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Review Article

Proteomics methods for discovering viral–host interactions

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A R T I C L E I N F O

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The functions of many viral proteins involve direct interactions with specific host proteins. Therefore considerable insight into the functions of a viral protein and its mechanisms of action can come from applying proteomics approaches to viral proteins in order to identify their cellular binding partners. In this chapter we describe proteomics approaches that have proven to be the most useful in identifying host interactions of viral proteins in human cells. Caveats and potential alternatives for each step are also discussed.

1. Introduction

Identifying the cellular proteins with which a viral protein interacts is an extremely valuable approach for determining the function(s) or mechanism(s) of action of a viral protein. Most viral proteins manipulate a cellular process and/or use one or more cellular proteins to carry out their viral functions. In both cases, considerable insight into viral protein function can be revealed by using proteomics methods to identify host protein interactions. These methods are powerful because they can reveal interactions with proteins for which there were no previously known connections, and they allow for detection of cellular proteins for which there are no suitable antibodies. In addition, these methods provide an unbiased view of the relative frequency with which interactions occur. The types of viral–host interactions that can be revealed by these proteomics methods include (1) scenarios in which a viral protein sequesters a cellular protein, preventing it from performing its usual function, (2) interactions of a viral enzyme with a cellular substrate, thereby revealing targets of the viral catalytic activity, and (3) interactions of a viral protein with a cellular protein modifying enzyme, which can reflect regulation of the viral protein by that protein modification or a mechanism by which the viral protein directs the enzymatic activity of the cellular protein to alternative substrates. Viral–host interaction proteomics can also provide the first insights into functions of the bound cellular proteins. For example, it was due to their interactions with viral proteins that the cellular functions of p53, pRb, E6AP and USP7 were revealed. Therefore proteomics experiments for viral–host interactions both teach us about viral protein function and reveal the cellular proteins and pathways that are instrumental in viral infection. Here we will outline the most useful proteomics approaches for identifying viral–host protein interactions, concentrating on the experimental design. In particular, we will concentrate on affinity purification coupled with mass spectrometry (AP-MS) and tandem affinity purification (TAP-tagging) approaches, in which the viral protein is recovered by virtue of a specific antibody or affinity resin and co-purifying proteins are identified by mass spectrometry.

2. Expression methods

The first consideration in designing viral proteomics experiments is how to express the viral protein. In some instances it may be possible to do the proteomics experiment in the context of endogenous viral infection. However this depends on the availability of an antibody against the viral protein that is of high specificity and avidity. This antibody must be affinity purified in the absence of BSA or other protein additives, then coupled to resin prior to use (see Rowles et al. [1] for coupling procedures). Moreover, the purified antibody must not be in Tris buffer since coupling occurs through primary amines. The advantages of this approach are that it allows for isolation of the viral protein at endogenous levels and in the context of any stage of viral infection, and can also provide information on interactions with other viral proteins. The caveat of the specific antibody is that the epitope recognized by the antibody might also be a protein interaction site. In that case, the antibody would preferentially recover viral proteins that are not in that protein complex and protein interactions at the

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epitope site would be missed. This specific antibody approach was used successfully by Youn et al. [2] to identify viral protein interactions with the NS1 protein of West Nile virus.

Another way that protein interaction proteomics experiments can be performed in the context of viral infection is by generating a recombinant virus in which an affinity tag is fused to the viral protein of interest, and using this virus to infect cells. However, depending on the virus and the arrangement of the viral genes, it may not be possible to generate such a recombinant virus without disrupting expression of other viral proteins. For example HIV genomes contain overlapping open reading frames (ORFs), such that adding a tag to one of these ORFs may alter the proteins expressed, which may in turn affect viral infection. In addition, the properties of some viruses make generating recombinant viruses difficult. For example, the large genome size, tendency to remain latent and low infectivity of Epstein-Barr virus makes the generation of recombinant viruses a laborious process. If the virus is amenable to generating recombinants, then one first has to verify that the tagged protein retains its expected cellular localization and function. At that point viral and host interactions of the tagged viral protein can be readily determined at various stages of infection. The affinity tag would typically not interfere with protein interactions unless the interaction occurs with sequences very close to the tag. For that reason, ideally the same protein should be tagged on either the N- or C-terminus (further discussed in Section 3 below).

Expressing an affinity tagged viral protein on its own in the absence of viral infection is the most popular approach, since it can be applied to any viral protein and is less laborious than generating recombinant viruses. While this approach has been very useful for many viral proteins, it is important to keep in mind that some viral proteins may normally interact with other viral proteins to form functional complexes. In these cases, functional host interactions may only be revealed in the context of viral infection. Two methods are commonly used to express tagged viral proteins individually. The fastest and simplest method is to transiently express the viral protein by transfection of a mammalian expression plasmid. The main caveat of this method is that, due to the uptake of many plasmids per cell, protein expression levels tend to be considerably higher than would normally occur in viral infection, which could promote interactions that are not physically relevant. In addition, this method requires cells with high transfection efficiencies, prompting many investigators to use HEK293 or 293T cells [3–8] (due to their high transfectability even with the inexpensive calcium phosphate transfection method) over cell lines that are more physiologically relevant for the virus in question. Note that with the wide variety of transfection reagents currently available, many cell lines can now be transfected efficiently enough to be used for such experiments (albeit at higher cost), overcoming the need to use 293 cells. Despite these caveats, viral proteomics performed by transient transfection in 293 cells has led to discovery of a plethora of viral–host interactions which have been subsequently verified under more physiological conditions [3–5,7–12]. For example, Germain et al. [3] identified 426 interactions with HCV core and nonstructural proteins, 37 of which were shown to modulate HCV replication. Jager et al. [6] systematically identified interactions with all 18 HIV-1 proteins in both 293 and Jurkat T-cells and found that 40% of these interactions with 235 host proteins were conserved in both cell types. Proteomic studies on the host interactions of the EBV EBNA1 protein have also been performed in 293 cells in addition to gastric and nasopharyngeal carcinoma cell lines relevant for EBV infection [8,13]. Most of the interactions were found to be conserved in all cell lines including strong direct interactions with USP7 and CK2, which are hijacked by EBNA1 for multiple purposes including disruption of PML nuclear bodies and chromatin modifications at the EBV latent origin of replication [14–16].

Another common method for expressing single affinity-tagged proteins for proteomics is to integrate an inducible expression cassette for the affinity-tagged protein in the cellular genome. Most studies have used a tetracycline-regulated mammalian expression system (T-REx™ system by Life Technologies™), in which expression of the target gene is suppressed by a tetracycline repressor protein present in the cell line or provided on another plasmid, and then induced by the addition of tetracyclin to the medium [6,17]. For example, Jager et al. [6] used a T-REx Jurkatt cell line to individually express 18 HIV proteins and identify their host interactors. This method is somewhat more laborious than transient transfection due to the need to select for cells that have integrated the plasmid, and is often restricted to cell lines that have already been engineered to express the Tet repressor. However, it is advantageous in that expression levels of the target protein are typically lower than in transient transfection (due to the presence of only one target gene per cell) and can be adjusted to some degree by the amount of tetracyclin and induction time used.

Another way to express epitope-tagged viral proteins in cells is to use either a lentivirus or adenovirus delivery system. Both can be used to deliver expression cassettes at low copy number such that the target protein is expressed at levels considerably lower than transient transfection. Both lentiviruses and adenoviruses can infect a wide variety of cell types and cell lines, and therefore these systems are particularly useful for enabling proteomics experiments in cells that cannot be efficiently transfected. Commercial kits are available for generating recombinant replication-incompetent adenovirus and lentiviruses, including Virapower™ adenoviral and lentiviral expression system by Life Technologies. Since the lentivirus integrates, expression can be stably maintained for extended periods of time and, after selection for the integrated virus, virtually all cells are expected to express the delivered protein. For adenovirus delivery, the expression level of the target protein can be adjusted by varying the multiplicity of infection while maintaining efficient cell delivery. White et al. [18] used retrovirus delivery to stably express HA-tagged E7 proteins from various HPVs to compare how sequence changes affects their host interactions. Malik-Soni et al. [13] used an adenovirus system to deliver FLAG-tagged EBNA1 from EBV to several carcinoma cell lines including EBV-positive cells. This enabled EBNA1 proteomic experiments both in the presence and absence of EBV infection providing a less laborious method for studying viral–host interactions in the context of infection than generating a recombinant EBV. This method verified EBNA1-host interactions that were previously observed by transient transfection in 293 cells, and also identified a novel interaction of EBNA1 with nucleophosmin, which was subsequently shown to be important for the transcriptional activation function of EBNA1 [19].

3. Affinity purification methods

For proteomics experiments, the viral protein of interest must be recovered from the cell in which it is expressed in a manner that also recovers co-purifying proteins. As mentioned above, this could be done using an antibody specific to that viral protein if a high stringency antibody exists. However, in most cases the viral protein is fused to an affinity tag that allows for efficient recovery of the protein. The tag can be fused to either the N- or C-terminal end of the viral protein, but one should avoid fusion to an end that is known to mediate protein interactions since the tag could interfere with such interactions. Alternatively a protein bound very close to the tag could interfere with recovery of the tagged protein.