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Viperin—taken down with a pinch of salt

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What to eat when you have a cold has always been the subject of much debate and advice, usually informed by very little science. However, in this issue of *EMBO Reports*, Yuan *et al* (2021) uncover an intriguing link between a high salt diet and a susceptibility to viral infection. Mice fed on a short-term high salt diet were found to carry a higher viral load than control mice fed a normal diet. The researchers trace this effect back to a salt-induced decrease in cellular levels of the antiviral protein, viperin. More generally, these studies provide further insights into the regulation of proteins involved in the cellular antiviral response.

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See also: **Y Yuan *et al*** (January 2022)

Viperin (**v**irus-**i**nhibitory **p**rotein, **e**ndoplasmic **r**eticulum-associated, **i**nterferon-**i**nducible) is an interferon-stimulated gene that is highly upregulated in response to viral infection and has been shown to restrict the replication of a broad range of viruses (Helbig & Beard, 2014). Since its discovery (Chin & Cresswell, 2001), viperin has puzzled enzymologists, immunologists, and virologists alike. Only recently have some of the puzzle pieces begun to fall into place as the biochemistry underpinning viperin's antiviral effects has begun to be understood, and a greater appreciation of viperin's multifaceted role in the innate immune response has immerged.

The puzzle initially raised upon viperin's discovery was: What reaction did it catalyze? Its sequence showed it to be a member of the radical *S*-adenosyl-L-methionine (SAM) superfamily, but at the time radical SAM enzymes were generally thought to be confined to microbes. Radical SAM enzymes are very oxygen sensitive and catalyze a truly remarkably array of challenging chemical reactions involved in vitamin and natural products biosynthesis, and the fermentation of a wide range of carbon sources (Broderik *et al*, 2014). However, none of these kinds of chemical reactions occur in animals.

This puzzle was only solved in 2018, when it was shown that viperin synthesizes the antiviral nucleotide 3′-deoxy-3′,4′-didehydrocytidine triphosphate (ddhCTP) from CTP through a radical mechanism with SAM as a co-substrate (Gizzi *et al*, 2018). ddhCTP acts as a chain terminating nucleotide when misincorporated by viral RNA-dependent RNA polymerases (Gizzi *et al*, 2018). Viperin-like enzymes are now known to occur in all kingdoms of life and the synthesis of ddhNTPs appears to be an ancient and universal defense against viruses (Bernheim *et al*, 2021).

However, the solution to this first puzzle creates a second. ddhCTP is only effective against RNA viruses, and even then, it that appears not all viral RNA-dependent RNA polymerases are sensitive to it. But viperin is known to restrict replication of a broad range of viruses, including DNA viruses and retroviruses. This puzzle has partly been solved through extensive work from multiple laboratories which reveals that in higher animals viperin has become centrally integrated into the broader cellular innate immune response through a network of protein–protein interactions (Ghosh & Marsh, 2020). Indeed, the number of proteins reported to bind viperin is remarkably large. Broadly, they may be divided into three groups: cellular proteins involved in innate immune signaling; proteins involved in cellular metabolic pathways that are exploited during the viral life cycle; and structural and nonstructural viral proteins (Ghosh & Marsh, 2020).

How viperin, a compact, single domain protein, is able to interact with so many other proteins to modulate their activity remains largely mysterious: in some cases, proteins are activated and in other cases inhibited by binding to viperin. In many cases, but not all, viperin accelerates the proteasomal degradation of its targets. The prevailing hypothesis is that viperin promotes ubiquitin-dependent degradation of target proteins by recruiting an (as yet undefined) E3 ubiquitin ligase to polyubiquitinate the target, thereby marking it for degradation. This hypothesis chimes with the recent observation that viperin stimulates Lys63-linked auto-ubiquitination of the E3 ubiquitin ligase, TRAF6 (Patel & Marsh, 2021).

This brings us to the third puzzle surrounding viperin: How is it regulated? It is this question that the authors of the current paper have concerned themselves with. As its name implies, viperin expression is strongly induced by interferons, although it can also be directly induced by viruses. However, in a previous study the same researchers found that viperin expression differed markedly between tissues, and moreover that protein levels did not correlate well with mRNA levels (Yuan *et al*, 2020). This led to the discovery of a pathway by which viperin is proteolytically degraded. The pathway involves the acetyltransferase, HAT1, which is itself upregulated by interferons and viruses, and which specifically acetylates Lys197 on viperin. This modification renders viperin a substrate for the ubiquitin ligase UBE4A, which polyubiquitinates viperin on Lys206 and thus marks viperin for proteasomal degradation (Fig 1A).

The authors have refined this mechanism in their current paper by identifying a deubiquitinase, ubiquitin-specific protease 33 (USP33), that removes ubiquitin from viperin, thereby counteracting the effect of UBE4A (Yuan *et al*, 2021). This sets up a proteostatic loop that regulates the cellular level of viperin (Fig 1A). Salt, however, turns out to perturb this delicate balance by causing USP33 itself

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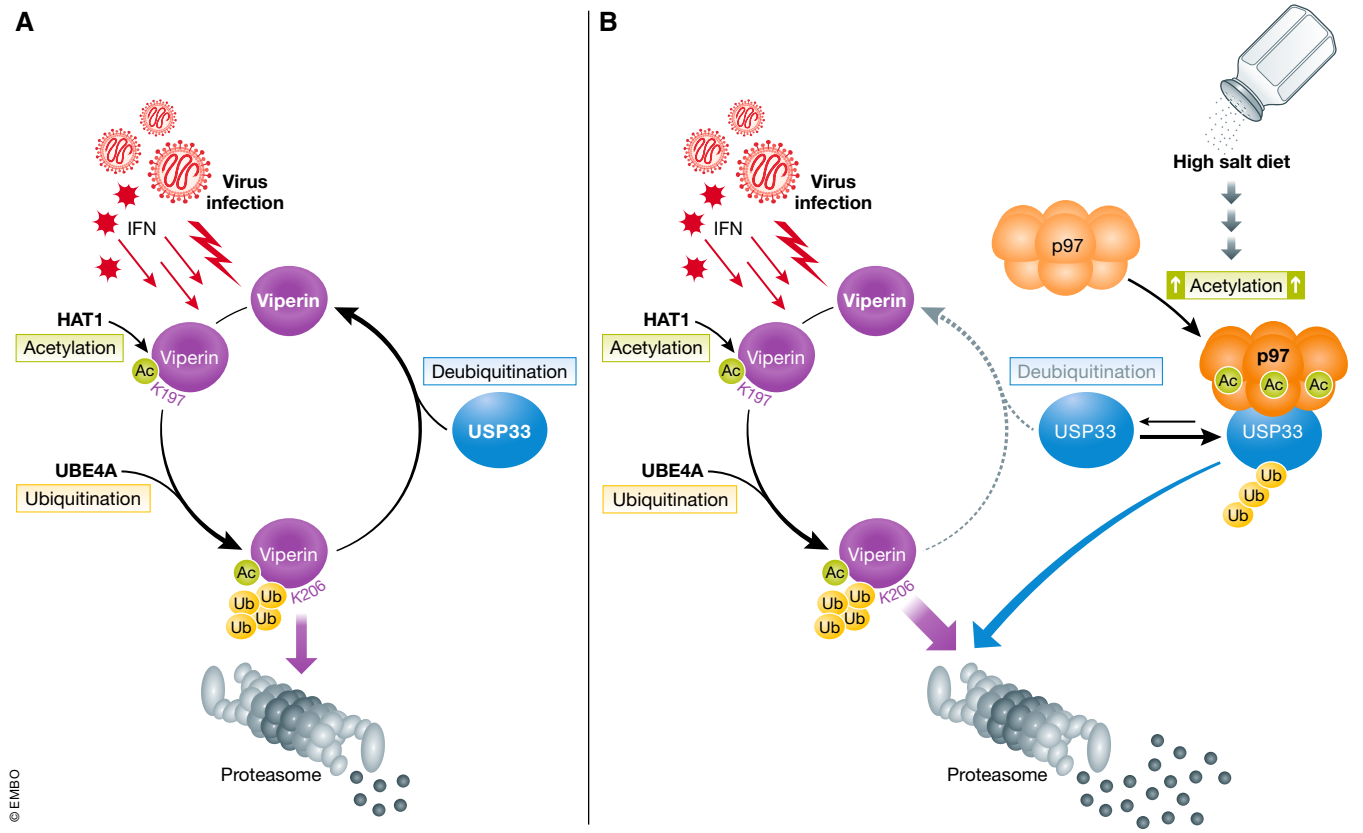

Figure 1. The regulation of viperin levels is disrupted by a high salt diet.

(A) Viperin expression is induced by interferons and its cellular levels regulated by a proteostatic loop involving the ubiquitin ligase, UBE4A and the deubiquitinase USP33. (B) Salt perturbs viperin regulation by indirectly activating the protein remodeling chaperone, p97, through acetylation. p97 recognizes ubiquitinated forms of USP33 and accelerates their proteasomal degradation.

to be degraded, thereby leaving UBE4A unchecked. High salt does not act directly on USP33, rather salt induces the activation of p97, also known as valosin-containing protein (Meyer *et al*, 2012), which is activated by acetylation on Lys663 (Fig 1B). The question of how p97 acetylation is linked to salt concentration is not addressed by this study.

p97 is an ATP-dependent chaperone that plays an important and wide-ranging role in regulating proteasome-mediated degradation by recognizing ubiquitinated proteins and remodeling them to facilitate their degradation (Meyer *et al*, 2012). The authors propose that by more efficiently clearing ubiquitinated USP33 from the cell, p97 accelerates to the degradation of viperin, leading, in turn, to the higher viral titers observed in mice fed a high salt diet (Yuan *et al*, 2021).

How this interesting study, conducted using mice and cultured cell lines, may play out in approaches for treating human viral infections remains to be seen. In the meantime, salty foods may be one more enjoyment to forgo during the current viral pandemic.

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