Liquid chromatin Hi-C characterizes compartment-dependent chromatin interaction dynamics

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Nuclear compartmentalization of active and inactive chromatin is thought to occur through microphase separation mediated by interactions between loci of similar type. The nature and dynamics of these interactions are not known. We developed liquid chromatin Hi-C to map the stability of associations between loci. Before fixation and Hi-C, chromosomes are fragmented, which removes strong polymeric constraint, enabling detection of intrinsic locus–locus interaction stabilities. Compartmentalization is stable when fragments are larger than 10–25 kb. Fragmentation of chromatin into pieces smaller than 6 kb leads to gradual loss of genome organization. Lamin-associated domains are most stable, whereas interactions for speckle- and polycomb-associated loci are more dynamic. Cohesin-mediated loops dissolve after fragmentation. Liquid chromatin Hi-C provides a genome-wide view of chromosome interaction dynamics.

Genomic and imaging approaches now produce high-resolution descriptions of the conformation of chromosomes in cell populations, in single cells, across the cell cycle, and during development1–10. Hi-C interaction maps display a ‘plaid’ pattern, which reflects the segregation of the genome in two major spatial compartments, A and B, that correspond to active chromatin and silent chromatin, respectively11–17. Compartments can be split into 5 subtypes (A1, A2, B1, B2 and B3) that differ in interaction patterns and chromatin state7. At the scale of tens to hundreds of kb, topologically associating domains (TADs) are separated by CTCF-bound boundaries. Higher resolution Hi-C, ChIA-PET (chromatin interaction analysis by paired-end tag sequencing)18 and 4C (circularized chromosome conformation capture) data19–21 have shown that convergent CTCF sites can engage in looping interactions.

Key questions remain unanswered regarding the molecular and biophysical processes that drive chromosome compartmentation. Compartmentalization has been proposed to be the result of phase separation driven by attraction between chromatin domains of similar status12–15. Polymer models simulating such attractions can reproduce the plaid pattern of chromatin interaction maps16–18.

Hi-C interaction maps do not reveal the biophysical nature of the interactions that drive compartment formation or the dynamic mobility of loci within them. Live cell imaging studies have shown that loci are constrained in their motion and that there is variation in the dynamics and mobility of loci; for example, between euchromatic and heterochromatic loci, and between loci tethered to the nuclear periphery and loci located in the nuclear interior17–41. However, imaging-based studies are limited in scale, and analysis of whole-genome dynamics by microscopy (for example, see ref. 42) has not allowed for determination of the positions of specific sequences. Therefore, new approaches are required to identify and quantify the molecular processes and biophysical forces involved in chromatin interactions and nuclear compartmentalization. Here, we describe liquid chromatin Hi-C, a Hi-C variant that quantifies stability of chromatin interactions genome-wide.

Results

Measuring stability of chromatin interactions and nuclear compartmentalization. The formation of spatially segregated heterochromatic and euchromatic domains can be viewed as microphase separation of a ‘block copolymer’. A block copolymer is a polymer that contains a series of alternating blocks (for example, A-type and B-type, or blocks of euchromatin and heterochromatin), each composed of multiple monomers (A monomers and B monomers; Fig. 1a). When As attract As, and Bs attract Bs, such polymers can fold into spatially segregated domains of As and Bs (Fig. 1a)46–48. Applied to chromatin, microphase separation may underlie the formation of segregated compartments.

Whether microphase separation of a block copolymer occurs depends on interaction strengths between monomers as well as the lengths of the blocks of each type (Fig. 1a). Flory–Huggins polymer theory predicts that spatial segregation will occur when the product of the length of the blocks (N, the number of monomers that make up blocks) and their effective preferential homotypic interaction strength (χ, a parameter that represents the difference in the strength of homotypic interactions, compared to heterotypic (A–B) interactions) is larger than a critical value C (refs. 46–48) (Supplementary Information). Large blocks of a polymer...
can spatially segregate even when attractive interactions among monomers are weak, while short blocks will only phase separate when interactions are sufficiently strong. This dependence suggests an experimental approach to quantify the strength of interactions that drive chromosomal compartmentalization (Fig. 1a,b). It is possible to start with a genome in the compartmentalized state and fragment the chromosomes by digestion to identify conditions in which fragments become so short that their interaction strength is not sufficient to maintain a phase-separated state. As a result, compartments will disassemble over time and chromosomal fragments of different types will become mixed; that is, chromatin becomes liquid-like. The kinetics of this process can then be assessed by Hi-C at different times after chromatin fragmentation (Fig. 1a,b). Here, we describe such a strategy, which we call liquid chromatin Hi-C.

Chromosome conformation in isolated nuclei. To facilitate fragmentation of chromosomes, we isolated nuclei from K562 cells. We performed four analyses to demonstrate that chromosome conformation in isolated nuclei was the same as that in cells (Methods and Supplementary Methods). First, imaging showed intact lamin A rings at the nuclear periphery (Fig. 2a). Second, using 3C (chromosome conformation capture) (ref. 49) we detected known loop–ring structures at the nuclear periphery (Fig. 2a). Next, imaging with lamin A staining, and large buds of apparently liquid chromatin (not surrounded by lamin A) protruding from the nuclear periphery (Fig. 2a, arrow). After spinning down nuclei, we detected no DNA in the supernatant, indicating that liquified chromatin remains largely within the nuclear envelope.

We next tested whether different chromatin fragmentation levels had an effect on nuclear stiffness. Nuclei were isolated from K562 cells, attached to two micropipettes at opposite ends, and the nucleus was extended by moving an extension micropipette (Methods and Supplementary Methods). The deflection of a force micropipette provides a measure of the force (Fig. 2c and Supplementary Videos 1 and 2). These data provide a force versus extension plot (Fig. 2d, left) in which the slope of the line fitted to the data is the nuclear spring constant in nN μm−1 (Fig. 2d, right). Extension between 0% and 30% strain measures the chromatin-dominated regime of the nuclear force response56–58. Isolated single nuclei were measured for their native spring constant before treatment. Stiffness can vary between individual nuclei. We then measured the stiffness of the same nuclei 60 min post treatment. Control nuclei (for which only restriction buffer was added to media) showed a slight stiffening of the nucleus (Fig. 2d). Treatment of nuclei with HindIII did not decrease their stiffness. By contrast, DpnII-treated nuclei displayed a large decrease (~75%) in stiffness, consistent with previous experiments56. We conclude that chromosome and nuclear organization can tolerate fragmentation to ~10–25 kb segments. By contrast, fragmenting the genome to <6-kb segments results in loss of chromatin-mediated stiffness.

Fig. 1 | Approach for measuring chromatin interaction stability. a, Block copolymer composed of a series of alternating A and B blocks, each composed of a number of monomers (left). The polymer can fold into spatially segregated domains of As and Bs (middle). Flory–Huggins polymer theory predicts that spatial segregation will occur when the product of the length of the blocks, N (the number of monomers that make up blocks), and their effective preferential homotypic interaction strength, χ (difference in the strength of homotypic interactions relative to heterotypic (A–B) interactions), is larger than a critical value. C, b, Workflow to determine the stability of chromatin interactions genome-wide. DNA is shown in black. Various chromatin features or proteins maintaining DNA conformation are shown as gray ovals. Dashed arrows indicate that samples were taken at different points for analysis.
Fig. 2 | Extensive fragmentation of chromatin leads to liquefied chromatin. a, Nuclear and chromatin morphology before and after chromatin fragmentation. Top row, control nuclei in restriction buffer; middle row, nuclei digested for 4 h with HindIII; bottom row, nuclei digested for 4 h with DpnII. Nuclei were stained with DAPI (left column), with antibodies against lamin A (middle column). The right column shows the overlay of the DAPI and lamin A stained images. HindIII digestion did not lead to major alteration in nuclear morphology and chromatin appearance, while DpnII digestion led to the appearance of DAPI stained droplets (arrow) budding from the nuclei. Representative images are shown, experiment repeated twice, with a dozen nuclei images per experiment. b, Top, DNA purified from undigested nuclei, and nuclei pre-digested with DpnII and HindIII run on a fragment analyzer. Bottom, cumulative DNA length distributions calculated from the fragment analyzer data. Representative data are shown for 1 out of 2 replicates. c, Micromanipulation of single nuclei. Isolated nuclei were attached to two micropipettes at opposite ends. Nuclei were extended by moving the right micropipette (extension micropipette) and the force required was calculated from the deflection of the calibrated ‘force’ (left) pipette. Blue and orange lines indicate the position of the force pipette before and after extension for control nuclei. After digestion of nuclei with DpnII (bottom), extension required less force as indicated by the much smaller deflection of the force pipette than for control nuclei (see also Supplementary Videos 1 and 2). d, Force-extension plots (left) for control nuclei before and 60 min after incubation in restriction buffer (pre and post control), for nuclei before and after digestion with DpnII, and for nuclei before and after HindIII digestion. Right panel, relative change in nuclear spring constants, calculated from the slopes of the force-extension plots shown on the left. Error bars indicate s.e.m. (n = 5 DpnII pre-digested nuclei, and n = 4 HindIII pre-digested nuclei). Bars show the average of all nuclei, dots indicate values obtained with individual nuclei.
Compartmental segregation requires chromatin fragments larger than 6 kb. We applied conventional Hi-C before and after chromatin liquefication (liquid chromatin Hi-C). All Hi-C datasets and mapping statistics are summarized in Supplementary Table 3. Nuclei were digested with HindIII or DpnII for 4 h followed by formaldehyde fixation and Hi-C (Extended Data Fig. 2a). Liquid chromatin Hi-C interaction maps obtained from nuclei that were pre-digested with HindIII were remarkably similar to those obtained with control nuclei (Fig. 3a). The relationship between interaction frequency and genomic distance, and the ratio of intra- to interchromosomal interactions were largely unaffected (Fig. 3b). Compartment positions (PC1) were unaffected (Spearman $\rho = 0.99$).

Chromosome compartment strength can be quantified by plotting interaction frequencies between pairs of 40-kb loci arranged by their PC1 to obtain compartmentalization saddle plots (Fig. 3c). In nuclei pre-digested with HindIII, the strength of preferential A–A and B–B interactions (the ratio of the frequency of A–A and B–B interactions divided by the frequency of A–B interactions) was similar to that in untreated nuclei (Fig. 3c; see Extended Data Fig. 2c for a replicate).

Extensive changes were observed when nuclei were pre-digested for 4 h with DpnII (Fig. 3a) followed by formaldehyde fixation and Hi-C. We observed a loss of short range (<10 Mb) intrachromosomal interactions, and a gain of longer range (>10 Mb) interactions and interchromosomal interactions (Fig. 3b). The gain in interchromosomal interactions appeared to be the result of random mixing of As and Bs from different chromosomes as the preference for interchromosomal A–A and B–B interactions decreased (Extended Data Fig. 2e). Moreover, compartment strength in cis was greatly reduced with a greater relative reduction evident in the A compartment (Fig. 3c).

Quantification of chromosome conformation dissolution. Loss of chromosome conformation and dissolution of chromosomal compartments will result in random mixing of previously spatially separated loci both in cis and in trans. In Hi-C this appears as a redistribution of contacts from short-range interactions towards longer range and interchromosomal interactions. We developed a metric that represents the percentage change in short-range intrachromosomal interactions (in a 6-Mb window centered on each 40-kb bin), and the concomitant increase in long-range and interchromosomal interactions after fragmentation relative to control nuclei, which we call 'loss of structure' (LOS) (Extended Data Fig. 2b).

We first calculated LOS after 4 h for chromatin fragmented with HindIII. In general, short-range interactions were only slightly reduced (less than 5% (Fig. 3b). When LOS was plotted along chromosomes (Fig. 3d), LOS was very weakly negatively correlated with PC1 (Fig. 3d,e, left panel).

We performed the same analysis for nuclei pre-digested with DpnII for 4 h. We found that fragmenting chromatin to <6-kb fragments led to extensive loss of chromatin interactions, with LOS of >80%. LOS varied along chromosomes and was strongly positively correlated with PC1, with loci in the A compartment displaying the largest loss (Fig. 3d,e).

Corrections for differential fragmentation. One explanation for the greater effect of fragmentation on chromatin interactions in the A compartment could be that DpnII cuts more frequently in the A compartment, producing smaller fragments. We performed two experiments to assess differential fragmentation. First, we determined the cutting frequency of DpnII in isolated nuclei across the genome by sequencing the ends of the DNA fragments (DpnII-seq; Extended Data Fig. 3, Methods and Supplementary Methods). Second, we directly determined the average fragment size along the genome by purifying and sequencing DNA of different sizes after pre-digestion with DpnII, and using the data to calculate for each 40-kb bin the average fragment size (Methods). The average fragment size for most bins ranged from 2.7 to 3.7 kb and was on average slightly smaller for A compartments than for B compartments (3.1 and 3.2 kb, respectively). Cutting frequency and average fragment size were both correlated with PC1 and with LOS (Fig. 3d,f, left panel, and Extended Data Figs. 2d,4).

Next, we corrected LOS for the differential efficiency of DpnII digestion by calculating the partial correlation between LOS and PC1 after correcting for the correlations of PC1 and LOS with DpnII digestion frequency (Methods). We found that the residuals of PC1 and LOS remained correlated (Spearman $\rho = 0.38$ for chromosome 2; Fig. 3g). Similarly, when we corrected LOS for differences in average fragment size we found that the residuals of LOS remained highly correlated with residuals of PC1 (Spearman $\rho = 0.83$ for chromosome 2; Extended Data Fig. 4). To illustrate the correlation between LOS and PC1 independent of fragmentation level directly, we selected a set of loci along chromosome 2 that are all cut to the same extent (1,000–1,100 reads in the DpnII-seq dataset). When we plotted LOS against PC1 for this set we found a strong correlation (Fig. 3f, right panel, Spearman $\rho = 0.46$). Finally, we repeated the entire liquid chromatin Hi-C procedure using a different restriction enzyme, Fatl, which has a different pattern of digestion across the genome as compared to DpnII but produces fragments that are similarly small (Extended Data Fig. 5). We calculated LOS and corrected for differential Fatl digestion along the genome using Fatl-seq, exactly as above for DpnII. A high correlation between residuals of LOS and...
PC1 was again observed (Spearman $\rho = 0.64$ for chromosome 2; Extended Data Fig. 5). We conclude that LOS is correlated with compartment status, and that this result is robust for different enzymes and different methods for correcting for digestion efficiency.

Dissociation kinetics of chromatin interactions and compartments. The loss of conformation after DpnII pre-digestion allowed us to measure the dissociation kinetics of compartments and stability of chromatin interactions. We first determined the kinetics of chromatin fragmentation (Extended Data Figs. 6a,7a). We digested nuclei with DpnII for a minimum of 5 min and up to 16h. After 5 min, the size range of fragments was between 3 and 15 kb (80% of fragments; Fig. 4a). After 1 h, 80% of DNA fragments were smaller than 7 kb and after 16 h 85% of fragments were smaller than 3.5 kb. We sequenced DNA ends to determine the distribution of DpnII cuts across the genome (Fig. 4b). At all time points
the number of DpnII cuts per 40-kb bin was correlated with PC1 (Fig. 4b), but the pattern did not change over time (Fig. 4b, correlation matrix; Extended Data Fig. 6a).

Micromanipulation was again used to measure the nuclear spring constant corresponding to nuclear stiffness. Nuclei displayed a significant loss in stiffness within 5 min. Loss of stiffness leveled off at 30 min of digestion and showed a 60% decrease in nuclear rigidity, similar to previous experiments in other cell types (Fig. 4c). Together, these analyses show that the bulk of DNA fragmentation and chromatin liquefaction occurs within the first hour.

Next, we performed liquid chromatin Hi-C in which nuclei were pre-digested with DpnII for a minimum of 5 min and up to 16 h (Extended Data Fig. 7a). After 5 min of pre-digestion, chromosome conformation and compartmentalization were intact, even though chromatin was fragmented to 3–15 kb segments before fixation and nuclear stiffness was significantly reduced (Fig. 4c,d). The percentage of intrachromosomal interactions increased, particularly for loci separated by <1 Mb (Fig. 4e).

At subsequent time points, when most chromatin fragments were <7 kb long we observed fewer intrachromosomal interactions and a concomitant increase in interchromosomal interactions genome-wide (Fig. 4d,e). Compartmentalization was progressively lost (Fig. 4d, lower row of heatmaps, and Extended Data Fig. 7b). A–A interactions disappeared faster than B–B interactions. After 16 h, only a low level of preferential B–B interactions remained.

Quantification of the half-life of chromosome conformation across the genome. To quantify the kinetics of loss of chromosome conformation and compartmentalization, we calculated LOS genome-wide for each time point (Fig. 4f). At $t=5$ min, LOS was negative indicating a ~25% gain of intrachromosomal interactions, consistent with the initial increase in overall intrachromosomal interactions described above (Fig. 4e). LOS was inversely correlated with PC1, indicating that loci located within A compartments gain more intrachromosomal interactions than loci located within B compartments (Spearman $\rho = -0.53$ for chromosome 2; Spearman $\rho = -0.49$ genome-wide). A block copolymer model predicts that partial DNA digestion can lead to a strengthening of compartmentalization by removing covalent linkages between A and B blocks, as long as the fragments are still large enough so that attractive forces between them are sufficient for phase segregation (Supplementary Note). At subsequent time points, LOS was increasingly positive as intrachromosomal interactions were progressively lost and interchromosomal interactions were gained. LOS was the highest for loci located in the A compartment. At $t=16$ h, LOS was as high as 90%, intrachromosomal interactions were low (<20% of total), and only preferential B–B interactions were still observed in the Hi-C interaction map (Fig. 4d). Very similar results were obtained with an independent replicate time course experiment (see below).

Next, for each 40-kb locus, we determined the time at which LOS reached 50% of its maximal value at $t=16$ h. We refer to this as the half-life, $t_{1/2}$ (min), of chromatin interactions at each locus (Fig. 4f and Extended Data Fig. 7c). When $t_{1/2}$ was examined along chromosomes, we found a strong inverse correlation with PC1 (Spearman $\rho = -0.87$; Extended Data Fig. 7f): interactions in the A compartment dissolve relatively fast ($t_{1/2} = 40–80$ min) and interactions in the B compartment dissolve slower ($t_{1/2} = 60–120$ min; Extended Data Fig. 7d). We also calculated $t_{1/2}$ genome-wide for the second independent time course experiment and found a strong correlation between $t_{1/2}$ calculated from the two datasets (Spearman $\rho = 0.78$ for chromosome 2; Spearman $\rho = 0.76$ genome-wide; Extended Data Fig. 7e). The value of $t_{1/2}$ is proportional to a dissociation rate constant and thus independent of the initial level of intrachromosomal interactions for a given locus. $t_{1/2}$ remained highly correlated with PC1 after correction for the initial level of intrachromosomal interactions for each bin (Spearman $\rho = -0.82$; Extended Data Fig. 7g).

Similar to LOS, $t_{1/2}$ correlated with DpnII digestion frequency at all time points (Extended Data Fig. 4f,6a). We calculated the partial correlation between $t_{1/2}$ and PC1 after correcting for correlations between PC1 and $t_{1/2}$ with DpnII cutting frequency. We found that $t_{1/2}$ and PC1 remained strongly correlated (Fig. 4f), regardless of which DpnII fragmentation dataset (genome-wide Spearman $\rho$ ranging from $-0.41$ to $-0.60$, $t=5$ min to 16 h) was used for the calculation of the partial correlation (Extended Data Fig. 6a,b). Although loci in the A compartment were often cut more frequently than loci in the B compartment, when loci cut with similar frequency were compared, loci in the A compartments had shorter half-lives (Extended Data Fig. 6c). Similar results were obtained when $t_{1/2}$ was corrected for the average fragment size for each bin (Extended Data Fig. 4i, Spearman $\rho = -0.85$ for chromosome 2, Spearman $\rho = -0.76$ genome-wide).

We considered whether we could have overestimated the $t_{1/2}$ for the B compartment because fragmentation of these loci could be slower than for loci in the A compartment. We reasoned that because after 1 h DpnII digestion is largely complete, calculation of LOS using the Hi-C data at $t=1$ h as the starting condition would provide an estimate of dissolution kinetics starting at a time point when A and B compartments are both extensively fragmented. We found that LOS, and $t_{1/2}$ calculated in this way were still strongly correlated with PC1, and this correlation remained strong after correction for fragmentation level (Extended Data Fig. 6d,e,f).

Dissociation kinetics of chromatin interactions at different sub-nuclear structures. The A1 sub-compartment is enriched in active histone modifications and found near nuclear speckles. B2 and B3 are located near the lamina and the nucleolus (B2) and the nucleolus (B3). B1 is enriched in H3K27me3 and polycomb proteins. To relate sub-compartment status to chromatin dissociation rates, we compared...
the residuals of $t_{1/2}$ for loci located in the five sub-compartments defined for K562 cells\(^6\) (Fig. 5a). We found that residual $t_{1/2}$ varied among sub-compartments: $t_{1/2}^{(A1)} < t_{1/2}^{(B1)} < t_{1/2}^{(A2)} < t_{1/2}^{(B2)} < t_{1/2}^{(B3)}$.

We split $t_{1/2}$ residuals into ten intervals and then explored the enrichment for varying chromatin features for each $t_{1/2}$ residual interval (Fig. 5b and Supplementary Table 4). Chromatin interactions for early replicating domains had short half-lives, whereas
interactions for loci in later replicating domains were more stable (Fig. 5b). Loci near the speckle-associated proteins pS3C5 or SON were engaged in the most unstable interactions. Similarly, transcriptionally active loci, containing H3K4me3 and RNA polymerase II, were also involved in relatively unstable chromatin interactions.

Interactions for loci bound by polycomb complexes were as unstable as active speckle-associated loci (Fig. 5b and Extended Data Fig. 8b). Half-lives differed for loci bound by different polycomb subunits. Loci with the shortest $t_{1/2}$ residual values were enriched for binding the CBX8 subunit. An example of a large polycomb-bound domain in K562 cells is the HoxD cluster. The half-life of chromatin interactions for loci in the HoxD cluster was relatively short (Extended Data Fig. 8c).

Silent and closed chromatin loci around the nucleolus or at the nuclear lamina were engaged in the most stable interactions (Fig. 5b). Chromatin interactions for loci associated with HP1$\gamma$ (CBX3) were relatively unstable, whereas interactions for loci associated with HP1$\beta$ (CBX1) or HP1$\alpha$ (CBX5) were more stable. This variation is in agreement with the chromosomal locations and dynamics of these three HP1 proteins. HP1$\gamma$ is associated with active chromatin and mobile, whereas HP1$\alpha$ and HP1$\beta$ are typically found in constitutive heterochromatin near pericentromeres and are much less mobile.

For each sub-compartment, we split loci into expressed (FPKM $\geq 1$) or not expressed (FPKM < 1) categories (Fig. 5c). We found that sub-compartment status is the major determinant of chromatin interaction stability, with transcriptional status having no or only very minor effects.

The differential stability of pairwise chromatin interactions at different sub-nuclear structures can be quantified by plotting interaction frequencies between pairs of 40-kb loci arranged by their level of factor binding to obtain homotypic interaction saddle plots (Fig. 5d). After chromatin fragmentation we observed loss of preferential interactions between speckle-associated loci, while preferential interactions between non-speckle-associated loci were observed even after 16 h. Conversely, preferential interactions between lamin-associated loci remained detectable even at late time points, whereas interactions between loci not at the lamina disappeared relatively fast.

**Chromatin loops dissociate upon chromatin fragmentation.** We aggregated Hi-C data at pairs of sites that had previously been shown to engage in looping interactions in K562 cells. We readily detected these loops in intact purified nuclei (Fig. 6a). After fragmentation with HindIII for 4 h, loops appeared to become slightly stronger. Fragmenting chromatin with DpnII resulted in loss of loops over time.

We assessed whether CTCF and cohesin binding to chromatin is affected by chromatin fragmentation. We fractionated proteins in chromatin-bound and soluble fractions (Methods). In intact nuclei, most of the CTCF and cohesin is associated with chromatin (Fig. 6b,c). Digesting chromatin with HindIII did not lead to dissociation of CTCF or cohesin. However, fragmenting chromatin with DpnII led to dissociation of cohesin after 1 h, while CTCF binding was only weakly affected. We conclude that DNA fragmentation to <6-kb fragments, but not to 10–25-kb fragments, leads to loss of cohesin binding and loss of looping interactions. These results are consistent with earlier observations in yeast that stable chromatin binding by cohesin requires intact DNA. These data can be interpreted in the context of the model in which cohesin rings encircle DNA pseudotopologically. It is possible that, when DNA is fragmented, the cohesin ring can slide off nearby free ends.

**Discussion**

Using liquid chromat Hi-C, we gained insight into the dynamics of chromatin interactions throughout the nucleus and the genome (Fig. 7a). Previously, live cell imaging experiments found differences in mobility dependent on sub-nuclear position and chromatin state and activity. A previous study by Gartenberg et al., which inspired the current work, aimed to identify factors that determine intrinsic locus–locus interactions and locus mobility by removing the polymeric constraint due to linkage. In that work, a silent locus was excised from the chromosome and its mobility and preference for association with other silent loci and the nuclear periphery was found to depend on specific silencing complexes. In our liquid chromat Hi-C experiments, the polymeric constraint on movement was removed for all loci simultaneously, resulting in a genome-wide variant of the experiments by Gartenberg et al.

Chromosomal compartmentalization tolerates genome-wide fragmentation with HindIII in >10–25-kb fragments. Micromechanical measurements also show that chromosomes remain mechanically fully connected. We conclude that stable chromosome conformation and phase segregation can occur when blocks of a particular chromatin state are at least 10 kb. The results that we obtained using DpnII digestion, with fragments <6 kb in size (average ~3.1 kb), show that these fragments are too short to maintain phase-segregated domains. The stability of interactions between <6-kb fragments depends on their chromatin state and association with sub-nuclear structures: interactions at the nuclear lamina are relatively stable, those near nuclear speckles and polycomb complexes are highly unstable, while interactions for loci associated with different heterochromatin proteins and the nucleolus displayed a range of intermediate stabilities. The dynamics of associations between loci are therefore determined by chromatin-related factors, and may also be determined directly by the biochemical properties of histone tail modifications. For instance, the Rosen laboratory found that chromatin fragments can form droplets in vitro and that the dynamics of chromatin fragments within these droplets are dependent upon both H1 binding and histone acetylation.46
Liquid chromatin Hi-C identified differences in chromatin interaction stability between facultative heterochromatic domains marked by polycomb and constitutive heterochromatic domains marked by lamina association or binding of HP1α/HP1β proteins. Although many chromatin contacts in constitutive heterochromatin were maintained even after 16 h of digestion, the half-life for chromatin contacts at polycomb-bound regions was shorter. The compacted states of polycomb and HP1α-bound chromatin appear to
form via a similar phase-separation mechanism. CBX2 (polycomb subunit) and CBX5 (HP1α) are capable of forming condensates. Our data indicate that these different condensates and associated chromatin have very different properties: the stability of interactions between loci mediated by these factors is distinct, possibly related to differences in affinity between CBX proteins and chromatin: the binding affinity of CBX5 for H3K9me3 is higher than the affinity of CBX2 for H3K27me3.

Our results allow a crude estimate of the Flory–Huggins χ parameter for A/B segregation of chromatin. Given that HindIII and...
DpnII cut chromatin into segments of approximately 17 ± 7 kb and 3 ± 1 kb, respectively, a reasonable estimate of the minimum length of fragments necessary to drive A/B segregation is \( N = 10 \pm 4 \) kb. For homopolymers, the Flory–Huggins model predicts a critical length needed for phase separation of \( N = \frac{2\gamma}{c} \) (ref. 41), indicating \( \gamma = 0.20 \pm 0.07 \) per kb (\( \gamma = 0.036 \pm 0.013 \) per nucleosome). More details and the assumptions underlying this calculation are given in the Supplementary Note.

It is important to note that during the liquid chromatin Hi-C procedure, some chromatin factors and RNAs may dissociate from the purified nuclei, and this could affect the locus-mixing behavior that we observe. The current work analyzed the intrinsic chromatin interaction strengths and dissolution kinetics of chromosome conformation within inactive nuclei. Future work may focus on how these kinetic properties change in cells or nuclei, where active processes such as transcription, replication, chromatin compaction and condensation, and loop extrusion are also acting, and on determining the roles of RNAs, proteins and histone modifications in modulating the attractive forces between loci and the dynamics of genome folding in general.

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Methods

K562 nuclei purification. Three sucrose cushions were made before starting nuclei purification: 30% of sucrose (10 mM PIPES pH 7.4, 10 mM KCl, 2 mM MgCl₂, pH adjusted to 7.4 using 1 N KOH, 30% sucrose, 1 mM DTT (dithiothreitol; added prior to use) and 1:100 protease inhibitor (Thermo Fisher 784383; added prior to use)) was transferred to a 50-ml tube, and then 5 ml of 10% sucrose (10 mM PIPES pH 7.4, 10 mM KCl, 2 mM MgCl₂, 10% sucrose, 1 mM DTT (added prior to use) and 1:100 protease inhibitor (added prior to use)) was slowly loaded on top of the 30% sucrose, and the tubes were incubated at 4 °C until needed. K562 cell pellets (100 million cells) were lysed using the following nuclear isolation procedure. After the cells were spun, the pellets were washed twice with 10 ml HBSS (Hanks’ balanced salt solution), then pelleted after each wash at 300 r.p.m. for 10 min at 4 °C. Cell pellets were dissolved in 15 ml nuclear isolation buffer (10 mM PIPES pH 7.4, 10 mM KCl, 2 mM MgCl₂, 1 mM DTT (added prior to use), 1:100 protease inhibitor (added prior to use), pH adjusted to 7.4 using 1 M KOH). Then, cells were lysed on ice in a 15-ml Dounce homogenizer with pestle A (KIMBLE Kontes 885002-0015) by moving the pestle slowly up and down 20 times, followed by incubation on ice for 20 min and another 20 strokes. Next, each 5 ml of lysed extract was loaded slowly on top of a sucrose cushion prepared earlier. Then the tubes were spun for 15 min at 800 g at 4 °C. The supernatant was removed carefully for a good recovery of the nuclei pellet in the bottom of the tube. Nuclei pellets were resuspended in 1 ml of HBSS, then spun for 5 min at 5,000 g at 4 °C using a benchtop refrigerated centrifuge. Then, the nuclei pellet was resuspended in 3 ml HBSS, and 1 µl was taken to quantify the nuclei before the 3 ml was split over two microtube tubes and spun for 5 min at 5,000 g at 4 °C using a benchtop refrigerated centrifuge. Finally, the nuclear lysates were dissolved into an equal absolute total volume to obtain 1 million nuclei per 0.1 ml of nuclear storage buffer (NSB) (10 mM PIPES pH 7.4, 10 mM KCl, 2 mM MgCl₂, 50% glycerol, 8.5% sucrose, 1 mM DTT (added prior to use), 1:100 protease inhibitor (added prior to use)). Each 0.5 ml of NSB containing 5 million nuclei was transferred to a microtube and stored at −80 °C.

3C. The 3C method was performed as described in ref. 1 (for details see Supplementary Methods). The 3C primers are listed in Supplementary Table 1. BAC library for 3C PCR. BAC (bacterial artificial chromosome) DNA was generated as described previously11. A control ligation library covering the beta-globin locus (ENCODE region EN0n09) was generated using Bacs overlapping the region. Starting with a mixture of DNA of seven BACs (CTC-775N15, RP11-715G8, CTD-3048C22, CTD-2643I7, CTD-3234J1 and RP11-589G14) (Invitrogen), mixed in equimolar ratios, we used the same steps described in the 3C protocol above, starting from the digestion step. BAC clones (ig010905, NEB2.1) were digested with EcoRI, then randomly ligated and the DNA was purified. The BAC ligation library reflects random ligation of EcoRI fragments throughout the beta-globin locus, so any difference in PCR signal for 3C primer pairs along the beta-globin locus due to differences in primer efficiency can be corrected by normalizing the amount of PCR product obtained with the 3C library to the amount obtained with the BAC ligation library.

3C. Experimental design. Probes were designed as described previously14, and 213 3C probes were designed for a ~1 Mb region (chr1:1370996-5729937; hg18) around the beta-globin locus at EcoRI restriction sites using publicly available 3C primer design tools12. Probes were designed according to a single alternating scheme exactly as described before14 and the genomic uniqueness of all primers was verified with the SSAHA algorithm. For each EcoRI fragment at the 1-Mb target region a primer was designed; 104 5′ forward (FOR) and 109 5′ reverse (REV) primers were designed. The 3C primers are listed in Supplementary Table 2.

Generation of 3C libraries. The 3C libraries were generated as described previously14, with three modifications. First, we skipped the gel purification after the pre-digestion procedure described above. The pre-digested nuclei were then treated with proteinase K. DNA was purified and DNA ends were filled in with a mixture of dNTPs (deoxynucleotide triphosphates) containing biotin-dATP. Biotin containing DNA was then purified using streptavidin coated beads, and prepared for Illumina paired-end sequencing (see Supplementary Methods for protocol details).

Fragment size determination after pre-digestion with DpnII. Four million cells were pre-digested for 4 h using the DpnII procedure described above and in the Supplementary Methods (Pre-digestion of nuclei). Nuclei were then treated with protease, DNA was isolated and size fractionated on an agarose gel. DNA was isolated from the gel in three size ranges: smaller than 1 kb, 1–3 kb and larger than 3 kb. DNA in each fraction was then sheared and prepared for Illumina paired-end sequencing (see Supplementary Methods for protocol details).

DpnII-seq and FatI-seq. For each DpnII-Seq library, 10 million nuclei were used immediately after the pre-digestion procedure described above. The pre-digested nuclei were then treated with proteinase K. DNA was purified and DNA ends were filled in with a mixture of dNTPs (deoxynucleotide triphosphates) containing biotin-dATP. Biotin containing DNA was then purified using streptavidin coated beads, and prepared for Illumina paired-end sequencing (see Supplementary Methods for protocol details).

Lamin A immunofluorescence and DAPI. For nuclei immunofluorescence, we prepared a coverslip by adding 1 ml of 0.1% poly-l-lysine solution (Sigma, SIGMAALDRICH, P3897) to 1 ml of 1% gelatin. According to a standard protocol, each coverslip was transferred to a single well of an eight-well plate. The coverslips were washed twice using PBS. Next, 500 µl of 30% sucrose with 1 mM DTT was added on top of the coverslips to protect nuclei from an abrupt contact with coverslip during spinning. One million control nuclei or nuclei after chromatin digestion were crosslinked for 20 min using a 4% final concentration of paraformaldehyde immediately after pre-digestion. Next, nuclei were added slowly on top of the sucrose solutions on the coverslips and spun for 15 min at 2,500 g at 4 °C. Nuclei were assumed to be attached to the coverslips, which were then transferred to a new eight-well plate. The coverslips were washed five times with PBS. Then, non-specific binding of the primary antibody was blocked by adding 500 µl of the blocking buffer (3% BSA, 1x PBS, 0.1% Triton X-100 (Sigma 9002-91-1) and incubating for 60 min at room temperature (20 °C). Subsequently, lamin A antibody (ab26300) was diluted 1:1,000 in blocking buffer, and the coverslip was incubated face-down on top of a 250 µl of lamin A antibody droplet that was placed on parafilm for 120 min at room temperature. The coverslip was then placed back in the well of a new plate face-up and washed five times with washing buffer (1x PBS, 0.1% Triton X-100). The secondary antibody goat anti-rabbit IgG (ab150077) was diluted 1:1,000 in blocking buffer, and the coverslip was incubated face-down on top of a 250 µl droplet of the secondary antibody (goat anti-rabbit IgG (ab150077)) that was placed on parafilm for 60 min at room temperature. Next, the coverslip was placed back in the well of a new plate face-up and washed five times with washing buffer (1x PBS, 0.1% Triton X-100) and twice with 3x PBS. The slide was mounted and sealed using 10 µl of paraformaldehyde mountant with DAPI (Invitrogen, P36931).

For image acquisition, we used a Nikon Eclipse Ti microscope. Imaging was performed using an Apo TIRF, N.A. 1.49, ×60 oil immersion objective (Nikon).
Chromatin fractionation assay. Chromatin-bound proteins were isolated and separated from free proteins. A sample of 2 million control nuclei or pre-digested nuclei (obtained as described above) was centrifuged at 5,000g for 5 min at 4°C. The supernatant was transferred to an Amicon column to reduce the volume from 500 µl to 100 µl by centrifugation for 4 min at 14,000g. This sample contained the free protein fraction. Next, 26 µl of glycerol and 1.3 µl of 100X protease inhibitor cocktail were added to the 100 µl free protein sample. The pellet containing the nuclei was resuspended in 100 µl of nuclei purification buffer with Triton (10 mM PIPES pH 7.4, 10 mM KCl, 2 mM MgCl2, 0.25% Triton, 1% protease inhibitor, 1 mM DTT) and incubated for 10 min on ice. Then, to protect protein structure during sonication, 25 µl of glycerol was added to the 100-µl pellet sample to give a 20% final glycerol concentration. The sample was sonicated using a Covaris instrument at 4°C with the following settings: duty cycle 10%, intensity 5, cycles per burst 200, set mode frequency sweeping, continuous degassing, process time 60s, 4 cycles. The pellet sample containing chromatin-bound proteins was transferred to a 1.5-ml tube. All samples were stored at −20°C. These samples contain the protein-bound CTCF and cohesin. Note that when these samples were centrifuged after the Triton solubilization, we found that no SMC3 or CTCF could be detected in the supernatant. These results indicate that non-chromatin-bound proteins exit the nuclei and were recovered in the supernatant prior to the Triton solubilization step.

For analysis of CTCF and SMC1 chromatin binding, 15 µl from each protein sample (supernatant or pellet) was mixed with 5 µl of 5× Lane Marker Reducing Sample Buffer (Thermo Fisher 39000), then the mix was boiled for 10 min. The samples were cooled to room temperature before loading on 3–8% Tris-Acetate Protein Gels (Invitrogen EA0375PK2). Next, the gel was run in 1× Tris-Acetate SDS Running Buffer (Invitrogen LA0041) for 75 min at 150 V. For histone H3, 1 µl of protein sample was mixed with 14 µl of PBS containing 1% protease inhibitor, 5 µl of 5× Lane Marker Reducing Sample Buffer was added to the mix and boiled for 10 min. The samples were cooled to room temperature before loading in Tris-Base 4–12% (Invitrogen NP0322BOX), and then the gel was run in 1× MES–SDS running buffer (Invitrogen B0002) for 60 min at 150 V. The proteins were transferred from the gel to a nitrocellulose membrane using 1× western blot transfer buffer (Thermo Scientific 35040). The transfer was 120 min for SMC1 and CTCF, and 75 min for histone H3. The nitrocellulose membranes were washed using 1× TBST (50 mM Tris-CL pH 7.6; 150 mM NaCl, 0.1 ml of Tween 20), then blocked for 120 min using 5% milk (1:1,000 CTCF antibody cell signaling (Active Motif 61311), 1:2,000 SMC1 (Bethyl Antibody, A300-055A), 1:4,000 histone H3 Abcam (ab1791)). Next, the membranes were washed six times for 10 min per wash using 1× TBST. The secondary antibody anti-rabbit IgG HRP from cell signaling was diluted using 5% milk for CTCF and SMC1 (1:10,000 for CTCF, 1:2,000 SMC1) and 1% milk for histone H3 (1:5,000). Membranes were incubated for 120 min at room temperature. Finally, membranes were washed six times for 10 min using 1× TBST. The membranes were developed using luminol-based enhanced chemiluminescence (Thermo Scientific 34076).

Micromanipulation force measurements and treatments of isolated nuclei. Micromanipulation force measurements were conducted as described previously by Stephens et al. K562 cells were grown in microscope slide wells and treated with 1 µg ml−1 latrunculin A (Enzo Life Sciences) for ~45 min before single nucleus isolation. The nucleus was isolated by using small amounts of detergent (0.65% Triton X-100 in PBS) locally sprayed onto a living cell via an ‘isolation’ micropipette. This gentle lysis allows the use of a second micropipette to retrieve the nucleus from the cell, using slight aspiration and non-specific adherence to the inside of the micropipette. A third micropipette was then attached to the opposite end of the nucleus in a similar fashion. This last ‘force’ micropipette was pre-calibrated for its deflection spring constant, which is on the order of 2 nN m−1. A custom computer program written in LabView was then run to move the ‘pull’ micropipette and track the position of both the pull and force pipettes. The pull pipette was instructed to move 5 µm at 45 nm s−1. The program then tracked the distance between the pipettes to provide a measure of nucleus extension ~3 µm. The distance that the force pipette moved (or deflected) multiplied by the pre-measured spring constant provides a calculation of force exerted. Calculations were performed in Excel (Microsoft) to produce a force-extension plot from which the best-fit slope of the line would provide a spring constant of the nucleus (nN m−1). Isolated nuclei were measured twice initially to establish the native spring constant prior to treatment. After 50 µl of buffer only (control), 100 units DpnII (I.GACTC) with NEB buffer 3.1, or 100 units HindIII (AAGCTT) with NEB buffer 2.1 was added to the 1.5-ml imaging well and mixed gently. Force measurements were performed 5 min, 30 min, and 60 min post treatment. Example movies of micromanipulation experiments can be found in Supplementary Videos 1 and 2.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All sequencing data have been submitted to a public data repository (GEO, accession number GSE134590). Data are available through the 4D Nucleome data portal. Source data are provided with this paper.

Code availability
All code for data processing and analysis, described in detail in the Methods, is available through the following GitHub accounts:
https://github.com/tborman/liquid-chromatin-Hi-C
https://github.com/tborman/DpnII-seq
https://github.com/dekkerlab/5C-CBFb-SMMHC-Inhib
https://github.com/dekkerlab/CMapping
https://github.com/dekkerlab/world-dekker
https://github.com/hms-dbmi/hic-data-analysis-bootcamp
https://github.com/mirnylab/cooltools/tree/master/cooltools

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Author contributions
J.D. conceived the study. H.B. performed all 3C, 5C, Hi-C and liquid chromatin Hi-C and chromatin fractionation experiments. D.L.L. performed restriction digestion efficiency (DpnII-seq) experiments. A.D.S. performed micromechanical studies and analyzed the data. T.B. and H.B. analyzed data. S.V. contributed analysis tools for liquid chromatin: capturing snapshots of genome organization with 5C technology. Methods 58, 255–267 (2012).

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Chromosome conformation in isolated nuclei. **a**, Hi-C 2.0 intra-chromosomal interaction maps for K562 cells (top) and purified nuclei (bottom). **b**, 5C interaction map of 1Mb region surrounding the beta-globin locus in K562 cells. Top: cells. Bottom: purified nuclei. CTCF-mediated interactions are preserved in purified nuclei. Red circles: positions of CTCF sites; purple square Beta-globin locus control region (LCR). **c**, Representative 3C-PCR (out of two experiments) for a 44,120 kb region surrounding the beta-globin LCR on chromosome 11, detects at high resolution the known looping interactions between the LCR and the expressed gamma-globin genes (HBE1, HBG2) in K562 cells. Looping interactions are not detected in GM12878 cells that do not express these genes. Top: cells. Bottom: purified nuclei. Each data point is the average of 3 PCR reactions; error bars indicate standard error of the mean. **d**, Compartmentalization saddle plots: average intra-chromosomal interaction frequencies between 100 kb bins, normalized by genomic distance. Bins are sorted by their PC1 value derived from Hi-C data obtained with K562 cells. In these plots preferential B-B interactions are in the upper left corner, and preferential A-A interactions are in the lower right corner. Numbers in the corners represent the strength of AA interactions as compared to AB interactions and BB interactions over BA interactions. Left: cells. Right: purified nuclei. **e**, Spearman correlation of PC1 in cells vs PC1 in nuclei for chromosome 2 at 100 kb resolution ($\rho = 0.99$).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Chromosome conformation dissolution upon chromatin fragmentation. a, Workflow for Liquid chromatin Hi-C. b, Illustration of loss of structure metric using a pre-digested sample and a control. c, Hi-C interaction maps and compartmentalization saddle plots for a second replicate of control nuclei (incubated for 4 hours in restriction buffer) and nuclei pre-digested with HindIII for 4 hours. d, Left: Spearman correlation of DpnII restriction digestion efficiency (DpnII-seq) and PC1 for chromosome 2 at 40 kb resolution. Right: Partial correlation of LOS (LOS residuals) with PC1 after controlling for restriction efficiency (DpnII-seq), for chromosome 2 at 40 kb resolution. Spearman correlation is indicated. e, compartmentalization saddle plots for the corresponding conditions. Numbers indicate strength of A-A and B-B interactions for inter-chromosomal interactions.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Experimental protocol and computational workflow for DpnII-seq. a, Schematic of DpnII-seq experimental protocol for recovering DNA fragments digested by the restriction enzyme DpnII. b, Directed graph of DpnII-seq computational pipeline. c, Histogram of distance to nearest DpnII recognition site for each recovered DpnII digested fragment. d, Raw DpnII-seq signal displaying multiple copy number states (2N, 3N, 4N) within chromosome 3 (data binned at 40 kb). e, Copy number corrected DpnII-seq signal displaying single copy number state (2N) across chromosome 3.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Average fragment size per bin and correlation with chromatin stability. **a**, DNA purified from nuclei pre-digested with DpnII for 4 hours were separated into slices of three sizes and run on a Fragment Analyzer. One experiment was performed. **b**, Fragment Analyzer distributions of DNA fragment sizes for the three separated slices (RFU: relative fluorescence unit, LM: lower marker, fragment sizes at distribution peaks are given in blue). **c**, Top plot: Eigenvector 1 values (PC1, 40 kb resolution) across a section of chromosome 2, representing A (red) and B (blue) compartments. Bottom three plots: Normalized coverage of fragments from given slice size across a section of chromosome 2. **d**, Percentages of fragments mapped to each subcompartment for given slice size. **e**, Top plot: LOS along chromosome 2 at 40 kb resolution for nuclei pre-digested with DpnII. Middle plot: Average fragment size estimated for every 40 kb bin after pre-digestion with DpnII (Methods). Bottom plot: LOS-residuals for nuclei pre-digested with DpnII after correction for average fragment size. **f**, Boxplot of average fragment size for A compartment bins (n = 35836) and B compartment bins (n = 33252). Significance determined by two-sample two tailed t-test (p < 2.2e-16, t = -80.535, d.f. = 67270, 95% CI = -0.1228385, -0.1170014). Boxplot middle line is the median, the lower and upper edges of the box are the first and third quartiles, the whiskers extend to interquartile range (IQR) × 1.5 from the box. Outliers are represented as points. **g**, Left plot: correlation between LOS for nuclei pre-digested with DpnII and average fragment size. Grey line indicates moving average used for residual calculation. Right plot: partial correlation between residuals of LOS for nuclei pre-digested with DpnII and residuals of PC1 after correcting for correlations between LOS and average fragment size and PC1 and average fragment size (for chromosome 2, Spearman correlation values are indicated). **h**, Left plot: Correlation between DpnII-seq signal and average fragment size. Right plot: correlation between residuals of LOS after correcting for average fragment size and residuals of LOS after correcting for DpnII-seq signal (for chromosome 2, Spearman correlation values are indicated). **i**, Partial correlation between residuals of t1/2 and residuals of PC1 after correcting for correlations between t1/2 and average fragment size and PC1 and average fragment size.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Liquid chromatin Hi-C results are reproducible using the restriction enzyme FatI. **a.** Restriction sites for the selected restriction enzymes. Black triangles denote cut sites. **b.** Top plot: Eigenvector 1 values (PC1, 40 kb resolution) across a section of chromosome 2, representing A (red) and B (blue) compartments. Bottom three plots: Coverage of restriction sites (40 kb resolution). Spearman correlation between restriction site coverage and PC1 is given for each restriction site track. **c.** Third replicate of DpnII predigest liquid chromatin Hi-C. Hi-C interaction maps of chromosome 2 binned at 500 kb. Bottom left: control nuclei in restriction buffer for 4 hours. Top right: nuclei digested for 4 hours with DpnII prior to Hi-C. Left track: Eigenvector 1 values (PC1, 40 kb resolution) across a section of chromosome 2, representing A (red) and B (blue) compartments. **d.** Top plot: LOS along chromosome 2 at 40 kb resolution for nuclei pre-digested with DpnII. Middle plot: DpnII-seq signal. Bottom plot: LOS-residuals for nuclei pre-digested with DpnII after correction for DpnII-seq signal. **e.** FatI predigest liquid chromatin Hi-C. Hi-C interaction maps of chromosome 2 binned at 500 kb. Bottom left: control nuclei in restriction buffer for 4 hours. Top right: nuclei digested for 4 hours with FatI prior to Hi-C. Left track: Eigenvector 1 values (PC1, 40 kb resolution) across a section of chromosome 2, representing A (red) and B (blue) compartments. **f.** Top plot: LOS along chromosome 2 at 40 kb resolution for nuclei pre-digested with FatI. Middle plot: FatI-seq signal. Bottom plot: LOS-residuals for nuclei pre-digested with FatI after correction for FatI-seq signal. **g.** Left plot: Correlation between LOS for nuclei pre-digested with DpnII and PC1. Right plot: partial correlation between residuals of LOS for nuclei pre-digested with DpnII and residuals of PC1 after correcting for DpnII-seq and PC1 and DpnII-seq signal (for chromosome 2, Spearman correlation values are indicated). **h.** Left plot: Correlation between LOS for nuclei pre-digested with FatI and PC1. Right plot: partial correlation between residuals of LOS for nuclei pre-digested with FatI and residuals of PC1 after correcting for DpnII-seq and PC1 and FatI-seq signal (for chromosome 2, Spearman correlation values are indicated). **i.** Correlation between residuals of LOS for nuclei pre-digested with FatI and residuals of LOS for nuclei pre-digested with DpnII after correcting for correlations between FatI LOS and FatI-seq and DpnII LOS and DpnII-seq (genome wide, Spearman correlation values are indicated).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Variations in Half-life and LOS are not explained by DpnII digestion kinetics. 

a, DpnII-seq signals along chromosome 2 after indicated times of digestion. Spearman correlations between DpnII-seq and t_{1/2} at each timepoint is indicated. 

b, t_{1/2} residuals along chromosome 2 after correcting t_{1/2} values by the correlation between t_{1/2} and DpnII-seq signals shown on the left obtained after the indicated times of digestion. Spearman correlation between t_{1/2} residuals and PC1 residuals are indicated. 

c, Top: Genome wide scatterplot of t_{1/2} versus 1 hour DpnII-seq signal. Gray line: moving average. Bar plot above shows the number of loci displaying various levels of DpnII-mediated cuts. Bottom: residuals of t_{1/2} calculated by subtracting t_{1/2} from the corresponding average t_{1/2} (gray line in top plot) plotted vs. number of DpnII cuts. Red dots: loci in the A compartment; Blue dots: loci in the B compartment. The majority of loci have 500-1100 cuts. When comparing loci with similar number of DpnII cut we observe that loci in the A compartment have shorter t_{1/2} values as compared to loci in the B compartment. 

d, Top: LOS along chromosome 2 at the indicated timepoints of digestion and calculated by comparison to Hi-C data obtained after 1 hour of digestion. Middle: calculation of t_{1/2} from LOS at different timepoints. Bottom: t_{1/2} along chromosome 2. This t_{1/2} is calculated using the Hi-C data obtained after 1 hour of pre-digestion as starting point. 

e, Partial correlation between LOS and PC1 after correcting for their correlations with DpnII-seq. LOS (at 2 hours) is calculated as in panel C using the Hi-C data obtained after 1 hour of pre-digestion as starting point. 

f, Partial correlation between t_{1/2} and PC1 after correcting for their correlations with DpnII seq. t_{1/2} is calculated as in panel D using the Hi-C data obtained after 1 hour of pre-digestion as starting point. Spearman correlations are indicated.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Liquid chromatin-Hi-C protocol and quantification of loss of structure after chromatin pre-digestion. a, Workflow for Liquid chromatin Hi-C timecourse. CL = cross-linking step. b, Compartment strength derived from compartment saddle plots (See Methods). Left: Diagram depicting compartment strength calculation for B-B interactions. Plot to the right of diagram: B-B interaction strength as a function of bin number for all timepoints of the time course. Right: Diagram depicting compartment strength calculation for A-A interactions. Plot to the right of diagram: A-A interaction strength as a function of bin number for all time points of the time course. c, Top: LOS signal across a 40 Mb region on chromosome 2 calculated for indicated timepoints in the digestion timecourse. Line colors as in Fig. 4b. Bottom: Exponential curve fit to LOS timepoints for a single 40 kb bin. \( t_{1/2} \) (dashed vertical blue line) representing time elapsed to reach half saturation of LOS signal. d, Left: Density distributions of \( t_{1/2} \) for A and B compartments. Right: \( t_{1/2} \) saddle plots: average intra-chromosomal interaction frequencies between 40 kb bins, normalized by genomic distance. Bins are sorted by their \( t_{1/2} \) value derived from digestion timecourse. Bins with high \( t_{1/2} \) preferentially interact (bottom right of heatmap) and bins with low \( t_{1/2} \) preferentially interact (top left of heatmap). e, Scatterplot of \( t_{1/2} \) vs \( t_{1/2} \) for two timecourse replicates (R1 and R2) on chromosome 2. Regression line (red). Spearman correlation is indicated. f, Scatterplot of PC1 vs \( t_{1/2} \) for chromosome 2. A compartment (red); B compartment (blue). g, Left: Scatterplot of percent interactions occurring in cis within a 6 Mb distance out of total genome wide interactions for each 40 kb bin in control Hi-C map (Cis %) vs PC1. Middle: Cis % vs \( t_{1/2} \). Right: Scatterplot of partial correlation between PC1 and \( t_{1/2} \) controlled by Cis %. A compartment (red); B compartment (blue). Solid red lines are regression lines. Spearman correlations are indicated.
Extended Data Fig. 8 | Associations between sub-nuclear structures and chromatin interaction stability. a, Spearman correlation matrix between signals for various chromatin state markers of various sub-nuclear structures, chromatin remodellers and histone modifications with row order determined by hierarchical clustering. b, The genome was split into 16 bins, where each bin corresponds to sets of loci that share the same $t_{1/2}$ interval. For each $t_{1/2}$ interval the mean z-score signal enrichment for various markers of sub-nuclear structures, chromatin remodellers and histone modifications was calculated and shown as a heatmap. Row order determined by hierarchical clustering. c, 3Mb region surrounding $HoxD$ locus. Top: Hi-C contact map for K562 control nuclei showing the position of the $HoxD$ locus. Tracks: ChIP-seq tracks for polycomb subunits (cyan) and the polycomb associated histone modification $H3K27me3$ (green). $t_{1/2}$ (blue). Minus strand and plus-strand signal of total RNA-seq (red). Refseq Genes (blue/black). The polycomb-bound domain displays shorter half-life compared to expressed genes in flanking regions.
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☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

☐ No software was used for data collection.

Data analysis

All code for data processing and analysis, described in detail in the manuscript, is available through the following GitHub accounts:

https://github.com/tborrman/liquid-chromatin-Hi-C
https://github.com/tborrman/DpnII-seq
https://github.com/dekkerlab/SC-CBFb-SMMHC-Inhib
https://github.com/dekkerlab/cMapping
https://github.com/dekkerlab/cworld-dekker
https://github.com/hms-dbmi/hic-data-analysis-bootcamp
https://github.com/mirnylab/cooltools/tree/master/cooltools

Specific code usage:

SC data processing
- https://github.com/dekkerlab/SC-CBFb-SMMHC-Inhib/blob/master/data_processing_steps.md
- novocraft/V3.02.08
- https://github.com/dekkerlab/cMapping
- cMapping/scripts/utilities/processFlowCell.pl
- cMapping/scripts/utilities/combine5C.pl
- https://github.com/dekkerlab/cworld-dekker
- cworld-dekker/scripts/perl/singletonRemoval.pl
- cworld-dekker/scripts/perl/column2matrix.pl
- cworld-dekker/scripts/perl/anchorPurge.pl
- cworld-dekker/scripts/perl/scaleMatrix.pl
Hi-C data processing
- https://github.com/dekkerlab/cMapping
- python 2.7.9
- bowtie2 2.3.0
- numpy 1.13.3
- hSpy 2.6.0
- cMapping/scripts/utilities/processFlowCell.pl
- cMapping/scripts/utilities/combineHiC.pl
- https://github.com/blajoie/tab2hdf
- tab2hdf/scripts/tab2hdf.py
- https://github.com/blajoie/hdf2tab
- hdf2tab/scripts/hdf2tab.py
- https://github.com/dekkerlab/balance
- balance/scripts/balance.py

A/B compartments
- https://github.com/dekkerlab/cworld-dekker
- cworld-dekker/scripts/perl/matrix2compartment.pl

LOS and half-life calculation
- https://github.com/tborrman/liquid-chromatin-Hi-C
- python 2.7.9
- R 4.0.0
- liquid-chromatin-Hi-C/src/liquid_chromatin_HiC/hdf2RangeCisPercent.py
- liquid-chromatin-Hi-C/src/scripts/LOS.R
- liquid-chromatin-Hi-C/src/scripts/make_LOS_table.R
- liquid-chromatin-Hi-C/src/scripts/get_half_life_exponential.R
- liquid-chromatin-Hi-C/src/scripts/get_halflife_residuals_moving_average.R

DpnII-seq data analysis
- https://github.com/tborrman/DpnII-seq
- python 2.7.9
- bowtie 1.0.0
- samtools 0.1.19
- bedtools 2.17.0
- r 3.4.3
- DpnII-seq/Snakefile

DpnII pre-digestion average fragment size analysis
- https://github.com/tborrman/liquid-chromatin-Hi-C
- R 4.0.0
- bowtie 1.0.0
- samtools 0.1.19
- bedtools 2.17.0
- liquid-chromatin-Hi-C/src/scripts/average_fragment_size.R

Sub nuclear structures
- https://github.com/tborrman/liquid-chromatin-Hi-C
- liquid-chromatin-Hi-C/src/liquid_chromatin_HiC/generate_feature_matrix_40kb.py
- liquid-chromatin-Hi-C/figures/figure5/RepliSeq_heatmap.R
- liquid-chromatin-Hi-C/figures/figure5/feature_halflife_heatmap.R

Gene expression
- https://github.com/tborrman/liquid-chromatin-Hi-C
- liquid-chromatin-Hi-C/src/liquid_chromatin_HiC/get_gene_positions.py
- liquid-chromatin-Hi-C/figures/figure5/subcompartment_halflife_FPKM_boxplot.R

Saddle plots
- https://github.com/hms-dbmi/hic-data-analysis-bootcamp/blob/master/notebooks/04_analysis_cooltools-eigenvector-saddle.ipynb
- https://github.com/open2c/cooltools

Scaling plot
- https://github.com/hms-dbmi/hic-data-analysis-bootcamp/blob/master/notebooks/02_analysis_scaling-curves.ipynb
# Data

- Catalogue of Somatic Mutations In Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cell_lines/download)
- SNIPER subcompartments (Xiong and Ma, 2019)
- ENCODE datasets (Supplementary Table S4)
- NADs state track for the human embryonic fibroblast IMR90 cell line (Dillinger, Straub and Nemeth, 2017; PMID: 28575119)
- TSA-seq data: Nuclear Speckle, nuclear lamina, and PolII (Chen et al., 2018; PMID: 30154186)
- ENCODE RNA-seq (Accession ID: ENCFF782PCD)
- hg19 ensGene table from UCSC Table Browser

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

### Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: All data was obtained for two independent biological replicates. Analysis of Hi-C statistics (Supplemental Table 3), Loss of structure and half-life calculations for the two replicates where concordant (Pearson Correlation r=0.78; Extended Data Figure 2E).
- **Data exclusions**: No data were excluded from any analysis.
- **Replication**: All results were observed in the two independent replicates, all effort to assess replication were successful.
- **Randomization**: No analyses involved any randomization, as all statistical tests were based on normal distributions.
- **Blinding**: No analyses involved blinding as no identifiable personal data was available for any samples or datapoints.

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
## Antibodies

| Antibodies used                                                                 | Validation                                                                                           |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Lamin A antibody (ab 26300) Abcam; CTCF antibody cell signaling (activeMotif 61311); SMC1 antibody (Bethyl Antibody A300-055A); H3 antibody Abcam (ab1791). Anti-Rabbit IgG, HRP-linked antibody #7074 Cell Signaling Technology. CST states the product is thoroughly validated. | Lamin A antibody (ab 26300) Abcam: knock-out validated (no reactivity with 76 Kda band when lamin A is knocked out). Abcam reports that the antibody cross-reacts with several smaller proteins. Dekker lab validated antibody for use in immunofluorescence by showing it stains nuclear periphery, and this staining disappears in mitosis. CTCF antibody cell signaling (activeMotif 61311): Validation listed by the manufacturer: Western blot validated, showing a single band with the correct size. Chromatin immunoprecipitation DNA was sequenced and showed CTCF binding sites. SMC1 antibody (Bethyl Antibody A300-055A): Validation listed by manufacturer: In Western blot a protein of the correct size is detected, with minor recognition of several smaller proteins. Dekker lab has validated this antibody for chromatin immunoprecipitation and found that this antibody identifies known cohesin binding sites that overlap CTCT sites as expected. H3 antibody Abcam (ab1791): Validated by Abcam: website lists these validations: Western blot (correct size, competed with H3 peptide), by immunofluorescence (localized in nucleus), by cell fractionation (localized in nucleus). Also validated in Dekker lab by chromatin fractionation (associated with chromatin) and reactivity in Western blot with protein of correct size. Anti-Rabbit IgG, HRP-linked antibody #7074 Cell Signaling Technology. CST states the product is thoroughly validated. |

## Eukaryotic cell lines

| Policy information about [cell lines](https://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=CellLines) | Cell line source(s) | Authentication | Mycoplasma contamination | Commonly misidentified lines *(See [ICCLAC](https://www.icclac.org) register)* |
|-------------------------------------------------------------------------------------------------|---------------------|-----------------|--------------------------|--------------------------------------------------|
| K-562 (ATCC, CCL-243), GM12878 (Coriell Institute)                                              | K-562 (ATCC, CCL-243), GM12878 (Coriell Institute) | Karyotyping using Hi-C (K-562); cell morphology, and conformation of the beta-globin locus (GM12878, K-562). | Cells were confirmed free of mycoplasm.             | None.                                             |