

[13] Selection of Genetic Agents from Random Peptide Aptamer Expression Libraries

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Peptide Aptamers as Genetic Agents

Most cellular processes are regulated by networks of interactions among proteins. Classic “forward” transmission genetic approaches have succeeded in identifying proteins involved in such networks and provide information on the relationships between them. Approaches such as (1) isolation of mutants and mapping the genes responsible for mutant phenotypes, (2) epistasis analysis to determine the order in which gene products might act on each other, (3) dependency analysis to determine the relationship between gene products with respect to the completion of a cellular process,¹ and (4) allele-specific suppression analysis to give clues as to the possible physical interaction between two proteins² provide detailed information about genes and their positions and interactions within networks. Although these approaches are often highly informative, they are often difficult to perform, especially in diploid organisms. Their full application is thus generally limited to a few tractable organisms such as phage, bacteria, yeast, worms, and flies.³

The ability to manipulate individual genes by recombinant DNA approaches allowed the development of “reverse” genetic techniques, in which investigators mutate the function of individual genes and monitor the resulting phenotypes. Reverse genetic approaches that mutate or knock out the function of genes at the DNA level suffer from the following limitations: (1) if the gene under study is essential, then mutations in it will yield nonviable organisms, but often no other information; (2) gene knockouts provide information about the phenotypic consequences of abolishing all the protein interactions in which the knockout gene product participates; (3) gene knockouts typically provide information related only to those phenotypes caused by the initial function of the gene in the development of the organism; and (4) in diploid organisms, as for forward genetic analysis, the observation of recessive phenotypes requires the generation of organisms homozygous for particular mutations, typically in the second generation.

¹ L. M. Hereford and L. H. Hartwell, *J. Mol. Biol.* **84**, 445 (1974).

² P. E. Hartman and J. R. Roth, *Adv. Genet.* **17**, 1 (1973).

³ J. H. Nadeau and P. J. Dunn, *Curr. Opin. Genet. Dev.* **8**, 311 (1998).

More recently, dominant reverse approaches have been devised that affect gene products rather than genes. These dominant reverse approaches rely on generating "mutagenic" agents that inactivate gene products in *trans* without affecting their coding DNA. Examples of dominant genetic agents include small molecule inhibitors,⁴ dominant negative proteins,⁵ injection of antibodies,⁶ antisense RNA,⁷ ribozymes,⁸ and nucleic acid aptamers.⁹ These methods are particularly useful for studying gene function in diploids; however, they too have various weaknesses. Dominant negative proteins and small molecule inhibitors may not exist for all gene products, thus restricting their universality. Antibodies, antisense RNA, ribozymes, and nucleic acid aptamers can in principle be generated to inactivate almost any gene product. Antibodies, however, are not cell permeable and their injection into cells is time consuming and not practicable in all organisms. RNA-based agents are generally not stable in intracellular environments. Although RNA stability can be partly overcome by using RNAs with different chemistry, it remains difficult to predict the sites on the target RNA that are exposed for antisense inhibition. Also, the stability of the protein product of the antisense and ribozyme target gene affects the timing (perdurance) and extent (penetrance) of the mutant phenotype. Moreover, like DNA mutagenesis, mutagenesis at the RNA level results in phenotypes that are caused by the abolishment of all protein interactions in which the target gene product is involved.

A new class of dominant agents has been developed to facilitate the analysis of processes in diploid and genetically intractable organisms. We termed these molecules "peptide aptamers," because of their similarity to nucleic acid aptamers.¹⁰ We define peptide aptamers as antibody-like recognition agents that consist of conformationally constrained peptides displayed on the surface of scaffold proteins, and distinguish such molecules from peptides of variable sequence displayed that are not constrained at both ends by the scaffold. Peptide aptamers are designed to inhibit cellular processes by interacting with proteins and disrupting their biological functions. Combinatorial libraries of peptide aptamers in principle contain aptamers that bind almost any protein target. Peptide aptamers specific for numerous proteins have been isolated by using the yeast two-hybrid system.

⁴ T. J. Mitchison, *Chem. Biol.* **1**, 3 (1994).

⁵ I. Herskowitz, *Nature (London)* **329**, 219 (1987).

⁶ G. J. Gorbsky, R. H. Chen, and A. W. Murray, *J. Cell Biol.* **141**, 1193 (1998).

⁷ A. D. Branch, *Trends Biochem. Sci.* **23**, 45 (1998).

⁸ B. Bramlage, E. Luzi, and F. Eckstein, *Trends Biotechnol.* **16**, 434 (1998).

⁹ M. Thomas, S. Chedin, C. Carles, M. Riva, M. Famulaok, and A. Sentenac, *J. Biol. Chem.* **272**, 27980 (1997).

¹⁰ A. E. Ellington and J. Szostak, *Nature (London)* **346**, 818 (1990).

