Peptides or small molecules able to modulate protein-protein interactions hold promise as tools with which to probe and manipulate biological pathways. An important issue in this nascent field is to evaluate different methods with which to search libraries for molecules that modulate the function of specific target proteins. One strategy is to screen libraries for molecules that bind specifically to a protein known to be critical in the pathway of interest, with the expectation that the molecules isolated will recognize regions of the target protein important for its function and thereby exhibit biological activity. Here, a peptide library was screened using a two-hybrid-like system for molecules able to bind human CDC6 protein (CDC6p), required for the initiation of DNA replication in eukaryotic cells. From a collection of over a million peptides, a single species that exhibited good affinity and specificity for binding CDC6p was obtained. When expressed in human cells, the peptide inhibited cell cycle progression and exhibited other properties expected of a CDC6p inhibitor. This approach, which does not require detailed knowledge of the mechanism of action of a protein target, may be generally useful for isolating peptides capable of manipulating biological pathways.

Pharmacologically active compounds are essential tools for probing the cell and molecular biology of organisms that are not amenable to genetic manipulation, including humans. The availability of such reagents is limited by the number of natural products with specific, well characterized activities. In an effort to circumvent this limitation, many laboratories have turned to combinatorial libraries and high throughput screens to identify compounds with which to manipulate biological pathways. Critical issues in such studies include the type of library employed (peptides, synthetic small molecules, etc.); the diversity of the library; and the sensitivity, specificity and throughput capacity of the screen used to identify molecules with the desired properties.

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A CDC6 Protein-binding Peptide Selected Using a Bacterial Two-hybrid-like System Is a Cell Cycle Inhibitor*

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The abbreviations used are: CDC6p, human CDC6 protein; ORC, origin recognition complex; MCM, human minichromosome maintenance; DBD, DNA-binding domain; GST, glutathione S-transferase; HA, hemagglutinin; PBS, phosphate-buffered saline; EGFP, enhanced green fluorescent protein; PAGE, polyacrylamide gel electrophoresis.
crucified by the origin recognition complex (ORC), which in turn promotes loading of the MCM proteins on chromatin (23). Immunodepletion of CDC6p in living cells arrests the cell cycle in G1, indicating the essential role of CDC6p in human cell proliferation (24). These results suggest that compounds able to block the interaction of CDC6p with other replication proteins should be efficient cell cycle inhibitors. We report here the isolation of a CDC6p-binding peptide using a bacterial two-hybrid-like system. This peptide binds specifically to CDC6p with a $K_d$ of approximately $10^{-7}$ M and is capable of reducing CDC6p loading on chromatin in G1 phase and blocking entry of human cells into S phase.

These results are of general interest because no particular activity of CDC6p was targeted, nor did the assay specifically seek to disrupt a particular protein-protein interaction. Indeed, the detailed mechanism of action of CDC6p is not yet understood to a point that would permit the rational design of functional screens for a CDC6p-targeted inhibitor. These findings suggest that useful reagents for functional manipulation of important pathways of cell regulation can be obtained from simple binding studies, even in the absence of detailed knowledge of the mechanisms of action of the target protein.

**EXPERIMENTAL PROCEDURES**

**Library Construction**—In order to detect the peptide encoded by genomic DNA fragment in later experiments, a T7 T10 epitope tag was introduced downstream of the cI repressor DNA binding domain (DBD) in vector pJH391 (25) by a pair of oligonucleotides as a HindIII/BamHI fragment. The resulting plasmid was named pJH391S. Chicken genomic DNA (CLONTECH) was completely digested with SacAI and cloned on masse into BamHI-digested and dephosphorylated pJH391S. The ligation products were transferred into Escherichia coli DH12S supercompetent cells (Life Technologies, Inc.), and cells were grown on one hundred 55-mm Luria broth plates supplemented with 50 g/ml carbenicillin at 37 °C overnight. Analysis of a large number of colonies picked from these plates indicated that there are approximately 5.6 × $10^6$ peptide-expressing plasmids in the library.

**Bacterial Two-hybrid Screening**—The full-length CDC6p gene was amplified by polymerase chain reaction and cloned as a SacI/BamHI fragment into pJH391. A 2.4-kilobase EcoRI and EcoRV fragment containing the cI DBD and CDC6p was released from the resulting plasmid and cloned into EcoRi- and ProII-digested pACYC184 containing a tetracycline resistance gene. The final construct, pC6C6, was transformed into kanamycin-resistant E. coli (JH372/RN/kn') for later experiments. The purified library plasmids were transformed into strain JH372 (25) containing pC6C6 via electroporation. After a 30-min recovery, the cells were harvested by low speed centrifugation and resuspended in SDS-PAGE sample buffer supplemented with 50 mM kanamycin at 37 °C overnight. Analysis of a large number of colonies picked from these plates indicated that there are approximately 5.6 × $10^6$ peptide-expressing plasmids in the library.

**Protein Expression and Purification**—The genomic DNA fragments in candidate colonies were amplified by polymerase chain reaction and cloned into the plasmid and immediately plated on a Luria broth plate supplemented with carbenicillin (50 g/ml) at 37 °C overnight. Analysis of a large number of colonies picked from these plates indicated that there are approximately 5.6 × $10^6$ peptide-expressing plasmids in the library.

**In Vitro Binding Assays**—The genomic DNA fragments in candidate colonies were amplified by polymerase chain reaction and cloned into the NcoI and EcoRI sites of pGEX-Cs. The resulting recombinant plasmids were transformed into E. coli BL21. Production of fusion protein was induced by adding IPTG (0.2 mM) at A$_{600}$ 0.4 followed by shaking at 37 °C for 5 h. The cells were harvested after centrifugation, resuspended in 1 × PBS buffer (138 mM NaCl, 2 mM KCl, 10 mM Na$_2$PO$_4$, and 5 mM K$_2$PO$_4$ containing protease inhibitor mixture (Roche Biochemicals) and lysed by sonication. Fusion protein was purified over glutathione-Sepharose 4B beads according to the manufacturer’s instructions (Amersham Pharmacia Biotech).

Overexpression of CDC6p in HeLa cells was achieved by transient transfection of HeLa cells with pGCM-SHC42FL in which HA-tagged CDC6p was under the control of the CMV promoter. 24 h posttransfection, cells were washed two times with ice-cold PBS buffer. 0.5 ml of lysis buffer (20 mM Tris, pH 7.5, 0.2% Nonidet P-40, 100 mM NaCl, 20% glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, plus protease inhibitor mixture) was added to each 100 mm plate. The cells were scraped off the plates and left on ice for 30 min to allow for complete lysis. The supernatant was collected after centrifugation at 4 °C for 10 min and used for the in vitro binding assay.

Equal volumes of cell lysate containing human CDC6p was added to glutathione-Sepharose 4B beads bearing equal amounts of the various GST-epitope fusion proteins. The mixtures were tumbled at 4 °C for 2 h and beads were washed with ice-cold 1 × PBS twice, 10 min tumbang at 4 °C for each wash. The proteins retained on the beads were denatured and reduced by treatment to SDS-PAGE and Western blotting. Mouse monoclonal antibody against the HA epitope was used for detection of HA-CDC6p protein, mouse monoclonal against GST was used for blotting GST fusion protein. Goat anti-mouse IgG horseradish peroxidase conjugate was used as the secondary antibody. The bands were visualized by SuperSignal West Pico chemiluminescent solution (Pierce).

**Scrambled Mutants of CDC6-BP35**—Scrambled mutants of CDC6-BP35 have identical amino acids composition but different sequence. To achieve this, five pairs of oligonucleotides encoding the new peptide were synthesized for each mutant. These oligos were annealed, ligated, and cloned into NcoI/EcoRI-digested pGEX-C5S. The constructs were confirmed by DNA sequencing. The peptide sequences of the scrambled mutants were SDKFPYCVKSSKFYTHCKIKQGGKKSKCLK for CDC6-BP55S5 and KSLIKSHIKGYKFDKVKQIKICTKISKIFC-RIL for CDC6-BP55S5.

**Estimation of Dissociation Constants**—The experiment was performed essentially as described by Zhang et al. (26). Different amounts of the GST-CDC6-BP35 fusion protein were added to the same amount of extract made from HeLa cells transfected with the CDC6p expression plasmid to determine the lowest amount of beads that could be employed. Then, this fixed amount of bead-bound fusion protein (5 ng) was mixed with HeLa cell extract containing different amounts of CDC6p protein (5, 10, 20, 30, 50, and 60 ng); the final volume was 100 μl in 1× lysis buffer. The mixtures were incubated at 4 °C for 2 h and washed with ice-cold PBS twice. The amount of CDC6p retained was determined by SDS-PAGE/Western blot analysis of the bead-bound material. The amount of HA-tagged CDC6p in the HeLa cell lysate was estimated by quantitative Western blot using purified HA-PLP as standard (provided by L. Sun, University of Texas Southwestern Medical Center, Dallas, TX). The data were plotted as described by Freifelder (27), using the equation $1/r = K_r/[C_{6p}]_{bound}$, where $r = [C_{6p}]_{bound}/[GST-CDC6-BP35].$ The slope of the line obtained in a double r plot provides the $K_r.$

**FACS Analysis**—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum at 37 °C, 5% CO$_2$ incubator and transfected with either pEGFP, pEGFP-CDC6-BP35, or pEGFP-C2 using LipofectAMINE PLUS™ (Life Technologies, Inc.). Cells were trypsinized from plates 24 h later, washed with ice-cold PBS, and fixed with 4.0% paraformaldehyde at 4 °C for 10 min. After being washed with ice-cold PBS, cells were permeabilized with 0.1% Triton X-100 at 4 °C for another 10 min. Finally, cells were washed with ice-cold PBS twice and treated with 20 μg/ml propidium iodide and 2 mg/ml RNase for more than 30 min before the FACS assay. CellQuest was used for the FACS data acquisition and analyses.

**Cell Synchronization**—HeLa cells were transfected with pEGFP or pEGFP-CDC6-BP35. 6 h after transfection, cells were cultured in growth medium containing thymidine (2 mM) for 18 h or aphidicolin (5 μg/ml) for 24 h. HeLa cells were released from the block by washing with growth medium containing aphidicolin (5 μg/ml) and incubation with 80 μg/ml aphidicolin for 2 h. The cell cycle stage at G1/S boundary was washed twice in growth medium. At different time points after release, cells were harvested and processed for FACS assay as above.

**Chromatin Isolation**—HeLa cells were transfected with pEGFP, pEGFP-C2, or pEGFP-CDC6-BP35. 48 h after transfection, cells were stained with Hoechst (10 μg/ml) at 37 °C for 10 min and harvested for
CDC6-binding Peptide

FIG. 1. Schematic representation of the protein interaction assay used in this study. A, basic structure of the plasmids employed. B, if the library-encoded peptide binds the CDC6p with sufficient specificity and affinity, a dimeric, active Repressor will be reconstituted, B, if the library-encoded peptide binds the CDC6p with sufficient specific- 

RESULTS

Isolation of a CDC6p-binding Peptide Using a Bacterial Two-

hybrid-like System—To isolate a CDC6p-binding peptide from a library, we employed a genetic assay based on reconstitution of the activity of a λ Repressor fragment that includes the entire DBD but lacks the C-terminal dimerization domain (19, 25, 29). Two Repressor DBD fusion proteins are introduced into bacteria, one fused to the target (in this case CDC6p) and the other fused to a peptide library. Binding of the library-encoded peptide to the target protein reconstitutes Repressor dimeriza-

 CDC6-binding Peptide
harbor an integrated Repressor-controlled assay were isolated and transformed into fresh JH372 cells, which
Library plasmids (see Fig. 1A, in bacteria.

tained with the another scrambled peptide CDC6-BP35S2 of CDC6p to GST-CDC6-BP35. An identical result was ob-
at concentrations 20-fold higher than that required for binding
did not bind CDC6p in a manner detectable in this assay, even
did not also harbor the target plasmid. The
b
 expressing EGFP fused to CDC6-BP35 exhibited a markedly dif-
(data not shown). We conclude that binding of CDC6-BP35 to
CDC6p is dependent on the primary sequence of the peptide
and not solely on the charged nature of peptide.

The CDC6-BP35-CDC6p interaction was examined in somewhat more detail. The complex is stable over a 20-min period in PBS containing up to 350 mM sodium chloride (data not shown). This also argues against an entirely ionic interaction. The equilibrium dissociation constant ($K_d$) of the GST-CDC6-BP35/CDC6p complex under the test conditions was approximately $10^{-7}$ M, as determined by the titration experiment (27) shown in Fig. 6.

**CDC6-BP35 Blocks the Cell Cycle When Overexpressed in HeLa Cells**—One of major aims of this study was to determine whether a peptide selected simply on the basis of its ability to bind CDC6p could perturb the cell cycle. Because CDC6p is known to be critical for the initiation of DNA replication, a functional effect of the peptide most likely would be observed at the G1/S boundary. To test this possibility, an expression plasmid was constructed that encoded a fusion of the CDC6-BP35 to the C terminus of enhanced green fluorescent protein (EGFP) under control of the CMV promoter. Human HeLa cells were harvested 24 h after transfection with this construct, or control DNAs, and incubated with propidium iodide. The cell population was subjected to FACS analysis in order to segregate cells expressing the fusion protein, and DNA content was measured in this subpopulation of cells. Within an asynchronously growing cell population, 2n, 4n, or intermediate levels of DNA identify cells in G1, G2/M, or S phases of the cell cycle, respectively. Conditions that block entry into S phase increase the fraction of cells with a 2n content of DNA, with corresponding declines in the fraction of cells with a DNA content greater than 2n.

Cells transfected with plasmids that express EGFP alone or EGFP fused to a control peptide that does not interact with CDC6p (C2, see Fig. 3) exhibited a population distribution of about 61–64% in G1, 16 ± 3% in G2/M, and 18–19% ± 3–4% in G2/M 24 h after transfection. These values that are typical for asynchronously growing HeLa cells under standard culture conditions. In contrast, cells transfected with a plasmid expressing EGFP fused to CDC6-BP35 exhibited a markedly dif-

BP35 peptide and CDC6p.

As another test of binding specificity, a “scrambled” derivative of CDC6-BP35 was created, CDC6-BP35S1, in which the identical amino-acid composition was retained, but the order of residues was altered randomly. This control was deemed particularly important because CDC6-BP35 is a highly basic peptide and relatively nonspecific binding to CDC6p through ionic contacts was a concern. As shown in Fig. 5, GST-CDC6-BP35S1 did not bind CDC6p in a manner detectable in this assay, even at concentrations 20-fold higher than that required for binding of CDC6p to GST-CDC6-BP35. An identical result was obtained with the another scrambled peptide CDC6-BP35S2
These experiments contained some GST lacking the peptide fusion.

Centrifugation of extracts made from cells transfected with expression plasmid for HA-CDC6p, HA-MNFα, HA-PR48, or HA-Dral. Retention of these proteins on the beads was analyzed by SDS-PAGE and Western blotting with the appropriate antibodies. Binding of the peptide to proteins other than CDC6p was not observed.

\[ \text{GST-CDC6-BP35} \text{ retained a constant ratio of cells in G, S, and G2/M throughout the time course. This result is consistent with these cells being prevented from continuing through the cell cycle after release of the block. In other words, most of these cells appear to be unable to initiate DNA replication.} \]

FIG. 4. The selected CDC6p-binding peptide does not associate with other proteins tested. Equal amount of GST-CDC6-BP35 was incubated with various extracts made from cells transfected with different expression plasmids for CDC6p, MNFα, PR48, or Dral. Retention of these proteins on the beads was analyzed by SDS-PAGE and Western blotting with the appropriate antibodies. Binding of the peptide to proteins other than CDC6p was not observed.

FIG. 5. A scrambled derivative of CDC6-BP35 does not bind CDC6p in vitro. Increasing concentrations of bead-bound GST-CDC6-BP35 or of GST fused to a scrambled version of CDC6-BP35 that had the same amino acid composition but a different primary sequence were incubated with a HA-CDC6p-containing extract. Retention of the tagged CDC6p was analyzed by SDS-PAGE and Western blotting. In each set of experiments, equal amounts of the two fusion proteins were employed: lanes 2 and 6, 10 ng; lanes 3 and 7, 20 ng; lanes 4 and 8, 100 ng; lanes 5 and 9, 200 ng. The preparations of fusion proteins used in these experiments contain some GST lacking the peptide fusion.

Different ratio. About 83 ± 5% of the cells were in G1, 5 ± 2% in S1, and 7 ± 2% in G2/M (Fig. 7A). Similar differences were also observed 30 and 36 h after transfection (data not shown). Expression of the CDC6-BP35 GFP fusion seemed lower than expression of the control proteins as visualized by fluorescence microscopy (Fig. 7B). Furthermore, more pEGFP-CDC6-BP35-transfected cells were dead 24 h after transfection than was the case in the two control populations.

To examine the effect of the CDC6-binding peptide on cell cycle progression more carefully, cells transfected with pEGFP or pEGFP-CDC6-BP35 were synchronized with either aphidicolin or thymidine 6 h after transfection. The block was later released, and the progression of the two cell populations through the cell cycle was followed by FACS as a function of time. As shown in Fig. 8, the results in the control population and the cells transfected with the CDC6-binding peptide were dramatically different. The fraction of the control population in S phase increased greatly after 3 h, as expected, and after 6 h, approximately 55% of the cells were in G1, 17% were in S1, and 28% were in G2/M. In contrast, the population transfected with pEGFP-CDC6-BP35 maintained a constant ratio of cells in G1 (79–82%), S1 (7–11%), and G2/M (7–10%) throughout the time course. This result is consistent with these cells being prevented from continuing through the cell cycle after release of the block. In other words, most of these cells appear to be unable to initiate DNA replication.

FIG. 6. Determination of the equilibrium dissociation constant of the CDC6p-CDC6-BP35 complex. A, results of an in vitro protein interaction experiment using a constant amount of bead-bound GST-CDC6-BP35 and increasing amounts of extract containing HA-tagged CDC6p. The amount of tagged CDC6p in the input and the retained fraction was quantitated by comparison to a known standard containing the same tag (not shown). B, double reciprocal plot of the data in A indicates a Kd of approximately 1 × 10⁻⁷ M. 1/r = Kd/[CDC6p]bound + 1, where r = [CDC6p]bound/[GST-CDC6-BP35].

CDC6-BP35 Inhibits CDC6p Binding to Chromatin in G1 Cells—Although the mechanism of action of human CDC6 protein in cell cycle progression is not understood in detail, studies in yeast cells indicate that the CDC6 protein binds to the ORC and then recruits MCM proteins to the prereplication complex in late G1 phase (23). Given the propensity of library-derived molecules to recognize native interaction sites in intact target proteins (see Introduction), we hypothesized that overexpression of CDC6-BP35 might disrupt the interaction of CDC6p with other proteins, such as the ORC or MCM, factors critical for initiation of DNA replication. Therefore, HeLa cells were transfected with pEGFP, pEGFP-C2, or pEGFP-CDC6-BP35. One million fluorescent cells in G1 phase were sorted. A chromatin-containing fraction was isolated biochemically from each collected sample. The chromatin-associated proteins were separated by SDS-PAGE, and the levels of ORC2, MCM3, and CDC6p in this fraction were assessed by Western blotting with the appropriate antibodies. As shown in Fig. 9, the amount of ORC2 protein associated with chromatin was indistinguishable in all three samples. However, the amount of CDC6p and MCM3 was reduced to about 50% in cells transfected with pEGFP-CDC6-BP35 relative to the two controls. This observation is consistent with the idea that CDC6-BP35 inhibits CDC6p function and, specifically, represses its loading onto the chromatin, which would be predicted to block the initiation of DNA replication and cell cycle progression.

DISCUSSION

The major conclusion of this study is that a peptide selected to simply bind to a given target protein can have interesting biological activities consistent with modulating the function of the target protein. A more complicated functional screen was unnecessary to obtain the active compound. A peptide selected in bacteria to bind full-length human CDC6p exhibits the characteristics expected of an inhibitor of cell cycle progression in human cells (Figs. 7 and 8). A specific assay for loading of CDC6p on chromatin reveals that the presence of the CDC6-BP35 peptide reduces CDC6p-chromatin association by about
50% compared with controls (Fig. 9), so it seems that the peptide interferes with some process important for CDC6p loading onto chromatin. Consistent with this interpretation is the fact that MCM3 association with chromatin is also inhibited by approximately the same amount, and this event is thought to be CDC6p-dependent. Quantitatively, the effect of CDC6-BP35 on CDC6p-chromatin association (Fig. 9) is more modest than the strong block to cell cycle progression (Fig. 8). Although we do not understand this completely, a number of simple models can be proposed. For example, the peptide may have an effect on CDC6p function over and above the inhibition of chromatin loading that accounts for the strong block to cell cycle progression. Alternatively, because CDC6p must operate at many thousands of replication origins in a cell, it may be that a 2-fold inhibition of chromatin loading is sufficient to provide a strong overall block to the global initiation of DNA replication. Advances in characterizing the specific biochemical functions of CDC6p will be required before such specific hypotheses can be tested rigorously, but the peptide identified in this study may prove useful in this process.

These results are of general interest because the selection was conducted in a mechanistically naïve fashion. Peptides are often employed to block protein-protein interactions or to inhibit enzymatic activities of target proteins for which detailed knowledge of the interacting domains is already available. We did not demand disruption of a specific CDC6p function or require binding to a particular domain known to be critical for protein-protein interactions. Indeed, it would have been difficult to do so with confidence, given the nascent state of work in unraveling the mechanisms of human CDC6p function. Thus, a novel feature of this study is not that a peptide can bind to a protein and inhibit its function; this is well established. Rather, it is that one can carry out a simple, unbiased binding assay using a protein target about which one has little mechanistic knowledge and have a reasonable expectation of identifying peptides able to modulate the function of that protein. Even though only a single specific CDC6p-binding peptide was isolated in the selection reported here, it proved to have the biological activity expected of a CDC6p inhibitor, thereby supporting the notion that functionally important surfaces of proteins are preferential sites for binding in library screening experiments. Of course, it is important to point out that although the biological data are consistent with the peptide blocking CDC6p activity, this cannot be proven without the definitive evidence of interaction between this peptide and CDC6p in vivo.

The \( \lambda \) Repressor reconstitution assay used here is one of many different kinds of two-hybrid-like systems that can be used for screening peptide libraries genetically. We have adopted the Repressor system for general use, because in re-
lated work we are interested in targeting specific epitopes on proteins (26, 35). An advantageous feature of this E. coli-based system relative to yeast- or mammalian cell-based assays is the ability to handle larger libraries due to the high transformation efficiency (19). Although in vitro binding systems, such as phage display, can accommodate even larger libraries and permit manipulation of binding conditions to favor high affinity interactions, peptides identified by in vitro screens, in which the target protein is (usually) the only polypeptide present may lack the specificity required of biologically useful peptides. On the other hand, only one specific CDC6p-binding peptide was isolated in this study from a library estimated to include more than 1 million peptides. Thus, it might be that binding peptides will be isolated for some targets but not others and a technique such as phage display will have to be considered.

Another point of potential variation in the use of this general approach to the isolation of biologically active peptides is the context in which the library is displayed. Prior to our work, Brent and co-workers (36) developed an elegant “peptide aptamer” system in which a library of peptides is displayed in the context of a surface loop of thioredoxin. Using the appropriate fusion proteins, the aptamer library is then screened in a yeast two-hybrid experiment for peptides that recognize a target protein of interest. These have generally been enzymes such as protein kinases and the aptamers obtained indeed prove to be potent inhibitors of the target enzymes in their native cellular environment (37–39). A particularly impressive feature of thioredoxin-based aptamers are their high binding affinities (KD values are often in the range of 10⁻⁹ M), whereas the CDC6-BP35/CDC6p dissociation constant was approximately 10⁻⁷ M. Tight binding is probably due to the fact that constraining the peptide into the surface loop makes it “cyclic peptide-like” in the sense that it has significantly less conformational freedom than is the case for a linear peptide. Of course, this means that the selected peptides may not work well outside the context of thioredoxin (37) or at least may exhibit much lower affinities, but fortunately, these thioredoxin-based aptamers express well in a variety of cell types. Our method uses unconstrained peptides displayed as a C-terminal extension on a monomeric protein. Thus, peptides selected using this technology should be highly “portable” in terms of retaining function when presented as a free peptide or following fusion to a variety of other proteins, and our experience to date confirms this prediction (26) (this study).2 Aptamers are likely to be the reagents of choice when high affinities are required and the use of the modified thioredoxin protein does not present a problem. A recent report from Benkovic and co-workers (28) of an intein-based method by which libraries of cyclic peptide can be produced in E. coli is also of interest. If this technology could somehow be married to a suitable two-hybrid-like assay, it might combine the best facets of free peptides and thioredoxin-based aptamers. In any case, the

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2 W. Zhu, R. S. Williams, and T. Kodadek, unpublished results.
work of Brent and co-workers (36) and now this study indicate that genetic protein binding assays using a suitable target appear to be an excellent strategy for the isolation of biologically active peptides. In the future, aptamers and peptides selected by these means are likely to play an increasingly important role as tools with which to manipulate and study a variety of biological pathways.

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