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Perspective

Methods to Determine Protein- Protein Interactions in Bacteriophage

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DESCRIPTION

Bacteriophages have been extremely important model systems in molecular biology. They were critical to numerous advance discoveries, similar as the nature of the genetic material, the structure of genes and genomes and the first genome sequence, recombination, transcriptional regulation and numerous others. In addition, phages have become important specialized tools in molecular genetics and nanobiology.

Methods used to study protein – protein interactions in phages have evolved over the decades. In the beginnings of phage molecular biology, discovery of genetic interactions dominated. For instance, certain mutants in the λ J gene abolished virion binding to host *Escherichia coli* cells. Similarly, mutants of the *E. coli* gene lamB made the cells resistant to λ infections. From similar trials it was concluded that J protein bound to LamB and that LamB was the λ receptor. This thesis has been verified by segregating compensatory mutations in both genes that regenerate the interaction.

Another important method for the investigation of physical relationships between phage components is electron microscopy, especially in combination with inheritable analysis. Mutants frequently have an aberrant morphology. However, it can be concluded that the mutated gene encodes this structure or is at least involved in its assembly, if certain structures are missing in the mutant virion. For case, many laboratory strains of λ harbor a frame shift mutation in the stf gene, and these mutants lack side tail fibers. Numerous similar studies not only showed which genes encode which proteins, but also indicated how the proteins interact (e.g., when particular structures could be "removed" from the virion by defined gene knockouts). More recently, numerous bacteriophage capsids and phage protein complexes have been analyzed by cryoelectron microscopy and three-dimensional reconstructions. This technique gives resolutions approaching that of X-ray crystallography, especially for proteins present in symmetric arrays in the virion, and has proven ideal for assaying protein – protein interactions in large complexes, especially in combination with high- resolution structural data from other ways, similar as X-ray crystallography.

More lately, the yeast two- hybrid system (Y2H) has become an important system to discover pairwise protein interactions. Interaction screens of phage proteins can be carried out easily by cloning all open reading frames in appropriate vectors and also testing these in a systematic pairwise fashion. This has been done for several phages, including coliphages T7 as well as Streptococcus pneumoniae phages Cp-1 and Dp-1. The whole process can also be automated.

One disadvantage of the Y2H system is that a typical pairwise screen, also called an "array screen" because of the methodical arrangement of clones in micro titer plates, may only detect 20 – 30 of all interactions. That is, the rate of false negatives is on the order of 70 – 80. This can, in theory, be overcome by using multiple vectors that differ in the structure of the fusion proteins, for instance in the production of N-or C-terminal fusion proteins. A combination of multiple vectors can achieve interaction detection rates of up to 80 (i.e., false negative rates as low as 20).

Y2H also suffers from false positives that can only be understood with additional trial. Nevertheless, a multivector Y2H strategy can help in the recognition of false positives. For instance, if multiple vector combinations are used, interactions found in all or utmost cases are more likely to be "true positives," whereas interactions detected in a lower bit of cases are less reliable and may be false positives.

CONCLUSION

Only atomic structures of all phage proteins and their interactions will give a mechanistic understanding of phage biology, and progress in this area has been accelerating. Individual proteins and protein complexes, if not the whole phage particle, can be crystallized and the structure of such proteins answered by X-ray analysis. Alternately, protein structures can be solved by nuclear magnetic resonance (NMR) if high enough concentrations can be achieved. Unfortunately, size restrictions on NMR structural determinations limit the analysis of large protein complexes, but monomeric structures can be extremely useful in devising testable models for the interactions proteins may undergo. Still, many phage proteins are refractory to structural analysis (especially those involved in virion assembly, as they frequently assemble only at high concentrations), and indeed in classical model systems similar as phage λ less than a third of all phage- encoded proteins have been crystallized — most of these as pure proteins rather than as protein complexes.

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