Going full circle: Validation of P-body dispersion in hepatitis C virus-infected patients

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Stability of biologically active molecules is a tightly regulated process that plays a key role in cellular homeostasis. This regulation is mediated by multiple mechanisms including the degradation of proteins by the proteasome or turnover of messenger RNAs by high-molecular-weight mRNA-protein complexes called Processing (P) bodies [1]. These are microscopically distinct aggregates of proteins and RNAs serving multiple purposes including, in addition to mRNA degradation, mRNA storage and microRNA (miRNA)-mediated suppression of mRNA translation. Degradation of mRNAs in P-bodies requires numerous enzymes such as deadenylases that remove the poly(A) tails at the 3′ end of the mRNA, the Dcp enzymes removing the cap-structure at the 5′ end and ribonucleases such as the exoribonuclease Xrn1, catalyzing nucleolytic degradation. This complex protein composition earned various names to these aggregates such as “Xrn1 foci”, “DCP-bodies”, or “GW-bodies”, but nowadays P-bodies is a widely accepted term referring to mRNA-protein aggregates involved in mRNA turnover. Assembly of P-bodies is a dynamic process that is linked to the translation status of the cell, requiring on the one hand the presence of some “core” protein components such as the GW182 scaffolding proteins and on the other hand the accumulation of a large pool of untranslated mRNAs in a cell. Thus, inhibition of mRNA translation by different stresses leads to P-body accumulation. Interestingly, P-body abundance is also influenced by the cell cycle with proliferating cells containing bigger and more abundant P-bodies than quiescent cells. P-bodies are also motile structures which can, under stress conditions, dock transiently to stress granules (SGs) and exchange some components. This might be involved in the degradation of transcripts stored in SGs.

Owing to their role in mRNA decay, P-bodies represent a potential threat to efficient replication of RNA viruses. Consequently, many RNA viruses such as West Nile virus, Poliovirus and Adenovirus redistribute, sequester or degrade components of P-bodies [2] whereas other viruses such as Hepatitis C virus (HCV) divert P-body components to increase viral replication. However, in spite of this inhibitory role, distinct components of P-bodies such as Xrn1, the mRNA decapping enhancer Hedls, the miRNA effector Argonaute 2 (Ago2), the RNA helicases DDX6 (also Rck/p54) and DDX3 as well as two factors involved in mRNA decay, PatL1 and Lsm1-7 were shown to increase HCV replication [3–8]. The underlying molecular mechanisms are poorly understood and only in few cases we begin to understand the mode of action. For instance, for Lsm1 it was shown that this factor enhances HCV RNA translation via the internal ribosome entry site in a microRNA-122- (miR-122) dependent manner [7,9]. In addition, it was shown that in HCV-infected cells Ago2 is recruited to lipid droplets, which are cellular lipids storage organelles playing a central role in both RNA replication and virus assembly [4,5,10]. Ago2 is an important host cell factor promoting HCV RNA translation and replication, presumably by forming a complex with miR-122 at the 5′ end of the HCV RNA genome (reviewed in [11]). For DDX3X, a presumed P-body component, it was found that this RNA helicase acts as a pattern recognition factor; it is recruited to lipid droplets and activated by HCV RNA [12]. This induces a signaling cascade finally leading to activation of sterol regulatory element–binding proteins and enhanced transcription of lipogenic genes. These examples illustrate that HCV recruits individual components of P-bodies to sites of viral RNA translation, replication and assembly to promote the viral replication cycle. It appears that these P-body components, rather than the structures themselves are exploited by HCV since ablation of P-body formation by depletion of Rap55, a translation repressor, neither affects HCV RNA translation nor particle production [13]. Moreover, the recruitment of individual P-body components to viral replication sites would easily explain why P-body composition in HCV-infected cells is altered and why their abundance is profoundly reduced [13,14].

While these studies provide conclusive evidence for P-body disruption in cultured cell lines, the in vivo relevance remained to be clarified and it was unclear whether P-body dispersion also occurs in HCV-infected humans. A major hurdle for such in vivo studies is the low abundance of HCV antigen and RNA in infected cells. Thus, highly sensitive methods are required, which are
however technically challenging and error-prone. Detection of viral antigens was first achieved by conventional immunohistochemistry or fluorescence microscopy ([15] and references cited therein). However, these methods were still limited by low sensitivity and high autofluorescence of liver tissue. An enormous improvement was the establishment of two-photon microscopy in combination with semiconductor quantum dot probes [16]. Although this technique overcomes several drawbacks of conventional fluorescence microscopy, it remains of restricted access due to equipment costs, is technically very challenging and clearly not adapted to a diagnostic setting. With respect to the detection of HCV RNA in infected tissues, various techniques have been developed such as in situ hybridization or in situ reverse-transcription polymerase chain reaction ([17] and references cited therein). However, the harsh staining conditions necessary for RNA detection do not allow simultaneous detection of viral antigen.

In this issue of the Journal of Hepatology, Perez-Vilaro et al. demonstrate that P-body disruption also occurs in hepatocytes of HCV-infected patients [18]. A total of 55 individuals were enrolled: 19 patients with chronic hepatitis C and biopsied before antiviral therapy, 10 healthy donors for living donor liver transplantation, 8 patients with chronic hepatitis B and 10 HCV- and HBV-negative liver transplant patients with inflammatory changes caused mainly by transplant rejection. The authors adapted an immunofluorescence technique described recently by this group [19] and applied it to paraffin-embedded liver sections. By staining two different P-body components, DDX6 and Dcp1 as well as HCV core, the authors observed that the decrease in P-body abundance was independent of HCV genotype and not influenced by the grade of liver inflammation in chronically HCV-infected patients. P-body dispersion was specific to HCV since it was not detectable in livers from patients chronically infected with HBV or with non-viral inflammation. Moreover, this decrease was reversible upon antiviral therapy and the amount of P-bodies reached levels comparable with healthy donors once sustained virological response and HCV clearance was achieved. While these results validated what was previously observed in hepatoma cell lines, three-dimensional reconstruction of confocal images revealed an intriguing difference: as expected, HCV core was found to localize around lipid droplets, but in contrast to previous reports, DDX6 did not [4]. This observation raises the question about the real localization of P-body components involved in HCV replication in vivo.

The study by Perez-Vilaro and co-workers provides the missing in vivo demonstration that P-body composition and abundance is affected by HCV infection, thus supporting previous observations obtained in human hepatoma cell lines [13,14] (Fig. 1). Importantly, the collection of human liver biopsies used in this study is comprehensive and the diversity of analyzed patient samples allowed a comprehensive comparison of P-body properties induced in different phases of HCV infection: acute, chronic or after therapeutic clearance. Importantly, by using liver sections obtained after liver transplantation and graft re-infection, the authors could study P-body properties in acute HCV infection, which is a situation rarely monitored in the clinic.
Editorial

Altogether the work by Perez-Vilaro and colleagues highlights the importance of the in vivo validation of observations made in cultured cell lines. The discrepancy with respect to recruitment of DDX6 to lipid droplets calls into question the physiological relevance of some of the in vitro observations. Unfortunately, in the absence of broadly available primary cell culture systems and owing to low replication of HCV especially in interferon-competent cell systems, we have to rely, to the most part, on (interferon-incompetent) immortalized or tumor-derived cell lines. This hurdle might be overcome, at least in part, by using methods as reported by Perez-Vilaro and co-workers that allow visualization of HCV in infected tissues. Along these lines, we note that a highly sensitive in situ hybridization method was recently established, which allows, at the single cell level, the detection of HCV RNA together with mRNAs of interferon-induced genes in human liver biopsies [20]. It is tempting to speculate that in the near future, the combination of this method with the fluorescence technique reported by Perez-Vilaro and co-workers will become available to enable the simultaneous detection of both HCV RNA and viral proteins or host cell factors at the single cell level in human liver tissues.

Conflict of interest

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References