We describe here a new technology, selectively infective phages (SIP)\(^1\), which can be used in screening large libraries and in driving molecular evolution within these libraries. SIP is related to phage display technology using filamentous phages\(^2\), where a protein or peptide of interest is fused to a phage coat protein and, thus, displayed on the outer surface of the phage, while its genetic information is contained in the phage DNA. In contrast to phage display, the phage particles can be used in screening large libraries only Nl, is stimulated by high Ca\(^{2+}\) concentrations, does not require F-pili and must therefore involve direct binding of Nl to the bacterial surface\(^3\). The second requires N2 and F-pili, but no Ca\(^{2+}\), suggesting a docking of N2 to the pilus\(^4\). Both pathways can be used in SIP; the second one is much more efficient, however\(^5\).

The influence of different g3p domain rearrangements in SIP has been studied. In a fluorescein/anti-fluorescein scFv model system, purified N1 or N1-N2 domains, chemically coupled to the antigen fluorescein (N1-Ag and N1-N2-Ag), were used in combination with scFv-"short" (Fig. 2a) or scFv-"medium" phages (Fig. 2b) to recreate a wt g3p interrupted at various positions\(^6\). As shown in Fig. 2c, infectivity as a function of adapter concentration was different in the two cases; furthermore, N1-N2 inhibits the entry of wt phage into cells with a half-inhibitory concentration two orders of magnitude lower than N1 alone\(^6\). At low N1-N2-Ag concentrations the phage is not sufficiently saturated with ligand-adapter to mediate infectivity in SIP selection, while at high concentrations the bacteria themselves become saturated, blocking infection\(^5\). Therefore, very low concentrations of N1-N2-Ag are needed and the system will select for high affinity binders provided, of course, such molecules exist in the library under study. In the case of the N1-Ag adapter molecule, higher concentrations can and must be used in order to overcome the lower infection efficiency of the system\(^4\), while possibly allowing selection for lower binding affinity pairs. This requirement for a high concentration of N1 may reflect a low-affinity binding to Escherichia coli in the absence of pili and/or a necessity for multivalent binding to the bacteria requiring simultaneous...
ous occupation of all cognate ligand-binding sites on the phage. In the absence of coupled antigen, no infectivity was observed, demonstrating that the N-terminal domains must be physically coupled to the phage for infection.

For SIP to work, the phage must be strictly non-infective. Using a phagemid/helper phage system, an unpackageable g3p expression plasmid must be used in combination with a g3p-deleted helper phage, but all tested plasmids appeared to contain cryptic packaging signals and thus gave rise to background infectivity. These problems are overcome by introducing the g3p fusion protein directly into a single genetic package, the phage genome.

SIP experiments can be carried out in vivo or in vitro (Fig. 3): In the former approach the phage or phagemid encodes both interacting molecules (for example, antibody-antigen) in the same vector14-16 (Fig. 3a), and the protein-ligand interaction occurs in the bacterial periplasm; the infective phage particle is extruded from the cell and can be harvested and used to infect different bacteria. The simplicity of design of the experiment is counteracted to some extent by the lack of experimental control over parameters such as concentrations, incubation times and the infection process itself.

These factors can be controlled in the in vitro SIP experiment (Fig. 3b). In this case, the adapter protein is prepared from a separate E. coli culture, either by secretion or by in vitro refolding. This approach broadly extends the range of applications of SIP, as, in addition to genetically fused peptidic antigens, non-peptidic antigens can now be chemically coupled to the N-terminal domains. The adapter is then incubated with the non-infective phage library, and added to the bacteria.

Examples of SIP studied

The SIP system has been tested with both proteins and peptides. The leucine-zipper motifs of the jun and fos proteins, with both helices flanked by cysteine residues, were used as a model system in which the heteromeric interaction between the two peptides restored the infectivity of the phage. An enrichment of up to 200-fold over non-interacting pairs was observed after one round. Furthermore, zipper-like domains that pair with the jun-zipper domain have been selected from a human cDNA library.

With an anti-lysozyme Fab fragment model system, in association with an N1-lysozyme adapter molecule, a specific enrichment factor of approximately 10^5 was observed after only two rounds of selection. The antibody 17/9, specific for a hemagglutinin peptide, was used as a model where both peptide and antibody were encoded in the same phage genome, resulting in a very clean selection of the desired Ab–Ag pair. An anti-fluorescein antibody/antigen system was used as the first example of a non-peptidic antigen being coupled to purified g3p N-terminal domains. In the latter case, up to 10^8 antigen-specific infection events were observed from 10^10 input phages, compared to only one antigen-independent event. No background infection was observed in the absence of antigen or in the presence of an antibody of different specificity in the latter two examples, presumably due to the use of phage vectors as the sole replicons and thus in strict absence of any wt g3p.

In a Fab library made from B cells of gp120 seronegative donors, with only the N1 domain fused to the V3 loop peptide, three rounds of traditional phage panning yielded no antibodies against the antigen, but half of the phages gave positive ELISA signals after three rounds of SIP. The ability to enrich for affinity or even kinetic constants has been tested using six different Fab fragments against the V3 loop peptide as a model library, with high-affinity antibodies being preferentially selected. High-affinity antibodies were also enriched from a library that had been made from B cells after in vitro immunization.

Further prospects

A requirement of SIP appears to be a high affinity between protein and ligand, and all of the systems studied thus far have had dissociation constants of at least 10^{-10} M. It is not yet clear, however, what the minimum affinity necessary for significant infectivity will be. Further investigations will also have to be carried out to test the, initially encouraging, apparent ability to select for kinetic parameters of ligand binding, and to investigate the dependency of infectivity on properties of the proteins, such as thermodynamic stability and expression rate, as well as the size limits of protein–ligand complexes usable in SIP.

Since SIP is definitely compatible with disulfide formation, it complements the two-hybrid system well for library screening, as the latter is designed for use with nuclear and cytoplasmic proteins. The toxicity of certain proteins, when displayed on phage, is a limitation SIP shares with phage display. Furthermore, in SIP care must be taken, especially during in vivo SIP experiments, in phage handling and in controlling rare recombination events in the phage that could restore infective wt-like g3p (ref. 4).

These challenges are to be contrasted with the numerous ad-
vantages that SIP has over phage display. No solid-phase interaction with any support is necessary in SIP, limiting the potential of the occurrence of non-specific interactions and eliminating the need for elution. In addition, the low background infectivity observed with some of the SIP systems demonstrates that SIP can be an extremely effective and highly specific method of selecting for cognate interaction events. Furthermore, the exciting possibility of simultaneous screening of two interacting libraries by in vivo SIP might broaden the ability to identify interacting receptor–ligand pairs.

Acknowledgments

The authors wish to thank G. Wall and C. Krebber for helpful suggestions and critical reading of the manuscript.