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Commentary & View

The peculiar interaction between mammalian prion protein and RNA

Mariana P.B. Gomes,[1] Yraima Cordeiro[2] and Jerson L. Silva[1,*]

[1]Programa de Biologia Estrutural; Instituto de Bioquímica Médica; [2]Faculdade de Farmácia; Universidade Federal do Rio de Janeiro; Rio de Janeiro Brazil

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In the past decade, the interaction between prions and nucleic acids has garnered significant attention from the scientific community. For many years, the participation of RNA and/or DNA in prion pathology has been largely ruled out by the "protein-only" hypothesis, but this is now being reconsidered. Experimental data now indicate that nucleic acids (particularly RNA), besides being carriers of genetic information, function as important key components during development, physiological responsiveness and cellular signaling. This revelation has brought a new perspective to prion pathology. Here we discuss the role of RNA molecules in prion protein aggregation and the resulting cellular toxicity. We combine our most recent findings with existing literature to shed new light on this exciting field of research.

The "protein-only" hypothesis postulates that PrP^{Sc} 'multiplies' by catalyzing the conversion of PrP^{C} into a molecule resembling itself, thereby leading to its own propagation.[1,2] This theory has excluded the participation of nucleic acids in prion propagation. However, the suggestion that an additional unknown factor could influence the PrP^{C} to PrP^{Sc} conversion has brought back the possibility of nucleic acid involvement in prion diseases, where evidence for this has been accumulating over the past decade.[3-8] Instead of encoding genetic information, nucleic acid molecules would act by lowering the free energy barrier between PrP^{C} and PrP^{Sc} and, consequently, triggering conversion.[9,10]

In the last decade, our group has studied the interaction between prion proteins and nucleic acids. We have demonstrated that PrP interacts with nucleic acids in vitro, binds small sequences of double stranded DNA, acquires β-sheet secondary structure according to spectroscopic measurements, and presents some PrP^{Sc}-like characteristics.[4,11] Studies on the interaction of prion protein with RNA molecules have demonstrated that PrP can acquire protease resistance upon RNA binding, and that scrapie and cellular PrP isoforms bind with different affinities to some highly structured RNA sequences.[12-15]

In our latest report, we presented experimental data on the interaction between murine recombinant prion protein (rPrP23-231) and RNA molecules.[16] We used total prokaryotic and eukaryotic RNA extracts from cultured cells along with small synthetic oligonucleotides and looked for changes in the secondary and tertiary structures of PrP. We also used two mutants lacking the N-terminal domain to demonstrate the importance for RNA binding, and evaluated the toxicity of the PrP:RNA complex in cultured mouse neuroblastoma cells (N2a).[17]

Our findings show that the heterogeneous mixture of RNA extracted from neuroblastoma cells was the only sample capable of triggering toxicity and massive aggregation. rPrP23-231 loses most of its secondary structure and immediately aggregates upon interaction with RNA. Interaction with small RNA sequences also leads to changes in the size of the complex and some aggregation, but the oligomeric species did not lead to toxicity. NMR investigations of this complex revealed that full length PrP partially recovers its native fold 72 h after RNA addition, and that the changes in the HSQC spectrum suggest that RNA binding causes some subtle changes in PrP structure. RNA does not induce aggregation of the PrP N-terminal deletion mutants, indicating that the N-terminal region is important for this process. We also observed that the rPrP23-231:N2aRNA complex protects both protein and RNA from degradation with proteinase K and Rnase A, respectively.

Moreover, we have seen that the PrP interaction with DNA, though identical in some respects, displays substantial differences from the interaction with RNA, and we try to correlate these differences with a possible physiological role for these binding events. We summarize the findings obtained when the prion protein binds DNA or RNA in Figure 1. The heterogeneous mixture of RNA extracted from neuroblastoma cells was the only sample that triggered toxicity and massive aggregation. The small RNAs were able to bind PrP but formed only small, non-toxic oligomeric species.

The component of the N2a RNA extract that facilitates aggregation and induces toxicity remains unidentified. In additional, several other questions remain unanswered. How do PrP:RNA aggregates exercise their toxicity? Is there a cellular receptor for prion aggregates? Is the observed cellular death caused by apoptosis or necrosis? Is it possible that a non-self RNA could produce toxic aggregates in other cell types?

All of these questions are becoming more relevant with the emerging evidence that RNAs can participate as regulatory molecules.[18] The physicochemical properties of RNA allow it to participate in a diverse range of structural and catalytic roles through various mechanisms, including cell-to-cell communication.[19]

*Correspondence to: Jerson L. Silva; Universidade Federal do Rio de Janeiro; Instituto de Bioquimica Medica; IBqM–Centro de Ciencias da Saude Sala E-10; Rio de Janeiro RJ 21941-590 Brazil; Email: jerson@bioqmed.ufrj.br

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Studies in plants first have shown that the phenomenon of co-suppression in response to transgene expression is mediated by RNA signaling and that this effect, which involves the RNA interference (RNAi) pathway, can be transmitted throughout the entire plant.[8,20-25] There is also evidence for systemic RNA signaling in animals. The discovery of RNAi in *Caenorhabditis elegans* showed that RNA signals can be transmitted both from the environment (via ingestion) and systemically throughout the organism.[26]

RNA molecules can act as molecular adaptors to connect incoming analog signals to sequence-specific outcomes.[18] This is exemplified by 'riboswitches,' which are mRNA elements that alter their structure in response to ligands, enabling them to sense and react to environmental parameters.[27,28] This capacity enables the creation of new classes of RNA signaling molecules that vary in their target specificity but utilize a common infrastructure and output, thereby providing latitude to explore new connections in RNA-based regulatory networks.[29,30] These findings place the PrP:RNA interaction in a new context since RNA is no longer confined within the cell.

Since both aggregation and nucleic acid binding occur with decreased hydration, a bypass of the unfolded state can arise in the conversion of PrPc into a PrPSc-like structure.[11,31] The interconversion might relate to an additional function of the PrP, such as a nucleic acid chaperone.[8] One of the main characteristics of a nucleic acid chaperone is the occurrence of mutual coupled rearrangements.[32] Nucleic-acid binding of the prion protein appears with the ordering and structural modification of the N-domain.[33] Thus, PrP might exert its function by participating as a NA chaperone in the gene regulation array at the post-transcriptional level, especially because of its capacity to bind both small RNAs and DNAs. In fact, PrP has been shown to interfere with the synthesis of HIV-1 proteins, implying an interaction with retroviral RNA.[34] The possibility of a functional role for PrP in nucleic acid processing has recently been corroborated by the demonstration that PrP participates in the control of activated endogenous retroviruses.[35] In the years ahead, we expect substantial progress in the breathtaking field of prion-RNA interactions.

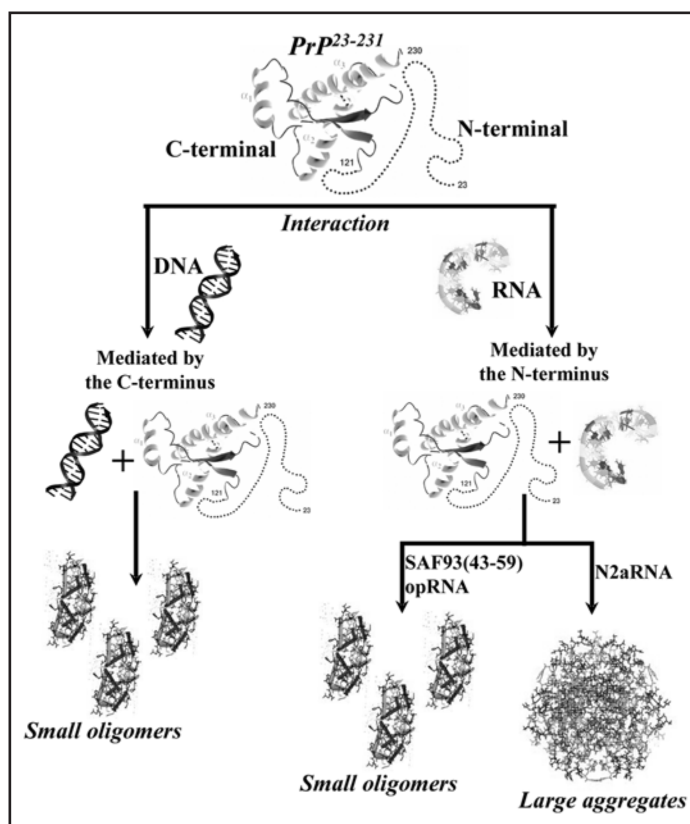

Figure 1. Diagram summarizing the findings obtained when rPrP binds either DNA or RNA. While rPrP binds DNA mainly through the C-terminal domain (left), it binds RNA through its N-terminus (right). Small RNAs (SAF93[43-59] and opRNA) bind to PrP but only form small, non-toxic oligomeric species in contrast to N2aRNA binding, which forms large oligomers.

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