Repairing subtelomeric DSBs at the nuclear periphery

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Nuclear organization creates microenvironments favoring distinct nuclear functions. In budding yeast, silent chromatin regions such as telomeres are clustered at the nuclear periphery, creating zones of transcriptional repression. Recently, in the Journal of Cell Biology, Therizols et al. report that ‘telomere tethering at the nuclear periphery is essential for DNA double strand break repair in subtelomeric regions’. Here, we discuss these results and their functional implications.

Introduction

In yeast, flies and humans, the protective ends of chromosomes, known as telomeres, nucleate the formation of an altered chromatin structure that represses transcription of adjacent RNA pol II genes in a heritable fashion (termed telomere position effect (TPE)) [1]. In budding yeast, the 32 telomeres cluster at the nuclear periphery in four to five groups, forming discrete focal compartments that sequester the silencing factors Sir2, Sir3 and Sir4 [1]. These histone-binding proteins mediate repression and are recruited to telomeres by protein–protein interactions. Notably, the yKu heterodimer, which has a conserved function in non homologous-end joining (NHEJ) as well as protecting native chromosome ends [2], cooperates with the telomere-repeat binding protein Rap1 to nucleate Sir complex binding. The Sir complex can then spread along the chromatin fiber through interactions with histone tails, leading to the variegated and heritable repression of telomere proximal genes [1].

Although we know many molecular details about TPE, the mechanisms that anchor telomeres are not yet completely elucidated. Sir4 and the yKu heterodimer, which bind chromatin and DNA, respectively [3], are necessary for telomere anchoring (Figure 1). But because yeast has no nuclear lamina homologue and because neither yKu nor the Sir proteins have membrane spanning domains, nuclear envelope (NE) components must also be implicated in yeast telomere tethering. One of these is the enhancer of silent chromatin 1 (Esc1), which is found on the nucleoplasmic surface of the inner bilayer of the NE, primarily between pores [3]. Esc1 interacts directly with Sir4. Many other mutations have been identified in yeast that result in telomere delocalization from the NE, although these often act indirectly by influencing the binding or abundance of Sir4 or yKu.

Telomere anchoring and TPE are sensitive to mutations in the Nup84 nuclear pore subcomplex

Earlier work suggested that components of the nuclear pore complex (NPC) are involved in telomere anchoring, although these results were not generally reproducible [4]. Therizols et al. [5] now investigated the role of the Nup84 nuclear pore subcomplex (Box 1) in telomere anchoring. The authors show that by deleting one of the components of the Nup84 complex, telomere XIL is clearly delocalized from the nuclear periphery and the silencing of a reporter gene inserted on the XIL subtelomeric region is reduced (Table 1). Both the anchoring and TPE defects could stem from an impaired recruitment of the Sir proteins, because this telomere is completely delocalized in a sir4 mutant. Accordingly, they find that Sir3–GFP is partially delocalized from telomeric foci in the nup145C mutant, a truncation that eliminates an essential component of the Nup84 complex [5].

These results suggest that some telomeres could interact directly with pores, although several lines of evidence suggest that pores are not universally required for telomere anchoring. Fluorescence imaging can clearly distinguish nuclear pores from telomeric foci in wild-type cells [3] and telomeres remain evenly distributed along the NE in a nup133A mutant, despite a clustering of nuclear pores [4].

Nup84 complex is required for efficient DSB repair in subtelomeric regions

The efficiency of double strand break (DSB) repair in haploid yeast correlates with the distance of the damaged site from the chromosome end (Box 2). To test whether this could be related to telomere position, Therizols et al. [5] analyzed survival rates after induction of a DSB in cells with mutant Nup84 complexes, in which telomeres were delocalized.

DSBs were generated at either a subtelomeric or an internal position along the left arm of chromosome XI (Figure 2). As previously reported, in a wild-type haploid strain most of the cells could not form colonies when the DSB was induced at internal sites, but survival rates increased 20-fold when the break was targeted to a subtelomeric zone [6]. This increase is due to repair events that involve either telomere addition or nonreciprocal recombination, events that cannot be tolerated at internal positions because they lead to the loss of essential genes (Figure 2).

Strikingly, mutants of the Nup84 complex specifically compromised survival when the breaks were subtelomeric, but not when they were located at a more...
unchanged by the.

of the cell to repair linearized plasmids by NHEJ was.

analysis of repair events in.

might influence efficient subtelomeric repair. However,

localization and silencing of telomere XIL, it was not clear

because mutations in the Nup84 complex affect both

localization and silencing of telomere XIL, it was not clear

which, if either, of these defects correlates with the

inefficient subtelomeric repair. To test whether telomere

anchoring is responsible for the repair defect, the authors

tested repair efficiency in an

mutant, indicating that all repair

events were equally affected in this mutant.

Importantly, homologous recombination functions

properly at internal chromosomal positions and the ability

to repair linearized plasmids by NHEJ was

unchanged by the

mutant. Therefore, one cannot exclude that

anchoring defects [3]. The rate of survival after the

induction of a subtelomeric DSB was reduced to half in

this mutant, arguing that proper telomere

localization. This indicates that there is no

linear correlation between the efficiency of telomere

anchoring and subtelomeric repair defects (Table 1), and

therefore suggests that the Nup84 complex has additional

roles in subtelomeric repair. Alternatively, subtelomeric

chromatin structure, which is more severely altered in the

Nup84 complex mutant than in the

esc1

mutant, could

have a role in repair.

Figure 1. Schematic representation of the dual pathways of anchoring mediated by Ku and an unknown protein (x) and Sir4-Esc1 at yeast telomeres: Rap1-bound Sir4 can

bring a telomere to the NE through interaction with either Ku or Esc1. Cryptic mating type loci, which are also silenced, associate with the nuclear periphery probably through

the same pathways. Adapted, with permission, from [3].

Box 1. Nup84 complex

The nuclear pore complex is a large protein assembly with an estimated mass of 60–125 MDa in vertebrates and 44–65 MDa in Saccharomyces cerevisiae. It is thought to be composed of <100 different proteins [18]. The Nup84 subcomplex is a well characterized building block of the NPC, which is evolutionary conserved. In budding yeast, it contains Nup84, Nup85, Nup120, Nup145-C (a natural COOH-terminal cleavage product of Nup145), Seh1 (Sec13 homologue 1) and a fraction of Sec13 (Table 1). This complex can be reconstituted in vitro and functions in nuclear mRNA export and NPC biogenesis [18].

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of Tel XIL at the nuclear periphery</th>
<th>Rate of cell survival</th>
<th>TPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central XI</td>
<td>Subtelo XIL</td>
<td>% FOAR</td>
</tr>
<tr>
<td>Wild type</td>
<td>65</td>
<td>0.16 ± 0.06</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>nup145C</td>
<td>53</td>
<td>0.19 ± 0.09</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>nup84Δ</td>
<td>48</td>
<td>0.12 ± 0.06</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>nup133Δ</td>
<td>45</td>
<td>ND</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>nup120Δ</td>
<td>45</td>
<td>ND</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>sir4Δ</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>nup170Δ</td>
<td>ND</td>
<td>0.17 ± 0.03</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>esc1Δ</td>
<td>48</td>
<td>ND</td>
<td>2 ± 0.5</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

% of cells in which the GFP tagged telomere XIL is found in the outermost of the nucleus corresponding to 50% of the total volume, for which the proportion expected for a randomly localization is 50%. Note that there is no linear correlation between deficiency in localization and rates of subtelomeric repair.

% of cells not expressing the URA3 (as measured by resistance to FOA) reporter gene inserted in telomere XIL subtelomeric region.

Below detection level [20].

Cited as minor defect [5].

NPC and DNA repair

How might the NPC be specifically involved in subtelo-

meric repair? Screens for genes affecting resistance to

DNA-damaging agents [10,11] identified Nup84 complex

mutations affecting repair, such as the nup145C

mutants, indicating that all repair

functions by affecting the localization or function of one of

nuclear pore proteins (see below), because the nuclear

envelope is visibly distorted in esc1 mutants [3,7].

The defect in subtelomeric repair is less striking in the

esc1Δ mutant than in the nup145C mutant [3,7], although the two mutations have similar effects on
telomere localization. This indicates that there is no

linear correlation between the efficiency of telomere

anchoring and subtelomeric repair defects (Table 1), and

therefore suggests that the Nup84 complex has additional

roles in subtelomeric repair. Alternatively, subtelomeric

chromatin structure, which is more severely altered in the

Nup84 complex mutant than in the esc1Δ mutant, could

have a role in repair.

Table 1. Summary of the phenotypes analyzed by Therizols

et al. [5]
Box 2. Repair mechanisms according to the position of DSBs

Repair mechanisms differ according to the position of the DSB on a chromosome [6]. The absolute frequency of NHEJ, which is the exclusive mode of repair that leads to survival of DSBs at internal chromosomal sites, remains constant along the chromosome (Figure 2). By contrast, other repair mechanisms, which generally involve the loss of the distal chromosomal fragment, occur in subtelomeric regions, leading to a reduction in the relative rates of NHEJ close to telomeres. Thus, the ability of a cell to survive is higher when the DSB occurs in subtelomeric regions because the alternative modes of repair cannot be tolerated at internal positions (Figure 2). Although it is not surprising that such events are only detected beyond the last essential gene, the observation that these repair events increase continually as the DSB gets closer to the telomere was unexpected. Chromatin structure, the association of specific factors with chromosome ends and telomere localization have been proposed to account for this phenomenon [6].

NHEJ is the predominant repair mechanism at subtelomere positions, involving the repair of DSBs within the telomere environment. This is consistent with the observation that the efficiency of NHEJ decreases specifically in subtelomeric regions [5]. Chromatin structure could be responsible for this apparent bias for certain types of repair. Indeed, the telomere binding protein Rap1 inhibits NHEJ between telomere ends [16]. Conditional Rap1 mutants, as well as other mutants affecting telomere chromatin structure (for example sir2Δ), would be important to test for effects on subtelomeric repair events. It is possible that anchoring to the nuclear periphery enables the cell to compensate for this ‘resistance’ to repair. In this model, NPC could serve as anchoring sites for DNA factors that favor repair of sequences located in close proximity. This could be tested by artificially targeting a chromatin domain that contains an inducible DSB to the nuclear periphery. Moreover, in this context, it is necessary to determine the location of DSBs during the process of repair by the use of in vivo imaging techniques.

Nuclear organization could have a role in homologous recombination during meiosis, as suggested by the universally conserved chromosome bouquet arrangement (within which telomeres cluster at the nuclear envelope) [17]. Whether this is related to the role of the nuclear periphery in the repair of subtelomeric DSBs in yeast remains to be investigated.

The exciting study by Therizols et al. [5] paves the way for experiments that will dissect the pathways underlying their observation that subtelomeric repair is specifically affected by mutations in the Nup84 complex. This might lead us to understand the importance of nuclear organization for DNA repair.

Acknowledgements

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