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> ONE MONTH, ONE PROTEIN <

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on guard

Vivienne Baillie Gerritsen

Picture a sentry watching out for the enemy from a castle turret. Suddenly, in a growing cloud of dust, a cavalcade of horsemen emerges, moving towards him fast. Seeing the enemy is one thing, but if our sentry does not act upon it, there is a fair chance the horsemen will enter the castle grounds before the Queen has been notified. So the watchman grabs his bugle and blows as hard as he can, setting off an alarm that prompts his fellow soldiers to prepare all the means of defence they have – cannons, crossbows, longbows, arrows, catapults, swords… you name it. This is very similar to what happens when a virus infects a cell. Molecular sentries sense the invader, causing them to set off an alarm which sparks off a myriad of metabolic pathways that will ultimately ward off the enemy. All these events are an integral part of our innate immune system. One of its most important bugles – both in number and in effect – is a protein known as ISG15, whose call is not only surprisingly varied but central to our immune response.

Fire in the Sauerkraut Factory

Arthur Garfield Dove (1880-1946)

The immune response is a singularly intricate and complex process. If it were to be painted, it would be one of the most colourful and majestic fireworks you have ever seen – and the more complex the organism, the greater the firework. Take humans. When a virus finds its way into our cells, one of our body's first lines of defence – of which there are many – is known as the innate immune response. This is triggered off by molecules within our cells that are able to rapidly sense infection, like the genetic material a virus injects for example. As a result, immune cells capable of ingesting and destroying the invading pathogen are ushered to the site of infection. Small signalling proteins known as cytokines are also rapidly called into action to spark off a diversity of fine-tuned metabolic pathways, which all have one aim: kill the enemy. Cytokines also happen to be what cause the classical symptoms of fever and muscle ache we are all familiar with when struck down by the flu.

Interferons (IFNs) are among the first critical cytokines to be summoned when a virus infects a cell. Not only do they stimulate immune cells, but they also tell neighbouring cells that it is time to jack up their anti-viral defences. IFNs are also responsible for activating the expression of ISG15, otherwise known as Interferon-Stimulated Gene Product 15. ISG15 is central to the innate immune response – so central, in fact, that it is one of the most solicited proteins involved in viral pathogenesis. Why is it central? Because – like a match lighting the mesh of a firework display – it sparks off a wide array of cellular pathways. Each pathway is part of the overall immune response and fights off a pathogen in numerous ways: by interfering with the machinery viruses hijack in our own cells to replicate, by silencing the genetic material viruses inject into our cells so that they cannot synthesize the proteins they need, by hindering viral protein oligomerization which impedes viral growth,

by interfering with viral protein function, by degrading viral proteins, by stopping nascent virions from budding… There seems to be no end to our immune system's determination to curb infection – and yet, sometimes, as we all know, it fails.

ISG15 is what is known as a ubiquitin-like protein. This is because it is similar, in structure and in function, to ubiquitins. Ubiquitins are proteins that bind – or conjugate – to other proteins to change their function or fate. They do this via a peptide bond; more specifically between a C-terminal glycine on ubiquitin and, more often than not, a lysine residue on the target protein. This reaction requires the assistance of three conjugation enzymes: E1, E2 and E3. Besides similar structural folds, ubiquitin-like proteins share this enzymatic conjugation feature with ubiquitins. It is the act of conjugation – called ISGylation – that enables ISG15 to crosstalk with many other proteins, each of which is involved in a different part of the immune response. This said, ISG15 also has many immunological roles in its unconjugated form – both inside and outside the cell – where it can initiate the production of numerous antimicrobial factors, not to mention IFN itself! In particular, ISG15 may also be called in to downregulate IFN signalling – which may sound counterproductive, but this would avoid excessive IFN signalling that can cause autoinflammatory pathogenesis.

The structural feature ISG15 shares with ubiquitins are two domains known as beta-grasp folds, each of which contain four beta-sheets huddled around one alpha-helix. The folds connect via a small polypeptide sequence, or hinge, which limits the way the protein can twist and move. The C-terminal of the protein ends with a short sequence – LRLRGG – essential for protein conjugation. Besides the beta-grasp fold, it is the three-enzyme mechanism required for ISGylation that mirrors ubiquitination. E1 activating enzyme, E2 conjugating enzyme and E3 ligase enzyme work together to bond the C-terminal glycine on ISG15 to one lysine on any of ISG15's many target proteins. It is once this bond has been made that as many pathways, each involved in the overall immune response, will be set into motion. The net result being a reduction of viral replication.

Inside a cell, ISG15 is able to fight off viral infection on the spot as well as set off anti-viral signalling pathways. Outside the cell, ISG15 interacts directly with viruses while activating immune cells to destroy them. It is hardly surprising, then, that viruses have found strategies to impede the action of a protein as critical as ISG15. These fall into two categories: 1) preventing or reversing ISGylation, and 2) sequestering ISGylated proteins. In both cases, immunity is drastically weakened. ISG15 is activated by a range of viruses, among which the now infamous SARS-CoV-2 coronavirus. Could ISG15 have potential for therapeutic use? Perhaps. Time will tell. Its diversity – both in sequence and in structure – do not make it an easy molecule to deal with. Like so many other proteins, it seems that the more scientists understand, the more there remains to be understood.

Cross-references to UniProt

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