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Chromatin-Bound Nuclear Pore Components Regulate Gene Expression in Higher Eukaryotes

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SUMMARY

Nuclear pore complexes have recently been shown to play roles in gene activation; however their potential involvement in metazoan transcription remains unclear. Here we show that the nucleoporins Sec13, Nup98, and Nup88, as well as a group of FG-repeat nucleoporins, bind to the *Drosophila* genome at functionally distinct loci that often do not represent nuclear envelope contact sites. Whereas Nup88 localizes to silent loci, Sec13, Nup98, and a subset of FG-repeat nucleoporins bind to developmentally regulated genes undergoing transcription induction. Strikingly, RNAi-mediated knockdown of intranuclear Sec13 and Nup98 specifically inhibits transcription of their target genes and prevents efficient reactivation of transcription after heat shock, suggesting an essential role of NPC components in regulating complex gene expression programs of multicellular organisms.

INTRODUCTION

In order to accommodate transport between the nucleoplasm and the cytoplasm, the nuclear envelope (NE) is fenestrated by nuclear pore complexes (NPCs), large multiprotein channels consisting of multiple copies of ~30 different nucleoporins (Nups) (Alber et al., 2007a; Hetzer et al., 2005; Wente, 2000). Nups can be classified into two categories: (1) scaffold Nups, which mainly consist of the large Nup107/160 and Nup93/205 complexes (Debler et al., 2008), and (2) peripheral Nups. The latter extend from the membrane-embedded scaffold either into the pore channels or as filaments into the cytoplasm or the nucleoplasm (Alber et al., 2007b; Beck et al., 2004; Brohawn et al., 2009). Whereas the scaffold is thought to provide structural integrity to the highly curved pore membrane, the peripheral Nups, many of which contain phenylalanine-glycine (FG)-repeats, are responsible for establishing the permeability barrier (D'Angelo et al., 2009) and mediating nuclear trafficking (Weis, 2002).

In addition to their role as transport channels, NPCs have been implicated in chromatin organization and gene regulation (Akhtar and Gasser, 2007; Capelson and Hetzer, 2009). Studies in yeast revealed that Nups can associate with promoters of active genes (Schmid et al., 2006) and that the expression of inducible genes is increased by interactions with nuclear pores (Taddei et al., 2006). Furthermore, a genome-wide analysis in *S. cerevisiae* demonstrated that a subset of Nups can occupy regions of highly transcribed genes (Casolari et al., 2004). Additionally, Nups have been shown to function as chromatin boundaries in *S. cerevisiae* (Dilworth et al., 2005; Ishii et al., 2002). Boundary activity involves protection from nearby activating or repressive signals and constitutes another plausible function for NPCs in the organization of the genome into discrete chromatin domains. As further evidence for the role of the NPC in regulation of active chromatin, Nups have been found to participate in X chromosome transcriptional hyperactivation in dosage compensation of *Drosophila melanogaster* (Mendjan et al., 2006).

Interestingly, the only genome-wide study of Nup-chromatin association in animal cells revealed a correlation between the binding sites of Nups and regions enriched in repressive histone modifications (Brown et al., 2008), which exhibited characteristics of sequences known to associate with the nuclear periphery in human cells (Guelen et al., 2008). The observed discrepancy between yeast and human data suggests that the genome-binding pattern of the NPC may be quite different or more complex in metazoa. Furthermore, many of the peripheral Nups in mammalian cells have been shown to be mobile and to move dynamically on and off the pore (Rabut et al., 2004). Therefore, it seems possible that Nup-chromatin interactions could occur at distant sites from the NE, a notion that has some experimental support from the observation of intranuclear Nups (i.e., not associated with the NE) in mammalian cells (Enninga et al., 2003; Griffis et al., 2002). Thus, the functional role of Nup-chromatin interactions and whether they occur exclusively at the nuclear periphery remain unresolved.

Given the functional implications of yeast Nups in gene regulation, we wanted to test whether NPC components play a role in gene expression of multicellular organisms. Here we demonstrate that different Nups bind to distinct regions of the *Drosophila* genome and that many of these interactions can occur at off-pore locations. More significantly, we show that a subset of NE-independent NPC proteins play an essential

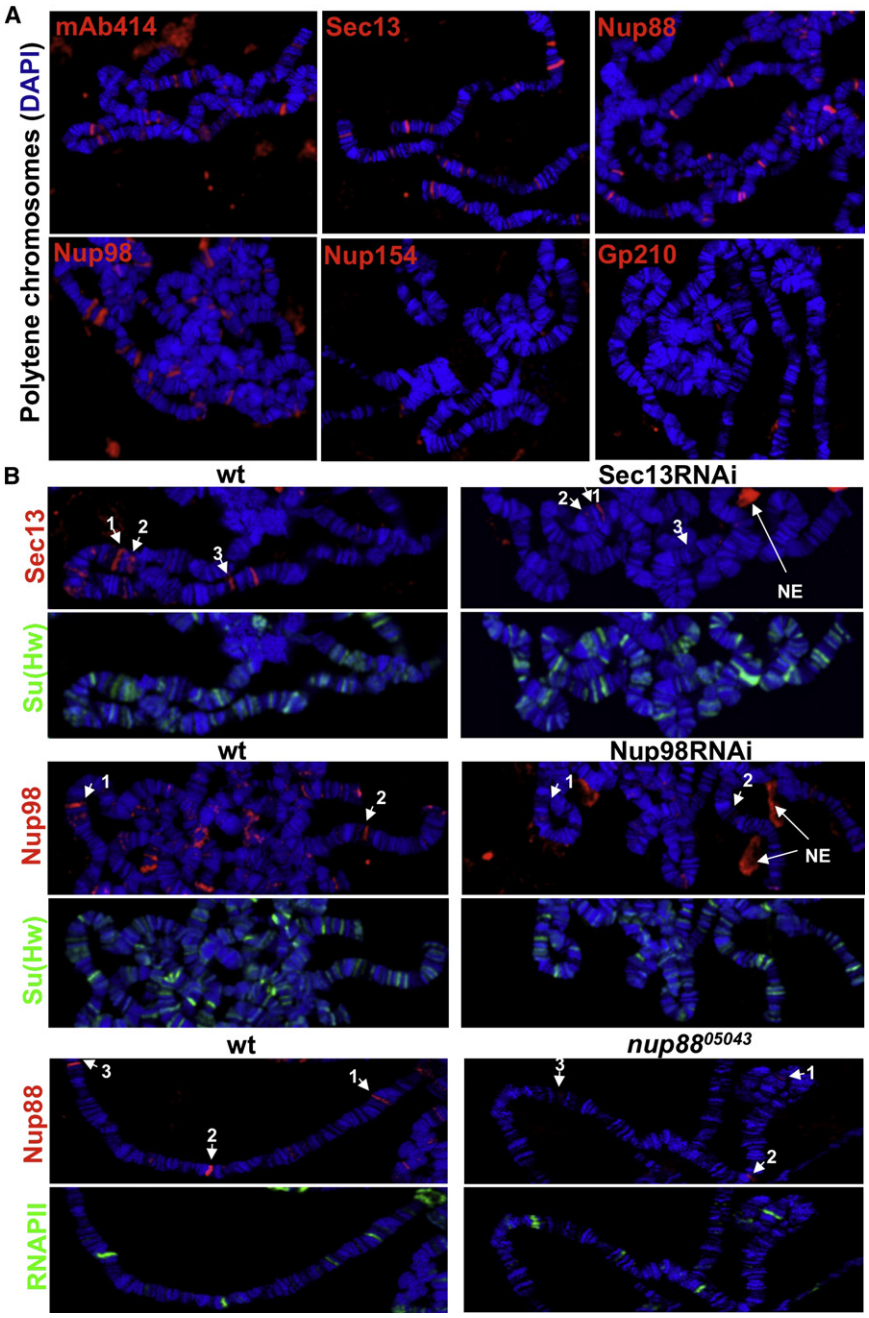

Figure 1. A Subset of Nuclear Pore Components Binds Specific Sites in the *Drosophila* Genome

(A) Polytene chromosome spreads were stained with mAb414, anti-Sec13, anti-Nup88, anti-Nup98, anti-Nup154, and anti-gp210 as indicated; chromosomes are stained with DAPI (blue) here and throughout the paper, unless otherwise indicated.

(B) Polytene chromosomes of third-instar larvae of wandering LB stage from WT and Sec13 RNAi (top), Nup98 RNAi (middle), or *nup88*^{05043} homozygotes (bottom) were stained with anti-Sec13 and anti-Su(Hw), anti-Nup98 and anti-Su(Hw), anti-Nup88 and anti-RNAP II, respectively. White arrows with numbers denote the same genomic locations for a particular Nup between chromosomes of WT and mutant larvae in a given panel. See also Figure S1. Nuclear envelope remnants are indicated as NE.

role in the induction of transcription of its target genes during *Drosophila* development, suggesting a direct function for Nups in the regulation of gene expression in metazoa.

RESULTS

A Subset of Nups Binds to Specific Sites of the *Drosophila* Genome

To study the potential role of Nups in metazoan gene regulation, we performed indirect immunofluorescence on polytene chromosome spreads using the antibody mAb414, which is a widely used marker of NE-associated NPCs and has been shown to react with the FG-repeat domain of Nup62, Nup153, Nup214, and Nup358 (Davis and Blobel, 1987). Strikingly, mAb414 stained dozens of specific sites on the chromosomes (Figure 1A). The staining pattern was highly reproducible among chromosomes of the same animal but varied among larvae of different developmental stages (see below). To determine whether additional Nups might exhibit chromatin-binding behavior, we used specific antibodies against representative components of the major subcomplexes of the NPC (Figure S2A available online), including Sec13, a component of the stable Nup107/160 subcomplex (Siniossoglou et al., 1996; Vasu et al., 2001), and Nup154, a member of the Nup93/205 subcomplex (Gigliotti et al., 1998; Hawryluk-Gara et al., 2005). In addition, antibodies against the cytoplasmic filament component Nup88 (Roth et al., 2003), the nucleoplasmic pore component Nup98, and the transmembrane nucleoporin gp210 (Greber et al., 1990) were analyzed. As expected, all antibodies were able to stain nuclear pores at the NE in cells of fly imaginal discs (Figure S1A). On chromosome spreads, we detected specific binding patterns with anti-Sec13, anti-Nup88, and anti-Nup98 antibodies (Figure 1A), suggesting that various pore components are targeted to particular genomic sites. In contrast, the scaffold Nup154 and the transmembrane gp210 did not associate with chromatin.

To confirm the specificity of Nup-chromatin interactions, we knocked down Sec13 and Nup98 using inducible UAS-RNAi transgenic lines directed against the *sec13* and *nup98* gene transcripts (Dietzl et al., 2007), which can be activated in