Gene expression within cells is primarily regulated by binding of transcription factors to genomic DNA target sequences, where they either recruit or block the general transcription machinery to activate or repress transcription, respectively. Identifying both the full spectrum of target sites bound by a transcription factor and the strength of these binding interactions is an important step toward being able to predict how the binding of transcription factors affects gene expression in vivo.

Basic leucine zipper (bZIP) transcription factors contain the most structurally simple DNA-recognition motifs, and are involved in a wide range of cellular processes, including neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation). Here, we used Hac1, a Saccharomyces cerevisiae bZIP transcription factor which is involved in neuronal differentiation, immune responses, and oncogenesis (i.e., tumor formation).

How Hac1 recognizes target gene promoters has long remained a mystery. Initial searches through the promoters of several known UPR target genes revealed a 22-bp unfolded protein response element (UPRE-1) that was both necessary and sufficient for UPR-dependent transcription, and further experiments demonstrated that mutations in the central 7 bp of this sequence (here termed the “core” UPRE-1, or cUPRE-1) abolished activity (1). However, searches for likely response elements within a much larger set of target genes identified in a subsequent genome-wide study failed to uncover this cUPRE-1, and instead identified an alternate 6-bp sequence capable of driving UPR-dependent transcription (UPRE-2) (2). Although initially proposed to represent an alternate response element bound by Hac1/Gcn4 heterodimers (2), in vitro binding studies of Hac1 homodimers recovered UPRE-2 binding alone (3). Thus, despite years of research, both the identities of the response elements bound by Hac1 and the roles played by any binding partners have remained unclear.

To resolve this mystery, we used a recently developed microfluidic technique for characterizing transcription factor binding preferences [MITOMI (4, 5), Fig. P1A]. In this
technique, microfluidic devices containing many small reaction chambers are aligned to spotted DNA microarrays, allowing the measurement of equilibrium binding by a single transcription factor to a large number of DNA sequences in parallel. Surprisingly, initial experiments that assessed binding to a synthetic DNA library containing all possible 8-bp nucleotide combinations revealed that Hac1 bound to UPRE-2 alone, with no evidence of binding to cUPRE-1. Subsequent detailed investigations confirmed a lack of binding to cUPRE-1, but demonstrated that binding could be restored through the addition of two conserved nucleotides up- and downstream of this motif to yield an 11- or 12-bp binding site, which we designated as the “extended core” UPRE-1, or xcUPRE-1. Through a genetic screen, we established that a region of extended homology N terminal to the basic DNA-binding region is required for the recognition of both target sites. These results unify previously discrepant observations of Hac1 binding by establishing that Hac1 is capable of binding to both a short UPRE-2 site and a significantly longer xcUPRE-1 site without other binding partners. This evidence of a single native bZIP transcription factor binding multiple genomic recognition sites of very different composition and length is unprecedented.

High-affinity binding of multiple distinct genomic target sites by a structurally simple transcription factor has several important implications for our understanding of transcriptional regulation. First, this binding indicates that even simple transcription factors may target specific subsets of genes under different conditions to drive complex transcriptional responses. Second, this binding implies that DNA may drive conformational changes in transcription factors more often than previously thought.