nature structural & molecular biology

Article

https://doi.org/10.1038/s41594-024-01286-7

DNA double-strand break–capturing nuclear envelope tubules drive DNA repair

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Supplementary Discussion

Our findings support a model where upon the induction of DNA damage, the DDR signaling kinases intersect with ATAT1-dependent microtubule acetylation (Fig. 6). The modified microtubules cooperate with the plus-end-directed kinesins KIF5B and KIF13B and nuclear envelope factors such as LINC and NPCs. This allows the cytoplasmic motors and microtubules to push onto the nuclear envelope, driving the formation of a network of nucleus-infiltrating tubular invaginations, or dsbNETs. The latter associate with DSBs away from or onto nuclear actin filaments, which partly promote dsbNETs-DSB association and partially stabilize the tubules. The perinuclear anchoring circadian factor PER1 also partly supports tubule formation. Overall, the dsbNETs are transient, and their reversal is mediated by the microtubule minus-end-directed kinesin-14 KIFC3. The dsbNETs can bring the nuclear envelope with its resident proteins to DSBs throughout the nucleus, promoting the sequestration of DSB ends within repair centers, optimizing repair protein engagement, and facilitating the reconnection of break ends to each other.

Our model is consistent with the ability of the human nuclear envelope to promote NHEJ repair[22,23] and resolve replication stress[84]. For instance, the transmembrane nuclease NUMEN, which specifically promotes NHEJ and limits HR, is enriched at the nuclear boundary and tubule-like lamin signals[23]. Also, evolutionarily conserved factors linked to the SUMOylation of proteins at the yeast nuclear envelope during repair via different pathways[2,7,50,85,86] are enriched at the nuclear envelope and localize to and promote the repair of DSBs throughout the human nucleus[87-93]. For example, yeast KU70 SUMOylation and function is regulated by the SUMO protease Ulp1, which is enriched at the Nup84 and Nup60 NPC factors[86,87]. An equivalent human SUMO protease, SENP2, localizes to the envelope via interaction with NUP153 and promotes NHEJ and HR repair[24-26]. The function of human XRCC4 downstream of KU70 during NHEJ repair is also linked to SUMOylation[88,89]. Also, the non-perinuclear-enriched RNF4, the human equivalent of the yeast DNA repair-promoting and NPC-associated Slx5/8 SUMO-targeted ubiquitin ligase, is recruited to DSBs and promotes NHEJ and HR throughout the nucleus[90-93]. Jointly, our and these studies show that the human nuclear envelope and its proteome mediate NHEJ and HR.

Various lines of evidence support the significance of dsbNETs to DSB repair and genome stability. Super-resolution three-dimensional imaging showed the preferred localization of DSBs at the nuclear envelope tubules as compared to the nuclear boundary (Fig. 1f,h; Fig. 3a,e) and live-cell imaging indicated the faster resolution of tubule-associated DSBs (Extended Data Fig. 2c). The disruption of dsbNETs increased the number and size of 53BP1 foci in three-dimensionally imaged nuclei and total intensity of 53BP1 and γH2AX signals in dsbNETs-deficient cells (Fig. 1f,g; Fig. 2b,d-g,j; and Extended Data Figs. 2e, 4l, 5e-g, and 9j). Abrogating dsbNETs resulted in slower repair kinetics as assessed by both the relative rate of 53BP1 foci resolution and the detection of DSB ends using neutral comet assays (Extended Data Fig. 5d-g) and chromosomal DSB repair efficiency assays specifically assessing HR and NHEJ (Extended Data Fig. 8k,l). Imaging showed the increased splitting of DSB ends, that DSB ends tethering can be rescued in dsbNETs-deficient cells by enlarging the DNA repair centre (Fig. 4g-i; Extended Data Fig. 8f-h), and that knockdown of DSB ends-tethering factors NBS1 or RAD50 phenocopies the DSB ends splitting phenotype observed in dsbNETs-deficient cells (Fig. 4h; Extended Data Fig. 8c). We observed decreased localization of DNA repair factors to DSB ends in dsbNETs-deficient cells and the rescue of such localization upon enlarging the DNA repair center (Fig. 4g; Extended Data Fig. 8d-h). Microtubule acetylation and dsbNETs showed dependence on the DNA damage response kinases ATM, ATR, and DNAPKcs (Figs. 1i, 2h-j; Extended Data Fig. 4m) and dsbNETs were induced upon interfering with endogenous DSB repair via knockdown of the DSB ends-

tethering factors RAD50 or NBS1 (Extended Data Fig. 2k). BRCA1/HR-deficient cells more readily induced dsbNETs (Fig. 5e-g). Gene expression profiling showed the positive correlation of the dsbNETs-promoting KIF5B and the negative correlation of the dsbNETs-reversing KIFC3 with DNA repair gene expression, and HR and especially NHEJ signatures (Fig. 5a,c-d; Extended Data Fig. 9a-c). We also observed genetic interactions between *BRCA1* and genes encoding dsbNETs regulators, that dsbNETs promotion drives chromosomal defects in BRCA1-deficient cells treated with the PARPi olaparib, and that KIF5B knockdown confers resistance to olaparib (Fig. 5h-k; Extended Data Figs. 9i,j and 10a-e). Finally, expression of the premature aging-linked Progerin induces DSBs and dsbNETs while also exerting additive effects with etoposide treatment with respect to both DSB buildups and dsbNETs induction (Extended Data Fig. 10f).

In etoposide-treated cells, more than half of DSBs localized to dsbNETs throughout the nucleus and DSBs showed a bias toward association with the tubules compared to the nuclear boundary. In the context of the FokI-DSB, the possibility that the single DSB is preferentially located away from the nuclear edge due to lower induction of DSBs at perinuclear heterochromatin was ruled out by data indicating that de-compacting chromatin using the histone deacetylase inhibitor SAHA does not decrease, but in fact increases, the distance between the DSB and nuclear edge (Extended Data Fig. 6j). So, the data indicate that the FokI-DSB, similar to the drug-induced DSBs, is more likely to associate with the nuclear envelope at the tubules than at the nuclear edge.

We quantified dsbNETs via the tubular score from reconstructed nuclei, machine learning-based tubular scores from imaging stacks, percent tubules-positive cells, tubular width, and tubular bodies crossing the nuclear midplane. Damage-dependent induction of tubules-positive cells was ~2.8 fold in osteosarcoma cells and ~1.7 fold in TNBC cells, with full dependence on key regulators. Due to the tubules' interconnectedness in etoposide-treated cells, we could not determine the precise total number of individual tubules. Also, the DSBs and tubules were highly dynamic. So, we could not image or reconstruct the full network of dsbNETs in single nuclei in vivo as we did for single nuclei in fixed cells. Moreover, the tubules, especially with a single damaged locus, collectively occupy a volume that constitutes a small fraction of the total nuclear volume (Fig. 3a). So, it is experimentally unfeasible to catch a single damaged locus co-localizing with a tubule in all cells at the specific time point at which the samples were imaged.

Although dsbNETs depended on NUP153 and NUP50 but not NUP98, future research should assess the extent to which NPCs may still partly operate via nuclear-cytoplasmic trafficking to promote repair. Also, ~20-35% of unchallenged cells were tubules-positive across our study, though they did not significantly increase the tubular score (such as in Fig. 1b,c). This baseline of tubules-positive cells represents less extensive tubules unrelated to DNA damage and preferentially and constitutively connected with nucleoli. The potential function of this baseline is outside of the scope of our study. As outlined above, we have detected dsbNETs and assessed their impact on DNA repair via numerous approaches. DsbNETs efficiently formed in G_1 and S/G_2 cells, colocalized readily with 53BP1 foci, and at least to some extent with RAD51 foci, and the disruption of dsbNETs compromised NHEJ and HR repair of chromosomal DSBs. HR/BRCA1-deficient cells further induced dsbNETs and showed higher sensitivity to their disruption. Also, in PARPi-treated HR-deficient cells, KIF5B promoted chromosome fusions, a process operating via SUN1, SUN2, and microtubule-dependent NHEJ[15,55]. In addition, KIF5B knockdown increased the ability of *BRCA1*-mutant cells to resist olaparib. Future research may benefit from further assessing the potential role of dsbNETs in DNA repair pathway choice using different breaks and chromosomal contexts as repair progresses over time. Nonetheless, our findings indicate that dsbNETs can promote NHEJ and HR repair within diverse biological and health-related settings.

Supplementary Table 1. TCGA cancer types studied.

Abbreviation	Name
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CLLE	Chronic lymphocytic leukemia
COAD	Colon adenocarcinoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
HNSC	Head and Neck squamous cell carcinoma
KIRC	Kidney renal clear cell carcinoma
LAML	Acute Myeloid Leukemia
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MALY	Malignant lymphoma
NBL	Neuroblastoma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PRAD	Prostate adenocarcinoma
RT	Retinoblastoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid carcinoma
UCEC	Uterine Corpus Endometrial Carcinoma
WT	Wilm's tumor

Supplementary Table 2. Antibodies used in this study.

Antibodies	Concentration	Company	Catalogue #	Application
KU70	1 mg/ml	Abcam	Ab83501	IF, WB
RAD51	1 mg/ml	Abcam	Ab133534	IF
BRCA1	2 mg/ml	Millipore	OP92	IF, WB
γH2AX	1 mg/ml	Abcam	Ab11174	IF
	1 mg/ml	Abcam	Ab22551	WB
SUN1	1 mg/ml	ProteinTech	24568-I-AP	IF, WB, IP
SUN2	1 mg/ml	Millipore	MABT880	IF, WB
SYNE1	0.6 mg/ml	Sigma Prestige	HPA019113-25UL	IF
NUP98	1 mg/ml	Invitrogen	MA5-14907	IF, WB
NUP153	0.2 mg/ml	Bethyl	A301-788A	IF, WB
LMNB1	1 mg/ml	Abcam	Ab16048	IF, WB, IP
	1 mg/ml	ProteinTech	66095-1-lg	IF
LMNA/C	7 mg/ml	Cell Signaling	4777	IF
KIF5B	1 mg/ml	Abcam	Ab167429	IF, WB
KIFC3	0.1 mg/ml	Thermo Fisher	PA5-54359	IF, WB
β-actin	Not provided	Thermo Fisher	AM4302, MA1-744	IF, WB
NUP98	1 mg/ml	Invitrogen	MA5-14907	IF, WB
NUP153	0.2 mg/ml	Bethyl	A301-788A	IF, WB
53BP1	1 mg/ml	Abcam	Ab36823	IF, WB
	1 mg/ml	Bethyl	A300-272A	IF, WB
	1 mg/ml	Millipore	MAB3802	IF
RNF8	Not provided	Santa Cruz	Sc-271462	IF, WB
RAD50	Not provided	Novus	NB100-1487	IF, WB
NBS1	2 mg/ml	Bethyl	A301-290A	IF, WB
Acetylated α-tubulin	317 µg/ml	Cell Signaling	5335S	IF
DNAPK-p2056	0.448 mg/ml	Abcam	ab124918	WB
phospho-Chk1 S345	141 µg/ml	Cell Signaling	2341S	WB
phospho-Chk2 T68	0.2 mg/ml	R&D Systems	AF1626	WB
Vinculin	Not provided	Millipore	V9131	WB
Phosphoserine	Not provided	Millipore	AB1603	WB
Ubiquitin	200 µg/ml	Santa Cruz	Sc-8017	WB
Alexa-488 anti-mouse	2 mg/ml	Invitrogen	A11001	IF
Alexa-488 anti-rabbit	2 mg/ml	Invitrogen	A11008	IF
Alexa-568 anti-mouse	2 mg/ml	Invitrogen	A11004	IF
Alexa-568 anti-rabbit	2 mg/ml	Invitrogen	A11011	IF
Alexa-647 anti-mouse	2 mg/ml	Invitrogen	A21235	IF
Phalloidin-iFluor reagent 488	Not provided	Abcam	76753	IF
anti-rabbit IgG	1 mg/ml	Abcam	Ab171870	IP
HRP-mouse-IgG	Not provided	Amersham	NA931-1ML	WB
HRP-rabbit-IgG	Not provided	Amersham	NA934-1ML	WB
α-Tubulin	1 mg/ml	Abcam	ab7291	WB
XRCC4	0.2 mg/ml	Santa Cruz	sc-271087	WB, ChIP

Supplementary Table 3. sgRNAs used in this study.

sgRNAs	Sequence (5' → 3')
sgRNA1	AUGGACGAGCUGUACAAGUC
sgRNA2	CAAGAUCCGCCACAACAUCG
sgRNA3	GUCGCCCUCGAACUUCACCU
Non-targeting control	AAAUGUGAGAUCAGAGUAAU

Supplementary Table 4. siRNA and shRNA sequences used in this study

Target	Type	Sense Sequence (5' → 3')	Anti-sense Sequence (5'→3')
SUN1	siRNA	GUGUUGAACUGGGCAAGCAtt	UGCUUGCCCAGUUCAACACgg
SUN2	siRNA	CGUAUGGUGCUUGGUAUUUtt	AAAUACCAAGCACCAUACGtc
KIFC3	siRNA	CCAAUGCUGUGACUUUCGAtt	UCGAAAGUCACAGCAUUGGtg
KIF5B	siRNA	CCAAUGCUGUGACUUUCGAtt	AUAACUCCAAUUGCGGUUct
NUP98	siRNA	GGAUUGUUUGGAACCAGUUtt	AAGUGGUUCCAAACAAUCCtc
NUP153	siRNA	CUUUAUUUUCUGGCCAGCGtg	CUUUAUUUUCUGGCCAGCGtg
RNF8	siRNA	GAGGAUUUGGUGUCACAUAtt	UAUGUGACACCAAAUCCUCgt
RAD50	siRNA	GGCCUUUAAGUGAAGGAAAtt	UUUCCUUCACUUAAAGGCCaa
NBS1 (NBN)	siRNA	GGAAAAACUGUGCCAUUCUtt	AGAAUGGCACAGUUUUUCCtt
BRCA1	siRNA	CAACAUGCCCACAGAUCAA CCAAAGCGAGCAAGAGAAU UGAUAAAGCUCCAGCAGGA GAAGGAGCUUUCAUCAUUC	Not applicable
ATAT1 (C6orf134)	siRNA	GGCUCAUAAUGAGGUAGAAtt	UUCUACCUCAUUAUGAGCCtc
KIF13B	siRNA	GGAUGAUGCUGACCGUGAAtt	UUCACGGUCAGCAUCAUCCtc
PER1	siRNA	AGCGCGUCAUGAUGACCUAtt	UAGGUCAUCAUGACGCGCUgg
NUP50	siRNA	GAAGGACUGUCGAAUGGAAtt	UUCCAUUCGACAGUCCUUCca
CTL	siRNA	Silencer™ Select Negative Control No. 1 siRNA (Cat# 4390843, Ambion) / For BRCA1 experiment: UGGUUUACAUGUCGACUAA UGGUUUACAUGUUGUGUGA UGGUUUACAUGUUUUCUGA UGGUUUACAUGUUUUCCUA	Not applicable
KIFC3 (shRNA)	shRNA	TGAAGGCTGTGCACGAGAATC	GATTCTCGTGCACAGCCTTCA
KIF5B (shRNA)	shRNA	CAACCGCAATTGGAGTTATAG	CTATAACTCCAATTGCGGTTG

Supplementary Table 5. FokI-DSB-related primers used in this study.

Primer	Forward (5'→3')	Reverse (5'→3')
1	GCTGGTGTGGCCAATGC	TGGCAGAGGGAAAAAGATCTCA
2	GGCATTTCAGTCAGTTGCTCAA	TTGGCCGATTCATTAATGCA
3	CCACCTGACGTCTAAGAAACCAT	GATCCCTCGAGGACGAAAGG

Supplementary Table 6. RT-qPCR primers used in this study.

Primer	Sequence (5'→3')
ATAT1 FWD	CAATAACCTTAAGGGAGGAG
ATAT1 RVS	GATAGGAGCGGAAAGATTCT
KIF13B FWD	CTGACTTGCATACCAAATGT
KIF13B RVS	ATCATAAGCAAACACCTTCG
PER1 FWD	ACTCAGAAGGAACTCATGAC
PER1 RVS	TGCTGGTAGTATTCCTGGTT
Vinculin FWD	CAACTCACCTCTTGGGATGAAG
Vinculin RVS	CCTGGTTCAGTTTGGAGTCTATG

Supplementary Table 7. Plasmids used in this study.

Plasmid	Source
GFP-Lamin B1	Kind gift from J. Lammerding
EB1-tdTomato	Addgene cat# 50825
RFP-LMNB1	Kind gift from J. Lammerding
GFP-53BP1	Kind gift from D. Durocher
KIF5B-YFP	Kind gift from S. Linder/C. Gu
KIF5B-T92N-YFP	Kind gift from S. Linder/C. Gu
dCas9-GFP	This study
KU70-GFP	This study
KU70-GFP-SUN1ΔN	This study
GFP-SUN1	This study
GFP-SUN1ΔN	This study
NLS-Actin	Addgene cat# 118380
NLS-Actin-R62D	Addgene cat# 118381
Lamin A minigene	Addgene cat# 20291
G606G:GGC>GGT Lamin A minigene	Addgene cat# 20292
Tet-pLKO-puro	Addgene cat# 21915
pLKO.1 Puro shRNA scramble	Addgene cat# 162011
psPAX-2	Addgene cat# 12260
pMD2.G	Addgene cat# 12259
pCBASceI	Addgene cat# 26477
pcDNA3-mRFP	Addgene cat# 13032

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