Cell, Vol. 117, 427–439, May 14, 2004, Copyright ©2004 by Cell Press

Genome-Wide Localization of the Nuclear Transport Machinery Couples Transcriptional Status and Nuclear Organization

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Summary

The association of genes with the nuclear pore complex (NPC) and nuclear transport factors has been implicated in transcriptional regulation. We therefore examined the association of components of the nuclear transport machinery including karyopherins, nucleoporins, and the Ran guanine-nucleotide exchange factor (RanGEF) with the *Saccharomyces cerevisiae* genome. We find that most nucleoporins and karyopherins preferentially associate with a subset of highly transcribed genes and with genes that possess Rap1 binding sites whereas the RanGEF preferentially associates with transcriptionally inactive genes. Consistent with coupling of transcription to the nuclear pore, we show that transcriptional activation of the *GAL* genes results in their association with nuclear pore proteins, relocation to the nuclear periphery, and loss of RanGEF association. Taken together, these results indicate that the organization of the genome is coupled via transcriptional state to the nuclear transport machinery.

Introduction

Transcriptional regulation has been shown to correlate with the intranuclear position of genes in a variety of species. For example, proper silencing of genes involved in B cell and T cell development is dependent upon the ability of the genes to relocate to centromeric heterochromatin (Brown et al., 1997, 1999). In *Drosophila melanogaster*, the degree of position effect variegation is correlated with the subnuclear localization of a reporter gene (Dernburg et al., 1996). Additionally, proper silencing of the mating-type loci in *Saccharomyces cerevisiae*, *HML* and *HMR*, is dependent upon their ability to associate with the nuclear periphery (Andrulis et al., 1998, 2002; Feuerbach et al., 2002). Furthermore, the *HML* locus is derepressed when relocated from its subtelomeric site to a more centromere-proximal location or to the arm of a different chromosome (Maillet et al., 1996; Marcand et al., 1996; Stavenhagen and Zakian, 1994; Thompson et al., 1994). Thus, the spatial context of a gene within the nucleus as well as within a chromosome appears to be critical in the epigenetic control of heterochromatin formation.

A number of nuclear proteins important for the maintenance of higher order genomic organization have been identified, including components of the nuclear pore complex (NPC; Feuerbach et al., 2002). The *S. cerevisiae* NPC is ~50 MDa in size, comprised of ~30 nucleoporin proteins, and is the channel through which proteins traverse the nuclear envelope. The nuclear face of the NPC is arranged in a basket-like structure, extending ~95 nm into the nucleoplasm (Fahrenkrog et al., 1998). Several studies have demonstrated that nucleoporins are essential for mediating epigenetic control of transcription in yeast. Feuerbach et al. have shown that the nucleoporins *NUP60* and *NUP145* are genetically required for full repression of the *HMR* locus. The deletion of myosin-like protein genes *MLP1* and *MLP2*, which are nuclear pore associated (Galy et al., 2000; Kosova et al., 2000; Strambio-de-Castillia et al., 1999), also causes derepression of a reporter gene at the *HMR* locus (Feuerbach et al., 2002). Furthermore, *MLP1* and *MLP2* have been implicated in the control of telomere anchoring and repression (Galy et al., 2000; Hediger et al., 2002). A recent screen for boundary proteins that prevent the spread of silencing at the *HML* locus identified several components of the nuclear transport machinery including the exporter, Cse1, and the nucleoporin Nup2 (Ishii et al., 2002). Nup2 is a known docking site for Cse1 (Hood et al., 2000), and the absence of Nup2 from the NPC abolishes the boundary activity of Cse1, highlighting the influence of nuclear transport factors on transcriptional state (Ishii et al., 2002). These results have suggested that association of genes with the NPC may play a key role in transcriptional regulation, possibly by topologically constraining repressed DNA segments.

The mammalian Ran guanine-nucleotide exchange factor, or RanGEF, is known to associate with a nuclear basket nucleoporin, Nup98, and with chromatin (Fontoura et al., 2000; Nemergut et al., 2001). RanGEF mutants exhibit defects in nuclear structure, chromosome stability, and chromatin condensation as well as perturbation of nuclear transport (Aebi et al., 1990; Azuma and Dasso, 2000; Clark et al., 1991; Forrester et al., 1992; Kadowaki et al., 1994; Ohtsubo et al., 1987). As such, NPC components and the RanGEF are well-established contributors to genomic organization in *S. cerevisiae*.

Using genomic location analysis (Ren et al., 2000), we have examined all genes bound by the NPC, several karyopherins, and the RanGEF in *S. cerevisiae*. We find that the silent mating-type loci, subtelomeric genes, and many transcriptionally active genes can be found in association with the NPC. Further analysis demonstrates that NPC-associated genes are significantly enriched for the binding site of the transcriptional regulator Rap1. Additionally, members of nuclear transport complexes with similar functional roles have similar occupancy profiles. Finally, we find both by chromatin immunoprecipitation and microscopy that genes relocate from the nucleoplasm to the nuclear pore upon transcriptional induction.

Results

The nucleoporins Nup2, Nup60, and Nup145, the karyopherin Cse1, and the nuclear pore-associated proteins

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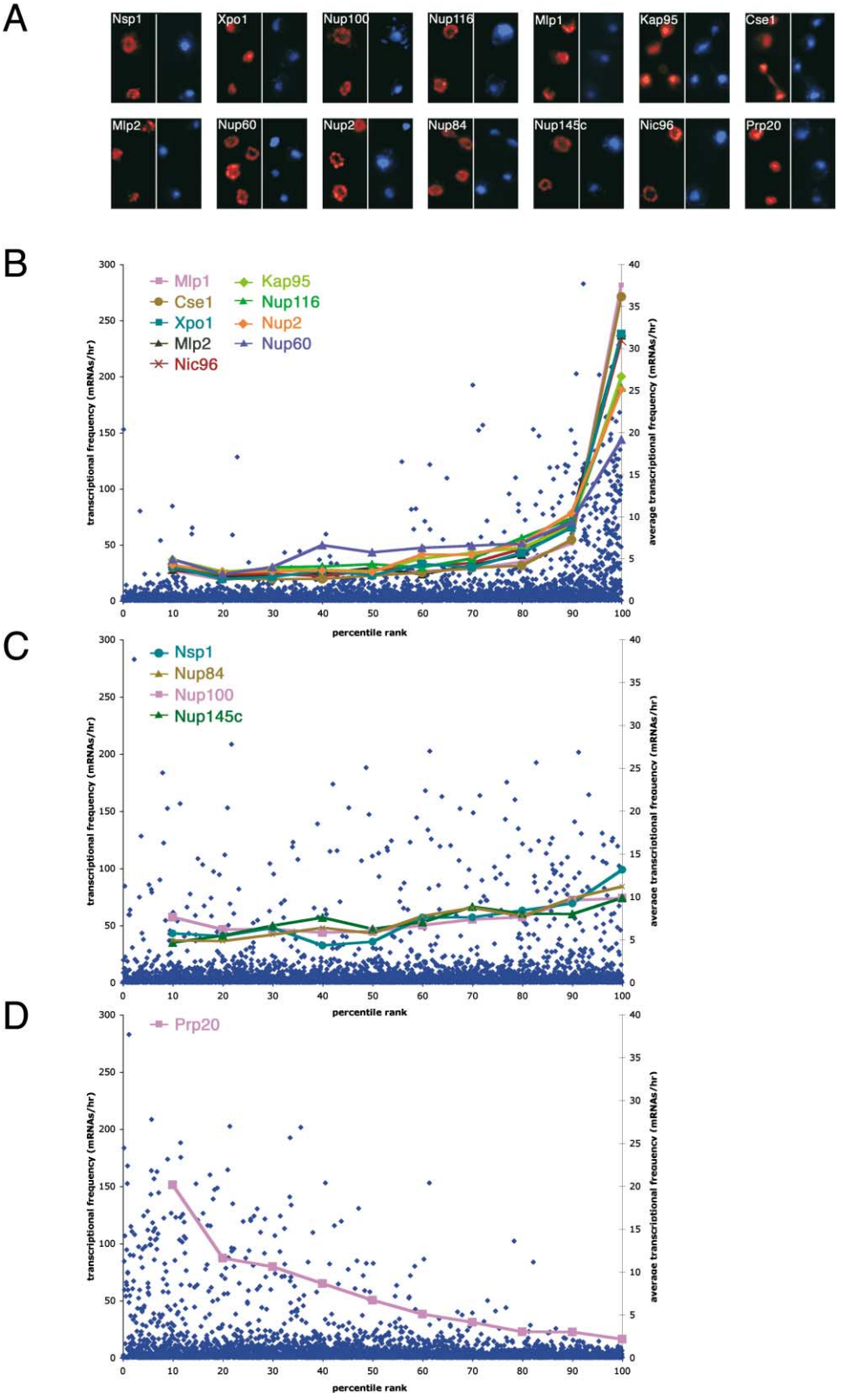


Figure 1. Correlation between Genome Occupancy and Transcriptional Frequency

(A) Localization of C-terminally Myc-tagged proteins as demonstrated by indirect immunofluorescence. Proteins were visualized with Alexa Fluor 594 (left) while DNA was stained with DAPI (right).

(B) ORFs are graphed as the percentile rank in each genomic localization experiment versus the experimentally determined transcriptional frequency for that gene (mRNAs/hr, Holstege et al., 1998). The average transcriptional frequency is graphed as a function of binding level

Mlp1 and Mlp2 were chosen for genome-wide location analysis based upon their previously identified roles in *HML* and *HMR* silencing (Feuerbach et al., 2002; Galy et al., 2000; Ishii et al., 2002; Ren et al., 2000; Strambiode-Castillia et al., 1999). We also obtained the genomic location profile of a set of nucleoporins from the central pore and nuclear basket of the NPC including Nic96, Nsp1, Nup84, Nup100, and Nup116, which were not previously shown to interact with genes in any specific way. In addition to Cse1, we chose to compare the behavior of two other members of the karyopherin family: Kap95, a nuclear localization sequence (NLS) binding importer, and Xpo1/CRM1, a nuclear export sequence (NES)-recognizing exporter (Weis, 2003). Finally, we obtained the genomic localization profile of the RanGEF, termed Prp20 in yeast, an essential regulator of the nuclear transport process that associates with histones (Huang et al., 2002; Nemergut et al., 2001; Weis, 2003).

To aid in chromatin immunoprecipitation (ChIP), Myc epitopes were added to the C terminus of the factors examined. In the case of Nup145, the previously observed auto-proteolytic cleavage (Emtage et al., 1997; Teixeira et al., 1997, 1999) was retained in the Myc-tagged strain (data not shown) and therefore the C-terminal portion of the protein used for our experiments will be hereafter referred to as Nup145c. All strains showed wild-type localization of the C-terminally Myc-tagged proteins, as demonstrated by immunofluorescence (Figure 1A). For ChIP, cells were grown in glucose to mid-logarithmic phase and then treated with formaldehyde to produce DNA-protein crosslinks. Chromatin was isolated, sheared to an average size of $\sim$350 bp, and DNA fragments associated with the protein of interest were enriched by anti-Myc IP. DNA fragments from the IP sample and a control whole-cell extract (WCE) were amplified by ligation-mediated PCR followed by incorporation of fluorophores. The samples were then competitively hybridized to at least three microarrays containing all *S. cerevisiae* ORF sequences.

Nuclear Pore-Associated Proteins Bind Preferentially to Transcriptionally Active Genes

We first sought to ascertain whether there was a correlation between transcriptional state and nuclear transport factor occupancy since previous studies have indicated that the mating-type loci require association with the nuclear periphery to achieve proper silencing (Feuerbach et al., 2002; Ishii et al., 2002). We therefore compared the degree to which all genes were bound and the transcriptional frequency of those genes, as determined by Holstege et al. (1998). Surprisingly, the karyopherins Xpo1, Cse1, and Kap95 as well as the nuclear pore components Nic96, Nup116, Nup2, and Nup60 and the NPC-associated proteins Mlp1 and Mlp2 showed a strong correlation between occupancy and transcriptional frequency (Figure 1B). Despite the apparent strong bias toward occupancy at highly transcribed genes, several infrequently transcribed genes are found in the 90th percentile or greater of the bound population (Figure 1B, see below). The nucleoporins Nsp1, Nup84, Nup100, and Nup145c, however, do not show a significant correlation between transcriptional frequency and occupancy throughout their genomic localization profiles (Figure 1C). These differences may reflect functional units of the nuclear pore, which will be discussed later.

The Prp20 genomic localization profile was strikingly contrary to that of the other factors examined. While sharing occupancy at the silent mating-type loci with all other components of the nuclear transport machinery examined (Supplemental Data at http://www.cell.com/cgi/content/full/117/4/427/DC1), Prp20 binds preferentially to transcriptionally inactive portions of the genome (Figure 1D).

Specific Functional Gene Classes Are Enriched for Binding to the NPC and Karyopherins

We next sought to determine if any particular gene classes were responsible for the apparent transcriptional bias seen in the occupancy profiles. To achieve this, gene spots with fluorescence intensities deemed statistically significant and with an IP/WCE ratio > 1.0 were examined for over- and under-enrichment of particular gene classes from the Gene Ontology Consortium for *S. cerevisiae* (Ashburner et al., 2000; Berriz et al., 2003). Mlp1, Mlp2, Cse1, Nic96, Nup116, Xpo1, and Nup2 showed strong enrichment for binding to genes involved in glycolysis and protein biosynthesis (p value $<$ 0.001; Table 1). The glycolysis and ribosomal protein (RP) gene categories comprise some of the most highly transcribed genes in yeast and are largely responsible for the transcriptional bias seen in Figure 1B. However, enrichment for these gene classes was not universal among nuclear transport factors: the karyopherin Kap95, the nucleoporins Nup60 and Nup100, and the RanGEF Prp20 did not show significant occupancy at glycolysis-associated genes (Table 1). The variation in genomic localization profiles is most notable in the case of Prp20, which showed significant under-enrichment for genes involved in protein biosynthesis and resides at infrequently transcribed genes that are largely uninhabited by the karyopherins and nucleoporins examined in this study. The Nup84, Nup145c, and Nsp1 genomic localization profiles did not show enrichment for specific gene classes and are not shown in Table 1. The lack of gene class enrichment may reflect either a reduced ability to crosslink these components to chromatin or an absence of Nsp1, Nup84, and Nup145c at the NPCs bound to the previously mentioned gene classes.

Rap1 Binding Sites Are Enriched for Binding by Nuclear Transport Factors

The karyopherin and nucleoporin enrichment at the silent mating-type loci and RP and glycolysis genes is

with a bin size of 10%. The percentile rank reflects relative binding level from 0% (not bound) to 100% (highest binding). Binding trends for Mlp1, Cse1, Xpo1, Mlp2, Nic96, Kap95, Nup116, Nup2, and Nup60 versus transcriptional frequency are displayed with individual Nic96 ORFs shown (blue diamonds).

(C) Nsp1, Nup84, Nup100, and Nup145c binding versus transcriptional frequency. The Nup100 ORF distribution is displayed.

(D) Prp20/RanGEF genomic binding versus transcriptional frequency.

Table 1. Gene Class Enrichment in Nuclear Transport Factor Genomic Location Profiles

	Mlp1	Mlp2	Cse1	Nic96	Nup116	Xpo1	Nup2	Kap95	Nup60	Nup100	Prp20
Metabolism											
Energy pathways											
Carbohydrate biosynthesis	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
Glycolysis	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
Fermentation	<0.001	0.007	0.019	0.02	0.005	0.006			0.005		
Amine biosynthesis											
Amino acid biosynthesis		0.049	<0.001	0.032	<0.001						
Amino acid metabolism		0.025	0.021		<0.001						
Protein metabolism											
Protein biosynthesis	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
Large ribosomal subunit	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.038	0.030	
Small ribosomal subunit	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.029	
Translation elongation factor	<0.001	<0.001	<0.001	<0.001	<0.001	0.001			0.035		
Heat shock protein			0.004		0.012						
Cell wall	<0.001	<0.001	0.001	<0.001	<0.001	0.001					
Nucleosome	<0.001		0.01	0.001		<0.001	<0.001	0.018			
Lipid particle		0.029		0.025							
Cytokinesis, completion of separation				0.014							
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	*<0.001*	*0.022*	*<0.001*								
Cell cycle	*0.012*	*0.026*									
RNA metabolism	*0.034*										
rRNA metabolism										*0.006*	
Protein metabolism											
Protein biosynthesis											*<0.001*
Large ribosomal subunit											*<0.001*
Translation elongation factor										*<0.001*	*<0.001*

Target genes from the nuclear transport factor genomic location profiles (IP/WCE $\geq$ 1.0, p value $\leq$ 0.02) were examined for gene category enrichment within the *S. cerevisiae* Gene Ontology Consortium (Ashburner et al., 2000) using Funcassociate (Berriz et al., 2003). The significance of enrichment for the listed functional categories was determined by Fisher's exact test, corrected for multiple hypothesis testing, and is displayed as a p value. The values in italics represent statistically significant under-enrichment for gene categories in each profile.

strikingly similar to the profile of genes bound by the transcriptional regulator Rap1 (Lieb et al., 2001). Rap1 is a yeast DNA binding protein that associates with telomeres, the silent mating-type loci, and many transcriptionally active genes and exhibits boundary activity (Klein et al., 1992; Lieb et al., 2001; Shore, 1997; Yu et al., 2003). We reasoned that binding of factors such as Rap1 to *cis*-regulatory sequences might contribute to the recruitment of genes to the nuclear periphery for association with the Mlp proteins and the NPC. We used AlignAce (Hughes et al., 2000; Tavazoie et al., 1999) to search for motifs in the sequences 500 bp upstream of genes bound by Mlp1, Mlp2, and Nic96 (Supplemental Data on *Cell* Website). Figure 2 shows that the Rap1 binding site (Buchman et al., 1988; Graham and Chambers, 1994; Idrissi and Pina, 1999; Lascaris et al., 1999) was strongly over-represented in the datasets examined. In addition, two A+T rich motifs are over-enriched in the promoters of genes, one of which has been previously characterized as the cell cycle activation (CCA) motif present in histone promoters (Freeman et al., 1992). Intriguingly, previous studies aimed at identifying looped chromosomal domains and nuclear scaffolds in yeast also show enrichment for A+T-rich regions of DNA (Amati et al., 1990; Amati and Gasser, 1988). Finally, two G+C-rich sequences lacking cognate transcription factors were identified as well.

Genome-wide location analysis has been previously performed on Rap1 (Lieb et al., 2001), so we compared the Rap1 genomic binding dataset and our yeast nuclear transport factor occupancy profiles. The transport factors Cse1, Mlp2, Mlp1, Xpo1, Nic96, Nup116, and Nup2 appear to bind preferentially to genes with Rap1 sites, which constitute about 8% of all yeast genes. In Figure 3, we display those genes that are bound by at least five of these seven nuclear transport factors. The propensity of nuclear transport factors to bind to genes containing Rap1 sites does not appear to be dependent upon enrichment of Rap1 sites in highly transcribed genes. Of the 193 genes bound by at least five such factors, 46 are infrequently transcribed (Holstege et al., 1998). Of these 46 genes, 14 have Rap1 sites (30%), far more than the 8% expected by chance ($p = 1.3 \times 10^{-7}$). In addition, of the 147 more highly transcribed genes in this set, half (74) have Rap1 sites. Thus, Rap1 occupancy also appears to significantly contribute to gene association with nuclear transport factors in addition to the influence of transcriptional status upon binding.

Nuclear Transport Subcomplexes Show Similar Specificities for Genome Occupancy

Gross inspection of the datasets led to a grouping of nuclear transport factors into three sets: proteins that bound preferentially to highly expressed genes (Mlp1, Cse1, Xpo1, Mlp2, Nic96, Kap95, Nup116, Nup2, and Nup60), proteins that showed no preference with respect to highly expressed genes (Nsp1, Nup84, Nup100, and Nup145c), and a protein that preferentially bound to infrequently expressed genes (Prp20). Highly expressed genes constitute only about 15%–20% of the yeast ge-

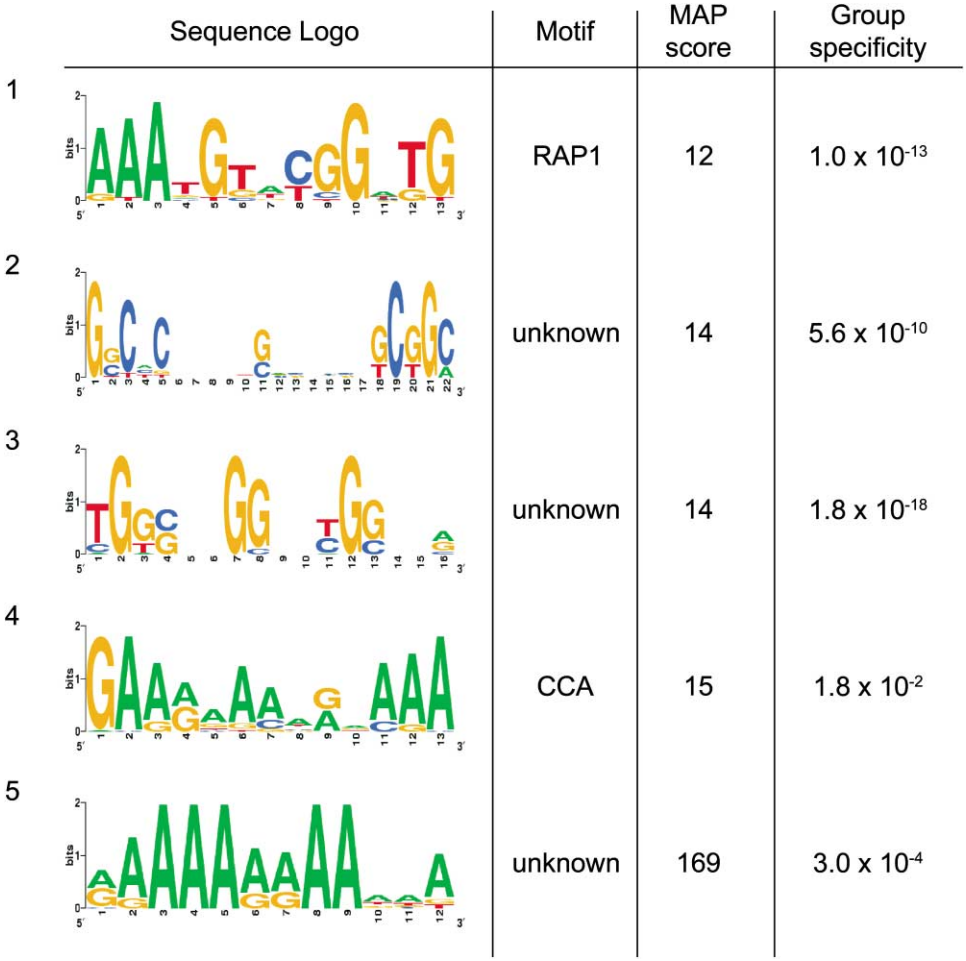


Figure 2. DNA Sequence Motifs Found among Mlp1, Mlp2, and Nic96 Target Genes

Sequence logos representing the motifs discovered in the combined Mlp1, Mlp2, and Nic96 genomic localization profiles. The overall height of each letter reflects its information content (0–2 bits). MAP scores are AlignAce internal metrics used to determine the significance of alignment. Group specificity scores measure the enrichment of this motif within our dataset relative to its abundance in the entire genome.

nome (Holstege et al., 1998), so we used hierarchical clustering (Figure 4A) and principal component analysis (PCA; Figure 4B) to identify underlying gene binding patterns. As shown by Alter et al., PCA can be used to sort microarray experiments into groups with similar regulation and functional roles (Alter et al., 2000). We extended this analytical technique to whole genome location analysis, in which the analog of coregulated groups would be protein complexes. The advantage of using such statistical grouping methods is that they do not depend on previously established gene categories.

We found that the PCA analysis led to groupings of the nuclear transport factor datasets in a way that could be correlated with known physical and functional interactions (Figure 4). The myosin-like proteins Mlp1 and Mlp2 are known to colocalize at the nuclear periphery and share common biochemical characteristics (Galy et al., 2000; Strambio-de-Castillia et al., 1999). As shown in Figure 4, Mlp1 and Mlp2 are in the same PCA cluster and have a distance metric of 0.8 by hierarchical clustering, indicating a high degree of overlap in their occupancy profiles. Other groupings based on both statistical measures include Nup145c and Nup84, Prp20 and Nup100, and a large group consisting of Mlp1, Mlp2, Xpo1, Nic96, Cse1, Nup116, Nup2, and Nup60. These data can be generally correlated with a map of the known and hypothesized physical and functional interactions of nuclear transport factors (Figure 4C) and may be used to argue for various interactions in vivo (see Discussion).

Nucleoporin and RanGEF Genomic Localization Changes in Response to Transcriptional Stimulus

As the nuclear transport factors bound to specific genomic loci and gene classes, we asked whether the localization profiles would change in response to a transcriptional stimulus. The response to galactose in yeast is well characterized, with an approximately 20-fold induction of the genes *GAL1*, *GAL2*, *GAL7*, and *GAL10* (Lashkari et al., 1997). The four *GAL* genes are distributed randomly in the genomic localization profiles for Nup116, Mlp1, Nup60, Cse1, Xpo1, and Nup100 in cells grown in glucose (Figure 5). Upon galactose induction, we found that the four *GAL* genes shifted to the 97th percen-

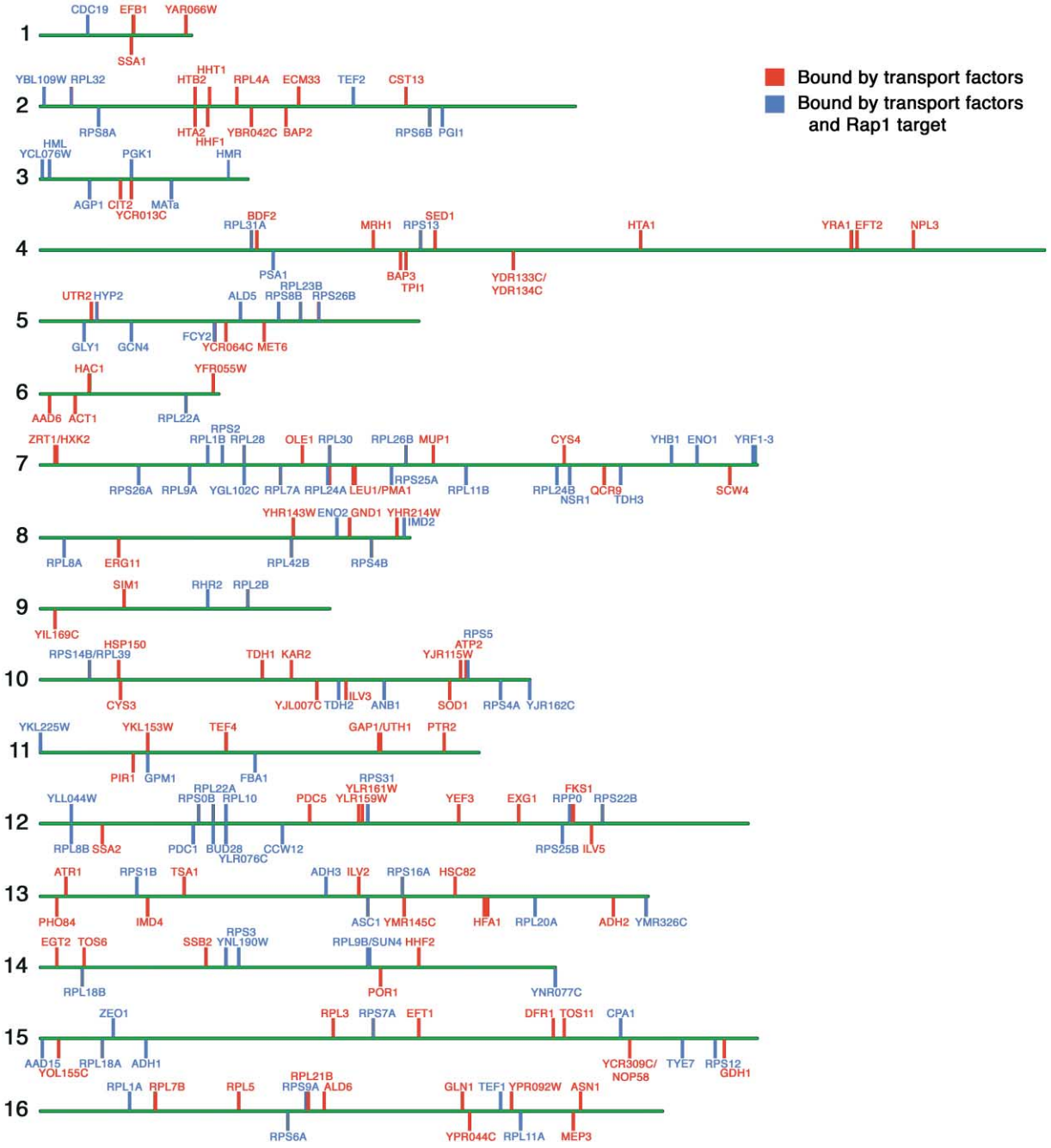


Figure 3. Map of the Overlap between Nuclear Transport Factor and Rap1 Occupancy in the *S. cerevisiae* Genome

Each of the 16 yeast chromosomes is displayed with lines representing the position of individual ORFs on the top (Watson) and bottom (Crick) strands. All ORFs displayed are bound by at least five nuclear transport factors showing enrichment for Rap1 targets. ORFs bound by nuclear transport factors but not Rap1 are displayed in red, while genes bound both by nuclear transport factors and Rap1 are displayed in blue.

tile or greater for these genomic binding profiles. Except for the *GAL* genes, the genomic localization profiles for these nuclear transport factors are largely similar in cells grown in glucose and galactose (Supplemental Data online), indicating that only localized changes in nuclear organization have taken place.

In contrast, the four *GAL* genes were found between the 68th and 97th percentiles in the Prp20 genomic localization profile when cells are grown in glucose, reflecting the Prp20/RanGEF occupancy at transcriptionally inactive genes. Upon shift to galactose, *GAL* gene binding by Prp20 was abolished, with the four genes at the 26th percentile or lower (Figure 5). This indicates that Prp20 is excluded from transcriptionally active genes. As with the other nuclear transport factors, the overall Prp20 genomic localization profile in cells grown in glucose and galactose are similar (Supplemental Data).

The change in nucleoporin association with the *GAL*

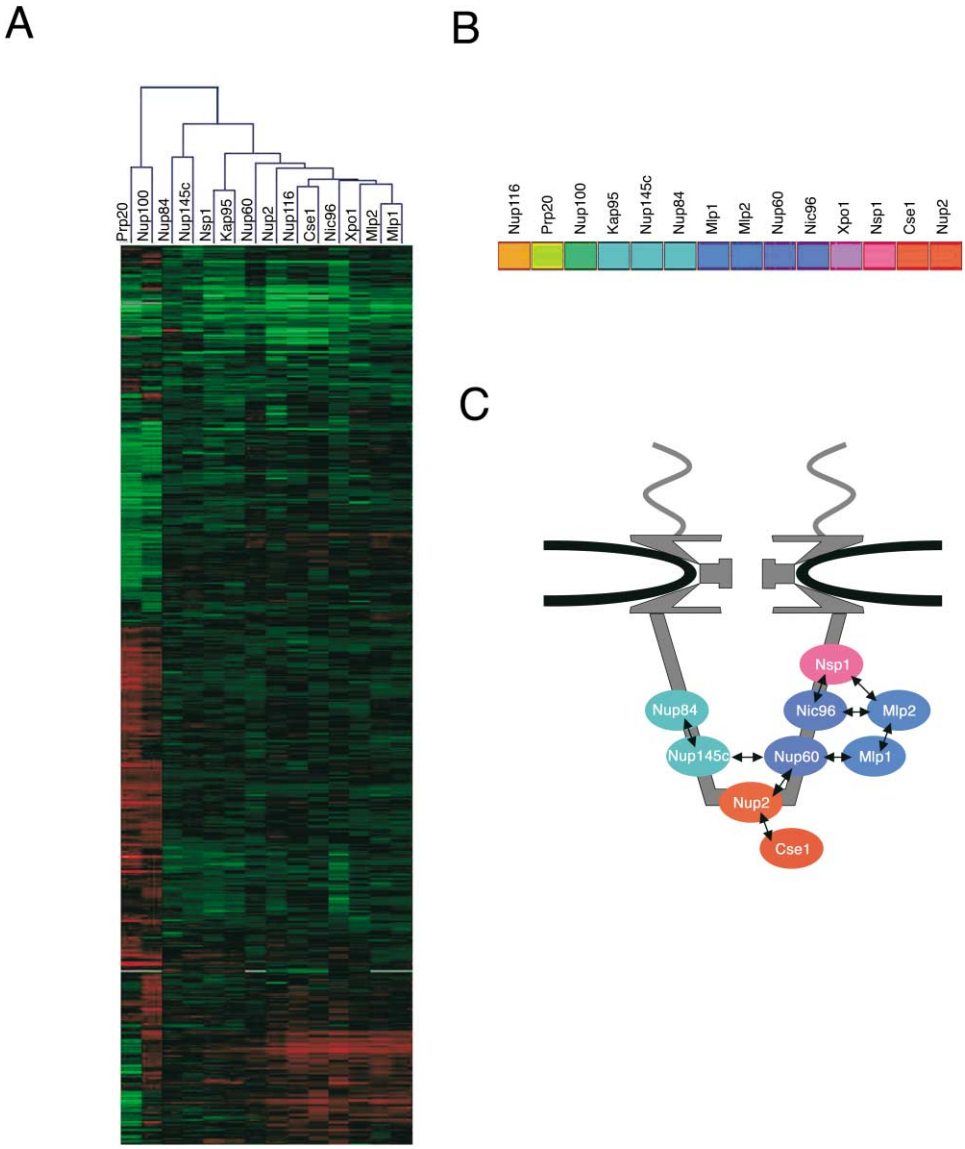


Figure 4. Clustering of Genomic Localization Profiles Reflects Known Physical and Functional Interactions at the NPC

(A) Hierarchical clustering of the genomic localization profiles was generated using the Rosetta Resolver algorithm for correlation without mean subtraction weighted by error. Genes with a p value ≤ 0.002 were considered in generating the cluster. The color scale ranges from saturated red for log ratios ≥ 0.5 to saturated green for log ratios ≤ -0.5.

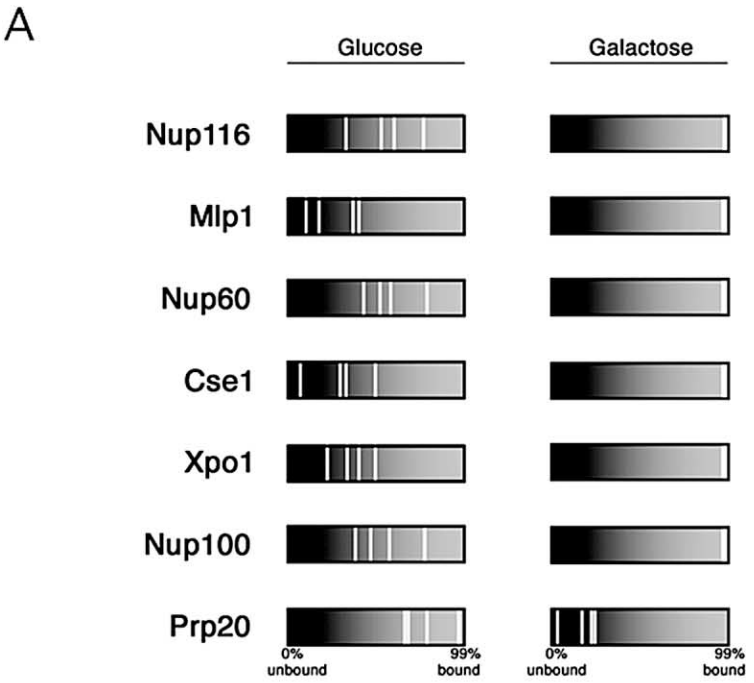
(B) Principal component analysis was performed using Rosetta Resolver with data reduction at 90% variation and sequences with a p value ≤ 0.002.

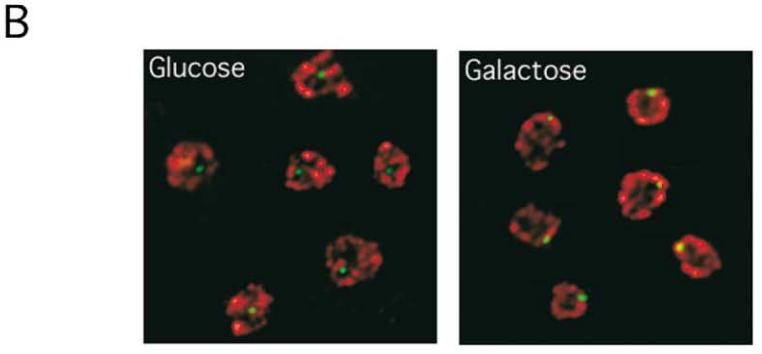
(C) Known subcomplexes of the nuclear pore complex are depicted (Booth et al., 1999; Grandi et al., 1993; Hood et al., 2000; Kosova et al., 2000; Suntharalingam and Wente, 2003) and colored according to principal component in (B). A direct or functional interaction between proteins is represented by a double-headed arrow.

genes indicates a change in the intranuclear location of the genes themselves upon galactose induction. Using a 5.3 kb digoxigenin-labeled probe that includes the *GAL1*, *GAL7*, and *GAL10* genes on chromosome II, we performed fluorescence in situ hybridization (FISH) combined with immunofluorescence (IF) of the nuclear pore complex. As illustrated in Figure 5, the *GAL* region is found associated with the nuclear periphery in 63% of cells after galactose induction versus just 21% of cells when grown in glucose. These data show that the intranuclear position of the GAL region itself changes in response to galactose to favor colocalization with the NPC.

Discussion

A number of previous studies have connected the association of genes with the nuclear periphery and the role of a few nuclear pore components with transcriptional repression of certain genes in *S. cerevisiae* (Andrulis et al., 1998, 2002; Feuerbach et al., 2002; Galy et al., 2000; Gotta and Gasser, 1996; Hediger et al., 2002; Ishii et al.,





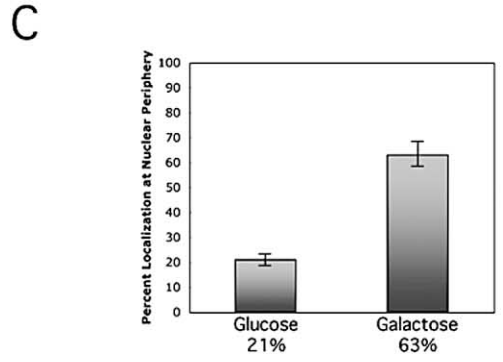


Figure 5. Change in *GAL* Gene Binding at the Nuclear Periphery upon Stimulation with Galactose

(A) The distributions of *GAL1*, *GAL2*, *GAL7*, and *GAL10* are displayed as white bars, representing the genes' percentile rank, on the distribution of binding for each nuclear transport factor in glucose (left) and galactose (right). The gradient of binding ranges from 0% (unbound) to 100% (bound). The four genes are combined into a single white bar after galactose stimulation for Nup116, Mlp1, Nup60, Cse1, Xpo1, and Nup100 as all were above the 97th percentile.

(B) Combined IF/FISH of the *GAL1*, *GAL7*, and *GAL10* region and the nuclear pore complex in haploid cells grown in glucose (left) and galactose (right). The 5.3 kb *GAL* region is visualized as a single green spot with nuclear pore staining in red.

(C) Localization of the *GAL* region relative to the nuclear pore complex was scored for 120 cells grown in glucose and galactose. Cells were scored as being at the nuclear periphery only if they entirely overlapped with nuclear pore staining and are represented as the percentage of cells showing peripheral localization in glucose (left) and galactose (right).

2002; Kosova et al., 2000; Strambio-de-Castillia et al., 1999). To address the generality of these effects, we undertook a genome-scale analysis of DNA binding patterns for 14 nuclear pore components and associated factors. Specifically, we examined DNA segments bound by the nuclear pore components Nup116, Nup100, Nup145c, Nup60, Nic96, Nsp1, and Nup2; the import and export factors Kap95, Xpo1, and Cse1; the pore-associated myosin-like proteins Mlp1 and Mlp2; and Prp20, the GEF for the Ran GTPase. In contrast to previous studies (Feuerbach et al., 2002; Ishii et al., 2002), our experiments were carried out in wild-type yeast cells and thus represent the association of nuclear transport factors with the genome in a natural cellular state. Surprisingly, most of the nuclear transport factors, including factors that had been implicated in transcriptional re-

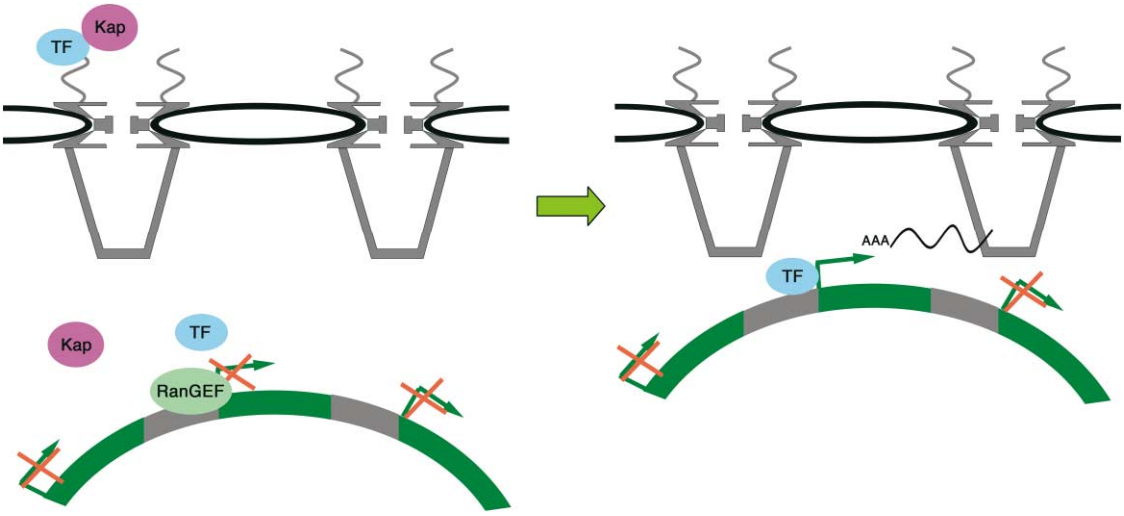


Figure 6. Models of Nuclear Transport Factor Association with the Genome

The RanGEF is shown binding to transcriptionally inactive portions of the genome that are not bound by nucleoporins, karyopherins, or the Mlp1 and Mlp2 proteins. RanGEF/RanGTP stimulated dissociation of transcription factors (TF) from kayropherin proteins (Kap) at transcriptionally inactive genes may lead to rapid and localized induction. Upon induction of transcription, active genes are recruited to the pore, which may facilitate proper boundary formation and/or promote direct entry of pre-mRNAs into the processing and export pathways.

pression, associated with a set of genes that was enriched for transcriptionally active sequences.

These experiments have led to several major conclusions. First, different nuclear transport proteins bind different subsets of genes: a large class of nuclear transport factors (class I; Mlp1, Cse1, Xpo1, Mlp2, Nic96, Kap95, Nup116, Nup2, and Nup60) preferentially associates with highly transcribed genes; a second class consisting of four nuclear pore proteins (class II; Nsp1, Nup84, Nup100, and Nup145c) showed no preference with respect to transcription levels; and one factor, the RanGEF, preferentially associates with infrequently transcribed genes. Second, genes with binding sites for Rap1 are associated with a set of nuclear transport factors. Our data therefore suggest a role for Rap1 as a general factor that might mediate anchoring of DNA to the nuclear periphery. Third, the interaction between highly transcribed genes and a subset of nuclear pore proteins predicts that at least some genes move to the pore upon transcriptional activation. Using FISH and confocal microscopy, we confirmed that the *GAL* genes move to the nuclear periphery upon galactose induction, providing validation of our genomic data.

The NPC in Genomic Organization

In yeast, reporter genes incorporated into the silent mating-type loci *HMR* and *HML* have been used to identify genes involved in the creation and maintenance of repression. Feuerbach et al. (2002) found that mutant versions of the nuclear pore components Nup60 and Nup145 and the pore-associated proteins Mlp1 and Mlp2 affected gene expression of artificial constructs with active genes inserted into the silent mating-type loci. In addition, Cse1 and Nup2 fusion proteins were shown to exhibit "boundary activity," preventing the spread of silencing to a reporter gene inserted at the *HML* locus (Ishii et al., 2002).

Our results go beyond these observations by identifying the genes that normally associate with pore proteins in wild-type yeast, including specific gene classes that are enriched for occupancy. These functional groups included ribosomal protein genes and genes involved in glycolysis and fermentation, leading to the more general observation that most of the nuclear transport factors associate with highly expressed genes. This finding may reflect the need for boundary elements between transcriptionally inactive and active portions of the genome to prevent the spread of transcriptional activation; alternatively, highly transcribed genes could associate with pores to facilitate efficient mRNA export (Figure 6). These two models are not mutually exclusive and would suggest that the NPC plays multiple roles in nuclear organization.

Rap1 Site Enrichment in Genes Bound by the NPC

We analyzed the 5′ noncoding sequences of preferentially bound genes for common sequence motifs (Figure 2). This analysis identified five potential motifs: the Rap1 binding site, the cell cycle activation (CCA) motif, and three motifs without known function. The CCA motif is present in genes induced during S phase, such as the highly expressed histone genes (Freeman et al., 1992). A significant portion of the cellular population was in S phase at the time of crosslinking and thus the occupancy at these genes may reflect their transcriptional activation at this stage of the cell cycle.

Rap1 is a yeast DNA binding protein that exhibits boundary activity and associates with telomeres, the silent mating-type loci, and many transcriptionally active genes (Klein et al., 1992; Lieb et al., 2001; Shore, 1997; Yu et al., 2003). Rap1 target genes are abundant in *S. cerevisiae,* comprising ~8% of all genes (Lieb et al., 2001). However, the enhanced frequency of Rap1 sites

in genes occupied by nucleoporins, karyopherins, and Mlp1 and Mlp2 does not appear to be a trivial consequence of the preferential association of highly expressed genes with nuclear transport factors. Of the genes bound by class I nuclear transport factors that are highly expressed, about 50% have Rap1 sites. Additionally, of the genes bound by class I nuclear transport factors that are infrequently expressed, about 30% have Rap1 sites. Therefore, it appears that the association of nuclear transport factors with genes containing Rap1 binding sites is not strictly dependent upon transcriptional state. In addition, the alignment program (AlignAce) used to identify the Rap1 sequence motifs in our datasets is unbiased with respect to known motifs, so the identification of the Rap1 binding site in the bound gene sets was not biased by the original rationale for our experiments. Moreover, this provides an independent confirmation of previous biochemical and cell-biological evidence for Rap1 association at the nuclear periphery (Klein et al., 1992). Finally, the identification of Rap1 sites among genes binding to nuclear transport factors independently suggests a relationship between DNA boundaries and nuclear pore components.

Functional Complexes of the Nuclear Pore

The patterns of association between the genome and various nuclear transport factors can be correlated with known physical and functional interactions at the pore. The NPC may bind to some transcriptionally active genes through mRNA; Mlp1 is known to interact with Nab2, an mRNA binding protein (Green et al., 2003) and appears to be involved in mRNA export (Galy et al., 2004; Kosova et al., 2000). Additionally, this correlation may reflect the manner in which NPC subcomplexes bind the genome. For example, Nup145c and Nup84 are known to exist in an NPC subcomplex (Teixeira et al., 1997) and fall adjacent in the hierarchical and PCA clusters (Figure 4). In addition, Feuerbach et al. (2002) proposed a model for the interaction between nuclear pores and DNA features such as telomeres and boundary elements in which the DNA interacts most directly with Mlp1 and Mlp2 in a hierarchical manner with Nup60 and Nup145. We found that Mlp1 and Mlp2 had very similar occupancy profiles, while Nup60 had a somewhat related profile and Nup145 was related to a lesser degree (Figure 5). These relationships among datasets may arise because there is a certain probability that proteins in a complex will become covalently attached and coprecipitate during crosslinking. Alternatively, the observed differences in the genome binding pattern of various nuclear transport factors could arise from different genes binding to different pore types or to different positions within the same pore. The yeast nuclear pore is a massive structure of approximately 50 MDa that extends almost 0.1 microns into the nuclear interior (Fahrenkrog et al., 1998). The vast size of the NPC makes it likely that proteins at the tip of the nuclear basket can interact with DNA differently than pore proteins near the nuclear membrane or with different DNA sequences entirely.

The Contrast between the Genome Occupancy of the RanGEF and the NPC

The binding pattern for the RanGEF, Prp20, stands in contrast to the binding pattern of all of the other nuclear transport factors examined here. The enzymatic nature of Prp20 suggests a rationale for these distinctions. The exchange of GDP for GTP on Ran promotes the dissociation of import cargoes from karyopherins (Kuersten et al., 2001). It is possible that Prp20 is favoring localized release of import cargoes, such as transcription factors, at genes requiring activation (Figure 6). Once activated, the genes then move to the NPC and lose association with Prp20 (Figure 5). Moreover, the finding that transcriptional activation of the *GAL* genes causes loss of Prp20 association further highlights the specificity shown by the RanGEF for transcriptionally inactive genes. Recent kinetic studies of export complexes have suggested that RanGTP-cargo complexes might sample the nuclear face of the NPC with some regularity, potentially affecting signaling and gene expression in the nucleus (Becskei and Mattaj, 2003). As a result, the genes bound by the RanGEF might be of special importance to the cell in terms of their proper activation and regulation.

In summary, we found that transcriptionally active genes are preferentially associated with nuclear pores. The tendency of nuclear transport factors to associate with genes with Rap1 binding sites appears to be independent of transcriptional activity, as both highly and lowly transcribed genes with Rap1 sites are preferentially bound. Indeed, based upon our data, transcriptional activation and the presence of Rap1 appear to be two different mechanisms by which genes can associate with the nuclear transport machinery.

Finally, our findings are consistent with some aspects of the "gene-gating" hypothesis, which suggests that the interaction of NPCs with different genes might serve as a level of gene regulation (Blobel, 1985). Although this hypothesis was originally put forth many years ago, its central tenet that different genes will be bound by the NPC in different ways has remained untested. This work provides support for this hypothesis, as well as leading to a model for gene regulation by the nuclear pore and nuclear transport machinery.

Experimental Procedures

Strains

The haploid yeast strain PSY2156/Z1256 (MATa, *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3*, GAL+, psi+; Ren et al., 2000) was transformed using a PCR-mediated tagging method described previously (Knop et al., 1999). The 9x MYC C-terminal tagging sequence was inserted into the yeast genome by homologous recombination at the endogenous loci to create *prp20:Myc* (PSY2671), *nup100:Myc* (PSY2774), *mlp1:Myc* (PSY2823), *mlp2:Myc* (PSY2824), *nup60:Myc* (PSY2825), *nup145:Myc* (PSY2826), *nup116:Myc* (PSY2945), *nup2:Myc* (PSY2946), *cse1:Myc* (PSY2947), *kap95:Myc* (PSY2951), *nic96:Myc* (PSY2952), *nsp1:Myc* (PSY2955), *nup84:Myc* (PSY2956), and *xpo1:Myc* (PSY2958). Strains used for expression analysis include *nup116:HIS3* (PSY1634), PSY1634 with the plasmid pPS944 <2μ *LEU2 NUP116*>; *nup60:KanMX4* (PSY2705) and *nup2:KanMX4* (PSY2963), and the corresponding wild-type parent BY4741/PSY1930.

Immunofluorescence

Indirect immunofluorescence was performed as previously described (Krebber et al., 1999) using a 1:200 dilution of 9E10 anti-Myc mouse monoclonal antibody (Santa Cruz Biotechnology) and goat anti-mouse Alexa594-conjugated secondary antibodies diluted 1:1000 (Molecular Probes). Chromatin was stained using approximately 15 μg of 496-diamidino-2-phenylindole (DAPI) per well.

Genomic Localization Analysis and Microarray Hybridization

The experimental procedure was performed as previously described (Damelin et al., 2002) with modification of the labeling protocol (Ren et al., 2002). Chromatin-immunoprecipitation was performed using 9E11 anti-c-*myc* monoclonal antibody (LabVision). One-third of the resulting DNA was used as template for one round of random priming with the BioPrime DNA labeling kit (Invitrogen) and 3 μl of either 1 nM Cy5-dUTP or Cy3-dUTP (PerkinElmer). Samples were hybridized to 6.2k and 6.4k yeast ORF (coding region) microarrays from University Health Networks, Toronto, following manufacturer's recommendations. Microarrays were briefly washed in 1× SSC to remove the coverslip. The slides were then washed three times at 50°C for 10 min in 1× SSC/0.1% SDS before a final wash in 1× SSC and drying.

Microarray Data Analysis

Microarrays were scanned and fluorescence intensities quantified using the Axon Genepix 4000B scanner and software. Arrays were repeated in triplicate or more in all cases and were uploaded into the Rosetta Resolver microarray analysis platform. Individual arrays were weighted by error and combined. All sequences with a p value ≤ 0.02 and a Cy5/Cy3 ratio > 1.0 were considered bound and therefore used for examination of gene class enrichment (Berriz et al., 2003). Lists ranked by the Cy5/Cy3 ratio were compared to previously determined transcriptional frequencies (Holstege et al., 1998). Clustering performed with Rosetta Resolver used an agglomerative algorithm with average link heuristics and correlation without mean subtraction. Principal component analysis was performed using Rosetta Resolver with settings for data reduction at 90% variation and inclusion of all sequences with $P \leq 0.002$.

Combined FISH/IF

A 5.3 kb fragment spanning the coding sequence of *GAL1*, *GAL7*, and *GAL10* was amplified from genomic DNA using the following primer sequences: 5′-CATTTGGGCCCCCTGGAACC-3′ and 5′-GGGGCTAAAACATATGACGAAACA-3′. The digoxigenin-dUTP derivatized *GAL* probe was resuspended in hybridization solution (50% formamide, 10% dextran sulfate, 2x SSC) to a final concentration of ~10 ng/ml. Combined IF/FISH was performed using a modified protocol based on a previously described technique (Gotta et al., 1999). Cells were grown in rich media containing either 2% glucose or 2% galactose at 30°C to a density of $\sim 1 \times 10^7$ cells/ml then fixed in 4% paraformaldehyde before spheroplasting to prevent nuclear spreading. The anti-nucleoporin antibody, MAb414 (Covance), was used at a 1:5000 dilution. Pre-absorbed Alexa Fluor 594 goat anti-mouse (Molecular Probes) and sheep anti-digoxigenin-fluorescein (Roche) were used at 1:50 dilutions. Cells were imaged using a Nikon TE2000U inverted microscope with PerkinElmer ultraview spinning disk confocal. Confocal *z* sections were encoded by the authors before being blindly evaluated by others. A stringent requirement for complete overlap with the NPC was used to score *GAL* region localization at the nuclear periphery. Error bars represent variation in scoring in multiple blind tests.

Acknowledgments

The authors would like to thank I. Simon for help in developing our genomic localization procedure; P. Grosu, R. Gali, and the Bauer Center for Genomics Research, Harvard University, for help with microarray analysis; Rosetta Biosoftware for help with the Rosetta Resolver microarray analysis platform; J. Waters Shuler and the Nikon Imaging Center at Harvard Medical School for help with confocal imaging; and K. Auld, J. Hurt, T. Kau, A. McKee, and J. Way for comments on the manuscript. This work was supported by a training grant from the National Eye Institute to J.M.C., a training grant from the US National Institutes of Health to C.R.B., a pre-doctoral fellowship from the Howard Hughes Medical Institute to H.H., and grants from the US National Institutes of Health to P.A.S.

Received: October 23, 2003
Revised: March 9, 2004
Accepted: March 16, 2004
Published: May 13, 2004

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