Commentary

On RNA interference as template immunity

The humoral immune response of vertebrates is able to generate antibodies that bind with exquisite specificity to novel antigens displayed by a pathogen. These antibodies target the pathogen for clearance. By the 1930's the experiments of Landsteiner and others (Clark 1991) demonstrated the enormous repertoire of possible responses: individuals were capable of producing antibodies that distinguish between protein antigens differing in a single amino acid, between the D- and L-chiral forms of organic molecules, and between ortho- and para-substitutions on benzene rings. Biologists spent the next half-century trying to understand the biological mechanisms underlying this extraordinary specificity.

Between the 1930's and 1950's, two leading hypotheses had emerged (Pauling 1940; Jerne 1955; Burnet 1959; Silverstein 1985). The *instructive* or *template hypothesis* postulates that B-cells produce antibodies that are plastic receptors capable of adopting a wide range of conformations. When an antibody encounters antigen, the antibody moulds to it and subsequently the B-cell is instructed to synthesize more antibodies with this conformation. In this way, the antigen serves as a template upon which the specific antibody response is constructed. The *clonal selection* hypothesis postulates that a vast repertoire of different B cells, each encoding antibodies with a predetermined shape and specificity, is generated prior to any exposure to an antigen. Exposure to an antigen then results in the proliferation or clonal expansion of only those B cells with antibody receptors capable of binding that antigen (see figure 1). Elegant experiments by Talmage (1959) and Ada and Byrt (1969) showed that the immune response of vertebrate hosts works by clonal selection.

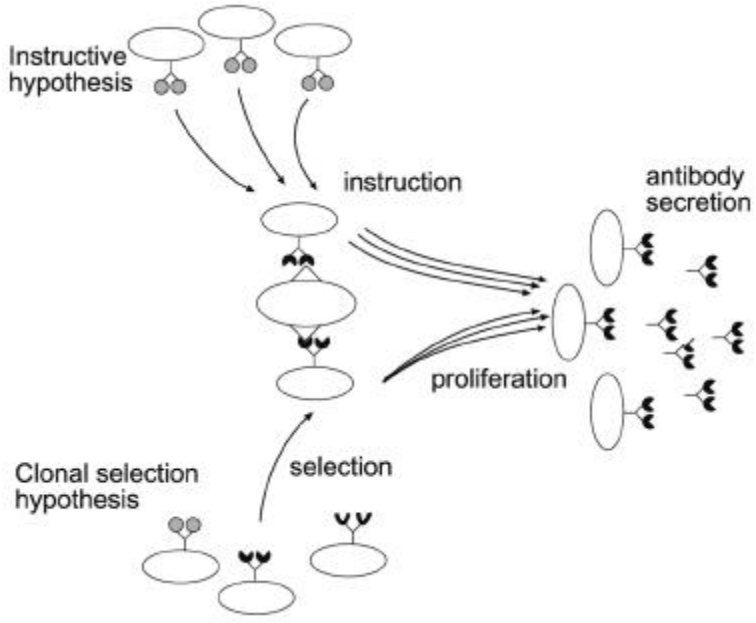

Figure 1. A comparison of the instructive hypothesis and the clonal selection hypotheses for the generation of antibody responses. In the instructive hypothesis, antigen can instruct any B-cell to generate antibodies which bind to the antigen. In the clonal selection hypothesis, individual B cells have predefined specificities and antigen selects for the expansion of only those B cells which produce antibodies capable of binding to the antigen.

Compared to the template hypothesis, the clonal selection hypothesis proposes a seemingly inefficient mechanism for the generation of immune responses. A enormous number of B cell clones must be generated, and the vast majority of these are either deleted immediately (if they are self-reactive) or maintained indefinitely without ever being needed. The luxury of hindsight suggests that the rules of protein folding prevent proteins from serving as plastic templates – a protein can have a few conformations; unlike plasticine, protein cannot stably adopt and maintain the tremendous diversity of conformations which would be required to specifically target the breadth of recognizable antigens.

Problematic as template immunity is for antibody responses, the basic conceptual idea is sound. We merely need the proper building blocks. Nucleic acids, with their complementary base-pairing structure, are ideal. Several billion years of adaptive evolution have settled upon nucleic acids as the heredity material precisely because they have the properties required for template immunity: given any 'model' strand, it is easy (using a nucleic acid polymerase) to synthesize a highly specific complement that will bind properly to this sequence and no other.

Systems of RNA interference (RNAi) (also known as RNA silencing, post-transcriptional gene silencing, or quelling) in unicellular eukaryotes (such as yeast), invertebrates (such as nematodes and *Drosophila*) and plants exploit these properties to provide template-based adaptive immunity directed against nucleic acids instead of proteins. RNAi operates within individual cells, using double stranded RNA (dsRNA) as a signal of non-self. (Long segments of dsRNA should not be present in properly-functioning eukaryotic cells, but do occur as the genetic material or replicative intermediates of many RNA viruses.) As described beautifully by Plasterk (2002), RNA interference confers immunity against such pathogens by destroying these double-stranded RNAs and using the fragments from these dsRNA molecules as the specific templates in template-based immunity. These fragments allow the system to identify and post-transcriptionally suppress the corresponding virus mRNAs. Furthermore, the templates are amplified in a manner that retains their specificity. Once appropriate the 'target' mRNAs

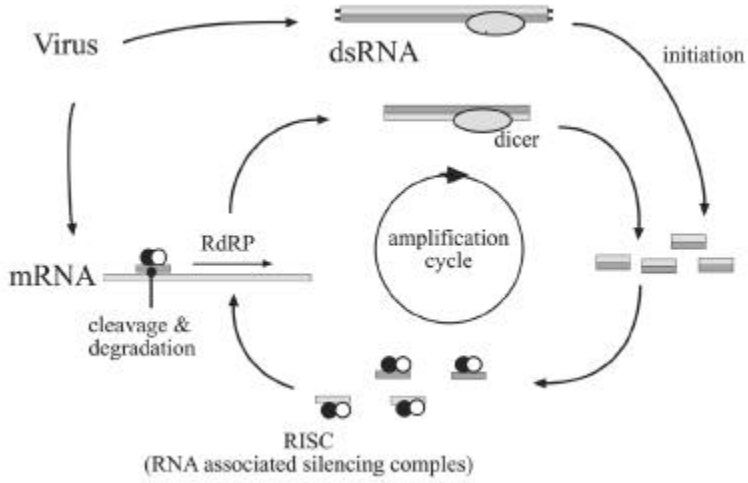

Figure 2. Schematic of the RNAi silencing pathway. Viral infection acts as a source of both mRNA and dsRNA. The viral dsRNA is cleaved by the Dicer enzyme to produce short but highly-specific RNA fragments called short interfering RNAs (Bernstein *et al* 2001). This induces an amplification cycle: The short interference RNA fragments are stabilized by the RISC proteins and play the role of template 'antibodies' in binding specifically to viral mRNAs (Sijen and Kooter 2000). The viral mRNAs are then either (i) targeted for degradation (Sontheimer and Carthew 2004) or alternatively, (ii) used to generate further templates with identical specificity, through a polymerization reaction (Lipardi *et al* 2001). As a result, a small number of dsRNAs corresponding to an viral gene can set off a massive reaction targeted against corresponding mRNA molecules and resulting in post-transcriptional silencing of viral protein expression.

are located, a host-encoded RNA-directed RNA polymerase (RdRP) is used to synthesize additional template copies by a standard polymerization reaction. This process is summarized in figure 2.

Interestingly the amplification cycle of the RNAi pathway is absent in vertebrates, perhaps because these have an adaptive immune system based on lymphocytes and antibodies.

The template hypothesis for immune specificity is an elegant – even beautiful – idea. In biology, with its spectacular diversity of form and pathway, few beautiful ideas are absolutely wrong. They merely wait for us to discover the system or systems to which they apply. For template immunity, RNAi was the missing discovery.

Acknowledgements

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