A phage-based system to select multiple protein-protein interactions simultaneously from combinatorial libraries

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Abstract Selectively infective phage (SIP) can be used to identify protein-protein interactions. SIP was modified to facilitate the simultaneous selection of interacting protein pairs from large combinatorial libraries. An interference-resistant phage was constructed which non-covalently, but stably links the genetic information of an interacting pair, encoded separately on phage and phagemid vectors, by co-packaging into hetero-polyphages. In a model system, the interaction between a SIP-selected peptide and the intracellular domain of the p75 neurotrophin receptor was detected in the presence of a 10^4-fold excess of a non-interacting control pair (jun leucine zipper and p75 intracellular domain) via SIP hetero-polyphage transductants. To minimize the redundancy of transductants and to minimize possible ligand exchange generated in a solution-based SIP screening, a filter-based in situ infectivity screening was developed. The combination of the above techniques may provide a powerful system for rapid screening of very large sequence spaces.

Key words: Protein-protein interaction; Selectively infective phage; Combinatorial expression library; Phage display; Peptide; Neurotrophin receptor

1. Introduction

Protein-protein interactions are important for virtually all aspects of cellular function, including signal transduction, gene regulation, cell-cell communication and cell division, particularly as highlighted by work on cancer cells [1]. The identification of physical interactions between cognate protein partners is often achieved by protein purification strategies followed by protein sequencing [2,3]. In recent years genetic selection strategies, which circumvent the need for purified protein, have partly replaced this more traditional approach. The use of the yeast two-hybrid system [4] has led to the identification of many important, physiological protein-protein interactions, yielding insight into cell regulatory processes, such as apoptosis [5,6]. The genomics era has already accumulated a massive amount of structural sequence data. Therefore, tools are required in the post-genomics era to ascribe functions to genes. By identifying the ligand to a particular gene product, important clues about gene function and positioning of the gene product within complex regulatory networks can be obtained [7,8]. In the light of the large number of genes to be analyzed an approach which can simultaneously identify a wide variety of protein interactions would be highly desirable [9]. We have developed a two-vector selection system, based on selectively infective phage (SIP) technology [10–13] in combination with polyphages [14,15], which in principle could be used to simultaneously select multiple protein-protein interactions from two expression libraries.

2. Materials and methods

2.1. Cloning of phage and phagemid constructs

Phage vectors are derived from the fd phage vector fjun-1B [16], which was constructed from phage fCKC [11]. fIR3 contained full length gIII [17] in place of the jun-CT fusion (amino acids 216–406) and was generated by replacement of a 1.7-kb *Bsr*GI/*Dra*III fragment (see Fig. 2A) with the corresponding fragment of the interference resistant (IR) helper phage R408 [18]. fpep3-1B-IR3 and fjun-1B-IR3 were derived from fIR3 by replacing full length gIII via *Xba*I/*Hin*dIII digest with the pep3-CT or jun-CT fusions from fpep3-1B [16] and fjun-1B, respectively. The SIP selection from which the p75 neurotrophin receptor intracellular domain (p75-ICD)-binding peptide 3 (CIVYHAHYLVAKC) was obtained is described in Ilag and Ge [16] and Ilag et al. (submitted). Phagemid pIG10.3-IMPp75-ICD, containing the C-terminal 152 amino acids of p75 [19], was constructed from pUC18/IMP-p75 [16] by excision of the IMP-p75-ICD cassette via *Xba*I/*Hin*dIII and cloning into the corresponding sites of pIG10.3 [20].

2.2. Native gel electrophoresis of phages

Gel fractionation of phage populations was done essentially as descibed by Russel and Model [21]. Phages produced by the indicated co-transformants were precipitated with 0.2 volumes of 20% PEG/2.5 M NaCl and resuspended in 1× TBS. Phage aliquots were then mixed with an equal volume of loading buffer (0.74 M Tris-glycine, pH 9.5; 8% sucrose) and separated on a 1.8% agarose gel (0.37 M glycine, 37 mM Tris-HCl, pH 9.5) at 3–4 V/cm and 4°C overnight. Identical loading schemes were duplicated on the gel. One half of the gel was treated for 60 min at room temperature under denaturing conditions (0.2 M NaOH), neutralized (0.25 M Tris-HCl, pH 7.5) for 60 min and then used for staining (1 h, room temperature) with SYBR Gold (Molecular Probes) to visualize phage DNA in situ. The identical other half of the gel was used to excise regions with phages from the gel. Phages were eluted from the agarose in 1 ml LB medium overnight at room temperature with constant agitation. An aliquot of the eluate was taken and centrifuged at $10\,000 \times g$, before titering the phages on the *E. coli* F^+ strain JM103 (str^R, Stratagene).

2.3. Co-culture and transduction assay

Co-transformants of the combinations fpep3-1B-IR3/pIG10.3-IMPp75-ICD and fjun-1B-IR3/pIG10.3-IMPp75-ICD were generated by electroporation of the mixed DNA into *E. coli* strain DH5α. After confirmation by restriction analysis, individual clones were grown at 30°C overnight. For co-cultures, 100 μl of fjun-1B-IR3/pIG10.3-IMPp75-ICD cells were mixed with 10 μl from serial dilutions (in LB medium) of fpep3-1B-IR3/pIG10.3-IMPp75-ICD cells in 3 ml LB medium containing 100 μg/ml ampicillin and 34 μg/ml chloram-

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Abbreviations: ISIS, in situ infectivity screening; IR, interference-resistant; p75-ICD, p75 neurotrophin receptor intracellular domain; SIP, selectively infective phage

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phenicol. After overnight incubation at 30°C bacteria were centrifuged at 10 000×*g* for 5 min and the supernatants either filtered through a 0.45-μm filter (Sartorius) prior to infection of K91 cells or used directly for infection of JM103 cells. Infection was performed at 37°C for 20 min with 100–200 μl of log-phase bacteria. Hetero-polyphage transductants were selected on LB cam/amp plates for K91 or LB cam/amp/str plates for JM103 cells. Transductants were analyzed by restriction digest and PCR to determine the identity of phage and phagemid constructs.

2.4. In situ infectivity screening (ISIS)

Co-transformants of the combinations fpep3-1B-IR3/pIG10.3-IMPp75-ICD and fjun-1B-IR3/pIG10.3-IMPp75-ICD were streaked onto isopore polycarbonate membranes (0.4 μm, Millipore) on LB cam/amp plates (supplemented with 1 mM IPTG) and grown at 30°C overnight. The membranes were transferred (with bacteria on top) to LB cam/amp/str plates onto which 100 μl log phase JM103 cells had been evenly spread immediately prior to transfer. For in situ infection through the membrane, plates with membranes were incubated for 3 h at 37°C. The membranes were removed and the plates incubated at 30°C overnight to visualize transductant colonies.

3. Results

3.1. A compatible phage/phagemid system for SIP-mediated 'library vs. library' screening

SIP is a method to select for interacting proteins, based on the infectivity process of filamentous phages [10–13,22,23]. In SIP, infectivity of the phage is made dependent on the interaction of heterologous proteins (see Fig. 1). This is achieved by separation of the N-terminal from the C-terminal domains of the infectivity-mediating phage gene III protein (gIIIp). Heterologous proteins are fused to the N-terminus of the gIII C-terminal domain (X-CT, amino acids 216–406 of mature gIIIp) and to the C-terminus of the N1–N2 domains (amino acids 1–218) of gIII (infectivity-mediating particle (IMP)-Y). The optimal fusion sites in gIII for highest phage infectivity have been determined by extensive insertional mapping [23]. Interaction between the heterologous fusion partners restores gIII function, thus rendering such a phage selectively infective [10–13,22,23] (see Fig. 1B). In the original SIP system, both fusion proteins (X-CT and IMP-Y) were co-encoded on the same vector [11,13]. We have modified this system by encoding CT and IMP fusions separately on phage and phagemid vectors, respectively (Fig. 2), in order to facilitate the generation of large combinatorial libraries. For a proof-of-principle experiment, a phage-displayed peptide (pep3-CT, Fig. 2A) in combination with p75-ICD expressed as an IMP fusion (Fig. 2B) was used as an interacting pair which produces SIP phages (Fig. 2C). Peptide 3 has been previously selected by SIP from a peptide library for binding to p75-ICD (Ilag et al., submitted). The leucine zipper domain of the jun transcription factor (junLZ-CT, Fig. 2A) in combination with IMP-p75-ICD served as a non-interacting pair which produces non-infective phages (Fig. 2C).

Table 1
Sensitivity of SIP hetero-polyphage system for selection in solution

Co-transformant ratio			cam^R/amp^R transductants (t.u./ml)[a]
pep3/p75-ICD		jun/p75-ICD	
1	pos. contr.	–	6×10^5
–	neg. contr.	1	0
1		10^2	1.2×10^4
1		10^3	8.6×10^2
1		10^4	1.2×10^2
1		10^5	10[b]
1		10^6	1[b]

[a]SIP hetero-polyphages (in transducing units/ml) produced by co-cultures of co-transformants mixed at the indicated ratios.
[b]Extrapolated SIP titers.

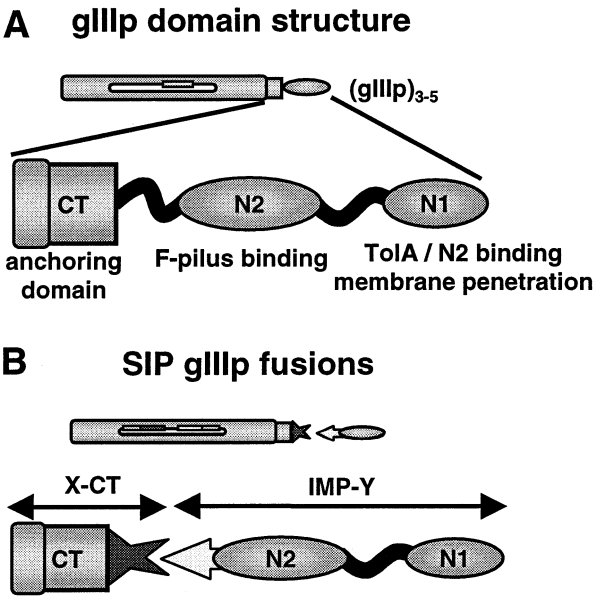


Fig. 1. The principle of SIP. A Constitutively infective phage displaying 3–5 copies of wild-type gIIIp. The domain structure of gIIIp with the corresponding domain function is indicated. Binding of N1 to TolA and N2 has been recently demonstrated [25] and the crystal structure of N1–N2 has been solved [39]. CT, C-terminal domain; N1, N2, N-terminal domains; thick lines, glycine-rich linkers. B: Selectively infective phage. Heterologous proteins are expressed as fusions to the gIIIp C-terminal domain (X-CT) and N-terminal domains N1–N2 (IMP-Y). Upon binding of IMP-Y to X-CT, gIIIp function is restored resulting in a selectively-infective phage.

3.2. Polyphages to co-package the genetic information of interacting pairs

In order to co-express the respective fusion protein pairs, phage and phagemid vectors have to stably co-exist in the same bacterial cell. This is not the case with a wild-type phage and a phagemid, due to the phenomenon of interference from two phage origins of replication [24]. Therefore, an IR phage had to be generated. This was achieved by replacing a part of the wild-type phage genome which covers the phage origin of replication and gene II with the corresponding part of the IR helper phage R408 [18]. The resulting chimeric phage, fIR3, could be stably co-transformed with the phagemid pIG10.3-IMPp75-ICD.

A SIP-based library vs. library approach not only necessitates stable co-existence of both library vectors to encode both components which are tested for interaction, but also has to allow for recovery of the genetic information from such an interacting pair. This could be achieved by co-packaging of both library vectors into polyphages. Polyphages are phages of more than unit length which have more than one genome packaged [14,15] and which occur at a frequency of about 1–5% with wild-type phages [21]. This is a consequence of failure of 'capping' the phage with gIIIp/gVIp, and thus appears to increase with certain gIIIp fusions [25] or variants [21,26]. The generation of homozygous and heterozygous 'diploid' phages

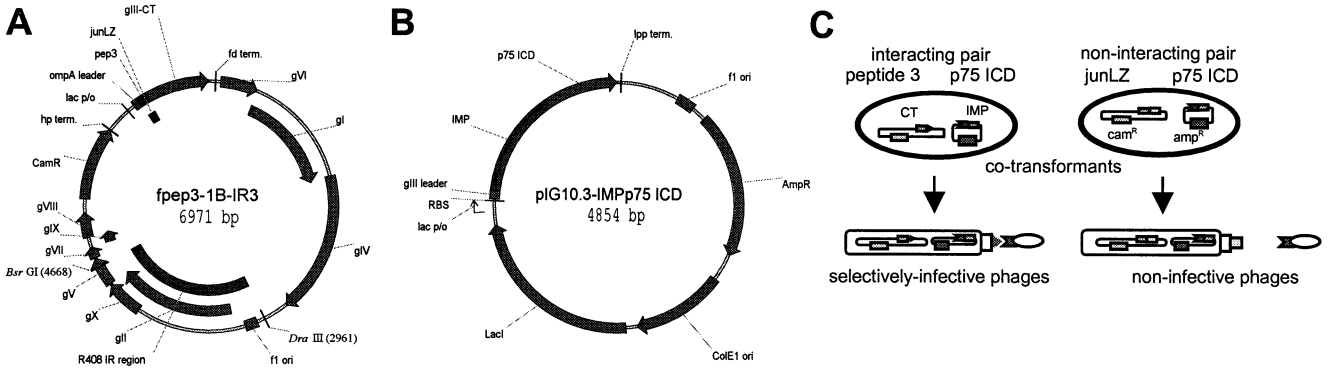


Fig. 2. SIP vectors and principle of 'library vs. library' screening. A: Vector map of the basic fd IR phage containing a fusion of either peptide 3 (pep3) or the jun leucine zipper (junLZ) to the C-terminal domain of gIII (gIII-CT). The IR region derived from f1 helper phage R408 and the other phage genes (labeled with roman numbers) are indicated. CamR, chloramphenicol acetyltransferase gene; lac p/o, lac promoter/operator; f1 ori, f1 phage origin of replication; fd/hp term., fd/hp terminator. B: Vector map of the pIG10.3 phagemid containing a fusion of p75-ICD to IMP. LacI, lac repressor; ColE1 ori, bacterial origin of replication; AmpR, β-lactamase gene. C: The principle of 'library vs. library' screening with SIP hetero-polyphages. Bacterial clones (i.e. combinatorial library members) containing an IR phage/phagemid combination which expresses an interacting protein pair (peptide 3/p75-ICD) produce SIP hetero-polyphages which contain the corresponding genetic information. This information can be selectively rescued by transduction after infection of F^+ bacteria. Cells which express a non-interacting pair (junLZ/p75-ICD) produce only non-infective phages.

has been demonstrated with phage mutants [26], and up to 30% co-encapsidation of a phagemid with an IR helper phage has been observed [21]. Since both vectors, IR phage and phagemid, contain packaging signals in their phage origins of replication, they should be co-packaged at some frequency into the same phage particle, resulting in a certain fraction of hetero-polyphages.

Native gel electrophoresis of intact phages with subsequent infection experiments was used to verify the production of hetero-polyphages with fIR3. The fIR3 vector, which carries a wild-type gIII (and thus produces constitutively infective phage) was transformed alone or together with the pIG10.3-IMPp75-ICD phagemid into bacteria. As a negative control, the non-IR, gIIIp N1–N2-deleted phage vector fjun-1B was co-transformed with the plasmid JB61 [27] carrying a complementing wild-type gIII (to produce infective phenocopy phages). Phages produced in the three different transformants were separated in an agarose gel under native conditions (Fig. 3A). This revealed that phages produced by fjun-1B in combination with a plasmid lacking a packaging signal, were predominantly faster migrating monophages (Fig. 3A, lane 2c) and to a much lesser extent polyphages (Fig. 3A, lane 2a/b). In contrast to that, the polyphage/monophage ratio was highly increased in case of fIR3 (Fig. 3A, compare lane 1a with 1c) and a packaging signal-containing phagemid (Fig. 3A, compare lane 3a with 3c), where the majority of polyphages did not enter the gel.

To assay for hetero-polyphages, homo-polyphages or mono-phages, phages of appropriate size were eluted from unstained regions of the gel and were subsequently used to infect bacteria. The presence of mono- and polyphages in different gel fractions was determined by transduction of phage- and phagemid-encoded antibiotic resistance markers into streptomycin-resistant JM103 cells and selection on double and triple antibiotics. The results indicated that slower-migrating phage populations, derived from the fIR3/pIG10.3-IMPp75-ICD co-transformants, contained significant numbers of hetero-polyphages which were able to co-transduce phage- and phagemid-encoded resistance markers (Fig. 3B, right panel, rows a and b). In contrast, very few hetero-polyphage transductants were obtained with the non-IR phage fjun-1B in combination with plasmid JB61, indicating that the plasmid is packaged very inefficiently (Fig. 3B, middle panel, rows a and b). In the fastest migrating gel fraction d, phage DNA was only visible with the combination fIR3/pIG10.3-IMPp75-ICD (Fig. 3A, lane 3). This fraction contained predominantly monophages which had only the phagemid vector packaged (Fig. 3B, right panel, row d) which demonstrated the need for a packaging signal for efficient encapsulation of vector DNA. Thus, even though fIR3 contains an intact gIIIp, it produces large amounts of polyphages, possibly because gIIIp may be expressed only at low levels from this phage construct.

3.3. Selection of protein interactions by SIP hetero-polyphages

Co-packaging of IR phage and phagemid into hetero-polyphages provides a way to non-covalently link the genetic information of separately-encoded, potentially interacting protein partners. The readout of whether they do interact is then the successful infection of a recipient cell. After the results obtained with the constitutively-infective fIR3, we wanted to test whether the IR genotype could be used to stably transduce the genetic information of an interacting pair in a SIP setting. For this purpose the wild-type gIII in fIR3 was replaced by either pep3-CT or jun-CT, rendering the resulting phages fpep3-IR3 and fjun-IR3 (Fig. 2A) non-infective. These phage vectors were individually co-transformed with pIG10.3-IMPp75-ICD and individual co-transformants confirmed by restriction analysis (Fig. 4, lanes 10 and 11). Co-transformants representing the interacting pair pep3/p75-ICD and the non-interacting pair jun/p75-ICD were mixed at equal amounts and the supernatant of the bacterial culture was tested for the presence of SIP hetero-polyphages. Restriction analysis of randomly picked, double-resistant transductants revealed that all transductants were derived from the interacting pep3/p75-ICD combination and contained the parental SIP vectors (Fig. 4, lanes 2–9). This result demonstrated the feasibility of using the IR phage to stably link and selectively co-transduce the genetic information of cognate protein partners via SIP hetero-polyphages.

We next wanted to test the sensitivity of the SIP/hetero-

polyphage approach under conditions which simulate selection from a library. Here, a small number of cells containing productive combinations of library members which can interact with each other and thus generate SIP would be diluted in a vast excess of cells containing non-productive combinations which would produce non-infective phages. Thus, serial dilutions of co-transformants containing the interacting pair pep3/p75-ICD were co-cultured with an excess of cells containing the non-interacting pair jun/p75-ICD. The presence and titer of correct SIP hetero-polyphages produced by these different co-cultures was determined by a transduction assay (Table 1). This showed that, down to at least a 10^{-4} dilution of pep3/p75-ICD co-transformants in jun/p75ICD co-transformants, it was possible to retrieve the pep3/p75-ICD interaction via SIP hetero-polyphage transductants. The SIP hetero-polyphage titer of positive control co-transformants expressing the pep3/p75-ICD interaction was approximately 6×10^5 t.u./ml whereas the non-interacting pair jun/p75-ICD did not produce any detectable SIP titer (Table 1). Swapping of pep3 and p75-ICD fusions, so that pep3-CT and IMP-p75-ICD were expressed on the pIG10.3 phagemid and IR phage, respectively, produced a similar SIP hetero-polyphage titer of 3×10^5 t.u./ml (data not shown).

3.4. In situ infection to reduce redundancy of transductants

Inevitably, the output of a 'library vs. library' screening performed in solution culture will have a certain degree of redundancy and bias in clone representation and possible 'swapping' of the adapter molecule. The existence of interacting pairs with different affinities and/or expression levels will generate different titers of SIP hetero-polyphages [28]. Thus, favored interactions will be over-represented, whereas weak interactions are likely to be under-represented or even lost. To address this problem, filter-based in situ infectivity screening (ISIS) was developed (Fig. 5). The primary combinatorial

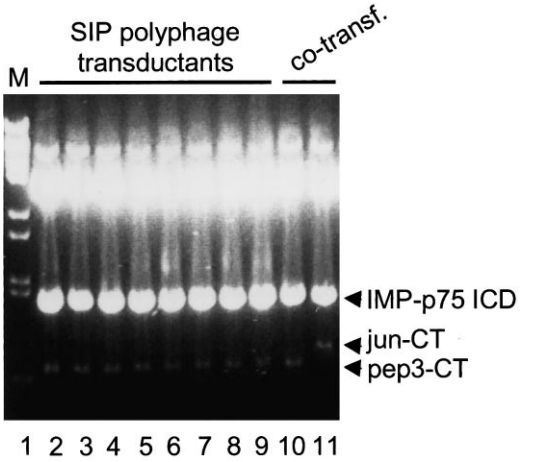
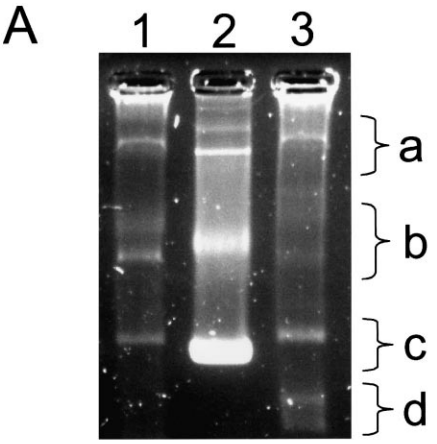


Fig. 4. SIP hetero-polyphages co-transduce the separately encoded genetic information for an interacting pair. Co-transformants containing the combinations fpep3-1B-IR3/pIG10.3-IMPp75-ICD (lane 10) and fjun-1B-IR3/pIG10.3-IMPp75-ICD (lane 11) were mixed and co-cultured. Phages produced by these co-cultures were used to infect K91 cells and transformants selected on LB cam/amp plates. Randomly picked transductants (lanes 2–9) were analyzed by restriction digest (*Xba*I/*Hin*dIII) and compared to the restriction pattern of the co-transformants. Migration of signature restriction fragments from the different vectors is indicated at the right of the gel. M, DNA molecular weight marker λ BstEII (lane 1).



B

	fIR3			fjun-1B + JB61			fIR3 + pIG10.3-IMPp75-ICD		
	a/s	c/s	c/a/s	a/s	c/s	c/a/s	a/s	c/s	c/a/s
a	6×10^2	7×10^5	$<10^2$	2×10^4	8.2×10^6	2×10^2	9.5×10^6	9.8×10^5	1.8×10^5
b	3×10^2	3.3×10^5	$<10^2$	9×10^3	1.9×10^7	10^2	4.4×10^6	9.6×10^5	6×10^4
c	$<10^2$	1.1×10^5	$<10^2$	2×10^3	2×10^8	9×10^2	1.2×10^6	2×10^6	8×10^2
d	10^2	10^3	$<10^2$	8×10^3	2×10^4	10^2	6.5×10^6	2×10^3	10^2

Fig. 3. Gel separation and typing of polyphages. A: Phenocopy phages (10^{10} t.u.), produced by gIII complementation of fjun-1B non-IR phages with the wild-type gIII-containing plasmid JB61 (lane 2), and phages (10^{10} t.u.) produced by the wild-type gIII-containing phage fIR3 in the presence (lane 3) or absence (lane 1) of phagemid pIG10.3-IMPp75-ICD were separated in a 1.8% agarose gel. After denaturation, phages were visualized in situ by SYBR Gold staining of phage DNA. B: Phage-containing areas of the gel (fractions a–d) were excised from unstained, parallel loadings and phages were eluted in LB medium. Titers (t.u./ml with respect to elution volume) of monophages and homo-/hetero-polyphages in the individual gel fractions were determined by infection of streptomycin-resistant JM103 cells and plating on double (a/s, c/s) or triple (c/a/s) antibiotics plates. a, ampicillin (for phagemid); c, chloramphenicol (for phage); s, streptomycin (for recipient strain).

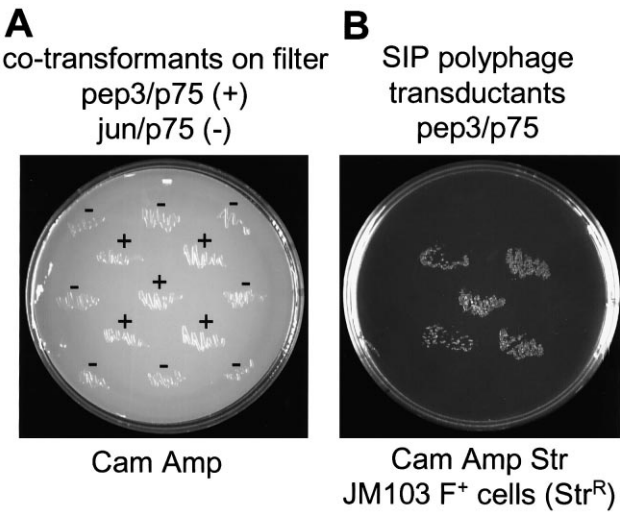


Fig. 5. In situ infectivity screening. A: Co-transformants containing the combinations fpep3-1B-IR3/pIG10.3-IMPp75-ICD (+) and fjun-1B-IR3/pIG10.3-IMPp75-ICD (−) were streaked in a defined pattern on a polycarbonate filter and grown on LB cam/amp plates. B: The filter was transferred to a second LB plate (cam/amp/str) containing streptomycin-resistant (Str^R) JM103 F^+ cells. After 3 h the filter was removed and the plate further incubated to visualize SIP hetero-polyphage transductants grown under triple-selective conditions.

library cells, i.e. co-transformants, are directly plated on a thin polycarbonate membrane (0.4 μm pore size) which is positioned on an agar plate. After growth of the combinatorial library, accompanied by hetero-polyphage production from the library members, the filter is transferred to a second plate containing F^+ recipient cells. SIP hetero-polyphages which diffuse through the pores of the filter can come into contact with the F^+ cells underneath and infect them. Since the primary combinatorial library is amplified only at colony level, this approach creates a non-redundant mirror image of only the productive combinations in the library via selective infection in situ. Fig. 5 shows the stringency and selectivity of ISIS with the model interaction pep3/p75-ICD in comparison with the non-interacting pairing jun/p75-ICD. This experiment demonstrated that only the interacting pair pep3/p75-ICD, but not jun/p75-ICD co-transformants were able to generate SIP hetero-polyphage transductants in situ.

4. Discussion

We have extended the SIP technology by encoding the two interacting partners on two different vectors which are co-packaged into phage particles. The system involves a combination of the SIP technology, to select protein interactions, with the use of polyphages to shuffle and link the genetic information contained in two libraries. SIP is a very sensitive technique to detect protein-protein interactions and inherently has a low background of false positives [11,23]. Using SIP, binders to diverse targets have been selected from antibody [10,29], peptide (Ilag et al., submitted) and cDNA [13,22] libraries. The lower affinity threshold required for successful in vivo SIP has not been extensively tested so far and may depend on the particular system studied. For example, Fos and Jun leucine zippers, which have a K_D of approximately 10^{-7} M [30], could be demonstrated to interact in in vivo SIP [13].

There are a number of alternative approaches to cloning protein interactions, some of which also address the problem of 'library vs. library' screening. The yeast two-hybrid system has been used in combination with a yeast mating/diploid replica plating approach for a genome-wide screen of the *E. coli* bacteriophage T7 to detect interactions among the 55 proteins encoded in the T7 genome [31]. Fromont-Racine et al. [32] have approached the problem of a genome-wide, comprehensive screen for protein interactions of yeast by using a modified mating strategy on filter. Starting from a defined set of 'anchor' sequences, successive rounds of yeast two-hybrid screenings were performed where selected prey sequences were chosen as new baits in subsequent screenings. It was stated that the efficiency of mating on filters (10–20%) would allow screening of more than 4×10^7 combinations on about 100 plates. Although there appear to be exceptions [33,34], a possible disadvantage of using the yeast two-hybrid system for a universal, exhaustive interaction screen could be the relative inaccessibility of the important group of secreted and transmembrane proteins as targets, since interactions take place in the reducing environment of the nucleus. In principle, this class of proteins can be targeted by SIP, since interactions are formed in the oxidizing environment of the periplasmic space of bacteria. This allows formation of disulfide bridges, important for structure and function of extracellular proteins, which is illustrated by functional expression of antibodies in bacteria [20]. However, in cases where eukaryotic post-translational modifications are required for interaction of two proteins, expression in bacteria will be a disadvantage.

Independent of expression criteria, it remains to be shown what combinatorial library sizes – and thus the number of possible combinations of two libraries – can be screened with the yeast approach. This will depend on cloning, transformation and mating efficiencies. With respect to library size, advantages for the SIP/polyphage approach are two-fold. (i) Very high transformation efficiencies are now achievable for bacteria by electroporation which greatly facilitates the generation of large individual libraries. (ii) In turn, highly efficient combinations of the individual libraries can then be achieved by infection with phenocopy phages rather than by transformation techniques. For this, the phage-encoded library is generated in a bacterial strain which has a complementing wild-type gIII stably integrated into the bacterial genome. Phenocopy phages which display wild-type gIIIp but have gIII-defective library phage DNA packaged (and thus are infective for only one round) are then used to infect bacteria which contain the phagemid-encoded library genome (results to be published). In this context, the IR polyphage approach could not only be used to generate large combinatorial libraries for SIP interaction screens but, in principle, also for other systems like Fab display libraries, where high combinatorial diversity is desired. The hetero-polyphage system offers a possible alternative to Cre-*lox*-mediated in vivo recombination [35] for generation of large and stable Fab libraries, and also offers itself for simultaneous mutagenesis of protein-protein interfaces (e.g. ligand-receptor). While recombinations generated via Cre-*lox* suffer to some extent from reversibility, this problem apparently does not occur with hetero-polyphages which could be regarded as a non-covalent, stable 'cross-over'.

Alternatively to yeast two-hybrid or SIP, a combination of bacterial and phage display which could also be used for 'library vs. library' screening has been developed recently [36]. Here, members of one library are expressed as fusions to the bacterial traA gene which codes for the building blocks of the F pilus. Members of the second library are co-expressed as fusions to the gIII C-terminal domain together with wt gIIIp, similar to conventional phage display. Interaction of a pilus-displayed protein with a phage-displayed cognate partner molecule then leads to a selective infection event, combining the genetic information for an interacting pair in the same bacterial clone. However, since infection was not absolutely dependent on interaction of cognate partners, the enrichment factor for a cognate pair was only about 1000-fold after one round of infection [36]. Moreover, with this approach it is likely that there are rather stringent limitations to fusion partner size for insertion into *traA* in order to maintain functional pilus assembly. So far, we have been able to express proteins of up to 65 kDa (UL84 transcriptional repressor [37] from cytomegalovirus) as CT fusions and up to 40 kDa (TrkA [38] extracellular domain; R. Kramer, unpublished results) as IMP fusions in the SIP system (data not shown). Preliminary results from a SIP 'library vs. library' screening of the human cytomegalovirus genome suggest that a range of eukaryotically-expressed proteins can also be expressed in the bacterial SIP system, at least to levels sufficient to produce SIP phages (C. Löhning and F.R., unpublished results).

In comparison with the different interaction cloning systems discussed above, SIP/polyphage combines several advantageous features to make it a promising candidate system for

high throughput interaction screening. We are currently employing this approach (i) to simultaneously select single chain Fv antibodies against multiple targets and (ii) to build a protein linkage map of the cytomegalovirus genome.

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