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Expression of anti-Z-DNA single chain antibody variable fragment on the filamentous phage surface

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Abstract

We describe the expression of an anti-Z-DNA single chain variable region antibody fragment (scFv) on a filamentous phage surface. Four vectors for phage display were constructed. Two of them are able to display multiple copies of the antibody fragment, and the others can be used to make monovalent libraries. The vectors use different promoter/leader sequences to direct the expression of the fused proteins. All were able to promote the assembly of fusion virion particles. In this paper we also show the affinity selection (biopanning) of those phage-antibodies based on the capacity of their products to recognize the antigen. We used biotinylated Z-DNA and the selection was performed in a solution phase fashion. The data presented here indicate that these vectors can be further used to construct anti-nucleic acid antibody fragment libraries that can be used to study the basis of nucleic acid-protein interaction and its role in autoimmunity mechanisms.

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- Phage display
- Biopanning
- scFv
- Z-DNA

Introduction

Anti-Z-DNA antibodies are found in serum of patients with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (1). It is also possible to induce them by immunization with Br-(dG-dC)$_n$, a stable Z-DNA conformation polymer (2). Thus, the study of the basis of nucleic acid recognition by this kind of antibodies can provide insights into the structural relationship between autoantibodies and vaccinal antibodies to this class of antigens. These studies must include the production of mutant forms of those antibodies and measurement of their residual affinity/specificity.

An important technology that arose in 1988 for studying macromolecular interactions is the display of foreign proteins and peptides on the surface of filamentous phages (3). This technique permits the generation of libraries of molecules with affinities for certain ligands, and when it is associated with biopanning and selection, new high-affinity forms can be isolated. Antibodies (particularly their Fvs - variable fragment and/or Fabs - antigen binding fragments) are the most common biomolecules expressed on the filamentous phage surface. The association of phage display libraries with biopanning can mimic the immune system clonal selection (4). The development of the antibody display on the filamentous surface has been the subject of extensive investigations (for a more extensive review, see Ref. 5). Using this approach it was possible to isolate

new high-affinity antibody forms (4,6), or even change specifities of well-characterized ones (7,8).

Phage display libraries are usually assembled in two different kinds of vectors. The initial studies were done using phage vectors derived from the filamentous phage genome, such as fd, f1 and M13 (9). More recently, the literature has proposed the use of phagemid vectors containing the filamentous phage intergenic region that comprises the rolling circle phage origin of replication which enables it to pack into the fusion virion particle (10). This last type of vector is used to transform the F^+ host strain and the library can be rescued by helper phage (11). The advantage of using phagemids is the easier manipulation of a plasmid DNA over a phage genome that facilitates cloning and DNA preparation.

A phagemid to be used for phage display library purposes must have some specific features. First, it should be able to produce N-terminal fusion molecules (library) with one of the phage coat protein. Usually, the most common are protein VIII, the major coat protein (pVIII), and protein III, one of the proximal end protein responsible for virus infectivity (pIII). For this purpose, a leader sequence (for secretion) and a weak promoter always precede the fusion system. Besides these characteristics, a phagemid must have a selection marker (to allow selection of the transformed strains) and both bacterial and viral replication origins (12).

The choice of gene VIII or III fusion is based on the experimental hypothesis addressed. Since gene VIII expresses the major coat protein, the fusion molecule with protein VIII is displayed on the virion surface in multiple copies, so the selection is also a function of the avidity of these repeated molecules. These libraries are called poly- or multivalent (13). If the choice is to construct a monovalent library, the fusion virion partner should be protein III since each virion has 5 to 6 copies of them (14) and the fusion particle would display 1 to 3 fusion molecules (13). Recent studies have proposed the use of multivalent libraries for initial screening experiments and in a second step, when refined ligands are desired, the monovalent libraries would be the choice (15).

In this paper we describe the display of an anti-Z-DNA antibody fragment (scFv) on the filamentous bacteriophage surface in a mono- or multivalent fashion. To produce these fusion particles we have constructed two new vectors and modified a third one to construct two additional ones. We also show that the particles that display anti-Z-DNA are selected by the interaction with biotinylated Z-DNA. This study represents the first step in identifying the amino acid residues that play an important role in the nucleic acid recognition by antibodies.

Material and Methods

Construction of pAIg 316 and 816 vectors

Multistep construction was carried out using standard molecular biology techniques (16). pAIg 316 was constructed in two steps: initially, the M13 gene III was amplified by PCR using a pair of primers (5' GCCCATGG CTCCGGTACCGGTACCGAAACTGTTGAA AGTTG 3' and 5' GCGAATTCTGGCATGA TTAAGACTC 3') designed to generate a fragment of 1.2 kb, encoding the mature form of pIII and flanked by *Nco*I and *Eco*RI restriction sites. The design was performed based on M13 mp18 sequence data obtained from the gene bank (access number X02513). The PCR product was digested with both restriction endonucleases and cloned into pIg 16, a vector that codes for the scFv fragment of the anti-Z-DNA Z22 antibody (17). This vector was named pIg 316. The next step was transfer the fusion (scFv/gene III) to the pGEM 3Z (f-) phagemid (Promega®, Madison, WI, USA). The recipient plasmid and the donor were digested with *Xma*I and *Eco*RI, and the fusion frag-

ment was ligated to pGEM 3z phagemid, giving origin to phagemid pGIg 316. The protein A promoter and leader sequence (PPLA) was initially obtained from the pGTT 15 plasmid (18) by PCR and subsequently digested with *Bcl*I and *Hin*dIII endonucleases. The sequences of the primers used were 5' GATTTAGGTGACACTATAG 3' and 5' CGCCCGGGCTTTTGTCACAGG 3' for the amplification of the promoter/leader sequence from upstream sequences and carboxyl terminus, respectively. The PPLA fragment was cloned into pGIg 316 *Bam*HI and *Hin*dIII unique sites, giving origin to phagemid pAIg 316, which is able to express Z22 scFv fused with pIII. Phagemid pAIg 816 was constructed by replacing the pAIg 316 gene III with gene VIII. Gene VIII was obtained by PCR, using a pair of primers (5' CCCATGGCTCCGGTACCGCTGAGGGT GACGAT 3' and 5' GAGCCTTGAATTCTA TCGGTTTATC 3') designed to amplify the mature portion of the M13 pVIII gene (approximately 150 bp), flanked by *Nco*I and *Eco*RI restriction sites. The primers were designed based on the same sequence as described above (access number X02513). The PCR product was ligated to the pAIg 316 vector gene III-less fragment, and the resulting phagemid was called pAIg 816. The two vectors were checked by nucleotide sequencing of the fusion regions: leader sequence/scFv and scFv/gene III and VIII.

Construction of pCIg 316 and 816 vectors

Phagemids named pCIg were derived from the pCANTAB 5E Pharmacia® vector (Uppsala, Sweden). To construct these vectors the linear recipient plasmid pCANTAB 5E was ligated to a DNA linker, harboring *Xma*I, *Bgl*II, *Xba*I and *Nco*I restriction sites, giving rise to the pCL vector. The oligonucleotides used to make the synthetic linker showed the following sequences: 5' CGGC CCGGGAAGATCTCTAGATCCCATGG TGC 3' and 5' GGCCGCACCATGGGATCT AGAGATCTTCCCGGGCCG 3'. The annealing procedure was performed by mixing equimolar quantities of both oligonucleotides, heating them and allowing them to cool down to room temperature under high salt conditions. The annealed linker harbors cohesive ends (*Not*I and *Sfi*I) that permit it to ligate to linearized pCANTAB 5E. The pCL vector was digested with *Xma*I and *Nco*I and ligated to the scFv fragment isolated from pGIg 316, giving origin to pCIg 316. Phagemid pCIg 816 was obtained by replacing the pCIg 316 gene III with gene VIII (the same used to construct pAIg 816), that was cloned into the pGEM-T vector, showing *Nco*I and *Eco*RI restriction sites. As a negative control for fusion protein production, the pCIg 16 plasmid was also constructed from pCL, introducing the fusion scFv/protein A from the pIg 16 vector instead of scFv/gene III or VIII at the same sites, *Xma*I and *Eco*RI. This last vector (pCIg 16) produces soluble Z22 scFv without a phage protein partner. The constructions were checked by nucleotide sequencing, mainly in their fusion regions: scFv/gene III or VIII.

Phage production and panning

Fusion phage particles were produced, titrated and selected using a protocol modified from the Recombinant Phage Selection Module (Pharmacia®). Briefly, transformed *E. coli* cells (strain TG1) were inoculated into 2X YT medium containing 2% glucose. The cultures were incubated at 37ºC with shaking at 250 rpm until they reached an A_{600} of 0.5. At this point ampicillin was added to a final concentration of 100 µg/ml and 3 x 10^{10} plaque-forming units of helper phage M13K07. The cultures were incubated for 1 h under the same conditions and then spun at 3,000 *g* for 10 min to sediment the cells. The cell pellets were resuspended in 2X YT medium containing ampicillin (100 µg/ml) and kanamycin (50 µg/ml) and incubated overnight at 37ºC with shaking at 250

rpm. The supernatants, containing fusion phages, were obtained by centrifuging the cell cultures at 3,000 *g* for 20 min. The phage preparations were titrated to determine the yield of ampicillin-transducing units per milliliter for each construction. These procedures were carried out by infecting log-phase *E. coli* TG1 cells with serial supernatant dilutions. A typical phage yield obtained was 10^{10} to 10^{11} ampicillin-transducing units/ml. The amount of transducing units used for solution phase selection was 3 to 4 x 10^{10} for each construction. In this experiment we used transducing units from all five constructions: pAIg 316, pAIg 816, pCIg 316, pCIg 816 and also pCIg 16 (virion particle without fusion protein) as a negative control.

The selection procedure was performed in a final volume of 1.4 ml containing the phage suspension diluted in PBS, blocking buffer (280 µl of 10% nonfat milk) and biotinylated Z-DNA (7 µl of a stock solution of 66 µg/ml prepared as described in Ref. 17). Initially, the fusion phage suspension was incubated with blocking buffer for 10 min followed by incubation with the antigen for 1 h at room temperature, with gentle rocking on a shaker table. At this point 60 µl of resuspended streptavidin-agarose resin was added and further incubated for 20 to 60 min under the same conditions. The samples were spun at 1,000 *g* for 1 min and the supernatant was removed. The pellet was washed 6 times with 0.05% PBS-Tween 20 by discarding the supernatant after centrifugation at 1,000 *g*. The washed agarose was transferred to 10 ml of log-phase TG1 *E. coli* cells to allow infection. Several dilutions of the transduced cells were plated onto SOBAG/ampicillin plates and incubated at 30°C, and the number of individual clones was determined. After this first round of selection, additional rounds were also performed. The estimated amount of transducing units per cycle was determined for each construction by subtracting the average number of colonies obtained with transformed pCIg 16 cells. We used the average number of pCIg 16 transducing units to represent the background of nonspecific binding of helper phage particles.

Results and Discussion

To produce virion particles harboring Z22 scFv we constructed two new vectors and modified the Pharmacia pCANTAB 5E vector, creating two new versions of the latter.

Vector pAIg 316 was assembled by amplifying and cloning both virus gene (gene III) and promoter and leader sequences of the *Staphylococcus aureus* protein A gene. Gene III was obtained from the M13 genome with the primers described in Material and Methods. These primers create restriction enzyme sites (*Nco*I at the 5' end and *Eco*RI at the 3' end). The 1.2-kb fragment was digested with both enzymes and cloned into the pIg 16 vector (17). The whole cassette (scFv/gene III) was then transferred to the Promega pGEM 3Z f(-) vector in order to receive the promoter and leader sequences of the *Staphylococcus aureus* protein A gene (fragment PPLA). This new vector (pGIg 316) was then digested with *Hin*dIII and *Bam*HI to receive the PPLA fragment. This last fragment was obtained by PCR of the *S. aureus* protein A gene cloned into the pGTT 15 vector (18). The PCR fragment was used instead of the original pGTT 15 to avoid the inhibitory effect of methylation of the *Bcl*I site. The PCR fragment (1.5 kb) was then cleaved with *Hin*dIII and *Bcl*I, giving origin to a 0.8-kb fragment that was purified and cloned into linear pGIg 316. This vector was named pAIg 316 (the whole strategy of construction of this vector is presented in Figure 1). The production of the fusion protein was observed in Southern-Western blot experiments by comparing the ability of the fusion and wild type particles to bind Z-DNA (data not shown).

To construct the pAIg 816 vector (the strategy is also presented in Figure 1), the M13 gene VIII was amplified by PCR and cloned between the *Nco*I and *Eco*RI sites of

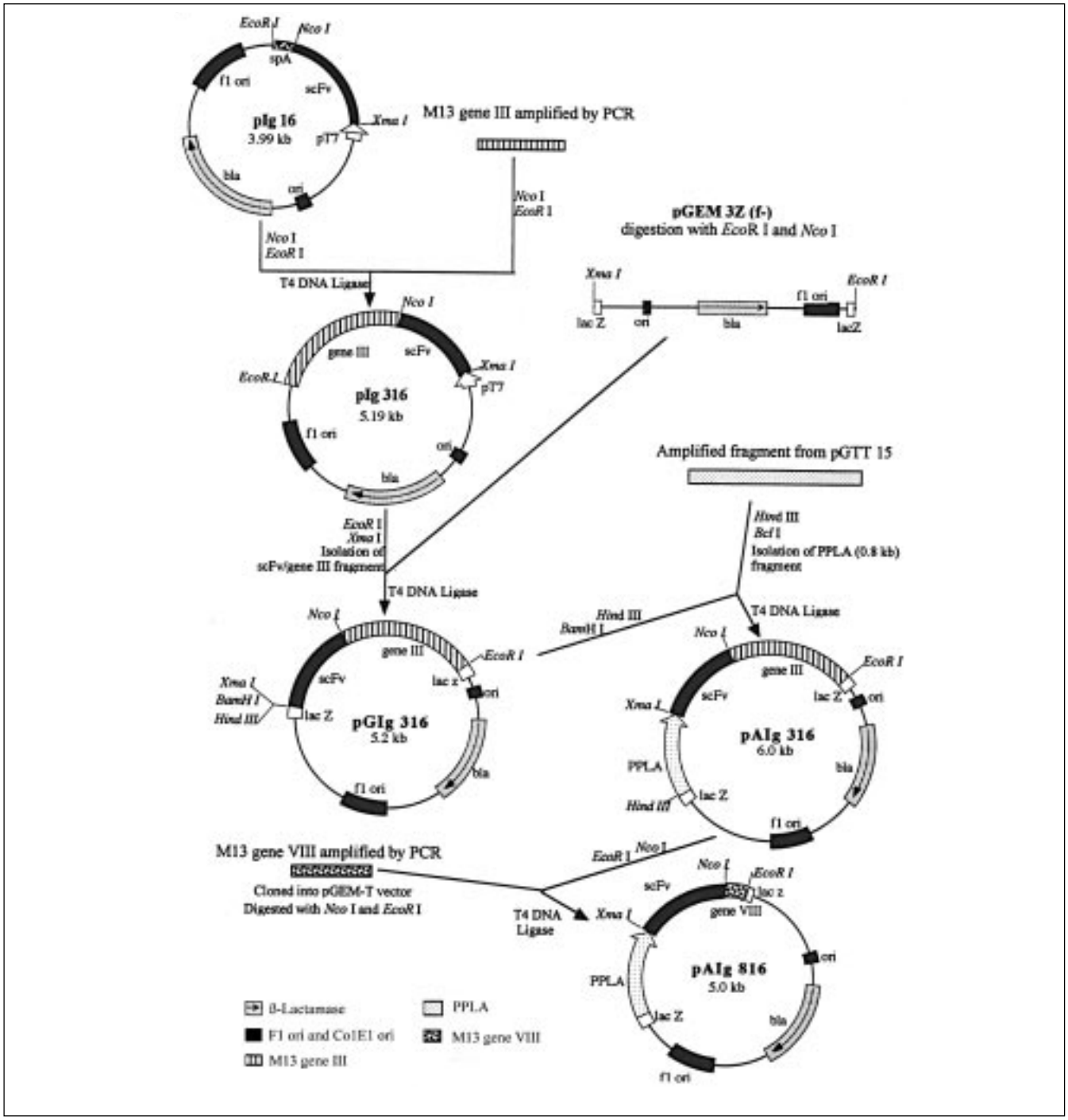

Figure 1 - Schematic representation of pAIg vector constructions. The M13 gene III was obtained by PCR with specially designed oligonucleotides (see Material and Methods) and then transferred to the pIg 16 vector (17). This originated the pIg 316 vector. The whole cassette single chain variable region antibody fragment (scFv)/gene III was cloned into *Xma*I and *Eco*RI sites of the Promega vector pGEM 3Z (f-), giving rise to the pGIg 316 plasmid. The fragment containing the *S. aureus* protein A promoter and leader sequence (PPLA) was obtained by PCR and a subsequent digestion with *Bcl*I and *Hin*dIII from the pGTT 15 vector (18). This insert was cloned into *Hin*dIII and *Bam*HI sites of pGIg 316, originating the pAIg 316 phagemid. To construct pAIg 816, the M13 gene III was replaced by the M13 gene VIII, which was obtained by PCR with specially designed primers from the M13 genome. Gene VIII was cloned into *Nco*I and *Eco*RI sites.

the gene III-less pAIg 316 phagemid.

The phagemids pCIg 316 and 816 were constructed from the pCANTAB 5E vector (Pharmacia). This plasmid is available commercially in linear form. To transfer Z22 scFv to this vector we constructed and cloned a synthetic linker and the resulting plasmid was called PCL. Between *Xma*I and *Nco*I PCL restriction sites we cloned the pIg 16 scFv, and this vector was called pCIg 316. Phagemid pCIg 816 was obtained by replacing pCIg 316 fd gene III with M13 gene VIII (obtained by PCR as described for pAIg 816). The strategies of these constructions are schematically presented in Figure 2. As a negative control, we also constructed pCIg 16, which is a vector that produces soluble scFv, without a virus partner. Thus, cells harboring pCIg 16 produce wild type phage particles.

The phagemids express a pIII (316) or pVIII (816) product fused with anti-Z-DNA scFv. In two of them (pAIgs) the production of fusion molecules is under the control of the *Staphylococcus aureus* protein A promoter and leader sequence, while the other two (pCIgs) are derived from the pCANTAB 5E Pharmacia® vector (which has the pLac-lactose operon promoter, and the fd phage gene III leader sequence).

The open reading frame-predicted sequences of the fusion proteins are shown in Figure 3. The nucleotide sequences of all four vector fusion regions were determined by sequencing procedures and are highlighted in the same figure. These determinations revealed that at least at these regions the open reading frames, as expected, were able to produce fusion proteins in a correct frame.

Fusion virion particles were produced by standard protocols and selected in a solution-phase assay: phage antibodies were mixed with biotinylated antigen (Z-DNA) and the complex was captured from solution with streptavidin-agarose resin. The washed streptavidin/biotinylated antigen/phage antibody complex was used to infect log-phase *E. coli* TG1 cells which could be rescued with the helper phage, selected again or plated onto SOBAG plates. All four vectors were able to produce phage-displaying scFv, since it was possible to select them using Z-DNA as antigen.

The results of three rounds of selection (Table 1) show a 100- to 10,000-fold increase in transducing units/ml in round 3 when compared with round 1 of selection. This is expected, since no diversity was introduced in the scFv sequence from one round to another, so that selection favored the virion particles able to bind to Z-DNA.

Using these four vectors one would expect a larger amount of colonies arising from 816 vectors than from 316 vectors due to their multivalent display of fusion proteins (19). According to our results, only pCIgs showed this feature: in round 3 we obtained about 20 times more transducing units/ml using pCIg 816 than using pCIg 316. The data for the pAIg vectors did not show this behavior; in fact pAIg 316 yielded 5 times more transducing units/ml than pAIg 816. This discrepancy may be the result of the expression level driven by two different promoters. The staphylococcal promoter (pAIg vectors) is a constitutive promoter while pLac (pCIg vectors) is an inducible one. In our experimental procedure for phage production we used a condition of no induction of pLac (medium without inductor), so that the expression of scFv/pIII or pVIII is a result of basal pLac expression, and, in this situation, the expression is considered to be weak (20). On the other hand, *Escherichia coli* cells harboring the original protein A expression vector, pGTT 15, express a constitutively significant amount of this staphylococcal protein (data not shown). Perhaps the expression driven by this promoter/leader sequence is high enough to affect virus production (21). Another aspect that should be considered is that our scFv-phage was selected against Z-DNA, which is a monotonous molecule, with repeated epitopes (22).

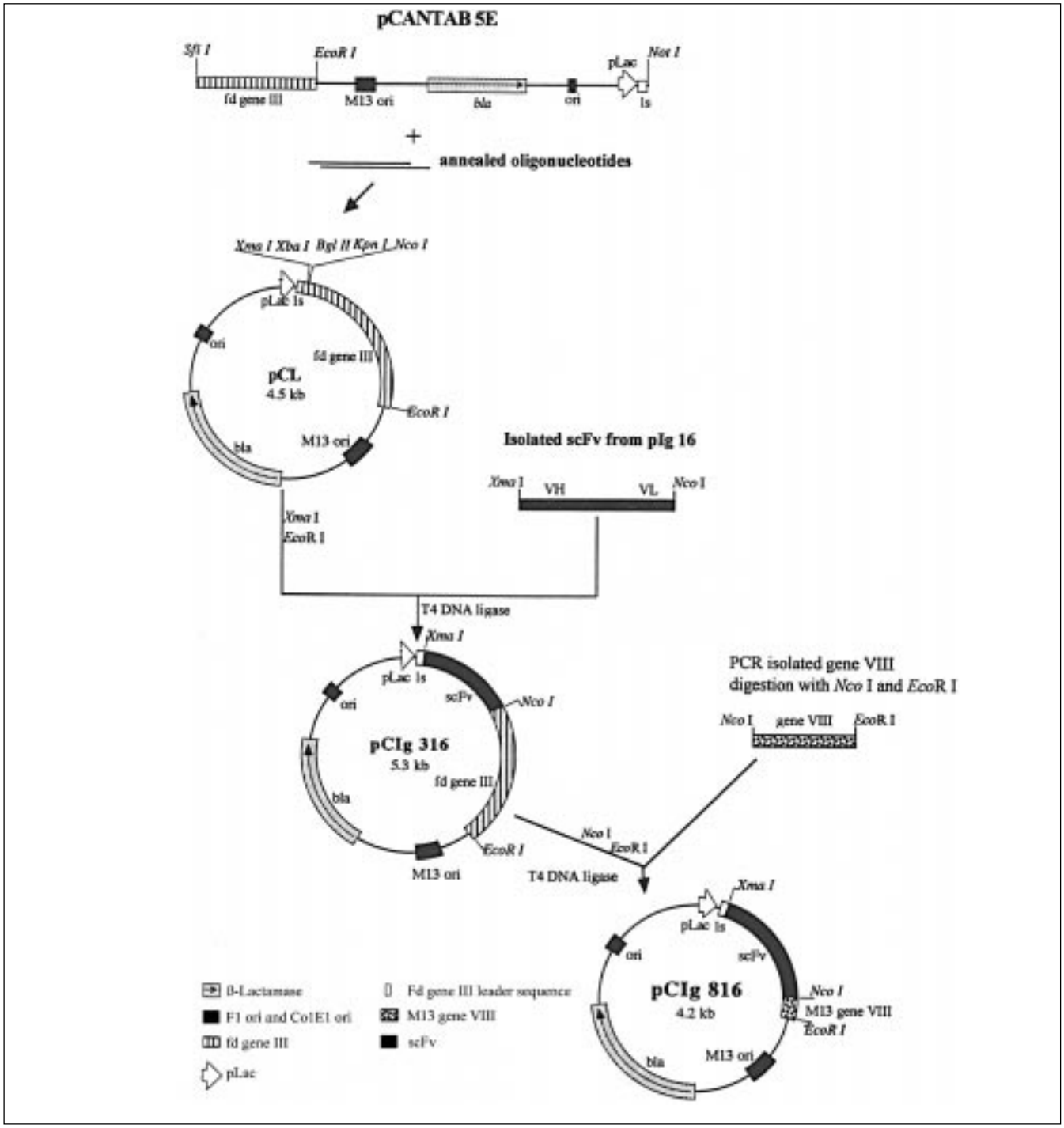

Figure 2 - Schematic representation of pCIg vector constructions. The Pharmacia pCANTAB 5E vector was modified to express the Z22 single chain variable region antibody fragment (scFv) fusioned with fd gene III. This commercial vector is available in a linear fashion. To clone the scFv, we designed self-annealed oligonucleotides to create a linker (see Material and Methods). The annealed oligonucleotides were cloned into the pCANTAB 5E vector, giving rise to the pCL plasmid. The scFv was obtained from the pIg 16 vector (17). This cloning procedure originated the pCIg 316 vector. To construct pAIg 816, fd gene III was replaced by M13 gene VIII, which was obtained by PCR with specially designed primers from the M13 genome. Gene VIII was cloned into the *Nco*I and *Eco*RI sites, giving rise to the pCIg 816 phagemid.

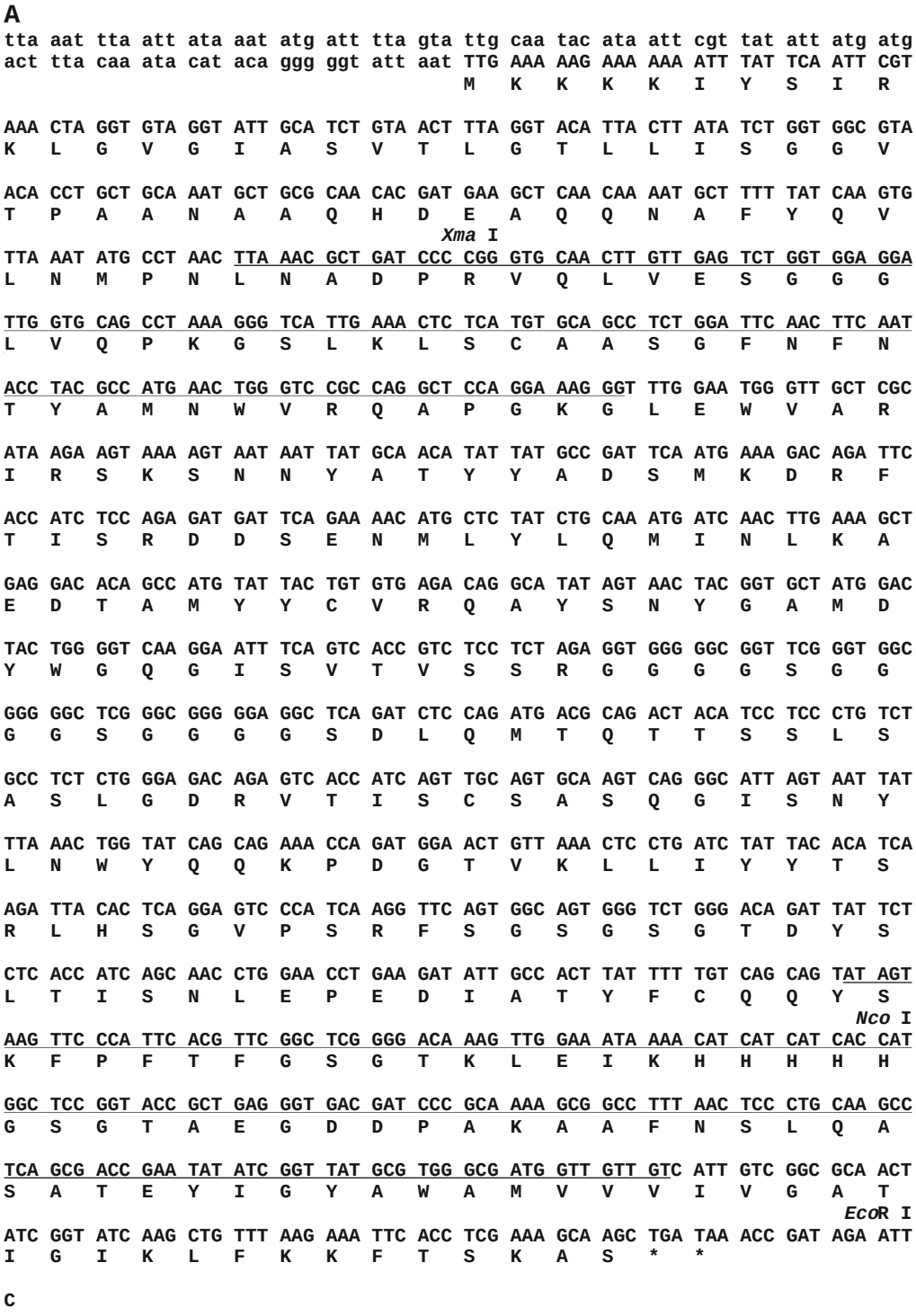

Figure 3 - Open reading frame and predicted fusioned polypeptides coded by phage display vectors. *A*, Open reading frame and amino acid prediction of scFv-M13 gene VIII fusion cloned into pAlg 816 vector. The non-coding sequence of PPLA fragment is in lower case. The restriction sites used for the cloning procedure are shown. The underlined nucleotides were checked by sequencing procedures. These regions were also sequenced for the other three vectors. *B*, Open reading frame of M13 gene III amplified by PCR from the M13 genome. The introduced sites are shown. *C*, Sequence of the pLac and fd gene III leader sequence of pCIg vectors. The introduced *Xma*I site is shown. This restriction site was used to clone Z22 anti-Z-DNA scFv. The non-coding region of pLac is in lower case.

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B

Figure 3 - continued.

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    NcoI
CAC CAT GGC TCC GGT ACC GAA ACT GTT GAA AGT TGT TTA GCA AAA CCC CAT ACA GAA AAT
H   H   G   S   G   T   E   T   V   E   S   C   L   A   K   P   H   T   E   N

TCA TTT ACT AAC GTC TGG AAA GAC GAC AAA ACT TTA GAT CGT TAC GCT AAC TAT GAG GGT
S   F   T   N   V   W   K   D   D   K   T   L   D   R   Y   A   N   Y   E   G

TGT CTG TGG AAT GCT ACA GGC GTT GTA GTT TGT ACT GGT GAC GAA ACT CAG TGT TAC GGT
C   L   W   N   A   T   G   V   V   V   C   T   G   D   E   T   Q   C   Y   G

ACA TGG GTT CCT ATT GGG CTT GCT ATC CCT GAA AAT GAG GGT GGT GGC TCT GAG GGT GGC
T   W   V   P   I   G   L   A   I   P   E   N   E   G   G   G   S   E   G   G

GGT TCT GAG GGT GGC GGT TCT GAG GGT GGC GGT ACT AAA CCT CCT GAG TAC GGT GAT ACA
G   S   E   G   G   G   S   E   G   G   G   T   K   P   P   E   Y   G   D   T

CCT ATT CCG GGC TAT ACT TAT ATC AAC CCT CTC GAC GGC ACT TAT CCG CCT GGT ACT GAG
P   I   P   G   Y   T   Y   I   N   P   L   D   G   T   Y   P   P   G   T   E

CAA AAC CCC GCT AAT CCT AAT CCT TCT CTT GAG GAG TCT CAG CCT CTT AAT ACT TTC ATG
Q   N   P   A   N   P   N   P   S   L   E   E   S   Q   P   L   N   T   F   M

TTT CAG AAT AAT AGG TTC CGA AAT AGG CAG GGG GCA TTA ACT GTT TAT ACG GGC ACT GTT
F   Q   N   N   R   F   R   N   R   Q   G   A   L   T   V   Y   T   G   T   V

ACT CAA GGC ACT GAC CCC GTT AAA ACT TAT TAC CAG TAC ACT CCT GTA TCA TCA AAA GCC
T   Q   G   T   D   P   V   K   T   Y   Y   Q   Y   T   P   V   S   S   K   A

ATG TAT GAC GCT TAC TGG AAC GGT AAA TTC AGA GAC TGC GCT TTC CAT TCT GGC TTT AAT
M   Y   D   A   Y   W   N   G   K   F   R   D   C   A   F   H   S   G   F   N

GAA GAT CCA TTC GTT TGT GAA TAT CAA GGC CAA TCG TCT GAC CTG CCT CAA CCT CCT GTC
E   D   P   F   V   C   E   Y   Q   G   Q   S   S   D   L   P   Q   P   P   V

AAT GCT GGC GGC GGC TCT GGT GGT GGT TCT GGT GGC GGC TCT GAG GGT GGT GGC TCT GAG
N   A   G   G   G   S   G   G   G   S   G   G   G   S   E   G   G   G   S   E

GGT GGC GGT TCT GAG GGT GGC GGC TCT GAG GGA GGC GGT TCC GGT GGT GGC TCT GGT TCC
G   G   G   S   E   G   G   G   S   E   G   G   G   S   G   G   G   S   G   S

GGT GAT TTT GAT TAT GAA AAG ATG GCA AAC GCT AAT AAG GGG GCT ATG ACC GAA AAT GCC
G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A   M   T   E   N   A

GAT GAA AAC GCG CTA CAG TCT GAC GCT AAA GGC AAA CTT GAT TCT GTC GCT ACT GAT TAC
D   E   N   A   L   Q   S   D   A   K   G   K   L   D   S   V   A   T   D   Y

GGT GCT GCT ATC GAT GGT TTC ATT GGT GAC GTT TCC GGC CTT GCT AAT GGT AAT GGT GCT
G   A   A   I   D   G   F   I   G   D   V   S   G   L   A   N   G   N   G   A

ACT GGT GAT TTT GCT GGC TCT AAT TCC CAA ATG GCT CAA GTC GGT GAC GGT GAT AAT TCA
T   G   D   F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N   S

CCT TTA ATG AAT AAT TTC CGT CAA TAT TTA CCT TCC CTC CCT CAA TCG GTT GAA TGT CGC
P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q   S   V   E   C   R

CCT TTT GTC TTT AGC GCT GGT AAA CCA TAT GAA TTT TCT ATT GAT TGT GAC AAA ATA AAC
P   F   V   F   S   A   G   K   P   Y   E   F   S   I   D   C   D   K   I   N

TTA TTC CGT GGT GTC TTT GCG TTT CTT TTA TAT GTT GCC ACC TTT ATG TAT GTA TTT TCT
L   F   R   G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V   F   S
                                                        EcoR I
ACG TTT GCT AAC ATA CTG CGT AAT AAG GAG TCT TAA TCA TGC GAA TTC
T   F   A   N   I   L   R   N   K   E   S   *
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Figure 3 - continued.

C

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ctc aca tgt tct ttc ctg cgt tat ccc ctg att ctg tgg ata acc gta tta ccg cct ttg

agt gag ctg ata ccg ctc gcc gca gcc gaa cga ccg agc gca gcg agt cag tga gcg agg

aag cgg aag agc gcc caa tac gca aac cgc ctc tcc ccg cgc gtt ggc cga ttc att aat

gca gct ggc acg aca ggt ttc ccg act gga aag cgg gca gtg agc gca acg caa tta atg

tga gtt agc tca ctc att agg cac ccc agg ctt tac act tta tgc ttc cgg ctc gta tgt

tgt gtg gaa ttg tga gcg gat aac aat ttc aca cag gaa aca gct ATG ACC ATG ATT ACG
                                                          M   T   M   I   T
CCA AGC TTT GGA GCC TTT TTT TTG GAG ATT TTC AAC GTG AAA AAA TTA TTA TTC GCA ATT
P   S   F   G   A   F   F   L   E   I   F   N   V   K   K   L   L   F   A   I
                                                  Xma I
CCT TTA GTT GTT CCT TTC TAT GCG GCC CAG CCG GCC CGG G
P   L   V   V   P   F   Y   A   A   Q   P   A   R
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Table 1 - Selection of phage-antibodies with biotinylated Z-DNA[a].

[a]Results of a typical experiment. [b]The number of ampicillin-transducing units (tu) was determined by plate counting and subtraction of the number of tu yielded by pCIg 16 (a vector that produces soluble scFv)-harboring cells, using the same selection procedures. The selection of all constructions was done in the same typical experiment. When it was possible to count two or more colony plates, the number shown is the corrected average of the dilutions.

Construction	Number of tu/ml in round 1[b]	Number of tu/ml in round 3[b]
pAIg 316	1.4×10^3	7.4×10^6
pAIg 816	1.7×10^3	1.6×10^6
pCIg 316	4.6×10^3	7.7×10^5
pCIg 816	2.9×10^3	1.5×10^7

This makes this antigen multivalent itself, so that a single Z-DNA molecule could bind to more than one phage, masking a multivalence library effect.

The vectors and protocols presented in this paper suggest a new experimental model for displaying libraries of anti-Z-DNA scFv. These vectors may help to investigate the basis of DNA-protein interaction and could be useful to generate specificities for nucleic acid such as those found in patients with autoimmune disease.

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