Nucleosomes affect local transformation efficiency

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ABSTRACT

Genetic transformation is a natural process during which foreign DNA enters a cell and integrates into the genome. Apart from its relevance for horizontal gene transfer in nature, transformation is also the cornerstone of today's recombinant gene technology. Despite its importance, relatively little is known about the factors that determine transformation efficiency. We hypothesize that differences in DNA accessibility associated with nucleosome positioning may affect local transformation efficiency. We investigated the landscape of transformation efficiency at various positions in the *Saccharomyces cerevisiae* genome and correlated these measurements with nucleosome positioning. We find that transformation efficiency shows a highly significant inverse correlation with relative nucleosome density. This correlation was lost when the nucleosome pattern, but not the underlying sequence was changed. Together, our results demonstrate a novel role for nucleosomes and also allow researchers to predict transformation efficiency of a target region and select spots in the genome that are likely to yield higher transformation efficiency.

INTRODUCTION

Genetic transformation occurs frequently in nature. Certain microbes are naturally able to incorporate DNA from their surroundings into their own genome, whereas some viruses and bacteria can inject (parts of) their genomes into host cells that subsequently integrate the foreign DNA into their genomes using the microbe's recombination machinery (1–3). This ability of microbes to take up foreign DNA is routinely used in current molecular microbiology. For instance, viruses are used to transform cells, and *Agrobacterium tumefaciens* is exploited to transform plants (4–6).

In some organisms, such as *Saccharomyces cerevisiae* (baker's yeast), foreign DNA is incorporated by homologous recombination at sites in the host genome with sequence similarity to the foreign DNA (7). In other cases, the foreign DNA is integrated semi-randomly in the host genome (8–10). However, not all insertion sites exhibit similar transformation efficiencies. For example, transformation efficiency differs greatly between organisms and even between different loci within the genome of the same organism. Despite the importance of transformation in biotechnology, little is known about the factors that influence local transformation efficiency (11–13).

In eukaryotic cells, DNA is wrapped around an octamer of four core histones to form a nucleosome. Nucleosomes are the basic packaging unit of chromatin. In recent years, genome-wide nucleosome maps have increased our understanding of nucleosome organization in yeast (14–20). These maps show that nucleosome positions are not random. Most nucleosome positions are highly stable and uniform across different individuals in a population; nucleosome positioning is inherited from one cellular generation to another.

Many studies have attempted to understand the factors controlling nucleosome positioning. Although this issue has not been fully resolved, DNA sequence, chromatin remodeling complexes, RNA polymerases, transcription factors, neighboring nucleosomes, higher order chromatin structure, post-translational histone modifications and histone variants are important (16,21–26). However, nucleosomes are not merely DNA packaging tools for the cells. Since the tightly wrapped nucleosomal DNA is less accessible to DNA-binding factors than naked DNA, nucleosome organization influences and regulates certain key cellular processes, including gene expression, DNA replication and recombination (27–36).

Here, we demonstrate that the altered DNA accessibility associated with nucleosome positioning affects local

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genetic transformation rates (i.e. the efficiency with which foreign DNA integrates into the genome).

MATERIALS AND METHODS

Microbial procedure

We used *S. cerevisiae* strain FY5 (Mat α, S288c background) for our experiments (37). Yeast cultures were grown as described previously and standard procedures for isolation and manipulation of DNA were used (38–40).

Target selection

We selected genomic regions with very well-positioned nucleosomes and targeted three consecutive peaks (positions in the genome that show a high nucleosome density) and valleys (positions showing a low nucleosome density) with two or more insertion sites in each peak or valley (41). We did not target genes or regions that are essential for cell growth or viability (see Supplementary Table S1).

Transformation protocol

We PCR (polymerase chain reaction) amplified the Hygromycin B (HYG) resistance cassette from plasmid pAG32 (42) using primers upstream and downstream of the cassette (HYG-specific primer sequence for the forward primer was 5′CAGCTGAAGCTTCGTACGC3′ and for the reverse primer 5′GCATAGGCCACTAGTG GATCTG3′). To target the HYG cassette to different positions in the genome, the primer pairs have 45-nucleotide tails corresponding to 90 nucleotides of the targeted integration site (see Supplementary Table S1). Integration sites were spaced seven nucleotides apart. Transformations were performed individually in 96-well deep well plates using an adapted version of the transformation protocol described by Gietz and Schiestl (43). Cells were grown overnight in 3 ml YPD and 0.5×10^8 cells were then inoculated in 250 ml pre-warmed YPD (12.5 ml for each transformation reaction) in the shaking incubator for 4 h. Cells were harvested by centrifugation at 3000g for 5 min and washed twice with water. Next, cells were resuspended in 0.1 M LiAc (Lithium Acetate) (25 μl for each transformation reaction). After 10 min incubation at room temperature, 25 μl of the cell suspension (10^7 cells) was added to each well of a 96-well deep well plate. The transformation mix was added to each transformation reaction (each well): 150 μl PLi [4.5 ml PEG 50%, 610 μl LiAc 1M, 57 μl Tris 1M (pH 7.5), 11.6 μl EDTA 1M (pH 7.4)], 2.5 μl boiled ss-carrier DNA (1 mg/ml) and 10 μg DNA (PCR product). The plate was incubated in a water bath at 42°C for 30 min. After the transformation, cells were recovered for 3 h in 1.5 ml YPD before plating on YPD-HYG (200 μg/ml HYG final concentration) plates. Plates were incubated for 2 days at 30°C and to confirm insertion in correct position, this was followed by either a counter-selection or PCR (see Supplementary Table S3). Each transformation was repeated three times independently and results were averaged. Transformation efficiency was calculated as described in the study by Gietz and Schiestl (43) (number of transformants/μg DNA/10^7 cells). To compare results from different loci, we calculated the relative transformation efficiency—i.e. the transformation efficiency of each insertion site divided by the highest transformation efficiency in the same locus.

GC content and melting temperature

To study the possible correlation between transformation efficiency and local DNA features, we calculated the GC content and melting temperature of sequences of homology (90 bp total) for each target position and plotted this against the observed transformation efficiency. This homology sequence corresponds to the 5′ and 3′ flanking sequences (45 bp each) of the HYG resistance marker in the integration cassette (see Supplementary Table S1).

Nucleosome mapping

We determined the nucleosome map for part of the *ADE2* gene. Mononucleosomal DNA was prepared using a previously published protocol (41). Nucleosomal DNA was analyzed using tiling qPCR; sequences of primers are listed in Supplementary Table S2. Each primer set generates a product of 50–58 bp, centered 14 bp away from the neighboring primer set. The position at the center of the PCR product was used as the point for plotting the value in the nucleosome positioning map. Nucleosome density was calculated as the log ratio of nucleosomal DNA to that of total genomic DNA and was normalized to the highest value. Peaks represent enrichment of the fragments covered by a nucleosome and valleys represent linker DNA (i.e. nucleosome-free DNA).

RESULTS

Transformation efficiency anti-correlates with nucleosome density

We hypothesized that nucleosome positioning may be an important factor affecting transformation efficiency. Several publicly available high-quality genome-wide nucleosome maps exist for *S. cerevisiae* (14,15,41,44,45). Thus, we were able to investigate transformation efficiency across various positions in the *S. cerevisiae* genome. We selected 40 positions in the *S. cerevisiae* genome with high nucleosome densities and 40 positions showing low nucleosome densities (see Materials and Methods). In each of these loci, we inserted a HYG resistance cassette and determined transformation efficiency (see Supplementary Table S1). Our results illustrate a significant difference in transformation efficiency between loci with low nucleosome density compared with loci covered by nucleosomes ($P < 1E-10$). Moreover, we also found a highly significant anti-correlation between transformation efficiency and nucleosome density for all 80 positions tested ($R^2 = 0.55$) (Figure 1A and B).

To ensure that nucleosome positions do not change during the transformation procedure, we collected cells from three different steps during a typical transformation
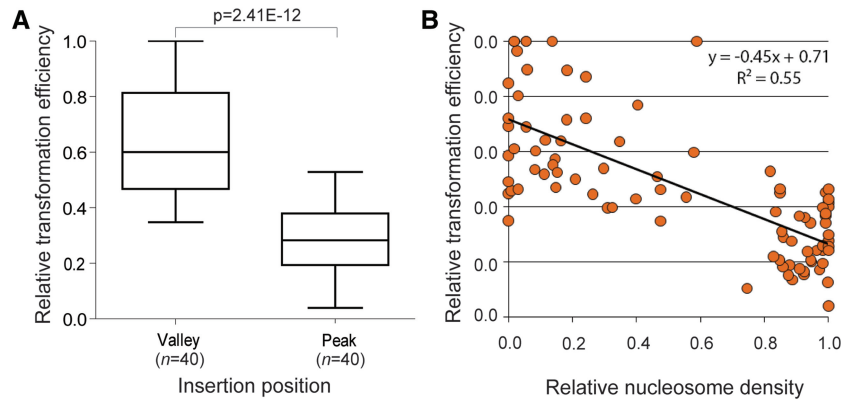
Figure 1. Nucleosome density affects transformation efficiency. (**A**) The transformation efficiency differs significantly between nucleosome-occupied (Peak: positions with a high nucleosome density) or nucleosome-depleted (Valley: positions with a low nucleosome density) positions. (**B**) Transformation efficiency anti-correlates with nucleosome density.
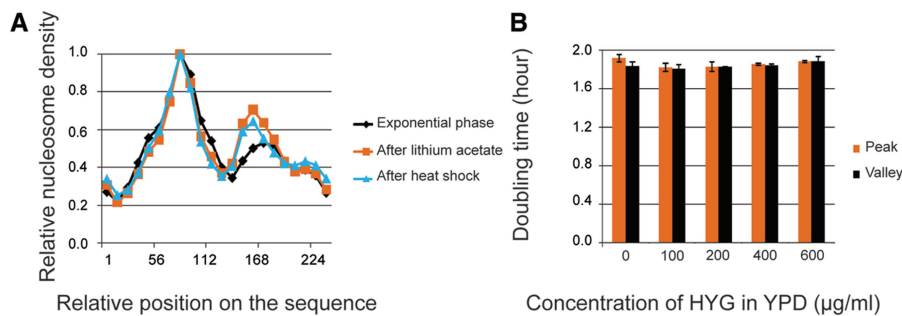

Figure 2. (**A**) Nucleosome positions do not change during the transformation procedure. We collected cells from three different steps during a typical transformation experiment and determined the nucleosome positions of a region with a well-positioned nucleosome pattern at these different stages of the transformation procedure. (**B**) Different transformants can grow equally well in different concentrations of hygromycin (HYG). We grew the transformants with HYG cassette inserted in different positions with high nucleosome density (referred to as Peak) ($n = 30$) or low nucleosome density (referred to as Valley) ($n = 30$) in different media (YPD containing different concentrations of hygromycin). Results show no variation in growth on selective media between the two groups.

experiment (exponential phase, after lithium acetate treatment and after heat shock) and determined nucleosome positioning in a region with a well-positioned nucleosome pattern in the *ADE2* gene (see Supplementary Table S2). The results of this test show that nucleosome positions do not change during the transformation procedure (Figure 2A). We also controlled for possible variation that might arise from possible differences in growth speed on the selective medium between the transformants (which could lead to misinterpretation of the transformation efficiencies). We measured the growth of 2 groups of 30 transformants with the HYG marker inserted in regions with either low or high nucleosome densities on medium with different hygromycin concentrations. There was no variation in growth in the presence of different concentrations of hygromycin (HYG) between the two groups (Figure 2B), showing that the difference we observe in colony counts are due to variation in transformation efficiency and not due to variation in growth on selective medium.

To increase the resolution of our study, we selected a 300 bp region with a well-positioned nucleosome pattern (beginning of *LYS2* gene) and inserted the same marker gene across 46 positions at 7 bp intervals (see Supplementary Table S1). The results show that

within this 300 bp tract, transformation efficiency varies over approximately one order of magnitude. These position-dependent differences in transformation efficiency exhibit a striking wave-like behavior that is inversely correlated with the nucleosome density (Figure 3A) whereas a control experiment in a 300 bp region (part of *SUC2* gene) with fuzzy nucleosomes (i.e. a region where nucleosomes change positions often within one cell and/or between cells in a population) did not show such a clear pattern for transformation efficiency (Figure 3B).

The effect of nucleosome positioning on transformation efficiency is independent of the local DNA sequence

Next, we aimed to check whether nucleosomes directly affect nucleosome positioning and transformation efficiency or whether it is an indirect effect caused by the local DNA sequence, which could influence both nucleosome positioning and transformation efficiency. In a control experiment, we attempted to find a correlation between transformation efficiency and properties of the local DNA sequence, i.e. GC content and melting temperature. However, these parameters weakly correlated to local transformation efficiency (Figure 4A). To
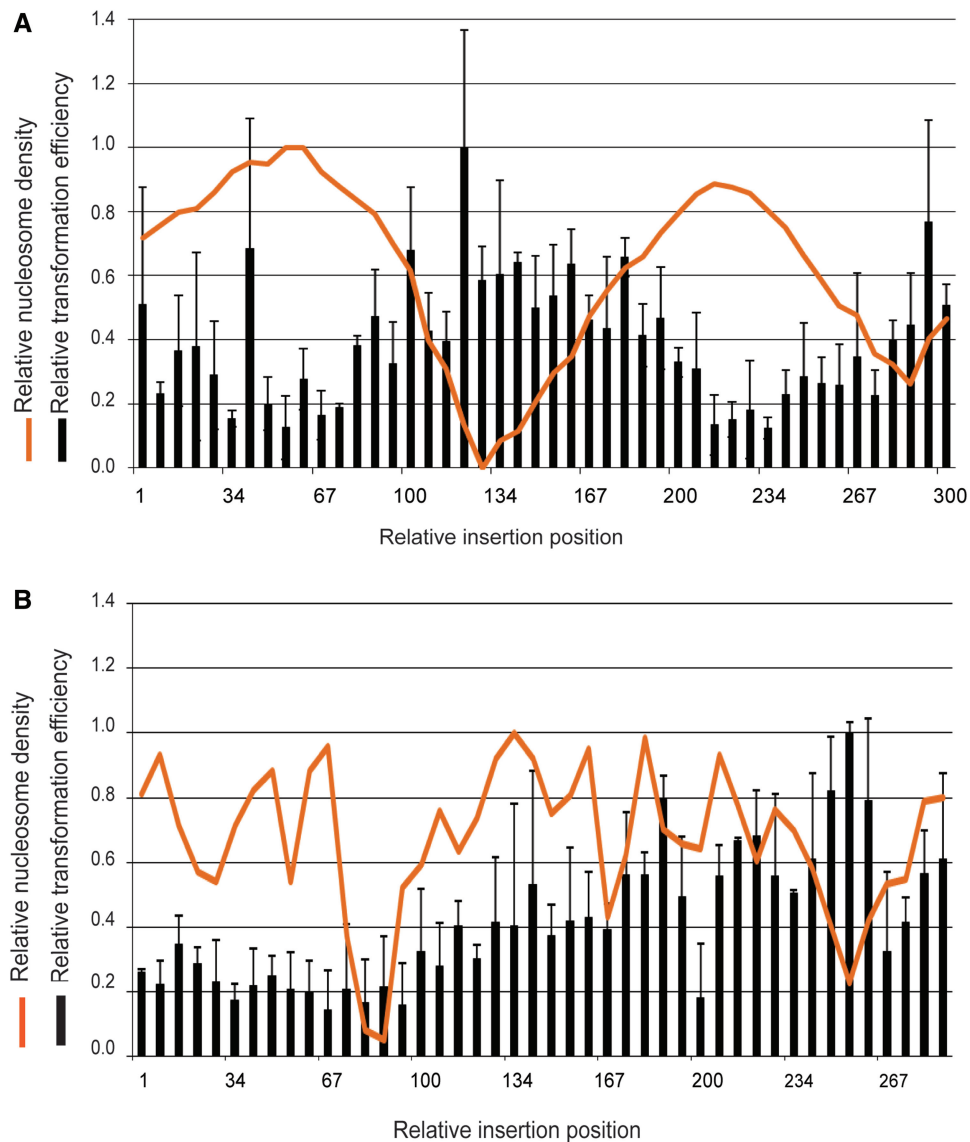
Figure 3. (A) Transformation efficiency anti-correlates with nucleosome density (in a region with well-positioned nucleosome pattern). Transformation efficiency (black bars) in a 300 bp region of the *S. cerevisiae* genome containing well-positioned nucleosomes depicts a wave-like pattern which anti-correlates with nucleosome density (orange line). In the nucleosome map (orange line), peaks represent the presence of a well-positioned nucleosome on the DNA sequence and valleys represent the nucleosome-depleted 'linker' DNA. (B) Insertion of the marker cassette (every 7 bp) in a 300 bp region of the *S. cerevisiae* genome lacking well-positioned nucleosomes shows that in this case, transformation efficiency (black bars) does not show a clear wave-like pattern as observed in panel A.

further explore the effect of local DNA sequence and nucleosome occupancy, we used two yeast strains that have different nucleosome occupancy across a specific region of their genomes without any differences in local DNA sequence. The first strain was FY5 (Mat α, S288c background) used in our other experiments whereas the second strain (AJY377) contained a changed sequence upstream of the *URA3* open reading frame; this resulted in a changed nucleosome map for the *URA3* ORF; from a well-positioned to a fuzzy map (46). We compared transformation efficiencies of these two strains in the same 100 bp region with 7 bp insertion intervals and found different transformation efficiency patterns for the two strains. Therefore, we concluded that changes in

nucleosome positions between these two strains lead to corresponding changes in the pattern of transformation efficiency, thereby demonstrating a direct effect of nucleosome positioning on transformation efficiency, independent of the local DNA sequence (Figure 4B).

DISCUSSION

Our results demonstrate that nucleosome positioning greatly affects transformation efficiency. In nucleosome poor regions, foreign DNA integrates 2- to 10-fold more efficiently than in regions covered by well-positioned nucleosomes. This study is the first to experimentally explore the correlation between nucleosome positioning and
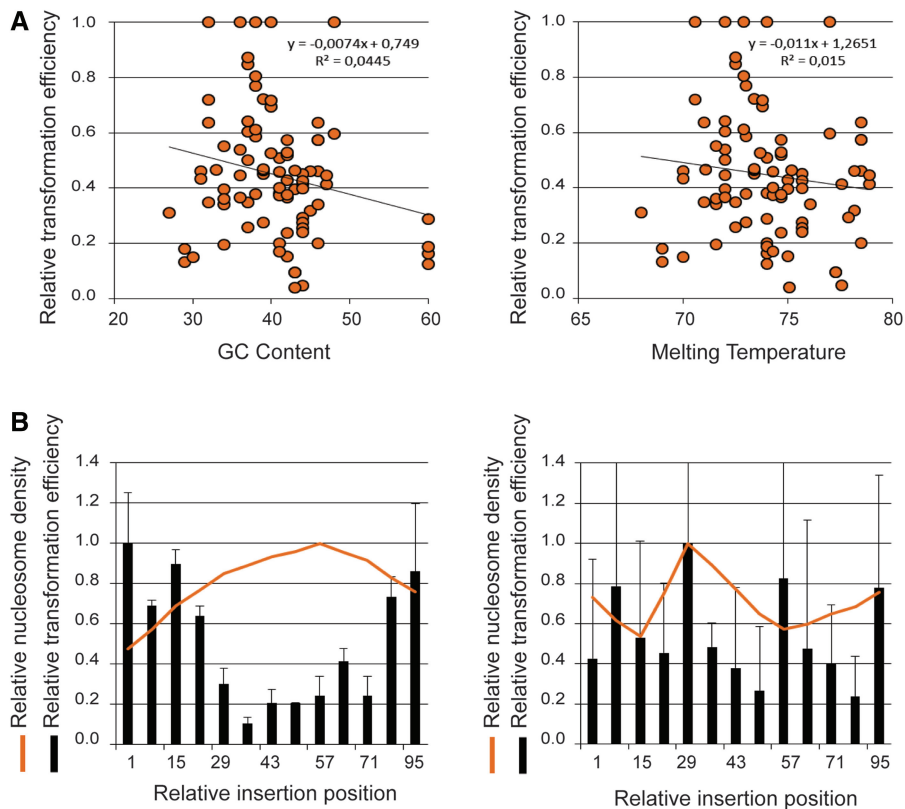
Figure 4. (**A**) Transformation efficiency does not correlate with properties of local DNA. The GC content and melting temperature of sequences of homology for each target position do not show correlation with local transformation efficiency. (**B**) Changes in the nucleosome pattern affect local transformation efficiency. Insertion of a marker cassette (every 7 bp) in a region of ∼100 bp in the same genomic locus in two strains that have a different nucleosome structure in this region reveals that nucleosome positions directly affect transformation efficiency. *Left*: the S288c strain shows a well-positioned nucleosome centered over the middle of the locus under investigation (seen as a peak in the relative nucleosome density, orange line). The local transformation efficiencies (black bars) show a strong anti-correlation with nucleosome density (orange line). *Right*: the same genomic region in a mutant derived from the same S288c strain that contains a different sequence upstream of the locus under investigation that disrupts the local nucleosome structure (orange line). In this mutant, the transformation efficiencies (black bars) do not show a clear pattern anymore. Moreover, despite both wild type S288c strain (left) and the mutant (right) have exactly the same DNA sequence at the locus under investigation, the transformation efficiencies differ greatly between these two strains.

transformation efficiency. Interestingly, a few previous studies have investigated the effect of nucleosome positioning on other DNA integration processes (i.e. retrotransposition or retroviruses). Although these processes involve different mechanisms, they all require insertion of DNA into the host genome. Our results are consistent with recent studies on Ty5 and Hermes transposon insertion in yeast (47,48). However, other recent studies show that not all DNA insertion events occur preferentially in nucleosome-depleted regions; yeast Ty1 retrotransposon targets nucleosomal DNA in regions upstream of Pol III-transcribed genes and integration of the HIV genome into the host genome seems to exploit nucleosome-induced bending of DNA and therefore occurs in nucleosome-occupied loci (49–53). Further research elucidating the precise molecular mechanisms underlying the DNA integration process in these different transposition events may shed light on the observed differences in the preference for naked or nucleosome-occupied insertion spots between transformation and transposition of certain (but not all) transposons. Another interesting issue to explore is whether a different nucleosome occupancy also influences expression

levels of the inserted construct. Studies have shown that different insertion sites can lead to different expression levels of the integrated gene (54–58). This is a major problem in plant transformation, where researchers cannot control the insertion site.

Though nucleosome density is not the only factor determining local transformation efficiency, nucleosomes clearly play a central role in directing foreign DNA to the target integration site. Apart from unveiling a novel function of nucleosomes, our results may also be very helpful for research projects suffering from low transformation efficiencies (e.g. genetics and synthetic biology projects in non-model organisms and industrial microbes that are difficult to transform). Since nucleosome-depleted sites show up to one order of magnitude higher transformation rates than nucleosome-occupied sites, selecting integration sites with low nucleosome densities may help overcome the problems associated with low transformation efficiencies. In this respect, it is important to note that other research teams have recently developed highly accurate models that are able to predict nucleosome positioning from the local DNA sequence (59,60). Although experimental *in vivo* nucleosome maps are only available

for some organisms, *in vitro* nucleosome maps have been shown to be very similar to *in vivo* nucleosome maps (22,45). This allowed the development of algorithms that can predict nucleosome occupancy, only based on DNA sequence (17,22,45,61). Since transformation efficiency is affected by DNA-directed nucleosome occupancy, these algorithms can be used to predict transformation efficiency based on target DNA sequence *a priori,* even for organisms and genomes for which nucleosome positions have not been determined experimentally. This will allow researchers to select the most efficient integration loci in the target genome.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1–3.

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