The activation domain of the yeast Gal4 protein binds specifically to the Gal80 repressor and is also thought to associate with one or more coactivators in the RNA polymerase II holoenzyme and chromatin remodeling machines. This is a specific example of a common situation in biochemistry where a single protein domain can interact with multiple partners. Are these different interactions related chemically? To probe this point, phage display was employed to isolate peptides from a library based solely on their ability to bind Gal80 protein in vitro. Peptide-Gal80 protein association is shown to be highly specific and of moderate affinity. The Gal80 protein-binding peptides compete with the native activation domain for the repressor, suggesting that they bind to the same site. It was then asked if these peptides could function as activation domains in yeast when tethered to a DNA binding domain. Indeed, this is the case. Furthermore, one of the Gal80-binding peptides binds directly to a domain of the Gal11 protein, a known coactivator. The fact that Gal80-binding peptides are functional activation domains argues that repressor binding and activation/coactivator binding are intimately related properties. This peptide library-based approach should be generally useful for probing the chemical relationship of different binding interactions or functions of a given native domain.

Most eukaryotic transcriptional activators contain an activation domain (AD) that is thought to interact with one or more coactivators in the RNA polymerase II holoenzyme (1–4) and/or chromatin remodeling machines (5–8). Although the mechanism of action of activators is not understood in detail, these AD-protein contacts are thought to be involved in recruitment of the holoenzyme or remodeling machines to the promoter targeted by the activator (9–12), which generally also contains a sequence-specific DNA binding domain. Most activators do not function constitutively but instead respond to changes in cellular conditions. In some cases, the activator is repressed under noninducing conditions by a protein that binds tightly to the AD and blocks its interaction with the transcription machinery. Well known examples of this type include the p53-MDM2 (13) and Gal4p-Gal80p (14, 15) complexes.

The chemistry of these competitive protein-protein interactions is poorly understood. Two limiting models can be considered. One is that the repressor and coactivator(s) would have more or less common AD binding surfaces, and the AD-repressor and AD-coactivator complexes would be structurally and chemically similar. The other is that completely different binding modes are employed to recognize the repressor and other transcription factors. For example, different regions of the AD could be employed for repressor and coactivator binding; or different faces of a single structure, such as a β-sheet (16), could be utilized for the different binding events.

Several studies have addressed this question for the Gal4 AD, but they have provided mixed results. Mutagenesis studies (16, 17) seem more consistent with different modes of binding. For instance, Gal4 AD binding to Gal80p is compromised severely by single point mutations in the AD, whereas activation is unaffected by these substitutions. Although no single substitution appears to affect the activation potential of the Gal4 AD greatly, multiple point mutants have been found which have a significant effect on activation (both up and down) but do not impair Gal80p binding. On the other hand, chemical cross-linking and "label transfer" experiments using Gal4 AD chemically modified at specific positions indicate that the same residues are in close proximity to Gal80p and to TATA-binding protein in the respective AD complexes (17). TATA-binding protein is considered to be a potential target of Gal4p in the activated state (18–21), although it is not clear if this is indeed the case.

We decided to address this issue in a novel fashion that makes use of powerful combinatorial methods developed over the last several years. Phage display (22–26) was used to select a peptide that binds to the Gal80 repressor specifically. This was the sole criterion used in the screen. This peptide was then fused to a sequence-specific DNA binding domain and expressed in yeast to determine if it could function as an AD in vivo. It is shown here that the Gal80-binding peptides indeed act as potent ADs. Furthermore, in vitro binding studies reveal that they also bind the Gal11 protein, a coactivator. These data argue that Gal80p binding and activation are fundamentally linked activities and in turn argue that Gal80p and certain coactivators have similar binding surfaces.

MATERIALS AND METHODS

Plasmids—The Escherichia coli expression vectors for His₆-Gal80, pProEX-L-Gal80, and the yeast expression vector for multicopy Gal80, YEp351Gal80, which has a native Gal80 promoter were kindly provided by Prof. Stephen Johnston (University of Texas, Southwestern). pGBl9 was purchased from CLONTECH. The expression plasmid encoding the GST-G80-Ba fusion was constructed by annealing the oligonucleotides (5'-CCG GAA TTC AAG CTT ACC GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3' and 5'-CCG GAA TTC AAG CTT ACC GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3') to form a complementary double-stranded DNA fragment. This fragment was cloned into the BstEII site of pGBl9 to replace the GST fragment and then digested with BamHI and BglII. The digested plasmid was then treated with Klenow polymerase to add a 5' extension to the fragment (5'-GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3') and digested with BamHI and BglII. The digested plasmid was then treated with Klenow polymerase to add a 5' extension to the fragment (5'-GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3') and digested with BamHI and BglII. The digested plasmid was then treated with Klenow polymerase to add a 5' extension to the fragment (5'-GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3') and digested with BamHI and BglII. The digested plasmid was then treated with Klenow polymerase to add a 5' extension to the fragment (5'-GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3') and digested with BamHI and BglII. The digested plasmid was then treated with Klenow polymerase to add a 5' extension to the fragment (5'-GAT GCC TTA GAT TGG TTT TGG AAG GAG GGG CAT CGG TAA GCT TGA ATT CCG G-3').
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for GST-G80B-B, except that the appropriate oligonucleotides to encode this peptide were used (5′-CCG GGA TAC CGC GGA TCC AAT GCT TGA ATT CCG G-3′ and 5′-CCG GAA TTC AAG CTT ACG CAC GAT TCA TCC ACA ACT TAT TTA). The resultant plasmid was named pGBT9-(G80BP-A) 2.

For the yeast expression plasmids encoding Gal4 1–147, fused to two copies of a peptide, the following protocol was employed (the example of G80BP-A is given). A pair of synthetic oligonucleotides encoding one copy of the 20-mer peptide G80BP-A (5′-CCG GGA ATT CTA TGA TCA TGA TCA TTA TGG TTT TGG AAG GAG GGG CAT CGG TAA CTG CAG AAA AAT TCC TCA AAA GTA TTA TTC TGC ATA TCC TGA TCA TAG AAT TCC GCA TAT CCG TCA TCC AAA ACA AAT CAT CAA AAG TAT TAT TCT GCA TAT

For the yeast expression plasmids encoding Gal4 1–147 peptide fusions, the same procedure was employed using duplex oligonucleotides encoding the desired peptide. For the yeast expression plasmids encoding Gal4 1–147 peptide fusions, two copies of a peptide, the following protocol was employed (the example of G80BP-A is given). A pair of synthetic oligonucleotides encoding one copy of the 20-mer peptide G80BP-A (5′-CCG GGA ATT CTA TGA TCA TGA TCA TTA TGG TTT TGG AAG GAG GGG CAT CGG TAA CTG CAG AAA AAT TCC TCA AAA GTA TTA TTC TGC ATA TCC TGA TCA TAG AAT TCC GCA TAT CCG TCA TCC AAA ACA AAT CAT CAA AAG TAT TAT TCT GCA TAT

Affinity Pull-down Experiments—E. coli BL21(DE3) pLysS cells transformed with pProEX-1/Gal80 were grown at 37 °C to O.D. 0.8 and then induced with isopropyl-1-thio-D-galactopyranoside. The harvested cells were lysed by sonication in PBS. After sonication, Triton X-100 was added to 1%, and the cell debris was removed by centrifugation at 20,000 × g for 25 min. The supernatant was used in the pull-down experiments.

To determine whether the Gal80-binding peptides and the native Gal4 AD compete for Gal80p, 2.5 μl His6-Gal80, about 10 μl labeled peptide, and the indicated amount of GST-34 were mixed in 200 μl of buffer (PBS with 0.2 mg/ml bovine serum albumin). The polarization values were measured. The GST protein was used as a negative control. An identical protocol was employed to see if the two selected peptides competed with one another for Gal80p, except that an unlabeled peptide was substituted for the native AD as the competitor.


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**RESULTS AND DISCUSSION**

**Isolation of Gal80p-binding Peptides by Phage Display**—As mentioned above, the central question that we wished to address was whether peptides selected solely on their ability to bind the Gal80 repressor would function as ADs in yeast when tethered to a sequence-specific DNA binding domain. To identify Gal80p-binding peptides, phage display was employed. An M13 phage library of approximately 10^12 20-mer peptides was screened against two different Gal80 fusion proteins, GST-Gal80 and His6-Gal80, adsorbed on different solid phases. Six rounds of screening were carried out. In various rounds, different Gal80p fusion proteins and different solid supports were employed. By changing the “background” throughout the course of the screen it was hoped that phage that bound to beads or linker arms, etc. could be reduced or eliminated from the final population. Furthermore, high Ionic strength washes were employed in the last two rounds to attempt to eliminate weakly bound phage from the population. After the final round, nine phage-infected colonies were selected, and the phage DNA in these colonies was sequenced.

**Two unique peptides were identified, one of which was found six times and the other three. These will be referred to as Gal80p-binding peptides (G80BPs) A and B, respectively. The peptide sequences deduced from the DNA sequences are shown in Table 1, as is that of the native core Gal4 AD for comparison. There may be some sequence similarity between the isolated peptides and the Gal4 AD; for example, each contains DD pairs, but no obvious extended consensus sequence is apparent. The affinities of G80BP-A and G80BP-B for head-bound Gal80p were estimated to be about 2,000 times higher than that of a sample was then placed into the cavity of a fluorescence spectrophotometer equipped to measure anisotropy (PanVera Beacon 2000). The polarization of the emitted light was recorded. A similar procedure was employed for the experiment using fluorescein-labeled native Gal4 AD, which was produced as recombinant polypeptide in E. coli and labeled at its NH2 terminus using the NHS ester of carboxyfluorescein. The polarization of the emitted light was recorded. A similar procedure was employed for the experiment using fluorescein-labeled native Gal4 AD, which was produced as recombinant polypeptide in E. coli and labeled at its NH2 terminus using the NHS ester of carboxyfluorescein. To determine whether the Gal80-binding peptides and the native Gal4 AD compete for Gal80p, 2.5 μl His6-Gal80, about 10 μl labeled peptide, and the indicated amount of GST-34 were mixed in 200 μl of buffer (PBS with 0.2 mg/ml bovine serum albumin). The polarization values were measured. The GST protein was used as a negative control. An identical protocol was employed to see if the two selected peptides competed with one another for Gal80p, except that an unlabeled peptide was substituted for the native AD as the competitor.

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Fig. 1. Determination of the equilibrium dissociation constants ($K_D$ values) of complexes between purified Gal80 protein and fluorescein-labeled G80BP-A (panel A) or a fluorescein-labeled peptide containing the 24 residue core native Gal4 AD (panel B) by fluorescence polarization. The titration shown in panel A reveals that the G80BP-A-Gal80 complex has a $K_D$ of approximately 300 nM under these conditions. Very weak binding of the peptide to GST was observed, indicating that the interaction with Gal80p is specific (see also Fig. 2A). The Gal4 AD-Gal80p complex has a $K_D$ of no more than 25 nM as evidenced by the fact that half-maximal saturation of the Gal4 AD was reached at this concentration, but under the conditions binding was clearly stoichiometric. Attempts to develop equilibrium conditions to determine the true Gal4 AD-Gal80p $K_D$ by fluorescence polarization were unsuccessful (see "Results and Discussion").

To address the specificity of binding of G80BP-A to Gal80p further, pull-down experiments were conducted (Fig. 2A). Gal80p with an NH$_2$-terminal His$_6$ tag was expressed in *E. coli*, and a cleared extract was incubated with glutathione agarose-bound GST-G80BP-A. His$_6$-Gal80p comprised about 1% of the total soluble protein in the extract. The column was washed thoroughly, and the bound proteins were then eluted with glutathione. The eluate was analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining. As shown in lane 7 of Fig. 2A, the G80BP-A-containing fusion protein retained only two proteins out of this mixture. One had an electrophoretic mobility identical to purified His$_6$-Gal80p, and the other was of slightly lower apparent molecular mass. Subsequent characterization revealed that the lower band is a proteolytic product of His$_6$-Gal80 lacking the NH$_2$-terminal tag (data not shown). No other significant bands were observed in the eluted fraction, indicating highly specific binding to Gal80p. An identical experiment using GST (lane 5, Fig. 2A) or other GST-peptide fusions not selected to bind Gal80p (data not shown) did not result in retention of Gal80p. The result of the pull-down experiment using G80BP-A was very similar to those obtained using a GST fusion protein containing the native Gal4 AD (lane 9, Fig. 2A). In this case as well, only the two forms of Gal80p were retained from the crude extract. A similar set of experiments carried out with G80BP-B provided results essentially identical to those obtained with G80BP-A (Fig. 2B). These data demonstrate that the selected peptides bind Gal80p with excellent specificity, apparently rivaling that of the native Gal4 AD.

Peptide G80BP-A and the Gal4 AD Compete for Binding to Gal80p—To determine if G80BP-A and the native AD bind Gal80p in a similar fashion, a competitive binding experiment was carried out using fluorescence polarization in which fluorescein-labeled G80BP-A was saturated with Gal80p, and then this solution was titrated with unlabeled GST-Gal4 AD fusion protein. If binding is competitive, a reduction in the polarization of the emitted light would be expected as the AD concentration is increased, reflecting dissociation of the bound peptide. As shown in Fig. 3, this was exactly the result obtained. When the G80BP-A-Gal80p complex was challenged with GST alone, no dissociation was observed.

This finding of competitive binding was not unexpected. It is
often the case that phage display or other selection protocols yield peptides that recognize a surface of the target protein that interacts with native partners (see Ref. 29 and references therein). This is probably because these surfaces are more “bindable” than other regions of the protein and represent hotspots for interactions. In any case, these data suggest that the selected peptide and the Gal4 AD bind the same or overlapping sites on Gal80p, although an allosteric model cannot be ruled out.

The same type of competition experiment was also done using unlabeled G80BP-B. As seen in Fig. 3, this also resulted in competition of labeled G80BP-A from Gal80p, indicating that the two selected peptides bind the same, or overlapping, sites on the repressor, as expected. The less efficient competition compared with the native AD reflects the lower affinity of the peptide for Gal80p.

AD with respect to binding Gal80p in vitro, although their affinity for the repressor is much lower. Having established this fact, we then proceeded to address the central point of this study, which was to determine if these peptides, selected solely on the basis of binding to Gal80p, can activate transcription in vivo when fused to a sequence-specific DNA binding domain.

DNA encoding G80BP-A or G80BP-B was inserted into the yeast expression vector pGBT9. The final construct encodes a fusion of the Gal4 DNA binding and dimerization domains (residues 1–147) (30) to the selected peptide. Expression was driven by the alcohol dehydrogenase promoter. The resultant plasmid was transformed into the gal4Δ gal80Δ strain Y190, which contains an integrated Gal4-responsive lacZ reporter gene. The activity of Gal4(1–147)-G80BP-A and some control constructs was assessed qualitatively by blue/white screening of colonies on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates (Table II).

Cells transformed with plasmids expressing Gal4(1–147) fused to G80BP-A formed blue colonies, indicating that this peptide can indeed function as an AD in vivo. A positive control in which the native Gal4 AD was fused to Gal4(1–147) also formed blue colonies, whereas a negative control in which only Gal4(1–147) was expressed formed white colonies, as expected. We also constructed plasmids that expressed fusions of Gal4(1–147) to two other peptides (“20un” and JZ15, see Table I), which were selected by phage display to bind proteins other than

Table II

| Polypeptide expressed | Color |
|-----------------------|-------|
| Gal4(1–147)            | White |
| Gal4(1–147)-G80BP-A    | Blue  |
| Gal4(1–147)-(G80BP-A)2 | Blue  |
| Gal4(1–147)-(G80BP-B)2 | Blue  |
| Gal4(1–147)-Gal4 AD    | Blue  |
| Gal4(1–147)-(20un)2    | White |
| Gal4(1–147)-(JZ15)2    | White |

The results of the blue/white screening assay to determine the activation potential of the Gal80p-binding peptides in yeast

In each case, Y190 cells (gal4, gal80) which contain a lacZ gene under the control of the GAL1 promoter, were transformed with a high copy number plasmid expressing the polypeptide indicated under the control of the alcohol dehydrogenase promoter. For each transformation, 8–10 colonies were transferred to fresh SC-Trp-glucose plates and grown overnight. A colony lift followed by a filter assay were employed to determine the color of the colonies in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

The Gal80p-binding Peptides Can Function as Repressive AIs in Yeast—The experiments presented above indicate that G80BP-A and G80BP-B mimic the properties of the native Gal4

**Fig. 2.** The selected peptides bind Gal80p specifically. Panel A, GST-G80BP-A and GST-Gal4 AD fusion proteins (for the purified preparations, see lanes 6 and 8, respectively) were incubated with a crude E. coli extract (lane 2) containing His6-tagged Gal80p (for a sample purified by metal ion affinity chromatography, see lanes 3 and 10). After washing and elution with glutathione, only two major bands other than the one representing the GST fusion were observed on this Coomassie-stained gel (lanes 7 and 9). One represented His6-Gal80p and the other a truncation product lacking the NH2-terminal His6 tag. Panel B, the same experiments as described for panel A were carried out using a GST-G80BP-B fusion protein (lane 3). Specific binding of Gal80p was again observed (lane 4), although in this case one other minor band was visible in the bound fraction.

**Fig. 3.** The Gal4 AD and G80BP-A bind to Gal80p competitively. Fluorescein-labeled GP80BP-A (10 nM) was mixed with enough Gal80p (2.5 μM) to saturate approximately 90% of the peptide. This complex (and the excess repressor) were then titrated with the indicated amount of unlabeled GST-Gal4 AD fusion protein, GST alone, or unlabeled GST-G80BP-B. The dissociation of the labeled peptide from Gal80p was monitored by fluorescence polarization spectroscopy.
Gal80 (Gal4p and human interleukin 1β, respectively). These peptides did not support detectable activation, as evidenced by the formation of white colonies. This demonstrates that not any protein-binding peptide can function as an AD.

To obtain a more quantitative feeling more the potency of the Gal80-binding peptides as ADs, several Gal4(1–147) fusion constructs were made, expressed in yeast, and their activity was measured using liquid β-galactosidase assays. These are shown in Fig. 4A. lacZ expression in cells containing Gal4(1–147) was barely detectable (0.06 unit/min), whereas cells containing Gal4(1–147) fused to the native Gal4 AD exhibited robust lacZ expression (45 units). lacZ expression mediated by Gal4(1–147)-G80BP-A was much weaker (1 unit) than that supported by the native AD. But this activity was well above the background level exhibited by Gal4(1–147) alone (~17-fold increase in lacZ expression over the basal level), consistent with the plate assay results. Gal4(1–147)-G80BP-B had an activity about half that of Gal4(1–147)-G80BP-A. Addition of one or two copies of the control peptide 20un to the COOH terminus of Gal4(1–147) did not result in any detectable activation.

It is often the case that the activity of weak ADs can be increased by fusing multiple copies to a DNA binding domain. This may reflect their ability to interact with more than one target in the holoenzyme and/or chromatin remodeling machines (31). To determine if this is the case for the selected peptides, two copies of G80BP-A or G80BP-B were fused to Gal4(1–147), and the activities of these fusions was determined. As shown in Fig. 4A, the direct repeat was a much more potent activator, exhibiting about one-third the activity of a single native Gal4 AD and driving transcription to levels about 200-fold higher than the basal rate. However, analysis of another two peptide-containing activators revealed that what appeared to be a synergistic effect instead seems to be the result of steric/spacing issues in these fusion proteins. For example, although two copies of 20un do not activate transcription, Gal4(1–147)-20un-G80BP-A is much more active than the analogous construct lacking the control peptide (10-fold difference; Fig. 4A). However, reversing the order of 20un and G80BP-A resulted in an activity slightly lower than that of Gal4(1–147)-G80BP-A. A similar conclusion can be drawn from the data using various G80BP-B-containing constructs (Fig. 4A). Thus, when the Gal80p-binding peptides are separated from Gal4(1–147) appropriately, they can act as quite potent

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2 Y. Han and T. Kodadek, unpublished results.
activators, increasing lacZ expression 100–150-fold above the basal level. This argues strongly that binding to Gal80p and the ability to activate transcription are closely linked properties.

To test the ability of the selected peptides to interact with Gal80 in vivo the yeast expression vector YEp351Gal80 (containing a native GAL80 promoter) was also transformed into Y190 cells. YEp351Gal80 is a multicopy plasmid. Fig. 4B shows that activation mediated by G80BP-A or G80BP-B in a number of different fusion protein contexts was repressed significantly when Gal80p was expressed, although for the single case of Gal1(1–147)-20un-G80BP-B, the effect was marginal. The level of Gal80p-repressed transcription supported by the selected peptides and the native Gal4 AD was similar. This result demonstrates that G80BP-A and G80BP-B are able to interact with Gal80p in vivo and that this interaction can inhibit transactivation.

G80BP-A Recognizes a Yeast Coactivator in Vitro—The fact that the G80p-binding peptides function as ADs in vivo implies that they can bind coactivators. To test this directly, we used fluorescence polarization to see if an association between G80BP-A and the NH2-terminal domain of Gal11 protein (Gal11p) could be detected. Gal11p is a component of the RNA polymerase II holoenzyme (32) and is required for the full expression of a large number of yeast genes (33, 34), including the GAL genes. Kim and co-workers have recently published evidence implicating Gal11p as the prime target of the yeast GCN4 transactivator (35), and we have evidence that this may also be the case for Gal4p, with the NH2-terminal region of Gal11p being the specific site of contact. 3

As shown in Fig. 5A, when fluoresceinated G80BP-A was titrated with recombinant Gal11(1–350), a strong increase in polarization was observed. Half-saturation occurred at approximately 1.2 μM Gal11(1–350), and the fact that a double reciprocal plot of these titration data was linear (Fig. 5B) suggested that this value is an accurate representation of the KD of this complex under these conditions. It is important to note that under these conditions G80BP-A does not bind any protein in a promiscuous fashion, as evidenced by the highly selective retention of Gal80p out of an E. coli extract (Fig. 2A) and the lack of binding to GST in a fluorescence polarization experiment (Fig. 1A). We therefore conclude that G80BP-A has a specific affinity for a coactivator. Although we cannot say that Gal11p is the target of G80BP-A in vivo, the important conclusion is that a peptide selected solely on the basis of Gal80p binding interacts directly with a known coactivator, suggesting that Gal80p and Gal11p have chemically related binding surfaces.

Recognition of the Gal80 Repressor and the Ability to Function as an AD Are Chemically Related Properties—The central result of this study is that two peptides, selected solely for their ability to bind the Gal80 repressor in vitro, are able to function as potent AD mimics in yeast. Thus, we conclude that the ability of a molecule to bind Gal80p and its ability to act as an AD are fundamentally related. It seems extremely unlikely that this concordance between Gal80p binding and activation potential is a coincidence, particularly because at least two other peptides selected to bind proteins other than Gal80p have no activation potential at all.

Of course, these experiments do not strictly speak to whether the native Gal4 AD uses similar chemistry to bind Gal80p and coactivators. It remains possible that the protein binding properties of the selected peptides and the native AD are distinct. This seems unlikely however, because G80BP-A binds a coactivator implicated in mediating transcription by Gal4p (Fig. 5), and both peptides appear to bind the same site on Gal80p recognized by the Gal4 AD. Nonetheless, an unequivocal resolution of this issue will require structural data on repressor and coactivator complexes of the native AD.

This study focused on using peptide library-based methods to probe the chemical relationship of the binding sites of particular coactivators and repressors, i.e. is it likely that the Gal4 AD employs similar chemistry to bind both kinds of partners? However, the same method could be applied in any case where a single domain of a polypeptide is known to interact with more than one other protein. In general, if a peptide selected to bind one protein binds others or exhibits an activity not obviously tied to its method of isolation, this provides strong preliminary evidence that these different binding events are related. Because the phage display-based technique is very rapid, it could be used as an initial screen prior to carrying out more time-consuming mutagenesis and chemical cross-linking or label transfer studies to probe the native system in more detail.

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