A combination of transcription factors mediates inducible interchromosomal contacts

Seungsoo Kim¹, Maitreya J Dunham¹, Jay Shendure¹,²,³*

¹Department of Genome Sciences, University of Washington, Seattle, United States; ²Howard Hughes Medical Institute, Seattle, United States; ³Brotman Baty Institute for Precision Medicine, Seattle, United States

Abstract The genome forms specific three-dimensional contacts in response to cellular or environmental conditions. However, it remains largely unknown which proteins specify and mediate such contacts. Here we describe an assay, MAP-C (Mutation Analysis in Pools by Chromosome conformation capture), that simultaneously characterizes the effects of hundreds of cis or trans-acting mutations on a chromosomal contact. Using MAP-C, we show that inducible interchromosomal pairing between HAS1pr-TDA1pr alleles in saturated cultures of Saccharomyces yeast is mediated by three transcription factors, Leu3, Sdd4 (Ypr022c), and Rgt1. The coincident, combined binding of all three factors is strongest at the HAS1pr-TDA1pr locus and is also specific to saturated conditions. We applied MAP-C to further explore the biochemical mechanism of these contacts, and find they require the structured regulatory domain of Rgt1, but no known interaction partners of Rgt1. Altogether, our results demonstrate MAP-C as a powerful method for dissecting the mechanistic basis of chromosome conformation.

DOI: https://doi.org/10.7554/eLife.42499.001

Introduction

The three-dimensional organization of the genome within the nucleus is structured but dynamic (Bonev and Cavalli, 2016). Although many features of this conformation are largely conserved across cell types and conditions (Rao et al., 2014; Schmitt et al., 2016a), some chromatin loops and contacts form specifically in response to signals such as differentiation (Bonev et al., 2017; Monahan et al., 2019; Schmitt et al., 2016a; Stadhouders et al., 2018), changes in nutrient availability (Brickner et al., 2015; Brickner et al., 2016), heat shock (Chowdhary et al., 2019; Chowdhary et al., 2017), drugs (D’Ippolito et al., 2018), meiosis (Muller et al., 2018), or circadian rhythms (Kim et al., 2018). This dynamic three-dimensional organization of the genome plays a role in regulating gene expression in diverse organisms. In multicellular organisms, active developmental gene promoters form long-range loops with specific enhancer elements (Bonev et al., 2017), and this looping is in some cases sufficient for transcriptional activation (Deng et al., 2014). In the budding yeast Saccharomyces cerevisiae, a well-studied model of genome conformation, genes targeted to nuclear pores are activated (Taddei et al., 2006), while those at the nuclear periphery are repressed (Andrulis et al., 1998).

Transcription factors (TFs) are attractive candidates for orchestrating such dynamic changes in chromatin conformation, given their site-specific DNA binding and changes in abundance or activity in response to differentiation and cellular signals (Lambert et al., 2018). For many conditions, it remains unknown exactly which TFs bind to any given locus. Although binding site motifs are known for many TFs, motif searches poorly predict TF binding (Guertin and Lis, 2010; Jolma et al., 2015; Le et al., 2018; Levo et al., 2015; Liu et al., 2006; Slattery et al., 2014). Even if the set of TFs...
bound to each locus is known, it is unclear which TFs are capable of forming chromosomal contacts. DNA-bound TFs can also recruit other cofactor proteins that can mediate chromosomal contacts (Deng et al., 2012; Monahan et al., 2019; Song et al., 2007), but our understanding of TF-cofactor interactions remains incomplete.

Among chromosomal contacts and loops, interchromosomal contacts are less well-understood. This is in part due to the relative paucity of interchromosomal contacts in Hi-C and other 3C (chromosome conformation capture) data, which results from their greater contact distance (Maass et al., 2018) and chromosomal self-association into territories (Cremer and Cremer, 2010). Nevertheless, many distinct classes of interchromosomal contacts are known, including clustering of transcriptionally active genes (Mitchell and Fraser, 2008; Osborne et al., 2004; Schoenfelder et al., 2010), associations with nuclear bodies (Quinodoz et al., 2018), interactions among developmental enhancers and promoters (Lomvardas et al., 2006; Monahan et al., 2019), and mitotic homologous chromosome pairing in organisms ranging from yeast (Burgess et al., 1999) to flies (Henikoff and Dreesen, 1989; Joyce et al., 2016; Morris et al., 1999) and mammals (Xu et al., 2006). However, our understanding of the molecular mechanisms of these contacts remains incomplete.

Many known mechanisms for establishing 3D chromosome conformation may act on both intrachromosomal loops and interchromosomal contacts. The emerging consensus model for such DNA-DNA interactions involves loop extrusion by cohesin and other Structural Maintenance of Chromosomes (SMC) factors, which is thought to primarily mediate intrachromosomal loops (Alipour and Marko, 2012; Rao et al., 2014; Rowley and Corces, 2018; Sanborn et al., 2015; Swygert et al., 2019). However, SMC complexes are also capable of mediating interactions between multiple DNA molecules, such as between sister chromatids (Michaelis et al., 1997). Interchromosomal contacts (Monahan et al., 2019) and intrachromosomal contacts (Weintraub et al., 2017; Deng et al., 2012)
can also be mediated by dimerization of structured proteins. In addition to these well-defined strong molecular interactions, weak interactions such as those underlying phase separation of nuclear factors such as transcription factors (Boija et al., 2018; Chong et al., 2018), coactivators (Cho et al., 2018), RNA polymerase (Boehning et al., 2018), and heterochromatin proteins (Larson et al., 2017; Strom et al., 2017) may play an important role in shaping 3D genome organization. How these and other mechanisms synergize remains an open question.

Mitotic (or somatic) homologous chromosome pairing is the preferential association of homologous pairs of loci in mitotically dividing cells. Homolog pairing occurs along the length of the genome in Drosophila (Joyce et al., 2016), but is more subtle in yeast and other organisms, where the association is often transient and/or genomically localized (Xu et al., 2006). Fluorescence in situ hybridization screens in flies have nominated various pairing and anti-pairing factors that modulate the strength of homolog pairing (Joyce et al., 2012), but the precise mechanisms by which these factors regulate pairing are largely unknown. In mammals, X chromosome pairing is mediated by CTCF and Oct4 (Donohoe et al., 2009), in conjunction with transcription (Xu et al., 2007). However, cases of highly localized homolog pairing remain rare. Furthermore, the distinctions between homolog pairing and non-allelic interactions between repetitive elements (Gladyshev and Kleckner, 2017; Mirkin et al., 2014) remain unclear.

We recently identified a novel example of an inducible, localized interchromosomal contact between homologous copies of the HAS1pr-TDA1pr locus in diploid Saccharomyces yeasts (Kim et al., 2017). This interaction occurs in saturated culture conditions, requires the 1 kb intergenic region between the HAS1 and TDA1 coding sequences, and is detectable by both Hi-C and microscopy. The condition-specificity and dependence on intergenic sequence led us to hypothesize that one or more TFs might mediate this pairing. Although yeast TF binding is well-characterized for standard growth conditions (Badis et al., 2008), TF binding has not been systematically measured in saturated culture conditions. Meanwhile, computational predictions of TF binding sites are insufficiently specific: for example, within the 1 kb region required for HAS1pr-TDA1pr pairing, dozens of TFs have at least one motif match. Furthermore, even if the TFs bound to this region were known, it would remain unclear which subset played a role in mediating inducible interchromosomal pairing.

Here we describe a method that enables the simultaneous testing of hundreds of cis or trans-acting mutations for their effects on a chromosomal contact of interest. As a proof of concept, we applied this method, which we call Mutation Analysis in Pools by Chromosome conformation capture (MAP-C), to characterize the molecular components mediating HAS1pr-TDA1pr pairing. We first perform saturating mutagenesis of the regulatory region that mediates the interchromosomal pairing (cis MAP-C) to identify sequence motifs required for pairing that potentially correspond to TF binding sites. We then test the effects of knocking out over one hundred TFs (trans MAP-C), and confirm that three—Leu3, Sdd4 (Ypr022c), and Rgt1—are necessary for inducible interchromosomal pairing. We verify their binding by chromatin immunoprecipitation, and find that HAS1pr-TDA1pr exhibits the strongest coincident, combined binding by all three factors across the genome in a condition-specific manner. We further use trans MAP-C to interrogate how interaction partners and domains of Rgt1 regulate pairing. Finally, we make an initial attempt to characterize the functional consequences of HAS1pr-TDA1pr pairing. Taken together, our results demonstrate how a combination of TFs can mediate inducible interchromosomal pairing. Furthermore, our study shows the utility of a pooled mutant approach to studying both the cis and trans dependencies of chromosome conformation.

Results

A pooled approach to systematically dissect chromosome conformation

In order to identify and dissect the molecular mechanisms underlying chromosome conformation, experiments involving perturbations (e.g. mutations) are needed. However, despite numerous advances in chromosome conformation capture (3C) technology over the last two decades (de Wit and de Laat, 2012; Schmitt et al., 2016b), each experiment characterizes a single sample, which limits the number of genes or cis-regulatory elements that can be disrupted (Monahan et al., 2019; Nora et al., 2017; Schwarzer et al., 2017; Weintraub et al., 2017).
To address this limitation and enable systematic screens, we developed MAP-C, an assay in which hundreds of mutations are simultaneously tested for their effects on a single chromosomal contact of interest (Figure 1A). In the cis version of MAP-C, which we describe first, these mutations are targeted to one of the regions involved in the chromosomal contact. In the trans version of MAP-C, mutations can be spread across the genome, as long as they are associated with a unique barcode sequence at the chromosomal contact site.

The first step of cis MAP-C is to generate an allelic series of a region of interest, which can be achieved in a cost-effective manner via array-synthesized oligonucleotide pools or error-prone PCR. Alternatively, if desired, variants can be generated individually and then pooled prior to conducting MAP-C. The resulting mutant pool is then integrated into the genome and subjected to the 3C assay (Dekker et al., 2002). The region containing the genetic variants is amplified using two different primer pairs: the first (3C library) amplifies a specific ligation product, and the second (genomic library) amplifies regardless of ligation. These amplification products are deeply sequenced to measure the abundance of each variant in the 3C library, which is normalized to its abundance in the genomic library. The relative extent to which sequence variants participate in the chromosomal contact of interest is proportional to their normalized representation in the 3C library.

**A cluster of TF motifs is necessary and sufficient for HAS1pr-TDA1pr pairing**

As a first test of cis MAP-C, we sought to systematically dissect the conserved pairing between HAS1pr-TDA1pr homologs in diploid Saccharomyces yeasts grown to saturation. We recently used Hi-C of *S. cerevisiae* x *S. uvarum* hybrids (<80% nucleotide identity) to discover this homolog pairing interaction, and furthermore identified a 1,038 bp noncoding region that was necessary and sufficient for pairing (Kim et al., 2017).

To find a minimal subsequence of the 1,038 bp HAS1pr-TDA1pr region that is sufficient to pair with other HAS1pr-TDA1pr alleles, we replaced the native *S. cerevisiae* HAS1pr-TDA1pr locus with a library containing each of 861 tiling 178 bp subsequences of the 1,038 bp region (along with a G418 resistance cassette and restriction site), in *S. cerevisiae* x *S. uvarum* hybrid yeast. We then performed cis MAP-C for pairing of the modified locus with the *S. uvarum* copy of HAS1pr-TDA1pr on a saturated culture of the pool, in two replicates (Figure 1B). Compared to the genomic libraries, the 3C libraries were highly enriched for a narrow region spanning ~500 to ~700 bp from the HAS1 coding sequence, with a plateau between ~525 to ~675 bp, consistent with that ~150 bp region being the only subsequence shorter than 178 bp sufficient for pairing (termed the ‘minimal pairing region’ below). To confirm that this pattern of enrichment is specific to HAS1pr-TDA1pr homolog pairing rather than underlying all of its chromosomal contacts, we repeated the assay with a pair of primers amplifying a ~10 kb intrachromosomal contact (Figure 1—figure supplement 1A and B). In this ‘off-target’ control, coverage from the 3C library matched that of the genomic control, suggesting that most variants are capable of intrachromosomal looping, but only those containing the minimal pairing region are capable of interchromosomal pairing with the other HAS1pr-TDA1pr allele.

Since our initial experiment was performed in the endogenous genomic context, other DNA sequences outside but near the HAS1pr-TDA1pr locus could be required in addition to the minimal pairing region. We therefore tested whether inserting a 184 bp sequence that included the minimal pairing region into an ectopic location, the gene FIT1 (*YDR534C*), would induce pairing with the native HAS1pr-TDA1pr locus in saturated cultures of haploid *S. cerevisiae* (Figure 1—figure supplement 1C). As a negative control, we inserted an equivalently sized subsequence insufficient for pairing into the same locus (Figure 1—figure supplement 1C). Indeed, insertion of the minimal pairing region led to a >30 fold increase in 3C signal for pairing with HAS1pr-TDA1pr as compared to the negative control (Figure 1C).

We next sought to obtain a base-pair resolution map of the DNA sequences necessary for pairing. We used error-prone PCR to generate variants of a 207 bp region (161 bp excluding fixed primer sequences) containing the minimal pairing region, with an average of 1.49 substitutions (range 0–14) per template (Figure 1—figure supplement 1D). We inserted this variant library in place of the native *S. cerevisiae* HAS1pr-TDA1pr sequence as before, and performed cis MAP-C. The ratio of total substitution abundance in the 3C and genomic libraries can be plotted at each mutagenized position. This identified six clusters of two or more adjacent positions showing strong depletion of substitutions in the 3C libraries, indicating that they are required for HAS1pr-TDA1pr...
Figure 1. MAP-C identifies DNA sequences necessary and sufficient for inducible pairing between HAS1pr-TDA1pr alleles. (A) In the cis MAP-C method, mutations in a ~250 bp segment of the genome are assessed for their effect on a specific 3D contact of that segment. Colored lines indicate mutant DNA sequences, and thin arrows indicate primers. (B) A ~150 bp region is sufficient for interchromosomal pairing. cis MAP-C was used to test 178 bp subsequences from the S. cerevisiae HAS1pr-TDA1pr region for pairing with the S. uvarum HAS1pr-TDA1pr. Shown are read coverage of the 3C (red) and genomic (gray) libraries, normalized to sum to 1. The two lines for each color represent technical replicates. Start positions and orientations of HAS1 and TDA1 coding sequences are shown on x-axis. (C) A minimal pairing region is sufficient for ectopic pairing. Shown are contact frequencies between HAS1pr-TDA1pr and a pairing (red) or non-pairing (gray) sequence (coordinates shown in Figure 1—figure supplement 1C) integrated at the FIT1 locus in haploid S. cerevisiae, as measured by 3C, normalized to contacts between FIT1 and HLR1 10 kb away. Bars indicate mean ± s.d. of technical triplicates. (D) Base-pairs necessary for pairing, shown as ratio of the total substitution frequency at each position in the 3C library compared to the genomic library. Error bars indicate the two technical replicates. Positions most strongly required for pairing (log₂3C/Genomic < −1.1) are Figure 1 continued on next page.
highlighted in red. (E–I) Selected regions from panel D are highlighted, with sequence logos for matching transcription factor motifs. See Figure 1—figure supplements 2 and 3 for full set of overlapping motifs. DOI: https://doi.org/10.7554/eLife.42499.003

The following figure supplements are available for figure 1:

Figure supplement 1. Design and controls for using cis MAP-C to dissect HAS1pr-TDA1pr pairing. DOI: https://doi.org/10.7554/eLife.42499.004

Figure supplement 2. Motifs overlapping positions required for HAS1pr-TDA1pr pairing. DOI: https://doi.org/10.7554/eLife.42499.005

Figure supplement 3. Lower-scoring motifs matching positions required for HAS1pr-TDA1pr pairing. DOI: https://doi.org/10.7554/eLife.42499.006

Figure supplement 4. Validation of TF motifs required for HAS1pr-TDA1pr pairing with a 3 bp substitution mutant library. DOI: https://doi.org/10.7554/eLife.42499.007

Figure supplement 5. Conservation of TF motifs in HAS1pr-TDA1pr. DOI: https://doi.org/10.7554/eLife.42499.008

pairing (Figure 1D). We inspected motifs in the region to identify candidate TFs that might mediate the pairing (Figure 1—figure supplements 2 and 3). Given the abundance of potential matches, we prioritized motifs with high-scoring matches, motifs with conserved positions corresponding to those most important for pairing, and motifs occurring in multiple clusters. The first two clusters together aligned to a Leu3 motif (Figure 1E), the third to several similar motifs, including Sdd4 (Ypr022c) and Mig1 (Figure 1F), and the last three to Rgt1 motifs in both orientations (Figure 1G–I). These motifs, which span 47 bp, include 23 of the 24 positions most depleted for mutations in the 3C libraries (Figure 1—figure supplement 1E), and all clusters of two or more adjacent such positions (Figure 1D). None of these mutations had a strong effect on intrachromosomal looping (Figure 1—figure supplement 1F), and all of the clusters were reproduced using an alternative mutagenesis strategy (programmed 3 bp substitutions) (Figure 1—figure supplement 4). Interestingly, a fourth Rgt1 motif and a second Sdd4/Mig1 motif mutagenized only in our validation experiment were not required for pairing, suggesting that not all motifs in this region are bound by the same TFs or not all bound TFs are involved in mediating homolog pairing at this locus.

Thus, using cis MAP-C, we identified a ~150 bp subsequence of HAS1pr-TDA1pr sufficient for pairing, containing five required TF motif occurrences. If these TF motifs are together sufficient for pairing, we would expect that 1) they are only observed in a cluster in the minimal pairing region and not elsewhere in the HAS1pr-TDA1pr, and 2) they are present in a cluster in the S. uvarum copy of this region, and potentially other Saccharomyces as well. Indeed, these motifs are clustered together only in the central region of HAS1pr-TDA1pr; remarkably, this pattern holds across all Saccharomyces species (Figure 1—figure supplement 5).

Three transcription factors are required for pairing

Although we identified the TF motifs required for HAS1pr-TDA1pr homolog pairing at base-pair resolution with cis MAP-C, the redundancy among TF motifs made it difficult to definitively identify the TFs involved (Figure 1F). To address this, we developed a modified version of MAP-C that is capable of assaying trans mutations spread across the genome, such as gene knockouts, for their effects on a specific chromosomal contact (trans MAP-C). With trans MAP-C, each mutation is uniquely associated with a short barcode sequence near a region involved in the chromosomal contact of interest, which is then assayed by 3C. Mutations that affect pairing frequency modulate the abundance of their corresponding barcodes in the 3C products, which can be readily quantified by deep sequencing. To study the trans requirements of HAS1pr-TDA1pr pairing, we ectopically integrated barcoded versions of the minimal pairing region, wherein the barcode identifies which TF is knocked out, and assayed their capacity to pair with the native copy of HAS1pr-TDA1pr (Figure 2A).

We first tested this approach in a pilot set of 10 TF knockouts by inserting the minimal pairing region (Figure 1—figure supplement 1C) into the common KanMX drug resistance cassette used to replace each deleted gene in the haploid yeast deletion collection (Giaever et al., 2002). We then assayed these constructs for interactions with the native HAS1pr-TDA1pr region, using the existing gene-specific barcodes to measure strain abundances in each library. In this approach, because the
Figure 2. Transcription factors Leu3, Sdd4, and Rgt1 mediate HAS1pr-TDA1pr pairing. (A) In trans MAP-C, the effect of TF knockouts or variants are assessed for their effect on a specific 3D contact by association with barcodes. Barcoded versions of the minimal pairing sequence were ectopically integrated and assessed for pairing with the native HAS1pr-TDA1pr in haploid S. cerevisiae. Red Xs indicate gene knockouts; red boxes indicate pairing sequence (Figure 1—figure supplement 1C); BC indicates barcode; double-headed arrows indicate the presence of chromosomal contacts. (B) Full TF knockout screen identifies Leu3 and Rgt1 as trans requirements for pairing. Histogram of relative abundance of each barcoded gene knockout strain in 3C library compared to the genomic library, excluding strains below a frequency of 0.3% of the pool. Barcode replicates are shown as separate squares in histogram. LEU3, RGT1, and MOT3 are highlighted individually; TF indicates other transcription factors; Nup indicates nuclear pore complex components; Neutral indicates fitness-neutral negative controls (Figure 2—figure supplement 2A). (C) Validation TF knockout screen confirms that Leu3, Sdd4, and Rgt1 are required for pairing. Bar plot of median relative abundance in 3C library compared to the genomic library, with overlaid scatter plot of individual barcoded strains. TF includes MIG1, VHR1, CBF1, and YGR067C. (D) Regions tested by ChIP-qPCR for TF binding. Bars show Leu3, Sdd4, and Rgt1 motif matches in S. cerevisiae HAS1pr-TDA1pr, and lines indicate regions (L and R) used for qPCRs in panel E. Bar heights indicate motif score as fraction of maximum possible score; all motifs with a score of at least 0.45 shown. (E) TFs bind HAS1pr-TDA1pr more strongly in saturated conditions. Chromatin immunoprecipitation qPCR results in exponentially growing (Exp) and saturated (Sat) cultures, normalized by input and to a negative control locus in the PRM1 coding sequence. L and R indicate two primer sets as shown in panel D. Bars show mean ± s.e.m. of biological triplicates. Dashed lines indicate a value of 1 (background enrichment). (F) Chromatin immunoprecipitation sequencing data near the HAS1pr-TDA1pr locus (coordinates chrXIII:844,705–862,314 in sacCer3 reference), shown as fold enrichment in IPs over input.

DOI: https://doi.org/10.7554/eLife.42499.009

The following figure supplements are available for figure 2:

**Figure supplement 1.** A pilot TF gene knockout screen for HAS1pr-TDA1pr pairing.

*Figure 2 continued on next page*
pairing sequences are inserted into different genes throughout the genome, the frequency of pairing is confounded by the potential effects of the genomic location of the pairing sequence. Therefore, for each of the 10 TFs we targeted, we included as controls up to six neighboring genes, which should have a similar genomic location effect as the targeted gene (Figure 2—figure supplement 1A and B). We hypothesized that due to the Rabl orientation of yeast chromosomes, in which centromeres are clustered together (Duan et al., 2010), centromere-proximal regions would interact less with HAS1pr-TDA1pr, which is centromere-distal. Indeed, the most centromere-distal gene knockouts interacted ~ 4 fold more with the HAS1pr-TDA1pr locus than the most centromere-proximal gene knockouts (Figure 2—figure supplement 1C). Of the 7 TF gene knockouts that were measured in our assay (three dropped out during library construction, including LEU3), six had no substantial difference in pairing strength compared to their genomic neighbors (Figure 2—figure supplement 1D). However, deletion of RGT1 led to a ~ 20 fold decrease in pairing strength, consistent with Rgt1 binding its cognate motifs in the minimal pairing region (Figure 2B and C).

Next, we expanded our trans MAP-C screen to include the majority of known nonessential TFs with known binding motifs (de Boer and Hughes, 2012). To avoid the confounding effect of genomic location, we inserted a barcoded pairing sequence construct into a fixed locus instead of into the gene knockout locations. We associated each of these barcodes with the cognate knockout by individually transforming each knockout strain with a unique barcode, in a 96-well plate format. We tested a total of 109 TF gene knockouts, as well as 15 nuclear pore complex components, eight fitness neutral negative controls, and a wild-type control, with multiple barcode replicates for controls and expected hits (Figure 2—figure supplement 2A). As expected, most barcoded strains were equally abundant in the 3C and genomic libraries, indicating that the corresponding knockout did not impact HAS1pr-TDA1pr pairing. However, LEU3 and RGT1 knockouts were depleted ~ 4 fold from the 3C libraries, suggesting that they are required for pairing (Figure 2B). In addition, deletion of MOT3 modestly decreased pairing (~2.5 fold); however, its binding motif is not present in the HAS1pr-TDA1pr region, suggesting an indirect role. Two other knockouts, VHR1 and CBF1, appeared to also decrease pairing, but were at low abundances and might reflect noise (Figure 2—figure supplement 2B).

Surprisingly, none of the TFs required for pairing in either trans MAP-C experiment had a motif matching the sequence CCCCAC (the third cluster of positions required for pairing; Figures 1F and 2B, and Figure 2—figure supplement 1C). However, two putative TFs with high scoring motif matches, YPR022C (SDD4) and YGR067C, were excluded in the initial screens due to their lack of annotations. Therefore, we repeated our fixed-locus TF knockout screen with a limited set of genes, including the two putative TFs and additional replicates for the hits MOT3, VHR1, and CBF1. We found that SDD4 is indeed required for pairing, suggesting that it is the trans-acting factor that binds the CCCCAC motif (Figure 2C). MOT3 once again exhibited modest depletion, suggesting a minor, perhaps indirect, role in pairing, whereas VHR1 and CBF1 displayed no depletion (Figure 2C).

The strong concordance between the genomic base-pairs required for pairing (identified by cis MAP-C) and the DNA binding motifs of trans factors required for pairing (identified by trans MAP-C) suggests that Leu3, Sdd4, and Rgt1 bind the HAS1pr-TDA1pr to mediate pairing. To test this hypothesis, we performed chromatin immunoprecipitation (ChIP) for the tandem affinity purification (TAP) tagged versions of Leu3, Sdd4, and Rgt1 (Ghaemmaghami et al., 2003), in haploid S. cerevisiae yeast under both saturated and exponential growth conditions. By qPCR using two different primer pairs, all three TFs strongly bound to the HAS1pr-TDA1pr pairing region in saturated conditions, but near background levels in exponential growth (Figure 2D and E). We then performed
ChIP sequencing to determine TF binding genome-wide. Consistent with our quantitative PCR measurements, all three TFs showed robust ChIP-seq peaks at HAS1pr-TDA1pr in saturated conditions. In contrast, only Leu3 demonstrated a significant peak in exponential growth, with 20-fold weaker enrichment (2.5-fold vs. 51-fold in saturated conditions; Figure 2F). For all three TFs, saturated conditions produced ChIP-seq peaks with greater enrichments over the input controls and more robust enrichment of the expected motifs, suggesting generally more extensive DNA binding (Figure 2—figure supplements 3 and 4). These global trends could be a result of technical artifacts as well as biological differences; however, all samples were treated identically using a protocol not optimized for saturated culture conditions, and the HAS1pr-TDA1pr peak showed particularly strong condition-specificity (Figure 2—figure supplement 3).

Based on the convergence of cis MAP-C, trans MAP-C, and ChIP-seq data, we conclude that Leu3, Rgt1, and Sdd4 directly bind to both alleles of the HAS1pr-TDA1pr minimal pairing region under saturated growth conditions and thereby mediate inducible interchromosomal contacts between them.

**Combinatorial transcription factor binding specifies strong pairing**

We next explored whether the combination of Leu3, Sdd4, and Rgt1 binding explains the uniqueness of HAS1pr-TDA1pr pairing. If the clustered binding of Leu3, Sdd4, and Rgt1 is necessary and sufficient to cause pairing in saturated culture conditions, either 1) no loci other than HAS1pr-TDA1pr should have all three TFs bound on both the *S. cerevisiae* and *S. uvarum* copies, or 2) other loci that do have all three TFs bound should also exhibit pairing. Because DNA binding data was only available for the *S. cerevisiae* genome, we tested the first possibility by scanning the *S. cerevisiae* and *S. uvarum* genomes for clusters of the three motifs using permissive thresholds for motif matches and allowing up to 200 bp between motifs (see Materials and methods), and then assessed ChIP-seq data at these clusters. The promoters of four genes, TDA1 but also HXT3, YKR075C, and SKS1, harbored a motif cluster containing all three motifs in both *S. cerevisiae* and *S. uvarum* (Figure 3A). We also assessed two additional loci, MIG1pr and ILV2pr, that lacked one of the three motifs in *S. uvarum*. Of these six loci, TDA1pr exhibited the strongest total ChIP-seq signal in saturated conditions (Figure 3B and C), even when extending the search to motif clusters with only one or two of the three motifs in *S. cerevisiae* (Figure 3—figure supplement 1). Furthermore, YKR075Cpr was the only other locus with robust binding of all three TFs in *S. cerevisiae*, but the only Leu3 motif in the *S. uvarum* copy of the region overlaps a stronger Rgt1 motif and may not result in Leu3 binding. We also noticed that the TDA1pr cluster was the most compact (i.e. shortest maximum distance between motifs); whether this plays a role in pairing is unclear. We infer that the combinatorial binding of Leu3, Sdd4, and Rgt1 on both homologs specifies inducible homolog pairing in saturated cultures.

Although TDA1pr exhibits the strongest combinatorial TF binding, we wondered whether any other motif clusters pair inducibly like the HAS1pr-TDA1pr locus, albeit perhaps more weakly. To address this question, we leveraged our previously published Hi-C datasets ([Kim et al., 2017](https://doi.org/10.7554/eLife.42499)), along with new Hi-C experiments for the high-pairing strain background in which we performed our pooled mutant experiments, to compare the strength of pairing at each homologous motif cluster and assess whether this pairing is condition-specific (Figure 3—figure supplement 2). We observed that HXT3pr appears to form inducible homologous contacts in saturated culture conditions (Figure 3D), despite weak Sdd4 and no Leu3 binding. We did not detect pairing at any other loci (Figure 3—figure supplement 2). Across several strain backgrounds, the HXT3 promoters exhibited 1.6- to 2.7-fold increased interaction frequencies compared to other similar interchromosomal pairs of loci (at least 480 kb from a centromere and excluding subtelomeric regions) in saturated culture conditions (Figure 3E), but only at baseline levels during exponential growth in rich medium. This is weaker than the pairing between HAS1pr-TDA1pr alleles (Figure 3—figure supplement 2), suggesting that combinatorial TF binding is not strictly necessary for inducible pairing but may facilitate particularly strong pairing.

The identification of two pairs of loci, HAS1pr-TDA1pr and HXT3pr, exhibiting homolog pairing opened the possibility that there might be cross-pairing (nonhomologous contacts) between HAS1pr-TDA1pr and HXT3pr. To test this possibility, we extracted the interaction frequencies among the four loci (i.e. two pairs) from our Hi-C data. In all saturated culture datasets where homolog pairing was present, inter-locus interactions were substantially weaker and similar in frequency to
Figure 3. Combinatorial TF binding specifies inducible homolog pairing. (A) Top clusters of Leu3, Sdd4, and Rgt1 motifs in homologous loci of both S. cerevisiae and S. uvarum genomes containing all three motifs in S. cerevisiae, in order of lowest P-value from top to bottom. (B) Not all motif clusters are bound by all three TFs. ChIP-seq data for exponentially growing (Exp) and saturated (Sat) S. cerevisiae corresponding to motif clusters in panel A. (C) Combined TF binding is strongest at TDA1pr. Shown are the sum of the ChIP log2 fold enrichments over inputs in saturated conditions for motif clusters in panel A. (D) HXT3pr also exhibits inducible homolog pairing. Hi-C contact maps of interactions between regions centered on HAS1pr-TDA1pr and S. uvarum HXT3 at 32 kb resolution in exponentially growing and saturated cultures of a S. cerevisiae x S. uvarum hybrid strain (YMD3920). White arrows indicate interactions between homologous HXT3 promoters. (E) Strength of HXT3 promoter pairing across conditions and strain backgrounds, at 32 kb resolution (red lines) compared to similar interactions (gray violin plots; i.e. interactions between an S. cerevisiae locus and an S. uvarum locus, where both loci are ≥ 15 genomic bins from a centromere and ≥ 2 bins from a telomere, ≥ 2 bins from HAS1pr-TDA1pr, and not both on chrXII). WT represents strain ILY456 (YMD3259); +R indicates a restriction site added upstream of HAS1 (YMD3920), Δ20 indicates a 20 kb deletion centered at S. cerevisiae HAS1 (YMD3266), Δ3 indicates a 3 kb deletion centered at S. cerevisiae HAS1 (YMD3267), Δ2 indicates a 2 kb deletion of the S. cerevisiae HAS1 coding sequence (YMD3268), and Δ1 indicates a 1 kb deletion of the S. cerevisiae HAS1pr-TDA1pr intergenic region (YMD3269). DOI: https://doi.org/10.7554/eLife.42499.014

The following figure supplements are available for figure 3:

Figure supplement 1. HAS1pr-TDA1pr exhibits uniquely strong combinatorial binding of Leu3, Sdd4, and Rgt1.

Figure 3 continued on next page
those in non-pairing conditions (Figure 3—figure supplement 1). Together with our previous experiment demonstrating that two identical copies of the minimal pairing region can pair even at non-allelic locations in haploid S. cerevisiae (Figure 1C), these data suggest that the interchromosomal pairing mediated by Leu3, Sdd4, and Rgt1 is sequence-specific beyond the simple presence of the same TF binding sites.

**HAS1pr-TDA1pr pairing is regulated by Rgt1 abundance, recruitment of Tup1/Ssn6, and competing domains**

We next sought to explore the mechanisms that regulate pairing. We hypothesized that TF expression levels might regulate the strength of HAS1pr-TDA1pr pairing. To test this hypothesis, we analyzed RNA-seq data for haploid S. cerevisiae in pairing and non-pairing conditions (saturated and exponentially growing cultures, respectively) (Kim et al., 2017). RGT1 was upregulated in saturated cultures, ~2 fold and ~6 fold, respectively, whereas LEU3 transcript levels remained constant (Figure 4A). These results are consistent with the hypothesis that increased transcription of the TFs mediating pairing, particularly RGT1, regulates the strength of the pairing interaction.

Based on these results, we wondered whether overexpression of any one of the three proteins would be sufficient to produce pairing in non-saturated culture conditions. We used the ZjEV estradiol induction system (McIsaac et al., 2014) to individually overexpress S. cerevisiae Leu3, Sdd4, or Rgt1, and measured pairing between the native HAS1pr-TDA1pr loci using 3C in S. cerevisiae x S. uvarum hybrids growing exponentially in rich medium (Figure 4B). In all three strains, a 2 hr estradiol induction led to no increase in pairing strength despite between 2- and 10-fold increases in transcript levels (Figure 4—figure supplement 1A). As an alternative test, we used galactose induction to overexpress epitope-tagged RGT1, and observed a decrease in pairing strength relative to a strain lacking the overexpression cassette (Figure 4—figure supplement 1B). These results are consistent with no single TF being sufficient for pairing; however, Leu3 and Rgt1 are both known to change in conformation (Sze et al., 1992) or phosphorylation state (Kim et al., 2003) in different conditions, so it remains possible that overexpression of a single TF in the appropriate state suffices for pairing.

Rgt1 is known to interact with several cofactors that affect its DNA binding and transcriptional repression activities: the Tup1/Ssn6 co-repressor complex and the proteins Mth1 and Std1 (Polish et al., 2005). Our experiments thus far had not distinguished whether Rgt1’s pairing activity is directly mediated by physical interactions among molecules of Rgt1 or indirectly, through these or other interaction partners. To address this, we performed trans MAP-C for individual deletions of these four interacting partners of Rgt1, along with the same positive and negative controls as before (Figure 4C). In addition, based on the glutamine and asparagine-rich domains present in Sdd4 and Rgt1, we tested deletion of RNQ1, a Q/N-rich peptide known to influence the oligomerization of other Q/N-rich proteins (Derkatch et al., 2004). Deletion of RNQ1 had no effect on pairing, suggesting that HAS1pr-TDA1pr pairing is not mediated by Q/N-rich domains. Deletion of TUP1 or SNN6 both led to increased pairing, indicating that the recruitment of the Tup1/Ssn6 co-repressor complex inhibits pairing. This is consistent with its known inhibition of Rgt1’s DNA binding activity (Roy et al., 2013), which is presumably required for pairing. Deletion of MTH1 or STD1 had a minimal negative effect on pairing. These results suggest that the role of Rgt1 in pairing is not simply to recruit cofactors that mediate pairing; instead, its pairing activity may compete with its transcriptional repression activity.

We hypothesized that a particular domain of Rgt1, coupled with its DNA-binding activity, might be responsible for HAS1pr-TDA1pr pairing. To test this idea, we generated a series of 10 amino acid
**Figure 4.** Rgt1 expression, interaction partners, and competing domains regulate HAS1pr-TDA1pr pairing. (A) RGT1 and SDD4 are upregulated in saturated cultures. RNA-seq expression levels for LEU3, SDD4, RGT1 in exponentially growing (Exp) and saturated (Sat) cultures. FPKM = fragments per kilobase per million read pairs. Bars indicate mean ± s.e.m. of biological triplicates. **p<0.01, ***p<0.001 (Student's t-test). (B) Individual TF overexpression is insufficient for pairing. Shown are HAS1pr-TDA1pr homolog pairing frequencies with and without estradiol-induced overexpression of LEU3, SDD4, RGT1 in S. cerevisiae x S. uvarum hybrids during exponential growth, measured by 3C and normalized to contacts between HAS1pr-TDA1pr and LCB1 on S. cerevisiae chrXIII. Bars indicate mean ± s.d. of technical triplicates. (C) Effects of Rgt1 interaction partner deletions on ectopic HAS1pr-TDA1pr pairing in saturated conditions. Bar plot of median relative abundance in 3C library compared to the genomic library, with overlaid scatter plot of individual barcoded deletion strains. Retested controls are shown in same colors as in Figure 2; potential interaction partners are shown in shades of purple. (D) Regions of Rgt1 necessary for pairing in saturated culture conditions, with overlaid predicted disorder (black line, right y-axis) and secondary structure (pink and blue highlights). Each bar represents the ratio of the frequency of each 10 amino acid deletion in the 3C library compared to the genomic library. Error bars indicate the two biological replicates. (E) Domain deletions and phosphorylation site mutations tested in panel F. (F) Effects of Rgt1 domain deletions and phosphorylation site mutations on ectopic HAS1pr-TDA1pr pairing in saturated cultures, plotted as in panel C. Rgt1 indicates the strains with an ectopic wild-type (WT) or mutant copy of Rgt1 in addition to a deletion of the endogenous RGT1 locus.

DOI: https://doi.org/10.7554/eLife.42499.019

The following figure supplements are available for figure 4:

**Figure supplement 1.** Overexpression of RGT1 is not sufficient for HAS1pr-TDA1pr pairing.

DOI: https://doi.org/10.7554/eLife.42499.020

**Figure supplement 2.** Regions of Rgt1 required for pairing correspond to regulatory domains.

DOI: https://doi.org/10.7554/eLife.42499.021
deletions spanning most of the Rgt1 protein (positions 91–1030, excluding several deletions that dropped out during strain construction) and performed trans MAP-C to test the pairing function of the Rgt1 mutants (Figure 4D). Surprisingly, much of the C-terminal half of Rgt1 was required for HAS1pr-TDA1pr pairing. These ‘pairing domains’ are closely aligned to the regions of Rgt1 predicted to be highly structured, largely through alpha helices (Figure 4D). The regions of Rgt1 required for pairing also correspond loosely to those required for regulation of activation vs. repression function through allosteric changes in protein conformation in response to glucose-regulated phosphorylation (Polish et al., 2005) (Figure 4—figure supplement 2).

To further compare the roles of Rgt1 protein domains and phosphorylation in HAS1pr-TDA1pr pairing, we generated deletions of the Rgt1 zinc finger, Q/N-rich, and C-terminal domains, and phosphodepletion mutants S88A and S758A, and performed trans MAP-C to test their pairing function (Figure 4E and F). As expected, deletion of the zinc finger DNA-binding domain or the C-terminal domain led to background pairing levels, equivalent to lacking Rgt1 altogether. Surprisingly, deletion of the Q/N-rich domain led to stronger pairing than the wild-type control (with RGT1 integrated at the same ectopic location), suggesting that the Q/N-rich domain inhibits pairing. Both the S88A and S758A mutations had a weak decrease in pairing, indicating that although these mutations are capable of disrupting Rgt1 activator function and intramolecular interactions (Polish et al., 2005), they do not individually play major roles in regulation of pairing in saturated cultures.

Together, our results suggest multiple potential modes by which the transcription factor Rgt1 regulates HAS1pr-TDA1pr pairing: its own expression level, recruitment of Tup1/Ssn6, and the competing activities of its Q/N-rich and C-terminal regulatory domains.

**Pairing factors do not play major roles in HAS1 or TDA1 transcriptional regulation**

We have thus far characterized the molecular mechanism and regulation of HAS1pr-TDA1pr homologous pairing in saturated cultures. However, the question of why this pairing occurs (i.e. what biological function it serves, if any) remains outstanding. Now that we have identified the precise DNA base pairs and proteins involved in HAS1pr-TDA1pr pairing, we are equipped to test whether Leu3, Sdd4, and Rgt1 play a role in transcription at HAS1 or TDA1 in saturated cultures. Furthermore, we can employ S. cerevisiae x S. uvarum hybrids, in which we can discriminate the two homologous copies of each gene, in order to distinguish the effects on transcription of disrupting the pairing DNA sequence in cis (presumably through impaired cis regulation) vs. in trans (presumably through impaired pairing). To this end, we generated mutant hybrid strains carrying a wild-type copy of S. uvarum HAS1pr-TDA1pr and a copy of S. cerevisiae HAS1pr-TDA1pr with either a wild-type (WT) genotype, mutated Leu3 binding site (leu3), or two mutated Rgt1 sites (rgt1 × 2) (Figure 5A). We then performed RNA-seq in saturated culture conditions. Of note, neither of these mutants is expected to have complete loss of pairing, since the individual binding site or TF mutants disrupt pairing by ~ 4–10 fold (Figure 1D, Figure 2B,C), as opposed to the ~ 30 fold increase in pairing upon ectopic insertion of the pairing sequence (Figure 1C); however, if the Leu3 and Rgt1 binding site mutations cause the same effect on HAS1 or TDA1 transcription, the orthogonal nature of these perturbations would add confidence that any resulting molecular phenotype is an effect of disrupting pairing. The Rgt1 binding site mutations led to higher levels of S. cerevisiae TDA1, consistent with Rgt1 acting as a repressor under low glucose conditions (Figure 5B), but had no significant effect on either S. uvarum HAS1 or TDA1. Surprisingly, despite the conserved binding site and strong ChIP-seq signal for Leu3 in HAS1pr-TDA1pr, disrupting its binding site had no significant effect on the transcript levels of any gene (Figure 5C).

To further characterize the transcriptional roles of Leu3, Sdd4, and Rgt1 in saturated culture, we also performed RNA-seq on deletion strains for each of these genes, along with a wild-type control, grown to saturation. As expected, each deleted gene was highly down-regulated in the mutant strains (Figure 5—figure supplement 1A). In addition, many known targets of Rgt1 and genes downstream of ChIP-seq peaks were highly upregulated in rgt1Δ (Figure 5—figure supplement 2). Focusing on the HAS1-TDA1 locus, none of the deletion strains had altered HAS1 expression, and only the rgt1Δ strain had even slightly altered TDA1 expression (Figure 5D). These results are consistent with our earlier results from mutating TF binding sites, and together indicate that despite the importance of Leu3, Sdd4, and Rgt1 binding for pairing at the HAS1pr-TDA1pr locus in saturated
Figure 5. Pairing TFs play a minimal role in transcriptional regulation of the HAS1-TDA1 locus. (A) Mutations in S. cerevisiae HAS1pr-TDA1pr tested for transcriptional effects in S. cerevisiae x S. uvarum hybrids. Colored boxes indicate TF motifs. Wild type and mutated sequences are shown below boxes, with mutated bases in lowercase, red letters. (B) Rgt1 regulates TDA1 expression in cis. RNA-seq expression levels for S. cerevisiae (Sc) and S. uvarum (Su) copies of HAS1 and TDA1 in saturated cultures of strains shown in panel A. FPKM = fragments per kilobase per million read pairs. Bars indicate mean ± s.e.m. of biological triplicates. **p<0.01 (Student’s t-test). (C) Mutations in pairing region cause few transcriptional changes. Volcano plot of fold change vs. p-value in mutant strains compared to wild type, shown in (A). (D) RNA-seq expression levels for HAS1 and TDA1 in saturated cultures of wild type haploid S. cerevisiae and gene deletion strains. Bars indicate mean ± s.e.m. of biological triplicates. *p<0.05 (Student’s t-test).

DOI: https://doi.org/10.7554/eLife.42499.022

The following figure supplements are available for figure 5:

Figure supplement 1. Transcriptional effects of TF deletion in saturated culture and exponential growth.
DOI: https://doi.org/10.7554/eLife.42499.023

Figure supplement 2. Comparison of TF binding targets and differentially expressed genes upon TF deletion.
DOI: https://doi.org/10.7554/eLife.42499.024
culture conditions, they do not play a major role in transcriptional regulation as detected by standard polyA mRNA sequencing.

**Discussion**

In summary, we developed Mutation Analysis in Pools by Chromosome conformation capture (MAP-C), a method to simultaneously test hundreds of mutations for their effects on a chromosomal contact of interest. MAP-C can be used to identify the precise sequences that are necessary for a contact (cis MAP-C) as well as the factors that are necessary to mediate the contact (trans MAP-C). Here we applied both versions of MAP-C to dissect the mechanism of inducible interchromosomal pairing between HAS1pr-TDA1pr alleles in budding yeast. Using a combination of gain-of-function and loss-of-function screens, we demonstrate that a trio of transcription factors—Leu3, Sdd4, and Rgt1—mediates pairing between clusters of binding sites.

Our results begin to elucidate the mechanisms of condition-specific interchromosomal contacts and homolog pairing, which have often been elusive (Mirkin et al., 2013). We have not yet fully defined the biochemical mechanism of pairing—it is possible the TFs interact directly and/or indirectly—but so far, no known interaction partners or cofactors have proven essential for this interaction. Unlike more prevalent nuclear-pore mediated gene relocalization and homolog pairing (Brickner et al., 2012; Randise-Hinchliff and Brickner, 2016), HAS1pr-TDA1pr pairing in saturated cultures does not appear to require the nuclear pore complex (Figure 2B). Instead, the Tup1/Ssn6 repressor complex recruited by Rgt1 negatively regulates pairing (Figure 4C), perhaps by inhibiting the DNA-binding activity of Rgt1 (Roy et al., 2013). Within Rgt1, the zinc finger DNA-binding domain and C-terminal regulatory domain are required for pairing, while the Q/N-rich region, which contains the activation domain (Polish et al., 2005), inhibits pairing (Figure 4F). These results point toward specific interactions among structured domains mediating pairing, rather than phase separation by intrinsically disordered regions.

Intriguingly, Leu3 also contains a regulatory domain whose conformation responds to environmental cues (Sze et al., 1992; Wang et al., 1997). We speculate that these regulatory domains, which are capable of intramolecular interactions (Polish et al., 2005), may mediate interchromosomal pairing. Furthermore, if these regulatory domains are indeed capable of direct dimerization or oligomerization, they may mediate not only interchromosomal contacts, but also cooperative TF binding at clusters of binding sites (Kim et al., 2003). We note that of Leu3, Sdd4, and Rgt1, only Leu3 is known to form strong dimers at each of its binding sites. However, even weak homotypic intermolecular interactions could increase the duration of interchromosomal contacts as well as increase local TF concentrations, and thereby increase DNA binding activity. It remains unclear whether heterotypic interactions among Leu3, Sdd4, and Rgt1 occur.

Another question is whether the contacts are stoichiometric, for example one-to-one, or instead mediated by aggregation of one or more TFs via weak interactions. Notably, we observe homotypic pairing between homologs of HAS1pr-TDA1pr and HXT3pr but not heterotypic pairing (Figure 3—figure supplement 3), suggesting that Rgt1 does not indiscriminately form contacts between all of its binding sites. However, this is not because the entire homologous context is necessary for pairing, as we found that the pairing sequence from HAS1pr-TDA1pr is sufficient to induce ectopic pairing in haploid S. cerevisiae (Figure 1C). Instead, a possible explanation is that only pairs of loci with a series of motifs in similar orders and orientations form frequent contacts, consistent with a stoichiometric interaction model. This added specificity beyond the presence of TF motifs could explain the lack of pairing between HAS1pr-TDA1pr and other sites of Leu3, Sdd4, and Rgt1 binding (Figure 3—figure supplement 4).

Despite our detailed molecular characterization of HAS1pr-TDA1pr homolog pairing, we still do not know its biological function, if any. We hypothesized that it might contribute to transcriptional regulation at either HAS1 or TDA1. In particular, TDA1 is a kinase that phosphorylates Hxk2, the main hexokinase in yeast, and thereby inhibits its ability to mediate glucose repression together with Mig1 (Kaps et al., 2015; Kettner et al., 2012). We speculated that pairing might help activate TDA1, which is mildly upregulated at the transcriptional level in saturated conditions (Kim et al., 2017). However, we find that Leu3 and Sdd4 play no detectable role in regulating transcript levels of either gene in saturated culture conditions, while Rgt1 mildly represses TDA1 in cis but not in trans (Figure 5). There are several possible reasons why we may not have detected a phenotypic
effect from disrupting the pairing region or factors: (1) even low levels of pairing (~10–25% based on our MAP-C experiments shown in Figure 1D and Figure 2B, C) are sufficient to mediate its biological function; (2) its function is not in saturated culture conditions per se, but affects either exit from or re-entry into those conditions, similar to transcriptional memory at GAL1 and INO1 (Brickner et al., 2015; Soed et al., 2017); (3) its function only affects the dynamics of transcription (or other processes) but not steady-state RNA levels (Zhang and Bai, 2016). However, we also acknowledge the possibility that there is no function per se, but that pairing is instead a side-effect of conservation of biochemical features of the pairing TFs. If the same domains are required for both cooperative binding and pairing, then evolutionarily conserved pairing could result from selection on cooperative binding. Interestingly, the TF Hsf1, which forms trimers, and in flies also exhibits cooperativity between trimers (Xiao et al., 1991), was also recently shown to mediate interchromosomal contacts in yeast (Chowdhary et al., 2019). More studies of the biochemical basis of cooperative TF binding are required to test this hypothesis.

Is HAS1pr-TDA1pr pairing unique? Based on Hi-C, we previously found HAS1pr-TDA1pr to exhibit the strongest interchromosomal interactions in saturated cultures of S. cerevisiae x S. uvarum hybrids, excluding centromeres, telomeres, and the chromosomes carrying the rDNA arrays (Kim et al., 2017). Using ChIP-seq and motif analyses, we found that a requirement for robust adjacent DNA-binding by Leu3, Sdd4, and Rgt1 on both the S. cerevisiae and S. uvarum copies was sufficient to identify HAS1pr-TDA1pr. However, we also expect that the limited resolution of Hi-C and the sequence divergence in interspecific hybrids would both limit our sensitivity for detecting homolog pairing. We hypothesize that more cases of localized pairing exist, as we found with HXT3pr (Figure 3D and E). We imagine that other TFs capable of interchromosomal contacts, like Hsf1 (Chowdhary et al., 2019), are also capable of mediating homolog pairing, and the condition-specificity of these contacts suggests that other conditions may also exhibit similar contacts but have not yet been explored.

Although our study was focused on HAS1pr-TDA1pr pairing in budding yeast, both cis and trans MAP-C should be applicable to other loci and organisms. The main constraints in experimental design are that 1) the introduced mutations or associated barcodes must be included in the 3C PCR product, and 2) the region of interest should not be digested by the restriction enzyme. As we have implemented this approach, the region targeted by saturation mutagenesis was limited to < 250 bp to allow for Illumina sequencing, but this could be extended using either barcode association, similar to our trans knockout screens, or long-read sequencing methods. Also, we focused on a single pairwise interaction, but it is also possible to assay a mutant pool for multiple interactions, by using multiple primer pairs. MAP-C should be applicable to intrachromosomal contacts as well as interchromosomal ones, albeit with a potentially higher background of nonspecific contacts. It would be interesting to apply MAP-C to dissect the cis and trans regulators of enhancer-promoter loops in mammals, and thereby distinguish the contributions of cohesin and CTCF (Guo et al., 2012), general looping factors like YY1 (Weintraub et al., 2017), site-specific transcription factors (Nolis et al., 2009), and other cofactors. Trans MAP-C could also allow mutational scanning of TFs to clarify the biochemical mechanisms by which these transcription factors mediate chromosomal contacts.

MAP-C leverages the high throughput of saturation mutagenesis and mutant collections to allow systematic dissection of chromosome conformation. We tested up to ~1000 variants at a time, but with larger-scale experiments, it should be possible to test even more variants. A major potential strength of our approach is that unlike cellular high-throughput genetic screens (Fowler and Fields, 2014; Gasperini et al., 2016; Shalem et al., 2015), it resolves the functional consequences of mutations at the allelic level, and thus is not confounded by heterozygosity (Patwardhan et al., 2009). As we continue to map chromosome conformation at high resolution across ever-expanding numbers of cell types and conditions, MAP-C will provide a scalable approach to dissect the molecular mechanisms underlying specific contacts.

Materials and methods

Yeast strains and culture

Yeast strains used in this study are described in the Key Resources Table. Yeast were cultured at 30°C, with the exception of S. uvarum strains, which were grown at room temperature. Cultures were
grown shaking overnight to OD$_{600}$ > 5 for saturated culture samples, or diluted to OD$_{600}$ ~ 0.125 and grown to OD$_{600}$ = 0.5–0.8 for exponential growth samples. Estradiol inductions were performed by addition of beta-estradiol to 1 mM final concentration (or equivalent volume of ethanol for negative control) to OD$_{600}$ = 0.5 cultures grown in YPD (1% w/v yeast extract, 2% w/v peptone, 2% w/v dextrose) and grown for 2 hr. Galactose induction was performed by growth in synthetic complete medium (without uracil for selection for overexpression plasmid) with 2% v/v raffinose to OD$_{600}$ = 0.75 followed by addition of 2% galactose and subsequent growth for 1.5 hr. For comparison, yeast were grown in synthetic complete medium with or without uracil with 2% glucose or 2% raffinose. S. cerevisiae x S. uvarum hybrids were generated by standard mating and auxotrophic or drug selection procedures. Yeast transformations were performed using a modified Gietz LiAc method (Pan et al., 2004).

**Mutant library generation**

**Subsequences**
All 178 bp subsequences of the intergenic region between the S. cerevisiae HAS1 and TDA1 coding sequences were synthesized in an Agilent (Agilent Technologies, Santa Clara, CA) array-synthesized oligonucleotide pool (sequences included in Supplementary file 1), and then amplified and cloned into a vector just downstream of a KanMX cassette followed by a DpnII restriction site (GATC) using NEBuilder HiFi (New England Biolabs, Ipswich, MA). These plasmids, which contain homology to the S. cerevisiae HAS1 and TDA1 sequences, were linearized by restriction digestion and transformed into YMD3919 (deletion of S. cerevisiae has1pr-tda1pr). Transformants (typically ~ 5000 per experiment) were selected on G418 medium for a total of at least 4 days (including two rounds of scraping and replating a portion onto a fresh plate), and then used to inoculate a 50 ml culture in YPD (1% w/v yeast extract, 2% w/v peptone, 2% w/v dextrose).

**Error-prone PCR**
The primers TTCACCGCCTGCTATCATCC and GAATCGGCGGAATAACCTAACG were used in a PCR reaction using the Agilent GeneMorph II kit, using as template 0.1 ng of a PCR product generated with the same primers from S. cerevisiae genomic DNA. The error-prone PCR products were then cloned, transformed, and selected as described above. The library contained ~ 63,000 transformants.

**Programmed 3 bp substitutions**
For each set of 3 consecutive base-pairs between positions 532–675 (inclusive), we randomly chose three trinucleotide substitutions such that among them, each nucleotide change (e.g. A->C) was included once at each position and so that no DpnII restriction sites (GATC) were created. These sequences were synthesized on an array (sequences included in Supplementary file 1) and processed as described above. The library contained ~ 38,000 transformants.

**In-gene knockout screen**
The selected gene knockout strains from the MATalpha yeast deletion collection (Giaever et al., 2002), in addition to the knockouts of the three nearest genes on either side excluding those absent or known to be slow-growing in the yeast deletion collection (Figure 2—figure supplement 1), were pooled and transformed en masse using a PCR construct with homology arms for the TEFb promoter and terminator from the KanMX deletion cassette flanking the pairing sequence (Figure 1—figure supplement 1B), an EcoRI restriction site, and the URA3 selectable marker gene. The entire pool, containing ~ 12,500 transformants, was selected on synthetic complete medium without uracil and processed as described above.

**Fixed-locus knockout screens**
TF genes were defined as genes with motifs on YeTFaSCo (de Boer and Hughes, 2012) that are not annotated as being part of a complex. Genes with no non-systematic name in the GFF file from the Saccharomyces Genome Database (SGD; version R64.2.1) were excluded. In addition, we included knockouts of genes described as nuclear pore components in SGD, the wild type strain and knockouts of eight genes known to have minimal fitness consequences under multiple nutrient limitation. 
conditions (Payen et al., 2016). The selected gene knockout strains from the MATa yeast deletion collection carrying the synthetic genetic array (SGA) reporter (Tong et al., 2001) (excluding those failing quality control or known to grow slowly) were grown in separate wells of deep 96-well plates, and transformed in 96-well format (Supplementary file 3) with a PCR construct similar to that used in the in-gene knockout screen, but with a unique 12 bp barcode added upstream of the pairing sequence (primer sequences in Supplementary file 1), and homology to the YDR535C coding sequence. Colonies were picked and verified by PCR, and one successful clone from each strain was pooled together and then diluted and grown overnight in YPD. Colonies from positive and negative control strains were repooled with new strains for subsequent fixed-locus trans MAP-C experiments.

Interactor knockout screen
The selected gene knockout strains from the MATa yeast deletion collection with the SGA reporter (Tong et al., 2001) were each transformed with a cocktail of constructs (derived from fixed-locus screen construct) with 14 different barcodes each, and then at least eight colonies were Sanger sequenced, and three colonies from each strain carrying different barcodes were pooled and processed as described above, with positive and negative control strains included.

Rgt1 deletion scan
The S. cerevisiae RGT1 gene was amplified with 537 bp of upstream promoter sequence and 221 bp of downstream terminator sequence, and cloned downstream of URA3 in the same vector used for the fixed-locus knockout screens. We attempted to create all 10 amino acid (30 bp in-frame) deletions between amino acids 91 and 1040; we successfully created 87 of the 96 attempted deletions as plasmids. The wild-type RGT1-containing plasmid was amplified in two pieces, each using one primer in the ampicillin resistance gene and one within RGT1 so that the resulting pieces each contain homology for the other. These pieces were combined using NEBuilder HiFi, and then transformed into E. coli, extracted, and verified for the deletion junction by Sanger sequencing. Each deletion plasmid was amplified with the same primers used to create the fixed-locus knockout constructs, with a different chosen barcode for each deletion. These PCR products were pooled and transformed en masse into the rgt1 deletion strain from the MATa yeast deletion collection with the SGA reporter, in two replicates. Each pool was selected on synthetic complete medium without uracil including G418 and processed as described above.

Rgt1 domain deletion and phosphorylation site mutations
Tested mutations included deletion of the zinc finger domain (amino acids 32–90), Q/N-rich domain (amino acids 293–459), C-terminal domain (amino acids 461–1163), and S88A (TCG->GCG) and S758A (TCC->GCC). Mutant versions of the wild-type RGT1-containing plasmid were amplified and cloned as for the 10 amino acid deletion scan of Rgt1, and then transformed using mixtures of 14 different barcodes per desired mutation and Sanger sequenced as in the Rgt1 interactor knockout screen. Five distinct barcoded versions of each mutant were pooled and processed as above, with positive and negative control strains included.

3C
Cells were crosslinked by addition of 37% formaldehyde to a final concentration of 1% (v/v) and incubation at room temperature for 20 min, quenched by addition of 2.5M glycine to a final concentration of 150 mM and incubation at room temperature for 5 min, and then washed in 1x Tris-buffered saline (TBS) and stored as a pellet at –80°C in aliquots of 50–100 µl dry pellets. Cells were lysed by vortexing in lysis buffer (TBS + 1% Triton X-100 with Pierce EDTA-free protease inhibitor tablet (Thermo Fisher Scientific, Waltham, MA)) with 500 µm acid-washed glass beads for 6 cycles of 2 min, with 2 min on ice between cycles. The lysate was collected by puncturing the bottom of each tube and then centrifuging the tube, stacked on top of an empty tube. The lysate was then washed in lysis buffer, then TBS, and finally resuspended in 10 mM Tris pH 8.0 to a volume of ~ 200 µl per 25 µl of starting dry pellet volume. A single 200 µl aliquot of lysate was then precleared by addition of 0.2% SDS and incubation at 65°C for 10 min, cooled on ice, quenched by addition of 1% Triton X-100 (v/v), and then digested overnight with at least 100U of restriction enzyme (200U DpnII for cis experiments and galactose induction, 400U EcoRI-HF for trans experiments, and 100U NlaIII for estradiol
inductions). The restriction digest was heat-inactivated at 65°C for 20 min in the presence of 1.3% SDS, and then chilled on ice and added to a dilute ligation reaction in 4 ml volume with 1% Triton X-100, 1x T4 DNA Ligase Buffer (NEB), and 10,000U of T4 DNA ligase (NEB) and incubated at room temperature for 4 hr. The ligation products were reverse-crosslinked with proteinase K at 65°C overnight, and then purified by phenol-chloroform extraction followed by clean-up on a Zymo DNA Clean and Concentrator-5 column (Zymo Research, Irvine, CA). The resulting 3C DNA was quantified using a Qubit. Each technical replicate was processed separately beginning with cell lysis.

**MAP-C library preparation and sequencing**

3C libraries were prepared by amplification of up to 8 reactions of 50 ng 3C DNA per replicate, using primer pairs specific to the chromosomal contact of interest (for pairing library) or a control off-target chromosomal contact (for off-target library), for 24–32 cycles. Genomic libraries were prepared by amplification of up to 4 reactions of 50 ng of either 3C DNA or genomic DNA using primers flanking the targeted mutations or barcodes, for 17–22 cycles. Reactions for each replicate were pooled, purified by Ampure XP beads (Beckman Coulter Life Sciences, Brea, CA), and then re-amplified with primers flanking the mutagenized or barcode region and including sequencing adapter sequences for 5–8 cycles, and then again with primers adding sample indices and Illumina flow-cell adapters for 6–9 cycles. All reactions were prepared with KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland) with recommended thermocycling conditions, and included 0.5x SYBR Green I to monitor amplification by quantitative PCR and minimize the number of PCR cycles. The final libraries were sequenced on an Illumina MiSeq or Nextseq 500 (Illumina, San Diego, CA) using paired-end sequencing. See Supplementary file 2 for detailed information on each library.

**MAP-C sequencing analysis**

Paired-end reads were merged and adapter-trimmed using PEAR (Zhang et al., 2014), except for trans knockout experiments, in which only read one was used. These reads were then trimmed of the first 4 bp (corresponding to a randomized region for Illumina clustering purposes) and mapped using Bowtie 2 (Langmead and Salzberg, 2012) Reads were mapped to the S. cerevisiae HAS1pr-TDA1pr region, and then the read coverage was calculated using bedtools (Quinlan and Hall, 2010).

**Error-prone PCR**

Reads were mapped to the wild-type sequence of the mutagenized region. The resulting alignments were scored for number of substitutions, insertions, and deletions, and the fraction of reads with a substitution at each position were calculated.

**3 bp substitutions**

Reads were mapped to the wild-type sequence of the mutagenized region. The resulting alignments were scored for number of substitutions, insertions, and deletions, and the fraction of reads with a substitution at each position were calculated, excluding reads with fewer than three substitutions (which correspond to PCR or sequencing errors of the wild-type sequence).

**Trans knockout screens, Rgt1 deletion scan, and Rgt1 mutant screen**

Reads were mapped to all 192 possible barcode sequences, and the normalized fraction of reads mapping to a given barcode with a MAPQ ≥ 20 in the 3C library compared to the genomic control was calculated for each replicate.

**3C qPCR**

3C DNA was amplified using the same conditions as MAP-C libraries, in three replicates per primer pair. The pairing 3C products were normalized to the off-target (intrachromosomal) 3C products, assuming 2-fold amplification per cycle.

**Chromatin immunoprecipitation**

Either 50 ml of exponentially growing (OD_{600} = 0.7–0.8) or 12 ml of saturated cultures of TAP-tagged strains (Ghaemmaghami et al., 2003) were crosslinked and lysed as for 3C, but for 15 min in
FA lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1x Pierce EDTA-free protease inhibitor tablet). Lysates were pelleted and resuspended in FA lysis buffer, sonicated using a Diagenode Bioruptor (Diagenode, Liege, Belgium) for 3 cycles of 10 min on the HIGH power setting, with 30 s cycles on and 30 s on, and cleared by centrifugation at 20000 g for 10 min. An aliquot of 50 µl supernatant was saved for input, and the remaining sample was incubated with 10 µl of Dynabeads Pan Mouse IgG magnetic beads (pre-washed with FA lysis buffer) rotating overnight at 4°C. The immunoprecipitations were washed twice with FA lysis buffer, once with high salt (500 mM NaCl) FA lysis buffer, twice with RIPA buffer (10 mM Tris-HCl pH 8, 250 mM LiCl, 0.5% Igepal CA-630, 0.5% sodium deoxycholate, 1 mM EDTA), and once with TE (50 mM Tris-HCl pH 8, 1 mM EDTA), and then eluted first with 100 µl TE + 1% SDS at 65°C for 15 min, and a second time with 150 µl TE + 0.67% SDS. Inputs were diluted with 200 µl of TE + 1% SDS, and all samples were treated with 50 mg RNase A at 37°C for 10 min and 100 µg proteinase K at 42°C for 1 hr, and then reverse crosslinked overnight at 65°C. DNA was purified with a Zymo ChIP DNA Clean and Concentrator-5 kit and eluted in 15 µl of 10 mM Tris-HCl pH 8.

qPCRs were performed using 5 µl of either 1:20 dilution of IP samples or 1:800 dilution of input samples, in 25 µl reactions with KAPA Robust 2G HotStart ReadyMix, using 0.5x SYBR Green I and standard cycling conditions for 40 cycles on a Bio-Rad C1000 Touch thermal cycler with a CFX96 Real-Time System (Bio-Rad Laboratories, Hercules, CA). Cq values were calculated using the Bio-Rad CFX Manager 3.1 software, using the single threshold mode for Cq calculation and baseline subtracted curve fitting. Primer efficiencies were calculated using a 5-fold dilution series of genomic DNA from S. cerevisiae BY4741 starting from 20 ng. Primer sequences are included in Supplementary file 1.

ChIP-seq libraries were prepared using Swift Accel-NGS 2S Plus (Swift Biosciences, Ann Arbor, MI) dual-indexed kits using 10 µl of IP samples or 1 ng of input samples, with 9 cycles of PCR for input samples and 12–15 cycles for IP samples. Libraries were sequenced to ~2.5–6 million read pairs per sample using 2 × 37 bp reads on an Illumina NextSeq 500.

ChIP-seq analysis

Sequencing reads were first pre-processed using cutadapt (Martin, 2011): reads were quality-trimmed (option -q 20), trimmed of adapter sequences, excluding any read pairs in which either read was shorter than 28 bp after trimming (option -m 28). Read pairs were mapped to the sacCer3 S. cerevisiae reference genome using Bowtie 2 (Langmead and Salzberg, 2012) with the –very-sensitive parameter set, requiring the reads in each pair to be within 2000 bp of each other (option -X 2000). Read pairs in which both reads had a mapping quality score of at least 30 were deduplicated using samtools rmdup. Replicates were merged prior to calling peaks and generating fold enrichment tracks using MACS2 (https://github.com/taoliu/MACS) (Zhang et al., 2008). Fold enrichment tracks were visualized using the UCSC Genome Browser (Karolchik et al., 2003).

RNA sequencing

Yeast strains were grown overnight in YPD in biological triplicate (independent colonies, or for newly generated transformants, independent transformants), pelleted and then stored at −80°C. RNA was purified using acid phenol extraction, and then treated with Turbo DNase and purified with a Qiagen RNeasy Mini kit (Qiagen, Hilden, Germany). Illumina libraries were prepared from 800 to 900 ng of total RNA, using the Illumina Truseq RNA Library Prep kit v2 (for TF binding site mutants) or the Illumina Truseq Stranded mRNA Library Prep kit (for TF knockouts). Libraries were sequenced to ~11–13 million read pairs per sample using 2 × 37 bp reads on an Illumina NextSeq 500.

RNA-seq analysis

Reads were preprocessed and mapped as with the ChIP-seq libraries, but with the -X 500 option for Bowtie 2. Read pairs in which both reads had a mapping quality score of at least 30 were overlapped with annotated genes using HTSeq (Anders et al., 2015). Global fold-change analyses were using DESeq2 (Love et al., 2014).
RT-qPCR

Yeast were grown as described above in Yeast strains and culture, and then pelleted and stored at −80°C. RNA was purified using acid phenol extraction, and then treated with Turbo DNase and purified with a Qiagen RNeasy Mini kit. For each sample, 1 ug of total RNA was annealed to oligo(dT)_{20} (13 μl reaction with 1 μl of 50 μM oligo(dT), 1 μl 10 mM dNTP mix) by incubating at 65°C for 5 min and then on ice for 1 min. Reverse transcription was performed with SuperScript IV by adding 4 μl of 5x SuperScript IV buffer, 1 μl SUPERase In RNase inhibitor, 1 μl 100 mM DTT, and 1 μl of SuperScript IV enzyme, and then incubating at 50°C for 10 min and then 80°C for 10 min. For qPCRs, 2.5 μl of each reverse transcription reaction was used for each 25 μl PCR, using KAPA Robust 2G HotStart ReadyMix with standard cycling conditions (except annealed at 55°C) for 40 cycles on a Bio-Rad C1000 Touch thermal cycler with a CFX96 Real-Time System. Cq values were calculated using the Bio-Rad CFX Manager 3.1 software, using the regression mode for Cq calculation and baseline subtracted curve fitting. Primer efficiencies were calculated using a 5-fold dilution series of genomic DNA from *S. cerevisiae* BY4741 starting from 10 ng. Primers were specific to *S. cerevisiae* (at least two substitutions to *S. uvarum*). See Supplementary file 1 for primer sequences.

Rgt1 protein annotations

Predicted intrinsic disorder was calculated using IUPred2 long disorder ([Mészáros et al., 2018](#)). Predicted secondary structure was calculated using Jpred4 ([Drozdetskiy et al., 2015](#)) separately on the first 400 amino acids and on the remaining 770 amino acids, as submissions are capped at 800 amino acids.

Motif analysis

Systematic scans of motifs were performed using YeTFaSCo ([de Boer and Hughes, 2012](#)) with the expert-curated no dubious motif set. Sequence logos were generated using ggseqlogo ([Wagih, 2017](#)).

Motif cluster analysis

Motif clusters were identified using MCAST ([Grant et al., 2016](#)) with a motif p-value threshold of 0.001, a maximum gap threshold of 200 bp, and an E-value threshold of 2, using the high-confidence motifs for Leu3 (#781), Rgt1 (#2227), and Sdd4 (#588) from YeTFaSCo ([de Boer and Hughes, 2012](#)). Individual motif occurrences for each TF were scored for the *S. cerevisiae* (version R64.2.1) and *S. uvarum* genomes (as revised in *Kim et al., 2017*) using FIMO ([Grant et al., 2011](#)) with a p-value threshold of 0.001 and the option —max-strand. Motif clusters were named based on the nearest downstream gene (on - strand if coordinate of gene < coordinate of motif cluster, and on + strand if coordinate of gene > coordinate of motif cluster). Motif clusters were defined to be homologous if they were upstream of homologous genes.

De novo motif discovery

De novo motif discovery was performed using MEME version 4.12.0 ([Bailey et al., 2006](#)). For all analyses, 100 bp centered at each ChIP-seq peak or tRNA gene was used. Motifs were allowed to be between 6 bp and either 10 bp or 20 bp, and the top three motifs were analyzed, with otherwise default settings.

Hi-C

Hi-C was performed and analyzed as in *Kim et al. (2017)* using the restriction enzyme Sau3AI.

Code availability

Code used to analyze data and generate figures are available at [https://github.com/shendurelab/MAP-C](https://github.com/shendurelab/MAP-C) (*Kim, 2019*; copy archived at [https://github.com/elifesciences-publications/MAP-C](https://github.com/elifesciences-publications/MAP-C)).

Data availability

All sequencing data have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE118118. Hi-C data from *Figure 3* and *Figure 3—figure supplements 2, 3 and 4* and RNA-seq data from *Figure 4* are from GEO accession number GSE88952. Processed microarray
data of gene expression in TF deletions under exponential growth from Figure 5—figure supplements 1 and 2 are from GEO accession number GSE4654 (Hu et al., 2007).

Acknowledgements
We thank K Xue, W Noble, and members of the Shendure and Dunham labs, particularly V Agarwal, J Tome, and S Domcke, for comments and discussion; I Liachko for help with Hi-C experiments; N Hanson for help with yeast deletion collections; and Y Zheng, M Sanchez, and RS McIsaac for strains.

Additional information

Funding

| Funders                        | Grant reference number | Author       |
|-------------------------------|------------------------|--------------|
| National Institutes of Health | U54 DK107979           | Jay Shendure |
| National Science Foundation   | Grad Research Fellowship DGE-1256082 | Seungsoo Kim |
| Howard Hughes Medical Institute | Investigator            | Jay Shendure |
| Canadian Institute for Advanced Research | Senior Fellow (Genetic Networks Program) | Maitreya J Dunham |

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions
Seungsoo Kim, Conceptualization, Investigation, Visualization, Methodology, Writing—original draft, Writing—review and editing; Maitreya J Dunham, Jay Shendure, Supervision, Writing—review and editing

Author ORCIDs
Seungsoo Kim [https://orcid.org/0000-0002-5559-5289](https://orcid.org/0000-0002-5559-5289)
Maitreya J Dunham [http://orcid.org/0000-0001-9944-2666](http://orcid.org/0000-0001-9944-2666)
Jay Shendure [https://orcid.org/0000-0002-1516-1865](https://orcid.org/0000-0002-1516-1865)

Decision letter and Author response
Decision letter [https://doi.org/10.7554/eLife.42499.037](https://doi.org/10.7554/eLife.42499.037)
Author response [https://doi.org/10.7554/eLife.42499.038](https://doi.org/10.7554/eLife.42499.038)

Additional files

Supplementary files
- Supplementary file 1. Oligonucleotide sequences used in this study.
  DOI: [https://doi.org/10.7554/eLife.42499.025](https://doi.org/10.7554/eLife.42499.025)
- Supplementary file 2. MAP-C libraries.
  DOI: [https://doi.org/10.7554/eLife.42499.026](https://doi.org/10.7554/eLife.42499.026)
- Supplementary file 3. 96-well yeast transformation protocol.
  DOI: [https://doi.org/10.7554/eLife.42499.027](https://doi.org/10.7554/eLife.42499.027)
- Supplementary file 4. Key resources table.
  DOI: [https://doi.org/10.7554/eLife.42499.028](https://doi.org/10.7554/eLife.42499.028)
- Transparent reporting form
  DOI: [https://doi.org/10.7554/eLife.42499.029](https://doi.org/10.7554/eLife.42499.029)

Data availability
All sequencing data have been deposited in GEO under accession code GSE118118.
The following dataset was generated:

| Author(s)          | Year | Dataset title                                                                 | Dataset URL                                      | Database and Identifier     |
|--------------------|------|-------------------------------------------------------------------------------|-------------------------------------------------|------------------------------|
| Kim S, Dunham MJ,  | 2018 | A combination of transcription factors mediates inducible interchromosomal contacts | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118118 | NCBI Gene Expression Omnibus, GSE118118 |
| Shendure J         |      |                                                                               |                                                 |                              |

The following previously published datasets were used:

| Author(s)          | Year | Dataset title                                                                 | Dataset URL                                      | Database and Identifier     |
|--------------------|------|-------------------------------------------------------------------------------|-------------------------------------------------|------------------------------|
| Kim S, Liachko I,  | 2017 | The dynamic three-dimensional organization of the diploid yeast genome        | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8952 | NCBI Gene Expression Omnibus, GSE8952 |
| Brickner DG, Cook K, Noble WS, Brickner JH, Shendure J, Dunham MJ |      |                                                                               |                                                 |                              |
| Hu Z, Killion PJ,  | 2007 | Genetic reconstruction of a functional transcriptional regulatory network      | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4654 | NCBI Gene Expression Omnibus, GSE4654 |
| Iyer VR            |      |                                                                               |                                                 |                              |

References

Alipour E, Marko JF. 2012. Self-organization of domain structures by DNA-loop-extruding enzymes. Nucleic Acids Research 40:11202–11212. DOI: https://doi.org/10.1093/nar/gks925, PMID: 23074191

Anders S, Pyl PT, Huber W. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169. DOI: https://doi.org/10.1093/bioinformatics/btu638, PMID: 25260700

Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R. 1998. Perinuclear localization of chromatin facilitates transcriptional silencing. Nature 394:592–595. DOI: https://doi.org/10.1038/29100, PMID: 9707122

Badis G, Chan ET, van Bakel H, Pena-Castillo L, Tillo D, Tsui K, Carlson CD, Gossett AJ, Hasinoff MJ, Warren CL, Gebbia M, Talukder S, Yang A, Mnaimeh S, Terterov D, Coburn D, Li Yeo A, Yeo ZX, Clarke ND, Lieb JD, et al. 2008. A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. Molecular Cell 32:878–887. DOI: https://doi.org/10.1016/j.molcel.2008.11.020, PMID: 19111667

Bailey TL, Williams N, Misleh C, Li WW, Ww L. 2006. MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Research 34:W369–W373. DOI: https://doi.org/10.1093/nar/gkl198, PMID: 16845028

Boehning M, Dugast-Darzacq C, Rankovic M, Hansen AS, Yu T, Marie-Nelly H, McSwiggen DT, Kocik G, Dailey GM, Cramer P, Darzacq X, Zweckstetter M. 2018. RNA polymerase II clustering through carboxy-terminal domain phase separation. Nature Structural & Molecular Biology 25:833–840. DOI: https://doi.org/10.1038/s41594-018-0112-y, PMID: 30127355

Boija A, Klein IA, Sabari BR, D’Alagnerse A, Coffey EL, Zamudio AV, Li CH, Shriniwas K, Manteiga JC, Hantinn NM, Abraham BJ, Afeyan LK, Guo YE, Rimel JK, Fant CB, Schuijers J, Lee TI, Taatjes DJ, Young RA. 2018. Transcription factors activate genes through the Phase-Separation capacity of their activation domains. Cell 175:1842–1855. DOI: https://doi.org/10.1016/j.cell.2018.10.042, PMID: 30449618

Bonev B, Mendelson Cohen N, Szabo Q, Fritsch L, Papadopoulos GL, Lubling Y, Xu X, Lv X, Hugnot JP, Tanay A, Cavalli G. 2017. Multiscale 3D genome rewiring during mouse neural development. Cell 171:557–572. DOI: https://doi.org/10.1016/j.cell.2017.09.043, PMID: 29053968

Bonev B, Cavalli G. 2016. Organization and function of the 3D genome. Nature Reviews Genetics 17:661–678. DOI: https://doi.org/10.1038/nrg.2016.112, PMID: 27739532

Brickner DG, Ahmed S, Meldi L, Thompson A, Light W, Young M, Hickman TL, Chu F, Fabre E, Brickner JH. 2012. Transcription factor binding to a DNA zip code controls interchromosomal clustering at the nuclear periphery. Developmental Cell 22:1234–1246. DOI: https://doi.org/10.1016/j.devcel.2012.03.012, PMID: 22579222

Brickner DG, Coukos R, Brickner JH. 2015. INO1 transcriptional memory leads to DNA zip code-dependent interchromosomal clustering. Microbial Cell 2:481–490. DOI: https://doi.org/10.15698/mic2015.12.242, PMID: 26688804

Brickner DG, Sood V, Tutucci E, Coukos R, Viets K, Singer RH, Brickner JH. 2016. Subnuclear positioning and interchromosomal clustering of the GAL1-10 locus are controlled by separable, interdependent mechanisms. Molecular Biology of the Cell 27:2980–2993. DOI: https://doi.org/10.1091/mbc.E16-03-0174, PMID: 27889341

Burgess SM, Kleckner N, Weiner BM. 1999. Somatic pairing of homologs in budding yeast: existence and modulation. Genetics & Development 13:1627–1641. DOI: https://doi.org/10.1101/gad.13.12.1627, PMID: 10385630

Cho WK, Spillie JH, Hecht M, Lee C, Li C, Grube V, Cisse II. 2018. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science 361:412–415. DOI: https://doi.org/10.1126/science.aa4199, PMID: 29930094
Chong S, Dugast-Darzacq C, Liu Z, Dong P, Dailey GM, Cattoglio C, Heckert A, Banala S, Lavis L, Darzacq X, Tjian R. 2018. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. Science 361:eaar2555. DOI: https://doi.org/10.1126/science.aar2555, PMID: 29930090

Chowdhary S, Kainth AS, Gross DS. 2017. Heat shock protein genes undergo dynamic alteration in their Three-Dimensional structure and genome organization in response to thermal stress. Molecular and Cellular Biology 37: DOI: https://doi.org/10.1128/MCB.00292-17, PMID: 28970373

Chowdhary S, Kainth AS, Pincus D, Gross DS. 2019. Heat shock factor 1 drives intergenic association of its target gene loci upon heat shock. Cell Reports 26:18–28. DOI: https://doi.org/10.1016/j.celrep.2018.12.034, PMID: 30605674

Cremer T, Cremer M. 2010. Chromosome territories. Cold Spring Harbor Perspectives in Biology 2:a003889. DOI: https://doi.org/10.1101/cshperspect.a003889, PMID: 20300217

D’Ippolito AM, McDowell IC, Barrera A, Hong LK, Leichter SM, Bartelt LC, Vockley CM, Majors WH, Saﬁ A, Song L, Gersbach CA, Crawford GE, Reddy TE. 2018. Pre-established chromatin interactions mediate the genomic response to glucocorticoids. Cell Systems 7:146–160. DOI: https://doi.org/10.1016/j.cels.2018.06.007, PMID: 30031775

de Boer CG, Hughes TR. 2012. YeTFaSCo: a database of evaluated yeast transcription factor sequence specificities. Nucleic Acids Research 40:D169–D179. DOI: https://doi.org/10.1093/nar/gkr993, PMID: 22102575

de Wit E, de Laat W. 2012. A decade of 3C technologies: insights into nuclear organization. Genes & Development 26:11–24. DOI: https://doi.org/10.1101/gad.179804.111, PMID: 22215806

Dekker J, Rippe K, Dekker M, Kleckner M. 2002. Capturing chromosome conformation. Science 295:1306–1311. DOI: https://doi.org/10.1126/science.1067799, PMID: 11847345

Deng W, Lee J, Wang H, Miller J, Reik A, Gregory PD, Dean A, Blobel GA. 2012. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell 149:1233–1244. DOI: https://doi.org/10.1016/j.cell.2012.03.051, PMID: 22682246

Deng W, Rupon JW, Krivega I, Breda L, Motta I, Jahn KS, Reik A, Gregory PD, Rivella S, Blobel GA. 2014. Reactivation of developmentally silenced globin genes by forced chromatin looping. Cell 158:849–860. DOI: https://doi.org/10.1016/j.cell.2014.05.050, PMID: 25126789

Derkatch IL, Song L, Gersbach CA, Crawford GE, Reddy TE. 2018. Pre-established chromatin interactions mediate the genomic response to glucocorticoids. Cell Systems 7:146–160. DOI: https://doi.org/10.1016/j.cels.2018.06.007, PMID: 30031775

Donohoe ME, Silva SS, Pinter SF, Xu N, Lee JT. 2009. The pluripotency factor Oct4 interacts with ctfc and also controls X-chromosome pairing and counting. Nature 460:128–132. DOI: https://doi.org/10.1038/nature08098, PMID: 19536159

Drozdetskiy A, Cole C, Procter J, Barton GJ. 2015. JPredd4: a protein secondary structure prediction server. Nucleic Acids Research 43:W389–W394. DOI: https://doi.org/10.1093/nar/gkv332, PMID: 25883141

Duan Z, Andronescu M, Schutz K, Mclwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS. 2010. A three-dimensional model of the yeast genome. Nature 465:363–367. DOI: https://doi.org/10.1038/nature08973, PMID: 20436457

Fowler DM, Fields S. 2014. Deep mutational scanning: a new style of protein science. Nature Methods 11:801–807. DOI: https://doi.org/10.1038/nmeth.3027, PMID: 25075907

Gasperini M, Starita L, Shendure J. 2016. The power of multiplexed functional analysis of genetic variants. Nature Protocols 11:1782–1787. DOI: https://doi.org/10.1038/nprot.2016.135, PMID: 27583640

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O’Shea EK, Weissman JS. 2003. Global analysis of protein expression in yeast. Nature 425:737–741. DOI: https://doi.org/10.1038/nature02046, PMID: 14562106

Gladyshev E, Kleckner N. 2017. DNA sequence homology induces cytosine-to-thymine mutation by a heterochromatin-related pathway in Neurospora. Nature Genetics 49:887–894. DOI: https://doi.org/10.1038/ng.3857, PMID: 28459455

Grant CE, Bailey TL, Noble WS. 2011. FIMO: scanning for occurrences of a given motif. Bioinformatics 27:1017–1018. DOI: https://doi.org/10.1093/bioinformatics/btr064, PMID: 21330290

Grant CE, Johnson J, Bailey TL, Noble WS. 2016. MCAST: scanning for cis-regulatory motif clusters. Bioinformatics 32:1217–1219. DOI: https://doi.org/10.1093/bioinformatics/btw750, PMID: 26704599

Guertin MJ, Lis JT. 2010. Chromatin landscape dictates HSF binding to target DNA elements. PLOS Genetics 6:e1001114. DOI: https://doi.org/10.1371/journal.pgen.1001114, PMID: 20844575

Guo Y, Monahan K, Wu H, Gertz J, Varley KE, Li W, Myers RM, Maniatis T, Wu Q. 2012. CTCF/cohesin-mediated DNA looping is required for protocadherin α promoter choice. PNAS 109:21081–21086. DOI: https://doi.org/10.1073/pnas.1219280110, PMID: 23204437

Henikoff S, Dreesen TD. 1989. Trans-inactivation of the Drosophila brown gene: evidence for transcriptional repression and somatic pairing dependence. PNAS 86:6704–6708. DOI: https://doi.org/10.1073/pnas.86.17.6704, PMID: 25052576

Hu Z, Killian PJ, Iyer VR. 2007. Genetic reconstruction of a functional transcriptional regulatory network. Nature Genetics 39:683–687. DOI: https://doi.org/10.1038/ng2012, PMID: 17417638
Iyer V, Struhl K. 1995. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. The EMBO Journal 14:2570–2579. DOI: https://doi.org/10.1002/j.1460-2075.1995.tb07255.x, PMID: 7781610

Jolma A, Yin Y, Nitta KR, Dave K, Popov A, Taipale M, Enge M, Kivoja T, Morgunova E, Taipale J. 2015. DNA-dependent formation of transcription factor pairs alters their binding specificity. Nature 527:384–388. DOI: https://doi.org/10.1038/nature15518, PMID: 26550823

Joyce EF, Williams BR, Xie T, Wu CT, -ting Wu C. 2012. Identification of genes that promote or antagonize somatic homolog pairing using a high-throughput FISH-based screen. PLOS Genetics 8:e1002667. DOI: https://doi.org/10.1371/journal.pgen.1002667, PMID: 2258973

Joyce EF, Ercig J, Wu CT, C-t W. 2016. Pairing and anti-pairing: a balancing act in the diploid genome. Current Opinion in Genetics & Development 37:119–128. DOI: https://doi.org/10.1016/j.gde.2016.03.002, PMID: 27065367

Kaps S, Kettner K, Migotti R, Kanashova T, Krause U, Rödel G, Dittmar G, Kriegel TM. 2015. Protein kinase Ymr291w/Tda1 is essential for glucose signaling in saccharomyces cerevisiae on the level of hexokinase isoenzyme SchHxk2 phosphorylation*. Journal of Biological Chemistry 290:6243–6255. DOI: https://doi.org/10.1074/jbc.M114.595074, PMID: 25953311

Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, Roskin KM, Schwartz M, Sugnet CW, Thomas DJ, Weber RJ, Haussler D, Kent WJ, University of California Santa Cruz. 2003. The UCSC Genome Browser Database. Nucleic Acids Research 31:51–54. DOI: https://doi.org/10.1093/nar/gkq129, PMID: 12519945

Kettner K, Krause U, Mosler S, Bodenstein C, Kriegel TM, Rödel G. 2012. Saccharomyces cerevisiae gene YMR291W/TDA1 mediates the in vivo phosphorylation of hexokinase isoform 2 at serine-15. FEBS Letters 586:455–458. DOI: https://doi.org/10.1016/j.febslet.2012.01.030, PMID: 22289182

Kim JH, Polish J, Johnston M. 2003. Specificity and regulation of DNA binding by the yeast glucose transporter gene repressor Rgt1. Molecular and Cellular Biology 23:5208–5216. DOI: https://doi.org/10.1128/MCB.23.15.5208-5216.2003, PMID: 12861007

Kim S, Liachko I, Brickner DG, Cook K, Noble WS, Brickner JH, Shendure J, Dunham MJ. 2017. The dynamic three-dimensional organization of the diploid yeast genome. eLife 6:e23623. DOI: https://doi.org/10.7554/eLife.23623, PMID: 28537556

Kim YH, Marhon SA, Zhang Y, Steger DJ, Won KJ, Lazar MA. 2018. Rev-erbα dynamically modulates chromatin looping to control circadian gene transcription. Science 359:1274–1277. DOI: https://doi.org/10.1126/science.aao6891, PMID: 29439026

Kim S. 2019. MAP-C. bcea752. GitHub. https://github.com/shendurelab/MAP-C

Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR, Weirauch MT. 2018. The human transcription factors. Cell 172:650–665. DOI: https://doi.org/10.1016/j.cell.2018.01.029, PMID: 29425488

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357–359. DOI: https://doi.org/10.1038/nmeth.1923, PMID: 22388286

Larson AG, Elnatan D, Keeney MM, Trnka MJ, Johnston JB, Burlingame AL, Agard DA, Redding S, Narlikar GJ. 2017. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. Nature 547:236–240. DOI: https://doi.org/10.1038/nature22822, PMID: 28636604

Le DD, Shimko TC, Aditham AK, Keys AM, Longwell SA, Orenstein Y, Fordyce PM. 2018. Comprehensive, high-resolution binding energy landscapes reveal context dependencies of transcription factor binding. PNAS 115:E3702–E3711. DOI: https://doi.org/10.1073/pnas.1715888115, PMID: 29588420

Levo M, Zalckvar E, Sharon E, Dantas Machado AC, Kalma Y, Lotam-Pompan M, Weinberger A, Yakhini Z, Roos R, Segal E. 2015. Unraveling determinants of transcription factor binding outside the core binding site. Genome Research 25:1018–1029. DOI: https://doi.org/10.1101/gr.185033.114, PMID: 25762553

Liu X, Lee CK, Granek JA, Clarke ND, Lieb JD. 2006. Whole-genome comparison of Leu3 binding in vitro and in vivo reveals the importance of nucleosome occupancy in target site selection. Genome Research 16:1517–1528. DOI: https://doi.org/10.1101/gr.1655606, PMID: 17053089

Lomvardas S, Barnea G, Pisapia DJ, Mendelsohn M, Kirkland J, Axel R. 2006. Interchromosomal interactions and olfactory receptor choice. Cell 126:403–413. DOI: https://doi.org/10.1016/j.cell.2006.06.035, PMID: 16873069

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550. DOI: https://doi.org/10.1186/s13059-014-0550-8, PMID: 25516281

Maass PG, Barutcu AR, Weiner CL, Rinn JL. 2018. Inter-chromosomal Contact Properties in Live-Cell Imaging and in Hi-C. Molecular Cell 70:178–189. DOI: https://doi.org/10.1016/j.molcel.2018.03.021, PMID: 29625035

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12. DOI: https://doi.org/10.1806/ej.17.1.200

McIsaac RS, Gibney PA, Chandran SS, Benjamin KR, Botstein D. 2014. Synthetic biology tools for programming gene expression without nutritional perturbations in saccharomyces cerevisiae. Nucleic Acids Research 42:e48. DOI: https://doi.org/10.1093/nar/gkt1402, PMID: 24445804

Mézsáros B, Erdos G, Dосztányi Z. 2018. IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding. Nucleic Acids Research 46:W329–W337. DOI: https://doi.org/10.1093/nar/gky384, PMID: 29860432

Michaëlis C, Ciosk R, Nasmyth K. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91:35–45. DOI: https://doi.org/10.1016/S0092-8674(01)80007-6, PMID: 9335333

Mirkin EV, Chang FS, Kleckner N. 2013. Dynamic trans interactions in yeast chromosomes. PLOS ONE 8:e75895. DOI: https://doi.org/10.1371/journal.pone.0075895, PMID: 24098740
Kim et al. eLife 2019;8:e42499. DOI: https://doi.org/10.7554/eLife.42499

Chromosomes and Gene Expression | Genetics and Genomics

Mirkin EV, Chang FS, Kleckner N. 2014. Protein-mediated chromosome pairing of repetitive arrays. Journal of Molecular Biology 426:550–557. DOI: https://doi.org/10.1016/j.jmb.2013.11.001, PMID: 24211468

Mitchell JA, Fraser P. 2008. Transcription factories are nuclear subcompartments that remain in the absence of transcription. Genes & Development 22:20–25. DOI: https://doi.org/10.1101/gad.454008, PMID: 18172162

Monahan K, Horta A, Lomvardas S. 2019. LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice. Nature 555:448–453. DOI: https://doi.org/10.1038/s41586-018-0845-z, PMID: 30626972

Morris JR, Geyer PK, Wu CT, Ct W. 1999. Core promoter elements can regulate transcription on a separate chromosome in trans. Genes & Development 13:253–258. DOI: https://doi.org/10.1101/gad.13.3.253, PMID: 9990850

Muller H, Scolari VF, Agier N, Piazza A, Thierry A, Mercy G, Descors-Declere S, Lazar-Stefanita L, Espeli O, Llorente B, Fischer G, Mozzinacci J, Kozul R. 2018. Characterizing meiotic chromosomes’ structure and pairing using a designer sequence optimized for Hi-C. Molecular Systems Biology 14:e8293. DOI: https://doi.org/10.15252/msb.201818829, PMID: 30012718

Nolis IK, McKay DJ, Mantouvalou E, Lomvardas S, Merika M, Thanos D. 2009. Transcription factors mediate long-range enhancer-promoter interactions. PNAS 106:20222–20227. DOI: https://doi.org/10.1073/pnas.0902454106, PMID: 19923429

Nora EP, Goloborodko A, Walton AL, Gibcus JH, Uebersohn A, Abdennur N, Dekker J, Mirny LA, Bruneau BG. 2017. Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell 169:930–944. DOI: https://doi.org/10.1016/j.cell.2017.05.004, PMID: 28525758

Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, Mitchell JA, Lopes S, Reik W, Fraser P. 2004. Activated genes dynamically colocalize to shared sites of ongoing transcription. Nature Genetics 36:1065–1071. DOI: https://doi.org/10.1038/ng1423, PMID: 15361872

Pan X, Yuan DS, Xiang D, Wang X, Sookhai-Mahadeo S, Bader JS, Hieter P, Spencer F, Boeke JD. 2004. A robust toolkit for functional profiling of the yeast genome. Molecular Cell 16:487–496. DOI: https://doi.org/10.1016/j.molcel.2004.09.035, PMID: 15525520

Patwardhan RP, Lee C, Litvin O, Young DL, Pe’er D, Shendure J. 2009. High-resolution analysis of DNA regulatory elements by synthetic saturation mutagenesis. Nature Biotechnology 27:1173–1175. DOI: https://doi.org/10.1037/nbt.1589, PMID: 19915551

Payen C, Sunshine AB, Ong GT, Pogachar JL, Zhao W, Dunham MJ. 2016. High-Throughput identification of adaptive mutations in experimentally evolved yeast populations. PLOS Genetics 12:e1006339. DOI: https://doi.org/10.1371/journal.pgen.1006339, PMID: 27727276

Polish JA, Kim JH, Johnston M. 2005. How the Rgt1 transcription factor of Saccharomyces cerevisiae is regulated by glucose. Genetics 169:583–594. DOI: https://doi.org/10.1534/genetics.104.035412, PMID: 15489524

Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26:841–842. DOI: https://doi.org/10.1093/bioinformatics/btp033, PMID: 20110278

Quinodoz SA, Olkikainen N, Tabak B, Palla A, Schmidt JM, Detmar E, Lai MM, Shishkin AA, Bhat P, Takei Y, Trinh V, Azaoureyan E, Russell P, Cheng C, Jovanovic M, Chow A, Cai L, McDonel P, Garber M, Gutmann M. 2018. Higher-Order Inter-chromosomal hubs shape 3D genome organization in the nucleus. Cell 174:744–757. DOI: https://doi.org/10.1016/j.cell.2018.05.024, PMID: 29887377

Randise-Hinchliff C, Brickner JH. 2016. Transcription factors dynamically control the spatial organization of the yeast genome. Nucleus 7:369–374. DOI: https://doi.org/10.1080/19491034.2016.1212797, PMID: 27442220

Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, Aiden EL. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159:1665–1680. DOI: https://doi.org/10.1016/j.cell.2014.10.021, PMID: 25497547

Rowley MJ, Corces VG. 2018. Organizational principles of 3D genome architecture. Nature Reviews Genetics 19:789–800. DOI: https://doi.org/10.1038/s41576-018-0060-8, PMID: 30367165

Roy A, Shin YJ, Cho KH, Kim JH. 2013. Mth1 regulates the interaction between the Rgt1 repressor and the Ssn6-Tup1 corepressor complex by modulating PKA-dependent phosphorylation of Rgt1. Molecular Biology of the Cell 24:1493–1503. DOI: https://doi.org/10.1091/mbc.e13-01-0047, PMID: 23468525

Sanborn AL, Rao SS, Huang SC, Durand NC, Huntley MH, Jewett AL, Bochkov ID, Chinnappan D, Cutkosky A, Li J, Geeting KP, Gnarke A, Melnikov A, McKenna D, Stamenova EK, Lander ES, Aiden EL. 2015. Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. PNAS 112:E6456–E6465. DOI: https://doi.org/10.1073/pnas.1518552112, PMID: 26499245

Schmitt AD, Hu M, Jung I, Xu Z, Qiu Y, Tan CL, Li Y, Lin S, Lin Y, Barr CL, Ren B. 2016a. A compendium of chromatin contact maps reveals spatially active regions in the human genome. Cell Reports 17:2042–2059. DOI: https://doi.org/10.1016/j.celrep.2016.10.061, PMID: 27851967

Schmitt AD, Hu M, Ren B. 2016b. Genome-wide mapping and analysis of chromosome architecture. Nature Reviews Molecular Cell Biology 17:743–755. DOI: https://doi.org/10.1038/nrm.2016.104, PMID: 27580841

Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, Kurukuti S, Mitchell JA, Umlauf D, Dimitrova DS, Eskiw CH, Luo Y, Wei CL, Ruan Y, Bieker JJ, Fraser P. 2010. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. Nature Genetics 42:53–61. DOI: https://doi.org/10.1038/ng.496, PMID: 20010836

Schwarzer W, Abdennur N, Goloborodko A, Pekowska A, Fudenberg G, Loie-Mye Y, Fonseca NA, Huber W, Haering C, Mirny L, Spitze P. 2017. Two independent models of chromatin organization revealed by cohesin removal. Nature 551:51–56. DOI: https://doi.org/10.1038/nature24281, PMID: 29094699

Shalem O, Sanjana NE, Zhang F. 2015. High-throughput functional genomics using CRISPR-Cas9. Nature Reviews Genetics 16:299–311. DOI: https://doi.org/10.1038/nrg3899, PMID: 25854182
Chromosomes and Gene Expression

Kim et al. eLife 2019;8:e42499. DOI: https://doi.org/10.7554/eLife.42499