cause these are the only mutations that will fix. This might help explain why similar beneficial mutation rates are estimated in very diverse organisms under very diverse environments. These estimates are obtained in populations with very large effective sizes (*4*, *5*, *9*, *16*), which are likely to produce strong underestimations of U_a.

It is plausible that, in natural habitats, population sizes will be large. If the effective size of a bacterial species is much higher than 10^4 (*25*), then our results imply that clonal interference plays a major role in limiting the adaptation of these asexual organisms. As such, if there is a chance for recombination, clonal interference will be much lower and organisms will adapt faster. This has been predicted theoretically (*14*), although the empirical evidence is still very preliminary (*26*, *27*). Given our results, we anticipate that clonal interference is important in maintaining sexual reproduction in eukaryotes. Notably, mutation accumulation experiments in *Saccharomyces cerevisiae* and *Arabidopsis thaliana* have detected a significantly large number of mutants with increased fitness (*28*, *29*).

Given the estimates for the overall mutation rate in *E. coli* (*30*) and its genomic deleterious mutation rate (*1*), our estimate of U_a implies that 1 in 150 newly arising mutations is beneficial and that 1 in 10 fitness-affecting mutations increases the fitness of the individual carrying it. Hence, an enterobacterium has an enormous potential for adaptation and may help explain how antibiotic resistance and virulence evolve so quickly.

References and Notes

1. T. Bataillon, *Heredity* **84**, 497 (2000).
2. R. A. Fisher, *The Genetical Theory of Natural Selection* (Clarendon Press, Oxford, UK, 1930).
3. H. A. Orr, *Nat. Rev. Genet.* **6**, 119 (2005).
4. M. Imhof, C. Schlotterer, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1113 (2001).
5. R. Miralles, P. J. Gerrish, A. Moya, S. F. Elena, *Science* **285**, 1745 (1999).
6. C. L. Burch, L. Chao, *Genetics* **151**, 921 (1999).
7. C. Zeyl, *Genetics* **169**, 1825 (2005).
8. S. Estes, M. Lynch, *Evol. Int. J. Org. Evol.* **57**, 1022 (2003).
9. R. D. Barrett, R. C. MacLean, G. Bell, *Biol. Lett.* **2**, 236 (2006).
10. D. R. Rokyta, P. Joyce, S. Caudle, H. Wichman, *Nat. Genet.* **37**, 441 (2005).
11. R. Kassen, T. Bataillon, *Nat. Genet.* **38**, 484 (2006).
12. M. Kimura, *The Neutral Theory of Molecular Evolution* (Cambridge Univ. Press, Cambridge, 1983).
13. A. J. Betancourt, J. P. Bollback, *Curr. Opin. Genet. Dev.* **16**, 618 (2006).
14. W. G. Hill, A. Robertson, *Genet. Res.* **8**, 269 (1966).
15. P. Gerrish, R. Lenski, *Genetica* **102–103**, 127 (1998).
16. D. E. Rozen, J. A. de Visser, P. Gerrish, *Curr. Biol.* **12**, 1040 (2002).
17. M. Hegreness, N. Shoresh, D. Hartl, R. Kishony, *Science* **311**, 1615 (2006).
18. C. Schlotterer, M. Imhof, H. Wang, V. Nolte, B. Harr, *J. Evol. Biol.* **19**, 1671 (2006).
19. Materials and methods are available as supporting material on *Science* Online.
20. J. F. Crow, M. Kimura, *An Introduction to Population Genetics Theory* (Harper & Row, New York, 1970).
21. $U_a = k/(N_e \cdot 2E(s_a) \cdot T)$, where k is the number of observed mutations, T is the number of generations and $E(s_a)$ is the mean selection coefficient. This assumes that there is no clonal interference. If its effect is major, the value of U_a will be greatly underestimated. Also, small effect mutations are likely to be missed because the time it takes for a mutation to increase in frequency is $\propto 1/s_a$.
22. The expected fitness increase over 1000 generations without clonal interference is $N_e \cdot U_a \cdot 2\ E(s_a) \cdot E(s_a) \cdot 1000 = 0.14$ in the small size populations.
23. H. A. Orr, *Genetics* **163**, 1519 (2003).
24. R. D. H. Barrett, S. P. Otto, L. K. M'gonigle, *Genetics* **174**, 2071 (2006).
25. J. Charlesworth, A. Eyre-Walker, *Mol. Biol. Evol.* **23**, 1348 (2006).
26. B. Grimberg, C. Zeyl, *Evol. Int. J. Org. Evol.* **59**, 431 (2005).
27. N. Colegrave, *Nature* **420**, 664 (2002).
28. S. B. Joseph, D. W. Hall, *Genetics* **168**, 1817 (2004).
29. R. G. Shaw, D. Byers, E. Darmo, *Genetics* **155**, 369 (2000).
30. J. W. Drake, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7160 (1991).

31. This work was supported by POCTI/BSE/46856/ 2002 and SFRH/BD/18161/2004 through Fundação para a Ciência e Tecnologia. We thank M. Imhof for help in the initial setup of this work and R. Azevedo, D. Bachtrog, A. Coutinho, F. Dionisio, K. Xavier, and anonymous reviewers for comments on the manuscript.

Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5839/813/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Table S1
References

8 March 2007; accepted 2 July 2007
10.1126/science.1142284

Divergence of Transcription Factor Binding Sites Across Related Yeast Species

Anthony R. Borneman,[1]* Tara A. Gianoulis,[2] Zhengdong D. Zhang,[3] Haiyuan Yu,[3] Joel Rozowsky,[3] Michael R. Seringhaus,[3] Lu Yong Wang,[4] Mark Gerstein,[2,3,5] Michael Snyder[1,2,3]†

Characterization of interspecies differences in gene regulation is crucial for understanding the molecular basis of both phenotypic diversity and evolution. By means of chromatin immunoprecipitation and DNA microarray analysis, the divergence in the binding sites of the pseudohyphal regulators Ste12 and Tec1 was determined in the yeasts *Saccharomyces cerevisiae*, *S. mikatae*, and *S. bayanus* under pseudohyphal conditions. We have shown that most of these sites have diverged across these species, far exceeding the interspecies variation in orthologous genes. A group of Ste12 targets was shown to be bound only in *S. mikatae* and *S. bayanus* under pseudohyphal conditions. Many of these genes are targets of Ste12 during mating in *S. cerevisiae*, indicating that specialization between the two pathways has occurred in this species. Transcription factor binding sites have therefore diverged substantially faster than ortholog content. Thus, gene regulation resulting from transcription factor binding is likely to be a major cause of divergence between related species.

Differences in related individuals are generally attributed to changes in gene composition and/or alterations in their regulation. Previous efforts to examine divergence of regulatory information have relied on the analysis of conserved sequences in putative promoter regions (*1*, *2*). However, these approaches are limited because transcription factor (TF) binding sites are often short and degenerate, making their computational detection difficult (*3*). In addition, requiring the conservation of motifs across species precludes the detection of sequences that are evolutionarily divergent.

The detection of binding sites with chromatin immunoprecipitation and microarray (ChIP-chip) analysis (*4*, *5*) offers the ability to globally map TF binding locations experimentally rather than computationally. For species such as yeasts, where genome sequences of numerous related species are available (*6*), this approach can allow for the evolutionary comparison of binding sites of conserved TFs across species.

We have used this approach to investigate evolutionary divergence in the targets of two developmental regulators in the *Saccharomyces* sensu stricto yeasts *S. cerevisae*, *S. mikatae*, and *S. bayanus*. In *S. cerevisiae* diploids, Ste12 and Tec1 act cooperatively to regulate genes during pseudohyphal development (*7*–*9*), whereas in haploid cells, Ste12 regulates mating genes (*10*). The binding sites of Ste12 and Tec1 were mapped in all three species under low-nitrogen

[1]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06511, USA. [2]Program in Computational Biology, Yale University, New Haven, CT 06511, USA. [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA. [4]Integrated Data Systems Department, Siemens Corporate Research, Princeton, NJ 08540, USA. [5]Department of Computer Science, Yale University, New Haven, CT 06511, USA.

*Present address: Australian Wine Research Institute, Glen Osmond, Adelaide, SA 5064, Australia.
†To whom correspondence should be addressed. E-mail: michael.snyder@yale.edu

(pseudohyphal) conditions with the use of triplicate ChIP-chip experiments and species-specific high-density oligonucleotide tiling microarrays (fig. S1) (*11*). Ste12 bound to 380, 167, and 250 discrete sites, whereas Tec1 bound to 348, 185, and 126 sites, in *S. cerevisiae*, *S. mikatae*, and *S. bayanus*, respectively (tables S1 to S6). For each species, the two factors bound to a high proportion of common regions (86, 80, and 87% for *S. cerevisiae*, *S. mikatae*, and *S. bayanus*, respectively), suggesting that the cooperative interaction observed between Ste12 and Tec1 in *S. cerevisiae* is conserved across the three *Saccharomyces* species.

Analysis of the signal tracks allowed for global comparisons in TF binding to be made among the species, revealing qualitative and quantitative differences in ChIP binding regions (Fig. 1A). To systematically perform interspecies comparisons, we removed regions that were not represented across all three yeast genomes (*12*). Comparison of the overlap in binding across species as a function of rank order revealed significant binding differences throughout the rank order, indicating that even strong targets from one species may not be bound in the others (Fig. 1B). As a control, replicate experiments from *S. cerevisiae* (*12*) displayed >98% concordance in binding.

Overall, three classes of TF binding events were observed: those conserved across all three species, those present in two of the three species, and species-specific binding events (Fig. 1C). Of the 221 and 255 targets bound in total by Ste12 and Tec1, respectively, only 47 (Ste12, 21%) and 50 (Tec1, 20%) targets were conserved across all three species (Figs. 1C and 2A). The conserved binding events were present throughout the rank order, indicating that both highly occupied and less-occupied regions are conserved (tables S7 and S8). To ensure that these binding differences were not due to the scoring threshold used, we calculated signal distributions for unbound orthologs of target regions (*12*). Of the unbound orthologous regions, 80% had signals similar to background, indicating that most will be unaffected by threshold changes (fig. S2). Even when identical binding regions were used, 23% differed in their intensity by at least 1.5 fold between species (0% between *S. cerevisiae* replicates), suggesting that quantitative differences exist in site occupation or binding strength between species (Fig. 2B and tables S9 and S10). Thus, most target genes were bound in only one or two of the three species, indicating considerable divergence in binding sites across

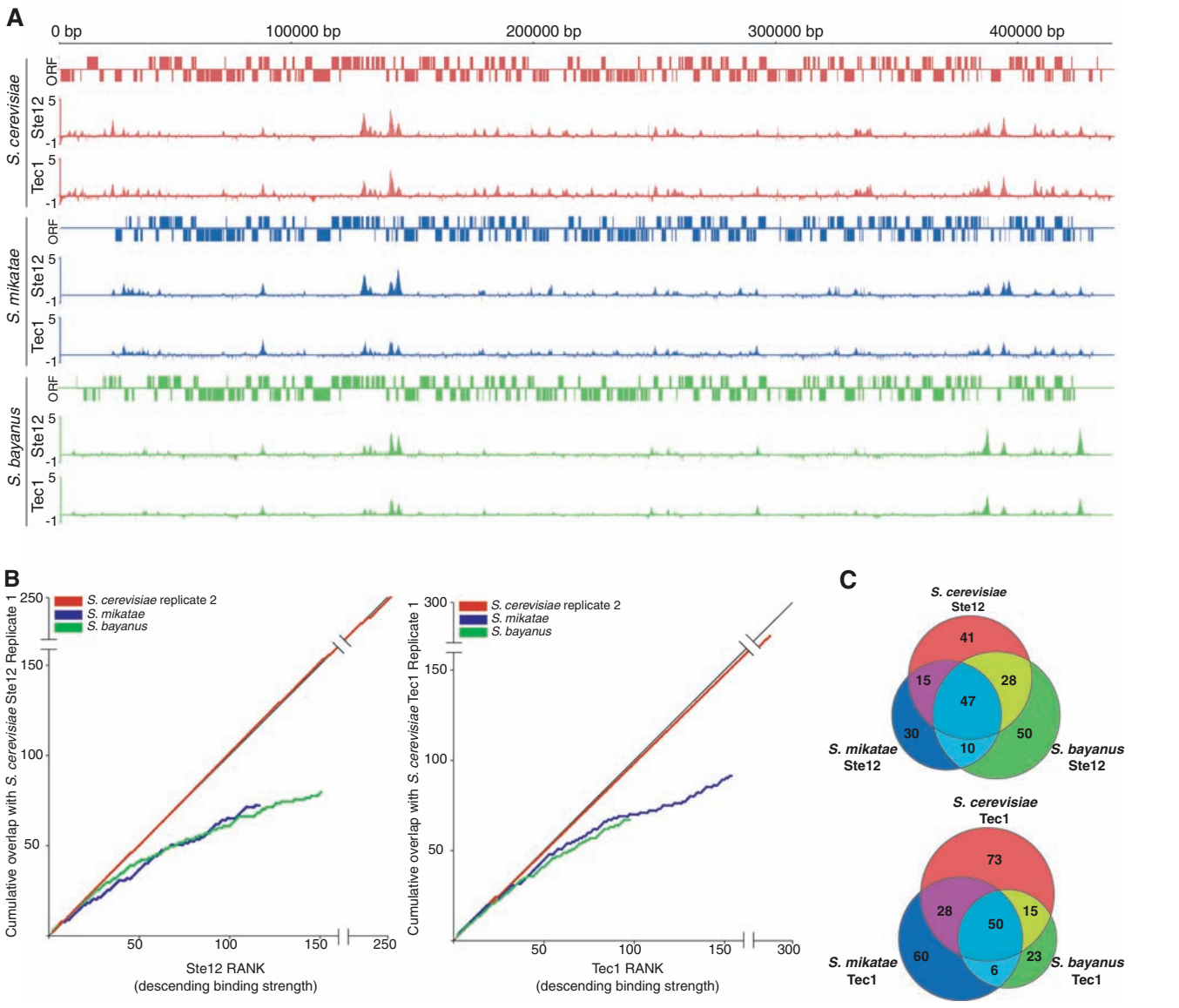


Fig. 1. (**A**) Ste12 and Tec1 bind to discrete regions of chromosome IX of *S. cerevisiae* and to orthologous regions of *S. mikatae* and *S. bayanus*. ChIP-chip enrichment by Tec1 and Ste12 (log 2 ratios) is shown relative to ORFs of *S. cerevisiae* (red), *S. mikatae* (blue), and *S. bayanus* (green). bp, base pairs. (**B**) Rank-order analysis of Ste12 and Tec1 ChIP-chip targets in *S. cerevisiae* (red), *S. mikatae* (blue), and *S. bayanus* (green) (*12*). (**C**) Gene target overlap across the three *Saccharomyces* species.

these yeasts (Fig. 2C). Because the fraction of nonconserved genes among *S. cerevisiae*, *S. mikatae*, and *S. bayanus* is less than 0.05% (*2*), the amount of variation in TF binding is substantially larger than that of gene variation.

One possible cause for the interspecies differences in the ChIP binding locations is divergence in binding site sequences. To examine this possibility, we investigated sequence motifs in both bound and orthologous unbound regions across the three *Saccharomyces* species. Position weight matrices (PWM), representing the putative binding motifs for Ste12 and Tec1, were generated from the ChIP-chip data (*13*). Analysis of the Tec1 targets of the three species revealed an overrepresented sequence motif that matched the known Tec1 consensus (*7*) (Fig. 3A), whereas the targets of Ste12 in *S. cerevisiae* and *S. mikatae* revealed a motif that was similar to the known binding sequence (*14*) (Fig. 3B). This sequence was not overrepresented in *S. bayanus*.

With the use of the PWM sequences, ChIP bound regions and orthologous unbound regions from each species were then scored for the presence of each motif (*15*). There were several significant classes of TF binding events, with those genes bound by all three factors present near the top of both the Tec1 (all bound, motif in all) and Ste12 (all bound, with and without motif) lists (Fig. 3, C and D). For promoter regions that displayed evolutionarily conserved ChIP binding in all three *Saccharomyces* species, 83% (Tec1) and 24% (Ste12) of the regions contained at least one significant occurrence of the PWM motif for that factor in each species (Fig. 3, E and F). In contrast, 2 and 62% of the promoters that displayed conserved ChIP binding did not contain a match to the PWM in at least two of the three species. Thus, the Ste12 motif is not present in a high proportion of pseudohyphal-responsive genes, implying that Tec1 may target Ste12 to these regulatory regions (*16*).

In contrast to the previous results in which experimentally determined binding correlated with the presence of predicted motifs, there were many examples where a species-specific loss of binding and/or a loss of sequence have occurred. There were 48 (Tec1, 14% of total binding events) and 35 (Ste12, 10% of total binding events) experimentally bound regions that contained a PWM match where the orthologous region in at least one other species neither was bound nor contained a motif match. For these loci, loss of ChIP binding is concordant with the loss of the motif for this factor, providing clear examples of regions where network evolution occurred through the gain or loss of regulatory sequences.

Furthermore, there were 45 (Tec1, 12%) and 9 (Ste12, 3%) instances where a PWM match occurred in all three species but where that region was experimentally bound in only two species (Fig. 2D). Either these loci are occupied at other times in the life cycle or they are not functional. Conversely, in 11 (Tec1, 3%) and 22 (Ste12, 6%) instances, genomic regions displayed conserved ChIP binding, but at least one species was missing a PWM motif match (Fig. 2E). Thus, sequence conservation does not readily predict binding.

To further examine the role of conserved versus nonconserved ChIP binding events and motifs, we compared these results with expression microarray studies of pseudohyphal formation in *S. cerevisiae* (*17*). Of the ChIP binding gene targets that had significantly altered expression

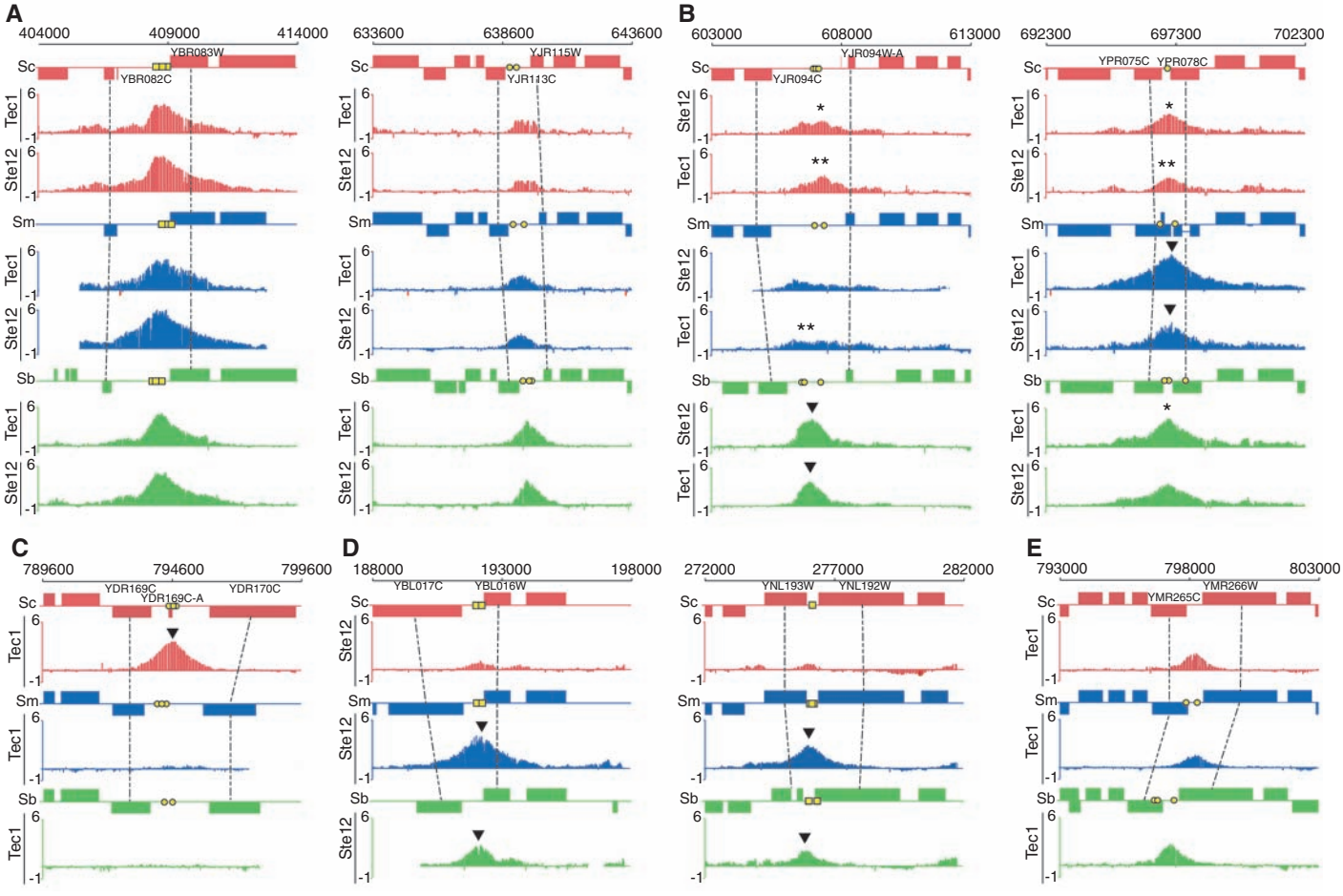
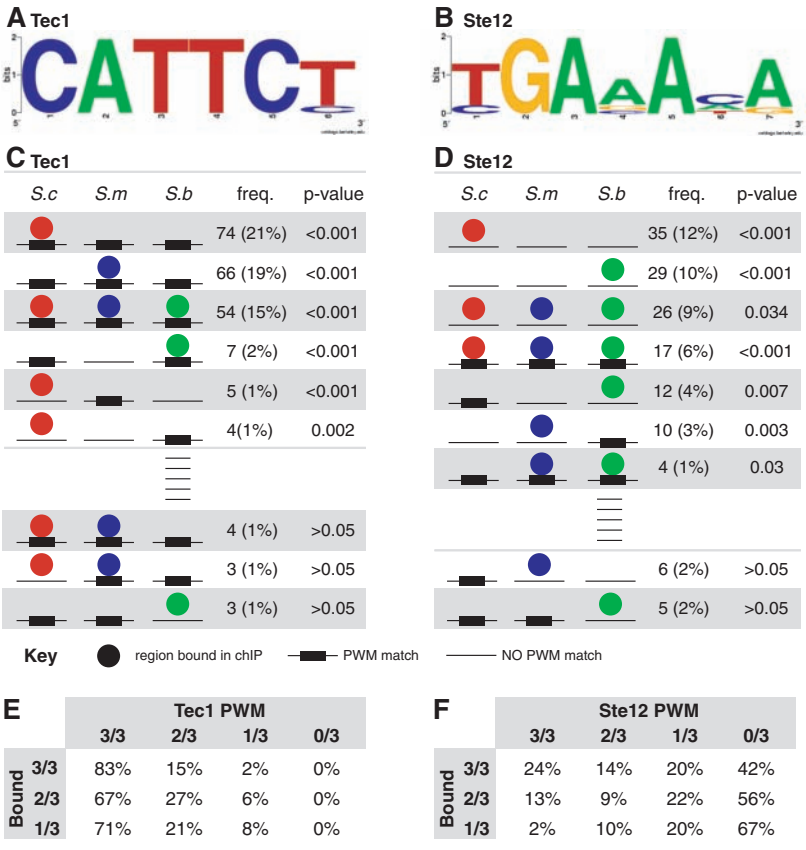


Fig. 2. Comparison of binding by Ste12 and Tec1 across *S. cerevisiae* (red), *S. mikatae* (blue), and *S. bayanus* (green). (**A**) Conserved binding. (**B**) Conserved binding with quantitative signal differences. (**C**) Conserved binding with loss of consensus sequences in one species. (**D**) Species-specific binding despite conserved consensus sequences. (**E**) Binding only in *S. mikatae* and *S. bayanus*. ChIp-chip enrichment signals are shown (log 2 ratios). Circles and squares represent matches to Tec1 PWM and Ste12 PWM, respectively. Triangles, nonconserved peaks; **, >2-fold difference in peak signal intensity; *, >1.5-fold difference in peak signal intensity.



E

Bound	Tec1 PWM 3/3	2/3	1/3	0/3
3/3	83%	15%	2%	0%
2/3	67%	27%	6%	0%
1/3	71%	21%	8%	0%

F

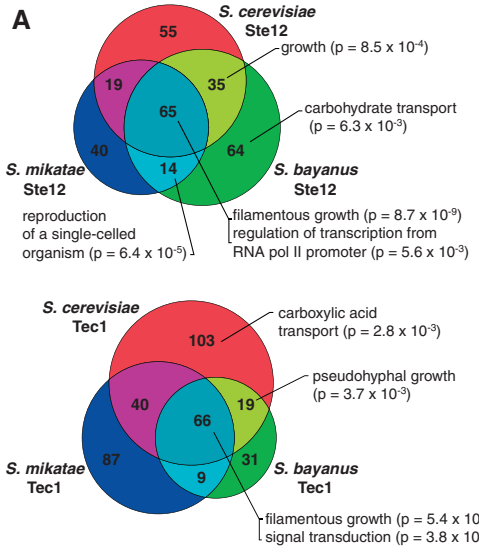
Bound	Ste12 PWM 3/3	2/3	1/3	0/3
3/3	24%	14%	20%	42%
2/3	13%	9%	22%	56%
1/3	2%	10%	20%	67%

Fig. 3. Motif analysis of ChIP binding targets. Logo representations of the PWM for Tec1 (**A**) and Ste12 (**B**) (*12*). (**C** and **D**) Classes of binding targets after classification by both the conservation of ChIP binding and the presence or absence of consensus motifs. (**E** and **F**) Compiled proportions of binding targets and PWM matches for Tec1 and Ste12.

(~20% of the ChIP targets, a several-fold enrichment), there was no enrichment for genes with conserved binding (11% bound versus 14% unbound) or PWM matches (12% with motif versus 16% without motif) (table S11). Thus, sequence-based motif analyses in the absence of experimentally determined binding data are not sufficient for the accurate prediction of TF binding profiles and gene function. In addition, the presence of nonconserved ChIP targets upstream of pseudohyphal-regulated genes at the same frequency as conserved targets indicates that nonconserved sites are likely to be functional.

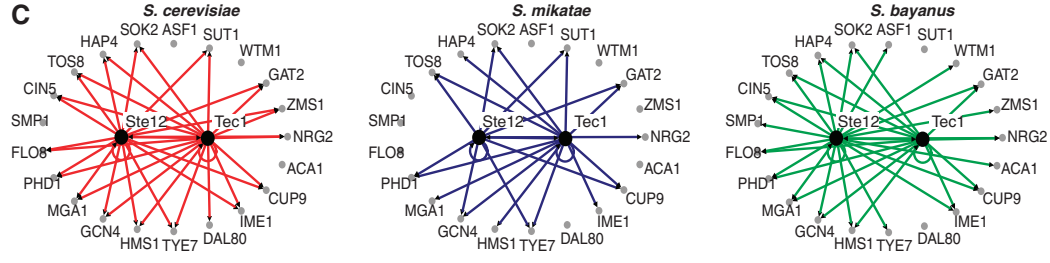
To elucidate the biological importance of both the conserved and species-specific gene targets, we mapped each bound region to its downstream target genes by identifying open reading frames (ORFs) that were 3′ of and directly flanking each ChIP binding event (tables S7 and S8). Conserved Ste12 and Tec1 gene targets displayed enrichment for two gene ontology (GO) (*18*) categories: "filamentous growth" and "regulation of transcription from RNA polymerase II promoters" (Fig. 4A). Because most of the genes from within the second category encode TFs, the predicted downstream TF networks of *S. bayanus* and *S. mikatae* were compared to those of *S. cerevisiae* (*19*) to determine which connections had been maintained during the evolution of the *Saccharomyces* sensu stricto group (Fig. 4C). The binding of Ste12 and Tec1 to downstream TFs was shown to be highly conserved (73% across the three species). The network of *S. mikatae* was most diverged and had several key

Fig. 4. (**A**) Ste12 and Tec1 bind to common and distinct sets of genes across the *Saccharomyces* sensu stricto lineage. Overrepresented GO terms are listed for each combinatorial category. (**B**) Mating genes bound specifically by Ste12 in *S. mikatae* and *S. bayanus*. (**C**) TF network conservation in *S. cerevisiae* (red), *S. mikatae* (blue), and *S. bayanus* (green).



B

	Gene	*S. cerevisiae* homologue	$\log_2$ Ratios
S. mikatae	ORFN:1359	FUS3	2.29
S. bayanus	ORFN:1436	(YBL016C)	2.13
S. mikatae	ORFN:18188	CHS1	2.21
S. bayanus	ORFN:20364	(YNL192W)	1.70
S. mikatae	ORFN:2230	KAR4	2.11
S. bayanus	ORFN:2394	(YCL055W)	1.60
S. mikatae	ORFN:2120	FUS1	1.79
S. bayanus	ORFN:2514	(YCL027W)	1.79
S. mikatae	ORFN:7184	MPT5	1.63
S. bayanus	ORFN:8405	(YGL178W)	1.31
S. mikatae	ORFN:10486	AXL2	1.45
S. bayanus	ORFN:11888	(YIL140W)	1.41
S. mikatae	ORFN:19760	RRI12	1.26
S. bayanus	ORFN:22091	(YOL117W)	1.28



regulatory omissions including Flo8 (not bound by either Ste12 or Tec1) and Mga1 (not bound by Ste12). Thus, although important differences can be found, TF binding to the promoters of other TFs was highly conserved between species relative to the level of conservation observed for other genes.

From those groups of genes that did not display conserved binding across the three species, one notable class was bound by Ste12 specifically in *S. mikatae* and *S. bayanus* and was enriched in genes involved in mating (GO category: "reproduction in single-celled organisms") in *S. cerevisiae* (Fig. 4, A and B). Unlike the gene targets in the diploid cells used in this study, these genes are targets of Ste12 in haploid *S. cerevisiae* cells (*20*, *21*), and this differential binding occurs despite the presence of conserved Ste12 binding motifs (fig. S3). Thus, Ste12 binding targets may be occupied under different conditions across related species. In *S. cerevisiae*, Ste12 binds to these sites only during mating, whereas in *S. mikatae* and *S. bayanus*, Ste12 binds to these same regions in diploid cells.

To extend this study outside of *Saccharomyces* yeasts, we also mapped the binding of the *Candida albicans* Ste12 ortholog, Cph1 (*22*). Cph1 functions in the dimorphic switch of this yeast, a process that shares many genetic components with pseudohyphal growth (*23*). A total of 52 significant Cph1 ChIP binding events (table S12) was detected under dimorphic growth conditions, with many residing upstream of known pathogenicity determinants (*24*–*27*). From these gene targets, 33 have recognizable orthologs in *S. cerevisiae*, and of these orthologs, 10, 10, and 13 displayed conserved binding with *S. cerevisiae*, *S. mikatae*, and *S. bayanus*, respectively. Although most gene targets of Cph1 in *C. albicans* are not conserved with the *Saccharomyces* species, the *C. albicans* orthologs bound by Ste12, like those from *S. mikatae* and *S. bayanus*, included a significant number of genes that function during reproduction and mating in *S. cerevisiae* ($P = 4 \times 10^{-3}$) (*18*). Thus, in *C. albicans*, like in *S. mikatae* and *S. bayanus*, the Ste12 ortholog also binds to genes required for mating in *S. cerevisiae* under filamentous growth conditions, raising the possibility that these genes have become more specialized in *S. cerevisiae*.

We find that extensive regulatory changes can exist in closely related species, which is consistent with a recent study that showed that distinct regulatory circuits can produce similar regulatory outcomes in *S. cerevisiae* and *C. albicans* (*28*). Furthermore, although *S. cerevisiae* and *S. mikatae* are quite similar to one another at the nucleotide sequence level, they are equally different to each other and to *S. bayanus* in their TF profiles. We expect that the extensive binding site differences observed in this study reflect the rapid specialization of these organisms for their distinct ecological environments and that differences in transcription regulation between related species may be responsible for rapid evolutionary adaptation to varied ecological niches.

References and Notes

1. P. Cliften *et al.*, *Science* **301**, 71 (2003).
2. M. Kellis, N. Patterson, M. Endrizzi, B. Birren, E. S. Lander, *Nature* **423**, 241 (2003).
3. M. Tompa *et al.*, *Nat. Biotechnol.* **23**, 137 (2005).
4. V. R. Iyer *et al.*, *Nature* **409**, 533 (2001).
5. B. Ren *et al.*, *Science* **290**, 2306 (2000).
6. J. Piskur, R. B. Langkjaer, *Mol. Microbiol.* **53**, 381 (2004).
7. H. D. Madhani, G. R. Fink, *Science* **275**, 1314 (1997).
8. V. Gavrias, A. Andrianopoulos, C. J. Gimeno, W. E. Timberlake, *Mol. Microbiol.* **19**, 1255 (1996).
9. H. Liu, C. A. Styles, G. R. Fink, *Science* **262**, 1741 (1993).
10. S. Fields, I. Herskowitz, *Cell* **42**, 923 (1985).
11. A. R. Borneman *et al.*, *Funct. Integr. Genomics*, in press; preprint available at www.springerlink.com/content/w65529354201666h/.
12. See supporting material on *Science* Online.
13. X. S. Liu, D. L. Brutlag, J. S. Liu, *Nat. Biotechnol.* **20**, 835 (2002).
14. J. W. Dolan, C. Kirkman, S. Fields, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5703 (1989).
15. T. L. Bailey, M. Gribskov, *Bioinformatics* **14**, 48 (1998).
16. S. Chou, S. Lane, H. Liu, *Mol. Cell. Biol.* **26**, 4794 (2006).
17. S. Prinz *et al.*, *Genome Res.* **14**, 380 (2004).
18. E. I. Boyle *et al.*, *Bioinformatics* **20**, 3710 (2004).
19. A. R. Borneman *et al.*, *Genes Dev.* **20**, 435 (2006).
20. C. T. Harbison *et al.*, *Nature* **431**, 99 (2004).
21. J. Zeitlinger *et al.*, *Cell* **113**, 395 (2003).
22. T. Jones *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 7329 (2004).
23. C. Sanchez-Martinez, J. Perez-Martin, *Curr. Opin. Microbiol.* **4**, 214 (2001).
24. C. E. Birse, M. Y. Irwin, W. A. Fonzi, P. S. Sypherd, *Infect. Immun.* **61**, 3648 (1993).
25. B. R. Braun, W. S. Head, M. X. Wang, A. D. Johnson, *Genetics* **156**, 31 (2000).
26. B. R. Braun, A. D. Johnson, *Genetics* **155**, 57 (2000).
27. T. C. White, S. H. Miyasaki, N. Agabian, *J. Bacteriol.* **175**, 6126 (1993).
28. A. E. Tsong, B. B. Tuch, H. Li, A. D. Johnson, *Nature* **443**, 415 (2006).

29. The authors would like to thank P. Chambers and D. Gelperin for comments on the manuscript. Funding was provided by NIH grants (to M.S. and M.G.) and by the Burroughs Wellcome Foundation. Detailed lists of binding regions, conservation information, and motif scores are available from www.gersteinlab.org/proj/regnetdiverge.

Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5839/815/DC1
Materials and Methods
Fig. S1 to S5
Tables S1 to S14
References

2 February 2007; accepted 20 June 2007
10.1126/science.1140748

High-Speed Imaging Reveals Neurophysiological Links to Behavior in an Animal Model of Depression

Raag D. Airan,[1]* Leslie A. Meltzer,[2]* Madhuri Roy,[1] Yuqing Gong,[3,4] Han Chen,[3] Karl Deisseroth[1,5]†

The hippocampus is one of several brain areas thought to play a central role in affective behaviors, but the underlying local network dynamics are not understood. We used quantitative voltage-sensitive dye imaging to probe hippocampal dynamics with millisecond resolution in brain slices after bidirectional modulation of affective state in rat models of depression. We found that a simple measure of real-time activity—stimulus-evoked percolation of activity through the dentate gyrus relative to the hippocampal output subfield—accounted for induced changes in animal behavior independent of the underlying mechanism of action of the treatments. Our results define a circuit-level neurophysiological endophenotype for affective behavior and suggest an approach to understanding circuit-level substrates underlying psychiatric disease symptoms.

The hippocampus, as an integral component of the limbic system, is a focus of depression research (*1*), drives other brain regions implicated in depression, and appears to serve as a primary site of action for antidepressants that inhibit pathological hyperactivity (*2*, *3*). Complicating this picture, however, is evidence suggesting that antidepressants can stimulate hippocampal activity. Antidepressant-induced hippocampal neurogenesis is linked to behavioral responses (*4*, *5*); moreover, excitatory hippocampal neurons are injured by chronic stress (*6*, *7*). Animal models have proven useful in identifying molecular and cellular markers relevant to depression (*8*–*10*) but have not identified neurophysiological final common pathways relevant to behavior. Voltage-sensitive dye imaging (VSDI) could allow analysis of disease-related neural activity on millisecond time scales, with micrometer spatial resolution and a scope spanning entire brain networks (*11*). We applied VSDI to hippocampal physiology in the chronic mild stress (CMS) model, a well-validated rodent

[1]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA. [2]Neuroscience Program, Stanford University, Stanford, CA 94305, USA. [3]Department of Electrical Engineering, Stanford University, Stanford, CA 94305, USA. [4]Department of Statistics, Stanford University, Stanford, CA 94305, USA. [5]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: deissero@stanford.edu

Divergence of Transcription Factor Binding Sites Across Related Yeast Species

Anthony R. Borneman, Tara A. Gianoulis, Zhengdong D. Zhang, Haiyuan Yu, Joel Rozowsky, Michael R. Seringhaus, Lu Yong Wang, Mark Gerstein, and Michael Snyder

Science, **317** (5839), .
DOI: 10.1126/science.1140748