Folia Microbiol 43 (3), 305–310 (1998)

Interaction of Vaccinia Virus with the Actin Cytoskeleton*

M. WAY

Cell Biology Programme, European Molecular Biology Laboratory, 69117 Heidelberg, Germany
E-mail Way@EMBL-Heidelberg.de

Received December 10, 1997

ABSTRACT. Vaccinia virus infection results in large rearrangements of the host actin cytoskeleton including the formation of actin tails that are strikingly similar to those seen in *Listeria, Shigella* and *Rickettsia* infections. Using actin polymerization as the driving force the intracellular enveloped form of the vaccinia virus (IEV) is propelled on the tip of actin tails at a speed of 2.8 μm/min, both intra- and intercellularly The similarities between the actin-based motility of the vaccinia virus, *Listeria, Shigella* and *Rickettsia* suggest that intracellular pathogens have developed a common strategy to exploit the actin cytoskeleton of the host to facilitate their intercellular spread This review focuses on our current understanding of the interactions between the vaccinia virus and the actin cytoskeleton.

CONTENTS

1 Introduction 305
2 Vaccinia virus morphogenesis 306
3 Vaccinia–actin interactions 306
4 An iActA homologue in vaccinia virus? 307
5 Vaccinia-encoded actin-binding proteins 308
5 1 The vaccinia F17R protein is an actin-bundling protein 308
5 2 The vaccinia A42R gene encodes a viral profilin 308
5 3 Vaccinia encodes a family of kelch like proteins 309
6 Concluding remarks 309
References 309

1 INTRODUCTION

It has long been recognized that viral transformation results in disruption of the actin cytoskeleton and the loss of actin stress fibers (Pollack *et al.* 1975; Verderame *et al.* 1980). In addition a number of observations also suggest that viruses are able to interact with or modify the actin cytoskeleton of the host during their life cycle (reviewed by Cudmore *et al.* 1997). For example, the formation of actin bundles or microvilli-like projections at the plasma membrane is a common theme during the release of many enveloped viruses. Virus-tipped microvilli have been observed for frog virus 3 (Murti *et al.* 1985; Murti and Goorha 1983), measles (Bohn *et al.* 1986), murine mammary tumor virus and other C-type retroviruses (Damsky *et al.* 1977; Iwasaki and Tsuchida 1978; Mortara and Koch 1986, 1989). Herpes simplex has also been shown to induce actin microvilli but the presence of a viral particle at their tip has not been demonstrated (Krempien *et al.* 1984). The role of the actin cytoskeleton during the viral budding process has been addressed by the disruption of the cytoskeleton with cytochalasin D. Disruption of actin filaments inhibits release of frog virus 3 (Murti *et al.* 1985), measles (Stallcup *et al.* 1983) as well as partially inhibiting HIV 1 release (Sasaki *et al.* 1995). Not all viruses induce the formation of actin projections late in infection. Epstein–Barr virus (EBV) induces rapid actin polymerization within a few minutes of infection while disruption of the actin cytoskeleton with cytochalasin D inhibits EBV-induced proliferation (Melamed *et al.* 1994; Mosialos *et al.* 1994). Cytochalasin D also inhibits the production of infectious baculovirus. Baculovirus infection also induces a rapid and dramatic effect on the actin cytoskeleton which results in the formation of virus-tipped actin cables in the cytoplasm immediately after viral entry (Charlton and Volkman 1991, 1993; Hess *et al.* 1989; Volkman 1988). Of all the viruses that interact with the host actin cytoskeleton the vaccinia virus is the most extensively studied and best characterized (Cudmore *et al.* 1995, 1996; Hiller *et al.* 1979, 1981; Hiller and Weber 1982; Krempien *et al.* 1981; Meyer *et al.* 1981; Payne and Kristensson 1982; Reckmann *et al.* 1997; Stokes 1976).

*Presented at the *1st International Minisymposium on Cellular Microbiology: Cell Biology and Signalization in Host–Pathogen Interactions*, Prague, October 6, 1997.

2 VACCINIA VIRUS MORPHOGENESIS

Vaccinia virus, the prototype member of the *Orthopoxviridae* and a close relative of smallpox virus, is one of the largest and most complex viruses known with a genome of 191 kbp encoding over 260 potential proteins (Johnson *et al.* 1993). The mechanism by which vaccinia virus enters the host is currently unknown, although recent data suggest that it is an actin dependent process (Moreau and Way, *unpublished results*). Once in the cell, the virus rapidly begins its replication cycle in specialized cytoplasmic viral factories in the host (Moss 1990). In contrast to most other viruses, vaccinia virus is able to replicate in the cytoplasm as it encodes its own transcriptional machinery. Assembly anu maturation of vaccinia virus is a complex process which is still largely not understood. An intriguing characteristic of the vaccinia virus assembly process is that it results in two different infectious forms. The first of these, the intracellular mature virus (IMV), is surrounded by a membrane cisterna derived from the intermediate compartment (Sodeik *et al.* 1993). IMV begin to appear about 4 h post infection a^n^d are released when the cell lyses due to the cytotoxic effects of infection. Additionally, a proportion of IMV, which varies according to virus strain and cell type, can undergo a second wrapping step by a cisternal domain derived from the *trans* Golgi network to form the intracellular enveloped virus (IEV; Payne 1980; Payne and Kristensson 1985; Schmelz *et al.* 1994). IEV are first seen 5–6 h post infection and are released from the cell by fusion of the outer viral membrane with the plasma membrane of the host, thereby liberating the second infectious form of vaccinia called the extracellular enveloped virus (EEV; Blasco and Moss 1991; Dales 1971; Morgan 1976; Payne 1980). EEV are responsible for the long-range dissemination of the virus *in vivo* (Payne 1980). During the fusion of IEV with the plasma membrane, a small proportion of EEV particles are not released into the medium but remain associated with the cell surface. These particles are refereed to as cell-associated enveloped virus (CEV) and are responsible for the cell-to-cell spread of the virus (Blasco and Moss 1992).

3 VACCINIA–ACTIN INTERACTIONS

The first indication of interactions between vaccinia virus and the actin cytoskeleton came from high-voltage electron microscopy studies which revealed single viral particles on the tip of large microvilli-like projections in infected cells (Stokes 1976). Subsequent immunofluorescence observations demonstrated that the formation of these large microvilli-like projections, which appear late in infection, was dependent on viral assembly (Hiller *et al.* 1979; Krempien *et al.* 1981). The same studies also showed that these virus-tipped structures contained actin as well as the actin cross-linking proteins α-actinin, fimbrin and filamin but not tropomyosin or myosin (Hiller *et al.* 1979; Krempien *et al.* 1981). While addition of cytochalasin D did not inhibit virus assembly it did prevent the release of the intracellular enveloped form of vaccinia virus (Payne and Kristensson 1982). More recently the earlier morphological studies on the effects of vaccinia on the actin cytoskeleton have been ascribed a function by the discovery that vaccinia virus uses actin polymerization as the driving force to move intra- and intercellularly (Cudmore *et al.* 1995). Vaccinia virus achieves this by promoting the polymerization of actin filaments directly behind the virus particle, in a manner reminiscent of the pathogenic bacteria *Listeria, Shigella* and *Rickettsia* (reviewed by Cossart 1995; Theriot 1995; Tilney and Tilney 1993; Fig. 1). In this way, the polymerization of actin is sufficient to propel the virus at a rate of 2.8 μm/min through the cytoplasm of the host cell and on the tips of extending microvilli-like projections that form when the virus particle reaches the cell surface (Cudmore *et al.* 1995, 1996).

Examination of vaccinia infected cells reveals that only a proportion of viral particles are able to induce the formation of actin tails, suggesting that this property is not universal for all forms of the virus (Fig. 1). Studies using a mutant virus which cannot form IEV, as well as infection with wild-type virus in the presence of a drug which prevents IEV formation, confirmed this suggestion and demonstrated that only the IEV is capable of inducing actin tails (Cudmore *et al.* 1995). Examination of the organization of actin filaments in the vaccinia tail in electron micrographs reveals that they show unidirectional polarity with their preferred end for assembly pointing toward the surface of the virus particle (Cudmore *et al.* 1996). This orientation is consistent with the motility of the virus being driven purely by actin polymerization and not by myosin motors. Rhodamine-actin incorporation experiments show that the first stage of actin tail assembly involves a polarized recruitment of G-actin, rather then preformed actin filaments, to the membrane surrounding the IEV (Cudmore *et al.* 1996). Furthermore, incorporation of actin into the tail occurs only from the viral surface, suggesting that filament ends in the tail are blocked against further actin addition and that actin tail formation is nucleated by one or more proteins on the IEV. In contrast to bacterial systems the protein(s) required for the nucleation of vaccinia actin tails, as well as the host proteins involved in the process, have not yet been identified. The vaccinia system is fundamentally different from bacterial systems

as the actin tail nucleating IEV surface is derived from the *trans*-Golgi of the host cell (Payne 1980; Payne and Kristensson 1985; Schmelz *et al.* 1994). Consequently the nature of the nucleation complex may reflect a situation closer to the events at the leading edge of cells than is found in bacterial systems. This hypothesis is supported by the observation that as virus particles fuse with the plasma membrane during the extension of projections, actin nucleation sites previously associated with the viral membrane now become localized to the plasma membrane where they are still able to nucleate actin polymerization (Cudmore *et al.* 1996). Thus vaccinia virus provides a model system to dissect actin-membrane interactions both within and at the leading edge of cells.

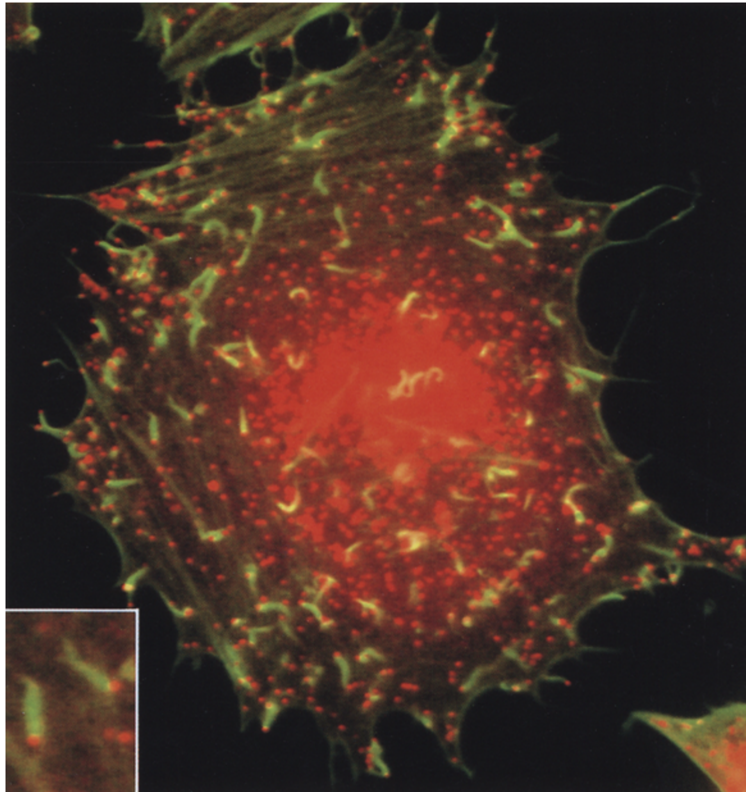

Fig. 1. Overall appearance of the actin cytoskeleton in a HeLa cell (8 h after infection with vaccinia virus, figure width corresponds to 150 µm) Virus particles are labeled with an antibody against the viral core protein P14 (*red*) while actin filaments are labeled with Bodipy phallacidin (*green*). Virus infection has resulted in the loss of actin stress fibers and the induction of actin tails directly from viral particles (*insert*)

4 AN iActA HOMOLOGUE IN VACCINIA VIRUS?

The bacterial surface protein iActA of *Listeria ivanovii* is responsible for recruiting host factors required to induce actin tails by this pathogenic bacterium (Gerstel *et al.* 1996; Gouin *et al.* 1995). The sequence of iActA contains seven copies of a proline-rich repeat of 47 amino acids that plays a critical role in initiating actin tail assembly (Gerstel *et al.* 1996; Gouin *et al.* 1995). Analysis of the vaccinia genome shows that the 65-amino-acid sequence of F8L vaccinia protein has a 38 % identity and a 53 % similarity with the proline-rich repeat of iActA (Gouin *et al.* 1995). Given this level of homology and the fact that proline-rich sequences have been shown to play an important role in actin tail assembly in both *Listeria* and *Shigella* (Higley and Way 1997*a*), we have characterized the product of the F8L gene to examine its possible role in vaccinia-induced rearrangements of the host actin cytoskeleton (Higley and Way 1997*b*). F8L is a 8-kDa protein that is expressed early during vaccinia infection and is found throughout the cytoplasm, with no association with viral particles, actin tails or any other discernible cellular structure. While the lack of F8L association with IEV or actin tails suggested that it plays no role in vaccinia actin tail formation it does not rule out the possibility that the protein is involved in actin stress-fiber disassembly prior to tail formation. To

address this question we deleted the F8L gene from the vaccinia genome (Higley and Way 1997*b*). The F8L deletion strain, WRΔFBL, is still able to form viral particles as well as actin tails that are indistinguishable from those seen in control vaccinia infections. Furthermore, the kinetics of actin stress-fiber disassembly early during infection are identical for WRΔFBL and controls. While the function of F8L during vaccinia infection remains to be established it is clear that it is not necessary for viral morphogenesis or actin rearrangements which occur during infection.

5 VACCINIA-ENCODED ACTIN-BINDING PROTEINS

5.1 The vaccinia F17R protein is an actin-bundling protein

During the initial characterization of the interaction of vaccinia virus with the actin cytoskeleton, a basic phosphorylated 11-kDa viral protein was identified that associated with viral induced microvilli and with the actin cytoskeleton when virus assembly was inhibited (Hiller and Weber 1982). Through a combination of studies involving immunoprecipitation, mapping and sequencing, p11 was finally identified as the product of the vaccinia F17R gene (Bertholet *et al.* 1985; Goebel *et al.* 1990; Johnson *et al.* 1993; Wittek *et al.* 1984). Given that F17R associates with the actin cytoskeleton we have recently examined its possible role in vaccinia actin-based motility (Reckmann *et al.* 1997). Not only is F17R associated with vaccinia actin tails in infected cells but it is also able to associate with the actin cytoskeleton in the absence of additional viral factors when transfected into non infected cells. Although F17R is able to associate with the actin cytoskeleton it plays no role in vaccinia actin tail formation as the recombinant vaccinia strain vRO11k is still able to induce actin tails that are indistinguishable from controls in the absence of F17R expression. Given that earlier work suggested that the primary function of F17R is in viral particle morphogenesis, the exact nature of which remains to be established (Zhang and Moss 1991*a*,*b*), why does this protein associate with the actin cytoskeleton?

Recently, it has been shown that basic polypeptides are able to induce the formation of actin bundles *in vitro* by virtue of their polyelectrolyte nature in an analogous manner to DNA condensation (Tang and Janmey 1996). We believe that the association of F17R with the actin cytoskeleton can be explained by a similar mechanism (Reckmann *et al.* 1997). Transfection of vaccinia F17R deletion constructs identified an extremely basic region of 50 amino acids in the N-terminal half of the molecule that is essential for its association with the actin cytoskeleton. This basic region is also conserved in the divergent myxoma virus F17R which is also able to associate with the actin cytoskeleton. Finally, *in vitro* a peptide corresponding to this basic region induces actin bundles at a peptide-to-actin concentration ratio comparable to the values for other positively charged peptides and not to functional actin-bundling proteins, such as of villin and fimbrin (Matsudaira 1991; Tang and Janmey 1996). The fact that F17R-induced actin bundles are not ordered reflects a lack of specific contact sites and filament packing constraints that are normally seen with functional actin-bundling proteins. F17R is not the first protein to have actin binding as a property in addition to its primary role. Elongation factor EF1-α, which is also basic with a p*I* of ≈9.0, bundles actin and associates with the actin cytoskeleton although it is critically required for translation (Liu *et al.* 1996). Our observations with F17R highlight the difficulty in distinguishing functional actin binding proteins from those that associate purely by virtue of their basic nature and may explain why so many actin-binding proteins have been identified.

5.2 The vaccinia A42R gene encodes a viral profilin

Another potential vaccinia actin-binding protein is a viral profilin homologue that displays ≈32 % identity to the actin-monomer-binding protein, profilin, in mammalian cells (Blasco *et al.* 1991). Interestingly, host profilin is recruited to the site of actin tail nucleation in *Listeria* although its role in the actin-based motility of this bacterium is controversial (Marchand *et al.* 1995; Smith *et al.* 1996; Theriot *et al.* 1994). Vaccinia profilin is expressed late during infection and its deletion does not affect the formation of virally induced microvilli, actin tails or the number of infectious viral particles (Blasco *et al.* 1991; Cudmore *et al.* 1995). This is not surprising given that vaccinia profilin has a considerably lower affinity for actin than mammalian profilin (Machesky *et al.* 1994), and endogenous profilins may be able to play a functionally equivalent role. A better understanding may be achieved when a profilin-deficient host is infected with the recombinant vaccinia virus strain lacking the A42R gene.

5.3 *Vaccinia encodes a family of kelch-like proteins*

In addition to profilin, the vaccinia genome encodes four other proteins, the products of the A55R, F3L, C2L and B10R gene, that show significant sequence homologies to the actin-bundling protein, scruin, which is found in the acrosomal process of the horseshoe crab *Limulus* (Way *et al.* 1995). All sequence homologies between the proteins are restricted to the ca. 50 amino acid residue motifs that are termed kelch repeats after their identification in the *Drosophila* protein kelch (Xue and Cooley 1993). Kelch is a structural component of actin rich ring canals that provide the intercellular conduits connecting nurse cells to the oocyte in *Drosophila* egg chambers. Although it has been suggested that kelch is an actin binding protein, the six tandem kelch repeats alone are able to localize to ring canals *in vivo*, it has not been demonstrated that the protein can bind directly to actin filaments *in vitro* (Robinson and Cooley 1997). While it is not clear whether kelch binds actin directly, recent data has localized one of the actin binding sites of scruin to the six kelch repeats in the C-terminal half of the molecule (Sun *et al.* 1997). Whether the vaccinia proteins A55R, F3L, C2L and B10R are able to interact with actin and play a possible role in the actin-based motility of vaccinia virus remains to be established.

6 CONCLUDING REMARKS

While the effects of vaccinia virus on the actin cytoskeleton were originally described more than 20 years ago, we have only recently begun to understand their purpose in viral infection. The task ahead is now to identify the viral and host components responsible for initiating the cascade of events that lead to vaccinia actin tail formation and motility.

The author would like to thank F Frischknecht, V. Moreau and K. Williams for comments and suggestions concerning the manuscript

REFERENCES

BERTHOLET C., DRILLIEN R., WITTEK R.: One hundred base airs of 5 flank in sequence of a vaccinia virus late gene are sufficient to temporally regulate late transcription. *Proc Nat.Acad.Sci USA* **82**, 2096–2100 (1985).

BLASCO R , COLE N B , MOSS B : Sequence analysis, expression, and deletion of a vaccinia virus gene encoding a homolog of profilin, a eukaryotic actin-binding protein *J.Virol* **65**, 4598–4608 (1991)

BLASCO R , MOSS B. Extracellular vaccinia virus formation and cell-to-cell virus transmission are prevented by deletion of the gene encoding the 37,000-dalton outer envelope protein *J.Virol.* **65**, 5910–5920 (1991).

BLASCO R., MOSS B Role of cell-associated enveloped vaccinia virus in cell-to-cell spread *J.Virol.* **66**, 4170–4179 (1992).

BOHN W , RUTTER G , HOHENBERG H., MANNWEILER K., NOBIS P · Involvement of actin filaments in budding of measles virus studies on cytoskeletons in uninfected cells. *Virology* **149**, 91–106 (1986)

CHARLTON C A., VOLKMAN L.E.. Sequential rearrangement and nuclear polymerization of actin in baculovirus-infected *Spodoptera frugiperda* cells. *J.Virol.* **65**, 1219–1227 (1991).

CHARLTON C A., VOLKMAN L E Penetration of *Autographa californica* nuclear polyhedrosis virus nucleocapsids into IPLB Sf 21 cells induces actin cable formation *Virology* **197**, 245–54 (1993)

COSSART P Actin-based bacterial motility *Curr.Opin.Cell Biol.* **7**, 94–101 (1995).

CUDMORE S , COSSART P., GRIFFITHS G , WAY M Actin-based motility of vaccinia virus *Nature* **378**, 636–638 (1995).

CUDMORE S., RECKMANN I., GRIFFITHS G , WAY M · Vaccinia virus a model system for actin-membrane interactions *J.Cell Sci.* **109**, 1739–1747 (1996)

CUDMORE S , RECKMANN I , WAY M Viral manipulations of the actin cytoskeleton *Trends Microbiol.* **5**, 142–148 (1997)

DALES S.. Involvement of membranes in the infectious cycle of vaccinia, pp. 136–144 in G W Richter, D.G. Scarpelli (Eds): *Cell Membranes: Biological and Pathological Aspects* Williams & Wilkins, Baltimore 1971.

DAMSKY C H., SHEFFIELD B.J , TUSZYNSKI G.P., WARREN L · Is there a role for cellular actin in virus budding? *J.Cell Biol.* **75**, 593–605 (1977)

GERSTEL B., GROBE L., PISTOR S., CHAKRABORTY T., WEHLAND J.. The ActA polypeptides of *Listeria ivanovii* and *Listeria monocytogenes* harbor related binding sites for host microfilament proteins *Infect.Immun.* **64**, 1929–1936 (1996).

GOEBEL S J., JOHNSON G.P., PERKUS M E , DAVIS S W., WINSLOW J P , PAOLETTI E.: The complete DNA sequence of vaccinia virus *Virology* **179**, 247–266 (1990)

GOUIN E., DEHOUX P , MENGAUD J., KOCKS C., COSSART P.. *iactA* of *Listeria ivanovii*, although distantly related to *Listeria monocytogenes actA*, restores actin tail formation in an *L. monocytogenes actA* mutant. *Infect.Immun.* **63**, 2729–2737 (1995).

HESS R.T., GOLDSMITH P A , VOLKMAN L.E.: Effect of cytochalasin D on cell morphology and AcMNPV replication in a *Spodoptera frugiperda* cell line *J.Invertebr.Pathol* **53**, 169–182 (1989).

HIGLEY S., WAY M.· Actin and cell pathogenesis. *Curr.Opin.Cell Biol.* **9**, 62–69 (1997*a*).

HIGLEY S., WAY M.: Characterization of the vaccinia virus F8L protein *J.Gen.Virol.*, in press (1997*b*)

HILLER G., JUNGWIRTH C., WEBER K . Fluorescence microscopical analysis of the life cycle of vaccinia virus in the chick embryo fibroblasts. *Exp.Cell.Res.* **132**, 81–87 (1981).

HILLER G., WEBER K A phosphorylated vaccinia virion polypeptide of molecular weight of 11,000 is exposed on the surface of mature particles and interacts with actin-containing cytoskeletal elements *J.Virol.* **44**, 647–657 (1982).

HILLER G., WEBER K., SCHNEIDER L , PARAJSZ C., JUNGWIRTH C : Interaction of assembled progeny pox viruses with the cellular cytoskeleton *Virology* **98**, 142–153 (1979)

IWASAKI Y., TSUCHIDA N : Transmission electron microscopy surveillance of retroviruses in tissue culture cells prepared by the critical-point drying method *J.Nat.Cancer Inst.* **61**, 431–436 (1978).

JOHNSON G P , GOEBEL S.J., PAOLETTI E.: An update on the vaccinia virus sequence. *Virology* **196**, 381–401 (1993).

KREMPIEN U., JOCKUSCH B.M., JUNGWIRTH C.: Herpes simplex virus induced cell surface protrusions. *Intervirology* **22**, 156–163 (1984).

KREMPIEN U., SCHNEIDER L., HILLER G , WEBER K., KATZ E., JUNGWIRTH C · Conditions for pox virus-specific microvilli formation studied during synchronized virus assembly. *Virology* **113**, 556–564 (1981)

LIU G B., EDMONDS T., CONDEELIS J : pH, EF-1α and the cytoskeleton. *Trends Cell Biol.* **6**, 168–171 (1996).

MACHESKY L M., COLE N.B., MOSS B., POLLARD T D.. Vaccinia virus expresses a novel profilin with a higher affinity for polyphosphoinositides than actin. *Biochemistry* **33**, 10815–10824 (1994).

MARCHAND J.B., MOREAU P , PAOLETTI E., COSSART P , CARLIER M.F., PANTALONI D.· Actin-based movement of *Listeria monocytogenes*. actin assembly results from the local maintenance of uncapped filament barbed ends at the bacterium surface. *J Cell Biol.* **130**, 331–343 (1995).

MATSUDAIRA P.: Modular organization of actin crosslinking proteins *Trends Biochem.Sci.* **16**, 87–92 (1991)

MELAMED I., STEIN L., ROIFMAN C M.: Epstein–Barr virus induces actin polymerization in human B cells. *J.Immunol.* **153**, 1998–2003 (1994).

MEYER R.K., BURGER M.M , TSCHANNEN R , SCHAFER R · Actin filament bundles in vaccinia virus infected fibroblasts. *Arch.Virol.* **67**, 11–18 (1981)

MORGAN C.. Vaccinia virus reexamined: Development and release. *Virology* **73**, 43–58 (1976).

MORTARA R.A., KOCH G.L . Analysis of pseudopodial structure and assembly with viral projections *J.Cell Sci.Suppl.* **5**, 129–144 (1986).

MORTARA R.A., KOCH G.L.E.:. An association between actin and nucleocapsid polypeptides in isolated murine retroviral particles. *J.Submicrosc.Cytol.Pathol.* **21**, 295–306 (1989)

MOSIALOS G , YAMASHIRO S., BAUGHMAN R.W., MATSUDAIRA P., VARA L , MATSUMURA F., KIEFF E., BIRKENBACH M.: Epstein–Barr virus infection induces expression in B lymphocytes of a novel gene encoding an evolutionarily conserved 55 kilodalton actin-bundling protein *J.Virol* **68**, 7320–7328 (1994)

MOSS B · Poxviridae and their replication, pp. 2079–2111 in B.N Fields, D.M Knipe (Eds) *Virology* Raven Press, New York 1990

MURTI K G , CHEN M , GOORHA R : Interaction of frog virus 3 with the cytomatrix *Virology* **142**, 317–325 (1985)

MURTI K G., GOORHA R : Interaction of frog virus-3 with the cytoskeleton. I. Altered organization of microtubules, intermediate filaments, and microfilaments. *J.Cell Biol.* **96**, 1248–1257 (1983).

PAYNE L.G.. Significance of extracellular enveloped virus in the *in vitro* and *in vivo* dissemination of vaccinia. *J.Gen.Virol.* **50**, 89–100 (1980)

PAYNE L.G , KRISTENSSON K.: The effect of cytochalasin D and monensin on enveloped vaccinia virus release. *Arch.Virol.* **74**, 11–20 (1982).

PAYNE L G , KRISTENSSON K Extracellular release of enveloped vaccinia virus from mouse nasal epithelial cells *in vivo*. *J Gen Virol.* **66**, 643–646 (1985)

POLLACK R., OSBORN M , WEBER K.. Patterns of organization of actin and myosin in normal and transformed cultured cells. *Proc.Nat.Acad.Sci.USA* **72**, 994–998 (1975)

RECKMANN I , HIGLEY S., WAY M.: The vaccinia virus F17R protein interacts with actin. *FEBS Lett* **409**, 141–146 (1997).

ROBINSON D , COOLEY L.: *Drosophila* kelch is and oligomeric ring canal actin organizer *J.Cell Biol.* **138**, 799–810 (1997)

SASAKI H , NAKAMURA M., OHNO T., MATSUDA Y., YUDA Y., NONOMURA Y.. Myosin-actin interaction plays an important role in human immunodeficiency virus type 1 release from host cells *Proc.Nat.Acad.Sci.USA* **92**, 2026–2030 (1995).

SCHMELZ M., SODEIK B , ERICSSON M., WOLFFE E.J , SHIDA H., HILLER G., GRIFFITHS G : Assembly of vaccinia virus: The second wrapping cisterna is derived from the *trans* Golgi network *J.Virol.* **68**, 130–147 (1994)

SMITH G.A., THERIOT J.A , PORTNOY D.A.· The tandem repeat domain in the *Listeria monocytogenes* ActA protein controls the rate of actin-based motility, the percentage of moving bacteria, and the localization of vasodilator-stimulated phosphoprotein and profilin. *J.Cell Biol* **135**, 647–660 (1996)

SODEIK B., DOMS R W , ERICSSON M., HILLER G , MACHAMER C.E., HOF W V.T , MEER G V., MOSS B., GRIFFITHS G.: Assembly of vaccinia virus: Role of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks. *J.Cell Biol* **121**, 521–541 (1993).

STALLCUP K C., RAINE C.S., FIELDS B.N.· Cytochalasin B inhibits the maturation of measles virus. *Virology* **124**, 59–74 (1983).

STOKES G.V.: High-voltage electron microscope study of the release of vaccinia virus from whole cells. *J.Virol.* **18**, 636–643 (1976)

SUN S , FOOTER M , MATSUDAIRA P.. Modification of Cys-837 identifies an actin-binding site in the b-propeller protein scruin *Mol.Biol.Cell.* **8**, 421–430 (1997).

TANG J X , JANMEY P.A · The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J.Biol.Chem.* **271**, 8556–8563 (1996).

THERIOT J.A., ROSENBLATT J., PORTNOY D.A., GOLDSCHMIDT-CLERMONT P.J , MITCHISON T J : Involvement of profilin in the actin-based motility of *L. monocytogenes* in cells and in cell-free extracts *Cell* **76**, 505–517 (1994).

THERIOT J.A.: The cell biology of infection by intracellular bacterial pathogens. *Ann.Rev.Cell Dev.Biol.* **11**, 213–239 (1995).

TILNEY L.G., TILNEY M.S.. The wily ways of a parasite· induction of actin assembly by *Listeria*. *Trends Microbiol* **1**, 25–31 (1993).

VERDERAME M., ALCORTA D , EGNOR M., SMITH K., POLLACK R.: Cytoskeletal F-actin patterns quantitated with fluorescein isothiocyanate-phalloidin in normal and transformed cells. *Proc.Nat.Acad.Sci.USA* **77**, 6624–6648 (1980).

VOLKMAN L.E *Autographa californica* MNPV nucleocapsid assembly: inhibition by cytochalasin D. *Virology* **163**, 547–553 (1988).

WAY M., SANDERS M., GARCIA C., SAKAI J., MATSUDAIRA P.: Sequence and domain organization of scruin, an actin-cross-linking protein in the acrosomal process of *Limulus* sperm. *J.Cell.Biol.* **128**, 51–60 (1995)

WITTEK R , HANGGI M., HILLER G.: Mapping of a gene coding for a major late structural polypeptide on the vaccinia virus genome *J.Virol.* **49**, 371–378 (1984).

XUE F., COOLEY L.: Kelch encodes a component of intercellular bridges in *Drosophila* egg cambers *Cell* **72**, 681–693 (1993).

ZHANG Y.F., MOSS B. Inducer-dependent conditional-lethal mutant animal viruses *Proc.Nat.Acad.Sci.USA* **88**, 1511–1515 (1991).

ZHANG Y.F., MOSS B. Vaccinia virus morphogenesis is interrupted when expression of the gene encoding an 11-kilodalton phosphorylated protein is prevented by the *Escherichia coli lac* repressor. *J.Virol.* **65**, 6101–6110 (1991).