Resistance is futile

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A new study of dominant negative functions in cells infected by a positive-strand RNA virus detects an array of locus- and allele-specific effects. Exploiting subunit defects in multi-component complex assemblies provides a new approach to identifying targets for antiviral therapies that may inhibit the emergence of drug-resistant RNA virus populations.

The replication fidelities of viruses are primary determinants of virus populations that we think of as a single species or defined isolate. For RNA viruses, this perception has been dispelled during the past several decades by the realization that their replication machineries are highly error-prone, primarily because they lack the editing functions normally associated with DNA replication enzymes. Thus, RNA virus isolates have been called swarms or quasi-species to reflect the fact that although there is a ‘consensus’ viral genomic sequence in plaque isolates, individual RNAs can have one, two or even more point mutations relative to this wild-type consensus sequence. As a result, the notion of allele-specific functions takes on additional complexities when analyzing RNA virus genomes.

In classic studies aimed at fine-structure mapping of the rII locus in bacteriophage T4, Seymour Benzer used coinfections of different mutant phage isolates to determine the nature of functional units in T4 genomic DNA. By applying the ‘cis-trans’ or complementation test, Benzer helped to define the functional units required for viral growth in bacteria and showed that specific alleles could show dominance in mixed infections. This phenomenon of dominant alleles in mixed viral infections provides the backdrop for the paper by Scott Crowder and Karla Kirkegaard on page 701 of this issue. Guided, in part, by the principles of gene inactivation through dominant negative mutations provided by Ira Herskowitz, Crowder and Kirkegaard describe a new approach to defining targets for antiviral chemotherapies directed at positive-strand RNA viruses.

Poliovirus replication

The authors chose poliovirus (a positive-strand RNA virus belonging to the picorna-virus family) for their analysis of defective viral genes that have a dominant inhibitory effect over wild-type viral alleles. The rationale behind these experiments is that identifying such genes may uncover features of viral replication that require cis or trans functions for specific viral proteins or RNAs. Given that there can be many variant forms of viral RNA in even a single human cell infected with poliovirus, knowing how proteins synthesized from one genome affect the replication cycle directed by another genome (in trans) is crucial for understanding viral dynamics.

The dominance map

Crowder and Kirkegaard approached the problem by cotransfecting cells with transcripts corresponding to wild-type poliovirus RNA and transcripts containing engineered mutations known to be lethal to virus growth (Fig. 2). These lesions were introduced individually at specific sites in the coding region corresponding to every known viral protein. Given the concept of poison subunits in multimeric complexes, it was not surprising that...
mutant viral capsid proteins showed trans dominance over wild-type virus growth. The mutated genomes would contribute a defective form of one of the capsid proteins, which would associate with ‘good’ proteins and render the resultant complexes defective in virus assembly or subsequent receptor binding or virion uncoating in the next round of infection. Likewise, the authors found that several defective alleles of the viral RNA-dependent RNA polymerase (a protein called 3D) could also have trans dominance. The viral RNA polymerase forms multimers in solution, although the precise domain interfaces of intermolecular interaction have not been completely defined.

Unexpectedly, Crowder and Kirkegaard also found that one of the viral proteinases (protein 2A) that carries out a single relevant polyprotein cleavage in cis (i.e., intramolecularly) also conferred trans dominance when its cleavage function was genetically abrogated. Loss of cleavage by this protein at the VP1-2A junction (Fig. 1) leads to an uncleaved capsid protein that would act as a poison subunit in assembly of viral capsids.

Of particular importance was the observation that dominance was not restricted to proteins, because a cis-acting RNA replication element (an RNA structure known to be required for initiation of poliovirus RNA synthesis) showed dominance. Notably, one specific allele of this RNA element seemed to be especially effective as an inhibitor of replication. To show that their trans-dominant genome assay was relevant to viral infections (rather than just RNA transfections), the authors used highly defective (but viable) mutant polioviruses in coinfection assays to validate further the locus- and allele-specific nature of dominance by defective poliovirus genomes.

Genomic RNA as a drug target
Beyond the inherent mechanistic insights one derives from generating a ‘dominance map’ of RNA virus functions, the approach described by Crowder and Kirkegaard could have applications in identifying targets for antiviral therapies, given that mixing drug-resistant subunits with drug-sensitive subunits might lead to dominance by the sensitive allele, thereby reducing the possible emergence of new drug-resistant viruses. The authors tested their ‘dominance over resistance’ hypothesis with a known capsid-binding inhibitor of poliovirus growth, using mixed infections of wild-type virus (sensitive to the drug) and mutant virus (encoding a drug-resistant allele of the capsid protein VP1). The results of this experiment showed that the drug-sensitive virus markedly interfered with the replication of the drug-resistant virus, leading the authors to conclude that the dominance of the drug-sensitive genomes was most likely caused by a chimeric or mixed capsid. This mixed capsid had both sensitive and resistant forms of capsid protein VP1, making the encapsidated mutated genomes sensitive to the effects of the inhibitor.

The implications of this latter finding are potentially far-reaching in targeting antiviral compounds against positive-strand RNA viruses that express some or all of their proteins in the form of polyprotein precursors, which, when cleaved, generate proteins that form multicomponent complexes. These include human rhinovirus, hepatitis C virus, SARS coronavirus, West Nile virus, HIV and a number of other viruses for which vaccination has not yet proved successful or practical. Even in mixed populations of sensitive and resistant RNA viruses that are likely to be generated in the presence of antiviral compounds, if the target is a subunit of a multiprotein complex, the dominance of the genomes encoding the sensitive forms of the protein might have a considerable effect on the rate of emergence of resistant genomes in viral populations. This desirable outcome warrants further exploration using the new approach described by Crowder and Kirkegaard.

Figure 2. Scheme for assaying protein dominance using cotransfection. HeLa cells are cotransfected with wild-type poliovirus RNAs and mutant RNAs and incubated for 10 h at 32.5 °C. Progeny virus titers are then determined by plaque assay.

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