# Species-Specific Interactions of Src Family Tyrosine Kinases Regulate *Chlamydia* Intracellular Growth and Trafficking

**Cherilyn A. Elwell,[a,b] Arlinet Kierbel,[c] and Joanne N. Engel[a,b,d]**

Departments of Medicine[a] and Microbiology and Immunology,[d] Microbial Pathogenesis and Host Defense Program,[c] University of California, San Francisco, California, USA, and Institut Pasteur de Montevideo, Montevideo, Uruguay[b]

**ABSTRACT Src family kinases (SFKs) regulate key cellular processes and are emerging as important targets for intracellular pathogens. In this commentary, we briefly review the role of SFKs in bacterial pathogenesis and highlight new work on the role of SFKs during the intracellular cycle of *Chlamydia* species.**

Src family tyrosine kinases (SFKs) are cytoplasmic tyrosine kinases that participate in a vast range of physiological functions, including cell proliferation and survival, regulation of the cytoskeleton, cell shape control, maintenance of normal intercellular contacts, cell matrix adhesion dynamics, motility, and migration (1). Although some family members are ubiquitously expressed (e.g., c-Src, Yes, and Fyn), others show more restricted patterns of expression (2). Many cell types express multiple SFKs. Selective tyrosine phosphorylation allows SFK members to switch between an inactive "closed" conformation and a catalytically active "open" configuration, releasing the Src homology (SH) domains, SH2 and SH3, from intramolecular interactions. In the open conformation, the SH2 and SH3 domains to bind to heterologous molecular partners and enables the kinase domain tyrosine phosphorylate substrates.

SFKs play key roles in the pathogenesis of diverse intracellular pathogens, including viruses, parasites, and bacteria. For example, SFK-mediated phosphorylation of cortactin plays a role in *Shigella* entry (3, 4) and in Internalin A/E-cadherin-induced internalization of *Listeria monocytogenes* (5). Upon binding of enteropathogenic *Escherichia coli* (EPEC) to host cells, the translocated EPEC receptor TIR is phosphorylated by c-Fyn, triggering actin polymerization and pedestal formation (6, 7). Src and Lyn have been shown to tyrosine phosphorylate the *Helicobacter pylori* type IV-secreted effector CagA, allowing it to bind and activate the tyrosine phosphatase SHP-2, resulting in changes in the host cell morphology (8–10). Thus, SFKs are targeted by numerous pathogens in order to achieve an array of consequences ranging from inducing local actin polymerization to altering cell morphology.

SFKs are also important during many steps of infection by the obligate intracellular parasite *Chlamydia*. Upon bacterial attachment, *Chlamydia trachomatis* uses its type III secretion apparatus to inject a multifunctional protein with actin-nucleating activity, called TARP (*t*ranslocated *a*ctin-*r*ecruiting phospho*p*rotein), which is phosphorylated by SFKs and other tyrosine kinases, such as Abl kinase (11–15). Although tyrosine phosphorylation of TARP is not required for bacterial internalization, it is postulated to allow the protein to function as a scaffold for recruitment of host proteins that modulate actin dynamics as well as for recruitment of host proteins that regulate signaling events necessary during early development.

Recent exciting studies by Mital and coworkers indicate that SFKs regulate the subsequent trafficking and intracellular development of different *Chlamydia* species (16, 17). Following entry, *Chlamydia* cells replicate within a membrane-bound compartment, the inclusion, which quickly divorces itself from the canonical endocytic pathway and is trafficked along microtubules to the microtubule-organizing center (MTOC) in a manner dependent on the minus-end motor protein dynein (18, 19). Upon arrival at the MTOC, the inclusion maintains a close association with centrosomes (20). The mechanism of interaction between the inclusion and dynein or centrosomes has remained elusive, since association of the inclusion with dynein is independent of p50 dynamitin, which activates dynein and links cargo to the dynein motor (19). These observations have led to the idea that one or more unknown bacterial effectors tether the inclusion to dynein and/or centrosomes.

Mital et al. made the observation that active Fyn and Src kinases localize with *C. trachomatis* and *Chlamydia pneumoniae* inclusion membranes in discrete microdomains (17). Furthermore, active Fyn and Src kinases colocalize with a subset of *C. trachomatis* bacterium-derived inclusion membrane proteins (Incs), including IncB, Inc101, Inc222, and Inc850, in these microdomains on the inclusion membrane, which display a close association with both centrosomes and dynein (17). One of these Incs, Inc850, colocalizes with centrosomes even when expressed in host cells in the absence of infection, suggesting a possible mechanistic link between the inclusion and centrosomes via Inc850. Cholesterol is also enriched in these microdomains, an intriguing observation given that SFKs are known to accumulate in membrane lipid rafts. Most notably, the authors discovered that SFK recruitment to the inclusion is not conserved in all species of *Chlamydiae*. Rather, SFK recruitment appears to be a feature shared only by the human-adapted species *C. pneumoniae* and *C. trachomatis* and not by *Chlamydia muridarum* or *Chlamydia caviae* (GPIC), which infect mice or guinea pigs, respectively (17).

In a recent issue of *mBio*, Mital and Hackstadt extended their studies and reported that the strain-dependent recruitment of SFKs to human-adapted chlamydial species exhibits unique requirements for SFKs throughout their developmental cycle (16). *Chlamydia trachomatis* infection of cultured cells leads to in-

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Address correspondence to Joanne N. Engel, jengel@medicine.ucsf.edu.

creased activation of SFKs. Although SFKs are not required for binding and entry of *C. trachomatis* or for Inc microdomain formation, the authors show that SFKs are required for microtubule-dependent trafficking of the inclusion to the MTOC and for intracellular growth. Of note, the requirement of SFKs for intracellular growth is separate from the requirement of SFKs for trafficking along microtubules. This observation suggests that SFKs may serve multiple functions during *C. trachomatis* infection. Remarkably, the two nonhuman species, *C. caviae* and *C. muridarum*, which fail to display active SFK recruitment to inclusions, also do not traffic to the MTOC, prompting the hypothesis that SFK recruitment correlates with trafficking to the MTOC. Furthermore, *C. caviae* and *C. muridarum* do not display the developmental defects observed with *C. trachomatis* in SFK-deficient cells. Instead, inhibition of SFKs actually enhances the intracellular replication of *C. caviae* and *C. muridarum*, suggesting that the growth of nonhuman strains may instead be restricted by SFKs. The authors speculate that species-specific utilization of SFKs may represent a novel mechanism for defining host tropism.

These new observations raise several interesting questions. First, how do SFKs regulate microtubule-dependent trafficking to the MTOC? Src and Fyn have been shown to interact with gamma-tubulin and to regulate microtubule nucleation from membranes (Fyn) and from centrosomes (Src) (21–23). It is possible that SFKs cooperate with both host and bacterial proteins within the specialized microdomains on the inclusion surface to control trafficking along microtubules and to promote proper positioning of the inclusion at the centrosome. Second, how are SFKs activated and recruited to the inclusion, and what accounts for their unusual distribution to a few discrete subdomains on the inclusion? Do the Incs interact directly with activated SFKs, or are other proteins involved in their interactions? Is SFK activity required for interaction with dynein and/or centrosomes? One attractive hypothesis is that one or more of the Incs present in the microdomains recruit active SFKs to the inclusion, thus promoting microtubule nucleation and dynein-dependent movement toward the MTOC, where upon arrival, Inc850 then binds centrosomes for proper positioning. Third, what accounts for the species specificity? The diverse requirements of SFKs for human versus nonhuman strains are particularly exciting because genomic comparisons of *Chlamydia* species have yielded little insight into specific virulence determinants associated with disease. It will be interesting to determine whether the specific Incs that colocalize with SFKs within the microdomains display species-specific binding and activation of Src and/or Fyn or are differentially expressed in the nonhuman strains, as this could account for the observed SFK dependencies. Fourth, what is the nature of the developmental defect observed in the absence of SFKs for the human-adapted strains? Finally, how do SFKs restrict the growth of nonhuman species? Do active SFKs enhance the innate immune response, affect accessibility to nutrients, or regulate general host protein trafficking during infection of the nonhuman strains?

Studying the role of SFKs during *Chlamydia* infection will likely lead to a better understanding of how SFKs regulate microtubule-dependent trafficking in normal host cells and shed light on the unique ways that pathogens subvert host cell signaling. Furthermore, the ability of *Chlamydia* to alter centrosome positioning and cause deregulation of centrosome duplication (20, 24) may also provide insights into the pathways leading to chromosome instability during deregulated states, such as cancer. Once again, pathogens serve as tutors to help us understand complex but fundamental cellular processes.

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