Characterizing nuclear pore complex components and their role in genome maintenance and stability

Maxime Kislanski
m.kislanski@student.rug.nl

Abstract

The nuclear pore complex (NPC) is one of the largest protein complexes within eukaryotic cells. Highly conserved between species, it is most well-known for its role in nucleocytoplasmic transport. However, more recent studies have illuminated its additional role in genome maintenance and stability, where defects in certain NPC components can lead to stem cell exhaustion, cancer, and aging due to compromised cellular functions. Previous investigations demonstrated how the deletions of specific nucleoporins (nups) in *Saccharomyces cerevisiae* led to heightened sensitivity to DNA damage. Prior investigations from our lab revealed this phenotype to be partially dependent on the background strain utilized, despite the strains 'supposedly' having an intact DNA damage repair machinery. This project aimed to create nup KO mutants from WT strains with known genetic backgrounds. We focused on the Y complex and nuclear basket, subcomplexes of the NPC. We assessed them on growth as well as DNA damage sensitivity. Additionally, we tagged nups with GFP to study their subcellular localization following the deletion of other nups. Results demonstrated DNA damaging agents have an effect on nup KO mutants, validating the NPCs role on genome maintenance. Additionally, DNA damage sensitivity of the *nup60Δ* mutant varied depending on strain background. Finally, GFP-tagging nups allowed visualization of the NPC components, however unexpectedly, also displayed differences in DNA damage sensitivity between the strains.

Introduction

A defining characteristic of eukaryotic cells is the presence of a nucleus, which is enclosed by a double lipid bilayer known as the nuclear envelope (NE). The NE acts as a physical barrier, separating the nuclear contents from the cytoplasm and vice versa. In it, are embedded sophisticated protein channels known as nuclear pore complexes (NPCs). These elaborate structures are more widely known for their function as key regulators of transport in and out of the nucleus (Hetzer, 2010), however more recently they've been shown to display additional functions related to genome stability and maintenance (Simon et al., 2024).

The nuclear pore complex (NPC) stands as the largest non-polymeric protein complex within the cell. It is an aqueous channel structured in a cylindrical form with 8-fold symmetry (Rout et al., 2000). Comprised of nuclear proteins, also known as nucleoporins or nups, the NPC encompasses various sub-structures. Among these are the inner and outer rings located on both the cytoplasmic and nuclear sides, serving as important anchor points for the rest of the NPC architecture (Hakhverdyan et al., 2021; Folz et al., 2019). Proteinaceous filaments extend from both of these rings. In the nucleus, eight filaments terminate in a cylindrical structure known as the nuclear basket, whereas in the cytosol, they have free ends. Additionally there are intrinsically disordered proteins called FG-nups that extend into the central channel, forming the diffusion barrier (Figure 1) (Hakhverdyan et al., 2021; Folz et al., 2019).

In order to delve deeper into the study of the NPC, researchers have extensively investigated this complex in the model organism *Saccharomyces cerevisiae*, commonly known as baker's yeast. Baker's yeast, a unicellular eukaryotic organism, shares highly similar biological and biochemical pathways with humans. For example, the NPC of both vertebrates and baker's yeast are composed of around 30 nucleoporins each, maintaining a highly conserved structure across species (Akey, et al., 2022)(Figure 1). Furthermore, its well-established genetic and physiological background has facilitated the creation of extensive molecular toolkits for research purposes. In addition, its short life cycle and ease of manipulation has made this species a valuable model organism to explore eukaryotic components, including the NPC (Vanderwaeren et al., 2022). With this in mind, there are slight alterations to the NPC when comparing baker's yeast against vertebrates. In baker's yeast, there are between 62 to 182 NPCs per cell (Winey, et al., 1997), while for vertebrates numbers go up to ~2000 (Adam SA, 2001). Finally, the size of the NPC in baker's yeast is also smaller (52 MDa) than that seen in vertebrates (109 MDa).

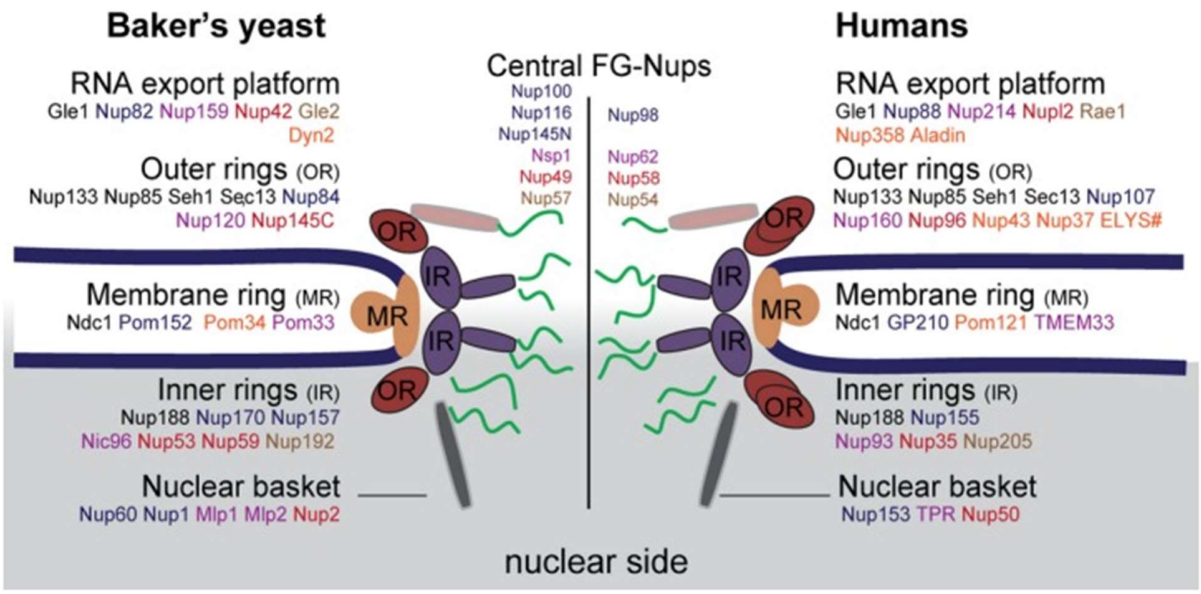

Figure 1. *Nuclear Pore Complex cartoon depicting both yeast (left) and human (right) positioning of nups (Rempel, et al., 2020).*

Transport function

As mentioned previously, one of the main roles of the NPC is its nucleocytoplasmic transport of molecules. To facilitate the transport of molecules in and out of the nucleus, they must traverse the central channel within the NPC. This channel is filled with intrinsically disordered proteins, named phenylalanine-glycine (FG)-nups, which form the diffusion barrier (Figure 1). The time duration for molecules to traverse the barrier depends on their size. Small molecules such as ions or metabolites pass through relatively quickly, whereas larger molecules take longer. To address this, macromolecules are tightly regulated by a more active mechanism, significantly reducing diffusion time. For larger macromolecules to traverse the NPC quickly, soluble nuclear transport receptors (NTRs) and transport signals are required. NTRs are proteins that bind to the transport signals found on their specific substrates, facilitating their passage through the NPC channel. These transport signals may comprise of short chain amino acids known as nuclear localization sequences (NLS) or nuclear export sequences (NES) (Strambio-De-Castillia, et al., 2010)(Figure 2).

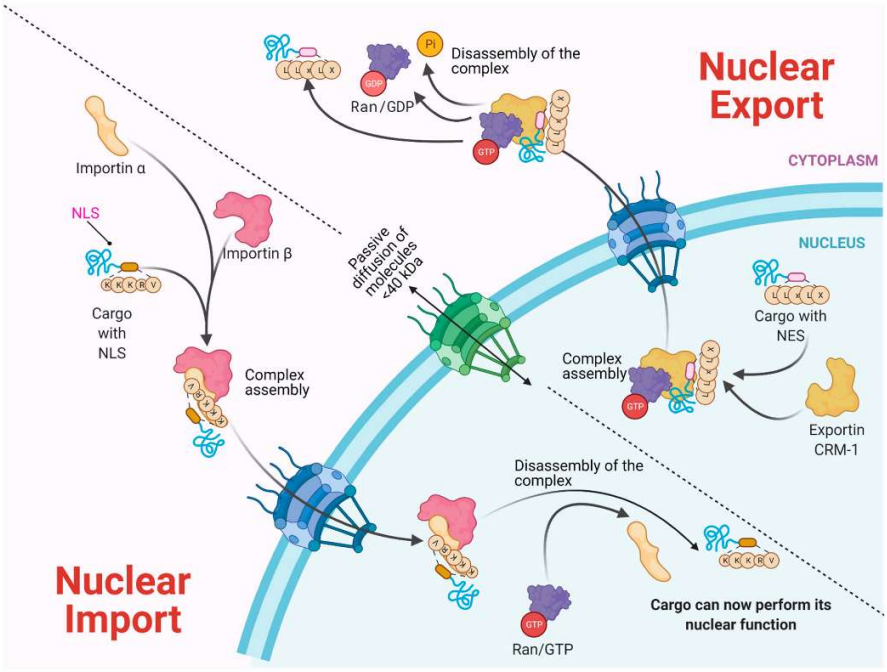

Figure 2. *Nucleocytoplasmic transport of NPC (Jesús-González, et al., 2021).*

Genome maintenance function

Dysfunctions in genome maintenance can have detrimental effects to the cell. One of these effects is the accumulation of DNA damage, contributing to cancer development, accelerated aging, and developmental defects (Schumacher & Wolters, 2013). Gaining insight into the mechanisms behind DNA integrity can lead to potential future therapeutic strategies. In addition to its function in nucleocytoplasmic transport, recent research has shed light on the involvement of certain nucleoporins in genome stability, particularly in the context of the DNA damage response (DDR). The Y complex of the NPC is notable for its impact on genome stability (Walther et al., 2003; Bennett et al., 2001). The outer rings of the NPCs consist of 8 of these Y complexes, arranged in a head-to-tail manner via beta propellers. Each Y complex consists of Nup84, Nup85, Nup120, Nup133, Nup145C, Sec13, and Seh1 (Lutzmann et al., 2002)(Figure 3). An earlier study demonstrated that knocking out certain nucleoporins from the Y complex, specifically Nup84, Nup120, and Nup133, resulted in heightened sensitivity to various DNA-damaging agents, including UV light, DNA methylation agents, and DNA strand break inducers (Bennett et al., 2001). Later studies have identified the Y complex being involved in SUMOylation processes to help repair DNA damage. One way this was observed was with the movement of SUMO protease Ulp1 to the NPC, where it regulates the SUMOylation status of other proteins that play important roles in DNA damage repair. Interestingly, Ulp1 was seen to only be able to associate to the NPC through Nup84 from the Y complex, and Nup60 from the nuclear basket (Palancade et al., 2007).

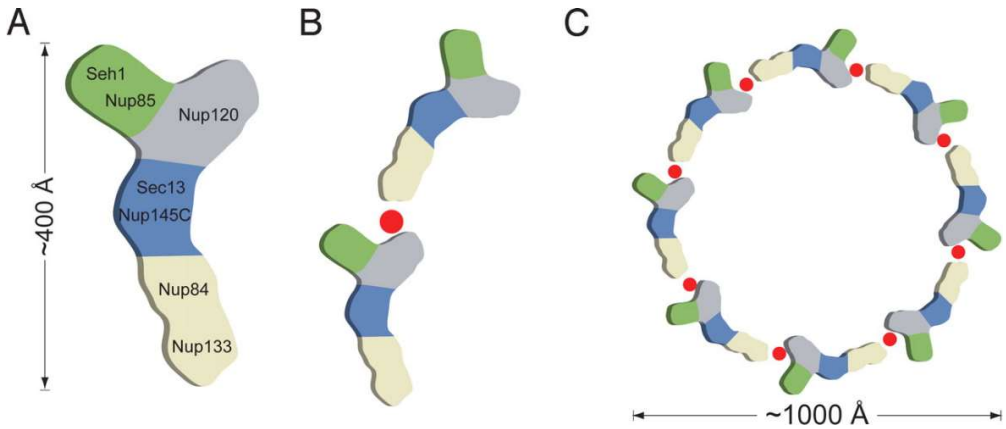

Figure 3. *Schematic depiction of the Y complex in the NPC. Here showing the head-to-tail fashion of forming the ring-like structure on both the cytoplasmic and nuclear side (Seo, et al., 2009).*

More in-depth studies have shown that various DNA structures involved in genome maintenance relocate to the NPC. The Y complex has been reported to relocate persistent DSBs to the NPC through a Mec1/Tel1 process (Nagai et al., 2008). Additionally, this complex, along with the Slx5/8 SUMO-dependent ubiquitin ligase, has been detected in the relocation of CAG/CTG repeats (Su et al., 2015). Apart from the Y complex, nuclear basket protein Nup1 has also been reported to be involved in various relocalization processes. Previous research has shown that disturbing the function of Nup1, either by truncating its C-terminal end or fusing it with the bacterial DNA-binding protein LexA, impairs the localization of replication forks stalled at expanded CAG/CTG repeats or eroded telomeres to the nuclear periphery (Aguilera et al., 2020).

Discrepancies in results between different yeast background strains

Previous research conducted by our lab delved into the sensitivity of DNA damage across mutant yeast strains characterized by truncations in the Y complex and deletions in basket components. In that study, a series of drug sensitivity assays were performed to assess the impact of the nups in DNA maintenance. However, an issue that the work had come across was differences in DNA damage sensitivity due to differences in strain background (W303 and BY). A notable example was *nup60Δ* in the W303 background being much more sensitive to topoisomerase inhibitor, campothecin (CPT), than *nup60Δ* in the BY background (Figure 4). This suggests the notion that genetic differences between the strain backgrounds may account for the observed variations in DNA damage sensitivity. However, it is unclear if these differences are actually due to background variations, as the original parental strains used in our previous lab work were not isogenic. If these differences are indeed accurate, identifying the relevant genetic variations responsible would provide further fundamental understanding to the underlying processes of genome maintenance and stability.

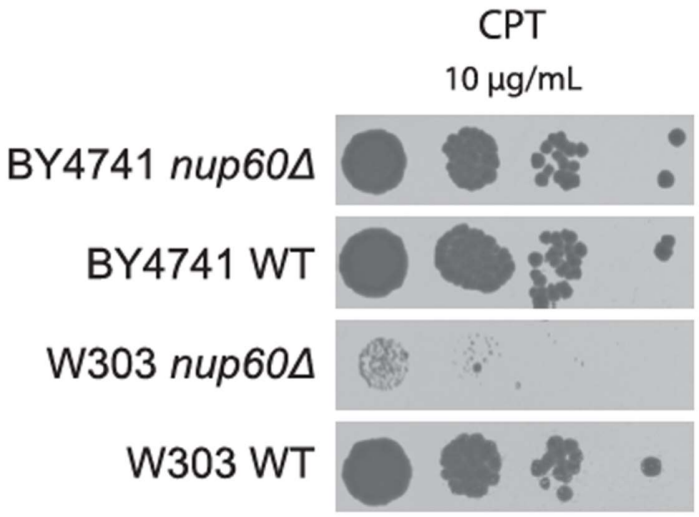

Figure 4. *DNA sensitivity growth test assay, performed on camptothecin (CPT, 10 µg/mL) with a 10-fold serial dilution on parental strains (W303 and BY4741), as well as their nup60Δ mutants (previous work from the lab).*

Additionally, little is understood of the structure of the NPC after mutation and deletion of certain components. Investigating this subject could play a crucial role in understanding how specific nup mutations impact the overall structure and integrity of the NPC. Previous research has demonstrated that the deletion of particular nups, such as Nup84 or Nup133, results in NPC clustering (Palancade et al., 2007; Niño et al., 2016). Conversely, strains carrying a Nup145 truncation mutation have been observed to exhibit nucleoplasmic mislocalization of Mlp1 and Mlp2 (Galy et al., 2000)(table 1). However it is not known if mutation of components of the basket affects components from the Y-complex, or how the rest of the complex looks once one component was deleted.

Goals of this project

Part of the goal of this project would be to continue the previous work in the lab and characterize Nup KO mutants by testing them in DNA damage sensitivity growth assays. To do this, wild-type W303 and BY strains, after thorough checking of their genetic background, will be used as the parental strains for the experiments. This will ensure that the differences observed in the assays are exclusively due to the genotypic changes realized in this study. The KO strains which will be created and used include *nup84Δ, nup120Δ, nup133Δ* (gene deletion of components of the Y complex), as well as *nup1Δct, nup60Δ and nup2Δ* (truncation/deletion of components of the basket). As previously discussed, the nucleoporins constituting the Y complex and nuclear basket have demonstrated significant importance for DNA damage sensitivity, making them key targets of interest to investigate NPC genome maintenance (Bennett et al., 2001)(Bukata et al., 2013)(Palancade et al., 2007). However, it is worth noting that *NUP85, NUP145C, SEC13, SEH1*,

and *NUP1* are each essential genes, therefore making the creation of their subsequent KO mutants not possible.

Furthermore, if differences are observed in the different assays, it would be interesting to investigate the localization of other nucleoporins upon deletion of others, since this information, as previously mentioned, is not yet very well characterized. For this reason, each of these constructed KO strains will be endogenously tagged with GFP on Nup1, Nup2, Nup60, Nup84, and Nup100 to observe their subcellular localization (Table 1).

Table 1. *Subcellular localization of certain nups in KO strains as reported in literature.*

Deletion	Nup1-GFP	Nup2-GFP	Nup60-GFP	Nup84-GFP	Nup100-GFP
nup1Δct	N/A	X	X	X	X
nup2Δ	X	N/A	X	X	X
nup60Δ	X	Nuclear (Cibulka 2022)	N/A	X	X
nup84Δ	X	X	Cluster (Niño 2016)	N/A	X
nup120Δ	X	X	Cluster (Palancade 2007)	X	X
nup133Δ	X	X	Cluster (Palancade 2007). Cluster (Niño 2016)	Cluster (Niño 2016)	X

X = has not been done.

Materials and Methods

Strains and growth conditions

Yeast strains were grown at 30°C in yeast extract peptone dextrose (YPD), supplemented with 2% (w/v) glucose. For spot assays, the samples were grown overnight and then diluted to achieve an OD of 0.5 for which successive 10X serial dilutions was performed four times. Subsequently, the samples were spotted onto YPD agar plates, and then incubated at 30°C. Scans were taken after both day 1 and day 2. For microscopy experiments, synthetic defined (SD) medium was used, supplemented with 2% (w/v) glucose. A list of all strains used in this work is found in the supplementary table (S1).

Strain construction and verification

Genomic DNA from either the YKO collection or GFP-collection was isolated using the wizard DNA purification Kit (promega) and used to PCR amplify the desired nup deletion or nup GFP tag. Transformation was done following the highly efficient yeast transformation protocol that uses

LiAc/SS carrier DNA/PEG, described in Gietz and Schiestl 2007. A listo of all the primers used are in table 2. To further verify the GFP-mutant strains, they were checked under fluorescent microscopy.

Table 2. *Primer list for amplification and verification of desired sequences for KO and GFP-tagging.*

Name	Sequence
NUP84Δ fwd	CTCTGGTATTGGGTGGTCCG
NUP84Δ rev	TTGGCGCATCATAGGGACAG
NUP60Δ fwd	CTGCTGCCTCGGGTGTTTT
NUP60Δ rev	TCTCGCTGTTACTTGTGGCT
NUP1ΔCT fwd	AATAATTCAACGCCTGGTGG
NUP1ΔCT rev	AAAGGAGCGCTTTGCAGATC
NUP120Δ fwd	GGAAGCACGGTATTGTGGAAG
NUP120Δ rev	GCCAAAGTGTAATTTCGGTGGA
NUP133Δ fwd	GCGCTTGCTTGACATTCTCT
NUP133Δ rev	TCGCAAGCAAGTTTGGGATTA
NUP2Δ fwd	TATCCTCCGTAGTTGTCG
NUP2Δ rev	GGGTGGATTAAACACAGAAGG
Nup1-GFP fwd	ACGCCATCAACAGTTCCT
Nup1-GFP rev	CAGGCTGTGTACTAGACC
Nup60-GFPfwd	CCCGTCCAACCAGATCTT
Nup60-GFPrev	AGACGCCTCTGAAGCTGT
Nup84-GFP fwd	TTCGCGGATTCGGCAGAT
Nup84-GFP rev	ATTGGCGCATCATAGGGA
Nup100-GFP fwd	GAGCCCGTTGATTTGTTG
Nup100-GFP rev	CAACCTTTCTGCATCTGG
Nup2-GFP fwd	TCAAGAGGCGTAGGCGAA
Nup2-GFP rev	CCTTGCAGGAAAGAAAGG
NUP84Δ fwd seq	TGATGAAGGGCCGAACCAAA
NUP60Δ fwd seq	TTAAGCACTAGGCGGTGGAC
NUP1ΔCTct fwd seq	TCAATAAACCTCCTTCCACG
NUP1ΔCTct rev seq 1	TCTGTTCGCCATAACACCAC
NUP1ΔCTct rev seq 2 (MX4rev)	AATTCAACGCGTCTGTGAGG
NUP120Δ fwd seq	GGCGACATGTCTTCGAAAGG
NUP133Δ fwd seq	GGCGACATGTCTTCGAAAGG
NUP2Δ fwd seq	TTTGGGGTCCCAAACTCT
KanMX rev seq	TAATGCCGAGGAGCGACGTC

Sporulation and tetrad dissection

Mutant diploid strains W303 were transferred to synthetic minimal MIN SPO (1% KOAc, 0.005% ZnOAc, and 2% agar) media plates and placed in a 22°C incubator for 5 days. Tetrads were treated

with zymolyase 0.1 mg/mL for 30 minutes. Dissection was then carried out under a dissection microscope, and the dissected cells are placed in a 30°C incubator for 2 days. Haploids were genotyped and scored.

Drug sensitivity assay

To investigate DNA damage sensitivity upon the newly constructed strains, a drug sensitivity assay was performed. The strains were left to incubate overnight at 30°C and then subsequently diluted to an OD_{600} of 0.5. Using a 96-well plate, four 10-fold serial dilutions were made. These were then spotted on YPD-agar plates treated with different genotoxic agents (10 µg/mL camptothecin (CPT), 100 mM hydroxyurea (HU), 2 mM hydrogen peroxide (H_2O_2), 0.04 µg/mL bleomycin, 0.02% methyl methanesulfonate (MMS)) as well as UV radiation (20 J/M^2 and 50 J/M^2). They were left to incubate for 48 hours at 30°C, or 72 hours for HU.

Fluorescence Microscopy

To perform fluorescence microscopy, the strains were left to incubate in SD complete media overnight at 30°C. Imaging experiments were done on a commercial DeltaVision Deconvolution Microscope (Applied Precision). The camera used was type EDGE / sCMOS_5.5 with speed 286000KHz and gain 1.00X. The objective used is Olympus 100X/1.40, UPLS Apo. Image-stacks were deconvolved using standard settings.

Results

Strain construction

The first objective of this project was to obtain the strains needed for the characterization and assays.

Table 3. *List of expected strains that were successfully constructed in W303*

W303						
		GFP				
Δ	**-**	**Nup1**	**Nup60**	**Nup84**	**Nup100**	**Nup2**
-	**WT**	✓	✓	✓	✓	✓
nup1Δct	✓	N/A	✓	✓	✓	✓
nup60Δ	✓	✓	N/A	✓	✓	✓
nup84Δ	✓	✓	✓	N/A	✓	✓
nup120Δ	✓	✓	✓	✓	**X***	✓
nup133Δ	✓	**X***	**X***	✓	✓	X*
nup2Δ	X	X	X	X	X	N/A

✓ = Successfully constructed.

X = Unable to obtain
X* = Unable to obtain due to synthetic lethality

Not all of the successfully constructed listed strains were obtained via tetrad dissection. Some of these strains were obtained via transformation of the WT haploid directly after failed tetrad dissection. These strains include *nup1Δct* Nup100- and Nup2-GFP; *nup60Δ* Nup2-GFP; *nup84Δ* Nup1- and Nup100-GFP; *nup120Δ* Nup1-GFP.

Growth assay

Prior to doing a DNA damage sensitivity assay, growth assays were first performed to gain insight into the difference in growth between the different strains in untreated conditions.

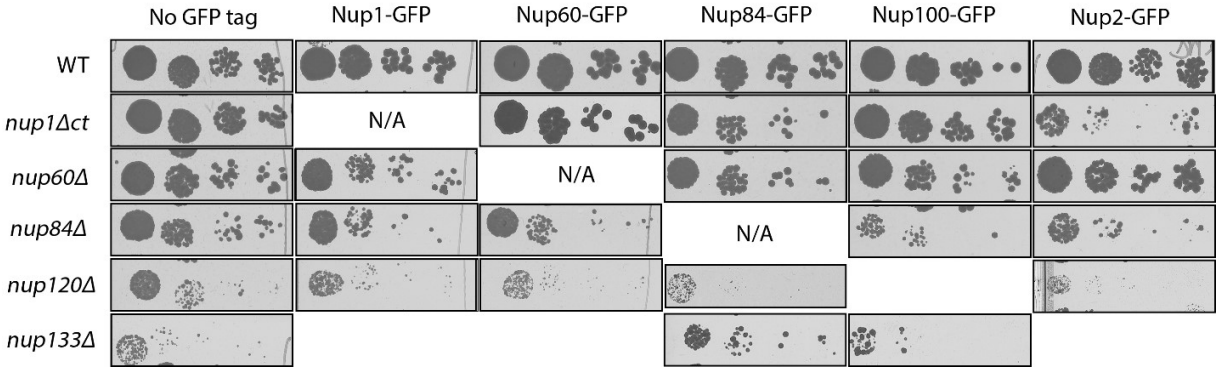

Figure 5. Growth assay displaying serial 10-fold dilutions for W303 KO mutant strains along with different GFP-tagged nup strains. Plates were left untreated to grow for 2 days at 30°C. Representative image of 3 different experiments.

Literature has demonstrated that knocking out Nup133, Nup120, Nup84, and Nup60 does have an effect on the growth rate of the strain when compared to the WT (Bennett et al., 2001; Folz et al., 2019; Loeillet et al., 2005). The following spot assay demonstrated that the mutants *nup133Δ* and *nup120Δ*, without GFP-tagging the nups, do display clear lower growth rates when compared to the WT (Figure 5). This is also seen with *nup84Δ* and *nup60Δ*, however less distinct than the latter. For *nup1Δct* no clear difference in growth rate is seen.

Furthermore, GFP-tagging of some nups has an additional deteriorating effect on some of the KO strains. For example, in *nup1Δct*, this is observed with GFP-tagged Nup2 and to a lesser degree Nup84. Moreover, for *nup84Δ* and *nup120Δ*, it can be seen that tagging any of the listed nups leads to a growth defect. Also, Nup100-GFP seems to be synthetically lethal in combination with *nup120Δ*, as I was unable to make this strain. Interestingly, it was observed that with *nup133Δ*, tagging any of the listed basket nups (Nup1, Nup60, and Nup2) with GFP also seemed to be synthetically lethal (Table 3).

DNA sensitivity assay

To gain greater insight into the roles of Y complex and basket nups on genome maintenance and stability, a DNA damage sensitivity assay was performed.

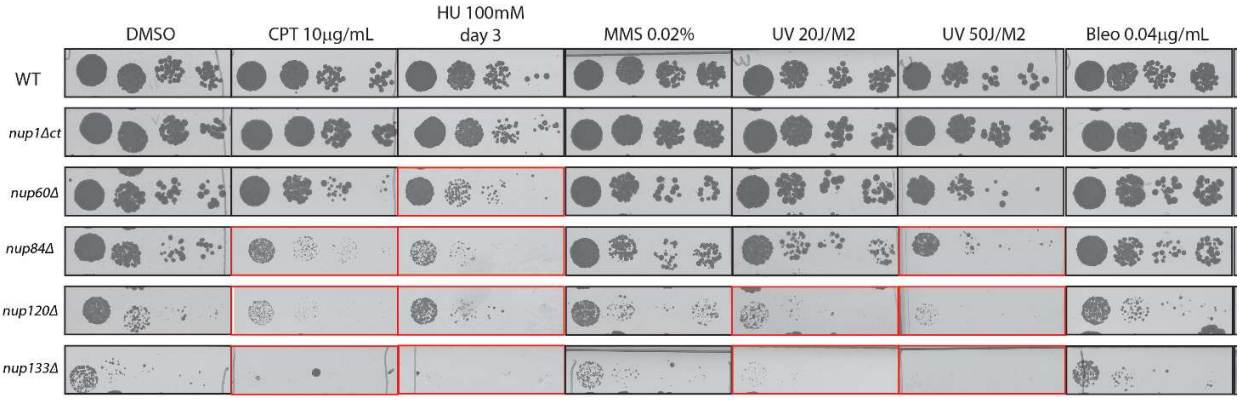

Figure 6. DNA damage sensitivity assay for various nups KO mutants using a 10-fold serial dilution in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days. In red are the strains that display higher sensitivity to DNA damage compared to the WT.

For *nup1Δct*, there are no observed differences in cellular fitness or DNA damage sensitivity compared to the WT strain. In contrast, Y complex nups KO strains *nup84Δ*, *nup120Δ*, and *nup133Δ* show markedly different results. These strains not only exhibit decreased growth rates under untreated conditions but are also more severely affected by genotoxic agents and treatments. CPT, HU, MMS, and UV all caused significant growth defects in these KO strains (Figure 6), consistent with what was observed in previous literature (Bennett et al., 2001). Furthermore, *nup60Δ* showed sensitivity to HU, but not other genotoxic agents and treatments. Additionally, bleomycin seems to have had no effect on the growth of any of the strains.

DNA sensitivity assays were also performed with the nup KO mutants and their subsequent GFP-tagged nups. This was done to see if tagging nups had any effect on overall DNA sensitivity. As an example, assay for *nup84Δ* and its GFP-tagged nups is displayed (Figure 7).

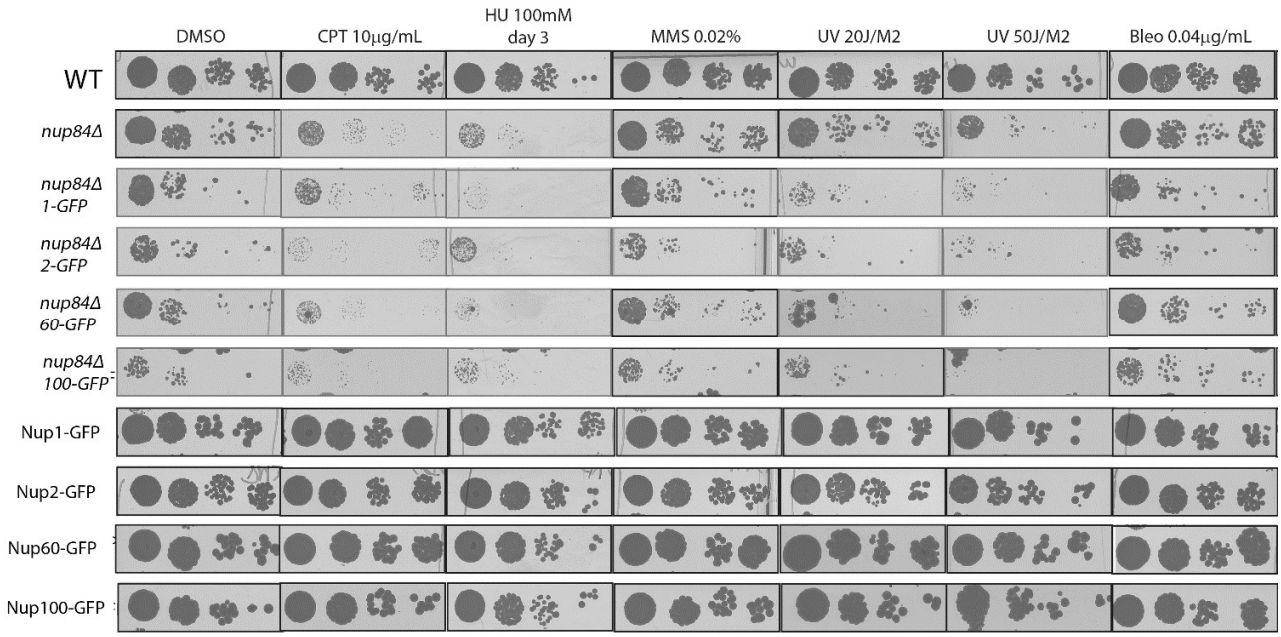

Figure 7. *DNA Damage sensitivity assay for nup84Δ and GFP-tagged nups using a 10-fold dilution series in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days, or 3 days for HU. NOTE: in figure 7 only nup84Δ was shown, nup60, nup1Δct, nup120 and nup133 were also checked (supplementary figure S2-S5).*

It can be seen for *nup84Δ* that tagging particular nups with GFP seems to increase DNA damage sensitivity. Genotoxic agent HU and UV treatment both display significant drops in growth for all of the GFP-tagged nups of *nup84Δ* compared to when it is left untreated.

Comparing BY and W303 strains

After receiving *nup60Δ* and *nup1Δct* strains in BY from another lab member, an additional drop test assay was performed to compare growth and DNA damage sensitivity with W303, aiming to gain further insight into our lab's previous findings.

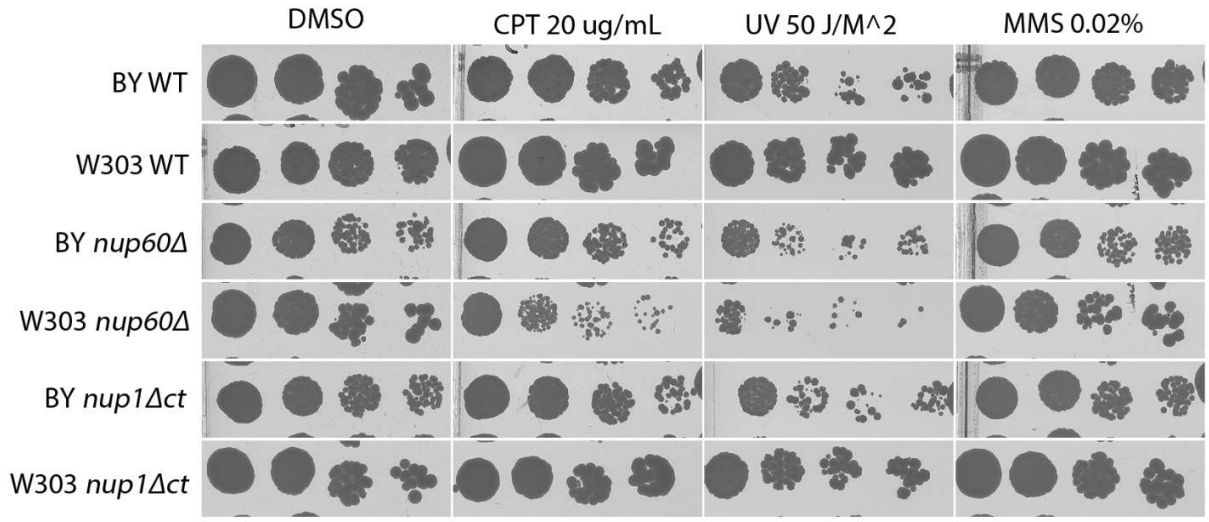

Figure 8. *DNA Damage sensitivity assay for nup60Δ, and nup1Δct in both W303 and BY strains. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days.*

In untreated conditions, the KO strains exhibit similar growth to the WT in both backgrounds. Interestingly, with CPT treatment, W303 *nup60Δ* shows slightly less growth than BY *nup60Δ*, and it seems that there is barely any difference between CPT-treated and untreated BY *nup60Δ*. This supports our lab's previous findings that W303 *nup60Δ* is more sensitive to CPT than BY *nup60Δ* (Figure 4). Additionally, the effect is different with UV treatment. UV exposure has no impact on W303 *nup1Δct*, while BY *nup1Δct* shows slightly reduced growth (Figure 8).

Fluorescence Microscopy

To further characterize the nups KO mutants, fluorescence microscopy was performed to gain greater insight into the mutants' subcellular localization of other nups. This information would provide further understanding on NPC assembly as well as structure.

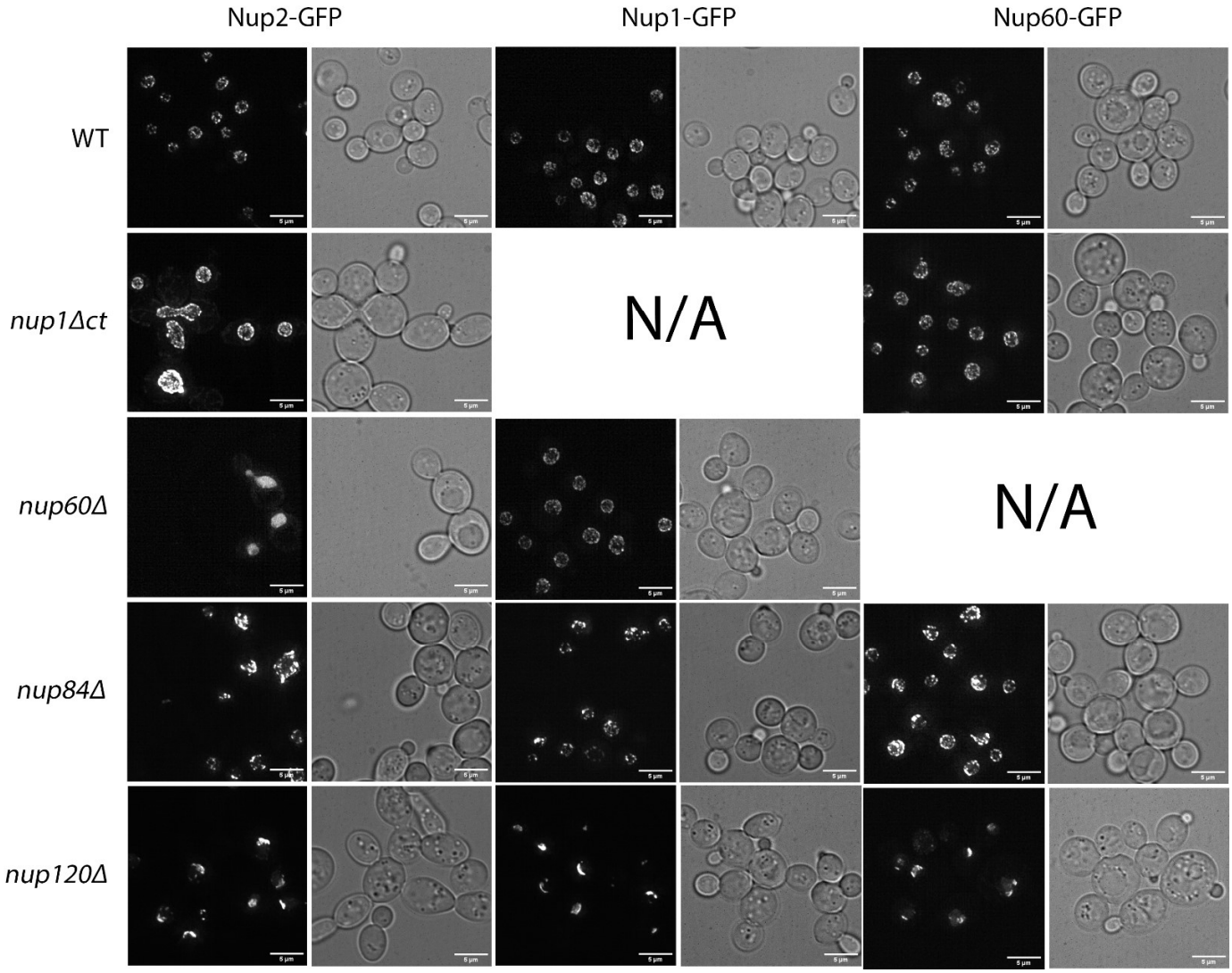

Figure 9. Fluorescent Microscopy images for listed basket nups (Nup1, Nup2, and Nup60) tagged with GFP in different nup KO mutants in W303 only. Scale bar is 5 µm.

Literature on the subcellular localization of certain nups upon the deletion of others, as summarized in Table 1, aligns accurately to the fluorescence microscopy results, validating these findings (Figure 9, 10). For the WT strains, NE signaling is observed as expected. In *nup60Δ* strains, the Nup2-GFP fluorescent tag is seen having a nuclear localization rather than just at the nuclear periphery, matching previous findings (Cibulka et al., 2022) (Figure 9). Other studies have shown that deletions of Nup84, Nup120, and Nup133 result in pore clustering, leading to clustered fluorescent foci around the nuclear periphery (Palancade et al., 2008; Niño et al., 2016), and similar findings were demonstrated in these experiments (Figure 9, 10). Nup100-GFP, a protein from the central channel was used to check a different sub-structure of the NPC when the Y complex or basket are mutated (Figure 10).

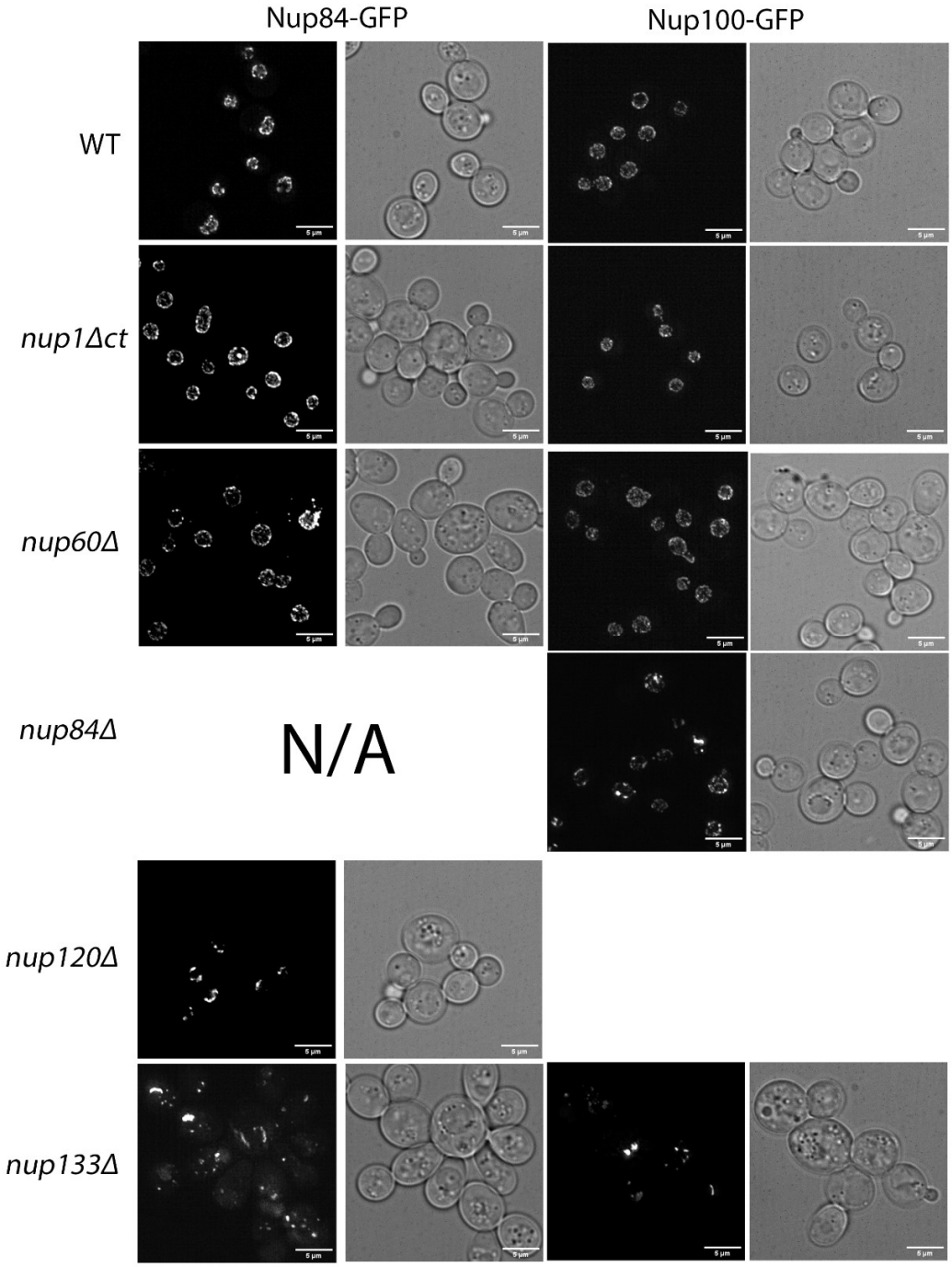

Figure 10. Fluorescent Microscopy images for listed nups (Nup84 and Nup100) tagged with GFP, tested on different nup KO mutants in W303 only. Scale bar is 5 µm.

Table 4. *Updated subcellular localization of certain nups in nup KO strains list.*

Deletion	Nup1-GFP	Nup2-GFP	Nup60-GFP	Nup84-GFP	Nup100-GFP
nup1Δct	N/A	NE	NE	NE	NE
nup60Δ	NE	Nuclear	N/A	NE	NE
nup84Δ	Cluster	Cluster	Cluster	N/A	Cluster
nup120Δ	Cluster	Cluster	Cluster	Cluster	X
nup133Δ	X	X	Cluster	Cluster	Cluster
Nup2Δ	X	X	X	X	X

Discussion

Strain construction

There were several difficulties in attempting to obtain each of the listed strains. For W303, it was observed that tagging certain nups with GFP in particular KO mutants leads to synthetic lethality. This was shown with tetrad dissections where the haploid containing the genotype of interest would fail to grow. Interestingly, for *nup133Δ* it was seen that tagging any of the basket nups (Nups 1, 2, and 60) would lead to this lethality. For *nup120Δ* this was seen instead with Nup100-GFP only. As previously mentioned, various successfully constructed strains were obtained via transformation directly instead of doing tetrad dissection. One potential reason as to why this ended up being successful, could be that during a transformation process you are engineering many more cells at once, increasing the chances that a viable KO survives. With *nup2Δ* we were unable to create any transformant, even in diploid form. This most probably has to be due to the genomic DNA isolated, or the primers that were used. Nevertheless, due to time constraints we could not continue working with this strain. For the BY strains there also simply wasn't enough time during the project to obtain all of them. (Table 3).

Growth and DNA sensitivity assays on nup KO mutants

Looking at the DNA sensitivity and growth assays for the nup KO mutants, clear differences can be observed between them. For *nup1Δct*, no differences in cellular fitness or DNA damage sensitivity were observed compared to the WT strain. This is noteworthy because, despite being an essential gene, several studies have shown that Nup1 plays a crucial role in genome stability and maintenance. It is required in relocalizing eroded telomeres, stalled replication forks, and expanded CAG/CTG triplet repeats to the NPC for repair (Aguilera et al., 2020; Whalen & Freudenreich, 2020). These processes are known to impact genome instability (Campbell, 2012; Cortez, 2015; Polleys, 2013). In contrast, a similar study done in our lab demonstrated *nup1Δct* displaying much higher sensitivity to DNA damage, suggesting that this particular *nup1Δct* strain might have picked up a suppressor (Figure 5 and 6). Suppressors are genes that inhibit the expression of mutant phenotypes (Hawthorne & Leopold, 1974). During colony selection, from

transformation or tetrad dissection, a *nup1Δct* strain with a suppressor may have been chosen. Investigating suppressors could enhance our understanding of the functional relationships, mechanisms, and modes of action in genetic pathways and one method to explore this is through Synthetic Genetic Array (SGA) analysis. SGA is a high-throughput genetic analysis where one investigates the functional relationship between genes. This is done by creating double mutants from a library, and analyzing their phenotypes.

In contrast, the Y complex nups KO, *nup84Δ*, *nup120Δ*, and *nup133Δ*, displayed clear growth disparities as well as higher sensitivity to most genotoxic agents and treatments. Under untreated conditions, a decrease in growth compared to the WT is not surprising (Figure 5). Knocking out Y complex nups leads to pore clustering (Figure 9), which can potentially disrupt nucleocytoplasmic transport and slow cellular replication. Additionally, earlier studies have demonstrated the Y complex's role in maintaining genome stability, particularly in double-strand break repair (Nagai et al., 2008) and handling of CAG/CTG triplet repeats (Su et al., 2015). When cells divide, they must replicate their genome, a process that naturally involves errors. If the mutants cannot effectively support the repair of these impacted sites, the damage accumulates, leading to further genome instability and then potentially cellular senescence.

This realization is brought closer to light when looking at the strains' response to the DNA damaging agents and conditions. Camptothecin (CPT), bleomycin (bleo), and ultraviolet light radiation (UV) are used to specifically induce DNA lesions. CPT is a topoisomerase type 1 (Top1) inhibitor, preventing re-ligation of the nicked DNA strand and therefore dissociation of Top1. Its toxicity arises from the conversion of single-strand breaks into double-strand breaks when the replication fork encounters the cleavage complexes formed by DNA and CPT (Li, et al., 2017). Bleo oxidatively damages DNA by binding to metal ions, including iron, forming metallobleomycin complexes. These complexes cause DNA single-strand and double-strand breaks, inducing a DDR (Petering, et al., 1990). Lastly UV is high energy, high frequency radiation that upon impact with DNA can lead to the formation of photoproducts, generation of free radicals, and strand breaks. These photoproducts cause perturbations to the DNA helix while for free radicals the main way they can damage DNA is by oxidizing nitrogen bases, causing different forms of damages that induce different DDR pathways (Kciuk, et al., 2020). When looking at the results, it can be observed that both CPT and UV treatment led to decreased growth of the Y complex KO mutants (Figure 6). However, this was not seen with bleomycin. This is surprising, as previous work from the lab demonstrated its effectiveness in disturbing DNA damage sensitive strains. Due to these unexpected results, it could be speculated that there was something wrong with the stock solution of bleomycin, since the concentration used for this experiment was also the same used for previous lab work where it previously was effective.

To specifically induce replication fork stalling, methyl methanesulfonate (MMS) was used. It is an alkylating agent that preferentially methylates guanine and adenine bases in DNA. These methylation processes can affect helix morphology, where at high dosages lead to stalled replication forks (Ovejero, et al., 2021). As previously mentioned, there is evidence that shows the Y complex nup KO mutants to have a role in stalled replication fork maintenance (Su et al., 2015). However this is not really hinted at when looking at the results. There is not much of a change in growth when comparing untreated and with MMS (Figure 6). One possible reason could be due to the concentration of MMS used being too low. To test, a serial dilution spot assay for one Y complex nup KO strain with various concentrations of MMS can be done.

Lastly, for the Y complex nup KO mutants, hydroxyurea (HU) treatment was additionally used. HU is a ribonucleotide reductase, an enzyme involved in transforming ribonucleosides into deoxyribonucleosides which serve as the building blocks for DNA synthesis. It reduces intracellular deoxynucleotide triphosphate pools inhibiting DNA synthesis therefore acting as an S-phase-specific agent (Agrawal, et al., 2014). It leads to both DSBs, as well as stalled replication forks. For this reason, it is not much of a surprise to see it having a clear decreased growth effect on the Y complex nup KO mutants (Figure 6). Overall, since most of these agents have an effect on replication fork stalling and DNA damage frequency, it can be speculated that the KO mutants cannot anchor the damaged sites for repair, leading to further genomic instability and cellular dysfunction.

This is similarly seen with *nup60Δ*, however to a lesser degree than with the Y complex nups. In untreated conditions, there was no significant difference in growth, and the increase in DNA damage sensitivity was also not as pronounced (Figure 5 and 6). While the Y complex nup KO mutants display similar results to what has been previously observed in studies (Bennett, et al., 2001; Loeillet, et al., 2005), this is not entirely the case with *nup60Δ*. With *nup60Δ* a much harsher response to genotoxic agents, particularly CPT, was expected in the W303 background strain (Folz, et al., 2019). One possible explanation, similar to *nup1Δct*, is that the strain also contains a suppressor. To further investigate and identify this gene, an SGA analysis can be done.

GFP-tagging certain nups negatively impacts particular KO nups

The growth assay as well as DNA sensitivity assay showed that tagging certain nups with GFP in specific KO mutants led to decreased growth in some strains and synthetic lethality in others (Figure 5 and 7). These findings are intriguing because many studies have demonstrated that GFP-tagging is an effective method for protein subcellular localization (Niedenthal et al., 1996). However, empirical evidence has also shown that GFP-tags can potentially impact protein function (Davidi et al., 2018; Shaner et al., 2004). The exact mechanisms by which GFP-tagging nups negatively affects cells are not yet fully understood, however speculations can be carried

out. Previous studies have demonstrated that popular fluorescent proteins, such as GFP, can be unreliable for fluorescent imaging. For example, it was observed that tagging certain proteins with GFP may lead to their mislocalization, aggregation, or impaired function during live-cell analysis (Constantini et al., 2015; Wiedenmann et al., 2009). In the context of the NPC, it can be speculated that tagging nups with a 28 kDa protein may interfere with the high specificity and complementarity required for proper NPC assembly. Given that nups frequently interact with each other, the presence of a GFP-tag at one end of a nup could disrupt these interactions, further impairing the cell's proper functioning. A potential method to address this limitation would be to use two different tags: mCherry, another fluorescent probe that can also be used to endogenously tag a protein and is commonly used as an alternative to GFP for live cell imaging. And a smaller tag, such as HA or Myc that could help to decrease possible interference with assembly.

Background strain has a minimal effect on *nup60Δ*'s DNA damage sensitivity

Previous work from our lab showed *nup60Δ* in BY having less sensitivity to DNA damage than W303 (Figure 4). In this new experiment, similar results were also observed, yet not to the same degree (Figure 8). However, as previously mentioned, W303 *nup60Δ* seems to be growing better in both untreated and treated conditions than observed in older literature, hinting at the idea that this strain might have picked up a suppressor. Nevertheless, it is not understood why there's a difference between the background strains even if their DDR pathways should technically be identical. Investigating why this is the case could give further clues on the mechanisms behind the NPC's role in genome maintenance and stability. To investigate this, an SGA analysis can also be performed.

Subcellular localization of nups upon KO strains agrees with current literature

As observed in the results, the fluorescence microscopy results align accurately to what has been previously observed in older literature (Table 1)(Figure 9 and 10). Knocking out Y complex nups leads to pore clustering, while this is not the case when knocking out the basket nups listed. Interestingly, there doesn't seem to be any visible difference for the GFP-tagged nups on each KO, with the exception of *nup60Δ* Nup2-GFP, where Nup2 is seen to be nuclear instead of the NE (Figure 9). One study has demonstrated that this is due to Nup60 being required for Nup2 to properly assemble to the NPC. Nup60 harbors an array of short linear motifs (SLiMs) where Nup2, along with other basket nups, are able to bind covalently (Cibulka, et al., 2022), which is surprising as Nup1 is at the NE in nup60Δ, while Nup2, Mlp1 and Mlp2 each become nuclear (Palancade & Doye, 2008).

Conclusion

Overall, this project demonstrated that DNA-damaging agents clearly affect nup KO mutants, confirming the NPC's role in genome maintenance and stability (Figures 6, 7, and 8). Additionally, DNA damage sensitivity may vary between different background strains upon the deletion of certain nups (Figure 8). Furthermore, GFP-tagging of nups proved useful for subcellular localization studies (Figures 9 and 10), though it is important to consider potential unexpected phenotypes, such as the growth defects observed in the assays (Figures 5 and 7). Consequently, it may be more optimal to use other fluorescent tags such as mCherry. It is hoped that this research could be used as a first approach method towards further understanding the mechanisms of genome maintenance by the NPC, however, there are still unknowns that arose from this research. It remains unclear why differences are observed between W303 and BY strains for *nup60Δ*. Looking into the molecular mechanisms behind these differences could provide new insights into genome maintenance processes. Additionally, it is uncertain whether the W303 *nup60Δ* and *nup1Δct* strains acquired suppressors during their making. Investigating this possibility could reveal potential interacting partners and their effects on the cell's phenotype for DNA damage sensitivity, contributing to a greater understanding of genome stability.

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Supplementary

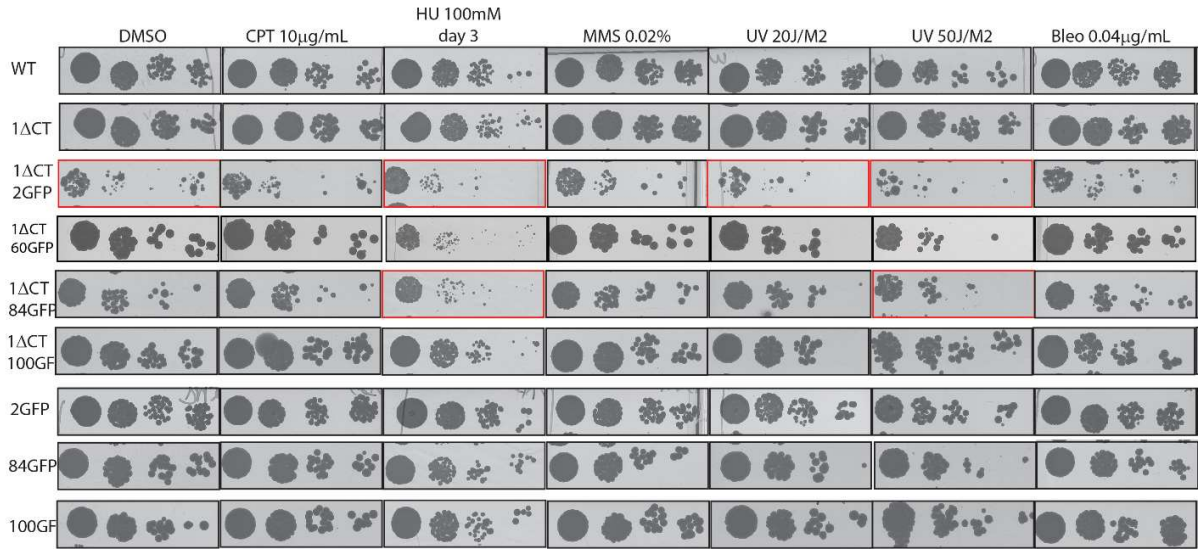
S1. Strain list used in this study

Strain	Name	Genotype
BY4743	-	*MAT**a**/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 LYS2/lys2Δ0*
nup60Δ	IKY101	*nup60Δ::kanMX his3Δ1 leu2Δ0 met15Δ0 * ura3Δ0 lys2Δ0 **
nup1Δct	IKY105	*nup1Δct::kanmx his3Δ1 leu2Δ0 met15Δ0 * ura3Δ0 lys2Δ0 **
W303	-	*MAT**a**/MATα leu2-3,112/leu2-3,112 trp1-1/ trp1-1 can1-100/ can1-100 ura3-1/ ura3-1 ade2-1/ADE2 his3-11,15/his3-11,15*
nup60Δ	MKY13	*MATα nup60Δ::kanmx leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup60Δ Nup1-GFP	MKY23	*MAT**a** nup60Δ::kanmx nup1-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup60Δ Nup84-GFP	MKY44	*MATα nup60Δ::kanmx nup84-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup60Δ Nup100-GFP	MKY54	*MATα nup60Δ::kanmx nup100-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup60Δ Nup2-GFP	MKY86	*MATα nup60Δ::kanmx nup2-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup84Δ	MKY11	*MATα nup84Δ::kanmx leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup84Δ Nup1-GFP	MKY26	*MATα nup84Δ::kanmx nup1-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup84Δ Nup60-GFP	MKY36	*MATα nup84Δ::kanmx nup60-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1his3-11,15*
nup84Δ Nup100-GFP	MKY56	*MATα nup84Δ::kanmx nup100-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup84Δ Nup2-GFP	MKY88	*MATα nup84Δ::kanmx nup2-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup1Δct	MKY16	*MATα nup1Δct::kanmx leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup1Δct Nup60-GFP	MKY32	*MATα nup1Δct::kanmx nup60-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup1Δct Nup84-GFP	MKY42	*MATα nup1Δct::kanmx nup84-gfp-his leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup1Δct Nup100-GFP	MKY52	*MATα nup1Δct::kanmx nup100-gfp-his30 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup1Δct Nup2-GFP	MKY84	*MATα nup1Δct::kanmx nup2-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup120Δ	MKY18	*MATα nup120Δ::kanmx leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
nup120Δ Nup1-GFP	MKY28	*MATα nup120Δ::kanmx nup1-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup120Δ Nup60-GFP	MKY38	*MATα nup120Δ::kanmx nup60-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ADE2 his3-11,15*
nup120Δ Nup84-GFP	MKY48	*MATα nup120Δ::kanmx nup84-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup120Δ Nup2-GFP	MKY90	*MATα nup120Δ::kanmx nup2-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*
nup133Δ	MKY20	*MATα nup133Δ::kanmx leu2-3,112 trp1-1 can1-100 ura3-1his3-11,15*
nup133Δ Nup84-GFP	MKY50	*MATα nup133Δ::kanmx nup84-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1his3-11,15*
nup133Δ Nup100-GFP	MKY59	*MAT**a** nup133Δ::kanmx nup100-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1his3-11,15*
Nup1-GFP	MKY65	*MATα nup1-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
Nup60-GFP	MKY66	*MATα nup60-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*

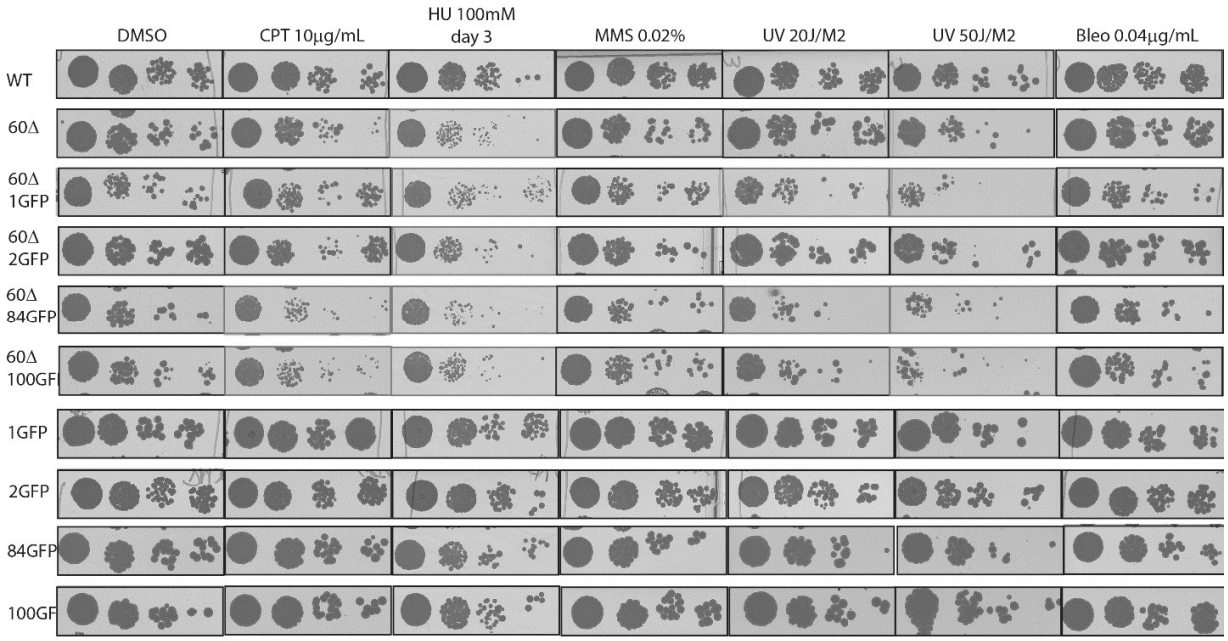
Nup84-GFP	MKY68	*MATα nup84-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
Nup100-GFP	MKY67	*MATα nup100-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*
Nup2-GFP	MKY81	*MATα nup2-gfp-his3 leu2-3,112 trp1-1 can1-100 ura3-1 his3-11,15*

(*) = Has not been genotyped yet.

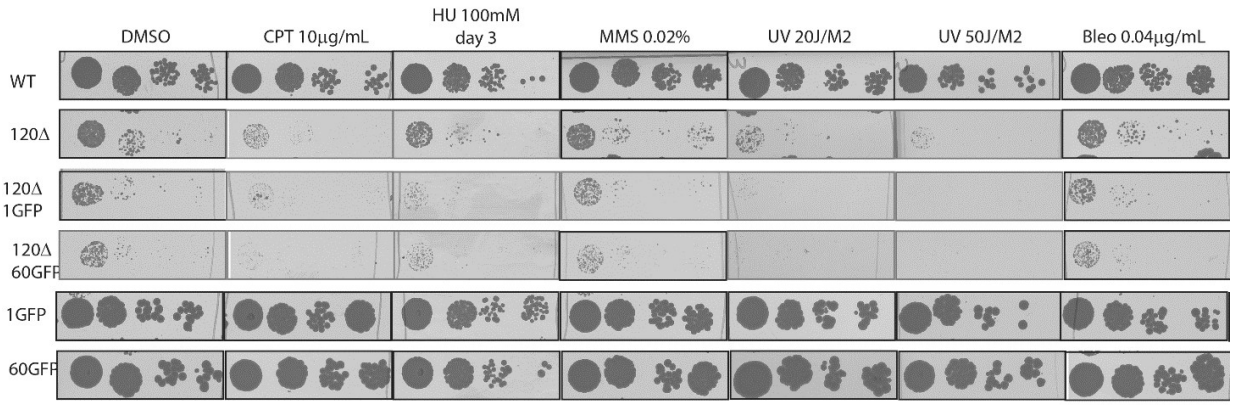
DNA Sensitivity assays



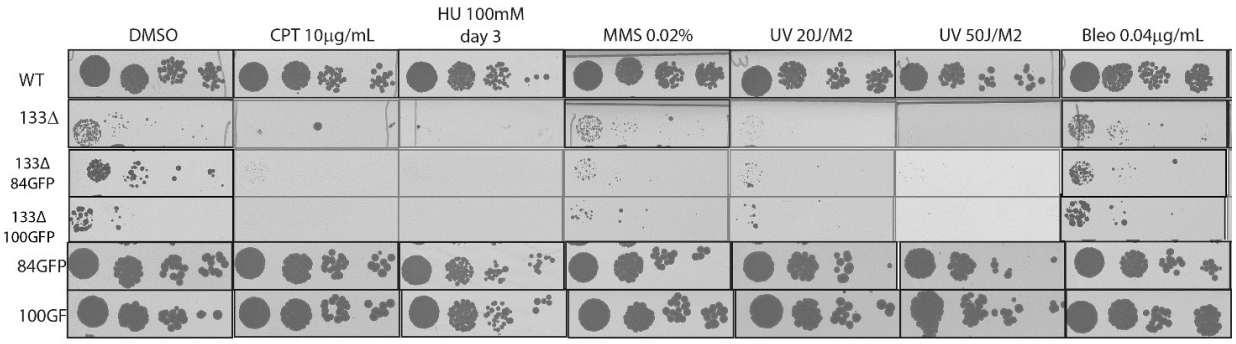
Supplementary 1. *DNA Damage sensitivity assay for Nup1Δct and GFP-tagged nups using a 10-fold dilution series in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days, 3 for HU.*



Supplementary 2. *DNA Damage sensitivity assay for Nup60Δ and GFP-tagged nups using a 10-fold dilution series in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days, 3 for HU.*



Supplementary 3. *DNA Damage sensitivity assay for Nup120Δ and GFP-tagged nups using a 10-fold dilution series in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days, 3 for HU.*



Supplementary 4. *DNA Damage sensitivity assay for Nup133Δ and GFP-tagged nups using a 10-fold dilution series in W303 background strain. Cells were spotted with different mutagenic treatments and agents at 30°C for 2 days, 3 for HU.*