Advances in Analyzing Virus-Induced Alterations of Host Cell Splicing

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Abstract

Alteration of host cell splicing is a common feature of many viral infections which is underappreciated because of the complexity and technical difficulty of studying alternative splicing regulation. Recent advances in RNA sequencing technologies revealed that up to several hundreds of host genes can show altered mRNA splicing upon viral infection. The observed changes in alternative splicing events can either be a direct consequence of viral manipulation of the host splicing machinery or result indirectly from the viral-induced innate immune response or cellular damages. Analysis at a higher resolution with single-cell RNAseq and a higher scale with the integration of multiple omics datasets in a systems biology perspective will be needed to further comprehend this complex facet of virus-host interactions.

Alteration of cellular splicing: a complex facet of virus-host interactions

In higher eukaryotic cells most genes are transcribed as precursor messenger RNAs (pre-mRNAs) that undergo splicing, a maturation process through which RNA sequences (introns) are removed and the remaining sequences (exons) are ligated together. Splicing occurs in the nucleus and is catalyzed by the spliceosome, a large and highly dynamic ribonucleoprotein complex [1, 2]. Most mammalian pre-mRNAs are subject to alternative splicing (AS), and human genes contain on average 8.8 exons and 7.8 introns per gene, giving rise to an average of 3.4 alternatively spliced isoforms [3, 4]. The most common types of AS events are the use of alternative donor and acceptor splice sites, exon skipping, alternative use of mutually exclusive exons, and intron retention. Alternative splicing expands the diversity of proteins that can be expressed from a given gene, and can also modify cis-regulatory elements that govern the stability and translation of mRNAs. In recent years, head-to-tail back-splicing events that result in the formation of non-coding circular RNAs (circRNAs) have also been observed to play key regulatory roles in a variety of biological processes, including antiviral immunity [5, 6]. Splicing is tightly coupled to transcription, and is controlled by cis- and trans-acting elements as well as through chromatin structure and signalling pathways [3, 5, 7]. The advent of high-throughput RNA sequencing technologies (RNAseq) has opened up a new era in studying how AS is regulated and shapes the cellular proteome in response to changes in environmental conditions (e.g. [8]).

Viruses modulate host gene expression in order to favor viral replication and evade antiviral responses. They have evolved mechanisms to affect cellular transcription, mRNA processing and nuclear export, mRNA decay, and translation [9-11]. RNAseq, proteomic and interactomic studies are now beginning to provide a global view of viral-induced alterations of cellular splicing and insights into how they may impact viral pathogenesis. Herein, we review the recent developments in the field, we discuss how current limitations could be overcome in the future, and what advances may be expected from the integration of splicing isoforms datasets into a systems biology perspective.

Viral manipulation of the host splicing machinery

Viruses that replicate in the nucleus of infected cells and gain access to the splicing machinery (e.g. adenoviruses, herpesviruses, and influenza viruses) have evolved an

expansion of their coding capacity by producing spliced viral mRNAs. However, manipulation of the host splicing machinery is not exclusive to nuclear viruses and has also been observed with viruses that replicate in the cytoplasm such as picornaviruses or flaviviruses (Table 1). This can be accounted for by the nucleo-cytoplasmic shuttling of some viral proteins (e.g. the dengue virus NS5 protein [12]) and splicing factors (e.g. SR proteins [13]), increased nuclear permeability upon viral infection [14] or signaling pathways triggered by viral infection [15]. Components of the splicing machinery commonly targeted across different virus families are the small nuclear ribonucleoproteins (snRNPs), serine/arginine-rich (SR) proteins, and heterogeneous nuclear ribonucleoproteins (hnRNPs). The U1 to U6 snRNPs are core components of the spliceosome, whereas SR and hnRNP proteins are involved in the regulation of constitutive and alternative splicing. Different viruses appear to induce similar types of alterations, *i.e.* changes in the level of expression, protein-protein or protein-RNA interaction pattern, localization, phosphorylation and/or intrinsic activity of splicing factors (Table 1).

Among the genes that appear differentially expressed upon viral infections in transcriptomic and proteomic datasets, the overall enrichment of splicing-related genes is generally not reported. However, particular splicing factors can undergo significant changes in expression upon viral infection. As an example, expression of EFTUD2, a U5-snRNP associated factor, was found to be decreased upon HCV infection of cultured cells and in liver samples from HCV-infected patients; downregulation of EFTUD2 impairs the splicing of RIG-I and MDA5 pre-mRNAs and therefore enables the virus to circumvent the innate antiviral response [16]. Phosphoproteomic profiling provides an additional layer of information by revealing viral-induced changes in the phosphorylation status of host proteins. Dynamic phosphorylation/dephosphorylation is known to regulate the function of splicing factors, most notably SR proteins [17]. HIV-1 entry triggers early changes in the phosphorylation of five SR proteins, including SRRM2 which regulates the splicing of HIV-1 transcripts [18]. Later in infection there is evidence that the HIV-1 protein Vpr modulates the activity of SR protein-specific kinases and the phosphorylation of SRRM2 [19]. In the pre-omics era, the herpesvirus ICP27, adenovirus E4-ORF4 and papillomavirus E1^E4 proteins have also been reported to regulate SR proteins phosphorylation in order to facilitate their replication [20-

22] (Table 1). Altogether these findings point to changes in SR phosphorylation as a mechanism commonly triggered by multiple viruses to co-opt the splicing machinery.

Interrogation of the VirHostNet 2.0 public database dedicated to virus-host protein-protein interactions (PPIs) [23] reveals that a large proportion of known splicing factors have at least one reported interaction with a viral protein, pointing to the spliceosome as a frequent viral target (Figure 1A and 1B). Most of the data derive from a yeast two-hybrid or affinity-purification mass spectrometry screen with no systematic experimental validation of the interactions and therefore cannot be assumed *a priori* as high confidence data [14]. However data integration helps reveal splicing factors which are found to be associated with multiple viral species and represent potentially relevant targets for onward studies, e.g. DDX5 and FUS (Figure 1C). Some PPIs have been identified in low throughput studies and are well documented such as the recently identified interaction between the reovirus protein μ2 and the SR protein SRSF2, which alters SRSF2 function and determines the virus ability to counteract the interferon response [24]. Additional PPIs and associated effects on the host splicing machinery are listed in Table 1. Furthermore, viral RNAs can act on the splicing machinery, as exemplified by the changes in AS events observed upon sequestration of the HuR protein by the 3'UTR of Sindbis virus [25], (Table 1).

Peering into viral-induced alterations of host alternative splicing events

To date there are only a few studies that performed transcriptome-wide micro-array or RNAseq analysis of cellular AS events in virus-infected cells (Table 2). They concern herpesviruses [26-28], reoviruses [24, 29], dengue viruses (DENV) [12, 30], Zika virus [31] and influenza viruses [32], and reveal several hundreds of host genes that show altered mRNA splicing upon infection. When examined, no correlation was found between changes in AS events and changes in mRNA expression levels. Validation by RT-PCR or RT-qPCR of a selected subset of the predicted differential AS events is compulsory and usually performed to sort out and confirm RNAseq findings. Validation rates can only be determined if a large enough number of AS events is assayed, and are likely to depend on the metrics and statistical analysis that are used. In the few studies which provide GO terms enrichment analysis on the list of differentially spliced genes in virus-infected cells, an enrichment in genes related to cell cycle, gene expression and/or RNA metabolism was reported [24, 27,

30-32]. Consistently with previous reports that the AS landscape varies between human tissues [33], neural progenitor cells infected with the human cytomegalovirus (HCMV) exhibited fewer AS changes compared to fibroblasts and only half of altered exon-skipping overlapped in both cell types [26]. Exon-skipping, which is most frequently detected in the human transcriptome [33] accounts for the largest share of infection-altered AS events in all except the DENV5 study [12]. Interestingly intron retention (IR) which, although identified early on in spliced viral mRNAs, was until recently considered a rare event in mammalian cells, was found to represent a substantial proportion (> 20%) of infection-altered AS events in herpes simplex virus-1 (HSV-1) and DENV5-infected cells [12, 27]. These observations align with recent findings that IR is actually a common AS event in mammalian cells, although the fate of intron-retaining mRNAs regarding nucleo-cytoplasmic export or nonsense mediated decay is not fully understood [34]. Taken together with a recent report that influenza virus NS1 protein primarily binds intronic sequences [35], they suggest that some viruses may have evolved specific mechanisms to alter host cell expression through increased IR. Finally, within the last couple of years the first reports of viral-induced alterations of cirRNAs were published. Notably, cirRNAs were found to be expressed and associated with the NF90/NF110 factor at lower levels upon Vesicular Stomatitis Virus (VSV) infection, which results in increased NF90/NF110 binding to viral mRNAs in the cytoplasm and thereby likely contributes to the antiviral immune response [36]. A global dysregulation of circRNAs was observed upon infection with HSV-1, which could potentially modulate the cellular transcriptional responses through the miRNA sponge function of circRNAs [37].

Splicing alterations observed upon infection potentially result from the combination of two types of mechanisms: i) they can be caused by a viral manipulation of the splicing machinery, as described above; or ii) they can be related to viral-induced cellular damages or innate immune responses (Figure 2). Indeed, there is increasing evidence that AS is a mechanism to regulate the immune responses to pathogens [38-40] (Box 1), as well as apoptosis [41], DNA damage response [42] and endoplasmic reticulum stress response [43]. Integration of multi-omics data will probably be key to distinguishing between direct and indirect effects of viral infection on the cellular splicing machinery and uncovering of the complex mechanisms by which the host-cell splicing landscape is modified (see below).

In some instances, differences in viral-induced splicing changes were reported when viral variants were compared. Reovirus strain T1L, compared to strain T3D which differs in that its µ2 protein does not localize to nuclear speckles, triggered more splicing changes *i.e.* 369 compared to 142, with an overlap of only 35 changes. The cellular processes which were most affected by T1L infection, *i.e.* gene expression and RNA post-transcriptional modification, were not strongly affected by T3D infection [24]. Comparison of a subset of AS events upon infection with DENV2 and DENV4 revealed differences, suggesting a possible serotype specificity in AS alteration [12]. However the molecular mechanisms that lead to specific alterations of the splicing landscape remain largely unknown. The only evidence for a sequence recognition mechanism comes from Tang et al. who showed that the ICP27 protein of HSV-1 mediates splicing alterations in genes that are GC-rich, with suboptimal splicing sites and cytosine-rich sequences close to the 5' splice site [44]. The presence of a conserved 41 nucleotide motif was observed in 93 out of 240 AS events that were dysregulated upon infection with the reovirus strain T3D [29], however this motif does not correspond to a predicted RNA regulatory motif and the significance of this observation remains to be demonstrated.

Importantly, transcriptomics studies revealed that not only splicing but also other features of cellular mRNAs can be altered upon viral infection. For instance, DENV1 was found to induce alterations in the relative usage of transcriptional start sites, in addition to splicing changes [30]. Infection with herpesviruses was shown to trigger widespread disruption of transcription termination of cellular mRNAs [26-28], which in the case of HCMV infection was attributed to a strong induction of the host RNA-binding protein CPEB1 [26]. Transcription extends over thousands of nucleotides beyond poly(A) sites and into downstream genes, it interferes with the analysis of transcriptional and splicing regulation of the downstream gene and can generate novel intergenic splicing between exons of two neighbouring genes [28]. Defective transcription termination of cellular mRNAs was also very recently reported in influenza virus-infected cells [45-47]. While most studies provide information about the steady-state levels of mRNA isoforms, a few of them rely on the NET-Seq method [45] or on 4sU tagging of mRNAs [28] to provide a real-time view of viral-induced changes in co-transcriptional RNA processing. As these methods allow to detect actively or newly transcribed mRNAs, they can also help avoid biaises due to isoform-specific

differences in the stability of mRNA. Transcriptomic analysis and mechanistic understanding of how viral infection impacts cellular mRNA co- and post-transcriptional processing is an expanding field, constantly evolving in response to progress in cellular biology (e.g. the recent findings on the biogenesis and function of circRNAs) and in technologies (as discussed below).

Towards a more accurate view: methodological challenges

RNAseq transcriptomic analysis of AS is a challenging issue, and even more so when performed on a virus-host system. Accurate quantification of isoform abundance requires a high read number (about 50 millions paired-end reads of at least 75 bp are recommended for the human transcriptome), which to date can only be provided by the Illumina technology. However a serious limitation of Illumina RNAseq is that it relies on short reads, so that the resolution of exon connectivity and full-length isoform structure cannot be achieved (Box 2). Upon viral infection the reads mapping to viral mRNAs may represent 5-75% of the total number of reads (Table 2), therefore the sequencing depth of virus- and mock-infected samples must be adjusted accordingly. The degree of variability of AS viral-induced perturbations also need to be taken into account when setting the sequencing depth and the number of biological replicates, in order to differentiate biologically relevant changes from transcriptional noise.

Many viruses induce host-cell transcription shut-off [10] although specific genes (e.g. IFN-stimulated genes, cytokines) can escape this shut-off and be up-regulated. Marked differences between the gene expression profiles of mock- and virus-infected cells poses a challenge for accurate comparison of isoform abundance. Commonly used RNAseq normalization methods [48] make the assumption that a core set of genes is not differentially expressed and mainly correct for sequencing depth. Normalization methods taking into account other variability factors (e.g. the average amount of cellular RNA per cell) besides sequencing depth for host-pathogen dual RNAseq experiments need to be developed [49].

Another methodological challenge of viral-host RNAseq lies in the ability to deal with cell-to-cell heterogeneity during infection. Usually high multiplicities of infection are used and it is verified or assumed that almost all cells are infected. However, single-cell RNAseq

(scRNAseq) experiments revealed extensive variability from one individual cell to another in terms of production of intracellular viral RNAs and cellular responses [50-52]. RNAseq experiments on polyadenylated mRNAs or circRNAs extracted from bulk cell cultures or tissues are providing an average measure of isoform abundance; they are potentially masking heterogeneity that occurs in the dynamics of infection and/or specific transcriptomic profiles in a subset of cells which could be relevant to the viral phenotype. Single cell RNAseq can be used for transcriptome-wide differential splicing isoform quantification, however this requires specific bioinformatics tools and normalization procedures to be developed, to cope with the low reads counts, the heterogeneity and noise in the datasets [53-55].

So far, most RNAseq analyses of splicing events in virus-infected cells have been conducted with cancer cell lines and/or viral laboratory strains. Such experimental conditions have practical advantages (ready availability, ability to infect almost 100% of the cells, and higher reproducibility), however they may not accurately reflect physiological infection. The development of scRNAseq, which allows discrimination between infected and uninfected cells, will probably encourage the use of field viral isolates and more relevant cellular systems such as primary cells or tissue explants. Novel perspectives will be opened by the use of induced human pluripotent cells (iPS) which upon differentiation can serve as convenient surrogates for primary cells that are difficult to isolate and culture [56], and 3D organoid cultures which can provide an accurate model for the micro-anatomy of an organ [57].

Integration of isoforms datasets in a systems biology perspective

The rapid advancement of high-throughput technologies has led to the development of the systems biology field, which aims at modeling the properties of complex biological systems, and predicting their response to biological or chemical perturbations. The commonly used "top down" systems biology approach turns RNAseq measurement of expression levels into a variable which can be included in a mathematical model, such as a generalized linear model or a multivariate analysis (Principal Component Analysis, Singular Value Decomposition, Partial Least Square). The variance across genes or isoforms, samples and conditions is computed to identify statistically significant transcriptomics signatures, *i.e*.

particular combinations of thousands of genes or isoform expression levels. The mechanistic interpretation of these signatures and the prioritization of candidate genes for downstream experimental validation remain challenging tasks. However, the use of transcriptomic signatures has already allowed genetically close viral strains to be robustly distinguished [58], species-specific responses to infection outlined [59] and the outcome of viral infections predicted [60]. It can also pave the way towards the discovery of relevant biomarkers [61]. Transcriptomic signature analysis has become a routine at the gene level, but little has been done so far at the isoform-level. Recent methodological developments - such as splicing signature comparison workflows for the discovery of candidate splicing regulatory elements - have been applied to psoriasis studies [62, 63] and could be transferred to the analysis of infected-cells in the future. Further progress in the field will also be enhanced by the accessibility and the integration of published RNAseq datasets within open access knowledge bases (e.g. ArrayExpress, Gene Expression Omnibus), which allows meta-analyses to be conducted.

The integration of RNAseq datasets with other omics datasets, so far mostly proteomics, interactomics and metabolomics datasets, has become a new way to rationalize the deconvolution of the transcriptomics signal, and has been applied to virus-infected cells [64-66] or patients [67]. To facilitate this integration step, network-based methods such as correlation network (e.g. WGCNA) or probabilistic models (e.g. MERLIN) are currently being investigated (reviewed in [68]). The main objective is to infer the transcriptional gene regulatory network and to prioritize a set of candidate genes, transcriptional factors or functional modules that are involved in viral infection. A growing number of studies combine newly generated experimental data with published datasets available in open access databases to identify the most relevant molecular pathways. For example, by combining proteomics and RNA-seq experimental data along with available gene-regulatory and protein-protein interaction networks Sychev *et al.* successfully implemented a computational model based on the Prize-Collecting Steiner Forest algorithm, which highlighted peroxisome lipid metabolism as an important function involved in KSHV (Kaposi's sarcoma associated herpesvirus) latent infection [65].

One should stress that integration is for now only performed at the gene-level. Gene annotation knowledge bases (GO, Interpro, and KEGG) are exploited to investigate

enrichment of specific biological or molecular functions (GSEA). Although they are continuously being improved, gene annotations are still far from being complete [69], and even more so at the isoform level and for non-model organisms. Gene annotations often relate to a “reference isoform” which is assumed to be the predominant one, whereas the relative proportion of splicing isoforms may differ from one tissue to another. Alternative splicing may lead to the gain or loss of functional domains, catalytic sites and/or protein-protein interfaces. However, the full set of alternative isoforms that effectively contribute to proteomic diversity and represent "functional alloforms” remains to be characterized [70, 71]. Available Exon Ontology resources can be used to readily identify enriched functions of gene isoform subsets but they restrict the analysis at the exon-level [72]. The systematic characterization of AS isoforms function is a challenging task and might be accelerated through bioinformatics prediction [72-74] or experimentally by using interactomics approaches [75, 76].

As molecular approaches to study virus-host interactions at a high level of mechanistic detail are also making steady progress, the “bottom up” systems biology approach also seem promising. In this approach the knowledge generated by the molecular and biochemical characterization of a subsystem and its response to perturbations is used to generate specific sub-networks, which can subsequently be integrated together with high-throughput data into larger molecular interaction networks [77-79]. In the case of the spliceosome machinery, subnetwork modeling is making progress through Bayesian probability modeling [80], food-web modeling [81], or deep-learning approaches [82], which offers advanced tools for studying its involvement and vulnerability upon viral infection. In return, research on the virus-spliceosome interplay will likely contribute to a better definition of the complex set of rules that can predict the splicing pattern of each isoform based on a comprehensive catalogue of cis-regulatory elements and their functional molecular interactions in various physiological and pathological conditions.

Concluding remarks

The importance of characterizing the transcriptome landscape of virus-infected cells at the splicing level is highlighted by recent studies, which reveal significant AS alterations in response to viral infections. The observed changes in AS events, which are regulated through

a very complex protein-RNA interaction network, can either be a direct consequence of viral manipulation of the host splicing machinery or result indirectly from viral-induced immune responses or cellular damages. Integration of multiple omics datasets in a systems biology perspective will be needed to comprehend this complex facet of virus-host interactions. Beyond proteomics and interactomics, which have been most commonly combined with RNAseq analysis so far, epigenomics and epitranscriptomics would also be relevant. Indeed, viral infections may induce epigenetic [83, 84] or epitranscriptomic [85] modifications, and both types of modifications may in turn affect splicing [86, 87]. Genetic mutations that affect AS, and consequently may determine phenotypic variability and individual susceptibility to viral diseases, is also an interesting direction for future research [88]. Species-dependent AS patterns of cellular genes could possibly be determinant for viral host-range, as suggested recently for influenza viruses [89]. As technological advances in RNA sequencing and RNA genomics will allow to study the interplay between cellular AS and viral infections at an increasing scale and resolution, major challenges in terms of computational analysis and storage of the corresponding datasets will need to be addressed, and the building of pluridisciplinary research teams along the lines of the European Virus Bioinformatics Center (http://evbc.uni-jena.de) [90] will be paramount. Integration of splicing isoform datasets with other omics data may well contribute to the development of personalized prognosis and management of infectious diseases and lead to therapeutic innovations.

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Box 1 : Role of alternative splicing in shaping immune responses.

In the recent years there has been growing evidence for a role of AS in shaping both innate and adaptative immune responses [38-40]. Different isoforms have been found for key players of the antiviral innate immunity, including pattern-recognition receptors (e.g. TLRs, RIG-I, and MDA5), downstream signaling proteins (e.g. MyD88, MAVS, STING, TBK1, and IRF3), and effectors (IFN Type I, IFNAR, cytokines and chemokines). The splicing factors involved are known in a few cases : EFTUD2 regulates the splicing of RIG-I, MDA5 and MyD88 [91], while SF3A1 affects the splicing of several genes of the TLR signaling pathway including MyD88 [92]. In several instances, splice variants exert a negative feedback loop on the signaling pathway, thereby probably controlling the intensity and duration of the antiviral and inflammatory responses. Notably, RIG-I and STING splice variants whose expression are up-regulated upon viral infection strongly inhibit RIG-I and STING signaling pathways, respectively [93, 94]. A viral-induced, alternatively spliced isoform of TPK-1 disrupts the interaction between RIG-I and MAVS and inhibits IFN-beta signaling [95]. Short isoforms of MAVS negatively regulate TLR3-mediated nucleic acid sensing [96], and limit self-aggregation of the full-length MAVS protein thereby preventing accidental antiviral innate immune signaling [97]. The contribution of AS to the regulation of humoral and cellular adaptative immune responses is also clearly recognized, however the mechanisms involved remain largely unknown. In B cells two mechanisms were recently uncovered : the HuR protein, by regulating mRNA splicing upon B cell activation, is essential for antibody response to a variety of antigens [98], while the ZFP318 factor regulates the AS-dependent balance between IgM and IgD immunoglobulins [99]. In T cells, one of the best documented examples is the AS of CD45 in response to antigen receptor-mediated signaling, which is differentially regulated depending on the T cell lineage and the stage of activation (reviewed in [39, 40]).

Box 2 : Resolving alternative splicing by short- or long-reads sequencing technologies.

Currently Illumina is the most commonly used RNAseq technology for transcriptome-wide analysis of AS. However Third Generation Sequencing (TGS) technologies, such as the Pacific Biosciences (PacBio) and Oxford Nanopore (ON) technologies, are emerging as alternative platforms for AS analysis [100].

The major advantage of Illumina over the TGS technologies is its higher sequencing depth (up to 400 million reads instead of 1 million for TGS), even more so in the case of dual RNAseq when both the viral and host transcriptome need to be sequenced. High depth is needed for the detection of minor isoforms and the robust quantification and statistical analysis of AS events [101]. Another advantage of Illumina resides in its higher sequencing accuracy (about 0.1% error rates instead of 10-15% for TGS), which is particularly advantageous when working on poorly annotated genomes of non-model organisms. However, a major limitation of Illumina is that it generates short reads (75-300 bp) which provides only local information about AS events and entails a challenging computational reconstruction of full-length isoforms. The strength of TGS is their read length (1-100 kb), which allows the direct characterisation of full-length transcripts with information not only on alternative splicing and the coordination of distant exons [102] but also on alternative transcription start and termination sites [103]. Moreover long reads allow to resolve repetitive sequences, that are posing a major challenge for sequence assembly or alignment from short-read datasets.

Future advances may lie in the Hybrid-Seq approach which combines short and long-read technologies [101], the Synthetic Long-Read technology which exploits the assets of Illumina with a short-read barcoding system to reconstruct full-length transcripts [104], or the very swift evolution of long-read technologies. Particularly promising is the perspective of direct RNA sequencing proposed by ON, which would avoid reverse-transcription- and amplification-related biases in isoforms quantification [105].

410 **Table 1. Viral targeting of the splicing machinery.**
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Virus	Viral factor	Cellular Target	Associated cellular changes [a]	References
HSV-1	ICP27	Binding to, relocalisation and inhibition of SRPK1	Altered phosphorylation of SR proteins	[22]
HSV-1	ICP27	Binding to SF3B2*	ND	[106]
HIV-1	Vpr	Binding to SF32B*	Altered splicing of pre-mRNAs	[107]
EBV	SM	Binding to SRSF3	Altered splicing of STAT1 pre-mRNA	[108]
HPV1	E1^E4	Binding to and inhibition of SRPK1	Altered phosphorylation of SR proteins	[21]
HPV16	E2	Transactivation of SRSF1-3 promoters	ND	[109]
Adenovirus	E4-ORF4	Binding to SRSF1, SRSF9	Modulation of pre-mRNA splicing	[20]
Influenza V	NS1	Binding to U6snRNA	Inhibition of pre-mRNA splicing	[110]
Influenza V	NS1	Relocalisation of SRSF2	ND	[111]
Influenza V	NS1	Binding to and relocalisation of NS1-BP	Altered splicing of some pre-mRNAs regulated by NS1-BP	[112, 113]
Poliovirus	2A	Relocalisation of HuR, TIA-1, TIAR	Modulation of Fas6 pre-mRNA splicing	[114]
Poliovirus	2A	Relocalisation of SRSF3	ND	[115]
EV-71	3D (Pol)	Binding to and inhibition of PRPF8**	Blockage of pre-mRNA splicing and mRNA synthesis	[116]
FMDV	3C (Pro)	Cleavage and relocalisation of Sam68	ND	[117]
Reovirus	µ2	Binding to and relocalisation of SRSF2	Altered splicing of pre-mRNAs	[24]
Rotavirus	NSP2, NSP5	Association with and relocalisation of hnRNPs and HuR	ND	[118]
Sindbis V	nsP2	Binding to hnRNPK	ND	[119]
Alphaviruses	3' UTR	Binding to and relocalisation of HuR	Altered stability, splicing and poly-adenylation of mRNAs	[25, 120]
HCV	3'UTR	Binding to and relocalisation of HuR	ND	[121]
DENV-1	NS5 protein	Binding to CD2BP2 DDX23**	Altered pre-mRNA splicing in vitro	[12]
VSV	M	Relocalisation of hnRNPH	ND	[122]

413 [a]ND: not determined, * component of the U2 snRNP, ** component of the U5 snRNP,
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Table 2. Transcriptome-wide analyses of AS in virus-infected cells.

	Rutkowski et al. 2015 [28]	Hu et al. 2016 [27]	Batra et al. 2016 [26]	Boudreault et al. 2016 [29]	Rivera-S et al. 2017 [24]	Sessions et al. 2013 [30]	De Maio et al. 2016 [12]	Hu et al. 2017 [31]	Fabozzi et al. 2018 [32]
Virus	HSV-1	HSV-1	HCMV	Reovirus	Reovirus	Dengue V	Dengue V	Zika V	Influenza V
Host-cell model[a]	Human Fibroblasts	Human Fibroblasts	Human Fibroblasts & NPC	Murine L929 cells	Murine L929 cells	Human HuH7 cells	Human A549 cells	Human NPC	Human BEAS-2B cells
Number of replicates	n=2	n=3 pooled	n=1	n=3	n>1 pooled	n=3	n=3	n=2	n=3
Sequencing library	ribo-depleted	polyA+	polyA+	polyA+	polyA+	polyA+	polyA+	polyA+	ribo-depleted
Sequencing platform	HiSeq 2500	HiSeq 2000	HiSeq	HiSeq 2000	NextSeq 500	HiSeq 2000/ GA II x	HiSeq 4000	NextSeq 500	HiSeq 2000
Reads features[a]	2 x 101 nt	2 x 90 nt	1 x 101 nt	2 x 100 nt	2 x 50 nt	2 x 75 nt	2 x 90 nt	2 x 75 nt	2 x 50 nt
Sequencing depth	> 25 x 10^6 reads	~ 26 x 10^6 reads*	~ 130-230 x 10^6 reads	> 40 x 10^6 reads	NA	~ 20-100 x 10^6 reads	> 20 x 10^6 reads	~ 7 x 10^6 reads	~ 30 x 10^6 reads
Fraction of viral reads[b]	27%	30%	22-68%	NA	NA	NA	30-40%	NA	~5-75%**
Mapping	Context Map	TopHat2	GSNAP	Bowtie2	TopHat	TopHat	TopHat2	TopHat2	TopHat2
Gene expression analysis	RPKM	Cufflinks	RPKM	RSEM	NA	Cufflinks	NA	Cufflinks	DESeq2
Alternative splicing analysis	Home-made scripts	Cufflinks ASD DaPars	Olego Quantas MISO	RSEM	MISO	Cufflinks MISO	ASpli edgeR	Cufflinks ASD	MISO
Data availability[b]	GSE59717	NA	GSE74250	GSE81017	NA	NA	GSE84285	GSE78711	GSE61517

[a] NPC: Neural Progenitor Cells
[b] nt: nucleotide
[c] NA: not available
* number of reads for the pooled samples, ** depending on the viral strain and time-point

Figure legends.

Figure 1. Virus-spliceosome protein-protein interaction network.

A. Summary of virus-spliceosome protein-protein interactions, as recorded in the VirHostNet database, January release 2018 [23]. For each viral family, the number of viral species (as defined by the International Committee on Taxonomy of Viruses and the National Center for Biological lnformation or NCBI) and viral proteins (as defined by the NCBI Reference Sequence and the UniProt databases) reported to interact with at least one cellular spliceosomal factor (among the list of 244 factors defined in [123]) is indicated. The number of viral proteins obtained upon merging of homologous proteins derived from different strains or isolates of a single viral species, grouped thereafter under the name "viral proteins types" and represented as a single node in B, are indicated into brackets.

B and C. Interaction network between viral proteins (B) or viral species (C) and cellular spliceosomal proteins. The network was built with Cytoscape (version 3.2.1). Nodes and edges between nodes represent protein and protein-protein interactions, respectively. Core and regulatory spliceosomal factors are represented by square and triangular black nodes, respectively. **B.** Viral proteins types, as defined in A, are represented by colored nodes (the color code is according to the Table in A). The size of the viral protein nodes is proportional to their degree of connectivity (i.e. the number of interacting partners of a protein) and the layout is done according to their centrality in the network. **C.** Viral species are represented by colored nodes (the color code is according to the Table in A). The size of the spliceosomal factor nodes is proportional to their degree of connectivity and the layout is done according to their centrality. The five spliceosomal factors showing the highest degree of connectivity (interaction with 11 to 15 distinct viral species) are indicated with a white star and edges representing their protein-protein interactions with viral species are highlighted in red.

Figure 2. Direct and indirect mechanisms for viral-induced splicing alterations.

Red arrows represent viral manipulation of the splicing machinery and the resulting AS changes, which have in turn the potential to modulate innate immunity [16, 95] or apoptosis [124]. Blue arrows represent viral-induced cell damages and innate immune responses and the subsequent AS changes in the infected cell [40-43]. Grey arrows represent viral-induced

457 B- and T-cell immune responses, which are subject to AS-mediated regulation [38-40]. UPR:
458 Unfolded Protein Response. DDR : DNA Damage Response.
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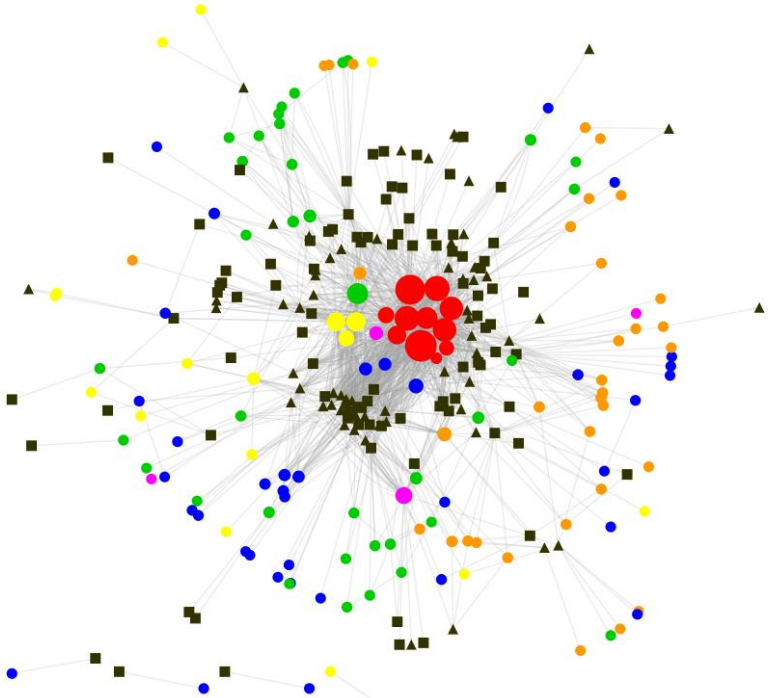
905

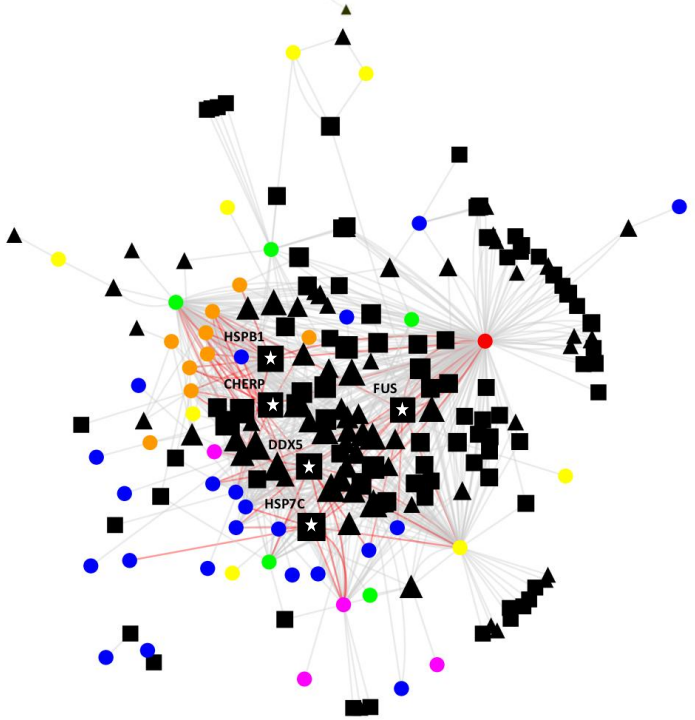
A

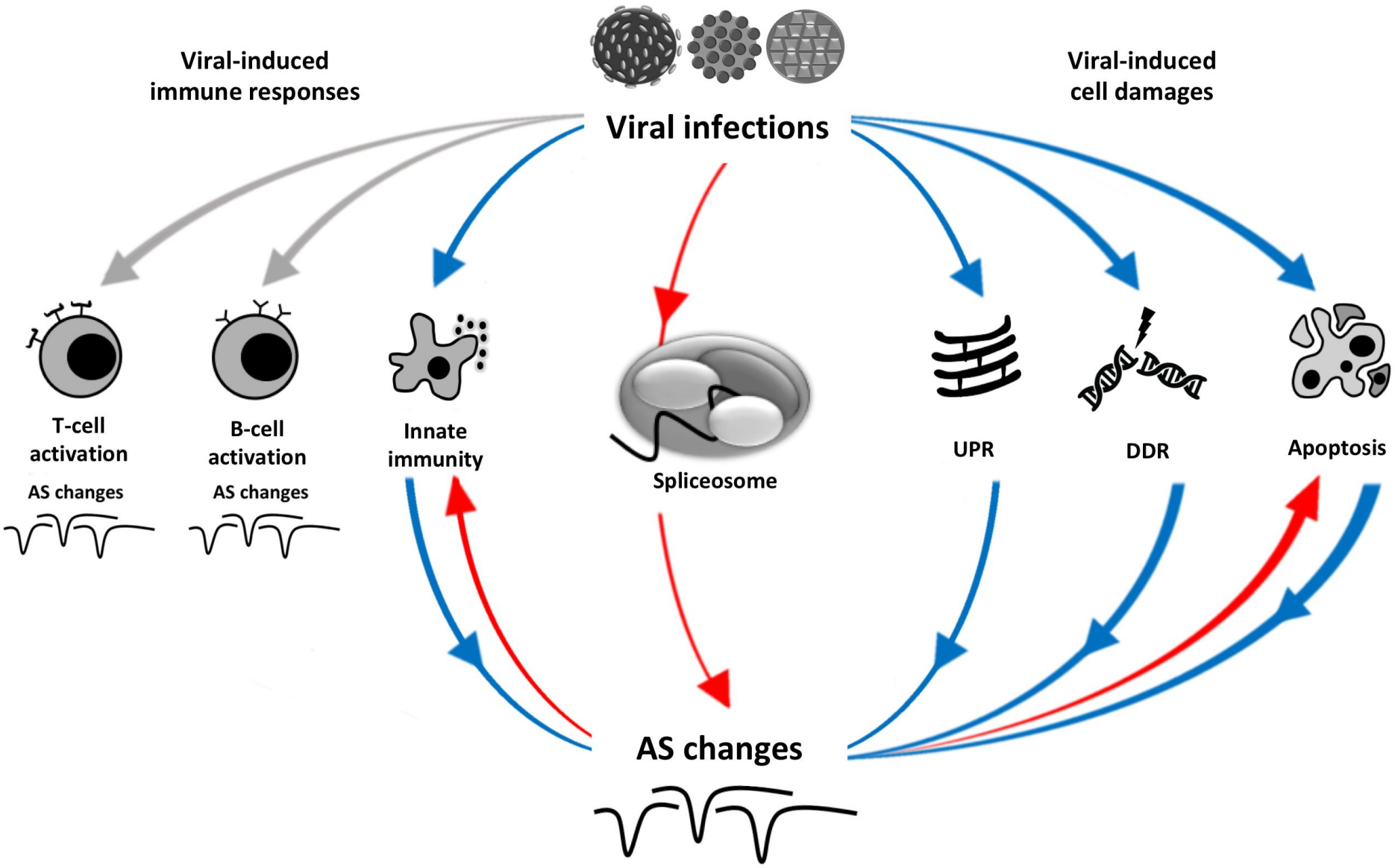
Viral family	Viral species	Viral proteins (non-redundant)
Papillomaviridae	**9**	**34(34)**
Retroviridae	**8**	**17(17)**
Herpesviridae	**5**	**49(36)**
Flaviviridae	**4**	**9(4)**
Togaviridae	3	3(3)
Paramyxoviridae	2	4(3)
Polyomaviridae	2	3(3)
Coronaviridae	2	2(2)
Parvoviridae	2	2(2)
Rhabdoviridae	2	2(2)
Orthomyxoviridae	**1**	**27(11)**
Poxviridae	1	13(12)
Filoviridae	1	2(2)
Pneumoviridae	1	2(2)
Adenoviridae	1	1(1)
Circoviridae	1	1(1)
Hepadnaviridae	1	1(1)
Peribunyaviridae	1	1(1)
Phenuiviridae	1	1(1)
Reoviridae	1	1(1)
Total	**49**	**175(139)**

B



C




Viral-induced
immune responses
Viral infections
Viral-induced
cell damages
T-cell
activation
AS changes
B-cell
activation
AS changes
Innate
immunity
Spliceosome
UPR
DDR
Apoptosis
AS changes