3 Discussion

3.1 Differences in the actin based motility of intracellular pathogens

3.1.1 Actin tail nucleators

A number of studies reported differences as well as similarities in the actin-based motility of intracellular pathogens by directly comparing the molecules involved in this process (Chakraborty *et al.*, 1995; Frischknecht *et al.*, 1999a; Gouin *et al.*, 1999; Heinzen *et al.*, 1999). From these studies it became clear that, with the exception of *Rickettsia*, intracellular pathogens rely on Arp2/3 complex mediated actin polymerization to achieve actin-based motility. *Listeria* and *Shigella* achieve the activation of Arp2/3 complex mediated actin assembly by different means (see introduction). My results show that the viral protein A36R is the 'nucleator' of actin polymerization for vaccinia and therefore plays an analogous role to ActA of *Listeria* (Domann *et al*., 1992; Kocks *et al.*, 1992; Kocks *et al.*, 1995) and IcsA of *Shigella* (Bernardini *et al.*, 1989; Goldberg and Theriot, 1995). However, there is no sequence homology between these proteins. All three proteins have in common that they possess at least two regions that are involved in the recruitment of host factors to the site of actin tail formation. For *Listeria* ActA, the major domain required for actin tail formation resides between residues 21 and 231, while the four proline rich repeats in the central region of ActA play a modulatory part in this process (Lasa *et al*., 1997; Smith *et al*., 1996). In *Shigella* IcsA, the glycine rich repeats of the β-domain are responsible for the recruitment of the actin polymerizing machinery while deletion of the α-domain results in a non-polar distribution of IcsA which leads to the inability of bacteria to form actin tails but does not inhibit actin polymerization on the *Shigella* surface (Suzuki *et al*., 1996). In vaccinia A36R, phosphorylation of two tyrosine residues mediates the actin-based motility of the virus (Figures 24, 25). Phosphorylation of tyrosine 112 in A36R is the major event while phosphorylation of tyrosine 132 plays a minor role. If tyrosine 112 is changed to phenylalanine only a small percentage of viruses are able to nucleate actin tails, while point mutation of tyrosine 132 has little or no effect (Figures 24, 25). Nevertheless, if both tyrosines are exchanged to phenylalanine not a single virus is able to assemble actin (Figures 24, 25).

3.1.2 Mechanisms of actin tail formation

How do these "nucleators" recruit the Arp2/3 complex? The N-terminus of ActA interacts directly with and stimulates the actin polymerization activity of the Arp2/3 complex (Welch *et al*., 1998). The glycine rich repeats of IcsA bind N-WASP which subsequently interacts with and activates the Arp2/3 complex (Egile *et al*., 1999). The phosphorylated tyrosine 112 of A36R recruits the adaptor protein Nck, which then binds N-WASP (Figures 28, 29). It is likely that N-WASP recruits and activates the Arp2/3 complex, although this has not been shown formally for vaccinia virus. Is this mechanism over simplified? It is possible that Nck binding to the proline rich domain of N-WASP could open up N-WASP and recruit the molecule, thereby activating the cascade leading to actin polymerization (Machesky and Insall, 1999; Rivero-Lezcano *et al*., 1995). Alternatively, Cdc42 could activate N-WASP by binding to the CRIB domain (Rohatgi *et al*., 1999). Finally, a protein such as the WASP-interacting protein WIP could link Nck and N-WASP (Ramesh *et al*., 1999). Although I demonstrated direct binding of Nck to phosphorylated A36R peptide, the pelleting experiment using the Nck-A36R complex does not show whether N-WASP is directly bound to Nck. Also, phosphorylated A36R peptide does not pull out N-WASP suggesting that there is another cellular or viral protein linking Nck and N-WASP. However, using extracts from uninfected cells I could show that the Nck-A36R complex still pellets N-WASP suggesting that no other viral protein is linking the two (data not shown).

Taken together, these results show that vaccinia entertains the most complex strategy of the three pathogens to achieve actin tail assembly. In conclusion, *Listeria* mimics the activation of Arp2/3 complex by N-WASP while *Shigella* mimics the Cdc42 dependent activation of N-WASP which then recruits and activates Arp2/3 complex (Egile *et al*., 1999 and introduction). Vaccinia mimics what appears to be a complete signal transduction pathway including the activation of tyrosine kinases, phosphorylation of a receptor-like protein and recruitment of adaptor proteins prior to recruitment of N-WASP and Arp2/3. As we only begin to unravel the actin-based motility of vaccinia, future studies will give us more insight into signal transduction pathways leading to actin polymerization.

3.2 Actin based motility of vaccinia and the activation of signalling cascades

Cells have evolved numerous mechanisms to link extracellular stimuli into intracellular effects. Of the many signal transduction pathways some affect the actin cytoskeleton (Bruckner and Klein, 1998; Carlier *et al.*, 1999; Machesky and Insall, 1999; Riese and Stern, 1998). Intracellular pathogens have independently developed a variety of strategies that subvert cellular signalling processes, especially during the entry process of the pathogen as they often bind to cellular receptor molecules (Dramsi and Cossart, 1998). Vaccinia virus activates host kinases early during infection (Figures 17, 39). Later during vaccinia infection at least one of the activated kinases is involved in actin-based motility (Figures 32, 34 and 35). Are there other effects? As vaccinia infection induces cell motility (Sanderson *et al.*, 1998b), it is very likely that a kinase is also involved during the process of cell migration. Whether the kinase that is responsible for phosphorylation of A36R is also involved in virus induced cell motility can only be answered when the kinase is identified. It is possible that A36R, having two phosphorylation sites, is not just phosphorylated by one kinase. When infected cells are treated with the src family kinase inhibitor PP1, cortactin is not phosphorylated, while A36R still is phosphorylated albeit at a lower level (Figure 36). The phosphorylation level of pTyr200 is unchanged. This indicates that c-Src is activated during viral infection, but shows that other kinases are also activated, which are responsible for the phosphorylation of A36R and pTyr200. Phosphorylation of cortactin has been implicated in cell motility (Huang *et al.*, 1998). Wound healing experiments are planned to address the role of src and cortactin in vaccinia induced cell motility using different viral strains, pE/L constructs and kinase inhibitors.

3.3 Is A36R the phosphotyrosine protein?

It is clear that a proportion of A36R is phosphorylated on tyrosine residues 112 and 132 (Figures 17, 18, 23 and 24). However, does A36R represent the phosphotyrosine signal observed by immunofluorescence microscopy at the tip of the actin tail? Or is there another protein involved in actin tail assembly that is also phosphorylated? I was only able to localize the phosphotyrosine signal to IEV when they had associated actin tails. This would suggest that either every IEV immediately forms an actin tail, or that on some IEVs A36R is not phosphorylated. Data obtained in methanol fixed cells show that the number of IEVs per cell is higher than those of IEVs having actin tails (data not shown). This would suggest that A36R is phosphorylated after IEV formation. However, A36R is phosphorylated even in the absence of IEVs or actin tails (Figure 17). Using immunofluorescence microscopy, I was not able to co-localize both, the A36R signal and the phosphotyrosine signal in cells infected with ΔA34R. This is not surprising, as A36R is localized throughout the cell similar to the localization of A33R in figure 16. As only a fraction of A36R is phosphorylated, a phosphotyrosine signal can possibly only be detected in immunofluorescence when there is a high local concentration of A36R.

Phosphorylation of A36R is detectable on western blots as early as 3 hpi, right after the protein is expressed and well before even IMVs are synthesized (data not shown). Does this phosphorylation already lead to the recruitment of the actin nucleating machinery? If this would be the case, the *trans*-Golgi network should stain for actin filaments, especially in cells infected with viral strains that do not make IEVs but express A36R. However, I could not observe such a staining pattern (see figures 13-15). This could indicate that A33R and/or A34R have assisting roles in A36R mediated actin tail formation, i.e. that A36R can only recruit the actin polymerizing complex when functionally mature IEVs are formed. Further, it could be possible that an equilibrium between phosphorylation and dephosphorylation of A36R exists. If so, the phosphorylated A36R might be stabilized (protected from possible phosphatases) only on IEVs by the actin tail nucleating complex. Such a dynamic equilibrium between the phosphorylated and unphosphorylated state would also make it hard to localize phosphorylated A36R by microscopical techniques as the localized concentration of phosphorylation might never be appreciably higher than the normal cellular background phosphorylation levels (see Figure 11).

All these observations do not answer the question whether the signal observed by immunofluorecence is phosphorylated A36R. Interestingly, however, when cells were transfected with dominant negative constructs of N-WASP lacking the WH2 domain, a phosphotyrosine signal could be observed on viral particles that had no actin tail associated. These particles did not have VASP associated indicating that the phosphotyrosine protein observed with immunofluorescence four colour technology is located upstream of N-WASP in the cascade that leads to actin polymerization of IEVs (Moreau, Frischknecht, Way, unpublished results). Both Nck and N-WASP have been reported to be phosphorylated on tyrosine (Guinamard *et al.*, 1998; Li *et al.*, 1992; Park and Rhee, 1992). However, I was not able to detect Nck and N-WASP in immunoprecipitates with anti-phosphotyrosine antibody (data not shown). This would be an indication that A36R is indeed corresponding to the phosphotyrosine signal observed by immunofluorescence microscopy, but is far from being proof. Finally, a molecule linking Nck and N-WASP could also be phosphorylated.

Additional confusion comes from observations of uninfected cells, where a phosphotyrosine signal is also observed on actin tail-like structures (LATs) nucleated from clathrin coated vesicles (see chapter 3.5) (Figure 42). As these cells have not been infected, A36R is absent and the phosphotyrosine signal has to indicate a different host protein. Whether this phosphotyrosine signal originates from a cellular A36R homologue or from a different protein that might also be implicated in actin tail formation of vaccinia remains to be established. Interestingly, these LATs recruit the same complement of host proteins to the actin tail as does vaccinia, including Nck and N-WASP (Figure 42 and Frischknecht *et al.*, 1999b). Possible functional roles of these and other cellular actin tails are discussed in chapter 3.5.

3.4 The role of accessory host proteins

A number of host proteins are localized to actin tails of intracellular bacteria as well as vaccinia and were reported to influence or to be required for their actin-based motility (see introduction). Most of those proteins are not required for actin tail formation but are likely to act in concert to enhance the efficiency of actin-based motility. However, whether small differences in intra-cellular speed or in the percentage of pathogens able to move reflects the ability of the respective pathogen to spread, remains to be established.

The localization of an actin binding protein to the actin tail suggests that the protein could be functionally required for tail formation or filament turnover. However, recruitment alone doesn't reflect functionality as the protein can localize to the tail simply because of the high concentration of actin, like it is the case for F17R (chapter 1.4.5). Therefore immuno-localization is only the first step in the investigation of the potential role of a cytoskeletal protein in actin-based motility of a pathogen.

3.4.1 The case of vinculin

Vinculin has been implicated in the actin-based motility of *Shigella* by two labs but it's role has been controversial (Dold *et al*., 1994; Goldberg, 1997; Laine *et al*., 1997; Suzuki *et al*., 1996) (see Introduction). Immuno-localization studies suggest that there is no role for vinculin in the actin-based motility of *Listeria* and vaccinia although one can never rule out accessibility problems with antibodies (Figure 10). Although not essential, vinculin may play a modulatory role in *Shigella* motility. Careful experiments using *in vitro* motility assays with pure proteins should be able to resolve this problem (Loisel *et al*., 1999). However, such experiments will not rule out a role for vinculin in establishing the cell-cell-adhesion like structures of inter-cellular protrusions which are important for efficient cell-to-cell spread of *Shigella* (Sansonetti *et al*., 1994). A possible role for vinculin in this process is suggested by the observation that vinculin staining is more prominent in actin tails projecting from *Shigella* infected cells than in intracellular tails (Sansonetti *et al*., 1994 and data not shown).

3.4.2 VASP

It is clear that VASP and profilin play an important accessory role in the actin-based motility of *Listeria* and *Shigella* (Laurent *et al*., 1999; Loisel *et al*., 1999). In the case of *Listeria* VASP binds to ActA, while no interaction between VASP and IcsA has been shown. Immunofluorescence analysis shows that VASP is found in actin tails of *Shigella* and vaccinia, while in *Listeria* it is only localized to the bacterial surface (Figure 9). A definite answer of how VASP enhances actin-based motility is still missing. Does VASP bind to A36R? There are no poly-proline rich regions in A36R which could bind to VASP, however the VASP signal observed with immuno-fluorescence microscopy is stronger at the vaccinia particle than in the tail (Figure 9). When vaccinia infected cells were transfected with N-WASP-ΔWA, the viruses were unable to form actin tails. Although the phosphotyrosine and Nck signals were still present on those viruses, no VASP signal could be found, suggesting that VASP recruitment to the site of actin tail formation needs at least full lenght N-WASP at this site. As VASP can bind to F-actin it is not surprising that it binds to the actin tail of both *Shigella* and vaccinia, which does not explain, however, why there is more VASP at the site of actin tail formation of vaccinia. That no VASP is found in *Listeria* tails could be due to the higher binding affinity of VASP to the proline-rich repeats than to F-actin. Future experiments using VASP mutants should answer how this molecule is recruited to vaccinia and if it plays a role in actin nucleation or filament bundling (Huttelmaier *et al.*, 1999). Using the recently available VASP knock-out cells one should also be able to determine the *in vivo* role of VASP in vaccinia actin-based motility (Aszodi *et al.*, 1999; Laurent *et al.*, 1999).

3.4.3 The art of micro-injection

Micro-injection of peptides or proteins into cells followed by video analysis potentially represents a good way to determine the role of a protein in the actin-based motility of a pathogen. However, such experiments have to be carefully controlled as they are full of pitfalls. Micro-injection of poly-proline peptides caused heavy retraction of the cell membrane which reflects dramatic effects on the cytoskeleton (Southwick and Purich, 1994). Given such changes it is hard to interprete what role a proline rich protein like zyxin or vinculin plays in the motility of an intracellular pathogen. An effect of such a peptide on actin-based motility might not be direct, but secondary in nature. One could also envisage that the retraction of cell protrusions is due to the effect of the peptide on many different regulatory systems affecting actin polymerization and not merely on one protein.

A striking example of how little micro-injection experiments can tell about the involvement of a protein in actin-based motility is given by a study on the barbed end capping and F-actin severing protein gelsolin (Laine *et al*., 1998). Starting with low quality immunofluorescence data the authors claim to localize gelsolin to the site of *Listeria* actin tail formation. They continue to show that bacteria move with twice the velocity in cells stably expressing gelsolin at 2.25 times the normal level. Is this acceleration due to an implication of gelsolin in actin-based motility or merely due to an effect of gelsolin on the actin cytoskeleton of the cell as a whole? Or does overexpression of gelsolin lead to a change in the expression of other actin binding proteins? When cells derived from gelsolin knockout mice were infected, *Listeria* exhibit the same speed as in wild type cells suggesting that gelsolin does not play a role in actin-based motility. These gelsolin null cells show prominent stress fibers indicating that the cells overexpressing gelsolin might have a more dynamic actin cytoskeleton. Therefore the higher speed of the bacteria could simply be an effect of a less constrained movement or the increased actin turnover as with ADF/cofilin (Carlier *et al.*, 1997, also see introduction). Alternatively, instead of being 'absorbed' by the cell cytoskeleton, the Arp2/3 complex could be more readily available in cells expressing higher levels of gelsolin as these cells have fewer filaments.

A similar critique can be addressed to my findings with micro-injecting anti-phosphotyrosine antibodies into vaccinia (Figure 12). Micro-injection of these antibodies resulted in the majority of cells in drastic changes of the cell shape over time and ultimately led to the loss of the injected cells (data not shown). Therefore I had to figure out a time frame of when to fix the injected cells in order not to have too unspecific effects on the cytoskeleton. However, micro-injection of cells usually took about one hour, meaning that when the cells were fixed one hour post injection, some cells were injected for two hours. Therefore, it is impossible to say without the appropriate controls, whether inhibition of vaccinia actin tails by micro-injected anti-phosphotyrosine antibodies is direct or due to more general effects on actin dynamics. As a control I choose to inject *Listeria* infected cells. Micro-injection of the antibody had no effect on *Listeria* actin tail formation although the *Listeria* infected and injected cells did ultimately also round up and detach (Figure 12 and data not shown). However, can one compare *Listeria* and vaccinia in such an experiment? Due to the size differences between the pathogens, *Listeria* actin tails are much larger than vaccinia induced actin tails. Could this difference mean that vaccinia actin tails are more delicate than *Listeria* actin tails? It's hard to say. On the other hand, a cell infected with vaccinia exhibits on average more actin tails than a cell infected with *Listeria* (Figures 8, 11 and 38). Therefore the method used to evaluate actin tail formation (see chapter 4.11) should discriminate against high numbers of cells showing *Listeria* tails and against low numbers of cells showing vaccinia tails (Figure 12). I am confident that these multiple layers of controls contribute to the validity of the experiment.

3.4.4 Actin cross linkers and actin tail formation

Are F-actin cross-linking proteins required for actin tail formation *in vivo*? A study injecting an actin filament cross-linking incompetent fragment of α-actinin into cells found that the cross-linking activity of α-actinin is required for actin tail formation of *Listeria* (Dold *et al*., 1994). However, no cross-linking protein is required for the *in vitro* movement of bacteria indicating that the formation of Arp2/3 induced dendritic networks provides sufficient rigidity within the actin tail (Mullins *et al.*, 1998a; May *et al.*, 1999 ; Loisel *et al.*, 1999; Machesky *et al.*, 1999) (see also

Figures 4, 7). However, so far no study was done on the ultrastructure of *in vitro* induced actin tails, which might differ greatly to those induced *in vivo*. I found the actin cross-linking protein cortactin localized to actin tails of *Listeria*, *Shigella* and vaccinia (Figure 38). A stimulatory role for cortactin in cell movement was found and linked to the phosphorylation of three tyrosine residues by c-Src kinase (Huang *et al.*, 1998). However, it is not clear how phosphorylated cortactin promotes cell motility. The same authors previously showed that phosphorylation of cortactin causes its dissociation from F-actin (Huang *et al*., 1997). Does cortactin play a role in the actin-based motility of pathogens? Again, the only answer can come from experiments where pure components are mixed together and from cells lacking or overexpressing cortactin (but see caveats in 3.4.2). I did not express cortactin and to date there is no cortactin knockout-mouse available. Therefore a role of cortactin in actin-based pathogen motility remains as speculative as for other cross-linking proteins.

3.4.5 The role of additional viral proteins in actin-based motility

The bacterial proteins ActA and IcsA are both necessary and sufficient for the actin-based motility of *Listeria* and *Shigella*, respectively (Dramsi and Cossart, 1998). To dissect if a specific vaccinia protein contributes to efficient actin tail formation is not easy as deletion of a protein often impairs IEV formation (Figures 13-16 and Röttger *et al*., 1999).

Is the A36R protein the only protein required for actin-based motility of vaccinia virus? It is well established that only the IEV form of vaccinia can induce actin tails (Blasco and Moss, 1992; Cudmore *et al*., 1995). From the results presented in Figures 13 to 16 and table 3 it is clear that A36R and A56R are the only proteins that are not needed for efficient IEV formation. A56R is not needed for actin tail assembly, leaving only A36R as a candidate for the viral actin tail organizer. However, one cannot exclude additional functional roles in actin tail formation of proteins primarily involved in IEV formation. Viruses lacking the proteins B5R or F13L can still induce actin tails late in infection when a small number of IEVs are formed ruling out that these proteins are directly involved in actin tail formation (Figure 15). The roles of the proteins A33R and A34R in actin tail formation are still ambiguous. Viruses lacking A33R seem to be deficient in the wrapping step leading to the formation of IEV (Roper *et al*., 1998), while viruses lacking A34R were reported to form IEVs as judged by electron microscopy (McIntosh and Smith, 1996; Wolffe *et al*., 1997). The studies I conducted in collaboration with Sabine Röttger, however, showed that in HeLa cells A34R did not form IEVs. We used both electron microscopy and immunofluorescence analysis over a range of infection times but limited our study to HeLa cells. Unfortunately we did not have access to the A33R deletion virus. To address the roles of A33R and A34R in actin tail formation one has to definitively show independently of cell type, whether viruses lacking these proteins are able to form IEVs. Only then can it be shown if the complex of A33R-A34R-A36R observed in WR infected cells has a functional role in actin tail formation (Röttger *et al.*, 1999).

Interestingly, performing rescue experiments using ΔA34R and pE/L-A34R deletion constructs shows that the cytoplasmic (N-terminal) domain of A34R (see figure 20) is not required for either IEV or actin tail formation (Röttger, Frischknecht, Way, manuscript in preparation). Also, point mutation of the N-glycosylation site allows the formation of IEV and actin tails. This is interesting as infections in the presence of tunicamycin, an inhibitor of N-linked glycosylation do not lead to formation of IEV and hence actin tails (Röttger, Frischknecht, Way, manuscript in preparation). However, considering that almost all IEV specific proteins are glycosylated (see chapter 1.1.3) such a result is not a surprise. Only a deletion of the very C-terminus of A34R abrogates formation of IEV and hence also formation of actin tails (Röttger, Frischknecht, Way, manuscript in preparation). It is not clear how a deletion of these residues could affect morphogenesis of IEV or interaction of A34R with A33R, A36R and/or B5R. Further studies using both, recombinant viruses and the rescue assay system will be needed to understand the interactions and relationships between A33R, A34R, A36R, IEV formation and actin tail assembly in more detail. Interestingly, quantitative analysis of the pE/L experiments showed that deletion of the C-terminus or the glycosylation site had effects on efficient actin tail formation, suggesting that these sites within A34R play some role in IEV and/or actin tail formation (Röttger, Frischknecht, Way, manuscript in preparation). Given the size of the vaccinia genome, it is also possible that more IEV proteins exist than have been identified to date.

From the results presented in this thesis and the problems discussed above it is nevertheless clear that A36R is the molecule required to nucleate the cascade of events leading to actin tail formation although it depends on other IEV proteins for correct localization and function. A33R, A34R and possibly other unidentified IEV proteins appear to play a supporting role in actin tail formation, for example by providing the appropriate structural scaffold on the IEV surface.

3.5 Actin-based motility of cellular organelles

In 1992 Heuser and Morisaki reported the actin-based motility of endosomes in macrophages stimulated with heavy metal salts (Heuser and Morisaki, 1992). I was the first to report actin tail like structures (LATs) in unstimulated cell (Frischknecht *et al.*, 1999a) (Figure 42). Clathrin coated vesicles derived from either the *trans*-Golgi network or the cell membrane can be observed in our HeLa cells associated with LATs. The appearance of LATs seems to be random and I could find no method to either consistently enhance or to abolish their presence (data not shown). Video microscopy of GFP-actin transfected cells showed that LATs are not moving like pathogens. The actin tails are rather being wagged at one site of the clathrin coated vesicle without moving the vesicle from its place. This is somewhat hard to reconcile with the observation that clathrin coated vesicles with associated actin tails can be found projecting from the cell surface (data not shown). Long term observation under the video microscope should give further insight into the function of these tail-like structures. An interesting functional role of these tails could be to serve as means to deliver their cargo (clathrin coated vesicles) to a target membrane thereby comprising a novel cellular transport system that has so far been overlooked.

Wolf Almers and collegues found that macropinocytosis in cultured mast cells results in the formation of an endocytic vesicle that is propelled towards the cytoplasma by actin-based motility (Merrifield *et al.*, 1999). Although it is not clear with what membrane compartment these actin-propelled vesicles are fusing, this highly interesting observation indicates that actin-based motility may play a role in membrane trafficking.

Evidence that actin is playing a crucial role in the transport of cellular vesicles comes from recent data describing a number of actin binding proteins as well as F-actin on the Golgi complex and Golgi derived vesicles (Heimann *et al.*, 1999). Further, a role for actin in endocytosis and exocytosis has been clearly demonstrated (Lamaze *et al*., 1997; Muallem *et al*., 1995; Vitale *et al*., 1991; Vitale *et al*., 1995). The question now is to what extent actin-based motility can play a role in vesicular trafficking.

3.6 Summary and outlook

The data presented and discussed show the elegant way used by vaccinia virus to directly facilitate its spread by hijacking a signalling pathway normally used by cells for actin polymerization at the plasma membrane. After vaccinia infects a cell it activates at least one kinase which causes the phosphorylation of several host and viral proteins. I succeeded in identifying two proteins that become phosphorylated on tyrosine during vaccinia infections, cortactin and A36R. While phosphorylation of cortactin could be involved in vaccinia induced cell migration (Sanderson *et al*., 1998b) a clear demonstration of its role during vaccinia infection remains to be established. Phosphorylation of tyrosine 112 and to a lesser degree of tyrosine 132 of A36R plays an essential role in the actin-based motility of vaccinia virus. Phosphorylation of these tyrosine residues results in the recruitment of the adaptor proteins Nck and Grb2 as well as N-WASP, which has been shown to stimulate the actin nucleation activity of the Arp2/3 complex. The high homology between vaccinia A36R and variola A39R suggests that smallpox used the same strategy as vaccinia when causing havoc throughout human history. It would, of course, be interesting to confirm this hypothesis. Of further interest is to dissect the roles played by the IEV proteins A33R and A34R in IEV and actin tail formation. The most interesting results, however, will come from the identification of the viral proteins that mediate the activation of the kinase(s) responsible for phosphorylation of A36R. This will allow the dissection of virus induced signal transduction pathways that lead to actin-based motility and possibly help to dissect the mechanisms of vaccinia induced cell motility.