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## Genomic nucleosome organization reconstituted with pure proteins

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### Summary

Chromatin remodelers regulate genes by organizing nucleosomes around promoters, but their individual contributions are obfuscated by the complex in vivo milieu of factor redundancy and indirect effects. Genome-wide reconstitution of promoter nucleosome organization with purified proteins resolves this problem and is therefore a critical goal. Here we reconstitute four stages of nucleosome architecture using purified components: Yeast genomic DNA, histones, sequence-specific Abf1/Reb1, and remodelers RSC, ISW2, INO80, and ISW1a. We identify direct, specific and sufficient contributions that in vivo observations validate. First, RSC clears promoters by translating poly(dA:dT) into directional nucleosome removal. Second, partial redundancy is recapitulated where INO80 alone, or ISW2 at Abf1/Reb1 sites, positions +1 nucleosomes. Third, INO80 and ISW2 each align downstream nucleosomal arrays. Fourth, ISW1a tightens the spacing to canonical repeat lengths. Such a minimal set of rules and proteins establishes core mechanisms by which promoter chromatin architecture arises through a blend of redundancy and specialization.

### Graphical abstract

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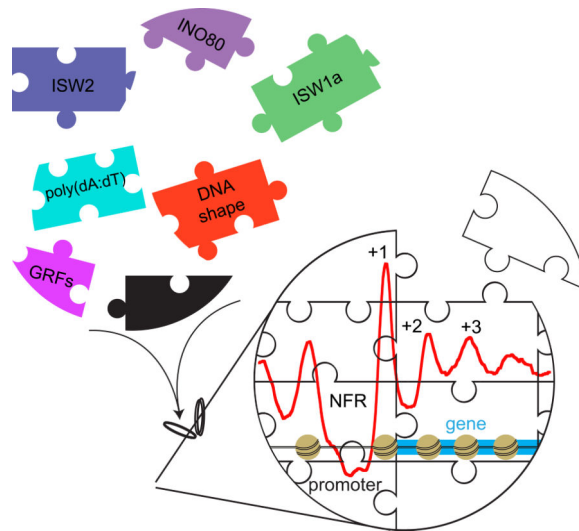
#### Author Contributions

N.K. performed the experiments, M.W. performed the bioinformatic analysis, S.W. purified the remodelers, B.P. mapped and managed the raw data, C.L.P. supervised the remodeler purification, B.F.P. and P.K. conceived and supervised the project and co-wrote the manuscript with contributions from N.K. and M.W.

#### Supplemental Information

Figures S1-S5

Tables S1-S3



## Keywords

chromatin; nucleosome positioning and remodeling; in vitro reconstitution; *Saccharomyces cerevisiae*; Isw; Chd; INO80; RSC; general regulatory factors (GRFs); Abf1; Reb1

## Introduction

Nucleosomes are the fundamental repeating unit of chromatin (Kornberg and Lorch, 1999). The precise positioning and unique composition of nucleosomes at transcriptional start sites (TSSs) regulate gene expression (Jiang and Pugh, 2009b). Immediately upstream of these +1 nucleosomes, relative to the direction of transcription, often resides a nucleosome-free promoter region (NFR). Downstream, genic nucleosomes align to the +1 nucleosome with regular spacing to form arrays. In metazoans, the TSS of active genes resides about 30-50 bp upstream of the +1 nucleosome edge (Schones et al., 2008), whereas in single-celled organisms like yeast the TSS resides just inside the nucleosome border (Tsankov et al., 2010). Dysregulation of chromatin is common in cancer cells (Wang et al., 2007), and so understanding the processes (i.e., a “parts list” and instructions) by which nucleosomes become organized into chromatin may provide insights into oncogenic mis-regulation of genes.

As with all massive cellular structures, a reasonable assumption is that proper nucleosome positioning across entire genomes arises from inordinately complex cellular processes. Indeed, no successful attempt has been made to reconstitute physiological nucleosome positions on a genomic scale with pure proteins. Nonetheless, many cellular structures and processes, like ribosomes, metabolic pathways or DNA replication, are able to self-organize from their constituents thus making their in vitro reconstitution possible. This allows individual assembly stages to be dissected, defined, and the role of individual factors deciphered. Current approaches involving in vivo removal or inactivation of one or a few nucleosome-related activities, for example chromatin remodeling enzymes, via genetic manipulation (Badis et al., 2008; Ganguli et al., 2014; Gkikopoulos et al., 2011; Hartley and

Madhani, 2009; Ocampo et al., 2016; Parnell et al., 2008; van Bakel et al., 2013; Whitehouse et al., 2007; Yen et al., 2012) have proven insightful for identifying factors involved, their contribution, and their genomic binding locations. Critically, however, direct versus indirect roles are not readily apparent due to the ever-present complex milieu of cellular proteins, and so cannot identify the minimal set of components and core mechanisms that directly establish the primary structure of chromatin.

Here, we take a biochemical approach towards reconstituting and understanding the basic pattern of physiological nucleosome positioning, simultaneously at >4,000 genes from budding yeast (*Saccharomyces cerevisiae*), using purified proteins. Importantly, our findings are validated by recapitulation of known in vivo effects involving mutants, but we go beyond in vivo approaches as we demonstrate which remodeler contributions are direct, sufficient and specific. We consolidate our observations into four biochemically identifiable architectural building stages regarding nucleosome organization at the 5' ends of genes, and identify the minimal sets of factors required to achieve each stage: 1) NFR formation, 2) +1 positioning, 3) downstream array alignment, and 4) physiological spacing. This provides a base framework by which the primary structure of chromatin self-organizes through the interplay of DNA sequence, histones, organizing factors, and chromatin remodelers, upon which other processes like transcription may work.

## Results

The success of in vitro reconstitution critically hinges on how closely the result matches its in vivo counterpart. A variety of metrics describes the extent to which reconstituted nucleosome organization across a genome coincides with the in vivo positions. These include NFR width and depth, nucleosome dyad location and fuzziness, and distance between adjacent nucleosomes (spacing). This, coupled to the combinatorial reconstitution with up to six factors (including remodelers and nucleosome organizing factors (called general regulatory factors or GRFs (Fourel et al., 2002))), presents a multi-dimensional graphical challenge wherein a separate representation of each of these metric risks losing contextual relationships with other linked metrics. Therefore, our analysis was conducted with three levels of abstraction: 1) Heat maps of individual genes aligned by a native feature whose reconstitution was being examined, and sorted by the relevant metric in the reference situation (e.g., intrinsic positioning by salt gradient dialysis), thereby preserving contextual relationships of metrics but being less quantitative; 2) An averaged profile, which provides a simpler more quantitative view, while preserving some context; 3) A quantitative assessment of individual metrics (Tables S1 and S2), but lacking in context, and so must be interpreted with caution.

### Purified Remodelers Reconstitute Genome-wide Nucleosome Organization

During salt gradient dialysis (SGD) of purified histones and genomic DNA, nucleosomes will assemble across genes at intrinsically preferred positions. Some will be positioned at their proper physiological locations, but most will not (graphs 1 vs. 2 in Figure 1A showing individual genes, Figure 1B showing gene averages, Figure 1C and Table S1 showing quantitated positions relative to Native, and Table S2 showing +1 nucleosome positioning

strength which is the reciprocal of fuzziness) (Kaplan et al., 2009; Zhang et al., 2009; Zhang et al., 2011). Less assembly occurs within promoters, which partially mimics the NFRs seen in vivo. As previously demonstrated (Zhang et al., 2011), addition of ATP and a crude whole cell extract having essentially a complex and undefined mixture of proteins, reconstitutes the basic pattern of canonically positioned NFRs, +1 nucleosomes, and arrays mainly downstream on a genomic scale (Figures 1A and 1B, graph 3). The +1 position was reconstituted to within 5 bp of the native position for essentially all (N=4,118) measured genes (Figure 1C), and with high precision (i.e., positioning strength at least as well as seen for Native, Table S2). However, there was some imprecision in the downstream arrays.

To gain confidence that proper chromatin organization could be reconstituted on a genome-wide scale with purified histones, DNA, and individual active chromatin remodelers (Figure S1 and Table S3), we first performed reconstitutions in extracts from *isw1 isw2 chd1* cells missing a large number of remodeler ATPases, i.e., they lacked four (ISW1a, ISW1b, ISW2, and Chd1) of the major chromatin-remodeling complexes in yeast (Flaus et al., 2006). We then added back purified remodelers. Inasmuch as different remodelers have distinct remodeling activities (Bartholomew, 2014), the relative amounts added were based on ATPase activity units, which all remodelers have in common. Similar to the respective in vivo phenotype (Gkikopoulos et al., 2011; Ocampo et al., 2016), this mutant extract failed to form well-aligned arrays (Figures 1A and 1B, graphs 3 vs. 4), although NFRs and +1 nucleosomes were largely reconstituted but with somewhat less accuracy (peak position, Figure 1C, Table S1) and precision (positioning strength, Table S2). Thus, formation of the NFR and +1 nucleosome is biochemically separable from array formation/alignment, and the former can be achieved in extracts without ISW1/2/Chd1 remodelers.

Importantly, adding back purified ISW2 improved the accuracy and precision of +1 reconstitution (Figures 1A-1C graphs 4 vs. 9a; Figure S2A, graphs 4 vs. 9a, b; and Table S2). ISW1a also had an effect (Figures 1A-1C graphs 8a and Figure S2A, graphs 8a, b) but to a lesser extent (based on relative +1 peak height in Figures 1B and S2A, and positioning strength in Table S2). Additionally, more regularly-spaced nucleosomes were aligned just downstream of the +1 nucleosome, akin to wild type extracts (Figures 1A and 1B, graphs 8a vs. 3; Figure S2A, graphs 8a, b). Strikingly, each remodeler generated a different, characteristic inter-nucleosomal repeat length: equivalent to Native (ca. 170 bp) for ISW1a, but up to 30 bp longer for ISW2 (Table S1), which is about the lower limit of what ISW2 requires for sliding nucleosomes and is consistent with previous observations (Kagalwala et al., 2004; Tsukiyama et al., 1999; Vary et al., 2003). When ISW1a was added together with ISW2 or other remodelers, ISW1a dominated in a concentration dependent manner, thereby creating more properly spaced nucleosomes to the extent seen with ISW1a added alone (Figures 1A and 1B, graph 10a, Figure S2A, graphs 10a, b, Figure S2B, graphs 10c-g and Figure S2C, graphs 5-7). The dominance of ISW1a may be explained by its utilization of shorter linkers compared to ISW2 (Gangaraju and Bartholomew, 2007a; Kagalwala et al., 2004); i.e., ISW1a may create short linkers that are poorer substrates for ISW2. That ISW1a generated tighter spacing than ISW2 from the same batch of chromatin is consistent with the “clamping activity” of ISWI type remodelers (Lieleg et al., 2015); i.e., these remodelers set a certain constant linker length independent of nucleosome density rather than equalize linker lengths according to nucleosome density.

Addition of purified RSC (which presumably was not depleted in the *isw1 isw2 chd1* extract), ISW1b or Chd1 did not reconstitute aligned arrays (Figures 1A and 1B, graphs 4 vs. 5-7), despite these remodelers being active (Figures S1B and S1C). This is consistent with RSC and ISW1b having nonexistent or weak nucleosome spacing activity in vitro (Gangaraju and Bartholomew, 2007a, b; Tsukiyama et al., 1999). RSC also antagonized +1 positioning by the mutant extract (Figures 1A and 1B, graphs 4 vs. 5, Tables S1 and S2), which fits in vivo observations (Parnell et al., 2015). The remodeling activities of both ISW1b (Smolle et al., 2012) and Chd1 (Lee et al., 2012; Park et al., 2014; Simic et al., 2003) have been linked to transcription, which was not reconstituted here, and may be mainly relevant in vivo for re-establishing nucleosomal arrays in the wake of RNA polymerase.

We conclude that in vivo-like basic nucleosome organization can be reconstituted across much of the 5' ends of yeast genes using purified remodelers in the context of a deficient crude extract. Remarkably, the distinct genome-wide nucleosome organizing activities in building NFR/+1/array patterns become evident for ISW1a and ISW2.

### RSC Recognizes the Directionality of Poly(dA:dT) to Make NFRs

We next moved to reconstitutions with purified components only. First, we examined the impact of individual purified remodelers on intrinsic genome-wide nucleosome organization in the absence of crude extracts. With the notable exception of INO80, which we address further below, none of the remodelers created +1 nucleosomes or even +1 aligned arrays on their own (Figure 2A, graphs 4-9). However, all remodelers widened the intrinsically weak NFR, with RSC generating NFRs of at least physiological average widths (Figure 2B, and Figure S3A). Surprisingly, this was not accompanied by canonical -1/+1 nucleosome positioning, which define NFR widths (Figure 2A, graph 5, Table S2). Thus, RSC on its own clears nucleosomes out of the NFR without the accuracy expected of canonical -1/+1 nucleosome positioning. This indicates that NFR clearance is biochemically distinct from canonical -1/+1 nucleosome positioning and array generation/alignment.

How does RSC on its own specifically target promoters (Lorch et al., 2011) so as to create NFRs? The Rsc3/30 subunits of the RSC complex may impart sequence specific DNA binding (Badis et al., 2008), but we did not observe enhanced NFR formation at their cognate CGCGCGG motifs located within promoters (Figure S3B). Another possibility is that poly(dA:dT) tracts present in promoters partially destabilize nucleosomes, directly or indirectly, as has been widely demonstrated in vivo (Hartley and Madhani, 2009; Iyer and Struhl, 1995; Kaplan et al., 2009; Segal and Widom, 2009; Sekinger et al., 2005; Struhl and Segal, 2013; Zhang et al., 2009). RSC might sample all nucleosomes but more effectively remove promoter nucleosomes because they are intrinsically unstable. Alternatively, nucleosome instability might play a minimal role in NFR formation in vivo, but instead poly(dA:dT) might directly activate RSC to displace nucleosomes (Kubik et al., 2015; Lorch et al., 2014). Since RSC translocates along one DNA strand in the 3'-5' direction (Saha et al., 2005), poly(dA) or poly(dT) on one strand could represent distinct directional signals for nucleosome removal by RSC. This concept has not been previously considered for RSC but could be tested now in the context of our data. Indeed, RSC led to greater nucleosome displacement (i.e., sliding or eviction) 5' of poly(dA) compared to its 3' side (and vice versa

for poly(dT)), by ~30 bp (Figure 2C). Asymmetric NFR widening did not occur with other remodelers, and only to a much lesser degree with SWI/SNF, which is highly related to RSC (Flaus et al., 2006) (Figure S3A). This suggests a mechanism whereby RSC, but not other remodelers, translates poly(dA:dT) orientation into directional displacement of nucleosomes (Figure 2D). Consequently, the canonical or average location of poly(dT) would be ~30 bp upstream of poly(dA) when genes are aligned at their +1 nucleosomes (Figure 2A, graph 2, and Figure S3C), as has been observed in vivo but has not been previously linked to directional action of RSC (de Boer and Hughes, 2014; Wu and Li, 2010).

As shown below, RSC may also be guided by GRFs like Abf1 and Reb1 (Hartley and Madhani, 2009; Kubik et al., 2015), whose binding sites occur on average at the same distance from +1 nucleosomes as poly(dT) elements (Figure S3C). So NFR formation need not involve poly(dA:dT) elements but can also be driven by DNA binding factors like GRFs, which may explain NFR formation in vivo on heterologous DNA regions without poly(dA:dT) (Hughes et al., 2012). Collectively, we suggest that RSC creates NFRs by recognizing poly(dA:dT) sequence elements and/or GRF-bound sites that make up organizing centers.

### **INO80 on its Own Properly Positions +1 Nucleosomes**

Since RSC and SWI/SNF did not position +1 nucleosomes at canonical locations (Figure 2A, graphs 4, 5), the positioning observed near +1 with *isw1 isw2 chd1* extracts alone (Figures 1A-1C, graph 4, Tables S1 and S2) may involve another remodeler present in the extract. As the INO80 complex was the only major chromatin remodeler with sliding activity remaining, we examined its ability to position +1 on its own. Strikingly, INO80 produced a highly accurate and robust -1/NFR/+1 organization at most genes in the complete absence of other factors besides DNA and histones (Figures 2A, graph 10, 2B, 3A and 3B, graph 4, Tables S1 and S2). This is remarkable because out of ~10 Mbp of yeast genomic DNA, INO80 selected the physiologically proper -1/+1 positions for nucleosome placement at most genes, in addition to excluding nucleosomes from most NFRs. As discussed below, these are the most critical chromatin structures at yeast genes and a global role of INO80 there concurs with its presence at >90% of all +1 nucleosomes in yeast (Yen et al., 2013). Importantly, the accuracy (0 bp difference in peak location compared to Native, Table S1) and also somewhat the precision (Table S2) of the reconstitution were similar to what is observed in vivo. In a control experiment, these effects were not observed with INO80 lacking its critical Arp8 subunit (Figures 3A and 3B, graph 3, Tables S1 and S2), and were largely unaffected by addition of other remodelers (Figures 3A and 3B, graphs 4 vs. 5, 6, Tables S1 and S2). Thus, INO80 is the only remodeler that we are aware of with an innate ability to position most +1 and -1 nucleosomes at their in vivo canonical location.

How does INO80 on its own position +1 nucleosomes at specific sequences? Since MEME analysis failed to detect a DNA motif enriched near +1, beyond promoter-associated poly(dA:dT), we examined DNA shape properties. As rather different DNA sequences may adopt similar DNA shapes, shared shape properties are different from classical consensus motifs. Genes were sorted by their fold enhancement of in vivo-like +1 positioning imparted by INO80 over the SGD chromatin (i.e., ratio of values in graph 4 to graph 2 in Figure 3A as

a measure of “INO80 effectiveness” in +1 positioning). This helps distinguish if +1 nucleosomes were properly positioned mainly by INO80 or mainly by SGD or by a combination of both. Such sorted genes were sectioned into quartiles (Q1-Q4). Consequently, Q1 generally represents intrinsically positioned +1 nucleosomes that were scattered by INO80, whereas Q4 represents intrinsically scattered nucleosomes that became positioned by INO80. Q2 and Q3 were intermediate. We first examined nucleosome positioning sequences (NPSs) within these quartiles. NPSs have strand-specific 10 bp periodicities of AA dinucleotides concentrated near nucleosome 5' ends (Ioshikhes et al., 2006; Mavrich et al., 2008). NPSs were overall enriched at +1 nucleosomes (Figure 3A, graph 7 and Figure 3C, red traces). However, they were stronger where intrinsic +1 positioning was generally the weakest and INO80 effectiveness was the greatest (Figure 3C, Q4 red vs. grey and violet traces). Thus, the NPSs in Q4 (and Q3) appeared to be ineffective, on average, for intrinsic proper positioning in SGD material, but became relevant with INO80 present.

We next examined DNA shape using shape analysis software (Zhou et al., 2013). Q4 genes in the average +1 region were calculated to be intrinsically (i.e., as free DNA) over-twisted relative to Q1-Q3 gene averages, all of which also trended directly with INO80 effectiveness in +1 positioning (Figure 3D, green Q4 trace; and Figures S4A-S4C for other linked DNA properties). Speculatively, this intrinsic over-twist at the +1 nucleosome position in Q4 might disfavor nucleosome formation (as seen in the NFRs of all quartiles, Figure 3D) despite the presence of strong NPSs, whereas the intrinsic twist at Q1 +1 might favor nucleosome formation even with weaker NPSs (Figure 3C, grey Q1 plot). If INO80 slightly untwists +1 DNA, it might make the NPS more (Q4) or less (Q1) effective (Figure 3E). This interpretation is based on correlations, which may not reflect causation, and so we emphasize the speculative nature of this hypothesis. Nonetheless, it offers a conceptual framework to rationalize INO80's innate ability to properly position +1 nucleosomes without apparent sequence specificity. Taken more generally, we observed some positioning of the +2 (and very weakly of +3) nucleosomes by INO80, albeit with inappropriately wide spacing (Figures 3A and 3B, graph 4, Figures S4D and S4E, Table S1). If genes were sorted according to the INO80 effectiveness of proper +2 or +3 positioning the intrinsic twist of the underlying DNA trended again with these effectivenesses (Figures S4F and S4G). Conversely, this average DNA shape trend between the quartiles was essentially nonexistent for regions not sorted by INO80 effectiveness (e.g., right side of Figure 3D).

INO80 alone increased the uniformity of +1/+2 spacing that was wider than seen in vivo (Figures 2A, 3A and 3B). This confirms on a genome-wide scale that INO80 has spacing activity (Udugama et al., 2011), but generating non-canonical repeat lengths (up to 205 bp, Table S1), and supports the in vivo observation that INO80 mutants have narrower spacing (Yen et al., 2012). The downstream spacing imparted by INO80 was diminished upon addition of other purified remodelers including ISW1a (Figures 3A and 3B, graphs 4 vs. 5, 6; Table S1), indicating that remodelers interfere with each other. However, addition of *isw1 isw2 chd1* mutant extract, which by itself did not create positioned arrays, restored spacing (Figures 4A, graphs 4 vs. 5a and 4B, graphs 4 vs. 5a, b Tables S1 and S2). As for the too wide spacing generated by ISW2 in the presence of the extract (Figures 1A and 1B), the spacing generated by INO80 was also narrowed to physiological repeat lengths upon further addition of ISW1a (Figures 4A, graphs 4 vs. 6a and 4B, graphs 4 vs. 6a, b Tables S1 and

S2). This suggests that an unknown factor(s) remaining in the *isw1 isw2 chd1* mutant extract allows INO80 to make +1-aligned downstream arrays that can be properly spaced by ISW1a. Combining INO80 and ISW1a in a purified system lacking this factor did not generate physiological spacing (Figures 4A, graph 6a and 4B, graphs 6a, b versus Figures 3A and 3B, graphs 5, 6; Tables S1 and S2). Collectively, these data show that INO80 by itself creates a physiological -1/NFR/+1 architecture, against which it can uniformly pack downstream nucleosomes. But proper spacing requires additional known (ISW1a) and unknown factors.

### Gene-specific GRFs and Remodelers Suffice to Organize Nucleosomal Arrays

The canonical array development observed with the addition of extracts, but not with remodelers alone, implicates the requirement of additional factors. We therefore purified and tested Abf1 and Reb1 (Figure S1). These GRFs are implicated in global nucleosome organization in vivo (Badis et al., 2008; Hartley and Madhani, 2009; Kubik et al., 2015; Raisner et al., 2005; Tsankov et al., 2010; van Bakel et al., 2013). Abf1 together with either ISW2 or ISW1a (or both) selectively and properly positioned +1 nucleosomes at Abf1-bound genes (as defined in vivo in rich media), and partially developed aligned nucleosome arrays having remodeler-specific spacing (Figure 5A, graphs 9a-11a and Figures S5A, graphs 9a-11a and S5B, graphs 9a-j, 10a-f, 11a-f, Tables S1 and S2). Other genes (Figure 5B), other remodelers (Figure 5A, graphs 6-8a and Figures S5A, graphs 6-8a and S5B, graphs 8a-c), or Abf1 alone (Figure 5A and Figure S5A, graph 5) on average lacked equivalent effects (Tables S1 and S2). Thus, a barrier like Abf1 is insufficient to organize nucleosomes on its own, but requires specific – not just any – remodelers. Interestingly, ISW1a and ISW2 generated well-positioned +1 nucleosomes on average ~10 bp upstream of the expected native location (Figure 5A, inset graphs 9a-11a, Table S1), which is a repressive position over the TSS, as observed in vivo (Parnell et al., 2015; Reja et al., 2015; Shivaswamy et al., 2008; Whitehouse et al., 2007). Thus, Abf1 and either ISW2 or to a lesser extent ISW1a (based on +1 peak height in Figure 5A, graphs 9a vs. 10a, and on +1 positioning strength in Table S2), suffice to position +1 nucleosomes into a proper repressive position in a gene-selective manner.

When RSC was included in the reaction with Abf1, ISW2 and ISW1a (and also ISW1b and Chd1, which have yet to display organizing activity in our assays and thus are presumed to be largely neutral), nucleosomes at Abf1-bound genes were placed at +1 in their canonical activating position, and aligned into mainly downstream arrays (Figure 5A, main and inset graphs 12a vs. 9a-11a, and Figures S5A, graph 12a and S5B graphs 12a, b). The accuracy and precision at +1 for Abf1-bound genes was similar as seen with Native chromatin (Tables S1 and S2). We surmise that the additional NFR clearance provided by RSC (Figure 2), which was even more pronounced in the presence of Abf1 (Figures 5A and 5B, graphs 8a vs. 3 and Figures S5A, graphs 8a vs. 3 and S5B, graphs 8a-c), contributed to ISW2 and ISW1a placing +1 into an activating position. This concurs with in vivo observations (Parnell et al., 2015). As INO80 did not cooperate with ISW1a in the purified system (Figures 3A and 3B, graphs 5, 6) we did not include INO80 in the reconstitutions with combinations of only purified remodelers and GRFs.



We conclude that Abf1, RSC, ISW1a and ISW2 suffice to produce in vivo-like nucleosome positioning that is relevant to gene regulation. They do so by toggling the +1 nucleosome into repressive and activating positions over the TSS, with RSC and the ISWI remodelers playing opposing roles consistent with in vivo findings (Parnell et al., 2015). When Reb1 was used in place of Abf1, similar results were obtained, but at Reb1-bound genes (Figure 6, Tables S1 and S2), including moving the +1 position dependent on the relative concentrations of ISW2/ISW1a versus RSC (Figure 6B, left, graphs 7, 8 versus 9, 10; Figure 6C, graphs 9a-c, Table S1). Thus, more generally, remodelers, GRFs, DNA sequence and histones suffice to provide gene specificity and regulation for the basic pattern of nucleosome organization at the 5' ends of genes.

## Discussion

### Four-stage Mechanism of Genomic Nucleosome Organization

The key conclusion of this study is that combinations of purified remodelers, GRFs, histones, and DNA sequence constitute a sufficient minimal system to create the basic physiological NFR/+1/array pattern at the 5' ends of yeast genes. This resonates with in vivo derived notions that remodelers are important for nucleosome positioning (Badis et al., 2008; Ganguli et al., 2014; Gkikopoulos et al., 2011; Hartley and Madhani, 2009; Ocampo et al., 2016; Parnell et al., 2008; van Bakel et al., 2013), via dynamic competition of different remodeling activities (Ganguli et al., 2014; Ocampo et al., 2016; Parnell et al., 2015; Yen et al., 2012), but now establishes their direct, specific and sufficient contributions.

Some of the in vitro patterns, especially the genic arrays, are not as robust or extensive as seen with native chromatin, and thus implicate additional factors and/or technical limitations. Nonetheless, we underscore the highly accurate reconstitution of the core -1/NFR/+1 architectures. These are the functionally most relevant features in vivo as there is no viable mutant known, only conditional mutants (Badis et al., 2008; Ganguli et al., 2014; Hartley and Madhani, 2009; Parnell et al., 2008; van Bakel et al., 2013), where these features were substantially impaired for most genes. In contrast, there are viable mutants with globally disrupted genic arrays (Gkikopoulos et al., 2011; Lee et al., 2012; Ocampo et al., 2016; van Bakel et al., 2013). For subsets of genes, the exact +1 position in vivo may slightly change according to gene expression state (activated or repressed, (Reja et al., 2015; Shivaswamy et al., 2008)) or in mutants (Parnell et al., 2015; van Bakel et al., 2013; Whitehouse et al., 2007; Yen et al., 2012). This range of +1 positions is reconstituted here by varying relative contributions of RSC versus ISW2/ISW1a, but may also involve transcription-linked mechanisms in vivo (Hughes et al., 2012; Weiner et al., 2010).

Our observations distill into a four-stage framework for conceptualizing how arrays become organized at the 5' ends of genes (Figure 7). We imply mechanistic contributions but not necessarily a temporal order. In stage 1, RSC translates the directionality of poly(dA)/(dT) tracts (option 1) into directionally-biased nucleosome displacement. RSC may also use GRFs bound at locations equivalent to those of poly(dA)/(dT) elements (option 2) for NFR generation. Both options do not lead to canonical positioning of flanking +1/-1 nucleosomes, and indeed may be rather disruptive. In agreement, ablation of RSC, Abf1 or Reb1 in vivo leads to NFR collapse (Badis et al., 2008; Ganguli et al., 2014; Hartley and

Madhani, 2009; Parnell et al., 2008; van Bakel et al., 2013). While poly(dA)/(dT) appears to be a direct effector of RSC, we do not exclude additional direct effects on nucleosome stability.

In stage 2, GRFs help set the register of +1 by serving as a base or barrier utilized by ISW2 and/or ISW1a (Li et al., 2015; McKnight et al., 2016) (options 1 and 2). Although their contributions appeared somewhat redundant, +1 positioning at GRFs was more pronounced for ISW2. This might involve a “ruler” function (Yamada et al., 2011) to measure out a precise distance against which the +1 nucleosome is packed into a default repressive position over the TSS, as seen in our data. In vivo studies support this notion (Whitehouse et al., 2007; Yen et al., 2012; Yen et al., 2013). Concurrent with this basic process is a dynamic interplay of multiple remodelers, including RSC (Parnell et al., 2015), that positionally adjusts +1 into a TSS-accessible activating position located on average 10-20 bp further downstream. Controlling the balance between active and repressive positions by varying the contribution of different remodelers may help regulate gene expression (Reja et al., 2015).

A third stage-2 option in the positioning of +1 nucleosomes involves INO80 (option 3) acting alone (in principle), probably after recognizing the adjacent NFR (Yen et al., 2013). In our conceptual framework, INO80 then locally untwists DNA that is otherwise over-twisted and thus less favorable for +1 formation, so that it now works in concert with other positioning sequences (NPSs) to properly position the +1 nucleosome. At the other extreme, untwisting properly-twisted DNA might delocalize +1, even counteracting otherwise intrinsically effective NPSs. The proper placement of +1 and also -1 nucleosomes by INO80 amounts to a third option also for NFR generation, i.e. stage 1. Our finding that RSC and INO80 generate NFRs and that INO80 also positions +1 nucleosomes may explain why these two features remained mostly unaffected in the *isw1 isw2 chd1* triple mutant, which retains these remodelers (Gkikopoulos et al., 2011; Ocampo et al., 2016). Redundancy in the mechanisms for NFR formation also explains why RSC ablation in vivo affects only about half of the NFRs (Hartley and Madhani, 2009).

In stage 3, ISW2 and/or INO80 (options 1 and 2) may create nucleosomal arrays aligned downstream to +1 with long linkers. In stage 4, these linkers are recognized by ISW1a and turned into short linkers of physiological lengths. This fits with the notion of ISW1a being able to make short linkers (Gangaraju and Bartholomew, 2007a), maybe due to a short “protein ruler” (Yamada et al., 2011) domain. A dominant role of *Isw1* in spacing concurs with in vivo findings (Ocampo et al., 2016).

While transcription is not reconstituted in our systems and thus is not likely to be required for establishing the ground state organizational pattern, we fully expect that transcription initiation and elongation modulates nucleosome positioning (Hughes et al., 2012; Ocampo et al., 2016; Struhl and Segal, 2013; Weiner et al., 2010). For example, transcription may bring remodelers, e.g., Chd1 and ISW1b (Lee et al., 2012; Park et al., 2014; Simic et al., 2003; Smolle et al., 2012), deeper into genes thus extending genic arrays much further in the direction of transcription, which we do not reconstitute here. Similarly, we do not address the impact of histone modifications or variants, which are likely to contribute in vivo, too, but do not seem to be essential for generating the basic nucleosome organization.

We suggest that genome-utilizing processes like transcription or replication act on a primarily self-organizing ground state of remodeler-driven nucleosome positioning. This basic nucleosome organization at the 5' ends of genes follows a definable set of rules that are implemented through several options and essentially depend on direct and specific remodeler contributions (Figure 7). This provides redundancy, robustness, and a means to independently regulate NFRs, +1 nucleosome positioning, and spacing within arrays. Because these remodelers are conserved across eukaryotic species, these assembly stages are likely applicable in other biological systems.

## METHODS AND RESOURCES

### Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-H3 antibody	Abcam	Abcam Cat# ab1791; RRID:AB_302613
Chemicals, Peptides, and Recombinant Proteins		
3xFlag peptide	Sigma-Aldrich	F4799
BSA	Biorad	500-0002
Ampure beads	Beckman Coulter	A63880
Proteinase K	Roche	03115801001
<i>E. coli</i> tRNA	Sigma-Aldrich	10109541001
RNaseA	Roche	10109169001
MNase	Sigma-Aldrich Aldrich	N3755-500UN
Formaldehyde	Sigma-Aldrich-Aldrich	F8775-500ML
Apyrase	NEB	M0398L
T4 DNA polymerase	NEB	M0203L
T4-PNK	NEB	M0201L
Klenow-exo	NEB	M0210L
USER™ Enzyme	NEB	M5505L
T4 Ligase	NEB	M0202L
T4 Ligase Buffer	NEB	B0202S
dNTPs	NEB	N0047S
Phusion Polymerase	NEB	M0530L
[ $\gamma$ - <sup>32</sup> P]-ATP	Perkin Elmer	BLU502Z
Magna CHIP™ Protein A Magnetic Beads	Millipore	16-661
Critical Commercial Assays		
NEBNext Adaptor	NEB	E7335L and E750L
Qubit® dsDNA HS Assay Kit	life technologies	Q32854
Freeze N Squeeze DNA Gel Extraction	Bio-Rad	732-6166
Bradford assay	Biorad	500-0002
Deposited Data		
Sequence data	This paper	<a href="http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72106">http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72106</a>
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
BY4741	EUROSCARF	<a href="http://www.euroscarf.de">www.euroscarf.de</a>
YTT227	Toshio Tsukiyama lab	Tsukiyama T et al., 1999, Genes & Dev.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
loc3-TAP=CY1875	Open Biosystems	
loc2-TAP=CY1874	Open Biosystems	
Chd1-TAP=CY1870	Open Biosystems	
Rsc2-TAP=CY1503	Open Biosystems	
Ino80-TAP=CY1868	Open Biosystems	
Isw2-2xFLAG = YTT480	Toshio Tsukiyama lab	Tsukiyama T et al., 1999, Genes & Dev.
BL21 (DE3)	Invitrogen	
BL21 (DE3) pLysS	Invitrogen	
BL21 (DE3) cd+	Stratagene	
DH5α	Invitrogen	
OregonR	Peter Becker lab	FBsn0000276
Recombinant DNA		
YCp50 yeast genomic plasmid library	via Steen Holmberg lab	Rose MD et al., 1987, Gene
601-25mer	Felix Müller-Planitz lab	Müller-Planitz F et al., 2013, Nat. Struct. Mol. Biol.
pProEx HTa A	Addgene	
pProEx HTa A -Reb1	This work	
pProEx HTa A -Abf1	This work	
Xenopus histone H2A/pET Xenopus histone H2B/pET Xenopus histone H3/pET Xenopus histone H4/pET	Karolin Luger lab	Luger K et al., 1997, J. Mol. Biol.
Sequence-Based Reagents		
primer Reb1 for CCATGGCTTCAGGTC	Eurofins	
primer Reb1 rev CTCGAGTTAATTTCTGTTTTTC	Eurofins	
primer Abf1 for CGAGGATCCCATGGACAAATTAGTCG	Eurofins	
primer Abf1 rev GTCTCGAGCTATTGACCTCTTAATTC	Eurofins	
Software and Algorithms		
BWA (version 0.6.2)	Li and Durban, 2009	<a href="http://bio-bwa.sourceforge.net/">http://bio-bwa.sourceforge.net/</a>
BWA-MEM (version 0.7.9a)	Li H, 2013 arXiv: 1303.3997v2 [q-bio.GN]	<a href="http://bio-bwa.sourceforge.net/">http://bio-bwa.sourceforge.net/</a>
GeneTrack	Albert et al., 2008	<a href="https://github.com/ialbert/chipexo">https://github.com/ialbert/chipexo</a>
Cluster 3.0	de Hoon et al., 2004	<a href="http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm">http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm</a>
Java TreeView 1.1.6r3	Saldanha AJ, 2004	<a href="http://jtreeview.sourceforge.net">jtreeview.sourceforge.net</a>
Other		
Ni-NTA Agarose	Qiagen	30210
HiTrap Heparin HP column	GE Healthcare	17-0406-01
HiTrap HP column	GE Healthcare	17-5247-01
Superdex 200 10/300 column	GE Healthcare	17-5175-01
IgG-Sepharose	GE Healthcare	17-0969-01
TEV protease	Invitrogen	12575
Calmodulin affinity resin	Agilent Technology	214303-52
VIVASPIN concentrators	Sartorius	VS0601
TLC PEI Cellulose	Millipore	1055790001
Q Sepharose	GE Healthcare	17-0510-01
SP Sepharose	GE Healthcare	17-1152-01
Superdex-200	GE Healthcare	17-1043-01

**CONTACT FOR REAGENT AND RESOURCE SHARING**—Further information and requests for reagents may be directed to, and will be fulfilled by the corresponding author Philipp Korber (pkorber@lmu.de).

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**—The yeast strains used for whole cell extract preparations were BY4741 (EUROSCARF) for wild type and YTT227 (Tsukiyama et al., 1999) for the *isw1 isw2 chd1* mutant. The histones were prepared from *Drosophila melanogaster* strain OregonR. The YCp50 yeast genomic plasmid library was transformed into and prepared from *Escherichia coli* strain DH5[.alpha]. The yeast remodelers were purified from the yeast strains CY1875 (Ioc3-TAP), CY1874 (Ioc2-TAP), CY1879 (Chd1-TAP), CY1503 (Rsc2-TAP), CY1868 (Ino80-TAP) (all from Open Biosystems) and YTT480 (ISW2-2xFLAG, Tsukiyama et al., 1999). Abf1 and Reb1 were purified from *E. coli* BL21 (DE3) cd+ (Invitrogen), and recombinant *Xenopus laevis* histones from BL21 (DE3) with or without pLysS (Invitrogen).

## METHOD DETAILS

**Plasmid library, embryonic *D. melanogaster* histones, whole cell extracts and salt gradient dialysis:** For detailed procedures of the following see also (Krietenstein et al., 2012). The plasmid library was as in (Zhang et al., 2011), i.e., derived from a library originally described in (Rose et al., 1987) where it was prepared from 10-30 kb yeast genome fragments generated by limited Sau3A digestion and ligated into the BamHI site of plasmid YCp50. We expanded the library as described (Krietenstein et al., 2012) via electroporation transformation into *E. coli* DH5 $\alpha$ , growing transformed cells first on plates and then in liquid culture, and preparing plasmid DNA (Qiagen Maxi kit). *D. melanogaster* histones were prepared from 12 h OregonR embryos as described (Krietenstein et al., 2012; Simon and Felsenfeld, 1979). Briefly, the embryos were dechorionated by hypochlorite and lysed in 15 mM HEPES-KOH, pH 7.5, 10 mM KCl, 5 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 0.5 mM EGTA, 17.5% (w/v) sucrose, 1 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium metabisulfite in a homogenizator. Nuclei were separated by centrifugation, suspended in 15 mM HEPES-KOH, pH 7.5, 10 mM KCl, 5 mM MgCl<sub>2</sub>, 0.05 mM EDTA, 0.25 mM EGTA, 1.2% (w/v) sucrose, 1 mM DTT, 0.1 mM PMSF and treated with CaCl<sub>2</sub> (3 mM final concentration) and 74 U MNase and protease inhibitors (Complete, Roche Applied Science) to mostly yield mononucleosomes. The mononucleosomes were bound to hydroxylapatite in 0.1 M potassium phosphate, pH 7.2, 0.63 M KCl, washed extensively with the same buffer and the histone octamers were eluted by 0.1 M potassium phosphate, pH 7.2, 2 M KCl. Histone containing fractions were pooled, concentrated by ultrafiltration (10 kDa MWCO), supplemented with protease inhibitors (Complete, Roche Applied Science) and 5 mM DTT, and an equal volume of 87% (v/v) glycerol was added. The histone batch used here and the corresponding full SGD assembly degree were exactly the same as described (Krietenstein et al., 2012). Briefly, 10  $\mu$ g YCp50 yeast genomic plasmid library DNA were mixed with the amount of *Drosophila* embryo histones that yields 0.9x of the highest assembly degree without precipitation and 20  $\mu$ g BSA (fraction V, Roth) in high salt buffer (10 mM Tris-HCl, pH 7.6, 2 M NaCl, 1 mM EDTA, 0.05% (w/v) IGEPAL CA630) in a dialysis mini chamber and a final volume of 100  $\mu$ l. After placing the dialysis mini chamber in 300 ml of high salt buffer plus 300  $\mu$ l of  $\beta$ -mercaptoethanol, 3 l of low salt

buffer (as high salt buffer but with only 50 mM NaCl and 300  $\mu$ l  $\beta$ -mercaptoethanol were added to the 3 l low salt buffer) were slowly added under stirring during the course of ca. 15 h using a peristaltic pump. After this the sample in the dialysis mini chamber was dialysed against 1 l low salt buffer plus 300  $\mu$ l  $\beta$ -mercaptoethanol for 1-2 h. The SGD chromatin was retrieved from the dialysis mini chamber, checked for DNA concentration by NanoDrop™ 1000 spectrophotometer (Thermo Scientific) and used directly or after storage at 4 °C in the reconstitution reactions. Whole cell extracts were prepared from logarithmically growing cells as described (Krietenstein et al., 2012; Wippo et al., 2011; Zhang et al., 2011). Briefly, logarithmically growing yeast cells (wild type strain was BY4741 and the *isw1 isw2 chd1* mutant was YTT227 (Tsukiyama et al., 1999)) were lysed under high salt conditions (200 mM HEPES-KOH, pH 7.5, 10 mM MgSO<sub>4</sub>, 20% (v/v) glycerol, 1 mM EDTA, 390 mM (NH<sub>4</sub>)SO<sub>4</sub>, 1 mM DTT) by grinding in liquid nitrogen and fractionated by ultracentrifugation. The soluble aqueous phase was concentrated by ammonium sulfate precipitation (337 mg / ml lysate), dialyzed against 20 mM HEPES-KOH, pH 7.5, 80 mM KCl, 10% glycerol, 1 mM EGTA, 5 mM DTT, 0.1 mM PMSF and stored at -80 °C.

**Purifications of chromatin remodeling enzymes:** Tandem affinity purification of ISW1a (Ioc3-TAP), ISW1b (Ioc2-TAP), Chd1 (Chd1-TAP), RSC (Rsc2-TAP), and INO80 (Ino80-TAP) was performed as follows (see also (Smith et al., 2003)). Cultures were grown in YPD media, and harvested cells were lysed in buffer E (20 mM HEPES-NaOH, pH 7.5, 350 mM NaCl, 10% glycerol, 0.1% Tween) and protease inhibitors (Leupeptin, Pepstatin, Aprotinin, and PMSF) by grinding in the presence of liquid nitrogen. Lysates were clarified at 40,000 g at 4 °C for 1 hour. Cleared lysates were incubated with IgG-Sepharose (GE Healthcare) at 4 °C for 2 hours. The sepharose was washed with buffer E and the remodelers were eluted by TEV protease (Invitrogen) cleavage. The elutions were incubated with calmodulin affinity resin (Agilent Technology) in buffer E plus 2 mM CaCl<sub>2</sub> and eluted in buffer E plus 10 mM EGTA.

ISW2 (Isw2-2xFLAG) was purified as follows: Cleared lysate was incubated with Anti-FLAG M2 affinity gel (Sigma) at 4 °C for 1 hour. The gel was washed with buffer E and the remodelers, were eluted with 0.1 mg/ml 3X FLAG peptide (Sigma). Buffer E was used during the entire purification. Purified proteins were concentrated with VIVASPIN concentrators (Sartorius) and dialyzed against buffer E with 1 mM DTT. Subunit compositions were confirmed by SDS-PAGE and mass spectrometry.

**ATPase assay of chromatin remodeling enzymes:** The ATPase activity of each remodeling enzyme was determined (see also (Smith and Peterson, 2005)) at 30 °C using 100  $\mu$ M ATP and 0.2  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P]-ATP (Perkin Elmer) in buffer A (10 mM Tris-HCl, pH 8.0, 70 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA, and 1 mM DTT). 0.1 mg/ml plasmid DNA was used as a substrate. Released phosphate was resolved from ATP by thin-layer chromatography PEI Cellulose (Millipore) in 750 mM potassium phosphate, pH 3.5. Analysis of hydrolysis rates was performed using a Molecular Dynamics PhosphorImager and Image-Quant software (GE Healthcare). ATP-hydrolysis rates were determined over three linear time points. The concentration of each remodeling enzyme was estimated relative to a standard SWI/SNF preparation.

**Purification of recombinant GRFs:** The coding sequences of Reb1 and Abf1 were amplified by PCR (primers: Reb1for CCATGGCTTCAGGTC, Reb1rev CTCGAGTTAATTTTCTGTTTTTC, Abf1 for CGAGGATCCCATGGACAAATTAGTCG, Abf1rev CGTCTCGAGCTATTGACCTCTTAATTC) from BY4741 genomic DNA and cloned into pProEx HTa A (Invitrogen) via NcoI/AvaI for Reb1 and via BamHI/HindIII for Abf1. This adds a His6-TEV tag to the N-terminus. Correct plasmid sequences were confirmed by Sanger sequencing. Expression plasmids were transformed into BL21 (DE3) cd+ cells (Stratagene). One liter LB medium with 600 mg/l ampicillin was inoculated with 20 ml of a logarithmically growing over-night culture. Cells were grown at 37 °C (Infors shaker, 120 rpm, 50 mm offset) to an OD<sub>600</sub> of 0.4-0.6 (Ultrospec 2000, Pharmacia), then induced by addition of IPTG (1 mM final concentration), incubated for 1-4 h, collected by centrifugation (Cryofuge 6000i, Heraeus), resuspended in 40 ml lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10 mM imidazole, pH 8.0) transferred to a 50 ml tube, collected by centrifugation, and stored at -80 °C. Pellets were resuspended in 10 ml lysis buffer per gram wet cell pellet followed by lysozyme treatment (1 mg/ml final concentration) for 30 min on ice and subsequent sonication on ice (Branson sonifier 250D, 6 cycles of 10 s burst and 10 s break at 50% peak power). Cell extracts were cleared by centrifugation (20 min, 20,000 g, SW34 rotor, Sorvall) and two filtration steps (45 and 20 µm, VWR).

Abf1 was purified by immobilized metal ion affinity chromatography (IMAC) using a 1 ml HisTrap HP column (GE, 17-5247-01) and an ÄKTA purifier system (GE). After loading the extract derived from 1 l cell culture, the column was washed with three column volumes of wash buffer (as lysis buffer but with 20 mM imidazole) and the protein eluted with elution buffer (as lysis buffer but with 250 mM imidazole). Abf1 containing fractions were determined by Coomassie SDS-PAGE, pooled and dialyzed over night against 1 l buffer C (20 mM Tris-HCl, pH 8.0, 0.25 M KCl, 1 mM EDTA, 10% glycerol), and applied to a HiTrap Heparin HP column (GE, 17-0406-01) pre-equilibrated with buffer C. After washing with three column volumes of buffer C, Abf1 was eluted with a gradient of KCl concentration from 0.25 to 1 M in buffer C. Abf1 eluted at approximately 0.5 M KCl as detected by Coomassie SDS-PAGE. Fractions containing Abf1 were pooled, dialyzed against E-buffer, snap frozen in liquid nitrogen and stored at -80 °C. Final Abf1 concentration was 0.1 mg/ml as determined by Bradford assay with BSA as standard (Biorad, 500-0002).

Reb1 was purified via IMAC using about 1 ml Ni-NTA Agarose (Qiagen, 30210) in a self-packed gravity flow column (Biorad, 737-4711). After loading the extract derived from 1 l cell culture, the column was washed with 5 ml wash buffer and Reb1 was eluted with 2 ml elution buffer. Reb1 containing fractions were detected by Coomassie SDS-PAGE, pooled, and loaded onto an E-buffer equilibrated 24 ml Superdex 200 10/300 column (GE, cat #17-5175-01).

Fractions containing purest Reb1 (Coomassie SDS-PAGE) were pooled, snap frozen in liquid nitrogen and stored at -80 °C. The final Reb1 pool had a concentration of 1.32 mg/ml as determined by Bradford as for Abf1.

**Reconstitution reactions:** Purified factors and whole cell extracts ( $\mu\text{g}$  extract protein according to BSA-calibrated Bradford assay (Biorad, 500-0002)) were added to SGD chromatin (usually corresponding to 1  $\mu\text{g}$  DNA reconstituted by SGD) as indicated in Table S3 and this reconstitution reaction was incubated for 2 h at 30 °C. The nucleosome concentration per reconstitution reaction was estimated to be 92 nM according to 1  $\mu\text{g}$  DNA assembled by SGD at the full assembly degree (Krietenstein et al., 2012). Remodelers were usually used at a molar ratio per nucleosome of 1:10 unless indicated otherwise (Table S3). Reconstitution reactions were usually in 100  $\mu\text{l}$  and with the following final buffer conditions: 1 mM Tris-HCl, pH 7.6, 2 mM HEPES-KOH, pH 7.5, 19.6 mM HEPES-NaOH, pH 7.5, 13% glycerol, 2.7 mM DTT, 3 mM  $\text{MgCl}_2$ , 0.6 mM EGTA, 0.1 mM EDTA, 85.5 mM NaCl, 8 mM KCl, 0.005 % Tween, 0.1 mM  $\text{Na}_2\text{S}_2\text{O}_5$ , 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 3 mM ATP, 30 mM creatine phosphate (Sigma), 20 ng/ $\mu\text{l}$  creatine kinase (Roche Applied Science).

**MNase treatment:** For MNase-anti-H3-ChIP-seq, reconstitution reactions were stopped by cross-linking with 0.05 % formaldehyde (Sigma-Aldrich, F8775-500ML) for 15 min at 30 °C followed by quenching with glycine (125 mM final concentration) at 30 °C for 5 min and treatment with 200 mU apyrase (NEB, M0398L) for 30 min. For MNase-seq, reconstitution reactions were stopped only by apyrase treatment at 30 °C for 30 min. After supplementation with  $\text{CaCl}_2$  (1.5 mM final concentration) digestions with various MNase (Sigma Aldrich, N3755-500UN) concentrations (Table S3) were at 30 °C for 5 min and stopped with EDTA (10 mM final concentration). MNase digestion degree was chosen to result in mainly mononucleosomal and some dinucleosomal products (see also (Weiner et al., 2010)).

**Restriction enzyme accessibility assay:** KpnI accessibility assays were performed by spiking SGD chromatinized 601-25mer designer array (still part of the circular plasmid (2,659 bp backbone plus 4,937 bp 601-25mer array)) into an aliquot of a reconstitution reaction (see also (Lieleg et al., 2015)). The aliquot was split again into two aliquots and after addition of either 60 or 150 U KpnI (NEB) incubated for 2 h at 30 °C. After DNA purification by proteinase K digestion, phenol extraction and ethanol precipitation, secondary cleavage was with XbaI and EcoRI and bands were detected by Southern blotting and hybridization with a probe spanning the “cut small” fragment, thus also recognizing the “cut large” and “uncut” fragments (Figure S1B).

**Nucleosome sliding assay:** Nucleosome sliding assays were performed as in (Watanabe et al., 2015). Recombinant *Xenopus* histones were expressed from pET based plasmids (Karolin Luger) in BL21 (DE3) pLysS *Escherichia coli* cells for histones H2A, H2B, and H3, and in BL21 (DE3) for H4. Expressed histones were purified as inclusion bodies, solubilized in unfolding buffer (7 M guanidinium hydrochloride, 20 mM Tris-HCl, pH 7.5, 10 mM DTT), and dialyzed against urea dialysis buffer (7 M urea, 10 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 1 mM EDTA, 0.2 mM PMSF, and 5 mM 2-mercaptoethanol). Samples were injected into tandemly connected Q Sepharose and SP Sepharose columns (GE Healthcare), washed with urea dialysis buffer, and eluted from SP Sepharose by a linear salt gradient (0.1 to 1 M NaCl). Histone fractions were dialyzed against water plus 0.2 mM PMSF and 5 mM 2-mercaptoethanol, and lyophilized.



The four histones were mixed in equimolar ratios in unfolding buffer, dialyzed against refolding buffer (2 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 5 mM 2-mercaptoethanol), and purified through a Superdex-200 column (GE Healthcare).

Nucleosomes were reconstituted by mixing octamers with a 245 bp <sup>32</sup>P-labeled DNA fragment containing the 601 nucleosome-positioning sequence at the fragment 5' end in 1:1 molar ratio in Hi buffer (2 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 5 mM 2-mercaptoethanol), and dialyzing against a linear salt gradient buffer from Hi buffer to Lo buffer (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 5 mM 2-mercaptoethanol) for 20 hours.

Mononucleosomes (1 nM) were incubated with Chd1 at 0, 0.125, 0.25, 0.375, 0.5 nM and 2 mM ATP in buffer A for 15 min at 30 °C. The reactions were quenched with 5% glycerol and 1 mg/ml salmon sperm DNA, incubated for 5 min at 30 °C, and resolved on 5% Native-PAGE in 0.5 X TBE. Gels were dried, exposed to film, and quantified by PhosphorImager analysis.

**Preparation of sequencing libraries:** MNase-anti-H3-ChIP-seq libraries were prepared as in (Wal and Pugh, 2012). MNase digested samples were brought to 500 µl with NPS buffer (0.5 mM spermidine, 0.075% (v/v) IGEPAL, 50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 1x Roche cOmplete EDTA-free (Roche diagnostics, 04693132001), sterile filtered) and 1.25 µl 20% SDS was added (final concentration 0.05%). 1 µg of anti-H3-antibody (Abcam, ab1791) was added per sample and incubated over night at 4 °C rotating. 20 µl Magna ChIP™ Protein A Magnetic Beads (Millipore, cat. # 16-661) were added and incubated for 1.5 h at 4 °C rotating. The bound nucleosomes were washed two times with 800 µl FA-lysis buffer (50 mM HEPES-KOH, pH 8, 150 mM NaCl, 2 mM EDTA, 1 % Triton X-100 (v/v), 0.1% sodium desoxycholate (w/v), 0.2x Roche cOmplete EDTA-free, sterile filtered) plus 0.025% SDS, resuspended in 800 µl FA-lysis buffer plus 0.025% SDS and incubated rotating at RT for 15 min. The samples were washed once with 800 µl FA-lysis buffer and once with 800 µl FA-high salt buffer (50 mM HEPES-KOH, pH 7.5, 1 M NaCl, 2 mM EDTA, 1% Triton X-100 (v/v), 1% sodium desoxycholate (w/v), 0.2x Roche cOmplete EDTA-free, sterile filtered), resuspended in 800 µl FA-high salt buffer and incubated rotating at RT for 15 min. The beads were washed two times with 800 µl FA-wash buffer 3 (10 mM Tris-HCl, pH 7.5, 25 mM LiCl, 2 mM EDTA, 1% Triton X-100 (v/v), 0.1% sodium desoxycholate (w/v), 0.2x Roche cOmplete EDTA-free, sterile filtered), once with 10 mM Tris-HCl, pH 8 plus 0.2x Roche cOmplete EDTA-free and resuspended in 500 µl H<sub>2</sub>O plus 0.2x Roche cOmplete EDTA-free. For sequencing library preparation, the beads were resuspended in 32 µl ddH<sub>2</sub>O, transferred to a fresh tube and prepared essentially according to the NEBNext® ChIP-Seq Library Prep Reagent Set for Illumina® protocol. Briefly, the purified DNA was end-repaired with Klenow (1 U, M0210L, NEB), T4 DNA polymerase (3 U, M0203L, NEB), and T4-PNK (10 U, M0210L, NEB) in 50 µl 1x ligation buffer (B0202S NEB) shaking at 20 °C for 30 min. Blunted, bead bound nucleosomes were washed once with 800 µl FA-high salt buffer and once with 800 µl Tris-HCl, pH 8 plus 0.2x Roche cOmplete EDTA-free, resuspended in 50 µl A-tailing reaction (5 U Klenow Fragment (3' to 5' exo-), M0210L, NEB, 1x NEBuffer 2, B7002S, NEB), and incubated shaking for 30 min at 37 °C. Blunted, A-tailed, bead bound nucleosomes were washed once with 800 µl FA-

high salt buffer, once with 800 µl Tris-HCl, pH 8 plus 0.2x Roche cOmplete EDTA-free, and resuspended in 20 µl ddH<sub>2</sub>O. NEBNext Adaptor (0.05 µM final concentration, E7335L and E750L, NEB) was ligated to A-tailed DNA with T4-Ligase (12 U, M0202L, NEB) in 30 µl 1x T4 Ligase reaction Buffer (B0202S, NEB) shaking at 16 °C overnight, then cleaved by addition of USER™ Enzyme (3 U, M5505L, NEB) for 15 min at 37 °C. Blunted, bead bound nucleosomes were washed once with 800 µl FA-high salt buffer, once with 800 µl Tris-HCl, pH 8, and resuspended in 100 µl ddH<sub>2</sub>O. The blunted, A-tailed, Adaptor ligated, bead bound nucleosomal DNA was eluted from beads and amplified by PCR in one step (NEBNext Index 1-16, 18-23, 25 or 27 Primer for Illumina (0.5 µM, E7335L and E750L, NEB) and NEBNext Universal PCR Primer for Illumina (0.5 µM, E7335L and E750L, NEB), Phusion® High-Fidelity DNA Polymerase (1 U, M0530L, NEB), and Deoxynucleotide (dNTP) Solution Mix (2.5 mM, N0047S, NEB) in a final volume of 50 µl Phusion® HF Buffer (1x M0530L, NEB) with the following protocol: 72 °C for 20 min (reverse crosslinking), 95 °C for 5 min (addition of 0.5 µl polymerase, hot start), 12 cycles (95°C for 15 s, 65 °C for 30 s, 72 °C for 30 s) and paused. The dsDNA content of 1 µl PCR reaction was measured by Qubit® dsDNA HS Assay Kit (Q32851, Invitrogen). If DNA concentration was higher than 3 ng/µl, the reaction was incubated for final elongation for 5 min at 72 °C. In rare cases of lower DNA concentrations, two additional amplification cycles were added and DNA concentration controlled again by Qubit until resulting DNA concentration was >3 ng/µl. Adaptor-ligated mono-nucleosomal DNA (supernatant, without beads) was purified by 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris-HCl, pH 7.6, 20 mM acetic acid, 1 mM EDTA). The DNA was extracted from agarose with Freeze N Squeeze DNA Gel Extraction Spin Columns (732-6166, Bio-Rad) and purified by 2-propanol precipitation. The pellet was resuspended in 12 µl 0.1x TE buffer and measured with Qubit® dsDNA HS Assay Kit (Q32851, Invitrogen). Concentrations were calculated assuming a DNA fragment length of 272 bp (147 bp mononucleosomal DNA and 122 bp sequencing adapter) and diluted to 10 nM. For sequencing, 10 nM solutions were pooled according to match sequencing lane requirements. Either the final pools or single samples were analyzed and quantified by BioAnalyzer (Agilent) or qPCR (using standard Illumina protocol).

For MNase-seq experiments, MNase digested samples were heated to 55 °C, supplemented with SDS (0.5% (w/v) final concentration), glycogen (0.25 mg/ml final concentration), and 200 µg ProteinaseK (BioLine or Roche) and incubated overnight. NaClO<sub>4</sub> was added to a final concentration of 1 M and the volume adjusted to 250 µl with ddH<sub>2</sub>O. In case of samples without WCEs, *E. coli* tRNA (Sigma) was added as carrier (2.1 µg/ml final concentration). DNA was phenol/chloroform purified, ethanol precipitated, resuspended in 100 µl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), treated with 1 µg RNaseA (Roche, 10109169001) for 3 h at 37 °C, 2-propanol precipitated, resuspended in 32 µl ddH<sub>2</sub>O and prepared for sequencing essentially according to the NEBNext® ChIP-Seq Library Prep Reagent Set for Illumina® protocol. Briefly, the purified DNA was end-repaired with Klenow (1 U, M0210L, NEB), T4 DNA polymerase (3 U, M0203L, NEB), and T4-PNK (10 U, M0210L, NEB), in 50 µl 1x ligation buffer (B0202S NEB) at 20 °C for 30 min. DNA was purified with 50 µl AMPureXP beads (Beckman Coulter) and 75 µl NaCl-PEG solution (20 % PEG-4000, 1.25 M NaCl), washed and eluted according to

manufacturer's instructions. The DNA was resuspended in 50  $\mu$ l A-tailing reaction (5 U Klenow Fragment (3' to 5' exo-), M0210L, NEB, 1x NEBuffer 2, B7002S, NEB), incubated for 30 min at 37 °C, rebound to the AMPureXP beads by addition of 125  $\mu$ l NaCl-PEG solution, washed, eluted in 20  $\mu$ l ddH<sub>2</sub>O and transferred without beads to a fresh tube. NEBNext Adaptor (0.05  $\mu$ M final concentration, E7335L and E750L, NEB) was ligated to A-tailed DNA with T4-Ligase (12 U, M0202L, NEB) in 30  $\mu$ l 1x T4 Ligase reaction Buffer (B0202S, NEB) at 16 °C overnight, then cleaved by addition of USER™ Enzyme (3 U, M5505L, NEB) for 15 min at 37 °C. DNA was purified using 30  $\mu$ l AMPureXP beads according to manufacturer's instructions. DNA was resuspended in 30  $\mu$ l ddH<sub>2</sub>O and amplified by PCR (NEBNext Index 1-16, 18-23, 25 or 27 Primer for Illumina (0.5  $\mu$ M, E7335L and E750L, NEB) and NEBNext Universal PCR Primer for Illumina (0.5  $\mu$ M, E7335L and E750L, NEB), Phusion® High-Fidelity DNA Polymerase (1 U, M0530L, NEB), and Deoxynucleotide (dNTP) Solution Mix (2.5 mM, N0047S, NEB) in a final volume of 50  $\mu$ l Phusion® HF Buffer (1x M0530L, NEB) with the following protocol: 98 °C for 30 s, 12 cycles (98 °C for 10 s, 65 °C for 30 s, 72 °C for 30 s) and paused. The dsDNA content of 1  $\mu$ l PCR reaction was measured by Qubit® dsDNA HS Assay Kit (Q32851, Invitrogen). If DNA concentration was higher than 3 ng/ $\mu$ l, the reaction was incubated for final elongation for 5 min at 72 °C. In rare cases of lower DNA concentrations, two additional amplification cycles were added and DNA concentration controlled again by Qubit until resulting DNA concentration was >3 ng/ $\mu$ l. Adaptor-ligated mono-nucleosomal DNA was purified by 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris-HCl, pH 7.6, 20 mM acetic acid, 1 mM EDTA). The DNA was extracted from agarose with Freeze N Squeeze DNA Gel Extraction Spin Columns (732-6166, Bio-Rad) and purified by 2-propanol precipitation. The pellet was resuspended in 12  $\mu$ l 0.1x TE buffer and measured with Qubit® dsDNA HS Assay Kit (Q32851, Invitrogen). Concentrations were calculated assuming a DNA fragment length of 272 bp (147 bp mononucleosomal DNA and 122 bp sequencing adaptor) and diluted to 10 nM. For sequencing, 10 nM solutions were pooled according to match sequencing lane requirements. Either the final pools or single samples were analyzed and quantified by BioAnalyzer (Agilent) or qPCR (using standard Illumina protocol).

**ChIP-exo:** ChIP-exo of Abf1-TAP bound in vivo was performed in duplicate as described (Rhee and Pugh, 2012). Briefly, cells were crosslinked using formaldehyde and isolated chromatin was subjected to sonication and IgG sepharose immunoprecipitation. Immunoprecipitated DNA was purified followed by ligation of sequencing adaptors and digestion by lambda exonuclease. The resulting DNA was used for sequencing library preparation.

**DNA sequencing:** Nucleosomal libraries were sequenced on either an Illumina Genome Analyzer Iix (LMU, single-end mode, 36 cycles), a HiSeq 1500 (LMU, single-end mode, 50 cycles), an Illumina HiSeq 2000 (PSU, single read mode, 40 cycles), or an Illumina NextSeq 500 (PSU, paired-end mode, 40 cycles, but only using Read1 for analysis after ascertaining that essentially the same patterns were observed using both reads). Sequences were mapped against the *S. cerevisiae* genome obtained from Saccharomyces Genome Database ([www.yeastgenome.org/download-data/sequence](http://www.yeastgenome.org/download-data/sequence): S288C\_reference\_genome\_R55-1-1\_10-

Nov-2006) using BWA (version 0.6.2) software (Li and Durbin, 2009) for single-end and BWA-MEM (version 0.7.9a; [arXiv:1303.3997v2](https://arxiv.org/abs/1303.3997v2) [q-bio.GN] ) for paired-end reads. Default settings were used for mismatch allowed to find the alignment to the reference genome. Sequencing statistics are reported in Table S3.

**Reference datasets:** In vivo +1 nucleosome dyad locations were reference points for most analyses and were from the MNase-anti-H4-ChIP-seq data of in vivo formaldehyde-crosslinked cells (Zhang et al., 2011). Tags were shifted in the 3' direction by 73 bp. Peaks (consensus nucleosome dyads) were called using GeneTrack software (Albert et al., 2008) and +1 nucleosomes were assigned according to their location in a +1 zone as defined in (Jiang and Pugh, 2009a). Native data were as in (Zhang et al., 2011).

Relevant to Figures 3A and 3C, NPS correlation data was retrieved from (Ioshikhes et al., 2006) but missing for 56 genes.

DNA shape features were calculated as in (Zhou et al., 2013). This distribution data was smoothed using a 20 nucleotide moving average.

Relevant to Figure S5A, graph 1, and Figure 6A, graph 1, genes bound by Abf1 or Reb1 in vivo were required to have a cognate recognition site and show Abf1 or Reb1 binding in ChIP-exo, respectively. ChIP-exo tag 5' ends were plotted and distributions binned in 25 bp intervals and smoothed using a 2 bin moving average. Rows/genes within heat maps were sorted based on Abf1 or Reb1 in vivo occupancy located <400 bp upstream of TSSs, which is where Abf1 and Reb1 are normally enriched.

Relevant to Figure 2A, graph 2, and Figure S3C, poly(dA) and poly(dT) tracts 6 bp in length were defined as described (Chang et al., 2012).

Relevant to Figures 2C and S3A, poly(dA:dT), poly(dT), and poly(dA) were defined to be at least 6 nucleotides: 5'-TTTTTT-3' and 5'-AAAAAA-3'. Unique poly(dT) and poly(dA) tracts were selected to be <200 bp upstream or between -200 and +80 bp, respectively, of the corresponding TSS and on the sense strand. These limits were based on the average poly(dA:dT) distribution around all TSSs. The RSC trace used RSC:nucleosome molar ratios of 1:10, 1:20, and 1:40.

Relevant to Figure S3B, Rsc3 binding motifs were as in (Badis et al., 2008) and selected to be within 500 bp upstream of TSSs.

**Data processing:** Data processing was essentially done as in (Zhang et al., 2011). For MNase-based maps, tag 5' ends were shifted in the 3' direction by a fixed calculated distance for each dataset (Table S3) to reflect dyad locations, and strand information was removed. Ribosomal proteins genes (3% of all genes) were excluded from the analysis since they organize their nucleosomes by a unique mechanism (Reja et al., 2015) that was not reconstituted here. Dyad locations were plotted relative to in vivo-defined +1 nucleosomes over a range of 500 bp upstream to 650 bp downstream. Distributions were binned in 25 bp intervals and smoothed using a 2-bin moving average. Except for Figure 2A, graph 2, where distribution was binned in 5 bp intervals.

Where nucleosome dyad density (tag counts) was plotted as a heat map, the individual genes were first adjusted to set the sum of squares for tag counts in each gene's  $\pm 1$  kb region flanking the +1 nucleosome to 1 using normalized genes option in Cluster 3.0 software. This minimized the gene copy number variation present in the genomic plasmid library (due to differential amplification when passed through *E. coli*). Thus, each gene contributed equally to heat maps and also composite traces in later steps. Row values were normalized to a mean of zero by centering genes to the mean in Cluster 3.0 software. Heat maps were generated using Java TreeView 1.1.6r3 software obtained from [jtreeview.sourceforge.net](http://jtreeview.sourceforge.net).

Where nucleosome dyad density was plotted as a composite plot, tag counts were normalized for area under each curve to be equal when comparing datasets over an interval  $\pm 1$  kb from the +1 nucleosome dyad location. Distributions were binned in 5 bp intervals and smoothed using a 9-bin moving average.

Unless specified otherwise, single representative datasets as opposed to averaged data are shown within each panel. The experimental details of each sample shown in each graph and trace are given in Table S3.

**Row sorting:** Relevant to Figure 1A, +1/NFR tag ratios for SGD sample were based on a merge of four SGD datasets and calculated using the following limits. For +1: number of tag 5' ends located  $\pm 30$  bp from in vivo defined +1 nucleosome dyads; for NFR: number of tag 5' ends located within a calculated in vivo NFR midpoint zone (108-188 bp upstream of the +1 dyad). In a very small fraction of genes (rows) the sum of tags for the +1 and/or NFR regions was zero. If both values were zero, the row was removed. If only one was zero, the sum was set to 1, which does not introduce a significant error as both zero and one are very small tag numbers, but it spares the respective gene from dropping out of the analysis.

Relevant to Figure 2B, NFR widths were determined as follows. First, from composite plots aligned separately by the in vivo  $-1$  and  $+1$  nucleosome location (smoothed using a bin size of 5 and a step size of 9), the X-axis values having a Y-axis local maximum around  $-1$  ( $\pm 15$  bp from  $-1$  peak center), and  $+1$  ( $\pm 15$  bp from  $+1$  peak center) was determined, respectively. Second, the X-axis value having a local Y-axis minimum within the NFR region (105-145 bp downstream of  $-1$  dyad for the  $-1$  aligned plot or upstream of  $+1$  dyad for the  $+1$  aligned plot) was determined as the NFR minimum. The X-axis values midway between the NFR minimum and the  $-1$  or  $+1$  maxima, respectively, defined the upstream and downstream borders of the NFR, respectively. The distance between these borders represented the NFR width, and the difference between this and the Native NFR width was reported.

Relevant to Figure 3A, nucleosome dyad density levels within  $\pm 30$  bp from in vivo-defined +1 nucleosome dyads were summed up, and the ratios between the corresponding sums for the sample in graphs 4 (SGD + INO80) and for SGD (four independent replicates were merged, one of them shown as graph 2) were determined and used for row sorting. A similar pattern was obtained for another independent replicate of INO80.

**QUANTIFICATION AND STATISTICAL ANALYSIS**—Relevant to Table S1, +1, +2 and +3 nucleosome dyads were determined from composite plots aligned to in vivo +1 nucleosome location (smoothed using a bin size of 5 and a step size of 9). The X-axis values corresponding to a Y-axis local maximum around Native +1, +2 and +3 peak centers were determined and gave the +1, +2 and +3 nucleosome positions. Table S1 also reports the distance between these +1, +2, +3 nucleosome positions relative to the respective Native positions as well as the distances between +1/+2 and +2/+3 nucleosome peak centers.

Relevant to Table S2, +1 nucleosome positioning strength was determined by the formula “ $b1/(a1+a2)$ ”. “b1” represents the summed up nucleosome dyad tag counts within  $\pm 15$  bp from in vivo-defined +1 nucleosome dyads. “a2” is same as b1 but at an interval located 85 bp downstream of +1 nucleosome dyad (i.e., linker midpoint located midway between +1 and +2 nucleosome dyads). “a1” is same as b1 but at an interval located 85 bp upstream of +1 nucleosome dyad (reflecting the predicted equivalent of an upstream linker interval). Each heatmap was split into quartiles (based upon the sort order of the respective heatmap in the respective figure) and average +1 nucleosome positioning strength was reported for each quartile.

#### DATA AND SOFTWARE AVAILABILITY

**Data Resources:** The data discussed in this publication are deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE72106 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72106>).

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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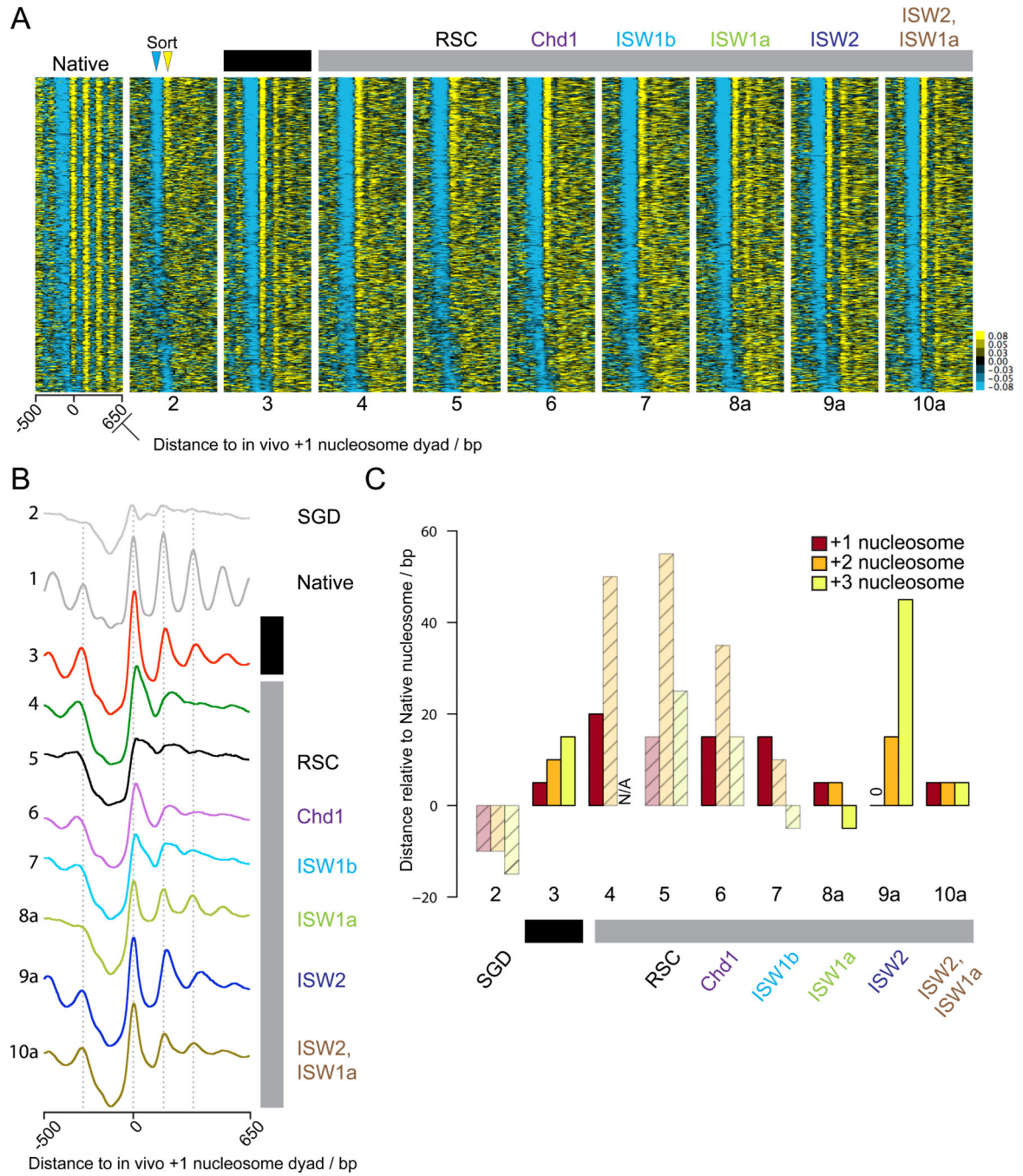
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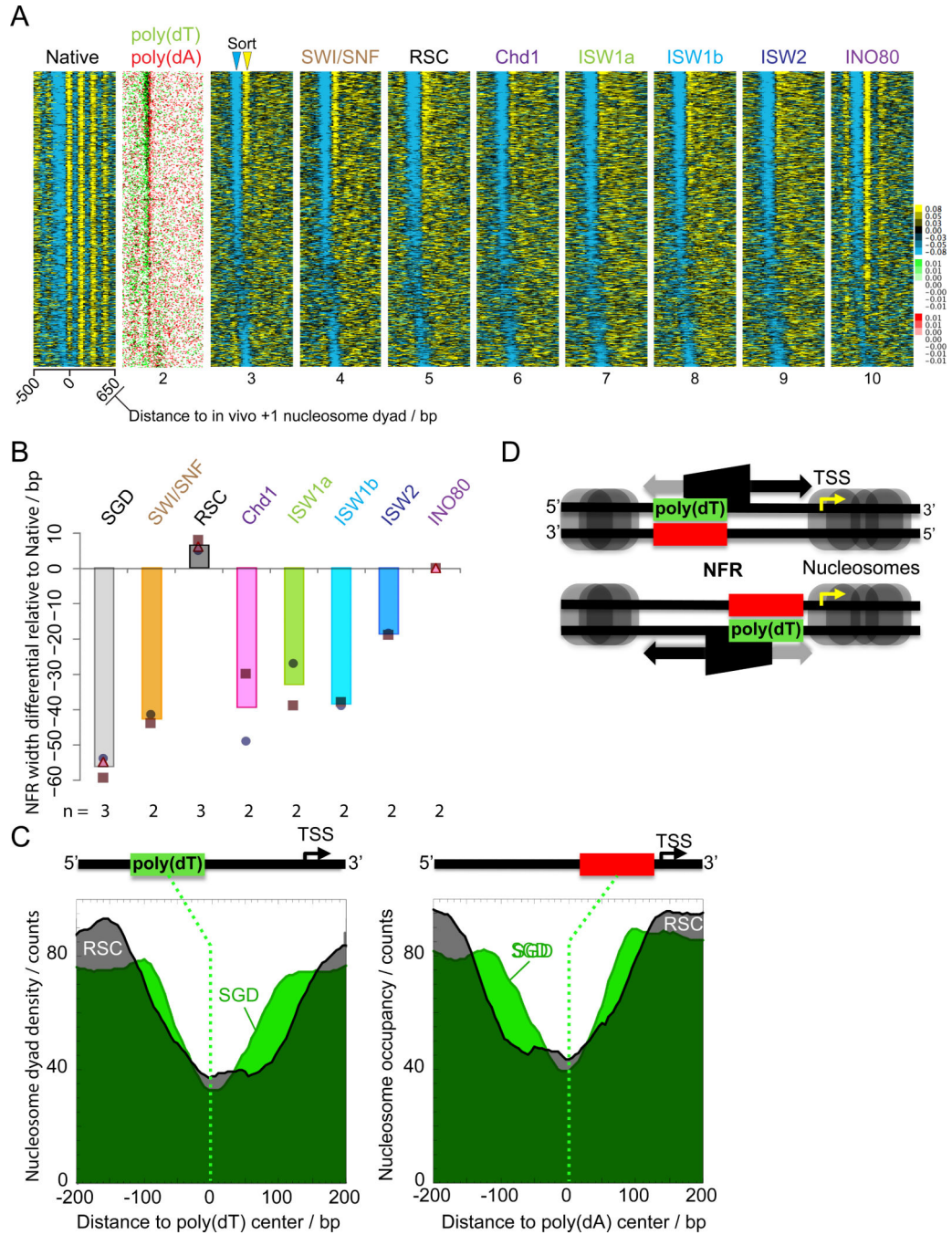
**Figure 1. Purified remodelers reconstitute genome-wide NFR/+1/array nucleosomal organizations in remodeler-depleted extract**

(A) Nucleosome dyad density along genes (4,118 rows) as detected by MNase-(anti-H3-ChIP)-seq were color-coded (yellow, black, and blue represent high, medium, and low tag density, respectively) and each gene aligned at in vivo-defined +1 nucleosome dyads. In all graphs, rows were sorted based on decreasing +1-to-NFR tag ratio in graph 2 (as indicated by the triangles). Throughout all figures, graph number/letter represents a dataset ID that is particular to each figure and its supplemental figure. The exact composition of each sample for all figures is given in Table S3. “Native” denotes chromatin isolated from cells, then

crosslinked in vitro so as to provide a “gold” standard of what can be achieved in vitro. Graph 2 shows the starting pattern consisting solely of histones assembled onto genomic DNA plasmid libraries by salt gradient dialysis (SGD). Purified remodelers were added along with whole cell extract, as indicated.

(B) Composite plots of data shown in panel (A), where the graphs were vertically separated but scaled identically. Grey dashed lines demarcate dyad peaks in the Native dataset.

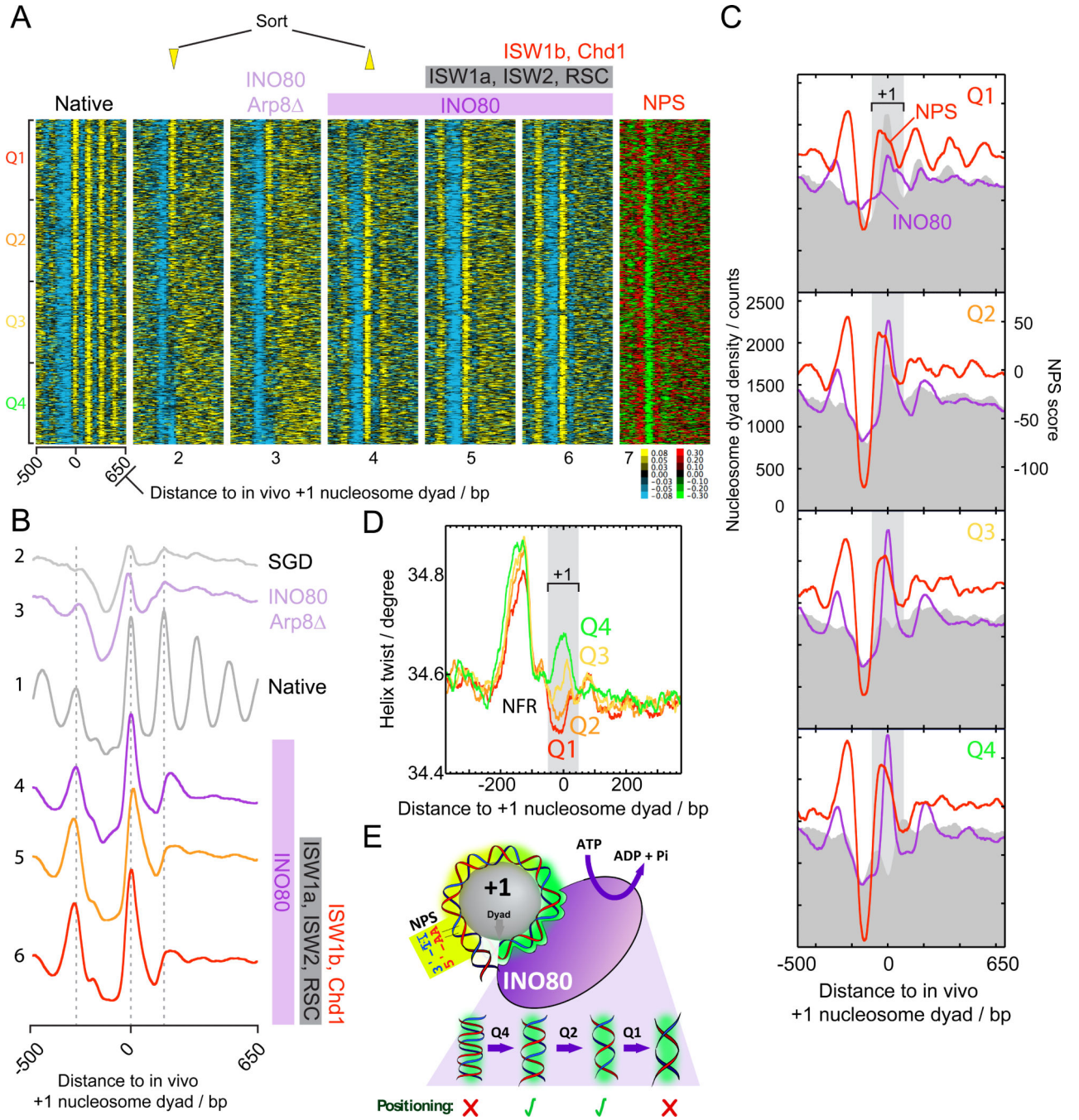
(C) Distances of the nucleosome +1, +2 and +3 peak positions relative to the respective Native positions for the traces in panel B. Transparent hatched bars show values that were not meaningful due to high nucleosome fuzziness. N/A, not applicable due to absence of peak. “0” denotes that the distance was zero. See also Figures S1 and S2, and Tables S1, S2 and S3.



**Figure 2. RSC creates physiological NFRs using strand-specific poly(dT)/(dA) tracts**  
 (A) See Figure 1A description but no extract was added. Graph 2: distribution of poly(dT) and poly(dA) tracts 6 bp in green and red, respectively. See also Figure S3C. Data for Native sample as in Figure 1A.  
 (B) Average NFR width difference between Native and SGD without or with the indicated remodelers. Bars show averages of “n” replicates, symbols show values of individual datasets. Data of one SGD replicate as in Figure 1A, and of one replicate for each sample with remodeler as in panel (A).  
 (C) Nucleosome dyad density (left) and occupancy (right) plots for RSC and SGD. The x-axis represents the distance to the poly(dT) or poly(dA) center in base pairs (bp), ranging from -200 to 200. The y-axis represents nucleosome dyad density or occupancy in counts, ranging from 0 to 80. The RSC plot shows a deep dip in density at the poly(dT) center, while the SGD plot shows a shallower dip. The occupancy plots show similar trends, with RSC showing a more pronounced dip at the poly(dA) center.  
 (D) Schematic diagram of a transcription start site (TSS) with a poly(dT) tract (green) and a poly(dA) tract (red) on opposite strands. The NFR (Nucleosome Free Region) is indicated by a yellow arrowhead, and nucleosomes are shown as grey cylinders.

(C) Composite nucleosome dyad distributions for SGD reconstituted without or with RSC (green and dark grey, respectively) relative to the midpoints of unique poly(dT) (left) or poly(dA) (right). These elements were defined as being 6 bp and occurring on the sense strand within NFRs. Only those TSS that had either poly(dT) or poly(dA) but not both on the sense strand were selected. Each trace corresponds to a merge of three replicates, one being the same as in panel (A). See also Figure S3A.

(D) Illustration emphasizing the orientation and relative position of poly(dT) and poly(dA) tracts upstream of TSSs. See also Figure S3C. Directional removal of nucleosomes by RSC is illustrated with long black arrows relative to the short grey arrows. See also Figures S1 and S3B, and Tables S1, S2 and S3.



**Figure 3. INO80 alone positions +1 nucleosomes, potentially through a combination of DNA sequence and shape features**

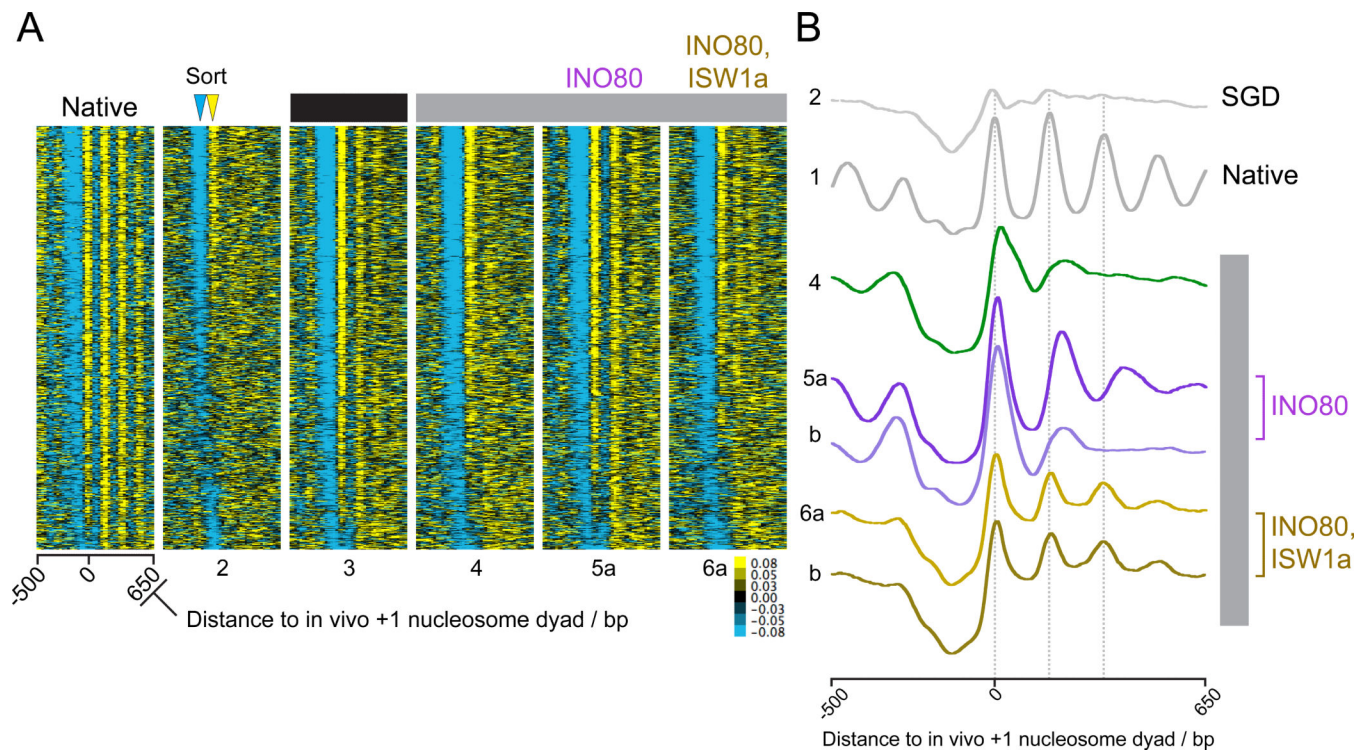
(A) See Figure 2A description. Rows (4,127) were sorted based by increasing effectiveness of +1 positioning by INO80 relative to SGD, i.e., ratio of tags in a 60 bp window centered on +1 dyad locations (defined in vivo) between reactions containing and lacking INO80 (graphs 4 vs. 2, indicated by linked triangles). Graph 7 shows corresponding NPS correlation scores (Ioshikhes et al., 2006) of 4071 genes. Red and green reflect positive and negative correlations, respectively. Data for the Native sample as in Figure 1A. Data for samples 2 and 4 as in Figure 2A. See also Figure S4D.

(B) Composite plots of data in panel (A).

(C) Composite plots of data shown in graphs 2, 4 and 7 in panel (A), separated into quartiles Q1-Q4 based on panel (A) sorting. In panels (C) and (D), the 147 bp region covered by +1 nucleosomes is shaded.

(D) Composite plot of intrinsic local DNA helical twist calculated for Q1-Q4, based on DNA sequence (Zhou et al., 2013). See also Figure S4A, B, C, F, G.

(E) Model of how INO80 might position +1 nucleosomes by using DNA sequence (e.g., AA dinucleotides constituting NPSs) and shape (e.g., over-/under-twist) features. The illustrated untwisting of DNA by INO80 is exaggerated for emphasis.

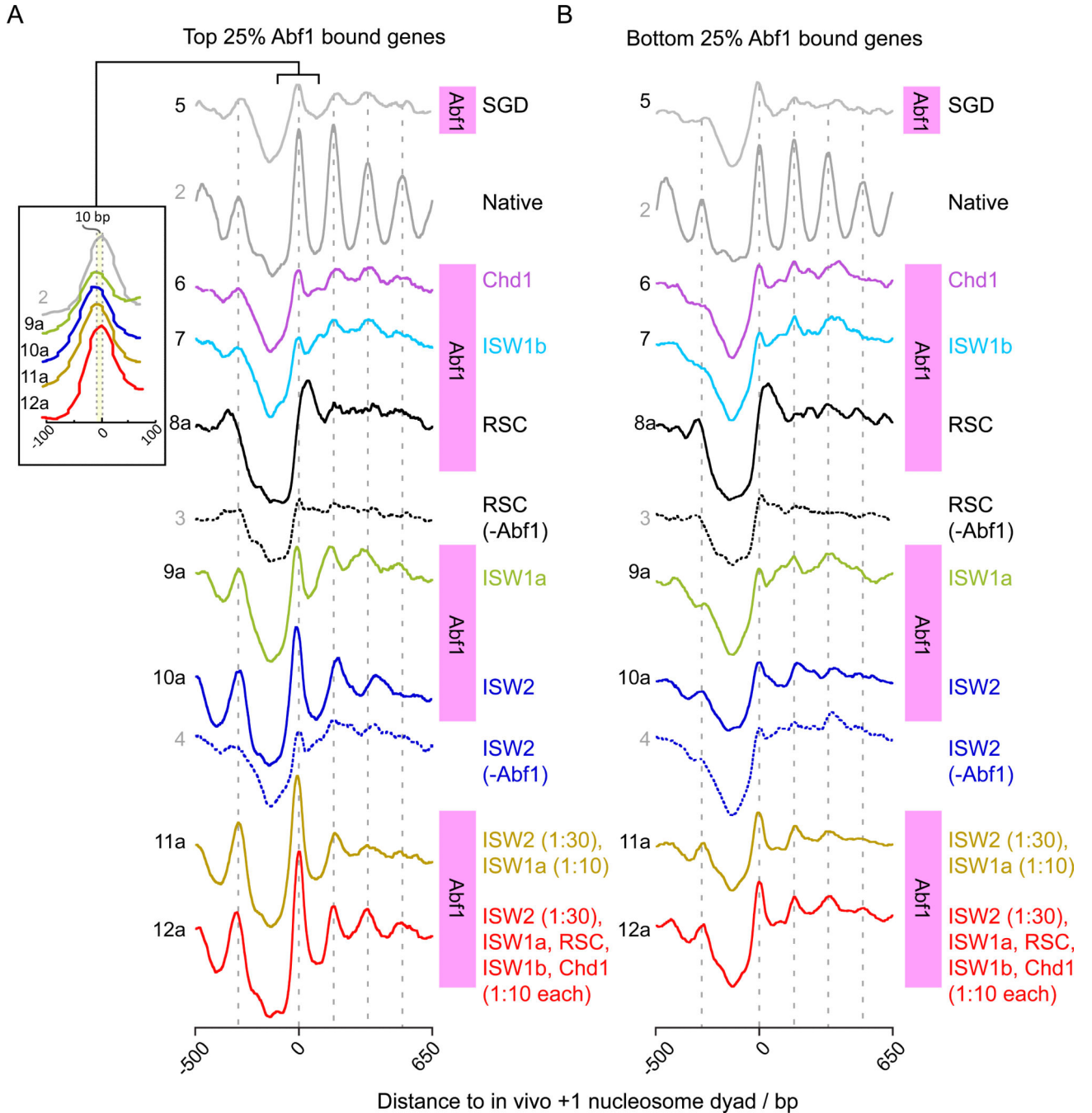


**Figure 4. INO80 shows enhanced spacing activity and cooperates with ISW1a in the context of extracts**

(A) See Figure 1A description. Data for samples Native, 2, 3 and 4 as in Figure 1A.

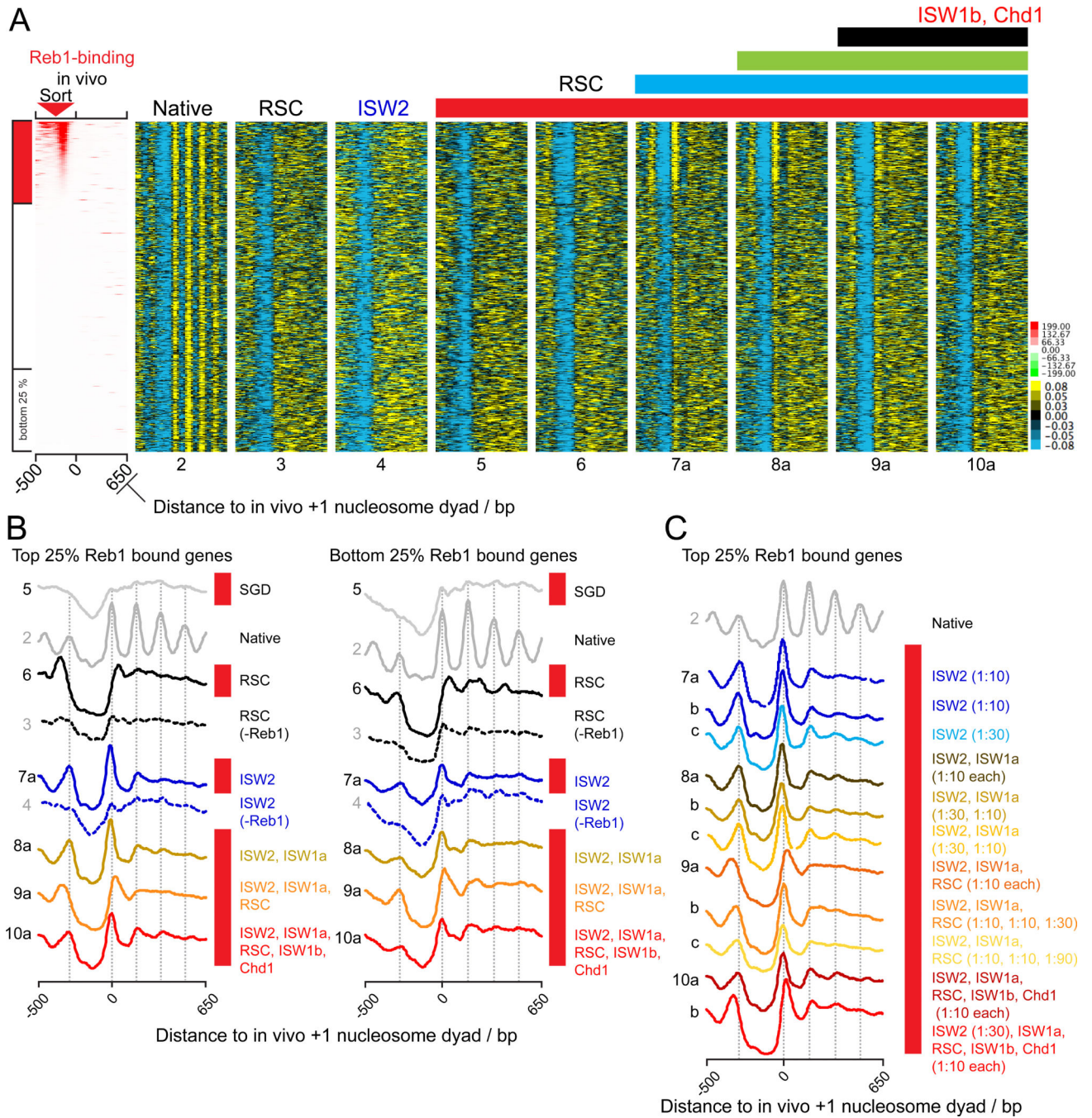
(B) Composite plots of data shown in panel (A) with independent replicates 5b and 6b. See also Figure S1, and Tables S1, S2 and S3.





**Figure 5. Abf1 and combinations of remodelers create near-canonical NFR/+1/array organization at Abf1-bound genes**

(A) and (B) Composite plots of indicated reconstitution reactions. Data for samples Native, 3, and 4 are as in Figure 2A. For corresponding heat maps see Figure S5A. Composites represent either the top (A) or bottom (B) 25% of all genes sorted by Abf1 ChIP-exo occupancy measured in vivo in YPD media (see also Figure S3C). The latter essentially being unbound in vivo, but potentially having some promiscuous binding in vitro. Dashed graphs lack Abf1. Inset shows a zoom-in of +1 nucleosomes for selected graphs. See also Figures S1 and S5B, and Tables S1, S2 and S3.



**Figure 6. Reb1 and combinations of remodelers create proper NFR/+1 organizations at Reb1-bound genes**

(A) See Figure 2A description (also organized as in Figure S5A, except using Reb1 instead of Abf1). Rows (4,168) were sorted by Reb1 ChIP-exo occupancy measured in vivo in YPD media (graph 1, red triangle, see also Figure S3C). Data for samples Native, 3 and 4 were the same as in Figure 2A.

(B) Composite plots of data in panel (A). Dashed graphs lack Reb1.

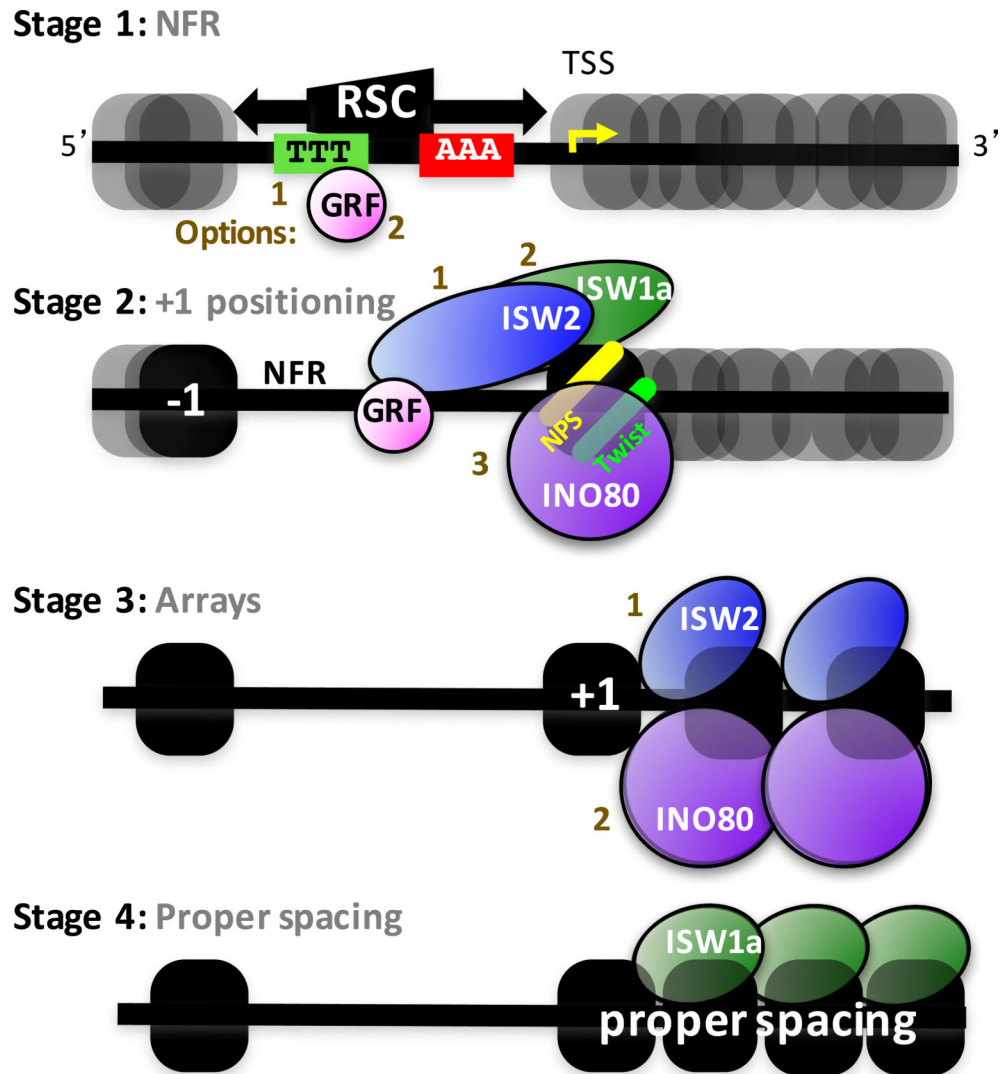
(C) Samples with same number as in panel (B) show same data, others independent replicates. Color intensity of graphs scales with amount of remodeler used (Table S3). See also Figure S1, and Tables S1, S2 and S3.

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**Figure 7. Model depicting the proposed four basic stages in nucleosome organization at the 5' ends of genes**

Brown numbers denote different options that may occur to varying degrees at each gene. GRFs and DNA sequence are gene-specific and so impart differing gene-selective utilization of remodelers and mechanisms. Nucleosomes are either depicted in black or grey signifying defined or fuzzy positioning, respectively. Stage 1, NFRs are formed through directional nucleosome displacement by RSC as guided by poly(dT)/poly(dA) tracts and/or by GRF-mediated RSC action. GRF binding is to cognate sites (not shown) rather than to poly(dT). INO80 may also generate NFRs (option 3, not depicted). Stage 2, the +1 nucleosome is set by ISW2 or ISW1a in cooperation with GRFs and/or by INO80 recognizing unique DNA sequence (NPS in yellow) and shape (helical twist in green) features at +1. Stage 3, both ISW2 and INO80 generate nucleosomal arrays aligned by the +1 nucleosome, but with non-canonically wide spacing. Stage 4, ISW1a properly spaces these nucleosomes leading to physiological arrays. At present, we make no assumption regarding the temporal order of events.