The inner nuclear membrane proteins Man1 and Ima1 link to two different types of chromatin at the nuclear periphery in *S. pombe*

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Metazoan chromatin at the nuclear periphery is generally characterized by lowly expressed genes and repressive chromatin marks and presents a sub-compartment with properties distinct from the nuclear interior. To test whether the *S. pombe* nuclear periphery behaves similarly, we used DNA adenine methyltransferase identification (DamID) to map the target loci of two inner nuclear membrane proteins, Ima1 and Man1. We found that peripheral chromatin shows low levels of RNA-Polymerase II and nucleosome occupancy, both characteristic of repressed chromatin regions. Consistently, lowly expressed genes preferentially associate with the periphery and highly expressed genes are depleted from it. When looking at peripheral intergenic regions (IGRs), we found that divergent IGRs are enriched compared with convergent IGRs, indicating that transcription preferentially points away from the periphery rather than toward it. Interestingly, we found that Ima1 and Man1 have common, but also separate target regions in the genome. Ima1-interacting loci were enriched for the RNAi components Dcr1 and Rdp1. This agrees with previous findings that Dcr1 is localized at the nuclear periphery. In contrast, Man1 target loci were bound by the heterochromatin protein Swi6, especially at subtelomeric regions. Subtelomeric chromatin was shown to form a unique chromatin type lacking both repressive and active chromatin features and containing low levels of the histone variant H2A.Z. Thus, we find that the fission yeast nuclear periphery shows similar properties to those of metazoan cells, despite the absence of a nuclear lamina. Our results point to a role of nuclear membrane proteins in organizing chromatin domains and loops.

Introduction

In the past decades, the understanding of the functional architecture of the cell nucleus has changed dramatically. Far from being a mere container for genetic material, the nucleus is now understood as a complex environment that influences cellular processes and is essential for proper organization of chromatin. Changes in nuclear topology and organization are hallmarks of diseases such as Hutchinson-Gilford progeria and various cancer types.

The nuclear periphery has long been thought to associate with silent chromatin, as dense heterochromatin was detected there in many cell types.1,2 In metazoan cells, a filamentous layer of lamin-proteins is located beneath the nuclear envelope. Chromatin that is in contact with the nuclear lamina contains repressive chromatin marks and silent or lowly expressed genes.3,4 To elucidate whether this is an active effect of the peripheral environment on gene transcription, different studies were published in which a reporter gene was tethered to the periphery.3,4 The effect on reporter gene expression varied; in some cases repression was observed, in some cases no effect was detected. Additionally, loss of lamin can lead to de-repression of peripheral genes.4

In contrast, interaction with nuclear pore complex components has been linked to active transcription, although it is not clear whether this interaction happens at the pore itself or in the nuclear interior.11,12 Therefore, the nuclear periphery could be comprised of different sub-environments which vary in their effect on gene transcription.

Less is known about the organization of the yeast nucleus. In contrast to metazoans, yeasts lack a nuclear lamina, but contain envelope proteins with similar interaction domains (LEM-domain proteins). The *S. pombe* nucleus is divided into a chromatin-rich and an RNA-rich hemisphere, the latter of which contains the nucleolus.13 The centromeres of the three chromosomes are attached to the periphery opposite the spindle pole body (SPB) throughout interphase.14 The telomeres are also found at the periphery, but not at a fixed location.14 There is some evidence for the existence of chromosome territories in fission yeast.15,16

As very little is known about the nuclear periphery in *S. pombe*, we performed DamID experiments with inner nuclear membrane (INM) proteins to map chromatin-periphery interactions. DamID is a method analogous to chromatin immunoprecipitation (ChIP), which has been successfully applied in metazoan

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systems to map regions associated with the nuclear lamina. This study aims to put existing genome-wide maps of chromatin features and gene expression states into a context of nuclear organization and radial positioning.

Results

Genome-wide mapping of the INM proteins Ima1 and Man1. To map interactions between the nuclear envelope and chromatin, we used DamID experiments. In DamID, a DNA adenine methylase (Dam) is fused to the protein of interest and methylates target loci in vivo (Fig. 1B). DNA marked in this way is later amplified by a methylation specific PCR. In contrast to ChIP, DamID does not rely on antibody specificity, can register transient interactions and does not show fixation artifacts. We also chose DamID over ChIP as solubilizing the formaldehyde-fixed membrane protein-chromatin complexes can be difficult.[17]

As a starting point, we chose two INM proteins, Ima1 (Integral membrane protein 1) and Man1 (formerly described as Heh2), which differ in their distribution and structure. Ima1 is involved in tethering pericentric heterochromatin to the SPB.[18] Man1 contains a LEM-like helix-extension-helix domain.[19] For both proteins, we engineered fusion constructs of the INM protein and the Dam methylase.

We verified the localization of the fusion protein by fluorescence microscopy and found it to be present mainly in the nuclear membrane (Fig. 1A). We also saw a few cytoplasmic spots and a weak staining of the plasma membrane which are probably due to protein overexpression for visualization purposes.

We performed DamID experiments for both membrane proteins, expressing the fusion constructs at low levels to prevent artifacts, and used Affymetrix tiling arrays to map targets in the fission yeast genome (Fig. 1C). For both INM fusion proteins, three biological replicates were performed in parallel with Dam-only control samples. One replicate of Ima1-Dam had to be excluded due to its aberrant intensity profile. All samples correlate well between replicates ($R^2 > 0.82$, average $R^2 = 0.90$), while arrays from different constructs correlate considerably less (average $R^2 = 0.66$). Furthermore, the different constructs separate in a Principal Component Analysis (PCA) (Fig. S1).

Using a Hidden Markov Model (HMM), we defined target loci and found about a third of the genome to be associated with either or both proteins (Fig. 1D). This is comparable to metazoan cells, where about 40% of the genome is found in lamina-associated domains (LADs).[4,20,21]

Ima1, which is enriched close to the SPB, was found to interact with a smaller portion of the genome than the more ubiquitously localized Man1. Both proteins share a proportion of their targets but also bind separately from each other (Fig. 1E).

Figure 1A (see next page for B-E). DamID of Ima1 and Man1. (A) Subnuclear localization of Ima1- and Man1-Dam fusion proteins and the Dam-only control. Immunofluorescence microscopy was performed using an anti-myc antibody against the 7myc-tags in the expressed proteins. A FITC-coupled antibody was used for secondary staining.
Looking at chromosome domains, we found the centromeres to be associated with both INM proteins (Fig. S2), which had been shown previously to be attached to the nuclear envelope.\(^{14,22}\) Interestingly, we observed a higher enrichment of Man1 and Ima1 over the central core of the centromeres and less on pericentric heterochromatin (Fig. S2). This agrees well with observations that the periphery attachment of the centromeres occurs through the central core domain, while the pericentric repeats are located further away from the INM.\(^{22,23}\) This we take as confirmation that our DamID maps represent in vivo periphery-chromatin interactions in fission yeast.

Properties of genes at the periphery. In metazoans, peripheral genes were found to be lowly expressed or silenced.\(^{24}\) Lamina associated domains in human fibroblasts contain genes that are mostly silent.\(^{14}\) To test whether this is also the case in fission yeast, we divided all genes into five groups of equal size depending on their expression status in minimal medium.\(^{25}\) When we averaged Man1 and Ima1 enrichment over the body of those genes, we found that lowly expressed genes showed significantly higher levels (p < 0.002, two-sided Wilcoxon-Mann-Whitney test) than the rest of the genes (Fig. 2A). Highly expressed genes on the other hand showed a significantly lower enrichment of both
Figure 2. Peripheral genes show lower expression levels and point preferentially away from the nuclear envelope. (A) Average enrichments of Ima1 and Man1 over the body of genes with different expression levels, represented by boxplots. Only those genes for which expression data was available are displayed (4884 of 5027). P-values are calculated using Wilcoxon-Mann-Whitney tests comparing the distribution of one gene expression level to the distribution of all remaining genes. (B) Average enrichments of Ima1 and Man1 over intergenic regions (IGRs) of different orientations. P-values are calculated using Wilcoxon-Mann-Whitney tests comparing the distribution of one IGR orientation to the distribution of all remaining IGRs.
proteins ($p < 0.002$, two-sided Wilcoxon-Mann-Whitney test). This indicates a tendency for lowly expressed and repressed genes to be associated with and for highly expressed genes to be excluded from the nuclear periphery. Interestingly, this periphery-interaction appears to be more pronounced for the gene body. For promoter regions (here defined as 350 bp upstream of the transcription start site), the anti-correlation between expression levels and Man1 or Ima1 binding was weaker (Fig. S3).

Next we looked at intergenic regions (IGRs), which we define as all non-coding sequence between two genes. Interestingly, we found that divergent IGRs showed significantly higher levels of Man1 and Ima1 than convergent IGRs, while tandem IGRs showed average enrichment (Fig. 2B). This is in agreement with the finding that promoters close to the nuclear lamina tend to point away from it in human fibroblasts. Taken together, these results suggest that genes at the periphery have low transcription levels and tend to orient away from rather than toward the periphery.

Properties of peripheral chromatin. Differences in expression levels can often be linked to differences in chromatin properties, such as histone modifications and binding of chromatin-associated proteins. Therefore, we compared our periphery interaction data with previously published maps of chromatin features. We found that loci associated with either or both Ima1 and Man1 show significantly lower levels ($p < 0.002$, permutation test, see Materials and Methods) of RNA Polymerase II (RNAPII) compared with the rest of the genome (Fig. 3A). This agrees with the findings in metazoans, where lamina associated regions showed the same property.4,20,21,26

In yeasts, nucleosome occupancy varies between euchromatic and heterochromatic regions. In S. pombe, heterochromatic regions show lower levels of nucleosomes compared with euchromatin.27 Consistent with this, we observed significantly lower nucleosome density ($p < 0.002$, permutation test) at loci associated with Ima1 and Man1 (Fig. 3B).

Furthermore, we found significantly higher levels of the histone variant H2A.Z ($p < 0.002$, permutation test) for Man1 targets (Fig. 3C). We also found that H2A.Z is significantly enriched at divergent and tandem IGRs ($p < 0.002$, two-sided Wilcoxon-Mann-Whitney test), while it was depleted at convergent IGRs (Fig. S4). In S. pombe, promoters of repressed genes have been shown to be enriched in H2A.Z binding.28 In S. cerevisiae, it has been reported that H2A.Z is involved in transcriptional regulation at the nuclear periphery.29 Thus, peripheral chromatin in S. pombe
shows features similar to those typically associated with repressed chromatin. Ima1 target loci are also targets of the RNAi machinery. It was previously shown that nuclear processes such as DNA replication and gene transcription occur at non-random positions in the nucleus. When looking at genome-wide maps of components of the RNA interference (RNAi) machinery, we found a strong correlation between localization of Dicer (Dcr1) and Ima1 occupancy (Fig. 4A). Interestingly, this strong correlation was only present for loci associated with Ima1, not those associated only with Man1. The RNA-dependent RNA Polymerase Rdpl showed a similar behavior toward Ima1 (Fig. 4B). This observation agrees with the previous finding that Dcr1 localizes to the nuclear periphery when overexpressed and suggests that RNAi occurs in distinct compartments of the nuclear periphery. Man1 associates with subtelomeric chromatin. Previous studies have identified an unusual type of chromatin at the subtelomeres of chromosomes 1 and 2 in fission yeast. This subtelomeric chromatin (ST-chromatin) is characterized by low levels of H2A.Z and transcription, while neither the repressive chromatin mark H3K9me2, nor the active chromatin mark H3K4me2 is present in high abundance. The boundaries of these regions are marked by binding of the SNF2 family ATP-dependent chromatin remodeling factor Fes3 (Fission yeast fun 30), which is required for maintenance of the repressed chromatin state in this region.

Figure 4. Ima1 shares common targets with the RNAi components Dcr1 and Rdpl. Overview boxplots for (A) Dcr1 and (B) Rdpl occupancy. The boxplots show score distributions over target loci, depending on how they are annotated by the Hidden Markov Model. The P-values were calculated by permutation test. Number of loci plotted per box and genome portion covered: genome wide, 29,697 loci (12.30 Mb); Man1 only, 6,429 loci (3.49 Mb); Ima1 only, 645 loci (0.24 Mb); both, 826 loci (0.41 Mb); neither, 21,797 loci (8.16 Mb).
When studying the binding profiles of Man1 and Ima1 at the subtelomeres, we found a strong enrichment of Man1, while Ima1 was absent from these regions (Fig. 5A). The same regions were found to be enriched for Swi6. In addition, Man1 and Swi6 show significant co-localization genome-wide, while Ima1 does not show a correlation to Swi6-localization (Fig. 5B).

**Discussion**

Many studies have described the metazoan nuclear periphery as an environment repressive of gene transcription. This repressive effect has been linked to the nuclear lamina and lamina-associated proteins. As yeasts lack homologs of the lamin genes and therefore do not have a nuclear lamina, it is not to be readily expected that they would show a similar behavior. It was therefore surprising that tethering the HM mating-type locus to the nuclear envelope is sufficient for its silencing in *S. cerevisiae*. Our results demonstrate that the *S. pombe* nuclear periphery shows similar properties.

Our analysis indicates a preference for poorly expressed and repressed genes to be located at the nuclear periphery, while highly expressed genes appear to be enriched in the nuclear interior (Fig. 6A). This agrees well with previous studies in fruit fly and human cells. However, it is still unclear how this organization of chromatin close to the nuclear periphery occurs. On the one hand, the periphery itself appears to have silencing effects on genes placed there, at least under some conditions.

![Figure 5](image)

**Figure 5.** Man1 is enriched at sub-telomeres and binds to Swi6-targets. (A) Target distribution along sub-telomeres. Genome browser views showing the distributions of Man1, Ima1, Swi632 and H2A.Z28 as log2-enrichment, as well as H3K9me2 and H3K4me2 enrichment over the sub-telomere of chromosome 2. Genes are colored by their expression status when available, otherwise shown in white. LTR elements are depicted as black boxes. (B) Overview boxplot for Swi6. The boxplot shows score distributions over target loci, depending on how they are annotated by the Hidden Markov Model. P-values are calculated by permutation test. Number of loci plotted per box and genome portion covered: genome wide, 29,697 loci (12.30 Mb); Man1 only, 6,429 loci (3.49 Mb); Ima1 only, 645 loci (0.24 Mb); both, 826 loci (0.41 Mb); neither, 21,797 loci (8.16 Mb).
Furthermore, gene clusters in different mammalian tissues were shown to move away from the periphery upon activation during differentiation (reviewed by Lanctôt et al.). On the other hand, several inducible genes relocalize to the nuclear envelope in *S. cerevisiae* when they are activated. Overall, transcriptional regulation at the nuclear periphery appears to depend on multiple factors and follows no straightforward rule.

Surprisingly, we have found that the two INM proteins Ima1 and Man1 have both common and separate target loci in the genome (Fig. 6C). Assuming free diffusion of both proteins in the INM, one would expect them to be uniformly distributed along the membrane and therefore to have roughly identical targets. This suggests that the nuclear envelope is not uniform in its protein localization, but rather contains patches that vary in protein composition. The size and distribution of those patches is still unclear, as well as their mechanism of formation. It is possible that the differential distribution of Ima1 and Man1 could be caused by a passive clustering reflecting the positioning of target loci close to the periphery. Alternatively, an active organization of both INM proteins in the nuclear envelope into patches could affect the positioning of chromatin interacting with it. In any case this shows that anchoring points of chromatin at the nuclear periphery are non-randomly organized. Further research into this area will be necessary to determine cause and effect.

Genes at the borders of lamina-associated domains in human fibroblasts tend to point away from the periphery rather than toward it. Likewise we find an enrichment of divergent IGRs at the periphery in *S. pombe* in this study. This raises the question of whether chromatin loop formation is used at the periphery to organize transcription units. In *S. cerevisiae*, it has been previously shown that single genes can form loop structures connecting the 3' end of a gene with its promoter. A divergent IGR at the nuclear periphery could position two transcription units in a loop, both pointing away from the periphery. This could happen if parts of the transcription process are excluded from the periphery, such as elongation or termination.

**Figure 6.** Putative model for chromatin organization at the fission yeast nuclear periphery. (A) Gene expression at the nuclear periphery. Genes with low expression levels are more commonly associated with the nuclear periphery, while highly expressed genes tend to reside in the nuclear interior. (B) A potential role for H2A.Z at the nuclear envelope. Localization of divergent IGRs and H2A.Z at the nuclear envelope could present a mechanism for anchoring the promoters of convergent gene pairs at the periphery. The convergent IGR would then be located in the nuclear interior, where the RNA surveillance machinery can act to suppress anti-sense transcription. (C) Differential localization of Ima1 and Man1. The INM proteins Ima1 and Man1 are not equally distributed at the nuclear periphery, but rather occupy distinct areas that interact with different chromosomal regions. The subtelomeric chromatin is associated with Man1-rich peripheral regions, where Swi6 is also located. In contrast, Ima1 is absent from subtelomeric chromatin, but tends to associate with Dcr1 and Rdp1 target loci.
Another possibility is that the interactions of divergent IGRs cause position loops containing convergent IGRs toward the nuclear interior (Fig 6B). In S. pombe, a process called “RNA surveillance” prevents accumulation of anti-sense RNA originating from read-through transcription at convergent gene pairs.40 Although the mechanistics of this process are still largely unclear, it has been shown that components of the histone methyltransferase complex ClrC, the RNAi component Ago1 and the mRNA export factor Mio3 are required for suppression of anti-sense transcription.41,42 Additionally, presence of the histone variant H2A.Z at the promoters of convergent gene pairs is necessary for anti-sense suppression. Based on our data, H2A.Z at divergent IGRs at the nuclear periphery could potentially play a role in positioning convergent IGRs in the nuclear interior and thus organize RNA surveillance in the nucleus.

We also show a strong correlation between Im1-association and binding of the RNAi components Dcr1 and Rdp1. The peripheral localization of Dcr1 has previously been shown.39 While the targeting of Dcr1 to the nucleus is necessary for formation of siRNAs and heterochromatin formation at pericentric repeats, it is unclear whether this distinct subnuclear localization at the periphery is important for its function.

Our finding that Man1 is enriched in subtelomeric chromatin is especially interesting as its nucleoplasmic tail contains a LEM-like Helix-loop-helix domain. In metazoans, INM proteins such as MAN1, LEM2 and Emerin interact with chromatin associated proteins such as BAF (barrier-to-autointegration factor) through their LEM domains.44 As no BAF-homolog has been found in unicellular organisms, it is unclear whether proteins with LEM-like domains could interact with chromatin in a similar fashion. However, it is likely that proteins like Man1 have functions analogous to those of metazoan LEM-DOM-domain proteins, especially as there appears to be an association with specific chromatin loci at the nuclear periphery. The C. elegans homolog of LEM2 was found to interact mainly with the arms of chromosomes and was absent from central regions.45 This unusual organization of chromosomes is not found in the other systems where periphery-chromatin interactions have been studied46-48 and is also absent in our data.

In S. cerevisiae, the Man1 ortholog Scel1 was also found to associate with subtelomeric genes and to affect their expression status.46 Interestingly, both the fusion yeast Man1 and Lem2 have been shown to be involved in anchoring of telomeres at the nuclear envelope.46 This might point to a conserved role for LEM-like domain proteins in subtelomeric chromatin organization.

In conclusion, we find that the fusion yeast nuclear periphery shows remarkable similarity to that of metazoans despite its lack of a nuclear lamina. We show that two INM proteins interact with common and separate targets, indicating the presence of subdomains in the nuclear envelope that vary in protein content. Our results point to a role of the nuclear periphery in organization of large-scale chromosomal domains such as subtelomeric chromatin, as well as in localized gene loop formation. Taken together, our study shows that basic mechanisms of genome organization are conserved across species and do not depend on the presence of the nuclear lamina.

Materials and Methods

Yeast strains and cell culture. The following strains were used in this study:
Hu2076 h- Chr3-171385-dam-7myc-im1:NAT
Hu2078 h- Chr3-171385-dam-7myc-NLS:NAT
Hu2130 h- Chr3-171385-man1-7myc-dam:NAT

For both INM proteins, we engineered fusion constructs containing the target proteins, an array of seven myc-tags and the Dam methylase. The tags and methylase were attached to the C-terminus of Im1 and to the N-terminus of Man1, respectively. The resulting construct was placed under the control of the repressible mnt41 promoter and stably integrated into the fusion yeast genome at a locus on chromosome three with no background expression (Dorota Feret, Agnes Grallert, Kuan Yoo-Chan, Aviash Patel and Jain Hakan, unpublished). The endogenous copy of each gene was not altered.

In a control strain (Hu2078) we expressed the Dam methylase alone under the same promoter and in the same locus. We added seven myc-tags for visualization and an additional nuclear localization signal to ensure targeting to the nucleus. Cells were grown in minimal medium (PMG + EMMG).39 We found that the leaky expression from the repressed mnt41 promoter (in PMG medium supplemented with 15 μM thiamine) resulted in sufficiently high adenine methylation levels for DamID, while preventing overexpression artifacts, and performed all subsequent DamID experiments under these conditions.

Cytology. Immunolocalization experiments were performed as previously described.49 Cultures were grown in PMG without thiamine and fixed with 1.75% Para-Formaldehyde for 30 min. The myc-epitope tags were detected using anti-myc 9E10 antibody (Sigma) and FITC-coupled anti-mouse antibody (Sigma). Microscopy pictures were recorded in TIFF-format and brightness/contrast were adjusted using Adobe Photoshop.

DamID. Yeast cells were grown in PMG with 15μM thiamine to a density of ~7 × 10^6 cells/ml. Genomic DNA was isolated as described by Moreno et al.47 DpnI digest, adaptor ligation, DpnII digest and PCR amplification were performed as previously described.46 Samples were then purified using the Qiagen PCR purification kit and fragmented with DNase.

Labeling of DNA and hybridization to the Affymetrix GeneChip S. pombe Tiling 1.0FR was performed using standard protocols by the Affymetrix core facility at Novum (http://apt.bea.ki.se). Raw and processed microarray data have been submitted to the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE30476.

Data analysis. All analysis was performed in R (http://www.r-project.org) using the Bioconductor (http://www.bioconductor. org) packages “affy,” “affyarray” and “preprocessCore” with standard parameters. In short, CEL files for each array were imported into R and probe scores were mapped to the genomic sequence of S. pombe (Chromosome contigs were downloaded from ftp://ftp.sanger.ac.uk/pub/yeast/pombe/Chromosome_contigs/ on Nov. 19, 2009). We excluded all tiling array probes that mapped to more than one region of the genome, as it is not possible to determine the origin of the signal for these probes. Signal intensities
for all arrays were normalized using Quantile Normalization.\textsuperscript{48} DamID scores were computed as the INM-protein-Dam signal (averaged across replicates) divided by the Dam-only signal (averaged across replicates). Microarray data published elsewhere was used as processed data when available. All data sets only available as raw data were processed like the DamID data described above.

Box-and-whisker plots were drawn in R using the “boxplot” function with standard parameters. Significance tests for gene and RCR comparisons were performed using the Wilcoxon-Mann-Whitney test function “wilcox.test,” also with standard parameters.

Target definitions. Profiles were discretized in three states (“depleted,” “neutral” and “enriched”) by a Hidden Markov Model (HMM) published elsewhere.\textsuperscript{49} In short, the HMM accommodates non-uniform sampling rates and it assumes that emissions follow a Student’s t distribution. Parameters are fitted through a Baum-Welch algorithm and state inference is performed through the Viterbi algorithm. We defined targets as regions in the “enriched” state.

The Viterbi algorithm, used in HMM, differs from cut-off based classifiers in the sense that it is optimal. Intuitively, it optimizes the false discovery rate as well as the false non-discovery rate. This means that every other segmentation has a larger misclassification rate (assuming that the hypotheses of the HMM framework hold).

The probabilistic framework of HMMs allows to estimate the probability that a record is in a certain state, given the whole data set. We can use this information to estimate the probabilities of misclassification and found that the probability of misclassification of targets and non-targets is way more likely to be small than large. For a random target of Man1, the average probability of misclassification is 0.165, and 0.072 for a non-target, showing that the classifier is more prone to generate false positives than false negatives. The estimated false negative and false positive rates for Man1 are respectively 0.100 and 0.329, for an average misclassification rate of 0.156.

The average estimated misclassification rate for Ima1 is 0.0958. If we were to set a 5% FDR cut-off on targets (i.e., reporting only those with a probability of misclassification less than 0.05), the average misclassification rate for Ima1 would raise from 0.093 to 0.182. Nothing motivated the introduction of a bias by decreasing false discovery at the expense of false non-discovery, we simply used the output of the Viterbi algorithm which is globally optimal and thus the best starting point for our downstream analyses of ‘targets’ vs. ‘non-targets.’

Permutation test. All binding profiles mentioned in the text should high levels of dependence, as shown by plotting the auto-covariance. The Wilcoxon-Mann-Whitney test cannot be performed on such signals because it assumes IID (Independent and Identically Distributed) sampling. To compare average signal from different genomic regions, we therefore used a resampling approach: we compared the observed W statistic of the Wilcoxon-Mann-Whitney test to 1,000 resampled values obtained after random circular permutations of the signal. The p values reported in the text are computed using the resampled values as the approximate null distribution of a two-sided test.

Disclosure of Potential Conflicts of Interest

The authors declare that they have no conflict of interest.

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Supplemental Material

Supplemental material may be downloaded here: http://www.landesbioscience.com/journals/nucleus/article/18825/
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