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The C-terminus of Nej1 is critical for nuclear localization and non-homologous end-joining

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Abstract

Nej1 is an essential factor in the non-homologous end-joining (NHEJ) pathway and interacts with the DNA ligase complex, Lif1-Dnl4, through interactions with Lif1. We have mapped K331-V338 in the C-terminal region of Nej1 to be critical for its functionality during repair. Truncation and alanine scanning mutagenesis have been used to identify a motif in Nej1, KKRK (331–334), which is important for both nuclear targeting and NHEJ repair after localization. We have identified F335-V338 to be important for proper interaction with Lif1, however this region is not required for Nej1 recruitment to HO endonuclease-induced DNA double-strand breaks in vivo. Phenylalanine at position 335 is particularly important for the role of Nej1 in repair and the loss of association between Nej1 and Lif1 correlates with a decrease in cell survival upon either transient or continuous HO expression in *nej1* mutants.

Keywords

DNA repair; Nej1p; Lif1p; Non-homologous end-joining; double-strand breaks

1. Introduction

In *Saccharomyces cerevisiae*, DSBs can be repaired by homologous recombination (HR), or by non-homologous end-joining (NHEJ). NHEJ is conserved in yeast and mammals and requires the core components: Yku70/80 (Ku70/80), Lif1-Dnl4 (XRCC4-DNA ligase IV) and Nej1 (XLF), as well as the Mre11/Rad50/Xrs2 (MRX) complex in yeast (reviewed in [1]). The initial step in the NHEJ pathway is the localization and binding of the Yku70/80 heterodimer to the DNA double-strand ends. Yku70/80 is crucial for recruitment of additional NHEJ factors to breaks, including the Lif1-Dnl4 complex and Nej1 [2–4].

Lif1-Dnl4 ligates DNA ends [4–8] together and interacts with Nej1 [9–11]. Nej1 stimulates Dnl4 activity [4] and this effect is presumably mediated by the interaction of Nej1 with Lif1, as Nej1 does not directly interact with Dnl4 [9]. The Nej1-Lif1 interaction has been mapped

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to the N-terminal region of Lif1 (1–157) and the C-terminal region of Nej1 (173–342) [9–11]. The C-terminus of Nej1 also binds DNA, which is important for NHEJ in vivo [12]. Lastly, in response to damage, Nej1 is phosphorylated on S297 and S298 by the Dun1 checkpoint kinase. Phosphorylation at S297/S298 does not affect the Nej1-Lif1 interaction, however it does regulate NHEJ and/or repair by single-strand annealing [13, 14].

Here, we demonstrate that the C-terminal region (CTR) of Nej1 between K330 and V338 is critical for Nej1 localization to the nucleus, for its interaction with Lif1 and for NHEJ repair in vivo. Following recruitment to the site of damage, residues F335-V338 in Nej1 mediate interactions with Lif1 that are critical for repair. The degree of interaction between Lif1 and Nej1, wild type and mutants, correlates with survival following DSB induction, strongly suggesting that Nej1 binding to Lif1-Dnl4 is central for NHEJ.

2. Materials and Methods

2.1 Yeast strains

Yeast strains used in this study are listed in Table 1.

2.2 Plasmids

A LacZ reporter plasmid, pSH18034, containing lexAgal1-lacZ, was used to monitor protein interactions quantitatively in yeast two-hybrid experiments. Prey vectors were created by the ligation of *NEJ1* into pJG4-6 and the bait plasmid was generated by the ligation of *LIF1* into pGAL-lexA [15]. Mutants were created by site-directed mutagenesis using Stratagene QuickChange™ mutagenesis. Nej1 integration plasmids were created by ligation of the Nej1 gene sequence along with its endogenous promoter and 3' untranslated region (UTR) into a pRS306-derived plasmid carrying a *URA3* marker. Integration plasmids containing the SV40 NLS were created by PCR of the Nej1 promoter with a reverse primer containing the SV40 NLS sequence. This fragment, a PCR product containing the ORF of Nej1, and a 13-Myc::TRP fragment, with complementary ends were ligated into pRS306 integration plasmid. The SV40 NLS nej1-1 was created by GenScript USA Inc (Piscataway, NJ). Wild type and mutants of Nej1 were reintegrated into the genome of JC-1342 and confirmed by DNA sequencing.

2.3 Media

For experiments with strains containing the galactose inducible HO-endonuclease, cells were grown in YPLGg media consisting of 1% yeast extract, 2% bactopectone, 2% lactic acid, 3% glycerol and 0.05% glucose. For yeast two-hybrid experiments cells were grown in standard amino acid dropout media lacking histidine, tryptophan and uracil (SC-His/-Trp/-Ura) and containing 2% raffinose as the carbon source.

2.4 Antibodies

Primary antibodies were anti-LexA (mouse; Santa Cruz, 2–12), anti-HA (mouse; Sigma, HA-7), anti-Myc (mouse; Santa Cruz, 9E10), and anti-actin (rabbit; Sigma, A2066). Secondary antibodies were coupled to horseradish peroxidase (Biorad) or to Alexa 594 (goat anti-mouse; Molecular Probes, Eugene) for immunofluorescence.

2.5 Western blotting

Whole cell extracts were prepared by silica bead beating in lysis buffer (50 mM Hepes, 140 mM NaCl, 1mM EDTA, 1% triton, 1 mM PMSF and protease inhibitor cocktail (Complete pellet, Roche)). For Nej1-Myc detection, cell debris was removed by centrifugation at 3000 rpm for 10 min at 4°C.

2.6 Indirect immunofluorescence

Overnight cultures were fixed with 3.7% formaldehyde for 1h at room temperature (RT). Cells were incubated in SK solution (0.1M KPO₄/1.2M sorbitol) containing zymolase (0.4 mg/mL; US Biological) at RT until spheroplasted. Spheroplasted cells were adhered to poly-lysine coated coverslips and treated as described in [16]. Coverslips were blocked with 1% BSA in phosphate buffered saline (PBS) for 30 min at RT and then incubated with primary antibody for 1h followed by a 30 min incubation secondary antibody. Coverslips were stained with 4', 6-diamidino-2-phenylindole (DAPI in 1 µg/mL of PBS) and mounted on slides with Vectashield (Vector Labs). Images were obtained on a Leica DMIRE2 fluorescent microscope at 100× magnification (Leica Microsystems Inc.).

2.7 HO endonuclease survival assays

Cell pellets from overnight cultures grown at 25°C in YPAD were washed and resuspended in water. Cells were plated on solid agar YPLG containing either 2% glucose or 2% galactose and incubated at RT for 3–4 days. In transient assays, HO-endonuclease expression was induced in exponentially growing cultures by the addition of galactose. Cultures were incubated for 3h at 30°C. Samples were plated on YPAD at time 0 and after 3h of induction. Plates were incubated at 30°C for 2 days. Survival was calculated relative to uninduced cells. All the survivors that were tested after transient induction were MAT α , indicating that their repair was precise.

2.8 Plasmid repair assays

Plasmid repair assays were performed with pRS414 as previously described [12–14]. Briefly, cells were transformed using the standard lithium acetate method with either 10 ng uncut plasmid to correct for transformation efficiency or 0.1 µg XhoI digested plasmid and plated on SC-TRP plates. Cells were grown at 30°C for 3 days. Survival was determined relative to cells expressing wild type Nej1.

2.9 Chromatin immunoprecipitation (ChIP) and Real-time PCR

ChIP was performed as described in [17] with the addition of a chromatin fractionation step following cell lysis at 13,000 rpm for 15 min. Chromatin pellets were resuspended in lysis buffer before sonication. Nej1-Myc was immunoprecipitated with anti-mouse Dynabeads (Invitrogen) uncoupled or coupled to anti-Myc antibody (mouse; Santa Cruz, 9E10) for 2h at 4°C. Following crosslink reversal, DNA was obtained by phenol/chloroform extraction and precipitated with ethanol. Quantitative real-time PCR was performed on an ABI 7900 sequence detector system. Reactions contained PerfeCTa qPCR supermix (Quanta Biosciences Inc.), primers and 5' FAM-labelled/3' TAMRA labelled probes specific to HO1 (1.6 kb away from cut site), HO2 (0.6 kb away from cut site), HO6 (at the cut site) or *SMC2*

(control). Enrichment was calculated relative to *SMC2* and corrected for cut efficiency, which was determined from the loss of PCR product produced at HO6 after 3h induction compared to time 0 as previously described in [17]

2.10 Yeast two-hybrid assays

Bait and prey plasmids, as well as the reporter plasmid were transformed into JC1280. Transformed cells were grown in SC-His⁻/Trp⁻/Ura media containing 2% raffinose overnight and used to inoculate fresh media containing either 2% glucose or 2% galactose. Cultures were grown for 6h at 30°C. β -galactosidase activity was measured in permeabilized cells from induced and uninduced cultures [18].

3. Results and Discussion

3.1 The C-terminus of Nej1

The C-terminus of Nej1 is highly conserved amongst family members (Figs. 1A and S1). To assess the importance of the last 12 amino acids of Nej1 for repair in a physiologically relevant setting, we determined survival upon HO endonuclease induction in a series of Nej1-13Myc epitope-tagged truncation mutants (Fig. 1B; [20]). Consistent with previous reports [12, 19, 21, 22], cells lacking *NEJ1* exhibited a marked decrease in cell survival (Fig. 1B). The survival of both Nej1-330-Myc and Nej1-334-Myc mutants was similar to *nej1* cells with a 50 to 100-fold reduction in survival. Nej1-335-Myc and Nej1-336-Myc mutants also exhibited reduced survival, albeit to a lesser degree, however survival of the Nej1-338-Myc truncation appeared similar to, or better than, wild type (Fig. 1B). The addition of a C-terminal Myc epitope did not impact the functionality of full-length Nej1 dramatically, as survival was only slightly reduced compared to the untagged strain (Fig. 1B).

The Nej1 truncation proteins expressed at similar levels to wild type ruling out defects attributed to a decrease in protein expression levels (Fig. 1C). Taken together, our data suggest residues between K331 and V338 are critically important for Nej1 function in NHEJ. As previously reported [13], Nej1-Myc displayed a phosphorylation dependent mobility shift (Figs. 1C and S2). With the exception of Nej1-330-Myc, all truncation mutants showed a dramatic shift in mobility that was indicative of phosphorylation. Although Nej1-330-Myc displayed a more subtle shift attributable to phosphorylation, which was only visible on 10% SDS-PAGE gels, its mobility was distinct from wild type and the other mutants (Fig. 1C and S2). It is possible that the Nej1-330-Myc truncation disrupts phosphorylation at S297 or S298, however the C-terminus may direct the phosphorylation of other unidentified site(s) as the mobility shift we detect appeared to be independent of Dun1 kinase and DNA damage, which have been shown previously to be important for phosphorylation at these sites (Fig. S2; [13]).

3.2 Nuclear localization and repair with Nej1 NLS mutants

The KKRK motif (331–334) of Nej1 binds DNA [12], however it is also a putative nuclear localization signal, thus we compared the subcellular localization pattern of Nej1 and Nej1-330 by indirect immunofluorescence (Fig. 2A). Full-length Nej1 was primarily nuclear and colocalized with DAPI, however the Nej1-330 truncation displayed a dramatically

different pattern, appearing mostly cytoplasmic (Fig. 2A). To artificially target Nej1-330 to the nucleus we generated a fusion with an SV40-NLS. Indeed, localization was restored as the resulting ^{NLS}Nej1-330 truncation looked indistinguishable from wild type (Fig. 2A). This indicated that the KKRK sequence contributes to Nej1 localization in the nucleus. The phosphorylation pattern of Nej1-330 and ^{NLS}Nej1-330 looked similar to each other and distinct from wild type (Fig. 2B), therefore targeting Nej1-330 to the nucleus did not seem restore wild type levels of phosphorylation. The influence of the C-terminal region on phosphorylation must be indirect as there are no serine, threonine or tyrosine residues present in the terminal amino acids that are removed in Nej1-330.

When Nej1-330 was targeted to the nucleus, as with ^{NLS}Nej1-330 (Fig. 2A), there was a slight increase in repair above the truncation alone, however survival remained markedly down (Fig. 2C). Additionally, when we targeted a Nej1 mutant KKRK/AAAA (331–334) to the nucleus by fusion with SV40-NLS, ^{NLS}Nej1-1 (Fig. 2A), we also observed that survival after damage remained down to the extent of *nej1* and the 1–330 truncation (Fig. 2C) even as phosphorylation was restored (Figs. 2B and E). This suggests that KKRK (331–334) is important for repair in addition to nuclear targeting [12], but that residues between 334 and 338 are also critical for NHEJ (Figs. 1B and 2C).

The continuous induction of the HO-endonuclease (as in Fig. 1B and 2C) allows for the measurement of imprecise NHEJ as cells are only able to survive and form colonies if they repair the cut site and introduce a mutation into the HO recognition site (or if a mutation is acquired that affects HO-endonuclease expression [24]). To investigate precise NHEJ in these mutants, we transiently induced the HO-endonuclease for 3h at which time we obtain over 95% cut efficiency (data not shown) before plating the cells on YPAD to repress endonuclease expression (Fig 2D; as in [25]). Under these conditions overall cell survival was about 100 times greater than with continuous induction (Fig. 2C and D). The addition of SV40 NLS to wild type Nej1, ^{NLS}Nej1, resulted in a slight enhancement in survival upon HO induction (Figs. 2C and D), however this might be a result of the protein expression level (Fig. 2B). In general, the same repair patterns during continuous induction were observed with the *nej1* mutants upon transient HO induction (Figs. 2C and D). The KKRK motif was previously shown to mediate DNA binding and be required for NHEJ [12] and our results substantiate its importance, but further indicate that this region also serves as a NLS.

Consistently, nuclear localization was restored in cells harboring the Nej1-334-Myc construct in which the KKRK motif was reintroduced (Figs. 3A, and B). In mammalian cells, the C-terminal 12 amino acids of the Nej1 homolog, XLF, are required for its recruitment to DSBs (Fig. S1; [23]). Thus, to determine whether the repair defect observed for Nej1-334-Myc could be explained by a lack of recruitment to DSBs, we performed ChIP on Nej1-Myc and Nej1-334-Myc at an HO endonuclease-induced DSB (Fig. 3C). After 3 hours of induction, Nej1-Myc and Nej1-334-Myc were similarly recruited to sites 0.6 kb (HO2) and 1.6 kb (HO1) away from the DSB (Fig. 3C). Taken together, our data confirms the importance of the Nej1-CTR for NHEJ in vivo, however, and in contrast to XLF, the CTR of Nej1 beyond 334 is not critical for recruitment to DSBs. This is consistent with the Yku70/80-Nej1 interaction being mediated by the N-terminal region of Nej1 [4].

3.3 Site-direct mutagenesis of Nej1

Although nuclear localization, phosphorylation, and recruitment to the site of damage were observed with Nej1-334-Myc (Figs. 1C and 3), this mutant still exhibited defects in NHEJ similar to that of *nej1* cells, while Nej1-338-Myc supported wild type levels of repair (Fig. 1B). To identify critical residues between F335 and V338 for Nej1 function in NHEJ repair we integrated Nej1 sequences, either wild type or alanine substitution, into the *URA3* locus of *nej1* cells. *NEJ1* reintegration restored NHEJ (Fig. 4A and B), however the *nej1* mutants, with alanine substitutions at F335, G336, K337 or V338, compromised survival to varying degrees. The most striking defects were seen with F335A and Nej1-2, FGKV/AAAA (335–338), as both mutants approached *nej1* levels of repair (Fig. 4A and B). The other alanine point mutants at positions G336-V338 displayed significant, but more moderate defects, at 3 to 5-fold below that of wild type NEJ1 upon continuous HO induction (Fig. 4A). During transient HO-endonuclease cutting, the G336A and K337A mutants displayed partial activity with survival at ~50% of wild type. Cells expressing F335A, V338A, or Nej1-2, displayed a repair defect similar to that of *nej1* cells with less than 30% survival relative to wild type (Fig. 4B). All alanine mutants were expressed and targeted to the nucleus, looking indistinguishable from wild type Nej1 (Figs. 4C and D). We note that overexpression of any one of the mutants in *nej1* cells partially rescued the loss of viability phenotype and resulted in survival above those levels observed when the mutants were expressed endogenously (Fig. S3A). This suggested the point mutants behaved as hypomorphic alleles rather than exhibiting a dominant negative effect.

To further verify the importance of F335-V338 in NHEJ we carried out in vivo plasmid repair assays. Compared to wild type, the G336A mutant showed a mild defect, however the K337A and V338A mutants repaired similarly to *NEJ1* (Fig. 4E). The F335A mutant exhibited a 3-fold reduction in end-joining repair (Fig. 4E), which was less severe than the “null-like” defect observed after HO induction (Fig. 4A and B). This allowed us to determine if the individual defects of the alanine mutants were additive. Similar to F335A, the *nej1-2* mutant retained some end-joining activity above a complete loss of *NEJ1* (Fig. 4E). In all, our results indicate that amino acids F335-V338 of Nej1 are individually important for NHEJ repair in vivo and that F335 is particularly important for Nej1 function.

3.4 Interaction between Lif1 and Nej1 mutants

The primary function of Nej1/XLF is thought to be the stimulation of DNA ligase activity through the interaction of Nej1/XLF with the Lif1/XRCC4-Dnl4/DNA ligase IV complex [4,27]. Mutations in XLF that disrupt XRCC4 binding have been shown to impair NHEJ in vitro and in vivo [28, 30, 31]. However, in yeast the importance of Nej1-Lif1 interactions for repair has not been fully characterized. We reasoned that defects observed with the alanine-scanning mutants were likely subsequent to Nej1 targeting because the Nej1-334-Myc truncation was still recruited to the break site (Fig. 3B). Previously yeast two-hybrid analysis was performed that mapped the interaction between Nej1 and Lif1 to be between the C-terminus of Nej1 and the N-terminus of Lif1 [9, 10, 11], thus we utilized this approach for consistency and to obtain quantitative measurements for interactions between Lif1 and Nej1, wild type and mutants.

Wild type Nej1 or alanine point mutants were expressed as HA-tagged prey and LexA-tagged Lif1, was the bait. All Nej1 proteins expressed at similar levels (Fig. S3B) and exhibited interaction with Lif1, however none interacted to the level of wild type Nej1 (Fig. 4F). G336A and K337A displayed a 20–40% decrease in their interactions with Lif1 while the F335A and V338A mutants displayed the most dramatic defects with an ~80% reduction (Fig. 4F). The level of Lif1 interaction with the different Nej1 mutants correlated to the level of survival observed for cells expressing these mutants following HO-endonuclease induction (Figs. 4A and B). Although we cannot rule out additional functionality for the F335-V388 region of Nej1 during repair, one primary function appears to be in mediating interaction with Lif1.

3.5 Conclusions

In conclusion, Nej1 and its mammalian homolog XLF are required for efficient NHEJ both in vitro and in vivo (reviewed in [1, 32]). Here we define residues in the C-terminus of Nej1 that are critical for nuclear localization, interaction with its binding partner, Lif1, and NHEJ. Similar to the interaction between XLF and XRCC4, our work supports the hypothesis that Nej1 binding to Lif1 is essential for efficient NHEJ in vivo. While the N-terminal regions of XLF and XRCC4 interact, it is the CTR of Nej1 that interacts with Lif1. Thus, our results help explain the difference between the CTR of mammalian XLF, which is not necessary for NHEJ or V(D)J recombination in vivo [33], and the CTR of yeast Nej1, which is critical for NHEJ-mediated repair.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CTR	C-terminal region
DAPI	4',6-diamidino-2-phenylindole
DSBs	double-strand breaks
NHEJ	non-homologous end-joining
PBS	phosphate buffered saline
UTR	untranslated region

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Highlights

- Nej1-CTR is essential for NHEJ-mediated DNA repair
- Residues K331-K334 serve as a nuclear localization signal in Nej1
- F335 is critical for interactions with Lif1 and NHEJ

Fig. 1. The C-terminal region of Nej1 is required for Nej1 function

(A) The C-terminus of Nej1 (amino acids 331–342) with the products of *nej1-1* and *nej1-2* mutants indicated. (B) Survival upon continuous expression of the HO-endonuclease in wild type (JC-727), *nej1* cells (JC-1342), *nej1*-Myc (JC-1687), *nej1*-330-Myc (JC-1691), *nej1*-334-Myc (JC-2505), *nej1*-335-Myc (JC-2530), *nej1*-336-Myc (JC-2669), or *nej1*-338-Myc (JC-2668) cells. Survival was determined by colony number on galactose relative to uninduced cells plated on glucose. Error bars represent the standard deviation of at least 3 independent experiments. (C) Western blot analysis from a 12% SDS-PAGE gel with an

anti-Myc antibody to detect Nej1 was performed on the indicated strains with a non-specific band serving as a loading control.

Fig. 2. Nej1 KKRK serves as an NLS and is critical for DNA repair
(A) Indirect immunofluorescence was performed using an anti-Myc antibody to detect Nej1 and DNA was stained with DAPI. (B) Western blot analysis from a 10% SDS-PAGE gel with an anti-Myc antibody was performed to detect Nej1 with actin serving as a loading control. (C) Survival as described in Fig. 1B upon continuous induction of the HO endonuclease or (D) following transient induction of the HO endonuclease where strains were plated on YPAD after a 3h incubation in 2% galactose. Error bars indicate the standard deviation of 3 independent experiments. Values shown indicate percent survival relative to *NEJ1*. Strains used are Nej1-Myc (JC-1687), Nej1-330-Myc (JC-1691) and Myc-tagged mutants integrated at the *URA3* locus of *nej1* cells including, Nej1-Myc (JC-3209), SV40-

NLS-Nej1-Myc (JC-3193), SV40-NLS-Nej1-330-Myc (JC-3194), and SV40-NLS-Nej1-1-Myc (JC-3215). (E) Phenotypic summary of wild type and NLS mutants for nuclear localization in Fig. 2A, reduced mobility/phosphorylation in Fig. 2B, and survival as a measure of repair in Fig. 2C.

Fig. 3. The C-terminal region of Nej1 beyond K334 is not required for Nej1 recruitment to DSBs (A) Indirect immunofluorescence was performed using an anti-Myc antibody on wild type (JC-727), Nej1-Myc (JC-1687), and Nej1-330-Myc (JC-1691), Nej1-334-Myc (JC-2505), Nej1-335-Myc (JC-2530), Nej1-336-Myc (JC-2669), Nej1-338-Myc (JC-2668) truncation mutants. DNA was stained with DAPI. (B) Phenotypic summary of wild type and truncation mutants for nuclear localization in Fig. 3A, reduced mobility/phosphorylation in Fig. 1C, and survival as a measure of repair in Fig. 1B. (C) Recruitment of Nej1-Myc (JC-1687) and Nej1-334-Myc (JC-2505) to DSBs was determined in ChIP assays. Relative enrichment before (0h) and 3h after HO endonuclease induction was calculated as the PCR signal at HO1 (1.6kb away) or HO2 (0.6kb away) normalized to the signal at a control locus (*SMC2*) and corrected for cut efficiency. Error bars indicate the standard deviation of 3 independent experiments.

Fig. 4. Amino acids 335-338 of Nej1 are important for interactions with Lif1 and Nej1 function (A) Survival upon continuous induction of the HO endonuclease or (B) following transient induction of the HO endonuclease where strains were plated on YPAD after a 3h incubation in 2% galactose. Error bars indicate the standard deviation of 3 independent experiments. Values shown indicate percent survival relative to *NEJ1*. *NEJ1* (JC-2789), *nej1*-F335A (JC-2648), -G336A (JC-2651), -K337A (JC-2655), -V338A (JC-2659), or *nej1*-2 (JC-2844) driven by the *NEJ1* promoter, were integrated at the *URA3* locus in *nej1* cells (JC-1342). (C) Indirect immunofluorescence was performed using an anti-Myc antibody on Nej1-F335A-Myc (JC-3133), Nej1-G336A-Myc (JC-3149), Nej1-K337A-Myc (JC-3159), Nej1-V338A-Myc (JC-3160), and Nej1-2-Myc (JC-3134). DNA was stained with DAPI. (D) Western blot analysis from a 10% SDS-PAGE gel with an anti-Myc antibody was performed to compare expression of the indicated Nej1 mutants with Nej1-Myc (JC-3209) with actin

serving as a loading control. (E) Plasmid repair assay with *nej1* cells (JC-1342) complemented with *NEJ1* (JC-2789), *nej1*-F335A (JC-2648), -G336A (JC-2651), -K337A (JC-2655), -V338A (JC-2659), or *nej1*-2 (JC-2844). Cells were transformed with undigested or XhoI (5' overhand, MCS) digested pRS414 before plating on SC-TRP. Error bars indicate standard deviation for three independent experiments. (F) Yeast two-hybrid analysis of the Nej1-Lif1 interaction where β -galactosidase activity was measured in strain JC-1280 transformed with vectors allowing for the expression of LexA-Lif1 as the bait and wild type Nej1 (Nej1), Nej1-F335A, -G336A, -K337A, -V338A or Nej1-2 (FGKV/AAAA) as the prey. Values shown are the average β -galactosidase measurements of three independent experiments and error bars represent the standard deviation.

Table 1

Yeast strains used in this study.

Strain	Genotype	Reference
JC-727	MATa; <i>hml::ADE hmr::ADE ade3::GAL-HO ade1 leu2-3, 112 lys5 trp1::hisG ura3-52</i>	JKM179, [34]
JC-1280	MATa <i>leu2::proLEU2-lexAop6 his3 trp1 ura3-52</i>	[35]
JC-1342	JC-727 with <i>nej1::KanMX6</i>	MAV015, [36]
JC-1687	JC-727 with <i>nej1-13Myc::trp1</i>	This study
JC-1691	JC-727 with <i>nej1-(1-330)-13Myc::TRP1</i>	This study
JC-2505	JC-727 with <i>nej1-(1-334)-13Myc::TRP1</i>	This study
JC-2530	JC-727 with <i>nej1-(1-335)-13Myc::TRP1</i>	This study
JC-2668	JC-727 with <i>nej1-(1-338)-13Myc::TRP1</i>	This study
JC-2669	JC-727 with <i>nej1-(1-336)-13Myc::TRP1</i>	This study
JC-2648	JC-1342 with <i>nej1 F335A::URA3</i>	This study
JC-2651	JC-1342 with <i>nej1 G336A::URA3</i>	This study
JC-2655	JC-1342 with <i>nej1 K337A::URA3</i>	This study
JC-2659	JC-1342 with <i>nej1 V338A::URA3</i>	This study
JC-2789	JC-1342 with <i>NEJ1::URA3</i>	This study
JC-2844	JC-1342 with <i>nej1-F335A/G336A/K337A/V338A::URA3</i>	This study
JC-3133	JC-2648 with <i>nej1 F335A-13Myc::TRP::URA3</i>	This study
JC-3134	JC-2844 with <i>nej1 F335A/G336A/K337A/V338A-13Myc::TRP::URA3</i>	This study
JC-3149	JC-2651 with <i>nej1 G336A-13Myc::TRP::URA3</i>	This study
JC-3159	JC-2655 with <i>nej1 K337A-13Myc::TRP::URA3</i>	This study
JC-3160	JC-2659 with <i>nej1 V338A-13Myc::TRP::URA3</i>	This study
JC-3193	JC-727 with <i>SV40-NLS-NEJ1-13Myc::TRP::URA3</i>	This study
JC-3194	JC-727 with <i>SV40-NLS-nej1-330-13Myc::TRP::URA3</i>	This study
JC-3209	JC-2789 with <i>NEJ1-13Myc::TRP::URA3</i>	This study
JC-3215	JC-727 with <i>SV40-NLS-nej1 K331A/K332A/R333A/K334A-13Myc::TRP::URA3</i>	This study

All strains except JC-1280 that was used for yeast two-hybrid analysis are derivatives of JKM179.