[Frontiers in Bioscience 16, 2910-2920, June 1, 2011]

Crosstalk between viruses and PML nuclear bodies: a network-based approach

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TABLE OF CONTENTS

1. ABSTRACT

Due to the recent advances in instrumental and scientific methods, cell biology data are generated with increasing speed and quantity. One of these fast developing fields is the crosstalk between promyelocytic leukemia protein nuclear bodies (PML-NBs) and viruses. PML-NBs are dynamic nuclear protein aggregates which are targeted by entire viral particles, viral proteins or viral nucleic acids. Their possible anti-viral properties motivated researchers to investigate the interaction between PML-NBs and viruses in depth. Based on extensive literature data mining, we created a comprehensive PML-NB/virus crosstalk Cytoscape network, which groups not only the most common relations but also less well described findings. The network is easy to navigate and provides a biologically relevant overview which can help finding interesting case studies.

2. INTRODUCTION

Promyelocytic leukemia protein (PML) is the founding constituent of the PML-nuclear bodies (PML-NB, also known as ND10, POD or Kremer bodies) (1). To date, 173 different proteins have been identified as PML-NB components (reviewed in (2)). The localization of these protein partners has implicated PML-NBs in a wide variety of cellular processes such as transcription regulation, cell cycle, apoptosis and senescence (3-12). Soon after the first cellular protein partners were identified, viral proteins were also reported to reside at the PML-NBs and, sometimes, to drastically influence their existence or composition (13;14). A link between viruses and PML-NBs is not surprising since the latter are now known to be a conversion point of different apoptotic pathways (5-7), a process often circumvented by viruses. In addition, several PML-NB components are interferon regulated (15-22) suggesting a

role for PML-NBs as an anti-viral protein complex that is deliberately targeted by viruses and their components.

Viruses can affect the PML-NBs in different ways and on various hierarchical levels. The most drastic effect is the complete disruption of the bodies, for example, through proteasomal degradation of the scaffold protein (23-25) or by interference with PML SUMOylation (26;27). Other viruses spare the PML scaffold from degradation but relocate it to the cytoplasm or in a nuclear diffuse pattern (28;29); both relocalizations entail the destruction of the PML-NBs. Alternatively, the PML-NBs remain intact but their constitution is altered by the removal or addition of cellular (30) and viral proteins (31-34). Many researchers are also intrigued by the anti-viral properties of PML and other PML-NB components. In fact, when PML is knocked out in cells, some viruses produce a higher viral yield and, in agreement with these results, PML negative animals are more susceptible to viruses and their symptoms (for review see (35)).

The crosstalk between PML-NBs and viruses has been extensively studied. Crosstalk is defined as any effect that viruses and PML-NBs have on each other such as physical interactions, functional interactions or effects such as impact on mRNA or protein levels.

An impressive amount of data has been gathered about different viruses and their crosstalk with PML-NBs. Although excellent reviews (e.g. (35;36)) have been written about the subject, most focus on the most common and well-described cases. Unfortunately, a lot of valuable information about less well-known processes and especially about negative findings is scattered throughout literature and is difficult to find. To maintain the overview on this fast-changing topic it would be useful to group information in an easy-to-navigate tool which would provide one with a complete picture of what has been discovered, which case studies were fruitlessly pursued or which topics remain unexplored as yet.

In the present study we present a network which not only shows physical protein-viral protein interactions but also highlights the influence that PML has on viral proteins (and viruses) and vice-versa. This network is currently the only reference database for "PML-omics" researchers with a specific interest in viral (inter)actors and is publicly available at the Proteomics division on http://www.ua.ac.be/ppes (open source network analysis software Cytoscape (37)).

3. COMPILATION OF A PML-NB/VIRUS CROSSTALK NETWORK

To compile a map of the crosstalk between viruses and PML-NB components, thorough manual data mining of international literature was performed.

Pubmed was used as a reference database to find peer-reviewed publications written in English. The first search syntax used was "PML and VIRUS". Since PML-NBs have alternative names such as ND10 or POD, these were incorporated in different searches ("POD AND VIRUS" / "ND10 and VIRUS"). Further, each virus found in the first search was brought into a new search query (e.g. "Cytomegalovirus AND PML") to obtain additional publications. Each of the publications which were retrieved after this search was were manually parsed for information about PML-NB/virus crosstalk and implemented in the database

After the searches for PML or PML-NBs, all other PML-NB components which can be found in the PML-NB interactome (2) were used in the syntax "PML and VIRUS and proteinX". An important criterion for a PML-NB component (other than PML) to be considered for the network was that at the time of infection the protein resides at the nuclear bodies, leaves the PML-NBs or is recruited to the PML-NBs.

The information from the publications retrieved after these searches was embedded in a network built using the Cytoscape network program (open source; www.cytoscape.com). The data embedded in the network include physical interactions, functional interactions and effects of the virus on PML-NBs and vice versa. Some of these observations yielded information about the lack of involvement between a virus and PML-NBs. In order to give an overview which is as complete as possible of the knowledge about this subject, these findings were included in the network as well. However, these results have to be interpreted with caution due to possible experimental design flaws.

The network consists of nodes and edges. Nodes represent a PML-NB component, a virus, a viral protein or a viral nucleic acid. General and detailed information about the virus or viral protein was added in node attributes (Table 1). Edges which connect nodes give information about the relation between a certain viral component and PML-NBs (Table 1). Each publication used to construct the network was attributed one edge. Thus, if two nodes are connected by multiple edges, the relation between these two components is better described than nodes connected by a single edge.

The crosstalk network developed in the present study and instructions on how to use the program to explore it, can be downloaded from the Laboratory of Protein Science, Proteomics and Epigenetic Signaling website (www.ua.ac.be/ppes).

4. GENERAL CONTENTS OF THE PML-NB/VIRUS CROSSTALK NETWORK

Whereas most review publications only mention the best described effects of viruses on PML-NBs, this set-up aims to gather available information on PML-NB/viral component crosstalk obtained from literature in a transparent and comprehensive network. Depending on the researcher's interest, the network can be analyzed from different angles, focusing for example on protein-protein interactions or the crosstalk between PML-NB and viruses. In addition, if desired, additional tools such as Cytoscape plug-ins can be used.

Table 1. Overview of the different node and edge attributes in the PML-NB/virus interaction network

Field	Description
Node Attributes	
ID[1]	Simple annotation of the virus, protein or nucleic acid in the network. The alias appears as a node label when visualizing the network.
Alias[1]	
UniProtID[2]	If an entry exists in UniProt we added this information. Often more than one UniProtID is filled in due to the existence of different viral strains. Some IDs are unreviewed by UniProt (marked by *).
GO Biological Process[2]	GO annotation concerning the biological function of the viral protein.
GO Molecular Function[2]	In addition to the biological function of the protein, a GO molecular function also exists. Molecular function describes activities such as catalytic or binding activities which occur at the molecular level. The difference between GO biological process and GO molecular function is sometimes difficult to make but the general rule is that a process must have more than one distinct step (as stated on the GO website).
GO Cellular Component[2]	Subcellular localization differentiates amongst the several organelles in the nucleus and cytoplasm. Also annotated with information about the virion.
Order[3]	Taxonomy of the virus as proposed by the International Committee on Taxonomy of Viruses (ICTV) (63)
Family[3]	
Subfamily[3]	
Genus[3]	
Species[3]	
Popular Name[1]	Often the species name of the virus is not the designation commonly used in literature. The popularly used name is embedded in this attribute.
Virus Group[4]	Information about the classification of viruses based on their genome as described by Baltimore (50).
Remarks[1, 2]	Potentially important details about the protein.
Edge Attributes	
Author (s) [1]	Reference to the first author, year of publication and PubMedID.
ID[1]	Identification tag of a certain interaction. It is generally written as a string with the following format: "viral component PMID000000 PML-NB component".
Methods[1]	Overview of the methods used to detect the physical or functional interaction. As a designation for these methods we used the same evidence code as used on the BIOGRID cellular protein database (64).
Fluorescence Microscopy[1]	Details about the conditions for co-localization studies e.g. endogenous levels, overexpression data and or partial overlap (if mentioned in the publication).
Effect[1]	The "effect" attribute gives detailed information about the nature of crosstalk whereas the "Interaction/disruption/…" attribute provides the same information using keywords.
Interaction/disruption/...[1]	
Cell Type[1]	Cell type used for PML-NB/virus crosstalk studies.
PML Isoform[1]	For PML interactions it is important to make reference to the PML isoform used, if this information is available.
MOI[1]	Multiplicity of infection used in experiments.
Responsible Domain[1]	Many authors carried out mutation studies to determine the vital domain of viral proteins or PML-NB component in order to forge an interaction or elicit an effect. This domain and (if mentioned) the domain of the PML-NB component, is given by this attribute.
Resistance[1]	Reference to a possible resistance conferred by PML-NB components against viruses.
Remarks[1]	Potentially important details about the interaction.

In the 'field' column we present the attribute as it is assigned in the network. The information in network database of these fields was retrieved from different sources. The information in node and edge attributes marked with ([1]) was retrieved after literature data mining of 228 publications since each edge stands for one publication of which each individual reference can be found in the edge attribute 'Author(s)'. Node and edge attributes indicated with ([2]) were retrieved from UniProt's (61) Gene Ontology (GO) (62) keywords or other UniProt features. To catalog virus taxonomy, we used the designation for virus order, family, subfamily, genus and species as proposed by the International Committee on Taxonomy of Viruses (ICTV) (63) (marked by [3]). In addition, for each virus the Baltimore classification was embedded in the network's database ([4]). In the 'description' column we provide a brief explanation of what is embodied by the attribute.

The complexity of the interplay between viral components and PML-NBs is once more highlighted by the visual representation in the network. The network contains the efforts of researchers spanning almost 20 years (228 papers from December 1993 to August 2010). From these data, 273 nodes were connected with 640 edges which contain detailed information. Table 2 summarizes the general information embedded in the edges and nodes of the network.

As indicated on the fourth row of Table 2, edges can exist between a virus, a viral nucleic acid or a viral protein and a PML-NB component.

When an edge connects a virus to a PML-NB component, this edge mostly represents an effect of viral infection on PML-NBs or vice versa (Figure 1). Figure 1 (B) is a pie chart of the effects viruses have on PML-NBs such as disruption of the PML-NB complex or changes in composition.

PML-NB components have also been reported to exert anti-viral activity and a wide spread interest for the PML-NB as anti-viral NB was raised. Given the number of edges leaving from PML it is clear that it is mostly the scaffold protein PML that shows anti-viral behavior. Several modes of action have been described in respect to the anti-viral properties of PML. First, PML can interfere directly with the function of viral proteins by acting as a repressor in vital processes of the virus (38;39). Second, overexpressed PML can prevent the relocalization of PML-NB components out of the PML-NBs (40). Finally, PML can act as a stable scaffold of the PML-NBs, keeping other cellular proteins such as SP100 and DAXX in the correct localization to work in synergy with PML in an anti-viral defense (41). Conversely, many authors have observed destructive influences of viruses on PML-NBs (23-25;28;29;31-34;42) which may indicate a pathway of viruses to deregulate an anti-viral body.

Based on the available reports, it is not possible to determine if a specific group of viruses (e.g. dsDNA,

Table 2. General information embedded in the PML-NB/virus crosstalk network

Taxonomy	4 Orders : • Herpesvirales • Mononegavirales • Picornavirales • Unassigned	17 Families: • Herpesviridae • Filoviridae • Paramyxoviridae • Rhabdoviridae • Picornaviridae • Adenoviridae • Arenaviridae • Baculoviridae • Flaviviridae • Hepadnaviridae • Orthomyxoviridae • Papillomaviridae • Parvoviridae • Polyomaviridae • Retroviridae • Circoviridae • Unassigned			30 Geni – 38 viruses • Simplexvirus –HHV1, HHV2 • Varicellovirus – HHV3, EHV1, BHV1 • Roseolo – HHV6 • Cytomegalovirus – HHV5 • Muromegalovirus – MHV1 • Lymphocryptovirus – HHV4 • Rhadinovirus – HHV8, MHV • Ebolavirus • Pneumovirus -hRSV • Lyssavirus -Rabies • Vesiculovirus - VSV • Cardiovirus - ECMV • Enterovirus - Polio • Mastadenovirus - adenovirus C • Arenavirus – Lassa, LCMV • Alphabaculovirus – AcMNPV • Hepacivirus – HCV • Orthohepadnavirus – HBV • Influenzavirus A • Human Papilloma Virus - HPV • Parvovirus – Parvovirus H1, MVM • Polyomavirus – SV40, JC Polyoma, BKV • Lentivirus – HIV1 • Deltaretrovirus –HTLV1 • Spumavirus - HFV • Gyrovirus - CAV • Deltavirus – HDV • Gammaretrovirus –MLV • Dependovirus – AAV2 • Deltapapillomavirus - BPV	
Group	3 (+)ssRNA viruses	8 (-)ssRNA viruses	19 dsDNA viruses	3 ssDNA viruses	4 ssRNA-RT viruses	1 dsDNA-RT viruses
Nodes	44 PML-NB components	35 virus entries		29 nucleic acid entries	165 viral protein entries	
Edges	136 edges containing information about PML-NB/virus crosstalk	45 edges containing information about PML-NB/viral nucleic acids crosstalk			453 edges containing information about PML-NB/viral protein crosstalk	

The first row gives information about the taxonomy of the viruses embedded in the network. The second row provides the virus group according to the Baltimore Classification. The third row provides the number of nodes which represent a PML-NB component, a virus, a viral nucleic acid or a viral protein. The fourth row gives information about the number of edges connecting the PML-NB/virus, PML-NB/viral nucleic acid and PML-NB/viral protein. (*) Since several authors made distinct contributions, we chose to provide an edge for each of these contributions. Such a representation accentuates that crosstalk between highly connected nodes is studied more extensively as compared to crosstalk between nodes connected with a single edge (e.g. CMV-IE1 in Figure 2).

(+)ssRNA, (-)ssRNA,…) is specifically targeted by PML. A more accurate picture could be obtained after extensive screening for the influence of PML on a large number of different viruses. Further, it may be interesting to have a platform to report negative results. Since this kind of data is notoriously difficult to publish, it often disappears in oblivion whereas this data might contribute to give a more complete picture of the actual role of PML in an innate immunity response.

In addition to all the data mentioned earlier, viral nucleic acids were added to the network. This information can give an indication of replication processes at the PML-NBs (Figure 2 (A)). The graphical representation (Figure 2 (B)) shows that the majority of the viral nucleic acids which were published, reside at or near the PML-NBs for at least a portion of the viral cycle (43;44). However, similarly to the effects that viruses have on PML-NBs, the actual significance of viral replication at the PML-NBs or anti-viral defense through nucleic acids might be biased to the due low number of viruses that have been researched and/or published.

Finally, viral proteins were also included in the network (Figure 3). As demonstrated by the pie diagram of Figure 3 (B), several viral proteins are a part of the PML-NB interactome by co-localization or interaction (34;38). In addition, viral proteins often forge a functional interaction. Three kinds of functional interactions are visible in the network. First, a virus may specifically recruit PML-NB components and thereby alter PML-NB composition (45). Secondly, viral proteins can have a far-fetching influence on the integrity of PML-NBs and may eventually destroy them (46). Third, viral proteins can modulate the expression, function or SUMOylation of PML-NB components (26;31;47;48). Finally, some edges correspond to functional interactions which represent a pronounced anti- or pro-viral effect of a certain PML-NB component on a viral protein (49).

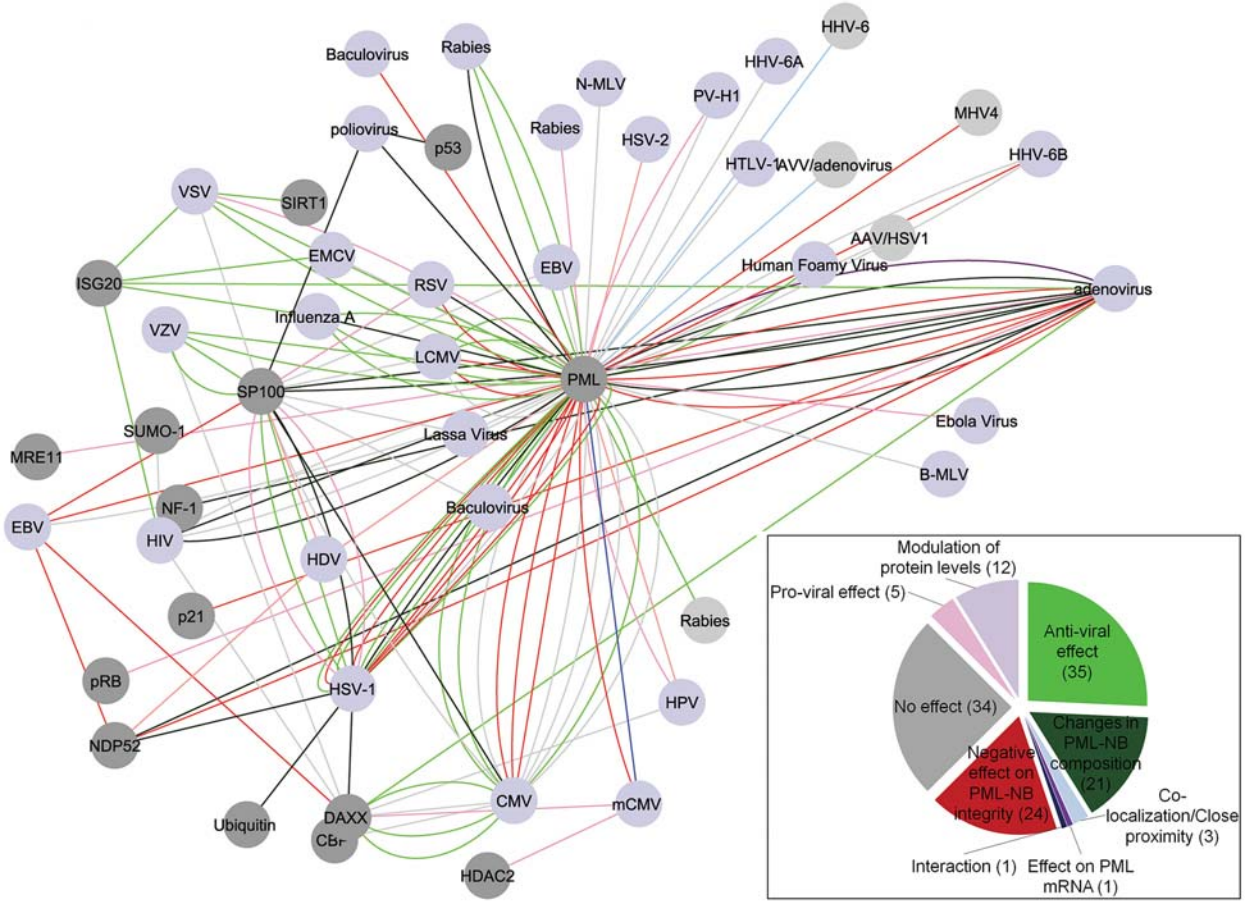

Figure 1. (A) A manually curated crosstalk network representing the influence of viruses on PML-NBs and vice versa. The nodes in gray represent PML-NBs components researched in regard to viral infection. Each node in the network is labeled with the canonical name of the protein or virus.Some nodes are connected with several edges, each representing a unique publication that reported a certain type of crosstalk. The edges are colored according to the effect on/by the PML-NBs: anti-viral activity of PML-NB component (light green), changes in PML-NB composition (dark green), co-localization/close proximity (army green), effect on PML mRNA (dark purple), interaction (dark blue), negative effect on PML-NB integrity (red), no effect of virus or virus component on PML-NBs (grey), pro-viral effect (pink), modulation of protein levels (light purple). (B) Graphical representation manually retrieved from the 'interaction/disruption/…' edge attribute from the network in (A), created to directly visualize the relative numbers of nucleic acids in each category. Color-coded as in Figure 1 (A).

5. TARGETED ANALYSIS OF THE NETWORK

The network can be used as an easy-to-navigate overview of literature to help focus on particular aspects of the crosstalk between viruses and PML-NBs. Here, three examples of a targeted analysis of the network are presented.

The viruses present in the network can be sorted based on their nucleic acid content (50). After the DNA viruses, the second largest group are the (-)ssRNA viruses. Interestingly, PML confers resistance against several of these viruses such as Influenza A, Lymphocytic Choriomeningitis Virus (LCMV), Rabies (Lyssavirus) and Vesicular Stomatits Virus. In experiments with the latter three viruses, it appeared that PML had an influence on the mRNA levels coding for viral proteins (39;51-53). Infection with two other (-)ssRNA viruses, the Ebola virus or Respiratory Syncytial Virus (RSV), induces PML upregulation (54;55). Efforts were made to elucidate the pathway behind these events and several proteins of these viruses have been investigated for their interaction, co-localization or effect on PML-NBs. However, the subnetwork depicted in Figure 4 shows that the knowledge on the crosstalk between viral proteins of (-)ssRNA viruses and PML-NBs is far from complete. Moreover, to date, the underlying mechanism for anti-viral activity conferred by PML remains obscure.

On the other hand, after sorting by taxonomy, it is immediately clear that the family of the Herpesviridae has been researched extensively regarding PML-NBs. Table 3, extracted from the network database, shows which herpesvirus protein products forge a physical or functional interaction with PML-NBs. Considering the estimated number of gene products, a large number of HHV proteins

Table 3. Herpesviridae protein products investigated in relation to PML-NBs

Species	Popular name	Protein products	# protein coding genes (reviewed by (65))
HHV-1	HSV-1	**ICP0, ICP4, ICP8, ICP27, UL5, UL8, UL8.5, UL9, UL14, UL30,** *UL42*, **UL52, US10**	74
HHV-2	HSV-2 (*)	*ICP0, ICP22*, **ICP27**, *UL1, UL3, UL4, UL7, UL11, UL12, UL13, UL18, UL20, UL23, UL31, UL34, UL35, UL40, UL41, UL42, UL45, UL48, UL48, UL50, UL50, UL56, US2, US9*	74
HHV-3	VZV	*ICP0, p61*	70
HHV-4	EBV	**BDLF1, BLLF2, BRLF1, BFLF2, BKRF4,** *BMRF1*, **BZLF1, EBNA-1,** *EBNA-2,* **EBNA-3,** *EBNA-4, EBNA-6,* **EBNA-5 (-LP), EBNA3B**	80
HHV-5	CMV	**IE1, IE2, TRL9, UL112/113, UL29, UL3, UL30, UL35,** *UL44,* **UL60, UL68, UL69, UL76, UL80a, UL97, UL98, US25, US32, pp65, pp71, ppUL69,** *pUL26,* **UL137**	165
HHV-6	HHV-6	**HHV-6A-IE1, HHV-6B-IE1, HHV-6B-p41, HHV-6B-U19, HHV-6B-IE1B**	86
HHV-7	HHV-7	None	84
HHV-8	KSHV	*K2, K8.1, K10, K11,* **LANA2, K8,** *ORF26, ORF50, ORF59, ORF 65, ORF73,* **ORF9, LNA1**	86

The first column gives the species of the herpesvirus whereas the second column provides the most commonly used name. The third column sums up the protein products which have been investigated in regard to PML-NBs (in bold). Indicated in italic are the proteins which have been found to have no influence on PML-NB components. The fourth column gives the estimates for the number of protein coding genes as reviewed by (65). Abbreviations - HHV: Human Herpes Virus, HSV: Herpes Simplex Virus, VZV: Varicella Zoster Virus, EBV: Epstein Barr Virus, CMV: Cytomegalovirus. (*) For HSV-2 only the influence on PML splicing was investigated.

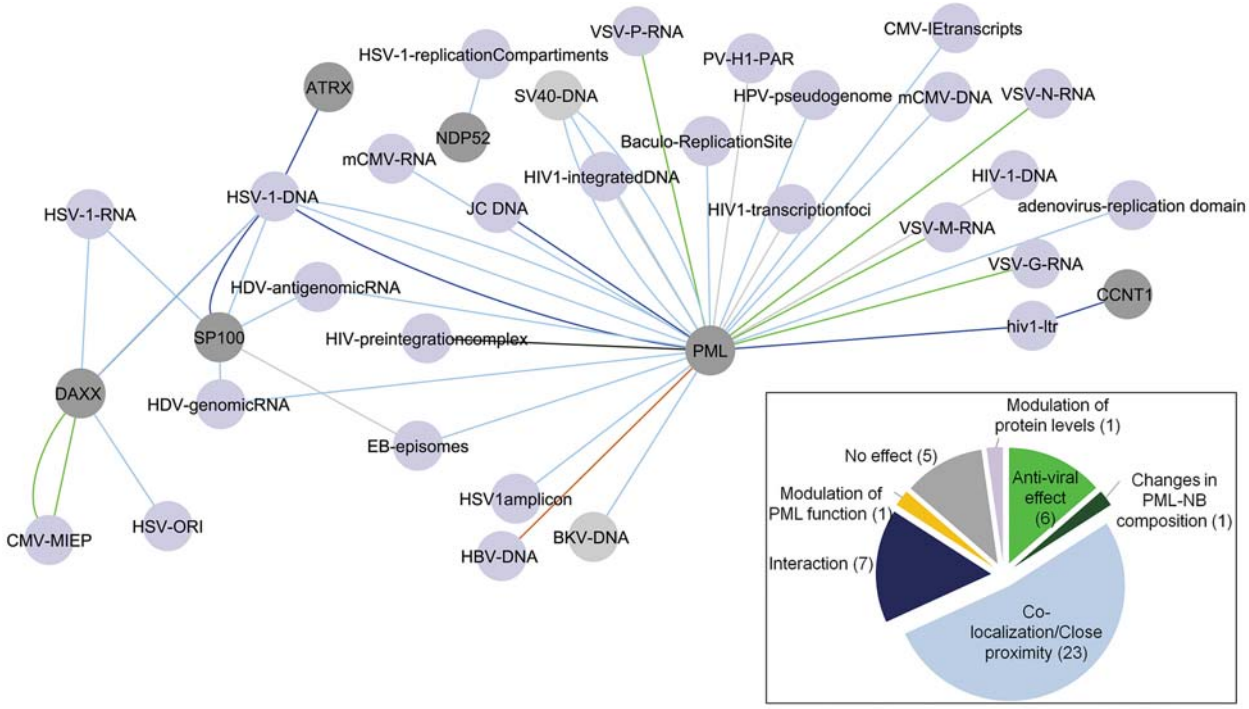

Figure 2. (A) A manually curated crosstalk and interaction network composed of viral nucleic acids and PML-NB components. The nodes in gray represent PML-NBs components researched in regard to viral infection. Each node is labeled with the canonical name of the viral nucleic acid or cellular protein.Since each edge represents a publication, nodes which are connected with more than one edge are described multiple times in literature. The edges are colored according to the effect on or by the PML-NBs: anti-viral effect (light green), changes in PML-NB composition (dark green), co-localization/close proximity (light blue), protein-nucleic acid interaction (dark blue), modulation of PML function (orange), no effect of PML-NB component on viral nucleic acid or vice versa (gray), modulation of protein levels (light purple). (B) Graphical representation manually retrieved from the 'interaction/disruption/…' edge attribute from the network in (A), created to directly visualize the relative numbers of nucleic acids in each category. Color-coded as in Figure 2 (A).

has not been investigated (or published) to date and are interesting research targets. Moreover, thus far no research has been published concerning the role of PML-NBs in HHV-7 infection.

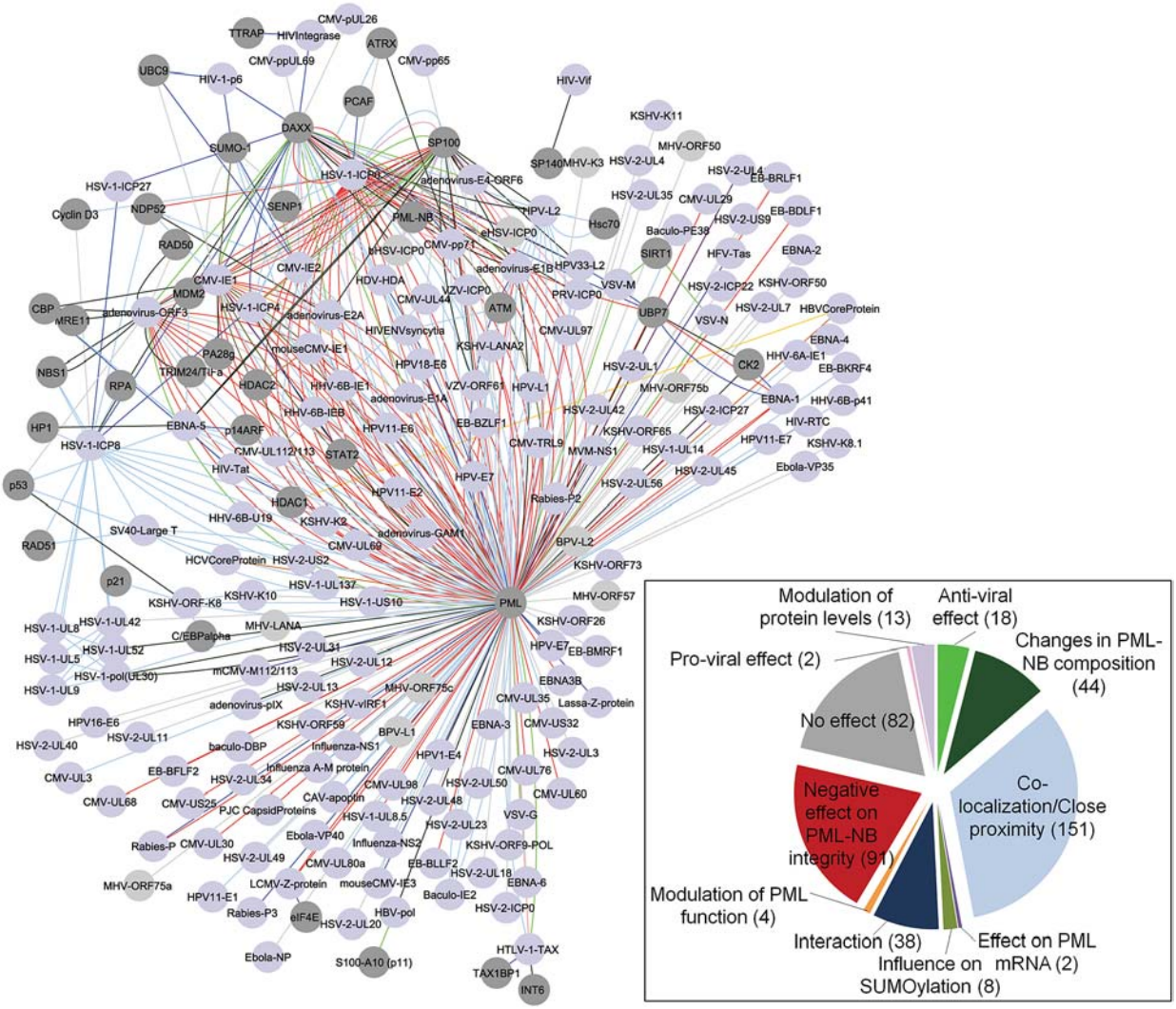

Figure 3. (A) A manually curated protein-protein crosstalk network between viral proteins and PML-NBs. The nodes in gray represent PML-NBs components researched in regard to viral infection. The canonical name is provided for each node. Viral protein nodes which are connected with multiple edges are better described in literature. The edges are colored according to the effect on, or by the PML-NBs: anti-viral activity of PML-NB component (light green), changes in PML-NB composition (dark green), co-localization/close proximity (light blue), effect on PML mRNA (dark purple), influence on SUMOylation (olive green), protein-protein interaction (dark blue), modulation of PML function (orange), negative effect on PML-NB integrity (red), researched protein which does not influence PML-NBs (grey), modulation of protein levels (light purple), pro-viral effect (pink). (B) Graphical representation manually retrieved from the 'interaction/disruption/…' edge attribute from the network in (A), to directly visualize the relative numbers of proteins, nucleic acids or virus in each category. Figure 3 (B) is color-coded as in Figure 3 (A).

Finally, the network can also be used to analyze existing specific knowledge about a certain virus. PML belongs to the TRIM protein family. Since several members of this family have been shown to have anti-HIV activity (56;57), PML was a logical candidate for further research. However, the network shows that the role of PML in HIV infection remains disputable, as contradictory results have been reported. Turelli and colleagues (58) have found that during early infection PML was momentarily relocated to the cytoplasm. When forcing the disappearance of PML-NBs by arsenic treatment, the efficiency of HIV-mediated transduction markedly increased. However, Berthoux *et al.* (59) could not detect such a translocation nor did they gather any proof that PML is involved in HIV infectivity. In addition, no HIV nucleic acids have thus far been found in the vicinity of the PML-NBs (60). Nevertheless it appears from network analysis that PML-NBs might remain interesting in the context of HIV. Several PML-NB components such as TTRAP, CyclinT, CBP, DAXX, ISG20 and SP140 have been attributed different roles in HIV infection and the PML scaffold possibly plays a vital part for the accumulation of these proteins.

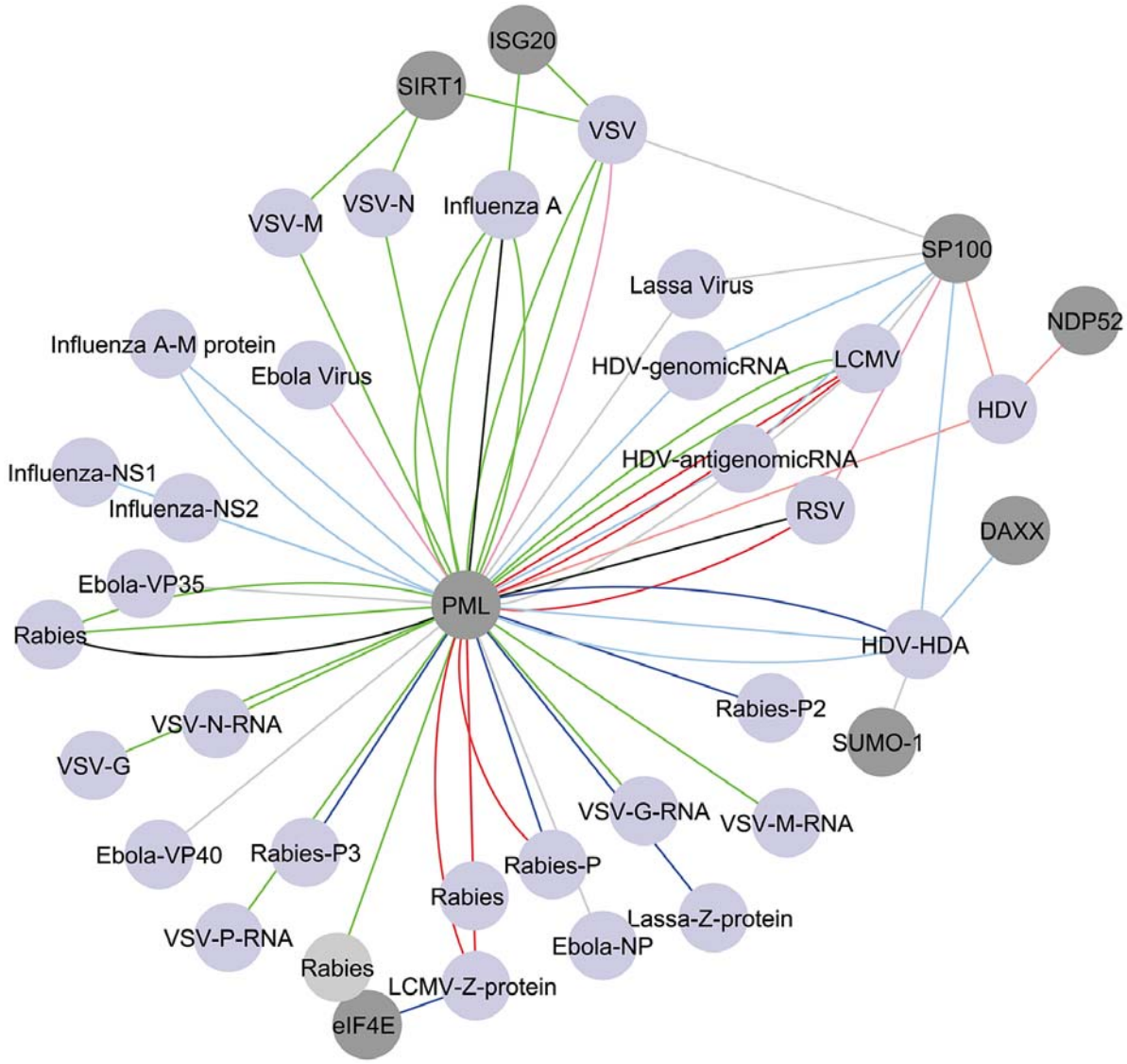

Figure 4. Subnetwork depicting (-)ssRNA virus components which have been under investigation (and published) in relation to PML-NBs.

6. CONCLUSION

In the present study a comprehensive network-based overview is created which easily allows researchers to explore particular aspects of the interactions between viral components and PML-NBs, destructive and non-destructive effects on PML-NBs or anti-viral properties. Without a common platform, gathered data is easily forgotten in the constant stream of information and valuable, even negative, research results might be lost. Therefore, the network presented here aims at being the first effort to integrate as much available information as possible from literature. Using the network to detect "white spots" in knowledge could precede experimental investigation and the results of this information could in turn be embedded in the network for other researchers. Then, based on this information, the true relevance or role of PML during virus infection could be assessed.

7. ACKNOWLEDGEMENTS

This work was supported by an NOI grant (BOF-UA-2005) from the University of Antwerp.The authors would like to thank Prof. Dr. Guido Vanham, Dr. Inge Brouns and Dr. Isabel Pintelon for the meaningful discussions and careful reading of the manuscript.

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Abbreviations: PML-NB: Promyelocytic Leukemia Protein Nuclear Body; POD: Promyelocytic Onceogenic Domain; ND10: Nuclear Domain 10; (-)ssRNA: negative single stranded RNA; HHV: Human Herpes Virus; HIV: Human Immunodeficiency Virus; TRIM: Tripartite Motif

Key Words: PML-NB, virus infection, Promyelocytic Leukemia protein, ND10, Review

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