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Divergence of Transcription Factor Binding Sites Across Related Yeast Species

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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 <i>Saccharomyces cerevisiae</i>, <i>S</i>. <i>mikatae</i>, and <i>S</i>. <i>bayanus</i> 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 <i>S. mikatae</i> and <i>S. bayanus</i> under pseudohyphal conditions. Many of these genes are targets of Ste12 during mating in <i>S. cerevisiae</i>, 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 development regulators in the <i>Saccharomyces</i> sensu stricto yeasts <i>S. cerevisiae</i>, <i>S</i>. <i>mikatae</i>, and <i>S</i>. <i>bayanus</i>. In <i>S. cerevisiae</i> 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 conditions.

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References and Notes
19. Materials and methods are available as supporting material on Science Online.
21. \( U_r = \frac{k\epsilon^2}{(N_r+2\epsilon)\cdot T_r}\), where \( k \) is the number of observed mutations, \( T \) is the number of generations and \( \epsilon \) is the mean selection coefficient. This assumes that there is no clonal interference. If its effect is major, the value of \( U_r \) 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 \( \approx 10^5 \).
22. The expected fitness increase over 1000 generations without clonal interference is \( N_r\cdot U_r -2\epsilon (-\epsilon + \epsilon^2) = 0.14 \) in the small size populations.
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Supporting Online Material
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Materials and Methods
SOM Text
Figs. S1 to S4
Table S1
References
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(pseudohyphal) conditions with the use of tripli-
cate 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 spe-
cies as a function of rank order revealed sig-
nificant 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 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 cal-
culated 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 thresh-
old 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 quantita-
tive 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

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**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.
Motif analysis of ChIP binding targets. Logo representations of the PWM for Tec1 (A) and Ste12 (B) (I2). (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.

Fig. 3. Motif analysis of ChIP binding targets. Logo representations of the PWM for Tec1 (A) and Ste12 (B) (I2). (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.

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).

(-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) (I8) 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 (I9) 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 (75% across the three species). The network of S. mikatae was most diverged and had several key
High-Speed Imaging Reveals Neurophysiological Links to Behavior in an Animal Model of Depression

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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 (4–7); 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 model of depression. Antidepressant-induced hippocampal neurogenesis is linked to behavioral responses (4–7); 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 model of depression.

References and Notes

11. 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.
12. 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

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