

Base-pairing in RNA virus replication and host plant defence

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Recent research has shown that host defence against plant virus infection can be 'switched on' by replication of the viral RNA, as Mike Mayo describes.

The fundamental basis of biological replication, for RNA viruses just as much as for their hosts, is base-pairing between complementary nucleotides, pretty much as predicted for DNA by Watson and Crick. Despite their initial doubts, it is clear that ribonucleotides in RNA can base-pair to form double-stranded structures just as deoxyribonucleotides can in DNA. Indeed, we now speak of an 'RNA world' as being the forerunner of the current 'DNA world'; replication based on RNA copying being improved on by evolving to DNA-based copying. However, in virology, RNA-based replication has not been superseded. Depending on the virus species, either RNA or DNA can be the genetic material in virions, or infectious particles, of viruses, with perhaps RNA being the commoner. Of the 245 genera of viruses currently recognized, 147 (60%) contain viruses with RNA genomes. Mostly this is due to plant virus genera (73% RNA genomes) compared to 53% RNA genomes for genera of vertebrate viruses.

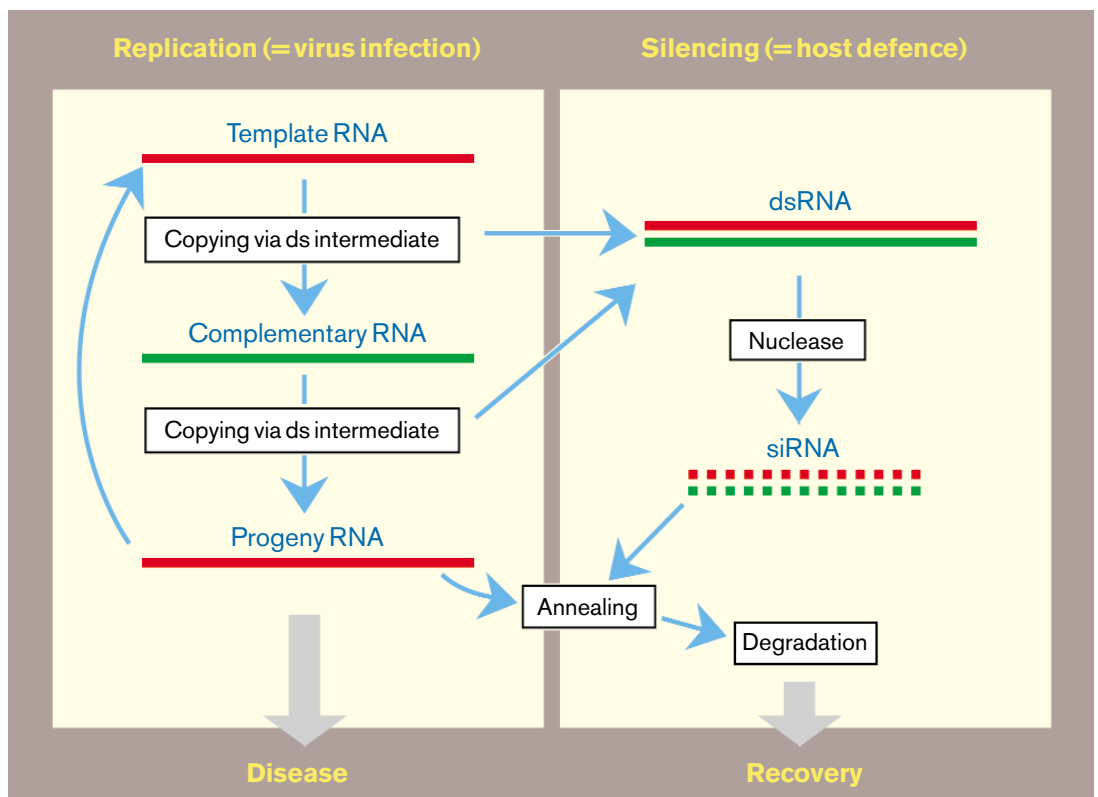
● RNA genomes

One important difference between DNA-based replication and that based on RNA is that DNA copying is much less error-prone than that of RNA. It is estimated that DNA replication is a million times more faithful than RNA replication. The result is that RNA genomes can accumulate point mutations

very much more rapidly (around 10^{-3} per nucleotide position per replication cycle) than DNA genomes. Thus populations of RNA viruses will always contain many variants and are thought of as existing as quasi-species. This may be seen as a disadvantage because of the resulting genetic instability, but one positive effect is that RNA viruses can evolve very rapidly in response to any novel selection pressures or new niche opportunities that may arise – a very useful property to have as a pathogen when hosts are continuously exerting pressure.

However, for RNA viruses there is a law of diminishing returns as genome size increases. The larger a genome is, the greater is the chance of a lethal copying error. Perhaps this is why another difference between viruses with either type of genome is their size. Whereas many species have RNA genomes of around 10 kb in size, and the largest RNA genomes are the 30 kb genomes of coronaviruses, many DNA virus species have genomes of greater than 100 kb and several exceed 300 kb. Possibly, RNA virus genome size has been constrained by the increased hazard of lethal mutation in a large RNA genome. Another way of decreasing this mutation risk would be to divide the genome into several components and this genome design is indeed more common for RNA viruses; with segmented genomes in about 8% of DNA virus genera but about 36% of RNA virus genera.

LEFT:
Fig. 1. Diagram illustrating the role of base-pairing in the replication of an RNA virus (left panel) and in the silencing response of the host (right panel). COURTESY SCOTTISH CROP RESEARCH INSTITUTE





RIGHT:
Fig. 2. A *Nicotiana benthamiana* plant about 10 days after infection with *Tobacco rattle virus* that has been engineered so as to carry sequence corresponding to the PDS gene. Regions in which virus replication has stimulated silencing are white because PDS mRNA has also been destroyed. The plant on the left is an uninfected control plant.
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● Infection by RNA viruses

Virus infection of a host can be thought of as what happens when a virus deploys the ability to invade and to multiply in a particular host environment. Multiplication is the result of cycles of replication in which the template RNA, initially in the infecting virion, is copied into a complementary strand that is then copied back into many copies of the virion-sense strand, some of which move out of the cell to infect new cells or new hosts (Fig. 1, left panel). The template and progeny RNA can be either positive-sense (i.e. message-sense) or negative-sense (complementary to message-sense).

During this process the RNAs that are complementary anneal to form double-stranded RNA (dsRNA) and recent research has found that this can provide a switch that provokes host defence against virus infection. In plants (as in fungi, invertebrates and vertebrates) there is a post-transcriptional gene silencing mechanism that involves dsRNA. In essence, the activity results in a nucleolytic attack on dsRNA that generates small interfering RNA molecules (siRNA) that can base-pair with a target (necessarily homologous) RNA. Following this base-pairing, the newly formed dsRNA is destroyed by nucleolytic activity (Fig. 1, right panel). Special nucleases are involved at each of the degradation steps.

● Gene silencing

When the initiating dsRNA structure is that of an invading virus, the result is that the host is defended against the virus by its capacity to degrade the template RNA. This effect is silencing. The process can be illustrated dramatically by engineering a virus to contain sequences of a host plant gene. Fig. 2 shows the result of silencing in plants that are infected by *Tobacco rattle virus* that carries part of the mRNA for the plant enzyme phytoene desaturase (PDS). When the silencing activity is stimulated by the virus infection, some of the siRNA is complementary to PDS mRNA. This targets the

homologous host mRNA which is destroyed at the same time as the invading virus RNA. Infected cells thus lack PDS and turn white on exposure to light.

Effective silencing would, of course, make all hosts immune to virus infection. This is obviously not so and it has become clear in recent years that at least some viruses encode functions (in some instances, identifiable proteins) that can suppress the gene silencing defence

mechanisms of their host plants. Silencing suppressors of different plant viruses differ in their effects and the ways in which they work. So, it is not surprising that those from taxonomically distinct viruses show no phylogenetic similarities; probably suppression has arisen independently during the evolution of distinct virus lineages.

It is thought-provoking that much of the understanding of this novel field in plant biology has been the result of experimentation with genetically manipulated plants and viruses, even though what has been revealed is a process (silencing) possessed by normal plants and one that is possibly fundamental to the functioning and development of the healthy plant.

● Conclusions

The process of base-pairing of ribonucleotides to form a double-stranded polynucleotide has turned out to be a key one in several ways. Not only (and unsurprisingly) is it the central event in RNA virus replication, but it also provides the variation needed for evolution by natural selection and it is fundamental to at least one host defence mechanism against infection.

So it is in the end fitting that the 1953 *Nature* papers by Watson and Crick on DNA structure carried acknowledgements of financial support from the National Foundation for Infantile Paralysis, a disease now known to be the result of infection by the RNA genome polioviruses.

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Further reading

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