A Biochemical Genomics Approach for Identifying Genes by the Activity of Their Products

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For the identification of yeast genes specifying biochemical activities, a genomic strategy that is rapid, sensitive, and widely applicable was developed with an array of 6144 individual yeast strains, each containing a different yeast open reading frame (ORF) fused to glutathione S-transferase (GST). For the identification of ORF-associated activities, strains were grown in defined pools, and GST-ORFs were purified. Then, pools were assayed for activities, and active pools were deconvoluted to identify the source strains. Three previously unknown ORF-associated activities were identified with this strategy: a cyclic phosphodiesterase (CPDase) (Fig. 1B), an otherwise uncharacterized enzyme that acts on adenosine diphosphate–ribose 1'-2' cyclic phosphate (Appr-p), and a cytochrome c methyltransferase.

A major task in the analysis of any biochemical activity is the purification and identification of the polypeptide or polypeptides responsible for that activity. Purification is often difficult, time consuming, and expensive, yet it is often a necessary prerequisite for cloning of the gene and subsequent detailed biochemical and genetic study. An alternative to purification is expression cloning: the introduction of cDNA pools into various host cells, followed by screening for activity and identifying the responsible cDNA (1). This method is inherently limited to those proteins that are easily detectable in the background of host cell proteins. Yet, given the accumulation of complete genome sequences, such as that of the yeast Saccharomyces cerevisiae, the sequences of genes encoding every biochemical activity of these organisms are already available. The challenge is how to use this information to connect biochemical activity with a specific gene.

We developed a rapid and sensitive genomic method for identifying yeast genes encoding biochemical activities, which is applicable for almost any detectable activity. We first constructed an array of 6144 strains, each of which bears a plasmid expressing a different GST-ORF fusion under control of the P_CUP1 promoter (2). To identify genes encoding particular biochemical activities, we purified this genomic set of GST-ORFs in 64 pools of 96 fusions each (3). Then, we assayed the pools for a particular activity and deconvoluted active pools to identify the strain and ORF responsible for the activity. Assay of the GST-ORF pools demonstrates that each of two previously known tRNA splicing activities is detected only in the pools that contain their respective GST fusions: tRNA ligase (4) in pool 35! (Fig. 1A) and 2'-phosphotransferase (5) in pool 46! (Fig. 1B).

The GST-ORFs were used to identify three previously unknown genes by biochemical assay of their products. A highly specific cyclic phosphodiesterase (CPDase) (6) that could convert Appr-p, produced during tRNA splicing (7), to Appr-1'-p was localized to pool 4 (Fig. 2A), and an otherwise uncharacterized Appr-1'-p-processing activity was found in pool 6 (Fig. 2B). We further explored the usefulness of the pools by searching for a protein-modifying enzyme. Yeast cytochrome c is known to have a tri-methyllysine (8), and pool 23 has a methyltransferase that is active with horse cytochrome c, but not with bovine serum albumin (Fig. 2C).

To determine the strain responsible for each activity, we prepared and assayed the GST-ORFs from each of the 8 rows and 12 columns of strains from the corresponding microtiter plates. In this way, CPDase activity was associated with strain MRM 319 (expressing YGR247w) in row C and column 7! (position C7) of plate 4! (Fig. 3A), Appr-1'-p processing was associated with MRM 546 (expressing YBR022w) at position F6 of pool 6, and cytochrome c methyltransferase activity was associated with MRM 2122 (expressing YHR109w) at position A10 of plate 23.

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In all three cases, we separately confirmed that the GST-ORF preparation from the individual strain had the expected activity (Fig. 3A) and that the plasmid DNA contained the expected ORF. No homologs of these ORFs were detected by routine BLAST searches.

Although these GST-ORFs copurify with the corresponding activities, they may instead interact with or be a subunit of their respective enzyme. For example, conventional purification of CPDase from crude extracts of wild-type cells confirms that ORF YGR247w copurifies with activity (9). SDS–polyacrylamide gel electrophoresis (PAGE) analysis of fractions from the final column indicated that the upper band of the 25-kD doublet copurified with CPDase activity (Fig. 3B, arrow), and mass spectrometry indicated that this band is YGR247w. However, we infer that YGR247w requires some other limiting factor for CPDase activity because overexpression of the ORF (or the GST-ORF) in yeast or the His6 ORF in Escherichia coli does not result in increased activity in extracts. It is also conceivable that any of these three enzymes might use other substrates in vivo; more biochemical and in vivo analyses are required to fully assess their cellular roles.

In principle, this biochemical genomics approach can be used to identify the gene associated with any biochemical activity, provided that the GST-ORF is functional, is associated with any biochemical activity, and retains or other required components when purified. A large number of NH$_2$-terminal fusions are functional (for example, in two-hybrid screens), and simple modifications of our procedure could be used for the study of membrane proteins, protein complexes, and proteins that might be toxic when overproduced.

This biochemical genomics strategy has distinct advantages over conventional purification or expression cloning. First, it is rapid. The identification of an ORF-associated activity presently takes ~2 weeks, starting with the 64 purified GST-ORF pools, and with appropriate pooling strategies, identification could take 1 day. In contrast, months or years are required for purification or expression...
Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat
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In the rat, variations in maternal care appear to influence the development of behavioral and endocrine responses to stress in the offspring. The results of cross-fostering studies reported here provide evidence for (i) a causal relationship between maternal behavior and stress reactivity in the offspring and (ii) the transmission of such individual differences in maternal behavior from one generation of females to the next. Moreover, an environmental manipulation imposed during early development that alters maternal behavior can then affect the pattern of transmission in subsequent generations. Taken together, these findings indicate that variations in maternal care can serve as the basis for a nongenomic behavioral transmission of individual differences in stress reactivity across generations.

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Individual differences in personality traits appear to be transmitted from parents to offspring. A critical question, however, concerns the mode of inheritance. Concordance studies with mono- and dizygotic twins have provided evidence for a genetic mechanism of transmission even of complex traits (1). In addition, parental behavior influences the development of the offspring (2) and could therefore serve as a mechanism for a nongenomic behavioral mode of inheritance. In the Norway rat (Rattus norvegicus), variations in maternal care are associated with the development of individual differences in behavioral and endocrine responses to stress in the offspring (3, 4). In the studies reported here we have examined the possibility that such variations in maternal care might be the mechanism for a behavioral transmission of individual differences across multiple generations.

Mother-pup contact in the rat occurs primarily within the context of a nest bout that begins when the mother approaches the litter, gathers the pups under her, licks/grooms her pups, and nurses her offspring while continuing to occasionally lick/groom the pups, and terminates when the mother leaves the nest (3). Naturally occurring variations in maternal licking/grooming and arched-back nursing (LG-ABN) have been associated with the development of individual differences in hypothalamic-pituitary-adrenal (HPA) and behavioral responses to stress in the offspring (3, 4). As adults, the offspring of high LG-ABN mothers are behaviorally less fearful and show more modest HPA responses to stress than do the offspring of low LG-ABN mothers. The variation in maternal behavior may thus constitute a mechanism for the nongenomic behavioral transmission of fearfulness from parent to offspring. Alternatively, of course, the differences in fearfulness and those in maternal behavior may both be associated with a common genotype so that the observed continuity of individual differences from mother to offspring is mediated by a genetically based pattern of inheritance.

We found that the female offspring of high LG-ABN mothers showed significantly increased licking/grooming of pups in comparison with those of low LG-ABN mothers (12.9 ± 1.0 versus 6.9 ± 1.1; P < 0.001) (6),

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9. Crude extract from 430 g of strain JHRY-20-Ca (5) was chromatographed successively on Blue Sephrose, heparin agarose, hydroxyapatite, and Orange A Sephrose to purify cyclic phosphodiesterase activity 4000-fold in relation to crude extract.