

Figure 1: The life cycle of vaccinia virus. Replication and morphogenesis of vaccinia is depicted above, while the right shows the wrapping and late stages required to understand actin tail formation. Models were copied from Moss, 1996 and Strauss, 1996. Abbreviations: IV-immature virion, IMV-intracellular mature virus. IEV-intracellular enveloped virus, CEV-cell attached enveloped virus, EEV-extracellular enveloped virus, CGN/IC-*cis*-Golgi network/intermediate compartment, TGN-*trans*-Golgi network





Figure 2: Proteins of IEV. The topologies of IEV specific proteins. Arrows indicate interactions between the proteins. The membrane corresponds to the outer IEV membrane exposed to the cytoplasm. Domains are drawn to size except for F13L which is a membrane associated protein.





Figure 3: An idealized scheme of *in vitro* actin polymerization. The different graphs show the influence of two proteins on the kinetics of actin polymerization. 1: actin polymerization is stimulated. 2: actin polymerization is inhibited. See introduction for a detailed description.



Figure 4: Actin polymerization *in vivo*. A popular model of how actin polymerization occurs at the plasma membrane after receptor activation. See introduction for a description of the individual processes. Model copied from Machesky and Way, 1999.





Figure 5: Life cycle of *Listeria monocytogenes*. The schematic shows the intracellular stages of the *Listeria* life cylce. A bacterium enters the cell, escapes the phagosome, accumulates actin and is propelled on the tip of an actin tail into a neighboring cell where the cycle repeats. See introduction for a detailed description of the different stages. From Tilney and Portnoy, 1989.



Figure 6: Putative domain organization of the *Listeria* ActA, *Shigella* IcsA and rat N-WASP proteins. See introduction and discussion for more details on the respective domains. Models adopted from Dramsi and Cossart, 1998 (ActA, IcsA) and Miki et al., 1996 (N-WASP).



Insertion of actin, filament elongation and propulsion





Figure 7: Actin tail formation by *Listeria* and *Shigella*. The cartoons describe current models of how actin polymerization is thought to be achieved at the surface of *Listeria* (left) and *Shigella* (above). See introduction and discussion for details on the mechanisms. Models copied from Machesky and Cooper, 1999 (*Listeria*) and Egile et al., 1999.





Figure 8: Intracellular pathogens move on the tip of actin tails in infected cells. Time lapse confocal microscopy of stable with GFP-actin transfected 143TK osteosarcoma cells infected with the *Listeria monocytogenes* strain 10403S (upper panels), *Shigella flexneri* strain SC301 (middel panels) or vaccinia virus strain WR (lower panels). Time between frames: approximately 20 sec for the *Listeria* and *Shigella* infected cell and approximately 5 sec for the vaccinia infected cell. Large arrowheads indicate possition of selected bacteria at the start of the time lapse. Small arrowheads indicate the movement of these bacteria. The boxed area highlights the movement of two virally induced actin tails. The images on the

the movement of two virally induced actin tails. The images on the lower right site of the figure pages can be played as a 'thumb cinema' movie.





Figure 9: Localization of VASP in infected cells. The actincytoskeleton is visualized with fluorescent phalloidin and VASP with antibody labelling VASP is localized to

the tip of vaccinia induced actin tails as well as throughout the actin tails while it is only found on *Listeria* actin clouds but not in actin tails (see insets). In contrast to *Listeria* VASP localizes throughout the actin tails in *Shigella* infected cells. Arrowheads point to focal adhesions and the arrows points to a *Shigella* bacterium that has not yet formed an actin tail. Scale bar: 20  $\mu$ m.





Figure 10: Localization of Arp3, vinculin and N-WASP to actin tails of intracellular pathogens. Infected cells were fixed in methanol (for Arp3 staining) and labelled with antibodies against Arp3 and actin. Alternatively, cells were fixed in PFA and labeled with antibodies against vinculin or N-WASP while F-actin was visualized with Alexa-488-phalloidin. Arp3 localizes to actin tails of all pathogens and to actin clouds around *Listeria* and *Shigella*. Vinculin is not present in tails induced by *Listeria* although it is present at focal adhesions (arrows). It is, however, weakly seen on tails induced by *Shigella*. Vinculin staining is absent from actin

on tails induced by *Shigella*. Vinculin staining is absent from actin tails induced by vaccinia virus (data not shown). N-WASP localizes to the tip of actin tails induced by vaccinia and *Shigella*. N-WASP staining is absent from actin tails induced by *Listeria* (data not shown). Asterixes mark viral particles or bacteria. Scale bars: 5 µm.

