

## Screening of a Library of Phage-displayed Peptides Identifies Human Bcl-2 as a Taxol-binding Protein

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A random library of phage displayed peptides was screened for binding to a biotinylated derivative of paclitaxel (Taxol). Affinity-selected peptides were analyzed for similarity to human proteins. There was no significant similarity between the paclitaxel-selected peptides and tubulin. However, a subset of the peptides was identified that exhibits significant similarity to a non-conserved region of the anti-apoptotic human protein Bcl-2: ELISA assays confirmed binding of paclitaxel to Bcl-2, and circular dichroism spectroscopy demonstrated that a substantial conformational change accompanies this binding. *In vivo*, treatment with paclitaxel has been shown to lead to Bcl-2 inactivation with concomitant phosphorylation of residues in a disordered, regulatory loop region of the protein. Similarity between paclitaxel-selected peptides and this loop region implicate these residues in drug binding, and suggest that the apoptotic action of paclitaxel may involve the binding of paclitaxel to Bcl-2. These results demonstrate that peptides displayed on the surface of bacteriophage particles can mimic the ligand-binding properties of disordered regions of proteins.

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**Keywords:** phage display; paclitaxel; Bcl-2; apoptosis

### Introduction

Paclitaxel (trade name Taxol) is a highly effective anti-neoplastic agent known to halt mitosis and induce apoptosis. Its anti-mitotic activity is a direct effect of its induction of tubulin polymerization and suppression of microtubule dynamics (Schiff *et al.*, 1979). Its apoptotic activity is presumed to be a secondary effect brought on by the mitotic block. A number of other microtubule-active drugs are now known to act by mechanisms that appear similar (Haldar *et al.*, 1997). A key step in the induction of apoptosis by these drugs is the phosphorylation and concomitant inactivation of the anti-apoptotic protein Bcl-2 (Haldar *et al.*, 1995, 1996). Numerous recent papers have suggested the involvement of several cellular pathways in paclitaxel action, and characterization of these pathways is critical to a complete understanding of the biological activity of paclitaxel.

To characterize better the types of interactions paclitaxel makes with proteins, a phage displayed peptide library was screened for members with relatively high affinity for a derivative of paclitaxel biotinylated at C7 of the taxane ring. The dodecapeptides in this library are attached to the N terminus of pIII, the host-binding protein of bacteriophage M13. Their conformation and environment on pIII is unknown. Some of the displayed peptides may interfere with infection of host by the phage, resulting in censorship of the library. Other metabolic and structural effects also lead to censorship of phage libraries (Rodi & Makowski, 1997). For instance, cysteine is present at very low levels in the library because of the action of the *Escherichia coli dsb* system which catalyzes the formation of disulfide bonds in the periplasm, leading to the covalent dimerization of pIII which precludes their incorporation into the assembling phage particles (Makowski & Russel, 1997). In spite of these factors, the library has very substantial sequence diversity, containing a significant fraction of the theoretically possible sequences (A. Soares & L.M. unpublished results). Nevertheless, the resulting censorship patterns must be

Abbreviations used: TBST.1, Tris-buffered saline plus 0.1% Tween 20; GST, glutathione-S-transferase.

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taken into account in any statistical analysis of an affinity-selection process.

Screening of the library (Scott & Smith, 1990) by biopanning (Kay *et al.*, 1996) involves incubation of the library above an immobilized ligand, in this case a biotinylated derivative of paclitaxel bound to a streptavidin coated plate. Peptides selected by biopanning are a mixture of those exhibiting preferential affinity for paclitaxel, those exhibiting favorable growth properties, and those randomly binding non-specifically to the substrate. Identification of those peptides with preferential affinity for paclitaxel requires the observation of patterns of sequence that are not observed in unselected populations. If the affinity is relatively weak, identification of these sequence patterns may require sequencing of significant numbers of selected peptides.

It is unclear to what extent the sequences of phage-displayed peptides with affinity for paclitaxel may reflect the sequences of proteins that bind to paclitaxel. The binding properties of peptides depend heavily on their environment, and there is no reason to believe that the environment at the N terminus of pIII of a filamentous bacteriophage would mimic in any way that of a paclitaxel binding site on a naturally occurring protein. Furthermore, the binding sites for small molecule ligands usually involve several short stretches of peptide separated by widely varying lengths of peptide, and arranged in a protein scaffold to form a well-defined ligand binding site. These facts notwithstanding, many ligand-binding sites are disordered loops on the surface of a protein prior to interaction with the ligand (Dunker *et al.*, 1998; Romero *et al.*, 1998), and the importance of the surrounding scaffold and other aspects of the environment are less obvious in these cases. Therefore, a comparison of the sequences of phage-displayed peptides exhibiting affinity for paclitaxel with the sequences of proteins, such as  $\beta$ -tubulin, that are known to bind paclitaxel may be informative as to the ways in which this drug interacts with proteins.

## Results

### Library screening

Filamentous bacteriophage particles whose recombinant pIII proteins might bind to paclitaxel were isolated by biopanning (Kay *et al.*, 1996) as detailed in Materials and Methods using a derivatized paclitaxel with a biotin group covalently attached to C7 (see Figure 3) and immobilized on a streptavidin-coated plate. The five pIII structural proteins present at the tip of the virion each possess a random 12 amino acid extension of their amino terminus, coded for by a random synthetic oligonucleotide inserted into the corresponding position in the gene for pIII. The sequences of inserted oligonucleotides were determined and translated to obtain the sequence of the displayed peptide.

Because of the weak interactions between the peptides and the paclitaxel, no strong consensus emerged from this process. In order to evaluate the success of the screening process, the statistical properties of the peptide insert sequences of phage particles isolated after each of three rounds of affinity selection were examined. Amino acid frequencies in the unselected library are primarily due to growth characteristics of the virus and to host-virus interactions (Rodi & Makowski, 1997). Screening selects virions for their ability to bind to the substrate paclitaxel. Since binding to a particular substrate will, in general, be a property independent from the growth characteristics of the viral clone, the statistical properties of the sequences of inserts in the selected virions should differ from those of the unselected virions due to the ligand-binding requirements. The probability of occurrence of any given peptide sequence within the library population was estimated from the amino acid frequencies of 100 members selected at random from the parent library prior to screening, and assuming that the occurrence of amino acid residues in the insert are independent events (e.g. no nearest neighbor correlations). Self-information (defined as  $-\ln(\text{probability})$ ) was used as a convenient measure of the probability of occurrence of a particular sequence; larger self-information being associated with less-likely sequences (Gallagher, 1968).

Three rounds of biopanning were carried out, and the sequences of affinity selected phage were determined after each round. The amino acid sequences of peptides isolated by affinity selection had higher self-information than those of the parent library. The average self-information increased from 33.1 for unselected phage ( $n = 101$ ) to 35.4 for round I phage ( $n = 20$ ), 35.4 for round II phage ( $n = 70$ ) and 34.8 for round III phage ( $n = 23$ ). These numbers suggest that affinity for substrate was a significant factor in the selection process for all three rounds of selection. Round II did not appear to produce further enrichment for paclitaxel-binding phage over that of round I, and in the third round of selection, growth characteristics became a measurable factor in the composition of the phage population.

### Consensus sequences

Insert sequences of phage isolated from rounds I, II and III were determined and a search was made for repeated occurrences of oligopeptides of length three or more. All three rounds, as well as randomly chosen phage, possessed multiple copies of one or more trimers. Of the affinity-selected sequences, only the round II peptides exhibited multiple copies of oligopeptides longer than three residues. The pentapeptide HTPHP was present at identical positions in two inserts, and SHPST was present at different locations in two other inserts. The HTPHP-containing clones had relatively high self-information (35.9 and 36.3) compared with the

SHPST-containing clones (31.6 and 31.2). Statistically, this translates into random observation of the pair of HTPHP-containing clones in an unscreened library being approximately  $10^8$  times less likely than the observation of the pair of SHPST-containing clones, and strongly implicates affinity for paclitaxel as the reason for their isolation.

A sequence search using the composite database OWL identified two human proteins containing the sequence HTPHP: Bcl-2, a 239 amino acid anti-apoptotic protein, and ataxin-2, a 1312 amino acid protein associated with the pathogenesis of spinocerebellar ataxia type 2. A similar search with the consensus sequence SHPST yielded four distinct human proteins, with sizes ranging from 1035 to 4303 amino acid residues.

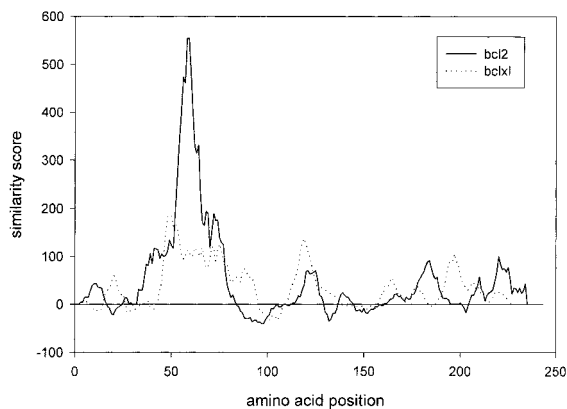
A more sophisticated similarity analysis was performed between these six proteins and the round II peptide sequences. All similarities between hexamers of amino acid residues in each protein and the round II selected peptides were compiled, and the total similarity score,  $H_s(x)$ , was calculated as a function of position along each protein sequence. A similar calculation was carried out using the set of 100 randomly selected clones,  $H_r(x)$ , and the difference in similarity scores,  $\Delta H(x) = H_s(x) - H_r(x)$ , was plotted for each protein. A value of  $\Delta H(x)$  significantly above background indicates that sequences with similarity to this region of the protein occur more frequently among paclitaxel-selected peptides than among randomly selected peptides.

### Similarity to Bcl-2

Of the six proteins identified by this process, only Bcl-2 demonstrated substantial similarity with the round II peptides over a region larger than the observed pentapeptide consensus. The region of high similarity between Bcl-2 and the selected peptides corresponds to a non-conserved region in Bcl-2 which, by analogy to the Bcl-2 homolog Bcl-X<sub>L</sub>, exists as a disordered surface loop (Muchmore *et al.*, 1996).

The absence of significant  $\Delta H(x)$  values between tubulin and round II peptides suggests that the paclitaxel-binding residues in tubulin have a scaffold structure functionally different from that on the surface of the phage, precluding their identification in this screen.

Similarity plots calculated for Bcl-2 and Bcl-X<sub>L</sub> are shown in Figure 1. The similarity between round II peptides and human Bcl-2 is not limited to the pentapeptide HTPHP, but extends across a 30 amino acid portion of the 60 amino acid long disordered loop. This loop plays a regulatory role for the protein, with upregulation of anti-apoptotic activity and increased cell proliferation accompanying genetic deletion of the region (Chang *et al.*, 1997; Uhlmann *et al.*, 1996). Figure 2 shows the alignment of selected peptides with the sequence of Bcl-2 in the loop region.



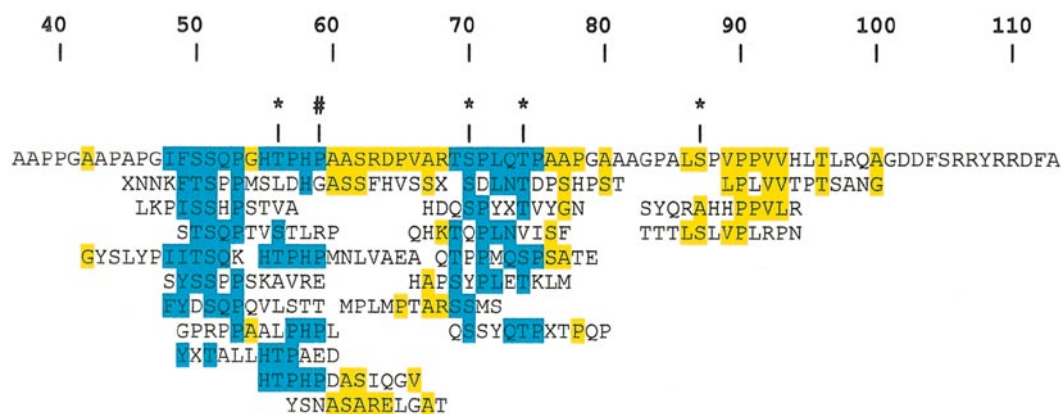
**Figure 1.** Measure of the relative similarity of Bcl-2 and Bcl-X<sub>L</sub> to round II paclitaxel-selected peptides plotted as a function of distance along the amino acid sequences of the two proteins. A high similarity score indicates that the similarity between the paclitaxel-selected peptides and the protein is greater than the similarity between the randomly selected peptides and the protein. A negative similarity score implies that sequences selected by screening against paclitaxel are less likely to have similarity to this region than are randomly selected peptides.

### ELISA binding assays

To test whether the observed pattern of similarity was predictive for paclitaxel binding, ELISA assays were carried out on Bcl-2 and Bcl-X<sub>L</sub>. As shown in Figure 3, human Bcl-2/GST fusion protein bound to immobilized paclitaxel in a concentration-dependent manner. Incubation of the protein with identical molar quantities of either immobilized biotin (to prove specificity for the paclitaxel moiety) or biotinylated dioxin (to rule out non-specific hydrophobic interactions) showed no detectable binding. Standard regression analysis showed the binding data to be consistent with a stoichiometric binding of paclitaxel to protein with a dissociation constant ( $K_D$ ) of  $400(\pm 105)$  nM (standard error).

Results of comparable experiments on the binding of paclitaxel to Bcl-X<sub>L</sub> are also shown in Figure 3. Binding is barely detectable at the highest concentrations of protein, indicating that binding of paclitaxel to Bcl-X<sub>L</sub> is at least two orders of magnitude weaker than paclitaxel binding to Bcl-2. These results confirm that the observation of similarity between affinity-selected round II peptides, and these two proteins are predictive for drug binding.

Competition experiments were carried out to demonstrate that paclitaxel could compete with biotinylated paclitaxel for binding to Bcl-2, and the results of these experiments suggested that the binding constant of paclitaxel to Bcl-2 is similar to that for biotinylated paclitaxel. Preliminary experiments in which docetaxel (taxotere) was used to compete biotinylated paclitaxel from Bcl-2



**Figure 2.** Alignment of round II insert peptide sequences and Bcl-2 in the regions of highest similarity. Blue highlighting indicates strongly conserved residues. The residues that are found to be phosphorylated in paclitaxel-treated cells are marked by (\*). The site of Pro59 is marked by (#). Mutations of Pro59 have been shown to prevent paclitaxel-induced phosphorylation of Bcl-2 (Reed & Tanaska, 1993; Aime-Sempe *et al.*, 1996; Reed, 1997).

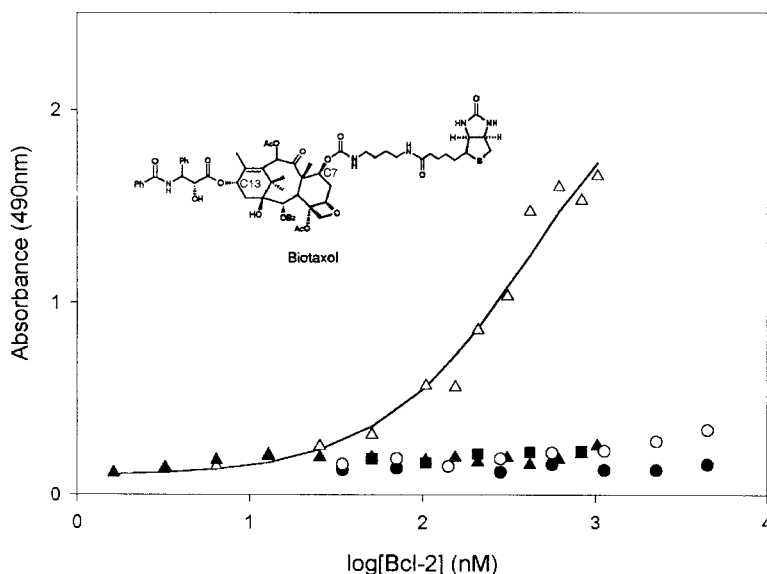
suggested that docetaxel binds to Bcl-2 with lower affinity than paclitaxel.

ELISAs were also carried out on synthetic peptides, one with sequence identical to the amino-terminal half of the flexible loop of Bcl-2; the other identical except for the single replacement P57A. These peptides were circularized by the formation of a disulfide bond between terminal cysteine residues. The first peptide exhibited affinity for paclitaxel 1.5-2 logs higher than the peptide containing the single amino acid replacement (data not shown), suggesting the involvement of Pro57 in paclitaxel binding. As seen in Figure 2, this residue is near the center of the Bcl-2 region that exhibits sequence similarity to the paclitaxel-selected peptides.

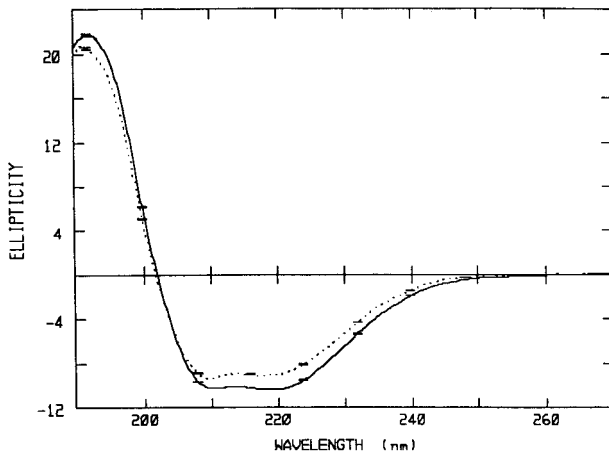
### Circular dichroism

Paclitaxel/Bcl-2 interactions were further characterized by circular dichroism spectroscopy, with the results demonstrating that the Bcl-2/gluta-

thione-S-transferase (GST) construct undergoes a substantial conformational change upon binding paclitaxel. The spectra of the fusion protein with and without paclitaxel are significantly different (see Figure 4). These differences are much larger than the standard deviations of these measurements (error bars in Figure 4), and involve a change in both the shape of the curve (e.g. ratio of the 220/210 nm peaks) and the peak positions. The secondary structures calculated for the Bcl-2 fusion protein with and without paclitaxel differ by approximately 4%. The CD experiments detected no change in the helical content of the molecule. Since the disordered loop is by far the largest non-helical region in Bcl-2, analysis of these spectra strongly suggest that it is the loop portion of Bcl-2 which is involved in paclitaxel binding. The magnitude of the difference suggests a net change in the conformation of Bcl-2 involving 10 to 12 amino acid residues. This net change provides a lower limit for the total number of residues changing



**Figure 3.** Results of ELISA binding assay of human Bcl-2/GST fusion protein to ( $\Delta$ ) paclitaxel; ( $\blacktriangle$ ) biotin and ( $\blacksquare$ ) biotinylated dioxin, and binding of human Bcl-X<sub>L</sub> to ( $\circ$ ) paclitaxel and ( $\bullet$ ) biotin. The X-axis is the log nM concentration of protein in solution (Bcl-2 or Bcl-X<sub>L</sub>); the Y-axis is the uncorrected optical density at 490 nm. The continuous line is the calculated best fit for data combined from four separate binding assays. The inset is the chemical structure of the biotinylated paclitaxel used in the selection of peptides.



**Figure 4.** Circular dichroism spectrum of human Bcl-2/GST fusion protein with (continuous line) and without (broken line) paclitaxel. The fusion protein and either buffer or paclitaxel in buffer were incubated together and analyzed as described in Materials and Methods. Each spectrum is the averaged result of five spectra and subtracted baselines with standard deviations as indicated.

conformation in response to paclitaxel binding. The region of the loop exhibiting high similarity to the paclitaxel-selected peptides includes 15-18 residues in extent, as shown in Figure 3. Consequently, the results of the CD experiments and the similarity calculations are completely consistent with one another.

In a control experiment where paclitaxel was added to purified GST, the GST spectra with and without paclitaxel are virtually identical (data not shown), meaning that were there any net structural change within the GST molecule alone, it would involve less than 0.5% of its total secondary structure. This data corroborates the non-significant  $\Delta H(x)$  values for round II peptides and GST (data not shown). The CD spectra make it clear that not only does paclitaxel bind to the Bcl-2/GST fusion protein, but the spectral differences observed for the protein must derive from the Bcl-2 portion of the molecule, and most probably from the loop region.

## Discussion

The results presented here demonstrate that similarity between the amino acid sequences of ligand-selected peptides and that of an intact protein can be predictive for the binding of the ligand to that protein. The disordered loop of Bcl-2 appears to have ligand-binding properties adequately similar to those of peptides displayed on the surface of a bacteriophage that similar sequences in the two environments result in similar binding properties. Lower similarity between the selected peptide and the disordered loop of Bcl- $X_L$  correlates with lower observed binding affinity between paclitaxel and Bcl- $X_L$ . Conversely, no sig-

nificant similarities were observed between the paclitaxel-selected peptides and any known tubulin. Phage-displayed peptides may not adequately mimic the relatively structured form of the paclitaxel-binding site of tubulin (Nogales *et al.*, 1998).

Binding sites in many proteins appear to be disordered prior to interaction with ligand (Dunker *et al.*, 1998; Romero *et al.*, 1998). The mechanism by which these disordered regions bind with high specificity to a given ligand is not understood. The results presented here suggest that phage-displayed peptides may represent a novel means for the study of protein-ligand interactions involving disordered regions.

The interaction between paclitaxel and Bcl-2 reported here has not been previously observed. The degree to which this interaction may be involved in the cytotoxic activity of paclitaxel is unclear, but the identification of residues in the loop region of Bcl-2 that are involved in both paclitaxel binding and in Bcl-2 inactivation suggests that the interaction may be relevant.

Inactivation of Bcl-2 is central to paclitaxel-induced apoptosis, and occurs concomitantly with the phosphorylation of hydroxyls in its loop domain, including those at Thr56, Ser70, Thr74 and Ser87 (Maundrell *et al.*, 1997; Ito *et al.*, 1997). As indicated in Figure 2, the first three of these sites are within the region with the highest similarity to the selected peptides. Mutation of Ser70 to alanine significantly reduces drug-induced phosphorylation of Bcl-2 (Haldar *et al.*, 1998). Other mutations in the loop region have been identified within numerous lymphoma-derived Bcl-2 proteins, with alterations at one common mutation site (the terminal Pro59 of the HTPHP<sub>59</sub> sequence) shown to result in reduced Bcl-2 phosphorylation in response to paclitaxel (Reed & Tanaska, 1993; Aime-Sempe *et al.*, 1996; Reed, 1997). This hot spot is at the very center of the similarity region shown in Figure 2. The loop domain is necessary for paclitaxel-induced phosphorylation of Bcl-2, as well as for inhibiting paclitaxel induced cytosolic accumulation of cytochrome *c* and apoptosis (Fang *et al.*, 1998). The high affinity of paclitaxel for the loop region of Bcl-2 demonstrated here, and the identification of paclitaxel-binding motifs in the region of the phosphorylated amino acid residues (residues 48-75) suggest that the change in phosphorylation state of these residues is mediated by direct interaction of Bcl-2 with paclitaxel.

Most studies of paclitaxel action have linked its induction of apoptosis to uncharacterized cellular events arising from microtubule polymerization and mitotic arrest. However, in a number of studies, paclitaxel-induced microtubule bundling and mitotic block have been functionally separated from its apoptotic and subsequent cytotoxic effects (Donaldson *et al.*, 1994; Woods *et al.*, 1995; Ibrado *et al.*, 1996; Jordan *et al.*, 1996; Milross *et al.*, 1996; Lieu *et al.*, 1997). Furthermore, paclitaxel appears to induce two forms of cell cycle arrest which, in turn, induce two independent apoptotic pathways:

a rapid-onset, p53 independent pathway, and a slow p53 dependent pathway (Woods *et al.*, 1995). It is possible that there are two sites of action for paclitaxel, leading to the induction of these two separate pathways. The results reported here suggest that Bcl-2 may represent a second molecular target for paclitaxel.

## Materials and Methods

### Library screening

Thirty micrograms of C7-biotinylated paclitaxel (molecular mass 1118 Da, unpublished synthesis), solubilized in HPLC-grade dimethylsulfoxide and diluted into Tris-buffered saline plus 0.1% (v/v) Tween 20 (TBST.1), was attached to a streptavidin-coated petri dish according to standard procedures (Kay *et al.*, 1996). A random 12 amino acid pIII display library was obtained from New England Biolabs (NEB PHD-12) and screened according to recommended instructions with the following modifications. Phage were propagated in *E. coli* JM109 and amplified subconfluent on 150 mm 2XYT agar plates (approximately  $10^5$  phage particles per plate), followed by extraction with TBST.1 and subsequent polyethylene glycol 8000 concentration as described (Kay *et al.*, 1996).

### Sequence determination

Aliquots of either the parent library, or paclitaxel-screened bacteriophage following one, two, or three rounds of screening, were used to infect log phase JM109 cells grown in 2XYT broth. Individual virions were amplified by infecting early-log phase JM109 cultures with a single plaque, followed by a ten hour 37°C growth. Double-stranded viral RF DNA was isolated with Qiagen spin columns according to manufacturer's instructions. Single strand nucleic acid sequences were determined by Commonwealth Biotechnology Inc., Richmond, VA using an ABI PRISM 377 DNA sequencer.

### Statistical analysis of peptides

Sequence complexity analyses of library peptides were performed as follows. The incidence of all 20 amino acid residues at each of the 12 positions within the 100 random peptides was determined. For every peptide, the incidence of each residue along the sequence was multiplied sequentially, giving an estimate of the likelihood of that particular sequence. No nearest neighbor effects were seen at the level allowed by the sample size. Consensus sequence determination was accomplished with a program that performed a comparison of all possible pairs of trimers, tetramers, etc. Proteins possessing a consensus sequence were identified by performing a DELPHOS sequence search of the OWL composite protein sequence database. For analysis of the similarity between peptides and selected protein sequences, the best match between each dodecapeptide and the protein was determined by an exhaustive search using a standard similarity matrix. Matches in which there were three or fewer identities in a continuous stretch of six amino acid residues were discarded as insignificant. Similarity scores were compiled as a function of distance along the protein chain and averaged over a 7 amino acid window to remove short, random matches. An analogous similarity function was calculated for the randomly chosen sequences and sub-

tracted from the calculated similarity function to remove library bias as a source of false similarity.

### ELISA assays

Santa Cruz Biotechnology, Inc. was the source of human Bcl-2 expressed as a fusion protein with *Schistosoma japonicum* GST, anti-Bcl-2 antibody, and anti-Bcl-X<sub>L</sub> antibody. SIGMA Chemical Corp. was the source of all secondary antibodies. Biotinylated dioxin was synthesized by Stanford Research Institute International, Menlo Park, CA. Biotinylated ligands were immobilized to streptavidin-coated Immulon II ELISA plate (Dynatech Corp.) wells. The wells were blocked by washing with TBST.1 four times. Increasing amounts of either human Bcl-2 fusion protein or human Bcl-X<sub>L</sub> were incubated with saturating amounts of ligand for three hours at room temperature. Monoclonal antibodies raised against either human Bcl-2 or Bcl-X<sub>L</sub> epitopes were used to detect the presence of bound protein, followed by an alkaline phosphatase-conjugated secondary antibody. Assays were developed with an ELISA Amplification System Kit (Life Technologies) as per manufacturers instructions, and analyzed at 490 nm on a Bio Tek Automatic ELISA Plate Reader.

### CD spectroscopy

The CD spectra were obtained in a 0.1 cm pathlength cell using an Aviv 62 ds spectropolarimeter over a wavelength range from 300 nm to 190 nm, at an interval of 0.2 nm. For each sample, five spectra and baselines were collected and averaged, and their standard deviations calculated (Mielke & Wallace, 1988) at all wavelengths (plotted as error bars in Figure 4). Both the Bcl-2/GST fusion protein and the GST sample contained approximately 0.2 mg/ml protein in 2.5 mM NaCl. Paclitaxel (CalBiochem) was added to each as a 1:100 dilution of a 90% (v/v) water/ethanol solution (1 mM paclitaxel); the samples without paclitaxel had a 1:100 dilution of a sample of 90% water/ethanol solution (without paclitaxel) added. The secondary structures were determined using a normalized constrained least squares algorithm (Wallace & Teeters, 1987); the NRMSD parameter was calculated as a measure of the quality of the fit of the calculated structure to the data.

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## Acknowledgements

We thank C. B. Thompson at the University of Chicago for purified human Bcl-X<sub>L</sub>, M. Glucksman at Mount Sinai School of Medicine for purified *S. japonicum* GST, Alex Soares at Florida State University for help with statistical analyses, and G. Myers at CBI for helpful discussions. This work was funded by a grant from the Lucille P. Markey Foundation and a grant from the National Science Foundation (L.M.).

## References

- Aime-Sempe, C., Kitada, S. & Reed, J. C. (1996). Investigations of paclitaxel-mediated phosphorylation of Bcl-2. *Blood*, **88**, 106.
- Chang, B. S., Minn, A. J., Muchmore, S. W., Fesik, S. W. & Thompson, C. B. (1997). Identification of a novel

- regulatory domain in Bcl-x<sub>L</sub> and Bcl-2. *EMBO J.* **16**, 968-977.
- Donaldson, K. L., Goolsby, G. L., Kiener, P. A. & Wahl, A. F. (1994). Activation of p34cdc2 coincident with paclitaxel-induced apoptosis. *Cell. Growth Differ.* **5**, 1041-1050.
- Dunker, A. K., Garner, E., Guilliot, S., Romero, P., Albrecht, K., Hart, J., Obradovic, Z., Kissinger, C. & Villagranca, J. E. (1998). Protein disorder and the evolution of molecular recognition: theory, predictions and observations. *Pac. Symp. Biocomp.* **3**, 473-484.
- Fang, G., Chang, B. S., Kim, C. N., Perkins, C., Thompson, C. B. & Bhalla, K. N. (1998). "Loop" domain is necessary for taxol-induced mobility shift and phosphorylation of Bcl-2 as well as for inhibiting taxol-induced cytosolic accumulation of cytochrome *c* and apoptosis. *Cancer Res.* **58**, 3202-3208.
- Gallager, R. G. (1968). *Information Theory and Reliable Communication*, p.23, John Wiley & Sons, New York.
- Haldar, S., Jena, N. & Croce, C. M. (1995). Inactivation of Bcl-2 by phosphorylation. *Proc. Natl Acad. Sci. USA*, **92**, 4507-4511.
- Haldar, S., Chintapalli, J. & Croce, C. M. (1996). Paclitaxel induces Bcl-2 phosphorylation and death of prostate cancer cells. *Cancer Res.* **56**, 1253-1255.
- Haldar, S., Basu, A. & Croce, C. M. (1997). Bcl2 is the guardian of microtubule integrity. *Cancer Res.* **57**, 229-233.
- Haldar, S., Basu, A. & Croce, C. M. (1998). Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. *Cancer Res.* **58**, 1609-1615.
- Ibrado, A. M., Huang, Y., Fang, G. & Bhalla, K. (1996). Bcl-X<sub>L</sub> overexpression inhibits paclitaxel-inducing Yama protease activity and apoptosis. *Cell. Growth Differ.* **7**, 1087-1094.
- Ito, T., Deng, X., Carr, B. & May, W. S. (1997). Bcl-2 phosphorylation required for anti-apoptosis function. *J. Biol. Chem.* **272**, 11671-11673.
- Jordan, M. A., Wendell, K., Gardiner, S., Derry, W. B., Copp, H. & Wilson, L. (1996). Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Paclitaxel) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res.* **56**, 816-825.
- Kay, B. K., Winter, J. & McCafferty, J. (1996). *Phage Display of Peptides and Proteins: A Laboratory Manual*, Academic Press, San Diego.
- Lieu, C. H., Yang, Y. N. & Lai, Y. K. (1997). Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. *Biochem. Pharmacol.* **53**, 1587-1596.
- Makowski, L. & Russel, M. (1997). Structure and assembly of filamentous bacteriophages. In *Structural Biology of Viruses* (Chiu, W., Burnett, R. M. & Garcea, R. L., eds), pp. 352-380, Oxford University Press.
- Maudrell, K., Antonsson, B., Magnenat, E., Camps, M., Muda, M., Chabert, C., Gillieron, C., Boschert, U., Vial-Knecht, E., Martinou, J. C. & Arkinstall, S. (1997). Bcl-2 undergoes phosphorylation by c-Jun N-terminal Kinase/Stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. *J. Biol. Chem.* **272**, 25238-25246.
- Mielke, D. L. & Wallace, B. A. (1988). Secondary structural analyses of the nicotinic acetylcholine receptor as a test of molecular models. *J. Biol. Chem.* **263**, 3177-3182.
- Milross, C. G., Mason, K. A., Hunter, N. R., Chung, W.-K., Peters, L. J. & Milas, L. (1996). Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. *J. Natl Cancer Inst.* **88**, 1308-1314.
- Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. L. & Fesik, S. W. (1996). X-ray and NMR structure of human Bcl-X<sub>L</sub>, an inhibitor of programmed cell death. *Nature*, **381**, 335-341.
- Nogales, E., Wolf, S. G. & Downing, K. H. (1998). Structure of the  $\alpha\beta$  tubulin dimer by electron crystallography. *Nature*, **391**, 199-203.
- Reed, J. C. (1997). Bcl-2 family proteins: strategies for overcoming chemoresistance in cancer. In *Apoptosis: Pharmacological Implications and Therapeutic Opportunities* (Kaufmann, S. H., ed.), pp. 501-532, Academic Press, San Diego.
- Reed, J. C. & Tanaska, S. T. (1993). Somatic point mutations in translocated bcl-2 alleles of non-Hodgkin's lymphomas and lymphocytic leukemias: implications for mechanism of tumor progression. *Leuk. Lymphoma*, **10**, 157-163.
- Rodi, D. J. & Makowski, L. (1997). Transfer RNA isoacceptor availability contributes to sequence censorship in a library of phage displayed peptides. In *Structure and Function of Macromolecular Assembly* (Namba, K., ed.), pp. 155-164, Matsushita Electric Industrial Co. Ltd.
- Romero, P., Obradovic, Z., Kissinger, C. R., Villafranca, J. E., Garner, E., Guilliot, S. & Dunker, A. K. (1998). Thousands of proteins likely to have disordered regions. *Pac. Symp. Biocomp.* **3**, 437-448.
- Schiff, P. B., Fant, J. & Horwitz, S. B. (1979). Promotion of microtubule assembly in vitro by taxol. *Nature*, **277**, 665-667.
- Scott, J. K. & Smith, G. P. (1990). Searching for peptide ligands with an epitope library. *Science*, **249**, 386-390.
- Uhlmann, E. J., D'Sa-Eipper, C., Subramanian, T., Wagner, A. J., Hay, N. & Chinnadurai, G. (1996). Deletion of a non-conserved region of Bcl-2 confers a novel gain of function: suppression of apoptosis with concomitant cell proliferation. *Cancer Res.* **56**, 2506-2509.
- Wallace, B. A. & Teeters, C. L. (1987). Differential absorption flattening optical effects are significant in the circular dichroism spectra of large membrane fragments. *Biochemistry*, **26**, 65-70.
- Woods, C. M., Zhu, J., McQueney, P. A., Bollag, D. & Lazarides, E. (1995). Paclitaxel-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. *Mol. Med.* **1**, 506-526.

Edited by I. A. Wilson

(Received 20 July 1998; received in revised form 2 October 1998; accepted 7 October 1998)