Molecular pathways of telomere maintenance

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DOI:
10.33612/diss.250234794

Appendix

Summary in English

My thesis aims to provide a better understanding of fundamental aspects of telomere biology. Telomeres are the physical ends of the linear eukaryotic chromosomes, protecting them against being recognised as double-stranded DNA breaks (DSBs) and preventing them from fusing with each other. Due to the progressive shortening of telomeric DNA during each round of DNA replication, telomeres eventually lose their protective capacity. This "end replication problem" is counteracted by a specialized reverse transcriptase called telomerase. Nevertheless, telomerase is not expressed at sufficient levels to prevent telomere attrition in most human somatic cells, which impacts two critical features of human health. On the one hand, progressive telomere shortening results in replicative senescence, which is a hallmark of ageing. In contrast, reactivation of telomerase is a characteristic in most cancer cells, allowing them to divide indefinitely. For these reasons, uncovering molecular details in different aspects of telomere biology is highly relevant.

Consequently, my work covers parts of three main features of telomere biology: telomere protection, the end replication problem, and telomere replication stress. Firstly, in terms of telomere protection, we investigated the role and consequences of telomeres depleted of the major protein associated with double-stranded telomeric DNA in budding yeast, Rap1. Our findings suggest that impairing Rap1 binding does not affect cell proliferation, but requires the presence of other components for the protection of the telomeres. These findings illustrate the flexibility and redundancy of telomere capping proteins and offer an explanation for the rapid evolution of telomeric sequences and telomere binding proteins in budding yeast species. In addition, the G-rich nature of the telomeres predicts the potential formation of secondary structures, such as G-quadruplexes (G4), which could contribute to telomere capping. Nevertheless, our data suggest that the ability to form telomeric G4s is not essential for telomere capping in *S. cerevisiae*. Secondly, to investigate how telomerase is recruited to telomeres to solve the end replication problem, we determined that Rap1-depleted telomeres dramatically increases the telomere extension frequency, where almost all telomeres are elongated in each cell cycle. This observation is consistent with Rap1 being a negative regulator of telomerase activity, so that all Rap1-depleted telomeres are recognised as short telomeres in need of elongation. To our knowledge, this is the first time that a mutant which such a characteristic has been reported. Finally, besides the end replication problem, there is a second and less-recognised problem where the DNA replication forks often stall and

collapse while traversing telomere sequences. To investigate these characteristics, we examined the properties of interstitial telomeric sequences (telomere sequences located internally in the genome). We focused on how telomere sequences are replicated and the consequences of replication fork collapse within telomeric sequences. Importantly, we identified genes that contribute to the stability of telomeric sequences and genes whose primary role is to suppress de novo telomere addition rather than replication fork collapses.

Samenvatting in het Nederlands

Mijn proefschrift heeft als doel een beter begrip te verschaffen van fundamentele aspecten van de biologie van telomeren. Telomeren zijn de fysieke uiteinden van de lineaire eukaryote chromosomen en beschermen de chromosomen tegen herkenning als dubbelstrengs DNA-breuken (DSB's) en voorkomen dat ze met elkaar fuseren. Door de progressieve verkorting van telomeer DNA tijdens elke ronde van DNA-replicatie, verliezen telomeren uiteindelijk hun beschermende vermogen. Dit "eindreplicatieprobleem" wordt tegengegaan door een gespecialiseerde reverse transcriptase genaamd telomerase. Desalniettemin wordt telomerase niet in voldoende mate tot expressie gebracht om slijtage van telomeren in de meeste menselijke somatische cellen te voorkomen, wat twee kritieke kenmerken van de menselijke gezondheid beinvloedt. Enerzijds resulteert progressieve telomeerverkorting in replicatieve senescentie, wat een kenmerk is van veroudering. Daarentegen is reactivering van telomerase een kenmerk van de meeste kankercellen, waardoor ze zich voor onbepaalde tijd kunnen delen. Om deze redenen is het zeer relevant om moleculaire details van verschillende aspecten van de telomeerbiologie te ontrafelen.

Daarom behandelt mijn werk delen van drie hoofdkenmerken van de telomeerbiologie: telomeerbescherming, het eindreplicatieprobleem en telomeerreplicatiestress. Ten eerste hebben we, wat betreft de telomeerbescherming, de rol en gevolgen onderzocht van telomeren die een van de belangrijkste eiwiten die geassocieerd is met dubbelstrengs telomeer DNA in knopvormende gist, Rap1 missen. Onze bevindingen suggereren dat het verslechteren van Rap1-binding de celproliferatie niet beïnvloedt, maar dat de aanwezigheid van andere componenten voor de bescherming van de telomeren vereist is. Deze bevindingen illustreren de flexibiliteit en redundantie van telomeerafdekkende eiwitten en bieden een verklaring voor de snelle evolutie van telomeersequenties en telomeerbindende eiwitten in knopvormende gistsoorten. Bovendien voorspelt de G-rijke aard van de telomeren de potentiële vorming van secundaire structuren, zoals G-quadruplexen (G4), die zouden kunnen bijdragen aan telomeerafdekking. Desalniettemin suggereren onze gegevens dat het vermogen om G4's in telomeren te vormen, niet essentieel is voor telomeerafdekking in *S. cerevisiae*. Ten tweede, om te onderzoeken hoe telomerase wordt gerekruteerd naar telomeren, om het eindreplicatieprobleem op te lossen, hebben we vastgesteld dat in telomeren die Rap1 missen, de frequentie van telomeerverlenging dramatisch is verhoogd, waarbij bijna alle telomeren in elke celcyclus verlengd zijn. Deze waarneming is consistent met het feit dat Rap1 een negatieve regulator is van telomerase-activiteit, zodat alle telomeren die Rap1 missen, worden herkend als korte telomeren die verlenging nodig hebben. Voor zover wij weten, is dit de eerste keer dat een mutant met een dergelijke eigenschap is gerapporteerd. Ten slotte is er, naast het eindreplicatieprobleem, een tweede en minder erkend probleem, waarbij de DNA-replicatievorken vaak vastlopen en ineenstorten terwijl ze de telomeersequenties doorlopen. Om deze kenmerken te onderzoeken, hebben we de eigenschappen van interstitiële telomeersequenties (telomeersequenties die intern in het genoom

zijn gelokaliseerd) onderzocht. We hebben ons gericht op hoe telomeersequenties worden gerepliceerd en op de gevolgen van het ineenstorten van de replicatievork binnen telomeersequenties. Belangrijk is dat we genen hebben geïdentificeerd die bijdragen aan de stabiliteit van telomeersequenties en genen waarvan de primaire rol is om de novo telomeertoevoeging te onderdrukken in plaats van het ineenstorten van de replicatievork.

Resumen en español

Mi tesis tiene como objetivo proporcionar una mejor comprensión de los aspectos fundamentales de la biología de los telómeros. Los telómeros son los extremos físicos de los cromosomas lineales eucariotas, protegiéndolos de ser reconocidos como rompimiento de doble cadena de ADN (DSB por sus siglas en inglés) y evitando que se fusionen entre sí. Debido al acortamiento progresivo del ADN telomérico durante cada ronda de replicación del ADN, los telómeros finalmente pierden su capacidad protectora. Este "problema de replicación en los telómeros" es contrarrestado por una retrotranscriptasa especializada llamada telomerasa. Sin embargo, la telomerasa no se expresa en niveles suficientes para prevenir el desgaste de los telómeros en la mayoría de las células somáticas humanas, lo que afecta dos características críticas de la salud humana. Por un lado, el acortamiento progresivo de los telómeros da como resultado la senescencia replicativa, que es un sello distintivo del envejecimiento. Por el contrario, la reactivación de la telomerasa es una característica de la mayoría de las células cancerosas, lo que les permite dividirse indefinidamente. Por estas razones, es muy relevante descubrir detalles moleculares en diferentes aspectos de la biología de los telómeros.

En consecuencia, mi trabajo cubre partes de tres características principales de la biología de los telómeros: la protección de los telómeros, el problema de la replicación al final de los cromosomas y el estrés de la replicación de los telómeros. En primer lugar, en términos de protección de los telómeros, investigamos el papel y las consecuencias de la ausencia de la principal proteína asociada con el ADN telomérico de doble cadena en la levadura, Rap1. Nuestros hallazgos sugieren que impedir la unión de Rap1 no afecta la proliferación celular, pero requiere la presencia de otros componentes para la protección de los telómeros. Estos hallazgos ilustran la flexibilidad y la redundancia de las proteínas que cubren los telómeros y ofrecen una explicación de la rápida evolución de las secuencias teloméricas y las proteínas que se asocian a ellos. Además, la naturaleza rica en guanina (G) de los telómeros predice la posible formación de estructuras secundarias, como G-quadruplexes (G4s), que podrían contribuir a la protección de los telómeros. Sin embargo, nuestros datos sugieren que la capacidad de formar G4s no es esencial para la protección de los telómeros en S. cerevisiae. En segundo lugar, para investigar cómo se recluta la telomerasa para resolver el problema de la replicación al final de los cromosomas, determinamos que los telómeros carentes de Rap1 aumentan drásticamente la frecuencia de extensión de los telómeros, donde casi todos los telómeros se alargan en cada ciclo celular. Esta observación es consistente con que Rap1 sea un regulador negativo de la actividad de la telomerasa, por lo que todos los telómeros carentes de Rap1 se reconocen como telómeros cortos que necesitan ser extendidos. Hasta donde sabemos, esta es la primera vez que se informa de una mutante con tal característica. Finalmente, además del problema de la replicación al final de los cromosomas, hay un segundo problema menos reconocido en el que las horquillas de replicación del ADN a menudo se estancan y colapsan mientras atraviesan

secuencias teloméricas. Para investigar estas características, examinamos las propiedades de las secuencias teloméricas intersticiales (secuencias de telómeros ubicadas internamente en el genoma). Nos enfocamos en cómo se replican las secuencias de telómeros y las consecuencias del colapso de la horquilla de replicación dentro de las secuencias teloméricas. Es importante destacar que identificamos genes que contribuyen a la estabilidad de las secuencias teloméricas y genes cuya función principal es suprimir la adición de telómeros de novo en lugar de colapsar la horquilla de replicación.

Curriculum Vitae

Research experience

The Francis Crick Institute — Sep 2022 – onwards
Postdoctoral researcher (laboratory of John Diffley) — London (UK)

European Research Institute for the Biology of Ageing — Sep 2017 – Aug 2022
PhD student (laboratory of Michael Chang) — Groningen (NL)

Johannes Gutenberg University Mainz — Oct 2015 – Jan 2017
Performed in pursuit of PhD before exiting the program — Mainz (GER)
International PhD program (IPP)

National Autonomous University of Mexico (UNAM) — Sep 2011- Sep 2015
Intern (laboratory of Jorge Vázquez) — Mexico City (MX)

Awards

Boehringer Ingelheim Travel grant — May 2017-July 2017
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Gabino Barreda Medal, for best student in generation 2008 — June 2013
Faculty of Chemistry, UNAM. — Mexico City (MX)

List of publications

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Yue Y, Fekete-Szücs E, **Rosas Bringas FR**, Chang M. *"Deletion of MEC1 suppresses replicative senescence of the cdc13-2 mutant".*

Acknowledgements

The most important factor for my PhD journey is the people who surround me. For this reason, I would like to express my deepest gratitude to all of you.

Firstly, I must start with **Michael**, not only because you were my supervisor but also because you became my friend inside and outside the lab. If I am proud of one of my decisions in life was to quit my previous laboratory and join your lab with you as my PhD supervisor. If you have to blame someone, go to Brian Luke, he inspired me to work in your field, get into the yeast system and ultimately search for you. Words cannot express my enormous gratitude for always maintaining your door open to me for discussion or even for jokes :). Thanks for always keeping a positive attitude, sharing your knowledge, being supportive, and listening to all types of complaints that I had when experiments did not work out. I appreciate that you take care of my scientific development and push me to my limits to improve in those abilities that I am still trying to perfect. I learned a lot from you, the congresses you allowed me to participate in, and the great memories I had at bouldering (even though you are already a pro). I admire you as a person and as a scientist; I learned how to have an open attitude towards everything, but it is mostly very inspiring that one day, I will perfect my writing skills as you do and understand when the text "flows" or not.

I am extremely grateful to my promotor **Floris Foijer** and my mentor **Liesbeth Veenhoff.** For sitting at all my annual assessment meetings, discussing my work, and taking care of my well-being. Both of you are extraordinary scientists that inspire me to become a successful PI if I have the opportunity to advance that step. At the same time, I would like to express my gratitude to **Liesbeth** for making me feel part of her group, for her feedback on every opportunity that she had, and for being a warm-hearted person inside and outside the lab.

I am also grateful to the members of the reading committee, **Teresa Teixeira**, **Liesbeth Veenhoff,** and **Marcel van Vugt**, for spending time and effort assessing this thesis.

I am deeply indebted to my paranymphs. I know the rule is to have only two, but I prefer Daniele Deluxe with an extra one. I cannot begin to express my thanks to **Eli, Marije,** and **Daniele** (even if he is busy changing diapers at the moment). Eli, I do not know if the "Latin blood" called us to be friends. Since the beginning of my stay in Groningen when I met you, we became immediate friends in the company of Jelmar. I like your sense of humour when things work out or not; thanks for being able to meet me any time (even in seasons when I was too busy). I think you are a fantastic person and PhD student who even had the energy to organise an immense quantity of Chilean scientific meetings. I wish you the best of luck in any path you decide to follow after your PhD. Marije, time allows us to become friends, join in our DnD sessions, dinners, board games, and even help me put the floor of my apartment (including Eli). I appreciate that you were aware all the time of my well-being. You are fantastic in everything you like and enjoy, and I am sure you will perform great once you find

that job. My best wishes for the next stage of your life. Finally, Daniele. Since my starting time as a PhD student, you have been an outstanding colleague. Unfortunately, you have to move to another lab, but after your return, I realised how fantastic it would be to spend more time in the same lab. I enjoyed a lot, our conversations about science, brainstorming, and even troubleshooting since we collaborated on your "simple" screen protocol :). Undoubtedly, you are an exceptional example to follow, not only as a person but also as a postdoc. Many thanks for all these years of friendship and for being an excellent colleague. Hopefully, next time you can win at squash. Best of luck with your new projects inside and outside the lab. Many thanks to the three of you for helping me with this event.

This work would not be possible without the enormous support of my family, especially my mom, brother, girlfriend (and her family), aunts, uncles, and grandfather. Even when I did not believe in myself many moments, you never doubted and supported me in good or bad circumstances. Many thanks, **mom,** for your unconditional support and for taking care of my brother and me without giving importance to adversity. Special thanks to my **brother**, basically my best friend. It is fantastic to discuss science or not with you. I enjoy how our paths are complementary; while I help you in your experimental troubleshooting, you are my bioinformatician "guru." It was pretty fun to share the apartment these last months. I am very proud of you and, I am sure you will do magnificent work with **John** (who I also express my thanks for the bouldering sessions, BBQs and whiskey). Many thanks to my aunts, uncles, and my grandfather for all your support in the distance. *"Muchas gracias mamá, por tu apoyo incondicional y por cuidar de mi hermano y de mi sin dar importancia a la adversidad. Agradezco especialmente a mi hermano, básicamente mi mejor amigo. Es fantástico discutir de ciencia o no, contigo. Disfruto enormemente como nuestros caminos ahora son complementarios; mientras te puedo brindar ayuda en los problemas experimentales que tienes, tu eres mi gurú bioinformático. Ha sido bastante divertido compartir el departamento contigo en los últimos meses. Estoy muy orgulloso de ti, estoy seguro que harás un trabajo excelente con John. De igual forma extiendo mi agradecimiento a mis tías, tíos y a mi abuelo, por todo su apoyo a pesar de la distancia".* Additionally, the completion of my dissertation would not have been possible without the support of my girlfriend, **Sara Hepko**. Even at a distance, you have always been for me on the good and bad days. You are the calmest, most supportive, and warmest person I have met. Being with you helped me a lot to forget the job, the stress, and the bad moments when something was not working out. I am deeply thankful to you for being in the important moments with me, especially in my interviews in London. I extend my gratitude to your family for making me feel at home all the time. To all of you, you are the best of the best.

I would like to express my sincere thanks to all my lab colleagues from **Veenhoff, LaCava, Berezikov, Paridaen, Guryev,** and **Chang** groups for making a magnificent work environment on our floor. From the current to the previous colleagues who crossed our paths, I can just say thank you. I

am extremely grateful to the technicians/postdoc on our floor (**Inge, Anton, Amarins, Leila, Lisa,** and **Yin Fai**). Without your help and efficiency, the work would not be possible. I want to make a special mention to Yin Fai; I am deeply indebted to you. Without your hard work and extreme efficiency, I would not progress at the speed that I did. Independently of your work superpowers, I will miss our small chats and your loud greetings XD. In addition, I do not want to forget thanking some other essential members of Eriba, including **Sylvia** and **Karin**, for making my life so much easier in terms related inside and outside the lab.

Besides being my colleagues, many of them became closer to me over the time that we could spend together, and I would like to extend my sincere thanks to them. Starting with **Sara M** and **Stijn**, which could also be my paranymphs, but I did not want to disturb your parental duties with princess Freya. Many thanks for integrating me into the group. I will miss our meetings at your place for playing, having dinner, chatting with cocktails, and exchanging beverages and food (Sara M, you and your parents are a life saviour with the chilis that you gave me). I am also grateful to **Gloria, Eniko, Thamar, Tatiana, Luciano, Sonia, Eike, Satya, Joscha, Chris, Ester, Hidde, Lin, Yue, Ziqing, Sandra, Tegan,** and **Tijmen** for the time that we spend at the institute and sometimes outside of it.

I spent part of this journey supervising students; without their help, I could not complete an essential part of the work. Thanks to my high school, bachelor and especially my master´s students. **Pien** and **Jonathan**, both of you were fantastic students. Pien, you were very efficient in doing key and laborious experiments in a very short time. I am very grateful to you for that and the fun time working together. Jonathan, I had a great time working with you. Not only for the progress of our work but also for the excellent time watching football matches or having "empanadas" for lunch. To both of you, thank you very much for your work.

I would like to thank my friends outside the institute, who are also responsible for the great memories I have of Groningen. Firstly, my astronomy-squash friends, for integrating me into your group and having an excellent time playing at the ACLO. Especially **Pavel**, who introduced me and even moved his influences to become neighbours in the same building :). You are a great friend with a bright future, and I will definitely miss our nocturnal squash sessions, ribs dinners, and horror movies. Many thanks to my board games gang (**Laszlo, Marteen, Vicky, and Ricardo**); I have excellent memories of our games, mainly when we defeated "Lord Bacon." I would like to thank Laszlo because we were roommates for a long time besides being friends. I enjoyed our nights of games, dinners, and chats about PhD life. I am also grateful to my friends and colleagues that I met in Mainz, particularly **Maxim** and **Hannah**. Maxim, despite the years and the distance, we are still good friends; many thanks for the support all this time. I admire your tenacity to overcome adversity with your positivity in facing trouble. Thanks for believing in me. I wish you the best of luck in your new stage as a postdoc, and take care not to break more bones XD.

Hannah Schwabl, I want to also dedicate this work to you and your family. I had very nice and funny memories of you when we were roommates, from the first time I met you until the last time I could talk with you. You were the only person who really knew my emotions when I was there. I do not have enough words to express my gratitude to you for being with me in challenging moments and encouraging me to move forward without regretting my decisions. Honestly, without your advice and support, I would not have the courage to never give up and be proud of the path that I decided to follow. I miss you a lot; you were a fantastic and warm friend, great daughter, and incomparable sister. Thanks for listening to me all that time and for also valuing my advice. I am grateful to have become part of your life, and you will always be with love in my memories.

Last but not least, my Mexican friends who were with me all this time, no matter the distance. Firstly, my Dutchified friends, **Jozz, Yess, Fernando G., Mariana, Jezabel, Rocio** and my Frenchified PI, **José P.**, many thanks for your support and for making me feel at home when we meet or talk. Finally, my home friends that despite the distance and the time, they were able to hang out when I am in Mexico. Many thanks, "**Muchachos**" XD, especially **Marco Ruiz**, who is a fantastic graphic designer, as you can appreciate his talent with the cover of this thesis.

A hug to everyone. Without you, this would not be possible. Many thanks!