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A comprehensive mapping of interactions among Epstein–Barr virus (EBV) proteins and interactions of EBV proteins with human proteins should provide specific hypotheses and a broad perspective on EBV strategies for replication and persistence. Interactions of EBV proteins with each other and with human proteins were assessed by using a stringent high-throughput yeast two-hybrid system. Overall, 43 interactions between EBV proteins and 173 interactions between EBV and human proteins were identified. EBV–EBV and EBV–human protein interaction, or "interactome" maps provided a framework for hypotheses of protein function. For example, LF2, an EBV protein of unknown function interacted with the EBV immediate early R transactivator (Rta) and was found to inhibit Rta transactivation. From a broader perspective, EBV genes can be divided into two evolutionary classes, "core" genes, which are conserved across all herpesviruses and subfamily specific, or "noncore" genes. Our EBV–EBV interactome map is enriched for interactions among proteins in the same evolutionary class. Furthermore, human proteins targeted by EBV proteins were enriched for highly connected or "hub" proteins and for proteins with relatively short paths to all other proteins in the human interactome network. Targeting of hubs might be an efficient mechanism for EBV reorganization of cellular processes.

herpesvirus | interactome | replication | yeast two hybrid

Epstein–Barr virus (EBV), like all herpesviruses, infects and replicates in epithelial cells and establishes latency in specific nonepithelial cells. EBV belongs to the gamma subfamily of herpesviruses and is similar to the other human gamma subfamily herpesvirus, Kaposi sarcoma-associated herpesvirus (KSHV). Both viruses establish latency and periodically replicate in B lymphocytes. EBV has 43 "core" genes, which are common to all herpesviruses. Of the remaining 46 "noncore" genes, 6 have orthologs in beta and gamma herpesviruses, 12 are specific to the gamma subfamily, and 28 are EBV-specific. Core herpesvirus proteins are necessary for genome replication, packaging, and delivery in all cells. Some EBV-specific proteins are important in latent B lymphocyte infection, lymphoproliferative disease, Burkitt lymphoma, Hodgkin disease, and nasopharyngeal carcinoma. The function of other EBV-specific and most gamma-specific herpesvirus proteins is less clearly understood (reviewed in refs. 1–4).

The goal of this study was to investigate interactions among EBV proteins and between EBV and human proteins in an unbiased manner. Such systematically derived protein–protein interaction or "interactome" maps could be combined with other gene-level biological information to formulate hypotheses about specific roles of EBV proteins of various functional and evolutionary classes and reveal more global properties of EBV and EBV–human interactomes. Noncore herpesvirus proteins are thought to adapt the core herpesvirus replication program to specific niches. In addition to the role of core herpesviruses proteins in replication, core proteins may interact with noncore proteins to adapt replication to specific cell types or enable more complex pathophysiologic strategies such as modification of innate or acquired immune responses. For example, recent studies of mature EBV virions revealed they contain many gamma-specific proteins in the tegument (5). Little is known about these proteins; therefore, a more detailed mapping of their interactions with core herpesvirus proteins, other gamma-specific proteins, and human proteins is important for understanding their assembly into the tegument and their role in infection. Furthermore, although much is known about the role of some EBV-specific protein interactions with lymphocyte proteins in cell growth and survival, the effects are well delineated for relatively few proteins (reviewed in refs. 3 and 4).

Specific hypotheses about the role of EBV and human protein interactions should emerge by correlating an unbiased EBV interactome data set with available functional annotations. Indeed, interactome network maps have revealed global topological and dynamic features that relate to biological properties (6). For example, proteins with a large number of interactions, or "hubs," in the interactome are more likely to be essential for yeast survival than proteins with a small number of interactions (7). Hub proteins can be further partitioned into those that function in specific biological modules and those that connect different modules (8). Furthermore, proteins somatically mutated in cancer tend to be interactome hubs (9). An unbiased EBV–human interactome map could be interrogated to address similar questions regarding system-level properties, which may be relevant to EBV pathogenesis and by extension, to other herpesviruses.

Systematic yeast two-hybrid (Y2H) interactome maps for viral proteins have been reported for Vaccinia virus (10) and more recently for Varicella-Zoster virus and KSHV (11). Although a preliminary viral–host interactome map has been produced for KSHV, this map was derived by experimental testing of predicted interactions and is limited by inspection bias toward specific proteins of high scientific interest in a literature-derived subset of viral–human protein interactions and by projection of

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The authors declare no conflict of interest.

Abbreviations: Y2H, yeast two-hybrid; KSHV, Kaposi sarcoma-associated herpesvirus; DB, DNA binding; AD, activation domain; AP, affinity purification; EBNA, EBV nuclear antigen; Rta, R transactivator; ET-HP, EBV-targeted human protein; TRAF, TNF receptor-associated factor.

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Fig. 1. EBV–EBV interactome network. Graph of the EBV protein interaction network identified by merging the interactions identified in this Y2H study with published interactions. Interactions identified in this study are shown as red lines, and previously published interactions are shown as purple lines. High confidence interactions (interaction that scored positive by either β-gal or co-AP assay) are shown as solid lines, and low confidence interactions are shown as dashed lines. Core herpesvirus proteins are shown as yellow circles, and noncore proteins are green circles. The network consists of 52 proteins linked via 60 interactions

the viral–human interactome onto a predicted, but not experimentally derived, human interactome (11). Here, we report systematically derived EBV and EBV–human interactome maps.

Results and Discussion

Map of the EBV Interactome Network. To map interactions among EBV proteins, a library or ORFeome of 80 full and 107 partial EBV ORFs, collectively representing 85 of the 89 known EBV proteins (1), was generated in a vector system that allows movement of ORFs to other vectors (12). Large ORFs were cloned as overlapping subclones, and the extracellular and cytoplasmic domains of transmembrane proteins were cloned separately. EBV polypeptides or proteins were expressed in fusion to the Gal4 DNA binding (DB) and activation domain (AD). The resulting constructs (187 DB-X and 187 AD-Y fusions) allowed systematic testing of ≈35,000 EBV protein combinations by using a Y2H mating-based interaction assay [see supporting information (SI) Fig. 5] (13). After elimination of autoactivators, the Gal4-inducible *GAL1*::*HIS3* reporter gene was used to identify 43 Y2H interactions (7 homodimers and 36 heterodimers) involving 44 EBV proteins (Fig. 1, SI Table 1, and SI Fig. 6). Twenty-four Y2H interactions also tested positive for the *GAL1*::*lacZ* reporter gene, which indicated that the overall quality of the data set was high (13).

Quality Assessment of EBV Interactome. To assess the specificity of our EBV interactome data set, interactions identified by Y2H were assayed by co-affinity purification (co-AP) using glutathione beads and expression of GST and EGFP fusion proteins in 293 cells (SI Fig. 5). Both GST-X and EGFP-Y proteins were sufficiently well expressed in 293 cells to enable testing of 32 pairs. From these, 15 (47%) EGFP-Y proteins copurified with the corresponding GST-X ORF fusion protein but not with GST alone (SI Fig. 5). In contrast, of 12 random EBV ORF pairs expressed as fusion proteins in 293 cells only one was co-AP-positive. Adjusting for this random positive rate, we obtained a co-AP validation rate of 42% for the Y2H interactions, which is similar to the 48% co-AP validation rate of the recently published KSHV interactome map (11). However, the validation rate in that study was not adjusted for the random positive rate of the co-AP (11). Furthermore, the inherent false negative rate of the co-AP assay is not included in our calculation, thus the estimated fraction of technically validated Y2H interactions in our data set is likely to be higher. Y2H interactions confirmed by β-gal or co-AP assay were included in the high confidence data set (Fig. 1, solid red lines and SI Table 1), and the remaining are reported as low confidence interactions.

To estimate the overall sensitivity of our EBV interactome map, we compared our data set with information available in the literature (SI Table 2) and interactions potentially conserved between different viruses (12, 14). Approximately 14% (3 of 22) of EBV interactions previously described in the literature were detected here, and this overlap is statistically significant (enrichment with $P = 10^{-4}$). Based on orthology between KSHV and EBV, we predicted 62 interologous protein pairs from the Uetz *et al.* (11) KSHV interactome data and identified interactions corresponding to three of these 62 predicted pairs in our EBV interactome map. Uetz *et al.* tested 54 of these pairs and confirmed 17 by co-AP. Additionally, our analysis identified 16 interactions between protein pairs with orthologs in KSHV, which were not present in the KSHV interactome map (11). The limited overlap between our data and other data sets is likely caused by limited effective sampling, as observed in existing cellular interactome maps (15, 16). False negatives can result from misfolding of fusion proteins, lack of other binding partners to stabilize an interaction, or dependence on posttranslational modification. Because these herpesvirus Y2H interactome maps are currently incomplete, the extent to which interactions are conserved between these gamma herpesviruses will need to be further assessed as larger interactome maps become available.

Among EBV protein–protein interactions in our map, homodimerization of SM/M and EBV nuclear antigen 1 (EBNA1) were known (17, 18), as was heterodimerization of BFLF2 and BFRF1 (19, 20). BFLF2 and BFRF1 and orthologs in other herpesviruses interact at the nuclear membrane and enable capsid egress (20–23). Interactions between the capsid proteins, BcLF1 and BFRF3, and homodimerization of BaRF1 were also anticipated from interactions of the herpes simplex virus and KSHV orthologs (11, 24, 25).

Testable hypotheses regarding several EBV proteins emerged from this version of the EBV–EBV interactome. A novel interaction between BNRF1 and BLRF2 is an important step in understanding the assembly of these gamma-specific proteins into the tegument. Further, a novel interaction between the EBV BGLF4 protein kinase and BXLF1 thymidine kinase could substantially alter substrate specificity or activity of these critically important kinases (26, 27).

Assay of the Functional Significance of the LF2–Early R Transactivator (Rta) Interaction. The potential functional significance of the Y2H interaction between the immediate early Rta protein, which is essential for replication, and the early LF2 protein was further assessed. The promoters of four EBV genes that have well characterized Rta responsive elements (28) were tested for an effect of LF2 on Rta activation (Fig. 2). Rta activated the four target promoters from 6- to 33-fold. LF2 alone had no effect. However, LF2 coexpression inhibited Rta activation of three promoters ≈50% and completely abolished Rta activation of the BALF2 promoter. LF2 expression did not affect Rta protein levels (Fig. 2). The mechanism by which LF2 impairs Rta activation is an important question for future study and may involve disruption of Rta binding near promoters or activator recruitment. Differences in the magnitude of the LF2 effect could reflect differential Rta impairment. LF2 down-regulation of Rta may abrogate or attenuate EBV replication.

LF2 and Rta are found only in gamma herpesviruses, and LF2 orthologs are specifically incorporated into the tegument of other gamma herpes virions (29, 30). Alpha herpesviruses have

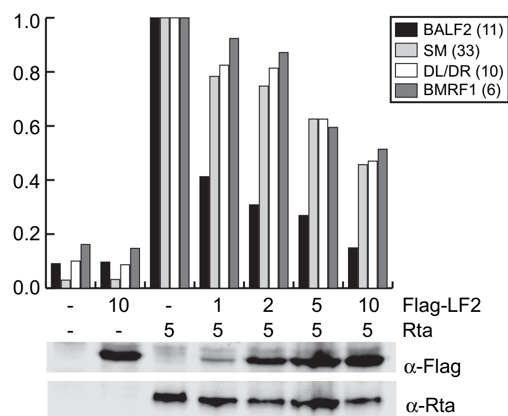

Fig. 2. LF2 represses Rta activation of four different EBV promoters. (*Upper*) BJAB cells were transfected with one of four pGL3-basic-Luc reporters containing the promoter region of an EBV gene with a well defined Rta response element (10 μg) along with 5 μg of Rta and increasing amounts (0–10 μg) of Flag-LF2. The luciferase activity shown represents the ratio of firefly luminescence over β-gal activity to normalize for transfection efficiency. The maximal response of each promoter to Rta in the absence of LF2 is shown at right (fold activation over vector only) and is normalized to 1 in the graph. The presence of LF2 significantly reduced Rta activation of all of the promoters in a dose-dependant manner. (*Lower*) Western blotting of cell lysates harvested 48 h posttransfection revealing that Rta expression levels were unaffected by LF2 cotransfection.

activators of immediate early gene transcription in their tegument, which promote replication in infected cells. Gamma herpesviruses may have LF2 orthologs in the tegument to impede initial replication and increase the propensity to latency in B lymphocytes.

Global Properties of the EBV Interactome. In addition to generating specific hypotheses of protein function, global properties of the EBV interactome were investigated. EBV proteins can be broadly divided into two evolutionary classes: herpesvirus core and noncore proteins. Of the 36 heterodimeric interactions identified in this screen, 21 (58%) were between proteins of the same evolutionary class. This statistically significant ($P = 0.004$) enrichment for preferential interactions between EBV proteins of the same conservation class may have resulted from the gradual divergence of herpesvirus lineages from an ancestral strain. As gamma herpesviruses evolved, they may have gradually acquired the proteins necessary to replicate and establish latency in B lymphocytes. These proteins appear to be more likely to form complexes with one another and interact to a more limited extent with herpesvirus core proteins.

Map of the EBV–Human Interactome Network. Interactions between EBV and human proteins were identified by Y2H screens using 113 DB-X nonautoactivating EBV hybrid proteins against a human spleen AD-cDNA library. In total, all or part of 85 distinct EBV proteins were screened against 10^5 to 10^6 human AD-Y fusion proteins (SI Fig. 5). Positive colonies were retested by using a stringent combination of Gal4-responsive reporter genes for uracil and histidine prototrophy, β-gal activity, and 5-fluoroorotic acid sensitivity to ensure a high level of specificity. Sequencing identified 306 AD-Y in-frame interaction sequence tags and yielded 173 different EBV and human protein interactions. This EBV–human interactome map includes 40 different EBV proteins and 112 human proteins (Fig. 3 and see also SI Table 3).

Emerging Hypotheses of Function of Specific EBV and Human Proteins. Analyses of our map of the EBV–human interactome suggests a number of hypotheses regarding functions of EBV proteins and their human target proteins (referred to as EBV-targeted human proteins or ET-HPs hereafter). The EBV early replication SM protein, which effects polyadenylation and cleavage of EBV DNA polymerase pre-mRNA and unspliced EBV RNA transport to the cytoplasm (18, 31, 32), interacts with the human promyelocytic leukemia nuclear body protein, Sp100, which is also known to interact with EBNALP and mediate its coactivation of EBNA2 (33). Despite N-terminal similarity among Sp100/Sp140 family members (34), SM functionally interacts with two nonhomologous Sp110b domains (35). The interaction of SM with Sp110b is hypothesized to stabilize EBV mRNAs, and SM may interact with a nonconserved Sp100 domain for similar purposes. SM also interacted with SFRS10, an arginine/serine-rich splicing factor that is involved in regulation of splicing of mRNAs encoding cancer-associated proteins, including p53 (36) and CD44 (37). SM interaction with SFRS10 could be important in SM inhibition of human mRNA splicing. EBNA1 (amino acids 320–386) interacted with p32/TAP and EBNA1, and the herpesvirus saimiri functional equivalent, ORF73, may interact with p32/TAP to affect mRNA transport (38, 39).

Two EBV proteins required for transformation of B lymphocytes into lymphoblastoid cell lines, EBNA3A and EBNA2, interacted with several human proteins. EBNA3A interacted with two human protein regulators of apoptosis, Nur77 and RPL4. Nur77 is a nuclear hormone receptor transcription factor that can translocate to mitochondria and induce apoptosis. Interaction of Nur77 with EBNA2 localizes Nur77 to the nucleus and protects cells from Nur77-mediated apoptosis (40, 41). EBNA3A may have a similar role in preventing Nur77-mediated apoptosis. EBNA3A interaction with RPL4 may also regulate programmed cell death, as RPL4 is expressed in cells before apoptosis and forced RPL4 expression induces apoptosis (42). EBNA2 may also be targeting two pathways that modulate intracellular Ca^{2+} ion levels. EBNA2 interacts with the B cell linker protein BLNK, which is a 65-kDa Src homology 2 domain protein that interacts with Igα or LMP2A (43) and regulates intracellular Ca^{2+} (44), and with sorcin, a regulator of ryanodine receptor Ca^{2+} release (45).

BHRF1, an EBV BCL-2 homolog expressed early in replication, interacted with the TNF receptor-associated factor 1 (TRAF1). TRAF1 is known to interact with the EBV LMP1 TRAF signaling domain (46) and has been implicated in promoting cell survival through NF-κB activation. TRAF1 interaction with BHRF1 is indicative of a direct role in cell survival during EBV replication.

Hypotheses Regarding Human Proteins Targeted by Multiple EBV Proteins. ET-HPs critical to the virus life cycle may be targeted by more than one EBV protein. For example, RBP-Jκ, a DB protein in the Notch signaling pathway, is targeted by four EBV nuclear proteins and three of these interactions are known to be critical for EBV-mediated B lymphocyte growth transformation (47–49). Twenty-four ET-HPs interacted with more than one EBV bait protein, accounting for 85 of 173 interactions. The high stringency used in these screens makes promiscuous binding unlikely, and highly connected protein hubs detected in other protein interaction networks analyzed to date can be biologically significant (50). For example, HSP90 interacts with a broad range of proteins and is important for specific processes such as NF-κB signaling (51).

Examination of the 13 ET-HPs with three or more Y2H interactions with EBV proteins revealed several interesting patterns. Human HOMER3 interacted with eight EBV baits, seven of which are transmembrane proteins. The apparent selectivity of HOMER3 for transmembrane proteins cannot be attributed to an ability to bind to hydrophobic transmembrane helices, as these were deleted in the EBV baits that retrieved HOMER3. Human HOMER3 localizes primarily to the endo-

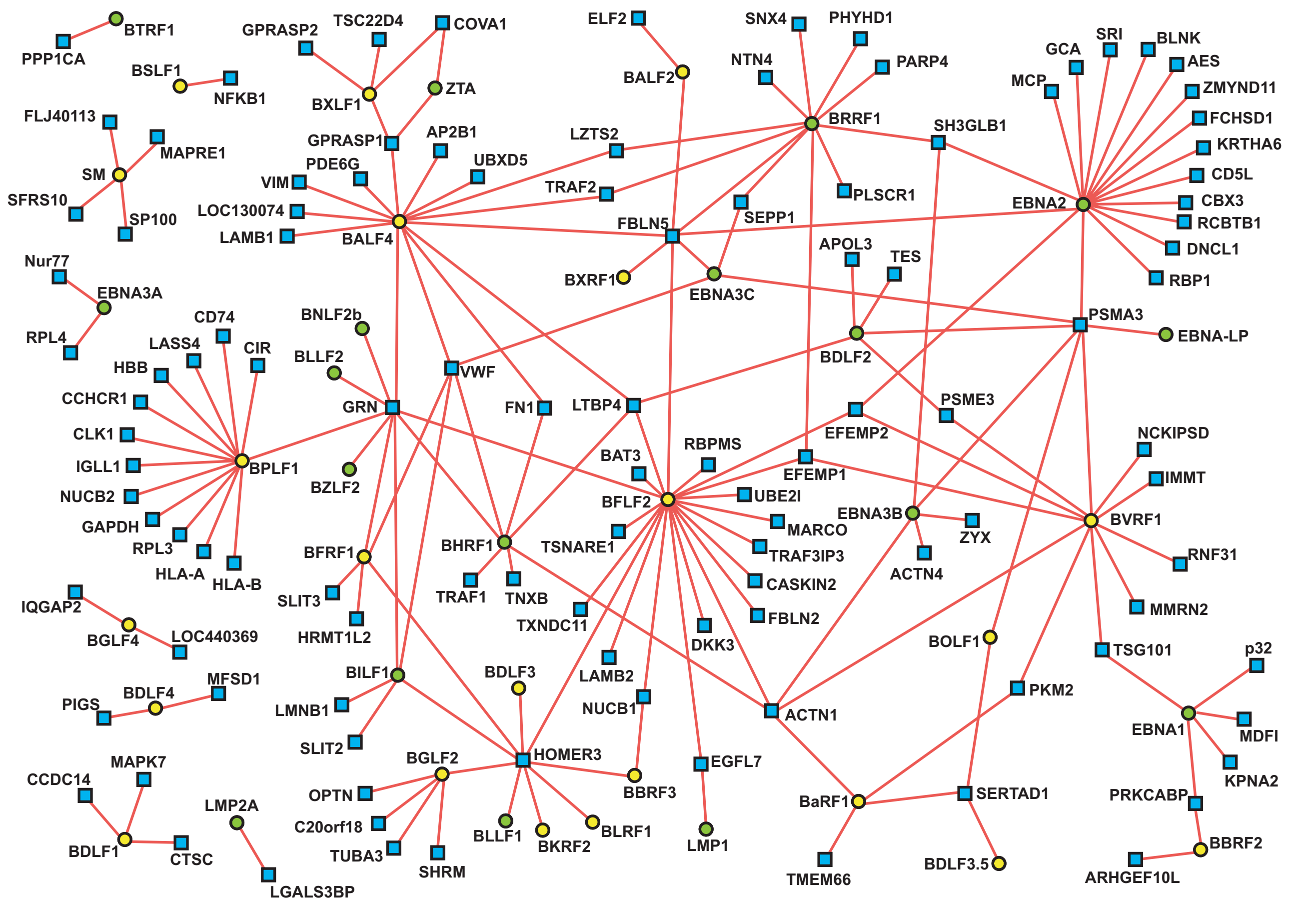

Fig. 3. EBV–human interactome network graph of the EBV–human protein interaction network as determined by our Y2H screen. Core herpesvirus proteins are shown as yellow circles, and noncore herpesvirus proteins are green circles. Human proteins are shown as blue squares. Interactions identified in this screen are shown as red lines. This interactome represents 40 EBV proteins and 112 human proteins connected by 173 interactions

plasmic reticulum (ER) and plasma membrane and is concentrated at immune synapses (52). In six of the seven cases, HOMER3 interacted with the ectodomain of the EBV membrane protein. Thus, it seems likely that HOMER3 interacts with common motifs in membrane proteins required for ER insertion or trafficking. As *Drosophila* HOMER is a synaptic scaffold that brings neurotransmitter receptors and other proteins to synaptic junctions, we cannot exclude a role for HOMER3 in EBV-mediated membrane fusion during viral entry or egress. In another instance, the interaction of proteasome alpha 3 subunit isoform 1 (PSMA3) with EBNA3A, EBNA3B, and EBNA3C was recently reported (53), and we observed PSMA3 interacting with seven EBV proteins, including four EBNAs. Recruitment of the 19S regulatory complex proteasome subunit mediates transcriptional activation of some eukaryotic promoters (54), and the observation that EBNA transcription factors interact with a 20S subunit component may extend this paradigm.

Network Analysis of the EBV–Human Interactome. Examination of topological characteristics of an interactome network can give insight into the dynamic operation or evolutionary constraints of the underlying biological system (6). The "degree" of a protein is defined as the number of interactions with other proteins in the network. The degree distribution has been investigated in various cellular interactome networks (50) and the KSHV viral network (11). Because of the small number of proteins in our EBV and EBV–human network, we could not fit the interactome degree distribution data to any specific model. Therefore, it is not clear whether the degree distribution of the EBV interactome departs from a power-law distribution as suggested for the KSHV interactome (11).

To elucidate the network topology of ET-HPs, the EBV–human interactome map was overlaid onto a set of currently available binary human interactome data sets corresponding to the union of high-quality, high-throughput Y2H interactions described by Rual *et al.* (15) and Stelzl *et al.* (55), with literature-curated interactions (assayed in low-throughput format) from the BIND (56), DIP (57), HPRD (58), MINT (59), and MIPS (60) protein interaction databases. Of the 112 ET-HPs identified here, 89 can be found in the current human interactome map. Comparison of these 89 ET-HPs with other proteins in the human interactome revealed interesting topological characteristics.

The average degree of ET-HPs in the human interactome (15 ± 2) was significantly higher than the average degree of proteins picked randomly from the human interactome (5.9 ± 0.1; Fig. 4*a*), indicating that ET-HPs tend to be highly connected or hub proteins (61) in the human interactome. Specifically, the fraction of proteins in the human interactome that are ET-HPs increases with increasing degree, k, with a sublinear dependence k^b, where $b \approx 0.64$ (Fig. 4*b*). As a consequence of this positive correlation for ET-HPs to be of higher degree in the human interactome, the subnetwork of ET-HPs and their direct human protein interactors (the ET-HP subnetwork) shows significantly more connected proteins and more interactions between them compared with similarly extracted subnetworks from randomly picked proteins in the human interactome (SI Table 4). The targeting of protein hubs was similar among latent, early-replication, and late-replication EBV proteins.

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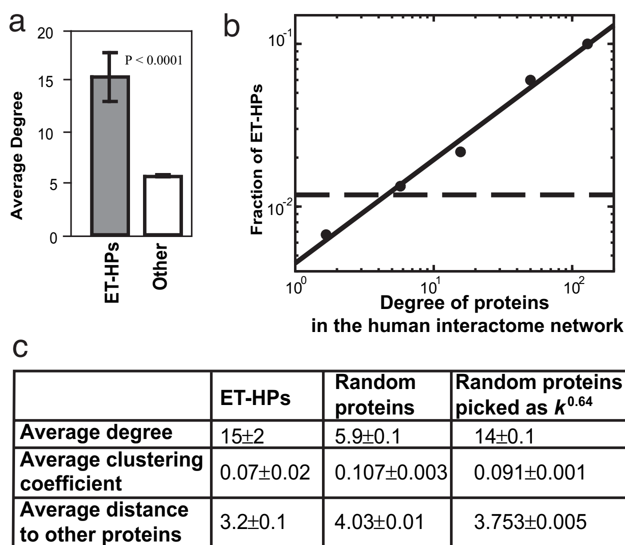

	ET-HPs	Random proteins	Random proteins picked as $k^{0.64}$
Average degree	15±2	5.9±0.1	14±0.1
Average clustering coefficient	0.07±0.02	0.107±0.003	0.091±0.001
Average distance to other proteins	3.2±0.1	4.03±0.01	3.753±0.005

Fig. 4. Systematic analysis of the topology and functional characteristics of ET-HPs. (*a*) Bar graph indicating the degree of ET-HPs in the human interactome as compared with the degree of other human proteins picked at random from the human interactome. (*b*) The circles represent the fraction of human proteins with degree *k* that are ET-HPs. The solid black line represents the best fit to Ak^b, resulting in $b = 0.64 \pm 0.1$. The dashed line represents the expected probability that a human protein selected at random is an ET-HP. (*c*) Various topological parameters of ET-HPs in the human interactome network compared with other human proteins picked randomly with uniform probability or with a probability proportional to $k^{0.64}$, where *k* is the degree of a protein in the network, are indicated.

To investigate the robustness of this correlation, we examined the average degree of both ET-HPs and random proteins that were present in various nonoverlapping subsets of the existing human interactome generated by different groups in two large-scale interactome maps (15, 55), each generated by using different Y2H technologies, as well as a data set of literature-curated interactions collated as described above. All three data sets are subject to technological biases of the assays used to detect specific interactions. Furthermore, the literature-curated data set is likely to be subject to inspection biases toward extensively studied proteins of high scientific interest. Therefore, these three data sets have different biases for detecting interactions involving particular proteins and overlap only partially in terms of protein coverage and interactions (ref. 15 and unpublished observations). Despite these biases and differences in coverage, we find that the average degree of ET-HPs present in each data set is significantly higher than the degree of other proteins. Using the Rual *et al.* (15) network, the average degree of ET-HPs is 15 ± 4.48, whereas the average degree of other proteins is 3.2 ± 0.16. In the Stelzl *et al.* (55) network, the average degree of ET-HPs is 8.38 ± 2.32, whereas the average degree of other proteins is 3.64 ± 0.17. With the literature-curated network, the average degree of ET-HPs is 9.74 ± 1.45, whereas the average degree of other proteins is 5.44 ± 0.11. This finding indicates that the preferential targeting of hubs we observed here is likely to be independent of the technical manner in which interactions were derived.

To assess the local connectivity of ET-HPs in the human interactome, we computed the average clustering coefficient, which represents the fraction of possible interactions among interactors of a given protein. The average clustering coefficient of ET-HPs is slightly smaller than that of human proteins selected at random (Fig. 4*c*). Because the degree of a protein in a power-law network such as the human interactome is inversely correlated with its clustering coefficient (62), we examined whether this decrease in clustering coefficient of ET-HPs is a consequence of degree bias. We also computed as a control the average clustering coefficient for human proteins selected with a probability proportional to $k^{0.64}$. This choice represents a reference distribution of proteins that maintains the same overall degree distribution as that of the ET-HPs. The clustering coefficient of these control proteins was close to that of ET-HPs (Fig. 4*c*), which indicates that the decrease in clustering coefficient of ET-HPs compared with randomly picked human proteins follows from their degree bias.

To evaluate the extent to which proteins are "centrally" located, we considered the minimum number of interactions required to connect any "probe" human protein to any other reachable protein in the network, i.e., the "distance" to any protein present in the largest connected component. When the probe protein is an ET-HP, this average distance is smaller than when the probe is a human protein selected at random (Fig. 4*c*). The average distance of a protein to any other protein is inversely correlated with its degree in a power-law network such as the human interactome network. However, the shorter distance to other proteins from ET-HPs cannot be completely explained by the bias of ET-HPs toward higher-degree proteins. This assertion is supported by the fact that the average distance from ET-HPs is still smaller than the average distance from proteins selected randomly while maintaining the same overall degree distribution as ET-HPs (i.e., proteins selected randomly with a probability proportional to $k^{0.64}$) (Fig. 4*c*). Thus, it appears that EBV proteins favor the targeting of hubs in the human interactome, and moreover, exhibit a bias toward more centrally located proteins in the human network.

Conclusion

In summary, we have undertaken an unbiased, systematic, proteome-scale mapping of EBV–EBV and EBV–human direct protein–protein interactions. Such maps represent a rich source of protein function hypotheses, which we illustrated by demonstrating that LF2 inhibits the critical immediate early replication protein Rta. This interaction may enable the efficient establishment of latent EBV infection. Importantly, we observed a preference for interactions between EBV proteins belonging to the same evolutionary class. Further, human proteins potentially targeted by EBV tend to be hubs in the human interactome, consistent with the hypothesis that hub protein targeting is an efficient mechanism to convert pathways to virus use. The same biological properties that result in proteins being hubs in the human interactome may also result in these proteins being preferentially targeted by EBV. Finally, ET-HPs have many different functions in diverse biological pathways, consistent with the breadth of cellular machinery targeted by the virus. Although our observations are derived from incomplete sampling of the EBV and EBV–human networks, they form an important basis for comparisons to similarly sampled networks from other organisms to investigate similarities and differences. This partial understanding of the network can guide further analyses of the expanded network. Ultimately, information gained from this and other virus and viral–human interactome mapping efforts may provide an important foundation to better understand the overall organization of both viral and host proteomes and the complex interplay between their molecular machinery.

Materials and Methods

The EBV and EBV–human interactome data sets were generated by using a high-throughput Y2H system. An EBV ORFeome, comprising 187 unique clones representing 85 of 89 EBV ORFs, was transferred from entry clones to both DB and AD vectors by Gateway recombinational cloning (12) (Invitrogen, Carlsbad, CA). The resulting constructs were transformed into haploid yeast cells. To assay EBV–EBV protein interactions the DB- and AD-transformed yeast were mated and assayed on selective media for their ability to grow in an interaction-dependent manner. The

identity of interactors was determined by PCR amplification and sequencing. For the EBV–human interactions haploid yeast containing EBV DB clones were transformed with a spleen cDNA library and selected as described above. Further details are provided in *SI Text*.

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