Present and not reporting for duty: dsRNAi in mammalian cells

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Double-stranded RNA interference (dsRNAi) represents a primary means of anti-viral defense in plants, worms, and insects, yet appears mostly supplanted by the protein-based interferon (IFN) response in vertebrates such as mammals. The degree to which dsRNAi is anti-viral in mammals has been contentious. Maillard *et al* (2016) find that dsRNAi retains sequence-specific silencing in mammalian cells incapable of triggering an IFN response, suggesting that dsRNAi is inhibited by the action of interferon-stimulated genes. Importantly, they observe that while dsRNA can “vaccinate” against the incoming cognate virus though dsRNAi silencing, no dsRNAi is observed with viral infection alone, suggesting that this evolutionarily conserved anti-viral pathway is present but functionally elusive in the cell types studied thus far.

See also: **PV Maillard *et al*** (December 2016)

RNA interference (RNAi) is a mechanism conserved in most eukaryotes that enables sequence-specific gene silencing via the use of small RNAs 22–30 nt in length. As an anti-viral defense system, RNAi operates by sensing and fragmenting viral dsRNA through the action of a Dicer RNase. The resulting small interfering RNAs (siRNAs) then guide Argonaute (Ago) effector proteins in an RNA-induced silencing complex (RISC) to cleave target RNA sequences bearing siRNA complementarity. This core system to sense and process dsRNA via Dicer into siRNAs that guide Agos is the hallmark of dsRNAi and is distinct but related to the role of these proteins in gene regulation via microRNAs

(miRNAs) (tenOever, 2016). In plants and invertebrates, dsRNAi plays a critical role in suppressing viral accumulation and provides a systemic immune response (tenOever, 2016). Attesting to the power of this nucleic acid-based immune system, numerous plants and invertebrate viruses have evolved viral suppressors of RNAi (VSRs) that are only dispensable in RNAi-defective hosts (tenOever, 2016).

While mammalian cells retain Dicer and Agos to regulate gene expression via miRNAs, it has been difficult to demonstrate a role for these proteins in executing a dsRNAi response against an RNA virus. This is in large part due to the IFN system, which has evolutionarily co-opted dsRNA sensing to induce a constellation of IFN stimulated genes (ISGs) to control infection (Schneider *et al*, 2014; tenOever, 2016). Indeed, genetic ablation of IFN components renders cells and animals critically susceptible to numerous viruses, despite presumably having intact RNAi. As Dicer and Ago retain numerous critical non-immune related functions via miRNAs, the reverse experiment has been less feasible; however, cell lines lacking Dicer have so far demonstrated that many RNA viruses are refractory to inhibition by endogenous miRNA or siRNAs, should the latter even be present (Bogerd *et al*, 2014). Indeed, studies have failed to discern appreciable levels of viral-derived siRNAs (Parameswaran *et al*, 2010), except in artificial instances where siRNA sites have been engineered into viral genomes by co-opting endogenous miRNAs (Barnes *et al*, 2008; Perez *et al*, 2009).

And yet, exceptions have emerged. In 2013, two reports provided the most

compelling but not conclusive evidence for anti-viral RNAi in mammalian cells by showcasing how embryonic stem cells (ESCs), lacking a developed IFN response, clearly produce siRNAs during encephalomyocarditis virus (EMCV), or Nodamura virus (NoV) infections (Li *et al*, 2013; Maillard *et al*, 2013). Moreover, NoV mutants lacking the B2 protein markedly accumulated viral siRNAs, even in differentiated cells, suggesting that VSR activities may be important in mammalian cells (Li *et al*, 2013; Maillard *et al*, 2013). These findings argue that cellular context plays a major role in determining the anti-viral efficacy of dsRNAi that may be masked by VSR. Compounded by the breadth of the IFN response, the myriad of RNA viruses and cell types studied has made it difficult to discern the general utility, or absence thereof, for dsRNAi in mammalian cells.

In this issue of *The EMBO Journal*, Maillard *et al* (2016) refreshingly simplify the problem and investigate whether the IFN response may mask anti-viral RNAi in somatic cells. As a means of removing virus-specific variables, the authors use dsRNA to silence coding reporters bearing complementary sequence. Notably, cells lacking the mitochondrial anti-viral signaling protein (MAVS), a key player in dsRNA sensing pathways that trigger downstream IFN responses, remain capable of inducing sequence-specific dsRNAi, whereas this effect is not seen in IFN-responsive cells. Sequence-specific dsRNAi was also observed in cells lacking the IFN receptor, where dsRNA sensing and IFN production are preserved but where no ISG induction can occur. Thus, it is likely that the action of one or more ISGs inhibit dsRNAi. This would be

Figure 1. A web of inhibition for anti-viral dsRNAi.

Numerous lines of evidence document the interplay between the RNAi pathway, the IFN response, and viral dsRNA as mutually inhibitory. Maillard *et al* (2016) propose that anti-viral roles for RNAi may be masked by interferon-stimulated genes (red line).

consistent with previous findings showing that triggering an anti-viral response using dsRNA-like agonists inhibits RISC activity via poly-ADP ribosylation (Seo *et al*, 2013). Overall, these results point to a multi-faceted inhibition of dsRNAi by the interferon response that may be further compounded by VSR activity (see Fig 1). How dsRNAi inhibition works in various contexts remains to be seen, though ISG inhibition is likely to act on Dicer, as RNAi from exogenous siRNAs was not affected by the presence or absence of the IFN response.

In addition to demonstrating that unmasked dsRNAi is Dicer and Ago dependent, Maillard *et al* (2016) also observe that dsRNAi can productively inhibit virus in MAVS deficient cells. By introducing dsRNA of a reporter virus into cells prior to infection, the authors observe a decrease in subsequent virus replication. Such a “vaccination” response suggests that dsRNAi can provide sequence-specific protection from incoming virus, and yet, no anti-viral dsRNAi was observed with infection alone. This failure to achieve anti-viral dsRNAi indicates that dsRNA sensing of viral replication intermediates do not feed into Dicer processing. This might be due to several virus-initiated possibilities: from previously mentioned VSR activity to replication schemes that shield dsRNA intermediates in compartments inaccessible for sensing.

Perhaps the most pressing question centers on cell determinants that favor anti-viral RNAi over the IFN response. RNAi is active in ESCs and potentially other types of undifferentiated cells, in which the IFN response is severely attenuated (Burke *et al*, 1978), similar to the cells lacking the IFN receptor used by the authors. Human ESCs display reduced expression of genes involved in the dsRNA response pathways, including pathogen recognition receptors (PRRs) that lead to IFN induction such as OAS1, PKR, MDA5, TLR3, and others. Some proteins such as PKR and RIG-I are expressed in ESCs, but fail to respond to dsRNA (Chen *et al*, 2010). As ESCs display attenuated cytoplasmic dsRNA sensing, similar to the cells lacking MAVS, this may suggest that anti-viral RNAi may only happen naturally in this context.

Why might dsRNAi be preferred over IFN in undifferentiated cells? There are several interesting possibilities: (i) pluripotent cells undergo rapid cell division and may mute the IFN response to avoid its antiproliferative effects (Hertzog *et al*, 1994); (ii) interferon has been shown to stimulate differentiation, suggesting that pluripotent cells may inhibit its expression as a means of maintaining potency (Hertzog *et al*, 1994); (iii) triggers of the IFN response (i.e., cytoplasmic dsRNA) are readily produced in pluripotent cells (Tam *et al*, 2008) and the suppression of the IFN response prevents terminal sacrifice of the lineage; (iv) it might be possible that RNAi serves as a more efficient defense against transposons than the IFN response; (v) finally, previous studies have shown that components of the endogenous small RNA processing machinery may have important functions in the maintenance of stem cell properties (Qi *et al*, 2009). Clearly, future studies are in store for this present but mostly silent RNA-based immune system.

Acknowledgements

The authors' work on innate immunity and small RNAs is supported by NIH grants R01 AI091707 and R01 AI116943. We apologize to colleagues whose work was not referenced due to space constraints.

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