

A Combined Yeast/Bacteria Two-hybrid System

DEVELOPMENT AND EVALUATION*

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Two-hybrid screening is a standard method used to identify and characterize protein-protein interactions and has become an integral component of many proteomic investigations. The two-hybrid system was initially developed using yeast as a host organism. However, bacterial two-hybrid systems have also become common laboratory tools and are preferred in some circumstances, although yeast and bacterial two-hybrid systems have never been directly compared. We describe here the development of a unified yeast and bacterial two-hybrid system in which a single bait expression plasmid is used in both organismal milieus. We use a series of leucine zipper fusion proteins of known affinities to compare interaction detection using both systems. Although both two-hybrid systems detected interactions within a comparable range of interaction affinities, each demonstrated unique advantages. The yeast system produced quantitative readout over a greater dynamic range than that observed with bacteria. However, the phenomenon of “autoactivation” by baits was less of a problem in the bacterial system than in the yeast. Both systems identified physiological interactors for a library screen with a *cl-Ras* test bait; however, non-identical interactors were obtained in yeast and bacterial screens. The ability to rapidly shift between yeast and bacterial systems provided by these new reagents should provide a marked advantage for two-hybrid investigations. In addition, the modified expression vectors we describe in this report should be useful for any application requiring facile expression of a protein of interest in both yeast and bacteria. *Molecular & Cellular Proteomics* 4: 819–826, 2005.

Yeast two-hybrid systems (1–4) are standard tools used to identify novel protein-protein interactions and to perform structure-function analysis on previously defined protein-pro-

tein interactions. Such systems are effective with a substantial fraction of eukaryotic proteins and have played an important role in high throughput proteomic analyses aimed at establishing sets of interacting proteins (e.g. Refs. 5–8). To increase the power of a two-hybrid approach to identify and analyze protein interactions in high throughput applications, one strategy has been to translate the basic components of the yeast two-hybrid system to a bacterial host organism (9, 10). To date, the relative effectiveness of protein interaction detection in bacterial and yeast backgrounds has not been directly compared. However, there are a number of reasons to anticipate that differences might be observed. Because yeast are eukaryotes, eukaryotic proteins used as “baits” in two-hybrid screens might be more likely to be appropriately folded and post-translationally modified in yeast than in bacteria, thereby increasing their chances of identifying physiological partners. However, certain proteins can be challenging as baits in the yeast two-hybrid system; for example, eucaryotic proteins that are normally excluded from the nucleus, that are potentially sequestered via interaction with an abundant partner evolutionarily conserved in yeast, or that stimulate transcription in yeast (i.e. that “autoactivate”). All of these potential issues would be expected to be less problematic in the bacterial two-hybrid system. To maximize chances of obtaining all relevant interactors for a protein of interest, it would be desirable to have the capability to rapidly test any given bait in both yeast and bacterial milieus.

In the current study, we have created and validated plasmids and strains that facilitate interconversion between yeast and bacterial protein interaction systems. We have designed a novel series of vectors in which a single plasmid containing a modified promoter drives the efficient expression of a bait protein in either yeast or bacteria, thereby permitting parallel studies in both organisms. In addition, we have constructed optimized yeast and bacterial two-hybrid reporter strains. Using these reagents, we have generated constructs that permitted us to test a series of leucine zippers with interaction constants ranging between K_d values of $\sim 10^{-4}$ and 10^{-15} M in both the yeast and bacterial systems using auxotrophic and quantitative reporters. We report that although both systems detect protein interactions within a comparable range of affinities, there are characteristic differences between the two systems; the yeast system possesses greater dynamic range

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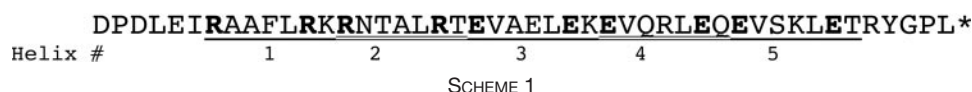
Received February 24, 2005

Published, MCP Papers in Press, March 20, 2005, DOI 10.1074/mcp.T500005-MCP200

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for signal, but the bacterial system seems to be less susceptible to the phenomenon of bait autoactivation. Using a well characterized protein (H-Ras) as a bait, we also show that the system is robust for library screening purposes and that its use in parallel in both organisms may increase coverage and accuracy in screening. We discuss particular applications for this novel yeast/bacterial two-hybrid system.

MATERIALS AND METHODS

Molecular and Microbiological Manipulation—The cloning of novel constructs was performed using conventional protocols. Details of the sequences and cloning sites encompassed in the plasmids described under “Results,” as well as other basic characterizations of expression properties of these plasmids, are available at www.fccc.edu/research/labs/golemis/InteractionTrapInWork.html. Media and growth conditions used are described in Ref. 11.

In brief, plasmid pGLS20 was constructed by replacing the *ADH1* promoter of pGKS9 with a combination of the *TEF1* promoter (from the pLexZeo plasmid, Invitrogen, Carlsbad, CA) and a *lpp/lacUV5* promoter (from the pBT plasmid; Stratagene, La Jolla, CA). To produce pGLS23, a *HIS5* Cm^R cassette was constructed in the pCR2.1 vector by combining a *HIS5* cassette from pJFK¹ and a Cm^R cassette from pMW108. This cassette was then used to replace the G418R cassette in pGLS20. The bacterial two-hybrid prey plasmid pAC-AMP- α LPL was constructed by replacing the chloramphenicol resistance gene present in plasmid pKJ1267² with the ampicillin resistance gene from plasmid pACYC177. To fuse the various leucine zippers to the amino-terminal domain and interdomain linker of the *Escherichia coli* RNA polymerase- α subunit, DNA fragments encoding the zipper variants were inserted into the plasmid using unique *NotI* and *XhoI* restriction sites. To fuse leucine zippers to λ cl and B42 moieties of the bait and yeast prey (pJG4–5) plasmids, DNA fragments encoding the zipper variants were inserted into the plasmid using unique *EcoRI* and *XhoI* restriction sites. Further information about cloning strategies used for plasmid construction or details of yeast or bacterial strain construction and characterization are available upon request.

Leucine Zippers—Leucine zipper sequences were chosen from among peptides described in Refs. 12–14. DNA was synthesized artificially to encode the described peptide sequences. All leucine zippers have the same length and differ only in the amino acids in positions *g* and *e* of the coiled coil (marked above sequence). Shown in Scheme 1 is the amino acid sequence of the zipper RR₁₂EE₃₄₅; helices 1, 3, and 5 are *underlined*, helices 2 and 4 are *double-underlined*, and the variable amino acids are shown in *bold*. Thus, in the example shown, there are Rs in positions *g* and *e* of helices 1 and 2 (hence the nomenclature of the molecule starts with RR₁₂), whereas E's in the corresponding positions of the helices 3–5 cause the nomenclature of the molecule to end with EE₃₄₅. Complete details are available upon request.

Bait and Prey Expression—The expression of the bait and prey proteins (except for bacterial RNA polymerase- α fusions, for which no antibody was available) was confirmed by Western analysis, with

primary antibody to cl for baits (1:5000) or hemagglutinin (1:1000) for preys expressed in yeast. To compare expression levels of cl proteins in *E. coli*, corresponding plasmids were transformed into the DH5 α strain and protein extracts prepared from exponentially growing cultures. Equal protein concentration was confirmed by Coomassie staining of a PAGE gel; then, equal volumes of 1:40 (for pGLS20) or 1:100 (for pBT) dilutions of extracts in sample buffer were loaded in parallel with the same volume of undiluted extract from pGLS10-bearing cells. Proteins were resolved on a PAGE gel, and Western blot analysis was performed, using anti-cl antibodies. To compare expression levels of cl proteins in yeast, corresponding plasmids were transformed in SKY191 strain and protein extracts prepared from exponentially growing cultures. Equal protein loading was confirmed by Coomassie staining samples resolved on a PAGE gel (data not shown). Then, equal volumes of extracts in sample buffer were loaded on the gel, and Western blot analysis was performed.

Reporter Assays—For yeast, the activity of quantitative reporters was determined on a plate reader using a technique modified from Serebriiskii *et al.* (15). In brief, to 50 μ l of cultures exponentially growing in the wells of 96-well plates was added an equal volume of 2 \times Z-buffer containing 2 mg/ml of *p*-nitrophenyl β -D-glucopyranoside and 50% Y-PER (Pierce). Activity was calculated as $(A_{420f} - A_{420i})$ divided by A_{600} , where the difference between A_{420i} and A_{420f} (*i* and *f* indicate initial and final readings, respectively) reflects the conversion of the colorless substrate into yellow product over a period of time from ~10–30 min, and A_{600} is a measure of cell density in a given sample. For each data point for each yeast experiment, activities of five to eight clones were measured and averaged. All readings were taken in a plate reader; it was shown previously (15) that plate reader measurements and derivative units are proportionally correlated with the optical density units taken on a spectrophotometer.

For fluorescence detection, an equal volume of 2 \times Z-buffer/50% Y-PER containing 0.8 mg/ml of 4-methylumbelliferyl- β -D-glucopyranoside was added. Increase in fluorescence (excitation wavelength, 355 nm; emission wavelength, 460 nm) reflected the conversion of the colorless substrate into fluorescent product over a period of time from ~3–10 min, whereas A_{600} was a measure of cell density in a given sample. The pRG61 plasmid was used as reporter in these experiments. For bacterial β -galactosidase reporter gene measurements, assays were performed essentially as described previously (16). In brief, cultures inoculated from a fresh single colony were grown to mid-log phase and lysed by adding 1/10 volume PopCulture (Novagen, Madison, WI). In a 96-well microtiter plate, 15 μ l of cell lysate was added to a mixture of 135 μ l of Z buffer and 30 μ l of 4 mg/ml O-nitrophenyl β -D-galactopyranoside to start the reaction. Kinetic assays were carried out by monitoring A_{415} from 0–30 min using a plate reader. Additional details can be found at www.zincfingers.org. All bacterial β -galactosidase assays were done in triplicate. Auxotrophic reporters were assayed as described in Ref. 11. Bait and prey plasmids were transformed into corresponding selection strain (*Saccharomyces cerevisiae* SKY191 or pRT50 or *E. coli* KJ1567). Growth on selection plates was measured over 5 days (yeast; note that all colonies that grew were prominent at 2 days) or 1 day (bacteria).

Library Screenings—Screening of the yeast two-hybrid library and analysis of primary isolates was done essentially as described in Ref. 11 using the pOR6 *lacZ* reporter. In brief, approximately 3.5×10^6 cells carrying plasmids from the HeLa cDNA library were plated. 130 clones appearing on the auxotrophic selection plates were further

¹ R. Hopkins, unpublished observations.

² J. Keith Jung, unpublished observations.

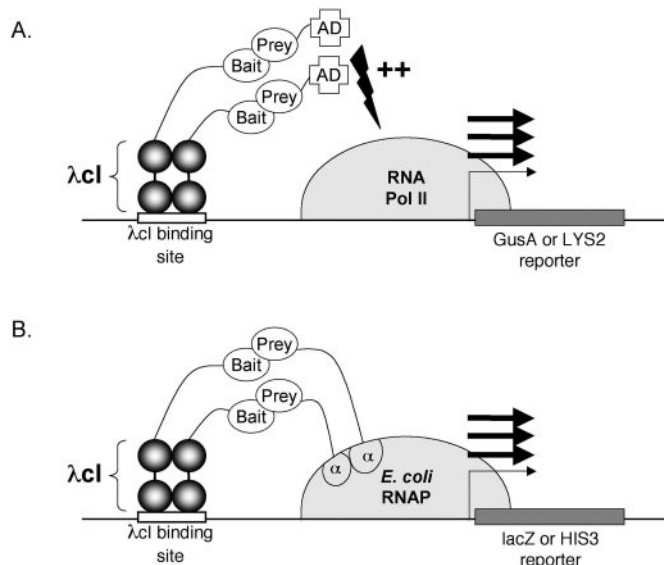


FIG. 1. Schematics of the yeast and bacterial two-hybrid systems. A, in the yeast two-hybrid system shown, a dimeric λ cl-bait hybrid protein interacts with an activation domain (AD)-prey hybrid protein, thereby stimulating transcription from an adjacent promoter that directs expression of a quantitative *gusA* or selectable *LYS2* reporter gene. B, in the bacterial two-hybrid system shown, a dimeric λ cl-bait hybrid protein interacts with an *E. coli* RNA polymerase (RNAP) α -subunit-prey hybrid protein, thereby recruiting RNAP to an adjacent promoter that directs expression of a quantitative *lacZ* or selectable *HIS3* reporter gene. Note that both systems use a λ cl-bait hybrid protein expressed from a single plasmid effective in either organism.

examined. Of 28 positives (in which an initial positive phenotype was repeated), 24 were sequenced. The yeast/bacteria two-hybrid system developed here is fully compatible with strains and library reagents from Stratagene (the Bacteriomatch system). To emphasize compatibility, screening of the BTH library and analysis of primary isolates was done with this system (see Table II) essentially as recommended by the supplier. In brief, approximately 5.5×10^6 cells carrying plasmids from the HeLa cDNA library (average insert size, 1.3 kb) were plated. 96 clones appearing on the auxotrophic selection plates were characterized, and 18 of 22 positives with a reproducible phenotype were sequenced.

RESULTS AND DISCUSSION

We have developed plasmids that allow the expression and parallel screening of a single bait protein in either a yeast or bacterial two-hybrid system using a single expression plasmid (Fig. 1). As shown in Fig. 1, bait proteins are expressed as fusions to the λ cl protein in both the yeast and bacterial two-hybrid systems. To enable this, we made several modifications to the plasmid pGBS9 (17), originally developed to express bait proteins as fusions to the λ cl repressor in a yeast two-hybrid system. The *ADH1* promoter from this plasmid was replaced with a tandem promoter in which the extremely powerful *TEF1* promoter (18) from *S. cerevisiae* and the *E. coli lpp/lacUV5* promoter both direct expression of a λ cl coding sequence and polylinker cloning site. The resulting plasmid, pGLS20, can be maintained in yeast or bacteria based on

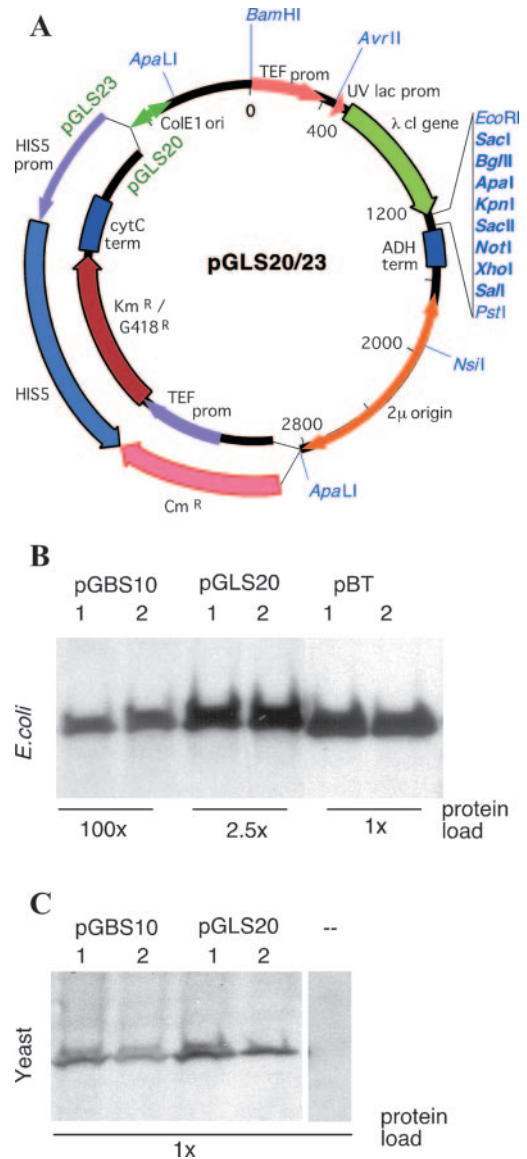


FIG. 2. Bait expression from a combined bacterial/yeast expression plasmid. A, plasmids pGLS20, pGLS22, and pGLS23 (pGLS22 and pGLS23 differ only in the presence of an extra *EcoRI* site in the *Cm^R* gene of pGLS22) use a combined *TEF1/lpp-lacUV5* promoter to express λ cl fused baits in yeast or bacteria. Plasmids are selected in yeast by selection for G418 resistance (pGLS20) or *HIS5* complementation (pGLS23), and in bacteria by selection for kanamycin resistance (pGLS20) or chloramphenicol resistance (pGLS23). B, relative expression of cl baits from these plasmids compared with the previously described pGBS10 (yeast two-hybrid (17)) or pBT (bacterial two-hybrid; Stratagene) vectors is shown in bacteria (center). To demonstrate relative bait levels, equal total protein concentration was confirmed by Coomassie staining of a PAGE gel loaded with equivalent amounts of cell lysate for bacteria expressing each plasmid (not shown). Then, equal volumes of 1:40 (for pGLS20) or 1:100 (for pBT) dilutions of extracts in sample buffer were loaded in parallel with the same volume of undiluted extract from pGBS10-bearing cells. Western blots using anti-cl antibodies are shown. C, pGBS10 and pGLS20 express comparable levels of λ cl baits in yeast, based on Western analysis with antibodies to λ cl. 1 and 2, two independent transformants in bacteria or yeast; -, yeast containing no bait plasmid.

TABLE I
Properties of leucine zippers used in this study

pl calculations were made using the site at us.expasy.org/tools/pi_tool.html. Leucine zippers for many of the baits and their *in vitro* interaction properties were described previously (12–14). N.D., not detectable.

Combination	Bait	pl	Prey	K_d for Bait-Prey
				<i>M</i>
1	EE ₁₂₃₄₅ L	4.2	EE ₁₂₃₄₅ L	N.D.
2	RR ₁₂ EE ₃₄₅ L	6.5	RR ₁₂ EE ₃₄₅ L	N.D.
3	EE ₃₄	5.3	EE ₃₄	8.1×10^{-4}
4	RR ₃₄	10.5	RR ₃₄	3.9×10^{-5}
5	RR ₁₂₃₄ L	11.8	RR ₁₂₃₄ L	2.5×10^{-7}
6	RR ₃₄	10.5	EE ₃₄	1.0×10^{-8}
7	EE ₃₄	5.3	RR ₃₄	1.0×10^{-8}
8	RR ₁₂ EE ₃₄₅ L	6.5	EE ₁₂ RR ₃₄₅ L	1.3×10^{-11}
9	EE ₁₂ RR ₃₄₅ L	10.4	RR ₁₂ EE ₃₄₅ L	1.3×10^{-11}
10	RR ₁₂₃₄₅ L	12.2	EE ₁₂₃₄₅ L	1.1×10^{-11}
11	RR ₁₂₃₄ L	11.8	EE ₁₂₃₄ L	1.0×10^{-15}
12	EE ₁₂₃₄ L	4.3	RR ₁₂₃₄ L	1.0×10^{-15}

G418 or kanamycin resistance, respectively (Fig. 2A). Other closely related plasmid derivatives (pGLS22, pGLS23) harbor the *HIS5* gene to confer selection in yeast and chloramphenicol resistance for selection in bacteria (Fig. 2A). As shown in Fig. 2B, expression of λ cl repressor using plasmid pGLS20 in bacteria is comparable with that obtained with plasmid pBT (a vector optimized for the bacterial two-hybrid system; Stratagene), and is more than 40-fold higher than that provided by the standard yeast two-hybrid expression plasmid pGBS10 (17). In yeast, expression of cl repressor fusions from pGLS20 and its derivatives is comparable with or exceeds that from pGBS10 (Fig. 2C).

We used these bifunctional pGLS plasmids to determine whether the yeast and bacterial two-hybrid systems exhibited any differences in their abilities to detect a series of interactions with differing affinities. To do this, we created a series of bait and prey fusion proteins using a set of previously characterized leucine zipper variants (12–14) with defined interaction affinities ranging from $K_d > 10^{-4}$ to 10^{-15} M as determined *in vitro* (Table I). For analysis in the bacterial two-hybrid system, plasmid pAC-AMP- α LPL (Table II) was used to express preys from the strong inducible *lpp/lacUV5* tandem promoter as fusions to the amino-terminal domain and linker of the *E. coli* RNA polymerase α subunit. For the yeast two-hybrid system, pJG4–5 (3) was used to express preys from the inducible *GAL1* promoter as fusions to the synthetic transcriptional activation domain B42 (Fig. 1). The ability of each zipper pair to activate transcription of a quantitative and an auxotrophic reporter was then assessed in bacteria and in yeast.

Our results in the yeast-based system demonstrate that zipper bait-prey combinations activate transcription of a quantifiable β -glucuronidase (*gusA*) reporter over a substantial range of affinities (Fig. 3, *bar graph*). In this assay, zipper pairs with reported interaction dissociation constants of $1 \times$

10^{-8} M or lower (*lanes 6–12*) strongly activated reporter gene expression, as detected using a colorimetric substrate (*p*-nitrophenyl β -D-glucopyranoside). Those with K_d values of 2.5×10^{-7} M or higher (with one exception; see below) did not strongly activate the reporter gene (Fig. 3A, *lanes 1–5*). β -Glucuronidase activity was generally induced ~ 30 – 180 -fold over baseline values with the higher affinity leucine zipper pairs. Additional testing of the lower affinity interacting pairs using a more sensitive fluorescent substrate for β -glucuronidase, 4-methylumbelliferyl- β -D-glucopyranoside (Fig. 3A, *inset*), indicated that it was also possible to convincingly detect interactions in the range of 10^{-7} M, although the stimulation of *gusA* gene expression seen in these samples is markedly less strong than those obtained with interactions in the 10^{-8} M range. With the auxotrophic reporter strain (Fig. 3, *panels below bar graph*), cells grew under selective conditions only if the interacting zippers possessed dissociation constants of $\leq 1 \times 10^{-8}$ M, paralleling the results obtained with the quantitative *gusA* reporter. The system did not have significant ability to discriminate between interactions with dissociation constants of $\leq 10^{-8}$ M, suggesting the expression of the reporter gene was saturated. It is noteworthy that for some of the baits examined, expression of the bait alone in the absence of the prey was sufficient to strongly activate transcription of the reporters, making it difficult to convincingly demonstrate protein interaction (see Fig. 3, *samples 1, 10, and 12*).

We next examined the abilities of the same zipper bait-prey combinations to activate transcription in the bacterial two-hybrid system (Fig. 1) using the quantifiable *lacZ* reporter (Fig. 4). Consistent with our results in the yeast-based system, leucine zipper pairs with reported dissociation constants lower than 10^{-8} M clearly stimulated expression of the *lacZ* reporter gene (Fig. 3, *samples 6–12*), whereas interaction pairs with dissociation constants 2.5×10^{-7} M or higher failed to stimulate *lacZ* expression (Fig. 3, *samples 1–5*). We also analyzed zipper-based activation of the auxotrophic reporter *HIS3* (Fig. 4, *panels below bar graph*). Results obtained using the auxotrophic *HIS3* reporter gene closely paralleled those obtained with the *lacZ* reporter; only cells harboring zipper pairs with dissociation constants of $\leq 1 \times 10^{-8}$ M showed growth after 24 h on selective plates. In contrast to the results obtained in the yeast-based system, none of the baits tested exhibited autoactivation in the absence of prey partners (compare *samples 1, 10, and 12* in Figs. 3 and 4).

These results suggest differential advantages for detecting protein-protein interactions in the yeast and bacterial two-hybrid systems. First, our results using quantifiable reporters suggest that the yeast-based system possesses a broader dynamic range for detecting interactions (contrast Figs. 3 and 4). In the yeast system, interactions characterized by dissociation constants as high as 10^{-8} M could be detected as an increase in *gusA* reporter gene expression (or as high as 10^{-7} M if a more sensitive substrate for GusA detection was used). In contrast, in the bacterial system, only interactions charac-

TABLE II
Strains and plasmids used in this study

pRG61 (17), pDR8 (17), SKY191 (24), SKY54 (17), and pJG4–5 (3) have been described previously. Bacteriomatch II reporter strain and library were from Stratagene.

Plasmids	Selection in		Comment/description
	Yeast	<i>E. coli</i>	
Baits			
pGLS20 ^a	G418 ^R	Km ^R	TEF1 promoter ensures expression of <i>cl</i> in yeast, while <i>lpp/lacUV5</i> promoter provides for expression in <i>E. coli</i>
pGLS22/23 ^a	<i>HIS5</i>	Cm ^R	Similar to pGLS20, see text for details
Reporters			
pRG61	<i>URA3</i>	Km ^R	λ cl operators direct transcription of the <i>gusA</i> gene; pRG61 is less sensitive and lower background reporter than pDR8.
pDR8	<i>URA3</i>	Km ^R	λ cl operators direct transcription of the <i>gusA</i> gene; pRG61 is less sensitive and lower background reporter than pDR8
pOR6	<i>URA3</i>	Ap ^R	λ cl operators direct transcription of the <i>lacZ</i> gene
Activation Domain Fusions			
pJG4–5	<i>TRP1</i>	Ap ^R	<i>GAL1</i> promoter provides efficient expression in yeast of a gene fused to a cassette consisting of nuclear localization sequence, transcriptional activation domain, and HA epitope tag
pAC-AMP- α LPL ^a pBR-UV5- α LP ^a	N/A	Ap ^R	Tandem <i>lpp/lacUV5</i> promoters provide efficient expression in <i>E. coli</i> of a gene fused to <i>E. coli</i> RNAP α subunit residues 1–248. Plasmids differ in replication origins (pACYC and ColE1, respectively)
Strains	Relevant Genotype		Comment/Description
<i>S. cerevisiae</i> SKY191	MAT α <i>trp1, his3, ura3, clop-LYS2</i>		Reporter strains in which the expression of the <i>LYS2</i> reporter gene is directed by a weak promoter bearing a λ cl DNA binding site
<i>S. cerevisiae</i> PRT475 ^a	MAT α <i>trp1, his3, his5, ura3, clop-LYS2</i>		
<i>S. cerevisiae</i> PRT50 ^a	MAT α <i>trp1, his3, his5, ura3, clop-LYS2</i>		
<i>E. coli</i> KJ1567 ^a	Δ <i>hisB463, \Delta (gpt-proAB-arg-lac)XIII</i> <i>zaj::Tn10 [F' lacI^R HIS3 aadA Kan^R]</i>		Reporter strain in which the expression of the <i>HIS3</i> and <i>aadA</i> reporter genes is directed by a weak promoter bearing a λ cl DNA binding site
<i>BacterioMatch II reporter strain (Stratagene)</i>	$\Delta (mcrA)183 \Delta(mcrCB-hsdSMR-mrr)173$ <i>endA1 hisB supE44 thi-1 recA1 gyrA96</i> <i>relA1 lac [F' laqI^R HIS3 aadA Kan^R]</i>		Reporter strain in which the expression of the <i>HIS3</i> and <i>aadA</i> reporter genes is directed by a weak promoter bearing a λ cl DNA binding site
<i>E. coli</i> AG58A(RP28) ^a	Δ <i>hisB463, \Delta (gpt-proAB-arg-lac)XIII</i> <i>zaj::Tn10 [F' lacI^R lacZ Kan^R]</i>		Reporter strain in which the expression of the <i>lacZ</i> reporter gene is directed by a weak promoter bearing a λ cl DNA binding site
Libraries	Vector		Comment/description
<i>YTH Human HeLa Cell Library</i>	pJG4–5		Hela S-3 cells, Primary size: 9.6×10^6 Primer: UdT, average insert size: 1.5 kb
<i>BacterioMatch II Human HeLa Cell Library</i>	pTRG: see Stratagene Manual for details		Hela S-3 cells, Primary size: 4.5×10^6 Primer: UdT, average insert size: 1.3 kb

^a Reagent produced in this study.

terized by dissociation constants 10^{-8} M or lower could be detected as an increase in *lacZ* expression. Second, we note that the experiments performed using bacterial two-hybrid system yield colonies on selective medium somewhat more quickly than those done in the yeast system (1 day versus 2). Third, our results also suggest that autoactivation by bait proteins may be less problematic in bacteria than in yeast, because at least some proteins that are autoactivators in the yeast two-hybrid system are not in the bacterial two-hybrid system (compare lanes 1, 10, and 12 in Figs. 3 and 4). This finding is not entirely surprising given the fundamental differences in mechanisms of gene activation and the evolutionary distance between

prokaryotes and eukaryotes. The ability to use some baits that are autoactivating (unusable) in yeast in the bacterial two-hybrid system is a potentially significant advantage.

Our data also suggest that the threshold interaction strength required for robust transcriptional activation is similar in both organisms. In both the yeast and bacterial systems, full activation seems to require an interaction affinity between bait and prey fusion proteins defined by a dissociation constant in the range of $\leq 10^{-8}$ M. Although our results demonstrate a sharp transition between no activation and full activation of the reporter genes, previous studies in both systems have demonstrated that the magnitude of transcriptional ac-

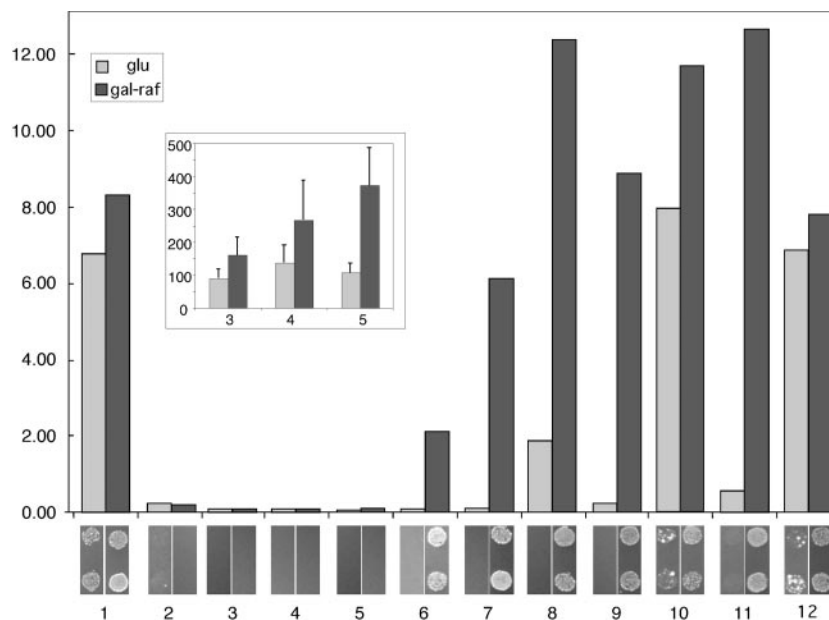
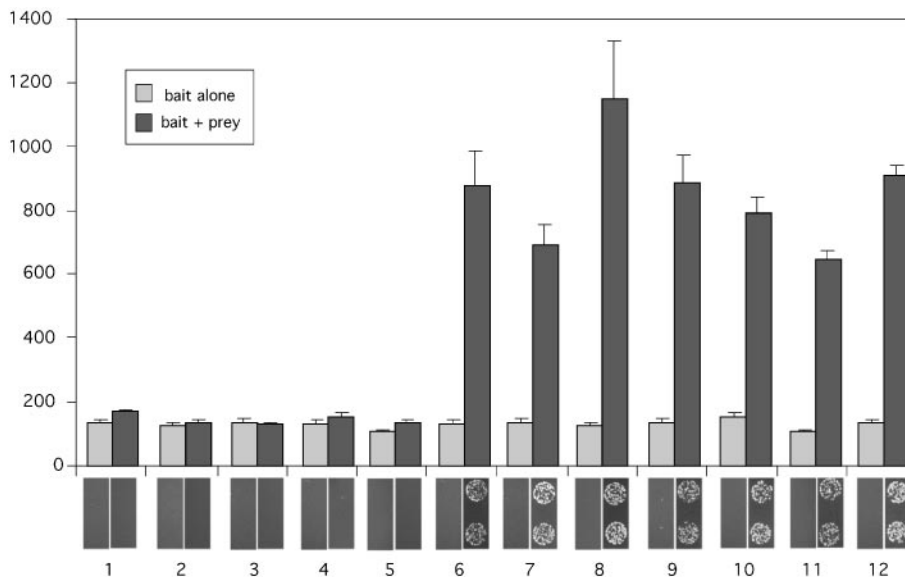


FIG. 3. **Activation of colorimetric and auxotrophic reporters by zipper interaction in yeast.** Lane numbers below bar graph represent pairs of samples defined in Table I. Bar graph reflects relative reporter activity measured by β -glucuronidase assay using *p*-nitrophenyl β -D-glucopyranoside as a substrate. Expression of the AD-fusion protein in yeast is inducible by galactose. Therefore, β -glucuronidase activity revealed upon the growth on glucose (light gray bars with *glu*) represents mainly contribution of bait alone, whereas activity upon the growth in the presence of galactose (dark gray bars with *gal-raf*) reflects the interaction between bait and prey. Results shown represent mean values for three independent experiments. Standard deviation (not shown) was variable but did not exceed 25% of the experimental value, as is typical for yeast two hybrid experiments. Inset, indicated samples re-analyzed using 4-methylumbelliferyl- β -D-glucopyranoside as a substrate. For context, values obtained for combination 6 (with a K_D of 1×10^{-8} M), were more than 10-fold higher than those with combination 5 with the 4-methylumbelliferyl- β -D-glucopyranoside substrate, indicating a significant discriminating function of the yeast two-hybrid system in this affinity range (data not shown). Shown below bar graph is the growth of two representative spots of colonies 2 days after plating to selective medium. Data shown are obtained using the SKY191 strain and pGLS20 as bait plasmids. Similar results were obtained using a combination of the PRT50 strain and pGLS22 bait plasmid (data not shown).

FIG. 4. **Activation of colorimetric and auxotrophic reporters by zipper interaction in bacteria.** Lane numbers below bar graph represent pairs of samples defined in Table I. Bar graph reflects relative reporter activity measured by β -galactosidase assay using *O*-nitrophenyl β -D-galactopyranoside as a substrate. β -Galactosidase values are expressed in Miller units and represent the mean of three independent measurements with S.E.M. shown. The panel below the bar graph presents the growth of two representative spots of colonies 24 h after plating to selective medium.



tivation observed can be correlated with the affinity of the bait-prey interaction (10, 19). Although we do not know the precise reason for this difference in our results compared with previous studies, we note that Estojak *et al.* (19) assessed

interactions using a series of reporters of varying stringency (*i.e.* containing differing numbers of binding sites for the baits) to expand the detection range; there is no technical limitation to using a similar strategy with this new system. Overall, our

TABLE III

Comparative results of yeast and bacterial two-hybrid screening
Accession numbers for clones are available on request.

	YTH Clones Total/Indep	BTH Clones Total/Indep
Clear Ras relevance		
Krit-1	1/1	N ^a
Araf	5/1	N
RGL-2	N	7/2
Ras relevance not known		
<i>Homo sapiens</i> suppression of tumorigenicity 13	2/1	N
Zinc finger protein 616	3/1	N
Hematopoietically expressed homeobox	1/1	N
RAS-related GTP binding dynein	1/1	N
Maspin	1/1	N
Clone CS0DM007YF21	1/1	N
Chromosome 1 open reading frame 37	1/1	N
General transcription factor III C	N	1/1
Glutathione peroxidase 4	N	3/1
G protein	N	1/1
γ -Interferon-inducible lysosomal thiol reductase	N	1/1
Latent transforming growth factor β binding protein 4	N	1/1
Previously described as common false positive		
CPEO mitochondrion	2/1	N
90-kDa heat shock protein 1	2/1	N
Ribosomal protein L10a	1/1	N
Ribosomal protein S7	1/1	N
T108 mitochondrion	1/1	N
Ribosomal protein L26	N	1/1
Ferritin	N	1/1
NADH dehydrogenase	N	1/1
Ribosomal protein S2	N	1/1

^a N, not isolated.

results strongly suggest that use of the current system as a selection tool will work best for detecting interactions with dissociation constants in the mid-nanomolar range (or lower).

The most demanding test of a protein interaction system is its ability to identify physiologically relevant interacting proteins from library screens. To test the YBTH system, we used a cI-Ras bait, because the interaction profile of Ras is well defined (20, 21). This bait was used to screen HeLa cDNA libraries in yeast and in bacteria, screening comparable numbers of primary transformants in each organism (see "Materials and Methods"). As shown in Table III, both screens identified at least some clearly relevant interactors for Ras; the yeast screen identified clones for A-Raf (4) and Krit-1 (22), and the bacterial screen identified RGL2 (23). Both screens also identified a number of clones with uncertain relevance toward Ras that may or may not represent nonspecific interactors, as well as a number of proteins frequently identified as false positives in the yeast two-hybrid screens ([search/labs/golemis/InteractionTrapInWork.html\). Somewhat surprisingly, given that both libraries were prepared from HeLa cell mRNA, there were no overlapping isolates in the two screens, even though multiple isolates of some clones were obtained and the number of primary transformants was in excess of \$3 \times 10^6\$ in each case. This may indicate that specific Ras interactions are more readily detected in one or the other organism. In theory, a protein requiring post-translational modification to interact with Ras may be more readily detected in yeast, whereas a protein that interacts not only with Ras but also with other eukaryotically conserved signaling partners may be more available to interact with Ras in bacteria. Together, these results indicate that this system is robust for screening purposes, and the facile use of the pGLS vectors in both organisms in parallel may increase coverage and accuracy in screening.](http://www.fccc.edu/re-</p>
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Finally, we note that (to our knowledge) this is the first description of a promoter combination that is potent in both yeast and bacterial milieus. In fact, we have found that our pGLS plasmids express sufficient levels of bait fusion proteins for activity in the bacterial two-hybrid system even without inducing the strong bacterial promoter (data not shown). Although this article focuses on the use of the pGLS plasmids in a two-hybrid context, we anticipate that our general promoter design might also be useful in other functional characterization studies.

Acknowledgments—We thank Dr. Paul Watt for critical review of the manuscript. We are grateful to Yijing Groeber for assistance in library screening. We also thank Mary Buchanan for the Bacteriomatch II HeLa cDNA library, Astrid Giesecke for bacterial strains, and Stacey Thibodeau for technical assistance.

* This work was supported by American Cancer Society pilot funding (to I. G. S.); awards from NCI Translational Pilot Project funding, National Institutes of Health Grant R01-CA63366, and the Pennsylvania Tobacco Health Research Formula Fund (to E. A. G.); National Institutes of Health Core Grant CA06927 (to Fox Chase Cancer Center); National Institutes of Health Grants K08-DK02883 and R01-GM069906, Massachusetts General Hospital Department of Pathology start-up funds (to J. K. J.); and the National Health and Medical Research Council of Australia (to R. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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