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Deciphering novel host–herpesvirus interactions by virion proteomics

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Over the years, a vast array of information concerning the interactions of viruses with their hosts has been collected. However, recent advances in proteomics and other system biology techniques suggest these interactions are far more complex than anticipated. One particularly interesting and novel aspect is the analysis of cellular proteins incorporated into mature virions. Though sometimes considered purification contaminants in the past, their repeated detection by different laboratories suggests that a number of these proteins are *bona fide* viral components, some of which likely contribute to the viral life cycles. The present mini review focuses on cellular proteins detected in herpesviruses. It highlights the common cellular functions of these proteins, their potential implications for host–pathogen interactions, discusses technical limitations, the need for complementing methods and probes potential future research avenues.

Keywords: herpes, virus, host–pathogen interactions, HSV, PRV, HCMV, EBV γHV68, KSHV

INTRODUCTION

Over the last decades, many host–pathogen interactions have been characterized using genetics, biochemical, and microscopy approaches. These discoveries relied on mutants, pharmacological reagents, immunoprecipitations, immunofluorescence, electron microscopy, cell fractionation, and Western blotting to name a few of the methods employed. These approaches provided much precious information but, given the typical focus of these approaches on individual molecules, likely only revealed a small portion of the proteins involved. Other methods such as high throughput two-hybrid and genetic screens, nucleic acid arrays, RNA interference, and proteomics are now proving essential tools to tackle the complexity of these interactions. The main advantages of mass spectrometry, for instance, are that it is a fast, sensitive and potentially a quantitative approach to identify putative novel players, particularly when coupled to efficient purification schemes. Already, proteomics revealed how viruses modulate the expression of host proteins (Rassmann et al., 2006; Sun et al., 2008; Tong et al., 2008; Antrobus et al., 2009; Pastorino et al., 2009; Thanthrige-Don et al., 2009; Zandi et al., 2009; Zhang et al., 2009, 2010; Coombs et al., 2010; Emmott et al., 2010; Lu et al., 2010, 2012; Munday et al., 2010; Bartel et al., 2011; Lietzen et al., 2011; Ramirez-Boo et al., 2011; Chou et al., 2012). A relatively new and interesting field is the characterization of host–pathogen interactions within mature purified virions. As reviewed on several occasions, several studies reported the presence of individual cellular proteins in viral particles (Bernhard et al., 2005; Maxwell and Frappier, 2007; Viswanathan and Fruh, 2007; Friedel and Haas, 2011; Zheng et al., 2011). This includes vaccinia virus (Krauss et al., 2002), influenza virus (Shaw et al., 2008), HIV (Gurer et al., 2002; Cantin et al., 2005; Ott, 2008), vesicular stomatitis virus (Moerdyk-Schauwecker et al., 2009), and several herpesviruses (see below). Though these cellular components have often been considered purification contaminants, the presence of similar proteins in both related and unrelated viruses suggests that some of them may be biologically relevant. The identification of virion-associated host proteins could thus lead to the discovery of novel therapeutic tools against viruses. The present review focuses on their identification and putative roles with respect to the proteomics of herpesviruses.

PROTEOMICS OF HERPESVIRIONS

Thus far, the protein composition of eight different herpesvirions has been studied by mass spectrometry. These studies include the alphaherpesvirinae herpes simplex virus type 1 (HSV-1) and pseudorabies virus (PRV; Loret et al., 2008; Kramer et al., 2011), the betaherpesvirinae human and murine cytomegaloviruses (HCMV and MCMV, respectively; Kattenhorn et al., 2004; Varnum et al., 2004) and the gammaherpesvirinae Kaposi sarcoma herpesvirus (KSHV), gamma herpesvirus 68 (γHV68), Epstein–Barr virus (EBV), and Alcelaphine (Bortz et al., 2003; Johannsen et al., 2004; Bechtel et al., 2005; Zhu et al., 2005; Dry et al., 2008). Interestingly, host proteins were detected in all herpesvirions analyzed so far, as summarized in **Table 1**. For instance, our laboratory previously reported the protein composition of mature extracellular HSV-1 viral particles and identified as many as 49 cellular proteins (Loret et al., 2008). Similarly, studies focusing on PRV and EBV reported up to 48 and 43 cellular proteins, respectively (Johannsen et al., 2004; Kramer et al., 2011). Meanwhile, Varnum et al. (2004) found as many as 70 different host proteins in extracellular HCMV virions. While fewer cellular proteins were reported for other viral particles, it is clear that herpesviruses can potentially incorporate many proteins from its host. Moreover, of the 173 different proteins detected in herpesvirions, nine protein groups are present in at least four distinct herpesvirions. This includes 14-3-3, actin, annexins, cofilin, translation factors, GAPDH, heat shock proteins, pyruvate kinase M2, and various Rab GTPases. These results indicate that, first of all, it is common for herpesviruses to incorporate cellular proteins into their viral particles and, secondly, that

Table 1 | Proteomics studies of herpesviruses associated host proteins.

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P31946	4507949	14-3-3 Protein beta (tyrosine 2-monooxygenase beta)		+	+				+?		
P62258	5803225	14-3-3 Protein epsilon (tyrosine-3-monooxygenase epsilon)	+	+	+		+		+		
P61981		14-3-3 Protein gamma (tyrosine-3-monooxygenase gamma)	+								
P31947		14-3-3 Protein sigma (tyrosine-3-monooxygenase sigma)		+							
P27348	5803227	14-3-3 protein theta (tyrosine-3-monooxygenase theta)		+	+						
P63104	4507953	14-3-3 protein zeta/delta (tyrosine-3-monooxygenase zeta/delta)	+		+		+		+		
P68133	1070613	Actin (alpha)	+?		+	+?					+?
P60709	4501885	Actin (beta)	+?		+	+?	+	+	+	+	+?
P63261		Actin (gamma)	+?			+?					+?
P12814	112959	Actin (alpha)		+	+		+				
P04075	49168540	Aldolase A					+				
P15144	113743	Aminopeptidase N (CD13)			+						
P08195.3	21361344	Amino acid transporter					+				
P80385	2507205	AMP-activated protein kinase subunit gamma-1							+		
P04083	4502101	Annexin A1	+		+	+?				+	+
P07355	4757756	Annexin A2	+		+				+	+	+
P08758	3212603	Annexin A5	+	+	+	+?					
P08133.3	113962	Annexin A6			+		+		+		
P13928		Annexin A8		+							
	1065361	Adp-ribosylation factor 1 chain A			+						
P84077		Arf1	+	+	+						
P61204	1351907	Arf3	+	+							
P18085		Arf4	+								
P84085		Arf5	+								
P00966.2	4557337	Arginosuccinate synthetase					+				
P61158.3	5031573	ARP3 (actin-related protein)					+				
P08243.4	19718772	Asparagine synthetase					+				
P11021.2	121567	BIP (GRP78)			+						
P27797.1	4757900	Calreticulin			+						

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P62158		Calmodulin		+							
P68400	4503095	Casein kinase 2	+	+	+						
P08195.2	112803	CD98 (lymphocyte activation antigen 4F2 large subunit)			+						
O00299		Chloride intracellular channel protein 1		+							
Q00610.5	4758012	Clathrin heavy chain			+		+				
P23528	5031635	Cofilin 1	+		+	+	+				
Q5G6V9		Cofilin 2		+							
P12277.1	21536286	Creatine kinase					+				
P62937	118102	Cyclophilin A	+		+				+		
P21291		Cystein-glycine rich protein 1	+								
Q92499.2	4826686	Dead box protein 1			+						
O00571	4503295	DDX3X (dead box protein 3; ATP dependant RNA helicase)	+	+	+						
P60981		Destrin		+							
	219588	DnaJ human homolog			+						
Q9H4M9.2	30240932	EH-domain containing protein(s)					+				
P58021.1		Endomembrane protein 70 (transmembrane 9 superfamily member 2 precursor)								+	
P14625.1	4507677	Endoplasmin precursor (tumor rejection antigen; gp96)			+						
	2135068	Enhancer protein			+						
P06733.2	4503571	Enolase 1 (alpha)			+		+	+	+		
P68104.1	4503471	Eukaryotic translation elongation factor 1			+		+				
	1169475	EF-1α (EF-Tu)				+					
P13639.4	4503483	Eukaryotic translation elongation factor 2			+		+	+	+		
P60842	4503529	Eukaryotic initiation factor 4A-I		+	+						
Q15056		Eukaryotic translation initiation factor 4H (eIF4H=WBSCR1)	+	+	+? (eIF4A)						
P63241		Eukaryotic initiation factor 5A-I		+							
P21333.4	4503745	Filamin 1 (actin binding protein 280)			+		+				
P04899.3	4504041	G protein alpha-I					+				

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P17931		Galectin 3		+							
P50395.2	13638228	Rab GDP Dissociation Inhibitor Beta (Rab GDI)			+						
P80031		Glutathione *S*-transferase		+							
P04406	7669492	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	+		+	+	+		+		
P62993	4504111	Growth factor receptor bound protein 2	+	+	+						
P62879		Guanine nucleotide-binding protein subunit alpha-2		+							
P08107.5	2119712	HSPa1L (DNAK-type molecular chaperone; Heat shock 70 kDa protein 1A/1B or 1/2; HSP70.1/HSP70.2)			+						
Q5S1U1		HSP27		+	+						
Q27965.1	2495339	Heat shock protein 70 (HSP70.2)							+		
P54652.1	1708307	Heat shock 70 protein 2			+						
P11021.2	16507237	Heat Shock 70 protein 5 (78 kDa glucose-regulated protein precursor; BIP?)					+				
P48741.2	1346317	Heat shock 70 protein 7 (heat shock 70 kDa protein B)			+						
P11142	5729877	Heat shock 70 protein 8 (HspA8; LAP1)	+				+				
P19120	5729877	Heat Shock 70 protein 10 (Hsc71)			+			+			
P07900.5	123678	Heat shock 90 protein 1 alpha (HSP 86)					+				
P08238.4	6680307	Heat shock 90 protein 1 beta			+		+				
P07900	6016267	Heat shock protein HSP90			+			+	+		
P22752.3	121961	Histone 2A				+?					
		Histone 2B				+?					+
		Histone 4									+
P22626		HNRNPA2B1		+							
P22626		HNRNPF		+							
P22626		HNRNPK		+							
P22626		HNRNPH3		+							
Q9GLP0		Integrin beta-1		+							
P04264		Keratin 1	+		+						
	1082558	Keratin 9			+						

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P13645		Keratin 10	+		+						
Q08431.2	422992	Lactadherin (milk fat globule protein)			+						
Q08431.2	5174557	Lactadherin isoform a preproprotein (Milk Fat Globule-EGF Factor 8 Protein)			+						
P00338.2	5031857	Lactate dehydrogenase A			+						
P07195.2	4557032	Lactate dehydrogenase B			+						
P28838.3	7705688	Leucine aminopeptidase (cytosol aminopeptidase)					+				
Q9GKE8		Leukocyte surface antigen CD47		+							
O60488		Long chain fatty acid CoA ligase 4		+							
P13796.6	4504965	L plastin (lymphocyte cytosolic protein 1; plastin-2)					+		+		
Q53EU6		Lung cancer metastasis-associated protein (1-acylglycerol-3-phosphate *O*-acyltransferase 9)	+								
P14174		Macrophage migration inhibitory factor (glycosylation-inhibiting factor; phenylpyruvate tautomerase)	+								
P28482		MAPK1		+							
P13987		Membrane attack complex inhibition factor (CD59)	+								
P30515.1	231348	MHC_I (Class I histocompatibility antigen, A alpha chain)					+				
AAH01112	4505257	Myosin			+		+	+			
P35579.4	6166599	Myosin heavy chain (myosin-9; non-muscle myosin heavy chain A)			+				+		
P60660		Myosin light polypeptide 6		+							
O00159		Myosin Ic		+							
P30101.4	1085373	PDI ER60 precursor (protein disulfide-isomerase A3; endoplasmic reticulum resident protein 57)			+						
Q06830		Peroxiredoxin-1 (thioredoxin-dependent peroxide reductase 2; natural killer cell-enhancing factor A)	+								
P32119		Peroxiredoxin-2	+								

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P00558.3	129902	Phosphoglycerate kinase 1 (primer recognition protein 2; PRP 2; cell migration-inducing gene 10 protein)			+		+		+		
P15531		NM23A (nucleoside diphosphate kinase A; granzyme A-activated Dnase; metastasis inhibition factor nm23)	+								
P22392		NM23B	+								
	1362872	Nuclear protein import factor			+						
Q15365		Poly(rC)-binding protein 1		+							
P07737		Profilin-1	+		+						
P13861.2	4758958	Protein kinase A (cAMP-dependent protein kinase type II-alpha regulatory subunit)					+				
P62136	4506003	Protein phosphatase 1 (catalytic subunit, alpha isoform)		+	+						
P61292		Protein phosphatase 1 (catalytic subunit, beta isoform)		+							
P30153.4	107300	Protein phosphatase 2 (alpha regulatory chain; PP2A subunit A isoform R1-alpha)			+						
O75340		Programmed cell death protein 6	+								
P14618		Pyruvate kinase			+		+	+	+		+
	107554	Pyruvate kinase M2							+		
P62820		Rab1a	?	+			+				
P61019		Rab2a	+	+							
Q8WUD1		Rab2b	+								
P61018		Rab4b	+?								
P51148		Rab5c	+								
P20339		Rab5a	+								
P61020		Rab5b	+								
P20340		Rab6a	+	+							
P20340		Rab6b	+								
Q9H0N0		Rab6c	+								
P51149		Rab7A	+	+					+		
P61026		Rab10	+								
P62491		Rab11a	+								
Q15907		Rab11b	+								
P61106		Rab14		+							

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P59190		Rab15	+								
Q9H082		Rab33B	+								
Q15286		Rab35 (Rab1c)	+								
Q5HYI8		Rab-like protein 3	+								
P26044		Radixin		+							
P46940.1	4506787	Ras GAP			+						
P61586		RhoA		+							
Q99PT1.3	21759130	Rho GDI				+					
P31949		S100 calcium protein binding A11 (calgizzarin)	+								
Q9UDX3		Sec14-like protein 4 (TAP3)	+								
P05023.1	114374	Sodium/potassium transporting ATPase subunit alpha 1			+		+		+		
Q86Y82		Syntaxin12		+							
P17987.1	13540473	T-complex (T-complex protein 1 subunit alpha isoform a)					+				
P49368.4	31542292	TCP1 subunit 3 [T-complex protein 1 subunit gamma isoform a; chaperonin containing TCP1, subunit 3 (gamma)]					+				
P48643.1	24307939	TCP1 subunit 5 (T-complex protein 1 subunit epsilon)					+				
Q99832.2	5453607	TCP1 subunit 7 (T-complex protein 1 subunit eta isoform a)					+				
P50990.4		TCP1 subunit 8 [T-complex protein 1, theta subunit; chaperonin containing TCP1, subunit 8 (theta)]					+				
P60174		Triosephosphate isomerase	+		+						
P68363.1		Tubulin, alpha, ubiquitous (tubulin alpha-1B chain)			+						
Q71U36.1		Tubulin alpha (tubulin alpha-1A chain)			+		+	+			
P07437.2		Tubulin beta-1 chain (tubulin beta-5 chain)			+		+				
P62987		Ubiquitin C (60S ribosomal protein L40)	+								
P62987		Polyubiquitin 3 (ubiquitin C; 60S ribosomal protein L40)			+						
P68036		Ubiquitin-conjugating enzyme E2 L3 (UBCH7)	+								

(Continued)

Table 1 | Continued

Uniprot/ Swissprot	NCBI	Host protein name	Alphaherpes		Betaherpes		Gammaherpes				
			HSV-1*	PRV*[2]	HCMV*[3]	MCMV*[4]	EBV*[5]	KSHV*[6]	KSHV*[7]	Murine γ HV68*[8]	Alcelaphine*[9] (cell-assoc)
P55072.4		Valocin containing protein (transitional endoplasmic reticulum ATPase)					+				
Q15836		VAMP3/cellubrevin		+							
O95857		Tetraspanin 13	+								
P82460		Thioredoxin (=peroxiredoxin?)		+	+						
P07996.2		Thrombospondin 1							+		
Q15654.3		Thyroid receptor interacting protein 6 (Zyxin-related protein 1; ZRP-1; Opa-interacting protein 1)			+						
P02786		Transferrin receptor protein 1 (CD71)	+								
P37802		Transgelin 2	+	+	+						
P29401.3		Transketolase			+						
Q3ZCQ8		Translocase of inner mitochondrial membrane 50 (TIMM50)	+								
P15311.4		Villin 2 (Ezrin)					+	+			
P08670.4		Vimentin			+						
	4507877	Vinculin			+						
O75083		WD repeat containing protein 1		+							
	1065111	Chain A, mixed disulfide intermediate between mutant human thioredoxin and A 13 residue peptide of NF-kB			+						
		Up to:	**49 proteins**	**48 proteins**	**70 proteins**	**11 proteins**	**43 proteins**	**9 proteins**	**20 proteins**	**4 proteins**	**6 proteins**

The table shows a compilation of the various host proteins identified in these mature herpesvirions enriched from the extracellular milieu. One notable exception is Alcelaphine, where cell-associated viral particles were analyzed. The study by Padula and colleagues, which found that annexin A2 is present in HSV-1 perinuclear virions, was omitted from the table since preliminary for the moment (Padula et al., 2009).

+: Detected in that study.

+?: Indicates an uncertainty as to the subtype (e.g., alpha, beta, gamma, a, b, c, ...).

All extracellular virions except the study by Dry (cell associated).

NB: The uniprot/swissprot numbers are not available for all proteins due to the constant evolving of protein and gene databases.

NB: Multiple accession numbers are sometimes possible and may therefore differ among the above studies.

**[1]: Loret et al. (2008)*

**[2]: Kramer et al. (2011)*

**[3]: Varnum et al. (2004)*

**[4]: Kattenhorn et al. (2004)*

**[5]: Johannsen et al. (2004)*

**[6]: Bechtel et al. (2005)*

**[7]: Zhu et al. (2005)*

**[8]: Bortz et al. (2003)*

**[9]: Dry et al. (2008).*

different viruses share similar host proteins. Most excitingly, it also suggests that these host proteins may play common roles throughout the herpesviral family. This defines an interesting and novel set of host–pathogen interactions taking place within the virus itself, rather than the cell.

It is tempting to speculate that some viruses might have a higher capacity to steal cellular proteins because of their size and symmetry. Herpesviruses are indeed large viruses containing a layer called the tegument between their capsids and envelopes that could accommodate non-viral proteins. Though some host proteins may randomly be incorporated into virions, others may rather be selected to insure the optimal replication of the viruses that carry them.

PUTATIVE FUNCTIONS OF HOST PROTEINS ASSOCIATED WITH HERPESVIRIONS

Bioinformatics databases such as the KEGG, Gene Ontology, or DAVID are useful tools to get an overview of the functional interplay of proteins (Ashburner et al., 2000; Huang Da et al., 2009; Kanehisa et al., 2010). As pointed out by Friedel and Haas (2011), complex statistical tools are available to quantitatively evaluate the implication of proteins in various processes but these are beyond the scope of the present review. Here an analysis of the proteins identified in herpesvirions was instead performed with the Ingenuity Pathways Analysis database (Ingenuity® Systems), which contains all the known physical and functional links among cellular proteins and defines their most significant functions. That analysis indicates that many of the cellular proteins found in herpesvirions normally modulate trafficking, cell proliferation, cell death, cell migration, cell metabolism, or the cytoskeleton (**Figure 1**, upper pie chart). Though subtle differences between family members are noticeable when looking at individual viruses, similar functions are found (**Figure 1**, other charts). Immune-related molecules are also important constituents for several viruses, including HSV-1, KSHV, γHV68, Alcelaphine, and MCMV. Altogether, this provides an overall picture whereby herpesviruses, not surprisingly, modulate all of the important aspects of the cell but where each virus might deploy its energies slightly differently. The main surprise is that so many cellular proteins are detected within assembled viral particles, which raises an important question as to their biological significance and mode of action.

IMPLICATIONS OF VIRION-ASSOCIATED HOST PROTEINS FOR HERPESVIRUSES

The overall picture that several important cellular functions might be modulated by the host proteins incorporated into viral particles is intriguing. This clever strategy is consistent with the parasitic nature of all viruses, including herpesviruses, which would presumably gain some replication advantage from stealing cellular modulators rather than coding for them in their own genomes. The most critical question is the benefit for the viruses to incorporate these cellular proteins in their assembled particles, particularly since these proteins also exist in the cells. While this is open to discussion, one possibility is that some of the incorporated cellular proteins may be remnants of the final capsid envelopment process. Alternatively, this may allow the prompt action of some of these proteins immediately upon viral entry. This could jumpstart the expression and/or duplication of the viral genome, as it is the case for the herpesviral VHS, VP16, ICP0, and ICP4 proteins that are present in virions (Lam et al., 1996; Everett, 2000; Halford and Schaffer, 2001; Ellison et al., 2005; Hancock et al., 2006; Loret et al., 2008; Sarma et al., 2008; Loret and Lippe, 2012). Other early potential sites of action are the process of viral entry itself, intracellular capsid transport, import of the viral genome through the nuclear pore or immune modulation, all common steps among herpesviruses. Whatever the case might be, the question remains as to why the cellular pool of these proteins would not suffice. Several options may be considered. First, it may be that the virions incorporate specific isoforms, splice variants or post-translationally modified proteins that could have properties or functions distinct than their cellular counterparts. Second, the incorporation of a host protein from one cell type might permit the infection of a different cell type that does not express such protein. For example, alpha herpesviruses initially infect mucosal cells and could acquire host proteins that are beneficial to infect dormant neuronal cells. Finally, the host proteins might be in complex with viral proteins and it is those complexes that are active to promote the infection. These possibilities are of course speculative at this point and need to be explored.

One aspect where the incorporation of host proteins in mature virions might be beneficial is molecules involved in intracellular trafficking. Work by numerous laboratories demonstrated that the transport machinery used to move cellular proteins is also employed by viruses (Simons and Warren, 1984; Lodish et al., 2000; Sollner, 2004; Greber and Way, 2006; Mercer et al., 2010). This is essential for their proteins and particles to reach their final destination, for example, the site of viral replication, assembly, and/or envelopment. Along with SNARES proteins, Rab and Arf GTPases are master regulators of molecular trafficking throughout the cell (Sollner and Rothman, 1996; Zerial and McBride, 2001; Mizuno-Yamasaki et al., 2012). So far, VAMP3, a SNARE, was identified in PRV virions (Kramer et al., 2011) but it may only be a matter of time until other SNARES are discovered in other members of the herpes family. This is relevant as another SNARE was reported to facilitate the envelopment of MCMV capsids (Cepeda and Fraile-Ramos, 2011). In contrast, a great number of Rab proteins have been identified in herpesvirions, particularly HSV-1 and PRV (**Table 1**). One stimulating option is that these proteins regulate the displacement of viral capsids in the cell, which could justify their incorporation in the viral particles. As Rab and Arf proteins collectively modulate several intracellular transport steps within the cell, it is anticipated they may be involved in various stages of the infection. For instance, Rab1, which is present in HSV-1 extracellular virions (Loret et al., 2008), and Rab43 were recently demonstrated to modulate the final envelopment of the virus (Zenner et al., 2011). Similarly, Rab6, found in HSV-1 and PRV (Loret et al., 2008; Kramer et al., 2011), is also necessary for the efficient assembly of the related HCMV (Indran and Britt, 2011). It will now be of interest to determine if the virion-associated pool of these GTPases actively participates in the viral life cycle. Interestingly, several Rab proteins have been implicated in autophagosome formation and maturation (Chua et al., 2011). While it is difficult to consider how virion-incorporated Rab proteins play a role at that stage, they might rather be incorporated into the virions as

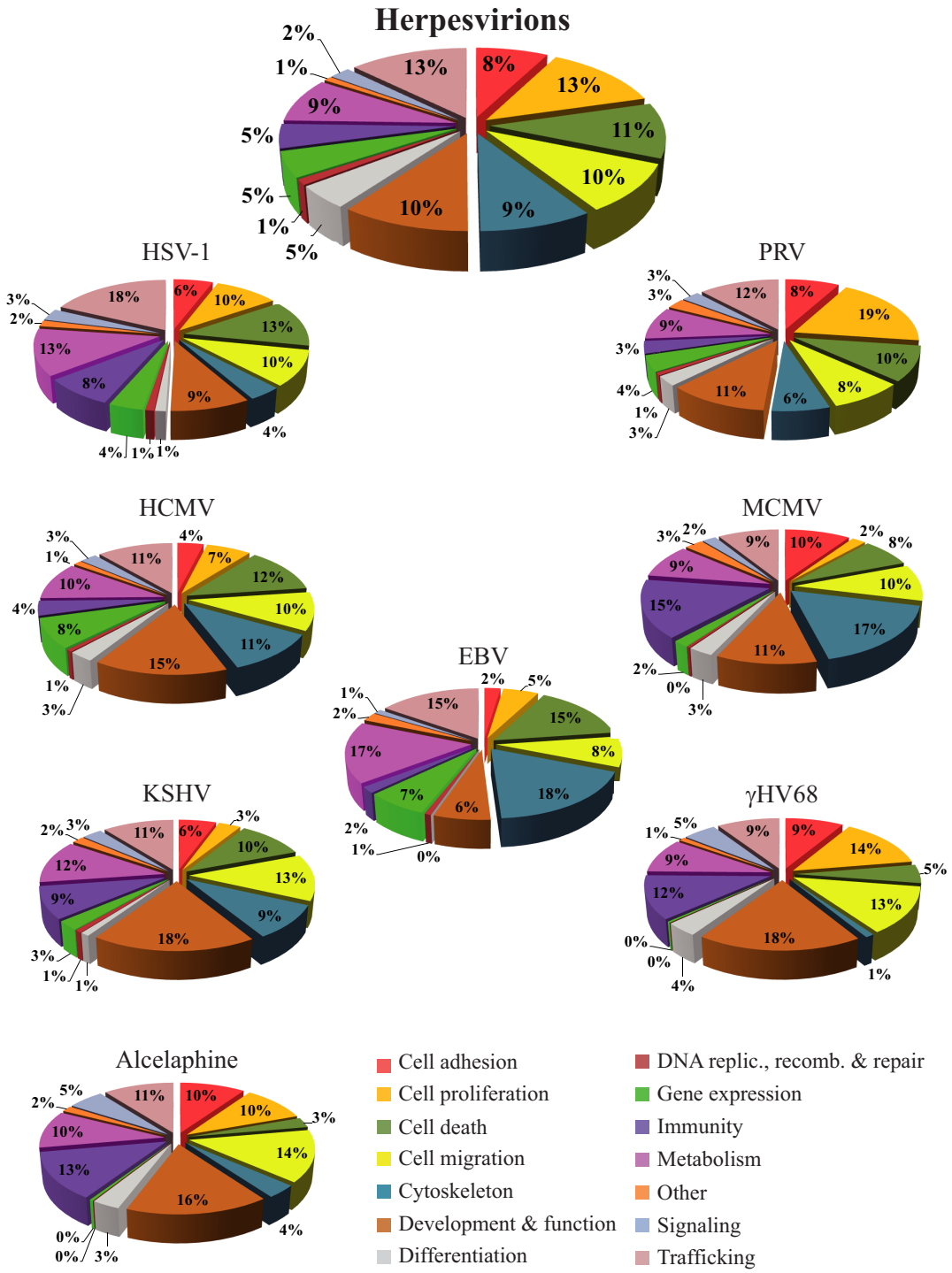

FIGURE 1 | The proteins from Table 1 were analyzed with the Ingenuity database to define their putative functions in the context of an infection. To this end, the protein accession numbers (or GI numbers) were queried from the Ingenuity database. For the purpose of this figure, all known functions associated with these proteins were exported to Microsoft Excel and regrouped. In the top pie chart, the cellular proteins found in all the herpesvirions were analyzed collectively, while the other pie charts depict the host proteins incorporated into each virus. Since each protein can be associated with multiples functions in the database, the results of those analyses are expressed as relative values instead of raw numbers, which consequently exceeds the original number of proteins analyzed. The percentages therefore represent the number of proteins falling into a given category with the total of each pie chart being 100%. A graphical legend of the categories is provided at the bottom right corner of the figure.

a consequence of their involvement in autophagosome formation and concomitant viral envelopment. Given the vast impact of Rab proteins on the cell, it will be a major challenge to decipher all their roles in the life cycle of herpesviruses, particularly for the pool present in mature virions.

Molecular trafficking is not only dependent on SNARES, Rab, and Arf proteins, it is also intimately linked to the cytoskeleton. It is thus not surprising that herpesviruses devote some of their resources toward regulating this central cellular machinery. For instance, herpesviruses significantly reorganize both cellular and nuclear actin as well as microtubules (Norrild et al., 1986; Avitabile et al., 1995; Sharma-Walia et al., 2004; Simpson-Holley et al., 2005; De Regge et al., 2006; Saksena et al., 2006). They also travel along microtubules during both entry and egress and interact with several cellular molecular motors (Sodeik et al., 1997; Smith et al., 2001; Dohner et al., 2002; Marozin et al., 2004; Lee et al., 2006; Wolfstein et al., 2006; Radtke et al., 2010) as well as cortical and nuclear actin filaments (Forest et al., 2005; Feierbach et al., 2006; Roberts and Baines, 2011). Furthermore, some members incorporate in their viral particles tubulin or actin-related components (**Table 1**; Wong and Chen, 1998; Grunewald et al., 2003). Actin has been reported to compensate the loss of various viral tegument proteins in PRV (del Rio et al., 2005; Michael et al., 2006) and may thus act as an abundant filling agent, so its significance in herpesviral particles remains enigmatic. Similarly, the relevance of intermediate filament components vimentin and keratins in some herpes virions (**Table 1**) is difficult to assess given these filaments are not as well characterized as other cytoskeletal elements. It may nevertheless be important for herpesviruses, particularly since they are not all associated with the common skin or hair contaminants often detected in mass spectrometry (Hertel, 2011).

Viruses tend to monopolize for their own purpose their host expression apparatus, including protein translation (Bushell and Sarnow, 2002). For example, the prototypic HSV-1 ICP27 viral protein regulates all aspect of mRNAs including transcription, splicing, nuclear export, and translation for the benefit of the virus (Rice and Knipe, 1988; Sekulovich et al., 1988; Sandri-Goldin and Mendoza, 1992; Smith et al., 1992; Hardwicke and Sandri-Goldin, 1994; Hardy and Sandri-Goldin, 1994; Brown et al., 1995; Soliman et al., 1997; Chen et al., 2002; Lindberg and Kreivi, 2002; Ellison et al., 2005; Larralde et al., 2006; Fontaine-Rodriguez and Knipe, 2008). As these cellular functions are highly regulated, the inclusion of DDX3X, a multifunctional RNA helicase that also regulates transcription, nuclear export, and translation that is used by several viruses (Schroder, 2010, 2011) may be relevant. Its incorporation into mature virions could thus accelerate viral gene expression in the early stages of the infection. Similarly, the presence of translation initiation or elongation factors in virions (**Table 1**) may also jumpstart gene expression in favor of the viruses.

Interestingly, HSV-1 does not require cells to be in the S-phase and even arrests the cell cycle at the G1/S transition step (Shadan et al., 1994; Song et al., 2000), which partly explains why it can grow in non-dividing neurons. While the precise mechanism of this arrest is unclear, it is known that the viral ICP0 protein and the VP16 cellular partner HCF modulate the cell cycle (Hobbs and DeLuca, 1999; Lomonte and Everett, 1999; Piluso et al., 2002). Moreover, ICP0 interacts with the host cyclin D3 (Kawaguchi et al., 1997). However, it was recently reported that stress, rather than the cell cycle *per se*, may be a critical feature (Bringhurst and Schaffer, 2006). Clearly, the interaction of herpesviruses with the cell proliferation apparatus is complex and likely involves several host and viral proteins. Identifying novel players that might be incorporated into mature virions may thus be very useful to clarify this process.

An interesting scenario is the possible regulation of apoptosis by host proteins loaded onto viral particles. Apoptosis is regulated both negatively and positively by several viruses (Teodoro and Branton, 1997; Goodkin et al., 2004), presumably to insure their survival at the early stages of the infection but their efficient release later on. Conceptually, the presence of anti-apoptotic proteins in herpes particles might thus provide a mean to quickly evade death upon entry, while the presence of pro-apoptotic proteins on newly assembled/enveloped viral particles may trigger or stimulate their extracellular release. Only further work will resolve this open question.

TECHNICAL LIMITATIONS

Several factors generally contribute to variation among proteomic studies. Hence, the preparation of the samples (e.g., in-gel trypsin digestion versus liquid digestion and chromatography) may lead to the detection of different populations of tryptic peptides. Moreover, the sensitivity of the mass spectrometers and the abundance of the proteins in the samples also impact peptide detection. The relative abundance of a peptide is itself influenced by the complexity of the samples, where some proteins may evade identification. Finally, each protein differs in its properties (ionization, resolution in SDS-PAGE gels), which will be reflected in their detection. This includes SNARES, which are transmembrane proteins resistant to SDS extraction (Yang et al., 1999; Kubista et al., 2004). It is thus likely that some of the proteins in **Table 1** are present in more viral particles than reported and that additional proteins are indeed incorporated in herpesvirions. More specific aspects regarding herpesviruses includes the purification schemes employed to enrich the viral particles, which will directly influence the purity of the samples and hence the potential detection of contaminants. One important caveat is that some host proteins may simply stick to the large viral particles. Another one is common contaminants such as some hair/skin associated keratins or as mentioned above actin, which may simply fill the virions. However, even potential contaminants cannot simply be discarded since actin and even some keratins may indeed participate in viral life cycles. Moreover, the relative abundance of all the cellular proteins within the cell is unknown, so it is not possible to rule out potential contaminants on the sole basis of abundance. It is thus critical to orthogonally validate all proteomics hits.

Various tools are available to define the biological relevance of host proteins identified in viral particles, including Western blotting, immuno-electron microscopy or functional screens. One powerful method is RNA interference. However, given the dual presence of the host proteins within the viral particles and the cell itself, this becomes a challenging task. RNA interference also has its own caveats (false positives and negatives). Another common step is the expression of dominant positive or negative mutants.

In all cases, one major difficulty is that the host proteins may be essential for the cells and their depletion may lead to cytotoxicity, thus proper controls are needed. In addition, the host proteins might be essential for the virus within the cells but only accessory within the virions. Consequently, depletion of a protein may have limited impact on the virus since complemented by the other pool of that protein in the virus or the cell. Small reduction or stimulation in viral yields may thus result. It such cases, it may be necessary to produce the virus on cells that lack these proteins to see if this makes a difference. One should also consider animal models since tissue culture based screens may miss important players, for instance modulators of the immune system or virulence factors. Clearly, multiple experimental strategies are needed to ultimately insure the biological significance of the host proteins found in viral particles.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The identification and functions of host proteins in viral particles is an important step toward the elucidation of novel host-pathogen interactions. In the case of herpesvirions, this is well under way with eight different family members analyzed so far. One main aspect is to sort biologically relevant cellular proteins from sticky contaminants. The orthogonal validation of the host proteins found in herpesvirions using biologically relevant assays is thus critical. As pointed out above, it will necessary to analyze all these proteins in the background of two pools, one cellular and one virion-associated, which are likely to complement one another. An interesting possibility is that some isoforms or specific post-translationally modified host proteins may be loaded into the capsids. Thus a detailed analysis of the host proteins present in viral particles will be important and a potential way to distinguish them from their cell-associated counterparts. Another issue is the expected variation among cell types. In that respect, it would be most interesting to examine the cellular protein content of HSV-1 produced in neurons in opposition to the virions produced on other cell types. Finally, the mechanisms by which all these host proteins are recruited to the viral particles will also need to be explored. Thus the proteomics of viral particles is only the beginning of the adventure, which should prove most exciting yet challenging.

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