Nuclear F-Actin and the DNA Damage Response Regulate Telomerase Recruitment

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Introduction

- Cancer cells achieve replicative immortality in part through the extension of their telomeres which would otherwise shorten, triggering senescence or cell death; in ~85% of cancer cells, telomeres are elongated by the ribonucleoprotein telomerase.
- While the function of telomerase is reasonably well characterised, less is known about the processes which regulate its function, including its recruitment to telomeres.
- The DNA damage response is important in regulating telomerase recruitment to telomeres, specifically via the regulatory kinases ATR and ATM, which coordinate the cellular response to DNA damage[1,2]. ATR is important for resolving DNA replication stress, any process which slows replication such as replication fork stalling.
- Recently it has been demonstrated that nuclear filamentous actin (F-actin) is important for relocalisation of stalled replication forks to resolve replication stress[3].
- We hypothesise that there is controlled interplay between the DNA damage response, nuclear actin polymerisation and telomere maintenance.

Actin polymerisation is required for telomerase recruitment to telomeres

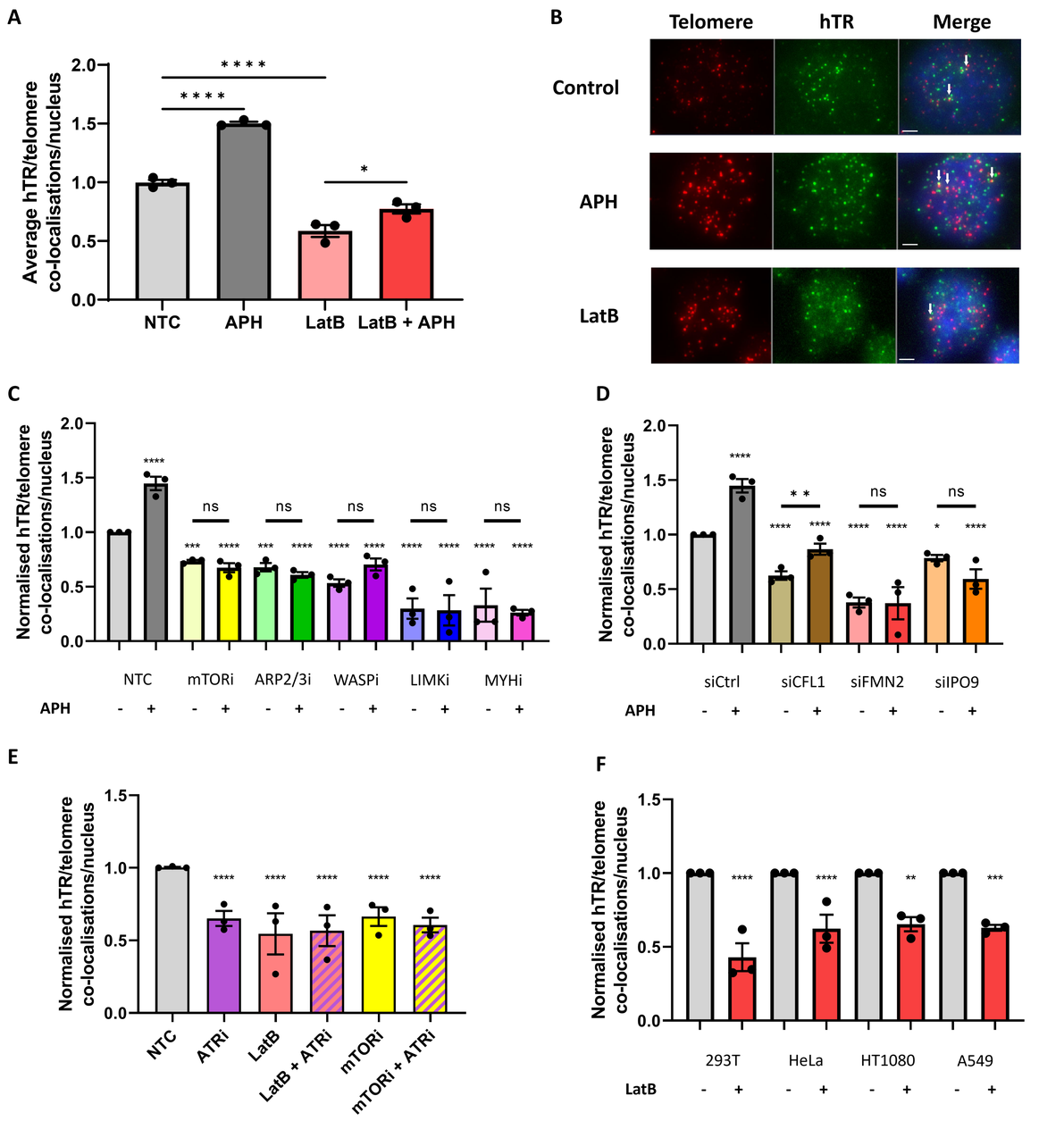

Figure 1. (A) Telomerase localisation at telomeres was measured using hTR-telomere FISH in 293T cells following latrunculin B (LatB; actin polymerisation inhibitor) treatment with and without aphidicolin (APH) to induce replication stress. **(B)** Representative FISH images showing co-localisation between telomeres (red) and hTR (green). Nuclei were stained with DAPI (blue) and labelled with EdU to identify cells in S phase. Scale bar = 2 µm. **(C)** Telomerase recruitment was also measured in 293T cells following inhibition of mTOR (INK128), ARP2/3 (CK666), WASP (wiskostatin), LIM kinase (LIMKi) and myosin (BTS) in the presence or absence of APH. **(D)** hTR-telomere FISH in 293T cells following siRNA knockdowns of the indicated proteins, with or without APH. **(E)** hTR-telomere FISH in 293T cells treated with inhibitors of actin polymerisation (LatB) or mTOR (INK128) in the presence or absence of ATR inhibition (VE822). **(F)** Quantitation of hTR-telomere co-localisations in 293T, HeLa, HT1080 or A549 cells treated with or without latrunculin B to inhibit actin polymerisation. Significance for each column is expressed relative to the relevant control (DMSO or siCtrl ± APH), determined by 2-way ANOVA. Data are presented as mean ± SEM; n = 3 for all experiments, counting ≥ 100 nuclei per replicate.

Telomerase recruitment is dependent on nuclear actin

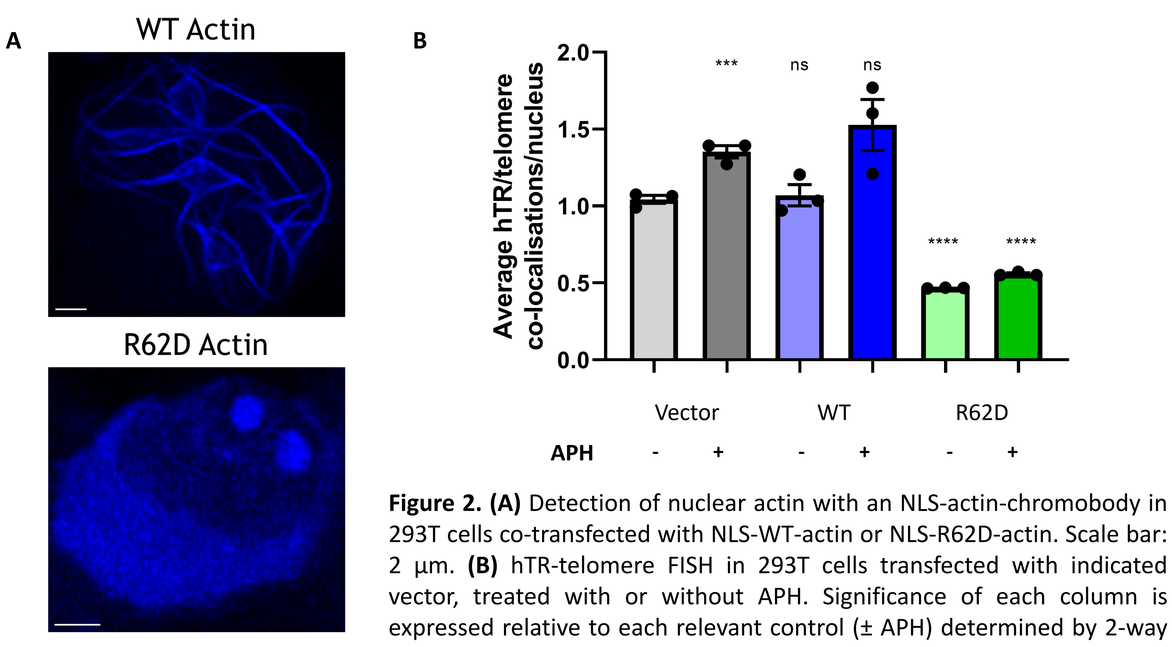

Figure 2. (A) Detection of nuclear actin with an NLS-actin-chromobody in 293T cells co-transfected with NLS-WT-actin or NLS-R62D-actin. Scale bar: 2 µm. **(B)** hTR-telomere FISH in 293T cells transfected with indicated vector, treated with or without APH. Significance of each column is expressed relative to each relevant control (± APH) determined by 2-way ANOVA. Data are mean ± SEM; n = 3.

Telomeres interacting with telomerase are located closer to F-actin

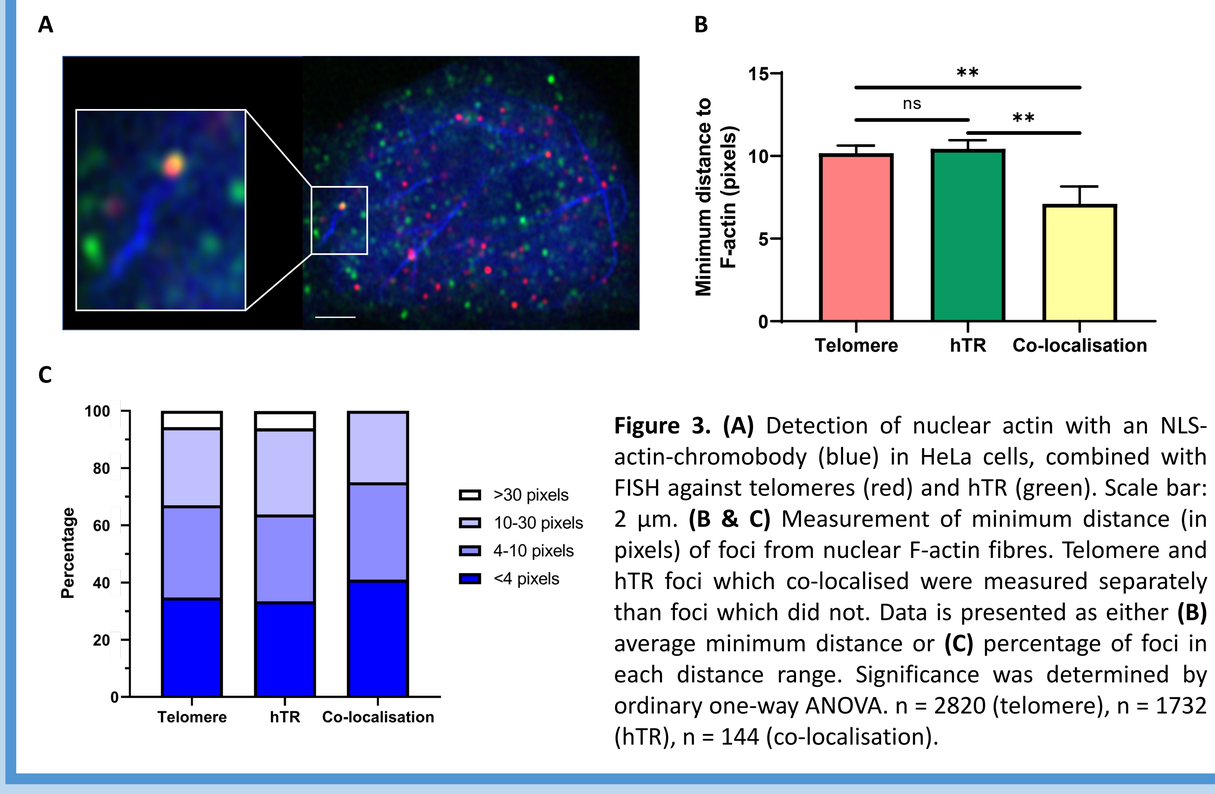

Figure 3. (A) Detection of nuclear actin with an NLS-actin-chromobody (blue) in HeLa cells, combined with FISH against telomeres (red) and hTR (green). Scale bar: 2 µm. **(B & C)** Measurement of minimum distance (in pixels) of foci from nuclear F-actin fibres. Telomere and hTR foci which co-localised were measured separately than foci which did not. Data is presented as either **(B)** average minimum distance or **(C)** percentage of foci in each distance range. Significance was determined by ordinary one-way ANOVA. n = 2820 (telomere), n = 1732 (hTR), n = 144 (co-localisation).

Telomerase recruitment to telomeres occurs in proximity to F-actin in live cells

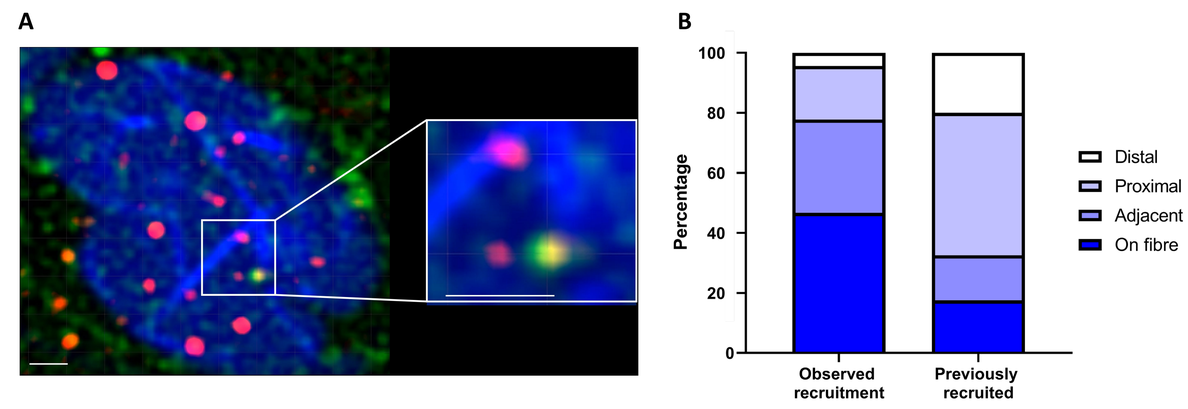

Figure 4. (A) Still image from live cell video of HeLa cells containing endogenously-tagged mEOS.2-TRF2 (red) and HALO-hTERT (green), transfected with a plasmid encoding the NLS-actin-chromobody (blue). Scale bar: 2 µm. **(B)** Classification of TRF2/hTERT co-localisation based on proximity to F-actin. Foci are grouped based on whether co-localisation was observed within a live cell video ('observed recruitment'; n = 45) or occurred prior ('previously recruited'; n = 40).

Summary

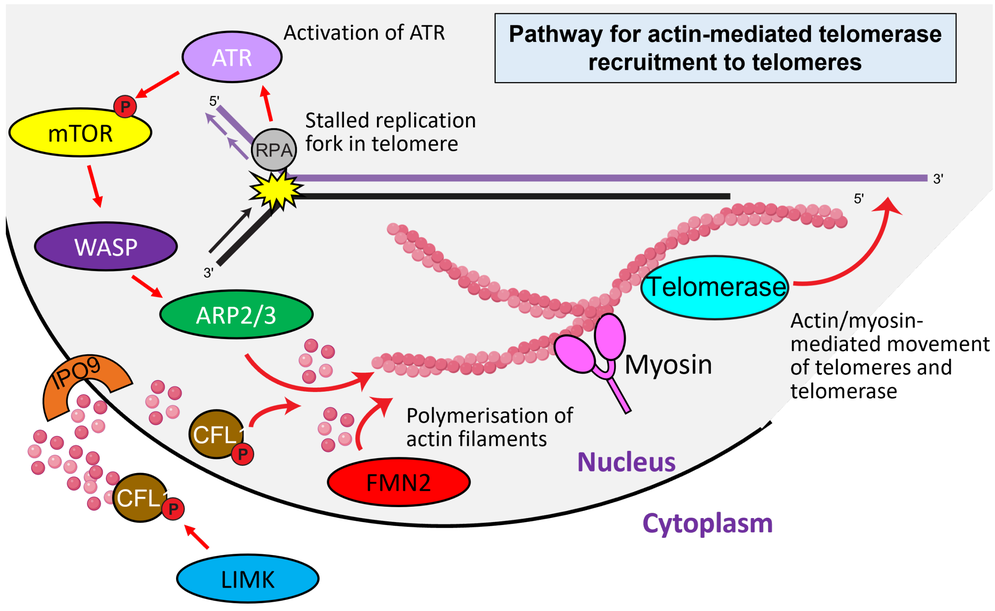

Figure 5. Proposed model of actin-mediated telomerase recruitment.

References

1. Tong, A.S., Stern, J.L., Sfeir, A., Kartawinata, M., de Lange, T., Zhu, X.D. and Bryan, T.M. (2015) *ATM and ATR Signaling Regulate the Recruitment of Human Telomerase to Telomeres.* Cell Reports, 13(8):1633-46.
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3. Lamm, N., Read, M.N., Nobis, M., Ly, D.V., Page, S.G., Masamsetti, P., Timpson, P., Biro, M. and Cesare, A.J. (2020) *Nuclear F-Actin Counteracts Nuclear Deformation and Promotes Fork Repair During Replication Stress.* Nature Cell Biology, 22:1460-1470.