synaptic complex formation with extended dsDNA despite the presence of numerous microhomology sites. Short-range sliding of presynaptic filaments on dsDNA substrates has also been observed, which may speed up the search process by ~200 fold (Ragunathan et al., 2012).

The advent of elegant single-molecule methods has allowed us to better understand the molecular mechanisms of homologous DNA recombination (Sanchez et al., 2014), but several questions remain. The crystal structure of ssDNA-bound RecA filament shows that the ssDNA has periodic base triplets in nearly B form, followed by an extended bond (Chen et al., 2008), so the structural basis for 8-nt microhomology recognition is not clear. Similarly, how the dynamics of the presynaptic filament are involved in recognizing dsDNA base pairing is not known. Finally, while the proposed mechanisms for accelerating the homology search may work in concert, they have not been observed simultaneously in a single study. Further work is needed to determine how, or if, the mechanisms function together, as well as the relative contribution of each to accelerating the process.

REFERENCES

Chen, Z., Yang, H., and Pavletich, N.P. (2008). Nature *453*, 489–484.

Forget, A.L., and Kowalczykowski, S.C. (2012). Nature *482*, 423–427.

Hsieh, P., Camerini-Otero, C.S., and Camerini-Otero, R.D. (1992). Proc. Natl. Acad. Sci. USA *89*, 6492–6496.

Jiang, L., and Prentiss, M. (2014). Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *90*, 022704.

Lusetti, S.L., and Cox, M.M. (2002). Annu. Rev. Biochem. *71*, 71–100.

Qi, Z., Redding, S., Lee, J.Y., Gibb, B., Kwon, Y., Niu, H., Gaines, W.A., Sung, P., and Greene, E.C. (2015). Cell *160*, this issue, 856–869.

Ragunathan, K., Joo, C., and Ha, T. (2011). Structure *19*, 1064–1073.

Ragunathan, K., Liu, C., and Ha, T. (2012). eLife *1*, e00067.

Renkawitz, J., Lademann, C.A., and Jentsch, S. (2014). Nat. Rev. Mol. Cell Biol. *15*, 369–383.

Sanchez, H., Reuter, M., Yokokawa, M., Takeyasu, K., and Wyman, C. (2014). DNA Repair (Amst.) *20*, 110–118.

ALT Telomeres Get Together with Nuclear Receptors

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http://dx.doi.org/10.1016/j.cell.2015.02.006

Nuclear receptors bind chromosome ends in "alternative lengthening of telomeres" (ALT) cancer cells that maintain their ends by homologous recombination instead of telomerase. Marzec et al. now demonstrate that, in ALT cells, nuclear receptors not only trigger distal chromatin associations to mediate telomere-telomere recombination events, but also drive chromosome-internal targeted telomere insertions (TTI).

Telomeres, the ends of chromosomes, would look just like the products of DNA double strand breaks if not for their specialized sequences and cohort of protective binding proteins. The cellular overproliferation characteristic of cancer requires some means of maintaining telomeric sequence through successive rounds of replication. For some cells, that involves reactivating telomerase, the enzyme that templates the characteristic telomere repeats. For others, it means relying on a homologous recombination-dependent mechanism termed alternative lengthening of telomeres (ALT). In this issue of *Cell*, Marzec et al. (2015) identify nuclear receptors as critical components in reprogramming normal telomeres toward ALT.

In most normal human, somatic cells, telomeres shorten with every round of DNA replication due to the DNA end replication problem and the absence of telomerase. Too short telomeres elicit a DNA damage response triggering a permanent cell-cycle arrest termed cellular senescence. Thus, the replicative potential of primary cells is limited, restraining the growth of pre-cancerous lesions that have lost normal growth control. However, mutations in cell-cycle regulators like p53 and pRB cause senescence bypass and restart the march toward malignancy. Replication under these conditions can lead to further telomere shortening and loss of the proteins that protect chromosome ends from fusion or "repair." In cases in which telomeres do fuse, cells enter a crisis state in which fused chromosomes that contain multiple centromeres become missegregated or become torn apart during mitosis. Cells can escape crisis either by re-gaining telomerase expression, for instance by mutating the promoter of the human

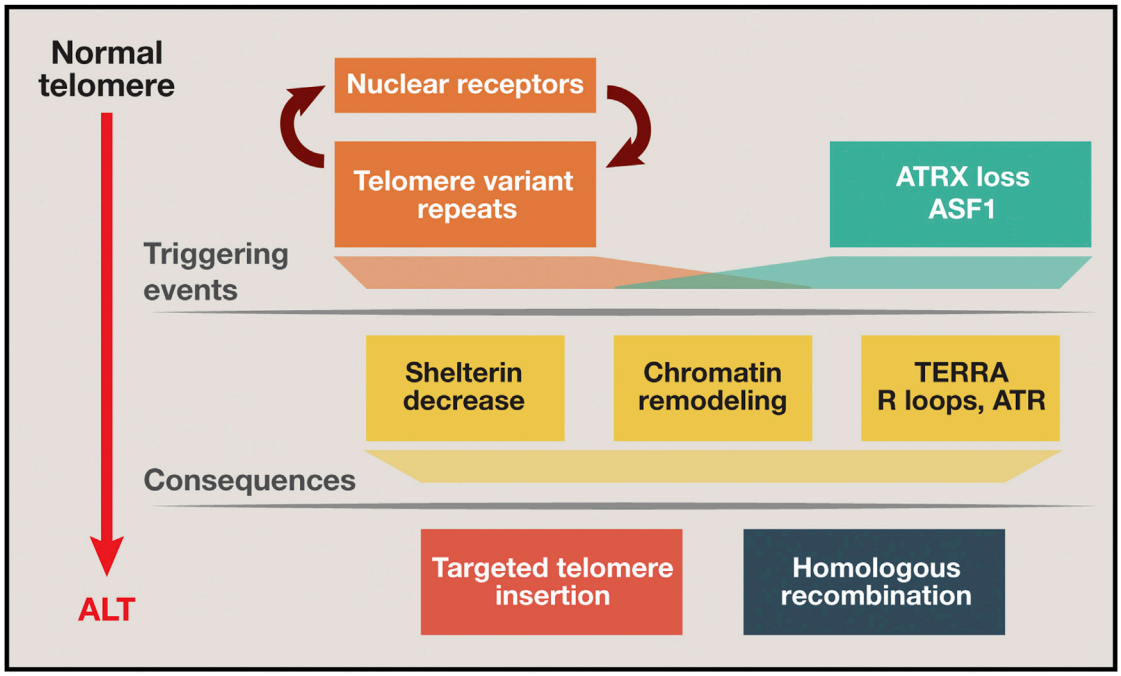

Figure 1. Molecular Events that May Trigger the Alternative Lengthening of Telomeres Pathway
At normal telomeres, HR is repressed by the high abundance of shelterin proteins. For ALT formation, nuclear receptors accumulate at telomeres, binding to variant telomeric repeat sequences. Nuclear receptor binding promotes formation of chromatin clusters that favor HR and spreading of telomeric variant repeats, which in turn will promote further nuclear receptor binding. Nuclear receptors may also promote recruitment of chromatin remodelers, which favor HR protein association and counteract the presence of shelterin. While ATRX loss will also promote telomeric chromatin remodeling, it upregulates the long noncoding RNA TERRA. Misregulated TERRA forms recombination-prone R loops that activate HR. TERRA also perturbs telomeric protein composition favoring association of RPA with telomeric DNA, which activates the ATR checkpoint kinase. Whether ATRX loss and nuclear receptor binding to telomeres are sufficient to trigger ALT remains to be tested.

telomerase reverse transcriptase (hTERT) gene, or by engaging the ALT pathway to maintain telomeres.

ALT is found in ~10% of cancers and is prevalent in sarcomas and glioblastomas. ALT telomeres are maintained by the homologous recombination (HR) machinery. Recombination occurs between telomeres of separate chromosomes. HR and telomere clustering are repressed at normal telomeres by the telomeric shelterin proteins (Sfeir and de Lange, 2012). Critical changes occur in ALT telomeric chromatin to overcome the shelterin-mediated repression of HR.

Orphan nuclear receptors of the NR2C/F class that classically regulate gene expression have also been found at ALT telomeres (Déjardin and Kingston, 2009). Subsequent work demonstrated that the nuclear receptors bind to variant telomeric repeat sequences (5′-GGGTCA-3′ instead of the canonical 5′-GGGTTA-3′ repeats) that are scarce in normal telomeres but that accumulate at ALT telomeres (Conomos et al., 2012). In the new study, Marzec et al. identified tandem 5′-GGGTCA-3′ repeats accumulating at ALT-telomeres bound by NR2C/F nuclear receptors. Importantly, nuclear receptor binding to telomeres induced telomere cluster formation, which is required for HR in ALT cells. Intriguingly, tethering a NR2C2-lacI fusion protein to a single *LacO* array not only led to colocalization of the array with telomeres, but also triggered its rapid amplification and spreading to other sites in the genome. It thus appears that the telomere clusters are sites of highly active recombination, and NR2C/F-mediated recruitment of DNA to this locus is sufficient to make this DNA recombine and spread elsewhere in the genome.

Consistent with this notion, Marzec et al. also discovered that a subset of chromosomal NR2C/F binding sites in ALT cells are locations of targeted telomere insertions (TTI). These newly inserted interstitial telomeric sequences may promote genome instability in ALT cells, as telomeric DNA is fragile and difficult to replicate. The association of chromosome-internal NR2C/F binding sites with telomeres may explain the spreading of the NR2C/F-bound 5′-GGGTCA-3′ repeats into telomeric tracts. Alternatively, the 5′-GGGTCA-3′ sequences may amplify from the rare telomeric copies. Thus, the new work suggests a sequence of molecular events that may occur during the evolution of ALT cells from normal cells. Critically short telomeres, missing key shelterin proteins, may expose the scarce 5′-GGGTCA-3′ sequences for NR2C/F binding. Binding of NR2C/F at telomeres and its binding at chromosome internal sites would then promote chromatin clustering, HR, and 5′-GGGTCA-3′ spreading, which in turn would facilitate further NR2C/F binding, telomere cluster formation, and recombination in a feedforward loop reaction (Figure 1). Thus, in this hypothetical scenario, HR would reinforce itself in ALT once it was triggered by rare initiating events.

Is nuclear receptor binding to telomeres sufficient to trigger ALT? Probably not. Spreading of the receptor binding sites at telomeres using a mutant version of telomerase did not trigger ALT, although it was sufficient to induce some ALT-specific features such as accumulation of single-stranded telomeric $(CCCTAA)_{(n)}$ DNA circles (C-circles) (Conomos et al., 2012). Recent work has pinpointed additional distinct events required for ALT. These include recruitment or mutation of distinct chromatin remodeling factors that contribute to displacement of shelterin, HR factor binding, and HR activation. Among these, binding of the histone deacetylase NuRD is sustained by nuclear receptors (Conomos et al., 2014). Mutations in other factors may support ALT through mismanagement of histone assembly. For example, depletion of the histone chaperone ASF1 induced ALT features, although this protein is not generally mutated in ALT-utilizing cancers (O'Sullivan et al., 2014). However, a strong correlation was found between ALT status and mutations in the SWI/SNF family ATP-dependent helicase ATRX (Lovejoy et al., 2012), and ATRX-loss was recently intimately linked to the onset of recombination at ALT telomeres (Flynn et al., 2015). ATRX loss leads to upregulation of the telomeric long noncoding RNA TERRA, which may perturb protein association with single-stranded telomeric DNA, coinciding with accumulation of replication protein A (RPA) at telomeres. RPA activates the DNA damage protein kinase ATR, which seems important for ALT as ATR inhibition led to selective killing of ALT cells (Flynn et al., 2015). At the same time, loss of ATRX and TERRA upregulation in S phase may promote the formation of telomeric R loops. In R loops, an RNA strand is base paired with the template DNA strand of a DNA duplex, leaving the displaced non-template DNA single stranded. Telomeric R loops are repressed at normal telomeres (Pfeiffer et al., 2013), but they become prevalent in ALT cells, where they promote recombination between telomeric repeats (Arora et al., 2014). Overall, nuclear receptor accumulation at telomeres and ATRX loss seem to represent two essential triggering events for ALT (Figure 1).

In summary, the new work by Marzec et al. elucidates critical roles for nuclear receptors in mediating telomeric chromatin associations in ALT that are essential for recombination. The work provides a model for how nuclear receptor binding sites spread at telomeres and uncovers TTI as a novel mechanism of genome instability in ALT cells. In combination with other recent results, these findings support the hypothesis that ALT activation depends on several molecular events. Finally, this complexity may explain why telomerase reactivation instead of ALT is the more frequently selected route toward immortality of cancer cells.

ACKNOWLEDGMENTS

We thank the Swiss Cancer League and the Swiss National Science Foundation for funding.

REFERENCES

Arora, R., Lee, Y., Wischnewski, H., Brun, C.M., Schwarz, T., and Azzalin, C.M. (2014). Nat. Commun. *5*, 5220.

Conomos, D., Stutz, M.D., Hills, M., Neumann, A.A., Bryan, T.M., Reddel, R.R., and Pickett, H.A. (2012). J. Cell Biol. *199*, 893–906.

Conomos, D., Reddel, R.R., and Pickett, H.A. (2014). Nat. Struct. Mol. Biol. *21*, 760–770.

Déjardin, J., and Kingston, R.E. (2009). Cell *136*, 175–186.

Flynn, R.L., Cox, K.E., Jeitany, M., Wakimoto, H., Bryll, A.R., Ganem, N.J., Bersani, F., Pineda, J.R., Suvà, M.L., Benes, C.H., et al. (2015). Science *347*, 273–277.

Lovejoy, C.A., Li, W., Reisenweber, S., Thongthip, S., Bruno, J., de Lange, T., De, S., Petrini, J.H., Sung, P.A., Jasin, M., et al.; ALT Starr Cancer Consortium (2012). PLoS Genet. *8*, e1002772.

Marzec, P., Armenise, C., Pérot, G., Roumelioti, F.-M., Basyuk, E., Gagos, S., Chibon, F., and Dejardin, J. (2015). Cell *160*, this issue, 913–927.

O'Sullivan, R.J., Arnoult, N., Lackner, D.H., Oganesian, L., Haggblom, C., Corpet, A., Almouzni, G., and Karlseder, J. (2014). Nat. Struct. Mol. Biol. *21*, 167–174.

Pfeiffer, V., Crittin, J., Grolimund, L., and Lingner, J. (2013). EMBO J. *32*, 2861–2871.

Sfeir, A., and de Lange, T. (2012). Science *336*, 593–597.