Viral proteins that regulate cellular signalling

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Introduction

Molecular details of viral pathogenesis have been explored at an amazing speed during the past decade. Before this, viruses had been viewed as genetic parasites that exploit cellular metabolism in order to propagate themselves. To usurp the infected cell, viruses have evolved various strategies to shut down the synthesis of cellular macromolecules and direct the cell machinery to produce viral material. During the sometimes millions of years of virus and host co-evolution, there has been plenty of time for the development of very complex and intimate interactions between the two. Studies on latent and persistent viruses in particular have shown that viruses have developed extremely sophisticated strategies to manipulate the host’s cellular regulation in order to transform host cells, to replicate or to persist. Cellular homeostasis in multicellular organisms is directed by extracellular signals. Not surprisingly, to exercise total control over host cells, viruses have evolved numerous genes that function as signal interceptors or activators. Some of these genes are of host origin as shown by homology searches; others may have arisen by convergent evolution (Murphy, 1994; Smith, 1994). In addition to helping understand viral pathomechanisms, these studies provide further insight into cellular regulation.

Cell growth and immunoregulation pathways are the two signalling networks most frequently targeted by viruses. This review focuses on the plasma membrane and cytoplasmic events in virally regulated cell growth and in immunoregulation signalling. The paradigm of viral gene products reprogramming cell growth is provided by retroviruses. This material has been extensively reviewed (Bishop, 1991; Kung & Liu, 1997; Varmus, 1989) and is therefore not addressed in this paper.

Cell death/immunoregulation signalling

A virus must penetrate the host’s antiviral immune defences before it can become absorbed by a host cell and redirect the host cell’s functions to its own replicative advantage. Most of the antiviral defences directed against infected cells are radical: complement, cytotoxic T lymphocytes (CTLs) and phagocytic cells kill the infected cells. In general, viral strategies to counteract cytotoxic immune defences fall into two groups: (i) prevention of recognition of infected cells and (ii) inhibition of cell death machinery.

A virus must first pass extracellular immune defences such as the complement cascade and antibody neutralization. Both defences directly mediate lysis or phagocytosis of free virus and virus-infected cells. The common theme is the highly efficient destruction of the target. Therefore, the most successful viral strategies to evade complement and antibody-mediated lysis are the ones that inhibit association of the virus-infected cell with complement and/or antibody-driven phagocytes. Vaccinia virus and herpesviruses have evolved proteins (Table 1) that block complement function either by inhibiting complement activation (Kotwal et al., 1990; Harris et al., 1990; Albrecht & Fleckenstein, 1992) or preventing deposition of the membrane attack complex (Rother et al., 1994). Expression of Fc receptors by herpesviruses (Bell et al., 1990; Thale et al., 1994) and varicella-zoster virus (VZV) (Olson et al., 1997) serves a dual function: it prevents activation of the classical complement pathway and may also protect against Fc-facilitated phagocytes.

CTLs have evolved dual strategies to kill virus-infected cells. Activated CTLs express membrane-bound Fas ligand that triggers Fas-mediated cell death. At the same time, CTLs introduce granzyme B, a serine protease, into the infected cell (Nagata, 1997). Both molecules activate the cascade of apoptotic proteases (caspases). The lytic attack of CTLs is highly efficient. Therefore, the counter-attack by many viruses is based on inhibition of major histocompatibility complex (MHC) class I-mediated presentation of viral peptides on the infected cells, in order to avoid recognition. The viral proteins can strike at any point in gene expression and transport of MHC class I antigen (Table 1). This strategy is applied by adenoviruses (Ad) (Schouten et al., 1995; Pääbo et al., 1983; Burgert & Kvist, 1985; Hermiston et al., 1993); cytomegaloviruses (CMV) (Campbell & Slater, 1994; Wiertz et al., 1990; Ahn et al., 1996; Jones & Sun, 1997; Ziegler et al., 1997); and human immunodeficiency virus type 1 (HIV-1) (Schwartz et al., 1996). Herpes simplex virus (HSV) ICP47 protein inhibits production of antigenic peptides or their translocation into the
### Table 1. Virus factors that regulate cell death/immunoregulation signalling

TAP, Transporter associated with antigen processing; SCR, short consensus repeat; MAC, membrane attack complex.

<table>
<thead>
<tr>
<th>Virus factor</th>
<th>Homologue in host</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complement</strong></td>
<td>Complement SCR</td>
<td>Binds C4b, blocks complement activation</td>
<td>Kotwal et al. (1990)</td>
</tr>
<tr>
<td>Vaccinia virus CP</td>
<td>Complement SCR</td>
<td>Binds C4b, blocks complement activation</td>
<td>Albrecht &amp; Fleckenstein (1992)</td>
</tr>
<tr>
<td>HVS ORF5</td>
<td>CD59</td>
<td>Inhibits formation of MAC</td>
<td>Rother et al. (1994)</td>
</tr>
<tr>
<td>HSV C-1</td>
<td>CD59</td>
<td>Binds C3b, blocks complement activation</td>
<td>Harris et al. (1990)</td>
</tr>
<tr>
<td>HSV gE-gI</td>
<td>CD59</td>
<td>Binds C3b, inhibits complement and phagocytosis</td>
<td>Bell et al. (1990)</td>
</tr>
<tr>
<td>Murine CMV early gene</td>
<td>CD59</td>
<td>Blocks function of MAC</td>
<td>Thale et al. (1996)</td>
</tr>
<tr>
<td>VZV gE</td>
<td>CD59</td>
<td>Binds C3b, blocks complement activation</td>
<td>Olsen et al. (1997)</td>
</tr>
<tr>
<td><strong>CTL</strong></td>
<td></td>
<td>Downregulation of MHC class I at transcriptional level</td>
<td>Schouten et al. (1995)</td>
</tr>
<tr>
<td>Ad12 E1A</td>
<td></td>
<td>Retains MHC class I in ER</td>
<td>Paabo et al. (1983); Burgert &amp; Kvist (1985); Hermiston et al. (1993)</td>
</tr>
<tr>
<td>AdE3 gp19K</td>
<td></td>
<td>Blocks MHC transport</td>
<td>Campbell &amp; Slater (1994)</td>
</tr>
<tr>
<td>Murine CMV m152</td>
<td></td>
<td>Downregulation of MHC class I at transcriptional level</td>
<td>Ahn et al. (1996)</td>
</tr>
<tr>
<td>Murine CMV early gene</td>
<td></td>
<td>Retains MHC class I in ER</td>
<td>Ahn et al. (1996); Wiertz et al. (1996); Jones &amp; Sun (1997)</td>
</tr>
<tr>
<td>Human CMV US3</td>
<td></td>
<td>Dislocates MHC class I from ER to cytosol</td>
<td>York et al. (1994)</td>
</tr>
<tr>
<td>Human CMV US6</td>
<td></td>
<td>Inhibits production and transport of antigenic peptides</td>
<td>Schwartz et al. (1996)</td>
</tr>
<tr>
<td>HSV ICP47</td>
<td></td>
<td>Inhibits endocytic degradation of MHC class I</td>
<td>Schwartz et al. (1996)</td>
</tr>
<tr>
<td>HIV-1 Nef</td>
<td></td>
<td>Induces expression of an IL-1 receptor antagonist, blocks IL-1 function</td>
<td>Schwartz et al. (1996); Dobbelstein &amp; Shenk (1996); Kettle et al. (1997)</td>
</tr>
<tr>
<td><strong>NK cytotoxicity</strong></td>
<td></td>
<td>Blocks MHC transport</td>
<td>Caspian &amp; Smith (1992)</td>
</tr>
<tr>
<td>Human CMV UL18</td>
<td>MHC class I</td>
<td>Binds NK KIRs (?), inhibits NK cytotoxicity</td>
<td>Beck &amp; Barael (1988); Rayburn et al. (1997)</td>
</tr>
<tr>
<td>Murine CMV m144</td>
<td>MHC class I</td>
<td>Binds NK KIRs (?), inhibits NK cytotoxicity</td>
<td>Rawlinson et al. (1996); Farrell et al. (1997)</td>
</tr>
<tr>
<td><strong>TNF/IL-1</strong></td>
<td></td>
<td>Induces expression of an IL-1 receptor antagonist, blocks IL-1 function</td>
<td>Roberge et al. (1996)</td>
</tr>
<tr>
<td>EBV gp350</td>
<td>IL-1 receptor</td>
<td>Sequesters IL-1 and blocks IL-1 function</td>
<td>Alcamì &amp; Smith (1992)</td>
</tr>
<tr>
<td>Vaccinia virus B15R</td>
<td>IL-1 receptor</td>
<td>Inhibits IL-1 convertase, blocks TNF- and Fas L-induced cell death</td>
<td>Dobbelstein &amp; Shenk (1996); Kettle et al. (1997)</td>
</tr>
<tr>
<td>Vaccinia virus SPI-2</td>
<td>TNF receptor</td>
<td>Sequesters TNF and blocks TNF function</td>
<td>Upton et al. (1994)</td>
</tr>
<tr>
<td>Shope fibroma virus T2</td>
<td>TNF receptor</td>
<td>Sequesters TNF and blocks TNF function</td>
<td>Hu et al. (1994)</td>
</tr>
<tr>
<td>Cowpox virus CmpB</td>
<td>TNF receptor</td>
<td>Sequesters TNF and blocks TNF function</td>
<td>Smith et al. (1996a)</td>
</tr>
<tr>
<td>Cowpox virus CmpC</td>
<td>TNF receptor</td>
<td>Sequesters TNF and blocks TNF function</td>
<td>Ray et al. (1992); Tewari &amp; Dixit (1995)</td>
</tr>
<tr>
<td>Cowpox virus CmpA</td>
<td></td>
<td>Blocks TNF-induced AA release, inhibits TNF cytotoxicity</td>
<td>Krajić et al. (1996)</td>
</tr>
<tr>
<td>Ad E3–14.7K</td>
<td></td>
<td>Blocks TNF-induced cPLA₂ translocation, AA release and TNF cytotoxicity</td>
<td>Dimitrov et al. (1997)</td>
</tr>
<tr>
<td>Ad E3–10.4K</td>
<td></td>
<td>Blocks TNF-induced cPLA₂ translocation, AA release and TNF cytotoxicity</td>
<td>Dimitrov et al. (1997)</td>
</tr>
<tr>
<td>Ad E1B–19K</td>
<td></td>
<td>Blocks TNF-induced cPLA₂ translocation, AA release and TNF cytotoxicity</td>
<td>Dimitrov et al. (1997)</td>
</tr>
<tr>
<td>EBV BHRF1</td>
<td>Bcl-2</td>
<td>Sequesters Bax, blocks TNF cytotoxicity</td>
<td>Han et al. (1996); Kawanishi (1996)</td>
</tr>
<tr>
<td>Human HBV</td>
<td>Bcl-2</td>
<td>Blocks TNF toxicity</td>
<td>Kawanishi (1996)</td>
</tr>
<tr>
<td>Herpesvirus, molluscipoxvirus v-FLIPs</td>
<td>FADD</td>
<td>Blocks TNF toxicity</td>
<td>Hsu et al. (1999); Berin et al. (1997); Thevenet et al. (1997)</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>FADD</td>
<td>Bind FADD or FLICE, inhibit recruitment of FLICE</td>
<td>Hsu et al. (1999); Berin et al. (1997); Thevenet et al. (1997)</td>
</tr>
<tr>
<td>EBV BCRF1</td>
<td>IL-10</td>
<td>Th2 inducer, suppresses inflammation</td>
<td>Hsu et al. (1999); Fleming et al. (1997); Karp et al. (1996)</td>
</tr>
<tr>
<td>ORF gene</td>
<td>IL-10</td>
<td>Blocks TNF cytotoxicity</td>
<td>Hsu et al. (1999); Fleming et al. (1997); Karp et al. (1996)</td>
</tr>
<tr>
<td>Measles virus</td>
<td></td>
<td>Downregulates pro-Th1 IL-12, suppresses inflammation</td>
<td>Hsu et al. (1999); Fleming et al. (1997); Karp et al. (1996)</td>
</tr>
<tr>
<td><strong>IFN</strong></td>
<td></td>
<td>Sequesters IFN-α and blocks its function</td>
<td>Upton et al. (1992)</td>
</tr>
<tr>
<td>Rabbit myxoma virus M-T7</td>
<td>IFN type I receptor</td>
<td>Sequesters IFN-α and blocks its function</td>
<td>Alcamì &amp; Smith (1992); Colamonici et al. (1995)</td>
</tr>
<tr>
<td>Poxvirus</td>
<td>IFN type I receptor</td>
<td>Downregulates pro-Th1 IL-12, suppresses inflammation</td>
<td>Leon &amp; Sen (1990); Bhattatharyya et al. (1996); Zhang et al. (1996)</td>
</tr>
<tr>
<td>Vaccinia virus B18R</td>
<td>IFN type I receptor</td>
<td>Represses STAT1, binds p300/CBP, blocks IFN-induced JAK/STAT pathway</td>
<td>Foster et al. (1999)</td>
</tr>
<tr>
<td>Ad E1A</td>
<td></td>
<td>Blocks IFN signalling</td>
<td>Foster et al. (1999)</td>
</tr>
</tbody>
</table>
endoplasmic reticulum (ER)/cis Golgi (York et al., 1994), and in another scenario CMV US6 gene product prevents peptide loading of MHC class I by inhibiting peptide translocation into the ER acting on the luminal side (Ahn et al., 1997; Hengel et al., 1997).

Orthopoxvirus SPI-2 (crmA in cowpox virus) is an exception to the rule since it blocks CTL-induced apoptosis by inhibition of the caspase cascade (Tewari et al., 1995). In some cell types, mutation of the SPI-1 gene in addition to SPI-2 was also necessary to completely abrogate inhibition, thus orthopoxviruses encode two genes that function independently to inhibit antiviral CTL cytotoxicity (Macen et al., 1996). The Bcl-2 family viral proteins [Ad E1B-19K, Epstein–Barr virus (EBV) BHRF1, human herpesvirus-8 (HHV-8) Ksbc1-2 (Sarid et al., 1997), HSV ORF16 (Nava et al., 1997), and African swine fever virus (ASFV) LMV5-HL (Neilan et al., 1993)] are also likely CTL signal interceptors, since Bcl-2 inhibits both the Fas and the perforin–granzyme branches of CTL cytotoxicity (Schroter et al., 1995).

Natural killer (NK) cells dominate cellular cytotoxicity early after infection. Patients with NK deficiency show increased susceptibility to herpesvirus infection (reviewed by Biron, 1997). However, while downregulation of MHC class I will prevent CTL-mediated lysis of infected cells, it renders them susceptible to NK-mediated killing (Ljunggren & Kärre, 1990). It has been hypothesized (Kärre & Welsh, 1997) that the MHC class I homologue human CMV UL18 (Beck & Barrel, 1988) and murine CMV m144 (Rawlinson et al., 1996) bind the inhibitory NK receptors (killer cell inhibitory receptors; KIRs) acting as a ‘viral decoy’. Although the proposed interaction of UL18 and m144 with the KIRs themselves has not been demonstrated, it has been shown that both viral proteins inhibit NK cytotoxicity (Reyburn et al., 1997; Farrell et al., 1997).

Cytokines are an important part of the immune response to many organisms. They contribute to the proliferation and differentiation of lymphocytes and other target cells, and also drive inflammation. Most of the infectious organisms seem to target local inflammatory mediators directly and avoid wholesale immunosuppression. In support of this idea, several infectious agents have been identified that produce macro-molecules which interfere with the function of the inflammatory cytokines interleukin-1 (IL-1) and tumour necrosis factor (TNF) (Table 1). The most direct approach to counter TNF action is taken by the Shope fibroma/myxoma virus (Upton et al., 1991) and cowpox viruses that encode secreted TNF receptors (Hu et al., 1994; Smith et al., 1996a). Similarly, vaccinia virus expresses a soluble IL-1 receptor (Alcami & Smith, 1992) that is specific for IL-1β (Spriggs et al., 1992). EBV has evolved a different strategy: the gp350 viral envelope protein induces expression of the IL-1 receptor antagonist which competitively inhibits IL-1 activity (Roberge et al., 1996). TNF and IL-1 are inducible functions of the immune defence. Expression of IL-1β is blocked by cowpox (Ray et al., 1992) and vaccinia viruses (Dobbelstein & Shenk, 1996; Kettle et al., 1997) that inhibit proteolytic maturation of IL-1. However, expression of these viral protease inhibitors has not been shown to inhibit inflammation, unlike the soluble IL-1β receptor that inhibits fever, a non-specific, systemic, inflammatory response to virus infection (Alcamí & Smith, 1996).

Many viruses encode proteins that can interfere with the intracellular phase of the cytokine signalling pathway. Ad have evolved a collection of proteins that target TNF cytotoxicity at the signalling phase (Wold, 1993). Specifically, Ad target TNF-induced arachidonic acid (AA) release (Krajcsí et al., 1996; Dimitrov et al., 1997), a signalling event crucial for TNF cytotoxicity (Hayakawa et al., 1993). AA metabolites are potent mediators of inflammation, thus inhibition of AA release suppresses TNF-induced inflammation as well as cell death. Another Ad protein, E1B-19K, which functions to prevent cell death inflicted by TNF, inactivates Bax (Han et al., 1996), a pro-apoptotic cellular protein (Olvai et al., 1993). EBV BHRF1 (Kawanishi, 1996), HSV ORF16 (Nava et al., 1997) and ASFV LMW5-HL protein (Neilan et al., 1993) are also Bcl-2 homologues, and so presumably utilize a similar strategy to inhibit TNF cytotoxicity. The most recently discovered family of inhibitors of TNF receptor family-mediated apoptosis is the viral FLICE inhibitory proteins (vFLIPs) (Thome et al., 1997; Bertin et al., 1997) that interact with FADD (an adaptor protein that links TNF receptor family proteins to FLICE) or FLICE and inhibit recruitment of FLICE (the most upstream element of the caspase cascade). vFLIPs have been found in gammaherpes-viruses, including HHV-8 and herpesvirus saimiri (HVS), and tumorigenic human molluscipoxvirus.

An indirect route to reduce the inflammatory response is to shift the ratio of T-helper 1 (Th1) and T-helper 2 (Th2) cells produced during immune responses. The IL-2- and interferon (IFN)-γ-producing Th1 cells stimulate cellular cytotoxicity and macrophage-mediated inflammatory responses, while Th2 cells boost antibody production (Abbas et al., 1996). The EBV BCRF1 gene product (Hsu et al., 1990) and a poxvirus orf virus gene (Fleming et al., 1997) are homologues of the Th2 inducer mammalian IL-10. These viral products are likely to be potent suppressors of inflammation. In a different scenario, measles virus infection downregulates production of pro-Th1 cytokine IL-12 (Karp et al., 1996) resulting in suppression of inflammatory and CTL responses.

The importance of the IFN system in the antiviral immune response has been demonstrated in vivo using IFN receptor knock-out mice. Mice lacking either the IFN-α/β-specific type I receptor or the IFN-γ-specific type II receptor showed no overt anomalies, but were unable to cope with virus infection (Müller et al., 1994). It has also been shown that both IFN systems are essential for antiviral defence (Müller et al., 1994). The anti-IFN strategies bear a resemblance to the anti-TNF/IL-1 defence: a soluble type I receptor homologue is encoded by vaccinia virus (Colamonici et al., 1995; Symons et al., 1995);
functional IFN type II receptors are secreted by cells infected with poxviruses (Upton et al., 1992; Alcamí & Smith, 1995; Mossman et al., 1995); and a gene homologue has been found in swinepox virus (Massung et al., 1993). Signalling through the IFN receptors activates the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signal transduction pathway (Darnell et al., 1994). The only signal interceptor known to target the JAK/STAT pathway of IFN signalling is the Ad transcription factor E1A. Ad E1A inhibits IFN-stimulated genes factor (ISGF) activity at two different levels: (i) by repression of the expression of the STAT1 subunit of ISGF (Leonard & Sen, 1996); and (ii) by binding p300/CBP, a transcriptional adaptor for STAT1 and STAT2 components of the IFN-α and IFN-γ signalling apparatus (Bhattacharya et al., 1996; Zhang et al., 1996).

The main outcome of the IFN response is the blockage of viral protein synthesis (Samuel, 1991). A number of viral gene products known to inhibit the IFN system, which interfere with the execution phase of the IFN response at a translational level (reviewed by Smith, 1994), will not be discussed here.

**Cell growth/activation signalling**

**Signal activators**

Subversion of normal growth factor signalling pathways is critical to the neoplastic processes. Consistent with this notion, a sizeable fraction of oncogenes have been shown to encode components of the cell growth machinery such as growth factors and growth factor receptors. The transforming proteins of many DNA viruses and retroviruses are known to interact with growth factor signalling pathways (Fig. 1).

A family of receptor tyrosine kinases that have been reported to transmit growth factor signals are targeted by DNA viruses and retroviruses. There is evidence that bovine papilloma virus E5 protein co-operates with colony stimulating factor receptor and epidermal growth factor receptor (EGF-R) to transform NIH 3T3 cells (Martin et al., 1989). The effect of E5 occurs in the absence of receptor stimulation by ligand. It is enhanced by the addition of ligand and is associated with inhibition of receptor degradation and persistence of activated receptors at the cell surface. A different approach is utilized by vaccinia virus. The virally encoded vaccinia virus growth factor (VGF) is homologous to EGF (Brown et al., 1985) and has been shown to stimulate the protein kinase activity of EGF-R (King et al., 1986). Although vaccinia virus is not considered a transforming virus, it induces hyperplastic responses of cells at the edge of growing lesions during active virus replication. It has been speculated that the mitogenic signal is beneficial to virus replication (Kim et al., 1995).

Other signal-transducing plasma membrane receptors do not harbour intrinsic tyrosine kinase activity. Instead, they associate with non-receptor tyrosine kinases that can serve as targets for viral intervention. Middle T antigen of the oncogenic mouse polyomavirus (Py mT) associates with and activates the cellular tyrosine kinases c-Src, c-Yes and Fyn...
Upon complex formation, middle T becomes phosphorylated, a prerequisite for binding phosphatidyl inositol-3-kinase (PI3K) (Whitman et al., 1985), the adaptor protein SHC (Campbell et al., 1994; Dilworth et al., 1994), and phospholipase C-γ1 (PLC-γ1) (Su et al., 1995). These interactions transduce mitogenic signals (fig. 1) normally triggered by growth factors and are essential for polyomavirus-mediated transformation of cells in culture and tumour formation in animals.

Tumorigenic activities of acute transducing retroviruses were found to reside in the transduced cellular sequences. However, not all retroviruses that induce cell proliferation contain such oncogenic cellular sequences. The acute erythroleukaemia-inducing virus (env) gene (Ruscetti et al., 1990) that encodes a 55 kDa glycoprotein (gp55) which interacts with the erythropoietin receptor (EPO-R) in the ER (Li et al., 1990) and at the plasma membrane (Li et al., 1995a). The results strongly argue that SFFV gp55 subverts normal requirements of erythroid cells for EPO by directly binding to and triggering EPO-R.

The major signalling pathway that transduces mitogenic signals from the plasma membrane to the nucleus is the Ras/mitogen-activated protein kinase (MAPK) network. MAPKs, also known as extracellular signal regulated kinases, are protein serine/threonine kinases that are rapidly activated upon stimulation of a variety of cell surface receptors. They control expression of genes essential for many cellular processes, including cell growth and differentiation (Marshall, 1995). These kinases play a central role in mitogenic signalling, as impeding their function prevents cell proliferation in response to a number of growth-stimulating agents (Pages et al., 1993). Furthermore, constitutive activation of the MAPK pathway is itself sufficient for tumorigenesis (Cowley et al., 1994; Mansour et al., 1994). MAPKs include extracellular signal-regulated protein kinase (ERK), c-Jun amino-terminal kinase/stress activated protein kinase (JNK/SAPK) and p38 subgroups. So far, only the ERK pathway has been implicated in oncogenesis, even though the c-Jun proto-oncogene is activated by JNK/SAPK phosphorylation and is thought to be required for Ras transformation (Johnson et al., 1996).

It is plausible that some oncogenic viruses encode proteins that activate the Ras/MAPK pathway. HVS STP-C488 was the first virus-encoded protein shown to associate with cellular Ras in transformed cells. STP-C488 can transform Rat-1 cells, and results in the formation of invasive tumours in nude mice (Jung et al., 1991). Expression of STP-C488 activated the Ras signalling pathway as evidenced by a 2-fold increase in the ratio of Ras-GTP to Ras-GDP and by the constitutive activation of MAPK (Jung & Desrosiers, 1995). HBx, the transcripational transactivating protein of hepatitis B virus (HBV), also enhances Ras-GTP formation (Benn & Schneider, 1994) stimulating both ERK and JNK/SAPK activities (Benn et al., 1996). The molecular interactions have not yet been elucidated, but both Ras and Raf-1 are required for activation of c-Jun transcriptional activity (Natoli et al., 1995). The oncogenic potential of HBx has been described as ‘atypical’ (Kekulé et al., 1993). The long latency between HBV infection and the development of hepatocellular carcinoma makes it unlikely that HBx alone directly causes hepatocyte transformation. Since the Ras–Raf pathway has also been implicated in prevention of apoptosis (Kinosita et al., 1995; Wang et al., 1996; Fernandes et al., 1996), it is tempting to speculate that HBx might prevent apoptotic cell death of hepatocytes. Simian virus 40 (SV40) small t antigen utilizes a different strategy to induce cell growth. Small t binds to protein phosphatase A (Sontag et al., 1993) and inhibits its activity to dephosphorylate a variety of phosphoproteins in vitro, including ERK2 and MAPK/ERK kinase (MEK). Expression of small t in SV40 permissive cell line CV 1 leads to an increase in the activity of ERK2 and MEK1 and ultimately causes these cells to proliferate (Frost et al., 1993; Sontag et al., 1993).

The other major pathway known to transmit mitogenic signals is the phosphatidylinositol/protein kinase C (PKC) system. The phosphoinositidase C (PI) family (otherwise known as phosphoinositide-specific phospholipase C or PI–PLC) is a receptor-controlled family of enzymes that catalyse the splitting of phosphatidylinositol-4,5-bisphosphate (PIP2) into 1,2-diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3) (Irvine, 1996). IP3 induces receptor-mediated release of Ca2+ from intracellular stores, and the elevated level of Ca2+ in concert with DAG induces translocation and activation of PKC. Sustained activation of PKC is essential for subsequent responses such as cell proliferation and differentiation (Nishizuka, 1992). Activation of this pathway by Ad (Shiroki et al., 1992), herpesviruses (Arvanitakis et al., 1997), HIV-1 (Zauli et al., 1995), HBV (Kekulé et al., 1993) and human T-lymphotropic virus (HTLV) (Lindholm et al., 1996) has been reported. However, the molecular details of these interactions have been investigated in only a few cases. Kaposis’s sarcoma-associated herpesvirus (HHV-8) encodes a G protein-coupled receptor (Cesarman et al., 1996) that is a bona fide signalling receptor which has constitutive activity in the phosphoinositide–inositolphosphate–PKC pathway, stimulating cellular proliferation and making it a candidate oncogene (Arvanitakis et al., 1997). Polyomavirus takes an entirely different approach to activate the PI–PKC pathway: the Py mT antigen forms a complex with PLC-γ1, a member of the PI–PLC family (Su et al., 1995). Tyrosine phosphorylation of PLC-γ1 is elevated in cells expressing middle T, suggesting activation of this enzyme. Another enzyme of the phospholipid metabolism that is known to associate with middle T is PI3K (Dahl et al., 1996). PI3K is a dual specificity kinase and is thought to be important in regulating cell proliferation. The Py mT–PI3K complex formation results in activation of pp70 s6 kinase (pp7056K) (Dahl et al., 1996). The induction of pp7056K activity most probably plays a role in the emergence of at least some transformation-related changes, given the known role of S6...
kinase in normal mitogenesis (Lane et al., 1993; Reinhard et al., 1994).

The oncogenic retrovirus HTLV-1 also usurps the PKC signalling pathway. Tax, a viral regulatory protein of HTLV-1, associates with PKC. This interaction stimulates PKC autophosphorylation in the absence of co-factors, leading to the cytosol-to-membrane translocation of the enzyme, suggesting that Tax, activates PKC (Lindholm et al., 1996).

Two other signal transducing protein complexes targeted by many viruses are the 14-3-3 proteins and the NFκB complex. The 14-3-3 family comprises at least seven isoforms in mammalian cells (Aitken et al., 1992). Some of the 14-3-3 proteins were recently reported to interact with cellular products, in particular Raf-1 (Fu et al., 1994; Li et al., 1995b), Bcr and Bcr-Abl (Braselmann & McCormick, 1995), PKC (Toker et al., 1992), cdc25 (Conklin et al., 1995), and Bad (Zha et al., 1996), and so they are thought to be connected with signal transduction and cell cycle control pathways. Py mT (Pallas et al., 1994), and more recently the non-structural protein NS2 of minute virus of mice (a parvovirus), have been found to associate with 14-3-3 proteins (Brockhaus et al., 1996). Autonomous parvoviruses are characterized by their dependence for growth on cellular factors that are expressed only in proliferating cells. These requirements result in the restriction of productive parvovirus infections to proliferating tissue, and are likely to account for the remarkable oncotropism exhibited by some of these viruses (Rommelaker & Cornelis, 1991; Salomé et al., 1990). Thus, both of these interactions seem to function in the proliferation or apoptosis inhibitory signalling pathways.

The transcription factor NFκB lies at the crossroads of signalling cascades. It has been implicated in many different cellular regulatory pathways, most notably transformation, inhibition of cell death, immunoregulation and activation of viral gene expression (reviewed by Bauerle & Baltimore, 1996). In resting cells, NFκB/Rel complexes are sequestered in the cytoplasm at latent precursors by physical association with a family of ankyrin motif-rich inhibitory proteins, named IκBα–κ (Beg & Baldwin, 1993). Cellular activation triggers IκB phosphorylation, the subsequent degradation of IκB, and the concomitant nuclear translocation of NFκB/Rel (Beg & Baldwin, 1993).

The Tax1 gene product of HTLV-1 is known to induce persistent nuclear expression of various NFκB/Rel factors. Recent studies have demonstrated that Tax induces NFκB activation either by phosphorylation and degradation of IκBx (Lacoste et al., 1995; Maggirwar et al., 1995) and IκBβ (Good & Sun, 1996; Kanno et al., 1994), or by direct interaction with α100/IκBα via the Rel homology domain and with p105/IκB through the ankyrin motif (Hirai et al., 1994). NFκB enhancers have been found in IL-2 and IL-2Rx promoters and Tax has been shown to induce expression of IL-2 and IL-2Rx. This sets up an autocrine growth loop that no longer requires external growth signals (reviewed by Kung & Liu, 1997).

HBV encodes two genes to manipulate NFκB signalling. HBx protein utilizes a Ras-dependent (Su & Schneider, 1996) and a Ras-independent pathway (Chirillo et al., 1996). The other protein, MHBs, an ER resident transactivator, acts through a radical-mediated mechanism to activate NFκB signalling (Meyer et al., 1992). EBV LMP1, in addition to induction of IκB degradation (Herrero et al., 1995), utilizes a second mechanism to turn on NFκB signalling. LMP1 complexes with TNF receptor-associated factor 2 (TRAF2), implicated in TNF-mediated NFκB activation. LMP1 recruits TRAF2 to the membrane and this complex triggers activation of NFκB (Kaye et al., 1996). Recombinant EBV genetic analyses have indicated that the TRAF binding domain is essential for primary B-lymphocyte transformation. In addition, it may protect the virus from TNF cytotoxicity, since NFκB blocks cytolytic action by TNF. The fact that different viruses have evolved three oncogenic proteins to turn on NFκB signalling is a strong argument for its role in oncogenesis.

A number of viruses express proteins that regulate nuclear events of mitogenesis (e.g. the cell cycle). The mechanism of these interactions has been reviewed elsewhere (Nevins, 1992, 1994; Voudson, 1995).

All DNA viruses share a common strategy of programmed gene expression and the necessity to induce DNA synthesis in host cells. Early viral proteins are thought to bring about both viral and host DNA replication. Small DNA viruses are especially dependent on cellular enzymes for replication. Blocking cell cycle progression by n-butyrate inhibited replication of the small DNA viruses polyomavirus and papillomavirus, thus supporting this notion (Shadan et al., 1994). Although the large T antigen of polyomavirus can by itself induce G1–S cell cycle transition due to its affinity for retinoblastoma protein and p53, it has been shown that the Py mT protein is also essential for efficient virus replication (Freund et al., 1992). Thus, mitogenic signalling may serve another function, boosting virus replication by induction of host cell macromolecular synthesis. Large DNA viruses encode a number of replicatory enzymes and so are less dependent on the cellular replication machinery. Nevertheless, a VGF-mutant of vaccinia virus replicated less efficiently in Swiss 3T3 cells and exhibited an attenuated phenotype in mice and rabbits (Buller et al., 1988).

**Signal interceptors**

Persistent/latent viruses may benefit from interception of signalling pathways transducing growth/activation signals, since induction of cell proliferation/activation pathways may lead to apoptosis of host cells (Gougeon & Montagnier, 1993) or activation of the lytic replication pathway in latently infected cells (Miller et al., 1994).

p56 Lck, a non-receptor tyrosine kinase, is an ideal target for signal interceptors since it has been found associated with cell surface receptors such as CD2, CD4, CD5, CD8, the IL-2
receptor and a number of downstream effectors, and thus functions in an array of growth/activation signalling pathways in lymphocytes (reviewed by Weiss & Litman, 1994). On the other hand, Lck has been implicated in T-cell receptor (TCR)-mediated or HIV-induced T-lymphocyte apoptosis (Oyaizu et al., 1995; Corbeil et al., 1996). It has been shown that HVS ORF1 (tip) forms a complex with Lck (Biesinger et al., 1995; Jung et al., 1995a). HVS tip has two domains homologous to src family kinases, a kinase homology domain and a proline-rich, src homology 3 (SH3) binding conserved motif, both of which are required for the interaction with Lck (Jung et al., 1995b). HVS tip may be responsible for the recruitment and sequestration of Lck. HVS transforms T-cells in vivo and in vitro resulting in immortalized growth; thus, it seems counter-intuitive that HVS tip should downregulate Lck-mediated signal transduction (Jung et al., 1995a). However, this virus is known to contain another gene, STP, that is capable of functioning as an oncogene in rodent fibroblasts and is required for viral T-cell transformation (Jung et al., 1991; Medveczky et al., 1993). Recent results suggest that STP binds and activates Ras (Jung & Desrosiers, 1995). A similar scenario has been found in transformation of B cells by EBV. LMP2A protein of EBV associates with B cell tyrosine kinases Lyn and Syk. LMP2A is a constitutive dominant negative regulator of surface immunoglobulin signalling through Lyn and Syk (Miller et al., 1995; Fruehling et al., 1996). The EBV equivalent of HVS STP is LMP1, a viral oncogene that transforms rodent fibroblasts in vitro and is required for viral transformation. These strategies may allow cellular immortalization by HVS and EBV to proceed, and at the same time prevent potential adverse effects that may be associated with Lck, Syk or Lyn activation, e.g. apoptosis or untimely virus replication.

Inhibition of cell proliferation/activation pathways has also been reported for non-oncogenic viruses. HIV-1 Nef has been shown to interact with cellular signalling apparatus at different points. Nef post-translationally downregulates CD4 protein via endocytosis (Garcia & Miller, 1991; Aiken et al., 1994) and forms a complex with Lck, thus inhibiting its kinase activity (Greenway et al., 1996) and protecting virus-infected cells against reinfection and activation through the TCR. Moreover, it has been shown that HIV-1 Nef associates with a member of the p21-activated kinase (PAK) family (Nunn & Marsh, 1996). The recruitment of PAK by membrane-associated Nef might facilitate the PAK activation pathways. PAK initiates a cascade leading to the activation of JNK (Coso et al., 1995; Minden et al., 1995). The significance of the activation of the PAK–JNK pathway for HIV pathogenesis is not known. However, CD28 co-stimulation, which inhibits TCR-induced apoptosis during a primary T-cell response (Radvanyi et al., 1996), is also known to activate JNK (Su et al., 1994), supporting the notion that the primary function of Nef is to inhibit apoptosis of infected cells. Finally, Nef complexes with PKCθ, inhibiting the usual translocation of PKCθ from the cytosol to the particulate fraction upon phorbol myristate acetate or phytohaemaglutinin stimulation. Thus, PKCθ is another target for Nef to impair T-cell function (Smith et al., 1996b). These observations support the concept that Nef may act as a positive factor in the HIV-1 life cycle by preventing the cell against virus-induced apoptosis and other cell activation processes (Greenway et al., 1995). Inhibition of apoptosis in HIV-infected cells enhances virus production and facilitates persistent infection (Antoni et al., 1995).

In another scenario, the Ad E3 10.4K/14.5K membrane protein complex associates with EGF-R (P. Krajcsi & W. S. M. Wold, unpublished data) and stimulates EGF-R internalization and degradation (Carlin et al., 1989; Tollefsen et al., 1991) without activating the EGF-R kinase activity (P. Krajcsi & W. S. M. Wold, unpublished data). This property initially desensitizes the infected cells to EGF. The biological significance of this effect is unclear. Since EGF is known to induce cystolic phospholipase A2 activity at both transcriptional (Maxwell et al., 1993) and post-transcriptional levels (Schalkwijk et al., 1996), and synergizes with TNF and IL-1 to activate prostaglandin biosynthesis (Modeer et al., 1993), this receptor downregulation could be another mechanism by which Ad inhibits inflammatory antiviral responses.

Concluding remarks

How can viruses gain these functions? The first alternative to consider is ‘molecular piracy’. A majority of the proteins with clear homology to cellular proteins are the ones involved in regulating cellular signalling. The sequence relationship of the host and virus homologues for the RNA viruses is very strong, i.e. in most cases about 80% amino acid identity, yet for the DNA viruses it is usually weak, i.e. generally less than 40% identity (Murphy, 1994). The other group of proteins have no sequence similarity, but serve a similar function. The second alternative for these viruses is convergent evolution (Murphy, 1994). This is, on the one hand, a potent selective force behind the generation of host defence protein diversity. On the other hand, since the virus mutates much more quickly than the cell, the absolute dependence of the virus on the host may ultimately be a limitation for the redesigning of the viral protein.

In summary, studies on viral proteins regulating cellular signalling shed more light on priorities of viruses in the battle against their hosts for survival. Firstly, the trimeric G protein–cyclic AMP signal transduction pathway, one of the major cellular signalling pathways, is clearly under-represented among cellular targets for viral products. The HTLV-1 Tax (Semmes et al., 1996; Kwok et al., 1996) and the hepatitis C virus non-structural protein 3 (Borowski et al., 1996) are the notable exceptions. Although herpesviruses encode trimeric G protein-coupled receptor homologues (Chee et al., 1990; Nicholas et al., 1992; Gompels et al., 1995), and the cellular receptor for HIV-1 also belongs to this family of signal
transducing proteins (Deng et al., 1996; Feng et al., 1996), the functional significance of these interactions has not been determined. The G protein–cyclic AMP–PKA signalling is the major regulatory pathway of cellular energy homeostasis. It seems that it is in the viruses’ vital interest to keep the cellular ATP pool intact. Secondly, viral strategies that affect cell growth and virus replication are mostly intracellular. In contrast, the major battlefield between antiviral immune functions and the viral defence is extracellular. In keeping with this, it has been shown that, due to co-evolutionary pressure, the interspecies divergence among pro-inflammatory and host-defence proteins is three times higher than the average (Murphy, 1994). Even more striking, the divergence of the extracellular domains of receptors for host-defence proteins is usually higher than the divergence of the intracellular domains (Gerard et al., 1992).

Finally, viral genomes are rich sources of host genes: analysis of the HVS genome has led to the discovery of a new cytokine, IL-17, which binds to a novel receptor (Yao et al., 1995). This is neither a surprise nor unprecedented, since a number of proto-oncogenes were explored through the cognate sequences found in transducing retroviruses.

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