Our findings suggest that PSA induces T_{regs} through TLR2 signaling to suppress T_H17 cell responses and promote mucosal colonization by *B. fragilis*. To test this model, we measured colonization levels of *B. fragilis* in *Rag1*$^{-/-}$ mice reconstituted with TLR2-deficient CD4$^+$ T cells. Tissue association by wild-type *B. fragilis* in the colon was reduced to the levels of *B. fragilis*ΔPSA in these mice (Fig. 3E and fig. S15). Moreover, Foxp3$^+$ T_{reg} ablation in *B. fragilis* mono-associated animals resulted in significantly reduced amounts of tissue-associated *B. fragilis* (Fig. 3F), without affecting bacterial numbers in the lumen of the gut (fig. S16). Finally, to functionally determine the role of IL-17 responses in mucosal association, we treated *B. fragilis*ΔPSA mono-associated animals with a neutralizing antibody to IL-17A. Whereas the amounts of *B. fragilis*ΔPSA in isotype control–treated animals remained low, neutralization of IL-17A resulted in a 1000-fold increase in tissue-associated bacteria (Fig. 3, G and H). These data indicate that IL-17 suppression by PSA is required by *B. fragilis* during association with its host. Therefore, unlike pathogens that trigger inflammatory responses through TLRs to clear infections, symbiotic colonization by *B. fragilis* is actually enhanced via the TLR pathway. We conclude that PSA evolved to engender host-bacterial mutualism by inducing mucosal tolerance through TLR2 activation of T_{reg} cells.

The gastrointestinal tract represents a primary portal for entry by numerous pathogens. Toll-like receptors recognize MAMPs (microbial-associated molecular patterns) expressed by bacteria and coordinate a cascade of innate and adaptive immune responses that control infections (*20*). Although TLRs have classically been studied on innate immune cells, recent reports have demonstrated their expression by T cells in both mice and humans (*4*, *21*–*23*). As bacteria contain universally conserved MAMPs, how do commensal microbes, unlike pathogens, avoid triggering TLR activation? It is historically believed that the microbiota is excluded from the mucosal surface (*24*). However, certain symbiotic bacteria tightly adhere to the intestinal mucosa (*9*–*11*), and thus immunologic ignorance may not explain why inflammation is averted by the microbiota. Our study provides new insight into the mechanisms by which the immune system distinguishes between pathogens and symbionts. The functional activity of PSA on T_{regs} contrasts with the role of TLR2 ligands of pathogens, which elicit inflammation, and thus reveals an unexpected function for TLR signaling during homeostatic intestinal colonization by the microbiota. Although engagement of TLR2 by previously identified ligands is known to stimulate microbial clearance of pathogens, TLR signaling by PSA paradoxically allows *B. fragilis* persistence on mucosal surfaces. These results identify PSA as the incipient member of a new class of TLR ligands termed "symbiont-associated molecular patterns (SAMPs)" that function to orchestrate immune responses to establish host-commensal symbiosis. On the basis of the importance of the microbiota to mammalian health (*25*), evolution appears to have created molecular interactions that engender host-bacterial mutualism. In conclusion, our findings suggest that animals are not "hard-wired" to intrinsically distinguish pathogens from symbionts, and that microbial-derived mechanisms have evolved to actively promote immunologic tolerance to symbiotic bacteria. This concept suggests a reconsideration of how we define self versus nonself.

References and Notes

1. R. E. Ley, D. A. Peterson, J. I. Gordon, *Cell* **124**, 837 (2006).
2. S. Manicassamy, B. Pulendran, *Semin. Immunol.* **21**, 185 (2009).
3. M. Fukata *et al.*, *J. Immunol.* **180**, 1886 (2008).
4. J. M. Reynolds *et al.*, *Immunity* **32**, 692 (2010).
5. I. Caramalho *et al.*, *J. Exp. Med.* **197**, 403 (2003).
6. P. B. Eckburg *et al.*, *Science* **308**, 1635 (2005).
7. I. I. Ivanov *et al.*, *Cell Host Microbe* **4**, 337 (2008).
8. K. Atarashi *et al.*, *Nature* **455**, 808 (2008).
9. V. Gaboriau-Routhiau *et al.*, *Immunity* **31**, 677 (2009).
10. I. I. Ivanov *et al.*, *Cell* **139**, 485 (2009).
11. K. Atarashi *et al.*, *Science* **331**, 337 (2011).
12. S. K. Mazmanian, J. L. Round, D. L. Kasper, *Nature* **453**, 620 (2008).
13. J. Ochoa-Repáraz *et al.*, *Mucosal Immunol.* **3**, 487 (2010).
14. J. L. Round, S. K. Mazmanian, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12204 (2010).
15. Materials and methods are available as supporting material on *Science* Online.
16. J. M. Kim, J. P. Rasmussen, A. Y. Rudensky, *Nat. Immunol.* **8**, 191 (2006).
17. S. K. Mazmanian, C. H. Liu, A. O. Tzianabos, D. L. Kasper, *Cell* **122**, 107 (2005).
18. Q. Wang *et al.*, *J. Exp. Med.* **203**, 2853 (2006).
19. C. A. Janeway Jr., R. Medzhitov, *Annu. Rev. Immunol.* **20**, 197 (2002).
20. R. Medzhitov, *Nature* **449**, 819 (2007).
21. H. Liu, M. Komai-Koma, D. Xu, F. Y. Liew, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 7048 (2006).
22. R. P. Sutmuller *et al.*, *J. Clin. Invest.* **116**, 485 (2006).
23. S. Babu, C. P. Blauvelt, V. Kumaraswami, T. B. Nutman, *J. Immunol.* **176**, 3885 (2006).
24. L. V. Hooper, *Nat. Rev. Microbiol.* **7**, 367 (2009).
25. K. Smith, K. D. McCoy, A. J. Macpherson, *Semin. Immunol.* **19**, 59 (2007).
26. W. Lin *et al.*, *Nat. Immunol.* **8**, 359 (2007).

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A Packing Mechanism for Nucleosome Organization Reconstituted Across a Eukaryotic Genome

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Near the 5′ end of most eukaryotic genes, nucleosomes form highly regular arrays that begin at canonical distances from the transcriptional start site. Determinants of this and other aspects of genomic nucleosome organization have been ascribed to statistical positioning, intrinsically DNA-encoded positioning, or some aspect of transcription initiation. Here, we provide evidence for a different explanation. Biochemical reconstitution of proper nucleosome positioning, spacing, and occupancy levels was achieved across the 5′ ends of most yeast genes by adenosine triphosphate–dependent trans-acting factors. These transcription-independent activities override DNA-intrinsic positioning and maintain uniform spacing at the 5′ ends of genes even at low nucleosome densities. Thus, an active, nonstatistical nucleosome packing mechanism creates chromatin organizing centers at the 5′ ends of genes where important regulatory elements reside.

Statistical positioning depends on the presence of a genomic barrier within a linear array of nucleosomes (*1*). Nucleosomes within the array will passively align at regular intervals from the barrier, independent of sequence or other external factors, rather than arrange randomly. Nucleosome organization in vivo displays patterns that are consistent with statistical

positioning (*2*–*4*). Yet studies have suggested that as much as half of all nucleosome positions are "encoded" in the DNA sequence (*5*, *6*), because nucleosome occupancy reconstituted in vitro with purified genomic DNA and histones is similar to that in vivo. However, occupancy and positioning are distinct metrics of nucleosome organization (fig. S1). Nucleosome positions around transcription start sites (TSS) in vivo are different from in vitro positions (*7*–*9*) (Fig. 1A), which has led to the suggestion that transcription promotes nucleosome organization in vivo (*7*, *10*).

To determine what is needed to reconstitute proper nucleosome positions across all genes, we added whole-cell extracts to nucleosomes reconstituted on genomic DNA (*11*). To facilitate visualization of nucleosome patterns, genes were clustered based on their in vivo nucleosome organization (Fig. 1B, left panel). We produced an equivalently ordered "native" nucleosome pattern (Fig. 1B, right panel), in which chromatin was first isolated from cells without prior cross-linking, then cross-linked in vitro, as a positive control for in vitro reconstitution. The native pattern was stable (fig. S4) and similar to the in vivo pattern (Fig. 1B).

We reevaluated the intrinsically DNA-encoded organization of nucleosomes in these five clusters in three ways: (i) existing datasets were re-examined (*6*, *7*), (ii) nucleosomes within native chromatin were allowed to redistribute to their thermodynamically favored DNA-guided positions by incubation in 600 mM NaCl, and (iii) purified *Drosophila* histones were deposited by salt gradient dialysis (SGD) onto recombinant plasmid libraries (1:1 histone/DNA ratio), containing 10- to 30-kb inserts of *Saccharomyces* genomic DNA.

These experiments recapitulated some of the more prominent features of the native patterns, including nucleosome-free promoter regions (NFRs) and nucleosome positions and occupancy at certain canonical locations, as evident by the similarity of some peaks and troughs between data sets (fig. S5). However, most positions were not predominantly sequence-intrinsic. Thus, sequence-intrinsic cues contribute to nucleosome exclusion at the 5′ ends of genes but are very limited in defining nucleosome occupancy and positioning in adjacent regions and are negligible for positioning further into the coding regions.

Poly(dA:dT) tracts are a major intrinsic determinant of low nucleosome levels in yeast promoters (*12*–*14*) but have not been linked to positioning of adjacent nucleosomes. We find a strong correlation between the consensus positions of poly(dA:dT) tracts and +1 nucleosomes

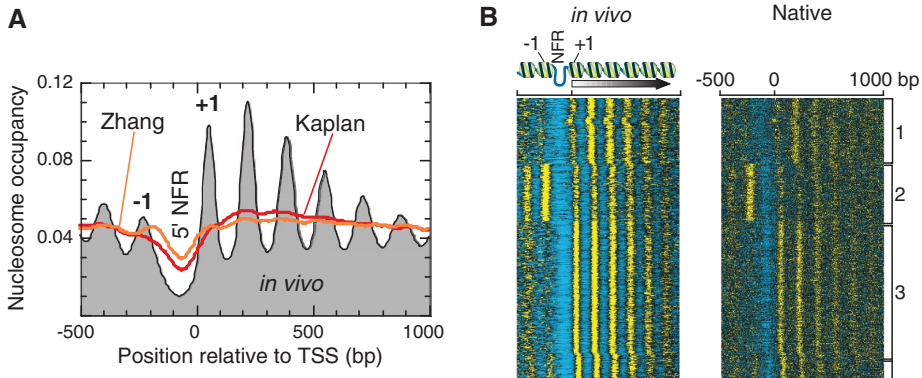

Fig. 1. Nucleosome organization around the 5′ ends of genes is not reconstituted in vitro with purified histones alone. (**A**) Composite distribution of nucleosome midpoints, assembled in vivo or in vitro (*6*, *7*), around transcriptional start sites. (**B**) Cluster view showing five in vivo patterns of nucleosome organization (left panel) and further characterized in fig. S3. All other cluster plots were ordered identically. A total of 4785 genes (rows) are aligned by their TSS. Yellow, black, and blue indicate a high, medium, and low occupancy level (tag counts), respectively.

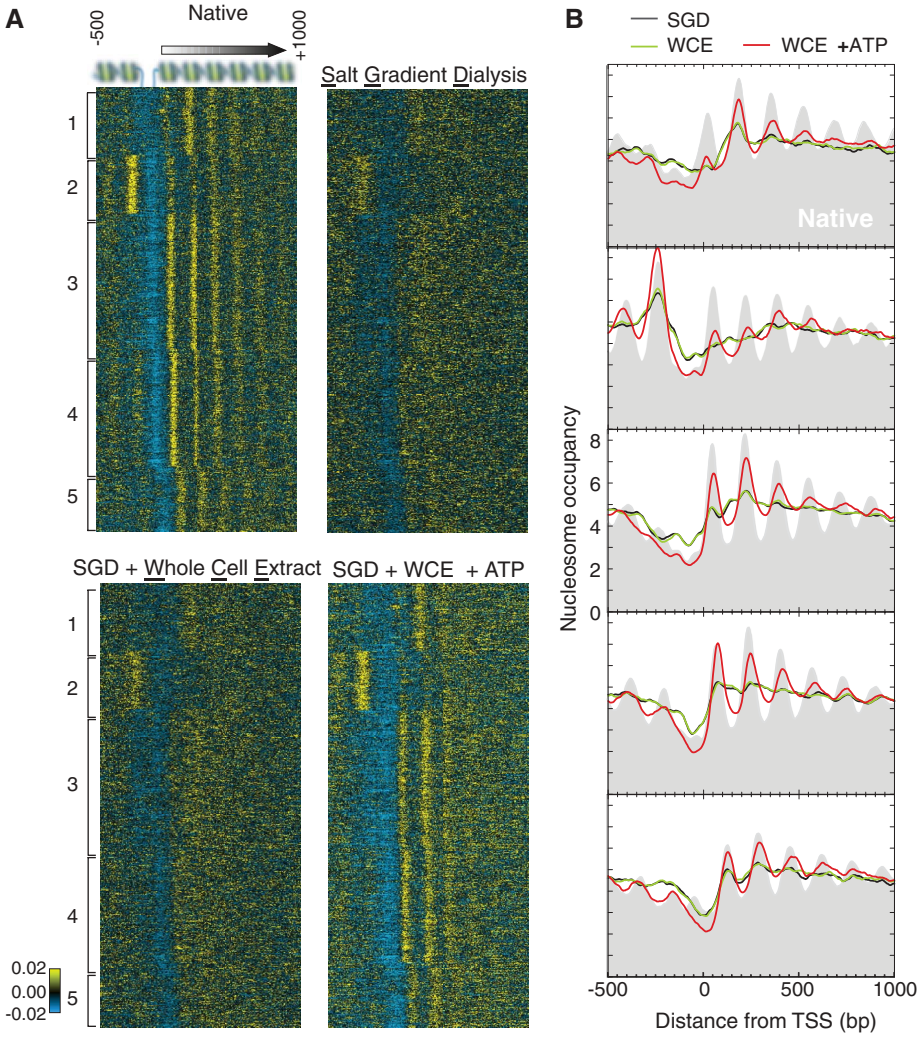

Fig. 2. Nucleosome organization around the 5′ ends of genes is reconstituted with whole-cell extracts and ATP. (**A**) Cluster plot and (**B**) corresponding composite plots of nucleosomes reconstituted by SGD. This reconstituted chromatin was either left untreated (SGD) or incubated with yeast whole-cell extracts in the absence (WCE) or presence (WCE+ATP) of ATP.

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(fig. S6). Thus, poly(dA:dT) tracts may contribute to positioning of the +1 nucleosome.

Statistical positioning requires fixed barriers as sole guides of nucleosome positioning and sufficiently high nucleosome density such that one nucleosome sterically restricts the position of a neighboring nucleosome (*1*). Three of the in vitro reconstitution experiments (SGD, 600 mM, and Zhang *et al.*) (fig. S5) seemingly met these criteria: (i) the NFRs, which may serve as barriers, were largely recapitulated, and (ii) the histone:DNA ratio was sufficiently high (1:1) to promote statistical positioning. Yet, in conflict with statistical positioning, no regular arrays aligned at the canonical +1 position were observed. Even thermal reequilibration of nucleosomes (*15*, *16*) did not allow statistical positioning to occur, because extended incubation of the SGD material at 55°C did not generate uniformly positioned arrays (fig. S7). The failure to achieve statistical positioning with only histones and DNA suggests that sequence-guided placement of each nucleosome predominates in vitro over statistical positioning.

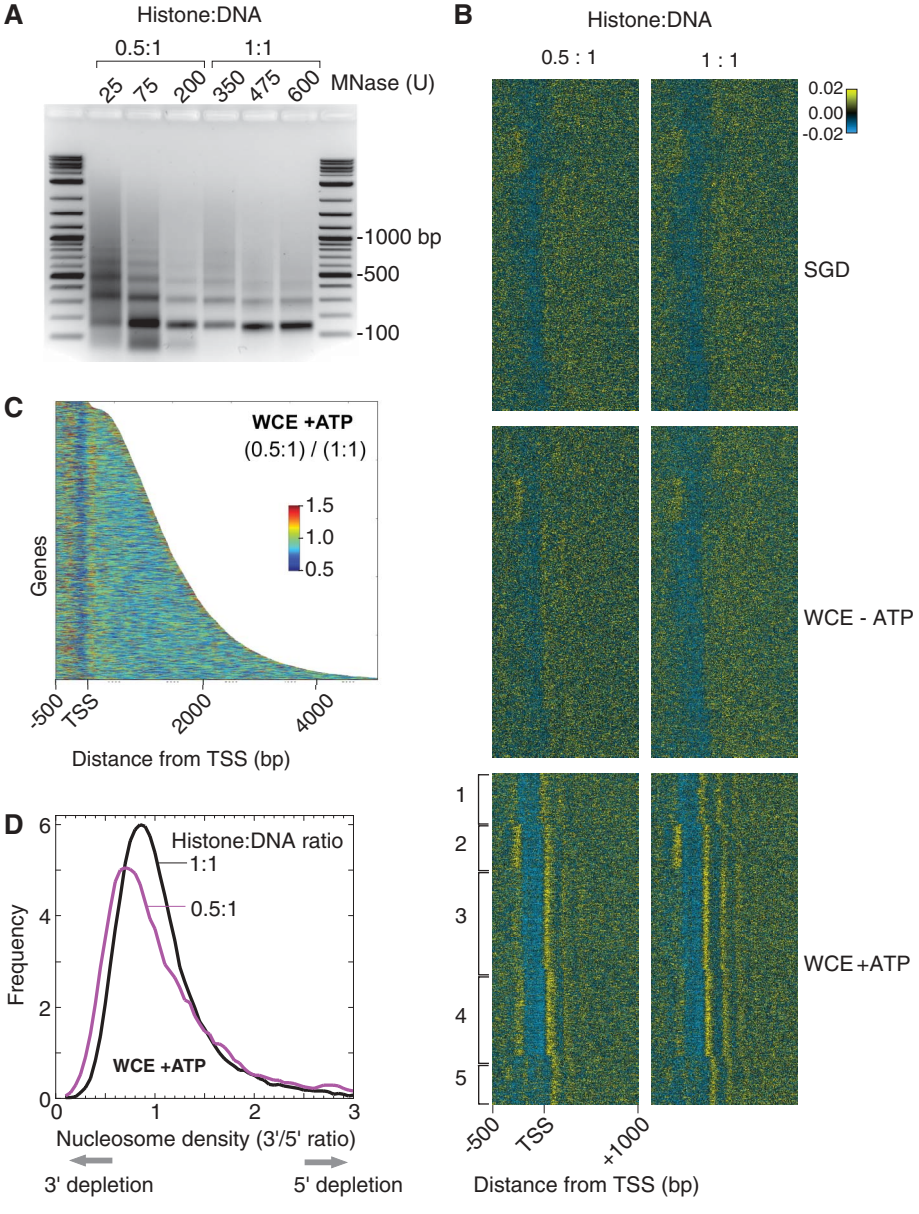

Fig. 3. Evidence that nucleosomes are actively packed against a barrier. (**A**) Ethidium bromide stained gel of SGD chromatin assembled at the indicated histone:DNA ratio (characterized in fig. S9), treated with whole-cell extracts and ATP, and then digested with micrococcal nuclease (MNase). (**B**) Cluster plots of nucleosomes reconstituted at 0.5:1 and 1:1 histone:DNA mass ratios. The bin-by-bin ratio of the bottom two panels to each other is shown in (**C**), but sorted by gene length. Data beyond the termination site is not shown. (**D**) Frequency distribution of 3′ histone density to 5′ histone density, on a per gene basis. The 3′ region is from +140 bp to the transcript termination site, whereas the 5′ region is from −20 to +140, relative to the TSS.

Given the central role adenosine triphosphate (ATP)–dependent chromatin remodeling complexes play in nucleosome organization (*17*), we considered that proper reconstitution of nucleosome positions might require ATP and trans-acting factors. The addition of whole-cell extracts plus ATP to the SGD material reconstituted nucleosome positions and occupancy levels around the 5′ ends of nearly all 4,785 tested yeast genes (Fig. 2). This was strictly ATP-dependent as incubation with extract in the absence of ATP had virtually no effect on nucleosome organization.

This reconstitution of in vivo–like nucleosome positioning did not require the other nucleoside triphosphates (*11*), indicating that transcription and DNA replication is not the predominant means by which nucleosomes become organized around genes, as originally demonstrated on *PHO5* (*18*). Moreover, the transcription initiation complex is not an obvious barrier against which nucleosomes are organized, because the TATA box position did not correlate with the position of the +1 nucleosome (fig. S8), and canonical nucleosome positioning is maintained in vivo at genes having little or no transcription (*3*). However, the binding site positions for Reb1, which is not part of the transcription machinery but functions similar to poly(dA:dT) tracts (*19*), did correlate with +1 positioning.

The data thus far argue against a DNA-intrinsic or transcription-based mechanism for organizing nucleosomes around the 5′ ends of genes but are entirely consistent with ATP-facilitated statistical positioning. For example, chromatin remodeling complexes could use ATP hydrolysis to override the DNA-intrinsic positioning landscape, thereby providing free bidirectional fluidity to nucleosomes that is only impeded by barriers. Although we favor the involvement of a remodeler adenosine triphosphatase (ATPase), we cannot formally exclude a kinase.

Statistical positioning predicts that internucleosomal spacing within arrays should be inversely related to nucleosome density (*1*), yet the cluster plots in Figs. 1 and 2 indicate that nucleosomal spacing is largely constant regardless of local nucleosome density (i.e., the periodicity of the yellow stripes is independent of the intensity of the yellow). As a direct test, we reconstituted ATP-dependent positioning on genomic DNA assembled at half the global histone:DNA density (0.5:1 instead of 1:1). Spacing remained largely unaltered [~165 base pairs (bp)], both globally (Fig. 3A) and in relation to a reference point like the TSS (Fig. 3B). Thus, a key test of statistical positioning failed.

Due to the bidirectional fluidity of nucleosomes inherent to the statistical positioning model, nucleosome density should remain relatively uniform, albeit periodic, outside of the barrier. This was not observed at the lower histone:DNA ratio. Instead, there was a decrease in nucleosome density in the NFR and internal to genes compared to the +1 nucleosome position. This was

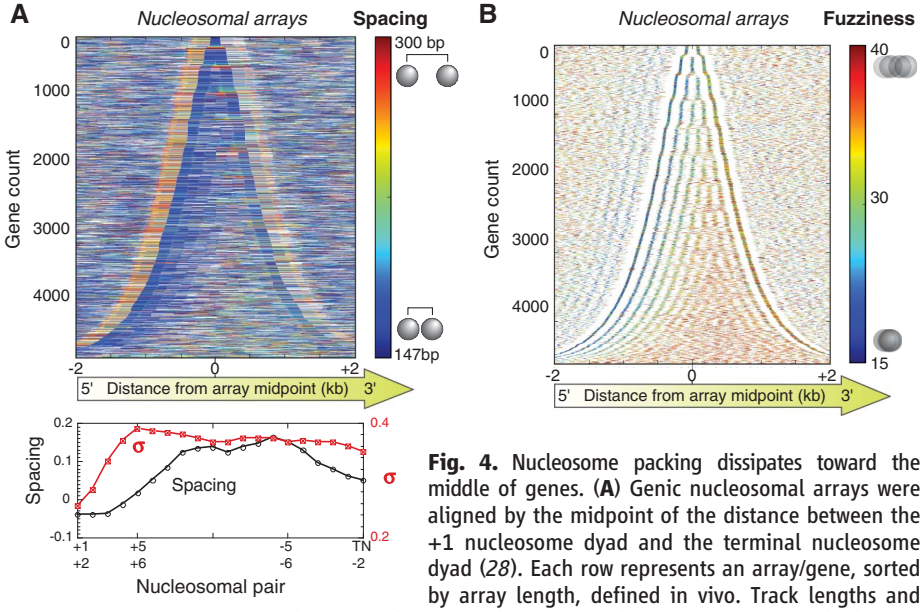

Fig. 4. Nucleosome packing dissipates toward the middle of genes. (**A**) Genic nucleosomal arrays were aligned by the midpoint of the distance between the +1 nucleosome dyad and the terminal nucleosome dyad (*28*). Each row represents an array/gene, sorted by array length, defined in vivo. Track lengths and coloring represent the spacing between adjacent nucleosome dyads measured in vivo. The bottom graph plots the median spacing (black) as well as its standard deviation, starting from the +1 nucleosome to the terminal nucleosome (TN). Median spacing is represented as the fractional change from the canonical 165 bp. (**B**) Same as (A), except that the track midpoints report the dyad position of each nucleosome measurement. Track lengths and heat map colors represent the standard deviation (fuzziness) of each cluster of tags measured in vivo.

evident on genes analyzed individually (Fig. 3C) or on aggregated data (Fig. 3D), suggesting that nucleosomes are actively packed against barriers at the 5′ ends of genes using ATP. This would occur at the expense of more distal nucleosomes under conditions of low nucleosome density. This model does not exclude bidirectional fluidity, but does implicate net directionality of nucleosome packing (fig. S10). This packing mechanism is consistent with previously proposed spacing mechanisms (*20*–*23*) but differs by the addition of a barrier and directionality. Together they provide constant spacing close to the barrier regardless of nucleosome density.

To analyze the packing mechanism further, we examined internucleosomal spacing in vivo along genic nucleosome arrays (Fig. 4A). The average spacing was relatively narrow and uniform from nucleosomes +1 through +4, and to a lesser extent also at the 3′ end. Spacing was, on average, wider but more variable toward the middle of longer genes, and thus less definable. This is not in conflict with the uniform spacing (peak-to-peak distances) in composite plots (e.g., Fig. 1A), because such measurements reflect modal internucleosomal distances (i.e., the most common spacing), rather than the average spacing. Modal internucleosomal distances are expected to remain constant along arrays until spacing activities and/or the influence of the barriers have fully dissipated. The wider and more variable spacing toward the middle of genes suggested that the active packing mechanism at 5′ barriers dissipates toward the middle of genes. The ATP-dependent packing activities may be constrained to position about four nucleosomes, because this was the extent to which ATP reconstituted proper positioning (Fig. 2).

More distally from barriers, nucleosome positioning may gradually transition to other mechanisms, for example through sequence-intrinsic preferences. If well-positioned nucleosomes resulted, then such positioning would be manifested as low fuzziness (standard deviation of sequencing tag positions) (*24*). However, nucleosome fuzziness increased toward the middle of genes, with some skewing toward the 3′ end (Fig. 4B). Thus, mechanisms outside the 5′ packing activity (and to a lesser extent at the 3′ end as well), whether active or passive, do not produce well-positioned nucleosomes.

Nucleosome positioning at the 5′ ends of most genes appears to be driven by ATP-dependent activities that directionally package nucleosomes against a 5′ barrier (and to a lesser extent 3′ barriers). Such nucleosome placement is not likely to be static and may involve dynamic exchange with free histones (*25*, *26*). Accordingly, the active nucleosome organization in vivo may be at steady state, under the continuous expense of energy, rather than at equilibrium (*27*). This barrier-packing combination may constitute an organizing center that operates for a limited distance to buffer nucleosome organization at the 5′ ends of genes from fluctuations in histone levels both globally and locally during DNA replication and transcription. If replication transiently decreases nucleosome density by half and if 5′ nucleosome packing operates faster than replication-dependent nucleosome assembly, old nucleosomes would be enriched toward the 5′ ends and new histones mainly would be deposited in the middle to 3′ ends of genes. A 5′ packing mechanism may also serve to regulate access to transcriptional start sites. Furthermore, the control of nucleosome positioning at each gene by a single organizing center would minimize evolutionary constraints on coding sequences that might otherwise occur if positioning was intrinsically encoded by the DNA sequence.

References and Notes

1. R. D. Kornberg, L. Stryer, *Nucleic Acids Res.* **16**, (14A), 6677 (1988).
2. G. C. Yuan *et al.*, *Science* **309**, 626 (2005).
3. T. N. Mavrich *et al.*, *Genome Res.* **18**, 1073 (2008).
4. W. Möbius, U. Gerland, *PLOS Comput. Biol.* **6**, e1000891 (2010).
5. E. Segal *et al.*, *Nature* **442**, 772 (2006).
6. N. Kaplan *et al.*, *Nature* **458**, 362 (2009).
7. Y. Zhang *et al.*, *Nat. Struct. Mol. Biol.* **16**, 847 (2009).
8. A. Stein, T. E. Takasuka, C. K. Collings, *Nucleic Acids Res.* **38**, 709 (2010).
9. A. Hughes, O. J. Rando, *J. Biol.* **8**, 96 (2009).
10. A. Weiner, A. Hughes, M. Yassour, O. J. Rando, N. Friedman, *Genome Res.* **20**, 90 (2010).
11. Materials and methods are available as supporting material on *Science* Online.
12. J. D. Anderson, J. Widom, *Mol. Cell. Biol.* **21**, 3830 (2001).
13. V. Iyer, K. Struhl, *EMBO J.* **14**, 2570 (1995).
14. R. M. Raisner *et al.*, *Cell* **123**, 233 (2005).
15. K. Luger, T. J. Rechsteiner, T. J. Richmond, *Methods Enzymol.* **304**, 3 (1999).
16. A. Flaus, T. Owen-Hughes, *Mol. Cell. Biol.* **23**, 7767 (2003).
17. G. R. Schnitzler, *Cell Biochem. Biophys.* **51**, 67 (2008).
18. P. Korber, W. Hörz, *J. Biol. Chem.* **279**, 35113 (2004).
19. P. D. Hartley, H. D. Madhani, *Cell* **137**, 445 (2009).
20. P. D. Varga-Weisz *et al.*, *Nature* **388**, 598 (1997).
21. G. LeRoy, G. Orphanides, W. S. Lane, D. Reinberg, *Science* **282**, 1900 (1998).
22. T. Ito, M. Bulger, M. J. Pazin, R. Kobayashi, J. T. Kadonaga, *Cell* **90**, 145 (1997).
23. T. Tsukiyama, J. Palmer, C. C. Landel, J. Shiloach, C. Wu, *Genes Dev.* **13**, 686 (1999).
24. I. Albert *et al.*, *Nature* **446**, 572 (2007).
25. A. Rufiange, P. E. Jacques, W. Bhat, F. Robert, A. Nourani, *Mol. Cell* **27**, 393 (2007).
26. M. F. Dion *et al.*, *Science* **315**, 1405 (2007).
27. E. Segal, J. Widom, *Trends Genet.* **25**, 335 (2009).
28. C. Vaillant *et al.*, *Genome Res.* **20**, 59 (2010).

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