to SifA the formation of SIF requires further SPI2-T3SS effector proteins including SseF, SseG, SopD2 and PipB2 (Brumell et al., 2002; Kuhle et al., 2004). Contributions of further SPI2-T3SS effector proteins SteA, SseJ and GtgE to manipulation of the host endosomal system and the SCV membrane were reported (see Jennings et al., 2017, for recent review).

SifA is the best characterized effector that is crucial for virulence in a murine model of systemic Salmonellosis, formation of SIF by aggregation of late endosomal/lysosomal compartments (Stein et al., 1996), and SCV stability (Beuzon et al., 2000), which all together underscore the importance of this protein to *Salmonella* pathogenesis. SifA is composed of two distinct domains, each harboring distinct functional motifs. The N-terminal domain has been proposed for secretion/

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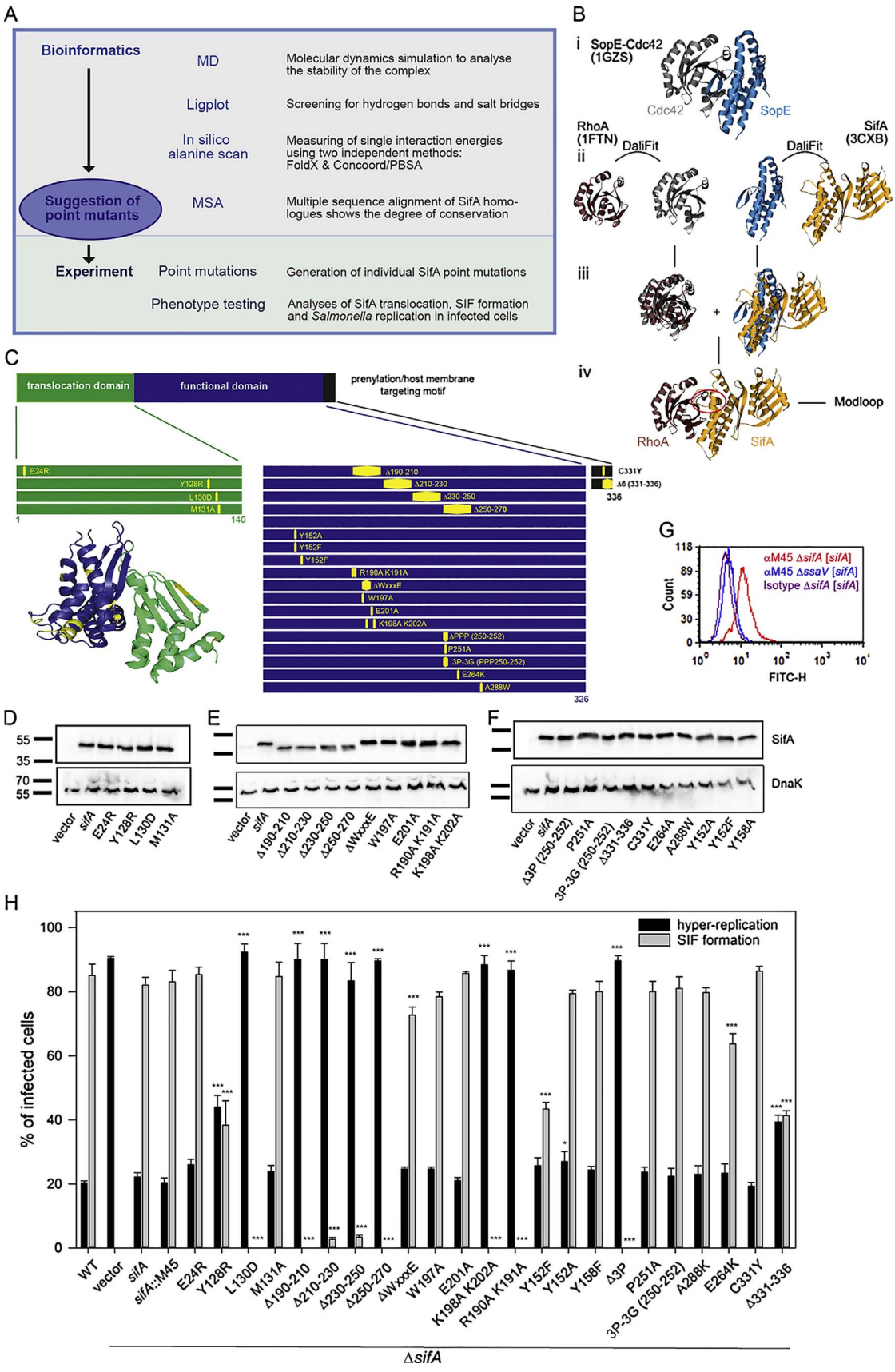

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