Long-term Effects of Cancer-Associated POT1 Mutations on Telomerase-Telomere Interactions and TPP1 Function

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Telomere maintenance plays a crucial role in cellular aging and cancer development. This research proposal aims to elucidate the intricate dynamics of telomere maintenance mechanisms in cancer-associated mutations, with a specific focus on the interactions between telomerase, POT1, and TPP1. By leveraging the advanced capabilities of the CoPixie algorithm for high-resolution single-molecule imaging analysis, I seek to address two critical gaps in our current understanding: The long-term effects of cancer-associated POT1 mutations on telomerase-telomere interactions across multiple cell cycles and the indirect effects of these mutations on TPP1 function and its interaction with telomerase. I will employ live-cell imaging techniques and the CoPixie algorithm to analyze telomerase-telomere interactions in HeLa cells expressing cancer-associated POT1 mutants. My experiments will extend beyond the current 300-second observation window to multiple cell cycles, allowing me to assess cumulative effects over time. Additionally, I will investigate how POT1 mutations impact TPP1's interactions with telomerase by overexpressing wild-type and mutant hTERT. I hypothesize that the effects of POT1 mutations on telomerase-telomere interactions will become more pronounced over multiple cell generations and that POT1 mutations will indirectly affect TPP1's ability to regulate telomerase activity at telomeres. This research has the potential to significantly advance our understanding of telomere biology in cancer, potentially identifying novel targets for therapeutic interventions. By elucidating the mechanisms through which cancer-associated mutations disrupt telomere maintenance, we may contribute to the development of more effective strategies for cancer treatment and prevention.

Telomeres and Telomerase

Imagine a world where our cells contain an internal clock that ticks away with each cell division. Special structures called telomeres compose this internal clock. These telomeres act like the plastic tips that protect the ends of our shoelaces, protecting the vital information stored in our DNA. The telomeres are tandem genetic repeat sequences at the end of our chromosomes, but what happens when these protective caps wear down? Telomerase is a remarkable enzyme that can turn back this clock, extending the lifespan of the cells in our bodies. This crucial interaction between telomeres and telomerase plays a vital role in aging, cancer, and various diseases (Chan & Blackburn, 2003).

Telomeres, located at the ends of our chromosomes, are like biological countdown timers. Every time the cell divides, these protective caps get slightly shorter and shorter. When telomeres become too short, cells stop dividing and enter a state of senescence or even die. This is a natural process of aging, but it also serves as a safeguard against uncontrolled cell growth, a key feature of many cancers.

Telomerase, on the other hand, is nature's way of hitting the snooze button on this internal cellular clock. This enzyme can add DNA back to telomeres, effectively resetting telomere shortening caused by cell divisions. While most of our cells do not produce telomerase, specific cells, such as stem cells, can activate this enzyme (Cifuentes-Rojas & Shippen, 2011). This ability of telomerase to maintain or even lengthen telomeres gives these cells the potential for indefinite division. Unfortunately, cancer cells can also activate telomerase, allowing them to divide indefinitely.

Protecting our genetic material is not the job of telomeres alone. They work alongside a group of proteins called the shelterin complex. Returning to our imaginary world, the shelterin complex would be best described as a group of security guards, each with a specific role in guarding the ends of our chromosomes. The shelterin complex comprises six proteins, but the two key players are POT1 and TPP1.

POT1 binds to single-stranded DNA at telomere ends, where it acts as a lock. It prevents other cellular mechanisms in our body from mistaking the single-stranded telomeres as broken DNA and prevents telomere elongation (Rice et al., 2017). TPP1, on the other hand, serves as a bridge between POT1 and the rest of the shelterin complex. Together, they form a protective cap that shields telomeres from damage and regulates telomerase's access to and extension of telomeres.

The intricate interactions between telomeres, telomerase, and the shelterin complex maintain a balance in our cells. When this balance is disrupted in our cells, it can lead to various health problems, including cancer. In many types of cancer, cells have evolved a mechanism to reactivate telomerase, allowing them to divide endlessly (Jafri et al., 2016). It is as if these cancer cells have found a way to keep their internal clocks wound continuously, avoiding the natural processes that usually limit their growth.

Interestingly, some cancers are associated with mutations in genes encoding shelterin proteins, particularly POT1. These mutations can affect how POT1 binds to telomeres and interacts with the other shelterin proteins. This causes abnormal telomere lengthening and compromises the cell's ability to regulate telomerase activity (Prince et al. 2024a, 2024b). This telomere dysfunction contributes to the genomic instability often seen in cancer cells, allowing them to accumulate even more mutations and grow unchecked. Mutations in POT1 also disrupt the natural state of the POT1-TPP1 complex, leading to chromosomal abnormalities and cancer.

Understanding these complex interactions between telomeres, shelterin proteins, and telomerase is crucial for developing new strategies to combat cancer and age-related diseases. By unraveling the mechanisms by which cells maintain their telomeres, researchers hope to find ways to selectively target cancer cells or, potentially, slow the aging process in healthy cells. As we delve deeper into telomere biology, we hope to uncover new and exciting possibilities for future medical research.

Review at a more scientific level

While the overview of telomeres and telomerase provides a foundation for the understanding of cellular aging and cancer, a deeper dive into the molecular mechanisms and recent scientific advancements reveals a more complex and nuanced picture of telomere length regulation. The intricate system between telomerase and shelterin proteins in telomere length regulation maintains genomic stability and cellular longevity, with implications for aging and cancer development.

Telomerase is a ribonucleoprotein complex composed of a catalytic subunit (TERT) and an RNA component (TR or TERC). The telomerase RNA component provides the template for telomeric DNA synthesis and also contains elements important for the enzyme formation and regulation. The TERT subunit contains several conserved domains critical for its function: the N-terminal extension, the reverse transcriptase domain, and the C-terminal extension (Wyatt et al., 2010). The human telomerase (hTERT) adds TTAGGG repeats to chromosome ends, counteracting telomere shortening that occurs during cell division and DNA replication. Diverse transcriptional, post-transcriptional, and post-translational mechanisms tightly regulate the expression and activity of telomerase. At the transcriptional level, multiple transcription factors that bind to the TERT promoter regulate TERT expression (Robinson & Schiemann, 2022). These transcription factors integrate signals from

*This author wrote this paper for Biology 470: Telomeres, Race, and Cancer taught by Dr. Karen Kirk.

numerous intracellular signaling pathways to either activate or repress TERT transcription. Accessory proteins such as dyskerin, TCAB1, and the shelterin complex regulate the assembly and localization of the full telomerase holoenzyme (Robinson & Schiemann, 2022).

The shelterin complex is a critical regulator of telomere structure and function. It is composed of six core subunits: TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 (Amir et al. 2020). These proteins interact with each other to protect telomeres from DNA damage response pathways and regulate telomere length by controlling telomerase access. POT1 (Protection of Telomeres 1) is a key component of the shelterin complex and contains two N-terminal OB-fold domains that bind directly to single-stranded telomeric DNA (Amir et al., 2020). This binding of POT1 helps facilitate telomerase access. The C-terminal region of POT1 interacts with TPP1, another subunit of shelterin. TPP1 serves as an important bridge between POT1 and the rest of the shelterin complex. The N-terminal OB-fold domain binds telomerase and recruits it to telomeres (Amir et al., 2020). This TPP1-POT1 complex enhances POT1's DNA-binding affinity and regulates telomerase access and activity at the telomere. Disruption of this POT1-TPP1 interaction, through genetic mutations or other means, can lead to telomere dysfunction and the development of various diseases like cancer.

Mutations in the POT1 gene have been identified in several types of cancer, including chronic lymphocytic leukemia, familial melanoma, cutaneous malignant melanoma, Coats plus syndrome, cardiac angiosarcoma, and familial glioma (Prince et al. 2024a, 2024b). Many of these cancer-associated POT1 mutations cluster within the OB-fold domains of the protein, which are critical for binding to single-stranded telomeric DNA. Researchers have shown that these POT1 mutations disrupt POT1's ability to bind and protect the telomeric single-stranded DNA overhang, leading to telomere lengthening and fragility (Prince et al. 2024a, 2024b). Mutations in POT1 fail to inhibit telomerase access and activity at telomeres, thereby promoting telomere elongation that drives cancer progression.

The advent of single-molecule imaging at nanometer-scale resolution has revolutionized our understanding of molecular dynamics in living cells. However, quantifying colocalization events between single molecules in living cells remains a challenge. Current strategies for analyzing colocalization often rely on manual scoring, which limits data collection. To address this, researchers developed a new algorithm, CoPixie, that rapidly and automatically quantifies colocalization events across an unlimited number of imaging channels (Prince et al. 2024a, 2024b). Researchers have used CoPixie to study the dynamic interactions between telomerase (labeled with the hTR component) and telomeres (labeled with mCherry-TRF1) in living HeLa cells. HeLa hTR5MS2 cells, which stably express MS2-tagged hTR and mCherry-TRF1, were used as a model system to image single telomerase particles and telomeres simultaneously. This dual-color single-particle tracking data was then analyzed using the CoPixie pipeline to quantify colocalization events and binding dynamics between telomerase and telomeres. The high-throughput capabilities of CoPixie enabled the researchers to explore how cancer-associated mutations in the telomeric protein POT1 impact telomerase access and residence time at telomeres. The manual nature of previous analysis had limited the approaches to this question (Prince et al. 2024a, 2024b).

While researchers have made significant progress in understanding telomere length regulation and the role of POT1 mutations in cancer, several key questions remain: the long-term effects of POT1 mutations and the effects on telomerase-telomere interactions over multiple cell cycles. Current studies have focused only on short-term effects, measuring for only 300 seconds within a single cell cycle (Prince et al. 2024a, 2024b). There is a need to investigate whether the effects of these mutations would be more pronounced in subsequent cell generations. Another knowledge gap is the indirect effects of cancer-associated POT1 mutations on TPP1 function. Exploring how the mutations alter the formation and stability of the POT1-TPP1 complex.

Future Experiments

The proposed investigation seeks to elucidate the dynamics of telomere maintenance mechanisms (TMMs) in cancer-associated mutations, with a specific focus on the intricate interactions between telomerase, Protection of Telomeres 1 (POT1), and TPP1. This study will use CoPixie, a novel object-based colocalization algorithm that integrates pixel- and trajectory-based overlap analysis, to visualize and quantify these molecular interactions with high precision. The research proposal is structured around two primary objectives:

Aim 1: Longitudinal Analysis of Cancer-Associated POT1 Mutations on Telomerase-Telomere Dynamics

I first aim to investigate the effects of cancer-associated POT1 mutations on telomerase-telomere interactions over extended time periods. Previous studies using CoPixie to investigate the impact of POT1 mutations on telomerase-telomere interactions have shown that cancer-associated POT1 mutants increase telomere elongation by increasing telomere accessibility and enhancing telomerase retention at telomeres (Prince et al. 2024a, 2024b). However, these were performed in only one cell cycle, and long-term interactions were measured for only 300 seconds. Another study used single-molecule tracking with MS2 stem loops and CRISPR/Cas9 gene editing to tag and visualize hTR dynamics with POT1 and showed that POT1 plays a regulatory role in telomerase retention at telomere ends, and that mutations enhance this retention (Laprade et al., 2020). I want to explore the effects of cancer-associated POT1 mutations on telomerase-telomere interactions over a more extended period and across multiple cell cycles to determine whether POT1 mutation effects become stronger in later generations. I will explore this by using CoPixie to analyze hTR-telomere interactions using single-molecule tracking images and movies. I hypothesize that specific POT1 mutations will significantly alter the frequency and duration of telomerase-telomere associations across multiple cell cycles. I believe that the effects of the POT1 mutation will worsen over time, causing even more dysfunction in telomerase and telomere interactions, leading to increased telomere elongation in cancer-associated mutated cells.

Aim 2: Elucidation of TPP1 Dynamics in the Context of POT1 Mutations and Telomerase Activity

Secondly, I aim to apply the CoPixie algorithm to examine the dynamics of TPP1 in relation to POT1 mutations and telomerase activity. Previous studies have shown that the shelterin subunit TPP1 requires another shelterin subunit, POT1, to interact stably with DNA (Wang et al., 2007). Given that POT1 stabilizes TPP1, I believe mutations in POT1 will affect the interaction between the two shelterin subunits, further reducing telomerase-telomere maintenance mechanisms. I will explore this by applying the CoPixie algorithm to analyze telomerase interactions with TPP1 in the presence of various cancer-associated POT1 mutations, to observe any differences in TPP1 functionality. I hypothesize that POT1 mutations will indirectly affect TPP1's interactions with telomerase, altering telomere homeostasis.

Experiment 1:

Cell culture:

I followed the same methods as in Prince et al. (2024a, 2024b), using HeLa hTR^5'MS2 cells, Express mCherry-TRF1 to detect telomere foci & mCherry-CDT1 for filming and particle tracking. Cells are then cultured in DMEM, supplemented with 10% fetal bovine serum, two mM L-glutamine, and 100 U/ml penicillin-streptomycin. Induction of myc-tagged POT1 mutants will be performed using lentiviral vector infection. A positive control will include cells expressing wild-type POT1. A negative control will include cells with POT1 knockouts. I will also include cells transduced with an empty lentiviral vector to control for the effects of the viral infection process.

Western blot analysis:

The western blot analysis will be used to validate the expression of myctagged POT1 proteins in HeLa^MS2 cells, as in Prince et al.

(2024a, 2024b).

Immunofluorescence:

Immunofluorescence will be used to visualize the localization of myc-tagged proteins at telomeres in HeLa^MS2 cells, as in Prince et al. (2024a, 2024b).

Live-cell imaging of hTR and telomeres:

This will allow me to investigate the impact of cancer-associated POT1 mutations on the dynamics of telomerase-telomere interactions, following the methods by Prince et al. (2024a, 2024b). It will allow live-cell imaging and 300-second movies to observe longer interactions, which will be repeated in the same cells to observe changes over a longer period of time.

CoPixie analysis of hTR–telomere interactions:

This will be used to quantify colocalization events between single hTR particles and telomeres, as in Prince et al. (2024a, 2024b).

Mathematical and statistical analyses:

I will be analyzing survival probability, which measures the dwell times of hTR particles at telomeres, as in Prince et al. (2024a, 2024b). This will allow me to quantify the dynamics of individual hTR-telomere interactions and telomerase interactions at telomeres.

Predicted Outcome:

From this experiment, I predict that the effects of the POT1 mutations will be greater over a longer period and in subsequent generations. POT1 mutant cells may show a higher frequency of telomerase-telomere interactions compared to wild-type cells. This increase could become more pronounced over multiple cell cycles, indicating a cumulative effect of the mutation. Telomerase will spend more time at the telomeres of POT1 mutant cells. Because telomerase interacts more frequently and spends more time at telomeres in POT1 mutant cells, this leads to continuous elongation over multiple cell cycles, accelerating in subsequent generations. This will ultimately lead to tumor formation, with accelerating growth with each cell division. These potential outcomes would be analyzed using the CoPixie algorithm to quantify changes in telomerase-telomere interactions over extended time periods and multiple cell cycles. The results would provide insights into the long-term effects of cancer-associated POT1 mutations on telomere maintenance mechanisms, potentially revealing how these mutations contribute to cancer progression over time.

Experiment 2:

Cell Culture:

I followed the same methods in Prince et al. (2024a, 2024b) using HeLa hTR^5'MS2 cells. Express mCherry-TRF1 to detect telomere foci & mCherry-CDT1 for filming and particle tracking. Cells are then cultured in DMEM supplemented with 10% fetal bovine serum, two mM L-glutamine, and 100 U/ml penicillin-streptomycin, and myc-tagged POT1 mutants are induced by lentiviral vector infection. However, I will also induce lentiviral particles overexpressing hTERT-WT and hTERT-K78E, the mutant variant of the telomerase catalytic subunit hTERT, which is unable to interact with TPP1, and expression did not affect POT1 mutants' ability to enhance telomerase retention at telomeres (Laprade et al., 2020). The POT1 wild-type-expressing cells will act as a control, as will empty lentiviral vectors. There will also be hTERT-WT overexpressing cells without any POT1 mutations to isolate the effects of hTERT. Cells overexpressing the hTERT-K78E without POT1 mutations were used to isolate the effects of this specific hTERT mutation.

Dynamics of TPP1 in relation to cancer-associated POT1 mutations:

The same methods of live-cell imaging of hTR as described in the previous experiment, as described by Prince et al. (2024a, 2024b). However, the addition of overexpressing hTERT-WT and hTERT-K78E will allow me to assess telomerase-TPP1 interactions as in Laprade et al. (2020). I will compare the interaction between TPP1 and telomerase in cells expressing POT1 mutations with that in POT1-WT cells to determine whether POT1 mutations affect the dynamics of TPP1 and telomerase.

Predicted Outcome:

I believe that cancer-associated POT1 mutations will have both direct and indirect effects on TPP1 and telomere maintenance. With TPP1 acting as a bridge between POT1 and the rest of the shelterin complex, I believe a mutated version will amplify its influence. I believe that the mutated POT1 will indirectly cause increased telomerase retention and recruitment to telomeres via TPP1. I should see increased telomerase dwell time at TPP1 and increased telomerase abundance. Alternatively, it is also possible that mutated POT1 variants will no longer bind or interact with TPP1, disrupting the shelterin complex as a whole and potentially causing cancer.

Limitations

If undergoing multiple cell cycles, the cells may die or enter cellular senescence before they can enter new cycles with cancer-associated mutations. Especially telomere-altering mutations. There is also the possibility that additional mutations accumulate in cells, which could influence telomere maintenance, complicating the interpretation of results from POT1 mutations. These factors could introduce variability in long-term experiments, making it challenging to isolate the effects of POT1 mutations from other cellular changes over time.

While CoPixie is an advanced algorithm, the resolution of single-molecule tracking may still be limited, especially over extended time periods. Long-term imaging could lead to photobleaching or phototoxicity, potentially affecting cellular behavior or protein dynamics. These limitations could affect the accuracy of quantifying telomere-telomerase interactions, especially in long-term studies, potentially leading to underestimation or misinterpretation of subtle changes in these interactions.

Another limitation is that I am using only HeLa cells, which are the most common in research but may not fully represent the diversity of cancer cells; exploring other cell lines may be helpful. I am also using specific POT1 mutations, for which the effects may not be generalized to all POT1 mutations. The cells were cultured in vitro, which may not fully replicate the complex interactions and signaling that occur within a three-dimensional tissue environment, potentially limiting the translational value of the findings to clinical applications. Using controls that overexpress specific proteins, such as hTERT, may lead to protein levels that do not reflect natural cellular conditions, potentially altering the dynamics of telomere maintenance complexes. Protein tags, while necessary for visualization, may interfere with normal protein function or interactions. These factors could lead to observations that do not accurately represent the natural behavior of telomere maintenance proteins, potentially skewing the interpretation of how POT1 mutations affect telomere dynamics.

Conclusion

In conclusion, this research proposal aims to elucidate the intricate dynamics of telomere maintenance mechanisms in cancer-associated mutations, with a specific focus on the interactions between telomerase, POT1, and TPP1. By leveraging the advanced capabilities of the CoPixie algorithm for high-resolution single-molecule imaging analysis, we seek to address two critical gaps in our current understanding. First, the long-term effects of cancer-associated POT1 mutations on telomerase-telomere interactions across multiple cell cycles. Secondly, the indirect effects of these mutations on TPP1 function and its interaction with telomerase.

Our proposed experiments will provide unprecedented insights into the molecular mechanisms underlying telomere dysfunction in cancer. By extending the observation period and analyzing interactions

across multiple cell generations, we expect to uncover the cumulative effects of POT1 mutations, which may become more pronounced over time. This approach could reveal how these mutations contribute to the progressive genomic instability characteristic of cancer cells.

Furthermore, by examining the dynamics of TPP1 in relation to POT1 mutations and telomerase activity, we aim to unravel the complex interplay within the shelterin complex. This investigation may shed light on how disruptions in a single component of the telomere maintenance machinery can have far-reaching consequences for overall telomere homeostasis.

The results of this study have the potential to advance our understanding of telomere biology in cancer significantly. By elucidating the mechanisms by which cancer-associated mutations disrupt telomere maintenance, we may identify novel therapeutic targets. Ultimately, this research could contribute to the development of more effective strategies for cancer treatment and prevention, particularly for cancers associated with telomere dysfunction.

Use of AI

Throughout the grant proposal writing process, I utilized various AI tools to enhance my research and streamline my work. One significant tool was Undermind AI, which I used to conduct an in-depth search of research articles on single-molecule imaging techniques. This tool's iterative search capability mimics a human researcher's process, enabling a comprehensive "deep search" across titles, abstracts, and full texts when available. As a result, I uncovered a more thorough and relevant set of research articles compared to traditional search methods, potentially identifying 30-80% more relevant papers on my topic.

In addition to the literature review, I also used PerplexityAI to help interpret the results of various research articles. This AI-powered tool provided clear answers with clickable citations for easy verification, enabling me to ask follow-up questions for deeper understanding. By leveraging PerplexityAI, I gained a comprehensive grasp of complex research findings, which informed my experimental design and sparked ideas for future experiments.

The combination of these AI tools not only facilitated my understanding of existing literature but also played a crucial role in generating innovative ideas for future experiments. By synthesizing information from multiple sources, AI helped me identify gaps in current research that my proposal could address, ultimately increasing the relevance and potential impact of my proposed experiments.

References:

1. Amir, M., Khan, P., Queen, A., Dohare, R., Alajmi, M. F., Hussain, A., Islam, A., Ahmad, F., & Hassan, Md. I. (2020). Structural features of nucleoprotein CST/Shelterin complex involved in telomere maintenance and its association with disease mutations. Cells, 9(2), 359. https://doi.org/10.3390/cells9020359

This review article provides a comprehensive overview of the structural features and functions of the shelterin and CST complexes, which are critical regulators of telomere structure and maintenance. The authors discuss the key components of each complex, their domain organization, and how they interact to protect telomeres and regulate telomere length. Particular emphasis is placed on the role of POT1, a core subunit of the shelterin complex, and its interaction with TPP1. Disruption of these protein-protein interactions, often due to disease-associated mutations, can lead to telomere dysfunction and the development of various diseases, including cancer.

2. Chan, S. R., & Blackburn, E. H. (2004). Telomeres and telomerase. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 359(1441), 109–122. https://doi.org/10.1098/rstb.2003.1370

This review article provides a comprehensive overview of telomere structure and function, as well as the telomerase enzyme. The authors discuss how telomerase solves the "end-replication problem" by using an RNA template to synthesize telomeric DNA and maintain chromosome ends. They also describe how telomeres and associated protein complexes, such as shelterin, protect chromosome ends from being recognized as DNA damage. The authors highlight how mutations in the telomerase RNA template can disrupt telomere function and lead to cellular senescence. Additionally, they discuss the protective role of telomerase in preventing catastrophic telomere shortening and fusion events, even in the absence of telomere lengthening. This suggests telomerase has functions beyond just maintaining telomere length. The review emphasizes the complexity of telomere regulation and the limitations of simply using telomere length as a readout of cellular proliferative potential.

3. Cifuentes-Rojas, C., & Shippen, D. E. (2012). Telomerase regulation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 730(1–2), 20–27. https://doi.org/10.1016/j.mrfmmm.2011.10.003

This review article provides a comprehensive overview of the regulatory mechanisms governing telomerase activity. The authors discuss the multiple layers of telomerase regulation, including transcriptional control of the TERT catalytic subunit, post-translational modifications of TERT, and regulation of the telomerase RNA component TR. They highlight how alterations in telomerase subunit gene dosage and alternative splicing isoforms can also impact enzyme activity. Additionally, the authors describe how telomerase recruitment to telomeres and its processivity at the chromosome terminus are tightly controlled, involving interactions with telomere-associated proteins and the long non-coding RNA TERRA. The review emphasizes the sophisticated networks governing telomerase function and the critical importance of these regulatory mechanisms for maintaining telomere homeostasis and preventing human diseases associated with telomerase dysfunction.

4. Jafri, M. A., Ansari, S. A., Alqahtani, M. H., & Shay, J. W. (2016). Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Medicine, 8(1). https://doi.org/10.1186/s13073-016-0324-x

This comprehensive review article provides an overview of the critical roles of telomeres and the telomerase enzyme in cancer. The authors discuss how telomere dysfunction and telomerase reactivation contribute to the initiation and progression of the majority of human cancers. Key topics covered include: the organization and function of telomeres and the shelterin complex; the mechanisms of telomerase assembly, trafficking, and recruitment to telomeres; the significance of recurrent TERT promoter mutations in activating telomerase in cancer; and the development of various telomerase-targeted therapeutic strategies, including small molecule inhibitors, immunotherapies, and nucleoside analogs.

5. Laprade, H., Querido, E., Smith, M. J., Guérit, D., Crimmins, H., Conomos, D., Pourret, E., Chartrand, P., & Sfeir, A. (2020). Single-molecule imaging of telomerase RNA reveals a recruitment-retention model for telomere elongation. Molecular Cell,79(1). https://doi.org/10.1016/j.molcel.2020.05.005

This article uses advanced live-cell imaging techniques, including single-molecule tracking of the telomerase RNA component hTR, to provide unprecedented insights into telomerase dynamics in human cancer cells. The authors developed an MS2-tagging approach to visualize single hTR particles and track their movement through the nucleus, Cajal bodies, and at telomeres. The key findings include: 1st, hTERT controls the exit of hTR from Cajal bodies, where telomerase assembly and maturation occur. 2nd telomerase recruitment to telomeres involves a two-step "recruitment-retention" model. TPP1-mediated recruitment leads to short, highly diffusive telomere-telomerase interactions. Subsequent retention at telomeres is dependent on base pairing between the hTR template region and the telomeric single-stranded DNA overhang. 3rd the DNA damage response kinases ATM and ATR regulate the recruitment, but not retention, of telomerase at telomeres. Finally, cancer-associated POT1 mutations that disrupt the OB-fold DNA-binding domain enhance telomerase retention, providing a mechanistic explanation for how these mutations lead to abnormally long telomeres. Overall, this study provides unprecedented mechanistic insights into the spatiotemporal regulation of telomerase trafficking and its engagement with telomeres, which is critical for cancer cell immortalization. The single-molecule imaging approach provides a powerful tool for dissecting the multi-

step process of telomere elongation.

6. Prince, S., Maguemoun, K., Ferdebouh, M., Querido, E., Derumier, A., & Chartrand, P. (2024). Single-Particle Track Colocalization Using Copixie Reveals the Impact of Cancer-Associated POT1 Mutations on Telomerase-Telomere Interactions. https://doi.org/10.1101/2024.02.19.580537

This article introduces CoPixie, a new software tool for high-throughput quantification of colocalization events between single-particle tracks and other imaging objects in live cells. The authors demonstrate CoPixie's capabilities by applying it to study the impact of cancer-associated mutations in the telomeric protein POT1 on the dynamic interactions between telomerase and telomeres. Key findings: CoPixie accurately reproduced previous manual colocalization quantification between the telomerase RNA component hTR and telomeres, significantly increasing analysis throughput. Expression of cancer-associated POT1 mutants, including K90E, Y223C, and D224N, increased the percentage of telomeres that colocalized with hTR particles and the cumulative dwell time of hTR at telomeres, suggesting that these mutations enhance telomerase accessibility to telomeres. Interestingly, the POT1-K90E and POT1-Y223C mutants also increased the duration of long-lasting interactions between hTR and telomeres, unlike the POT1-ΔOB mutant. This indicates these mutations may impact an activity that limits telomerase elongation at telomeres. Overall, this work provides an important methodological advance in single-particle colocalization analysis, as well as novel insights into how cancer-associated POT1 mutations mechanistically drive telomere elongation by modulating both telomerase accessibility and retention at chromosome ends.

7. Prince, S., Maguemoun, K., Ferdebouh, M., Querido, E., Derumier, A., Tremblay, S., & Chartrand, P. (2024). Copixie, a novel algorithm for single-particle track colocalization, enables efficient quantification of telomerase dynamics at telomeres. Nucleic Acids Research, 52(16), 9417–9430. https://doi.org/10.1093/nar/gkae669

This article introduces CoPixie, a new software tool for high-throughput quantification of colocalization events between single-particle tracks and other imaging objects in live cells. The key features of CoPixie include the ability to identify colocalization events across an unlimited number of imaging channels, combining spatial and temporal information from particle trajectories. Flexibility to accommodate both diffraction-limited single particles and larger irregular objects, using either centroid-based or mask-based colocalization. Demonstration of CoPixie's robustness and accuracy in reproducing previous manual quantification of colocalization between the telomerase RNA component hTR and telomeres. The authors then applied CoPixie to study the impact of cancer-associated mutations in the telomere-binding protein POT1 on the dynamics of telomerase interactions with telomeres. Key findings include: POT1 mutants K90E, Y223C, and D224N increase telomerase binding to telomeres. Unexpectedly, the POT1-K90E and POT1-Y223C mutants also enhance the duration of long-lasting interactions between telomerase and telomeres, unlike the POT1-OB deletion mutant. The distinct effects of these POT1 mutants on both telomere accessibility and telomerase retention suggest a dual mechanism by which they promote telomere elongation in cancer cells. Overall, this work provides a valuable methodological advance in single-particle colocalization analysis and new insights into how cancer-associated POT1 mutations mechanistically drive telomere dysfunction.

8. Rice, C., Shastrula, P. K., Kossenkov, A. V., Hills, R., Baird, D. M., Showe, L. C., Doukov, T.,Janicki, S., & Skordalakes, E. (2017). Structural and functional analysis of the human POT1-TPP1 telomeric complex. Nature Communications, 8(1). https://doi.org/10.1038/ncomms14928

This article presents a structural and functional analysis of the human POT1-TPP1 telomeric complex. The authors determined the atomic structure of the interacting portion of the human telomeric POT1-TPP1 complex and investigated how naturally occurring POT1 mutations contribute to cancer development. The key findings include: POT1C consists of an OB-fold and a holiday junction resolvase domain, which make extensive interactions with TPP1, forming a tight heterodimer. Several cancer-associated POT1 mutations (P446Q, C591W, and Q623H) partially disrupt the POT1-TPP1 complex, reducing its ability to bind telomeric DNA efficiently. Partial disruption of the POT1-TPP1 complex results in longer, more fragile telomeres, contributing to genomic instability and cancer. The article provides insights into the molecular mechanisms by which dysfunction of the POT1-TPP1 complex can drive carcinogenesis. This study advances the understanding of the structural basis and functional consequences of POT1-TPP1 complex formation, as well as the role of cancer-associated mutations in telomere maintenance and genomic stability.

9. Robinson, N. J., & Schiemann, W. P. (2022). Telomerase in cancer: Function, regulation, and clinical translation. Cancers, 14(3), 808. https://doi.org/10.3390/cancers14030808

This comprehensive review explores the multifaceted roles of telomerase in cancer biology, emphasizing its dual functions in telomere maintenance and extratelomeric regulation of cellular processes. The authors detail the transcriptional and post-transcriptional mechanisms regulating telomerase components, particularly the TERT and TR proteins, and their implications for tumorigenesis. Additionally, the review highlights the extratelomeric contributions of telomerase to cancer progression through pathways like Wnt/β-catenin and NF-κB signaling. Clinical applications discussed include telomerase as a prognostic biomarker and as a potential oncology therapeutic target. This article is a valuable resource for researchers investigating telomere dynamics and therapeutic interventions in cancer.

10. Wang, F., Podell, E. R., Zaug, A. J., Yang, Y., Baciu, P., Cech, T. R., & Lei, M. (2007). The pot1–TPP1 telomere complex is a telomerase processivity factor. Nature, 445(7127), 506–510. https://doi.org/10.1038/nature05454

This study investigates the role of the POT1-TPP1 protein complex in telomere biology, providing significant insights into its dual functions. It reveals that the complex not only protects telomeres by capping chromosome ends but also enhances telomerase processivity, a novel finding contrasting its presumed inhibitory role on telomerase. The authors use structural biology and biochemical approaches to elucidate the molecular mechanisms by which POT1-TPP1 stabilizes single-stranded DNA and promotes efficient telomerase-mediated telomere elongation. This research advances our understanding of telomere maintenance and its implications for cancer biology, as telomerase activation is a hallmark of cancer. The article serves as a foundational reference for those studying telomere dynamics and telomerase-related therapeutic strategies.

11. Wyatt, H. D., West, S. C., & Beattie, T. L. (2010). InTERTpreting telomerase structure and function. Nucleic Acids Research, 38(17), 5609–5622. https://doi.org/10.1093/nar/gkq370

This comprehensive review article provides an in-depth analysis of telomerase structure and function, with a particular focus on the telomerase reverse transcriptase (TERT) subunit. The authors discuss: The importance of telomeres and telomerase in genome stability and human diseases. The composition of the telomerase holoenzyme includes the TERT and TR (telomerase RNA) subunits, as well as species-specific accessory proteins. The structural organization of TERT includes its N-terminal extension, central catalytic RT domain, and C-terminal extension. Recent structural studies on TERT, including the controversial crystal structure of Tribolium castaneum TERT, and the evolutionary conservation and variation of telomere-associated proteins across different species. The article provides valuable insights into current understanding of telomerase biology. It highlights areas for future research, making it a valuable resource for researchers in molecular biology, genetics, and cancer research.