

A plant virus hijacks the cellular degradation pathway to knock-down the host defense

Veronique Ziegler-Graff & Pascal Genschik

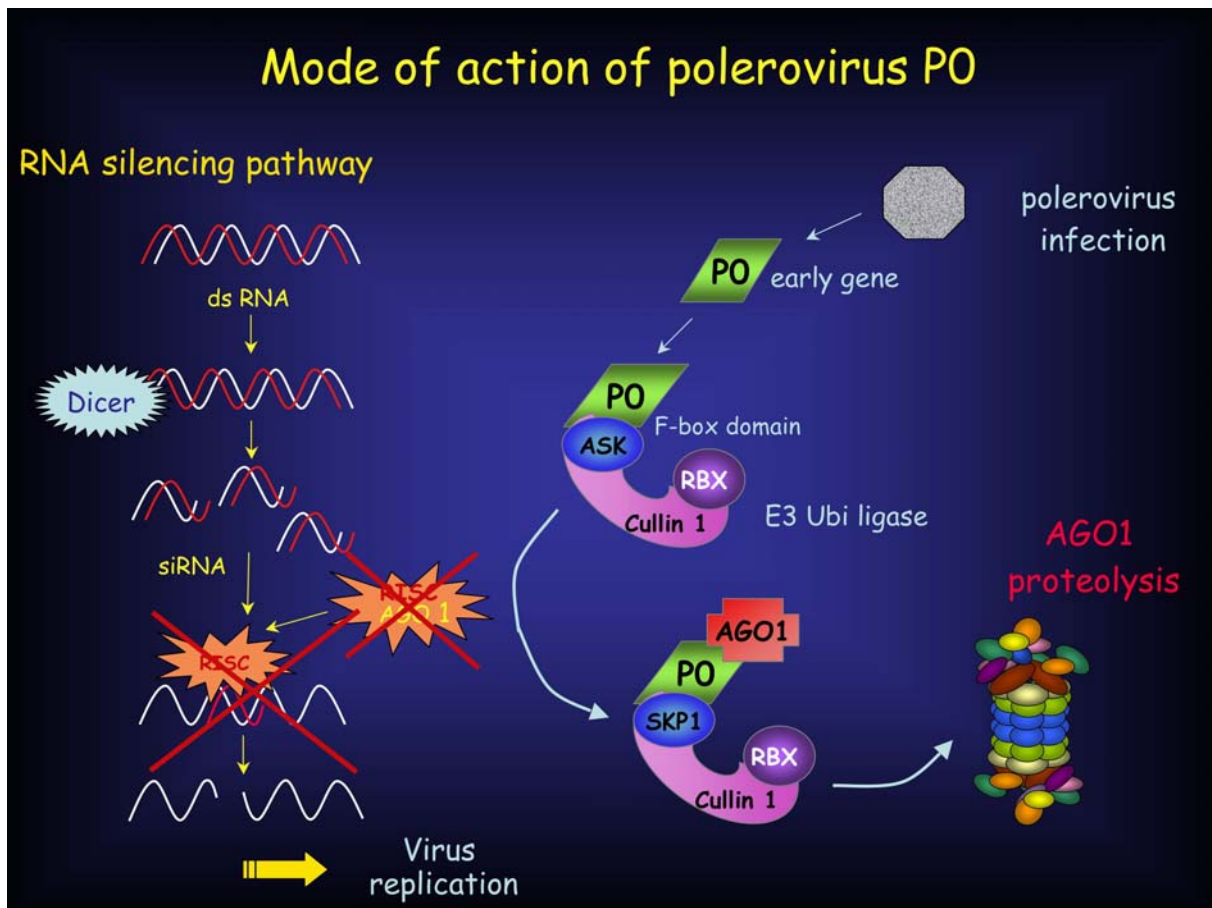
French National Center for Scientific Research (CNRS)

RNA silencing is an essential RNA-based regulatory mechanism of gene expression discovered about 15 years ago and rewarded in 2006 by a Nobel Prize to Andrew Z. Fire and Craig C. Mello. Organisms like plants, but also insects and worms take advantage of RNA silencing to defend themselves against viruses. This process is triggered by the perception by the cell of an unusual double stranded RNA molecule (characteristic of replicating viruses) that will be diced into pieces of around 25 nucleotides long siRNA (short-interfering RNA). One strand of siRNA can then be loaded into a larger complex containing a protein called ARGONAUTE (AGO) to guide specific cleavage of the complementary invading viral RNA. To counteract this defense mechanism, viruses have developed proteins able to inhibit RNA silencing at various levels. A common characteristic of these so-called silencing suppressor proteins is their ability to enhance the pathogenicity effect of the viruses.

One of the silencing suppressors studied in our laboratory is the P0 protein encoded by polioviruses. These viruses cause severe losses in production of a wide range of crops including barley, potato or sugar beet. We found that by mimicking the characteristics of specific cellular proteins, P0 can manipulate the central cellular machinery of destruction (the ubiquitin/proteasome pathway) to target one of the key components of RNA silencing to degradation.

Most proteins have their life cycle regulated by a process of proteasome-dependant degradation that requires addition of ubiquitin moieties by a cascade of three enzymes (E1 ubiquitin activation enzyme, E2 ubiquitin conjugating enzyme and E3 ubiquitin ligase). In order to restrict this process to only those proteins that have to be destroyed (the substrate), the E3 ubiquitin ligase complex contains a protein responsible for the selective recognition of the substrate. Among these sorting proteins are the so-called F-box proteins (more than 700 candidates were identified in Arabidopsis) that bind via their F-box motif to SKP1 protein (S-phase kinase-regulated) of an E3 ubiquitin ligase complex. The substrate specificity is determined by another domain of the F-box protein. The viral P0 is precisely able to mimic such an F-box protein by interacting with SKP1. As this interaction is essential to P0's suppression activity, a link between ubiquitination and RNA silencing could be drawn.

If P0 really functions like a cellular F-box protein, it has to bind a substrate that will be ubiquitinated. To identify its potential target, we analysed the effects of a conditional expression of P0 in transgenic plants. When producing P0, these plants displayed developmental alterations reminiscent to mutant plants affected in the miRNA pathway. miRNA are another species of short RNA that play a pivotal role in the regulation of many genes, some of them being essential to development. Importantly they can be loaded into the AGO1 containing complex to regulate the fate of cellular messenger RNA (mRNA). And we observed that the expression of P0 in plants led to over-accumulation of some cellular mRNA known to be regulated by miRNA, indicating that their cleavage was impeded. The second essential observation in these plants was a massive decrease of AGO1 protein, which was not explained by a drop of the AGO1 transcripts. Finally, a physical interaction between P0 and AGO1 was demonstrated by a fluorescent complementation approach.



The conclusion of these experiments is that P0 triggers in the cell the degradation of AGO1 to suppress RNA silencing. This mode of action is today the first example among plant viruses by which the virus hijacks the cellular ubiquitination process to inhibit the anti-viral plant defense response.