Important questions about SepF remain. For example, does SepF play a role in septation in divergent species such as cyanobacteria? Can purified SepF from other species also spontaneously self-assemble into rings and orient FtsZ protofilaments into tubules? SepF may be essential for cell division of cyanobacteria because they lack FtsA and/or EzrA homologs [13,16]. Other Gram-negative bacteria, which lack SepF, must also maintain Z-ring integrity to coordinate constriction, septum formation, and outer membrane invagination. For the γ-proteobacteria, evidence suggests that ZipA and the well-conserved FtsA mediate this coordination [1] and it is likely that other bacteria have as yet unidentified, functionally related factors. Although the basic theme of cell division is becoming clear, unraveling the plethora of variations in the most diverse group of organisms on Earth remains a challenge.

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Chromatin: Bind at Your Own RSC

Recent work has identified a novel RSC–nucleosome complex that both strongly phases flanking nucleosomes and presents regulatory sites for ready access. These results challenge several widely held views.

Nicolas E. Buchler[1,2,3,*] and Lu Bai[4,5]

Genome-wide experiments in yeast, fly and mammalian cells have identified the existence of nucleosome-depleted regions in promoters and enhancers [1–4]. Transcription factors are thought to bind to their cognate sites located in these nucleosome-depleted regions, subsequently recruit nucleosome-remodeling and modifying complexes, and evict or reposition flanking nucleosomes that block RNA polymerase assembly at the promoter. By using a novel, quantitative assay, recent work from the Ptashne lab has uncovered several striking insights into nucleosome occupancy at the *GAL1/10* promoter of budding yeast [5–7]. These results challenge current ideas of whether nucleosome-depleted regions are completely nucleosome-free, whether strongly positioned nucleosomes are always incompatible with the binding of regulatory proteins, and whether the occupancy of a DNA fragment by a nucleosome is mostly determined by its sequence.

Nucleosome occupancy at a particular genomic location is measured by assessing nucleosome-mediated 'protection' (often assumed to be the canonical, mono-nucleosome size of 147 bp) of that sequence from digestion by micrococcal nuclease (MNase). Typical nucleosome occupancy assays fix chromatin in cells, lightly digest chromatin at a single concentration of MNase, and quantify protected DNA fragments by quantitative PCR (qPCR), tiling microarrays, or next-generation sequencing. Unfortunately, DNA sequence itself influences digestion efficiency of MNase, a bias that can create a false apparent protection of 'naked' genomic DNA. Strikingly, recent papers show that MNase digestion of naked genomic DNA infers similar nucleosome occupancies to that obtained by MNase digestion of chromatin DNA [8,9].

Bryant *et al.* [5] developed a quantitative MNase protection assay that normalizes against such variability. The assay digests naked genomic DNA and fixed chromatin DNA over a wide range of MNase concentrations,

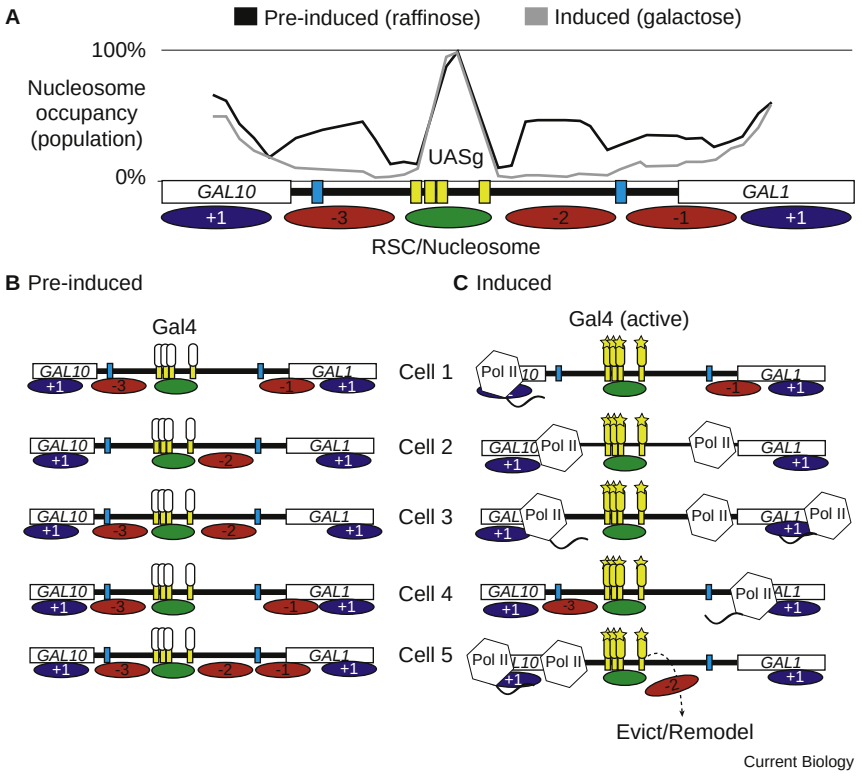

Figure 1. Nucleosome occupancy profile of the wild-type *GAL1/10* promoter in budding yeast before and after induction (redrawn from Wang *et al.* [7]).

(A) Construct of the divergent *GAL1/10* promoter, as well as the measured occupancy and inferred positioning of nucleosomes on the promoter. This promoter contains four Gal4 (activator) binding sites (yellow rectangle) in the *UASg*. Nucleosome occupancy (i.e., the fraction of cells that have a bound nucleosome at a particular genomic location) is determined by qPCR following MNase digestion of chromatin with a wide range of MNase concentrations [5,7]. Blue and red ovals represent nucleosomes over the coding/promoter DNA, which have regular-sized footprints. A smaller, green nucleosome over the *UASg* covers less DNA and is thought to interact with and be partially 'unwrapped' by RSC. In yeast, RSC has a DNA-binding subunit that strongly positions the RSC–nucleosome complex at *UASg*. (B,C) Nucleosome configuration in single cells before and after galactose induction (the notation is the same as (A)). Before activation, the red nucleosomes (–3 to –1) are only present in a fraction of cells. Upon activation, Gal4 recruits chromatin remodelers (Swi/Snf) and evicts the red nucleosomes, allowing efficient RNA polymerase assembly near the +1 transcription start site (TSS, cyan box). The small, green nucleosome is constitutively bound during induction.

followed by qPCR to quantify the relative amount of DNA. For any given amplicon (~50 bp) of chromatin DNA, the measured digestion rate of nucleosomes is usually biphasic. One fraction of chromatin is digested at a rate comparable to naked DNA; the other (nucleosome-bound) fraction is digested ~200-fold more slowly. Because of this separation of timescales, the occupancy of the nucleosome-protected DNA fragment is robustly determined by fitting a bi-exponential function to the MNase digestion series. Using this quantitative assay, Bryant *et al.* [5] illustrated that some unknown protective factor (not Gal4) is bound to the *UASg* in *GAL1/10* in 100% of yeast cells both before and after galactose induction.

In a follow-up study, Floer *et al.* [6] determined the identity of this factor. It is a 'small' RSC–nucleosome complex (containing all four histone components) that protects ~130 bp and binds strongly to specific sequences within the *UASg* (Figure 1A). RSC is a chromatin remodeling complex, and unlike its relative Swi/Snf, RSC is essential for yeast viability. The ~130-bp footprint of the RSC nucleosome was further validated by genome-wide 'paired-end' DNA sequencing of digested chromatin. The genome-wide data of Floer *et al.* show the existence of hundreds of small nucleosome footprints that overlap regulatory sites in other yeast promoters. This is a striking result because many protocols and algorithms used to analyze nucleosome occupancy presume that mono-nucleosomes always protect an invariant ~150-bp DNA fragment. Thus, the field may have been blind to a potentially important class of regulatory nucleosomes.

Floer *et al.* subsequently showed that formation of this unusual and strongly positioned nucleosome depends on both the DNA-binding and catalytic subunit of the RSC complex. Mutants deficient in the RSC nucleosome were significantly delayed in Gal4 binding to *UASg* and *GAL1* transcription, suggesting that a strongly positioned RSC nucleosome both prevents encroachment from flanking nucleosomes and facilitates the binding of Gal4 to the *UASg*. However, these encroaching nucleosomes do not prevent the eventual binding of Gal4 to *UASg* — they only make the process slower. To explain their results, Floer *et al.* proposed a structural model (based on [10]) in which the DNA is partially unwrapped on the histone surface (presumably by RSC), so as to accommodate the binding of Gal4.

Interestingly, a *UASg* ectopically inserted into the coding region of *GAL1* sufficed to strongly position the RSC/nucleosome (100% occupancy) and strongly phase the flanking nucleosomes [6]. One explanation could be that these nucleosomes are not strongly positioned by their underlying DNA sequence and are relatively 'fluid', such that the strongly bound RSC nucleosome at *UASg* forms a barrier that statistically positions or phases these nucleosomes [11,12].

In contrast to the ~100% occupancy of the RSC–nucleosome complex, the nucleosomes at positions -1, -2, and -3 in *GAL1/10* seem to be present in only ~50% of the population before galactose induction [5] (Figure 1A). What determines the occupancy, and does that have any effect on the dynamics of GAL1 induction? To address these questions, Wang *et al.* [7], as reported recently in *Nature Structural & Molecular Biology*, replaced the DNA occupied by nucleosomes at postions -1 and -2 with a series of non-natural DNA sequences that are predicted to bind canonical nucleosomes with increasing affinity. As predicted, nucleosome affinity increased; the measured occupancy at positions -1 and -2 increased up to 100%. Upon induction with galactose, the occupancy of these

strongly positioned 'super-binder' nucleosomes decreased (presumably by Swi/Snf), although eviction was less complete and induction occurred more slowly as nucleosome affinity increased. These data suggest that the wild-type *GAL1/10* promoter has likely evolved a promoter sequence with low nucleosome occupancy to allow for rapid eviction upon galactose induction (Figure 1B,C).

These studies raise important questions that will keep the chromatin field busy: how accurate are nucleosome occupancies derived from a single MNase digestion with no naked genomic DNA control? Do such artifacts change our current understanding of genome-wide nucleosome-depleted regions and whether nucleosome position is encoded in the DNA? How many other regulatory nucleosomes remain undiscovered because of our presumption that all nucleosomes protect ~150 bp of DNA? How does partial nucleosome occupancy keep wild-type *GAL1/10* transcription low? Is there a correlation in positioning and occupancy between adjacent nucleosomes at postions -1, -2, and -3? If this nucleosome depletion is a result of histone turnover, what is the on/off rate? Lastly, how does cell-to-cell variability in nucleosome configuration affect the noise in gene expression levels and dynamics? If we take our cue from Ptashne and co-workers, population-level and genome-wide assays may not be the best approach. Rather, biological insight will come from low-throughput approaches that measure nucleosome occupancy and gene expression of model genes in single cells [13,14].

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Animal Navigation: Longitude at Last

Newly hatched sea turtles exposed to artificially generated magnetic fields with parameters characteristic of two sites 3700 km apart, differing only in longitude, can distinguish the two apparent locations and orient appropriately.

James L. Gould

Humans establish their global position by separately determining latitude and longitude. The east–west parameter (longitude) is notoriously difficult to measure accurately, depending as it does on knowing the time with nearly impossible exactitude. While the global position systems (GPSs) of animals manage to ignore time [1], longitude looks at first glance to be nearly as impossible for them too [2]. As reported in this issue of *Current Biology* [3], however, new tests with sea turtles demonstrate that these creatures act as if they know their longitude, and infer this parameter on the basis of magnetic intensity and inclination.

A map sense is not necessary for many traveling creatures. For instance, some migrating animals simply fly a fixed compass vector (or a dogleg series of vectors); this is typical of many birds during their first autumn trip south. Some migrants and homing species depend instead on piloting, using their memory of landmarks observed during a previous journey to place themselves; many group-flying diurnal migrants such as geese use this approach. Other homing animals — most famously homing pigeons younger than 12 weeks — rely on inertial navigation, using cues monitored on the outward trip to judge the return bearing and distance back to the loft [4].

Most interesting of all, however, are the creatures capable of true navigation, who act as though they know their current position based on real-time cues. For example, members of at least some nocturnally migrating species can be captured *en route* to their breeding or wintering grounds and then displaced hundreds or thousands of kilometers to novel locations in apparent sensory isolation. When