MITOCHONDRIA AS FUNCTIONAL TARGETS OF PROTEINS CODED BY HUMAN TUMOR VIRUSES

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ABSTRACT

Molecular analyses of tumor virus-host cell interactions have provided key insights into the genes and pathways involved in neoplastic transformation. Recent studies have revealed that the human tumor viruses Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell leukemia virus type 1 (HTLV-1) express proteins that are targeted to mitochondria. The list of these viral proteins includes BCL-2 homologues (BHRF1 of EBV; KSBCL-2 of KSHV), an inhibitor of apoptosis (IAP) resembling Survivin (KSHV K7), proteins that alter mitochondrial ion permeability and/or membrane potential (HBV HBx, HPV E1^4, HCV p7, and HTLV-1 $p13^{II}$), and K15 of KSHV, a protein with undefined function. Consistent with the central role of mitochondria in energy production, cell death, calcium homeostasis, and redox balance, experimental evidence indicates that these proteins have profound effects on host cell physiology. In particular, the viral BCL-2 homologues BHRF1 and

KSBCL-2 inhibit apoptosis triggered by a variety of stimuli. HBx, p7, E1^4 and $p13^{II}$ exert powerful effects on mitochondria either directly due to their channel forming activity or indirectly through interactions with endogenous channels. Further investigation of these proteins and their interactions with mitochondria will provide important insights into the mechanisms of viral replication and tumorigenesis and could aid in the discovery of new targets for anti-tumor therapy.

I. INTRODUCTION

Viruses manipulate the anabolic and proliferative capacity of the host cell and counteract apoptotic cell death in order to maximize virion production and dissemination. However, cells that are driven to replicate and evade apoptosis are at risk of acquiring mutations activating oncogenes and disrupting tumor suppressor genes, suggesting that deregulation of host cell growth and death, 2 hallmarks of neoplastic transformation (Hanahan and Weinberg, 2000), can be viewed as side effects of tumor virus propagation. Accumulating evidence indicates that interference with mitochondrial function may play a role in the life cycle and pathogenesis of many viruses, including oncogenic viruses. The present review is focused on proteins encoded by human tumor viruses that are targeted at least in part to mitochondria and summarizes their possible function in the context of the viral propagation strategies and associated pathologies.

A. The Human Tumor Viruses

Table 1 summarizes the properties of the human tumor viruses identified to date: Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), hepatitis B virus (HBV), human papillomavirus (HPV), human T-cell leukemia virus type 1 (HTLV-1), and hepatitis C virus (HCV).

1. EPSTEIN-BARR VIRUS

EBV infects over 95% of the adult population worldwide, mainly through oral contact. While infection in early childhood is usually asymptomatic, infection later in life often leads to infectious mononucleosis, a self-limiting polyclonal B-lymphoproliferative disease. EBV is also linked to a number of lymphoid neoplasias (e.g., Burkitt's lymphoma, Hodgkin's disease, post-transplant lymphoproliferative disease, and AIDS-related lymphoma), epithelial neoplasias (nasopharyngeal carcinoma, gastric adenocarcinomas, oral hairy leukoplakia) and other tumors [reviewed by (Rickinson and Kieff, 2001; Young and Murray, 2003)].

EBV's oncogenicity *in vivo* is likely to be strongly influenced by a combination of environmental cofactors as well as the genetic background and immunological status of the host. Upon initial infection of naive B-cells, EBV impinges on the mechanisms controlling B-cell proliferation, survival and differentiation in order to persist in its host within long-lived memory B-cells [reviewed by (Thorley-Lawson, 2001)]. Productive or 'lytic' infection is detected mainly in the oral epithelium and in plasma cells; the other B-cell populations, as well as EBV-associated tumors, generally harbor the virus in a latent state characterized by expression of at least one of 9 latency-associated proteins (EBNAs 1, 2, LP, 3A, 3B, 3C; LMPs 1, 2A and 2B) along with 2 noncoding transcripts (EBERS1/2) [reviewed by (Kieff and Rickinson, 2001)]. Several of these proteins play important roles in providing growth and survival signals in infected B cells; EBNAs 2, 3A, 3C, and LMP1 are required for B-cell transformation [reviewed by (Dolcetti and Masucci, 2003; Klein, 1994; Klein, 2002; Kuppers, 2003; Young and Murray, 2003)].

Malignancies commonly associated with EBV (e.g., Burkitt's lymphoma) do not always harbor the viral genome, suggesting a 'hit and run' mechanism of tumorigenesis (Ambinder, 2000), with growth and survival stimuli initially supplied by viral genes such as the EBNAs and LMPs and then supplanted by host factors, e.g., upregulated c-Myc, a hallmark of Burkitt's lymphoma. A number of

EBV genes homologous to cellular genes might also play a role in tumorigenesis. These genes, which are thought to have been acquired from the host genome through a mechanism of "molecular piracy", include two BCL-2 homologues (vBCL-2) named BHRF1 and BALF1 (see Section II).

2. KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

KSHV, or human herpesvirus 8, is the most recent addition to the list of human tumor viruses [reviewed by (Dourmishev et al., 2003; Moore and Chang, 2003)]. Unlike EBV, KSHV is not ubiquitous, with infection most prevalent in Africa, southern Europe, the Middle East, and some Amerindian populations (Calabro et al., 2001; Schulz, 1999). KSHV infection is usually asymptomatic and most commonly spread via infected saliva or sexual contact (Calabro, et al., 2001; Schulz, 1999). In addition to Kaposi's sarcoma (KS), KSHV is linked to primary effusion B cell lymphoma (PEL), a monoclonal tumor often coinfected with EBV, and multicentric Castelman's disease (MCD), a polyclonal B-cell lymphoproliferative disorder; all 3 diseases have an increased incidence in immunosuppressed patients [reviewed by (Hengge et al., 2002a; Hengge et al., 2002b)].

Similar to EBV, KSHV-associated tumorigenesis is thought to arise through aberrations in the mechanisms used by the virus to promote cell proliferation, block apoptosis and evade immune surveillance. In addition, KSHV probably indirectly favors hyperplastic/neoplastic cell growth through the activities of inflammatory cytokines secreted by infected cells. This is consistent with the complex histopathogenesis of KS lesions, which start out as polyclonal expansions of endothelial cells, inflammatory cells, and "spindle" cells and progress to oligo-monoclonal spindle cell tumors (Sturzl et al., 2001). A peculiar feature of iatrogenic and AIDS-associated KS is its regression when the immune status is improved by suspension of immune suppressive therapy or by antiretroviral therapy (Cattelan et al., 2001).

The KSHV genome contains a variety of genes with immune modulatory, anti-apoptotic and cell cycle regulatory functions that may play a role in neoplastic transformation [reviewed by (Schulz, 2000)]; Table 1 lists genes demonstrated to transform cells *in vitro* (Bais et al., 1998; Gao et al., 1997; Lee et al., 1998; Muralidhar et al., 1998; Sun et al., 2003). KSHV homologues to cellular genes include vIL-6, interferon regulatory factors (vIRFs 1, 2, and 3), chemokine homologues (vMIP-I, II and III), an IL8-receptor-like G protein-coupled receptor (v-GPCR), a Cyclin (v-Cyc), a fas-ligand IL-1β converting enzyme inhibitory protein (v-FLIP), and three mitochondrial proteins: K7, an inhibitor of apoptosis (vIAP) resembling Survivin, KSBCL-2, an anti-apoptotic vBCL-2, and K15, which awaits functional characterization (see Section II).

3. HEPATITIS B VIRUS

About 380 million people are infected with HBV, with higher infection rates found in developing countries. The virus is transmitted through sexual contact and transfer of contaminated blood or other body fluids [reviewed by (Hollinger and Liang, 2001)]. In the majority of cases, infection is asymptomatic or limited to acute liver disease characterized by destruction and regeneration of hepatocytes and inflammation. However, failure of the immune system to clear the virus and consequent viral replication can give rise to chronic hepatitis B, characterized by liver injury, inflammation, and compensatory regeneration. Over a period of several decades, this chronic liver damage may evolve to cirrhosis, end-stage liver failure and hepatocellular carcinoma (HCC), a rapidly progressing tumor with a poor prognosis [reviewed by (Arbuthnot and Kew, 2001)]. Emergence of HCC in HBV-infected individuals is a multi-step process driven by chronic hepatocyte death and regeneration resulting from inflammatory and cell-mediated anti-viral immune responses, environmental and host cofactors, and viral proteins promoting high cell turnover and generation of cells with deregulating mutations. The viral protein most strongly implicated in HBV-associated

hepatocarcinogenesis is HBx, whose many activities include important effects on mitochondrial function (see Section II).

4. HUMAN PAPILLOMAVIRUS

HPV is an ubiquitous member of the *Papillomaviridae* family of small, nonenveloped DNA viruses [reviewed by (Lowy and Howley, 2001)]. HPV infection occurs through microlesions in the squamous or mucosal epithelium, where the virus initially infects basal cells. HPV completes its life cycle as the infected basal cells undergo terminal differentiation into squamous epithelium, with lower layers of the epidermis exhibiting restricted viral gene expression of early viral genes, and subsequent layers supporting high-level expression of late viral genes, massive replication of the genome, and virion assembly.

In addition to cervical carcinoma, HPV is associated with other anogenital carcinomas, upper airway carcinomas, and cutaneous tumors. The nearly 100 HPV genotypes identified to date can be classified according to their predominant target of infection (cutaneous, oral, genital, other mucosae), associated pathologies (e.g., flat warts, common warts, genital warts, other benign proliferative lesions of the skin and mucosa), and low- or high-risk association with carcinoma, with HPV 16 representing the most prevalent high-risk type (Walboomers et al., 1999). Transformation by HPV depends on viral, host and environmental factors [reviewed by (Fehrmann and Laimins, 2003; zur Hausen, 2002)]. Among the HPV genome's 8 major ORFs, E6 and E7 are most strongly implicated in HPV-associated carcinogenesis and are almost always maintained in HPV-associated tumors; coexpression of high-risk E6 and E7 (but not their low-risk counterparts) results in immortalization of human keratinocytes (Munger et al., 1989). E6 and E7 are multifunctional proteins which work in an interdependent manner to drive DNA synthesis by interfering with Rb (E7) and to prevent p53-induced apoptosis (E6), thus bypassing normal checkpoints and promoting cell proliferation [reviewed by (Longworth and Laimins,

2004)]. As described in Section II, the E4 ORF codes for a small protein that shows partial targeting to mitochondria and disrupts mitochondrial distribution and membrane potential.

5. HUMAN T-CELL LEUKEMIA VIRUS TYPE 1

HTLV-1 was the first human retrovirus to be identified and is the only one with a direct etiological link to cancer. HTLV-1 is classified as a 'complex' retrovirus, as its genome contains extra open reading frames (ORFs) in addition to the *gag*, *pol*, *pro* and *env* genes common to all retroviruses [reviewed by (Green and Chen, 2001)]. The extra ORFs in HTLV-1 code for a transcriptional transactivator (Tax), a post-transcriptional regulatory protein (Rex) and 3 accessory proteins whose functions are currently under study [reviewed by (Albrecht and Lairmore, 2002)]. HTLV-1 is the causative agent of adult T-cell leukemia/lymphoma (ATLL), an aggressive neoplasm of mature CD4+ cells, and tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM), a severe demyelinating neuropathy. Worldwide, 15-25 million people are infected with HTLV-1, with higher prevalences in southwestern Japan and the Caribbean basin. The virus is transmitted through blood, semen, and breast milk. While most infected individuals remain asymptomatic, about 5% eventually develop TSP/HAM or ATLL after a clinical latency of several years (TSP/HAM) to decades (ATLL) [for recent reviews on HTLV-1 pathogenesis, see (Franchini et al., 2003; Matsuoka, 2003)].

HTLV-1 is found mainly in CD4+ T-cells *in vivo*. Infection of PBMC with HTLV-1 yields IL-2-dependent immortalized T cells, some of which progress to a fully transformed phenotype with IL-2-independent growth. The immortalizing potential of HTLV-1 is attributable primarily to the viral protein Tax which, in addition to transactivating the viral promoter, affects the expression and function of cellular genes controlling signal transduction, cell growth, apoptosis and chromosomal stability. Although Tax is able to immortalize T cells, other undefined viral and host factors are necessary to achieve the transformed phenotype of ATLL cells. This and other unclear aspects of HTLV-1

pathogenesis (e.g., HTLV-1's *in vivo* tropism for T-cells and association with 2 distinct diseases, both with a long latency and low prevalence) are being addressed from a number of directions, including studies of the accessory proteins, one of which, $p13^{II}$, is targeted to mitochondria (see Section II).

6. HEPATITIS C VIRUS

HCV is classified in the *Hepacivirus* genus of *Flaviviridae*, a family of enveloped, positive-strand RNA viruses; unlike HTLV-1, the replication cycle of HCV does not include a DNA intermediate, and its genome thus is not integrated into that of the host [reviewed by (Major et al., 2001)]. Over 170 million people worldwide are chronically infected with the virus, with infection most often traceable to contact with infected blood. The high prevalence of chronic infection reflects the fact that the majority of HCV-infected individuals (50-80%) are unable to clear the virus due to its ability to escape the immune system. Although HCV is mainly hepatotropic and is associated with chronic hepatitis, cirrhosis and HCC, the virus may also infect lymphoid cells, and is linked with immune system disturbances, lymphoproliferative disorders (e.g., mixed cryoglobulinemia), B-cell lymphomas, and other extrahepatic manifestations [reviewed by (Pawlotsky, 2004; Poynard et al., 2003; Zuckerman and Zuckerman, 2002)].

The RNA genome of HCV is translated into a single polyprotein, which is then cleaved to produce at least 10 mature proteins comprising virion components, enzymes, and regulatory proteins. Studies of the role of individual HCV proteins in oncogenesis have paid particular attention to the core protein, which associates with the viral genome in the nucleocapsid, and the nonstructural protein NS5A, which forms part of the viral replicase complex. The core protein is also able to influence cell proliferation, senescence, apoptosis, and immune modulation [reviewed by (Ray and Ray, 2001)], and NS5A influences cell growth, intracellular calcium homeostasis, apoptosis, and the IFN-α-mediated

antiviral response [reviewed by (Reyes, 2002)]. As described in Section II, HCV also produces a small mitochondrial protein named p7 that displays channel-forming activity.

B. Mitochondria as Central Regulators of Energy Conservation, Signal Transduction, and Cell Death

Disruption of mitochondrial function is associated with a wide variety of phenotypes ranging from aging to neuromuscular diseases and cancer [reviewed by (Chinnery and Schon, 2003)]. Before describing how tumor viruses manipulate cell physiology through the expression of mitochondrial proteins, it is important to briefly summarize the major physiological activities of mitochondria.

1. ENERGY PRODUCTION AND THE WARBURG EFFECT

As the sites of pyruvate- and fatty acid oxidation, the citric acid cycle, and the electron transport system, mitochondria are the main power plants for ATP production in almost all eukaryotic cells. The hypothesis that perturbations in mitochondrial energy production can contribute to neoplastic transformation arose from studies initiated by Otto Warburg in the 1930s, which revealed that cancer cells exhibit elevated rates of glycolysis and lactate production even when supplied with sufficient oxygen to drive the electron transport system (Warburg, 1956). This metabolic reprogramming from oxidative phosphorylation to aerobic glycolysis, termed the "Warburg effect", has been confirmed in a very large number of human tumours. The Warburg effect is likely to result in part from the adaptation of cancer cells to hypoxia arising from the high proliferation and uncoordinated blood vessel neoformation that characterize tumor growth. This adaptative change is largely controlled by the hypoxia-induced factor (HIF) family of transcription factors, which activate expression of genes involved in glucose transport and glycolysis, angiogenesis, cell proliferation, pH regulation, and apoptosis [reviewed by (Acker and Plate, 2002)]. It is noteworthy that diversion of pyruvate from

oxidative metabolism to lactic acid formation is also favored by overexpression of the Myc oncogene (Shim et al., 1997), which induces expression of lactate dehydrogenase A, the enzyme responsible for conversion of pyruvate to lactate. Although current hypotheses to explain the Warburg effect place greater emphasis on alterations in the expression of glycolytic enzymes rather than mitochondrial defects as originally proposed by Warburg, the emerging connection between mutations in mitochondrial enzymes and some cancers [reviewed by (Eng et al., 2003)], together with protein expression profiles of normal vs. tumor cells (Unwin et al., 2003), support involvement of both glycolytic and mitochondrial enzymes.

2. INTRACELLULAR CALCIUM HOMEOSTASIS

Ca^{2+}-signalling controls a vast number of biological responses ranging from muscle contraction to secretion, control of transcription, and apoptosis. These signals are determined by the amplitude, number and duration of elevations in the cytosolic Ca^{2+} concentration, which result mainly from mobilization of Ca^{2+} from the endoplasmic reticulum (ER) via triggering of the IP3- and ryanodine receptors and by entry from the extracellular medium through plasma membrane channels. In this scenario, mitochondria act as key regulators through their ability to take up Ca^{2+} [reviewed by (Rizzuto et al., 2000)]. This process is driven by the mitochondrial transmembrane potential ($\Delta\psi_m$) and occurs through the Ca^{2+} uniporter, a high affinity Ca^{2+} channel located in the inner membrane (Kirichok et al., 2004) and/or via the 'rapid uptake mode' (Gunter et al., 2004). Ca^{2+} uptake by mitochondria is thought to occur mainly at points of close proximity with the ER and plasma membrane, which represent the major sites controlling elevations in cytosolic Ca^{2+}. The functional consequences of mitochondrial Ca^{2+} uptake are to spatially and temporally confine elevated cytosolic Ca^{2+} concentrations, and to modulate feedback controls of store-operated plasma membrane Ca^{2+} channels, thus controlling the duration of Ca^{2+} influx from the extracellular environment (capacitive entry). An increase in the mitochondrial

matrix Ca^{2+} concentration may also result in the activation of Ca^{2+}-dependent dehydrogenases, with a consequent increase in oxidative metabolism and ATP production [reviewed by (Hajnoczky et al., 2002)].

3. REDOX BALANCE

The mitochondrial electron transport chain is a major source of reactive oxygen species (ROS) production at the level of complex I and, more importantly, complex III. In particular, the complex III-catalyzed transfer of an electron to molecular oxygen generates superoxide anion, which is then converted to peroxide by superoxide dismutase and subsequently detoxified by the action of thioredoxine reductase or glutathione peroxidase. Superoxide and peroxide which have escaped detoxification can generate toxic peroxynitrite and hydroxyl anions, respectively. Overwhelming the cell's protective enzymes (e.g. superoxide dismutase) and antioxidants (e.g., glutathione) with ROS can result in lipid peroxidation, protein- and DNA oxidation, a shift in the thiol/disulfide redox state, and cell death by necrosis or apoptosis [reviewed by (Fleury et al., 2002)]. Aside from their potentially toxic effects, ROS, as well as the cell's general redox status, influence a variety of signalling pathways, including those involving JNK, p38 MAPK, NF-κB, and AP-1 [reviewed by (Droge, 2002)]. ROS can also trigger release of calcium from mitochondria, which can in turn affect the activities of calcium dependent enzymes (e.g., nucleases) and influence calcium-dependent signalling pathways. The close proximity of the mitochondrial genome to the electron transport chain, together with its limited repair capacity and lack of introns or protective proteins such as histones, render it particularly sensitive to ROS-induced damage [reviewed by (Mandavilli et al., 2002)].

4. CELL DEATH

Disruption of mitochondrial function can cause cell death through ATP depletion and deregulation of Ca^{2+} signaling pathways. In addition, mitochondria play a central role in apoptotic cascades by releasing pro-apoptotic proteins in response to extrinsic stimuli (e.g., engagement of the TNF receptor) and intrinsic stimuli (e.g., DNA damage). The following sections describe some of the major participants in the apoptotic events controlled by mitochondria [for a general review on apoptosis, see (Danial and Korsmeyer, 2004)].

a. The BCL-2 Family

Mitochondria are recruited to death signalling pathways in response to targeting/multimerization of proteins of the BCL-2 family in their outer membrane, followed by release of pro-apoptotic proteins located in the intermembrane space (e.g. cytochrome *c*). The more than 20 mammalian BCL-2 proteins described to date are classified based on their pro- or anti-apoptotic properties and on the presence of 4 domains termed BCL-2-homologous regions (BH) arranged in the order BH4-BH3-BH1-BH2; an additional C-terminal hydrophobic transmembrane (TM) domain mediates anchoring of the protein to the mitochondrial outer membrane and endomembranes [reviewed by (Scorrano and Korsmeyer, 2003)]. The BH1 and BH2 domains are the most highly conserved of the four, and are important for anti-apoptotic activity and formation of multimers between BCL-2 family members; the BH3 domain is important for pro-apoptotic activity; and the poorly conserved BH4 domain is required for the anti-apoptotic activities of BCL-2 and BCL-x_L. Most of the anti-apoptotic BCL-2 proteins (e.g. BCL-2, BCL-x_L) contain all 4 BH domains and a TM domain; a few lack a BH3 or TM domain. The pro-apoptotic BCL-2 proteins are distinguished by the absence of a BH4 domain and the presence of a BH3 domain with or without other domains; e.g., BH3-BH1-BH2-TM (BAX, BAK BOK), BH3-TM (e.g., BIK) or BH3 only (BID, BAD, NOXA).

The various BCL-2 proteins can accumulate in the ER membrane, mitochondrial outer membrane, nuclear envelope and/or cytosol depending on their domain structure, post-translational modifications, and interactions with other proteins. Structural analogies between BCL-x_L and the membrane insertion domains of bacterial toxins suggest that the multidomain BCL-2 proteins might form pores or channels that can influence apoptotic signals and bioenergetics (Muchmore et al., 1996). For example, multimerization of BAX and BAK in the outer mitochondrial membrane is thought to result in the formation of channels that allow release of cytochrome *c* and other pro-apoptotic factors. The BH3-only proteins activate this process through direct interaction of their BH3 domains with a hydrophobic pocket formed by the BH1, BH2 and BH3 domains of the multidomain pro-apoptotic proteins. One such BH3-only protein is BID, which, upon engagement of death receptors, is converted to its active form tBID through cleavage by caspase 8. tBID then relocates to the mitochondrial outer membrane (Li et al., 1998; Luo et al., 1998), where it promotes formation of BAX complexes, resulting in cytochrome *c* release (Eskes et al., 2000). Anti-apoptotic proteins such as BCL-2 are thought to sequester the BH3-only proteins in their hydrophobic pockets and thus interfere with activation of the multidomain pro-apoptotic proteins. Accumulation of anti-apoptotic BCL-2 proteins in the outer mitochondrial membrane may also interfere with oligomerization of BAX through a steric hindrance mechanism (Mikhailov et al., 2001). In addition, emerging evidence indicates that BCL-2 proteins can influence apoptosis by altering the ER's Ca^{2+} storage capacity (Scorrano et al., 2003). The recent finding that BAD forms a mitochondrial complex that includes glucokinase, a key enzyme controlling glucose transport into the cell and glycolysis, underscores the role of mitochondrial proteins in integrating metabolism and programmed cell death (Danial et al., 2003). As described in Section II, EBV, KSHV, and other gammaherpesviruses encode viral BCL-2 homologues.

Released cytochrome *c* contributes to assembly of the apoptosome, a multimeric complex of APAF-1, procaspase 9, cytochrome *c* and ATP that allows caspase 9 activation (Zou et al., 1999).

Caspase 9 in turn activates caspase 3, thus effectively amplifying the death signal triggered by engagement of the surface receptors (Zou, et al., 1999). In addition to cytochrome *c*, mitochondria release a variety of other apoptogenic proteins [reviewed by (Saelens et al., 2004)]. These include Smac-DIABLO [an antagonist of inhibitor of apoptosis proteins (IAP) that normally prevent procaspase 9 activation], apoptosis inducing factor, and endonuclease G; the latter two factors may activate nuclear degradation and thus amplify apoptosis independent of caspase 9 activation. As described in Section II, KSHV interferes with formation of a functional apoptosome through production of an IAP homologue.

b. The Permeability Transition Pore (PTP)

Pro-apoptotic signals are also determined by the activity of the PTP, a high conductance mitochondrial channel whose structure remains to be defined. (Klohn et al., 2003; Marzo et al., 1998; Susin et al., 1996). When stabilized in the open conformation, the PTP causes an increase in the inner mitochondrial membrane permeability to ions and solutes with an exclusion limit of approximately 1,500 Da, which causes dissipation of the mitochondrial membrane potential and diffusion of solutes down their concentration gradients, a phenomenon termed the permeability transition. Studies performed on isolated mitochondria showed that PTP opening is followed by osmotic water flux, passive swelling, outer membrane rupture, and cytochrome *c* release. The PTP is inhibited by cyclosporin A (CsA), and is regulated by a striking number of modulators (e.g., voltage, matrix Ca^{2+}, matrix pH, redox potential) and signalling molecules (such as arachidonic acid and complex lipids) that are also involved in cell death (Bernardi, 1999; Bernardi et al., 1999). Because of the ensuing depletion of ATP and Ca^{2+} deregulation, opening of the PTP had been proposed to be a key element in determining the cell fate before a role for mitochondria in apoptosis was discovered (Duchen et al., 1993; Imberti et al., 1993; Pastorino et al., 1993). Whether the PTP also plays a role in the BAX-

dependent release of pro-apoptotic factors and in apoptotic signalling through surface receptors has been the subject of considerable controversy (Appaix et al., 2002; Bradham et al., 1998; Di Lisa et al., 2001; Eskes et al., 1998; Kluck et al., 1999; Pastorino et al., 1998; Scorrano et al., 2001; Scorrano et al., 1999). These apparently conflicting reports may be resolved by studies of mitochondrial ultrastructure that suggest a functional separation between the intercristal and intermembrane spaces (Frey and Mannella, 2000), consistent with earlier observations that defined the existence of two pools of cytochrome *c* in mitochondria (Bernardi and Azzone, 1981). Thus, permeabilization of the outer mitochondrial membrane by tBID/BAX alone may not result in complete release of cytochrome *c*, which may demand a redistribution of the mitochondrial cytochrome *c* pools contained within the cristae to the intermembrane space. Another study demonstrated BAX-dependent inner membrane remodeling with cytochrome *c* release through an apparently intact outer membrane (Scorrano et al., 2002).

Three recent observations support the PTP's relevance to apoptosis *in vivo*: (i) inappropriate PTP opening results in cell death in a myopathic mouse model of collagen VI deficiency (Irwin et al., 2003); (ii) inhibition of PTP opening causes increased resistance to apoptosis prior to liver carcinogenesis by 2-acetylaminofluorene, the first example of tumor promotion by mitochondria *in vivo* (Klohn, et al., 2003); and (iii) desensitization of the PTP with cyclosporin A prevents activation of the mitochondrial pro-apoptotic pathway and liver damage induced by TNFα (Soriano et al., 2004).

c. The Voltage-Dependent Anion Channel (VDAC)

VDAC, the most abundant protein in the outer mitochondrial membrane, forms channels which are thought to function as the primary pathway for the movement of metabolites across the outer membrane [reviewed by (Colombini et al., 1996)]. When purified and reconstituted into planar lipid bilayers, VDAC forms anion-selective channels with an open channel diameter of ~ 3 nm. When either

positive or negative voltages are applied, VDAC closes to form cation-selective channels with a smaller diameter and lower conductance.

Conflicting reports have linked VDAC to the release of mitochondrial pro-apoptotic proteins. Tsujimoto and colleagues have proposed that cytochrome *c* is released from mitochondria through the direct association of VDAC with pro-apoptotic BCL-2 proteins to form a novel, voltage-independent pore of sufficient size to release cytochrome *c* (Shimizu et al., 1999). Anti-apoptotic BCL-2 proteins would block formation of this channel and drive closure of VDAC (Shimizu et al., 2000). In contrast, Thompson and co-workers proposed that the primary response of mitochondria to death signals is VDAC closure (Vander Heiden et al., 1999; Vander Heiden et al., 1997), resulting in a block in the exchange of metabolic anions with the cytoplasm and generation of ionic gradients across the outer membrane and hyperpolarization of the inner membrane, leading to matrix swelling and loss of outer membrane integrity (Vander Heiden et al., 2000). Anti-apoptotic BCL-2 proteins would prevent the loss of outer membrane permeability by the formation of a channel that collapses the hypothetical transmembrane potential across the outer membrane and thereby maintain VDAC in an open configuration, thus allowing the exchange of metabolic anions (Bernardi et al., 2001; Vander Heiden, et al., 2000).

At any rate, solid experimental evidence links VDAC to the PTP (Crompton et al., 1998; Le-Quoc and Le-Quoc, 1985; Szabo et al., 1993; Szabo and Zoratti, 1993). A functional screen for PTP inhibitors identified a novel agent, Ro 68-3400, which inhibits the permeability transition at submicromolar concentrations through a site distinct from that of CsA and binds to isoform 1 of VDAC (VDAC1) (Cesura et al., 2003). As described in Section II, VDAC has been identified as a target of the HBx protein of HBV.

d. The Adenine Nucleotide Translocator (ANT)

PTP opening is accompanied by inner membrane permeabilization, indicating that one or more inner membrane proteins must participate in this process. One candidate is the adenine nucleotide translocator (ANT) (Hunter and Haworth, 1979), the inner membrane protein that catalyzes the exchange of ATP for ADP, thus allowing the export of ATP from respiring, energized mitochondria (Pebay-Peyroula et al., 2003). The evidence linking the ANT to the permeability transition is essentially based on the effects of atractylate and bongkrekate. However, while both compounds inhibit ATP-ADP exchange catalyzed by ANT, the permeability transition is favored by atractylate and inhibited by bongkrekate, thus arguing against a causal link between ATP-ADP exchange and the permeability transition. A step forward on this topic was achieved through recent experiments performed on liver mitochondria obtained from ANT-knockout mice (Kokoszka et al., 2004). ANT(-) mitochondria underwent a Ca^{2+}-dependent permeability transition that could be triggered by all PTP inducers except atractylate, demonstrating that although ANT modulation is capable of affecting the permeability transition, it is not essential for this process. Furthermore, the permeability transition in ANT(-) mitochondria was fully inhibited by CsA, ruling out a role for ANT in CsA-dependent inhibition of the PTP.

Consistent with the central role played by the PTP in controlling cell death and tumor pathogenesis, many drugs acting directly or indirectly on the PTP exhibit anticancer effects (Costantini et al., 2000). These drugs act through a variety of mechanisms, including interaction with proteins and complexes involved in the control of the permeability transition, such as BCL-2, BAX, VDAC, and ANT, as well as cyclophilin D and the peripheral benzodiazepine receptor, two other possible components of the PTP. Interestingly, ANT is the target of several viral mitochondrial proteins with an impact on apoptosis, including HIV Vpr (see Section II).

II. MITOCHONDRIAL PROTEINS CODED BY HUMAN TUMOR VIRUSES

Mitochondrial proteins coded by human tumor viruses can be grouped into three major functional classes: (i) homologues of the BCL-2 family; (ii) proteins that interfere with effector caspases (vIAP); and (iii) proteins that alter mitochondrial ion permeability and/or membrane potential; insufficient information is available to allow functional classification of the K15 protein of KSHV (Table 1).

A. Viral BCL-2 Homologues (vBCL-2)

To date, viral BCL-2 homologues have been identified in fowlpoxvirus, African swine fever virus, human adenoviruses, and all members of the gammaherpesvirus subfamily of herpesvirues, including the human tumor viruses EBV and KSHV (Table 2) [for detailed reviews of vBCL-2s, see (Cuconati and White, 2002) and (Hardwick and Bellows, 2003)]. In addition, Hepatitis C virus might soon join this list based on the report that one of its products, named NS5A, contains BH1, BH2 and BH3 domains and exhibits vBCL-2-like activity (Chung et al., 2003).

vBCL-2 proteins are thought to counteract apoptosis triggered by the natural host immune response, by unscheduled growth signals provided by other viral proteins such as v-cyclins and transcriptional activators, and by stress signals resulting from hijacking of the host cell's machinery to synthesize viral products. The net effect of vBCL-2 activity during primary infection is therefore prolongation of the lifespan of host cells, resulting in higher numbers of viral progeny and spread of infection to new cells. Expression of vBCL-2 proteins also enables latently infecting viruses to make the transition to productive infection, thus favoring viral persistence. As described below, the EBV BALF1 protein might be an exception, as one of its tasks might be to counterbalance the anti-apoptotic effects of the virus's other vBCL-2, BHRF1.

The E1B 19K protein of human adenovirus represents the first vBCL-2 described and provides a clear example of how a vBCL-2 can contribute to viral transformation by counteracting apoptosis

triggered by an unscheduled viral proliferative stimulus. E1B 19K contains BH3 and BH1 domains, and although lacking a recognizable transmembrane domain, its modification with fatty acyl groups is likely to mediate its membrane targeting (McGlade et al., 1987; Smith et al., 1989), primarily in the nuclear envelope and ER (Boyd et al., 1994). Adenovirus mutants lacking functional E1B 19K display increased cytopathic effects indicative of apoptosis and decreased production of viral particles as well as a reduction in the ability to transform rodent cells (Pilder et al., 1984; Takemori et al., 1984; White et al., 1984). These effects arise from the activity of the viral protein E1A, whose normal task, to drive quiescent cells into the S phase, causes p53-dependent apoptosis in the absence of counterbalancing anti-apoptotic factors. This death pathway is blocked by E1B 19K (White, 1993; White, 2001) and by E1B 55K and E4orf6, which accelerate p53 degradation (Steegenga et al., 1998). The pro-apoptotic effect of E1B 19K-mutant adenoviruses can be overcome by expression of BCL-2 (Chiou et al., 1994; Subramanian et al., 1995; Subramanian et al., 1993) or by ablating expression of BAX and BAK (Cuconati et al., 2002). E1B 19K interacts with BAX, BAK, and several other pro-apoptotic BCL-2s (Boyd, 1994; Boyd et al., 1995; Boyd, et al., 1994; Chen et al., 1996; Farrow and Brown, 1996; Farrow et al., 1995; Han et al., 1996a; Han et al., 1996b), blocks a conformational change in BAX (Sundararajan and White, 2001), and interferes with formation of BAK/BAX complexes (Sundararajan et al., 2001). Interference with BAK/BAX multimerization blocks release of cytochrome *c* and Smac/DIABLO from mitochondria (Cuconati, et al., 2002; Cuconati and White, 2002; Henry et al., 2002). Although prolonged stimulation of the p53 apoptotic pathway eventually leads to release of cytochrome *c* and Smac/DIABLO, caspase 9 and caspase 3 are not activated and the cells do not undergo apoptosis (Henry, et al., 2002), suggesting that E1B 19K might have other as yet undefined postmitochondrial effects. E1B 19K also interferes with other apoptotic stimuli such as TNF-α, anti-Fas antibodies, and ectopic expression of BIK (Boyd, et al., 1995; Gooding et al., 1991; Hashimoto et

al., 1991). In addition to blocking apoptosis, E1B 19K was recently shown to inhibit senescence of bladder cancer cells resulting from p53-induced ROS accumulation (Jung et al., 2004).

While vBCL-2s and cellular BCL-2s share limited sequence homology, their secondary structures are predicted to be quite similar, consisting of a series of α-helixes and loops [reviewed by (Cuconati and White, 2002)]. NMR spectroscopy performed on BHRF1 and KSBCL-2 confirmed their substantial structural similarity with BCL-2 and BCL-x_L (Huang et al., 2002; Huang et al., 2003). However, BHRF1 does not contain the hydrophobic groove important for formation of a BAK-BCL-x_L complex (Sattler et al., 1997), and its BH1 domain presents a less accessible conformation and does not contain the characteristic sequence NWGR proposed to be important for the anti-apoptotic activity of BCL-2 and formation of BCL-2 family heterodimers (Yin et al., 1994).

With the exception of BALF0/BALF1, all of the vBCL-2s are anti-apoptotic, and, unlike their cellular anti-apoptotic homologues, apparently cannot be converted to pro-apoptotic forms. This property was first described by Cheng et al., who demonstrated pro-apoptotic conversion of BCL-2 following its caspase cleavage (Cheng et al., 1997). Among 5 herpesvirus vBCL-2s tested, only that of the murine gammaherpesvirus γ-HV68 was cleaved by caspase, although the truncated product did not exhibit pro-apoptotic activity (Bellows et al., 2000). This resistance to cleavage reflects the absence of a cleavage site that is found in the loop separating the first 2 α-helices (just C-terminal to the BH4 domain) present in BCL-2 and BCL-x_L (Cheng, et al., 1997). Another noteworthy difference between vBCL-2s and cellular BCL-2 is suggested by the finding that apoptosis induced by KSHV v-cyclin could be blocked by co-expression of KSBCL-2 but not by cellular BCL-2 (Ojala et al., 2000). This difference may reflect the ability of v-cyclin-CDK6 complexes to inactivate BCL-2 by phosphorylating it on serines in the loop that connects its first 2 α-helixes; KSBCL-2 lacks these serines, and thus is not subjected to this inactivating modification (Ojala, et al., 2000). These findings suggest that, unlike cellular BCL-2 proteins, the herpesvirus vBCL-2s are locked in an anti-apoptotic mode [reviewed by

(Hardwick and Bellows, 2003)]. Cells expressing vBCL-2 are therefore affected by both inappropriate expression of the anti-apoptotic factor and deregulated function.

1. The BHRF1 PROTEIN OF EBV

BHRF1 was the first viral gene found to share sequence homology with BCL-2 (Cleary et al., 1986). Early studies indicated that BHRF1 is abundantly expressed in the early lytic phase of infection and fractionates with endomembranes (Pearson et al., 1987). Subsequent analyses showed that BHRF1 partially colocalizes with BCL-2 in structures resembling the ER and mitochondria (Henderson et al., 1993), and later confirmed its targeting to mitochondria (Bellows et al., 2002; Hickish et al., 1994; Khanim et al., 1997; Yasuda et al., 1998).

The anti-apoptotic effects of BHRF1 were first demonstrated in studies carried out using EBV-positive Burkitt's lymphoma cell lines stably transfected with BHRF1, which revealed the ability of the protein to protect against apoptosis induced by serum withdrawal or the calcium ionophore ionomycin, without affecting the viral latency pattern or cell growth under standard culture conditions (Henderson, et al., 1993). Subsequent studies confirmed the ability of BHRF1 to protect many cell types from apoptosis induced by a broad spectrum of extrinsic and intrinsic apoptotic stimuli, including exposure to TNF-α-related apoptosis-inducing ligand (TRAIL) (Kawanishi et al., 2002), TNF-α (Foghsgaard and Jaattela, 1997; Kawanishi, 1997), anti-Fas antibody (Foghsgaard and Jaattela, 1997; Kawanishi, 1997), DNA damaging agents (Henderson, et al., 1993; Khanim, et al., 1997; McCarthy et al., 1996; Tarodi et al., 1994), gamma radiation (McCarthy, et al., 1996), deprivation of growth factors (Foghsgaard and Jaattela, 1997) or cytokines (Takayama et al., 1994), overexpression of BIK (Boyd, et al., 1995) or BOK (Hsu et al., 1997), expression of E1A plus p53 (D'Sa-Eipper et al., 1996), and infection with an E1B 19K-deleted adenovirus (Tarodi, et al., 1994) or with Sindbis virus (Cheng, et al., 1997). BHRF1 was shown to act downstream of BID activation and prevent mitochondrial

depolarization in response to treatment with the PTP opener t-BHP (t-butylhydroperoxide) (Kawanishi, et al., 2002).

Yeast 2-hybrid assays demonstrated that BHRF1 associates with several pro-apoptotic BCL-2 proteins, including BAK, BAX, BIK and BOK (Boyd, et al., 1995; Chittenden et al., 1995; Hsu, et al., 1997), suggesting that its anti-apoptotic effect involves interaction with these proteins (see Figure 1). Although interactions between BHRF1 and anti-apoptotic BCL-2 members have been detected in co-immunoprecipitations and GST-pulldowns, interpretation of these results is complicated by the tendency of BCL-2 family members to form non-specific complexes in the presence of nonionic detergents commonly used in such assays (Hsu and Youle, 1997). Yeast 2-hybrid screens also demonstrated the ability of BHRF1 to interact with R-Ras (Theodorakis et al., 1996), a plasma membrane GTPase that interferes with H-Ras, and with prenylated rab acceptor 1 (PRA1), a Golgi protein involved in vesicular trafficking (Li et al., 2001). Interestingly, coexpression of PRA1 interferes with the ability of BHRF1 to protect against apoptosis induced by DNA-damaging agents; in contrast, BCL-2 does not bind to PRA1 and is not inhibited by it (Li, et al., 2001).

a. BHRF1 and Tumorigenesis

The strong conservation of BHRF1 among EBV isolates (Khanim, et al., 1997) and EBV-related primate herpesviruses (Williams et al., 2001) points toward its functional relevance. The ability of BHRF1 to inhibit TNF- and Fas-induced apoptosis implies that it should promote viral replication in the infected host, given the role of these pathways in the anti-viral response. The higher viral loads achieved through the action of BHRF1 could in turn increase the odds of transformation. However, BHRF1 is not required for EBV-mediated transformation of primary B cells to lymphoblastoid cell

lines (LCL) or for completion of the EBV replication cycle (Lee and Yates, 1992; Marchini et al., 1991). In addition, BHRF1 is unable to transform primary baby rat kidney (BRK) cells by itself; however, its coexpression with the adenovirus E1A gene results in transformed foci (D'Sa-Eipper, et al., 1996), consistent with the hypothesis that the survival signal provided by anti-apoptotic BCL-2 proteins favors, but is not by itself sufficient for, a fully transformed phenotype. A study carried out using baby rat kidney (BRK) cells expressing a temperature-sensitive p53 mutant plus E1A revealed two BHRF1 mutants (one with a double substitution between the BH4 and BH3 domains; the other with a double substitution within the BH3 region) which, in addition to suppressing apoptosis, were defective for binding to R-Ras and promoted cell proliferation, suggesting the presence of a region with proliferation-restraining activity (Theodorakis, et al., 1996). Interestingly, cotransfection of BRK cells with E1A and the gain-of-function BHRF1 mutants produced more foci compared to E1A plus wild-type BHRF1 (Theodorakis, et al., 1996), suggesting that natural mutations in BHRF1 or perturbations in its interaction with R-Ras might result in both increased survival and proliferation, and thus magnify the contribution of BHRF1 to neoplastic transformation.

BHRF1 also protects Rat-1 fibroblasts from apoptosis induced by c-myc overexpression in combination with serum starvation or an S-phase block (Fanidi et al., 1998). This effect is particularly interesting, as most Burkitt's lymphomas exhibit deregulated c-Myc expression as a result of c-myc-Ig translocations, and suggests that Burkitt's lymphoma cells might need either BHRF1 or BCL-2 to overcome the pro-apoptotic effects of c-myc at some point on their way to transformation. Speculation regarding the role of BHRF1 in EBV-associated tumorigenesis must take into account the fact that EBV LMP1 upregulates expression of BCL-2 and BCL-x_L, and that some EBV-associated tumors express high levels of BCL-2. The importance of this event to EBV-associated lymphomagenesis is supported by the recent finding that exposure of EBV(+) LCLs to an antisense BCL-2 oligonucleotide increased their sensitivity to apoptosis induced by an anti-CD20 antibody (Loomis et al., 2003).

A possible role for BHRF1 in the pathogenesis of epithelial neoplasia is more evident. An examination of the effects of BHRF1 expression on the growth properties of a human squamous cell carcinoma cell line that responds to terminal differentiation signals indicated that the vBCL-2 is able to interfere with normal epithelial cell differentiation, a process that is dependent on apoptosis triggered as a consequence of downregulation of BCL-2, whose expression is limited to the basal cell layer (Hockenbery et al., 1991). When cultivated as monolayers, the BHRF1-expressing cell lines showed less cell-cell contact and did not completely cover the substratum when grown to confluency compared to control cells (Dawson et al., 1998; Dawson et al., 1995). BHRF1 disrupted the differentiated phenotype assumed by the cells when grown in organotypic raft cultures (Dawson, et al., 1995). BHRF1 expression also altered the morphological properties of the characteristic cysts formed by these cells after subcutaneous inoculation into nude mice, resulting in expansion of the differentiating epithelial cell layers and other aberrations. The BHRF1-expressing cell lines also showed a reduced propensity to terminally differentiate and enter apoptosis when grown as suspension cultures, and were less sensitive to apoptosis induced by cis-platin, required less fetal calf serum in order to grow, and exhibited increased proliferation (Dawson, et al., 1998). This latter effect might reflect functional inactivation of the 'proliferation control' domain identified by Theodorakis et al. (Theodorakis, et al., 1996). The ability of BHRF1 to interfere with differentiation would be expected to increase the pool of cells able to replicate the virus. It is also possible that some cells that enter the lytic phase and express early lytic proteins such as BHRF1 do not complete the replicative cycle and thus fail to die; such a cell population would be capable of propagating genetic changes favoring oncogenic transformation.

b. BALF0/BALF1 as Possible BHRF1 Antagonists

A second EBV vBCL-2, coded by the early lytic gene BALF1, was initially identified through a BLAST search (Marshall et al., 1999). BALF1 is predicted to contain all of the BH domains, including

BH4 (Marshall, et al., 1999). A subsequent study pointed out the presence of a TATA box upstream of a second ATG in the BALF1 ORF whose use would give rise to an mRNA coding for a 182-amino acid BALF1 isoform lacking most of the putative BH4 domain; the authors named this isoform BALF1 and renamed the longer (220-amino acid) product BALF0 (Bellows, et al., 2002). The BH1 domain of BALF0 and BALF1 differs from that of other BCL-2 proteins in that it contains a serine instead of a highly conserved glycine essential for the anti-apoptotic function of BCL-2. BALF0 and BALF1 also lack the C-terminal hydrophobic domain found in other gammaherpesvirus vBCL-2s. Initial studies indicated that BALF0 protects cells against a variety of apoptotic stimuli (Marshall, et al., 1999), while others then showed that it was unable protect against apoptosis induced by Sindbis virus infection or BAX overexpression, both of which can be blocked by BHRF1 (Bellows, et al., 2002). BALF0 and BALF1 antagonize the ability of BHRF1 (and its KSHV homologue KSBCL-2) to block apoptosis induced by BAX overexpression without interfering with the protective effects of BCL-X_L, suggesting that they act as specific inhibitors of vBCL-2s (Bellows, et al., 2002). BALF1 and BALF0 are detected in the cytoplasm rather than in mitochondria with BHRF1; furthermore, the 3 proteins do not change each others' distribution when co-expressed, suggesting that they do not form complexes (Bellows, et al., 2002).

2. THE KSBCL-2 PROTEIN OF KSHV

The presence of a vBCL-2 gene in KSHV was predicted based on sequence comparisons with the primate gammaherpesvirus Herpes Virus Saimiri, which encodes a vBCL-2 gene named ORF 16 (Cheng, et al., 1997; Sarid et al., 1997). Sequence comparisons revealed 15-20% homology between the KSBCL-2 protein and other BCL-2s, with its BH1, BH2 and TM domains showing the greatest degree of conservation (Cheng, et al., 1997).

While results of a yeast 2-hybrid study indicated that KSBCL-2 binds to BCL-2 (Sarid, et al., 1997), a second study failed to detect interactions with cellular BCL-2 family members (Cheng, et al., 1997). The KSBCL-2 mRNA is detected in KS lesions and PEL cell lines and is upregulated in PEL cells upon induction of lytic infection (Cheng, et al., 1997; Sarid, et al., 1997). Sarid et al. showed that KSBCL-2 accumulates in punctate cytoplasmic structures but did not identify them (Sarid, et al., 1997). KSBCL-2 protects cells from apoptosis induced by BAX (Sarid, et al., 1997) or by infection with Sindbis virus (Cheng, et al., 1997).

B. v-IAP - The K7 Protein of KSHV

The K7 ORF encodes a 126-amino acid protein with partial sequence homology to Survivin-deltaEx3, a cellular IAP (Wang et al., 2002). K7 contains an atypical amino-terminal mitochondrial targeting signal (MTS) partially overlapping with a putative transmembrane domain, a BIR (baculovirus IAP repeat), and BH2 domain in its carboxy-terminal portion, and is modified by N-glycosylation at arginine 108 (Wang, et al., 2002) (see Figure 2). K7 is detected in mitochondria, the ER, and nucleus (Feng et al., 2002; Wang, et al., 2002). Analysis of truncation mutants indicated that residues 23-73 are sufficient for mitochondrial targeting (Feng, et al., 2002), a puzzling result, since such a mutant would lack part of the putative MTS. K7-transfected HeLa cells are partially protected against apoptosis induced by BAX, TNF-α or anti-Fas antibody; the protective effect against the latter 2 stimuli is dependent on the presence of sequences lying beyond residue 72 that include the BIR and BH2 domains (Wang, et al., 2002). K7 expression partially protects against mitochondrial depolarization in response to treatment with anti-Fas, TRAIL, ceramide, and staurosporin. Pull-down experiments indicated that K7 interacts with BCL-2 through its BH2 domain and with caspase 3 through its BIR domain, resulting in the formation of a BCL-2/K7/caspase 3 complex that inhibits caspase 3 activity and blocks apoptosis (Wang, et al., 2002) (see Figure 1). Yeast 2-hybrid and co-

immunoprecipitation assays demonstrated that K7 also interacts with CAML (calcium-modulating cyclophilin ligand), an ER protein that controls intracellular Ca^{2+} homeostasis (Feng, et al., 2002). K7 and CAML display a similar ability to counteract the effect of thapsigargin, an inhibitor of the sarcoplasmic/ER Ca^{2+} ATPase (SERCA) that produces a transient increase in cytosolic Ca^{2+} concentration and depolarizes mitochondria. Upon treatment with thapsigargin, cells ectopically expressing either K7 or CAML exhibit a faster and more accentuated release of Ca^{2+} from the ER and capacitative Ca^{2+} entry (Feng, et al., 2002), an effect depending on the presence of residues 22-73 of K7 and the first 160 amino acids of CAML (Feng, et al., 2002). In contrast, K7 does not protect against mitochondrial depolarization induced by the Ca^{2+} ionophore A23187 (Feng, et al., 2002), suggesting that its primary effect is to modify ER Ca^{2+} loading/release. While the link between the effects of K7 on intracellular calcium levels and protection against apoptosis remains to be established, the observation that K7 is expressed very early after induction of lytic infection in PEL cells (Thome et al., 1997; Wang, et al., 2002) suggests that, similar to KSBCL-2, the protein might play a role in delaying cell death that would otherwise be triggered by viral replication, thus increasing virus persistence and propagation.

C. Proteins That Alter Mitochondrial Ion Permeability and/or Membrane Potential

1. THE HBx PROTEIN OF HBV

HBx is a 154 amino acid, 17-kDa protein that is produced from the X ORF of HBV. This ORF is conserved among all mammalian hepadnaviruses. Studies carried out using the woodchuck hepatitis virus model showed that the X ORF is required for the establishment of a productive infection *in vivo* (Chen et al., 1993; Zoulim et al., 1994). The fact that most HBV-associated HCCs selectively retain and express the X ORF points toward an important role for HBx in HCC development (Paterlini et al.,

1995; Unsal et al., 1994). The ability of HBx to transform NIH3T3 cells (Koike et al., 1989; Shirakata et al., 1989) and to produce tumors in some lines of transgenic mice [reviewed by (Koike, 2002)] also support its participation in neoplastic transformation. Interestingly, the X ORF is poorly conserved or absent in avian hepadnaviruses, which, unlike the mammalian viruses, show a much weaker association with HCC.

Immunohistochemistry performed on liver biopsies revealed accumulation of HBx in the nucleus of some cells and in both the nucleus and cytoplasm of others (Hoare et al., 2001). This localization pattern is also seen in HBx-transfected cells, with the cytoplasmic pattern consisting of dense granular structures (Doria et al., 1995) identified as mitochondria (Takada et al., 1999) and proteasomes (Sirma et al., 1998).

HBx has a variety of effects on the host cell that can positively or negatively influence growth and survival depending on the balance between viral, cellular and environmental signals [reviewed by (Bouchard and Schneider, 2004)]. HBX functions as a weak transcriptional activator of many promoters and enhancers, including the HBV EN1 enhancer, promoters of other viruses (e.g., HIV, SV40, HSV), and many cellular promoters. The transactivating ability of HBx is mediated by interactions with cellular transcription factors and components of signal transduction pathways that regulate cell growth, differentiation, and apoptosis, including the Ras-Raf-MAP kinase, JNK, Jak-STAT, NF-κB, PKC, PI3K, and Src pathways (Klein et al., 1999).

HBx has also been shown to bind to p53, inhibit its transcriptional activity, and protect against p53-mediated apoptosis (Feitelson et al., 1993; Takada et al., 1997; Wang et al., 1994). HBx induces retention of p53 in the cytoplasm, an effect that was mapped to carboxy-terminal sequences of both proteins (Takada, et al., 1997). The association between p53 and HBx, as well as abnormal cytoplasmic retention of p53, has also been observed in tumors arising in HBx-transgenic mice (Ueda et al., 1995). Furthermore, HBx has been shown to modulate nucleotide excision repair (NER) both through its

effects on p53 (Jia et al., 1999) and by binding to NER components (Becker et al., 1998; Capovilla and Arbuthnot, 2003; Jia, et al., 1999). HBx can also interfere with apoptosis by inhibiting caspase 3 (Gottlob et al., 1998; Lee et al., 2001).

Consistent with these properties, HBx can induces cells to exit quiescence, enter the S phase, and progress more rapidly through cell cycle checkpoints (Benn and Schneider, 1995; Koike et al., 1994). The growth-promoting activities of HBx are likely to have evolved to optimize viral replication in hepatocytes, which grow slowly under normal circumstances. As a side effect of bypassing cell cycle checkpoints, cells expressing HBx are more likely to accumulate transforming mutations.

Furthermore, HBx is able to increase the stability and activity of HIF-1α, one of the key mediators of the Warburg effect. HBx-induced upregulation of HIF-1α involves activation of the MAPK pathway (Yoo et al., 2003) as well as direct interaction with HIF-1α and inhibition of its ubiquitin-dependent degradation (Moon et al., 2004) and is linked to increased angiogenesis (Moon, et al., 2004; Yoo, et al., 2003).

On the other hand, after driving quiescent cells into the S phase, HBx can induce p53-mediated apoptosis (Chirillo et al., 1997). Subsequent studies confirmed the ability of HBx to interfere with cell growth (Bergametti et al., 1999; Kim et al., 1998) and promote apoptosis induced by serum deprivation, pro-apoptotic drugs, anti-Fas antibodies, or TNF-α, a cytokine implicated in HBV-associated liver damage (Kim and Seong, 2003; Su and Schneider, 1997). The ability of HBx to promote apoptosis triggered by TNF-α is linked to its binding to c-FLIP and consequent subtraction from death-inducing signalling complexes [DISC (Kim and Seong, 2003)]. Studies of HBx/BCL-2 bi-transgenic mice demonstrated that HBx can override BCL-2's protection against Fas-mediated apoptosis (Terradillos et al., 2002). Increased levels of spontaneous apoptosis have been detected in liver cells of HBx transgenic mice, indicating that the protein might play an important role in HBV-associated hepatocyte turnover (Koike et al., 1998; Pollicino et al., 1998; Terradillos et al., 1998).

HBx's transactivating function is required to induce apoptosis in some assays (Bergametti, et al., 1999) but not in others (Shirakata and Koike, 2003). Furthermore, while some studies support a role for p53 in the pro-apoptotic effect of HBx, others indicate that the phenomenon is p53-independent (Bergametti, et al., 1999; Sirma et al., 1999; Terradillos, et al., 1998). These discrepancies, as well as the fact that in some assays HBx only sensitizes cells to apoptotic stimuli rather that exhibiting intrinsic apoptotic activity, suggest that the protein might act through different mechanisms depending on the cell system and, perhaps, on its level of expression, which also affects its subcellular localization and function (see below).

Analysis of the HBx-p53 interaction demonstrated that HBx can relocalize p53 to mitochondria and induces clustering of mitochondria around the nucleus (Shintani et al., 1999; Takada, et al., 1997). The finding that HBx accumulates in mitochondria and changes mitochondrial architecture in p53-null cells, together with the observation that the carboxy terminal portion of HBx (residues 134-154) is not required for these effects, indicates that HBx targets to and alters mitochondria independently of p53 (Takada, et al., 1999). Additional studies confirmed that HBx accumulates in liver cell mitochondria (Henkler et al., 2001; Rahmani et al., 2000), disrupts their architecture (Chami et al., 2003; Henkler, et al., 2001) and depolarizes them (Rahmani, et al., 2000). Results of yeast 2-hybrid assays demonstrated that HBx binds to a VDAC isoform named VDAC3, suggesting that HBx's effects on mitochondrial morphology and $\Delta\psi$ might be mediated through VDAC3 (Rahmani, et al., 2000; Rahmani et al., 1998) (see Figure 1).

HBx-expressing cells with altered, depolarized mitochondria show signs of apoptosis (Takada, et al., 1999). Analyses of HBx-GFP fusion proteins showed that these effects require a region of HBx spanning residues 68-117 (Shirakata and Koike, 2003). Within the sequence spanning residues 68-117, substitution of 5 leucines and one cysteine with alanines interfered with mitochondrial targeting and

suppressed the death-inducing effects, whereas substitution of basic or acidic residues with alanines did not affect mitochondrial targeting (Shirakata and Koike, 2003) (see Figure 2).

Shirakata and Koike demonstrated that HBx does not induce release of cytochrome *c* or apoptosis-inducing factor (AIF) from isolated mitochondria (Shirakata and Koike, 2003). The effects of HBx on $\Delta\psi$ and cell death are attenuated in cells treated with PTP inhibitors (cyclosporin A or bongkrekic acid), a ROS scavenger (*N*-acetyl-L-cysteine), a caspase inhibitor, or by overexpression of BCL-2 or BCL-x_L (Chami, et al., 2003; Shirakata and Koike, 2003).

HBx-induced apoptosis can also be partially blocked by pharmacologically decreasing intracellular calcium levels, thus implicating calcium signaling in this process (Chami, et al., 2003). An investigation of the influence of HBx on intracellular calcium homeostasis carried out using aequorin chimeras demonstrated that HBx-expressing cells exhibit a greater increase in cytoplasmic Ca^{2+} levels in response to ATP or histamine, both of which trigger Ca^{2+} release from ER stores (Chami, et al., 2003). These differences appear to be linked to a reduction in the activity of the plasma membrane calcium ATPase (PMCA), leading to accumulation of Ca^{2+} beneath the plasma membrane. Reduced PMCA activity is likely to result from its cleavage by caspase-3, whose activity is increased in HBx-expressing cells. In this study, the mitochondria of HBx-expressing cells also showed reduced uptake of Ca^{2+}, possibly due to disruption of the interconnections between the ER and mitochondria. Although this study did not examine Ca^{2+} signaling in cells expressing HBx mutants that do not accumulate in mitochondria, the observations support a connection between HBx, mitochondrial alterations, altered calcium signaling (due to both reduced uptake by mitochondria and inactivation of the PMCA), and apoptosis.

HBx-expressing cells can also exhibit other signs of mitochondrial dysfunction, including a reduction in the levels of enzymes involved in oxidative phosphorylation and electron transport, and increased production of ROS and lipid peroxide (Lee et al., 2004). While the HBx-expressing

hepatoma cells used in this study did not exhibit mitochondrial depolarization or spontaneous apoptosis, they showed increased sensitivity to depolarization induced by inhibitors of Complexes I, III, IV, and V, as well as increased sensitivity to apoptosis inducing agents, which could be blocked by administration of anti-oxidants (Lee, et al., 2004). Interestingly, lipid peroxidation is also observed in liver tumors and non-tumor liver tissue of HBx-transgenic mice (Lee, et al., 2004). This observation provides evidence for a direct link between HBx expression and oxidative stress, a phenomenon with an important role in the pathogenesis of chronic liver diseases [reviewed by (Parola and Robino, 2001)].

Aside from these detrimental consequences, emerging data indicate that mitochondrial-associated HBx can exert positive effects on cell growth and viral replication through modulation of the NF-κB, STAT-3, and perhaps other signaling pathways through stimulation of ROS production (Waris et al., 2001). These effects, as well as mitochondrial accumulation and interaction with VDAC3, require HBx sequences lying beyond amino acid 99, thus suggesting a connection between mitochondrial HBx and its activation of the STAT-3 and NF-κB pathways.

Furthermore, HBx induces autophosphorylation and activation of Pyk2, a calcium-dependent tyrosine kinase that activates Src (Bouchard et al., 2001). In this study, Pyk2 activation by HBx was blocked by the cytosolic calcium chelator BAPTA-AM or by CsA, suggesting that Ca^{2+} and mitochondria are involved in this process. Interestingly, HBx-mediated activation of the AP-1 transcription factor was also blocked by CsA, thus implicating mitochondria and possibly calcium signaling in this transactivating property. In contrast, activation of the CREB transcription factor, which is mediated by a direct HBx-CREB interaction (Andrisani and Barnabas, 1999), was not affected by CsA. HBV reverse transcription and DNA replication were substantially inhibited by expression of a Pyk2 transdominant mutant or upon treatment with BAPTA-AM, CsA, or CGP37157, an inhibitor of the mitochondrial sodium-calcium pump (Bouchard, et al., 2001). Furthermore,

replication of an HBx-minus HBV could be restored by increasing intracellular Ca^{2+} levels with thapsigargin (Bouchard, et al., 2001). However, as this study did not test the ability of non-mitochondrial HBx mutants to affect calcium signaling, a direct connection between mitochondrial localization of HBx and modulation of Pyk2 through calcium signaling could only be hypothesized. A subsequent study reinforced the connection between the ability of HBx to modulate calcium homeostasis and its pleiotropic effects on signal transduction pathways (Oh et al., 2003).

HBx is thus a multifaceted protein at the level of mitochondrial function that can either promote apoptosis or influence signal transduction, with possible growth promoting consequences for both the cell and the virus. The fate of an HBx-expressing cell is likely to be determined by the baseline status of the cell's mechanisms controlling proliferation and death as well as by the intracellular location of the viral protein and availability of its different targets. Interestingly, HBx's nuclear targeting is favored at low levels of expression, while cytoplasmic and mitochondrial localization are observed as expression is increased, suggesting that biological activity might also be linked to HBx expression levels (Henkler, et al., 2001). This hypothesis is supported by results of transfection assays in which maximum transactivation was achieved with 10-fold less HBx plasmid that that needed to induce cell death (Shirakata and Koike, 2003). It will be of interest to determine if the dual outcome of HBx's effects on calcium homeostasis (i.e., stimulation of signal transduction pathways or apoptosis) is determined by the extent of its mitochondrial accumulation.

2. THE E1^E4 PROTEIN OF HPV

The E1^E4 protein is translated from spliced mRNAs that join the first 5 amino acids of the E1 ORF to the E4 ORF (Nasseri et al., 1987) and is detected at high levels in differentiated keratinocytes that are replicating the viral genome (Doorbar et al., 1997). The E1^E4 protein sequence is not very well conserved among different HPV types; for example, low-risk HPV 1 and high-risk HPV 16 code

for 125- and 92-residue proteins, respectively. The E1^E4 proteins of different HPV types share the ability to form multimers. Recombinant HPV 16 E1^E4 migrates as a 10-kDa monomer, 22-kDa dimer, 66-kDa hexamer, and other higher-order species in SDS-PAGE, with multimer formation dependent on residues 87-92 (Wang et al., 2004) (see Figure 2).

Initial functional studies of E1^E4 demonstrated that it interacts with the keratin network, resulting in its collapse (Doorbar et al., 1991). A comparison of E1^E4 proteins expressed by HPV types 1 and 16 showed that while both proteins colocalize with the keratin network, only type 16 E1^E4 results in its collapse (Roberts et al., 1993). The interaction between E1^E4 and keratins was mapped to a highly conserved motif spanning residues 12-16 (i.e., LLXLL, where X is K in HPV 16), with deletion of these residues resulting in accumulation of the protein in the nucleus (Roberts et al., 1994); the last 16 amino acids of HPV 16 E1^E4 were found to be required for collapse of the keratin network (Roberts et al., 1997). HPV 16 E1^E4 binds strongly to keratin 18 and weakly to keratin 8 (Wang, et al., 2004). Binding of E1^E4 multimers to keratin is proposed to stabilize the network and promote recruitment of proteins involved in viral DNA replication or virion production (Wang, et al., 2004). Eventual collapse of the keratin network in the presence of HPV 16 E1^E4 may aid in the release of virus particles from the keratin-rich terminally differentiated epithelial cells (Doorbar, et al., 1991). Although a yeast 2-hybrid screen failed to detect self-associations between HPV 16 E1^E4 molecules, several other binding partners were identified, including a putative RNA helicase which might play a role in post-transcriptional regulation of expression of the late HPV genes (Doorbar et al., 2000). E1^E4 expressed by some HPV types (e.g., 11, 16 and 18, but not 1) has also been demonstrated to arrest cells in G2; this property was mapped to a single threonine within a proline-rich domain spanning residues 17-45 of HPV 16 E1^E4 and is not linked to its ability to interact with keratins (Davy et al., 2002; Nakahara et al., 2002).

A detailed study of the intracellular distribution of HPV 16 E1^E4 carried out by Raj et al. (2004) demonstrated its ability to accumulate in mitochondria. In HeLa cells, E1^E4 initially colocalizes with the keratin network, causing its redistribution into a perinuclear cage followed by its collapse into a dense granule adjacent to the nucleus; upon collapse of the keratin network, E1^E4 accumulates in mitochondria. Saos-2 cells, which do not express keratins, show mitochondrial localization of E1^E4. Additional experiments carried out in keratinocytes and Saos cells revealed that accumulation of E1^E4 in mitochondria results in their redistribution in a perinuclear pattern, possibly as a result of their dissociation from microtubules. Mitochondrial targeting of E1^E4 was found to be directed by the same amino-terminal leucine-rich sequence (aa. 12-16, LLKLL) previously shown to be important for its association with keratin filaments; the leucine-rich sequence alone is able to direct mitochondrial targeting of GFP, indicating that this region functions as an MTS. In addition to exhibiting disrupted mitochondrial distribution, cells expressing high levels of E1^E4 show signs of depolarization, with prolonged E1^E4 expression leading to apoptosis (Raj et al., 2004). It will be interesting to determine whether the E1^E4 proteins coded by other HPV types accumulate in mitochondria and disrupt their architecture and function. It would also be useful to extend these analyses to the cottontail rabbit papillomavirus (CRPV) model, based on recent studies demonstrating that the CRPV E4 ORF is dispensable for development of papillomas but required for completion of the viral life cycle *in vivo* (Peh et al., 2004).

3. THE HTLV-1 $p13^{II}$ PROTEIN

$p13^{II}$, an 87-amino acid, 13-kDa protein coded by the x-II ORF, was the first retroviral protein demonstrated to be targeted to mitochondria (Ciminale et al., 1999). The x-II ORF gives rise to 2 protein isoforms differing in their amino terminal portion: a 241-amino acid protein, named $p30^{II}$/Tof, is produced from a doubly-spliced mRNA (Ciminale et al., 1992; Koralnik et al., 1992); $p13^{II}$

corresponds to the carboxy-terminal 87 amino acids of $p30^{II}$/Tof and is produced by a singly spliced mRNA (Berneman et al., 1992). $p30^{II}$/Tof, which is targeted to nuclei/nucleoli via a bipartite nuclear localization signal (NLS) (D'Agostino et al., 1997), acts both as a transcriptional modulator of CREB-responsive promoters (Zhang et al., 2000) and as a post-transcriptional repressor of the viral mRNA coding for Tax and Rex (Nicot et al., 2004; Younis et al., 2004). $p13^{II}$ lacks the NLS sequence, and, while occasionally detected in the nucleus (D'Agostino, et al., 1997; Koralnik et al., 1993), is predominantly mitochondrial (Ciminale, et al., 1999).

Functional mapping of $p13^{II}$ revealed that it contains a mitochondrial targeting signal (MTS) spanning amino acids 21-31 (see Figure 2). This sequence (LRVWRLCTRRL) is predicted to form an amphipathic α-helix (Ciminale, et al., 1999). CD spectroscopy demonstrated that α-helical folding of this region requires exposure to phospholipid bilayers (D'Agostino et al., 2002). $p13^{II}$'s targeting sequence also behaves as a potent MTS when attached to the N-terminus of heterologous proteins such as GFP (Ciminale, et al., 1999), or HIV-1 Rev, which contains a strong NLS (D'Agostino et al., 2000). Interestingly, while $p30^{II}$/Tof also contains the $p13^{II}$ MTS, it does not accumulate in mitochondria (Ciminale, et al., 1992); furthermore, an N-terminal truncation mutant of $p30^{II}$/Tof that initiated 56 residues before $p13^{II}$'s ATG (i.e., just C-terminal to the NLS of $p30^{II}$/Tof) showed little or no mitochondrial targeting (D'Agostino, et al., 1997), suggesting that the $p13^{II}$ MTS must be positioned near the N-terminus in order to function efficiently. The $p13^{II}$ MTS differs somewhat from canonical amphipathic MTS, as it is shorter and is not cleaved upon import (Ciminale, et al., 1999). Furthermore, site-directed mutagenesis of the $p13^{II}$ MTS revealed that replacement of arginines 22, 25, 29, and 30 with 2 alanines and 2 leucines or with 4 prolines did not abrogate mitochondrial targeting, indicating that the signal did not require the presence of positively charged amino acids; however, substitution of the arginines with aspartic acids disrupted mitochondrial targeting (D'Agostino, et al., 2002). Results of fractionation assays and immuno-electron microscopy indicated that $p13^{II}$ accumulates predominantly

in the inner mitochondrial membrane (D'Agostino, et al., 2002). Examination $p13^{II}$-expressing cells by immunofluorescence revealed that its accumulation in mitochondria disrupts the mitochondrial network into isolated clusters of round-shaped mitochondria, a pattern suggestive of mitochondrial swelling (Ciminale, et al., 1999). Electron microscopy confirmed these changes, including fragmentation of the cristae and swelling; interestingly, mitochondria exhibiting more prominent alterations often appeared in close proximity to endoplasmic reticulum cisternae (D'Agostino, et al., 2002). Two-hybrid and pull-down assays demonstrated binding of $p13^{II}$ to a protein of the nucleoside monophosphate kinase superfamily and to actin-binding protein 280 (Hou et al., 2000), as well as to farnesyl pyrophosphate synthase (Lefebvre et al., 2002b).

The marked changes in mitochondrial morphology and architecture noted in $p13^{II}$-expressing cells are supported by biochemical studies demonstrating that a $p13^{II}$ peptide spanning residues 9-41 induces changes in permeability of isolated mitochondria to K^+, Na^+ and Ca^{2+}. In contrast to HBV HBx and HIV Vpr (see below), these effects are not inhibited by CsA, suggesting that $p13^{II}$ does not exert these effects through the PTP. Furthermore, $p13^{II}$ does not induce release of cytochrome *c* or trigger apoptosis in the cell systems tested to date (Ciminale, et al., 1999; D'Agostino, et al., 2002). The $p13^{II}$-induced Ca^{2+} currents in isolated mitochondria are not inhibited by ruthenium red, arguing against a mechanism mediated through opening of the Ca^{2+} uniporter (Ciminale, et al., 1999; D'Agostino, et al., 2002). $p13^{II}$'s ability to alter mitochondrial ion transport *in vitro* and disrupt their morphology in intact cells require a "functional domain" (residues 9-41) that includes the MTS and is strictly dependent on the presence of arginines 22, 25, 29, and 30 constituting the charged face of $p13^{II}$'s α-helix. In line with these *in vitro* data, cell lines expressing $p13^{II}$ show increased sensitivity to Ca^{2+}-mediated stimuli (Silic-Benussi et al., 2004).

$p13^{II}$ might influence mitochondrial permeability through the control of endogenous mitochondrial channels, or by forming ion channels itself (see Figure 1). Regarding the second

possibility, membrane-directed folding of the $p13^{II}$ MTS into an amphipathic α-helix might force its multimerization in the inner mitochondrial membrane, with the hydrophobic face of the MTS exposed to the apolar phospholipid bilayer and the charged face forming an internal channel-like structure. As described in greater detail below, such structures are formed by viroporins, a recently described class of small viral proteins that includes HCV p7 (see below).

The emerging links between mitochondria and tumorigenesis prompted studies aimed at testing the impact of $p13^{II}$ on cell growth using *in vitro* and *in vivo* models. Tumorigenicity assays revealed that $p13^{II}$ reduces the ability of c-Myc and Ha-Ras to transform primary rat embryo fibroblasts; likewise, $p13^{II}$-expressing cell lines are less tumorigenic in nude mice and exhibit a proliferation defect *in vitro* (Silic-Benussi, et al., 2004). These observations suggest that $p13^{II}$ might counterbalance positive growth signals (e.g. those provided by the viral transactivator Tax), thus limiting the tumorigenicity of HTLV-1 and increasing its adaptation to the host.

An HTLV-1 molecular clone containing a mutation in the x-II ORF that disrupted $p30^{II}$/Tof and $p13^{II}$ was able to produce infectious virus and immortalize human T cells in tissue culture, indicating that $p30^{II}$/Tof and/or $p13^{II}$ are dispensable *in vitro* (Robek et al., 1998). However, studies of *in vivo* viral replication carried out by inoculating rabbits with T-cell lines immortalized by the wild-type HTLV-1 virus or the $p30^{II}$/Tof-$p13^{II}$ double mutant revealed that latter virus was less infectious and produced substantially lower proviral loads compared to the wild-type control, indicating that $p30^{II}$/Tof and/or $p13^{II}$ is important for efficient viral propagation *in vivo* (Bartoe et al., 2000). Characterization of a recently generated $p13^{II}$ knock-out virus (M.D. Lairmore and L. Ratner, personal communication) will complete this picture.

Insights into the functional role of $p13^{II}$ might also be gained from characterization of the bovine leukemia virus (BLV) G4 protein, which, in analogy with $p13^{II}$, is targeted primarily to mitochondria and causes similar changes in their morphology (Lefebvre et al., 2002a). BLV is closely related to HTLV-1 in

terms of genetic organization (Alexandersen et al., 1993); it causes a benign B-cell expansion (persistent lymphocytosis) in about 30% of infected cattle and fatal B-cell neoplasias in about 5%, after a 4- to 10-year period of latency [reviewed by (Willems et al., 2000)].

G4 is required for efficient viral propagation and development of BLV-associated neoplasias; furthermore, G4 cooperates with Ha-Ras to transform primary rat embryo fibroblasts (Kerkhofs et al., 1998; Willems et al., 1994). The G4 ORF codes for a 105-amino acid protein that accumulates in mitochondria and to a lesser extent in the nucleus (Lefebvre, et al., 2002a). The G4 sequence includes a hydrophobic amino-terminus (residues 1-24) and an arginine-rich region spanning residues 58-72 (Alexandersen, et al., 1993) and is predicted to contain 3 α-helices spanning residues 8-22, 63-69 and 76-89, with the second helix displaying amphipathic properties (Lefebvre, et al., 2002a) (see Figure 2). While truncation mutants lacking either residues 1-24 or 52-105 failed to accumulate in mitochondria, a mutant spanning residues 1-72 retained mitochondrial targeting (Lefebvre, et al., 2002a). The presence of potential proteolytic cleavage sites after residues 21 and 23 suggests that the amino-terminus of G4 might be removed upon mitochondrial import. Given the apparently similar effects of $p13^{II}$ and G4 on mitochondrial morphology, it will be of interest to further characterize the MTS of G4, determine its submitochondrial location, and compare its effects on mitochondrial ion permeability with those described for $p13^{II}$. The significance of G4's partial nuclear targeting, a property that is enhanced upon removal of the first 24 residues of the protein (Lefebvre, et al., 2002a), also merits further study.

Like $p13^{II}$, G4 interacts with farnesyl pyrophosphate synthase (FPPS) (Lefebvre, et al., 2002b), an enzyme that catalyzes condensation reactions yielding geranylpyrophosphate (GPP) and farnesyl pyrophosphate (FPP), precursors for the biosynthesis of steroids, cholesterol, ubiquinone, and other isoprenoid-derived molecules [reviewed by (Liang et al., 2002)]. In addition, FPP and geranylgeranylpyrophosphate (a GPP derivative) are transferred by prenyl transferases to cysteine residues of a variety of proteins controlling cell growth, differentiation, vesicle trafficking, and

cytoskeletal dynamics [reviewed by (Roskoski, 2003)]. One such substrate is Ras, whose association with the plasma membrane and function depends on farnesylation [reviewed by (Hancock, 2003)]; indeed, the application of inhibitors of Ras farnesylation as anti-tumor agents is currently under study [reviewed by (Midgley and Kerr, 2002)].

The oncogenic properties of G4 appear to depend on its ability to interact with FPPS, as deletion of 4 residues from its amphipathic α–helix abrogated both its binding to FPPS and its oncogenic potential (Lefebvre, et al., 2002b). On the contrary, deletion of G4's first 24 amino acids (corresponding to the first α–helix) favored its interaction with FPPS, suggesting that cleavage after residue 21 or 23 during mitochondrial import might stabilize the protein and enhance its interaction with FPPS. It is possible that G4 and FPPS might interact in different cellular compartments, as suggested by the distribution of FPPS activity (cytosol >>> mitochondria >> peroxisomes > lysosomes > microsomes > and nuclei) (Runquist et al., 1994) and the mixed mitochondrial and nuclear localization of G4.

The oncogenic potential of G4 suggests that it might function through the activation of FPPS-mediated Ras prenylation. While *in vitro*-based assays gave no indication for an effect of G4 on prenylation of Ras (Lefebvre, et al., 2002b), this possibility remains to be explored in intact cells. It would also be worth testing whether inhibitors of Ras farnesylation are able to interfere with the oncogenic potential of G4.

Although $p13^{II}$ was also found to bind to FPPS (Lefebvre, et al., 2002b), our observations suggest that $p13^{II}$ reduces tumorigenicity rather than potentiating it. Although it is possible that the distinct effects of $p13^{II}$ and G4 on cell transformation may reflect differences in the experimental systems used, the data taken at face value suggest that $p13^{II}$ and G4 might have opposite effects on FPPS function.

4. THE p7 PROTEIN OF HCV: A VIROPORIN

A recent study established that the p7 protein of HCV is mainly (although not exclusively) targeted to mitochondria (Griffin et al., 2004). p7 is a 63 amino acid, hydrophobic, integral membrane protein (Carrere-Kremer et al., 2002) that is required for viral infectivity (Harada et al., 2000; Sakai et al., 2003). p7 assembles into hexameric complexes both in artificial membranes and in cells and controls membrane permeability to cations (Griffin et al., 2003; Griffin, et al., 2004; Pavlovic et al., 2003) (Figure 1). In analogy to influenza M2 protein (see below), this latter function is inhibited by amantadine (Griffin, et al., 2003). The p7 sequence includes two putative transmembrane α-helical regions (residues 12-32 and 37-58) separated by a loop containing 2 positively charged residues that are critical for both virus infectivity and ion channel activity (Griffin, et al., 2004; Harada, et al., 2000) (see Figure 2).

Based on its structural and functional properties, p7 has been classified as a viroporin, a recently defined class of small viral proteins that, through oligomerization of amphipathic α-helical domains in phospholipid bilayers, form channels that alter membrane permeability to ions and small molecules [reviewed by (Gonzalez and Carrasco, 2003)] and promote viral replication by aiding entry and release of viral particles.

In addition to p7, the list of viroporins includes influenza A virus M2 protein, Chlorella virus Kcv protein, and HIV-1 Vpu and Vpr. Based on its small size, presence of an amphipathic α-helix, and effects on membrane permeability, it is tempting to speculate that HTLV-1 $p13^{II}$ may also act as a viroporin.

Influenza M2, a prototypic viroporin, contains a single 19-amino acid amphipathic α-helical transmembrane domain. Association of M2 into homotetramers forms a proton channel that, in the context of endosomal membranes, decreases the pH, thus favoring virion uncoating and nuclear targeting of the viral genome, 2 critical steps in the initial stages of infection. The Chlorella virus Kcv

protein assembles into a somewhat more complex structure consisting of two TM domains attached through a linker, and forms a K^+ channel. HIV-1 Vpu consists of an N-terminal amphipathic α-helical transmembrane helix, which may form membrane-spanning multimers with non-selective channel activity, followed by two amphipathic α-helixes exposed on the cytosolic face of the plasma membrane, which affect the CD4/envelope interaction. Vpu plays a critical role in budding of viral particles from infected cells and is thus important for viral infectivity. The properties of viroporins suggest that they might represent ancestors of more selective and regulated cellular channel proteins in which individual TM helices are linked in the context of a single protein rather than assembled into multimeric bundles.

HIV-1 Vpr shares some functional and structural links with both $p13^{II}$ and HBx. This 14 kDa multifunctional protein is detected in the nucleus, mitochondria, and mature viral particles. Vpr contains three α-helices spanning residues 17-33, 38-50 and 56-77 surrounded by flexible N and C-terminal domains, and folded around a hydrophobic core (Morellet et al., 2003) (Figure 2). Exposure of isolated mitochondria or intact cells to Vpr results in mitochondrial depolarization and release of pro-apoptotic proteins from mitochondria (Jacotot et al., 2000). Induction of cell death and permeability transition require the same region of Vpr (amino acids 52-96, which include the third α-helix and critical arginine residues) and are blocked by BCL-2 and PTP inhibitors. Experiments carried out using ANT or VDAC-defective yeast strains showed that Vpr-induced cell death is dependent on ANT and VDAC, suggesting that this effect is mediated through the PTP (Jacotot, et al., 2000). *In vitro* studies showed that Vpr binds to ANT and form channels with it in artificial membranes (Figure 1). Interestingly, BCL-2 is able to interfere with the Vpr-ANT interaction as well as Vpr-induced changes in permeability of synthetic membranes and isolated mitochondria (Jacotot et al., 2001). In addition to these effects, Vpr mediates nuclear targeting of the viral genome following reverse transcription (Gallay et al., 1996) and induces cell cycle arrest at the G2/M checkpoint through inactivation of the cyclin B-cdc2 complex (Bartz et al., 1996;

He et al., 1995; Jowett et al., 1995; Nishino et al., 1997; Re et al., 1995; Re and Luban, 1997). Interestingly, HIV-1 promoter activity and viral production are upregulated in G2 (Goh et al., 1998), suggesting that, by prolonging this phase, Vpr might increase viral replication. In addition to inducing apoptosis *in vitro* (Poon et al., 1998; Stewart et al., 1997; Stewart et al., 1999), Vpr exerts anti-tumor effects *in vivo*; this latter effect is seen in immunocompetent but not in nude or SCID mice, suggesting modulation of the immune response rather than a direct anti-proliferative/apoptotic action (Pang et al., 2001). Further studies should address the possible links between the effects of Vpr on mitochondria, the cell cycle and tumorigenicity, an issue that is of particular relevance in light of the effects of HTLV-1 $p13^{II}$. One obvious explanation for the multiple effects of Vpr might be linked to its multiple intracellular localizations. It would be particularly interesting to investigate the processes controlling Vpr's localization in the nucleus vs. mitochondria and determine if its effects on the cell cycle require nuclear targeting or reflect signalling from mitochondria to the nucleus (Liu et al., 2003; Sekito et al., 2000). This latter phenomenon, termed retrograde signalling, has recently emerged as an intriguing mechanism by which functional alterations of mitochondria change the pattern of nuclear gene expression; in mammalian cells this appears to result mainly through changes in mitochondrial membrane potential that alter Ca^{2+}-mediated signal transduction pathways controlling key transcription factors such as NF-AT, CREB, and NF-κB (Arnould et al., 2002; Biswas et al., 1999).

Similar to Vpr, the PB1-F2 protein of influenza virus A induces cell-type specific death and loss of inner membrane potential (Chen et al., 2001). These effects are proposed play a role in influenza virus pathogenesis by killing immune cells responding to infection (Chen, et al., 2001). PB1-F2 is an 87-amino-acid protein that accumulates in the nucleus, cytosol, and mitochondria. The PB1-F2 MTS was mapped to an amphipathic α-helical C-terminal region that is very similar to the $p13^{II}$ MTS in terms of both sequence and structure; furthermore, the PB1-F2 MTS can functionally replace the $p13^{II}$ MTS and is not cleaved upon import (Gibbs et al., 2003). However, unlike the case of $p13^{II}$, the MTS of PB1-F2 is C-terminal and

requires the presence of arginine residues for mitochondrial targeting, although placement of the PB1-F2 MTS closer to the amino terminus increases its efficiency in mediating mitochondrial targeting (Gibbs, et al., 2003).

C. A Mitochondrial Protein of Unknown Function: The K15 Protein of KSHV

The K15 ORF sequence is highly variable among different KSHV isolates, and has been classified into 2 major alleles having only 33% amino acid identity (Poole et al., 1999). The ORF is found in several alternatively spliced mRNAs containing up to 8 exons which are predicted to express protein isoforms whose sequences include 2 to 12 transmembrane domains at their amino terminus and a shared carboxy-terminal region containing SH2- and SH3-binding motifs (Choi et al., 2000; Glenn et al., 1999). In transfection assays, the 8-exon mRNA yields a minor 50-kDa band corresponding to the full-length, 489-residue protein, abundant 35- and 23-kDa proteins that are likely represent C-terminal products of proteolytic cleavage occurring at residues 164 and 288, respectively, and other minor products (Sharp et al., 2002). The smaller forms were not as evident in transfections carried out using another cell line (Choi, et al., 2000), suggesting that cleavage might be cell type-specific.

Only the 23-kDa K15 protein is detected in chronically KSHV-infected PEL cell lines (Sharp, et al., 2002). The levels of the 23-kDa species are not increased upon treatment of a PEL cell line with an agent that induces lytic replication, thus classifying this protein as a latent product (Sharp, et al., 2002). However, experiments carried out with other PEL cell lines indicated that some mRNAs containing the K15 ORF, including the full-length species, are more abundant after this treatment (Choi, et al., 2000; Glenn, et al., 1999). Although these conflicting observations could reflect differences in the experimental systems, they might suggest that expression of the K15 protein is regulated at the post-transcriptional level.

Immunofluorescence assays of cells transfected with the 8-exon mRNA or shorter mRNAs indicated that K15 accumulates in the plasma membrane, cytoplasm, and perinuclear region (Choi, et al., 2000); immunohistochemistry performed on PEL cell lines and a lymphoma from a patient with KSHV-positive multicentric Castleman's disease likewise revealed accumulation of K15 in the cytoplasm and nuclear periphery (Sharp, et al., 2002).

Evidence for mitochondrial targeting of K15 arose from studies of its interaction with HS1 associated protein X-1 (HAX-1) (Sharp, et al., 2002). This interaction was revealed in a yeast 2-hybrid screen carried out using the carboxy-terminal 144 residues of K15 as bait (Sharp, et al., 2002). Subcellular fractionation assays demonstrated that the 35- and 23-kDa K15 species colocalize with HAX-1 in mitochondria, with the 35-kDa band also detected in the ER (Sharp, et al., 2002). Consistent with these results, K15 is predicted to contain a mitochondrial targeting sequence spanning residues 345 to 374 of the full-length protein (Sharp, et al., 2002); the predicted cleavage events producing the 23-kDa and 35-kDa K15 proteins would place this MTS considerably closer to the N-terminus in the smaller protein.

The K15-HAX-1 interaction involves one of K15's putative C-terminal SH2-binding domains and the carboxy-terminal 170 amino acids of HAX-1, which include a PEST domain and transmembrane domain (Sharp, et al., 2002). HAX-1 is an ubiquitously expressed protein that accumulates in mitochondria, the ER and nuclear envelope (Suzuki et al., 1997). It was originally discovered based on its binding to hematopoietic lineage cell-specific protein 1 (HS1), a protein involved in BCR and TCR signaling (Suzuki, et al., 1997). Interestingly, HAX-1 possesses BH1 and BH2 domains near its amino-terminus and is able to inhibit apoptosis induced by overexpression of BAX (Sharp, et al., 2002). In these assays, K15 did not exhibit intrinsic pro-apoptotic activity, nor did it affect BAX-induced apoptosis or the anti-apoptotic effects of HAX-1 (Sharp, et al., 2002).

In addition to binding to HAX-1, K15 is able to activate the Ras/MAPK and NF-κB pathways as well as the AP-1 transcription factor upon phosphorylation of a tyrosine within one of its SH2-binding motifs (Brinkmann et al., 2003). K15 also exhibits interesting similarities to the LMP-1 and LMP-2A proteins of EBV. Full-length K15 and shorter isoforms are incorporated into lipid rafts, a property shared by both LMP-1 and LMP-2A (Brinkmann, et al., 2003). Similar to LMP-1, K15's C-terminal region is able to interact with TRAF1, TRAF2 and TRAF3 (Glenn, et al., 1999). This portion of K15 also inhibits BCR signaling when expressed as a chimera attached to the extracellular and transmembrane domains of CD8α, a property described for EBV LMP-2A (Choi, et al., 2000).

The sequence heterogeneity of the K15 ORF, its expression as different isoforms from alternatively spliced mRNAs, and possible post-transcriptional and post-translational regulation increase the complexity of studies aimed at understanding its functional significance. In addition, depending on its extent of proteolytic cleavage, a given K15 molecule might exert diverse, possibly hierarchical functions in distinct subcellular compartments, including mitochondria.

III. CONCLUDING REMARKS

Approximately 15% of human cancers are caused by tumor viruses. The study of oncogenic viruses has revealed how their replication strategies can lead to neoplastic growth and led to the identification of common genes and pathways involved in the molecular pathogenesis of cancer. Recent studies have shown that many viruses, including tumor viruses, encode proteins that are targeted to mitochondria and control a number of functions including apoptosis, cell growth, ion homeostasis and signaling pathways.

Defects in apoptotic signalling, including interference at the mitochondrial level, represent a hallmark in the development of the neoplastic phenotype contributing to tumor cell survival and escape from immune surveillance [reviewed by (Igney and Krammer, 2002)]. The fact that many tumors have

evolved mechanisms to resist apoptosis has prompted efforts aimed at restoring their response to apoptotic stimuli [reviewed by (Blagosklonny, 2004)], ranging from current radiation and chemotherapy regimens to newly developed drugs targeting BCL-2, FLIP and IAP (Costantini, et al., 2000; Holmuhamedov et al., 2002). The fact that some tumor viruses exploit strategies to inhibit apoptosis, including the expression of vBCL-2, vFLIP, and vIAP, makes them potentially vulnerable to such agents both in terms of inhibiting viral replication and blocking their neoplastic potential. Although this picture is complicated by ability of vBCL-2s and vIAP to escape normal cellular control, these features could also be exploited to specifically target the viral proteins while minimizing side effects due to interference with the host cell machinery. Further understanding of how viral mitochondrial proteins such as HBx and Vpr sensitize cells to apoptosis could also aid in designing new strategies to overcome apoptosis resistance.

Although the impact of tumor virus' mitochondrial proteins on apoptosis has thus far been characterized most thoroughly, further analysis of other mitochondrial functions that modulate signal transduction and cell proliferation are likely to provide important mechanistic insights. For example, it is intriguing that increased mitochondrial permeability to K^+, possibly through changes in the mitochondrial transmembrane potential and Ca^{2+} uptake, leads to suppression of tumor cell proliferation (Holmuhamedov, et al., 2002), a phenomenon reminiscent of the properties exhibited by the $p13^{II}$ protein of HTLV-1 (Silic Benussi et al., 2004). In addition, recent experimental evidence links the control of mitochondrial membrane potential to ROS production, signal transduction, and cell proliferation [reviewed by (Perl et al., 2004)]. For example, mitochondrial depolarization induced by K^+ channel openers was shown to increase mitochondrial ROS production, leading to an activation of the ERK signalling pathway (Samavati et al., 2002). Continued studies of the molecular mechanisms by which tumor viruses hijack the cellular machinery through the expression of mitochondrial proteins

are thus likely to cast light on basic cellular processes and provide new targets for ablating viral replication and treating cancer patients.

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REFERENCES

Acker, T., and Plate, K. H. (2002). *J Mol Med* **80,** 562-75.
Albrecht, B., and Lairmore, M. D. (2002). *Microbiol Mol Biol Rev* **66,** 396-406, table of contents.
Alexandersen, S., Carpenter, S., Christensen, J., Storgaard, T., Viuff, B., Wannemuehler, Y., Belousov, J., and Roth, J. A. (1993). *J Virol* **67,** 39-52.
Ambinder, R. F. (2000). *Am J Pathol* **156,** 1-3.
Andrisani, O. M., and Barnabas, S. (1999). *Int J Oncol* **15,** 373-9.
Appaix, F., Guerrero, K., Rampal, D., Izikki, M., Kaambre, T., Sikk, P., Brdiczka, D., Riva-Lavieille, C., Olivares, J., Longuet, M., Antonsson, B., and Saks, V. A. (2002). *Biochim Biophys Acta* **1556,** 155-67.
Arbuthnot, P., and Kew, M. (2001). *Int J Exp Pathol* **82,** 77-100.
Arnould, T., Vankoningsloo, S., Renard, P., Houbion, A., Ninane, N., Demazy, C., Remacle, J., and Raes, M. (2002). *Embo J* **21,** 53-63.
Bais, C., Santomasso, B., Coso, O., Arvanitakis, L., Raaka, E. G., Gutkind, J. S., Asch, A. S., Cesarman, E., Gershengorn, M. C., Mesri, E. A., and Gerhengorn, M. C. (1998). *Nature* **391,** 86-9.
Bartoe, J. T., Albrecht, B., Collins, N. D., Robek, M. D., Ratner, L., Green, P. L., and Lairmore, M. D. (2000). *J Virol* **74,** 1094-100.
Bartz, S. R., Rogel, M. E., and Emerman, M. (1996). *J Virol* **70,** 2324-31.
Becker, S. A., Lee, T. H., Butel, J. S., and Slagle, B. L. (1998). *J Virol* **72,** 266-72.
Bellows, D. S., Chau, B. N., Lee, P., Lazebnik, Y., Burns, W. H., and Hardwick, J. M. (2000). *J Virol* **74,** 5024-31.
Bellows, D. S., Howell, M., Pearson, C., Hazlewood, S. A., and Hardwick, J. M. (2002). *J Virol* **76,** 2469-79.
Benn, J., and Schneider, R. J. (1995). *Proc Natl Acad Sci U S A* **92,** 11215-9.
Bergametti, F., Prigent, S., Luber, B., Benoit, A., Tiollais, P., Sarasin, A., and Transy, C. (1999). *Oncogene* **18,** 2860-71.

Bernardi, P. (1999). *Physiol Rev* **79,** 1127-55.
Bernardi, P., and Azzone, G. F. (1981). *J Biol Chem* **256,** 7187-92.
Bernardi, P., Petronilli, V., Di Lisa, F., and Forte, M. (2001). *Trends Biochem Sci* **26,** 112-7.
Bernardi, P., Scorrano, L., Colonna, R., Petronilli, V., and Di Lisa, F. (1999). *Eur J Biochem* **264,** 687-701.
Berneman, Z. N., Gartenhaus, R. B., Reitz, M. S., Jr., Blattner, W. A., Manns, A., Hanchard, B., Ikehara, O., Gallo, R. C., and Klotman, M. E. (1992). *Proc Natl Acad Sci U S A* **89,** 3005-9.
Biswas, G., Adebanjo, O. A., Freedman, B. D., Anandatheerthavarada, H. K., Vijayasarathy, C., Zaidi, M., Kotlikoff, M., and Avadhani, N. G. (1999). *Embo J* **18,** 522-33.
Blagosklonny, M. V. (2004). *Oncogene* **23,** 2967-75.
Bouchard, M. J., and Schneider, R. J. (2004). *J Virol* **78,** 12725-34.
Bouchard, M. J., Wang, L. H., and Schneider, R. J. (2001). *Science* **294,** 2376-8.
Boyd. (1994). *Cell* **79,** 1121.
Boyd, J. M., Gallo, G. J., Elangovan, B., Houghton, A. B., Malstrom, S., Avery, B. J., Ebb, R. G., Subramanian, T., Chittenden, T., Lutz, R. J., and et al. (1995). *Oncogene* **11,** 1921-8.
Boyd, J. M., Malstrom, S., Subramanian, T., Venkatesh, L. K., Schaeper, U., Elangovan, B., D'Sa-Eipper, C., and Chinnadurai, G. (1994). *Cell* **79,** 341-51.
Bradham, C. A., Qian, T., Streetz, K., Trautwein, C., Brenner, D. A., and Lemasters, J. J. (1998). *Mol Cell Biol* **18,** 6353-64.
Brinkmann, M. M., Glenn, M., Rainbow, L., Kieser, A., Henke-Gendo, C., and Schulz, T. F. (2003). *J Virol* **77,** 9346-58.
Calabro, M. L., Gasperini, P., Fiore, J. R., Barbierato, M., Angarano, G., and Chieco-Bianchi, L. (2001). *J Natl Cancer Inst* **93,** 154-6.
Capovilla, A., and Arbuthnot, P. (2003). *Biochem Biophys Res Commun* **312,** 806-10.
Carrere-Kremer, S., Montpellier-Pala, C., Cocquerel, L., Wychowski, C., Penin, F., and Dubuisson, J. (2002). *J Virol* **76,** 3720-30.
Cattelan, A. M., Calabro, M. L., Gasperini, P., Aversa, S. M., Zanchetta, M., Meneghetti, F., De Rossi, A., and Chieco-Bianchi, L. (2001). *J Natl Cancer Inst Monogr***,** 44-9.
Cesura, A. M., Pinard, E., Schubenel, R., Goetschy, V., Friedlein, A., Langen, H., Polcic, P., Forte, M. A., Bernardi, P., and Kemp, J. A. (2003). *J Biol Chem* **278,** 49812-8.
Chami, M., Ferrari, D., Nicotera, P., Paterlini-Brechot, P., and Rizzuto, R. (2003). *J Biol Chem* **278,** 31745-55.
Chen, G., Branton, P. E., Yang, E., Korsmeyer, S. J., and Shore, G. C. (1996). *J Biol Chem* **271,** 24221-5.
Chen, H. S., Kaneko, S., Girones, R., Anderson, R. W., Hornbuckle, W. E., Tennant, B. C., Cote, P. J., Gerin, J. L., Purcell, R. H., and Miller, R. H. (1993). *J Virol* **67,** 1218-26.
Chen, W., Calvo, P. A., Malide, D., Gibbs, J., Schubert, U., Bacik, I., Basta, S., O'Neill, R., Schickli, J., Palese, P., Henklein, P., Bennink, J. R., and Yewdell, J. W. (2001). *Nat Med* **7,** 1306-12.
Cheng, E. H., Nicholas, J., Bellows, D. S., Hayward, G. S., Guo, H. G., Reitz, M. S., and Hardwick, J. M. (1997). *Proc Natl Acad Sci U S A* **94,** 690-4.
Chinnery, P. F., and Schon, E. A. (2003). *J Neurol Neurosurg Psychiatry* **74,** 1188-99.
Chiou, S. K., Tseng, C. C., Rao, L., and White, E. (1994). *J Virol* **68,** 6553-66.
Chirillo, P., Pagano, S., Natoli, G., Puri, P. L., Burgio, V. L., Balsano, C., and Levrero, M. (1997). *Proc Natl Acad Sci U S A* **94,** 8162-7.
Chittenden, T., Harrington, E. A., O'Connor, R., Flemington, C., Lutz, R. J., Evan, G. I., and Guild, B. C. (1995). *Nature* **374,** 733-6.
Choi, J. K., Lee, B. S., Shim, S. N., Li, M., and Jung, J. U. (2000). *J Virol* **74,** 436-46.
Chung, Y. L., Sheu, M. L., and Yen, S. H. (2003). *Int J Cancer* **107,** 65-73.

Ciminale, V., Pavlakis, G. N., Derse, D., Cunningham, C. P., and Felber, B. K. (1992). *J Virol* **66,** 1737-45.
Ciminale, V., Zotti, L., D'Agostino, D. M., Ferro, T., Casareto, L., Franchini, G., Bernardi, P., and Chieco-Bianchi, L. (1999). *Oncogene* **18,** 4505-14.
Cleary, M. L., Smith, S. D., and Sklar, J. (1986). *Cell* **47,** 19-28.
Colombini, M., Blachly-Dyson, E., and Forte, M. (1996). *Ion Channels* **4,** 169-202.
Costantini, P., Jacotot, E., Decaudin, D., and Kroemer, G. (2000). *J Natl Cancer Inst* **92,** 1042-53.
Crompton, M., Virji, S., and Ward, J. M. (1998). *Eur J Biochem* **258,** 729-35.
Cuconati, A., Degenhardt, K., Sundararajan, R., Anschel, A., and White, E. (2002). *J Virol* **76,** 4547-58.
Cuconati, A., and White, E. (2002). *Genes Dev* **16,** 2465-78.
D'Agostino, D. M., Ciminale, V., Zotti, L., Rosato, A., and Chieco-Bianchi, L. (1997). *J Virol* **71,** 75-83.
D'Agostino, D. M., Ranzato, L., Arrigoni, G., Cavallari, I., Belleudi, F., Torrisi, M. R., Silic-Benussi, M., Ferro, T., Petronilli, V., Marin, O., Chieco-Bianchi, L., Bernardi, P., and Ciminale, V. (2002). *J Biol Chem* **277,** 34424-33.
D'Agostino, D. M., Zotti, L., Ferro, T., Franchini, G., Chieco-Bianchi, L., and Ciminale, V. (2000). *AIDS Res Hum Retroviruses* **16,** 1765-70.
Danial, N. N., Gramm, C. F., Scorrano, L., Zhang, C. Y., Krauss, S., Ranger, A. M., Datta, S. R., Greenberg, M. E., Licklider, L. J., Lowell, B. B., Gygi, S. P., and Korsmeyer, S. J. (2003). *Nature* **424,** 952-6.
Danial, N. N., and Korsmeyer, S. J. (2004). *Cell* **116,** 205-19.
Davy, C. E., Jackson, D. J., Wang, Q., Raj, K., Masterson, P. J., Fenner, N. F., Southern, S., Cuthill, S., Millar, J. B., and Doorbar, J. (2002). *J Virol* **76,** 9806-18.
Dawson, C. W., Dawson, J., Jones, R., Ward, K., and Young, L. S. (1998). *J Virol* **72,** 9016-24.
Dawson, C. W., Eliopoulos, A. G., Dawson, J., and Young, L. S. (1995). *Oncogene* **10,** 69-77.
Di Lisa, F., Menabo, R., Canton, M., Barile, M., and Bernardi, P. (2001). *J Biol Chem* **276,** 2571-5.
Dolcetti, R., and Masucci, M. G. (2003). *J Cell Physiol* **196,** 207-18.
Doorbar, J., Elston, R. C., Napthine, S., Raj, K., Medcalf, E., Jackson, D., Coleman, N., Griffin, H. M., Masterson, P., Stacey, S., Mengistu, Y., and Dunlop, J. (2000). *J Virol* **74,** 10081-95.
Doorbar, J., Ely, S., Sterling, J., McLean, C., and Crawford, L. (1991). *Nature* **352,** 824-7.
Doorbar, J., Foo, C., Coleman, N., Medcalf, L., Hartley, O., Prospero, T., Napthine, S., Sterling, J., Winter, G., and Griffin, H. (1997). *Virology* **238,** 40-52.
Doria, M., Klein, N., Lucito, R., and Schneider, R. J. (1995). *Embo J* **14,** 4747-57.
Dourmishev, L. A., Dourmishev, A. L., Palmeri, D., Schwartz, R. A., and Lukac, D. M. (2003). *Microbiol Mol Biol Rev* **67,** 175-212, table of contents.
Droge, W. (2002). *Physiol Rev* **82,** 47-95.
D'Sa-Eipper, C., Subramanian, T., and Chinnadurai, G. (1996). *Cancer Res* **56,** 3879-82.
Duchen, M. R., McGuinness, O., Brown, L. A., and Crompton, M. (1993). *Cardiovasc Res* **27,** 1790-4.
Eng, C., Kiuru, M., Fernandez, M. J., and Aaltonen, L. A. (2003). *Nat Rev Cancer* **3,** 193-202.
Eskes, R., Antonsson, B., Osen-Sand, A., Montessuit, S., Richter, C., Sadoul, R., Mazzei, G., Nichols, A., and Martinou, J. C. (1998). *J Cell Biol* **143,** 217-24.
Eskes, R., Desagher, S., Antonsson, B., and Martinou, J. C. (2000). *Mol Cell Biol* **20,** 929-35.
Fanidi, A., Hancock, D. C., and Littlewood, T. D. (1998). *J Virol* **72,** 8392-5.
Farrow, S. N., and Brown, R. (1996). *Curr Opin Genet Dev* **6,** 45-9.
Farrow, S. N., White, J. H., Martinou, I., Raven, T., Pun, K. T., Grinham, C. J., Martinou, J. C., and Brown, R. (1995). *Nature* **374,** 731-3.
Fehrmann, F., and Laimins, L. A. (2003). *Oncogene* **22,** 5201-7.

Feitelson, M. A., Zhu, M., Duan, L. X., and London, W. T. (1993). *Oncogene* **8,** 1109-17.
Feng, P., Park, J., Lee, B. S., Lee, S. H., Bram, R. J., and Jung, J. U. (2002). *J Virol* **76,** 11491-504.
Fleury, C., Mignotte, B., and Vayssiere, J. L. (2002). *Biochimie* **84,** 131-41.
Foghsgaard, L., and Jaattela, M. (1997). *J Virol* **71,** 7509-17.
Franchini, G., Nicot, C., and Johnson, J. M. (2003). *Adv Cancer Res* **89,** 69-132.
Frey, T. G., and Mannella, C. A. (2000). *Trends Biochem Sci* **25,** 319-24.
Gallay, P., Stitt, V., Mundy, C., Oettinger, M., and Trono, D. (1996). *J Virol* **70,** 1027-32.
Gao, S. J., Boshoff, C., Jayachandra, S., Weiss, R. A., Chang, Y., and Moore, P. S. (1997). *Oncogene* **15,** 1979-85.
Gibbs, J. S., Malide, D., Hornung, F., Bennink, J. R., and Yewdell, J. W. (2003). *J Virol* **77,** 7214-24.
Glenn, M., Rainbow, L., Aurade, F., Davison, A., and Schulz, T. F. (1999). *J Virol* **73,** 6953-63.
Goh, W. C., Rogel, M. E., Kinsey, C. M., Michael, S. F., Fultz, P. N., Nowak, M. A., Hahn, B. H., and Emerman, M. (1998). *Nat Med* **4,** 65-71.
Gonzalez, M. E., and Carrasco, L. (2003). *FEBS Lett* **552,** 28-34.
Gooding, L. R., Aquino, L., Duerksen-Hughes, P. J., Day, D., Horton, T. M., Yei, S. P., and Wold, W. S. (1991). *J Virol* **65,** 3083-94.
Gottlob, K., Fulco, M., Levrero, M., and Graessmann, A. (1998). *J Biol Chem* **273,** 33347-53.
Green, P. L., and Chen, I. S. Y. (2001). *In* "Fields Virology" (D. M. a. H. Knipe, P.M., ed.), Vol. 2, pp. 1941-1970. Lippincott Williams and Wilkins, Philadelphia.
Griffin, S. D., Beales, L. P., Clarke, D. S., Worsfold, O., Evans, S. D., Jaeger, J., Harris, M. P., and Rowlands, D. J. (2003). *FEBS Lett* **535,** 34-8.
Griffin, S. D., Harvey, R., Clarke, D. S., Barclay, W. S., Harris, M., and Rowlands, D. J. (2004). *J Gen Virol* **85,** 451-61.
Gunter, T. E., Yule, D. I., Gunter, K. K., Eliseev, R. A., and Salter, J. D. (2004). *FEBS Lett* **567,** 96-102.
Hajnoczky, G., Csordas, G., and Yi, M. (2002). *Cell Calcium* **32,** 363-77.
Han, J., Sabbatini, P., Perez, D., Rao, L., Modha, D., and White, E. (1996a). *Genes Dev* **10,** 461-77.
Han, J., Sabbatini, P., and White, E. (1996b). *Mol Cell Biol* **16,** 5857-64.
Hanahan, D., and Weinberg, R. A. (2000). *Cell* **100,** 57-70.
Hancock, J. F. (2003). *Nat Rev Mol Cell Biol* **4,** 373-84.
Harada, T., Tautz, N., and Thiel, H. J. (2000). *J Virol* **74,** 9498-506.
Hardwick, J. M., and Bellows, D. S. (2003). *Cell Death Differ* **10 Suppl 1,** S68-76.
Hashimoto, S., Ishii, A., and Yonehara, S. (1991). *Int Immunol* **3,** 343-51.
He, J., Choe, S., Walker, R., Di Marzio, P., Morgan, D. O., and Landau, N. R. (1995). *J Virol* **69,** 6705-11.
Henderson, S., Huen, D., Rowe, M., Dawson, C., Johnson, G., and Rickinson, A. (1993). *Proc Natl Acad Sci U S A* **90,** 8479-83.
Hengge, U. R., Ruzicka, T., Tyring, S. K., Stuschke, M., Roggendorf, M., Schwartz, R. A., and Seeber, S. (2002a). *Lancet Infect Dis* **2,** 281-92.
Hengge, U. R., Ruzicka, T., Tyring, S. K., Stuschke, M., Roggendorf, M., Schwartz, R. A., and Seeber, S. (2002b). *Lancet Infect Dis* **2,** 344-52.
Henkler, F., Hoare, J., Waseem, N., Goldin, R. D., McGarvey, M. J., Koshy, R., and King, I. A. (2001). *J Gen Virol* **82,** 871-82.
Henry, H., Thomas, A., Shen, Y., and White, E. (2002). *Oncogene* **21,** 748-60.
Hickish, T., Robertson, D., Clarke, P., Hill, M., di Stefano, F., Clarke, C., and Cunningham, D. (1994). *Cancer Res* **54,** 2808-11.
Hoare, J., Henkler, F., Dowling, J. J., Errington, W., Goldin, R. D., Fish, D., and McGarvey, M. J. (2001). *J Med Virol* **64,** 419-26.

Hockenbery, D. M., Zutter, M., Hickey, W., Nahm, M., and Korsmeyer, S. J. (1991). *Proc Natl Acad Sci U S A* **88,** 6961-5.
Hollinger, F. B., and Liang, T. J. (2001). *In* "Fields Virology" (D. M. a. H. Knipe, P.M., ed.), Vol. 2, pp. 2971-3036. Lippincott Williams and Wilkins, Philadelphia.
Holmuhamedov, E., Lewis, L., Bienengraeber, M., Holmuhamedova, M., Jahangir, A., and Terzic, A. (2002). *Faseb J* **16,** 1010-6.
Hou, X., Foley, S., Cueto, M., and Robinson, M. A. (2000). *Virology* **277,** 127-35.
Hsu, S. Y., Kaipia, A., McGee, E., Lomeli, M., and Hsueh, A. J. (1997). *Proc Natl Acad Sci U S A* **94,** 12401-6.
Hsu, Y. T., and Youle, R. J. (1997). *J Biol Chem* **272,** 13829-34.
Huang, Q., Petros, A. M., Virgin, H. W., Fesik, S. W., and Olejniczak, E. T. (2002). *Proc Natl Acad Sci U S A* **99,** 3428-33.
Huang, Q., Petros, A. M., Virgin, H. W., Fesik, S. W., and Olejniczak, E. T. (2003). *J Mol Biol* **332,** 1123-30.
Hunter, D. R., and Haworth, R. A. (1979). *Arch Biochem Biophys* **195,** 453-9.
Igney, F. H., and Krammer, P. H. (2002). *Nat Rev Cancer* **2,** 277-88.
Imberti, R., Nieminen, A. L., Herman, B., and Lemasters, J. J. (1993). *J Pharmacol Exp Ther* **265,** 392-400.
Irwin, W. A., Bergamin, N., Sabatelli, P., Reggiani, C., Megighian, A., Merlini, L., Braghetta, P., Columbaro, M., Volpin, D., Bressan, G. M., Bernardi, P., and Bonaldo, P. (2003). *Nat Genet* **35,** 367-71.
Jacotot, E., Ferri, K. F., El Hamel, C., Brenner, C., Druillennec, S., Hoebeke, J., Rustin, P., Metivier, D., Lenoir, C., Geuskens, M., Vieira, H. L., Loeffler, M., Belzacq, A. S., Briand, J. P., Zamzami, N., Edelman, L., Xie, Z. H., Reed, J. C., Roques, B. P., and Kroemer, G. (2001). *J Exp Med* **193,** 509-19.
Jacotot, E., Ravagnan, L., Loeffler, M., Ferri, K. F., Vieira, H. L., Zamzami, N., Costantini, P., Druillennec, S., Hoebeke, J., Briand, J. P., Irinopoulou, T., Daugas, E., Susin, S. A., Cointe, D., Xie, Z. H., Reed, J. C., Roques, B. P., and Kroemer, G. (2000). *J Exp Med* **191,** 33-46.
Jia, L., Wang, X. W., and Harris, C. C. (1999). *Int J Cancer* **80,** 875-9.
Jowett, J. B., Planelles, V., Poon, B., Shah, N. P., Chen, M. L., and Chen, I. S. (1995). *J Virol* **69,** 6304-13.
Jung, M. S., Jin, D. H., Chae, H. D., Kang, S., Kim, S. C., Bang, Y. J., Choi, T. S., Choi, K. S., and Shin, D. Y. (2004). *J Biol Chem* **279,** 17765-71.
Kawanishi, M. (1997). *J Virol* **71,** 3319-22.
Kawanishi, M., Tada-Oikawa, S., and Kawanishi, S. (2002). *Biochem Biophys Res Commun* **297,** 682-7.
Kerkhofs, P., Heremans, H., Burny, A., Kettmann, R., and Willems, L. (1998). *J Virol* **72,** 2554-9.
Khanim, F., Dawson, C., Meseda, C. A., Dawson, J., Mackett, M., and Young, L. S. (1997). *J Gen Virol* **78 (Pt 11),** 2987-99.
Kieff, E., and Rickinson, A. B. (2001). *In* "Fields Virology" (D. M. a. H. Knipe, P.M., ed.), Vol. 2, pp. 2511-2574. Lippincott Williams and Wilkins, Philadelphia.
Kim, H., Lee, H., and Yun, Y. (1998). *J Biol Chem* **273,** 381-5.
Kim, K. H., and Seong, B. L. (2003). *Embo J* **22,** 2104-16.
Kirichok, Y., Krapivinsky, G., and Clapham, D. E. (2004). *Nature* **427,** 360-4.
Klein, G. (1994). *Cell* **77,** 791-3.
Klein, G. (2002). *Front Biosci* **7,** d268-74.
Klein, N. P., Bouchard, M. J., Wang, L. H., Kobarg, C., and Schneider, R. J. (1999). *Embo J* **18,** 5019-27.

Klohn, P. C., Soriano, M. E., Irwin, W., Penzo, D., Scorrano, L., Bitsch, A., Neumann, H. G., and Bernardi, P. (2003). *Proc Natl Acad Sci U S A* **100,** 10014-9.
Kluck, R. M., Esposti, M. D., Perkins, G., Renken, C., Kuwana, T., Bossy-Wetzel, E., Goldberg, M., Allen, T., Barber, M. J., Green, D. R., and Newmeyer, D. D. (1999). *J Cell Biol* **147,** 809-22.
Koike, K. (2002). *J Gastroenterol* **37 Suppl 13,** 55-64.
Koike, K., Moriya, K., Iino, S., Yotsuyanagi, H., Endo, Y., Miyamura, T., and Kurokawa, K. (1994). *Hepatology* **19,** 810-9.
Koike, K., Moriya, K., Yotsuyanagi, H., Shintani, Y., Fujie, H., Tsutsumi, T., and Kimura, S. (1998). *Cancer Lett* **134,** 181-6.
Koike, K., Shirakata, Y., Yaginuma, K., Arii, M., Takada, S., Nakamura, I., Hayashi, Y., Kawada, M., and Kobayashi, M. (1989). *Mol Biol Med* **6,** 151-60.
Kokoszka, J. E., Waymire, K. G., Levy, S. E., Sligh, J. E., Cai, J., Jones, D. P., MacGregor, G. R., and Wallace, D. C. (2004). *Nature* **427,** 461-5.
Koralnik, I. J., Fullen, J., and Franchini, G. (1993). *J Virol* **67,** 2360-6.
Koralnik, I. J., Gessain, A., Klotman, M. E., Lo Monico, A., Berneman, Z. N., and Franchini, G. (1992). *Proc Natl Acad Sci U S A* **89,** 8813-7.
Kuppers, R. (2003). *Nat Rev Immunol* **3,** 801-12.
Lee, H., Veazey, R., Williams, K., Li, M., Guo, J., Neipel, F., Fleckenstein, B., Lackner, A., Desrosiers, R. C., and Jung, J. U. (1998). *Nat Med* **4,** 435-40.
Lee, M. A., and Yates, J. L. (1992). *J Virol* **66,** 1899-906.
Lee, Y. I., Hwang, J. M., Im, J. H., Kim, N. S., Kim, D. G., Yu, D. Y., Moon, H. B., and Park, S. K. (2004). *J Biol Chem* **279,** 15460-71.
Lee, Y. I., Kang-Park, S., and Do, S. I. (2001). *J Biol Chem* **276,** 16969-77.
Lefebvre, L., Ciminale, V., Vanderplasschen, A., D'Agostino, D., Burny, A., Willems, L., and Kettmann, R. (2002a). *J Virol* **76,** 7843-54.
Lefebvre, L., Vanderplasschen, A., Ciminale, V., Heremans, H., Dangoisse, O., Jauniaux, J. C., Toussaint, J. F., Zelnik, V., Burny, A., Kettmann, R., and Willems, L. (2002b). *J Virol* **76,** 1400-14.
Le-Quoc, K., and Le-Quoc, D. (1985). *J Biol Chem* **260,** 7422-8.
Li, H., Zhu, H., Xu, C. J., and Yuan, J. (1998). *Cell* **94,** 491-501.
Li, L. Y., Shih, H. M., Liu, M. Y., and Chen, J. Y. (2001). *J Biol Chem* **276,** 27354-62.
Liang, P. H., Ko, T. P., and Wang, A. H. (2002). *Eur J Biochem* **269,** 3339-54.
Liu, Z., Sekito, T., Spirek, M., Thornton, J., and Butow, R. A. (2003). *Mol Cell* **12,** 401-11.
Longworth, M. S., and Laimins, L. A. (2004). *Microbiol Mol Biol Rev* **68,** 362-72.
Loomis, R., Carbone, R., Reiss, M., and Lacy, J. (2003). *Clin Cancer Res* **9,** 1931-9.
Lowy, D. R., and Howley, P. M. (2001). *In* "Fields Virology" (D. M. a. H. Knipe, P.M., ed.), Vol. 2, pp. 2231-2264. Lippincott Williams and Wilkins, Philadelphia.
Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). *Cell* **94,** 481-90.
Major, M. E., Rehermann, B., and Feinstone, S. M. (2001). *In* "Fields Virology" (D. M. a. H. Knipe, P.M., ed.), Vol. 1, pp. 1127-1162. Lippincott Williams and Wilkins, Philadelphia.
Mandavilli, B. S., Santos, J. H., and Van Houten, B. (2002). *Mutat Res* **509,** 127-51.
Marchini, A., Tomkinson, B., Cohen, J. I., and Kieff, E. (1991). *J Virol* **65,** 5991-6000.
Marshall, W. L., Yim, C., Gustafson, E., Graf, T., Sage, D. R., Hanify, K., Williams, L., Fingeroth, J., and Finberg, R. W. (1999). *J Virol* **73,** 5181-5.
Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vieira, H. L., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C., and Kroemer, G. (1998). *Science* **281,** 2027-31.
Matsuoka, M. (2003). *Oncogene* **22,** 5131-40.

McCarthy, N. J., Hazlewood, S. A., Huen, D. S., Rickinson, A. B., and Williams, G. T. (1996). *Adv Exp Med Biol* **406,** 83-97.
McGlade, C. J., Tremblay, M. L., Yee, S. P., Ross, R., and Branton, P. E. (1987). *J Virol* **61,** 3227-34.
Midgley, R. S., and Kerr, D. J. (2002). *Crit Rev Oncol Hematol* **44,** 109-20.
Mikhailov, V., Mikhailova, M., Pulkrabek, D. J., Dong, Z., Venkatachalam, M. A., and Saikumar, P. (2001). *J Biol Chem* **276,** 18361-74.
Moon, E. J., Jeong, C. H., Jeong, J. W., Kim, K. R., Yu, D. Y., Murakami, S., Kim, C. W., and Kim, K. W. (2004). *Faseb J* **18,** 382-4.
Moore, P. S., and Chang, Y. (2003). *Annu Rev Microbiol* **57,** 609-39.
Morellet, N., Bouaziz, S., Petitjean, P., and Roques, B. P. (2003). *J Mol Biol* **327,** 215-27.
Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. L., and Fesik, S. W. (1996). *Nature* **381,** 335-41.
Munger, K., Phelps, W. C., Bubb, V., Howley, P. M., and Schlegel, R. (1989). *J Virol* **63,** 4417-21.
Muralidhar, S., Pumfery, A. M., Hassani, M., Sadaie, M. R., Kishishita, M., Brady, J. N., Doniger, J., Medveczky, P., and Rosenthal, L. J. (1998). *J Virol* **72,** 4980-8.
Nakahara, T., Nishimura, A., Tanaka, M., Ueno, T., Ishimoto, A., and Sakai, H. (2002). *J Virol* **76,** 10914-20.
Nasseri, M., Hirochika, R., Broker, T. R., and Chow, L. T. (1987). *Virology* **159,** 433-9.
Nicot, C., Dundr, M., Johnson, J. M., Fullen, J. R., Alonzo, N., Fukumoto, R., Princler, G. L., Derse, D., Misteli, T., and Franchini, G. (2004). *Nat Med*.
Nishino, Y., Myojin, T., Kamata, M., and Aida, Y. (1997). *Biochem Biophys Res Commun* **232,** 550-4.
Oh, J. C., Jeong, D. L., Kim, I. K., and Oh, S. H. (2003). *Exp Mol Med* **35,** 301-9.
Ojala, P. M., Yamamoto, K., Castanos-Velez, E., Biberfeld, P., Korsmeyer, S. J., and Makela, T. P. (2000). *Nat Cell Biol* **2,** 819-25.
Pang, S., Kang, M. K., Kung, S., Yu, D., Lee, A., Poon, B., Chen, I. S., Lindemann, B., and Park, N. H. (2001). *Clin Cancer Res* **7,** 3567-73.
Parola, M., and Robino, G. (2001). *J Hepatol* **35,** 297-306.
Pastorino, J. G., Chen, S. T., Tafani, M., Snyder, J. W., and Farber, J. L. (1998). *J Biol Chem* **273,** 7770-5.
Pastorino, J. G., Snyder, J. W., Serroni, A., Hoek, J. B., and Farber, J. L. (1993). *J Biol Chem* **268,** 13791-8.
Paterlini, P., Poussin, K., Kew, M., Franco, D., and Brechot, C. (1995). *Hepatology* **21,** 313-21.
Pavlovic, D., Neville, D. C., Argaud, O., Blumberg, B., Dwek, R. A., Fischer, W. B., and Zitzmann, N. (2003). *Proc Natl Acad Sci U S A* **100,** 6104-8.
Pawlotsky, J. M. (2004). *Trends Microbiol* **12,** 96-102.
Pearson, G. R., Luka, J., Petti, L., Sample, J., Birkenbach, M., Braun, D., and Kieff, E. (1987). *Virology* **160,** 151-61.
Pebay-Peyroula, E., Dahout-Gonzalez, C., Kahn, R., Trezeguet, V., Lauquin, G. J., and Brandolin, G. (2003). *Nature* **426,** 39-44.
Peh, W. L., Brandsma, J. L., Christensen, N. D., Cladel, N. M., Wu, X., and Doorbar, J. (2004). *J Virol* **78,** 2142-51.
Perl, A., Gergely, P., Jr., Nagy, G., Koncz, A., and Banki, K. (2004). *Trends Immunol* **25,** 360-7.
Pilder, S., Logan, J., and Shenk, T. (1984). *J Virol* **52,** 664-71.
Pollicino, T., Terradillos, O., Lecoeur, H., Gougeon, M. L., and Buendia, M. A. (1998). *Biomed Pharmacother* **52,** 363-8.
Poole, L. J., Zong, J. C., Ciufo, D. M., Alcendor, D. J., Cannon, J. S., Ambinder, R., Orenstein, J. M., Reitz, M. S., and Hayward, G. S. (1999). *J Virol* **73,** 6646-60.

Poon, B., Grovit-Ferbas, K., Stewart, S. A., and Chen, I. S. (1998). *Science* **281,** 266-9.
Poynard, T., Yuen, M. F., Ratziu, V., and Lai, C. L. (2003). *Lancet* **362,** 2095-100.
Rahmani, Z., Huh, K. W., Lasher, R., and Siddiqui, A. (2000). *J Virol* **74,** 2840-6.
Rahmani, Z., Maunoury, C., and Siddiqui, A. (1998). *Eur J Hum Genet* **6,** 337-40.
Raj, K., Berguerand, S., Southern, S., Doorbar, J., and Beard, P. (2004). *J Virol* **78,** 7199-207.
Ray, R. B., and Ray, R. (2001). *FEMS Microbiol Lett* **202,** 149-56.
Re, F., Braaten, D., Franke, E. K., and Luban, J. (1995). *J Virol* **69,** 6859-64.
Re, F., and Luban, J. (1997). *Prog Cell Cycle Res* **3,** 21-7.
Reyes, G. R. (2002). *J Biomed Sci* **9,** 187-97.
Rickinson, A. B., and Kieff, E. (2001). *In* "Fields Virology" (D. M. Knipe, Howley, P.M., ed.), Vol. 2, pp. 2575-2628. Lippincott Williams and Wilkins, Philadelphia.
Rizzuto, R., Bernardi, P., and Pozzan, T. (2000). *J Physiol* **529 Pt 1,** 37-47.
Robek, M. D., Wong, F. H., and Ratner, L. (1998). *J Virol* **72,** 4458-62.
Roberts, S., Ashmole, I., Gibson, L. J., Rookes, S. M., Barton, G. J., and Gallimore, P. H. (1994). *J Virol* **68,** 6432-45.
Roberts, S., Ashmole, I., Johnson, G. D., Kreider, J. W., and Gallimore, P. H. (1993). *Virology* **197,** 176-87.
Roberts, S., Ashmole, I., Rookes, S. M., and Gallimore, P. H. (1997). *J Virol* **71,** 3554-62.
Roskoski, R., Jr. (2003). *Biochem Biophys Res Commun* **303,** 1-7.
Runquist, M., Ericsson, J., Thelin, A., Chojnacki, T., and Dallner, G. (1994). *J Biol Chem* **269,** 5804-9.
Saelens, X., Festjens, N., Walle, L. V., van Gurp, M., van Loo, G., and Vandenabeele, P. (2004). *Oncogene* **23,** 2861-74.
Sakai, A., Claire, M. S., Faulk, K., Govindarajan, S., Emerson, S. U., Purcell, R. H., and Bukh, J. (2003). *Proc Natl Acad Sci U S A* **100,** 11646-51.
Samavati, L., Monick, M. M., Sanlioglu, S., Buettner, G. R., Oberley, L. W., and Hunninghake, G. W. (2002). *Am J Physiol Cell Physiol* **283,** C273-81.
Sarid, R., Sato, T., Bohenzky, R. A., Russo, J. J., and Chang, Y. (1997). *Nat Med* **3,** 293-8.
Sattler, M., Liang, H., Nettesheim, D., Meadows, R. P., Harlan, J. E., Eberstadt, M., Yoon, H. S., Shuker, S. B., Chang, B. S., Minn, A. J., Thompson, C. B., and Fesik, S. W. (1997). *Science* **275,** 983-6.
Schulz, T. F. (1999). *Adv Cancer Res* **76,** 121-60.
Schulz, T. F. (2000). *J Antimicrob Chemother* **45 Suppl T3,** 15-27.
Scorrano, L., Ashiya, M., Buttle, K., Weiler, S., Oakes, S. A., Mannella, C. A., and Korsmeyer, S. J. (2002). *Dev Cell* **2,** 55-67.
Scorrano, L., and Korsmeyer, S. J. (2003). *Biochem Biophys Res Commun* **304,** 437-44.
Scorrano, L., Oakes, S. A., Opferman, J. T., Cheng, E. H., Sorcinelli, M. D., Pozzan, T., and Korsmeyer, S. J. (2003). *Science* **300,** 135-9.
Scorrano, L., Penzo, D., Petronilli, V., Pagano, F., and Bernardi, P. (2001). *J Biol Chem* **276,** 12035-40.
Scorrano, L., Petronilli, V., Di Lisa, F., and Bernardi, P. (1999). *J Biol Chem* **274,** 22581-5.
Sekito, T., Thornton, J., and Butow, R. A. (2000). *Mol Biol Cell* **11,** 2103-15.
Sharp, T. V., Wang, H. W., Koumi, A., Hollyman, D., Endo, Y., Ye, H., Du, M. Q., and Boshoff, C. (2002). *J Virol* **76,** 802-16.
Shim, H., Dolde, C., Lewis, B. C., Wu, C. S., Dang, G., Jungmann, R. A., Dalla-Favera, R., and Dang, C. V. (1997). *Proc Natl Acad Sci U S A* **94,** 6658-63.
Shimizu, S., Konishi, A., Kodama, T., and Tsujimoto, Y. (2000). *Proc Natl Acad Sci U S A* **97,** 3100-5.
Shimizu, S., Narita, M., and Tsujimoto, Y. (1999). *Nature* **399,** 483-7.
Shintani, Y., Yotsuyanagi, H., Moriya, K., Fujie, H., Tsutsumi, T., Kanegae, Y., Kimura, S., Saito, I., and Koike, K. (1999). *J Gen Virol* **80 (Pt 12),** 3257-65.

Shirakata, Y., Kawada, M., Fujiki, Y., Sano, H., Oda, M., Yaginuma, K., Kobayashi, M., and Koike, K. (1989). *Jpn J Cancer Res* **80,** 617-21.
Shirakata, Y., and Koike, K. (2003). *J Biol Chem* **278,** 22071-8.
Silic-Benussi, M., Cavallari, I., Zorzan, T., Rossi, E., Hiraragi, H., Rosato, A., Horie, K., Saggioro, D., Lairmore, M. D., Willems, L., Chieco-Bianchi, L., D'Agostino, D. M., and Ciminale, V. (2004). *Proc Natl Acad Sci U S A* **101,** 6629-34.
Sirma, H., Giannini, C., Poussin, K., Paterlini, P., Kremsdorf, D., and Brechot, C. (1999). *Oncogene* **18,** 4848-59.
Sirma, H., Weil, R., Rosmorduc, O., Urban, S., Israel, A., Kremsdorf, D., and Brechot, C. (1998). *Oncogene* **16,** 2051-63.
Smith, K. J., Gallimore, P. H., and Grand, R. J. (1989). *Oncogene* **4,** 489-97.
Soriano, M. E., Nicolosi, L., and Bernardi, P. (2004). *J Biol Chem*.
Steegenga, W. T., Riteco, N., Jochemsen, A. G., Fallaux, F. J., and Bos, J. L. (1998). *Oncogene* **16,** 349-57.
Stewart, S. A., Poon, B., Jowett, J. B., and Chen, I. S. (1997). *J Virol* **71,** 5579-92.
Stewart, S. A., Poon, B., Jowett, J. B., Xie, Y., and Chen, I. S. (1999). *Proc Natl Acad Sci U S A* **96,** 12039-43.
Sturzl, M., Zietz, C., Monini, P., and Ensoli, B. (2001). *Adv Cancer Res* **81,** 125-59.
Su, F., and Schneider, R. J. (1997). *Proc Natl Acad Sci U S A* **94,** 8744-9.
Subramanian, T., Tarodi, B., and Chinnadurai, G. (1995). *Cell Growth Differ* **6,** 131-7.
Subramanian, T., Tarodi, B., Govindarajan, R., Boyd, J. M., Yoshida, K., and Chinnadurai, G. (1993). *Gene* **124,** 173-81.
Sun, Q., Zachariah, S., and Chaudhary, P. M. (2003). *J Biol Chem*.
Sundararajan, R., Cuconati, A., Nelson, D., and White, E. (2001). *J Biol Chem* **276,** 45120-7.
Sundararajan, R., and White, E. (2001). *J Virol* **75,** 7506-16.
Susin, S. A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M., and Kroemer, G. (1996). *J Exp Med* **184,** 1331-41.
Suzuki, Y., Demoliere, C., Kitamura, D., Takeshita, H., Deuschle, U., and Watanabe, T. (1997). *J Immunol* **158,** 2736-44.
Szabo, I., De Pinto, V., and Zoratti, M. (1993). *FEBS Lett* **330,** 206-10.
Szabo, I., and Zoratti, M. (1993). *FEBS Lett* **330,** 201-5.
Takada, S., Kaneniwa, N., Tsuchida, N., and Koike, K. (1997). *Oncogene* **15,** 1895-901.
Takada, S., Shirakata, Y., Kaneniwa, N., and Koike, K. (1999). *Oncogene* **18,** 6965-73.
Takayama, S., Cazals-Hatem, D. L., Kitada, S., Tanaka, S., Miyashita, T., Hovey, L. R., 3rd, Huen, D., Rickinson, A., Veerapandian, P., Krajewski, S., and et al. (1994). *DNA Cell Biol* **13,** 679-92.
Takemori, N., Cladaras, C., Bhat, B., Conley, A. J., and Wold, W. S. (1984). *J Virol* **52,** 793-805.
Tarodi, B., Subramanian, T., and Chinnadurai, G. (1994). *Virology* **201,** 404-7.
Terradillos, O., de La Coste, A., Pollicino, T., Neuveut, C., Sitterlin, D., Lecoeur, H., Gougeon, M. L., Kahn, A., and Buendia, M. A. (2002). *Oncogene* **21,** 377-86.
Terradillos, O., Pollicino, T., Lecoeur, H., Tripodi, M., Gougeon, M. L., Tiollais, P., and Buendia, M. A. (1998). *Oncogene* **17,** 2115-23.
Theodorakis, P., D'Sa-Eipper, C., Subramanian, T., and Chinnadurai, G. (1996). *Oncogene* **12,** 1707-13.
Thome, M., Schneider, P., Hofmann, K., Fickenscher, H., Meinl, E., Neipel, F., Mattmann, C., Burns, K., Bodmer, J. L., Schroter, M., Scaffidi, C., Krammer, P. H., Peter, M. E., and Tschopp, J. (1997). *Nature* **386,** 517-21.
Thorley-Lawson, D. A. (2001). *Nat Rev Immunol* **1,** 75-82.

Ueda, H., Ullrich, S. J., Gangemi, J. D., Kappel, C. A., Ngo, L., Feitelson, M. A., and Jay, G. (1995). *Nat Genet* **9,** 41-7.
Unsal, H., Yakicier, C., Marcais, C., Kew, M., Volkmann, M., Zentgraf, H., Isselbacher, K. J., and Ozturk, M. (1994). *Proc Natl Acad Sci U S A* **91,** 822-6.
Unwin, R. D., Craven, R. A., Harnden, P., Hanrahan, S., Totty, N., Knowles, M., Eardley, I., Selby, P. J., and Banks, R. E. (2003). *Proteomics* **3,** 1620-32.
Vander Heiden, M. G., Chandel, N. S., Li, X. X., Schumacker, P. T., Colombini, M., and Thompson, C. B. (2000). *Proc Natl Acad Sci U S A* **97,** 4666-71.
Vander Heiden, M. G., Chandel, N. S., Schumacker, P. T., and Thompson, C. B. (1999). *Mol Cell* **3,** 159-67.
Vander Heiden, M. G., Chandel, N. S., Williamson, E. K., Schumacker, P. T., and Thompson, C. B. (1997). *Cell* **91,** 627-37.
Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., Snijders, P. J., Peto, J., Meijer, C. J., and Munoz, N. (1999). *J Pathol* **189,** 12-9.
Wang, H. W., Sharp, T. V., Koumi, A., Koentges, G., and Boshoff, C. (2002). *Embo J* **21,** 2602-15.
Wang, Q., Griffin, H., Southern, S., Jackson, D., Martin, A., McIntosh, P., Davy, C., Masterson, P. J., Walker, P. A., Laskey, P., Omary, M. B., and Doorbar, J. (2004). *J Virol* **78,** 821-33.
Wang, X. W., Forrester, K., Yeh, H., Feitelson, M. A., Gu, J. R., and Harris, C. C. (1994). *Proc Natl Acad Sci U S A* **91,** 2230-4.
Warburg, O. (1956). *Science* **123,** 309-14.
Waris, G., Huh, K. W., and Siddiqui, A. (2001). *Mol Cell Biol* **21,** 7721-30.
White, E. (1993). *Proc Soc Exp Biol Med* **204,** 30-9.
White, E. (2001). *Oncogene* **20,** 7836-46.
White, E., Grodzicker, T., and Stillman, B. W. (1984). *J Virol* **52,** 410-9.
Willems, L., Burny, A., Collete, D., Dangoisse, O., Dequiedt, F., Gatot, J. S., Kerkhofs, P., Lefebvre, L., Merezak, C., Peremans, T., Portetelle, D., Twizere, J. C., and Kettmann, R. (2000). *AIDS Res Hum Retroviruses* **16,** 1787-95.
Willems, L., Kerkhofs, P., Dequiedt, F., Portetelle, D., Mammerickx, M., Burny, A., and Kettmann, R. (1994). *Proc Natl Acad Sci U S A* **91,** 11532-6.
Williams, T., Sale, D., and Hazlewood, S. A. (2001). *Intervirology* **44,** 55-8.
Yasuda, M., Theodorakis, P., Subramanian, T., and Chinnadurai, G. (1998). *J Biol Chem* **273,** 12415-21.
Yin, X. M., Oltvai, Z. N., and Korsmeyer, S. J. (1994). *Nature* **369,** 321-3.
Yoo, Y. G., Oh, S. H., Park, E. S., Cho, H., Lee, N., Park, H., Kim, D. K., Yu, D. Y., Seong, J. K., and Lee, M. O. (2003). *J Biol Chem* **278,** 39076-84.
Young, L. S., and Murray, P. G. (2003). *Oncogene* **22,** 5108-21.
Younis, I., Khair, L., Dundr, M., Lairmore, M. D., Franchini, G., and Green, P. L. (2004). *J Virol* **78,** 11077-83.
Zhang, W., Nisbet, J. W., Bartoe, J. T., Ding, W., and Lairmore, M. D. (2000). *J Virol* **74,** 11270-7.
Zou, H., Li, Y., Liu, X., and Wang, X. (1999). *J Biol Chem* **274,** 11549-56.
Zoulim, F., Saputelli, J., and Seeger, C. (1994). *J Virol* **68,** 2026-30.
Zuckerman, E., and Zuckerman, T. (2002). *Blood Rev* **16,** 119-25.
zur Hausen, H. (2002). *Nat Rev Cancer* **2,** 342-50.

Table 1. The human tumor viruses and their mitochondrial proteins

Virus	Taxonomic family	Virion structure	Main cellular targets *in vivo*	Viral proteins implicated in transformation	Mitochondrial proteins and their activities
EBV	*Herpesviridae*	115-nm-diameter enveloped virion; 184-kbp linear, double-stranded DNA genome	B-cells, epithelial cells	[a]EBNA2, EBNA3A, EBNA3C, LMP1	**BHRF1** (anti-apoptotic vBCL-2)
KSHV	*Herpesviridae*	110-nm-diameter enveloped virion; 160-170 kbp linear, double-stranded DNA genome	B-cells, endothelial cells	[a]vGPCR, vIRF-1, vFLIP, K1, Kaposin	**KSBCL-2** (anti-apoptotic vBCL-2) **K7** (vIAP) **K15** (binds to HAX1)
HBV	*Hepadnaviridae*	42-47-nm-diameter enveloped virion; 3.2-kb circular, partially double-stranded DNA genome	hepatocytes	HBx	**HBx** (disrupts mitochondrial morphology, membrane potential and permeability; pro-apoptotic)
HPV	*Papillomaviridae*	55-nm-diameter naked virion; 7.9-kbp closed circular double-stranded genome	epithelial cells	E6, E7	**E1^E4** (alters mitochondrial distribution, disrupts membrane potential, pro-apoptotic)
HTLV-1	*Retroviridae*	110-nm-diameter enveloped virion; 9-kb RNA genome (packaged as dimer)	T-cells	Tax	**p13^{II}** (disrupts mitochondrial morphology and permeability; ion channel?)
HCV	*Flaviviridae*	50-nm-diameter enveloped virion; 9.5-kb single-stranded RNA genome	hepatocytes	Core, NS5A	**p7** (ion channel)

[a] EBV and KSHV express a large number of proteins that promote cell proliferation or survival; listed are the EBV proteins that are required for B-cell transformation *in vitro* (Young and Murray, 2003) and the KSHV proteins demonstrated to transform cells *in vitro* (Bais, et al., 1998; Gao, et al., 1997; Lee, et al., 1998; Muralidhar, et al., 1998; Sun, et al., 2003).

Table 2. Properties of human tumor virus vBCL-2s

Virus	**Protein**	**Size**	**Functional domains**	**localization**	***in vivo* interactions with BCL-2 family members**	**other *in vivo* interactions**
EBV	BHRF1	191 a.a	BH3, BH1, BH2, TM	endomembranes, mitochondria	BAX, BAK, BIK, BOK	PRA1, R-Ras
	BALF0	220 a.a.	BH4, BH3, BH1, BH2	cytoplasm	ND	ND
	BALF1	182 a.a.	BH3, BH1, BH2	cytoplasm	ND	ND
KSHV	KSBCL-2	175 a.a.	BH4, BH3, BH1, BH2, TM	punctate cytoplasmic pattern (mitochondria? see text)	BCL-2? (see text)	ND

The domain structures of BHRF1 and KSBCL-2 are according to Cuconati and White (2002) and those of BALF0 and BALF1 are according to (Marshall, et al., 1999) and (Bellows, et al., 2002). ND, no data.

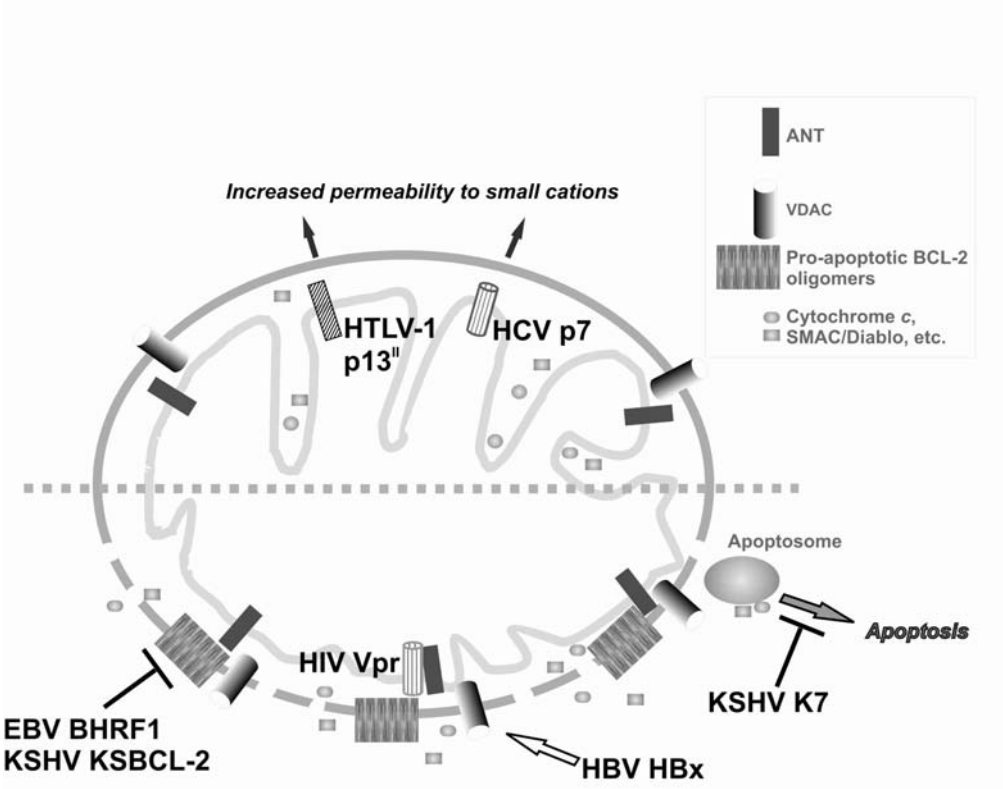

Figure 1. Interactions of tumor virus proteins with mitochondria. The portion of the mitochondrion drawn below the dotted line has received an apoptotic stimulus resulting in oligomerization of pro-apoptotic BCL-2 proteins, rupture of the outer membrane, and release of cytochrome *c* and other pro-apoptotic factors. EBV BHRF1 and KSHV KSBCL-2 are anti-apoptotic BCL-2 homologues that block the function of pro-apoptotic BCL-2 proteins, and KSHV K7 is a vIAP homologous to Survivin that inhibits activation of caspase 3. HBx interacts with VDAC, influences Ca2+ signalling, disrupts $\Delta\psi$ and permeability, and may trigger apoptosis. HIV Vpr is included as an example of a viroporin that can promote apoptosis; it interacts with ANT. HTLV-1 $p13^{II}$ and HCV p7 increase the permeability of mitochondria to small cations, with p7 classified as a viroporin. KSHV K15 has not functionally characterized, but does not appear to promote or inhibit apoptosis. HPV E1^E4 disrupts $\Delta\psi$ and promotes apoptosis through an unknown mechanism. Other properties of these proteins are described in the text.

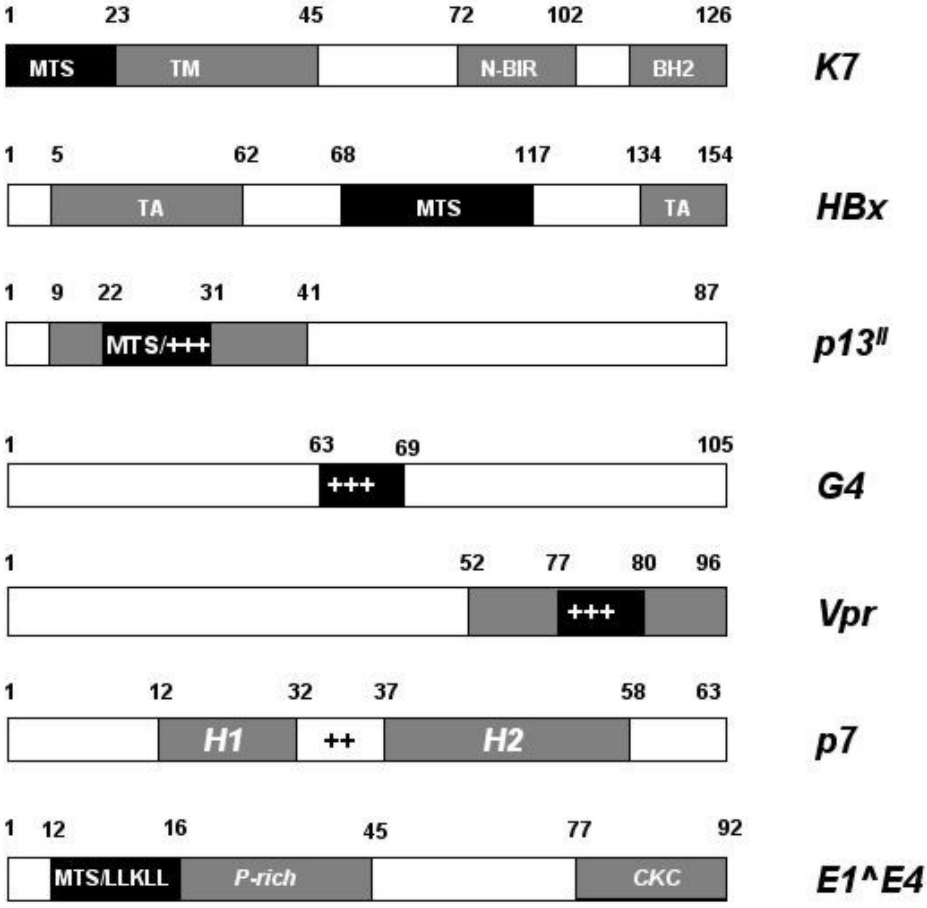

Figure 2. Domain structure of viral proteins acting through non-BCL-2 mechanisms. Indicated are the main functional domains identified in tumor virus proteins acting through mechanisms distinct from that of v-BCL-2. TA = transactivation domain; LLKLL = leucine-rich region; MTS = mitochondrial targeting signal; TM = transmembrane domain; N-BIR = baculovirus internal repeat-like domain; BH2 = BCL-2 homology region; +++ = positively-charged amphipathic α-helix; H1, H2 = α-helical regions; CKC = domain required to collapse the keratin network, which also contains sequences directing multimerization; The MTS/LLKLL sequence of HPV E1^E4 serves both for mitochondrial targeting and for keratin binding. The E1^E4 scheme refers to the HPV 16 protein. Drawings are not in scale.