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Microreview

Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells

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Summary

Intracellular survival and replication within eukaryotic host cells is of central importance for the pathogenesis of infections caused by *Salmonella enterica*. Intracellular *Salmonella* translocates a set of effector proteins by means of a type III secretion system (T3SS) encoded by *Salmonella* pathogenicity island 2 (SPI2) that manipulates normal host-cell functions. Intracellular survival and replication is linked to the function of the SPI2-T3SS, but recent observations show that many additional cellular functions are targeted by this virulence system. In this review, we focus on the recent observations on the interference of intracellular *Salmonella* with functions of the innate and adaptive immune system and the modification of endocytic and exocytic cellular transport. The common molecular basis of the different SPI2-dependent phenotypes could be the interference with cellular transport along microtubules.

Introduction

Salmonella enterica is a multitalented pathogen with the ability to adapt to highly different anatomical sites within infected host organisms. After ingestion of contaminated food or water, *Salmonella* is able to passage the stomach and to colonize the small intestine. *Salmonella* is a facultative intracellular pathogen that can invade non-phagocytic cells and survives internalization by phagocytes. Various virulence determinants are required for the intracellular phenotype. During intracellular life, *Salmonella* remains in a specialized membrane-bound compartment, referred to as the *Salmonella*-containing vacuole or SCV. The ability to survive and proliferate within eukaryotic host cells is considered as a major virulence factor of *S. enterica*.

Salmonella deploys two distinct type III secretion systems (T3SS) for the manipulation of eukaryotic cells. Both T3SS are encoded by genes located on *Salmonella* pathogenicity islands (SPI). The SPI1-encoded T3SS (SPI1-T3SS) is activated by extracellular bacteria in response to stimuli present in the environment of the intestinal lumen. SPI1-T3SS function is required for the invasion of non-phagocytic cells (Patel and Galan, 2005) as well as for the inflammatory response of intestinal cells (Wallis and Galyov, 2000). In contrast, the SPI2-encoded T3SS (SPI2-T3SS) is specifically active within eukaryotic cells and functions to translocate proteins across the membrane of the SCV.

In this review, we focus on the events during the intracellular phase of pathogenesis by *Salmonella*. A number of recent studies suggest that *Salmonella* actively manipulates host-cell transport processes resulting in a variety of phenotypes that, in combination, promote the intracellular survival and replication of the pathogen and also affect the immune responses raised against *Salmonella*.

The *Salmonella*-containing vacuole

Endocytosed material including microbes undergoes processing in the endocytic pathway that usually results in efficient killing of the microbes. Intracellular pathogenesis requires interference with this default process and generation of an intracellular niche that permits the intracellular survival and replication of the pathogen. In the case of *Salmonella*, intracellular bacteria reside in the SCV subsequent to uptake via either bacterial-mediated invasion or phagocytosis. The maturation process of the SCV in infected cells involves the sequential and highly selective interaction with the endosomal system (reviewed in Holden, 2002; Knodler and Steele-Mortimer, 2003), and is initially typified by the acquisition of marker proteins characteristic of the early and recycling endosomes, such as the early endosomal antigen 1 (EEA1) and transferrin receptor respectively. The association of the SCV with

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these proteins is, however, short-lived and they are rapidly replaced by markers of the late-endosomal system including Rab7, the lysosmal glycoproteins (lgp) LAMP-1, LAMP-2 and LAMP-3/LIMP-1 and the vacuolar ATPase responsible for phagosome acidification. While the SCV therefore displays certain characteristics of late-endosomes, such as lgp acquisition and diminished pH, it remains largely inaccessible to the lysosmal markers mannose-6-phosphate receptor and mature cathepsin D and cathepsin L. This selectivity suggests that the SCV does not directly fuse with late endosomes or lysosomes, but is instead capable of modulating its biogenesis in such a way that transit via the default phagosomal pathway, and therefore bacterial killing, is avoided.

Approximately 4–6 h after infection, *Salmonella*-infected epithelial cells are characterized by the formation of long tubular structures that extend from the SCV, termed *Salmonella*-induced filaments or SIF (Garcia-del Portillo *et al*., 1993). Similar to the SCV, SIF are enriched in lgps and the vacuolar ATPase, and are thought to arise because of the fusion of late endocytic compartments with the SCV. While the formation of SIF was originally observed exclusively in epithelial cells, subsequent studies using IFN-γ-primed macrophages revealed the formation of SIF in this cell type as well (Knodler *et al*., 2003). Although the physiological relevance of SIF has to date not been established, its importance for the intracellular pathogenesis of *Salmonella* is highlighted by the fact that multiple bacterial products translocated by the SPI2-T3SS are required for both its formation and downregulation.

The SPI2-encoded T3SS

The intracellular fate and the ability to cause systemic infections depend on the function of the SPI2-T3SS and its substrates, the so-called effector proteins [for a recent review on the SPI2 functions and effectors, refer to the paper by Kuhle and Hensel (2004)]. The SPI2 locus encodes the entire T3SS as well as a local regulatory system SsrAB controlling the co-ordinated expression of the SPI2 regulon. The expression of SsrAB itself is modulated by global regulatory systems. Only a subset of effector proteins is encoded by SPI2, and the majority of effector proteins are encoded by additional loci on the chromosome that present small insertions, further SPI or genes associated with bacteriophages. This complex organization might indicate the ongoing evolution of the repertoire of effector proteins translocated by the SPI2-T3SS. Another indication is the presence of the N-terminal conserved translocation domain of a subset of SPI2-T3SS effectors, referred to as '*Salmonella*-translocated effectors' or STE (Miao and Miller, 2000). At present 16 proteins, i.e. SpiC, SseF, SseG, SlrP, SspH1, SspH2, SifA, SifB, SseI, SseJ, PipB, PipB2, SseK1, SseK2, GogB, SopD2, have been described as effectors translocated by the SPI2-T3SS (see Table S1 for details on SPI2-effector proteins and further references). A subset of these proteins, namely SspH1, SlrP, SseK1 is also translocated by the SPI1-T3SS.

A remarkable feature of many SPI2-T3SS effector proteins is their association with the endosomal membrane system. These effectors include SseF, SseG, SseJ, SifA, SifB, PipB, PipB2 and SopD2, and a colocalization of these proteins with lgp was observed. Sequence analysis of the effectors reveals the presence of hydrophobic domains that could mediate the membrane association or integration of these effectors. For PipB and PipB2, the targeting to detergent-resistant membrane microdomains or lipid rafts, was also observed (Knodler *et al*., 2003). The targeting of effector protein SseG to Golgi membranes was reported (Salcedo and Holden, 2003), but other investigators found that this protein is predominantly associated with the SCV and SIF after translocation (Kuhle *et al*., 2004). The subcellular localization of SspH1 is rather unusual, as this effector is targeted to the host-cell nucleus (Haraga and Miller, 2003).

The molecular function of most SPI2-T3SS effector proteins is only partially understood, and experimental analysis is complicated by the large number and potential functional redundancy of the effectors. The functions of effectors studied in more detail will be described later in the context of the cellular phenotypes. Further effector proteins may be identified by new genetic screens, as recently exemplified by a screen using gene fusions to the reporter Cya that identified putative new SPI2 effectors (Geddes *et al*., 2005).

Interference with intracellular transport processes

An early study by Groisman's group identified the interference of intracellular *Salmonella* with cellular transport processes in a SPI2-dependent manner (Uchiya *et al*., 1999). SpiC has been proposed as the effector protein responsible for this phenotype, but the function of SpiC is still controversial, as it is also required for the translocation of effector proteins (Freeman *et al*., 2002; Yu *et al*., 2002). Salcedo and Holden (2003) observed that SPI2-effector protein SseG is targeted to the *trans*-Golgi network (TGN) and that the function of the protein is required to maintain *Salmonella* microcolonies in a juxtanuclear, Golgi-associated position within HeLa cells. Based on these observations, the authors suggested SseG as an effector that targets the SCV to the Golgi and an interference with the secretory pathway was postulated. Although the presence of the SCV in a Golgi-associated subcellular position does not necessarily imply an interference with the secretory pathway, new observations are supporting this model.

Our group recently observed that intracellular *Salmonella* interferes with exocytic transport events in a SPI2-dependent manner. Using the well-established marker vesicular stomatitis virus glycoprotein (VSVG), Kuhle *et al*. (2006) observed that vesicular transport from the Golgi to the cytoplasma membrane (CM) was redirected to the SCV. Similar observations were made with fluorescent derivatives of C5-ceramide that were preloaded in the Golgi for subsequent transport to the CM. This interference was dependent on SPI2 function and the effect could be narrowed down to a subset of effector proteins.

Interference with the host-cell actin cytoskeleton

The manipulation of the actin cytoskeleton is of central importance for the invasion of non-phagocytic cells and the molecular interaction of the effector proteins of the SPI1-T3SS and host-cell factors involved in organization of the actin cytoskeleton is well understood (reviewed in Patel and Galan, 2005). Also intracellular *Salmonella* interferes with the actin cytoskeleton in a SPI1-T3SS-independent manner. The virulence plasmid of *Salmonella* encodes SpvB, an ADP-ribosylating toxin that modifies G-actin and inhibits the formation of F-actin (reviewed in Guiney and Lesnick, 2005). However, there is also an involvement of the SPI2-T3SS in rearrangement of the actin cytoskeleton by intracellular *Salmonella*. The formation of an actin meshwork surrounding the SCV, also referred to as 'vacuole-associated actin polymerization' (VAP) (Miao *et al*., 2003), was observed and this phenotype was dependent on the function of the SPI2-T3SS (Meresse *et al*., 2001). The molecular mechanism by which VAP is organized is not understood, as typical cellular regulators such as GTPase dynamin and mDia1 are not involved in this phenotype (Unsworth *et al*., 2004). The work of Miao *et al*. (2003) showed that SPI2-T3SS effectors SspH2 and SseI interact with Filamin, an F-actin cross-linking protein. These activities may therefore contribute to the reorganization of the actin cytoskeleton by SPI2 functions, although an involvement of these effector proteins to the VAP phenotype was not observed (Miao *et al*., 2003).

Interference with the host-cell microtubule cytoskeleton

In addition to inducing actin rearrangements in infected host cells, several pathogenic microbes are known to interfere with the microtubule-dependent processes in order to establish a successful infection (reviewed by Gruenheid and Finlay, 2003). The importance of microtubules for *Salmonella* was initially established by studies that demonstrated that disruption of microtubules has an inhibitory effect on *Salmonella* replication (Garcia-del Portillo *et al*., 1993). Microtubules were subsequently shown to serve as a scaffold along which SIF are formed (Brumell *et al*., 2002), consistent with observations that depolymerization of microtubules leads to the abolition of SIF-formation (Garcia-del Portillo *et al*., 1993). The work by two different groups recently demonstrated that *Salmonella* induces the reorganization of the microtubule network in the vicinity of *Salmonella* microcolonies (Guignot *et al*., 2004; Kuhle *et al*., 2004). This reorganization is frequently accompanied by the formation of microtubule bundles in infected cells, a phenotype dependent on the SPI2-encoded effector proteins SseF and SseG (Kuhle *et al*., 2004). These two proteins belong to a subset of effectors that are targeted to microtubules following their translocation by the SPI2-T3SS, suggesting that they may play an important role in modulating microtubule-dependent processes in infected host cells (see also below).

The importance of the microtubule-associated motor proteins, dynein and kinesin, in controlling the intracellular positioning and proper biogenesis of the SCV has recently been demonstrated. Shortly after internalization, the SCV traffics to the juxtanuclear region and adopts an intracellular position in close apposition to the Golgi apparatus, where bacterial replication takes place (Salcedo and Holden, 2003). While the initial trafficking of the SCV to the juxtanuclear region depends on the Rab7-dependent recruitment of Rab-interacting lysosomal protein (RILP) and the dynein–dynactin complex (Harrison *et al*., 2004; Marsman *et al*., 2004) and appears to occur in a manner independent of the SPI2-T3SS (our unpublished observations), increasing evidence suggests that the steady-state positioning of the SCV within the juxtanuclear region at later time points post infection (> 4 h) depends on the activity of a subset of effectors translocated by the SPI2-T3SS. The work by Salcedo and Holden (2003) demonstrated that the effector protein SseG is required for the retention of the SCV in a juxtanuclear, Golgi-associated position in infected epithelial cells. A Golgi-targeting domain was identified within SseG and a role for this effector protein in tethering the SCV to Golgi membranes, either directly or via its interaction with additional SPI2-effector proteins, was postulated. We observed that SseF, in conjunction with SseG, is required to maintain the SCV in a juxtanuclear position (Fig. 1).

The SPI2-effector protein SifA has been shown to control the intracellular positioning of the SCV through its ability to prevent the excessive recruitment of the motor-protein kinesin to the SCV (Boucrot *et al*., 2005). SifA is capable of directly interacting with a host-cell protein–designated SKIP (SifA and kinesin-interacting protein), which in complex with SifA displaces kinesin from the vacuolar membrane. For the wild-type strain, this activity ensures that the majority of intracellular bacteria are retained in a juxtanuclear position in host cells. For a *sifA*

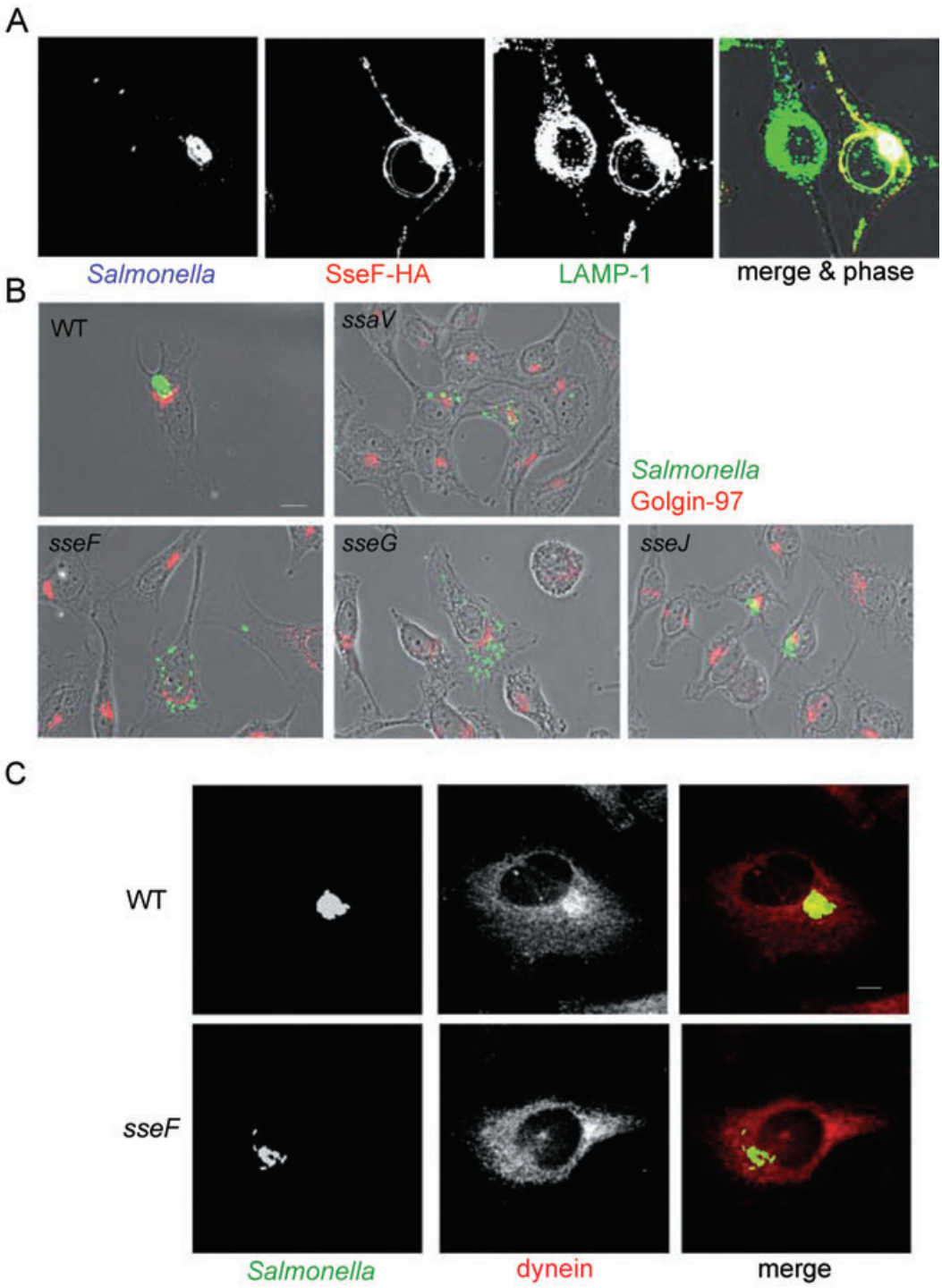

Fig. 1. Intracellular phenotypes of *Salmonella enterica*.
A. The formation of SIF is induced in *Salmonella*-infected cells. SIF are tubular aggregates of membrane vesicles containing lgp such as LAMP-1. Following translocation of effector proteins by the SPI2-T3SS, a subset of these effectors including SseF is targeted to SIF and the SCV.
B. The intracellular positioning of the SCV is dependent on SPI2-T3SS function. *Salmonella* wild type (WT) forms replicative clusters or 'microcolonies' in a juxtanuclear, Golgi-associated position (*trans*-Golgi marker Golgin-97, red). Mutant strains deficient in the SPI2-T3SS, i.e. the *ssaV* strain, are highly reduced in microcolony formation and frequently show a scattered intracellular distribution. A subset of effector proteins including SseF and SseG is required for formation of juxtanuclear microcolonies, while other effectors show no contribution to this phenotype, as represented by the *sseJ* strain.
C. Juxtanuclear microcolonies formed by wild-type *Salmonella* (WT, green) show a recruitment of microtubule motor dynein to the SCV. The number of microcolonies formed by the *sseF* strain is highly reduced but if microcolonies are formed, these are devoid of dynein recruitment.

strain, the absence of the SifA–SKIP complex results in excessive kinesin recruitment to the SCV resulting in its displacement to the cell periphery and loss of the vacuolar membrane due to the excessive pulling force of kinesin.

Consistent with the hypothesis that SPI2 effectors contribute to the intracellular positioning of the SCV via modulation of motor-protein recruitment, our group has observed that the function of the SPI2-effector protein SseF is required to retain the SCV in a juxtanuclear region in infected epithelial cells (G.L. Abrahams, P. Müller, and M. Hensel, in revision). Strains that lack SseF no longer show the characteristic juxtanuclear position displayed by a wild-type strain and are found dispersed throughout the host-cell cytoplasm. The inability of this strain to be retained in the juxtanuclear region was shown to correlate with a marked reduction in its ability to recruit the plus-end directed motor-protein dynein to the SCV, relative to a wild-type strain. Similar observations were made for a strain deficient in the translocation of SseG, suggesting that the combined action of these two effector proteins are required for efficient dynein recruitment to the SCV. Taken together, these observations suggest that *Salmonella* utilizes multiple effector proteins to modulate the recruitment or activities of both plus- and minus-end directed motor proteins to maintain its steady-state position in the juxtanuclear region of the cell.

The modulation of motor-protein recruitment by *Salmonella* is, however, likely to extend beyond a role in maintaining the intracellular positioning of the SCV. Dynein and kinesin activity has been shown to be required for intracellular bacterial replication, SIF formation and the loss of the vacuolar membrane from a *sifA* mutant strain (Guignot *et al*., 2004). Rab7 was demonstrated to be required for the recruitment of dynein to the SCV, and inhibition of the latter activity via disruption of the dynein–dynactin complex resulted in the formation of enlarged SCV in which several bacteria were accommodated. This was postulated to occur as a result of altered membrane detachment events that usually accompany the normal process of vacuole segregation. Functional Rab7 and kinesin motor-protein activity was also shown to be required for the centrifugal extension of SIF in infected cells (Harrison *et al*., 2004). This centrifugal extension was also dependent on the depletion of dynein from SIF, a process that was shown to be mediated by the local uncoupling of RILP and dynein from Rab7 by SifA (Harrison *et al*., 2004). The centrifugal extension of SIF has also recently been shown to be promoted by the activity of the SPI2 effector PipB2. While the molecular mechanism underlying this observation was not established, a role for the protein in modulating microtubule- or motor protein-dependent processes was proposed (Knodler and Steele-Mortimer, 2005).

Interestingly, Perrin *et al*. (2004) observed that a certain proportion of intracellular wild-type *Salmonella* is released into the cytoplasm of macrophages and subsequently recognized by the ubiquitin proteasome system. Further work has to reveal if this phenomenon is due to the inability of a subpopulation of intracellular *Salmonella* to translocate proper amounts of SPI2-effector proteins.

A major part of the work on the cellular phenotypes of intracellular *Salmonella* has been performed with HeLa cells or similar cell lines. Although these studies led to new and exciting insights into the manipulation of the host-cell functions by an intracellular pathogen, these infection models may only reveal a part of the consequences for *Salmonella* pathogenesis. It is possible that the interference with cellular transport of host cells is the molecular basis of some of the phenotypes described in the following section.

Interference of intracellular *Salmonella* with functions of the innate and adaptive immune system

The successful systemic pathogenesis of *Salmonella* depends on the avoidance and suppression of activities of both the innate and adaptive immune system. During intracellular life, *Salmonella* remodels the cell envelope in order to avoid damage by antimicrobial peptides. A central response regulator for the adaptation to the intracellular environment is the PhoPQ two-component system, which was recently shown to function as a sensor for antimicrobial peptides and subsequently activate genes mediating resistance against these agents (Bader *et al*., 2005). In addition to these defensive functions, there is evidence for direct interference of intracellular *Salmonella* with immune functions.

Avoidance of reactive oxygen and nitrogen intermediates by intracellular Salmonella

In addition to production of antimicrobial peptides, phagocytic cells generate an array of further antimicrobial activities that include reactive oxygen species (ROS) and reactive nitrogen species (RNS). Intracellular activities of *Salmonella* mediate protection against both ROS and RNS. Multiple systems are present that allow the detoxification of ROS (reviewed in Vazquez-Torres and Fang, 2001). In addition, two groups observed that SPI2 function enables intracellular *Salmonella* to avoid the formation of an active NADPH-oxidase (phagocyte oxidase, phox) on the membrane of the SCV, thereby preventing the localized generation of bactericidal concentrations of ROS (Vazquez-Torres *et al*., 2000; Gallois *et al*., 2001). Subunits of phox are constitutively expressed in macrophages and granulocytes, but only assemble into a functional enzyme upon specific stimuli. The assembly of phox sub-

units to a functional membrane-bound complex requires a functional actin cytoskeleton (Vazquez-Torres and Fang, 2001), but the molecular details of the interference of intracellular *Salmonella* with this process have not been reported to date.

The inducible nitric oxide synthase (iNOS or NOS2) is induced after uptake of microbes and contributes to their inactivation by synthesis of the highly diffusible and reactive NO. Abrogation of iNOS function by inhibitors or inactivation of the iNOS-encoding gene results in higher susceptibility of mice to *Salmonella* infections (reviewed in Chakravortty and Hensel, 2003). As for ROS, *Salmonella* has evolved various mechanisms to avoid RNS-mediated damages. Chakravortty *et al*. (2002) observed that SPI2 function contributes to the protection of intracellular *Salmonella* from iNOS-mediated damage. A reduced colocalization of iNOS with the SCV was dependent on the function of the SPI2-T3SS; however, no specific effector proteins have been identified that mediates the protection/redirection. Furthermore, wild-type bacteria showed less damage by peroxynitrite than SPI2-deficient strains. Peroxynitrite is a highly bacteriocidal reaction product of ROS and RNS. It is not understood how the distribution of iNOS is regulated in host cells. Earlier reports describe the association of a proportion of iNOS with membrane vesicles. Interestingly, a recent publication reported the microtubule-dependent intracellular transport of iNOS and the formation of iNOS aggresomes (Kolodziejska *et al*., 2005). If a similar situation applies to *Salmonella*-infected phagocytes, it is conceivable that a SPI2-mediated interference with microtubule organization or microtubule-dependent transport could also affect the distribution of iNOS and localization of iNOS in proximity to the SCV.

Salmonella *interaction with dendritic cells*

The ability to process and present antigens is of crucial importance for the development of an adaptive immunity against pathogens. The dendritic cell (DC) forms an important link between innate and adaptive immunity as this cell type is able to phagocytose pathogens in the periphery of the body, to migrate to lymph nodes and to present pathogen-derived antigens to T-cells. The role of DC in immunity to *S. enterica* infection has been investigated in various studies (reviewed in Sundquist *et al*., 2004). Although an initial study (Garcia-del Portillo *et al*., 2000) using a cell line exhibiting features of DC showed that *Salmonella* does not reside within a typical SCV, later investigation indicated that the bacteria are located in a membrane-bound, lgp-positive compartment (Jantsch *et al*., 2003; Petrovska *et al*., 2004). An atypical intracellular fate was observed for *Salmonella* in murine bone marrow-derived DC (BM-DC). Internalized bacteria form a static, non-replicating population, but are active in expression of SPI2 genes and translocation of SPI2-effector proteins (Jantsch *et al*., 2003). However, the function of many known virulence factors including SPI2 is not required for the intracellular survival. Jantsch *et al*. reported a delayed maturation of the SCV as a function of the SPI2-T3SS. Future studies have to reveal which host-cell factors lead to the arrest of bacterial proliferation, and how *Salmonella* modifies DC functions in order to persist in these cells. Interestingly, the function of SifA has been reported to be similar in DC and macrophages (Petrovska *et al*., 2004). The release of *sifA*-deficient bacteria into the cytoplasm was observed, resulting in the increased transfer of an eukaryotic expression vector from *Salmonella* to host cells. Other authors, however, observed that *Salmonella* does not replicate in DC. The persistence of *Salmonella* in DC might allow spread of the pathogen after uptake at intestinal sites. In such scenario, the migratory capacity of DC would turn these cells into 'Trojan Horses' for the systemic spread of the pathogen.

Another consequence of the *Salmonella*–DC interaction is the interference with the primary function of DC, the presentation of antigens. Cheminay *et al*. (2005) could demonstrate that viable *S.* Typhimurium reduce the capacity of BM-DC to present model antigens and to stimulate the proliferation of specific T-cells. This effect was found to require an intact SPI2-T3SS. It was shown that other features such as maturation, viability and expression of costimulatory molecules of DC were not affected. In vaccination experiments, a SPI2-deficient strain caused an increased T-cell proliferation when compared with an equally attenuated, SPI2-proficient strain. This result indicated that SPI2-mediated reduction of antigen presentation and T-cell proliferation might avoid the formation of a specific immune response towards *Salmonella* in a persisting infection.

While Cheminay *et al*. observed that the amount of MHC II molecules loaded with peptides is reduced on *Salmonella*-infected primary BM-DC, Mitchell *et al*. (2004) reported that *Salmonella* infection causes a reduced surface expression of MHC II molecules on MelJuSo and THP-1 cell lines. This phenotype required the function of the SPI2-T3SS. Further work has to reveal if reduced surface expression of MHC II due to SPI2 function is responsible for the suppression of the adaptive immune response. The molecular mechanisms underlying the SPI2-mediated interference of intracellular *Salmonella* with DC functions remain to be revealed.

Conclusions and perspectives

Salmonella has evolved a remarkable adaptation to its intracellular environment. There is a surprising complexity

of intracellular phenotypes, and many of these are dependent on the function of the SPI2-T3SS and its effector proteins. Although the molecular interactions sustaining these phenotypes are not as well characterized as those of the SPI1 effector proteins and their eukaryotic partners, we expect that SPI2 effectors will have an equally exciting biology. A future challenge will be the integration of the different effects of the SPI2-T3SS.

Current limitations to the understanding of Salmonella *intracellular pathogenesis*

Analysis of the intracellular virulence traits is affected by a number of biological and experimental problems: (i) different cell culture models are used to study intracellular phenotypes. Many studies, especially those with a focus on the modification of the cytoskeleton use non-phagocytic cells that are invaded by *Salmonella*, while other studies use macrophages or other phagocytic cell. Phagocytic cells can also be invaded in a SPI1-dependent manner, but this mode of internalization results in rapid apoptosis that excludes further phenotypic analyses. There is, however, increasing evidence for a temporal overlap in SPI1 and SPI2 function after invasion (Steele-Mortimer *et al*., 2002; Hernandez *et al*., 2004), as well as for the interdependence between the mode of entry/uptake and the biogenesis of the SCV (Drecktrah *et al*., 2006); (ii) systemic *Salmonella* infection of NRAMP1-deficient mice is generally considered as good animal model for human typhoid fever. However, the disease progression in the mouse model is very rapid, and even with low infectious doses mice succumb within 7 days, while human typhoid fever develops slower. Obviously, adaptive immune responses are missed in the current mouse model, and the interference of *Salmonella*, and in particular SPI2, with adaptive immune functions are not represented. The development of improved animal models that cover the adaptive immune response is required and (iii) the correlation of cellular phenotypes to specific effector proteins is complicated by the high number of these proteins. The presence of related effectors such as SifA/SifB, SspH1/SspH2, PipB/PipB2 may indicate functional overlaps or redundancies between these proteins. However, given that *S.* Typhimurium is a pathogen with a broad host spectrum, it is also conceivable that different repertoires of effectors have evolved for different host organisms. In this context, the information from comparative genomics of *Salmonella* serotypes will be very valuable. The data allow for the identification of pseudogenes for SPI2 effectors, and one example is *sseJ* that is defective in human-adapted *S. enterica* serovars Typhi and Paratyphi. Further studies are required with mutants deficient in multiple effectors and it will be essential to identify the entirety of effector proteins translocated by the SPI2-T3SS.

An integrated model and topics for future research

Although very different forms of interference of intracellular *Salmonella* with host cells have been observed, there may be a common molecular basis for most of these interactions (see Fig. 2 for a model). We propose that the SPI2-mediated interference with the host-cell cytoskeleton, in particular the microtubule network, is of crucial importance. Because microtubules and their associated motor proteins are essential for proper membrane organelle transport during endocytosis and exocytosis they frequently serve as targets for manipulation by intracellular pathogens (reviewed in Henry *et al*., 2006). Intracellular *Salmonella* selectively acquires membrane-bound cargo of both the endocytic and exocytic pathways, and the redirection of these vesicles towards the SCV may in large part be associated with alterations of the microtubule cytoskeleton or motor-protein activities. The fact that *Salmonella* utilizes multiple effector proteins to modulate the recruitment of motor proteins to the SCV suggests that the activities associated with these proteins play a fundamental role in determining the ultimate fate of the SCV. The motor protein-dependent positioning of the SCV in a predominantly juxtanuclear region may, for instance, facilitate the interaction of the SCV with both endocytic and secretory pathways, and in doing so promote the ability of *Salmonella* to acquire both membrane compartments for continued SCV biogenesis and nutrients for intracellular growth.

Microtubules and motor proteins are also required for the normal maturation of phagosomes to phagolysosomes (reviewed in Vieira *et al*., 2002) and an interference with this process may enhance the intracellular survival of *Salmonella*. Furthermore, *Salmonella* has been demonstrated to be capable of inhibiting the delivery of both NADPH-oxidase and iNOS to the SCV (Vazquez-Torres *et al*., 2000; Chakravortty *et al*., 2002). While the mode of transport of these proteins in host cells is not entirely understood, it is conceivable that *Salmonella* alters the delivery of the respective enzyme systems to the SCV by interfering with microtubule-dependent trafficking events. This possibility has, however, to be investigated further.

In antigen-presenting cells such as macrophages or DC, transport along microtubules is important for antigen processing, as well as the surface expression of peptide-loaded MHC II complexes and formation of immunological synapses for the stimulation of antigen-specific T-cells (Wubbolts *et al*., 1999; Boes *et al*., 2002). A possible explanation of the reduced capability of *Salmonella*-infected DC to stimulate T-cell proliferation could therefore be the reduced transport of loaded MHC II molecules in these DC. The redirection of secretory vesicles observed in other model systems would be in line with the effects on antigen presentation by professional phagocytes. The

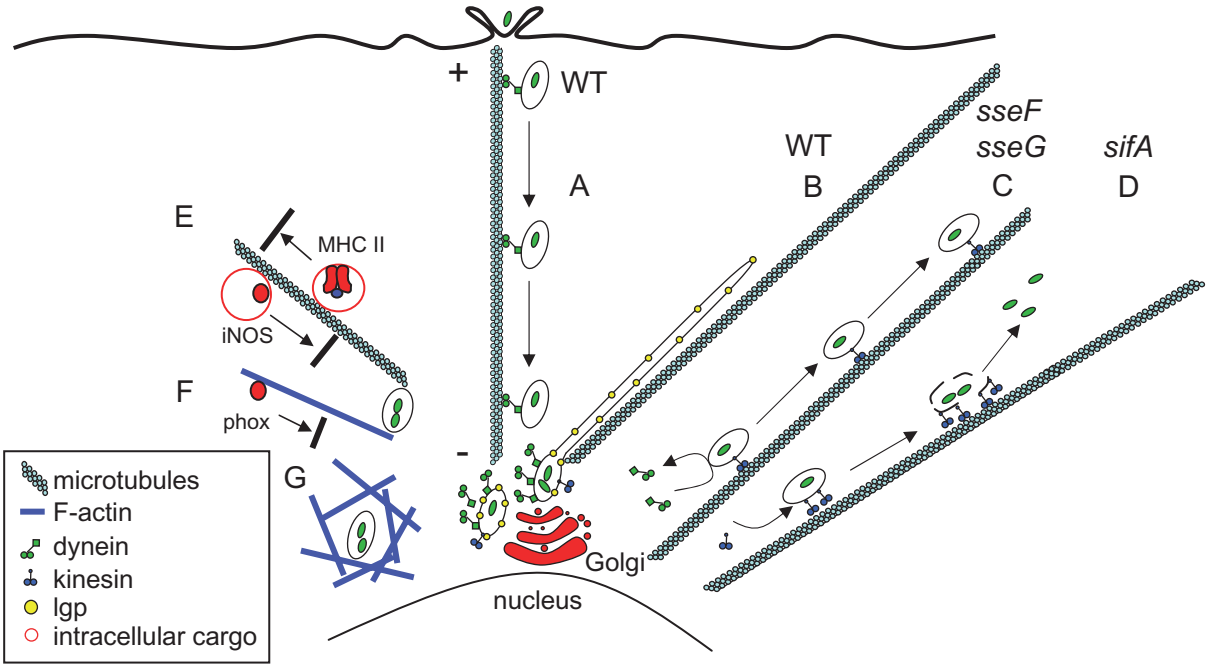

Fig. 2. Model for the intracellular activities of *Salmonella*. After internalization, the SCV is formed and assumes a juxtanuclear position (A). Effector proteins of the SPI2-T3SS are translocated by *Salmonella* residing in the SCV. A subset of effector proteins modifies the recruitment of microtubule motor proteins to the SCV. A balanced activity of dynein and kinesin results in the maintenance of the SCV in a juxtanuclear position and bacterial proliferation (B). The lack of SifA function causes an increased recruitment of kinesin to the SCV, resulting in a plus-end directed pulling force that ultimately disrupts the SCV and releases bacteria into the cytoplasm (C). SseF and SseG are also required for maintaining a juxtanuclear localization of the SCV. An *sseF* strain fails to recruit dynein to the SCV and the activity of kinesin results in the distribution of the SCV in the host-cell periphery (D). Intracellular *Salmonella* redirect exocytic events in a SPI2-dependent manner and this effect may also explain the reduced antigen presentation by DC (E). The avoidance of ROS- and RNS-mediated damages of intracellular bacteria may also be caused by interference with the transport of phox and iNOS, respectively, to the SCV (F). The formation of an F-actin meshwork surrounding SCV can be observed (G).

mechanisms of molecular interference, however, have to be unravelled.

One of the most challenging questions for future research will be the understanding of the interaction of *Salmonella* virulence proteins with cytoskeletal organization and cellular transport. What are the specific host-cell targets of the various effector proteins of the SPI2-T3SS? How do SPI2-effector proteins interfere with the function of microtubule motor proteins? The identification of SifA, as well as SseF and SseG, as effector proteins involved in the recruitment of motor proteins to the SCV, is likely to facilitate the understanding of mechanisms leading to many of the cellular phenotypes associated with the function of these proteins. It will be of future interest to determine whether any additional SPI2-effector proteins play a role in the modification of host microtubule- or motor protein-dependent processes, and if so, how these interactions contribute to the intracellular pathogenesis of *Salmonella*. Work in this direction is likely to give insight into an exciting field of biology.

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Supplementary material

The following supplementary material is available for this article online:

Table S1. Features of known effectors proteins of the SPI2-T3SS.

This material is available as part of the online article from http://www.blackwell-synergy.com

