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expert reviews in molecular medicine

Cell motility and cell morphology: How some viruses take control

Christopher M. Sanderson and Geoffrey L. Smith

Viruses replicate inside host cells, where they use host biochemical and structural components to facilitate the production of new virus particles. As a consequence of co-evolution with their hosts, viruses have acquired host genes and genetic mutations that confer dominance over normal cell function. Research on virus–cell interactions has focused on the identification of mechanisms of virus dominance in order to develop therapeutic strategies for preventing productive infection. Although such research remains an essential part of molecular virology, viruses are also important genetic tools that can be used to analyse cell function. Because virus genomes contain genetic information, some of which was derived from host cells, it is possible that the analyses of virus–host interactions might lead to the identification of functionally dominant virus genes and novel eukaryotic counterparts. In this article, we have described how transforming and non-transforming viruses can control cell motility (cell migration or membrane projection), and explained how the analysis of virus cytopathic effects (CPEs) led to the identification of a novel family of cellular genes that regulate diverse aspects of cell motility.

Cell motility is an essential part of many important biological processes. The co-ordinated migration of cells during embryogenesis facilitates correct morphological development and neuronal organisation. In addition, pathological processes, such as wound healing, angiogenesis, leukocyte invasion and tumour metastasis, all involve cell motility. In order to intervene rationally in any of these processes, it is necessary to have a comprehensive understanding of the molecular interactions that mediate cell motility and also of the highly complex pathways of signal transduction that regulate them. Despite significant progress, we are still some way off this goal. Many attempts to dissect the molecular mechanisms of cell movement are hampered by the inherent difficulty of genetically manipulating mammalian cells, and the technical problems of generating uniform populations of modified cells that can be used for biochemical analysis.

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An alternative approach is to use viruses as molecular tools to study the process of cell motility. Because some viruses have evolved mechanisms to control the motile state of the cell, it is possible to use functionally dominant virus genes to dissect biochemically the process of cell movement. Also, the identification of new virus genes that control aspects of cell movement might facilitate the identification of novel host counterparts. An understanding of the mechanisms by which viruses subvert host function is equally important to virologists because the functional characterisation of dominant virus genes might permit the rational design of attenuated viruses for use as therapeutic vectors in vaccines or gene therapy.

In this review, we have described how the analyses of retrovirus infection led directly to the identification of a novel family of eukaryotic genes that regulate a range of cellular functions including cell adhesion, motility and morphology. In addition, we have discussed recent data that show that non-transforming poxviruses, such as vaccinia virus, which was used for vaccination against smallpox, also encode genes that control cell migration and the formation of long neurite-like projections.

To understand fully the cytopathic changes that occur during virus infection, it is necessary first to define the basic components of cell adhesion and explain the sequential phases of cell movement. However, it was not our aim to provide a comprehensive guide to the molecular mechanisms of cell movement; this topic has been covered elsewhere and readers are referred within the text to excellent specialised reviews.

Cell adhesion

When adherent cells are cultured in vitro, they maintain a characteristic shape by adhesion both to neighbouring cells and to the extracellular matrix (ECM; Refs 1, 2, 3). In vivo, the ECM comprises a mixed population of proteins, including collagens, fibronectin, laminins and proteoglycans, which assemble into a complex 'fibrila' network that is found in close association with the plasma membrane. Cells bind to proteins of the ECM via transmembrane-surface receptors, which are a heterologous group of macromolecules that includes members of the integrin, cadherin, immunoglobulin, selectin and proteoglycan superfamilies. Of these, the cadherins, immunoglobulin-like receptors and selectins are involved predominantly in cell–cell adhesion (Ref. 1), whereas the integrins (with some exceptions) and proteoglycans are involved mainly in cell–ECM binding (Ref. 3). A common feature of cell-adhesion molecules is the ability to function as a molecular bridge between an external ligand and the cytoskeleton within the cell (Ref. 4). Over the past few years, it has become clear that receptors that mediate cell adhesion do not simply 'hold cells in place'; rather, the occupancy of cell-surface receptors results in the initiation of signal-transduction pathways that regulate many aspects of cell function (Refs 3, 4, 5, 6). These include transcription, proliferation, cytoskeletal organisation and receptor activation (Fig. 1; Ref. 7). One of the current goals of cell biology is to define the relationship between the occupancy of specific cell-surface receptors and cell function. This has been discussed later in this article, and comparisons drawn between the observed changes in cell–ECM adhesion that occur as a result of virus CPE and neuronal development.

Cell motility

In its broadest sense, cell motility can be defined as the movement of the whole cell (migration) or any part of the cell periphery (projection). Although the end results of these two processes are quite different, they share common features including polarisation (establishing the point and direction of movement), cytoskeletal re-organisation and the formation of new points of cell–ECM adhesion (Fig. 1; Ref. 8).

Sequential steps of cell migration

The process of migration begins with polarisation, which involves the creation of a clearly defined 'front–back' asymmetry in which actin and cell-surface receptors (including chemoattractant receptors, growth-factor receptors and adhesion receptors) are accumulated at the front of the cell (Refs 9, 10). Once polarity has been established, the second phase of migration is the protrusion of the plasma membrane from the front of the cell. This can occur as a result of either the formation of fine, tubular structures called filapodia or the extension of a broad, flat membrane sheet called a lamellipodium. Currently, it is believed that the force that is required to drive membrane extension is provided by the unidirectional polymerisation of actin (Refs 11, 12, 13). As yet, the precise molecular mechanism by which the polymerisation

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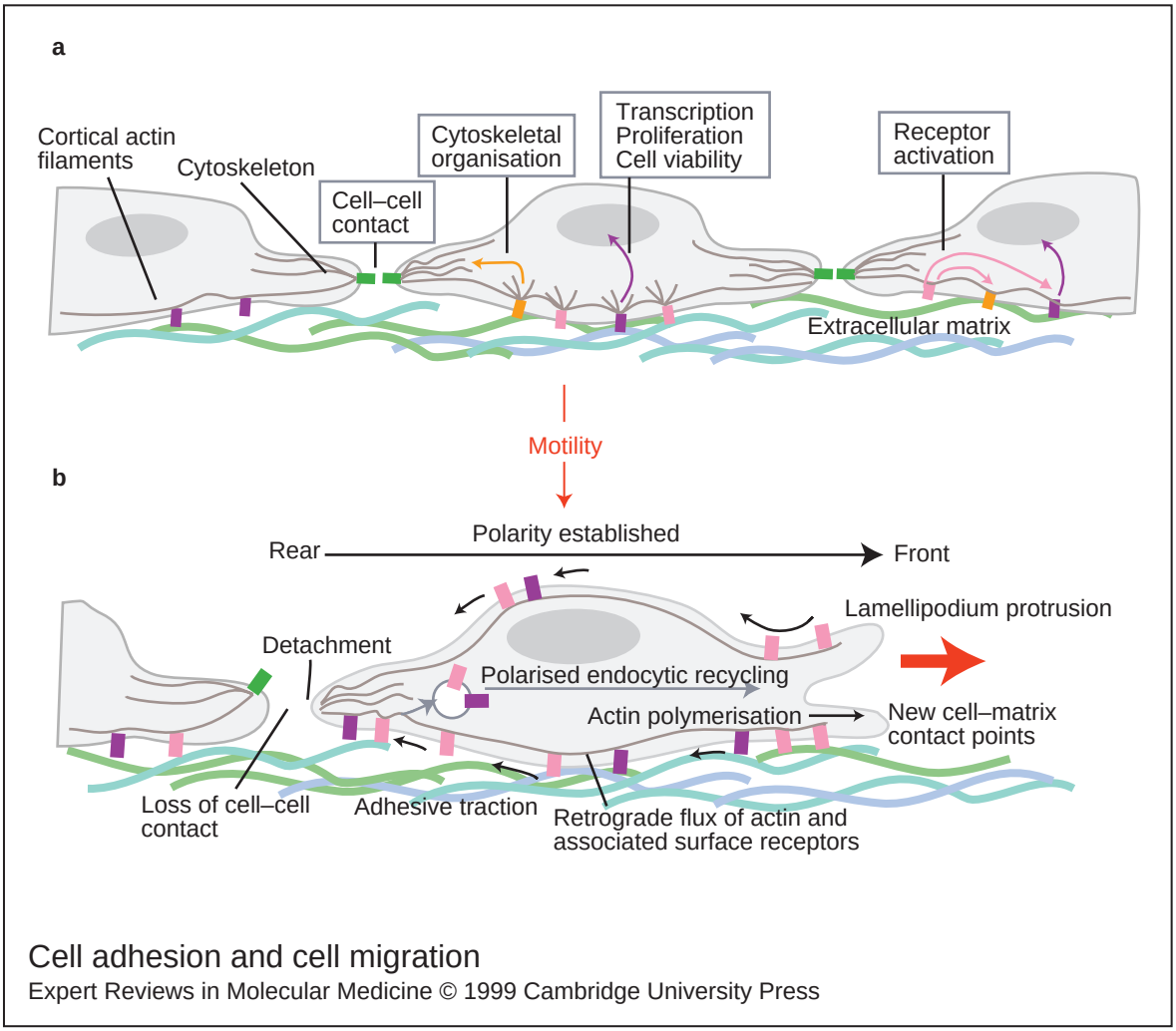

Figure 1. Cell adhesion and cell migration. (a) The shape and behaviour of cells is determined by a complex series of dynamic interactions that are mediated by a heterogeneous population of transmembrane adhesion molecules. In addition to mediating cell–cell and cell–extracellular-matrix attachment, cell-adhesion molecules facilitate indirectly cytoskeletal–membrane interaction and signal-transduction processes, which control cell viability, cytoskeletal organisation, cell motility and receptor activation. (b) In many cases, cell motility is physically and biochemically restricted by cadherin-mediated cell–cell interactions. Disruption of these interactions enhances the motility of cells in vitro and contributes to the invasive nature of tumour cells in vivo. The sequential phases of cell movement in vitro include the induction of a well-defined front–rear polarity, which determines the direction of motility. Membrane extension at the front of the cell is mediated by the combined effects of actin polymerisation and actin movement mediated by myosin. Receptor proteins (such as integrins) that are inserted into the front of the cell bind to components of the extracellular matrix (ECM), thus preventing membrane retraction and providing adhesive traction for cell movement. The physical process of cell movement results from the retrograde flux of polymerised actin (and associated membrane receptors) and the cyclical flux of membrane from the rear to the front of the cell. The final stage of cell movement involves the disruption or severing of matrix attachments at the rear of the cell and retraction of the trailing edge of the cell **(fig001cso).**

of actin drives membrane movement is unresolved; however, some bacteria (Refs 14, 15, 16) and poxviruses (Refs 17, 18) that can induce actin polymerisation to assist intracellular microbial movement and dissemination are being used as molecular tools to dissect the process of actin-driven motility. After membrane extension has occurred, new points of contact are established between the cell and the ECM. This prevents retraction of the newly extended membrane and

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provides 'grip' for the tractional force that is required to assist subsequent stages of cell movement. The two final stages of cell migration involve the flux of intracellular organelles into the newly extended area of the cell, and the retraction, or breaking off, of the trailing edge (Fig. 1; Ref. 8). The net result of this process is the directional movement of the cell body. In the case of projection formation, the underlying mechanisms of membrane extension and traction remain essentially the same as those for migration; however, extension is limited to localised areas of the plasma membrane and extension occurs from a 'static' cell body. The regulatory mechanism(s) that permit migration and projection to occur sequentially during neuronal development remain unclear. However, because poxviruses also elicit a transition from cell migration to projection formation, it is possible that vaccinia viruses can be used as a genetic tool to analyse the molecular events that are involved in the transition from migration to projection formation.

Before discussing specific examples of virus-induced changes in cell morphology and motility, it is important to illustrate how information about virus-induced CPEs can provide novel information about cell function.

Learning from a virus

Because viruses have evolved mechanisms to control host function, it is possible to use a virus or a virus gene as a molecular tool to analyse the host. It is also possible that the identification of virus genes that exert control over cell motility will provide a genetic 'short cut' to the identification of host genes that control similar processes in the uninfected cell. Possibly the best example of this approach is the identification of the *src* family of genes, which control many aspects of cell function including cytoskeletal organisation, cell adhesion and motility.

Rous sarcoma virus (RSV) is a retrovirus that belongs to the genus avian Leukosis-Sarcoma (Ref. 19). Early studies of RSV infection of fibroblasts from chicken embryos showed that infected cells became transformed, with a loss of contact between neighbouring cells and induction of a round, refractile cell morphology (Ref. 20). The isolation of temperature-sensitive mutants that were transformation defective at the non-permissive temperature, and analysis of the composition of the genomes of transforming and non-transforming strains (Refs 21, 22) showed that a single virus gene (*v-src*) was responsible for both transformation and changes in cell morphology. The protein product of the *v-src* gene was identified later as a 60-kDa phosphorylated protein called $pp60^{v\text{-}src}$ (Refs 23, 24).

Finding cellular src (c-src)

The use of genetic probes that were designed to identify the *v-src* gene led to the observation that *v-src* was closely related to *c-src*, a highly conserved cellular gene that is expressed ubiquitously; however, the level of expression of $pp60^{c\text{-}src}$ varies and some *src* family members are tissue specific (Refs 10, 25, 26). Although surprising at the time, these data confirmed the protovirus hypothesis, proposed by Temin (Ref. 27), which suggested that genes from transforming retroviruses were acquired from the host. Comparative analysis of $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$ revealed a deletion in the carboxyl terminal of the *v-src* gene, which removed a tyrosine residue at position 527 (Tyr527), thus rendering $pp60^{v\text{-}src}$ constitutively active (Fig. 2; Ref. 28). This unregulated ability to phosphorylate host proteins resulted in transformation of the host cell and functional dominance over $pp60^{c\text{-}src}$.

Using v-src to define c-src function

Owing to the dominant nature of $pp60^{v\text{-}src}$ and the existence of temperature-sensitive clones, *v-src* has been used extensively to study the function of *c-src*. This strategy led to the first description of phosphotyrosine kinase activity (Refs 29, 30) and the identification of a family of novel host proteins [$pp60^{c\text{-}src}$, fyn, yes (Ref. 25), yrk, blk, fgr, hck, lck, lyn (Ref. 31), frk/rak (Refs 32, 33) and fyk/bsk (Refs 34, 35)], all of which have similar amino acid sequences to that of $pp60^{v\text{-}src}$ (Fig. 3). In addition, many substrates for $pp60^{c\text{-}src}$ were identified first, following expression of the constitutively active $pp60^{v\text{-}src}$ (reviewed in Ref. 36). These substrates included several proteins that are associated with the cytoskeleton and have a role in cell adhesion and cell motility (Ref. 36).

v-src, c-src and cell motility

The ability of *v-src* to control cell motility has been demonstrated in several experimental systems. First, expression of *v-src* in the rat-adrenal pheochromacytoma cell line PC12 induced cell migration (Ref. 37) and the formation of

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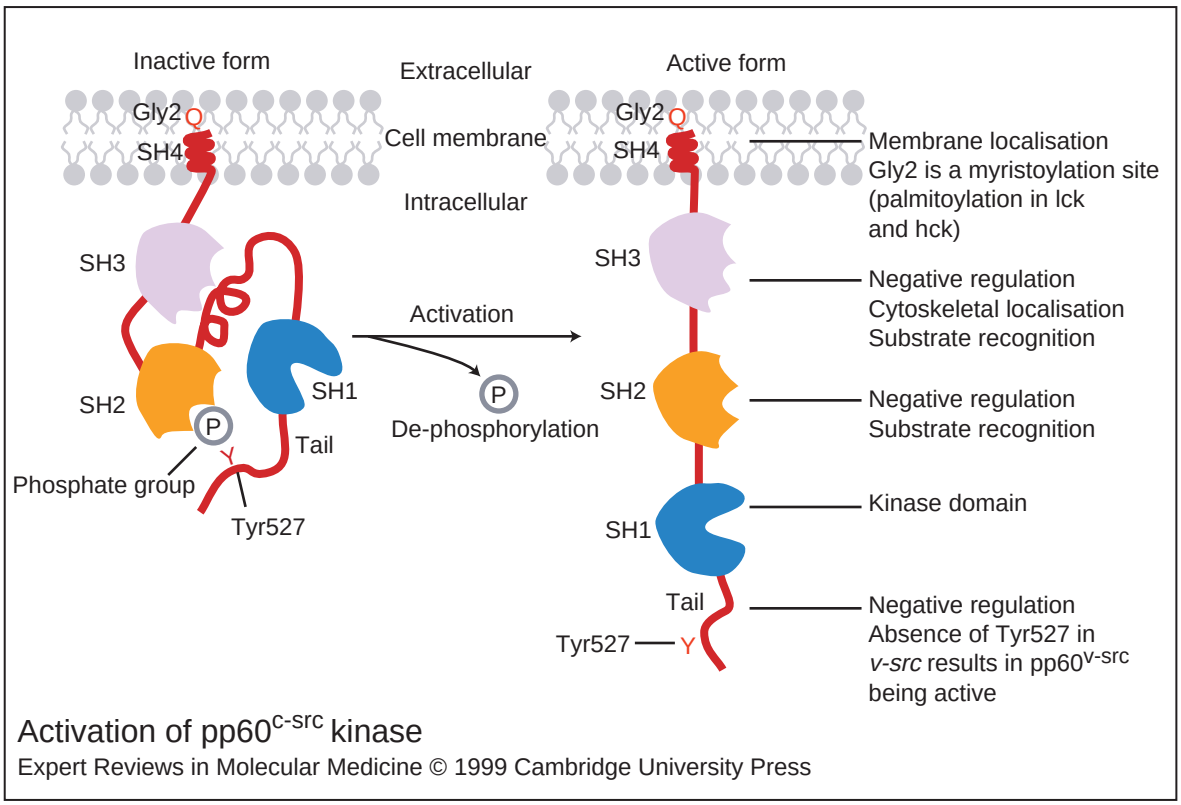

Figure 2. Activation of pp60$^{c\text{-}src}$ kinase. The inactive form of pp60$^{c\text{-}src}$ kinase is phosphorylated at Tyr527, promoting interaction between the carboxyl-terminal tail and the SH2 domain. De-phosphorylation of Tyr527 results in structural rearrangement and activation of kinase activity. Because Tyr527 is deleted in *v-src*, pp60$^{v\text{-}src}$ is constitutively active **(fig002cso).**

lamellipodia (Ref. 38). Second, expression of *v-src* in Madin–Darby canine-kidney cells (MDCK) resulted in the loss of differentiated epithelial status and gain of an invasive phenotype (Ref. 39). Third, expression of pp60$^{v\text{-}src}$ induced immature sympathetic neurones to initiate the outgrowth of neurites and to differentiate into neurone-like cells (Ref. 40). Subsequently, several groups have demonstrated the involvement of pp60$^{c\text{-}src}$ in similar processes. In particular, fibroblasts that had *c-src* knocked out (*c-src* -/-) exhibited an impaired motility, which was restored by the expression of an active form of the *c-src* gene (Ref. 41) but not by the expression of a kinase-deficient *src* or by a truncated *src* containing only functional SH2 and SH3 domains (Fig. 2; Ref. 41). Collectively, these data show how viruses can be used to identify novel host genes and define aspects of normal cell function.

The phenotypic similarities between transformed cells and embryonic cells are well known. However, it is surprising that non-transforming poxviruses can also elicit phenotypic changes that resemble the motile features of embryonic neuronal cells.

Clinical implications of poxviruses

The poxvirus family of DNA viruses is divided into two subfamilies, namely chordopoxviruses and entomopoxviruses, which productively infect vertebrates and insects, respectively. Historically, chordopoxviruses have played a seminal part in prophylactic medicine. In 1798, Edward Jenner used cowpox virus successfully as a live vaccine to confer protection against smallpox, a devastating human disease. During the 20th century, several strains of vaccinia virus were used in a worldwide vaccination campaign that led, in 1977, to the eradication of smallpox. To date, this remains the only example of the successful eradication of an infectious disease. Although smallpox is no longer a threat and other poxviruses cause little disease in humans, interest in the use of poxviruses as vaccine vectors has been maintained. The genetic

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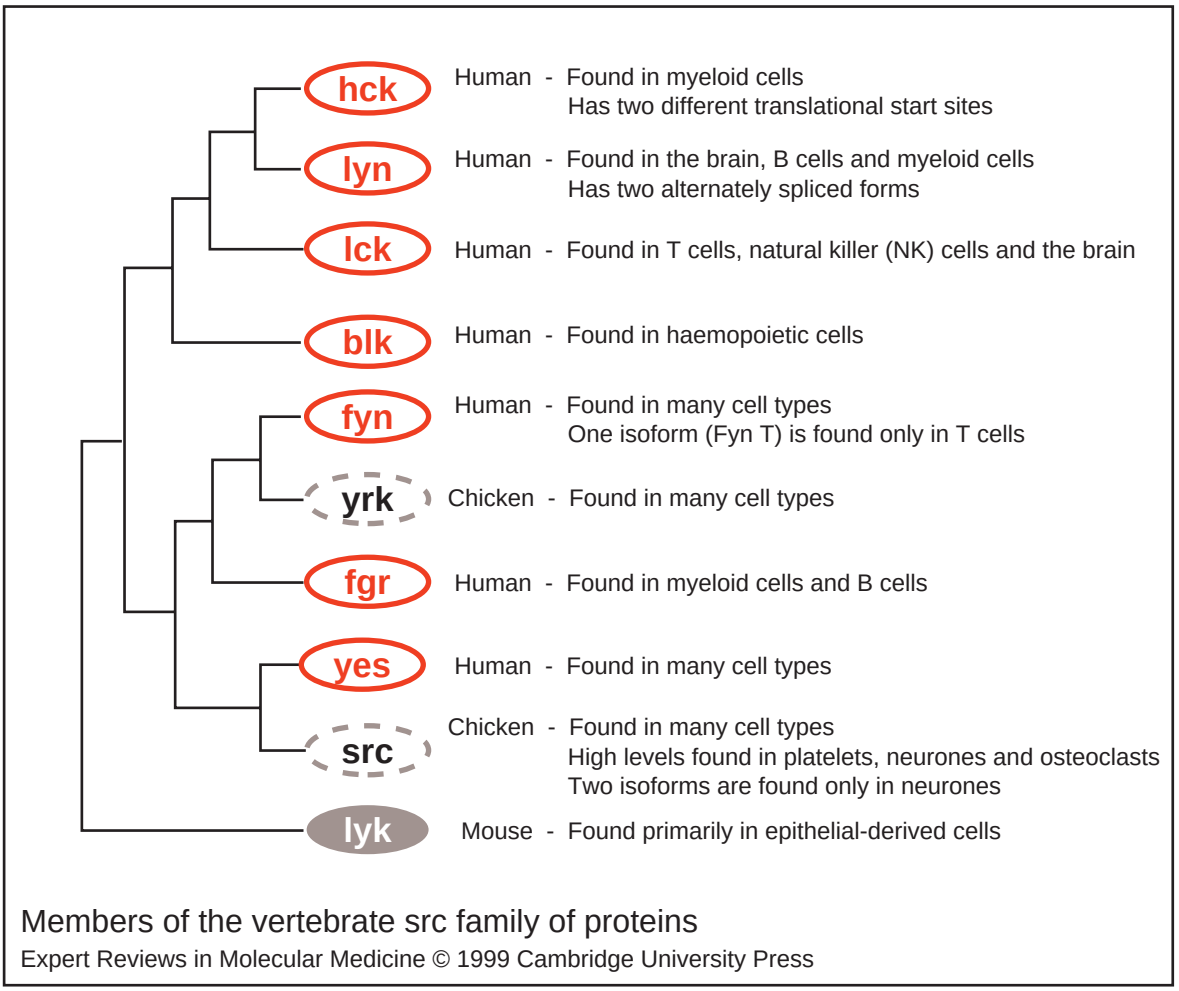

Figure 3. Members of the vertebrate src family of proteins. Following the identification of *v-src*, several vertebrate and invertebrate proteins that have similar amino acid sequences to that of pp60$^{v\text{-}src}$ have been identified and their tissue distribution characterised. Only vertebrate proteins that have SH2, SH3 and kinase domains have been listed in the figure; however, other, more divergent members of the family have been identified including abl, csk, matk, rak and iyk. The proteins are colour coded to denote species of origin **(fig003cso).**

manipulation of poxvirus genomes can be used to engineer recombinant poxviruses that express foreign proteins. Such recombinant viruses have been used successfully to elicit antigenic priming (an immune response) against several mammalian pathogens, such as rabies virus (Ref. 42), rhinderpest (Ref. 43), malaria protozoa (Ref. 44) and human immunodeficiency virus (Ref. 45), and against tumour-specific marker proteins (Ref. 46). The recent demonstration that entomopoxviruses undergo abortive infection of mammalian (including human) cells raises the exciting possibility that insect poxviruses might be useful non-pathogenic alternatives to standard strains of chordopoxviruses (Ref. 47).

Poxviruses and cell motility

Virus CPEs that are induced by infection with poxviruses such as vaccinia virus were viewed previously as a gradual degeneration of cell viability, function and architecture (Ref. 48). Ultimately this is true, but it is incorrect to assume that the virus simply 'shuts down' the host cell. Instead, poxviruses encode genes that induce the polymerisation of actin (Refs 49, 50, 51, 52), cell migration, the formation of long (>160 μm) cell projections (Ref. 53) and changes in the mechanism of cell–ECM adhesion (Ref. 54). In order to understand these CPEs, we must first explain the basic composition of the genome of vaccinia virus and the complex process of assembly of vaccinia virus.

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Vaccinia virus

Poxviruses replicate within the cytoplasm of the infected cell. The genome of vaccinia virus contains ~200 genes, which can be subdivided into three temporally distinct classes: early, intermediate or late. Early genes are transcribed by components that are contained within the invading virion, whereas intermediate and late genes are not transcribed until virus DNA replication has begun (Ref. 55). This sequence of gene expression can be uncoupled by the addition of the drug cytosine β-D-arabinofuranoside (ara-c), which inhibits DNA replication and permits only the early genes to be expressed.

Assembly of vaccinia virus

The assembly of vaccinia viruses is complex and produces two infectious forms of the virus (Fig. 4; Ref. 56). Virus morphogenesis is initiated within specialised areas of the cytoplasm called virus factories. Membrane crescents that contain virus proteins are the first visible structures to be formed. There is considerable debate about the origin and number of membranes within these structures. Early reports suggested that the crescents contain a single lipid bilayer that is formed by de novo membrane synthesis (Ref. 57); however, another report suggested that the crescents are derived from the intermediate compartment of the cell and contain a double lipid bilayer (Ref. 58). Ultrastructural data showing that virus crescents and the envelope of intracellular mature virus (IMV) particles are composed of only one lipid bilayer (Ref. 59) support a model of de novo synthesis; however, the mechanism of membrane formation, the source of lipids and the mechanism of protein incorporation remain unclear. As morphogenesis progresses, the crescents extend to form spherical immature virions (IVs). Although IV particles contain the viral genome, they are non-infectious until they undergo condensation to form infectious IMV particles. After leaving the cytoplasmic virus factory, some IMV particles become wrapped in membranes that are derived from either the trans-Golgi network (Ref. 60) or tubular endosomes (Ref. 61) to form intracellular enveloped viruses (IEVs). Components within the outer membrane of IEV particles promote the polymerisation of actin on one side of the virus particles (Refs 17, 18). The growing actin tail is thought to assist the intracellular movement of IEV particles in a manner that is similar to that described for the bacteria *Rickettsia*, *Shigella* and *Listeria* (Refs 14, 15, 16). The outer membrane of IEV particles then fuses with the plasma membrane to expose infectious cell-associated enveloped viruses (CEVs) on the surface of the cell. In the case of the Western Reserve (WR) strain of vaccinia virus, the majority of enveloped virus particles remains attached to the cell as CEVs, and only a few enveloped virions are released from the cell as extracellular enveloped viruses (EEVs). The outer membrane of CEV or EEV particles contains six virus-encoded proteins that are not found in infectious IMV particles: A56R (haemagglutinin gp86; Refs 62, 63), F13L (p37; Ref. 64), B5R (gp42; Refs 65, 66), A34R (gp22-24; Ref. 67), A36R (p45-50; Ref. 68) and A33R (gp23-28; Ref. 69). Few, if any, actin tails are made in the absence of any of these proteins (Refs 49, 50, 51, 52), with the exception of A56R, which is not required for the formation of EEV particles (G.L. Smith, unpublished observation) or actin tails (Ref. 50). In addition to virus-encoded proteins, EEV membranes contain host proteins that include complement-control proteins, which confer resistance to neutralisation by complement (Ref. 70).

Cell migration induced by poxviruses

When poxviruses infect confluent monolayers of cells, they generate holes or plaques in the cell monolayer, which are formed by virus-induced CPEs. Cells contained within these plaques are frequently polarised and show other signs of motility (Ref. 53). To test the possibility that poxvirus infection itself induces cell migration, an in vitro 'wound-healing' assay was used. Confluent monolayers of BS-C-1 cells were scratched (using a plastic pipette tip) to create 'wound areas', which were devoid of adherent cells. After washing twice, 'wounded' monolayers were infected with vaccinia virus at a concentration of five plaque-forming units (pfu) per cell; the migration of cells was assessed by their ability to move laterally into the wound area. Results showed that cells that were infected with vaccinia virus moved more rapidly than cells incubated without virus ('mock-infected' cells). In addition, the cell migration that was induced by poxviruses was found to be dependent upon the expression of early virus genes, but independent of the assembly of virus particles, the formation of actin tails or the secretion of proteins from virus-infected cells (Ref. 53). Time-lapse analysis of virus-induced

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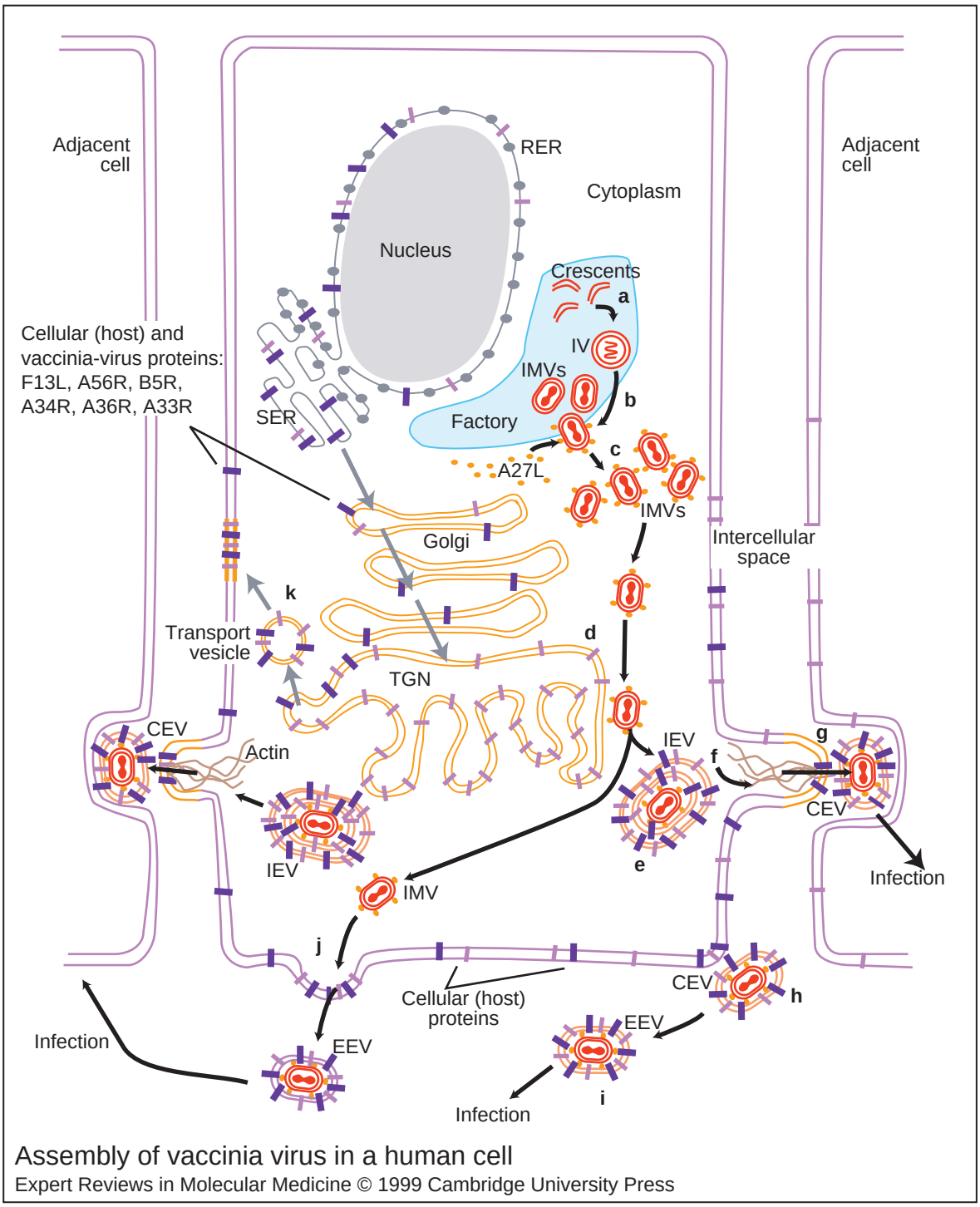

Assembly of vaccinia virus in a human cell

Figure 4. Assembly of vaccinia virus in a human cell *(see next page for legend)* **(fig004cso).**

wound healing (Ref. 53; Fig. 5) revealed that (1) cells migrate into the 'wound' laterally from each border; (2) cell migration is initiated 8–10 h after virus infection (the time of maximum release of virus particles from the cell); and (3) cells that are contained within the 'wound area' do not have a normal motile morphology (Ref. 53): cells infected with vaccinia virus develop long (often branched) cell projections, which extend from a static cell body (Ref. 53; Movie 1, HTML

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Figure 4. Assembly of vaccinia virus in a human cell. SER = smooth endoplasmic reticulum; RER = rough endoplasmic reticulum. The assembly of vaccinia virus is a complex process that results in the formation of two infectious forms of virus: intracellular mature virus (IMV) and extracellular enveloped virus (EEV). (a) Vaccinia-virus morphogenesis starts in cytoplasmic 'factories' with the formation of membrane crescents, which extend to form spherical immature virus (IV) particles. (b) IV particles are non-infectious until they undergo morphological condensation into 'brick-shaped' IMV particles. IMV particles recruit the virus A27L protein (p14 kDa) onto their surface. (c) During infection, most IMV particles remain within the cell until they are released by cell lysis. However, some IMV particles become enveloped by membranes derived from either the trans-Golgi network (TGN) or tubular endosomes (d) to form intracellular enveloped virus (IEV) (e). (f) Proteins within the outer membrane of IEV particles induce the polarised, unidirectional polymerisation of actin, which propels the particle towards the plasma membrane and assists the infection of neighbouring cells. (g) During this process, the outer membrane of the IEV particle fuses with the plasma membrane of the cell, exposing an infectious particle on the cell surface and leaving proteins that induce actin polymerisation within the plasma membrane. (h) If these particles remain attached to the cell they are called cell-associated enveloped virus (CEV); (i) however, if they are released from the cell they are called extracellular enveloped virus (EEV). (j) In addition to the classical mechanism of vaccinia-virus assembly described above, EEV particles can also form via direct budding of IMV particles through the plasma membrane. This alternative mechanism of EEV formation might be facilitated by the transport of viral glycoproteins from the TGN to the plasma membrane (k) **(fig004cso).**

version only). Unlike vaccinia-virus-induced cell migration, which is dependent on early virus gene expression, the formation of cell projections was inhibited by the addition of ara-c and was, therefore, dependent upon the expression of late virus genes (Ref. 53).

Kinetic analyses of poxvirus-induced changes in migration and projection formation showed that the two processes were sequential. First, infected cells dissociated from neighbouring cells and then they migrated. After a period of migration, movement of the cell body slowed and long projections formed from the cell body. These data show that poxviruses contain at least two genes that control different aspects of cell motility.

Similarities between the adhesive properties of embryonic neuronal cells and vaccinia-virus-infected cells

In addition to morphological similarities between the progression of poxvirus-induced CPEs and neuronal development, there are also biochemical similarities in the way poxvirus-infected cells and cranial neural crest cells adhere to the ECM.

Neural crest cells are a migratory population of embryonic cells that give rise to four distinct rostrocaudal populations (cranial, vagal, trunk and sacral). Cranial neural crest cells migrate through the cranial mesenchyme to produce facial cartilage and bone, as well as facial parasympathetic and sensory ganglia (Ref. 71). The motility of neural crest cells is mediated by integrin–ECM interactions. However, unlike classical integrin-mediated adhesion (Ref. 72), neural crest cells bind to ECM proteins (laminin and fibronectin) via adhesion that is independent of the presence of calcium ions (Refs 73, 74, 75). Interestingly, Ca^{2+}-independent ECM adhesion has been observed under conditions that favoured motility of neural crest cells (Refs 73, 76).

Infection with vaccinia virus also induced a conversion to Ca^{2+}-independent adhesion, and this change occurred during the motile phase of infection. As with neural crest cells, vaccinia-virus-infected cells adhered to ECM proteins via arginine–glycine–aspartic acid (RGD) motifs in the absence of extracellular Ca^{2+}, suggesting that in both cases adhesion was mediated by integrins (Ref. 54). Given the emerging correlation between Ca^{2+}-independent ECM adhesion and motility, it is interesting to note that transformed normal rat-kidney cells (Ref. 77) exhibited dramatic changes in cell morphology and Ca^{2+}-independent ECM adhesion (Ref. 54). More work is needed to determine if other motile or invasive cells exhibit similar changes in cell–ECM adhesion; however, there does seem to be a link between the type of ECM interaction and the motile state of the cell. Given the observed functional and biochemical similarities between poxvirus-induced CPEs, neuronal development and cell transformation, it is possible that further analyses of vaccinia-virus-induced CPEs might lead to the identification of novel cellular genes that are involved in cell motility, and also a better understanding of events that occur during malignancy and embryonic development.

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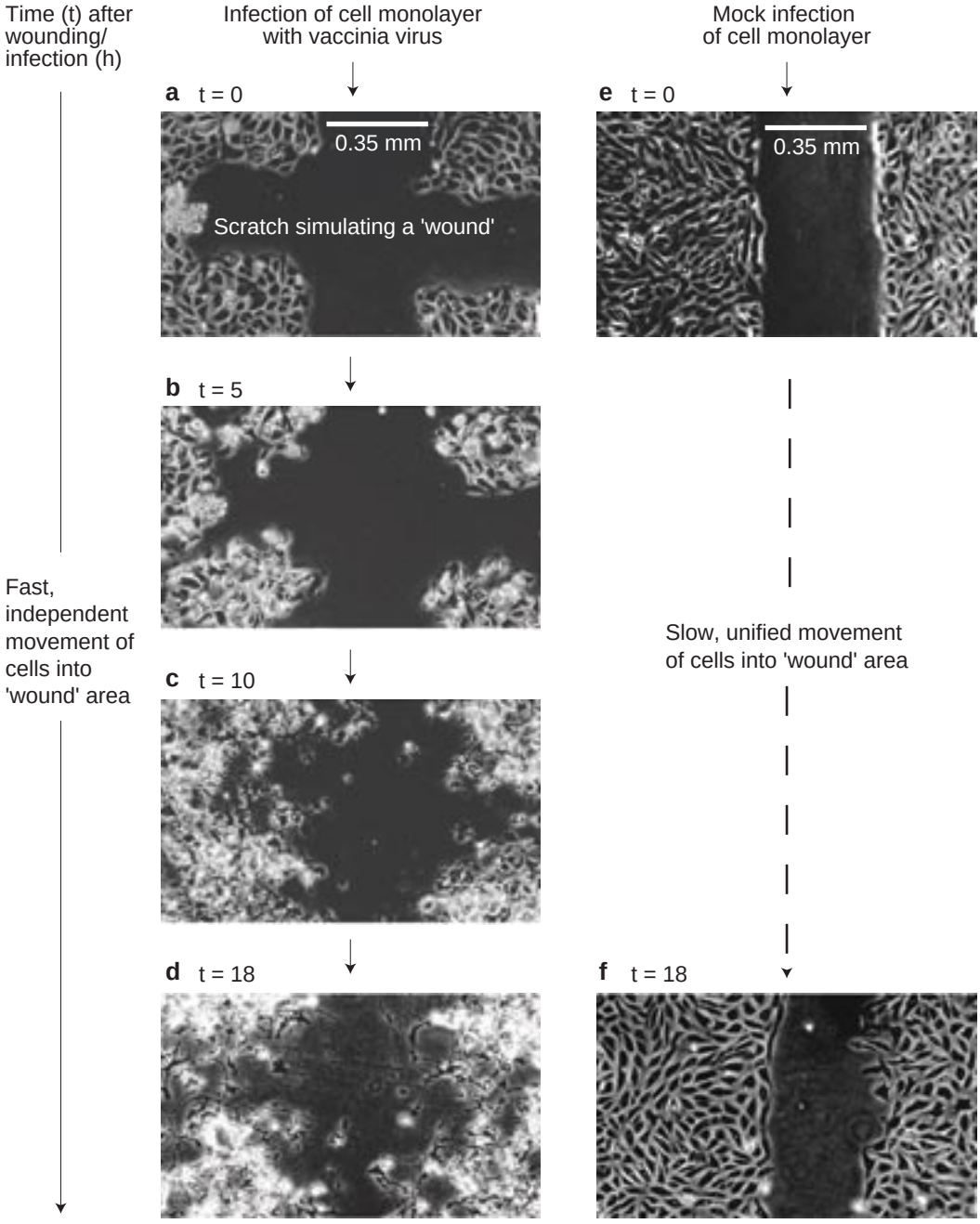

Cell migration induced by infection of a monolayer of cells with vaccinia virus

Figure 5. Cell migration induced by infection of a monolayer of cells with vaccinia virus *(see next page for legend)* **(fig005cso).**

Clinical implications/applications

More work is needed to define the significance of virus-induced cell motility in the mammalian host. However, it is clear that both transforming and non-transforming viruses encode proteins that induce migration and projection formation

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Figure 5. Cell migration induced by infection of a monolayer of cells with vaccinia virus. Confluent monolayers of African green monkey (*Cercopithecus aethiops*) kidney cells (BS-C-1) were scratched (using a plastic pipette tip) to generate wounds that were devoid of adherent cells. After wounding, monolayers were washed twice with phosphate-buffered saline (PBS); they were then either immediately infected with vaccinia virus at a concentration of five plaque-forming units (pfu) per cell (a–d) or mock infected (e and f). Cell movement was monitored by photography under phase-contrast microscopy. After 18 h, the wound size of the uninfected cells was marginally smaller than that observed directly after wounding (e and f), as a result of cells encroaching slowly as a unified 'wave' into the wound area. (d) In contrast, in the case of cultures that were infected with vaccinia virus, individual cells migrated rapidly into the wound area **(fig005cso).**

in vitro. Thus, we can and should use these viruses as genetic tools to dissect the complex cellular events that regulate cell migration and morphology. Finally, although poxviruses and the transforming RSV both induce migration and projection formation, poxviruses do not contain *src*-like genes. Consequently, the identification of the poxvirus genes that elicit *src*-like effects could, in turn, lead to the identification of novel eukaryotic genes that are involved in cell motility.

Clinically, viruses are being used for many therapeutic applications including vaccination, gene therapy, gene expression and selective cell killing. However, until we understand fully the subtleties of virus–host interactions, it is unlikely that we will be able to harness the full potential of virus vectors.

Outstanding research questions: points to ponder

It is the nature of research that one answer often generates many new questions. This is certainly true of those data reviewed here. Although it has been established that viruses can be used to analyse aspects of cell motility, the data also raise the question 'How do poxviruses acquire genes that are capable of controlling aspects of cell motility and cell adhesion?'

How do poxviruses acquire eukaryotic genes?

Because the genomes of poxviruses contain many genes that show amino acid sequence similarity to host genes, it is reasonable to presume that they were acquired originally from the host cells that they infected. However, how this happened is not understood. The genes of poxviruses lack introns, yet several cellular counterparts contain introns; therefore, it is probable that poxviruses acquired cellular genes via complementary DNAs (cDNAs). Although poxviruses lack reverse transcriptase, it is possible that a cell might have been co-infected by a retrovirus and a poxvirus, or that a poxvirus might have infected a cell containing a retrotransposon and hence reverse transcriptase activity. cDNAs that are present in poxvirus-infected cells might 'illegitimately' be ligated or recombined into replicating poxvirus DNA. Although inefficient, this process might be enhanced by the amplification of the copy number of cDNAs. In this regard, it is worth noting that (1) the DNA ligase of vaccinia virus can ligate single-stranded DNA that contains oligo dT (the complement of messenger RNA that contains poly A; Ref. 78); (2) non-virus circular DNA is replicated non-specifically in poxvirus-infected cells (Refs 79, 80); and (3) recombination can occur efficiently between virus DNA and single- (Ref. 81) or double-stranded linear or circular DNAs (Refs 82, 83). Thus, linear cDNA containing poly T ends could be ligated to form circular DNA, which would be amplified, thereby increasing the possibility of recombination with poxvirus genomic DNA.

Why do viruses induce cell motility or changes in morphology?

A simple answer to the question 'Why do viruses induce cell motility or changes in morphology?' might be that movement of infected cells enhances the spread of virus particles. It is easy to see how a motile infected cell could act like a 'molecular grenade'. The ability to move away from the initial site of infection to an area of uninfected cells would potentiate the rate of local infection. Motility-enhanced infection might be particularly effective for strains of poxviruses that form a higher proportion of CEV than EEV particles. Some evidence for the in vivo migration of poxvirus-infected cells in mice has been reported (Ref. 84). However, the origin of the motile cells (epithelial, leukocyte or macrophage) was not established in these studies, and more research is needed to define precisely how vaccinia-virus-induced CPE influences the rate of virus spread in vivo.

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An alternative answer to the question is that it is possible that cell movement might occur as a consequence of virus-induced changes in cell biochemistry. To ensure that the host cell is in an optimal state to support virus replication and dissemination, some viruses might have evolved mechanisms of up-regulating cellular metabolism and transcriptional capacity. If this were achieved by the activation of signal-transduction cascades that are linked to growth-factor receptors, members of the Ras protein superfamily (Rho, Rac and Cdc42) that control cell movement might also be activated (Ref. 85). Consequently, viruses that affect cell quiescence might benefit from both optimal replication status and enhanced local dissemination of particles via cell movement.

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Features associated with this article

Schematic figures

Figure 1. Cell adhesion and cell migration (fig001cso).
Figure 2. Activation of pp60$^{c\text{-}src}$ kinase (fig002cso).
Figure 3. Members of the vertebrate src family of proteins (fig003cso).
Figure 4. Assembly of vaccinia virus in a human cell (fig004cso).
Figure 5. Cell migration induced by infection of a monolayer of cells with vaccinia virus (fig005cso).

Movie

Movie 1. Morphological changes that result from vaccinia-virus infection of BS-C-1 cells (swf001cso).

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Further reading, resources and contacts

Molecular Biology of the Cell (1995) (Lodish, H. et al., eds), Scientific American Books, W.H. Freeman & Co., New York. Includes excellent introductory chapters on cell motility and cell adhesion.

Viral Pathogenesis (1997) (Nathanson, N., ed.), Lippincott-Raven, New York. Includes an excellent introductory chapter on virus pathogenesis.

Genes V (1994) (Lewin, B., ed.), Oxford University Press, Oxford, pp. 1218-1220. Includes an excellent introduction to the SRC gene and *src*-mediated transformation.

CELLS alive! This site provides wonderful images and video footage of cells in action.
http://www.cellsalive.com/

The NIH Motility Interest Group website provides details of the National Institute for Health (NIH) Cell Biology Interest Group (CBIG), including a useful directory of cell biology contacts and teaching tutorials in cell biology.
http://www.nih.gov/sigs/cellbio/index.html

The home page of the Cell Motility Club has been arranged by scientists from Johns Hopkins University and the National Institute for Health (NIH), who have a common interest in cell and subcellular motility; it includes lists of presentations and members.
http://www.bme.jhu.edu/~skuo/CellMotClub/index.html

Cell Motility and the Cytoskeleton is an on-line journal; pre-registration is required for access.
http://www.interscience.wiley.com/jpages/0886-1544

All the Virology on the WWW provides an excellent starting point for links to all aspects of virology.
http://www.tulane.edu/~dmsander/garryfavwebindex.html

The Institute for Molecular Virology, University of Wisconsin, Madison (home page). This is a well-conceived website with information about a range of viruses and virus evolution.
http://www.bocklabs.wisc.edu/Welcome.html

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