Chapter 5
Nuclear Pore and Genome Organization and Gene Expression in *Drosophila*

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Abstract Regulation of gene expression is central to the cell's ability to respond to external cues and to establish and maintain its developmental identity. The Nuclear Pore Complex (NPC) forms the nuclear envelope-spanning channel that mediates selective nucleo-cytoplasmic transport of macromolecules. In addition to contributing to gene expression via its transport functions, the NPC comes in close contact with the underlying chromatin and plays a role in regulation of gene expression of the associated gene targets. In recent years, studies in *Drosophila* and other organisms have identified numerous physiological roles for NPC components, including functions in immune response, tissue-specific development, epigenetic processes and neurodegeneration. This chapter focuses on the current knowledge of the physiological roles of NPC components and on the relationship between the NPC and chromatin regulation, obtained in the fly model. Findings, described here, demonstrate the far-reaching potential of NPC components to regulate gene expression via both transport and chromatin-binding mechanisms. Furthermore, they reveal *Drosophila* to be a useful experimental system for future dissections of the in vivo phenotypes and gene regulatory functions of the NPC.

Keywords nuclear pore · nucleoporin · genome organization · gene expression · drosophila · gene regulation

5.1 Introduction

The Nuclear Pore Complex (NPC) is a massive nuclear envelope (NE)-embedded protein complex that consists of components termed Nucleoporins (Nups) (D'Angelo and Hetzer 2008; Knockenhauer and Schwartz 2016). The classically

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characterized function of the NPC is to mediate nucleo-cytoplasmic transport. Via its transport function, the NPC is intimately tied to regulation of gene expression, since the entry of cell cycle regulators and transcription factors, and the exit of mature mRNAs are all critical steps of the gene expression regulatory cascade. Yet in addition to transport, the NPC has been linked to gene regulation directly, via interactions with the underlying genome. The link between chromatin and NPC was initially suggested by electron microscopy images of nuclei, which showed a juxtaposition of decondensed chromatin against nuclear pores (Blobel 1985). Such images formed the basis for the "gene gating hypothesis," which proposed that NPCs preferentially associate with decondensed and presumably active genomic regions to couple transcription to export of the generated mRNA. Multiple studies in several organisms have since supported the general idea that NPC components engage in interactions with active genes and contribute to regulation of transcription and chromatin (Ptak and Wozniak 2016; Sood and Brickner 2014). The core structures of the NPC, such as the NE-embedded channel-forming rings, are comprised of Nups that stably associate with the NPC, while many of the peripheral structures, such as the inner channel or the nuclear basket, consist of Nups that can move on and off the NPC relatively rapidly and are thus termed dynamic (Rabut et al. 2004). *Drosophila* contains clear homologues to the known verterbrate Nups, and the general NPC structure and assembly pathways are likely to be conserved. Although NPC biogenesis and structure have not been as thoroughly investigated in *Drosophila* as in yeast and verterbrates, the fly model system has been instrumental in expanding other areas of the NPC research field. One such contribution is revealing in vivo phenotypes and physiological functions of Nups, due to the availability of powerful genetic tools and the existence of well-characterized experimental models of organogenesis and physiology in *Drosophila*. Though mechanisms behind many of these phenotypes remain to be determined, the importance of various NPC components in development, disease models and evolutionary processes has been recently brought forth by studies in the fly system.

Another contribution of the *Drosophila* model system is the characterization of NPC-genome interactions in the context of a developing organism. This characterization has been facilitated by the presence of polytenized tissues such as salivary glands, which contain giant polytene chromosomes that allow for ready cytological analysis of chromatin-binding behaviors (Kuzin et al. 1994). One aspect of the NPC-genome relationship discovered in *Drosophila* is the ability of dynamic Nups to interact with chromatin in the nuclear interior, away from the NE-embedded NPCs (Hou and Corces 2010). This property appears to be conserved in mammalian cells and expands our understanding of the functional roles of Nups in regulation of gene expression, as discussed further below. In this chapter, we provide a brief summary of the known unique features of the fly NPC, outline reported *Drosophila* phenotypes of individual Nups, and discuss the current knowledge of the NPC-chromatin relationship gained from the fly system. Together, these studies highlight the notion that NPC components represent a far-reaching aspect of the gene regulatory cascade, with physiological roles in tissue-specific development and tissue homeostasis.

5.2 Unique Features of *Drosophila* NPC Structure and Assembly

The *Drosophila* genome possesses readily identifiable homologues to components of the mammalian outer ring Nup107-160 sub-complex and associated Nups Elys and Nup98, as well as to components of the cytoplasmic filament Nup88-Nup214 sub-complex, the transport channel Nup62 sub-complex and the nuclear basket Nups Tpr (termed Megator (Mtor) in flies), Nup153, and Nup50. Like in verterbrates, there appears to be three trans-membrane Nups in *Drosophila*, including homologues of Nup210 and Ndc1, as well as what appears to be a distantly related homologue of Pom121, termed *dumpy*. Surprisingly, the fly genome carries two versions of Nup93, the defining component of the inner ring Nup93-Nup205 sub-complex: Nup93-1 (CG11092) and Nup93-2 (CG7262), which share a 65% homology and are located on different chromosomes. Interestingly, although homozygous mutations, generated by P-element insertions into the 5′ Untranslated Transcribed Region (UTR) are adult lethal for the majority of fly *Nup* genes, such mutations in either *Nup93* gene still produce viable adults. This viability suggests that *Nup93* genes may compensate for each other's functions. On the other hand, the reported embryonic and adult expression patterns of *Nup93-1* and *Nup93-2* exhibit several drastic differences from each other (Chintapalli et al. 2007). For instance, while *Nup93-1* is expressed very highly in the adult heart, *Nup93-2* is not and is instead highly enriched in the adult testes, suggesting that the two Nup93 subtypes execute unique tissue-specific functions.

Although NPCs appear to disassemble during mitosis in *Drosophila* similarly to verterbrates (Katsani et al. 2008), mitosis occurs in a "semi-closed" fashion in fly cells, with some remnants of the NE persisting through early anaphase (Katsani et al. 2008; Kiseleva et al. 2001). Furthermore, the mitotic spindle in *Drosophila* embryos appears to be confined by a membranous structure, termed spindle envelope, which is required for faithful chromosome segregation (Harel et al. 1989; Kiseleva et al. 2001; Schweizer et al. 2015). This structure may be related to or associated with the spindle matrix, a conserved filamentous network supporting the mitotic spindle (Jiang et al. 2015; Qi et al. 2004), a defining component of which is the nuclear basket Nup Mtor (Qi et al. 2004). Interestingly, in developmental stages with rapid cell cycles, such as the early pre-gastrulation *Drosophila* embryo, NPC re-assembly has been recently shown to occur by a newly discovered method of pre-assembled nuclear pore insertion (Hampoelz et al. 2016). In such rapidly cycling cells, which rely on maternally supplied factors, ER-embedded storages of NPC scaffolds, termed annulate lamellae, are fed into the expanding NEs as cells progress through their shortened interphases. These findings demonstrate that unique pathways of NPC assembly can occur during specific developmental stages.

5.3 Phenotypes of *Drosophila* Nups

In this section, we highlight some of the best-characterized and recurrent phenotypes of *Drosophila* Nups in physiological and developmental processes. Studies in mammalian models have demonstrated unique functions of specific Nups in tissue-specific development, such as differentiation of neuronal and muscle lineages, as well as in maintenance of stem cell pluripotency (D'Angelo et al. 2012; Jacinto et al. 2015; Lupu et al. 2008; Raices and D'Angelo 2012). As discussed below, due to the widespread use of genome-wide screens in *Drosophila*, fly NPC components have been similarly identified as hits in a number of assays of organismal function and dysfunction. Some of the phenotypes defined for Nups in this manner have been unexpected, given the necessary function of the NPC as a transport channel and its identity as a highly structured protein complex. But identification of such roles in *Drosophila* offers a glimpse of the broad physiological significance of the NPC that is yet to be fully uncovered.

5.3.1 *Components of the Nup107-160 Sub-complex in* Drosophila *Speciation*

Speciation involves the evolution of incompatible gene interactions that cause sterility or lethality in hybrids between related populations, a phenomenon termed hybrid lethality or incompatibility (Presgraves 2007). For example, if two closely related *Drosophila* species are mated together, such as *Drosophila melanogaster* females crossed to *Drosophila simulans* males, the resulting hybrid progeny is inviable (Sawamura et al. 1993). To understand the molecular mechanisms driving speciation, there has been great interest in identifying genes that can rescue or alter hybrid incompatibility. Strikingly, a genome-wide screen, aimed at identifying genes that can reverse the hybrid viability phenotype of the *Lethal hybrid rescue (Lhr)* mutation, identified the *Nup96* gene as being able to restore hybrid inviability between *D. melangaster* and *D. simulans* (Presgraves et al. 2003). Although *Nup96* is encoded in the same gene as *Nup98*, the hybrid lethality phenotype has been narrowed down specifically to *Nup96*, and particularly to the most N-terminal ~100 amino acids of Nup96 (Presgraves et al. 2003).

Furthermore, comparison of amino acid substitutions between species demonstrated a high level of non-silent changes in *Nup96*, indicative of positive selective pressure and adaptive evolution in this NPC component. Given that Nup96 is a core component of the stable Nup107-160 sub-complex, its high degree of divergence between closely related species was somewhat unexpected. However, signs of rapid adaptive evolution were similarly observed in other components of this NPC sub-complex and in Nups known to interact with Nup96, such as Nup107, Nup160, Nup133, Nup75 and Nup98 (Presgraves and Stephan 2007; Tang and Presgraves 2009). Furthermore, another key Nup107-160 sub-complex component, *Nup160* has also been identified as a key speciation gene in subsequent

hybrid incompatibility screens (Tang and Presgraves 2009; Sawamura et al. 2010). Interestingly, the hybrid incompatibility effects of both *Nup96* and *Nup160* were found to depend on an unknown component of the *D. melanogaster* X chromosome, as for instance, hybrid lethality of *Nup96* mutations can be reversed if the *D. melongaster* X chromosome is replaced with the *D. simulans* version (Presgraves et al. 2003; Barbash 2007). It was hypothesized this factor could be the *Nup153* gene encoded on the X-chromosome, although this has not been proven.

Consistently with being components of the same NPC sub-complex, the roles of Nup96 and Nup160 in hybrid incompatibility appear to be linked, yet each Nup may have distinct contributions. For instance, homozygous *D.simulans* version of *Nup96* ($Nup96^{sim}$) in a *D. melanogaster* background restores hybrid inviability independently of the *D. melanogaster* X chromosome if $Nup160^{sim}$ is also hemizygous, indicating that their hybrid incompatibility phenotypes are functionally related (Maehara et al. 2012). However, $Nup160^{sim}$, but not $Nup96^{sim}$, in a *D. melanogaster* background, induces female sterility, and this phenotype is independent of species origin of the X chromosome (Sawamura et al. 2010; Maehara et al. 2012), suggesting a separate and distinct role of Nup160 in oogenesis or gamete compatibility.

Together, these studies revealed that components of the core NPC sub-complex are some of the key factors driving speciation in *Drosophila* evolution. However, how these Nups contribute to speciation or why these genes may be fast-evolving is not entirely understood. One proposed explanation is related to the role of the NPC in regulating viral and retrotransposon nuclear import (Sistla et al. 2007; Yarbrough et al. 2014), enabling diverging species to keep up with evolving pathogens in order to increase defense against them. Another proposed mechanism involves the chromatin-interacting roles of the NPC. Repetitive satellite DNAs within heterochromatin, especially at centromeres, are known to evolve rapidly, which is thought to correlate with rapid evolution of proteins that bind such DNA (Ferree and Barbash 2009; Sawamura 2012). In fact, *Lhr* and several other identified speciation genes code for proteins that bind repetitive heterochromatin (Sawamura 2012), suggesting an interesting possibility that Nup96 and Nup160 may similarly play a role in heterochromatin maintenance.

5.3.2 NPC Components and ALS/FTD Pathogenesis

The *Drosophila* model has been widely utilized for characterization of molecular pathways driving the pathogenesis of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (Mcgurk et al. 2015). Recently, several genome-wide screens looking for genetic modifiers of ALS and FTD pathogenesis, conducted in *Drosophila*, have identified multiple nucleocytoplasmic transport factors, including components of the NPC (Boeynaems et al. 2016a; Zhang et al. 2015; Jovicic et al. 2015; Freibaum et al. 2015). These screens primarily utilized the fly disease model based on the

hexanucleotide repeat expansion in the *C9orf72* gene, which is the most common genetic factor contributing to both ALS and FTD (Dejesus-Hernandez et al. 2011). Although these diseases have very different clinical presentations, one common factor is degeneration of neurons, to which repeat expansion in *C9orf72* gene is known to contribute. The molecular mechanism behind pathogenesis of expanded repeats in *C9orf72* proved to be complicated, with several different models of action in existence. One emerging theme however is the impairment of nucleocytoplasmic transport and of NPC components in ALS and FTD, both in human cells and *Drosophila* disease models (Boeynaems et al. 2016b; Jovicic et al. 2016).

In one model of pathogenicity, toxicity of expanded *C9orf72* repeats is based on non-canonical repeat-associated non-ATG (RAN) translation of the C9orf72 RNA, which produces toxic dipeptide repeat proteins (DPRs) (Ling et al. 2013). Expression of *C9orf72* repeats or specifically of DPRs in the *Drosophila* ALS/FTD model results in pronounced tissue degeneration when expressed in the eye. A screen for modifiers of this degenerative phenotype discovered multiple *Drosophila* Nups, RNAi-meditated depletion of which can suppress or enhance the phenotype (Boeynaems et al. 2016a). Specifically, knock-down of Nup62, Nup93-1 or Nup44A/Seh1 exacerbated DPR toxicity, while in contrast, knock-down of Nup107, Nup50 or Nup154 ameliorated the degenerative phenotype and dramatically rescued morphology in DPR-expressing eyes. Strikingly, other independent screens, using similar expanded repeat-based *Drosophila* models of ALS, also identified multiple Nups as strong modifiers of the degenerative phenotype (Freibaum et al. 2015; Jovicic et al. 2015; Zhang et al. 2015). Once again, RNAi against Nups such as Nup107, Nup160, and Nup96-Nup98 demonstrated powerful suppression of expanded repeat-induced toxicity (Freibaum et al. 2015).

Identification of NPC components as strong suppressors of the ALS-like phenotype offers exciting therapeutic potential, yet the mechanism by which certain Nups can alleviate ALS-associated toxicity remains unclear. This is due in part to the fact the mechanism behind degeneration, induced by expanded repeats, remains controversial. While repeat-produced DPRs are often considered the main source of toxicity, aberrant repeat-produced RNA has also been suggested to be a causal agent (Burguete et al. 2015; Freibaum et al. 2015; Ling et al. 2013). One hypothesis of *C9orf72* toxicity is based on the sequestration of RNA-binding proteins by the *C9orf72* RNA with expanded repeats, which could have a negative impact on mRNA export. In support of this hypothesis, depletion of mRNA export receptors, such as Gle1 and NXF1, enhanced the degenerative phenotype of *C9orf72* repeat-based *Drosophila* models, and RNA export defects have been reported in iPS cells-derived neurons, obtained from ALS patients (Freibaum et al. 2015). Interestingly, Nup50 was similarly identified as a modifier in a screen based on a distinct *Drosophila* ALS model, which overexpresses the nuclear RNA-binding protein TDP-43, cytoplasmic mis-localization of which is a canonical marker of ALS toxicity (Zhan et al. 2013). A loss-of-function mutation in Nup50 was found to result in suppression of toxicity, increasing lifespan of TDP-43-overexpressing flies. Furthermore, administering an inhibitor of mRNA export, Leptomycin B, to flies, overexpressing *C9orf72* repeats, enhanced the toxic effects

of the repeat expression (Freibaum et al. 2015), suggesting that RNA-based toxicity and mRNA export defects at least partly contribute to ALS pathogenesis.

A recent study offered a new insight into the etiology of ALS pathogenesis, showing that DPRs interact with endogenous proteins with low complexity sequence domains (LCDs), which are intrinsically disordered and can form phase-separated structures such as hydrogels (Kwon et al. 2014; Lee et al. 2016). LCDs are often components of membrane-less organelles, such as nucleoli and NPCs, and are exemplified by the Phenylalanine Glycine (FG) repeat domain of Nups. Interactions of DPRs with LCD proteins were shown to disrupt their phase separation and material properties, which may provide a mechanism for the observed transport defects associated with ALS/FTD pathogenesis (Lee et al. 2016). However, opposing effects of specific NPC components on ALS-based toxicity, observed in the fly model, are still to be explained, since for example, depletion of either the FG domain-containing Nup62 or the core Nup107-160 sub-complex component Nup44A/Seh1 enhance the ALS-like degenerative phenotype (Freibaum et al. 2015). Together, these findings expose the critical roles of NPC components in human neurodegenerative diseases and highlight the utility of the *Drosophila* model in identifying disease-relevant roles of NPC components.

5.3.3 Cytoplasmic Nups in Immune Response and Import of NF-κB

One of the initial demonstrations of the functional specificity of individual Nups was the discovery of the specific involvement of Nup88, the fly gene for which is termed *members only* (*mbo*), in *Drosophila* immune function. Nup88 expression was found to be tissue-specific in fly larvae: enriched in imaginal discs, trachea, CNS, and fat body, but low in epidermis, muscles and gut (Uv et al. 2000). While *mbo* loss-of-function mutants did not present obvious defects in nuclear morphology, NPC structure, mRNA export, or nuclear localization of multiple endogenous proteins, a specific defect in nuclear import of the *Drosophila* transcription factors Dorsal and Dif, homologues of the NF-κB/Rel transcription factors, was identified in *mbo* mutant cells (Uv et al. 2000).

NF-κB/Rel transcription factors are critical for response to immune insults in metazoan organisms. Upon immune activation, cytoplasmic inhibitors of NF-κB factors are degraded, physically releasing them and allowing for their translocation into the nucleus (Mitchell et al. 2016). Here NF-κB factors interact with chromatin and promote expression of target immune response genes, and successful nuclear translocation of these transcription factors during immune response is crucial for the expression of downstream immune response. When Nup88 mutant larvae were infected with bacteria, Dorsal and Dif NF-κB/Rel transcription factors were not effectively transported into the nuclei of cells within the fat body, which is the primary organ for anti-bacterial response, yet nucleocytoplasmic transport of several other tested proteins and mRNA products was completely unaffected in these mutants. Accordingly, the normally observed dramatic up-regulation of

target antimicrobial peptide genes *drosomycin* and *diptericin* is completely abolished in the Nup88 mutant background (Uv et al. 2000).

Further exploration has led to a likely mechanism, and implicated another NPC component Nup214, in this selective transport of NF-κB factors. Localization of Dorsal within the cell has been shown to rely directly on the nuclear export factor CRM1. In salivary gland cells of CRM1 mutant flies, or S2 cells with CRM1 activity chemically inhibited, the nuclear/cytoplasmic ratio of Dorsal protein is dramatically increased compared to wild type, demonstrating a clear defect in its nuclear export (Roth et al. 2003). CRM1, Nup214, and Nup88 appear to interact directly, and loss of either Nup88 or Nup214 induces re-localization of CRM1 from the nuclear periphery to the nuclear interior (Xylourgidis et al. 2006), suggesting that the Nup88/Nup214 complex normally sequesters CRM1 at the periphery and prevents it from performing its export functions. Without being anchored to the periphery, CRM1 is free to export Dorsal, Dif, and other protein cargo out of nuclei unabated, inhibiting their ability to act as transcription factors and induce expression of antimicrobial peptides. The specificity in transport targets in this system is thought to be accomplished by the previously demonstrated affinity of CRM1 for leucine-rich nuclear export sequences, including those possessed by Dorsal (Xylourgidis et al. 2006). These findings have introduced a mechanistic paradigm for how a specific gene expression program and the immune response can be directly influenced by transport activities of individual Nups. Interestingly, Nup98 has also been identified as a key player in *Drosophila* immunity, but is thought to function by a distinct and likely transport-independent mechanism, discussed further below (Panda et al. 2014).

5.3.4 Roles of Drosophila *Nups in Germ Cell Development*

Early genetic screens for female sterility have identified hundreds of genes that control germ cell development and maintenance in *Drosophila*, and have allowed for sophisticated mechanistic dissection of this critical developmental process. One hit from such screens was termed *tulipano* (*tlp*) and was subsequently found to encode the *Drosophila* homologue of the verterbrate Nup155, Nup154 (Gigliotti et al. 1998). Hypomorphic alleles of *Nup154* were found to affect both female and male sterility (Gigliotti et al. 1998), and stronger loss-of-function mutants demonstrated a severe phenotype, including failure to maintain spermatogenic stem cells in the male gonad and failure to progress into vitellogenic stages of oogenesis in the female gonad, with a complete loss of germ cells in the male testes by adulthood (Kiger et al. 1999).

One mechanistic insight into how Nup154 affects *Drosophila* germ cell development was obtained from the discovery of an interaction between Nup154 and another critical germ cell factor, the Cup protein (Grimaldi et al. 2007). Cup is a germline-specific protein, previously implicated in regulating several aspects of gametogenesis, translational repression and chromatin structure in the egg chamber (Piccioni et al. 2005). Nup154 and Cup were found to enhance each other's

sterility phenotypes and to co-immunoprecipitate together upon Nup154 overexpression (Grimaldi et al. 2007). Furthermore, Cup was found to associate with the nuclear periphery specifically in early stage (stage 4) egg-chambers. Strong mutants of either Nup154 or Cup both resulted in a very similar phenotype of developmentally stalled chromosome structure in stage 5 eggs, where chromosomes did not properly decondense from their polytenized state, suggesting that Nup154 and Cup co-function in regulating chromosome structure at this stage in egg development (Grimaldi et al. 2007). Interestingly, neither general mRNA export nor protein import of tested factors appeared impaired in *Nup154* mutant germ cells or somatic cells of the gonad (Gigliotti et al. 1998). Based on these findings, it was proposed that Cup and Nup154 cooperate at the nuclear periphery to possibly regulate transport of specific developmental factors important for chromatin structure and oogenesis (Grimaldi et al. 2007), though the precise role of Nup154 in these processes remains unclear. Coincidently, another critical regulator of germ cell development, *Germ cell less* (*Gcl*), was similarly found to localize to the nuclear periphery and co-localize with NPCs in very early germ cells (Jongens et al. 1994), suggesting that localization to the NPC is a recurrent regulatory mechanism utilized in germline maturation.

Another defect uncovered in *Nup154* mutant germ cells is a disruption of the TGFβ signaling pathway, manifested as a dramatically reduced nuclear import of active phosphorylated(p)-Mad in germ cells and primary spermatocytes of male testes (Colozza et al. 2011). Nup154 however was not found to interact physically with p-Mad, and the mechanism behind this import defect is not fully understood. Furthermore, this role does not appear to be unique to Nup154, as additional *Drosophila* Nups, such as Nup75, Nup93, Sec13, Nup205, and Nup50 were discovered in an RNAi screen for proteins required for nuclear Mad accumulation in *Drosophila* S2 culture cells (Chen and Xu, 2010). Levels of both p-Mad and Msk, the importin responsible for p-MAD import, were found to remain normal in cells depleted for these Nups, indicating that the observed loss of p-Mad from the nucleus was specifically due to reduced translocation and not protein stability. Similarly to the *Nup154* mutant phenotype in the germline (Gigliotti et al. 1998), general protein import was not disrupted upon knock-down of these Nups in culture cells. Interestingly, addition of a classical NLS to Mad rescued nuclear localization of p-Mad in Nup-depleted cells, suggesting that this select group of Nups play a role in a non-canonical mechanism of import utilized for activation-induced translocation of p-Mad.

In addition to Nup154, several other NPC components have been implicated in *Drosophila* oogenesis and germ cell development. A separate genetic screen for sterility associated with small gonads yielded a mutation in the *Nup96-nup98* gene, which disrupts both Nup96 and Nup98 proteins and results in a progressive loss of germ cells in both male and female gonads during adult lifespan (Parrott et al. 2011). This progressive loss was linked to increased rates of differentiation, at the expense of germ cell self-renewal, suggesting that either Nup96 or Nup98 regulate stem cell maintenance in the adult gonad. Additionally, a mutation in the *Drosophila* Nup107-160 sub-complex component Seh1 was found to specifically

affect oogenesis, similarly resulting in a differentiation defect, which led to an aberrant differentiation of oocytes into a related lineage of nurse cells (Senger et al. 2011). Whether the mechanisms behind these phenotypes are related to specific transport events, such as those suggested for Nup154, or to other Nup-associated functions, such as chromatin regulation, remains to be determined.

5.4 Chromatin-Binding Roles of *Drosophila* Nups in Gene Regulation

Multiple studies in yeast have identified the ability of Nups to interact with chromatin and to recruit inducible genes undergoing activation to the NPC (Ptak et al. 2014; Akhtar and Gasser 2007). Mechanistically, these investigations focused on the connection between transcription and mRNA export, proposed by the "gene gating hypothesis" and on the role of Nups in epigenetic memory of previous transcriptional activation, known as transcriptional memory (Tan-Wong et al. 2009; Rodriguez-Navarro et al. 2004; Light et al. 2013; Kohler et al. 2008; Cabal et al. 2006). These functional implications of yeast Nups in gene regulation have suggested that NPC-genome interactions may also play critical roles in the establishment of gene expression programs during multicellular development. Investigations in *Drosophila*, discussed below, have identified roles for NPC components in transcriptional and epigenetic phenomena, such as dosage compensation of the X chromosome, maintenance of tissue-specific expression by Trithorax Group (TrxG) proteins and transcriptional memory of hormone-inducible genes. Chromatin-binding roles of Nups are likely to underlie some of the reported phenotypes of Nups, described in the previous section, and below we discuss examples, where such roles have been suggested as the main mechanism by which Nups execute a particular physiological function.

5.4.1 Nups Interact with Chromatin On and Off the NPC

Analysis of chromatin binding behavior of *Drosophila* Nups, achieved by various genome-wide methods such as immunostaining of polytene chromosomes, ChIP and Dam-ID, revealed the presence of NPC components at a number of genomic locations, which showed enrichment for active genes (Capelson et al. 2010; Kalverda et al. 2010; Vaquerizas et al. 2010). These studies demonstrated that similarly to its yeast counterparts, multiple *Drosophila* Nups exhibit chromatin-binding behavior, but with an unexpected twist. Surprisingly, Nup-chromatin contacts were commonly found to occur in the nucleoplasm, away from the NE-embedded NPCs. This is possible because as discussed above, a subset of Nups has been classified as dynamic and able to exist in a nucleoplasmic pool (Rabut et al. 2004). Consistently, the Nups identified to participate in intra-nuclear chromatin binding were predominantly dynamic Nups, such as Nup98, Nup62,

Nup50, Nup153 and Tpr, as well as Sec13, which although not considered dynamic, has been reported to have an intranuclear population (Enninga et al. 2003). These findings were revealed by diverse methods in multiple cell types (Capelson et al. 2010; Kalverda et al. 2010; Vaquerizas et al. 2010), arguing for the generality of this phenomenon. For instance, an elegantly designed DamID approach, which mapped genomic binding of full-length Nup98 as well as Nup98 missing its C-terminal NPC-targeting domain in fly embryonic culture cells, was able to distinguish between genomic binding of NPC-associated versus nucleoplasmic Nup98 and to demonstrate the common occurrence of intranuclear Nup98-genome contacts (Kalverda et al. 2010). The ability of Nups to interact with genes in the nucleoplasm has now been also identified in mammalian cells (Franks et al. 2016; Jacinto et al. 2015; Liang et al. 2013; Light et al. 2013), thus it appears to be an evolutionarily conserved phenomenon and may be unique to metazoan cells.

Importantly, these reports also demonstrated a functional role of chromatin-bound Nups in transcriptional activity of its target genes. In polytenized tissues of *Drosophila* larvae, Nup98 and Sec13 were identified at loci of the *Ecdysone-induced protein 74* and *75* (*Eip74* and *Eip75*) genes, which are induced by a developmental steroid hormone ecdysone (Capelson et al. 2010). Tissue-specific RNAi knock-downs of Nup98 and Sec13 during the developmental stage when *Eip74* an *Eip75* are highly induced, resulted in reduced levels of phosphorylated RNA Polymerase II (RNAP II), decreased chromatin de-condensation and reduced mRNA output specifically at the *Eip74* and *Eip75* genes. Functional involvement of Nup98 in gene activation was similarly revealed by modulating levels of Nup98 in culture cells, via over-expression or RNAi depletion, which increased and decreased expression of a subset of its target intranuclear genes, respectively (Kalverda et al. 2010). Thus, the widespread genomic binding of *Drosophila* Nups, a large fraction of which occurs in the nuclear interior, is likely to contribute to functional regulation of genome activity.

Together, these studies suggested that Nups regulate gene activity independently of NPC localization, effectively extending the reach of the previously proposed "gene gating hypothesis." Since the majority of active genes are transcribed in the nuclear interior, these findings can overcome a major limitation of the "gene gating hypothesis," which argues that active genes have to be repositioned to the NPCs at the NE in order to be regulated by NPC components. Instead, dynamic Nups are able to come off the NPC to affect a larger pool of transcribing genes throughout nuclear space. On the other hand, the existence of Nup-chromatin contacts in the nucleoplasm calls into question one of the major premises of the "gene gating hypothesis": the coupling between transcription and mRNA export as the main cellular "reason" for recruiting genes to the NPC. It is less obvious how such a reason would apply to genes bound by Nups in the nuclear interior, suggesting that there is an additional, transport-independent function that Nups perform at active genes. Such functions have been proposed to involve regulation of chromatin, genome architecture and epigenetic memory, as discussed in the sections below.

5.4.2 Drosophila *Nups and Dosage Compensation Machinery*

Dosage compensation is a process by which organisms equalize expression differences derived from the unequal number of X chromosomes between the sexes. Dosage compensation in *Drosophila* occurs via a two-fold transcriptional upregulation of the male X chromosome (Mendjan and Akhtar 2007). The male X chromosome is maintained in this transcriptionally hyper-activated state by a chromatin regulatory complex Males Specific Lethal (MSL), which includes five core protein members and is associated with noncoding RNAs *rox*1 and *rox2*. The enzymatic component of the MSL complex is a HAT, termed Males absent On the First (MOF), which acetylates the histone H4 lysine 16 (H4K16) residue (Gelbart et al. 2009). The MSL core components MSL1, MSL2, MSL3, MOF and MLE were originally identified genetically, based on their phenotypes of being essential for dosage compensation in fly males (Gorman and Baker 1994). Interestingly, subsequent biochemical purifications of the tagged core components of the MSL complex, such as MOF and MSL3, from fly culture cells and embryos consistently yielded substantial amounts of several Nups, including Nup153, Mtor, Nup98, Nup160 and Nup154 (Mendjan et al. 2006). The interaction between MOF and Mtor was found to be evolutionarily conserved, since Tpr, the human homologue of Mtor, was similarly co-purified with MOF from HeLa cells extracts (Mendjan et al. 2006). Furthermore, depletion of either Mtor or Nup153 resulted in compromised localization of MSL proteins to the X chromosome and in lower expression of the X-linked genes in male culture cells, suggesting that Mtor and Nup153 are functionally involved in transcription-promoting activity of the MOF-containing complex (Mendjan et al. 2006). Together, these findings brought forth the possibility that metazoan cells utilize Nups to execute their complex epigenetic phenomena, and that similarly to yeast, these functions appear to involve physical interactions between nuclear basket Nups and HAT complexes.

A subsequent study of the MSL-driven dosage compensation in vivo failed to detect any obvious roles of Nup153 and Mtor in the recruitment of the MSL complex components to the X chromosome (Grimaud and Becker 2009). Specifically, RNAi against Mtor and Nup153 did not disrupt X chromosome targeting of at least one MSL component, MSL2 in several tested tissues of male fly larvae. Thus it remains unclear whether nuclear basket Nups functionally contribute to dosage compensation in the developing organism, or if perhaps reported conserved interactions between MOF and Nups are involved in non-dosage compensating activities of MOF. Interestingly, detailed biochemical characterization revealed that in addition to the MSL complex, MOF is present in a related but distinct chromatin-binding complex, termed Non Specific Lethal (NSL), in both *Drosophila* and human cells (Lam et al. 2012; Raja et al. 2010). The NSL complex contains several unique components, including NSL1 and Methyl Binding Domain Related 2 (MBD-R2), and importantly, targets autosomal genes, where it is involved in transcriptional regulation (Feller et al. 2011; Raja et al. 2010). The relationship between Nups and the MOF-containing NSL complex is supported by recent

findings that *Drosophila* Nup98 physically and genetically interacts with NSL components such as MBD-R2 (Pascual-Garcia et al. 2014). MBD-R2 and Nup98 appear to co-bind many of the same target genes in S2 cells and on polytene chromosomes, and both proteins bind autosomes to a similar degree as the X chromosome. Thus it appears that Nup98 and potentially other Nups may co-function with the NSL complex in regulation of transcription, independently of dosage compensation.

On the other hand, high-resolution chromatin binding analysis demonstrated that Mtor and Nup153 are particularly enriched on the male X chromosome relative to autosomes (Vaquerizas et al. 2010). This binding pattern at least indirectly suggests that Nups do indeed play a role in promoting the specialized transcriptional state of the male X. This notion is further supported by a recent study that remarkably, has also linked dosage compensation in *Caenorhabditis elegans* to the nuclear pore (Sharma et al. 2014). Dosage compensation in *C. elegans* occurs by an entirely different mechanism, where expression from both X chromosomes is down-regulated two-fold in the XX-bearing hermaphrodite, and requires a protein complex that includes condensins (Csankovszki 2009). Interestingly, the un-compensated and more active male X chromosome was found to associate with NPCs in nuclear space and to display enriched binding of the Elys homologue Mel-28, as assessed by genome-wide DamID (Sharma et al. 2014). The interaction between the X chromosome and the NPCs was antagonized by the presence of the silencing dosage compensating machinery in the hermaphrodite, suggesting that the NPC functions as an activity-promoting nuclear environment. A similar role for Nups in promoting global X chromosome activity via providing a permissive nuclear compartment or scaffold may be conserved in *Drosophila* (Fig. 5.1). In support of this idea, Nup153 and Mtor bind chromatin not as discrete peaks, but as large megabase-long domains, termed Nucleoporin Associated Regions (NARs) (Vaquerizas et al. 2010), representative of a potential scaffolding function. Beyond dosage compensation, these studies have highlighted the relationship between Nups and epigenetic machinery, and this connection is likely to be a component of tissue-specific gene regulation.

5.4.3 *Physiological Gene Targets of* Drosophila *Nups*

Genome-wide binding analysis of Nup98 by DamID and ChIP in culture cells identified on the order of 1,000 genes as binding targets of Nup98, which are frequently co-occupied by other Nups such as Nup50 or Nup62 (Capelson et al. 2010; Kalverda et al. 2010; Pascual-Garcia et al. 2014). Aside from the X chromosome dosage compensation mechanisms described above, a key question in the field is what defines the subset of genes that are subject to regulation by Nups. Gene ontology analysis has identified genes that regulate cell cycle and tissue-specific development as enriched categories among genome-wide targets of Nup98 (Kalverda et al. 2010). Consistently, Nup98 has been implicated in direct

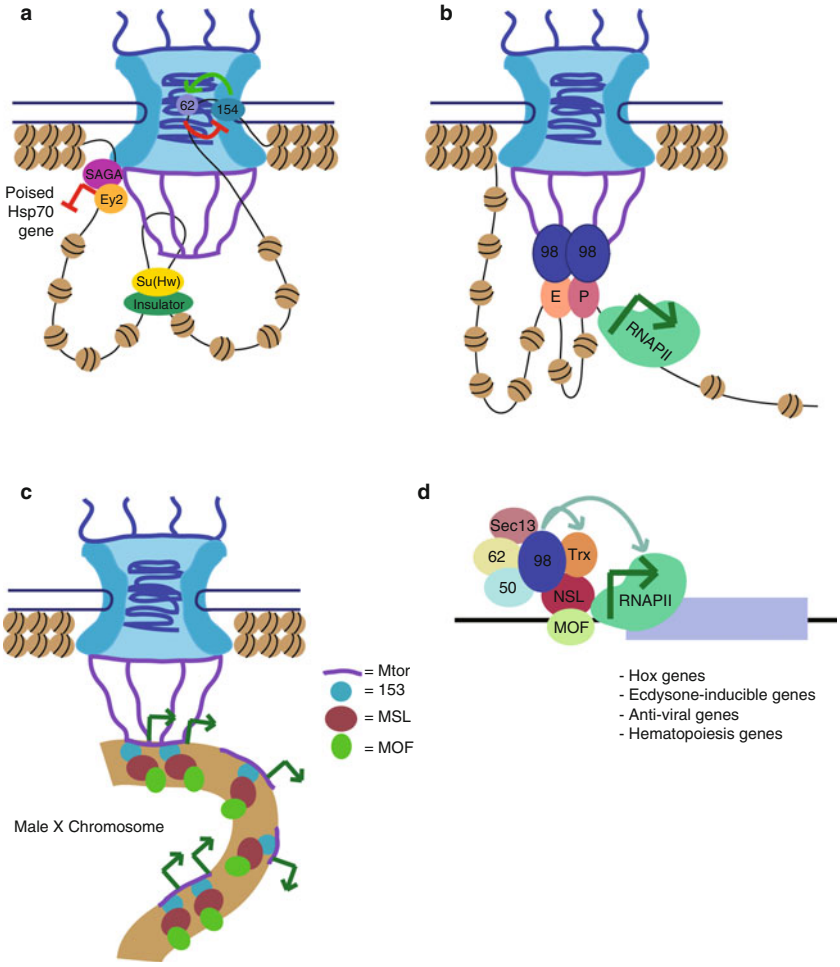

Fig. 5.1 Roles of NPC components in regulation of chromatin and transcription. (a) Schematic summary of NPC-genome interactions in non-transcribing processes, including tethering of poised *hsp70* gene to the NPC, targeting binding sites of insulator proteins, such as Su(Hw), and proposed chromatin binding of Nup154 being regulated by Nup62. (b) Model for the proposed role of Nup98 and the NPC in scaffolding activation-driven enhancer-promoter contacts of inducible genes. (c) Possible model for the relationship between nuclear basket Nups and dosage compensated male X chromosome, showing the binding of Mtor in extended domains along the X chromosome and the identified interactions between the MOF/MSL complex and Mtor and Nup153; these interactions may provide a permissive environment or structural scaffolding for the hyper-activated X chromosome. (d) Schematic summary for the roles of dynamic Nups in transcription, showing the identified interactions between Nup98 and the Trx and MOF/NSL complexes, and their proposed co-functioning in gene activation; below is the list of gene classes that are regulated by Nups in *Drosophila*. Beige circles represent the nucleosomes, green arrows represent active genes, and red lines represent silent genes. Please see text for specific references

regulation of at least two sets of developmentally critical genes. The first set is the ecdysone-inducible genes, discussed above, which exhibit robust binding of Nup98, Sec13 and mAb414-recognized FG Nups, and depend on Nup98 and Sec13 for normal transcriptional activation in larval development (Pascual-Garcia et al. 2017; Capelson et al. 2010). The second set of developmental genes regulated by Nup98 is the *Hox* or *Homeotic* genes, which are conserved regulators of tissue identity. During *Drosophila* development, *Hox* genes are expressed in precise tissue-specific patterns to define future identity of precursor structures known as imaginal discs, which will give rise to adult body parts. In particular, *Hox* gene *Ultrabithorax* (*Ubx*) is highly expressed in the haltere imaginal disc, and this expression is epigenetically maintained by a histone methyl transferase (HMT) Trithorax (Trx) (Schuettengruber et al. 2007; Ringrose and Paro 2007). It has been shown that depleting Trx during development, after the initial specification of *Hox* gene expression patterns, leads to cells "forgetting" the Hox expression profile of their lineage and to the resulting mis-specification of tissue identity, known as homeotic transformations (Grimaud et al. 2006). Interestingly, depletion of Nup98, but not of Nup107, in the haltere imaginal disc was found to result in down-regulation of *Ubx*, in a stochastic pattern reminiscent of the loss of Trx (Pascual-Garcia et al. 2014). Furthermore, over-expression of Nup98 was shown to genetically interact with *trx*, and to exhibit a homeotic transformation of the haltere into wing, a known consequence of insufficient levels of *Ubx* (Pascual-Garcia et al. 2014).

The identified role of *Drosophila* Nup98 in transcriptional regulation of *Hox* genes is particularly relevant to human cases of Acute Myeologenous Leukemia (AML), caused by translocation-derived oncogenic fusions of Nup98, which fuse the N-terminal half of Nup98 to a number of C-terminal partners (Wang et al. 2007; Gough et al. 2011). Aberrant *Hox* gene expression pattern, particularly of the *HoxA* cluster, is a hallmark of AML and is thought to underlie the loss of differentiating ability and the over-proliferation of hematopoietic precursor cells, observed in AML (Alharbi et al. 2013). In agreement with findings in the fly system, binding of fusion-derived Nup98 to *HoxA* gene clusters, as well as a functional role of Nup98 in regulation of *Hox* gene activity have been recently reported in mouse cells (Xu et al. 2016; Oka et al. 2016). These reports support the notion that Nup98 contributes to leukemigenesis and likely to hematopoietic development via direct regulation of *Hox* gene expression. Interestingly, Nup98 was identified as a key player in hematopoietic development in an unbiased screen for factors that regulate stem cell maintenance in the *Drosophila* hematopoietic organ, the lymph gland (Mondal et al. 2014). In this study, depletion of Nup98 was found to result in increased differentiation of hematopoietic stem cells and in decreased expression of key differentiation markers such as *Pvr*. The *Pvr* gene has been detected as a direct binding target of Nup98 in genome-wide studies (Capelson et al. 2010), leading the authors to suggest that Nup98 regulates transcription of key hematopoiesis genes to influence stem cell maintenance.

In addition to genes involved in regulation of tissue-specific development, Nup98 was recently shown to be required for the host-driven induction of

anti-viral genes in *Drosophila* cells (Panda et al. 2014). In response to viral infections, insect cells initiate a rapid transcriptional program, sharply up-regulating around 500 genes, as part of their host defense response (Xu and Cherry 2014). Nup98 was identified as a strong antiviral host factor, such that fly culture cells or adult organisms depleted of Nup98 were found to be more susceptible to infection against a panel of disparate RNA viruses, including human viruses Sindbis virus (SINV), vesicular stomatitis virus (VSV) and West Nile virus (Panda et al. 2014). It is of note that the life cycle of these RNA viruses takes place in the cytoplasm and does not involve nuclear import of the RNA genome or viral particles, making a transport-associated function of Nup98 less likely to play a major role in identified anti-viral activity. In support of this idea, single-molecule RNA FISH against mRNAs of antiviral genes in virus-infected Nup98-depleted cells did not identify a defect in their export, but instead revealed a reduction in their levels (Panda et al. 2014). Furthermore, Nup98 was detected at promoters of a subset of the rapidly induced anti-viral genes, and depletion of Nup98 was found to result in their compromised transcriptional up-regulation upon infection with SINV. Thus it appears that Nup98 functions as an anti-viral host defense factor via its chromatin-binding activity by promoting rapid up-regulation of an anti-viral transcriptional program.

5.4.4 *Roles of* Drosophila *Nups in Non-transcribing Chromatin*

In addition to regulation of actively transcribing genes, NPC components in yeast and mammalian cells have been implicated in binding and influencing non-transcribing regions of the genome (Jacinto et al. 2015; Van de Vosse et al. 2013; Casolari et al. 2004). *Drosophila* similarly exhibits examples of NPC-chromatin interactions that do not involve active genes (Fig. 5.1). Genome-wide DamID analysis revealed that while binding of nucleoplasmic Nups is enriched for highly transcribing genes, chromatin binding of the actual NPCs is not, and instead includes many silenced regions (Kalverda et al. 2010; Kalverda and Fornerod 2010). This observation seems counter to the idea of the NPC being a regulatory hub for actively transcribing genes, yet it is supported by similar findings in other organisms. Studies in yeast and human cells demonstrated binding of stable Nups such as Nup93, Nup155 and Nup107 at chromatin sites that show enrichment for repressive histone marks, silencing proteins or non-transcribing genes (Kehat et al. 2011; Brown et al. 2008; Casolari et al. 2004).

One possible explanation to reconcile these functionally opposing links to active vs. silent chromatin may be the binding of NPCs to poised genes that are held ready for future activation. This idea is supported by identification of certain poised genes, such as the heat shock response *hsp70* gene, being present at the NPC prior to activation in both *Drosophila* (Kurshakova et al. 2007) and yeast cells (Woolcock et al. 2012). Additionally, during developmental progression of the ecdysone-induced transcriptional program in salivary glands, Nup98 and Sec13 were located at the ecdysone-inducible genes before the appearance of

phosphorylated RNAP II, suggesting that they may have a role in pre-marking or poising these genes for upcoming activation (Capelson et al. 2010). Detailed genome-wide analysis in *Drosophila* embryonic S2 cells similarly identified robust binding of Nup98 at silenced ecdysone-inducible genes (Pascual-Garcia et al. 2017). Interestingly, these genes were found to be positioned at the NE-embedded NPCs before activation with ecdysone, and to remain associated with NPCs through activation, supporting the notion that stable NPCs function in tethering silenced genes that may be readily induced (Pascual-Garcia et al. 2017).

NPC binding at *Hox* gene clusters (Pascual-Garcia et al. 2017; Kalverda et al. 2010) also occurs in the context of completely silenced *Hox* genes, which are bound by the repressive Polycomb Group (PcG) proteins in embryonic Kc and S2 cells. Given the repeatedly reported binding of Nups to *Hox* genes, discussed above, it is tempting to speculate that the NPC-*Hox* gene interaction similarly represents the ability of the NPC to function as a scaffold for silent genes that are accessible for future activation, and that this function is a critical part of developmental gene regulation.

5.4.5 *Nups as Mediators of Epigenetic Memory and Genome Architecture*

Insights into the mechanisms by which Nups may regulate transcription have come form studies of a process known as transcriptional memory, which manifests as an enhanced transcriptional response of inducible genes after they have been previously activated by the inducing agent (Light et al. 2013). This enhanced transcriptional output in subsequent rounds of activation is thought to represent the primed or "remembered" state of recently transcribed genes, which may underlie the ability of cells to adapt to previous environmental stimuli. Importantly, the primed state of recently transcribed genes is maintained epigenetically, i.e. through cell divisions (Brickner et al. 2007). Mechanistically, it has been shown to involve the H3 K4 dimethyl (H3K4Me2) histone modification and the persistent binding of an un-phosphorylated poised form of RNAP II at gene promoters, and these events were found to depend on Nup98 in yeast and human culture cells (Light et al. 2013).

Identification of Trx as an interacting partner of *Drosophila* Nup98 (Pascual-Garcia et al. 2014) supports the reported role of Nup98 in H3K4Me2 deposition, since Trx and its human homologue Mixed lineage leukemia 1 (MLL1 or MLL) are specifically involved in catalyzing this modification (Rickels et al. 2016). In further support of this interaction, a sequence analysis of protein domain architecture of Nups across a large range of eukaryotes reported a repeated evolutionary occurrence of Nup98 homologues containing a SET domain, a conserved HMT domain that is present in Trx and MLL (Katsani et al. 2014). Interestingly, aberrant fusions of human MLL are another set of common genetic translocations that lead to leukemia phenotypes that are highly similar to those of Nup98 fusions

(Canaani et al. 2004; Hayashi 2000). Recently, the identified interactions of *Drosophila* Nup98 with the NSL and Trx/MLL complexes were found to be conserved in mammalian cells (Xu et al. 2016). Consistently, therapeutic agents developed against MLL-based leukemias have been shown to also effectively target Nup98 fusion-transformed cells (Deshpande et al. 2014). Together, these findings support the notion that Nup98 is involved in epigenetic regulation across species and that histone methylation is an integral part of the gene regulatory mechanisms of Nups.

Multiple studies have also linked NPC components to regulation of topological genome architecture, which is thought to be a key aspect of gene expression control. Genome architecture involves long-range genomic contacts, which lead to formation of gene loops and of topologically associated domains (Pombo and Dillon 2015). Interestingly, while *Drosophila* nuclear basket Nup Mtor binds chromatin in long NAR domains in fly cells (Vaquerizas et al. 2010), its yeast homologue Mlp1/2 was shown to be required for the formation of a transcriptional 5′-3′ gene loop at a galactose-inducible gene (Tan-Wong et al. 2009). In further support of an architectural role for NPC components, *Drosophila* Nups, such as Elys, Nup93 and Nup98, were identified at promoters and enhancers of multiple genes, and in line with this pattern of binding, Nup98 was found to be required for the formation of enhancer-promoter loops at ecdysone-inducible genes (Pascual-Garcia et al. 2017). This architectural role of Nup98 appears to be again linked to transcriptional memory, in this case of ecdysone-inducible genes in S2 cells, where Nup98 is specifically required for the higher levels of the later as opposed to initial inductions. Intriguingly, the high-frequency enhancer-promoter contact of the *Eip74* gene, formed as a result of ecdysone-driven activation, persisted after the transcriptional shut-off in an Nup98-dependent manner. This observation implies that stabilization of enhancer-promoter loops by NPC components may be a mechanism of epigenetic transcriptional memory (Pascual-Garcia et al. 2017). The role of Nup98 in enhancer-promoter looping is further supported by a recent study of *Drosophila* architectural proteins, which reported the prevalence of Nup98 at the bases of loops, detected by genome-wide chromosome conformation capture experiments (Cubenas-Potts et al. 2017). Furthermore, widespread targeting of strong tissue-specific enhancers, known as super-enhancers, to nuclear pores was recently observed in human cells (Ibarra et al. 2016), suggesting that the enhancer-looping function of Nups may be also be utilized in mammals.

The role of the NPC in genome architecture is also supported by previous links of Nups to insulators or boundary elements. Insulators are regulatory genomic elements that partition chromatin domains of different expression states and help establish topological genome architecture by scaffolding genomic loops (Yang and Corces 2012). Analysis of NPC binding sites in the *Drosophila* genome identified an enrichment of DNA binding motifs of a well-characterized insulator or architectural protein, Su(Hw), among NPC-genome contacts sites (Kalverda and Fornerod 2010). This link is in line with the previously discovered ability of ectopically tethered yeast Nup2 to function as a heterochromatin-impeding boundary (Ishii et al. 2002). Furthermore, ecdysone-dependent physical interactions between Nup98 and a number of architectural proteins, such as Su(Hw) and CTCF, have

been reported (Pascual-Garcia et al. 2017). The biophysical properties of FG domains within transcription-linked Nups, such as Nup98 may be particularly interesting to explore in this context, since recent studies have highlighted the importance of intrinsically disordered proteins in genome architecture and transcriptional control (Dunker et al. 2015; Hnisz et al. 2017). It remains to be determined how the architectural function of Nups is integrated with their connection to histone methylation, but together, these studies implicate Nups as key players in transcriptional memory via maintenance of gene looping conformations and of epigenetic marks.

5.5 Conclusions and Perspectives

Numerous studies in *Drosophila* and other organisms over the last 15 years have demonstrated the roles of Nups in physiological processes, such as tissue-specific development and immune response, and in regulation of transcription and chromatin. In the future, the field is likely to focus on the question of mechanism, i.e. which specific function of Nups, be it gene regulatory, transport-related or mitotic (Bukata et al. 2013), contributes to a given developmental or physiological role. This will be particularly relevant to cases of human disease that are linked to Nup mutations. For instance, while ALS pathogenesis appears to involve transport functions of Nups, AML is likely associated with chromatin-binding roles of Nup98. Specific *Drosophila* Nups have been identified as hits in genome-wide and targeted screens for regulators of key developmental signaling pathways, including TGF-β and Notch (Chen and Xu 2010; Saj et al. 2010). As described above, select Nups appear to contribute to these signaling pathways via their transport functions, but it remains possible that given the abundance of developmental genes among direct binding targets of the NPC, Nups also tune developmental signaling by binding to target genes of nuclear effectors. A particularly intriguing possibility is that metazoan NPCs couple the nuclear entry of signaling effectors to their respective target genes, thus integrating transport and chromatin-binding functions of the NPC, but this notion awaits future investigation.

One functional parallel that may be drawn between known gene targets of fly Nups is the need for rapid induction, suggesting that like in yeast, *Drosophila* NPC components preferentially target inducible genes that have to respond rapidly and robustly to external signals in their environment. In yeast cells, this signal can be an alternative carbohydrate source such as galactose, whereas steroid hormones or viral pathogens can constitute such signals in metazoan cells (Fig. 5.1). The proposed role of Nup98 and other Nups in epigenetic processes, discussed above, suggests that the primary function of Nups at inducible genes may be to enable an optimized transcriptional response and to facilitate the cellular memory of an external or developmental signal. This function of Nups appears to be linked to deposition of specific histone methyl marks and to stabilization of topological gene looping conformations. Whether these represent two distinct roles of NPC components or one role is a consequence of the other will be determined by future

studies, but the function of Nups in epigenetic memory is an intriguing emerging aspect of multicellular development and of organism-environment interactions.

Another question that remains is the dynamic relationship between NPC-bound and intranuclear gene targets of Nups. It is currently unclear whether Nups play a role in re-positioning of genes between the nuclear periphery and the nucleoplasm, or whether dynamic Nups actively shuttle between intra-nuclear chromatin binding sites and the NPC. Furthermore, which NPC components are responsible for gene recruitment to the NE-embedded NPCs is unknown. Interestingly, *Drosophila* Nup154 has been recently implicated as the primary tether of chromatin to the NE-embedded NPCs (Breuer and Ohkura 2015). It appears that recruitment of Nup62 by the Nup154-Nup93 stable sub-complex suppresses excessive chromatin attachment to the NPCs, since depletion of Nup62 in gonadal cells results in aberrant localization of chromatin to the nuclear periphery, while co-depletion of Nup154 rescues this phenotype (Breuer and Ohkura 2015). A related mechanistic question, crucial for further understanding of gene regulatory roles of Nups, is how Nups are actually recruited to chromatin. Since the majority of Nups, including Nup154, lack any identifiable chromatin-binding or DNA-binding domains, they likely employ adaptor proteins to mediate their targeting. Investigation of these questions will allow for better understanding of the functions of NPC components in tissue-specific development, evolutionary processes, immune responses and human disease, all of which appear to utilize Nups as regulatory modules.

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