2-15-2015

The Ino80 complex prevents invasion of euchromatin into silent chromatin

Yong Xue
University of California, Los Angeles

Christopher Van
University of Massachusetts Medical School, christopher.van@umassmed.edu

Suman K. Pradhan
University of California, Los Angeles

See next page for additional authors

Follow this and additional works at: http://escholarship.umassmed.edu/pmm_pp

Part of the Biochemistry Commons, Developmental Biology Commons, Molecular Biology Commons, and the Molecular Genetics Commons

Repository Citation
Xue, Yong; Van, Christopher; Pradhan, Suman K.; Su, Trent; Gehrke, Jason; Kuryan, Benjamin G.; Kitada, Tasuku; Vashisht, Ajay; Tran, Nancy; Wohlschlegel, James; Peterson, Craig L.; Kurdistani, Siavash K.; and Carey, Michael F., "The Ino80 complex prevents invasion of euchromatin into silent chromatin" (2015). Program in Molecular Medicine Publications and Presentations. Paper 67. http://escholarship.umassmed.edu/pmm_pp/67

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Program in Molecular Medicine Publications and Presentations by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
The Ino80 complex prevents invasion of euchromatin into silent chromatin

Authors
Yong Xue, Christopher Van, Suman K. Pradhan, Trent Su, Jason Gehrke, Benjamin G. Kuryan, Tasuku Kitada, Ajay Vashisht, Nancy Tran, James Wohlschlegel, Craig L. Peterson, Siavash K. Kurdistani, and Michael F. Carey

Keywords
Dot1, H3K79 methylation, Ino80, Sir proteins, heterochromatin, silencing

Comments
Copyright © 2015 Xue et al.; Published by Cold Spring Harbor Laboratory Press. This article is distributed exclusively by Cold Spring Harbor Laboratory Press for the first six months after the full-issue publication date (see http://genesdev.cshlp.org/site/misc/terms.xhtml). After six months, it is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at http://creativecommons.org/licenses/by-nc/4.0/.

Creative Commons License
This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 License

Rights and Permissions
Citation: Genes Dev. 2015 Feb 15;29(4):350-5. doi: 10.1101/gad.256255.114. Link to article on publisher's site

This article is available at eScholarship@UMMS: http://escholarship.umassmed.edu/pmm_pp/67
The Ino80 complex prevents invasion of euchromatin into silent chromatin

Yong Xue,²,³ Christopher Van,²,³ Suman K. Pradhan,¹ Trent Su,¹ Jason Gehrke,¹ Benjamin G. Kuryan,¹ Tasuku Kitada,¹ Ajay Vashisht,¹ Nancy Tran,¹ James Wohlschlegel,¹ Craig L. Peterson,²,⁴ Siavash K. Kurdistani,¹,⁴ and Michael F. Carey¹,⁴

¹Department of Biological Chemistry, David Geffen School of Medicine at University of California at Los Angeles, Los Angeles, California 90095, USA; ²Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA

Here we show that the Ino80 chromatin remodeling complex [Ino80C] directly prevents euchromatin from invading transcriptionally silent chromatin within intergenic regions and at the border of euchromatin and heterochromatin. Deletion of Ino80C subunits leads to increased H3K79 methylation and noncoding RNA polymerase II [Pol II] transcription centered at the Ino80C-binding sites. The effect of Ino80C is direct, as it blocks H3K79 methylation by Dot1 in vitro. Heterochromatin stimulates the binding of Ino80C in vitro and in vivo. Our data reveal that Ino80C serves as a general silencing complex that restricts transcription to gene units in euchromatin.

Supplemental material is available for this article.

Received November 20, 2014; revised version accepted January 16, 2015.

Euchromatin in the yeast Saccharomyces cerevisiae is characterized by Dot1-mediated H3K79 trimethylation [H3K79me3] [Briggs et al. 2002; van Leeuwen et al. 2002; Henry et al. 2003; Wood et al. 2003; Kao et al. 2004; Xiao et al. 2005; Pavri et al. 2006; Shilatifard 2006]. H3K79me3, a mark closely associated with active transcription, is typically present in the coding regions of genes but absent from intergenic regions as well as subtelomeric heterochromatin [Ng et al. 2003; Pokholok et al. 2005], both of which are transcriptionally silent. In heterochromatin, Sir3’s BAH domain binds over H3K79 [Armache et al. 2011] and blocks access of Dot1 to its substrate [Altaf et al. 2007], but whether this is the only mechanism preventing euchromatin invasion into heterochromatin is not known. It has also been unclear what mechanism prevents euchromatin invasion into heterochromatin, Sir3’s BAH domain binds over H3K79me3 and H3K79me4, with the caveat that Ino80C is clearly upstream of these peaks, which are expected to be enriched at the 5’ ends and the bodies of genes, respectively. Our positioning is somewhat consistent with previous studies, with the caveat that Ino80C is clearly upstream of both the TSS and H3K4 methylation, suggesting that it does not occupy the first nucleosome within the gene [Shimada et al. 2008; Yen et al. 2012].

The dense packing of genes in the S. cerevisiae genome raised the possibility that the Ino80C peaks could, in principle, be located upstream of the TSS, downstream from the TTS, or both. To distinguish between these, we plotted Arp5 upstream of the TSS of genes bearing di-
Ino80C enhances transcriptional silencing in the flanking extragenic regions

Figure 1. Ino80C is enriched at the boundaries of the gene body and anti-correlates with H3K79 methylation. (A) Distribution of Arp5, H3K79me3, and H3K4me3 across the gene through 1 kb upstream of the TSS to 1 kb downstream from the TTS at all genes. Log2 ratios of immunoprecipitation (IP) versus input at significantly enriched windows were used, and each row represents one gene. (B) Average of metagene profiles of Arp5, H3K79me3, and H3K4me3 from A. (C) Patterns of Arp5, H3K79me3, and H3K4me3 at representative genes. (D) Average distribution of H3K79me3 around peaks of Arp5. Log2 ratios of H3K79me3 versus input at all windows are plotted. (E) Metagene profiles of change of H3K79me3 in arp5Δ relative to wild type (WT) (BY4741). (F) Distribution of change of H3K79me3 in arp5Δ relative to wild type around the center of Arp5 peaks. (G) Metagene profiles of mRNA in Sir3 and Ino80 mutants alongside wild type. The log2 ratio of mRNA level in mutants versus wild type was plotted against the metagene. (H) Patterns of mRNA (log2 ratio) in Ino80 mutants versus wild type at chromosome XII 140,500–157,000.

Ino80C transcriptional silencing

Considering the strong correlation between H3K79me3 and transcription [Im et al. 2003; Jaskelioff and Peterson 2003; Martin and Zhang 2005; Vakoc et al. 2006; Guenther et al. 2007; Kitada et al. 2012], we next asked whether enhanced H3K79 methylation in the flanking extragenic regions correlated with their ectopic transcription. We performed RNA-seq of polyA-containing transcripts [i.e., mRNA-seq] in wild-type and mutant strains of Ino80C bearing deletions of either ARP5, IES6, IES2, or INO80. Figure 1G shows a metagene analysis revealing that extragenic transcription occurred in all deletion mutants and peaked at the locations of Arp5 binding in wild-type strains. Figure 1H shows an example with a browser track. Although the transcript levels were typically much lower than in the gene body, they nonetheless were clearly apparent and statistically significant. Importantly, arp5Δ, ies2Δ, ies6Δ, and ino80Δ elicited similar increases in transcription, arguing that Ino80C and not a subcomplex is suppressing extragenic transcription.

To determine whether the extragenic transcription was due to sense and/or antisense transcription, we performed strand-specific mRNA sequencing. To exclude transcriptional signals from nearby genes, we analyzed only those genes that are separated from their neighboring genes on the opposite strand by >1 kb either upstream of the TSS or downstream from the TTS. Although the transcript levels were much lower than in the gene body, they nonetheless were clearly apparent and statistically significant. Importantly, arp5Δ, ies2Δ, ies6Δ, and ino80Δ elicited similar increases in transcription, arguing that Ino80C and not a subcomplex is suppressing extragenic transcription.

Ino80C prevents H3K79 methylation and enhances transcriptional silencing within heterochromatin in vivo

Ino80C’s role in suppressing H3K79 methylation and transcription within euchromatin raised the possibility that Ino80C may perform a similar role in heterochromatin. Analysis of Sir3 showed that, on average, its binding is highest in the region closest to the telomere, decreases
gradually throughout the subtelomeric region, and reaches a minimum typically ~20 kb from the telomere (Fig. 2A; Sperling and Grunstein 2009). Conversely, Arp5 binding increases as Sir3 binding decreases, indicating that Ino80C occupies the less dense heterochromatin adjacent to euchromatin. For example, Figure 2B shows a browser track of chromosome VIII, where Ino80C binds poorly or not at all in dense heterochromatin near the telomere, but its binding becomes clearly apparent as the binding of Sir3 decreases.

On the basis of its transcriptional demarcation in euchromatin, Ino80C binding within heterochromatin suggested the possibility that it may function as a gatekeeper to prevent invasion of euchromatin into heterochromatin. Indeed, Figure 2C shows that, in the absence of Arp5, H3K79me3 increases throughout heterochromatin. Moreover, the greatest increase in H3K79me3 in an arp5Δ strain correlated with the average Arp5-binding peak observed in wild-type cells. Conversely, there was no measurable increase in subtelomeric levels of H3K4me3 (Supplemental Fig. S2).

Deletions of ARP5, IES6 (Fig. 2D), or INO80 (Fig. 2E) led to increased subtelomeric transcription similar to that observed in a strain bearing a deletion of SIR3 (Fig. 2D). Consequently, simultaneous deletions of SIR3 and ARP5 increased subtelomeric transcription in an additive manner (Fig. 2D). Interestingly, the enhanced transcription in the Ino80C mutants is greatest where the concentration of Sir3 decreases toward the euchromatic end of subtelomeric silent chromatin (Fig. 2F,G), and the effect of Sir3 deletion alone on transcription is greatest toward the telomeric end of subtelomeric silent chromatin. This point is illustrated clearly by plotting the increase in transcription in sir3∆, arp5∆, and ino80Δ strains on a browser track (Fig. 2G). These data suggest that Ino80C cooperates with the Sir complex to maintain heterochromatin gene silencing, especially in regions where the decreasing concentration of Sir3 may render it less effective in transcriptional silencing.

To determine whether this same silencing effect was observed at the Sir3-bound silent mating locus, we compared HMLa1 transcription in strains bearing deletions of Ino80C subunit genes and SIR3. Figure 2H shows a significant increase in transcription in all of the deletion mutants. We conclude that Ino80C and Sir3 contribute to transcriptional silencing in heterochromatin.

The Sir complex enhances Ino80C recruitment at heterochromatin in vivo and in vitro

The overlapping functions of Ino80C and Sir3 in silencing within heterochromatin raised the key question of whether they also enforce each other’s binding. To address this, we first asked whether Ino80C and Sir3 co-occupy genes within heterochromatin. Figure 3A shows that within 20 kb of the telomere, 80 genes bind significant levels of both Sir3 and Arp5, including HMLα located in the subtelomeric region at the left end of chromosome III. To determine whether Sir3 affects Ino80C-mediated chromatin silencing, we performed locus-specific ChIP of Ino80C at HMLα in wild-type and sir3Δ strains. Loss of Sir3 significantly affected binding of Ino80C at the E and I silencer elements of HML but not at ACT1, the control gene (Fig. 3B). These observations raised the
possibility that the Sir complex may promote Ino80C binding.

To address this question, we purified Ino80C to near homogeneity via tandem affinity purification (TAP) and titrated it onto immobilized, unmodified chromatin either containing or lacking a fixed amount of purified Sir2, Sir3, and Sir4 proteins in vitro (Fig. 3C). Conversely, the Sir2, Sir3, and Sir4 proteins were titrated onto chromatin in either the absence or presence of a fixed amount of Ino80C (Fig. 3D). In both experiments, Ino80C was recruited to chromatin at higher levels in the presence of the Sir proteins. Our in vivo and in vitro data lead us to conclude that the Sir proteins contribute to Ino80C recruitment at heterochromatin.

Ino80C blocks Dot1-mediated H3K79 methylation in vitro

To determine whether Ino80C in turn directly prevents Dot1-mediated H3K79 methylation, we employed purified Ino80C and recombinant Dot1 in nucleosomes modification assays. Figure 3, E and F, shows that Dot1 efficiently trimethylated H3K79 in the context of nucleosomes and free histone octamers. However, increasing amounts of Ino80C led to a dose-dependent inhibition of Dot1-mediated H3K79me3 on nucleosomes [Fig. 3E] but not on histone octamers [Fig. 3F]. We conclude that Ino80C can directly block Dot1-mediated nucleosome methylation.

Restricting gene expression to gene units

Our data showing that Ino80C prevents H3K79 methylation outside of gene boundaries and silences transcription are consistent with the results on noncoding transcription in Ino80C mutants by the Buratowski and Tsukiyama laboratories [Alicd and Tsukiyama 2014; Marquardt et al. 2014]. Importantly, we show that Ino80C is directly responsible for suppressing noncoding transcription within and around its binding site by blocking Dot1 and the Pol II machinery [Supplemental Fig. S3]. The previously described cryptic unstable transcripts and stable unannotated transcripts [Xu et al. 2009] extensively overlapped with the Ino80C-binding sites and were up-regulated in the Ino80C mutants [data not shown]. This observation further supports the idea that Ino80C prevents noncoding transcription in silent extragenic regions.

Previous studies have shown that Ino80C is important for removal of H2AZ incorporated either spuriously or as a dynamic process linked with gene activity [Papamichos-Chronakis et al. 2011; Yen et al. 2013]. Thus, it is plausible that H2AZ insertion is among the processes blocked by Ino80C. Mutants in other chromatin remodeling complexes such as ISW1 and RSC activate divergent noncoding transcription [Marquardt et al. 2014]. These complexes, however, do not typically colocalize with Ino80C, suggesting that they may operate independently by promoting regulated transcription and nucleosome stability within a gene [Yen et al. 2012]. We do not know how Ino80 localizes to the flanks of genes. The localization of H3K4me3 and H3K79me3 is directly linked with transcription, so perhaps such processes demarcate Ino80C outside of gene boundaries, where it serves to confine transcription to the gene-coding regions. It is plausible that ino80 also provides a crude insulator function to genes similar to that seen in higher eukaryotic genomes.

It is known that H4K16 acetylation prevents the Sir proteins from spreading from heterochromatin to euchromatin [Suka et al. 2002]. However, the mechanism of preventing euchromatin from invading heterochromatin has been unclear. Our previous study showed that H3K79 methylation is a key feature of reversing silencing in subtelomeric regions [Kitada et al. 2012]. We now demonstrate that heterochromatin can enhance Ino80C binding to prevent H3K79 methylation from invading into it. In principle, this binding works to enforce the silencing effect of the Sir complex within portions of heterochromatin where Sir3 levels are low. Collectively, these data suggest that Ino80C functions with the Sir proteins in some regions of heterochromatin to maintain silencing. Furthermore, Ino80C also directly blocks Dot1 in vitro and in vivo at silent regions flanking genes. Taken together, our in vitro and in vivo data argue that Ino80C globally inhibits H3K79
methyltransferase and any of its causative influences or consequences.

Materials and methods

Chromatin assembly and modification

The 601-containing nucleosomal template was prepared as described (Kuryan et al. 2012). His-Dot1 was purified using Talon beads and quantified via Coomassie blue staining. Increasing molar ratios of His-Dot1 and Ino80C were incubated for 2 hr at 30°C (Kuryan et al. 2012), diluted into methylation reaction buffer [10 mM Tris-Cl at pH 8, 5 mM MgCl2, 50 mM NaCl, 0.1 mg/ml BSA, 1 mM DTT, 80 μM S-adenosyl-methionine], incubated for 1 hr, and immunoblotted for H3K79me3 (antibody from the Grunstein laboratory), total Histone H3 [ab1791], and TAP-tagged Arp5 and Dot1 (Sigma, H1029).

ChIP-PCR

ChiP of Ino80-13myc was performed as described (Kitada et al. 2012) using anti-myc 9E10 from Millipore. Immunoprecipitated DNA was decross-linked overnight at 65°C. Each ChiP experiment was performed on a minimum of three biological replicates. Real-time quantitative PCR (qPCR) was conducted using a Stratagene MX3000P thermal cycler and a SYBR Green qPCR mix with ROX using Roche FastStart Taq DNA polymerase.

Histone modification

Histone modiﬁcations were evaluated using ChiP-Seq. ChiP-seq libraries were prepared from experimental and control samples. DNA was sheared to 200 bp using the Covaris M220, and ChiP-seq libraries were prepared using the NEBNext Ultra II kit. Adapters were added, and the libraries were purified using Agencourt AMPure XP beads. The libraries were size-selected using a Bio Stamp press at 150 bp, and the DNA was eluted and amplified using the NEBNext Ultra II DNA Library Kit. Duplex libraries were prepared using the SureSelect Human All Exon plus UTR Kit, and the libraries were validated by Agilent Bioanalyzer and Qubit measurement. Libraries were sequenced on an Illumina HiSeq 2500. The reads were aligned with BWA MEM to the S. cerevisiae genome, and mappings were generated using SnpEff.

RNA-seq

Libraries of mRNA were prepared with Illumina TrueSeq RNA sample preparation kit version 2 or stranded RNA sample preparation kit. Libraries were sequenced, and reads aligned as above, excluding an identical region between HML and HMR, using TopHat 2.0.8 with option -g 1 and -N 0 (Trapnell et al. 2009). Gene transcription levels were normalized to FPKM (fragments per kilobase of exon per million fragments mapped) using Cuffdiff 2.0.2 (Trapnell et al. 2010). For log2 ratio and log FPKM calculations, all transcripts with zero FPKM were replaced with 0.1. Mapped reads in the mutants were also normalized to reads in the wild type using a custom script. Because we were mapping changes in gene expression, windows without any overlapping reads in the wild-type cells were replaced with 0.1 for further treatment of the mutant expression patterns. The log2 ratios of mutants versus wild type at all windows were plotted against the distance from telomere ends or metagenes with a custom script. The data of the Arp5-enriched region with Sitepro 0.6.6 (Shin et al. 2009).

Purification of yeast proteins

Ino80C was puriﬁed from the TAP-tagged S. cerevisiae library as previously described (Li et al. 2003). The Sir proteins were puriﬁed as described (Kitada et al. 2012).

Immobilized template assay

Immobilized template assays were performed as described (Kitada et al. 2012) using G5E4T assembled into chromatin (Lin et al. 2011, Kuryan et al. 2012) and incubated with either Ino80C or a mix of Sir3 and Sir2/4 in 25 μl of binding buffer (100 mM KOAc, 20 mM HEPEs at pH 7.6, 1 mM EDTA, 10% glycerol) for 1 hr at 30°C with a subsequent 45-min incubation of the second protein. The beads were washed three times with 1 ml of binding buffer. Proteins were eluted in 200 μl of 50 mM Tris (pH 8.5) and 7 M urea, immunoblotted, and scanned using an Odyssey imaging system.

Acknowledgments

This work was supported by National Institutes of Heath grants R01 GM074701 and GM085002 to M.E.C., GM54096 to C.L.P., and CA178415 to S.K.K.

References


Ino80 transcriptional silencing


# The Ino80 complex prevents invasion of euchromatin into silent chromatin

Yong Xue, Christopher Van, Suman K. Pradhan, et al.

*Genes Dev.* 2015 29: 350-355  
Access the most recent version at doi:10.1101/gad.256255.114

<table>
<thead>
<tr>
<th><strong>Supplemental Material</strong></th>
<th><a href="http://genesdev.cshlp.org/content/suppl/2015/02/17/29.4.350.DC1.html">http://genesdev.cshlp.org/content/suppl/2015/02/17/29.4.350.DC1.html</a></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>References</strong></td>
<td>This article cites 41 articles, 18 of which can be accessed free at: <a href="http://genesdev.cshlp.org/content/29/4/350.full.html#ref-list-1">http://genesdev.cshlp.org/content/29/4/350.full.html#ref-list-1</a></td>
</tr>
<tr>
<td><strong>Creative Commons License</strong></td>
<td>This article is distributed exclusively by Cold Spring Harbor Laboratory Press for the first six months after the full-issue publication date (see <a href="http://genesdev.cshlp.org/site/misc/terms.xhtml">http://genesdev.cshlp.org/site/misc/terms.xhtml</a>). After six months, it is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at <a href="http://creativecommons.org/licenses/by-nc/4.0/">http://creativecommons.org/licenses/by-nc/4.0/</a>.</td>
</tr>
<tr>
<td><strong>Email Alerting Service</strong></td>
<td>Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or <a href="http://genesdev.cshlp.org/subscriptions">click here</a>.</td>
</tr>
</tbody>
</table>

---

Finally! Antibodies you can trust. Unparalleled ChIP & ChIP-seq with rigorously validated antibodies: **diagenode**

Learn more: [http://genesdev.cshlp.org/subscriptions](http://genesdev.cshlp.org/subscriptions)

© 2015 Xue et al.; Published by Cold Spring Harbor Laboratory Press