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REVIEWS

Spotlight

Surviving Telomere Attrition with the MiDAS Touch

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Cancer cells maintain telomere lengths through telomerase activity or by alternative lengthening of telomeres (ALT). Using an engineered model system, a recent study by Min *et al.* reveals that the combination of BLM-mediated DNA resection and telomere clustering, a characteristic of ALT telomeres, catalyzes RAD52-dependent mitotic DNA synthesis (MiDAS) specifically at telomeres to drive ALT activity.

Located at the chromosomal termini, telomeres are composed of double-stranded hexameric repeats ($TTAGGG_n$). To achieve unlimited replicative capacity, most cancers maintain their telomeres via telomerase, a telomere-specific reverse transcriptase. By contrast, a proportion of cancers utilize a telomerase-independent telomere maintenance mechanism, termed ALT. ALT telomeres are characterized by chronic replication stress, making them susceptible to spontaneous double-strand breaks (DSBs). These one-ended DSBs are repaired by break-induced replication (BIR), a type of homology-directed repair. BIR is initiated at ALT telomeres as a consequence of unresolved replication stress, thus resulting in telomere length maintenance by promoting DNA synthesis via the DNA polymerase, Polδ [1]. In addition to this chronic replication stress, ALT cells display G2/M checkpoint defects, overall telomere length heterogeneity, generation of circular extrachromosomal telomeric DNA species (C-circles), and notably the presence of ultra-bright telomeric DNA foci that often colocalize with promyelocytic leukemia protein (PML) to form ALT-associated PML bodies (APBs) [2].

APBs have been considered to be a platform for telomere recombination in ALT cells because defects in APB formation are associated with decreased ALT activity [3]. However, exactly what drives APB formation and whether the formation of APBs is necessary and/or sufficient to initiate ALT activity remains unclear. In normal cells, PML produces membraneless nuclear condensates which are thought to facilitate several integral cellular processes including transcription, apoptosis, senescence, and response to DNA damage. The formation of PML nuclear condensates is mediated by the SUMO pathway, suggesting that sumoylation may also contribute to the recruitment of telomeres to PML during APB formation.

In a recent issue of *Genes and Development*, Min *et al.* [4] attempted to functionally mimic PML nuclear body formation by generating a multivalent scaffold protein composed of human SUMO3 (polySUMO) repeats and SIM repeats from PIASx (polySIM) to drive the formation of condensates through liquid–liquid phase separation (LLPS) [5]. Through the addition of nuclear localization signals and telomere-specific functional domains, this artificially engineered model system generates APB condensates in the nucleus of live cells. However, as shown by the authors, APB formation alone is insufficient to drive ALT activity, implying that the initiation of ALT requires additional factors.

Recent studies have demonstrated that the 3′–5′ BLM helicase is essential for ALT telomere maintenance [6], providing Min *et al.* with a candidate to test in the new scaffolding system. Incredibly, APB formation combined with overexpression of the BLM helicase led to the rapid induction (24–72 h) of ALT phenotypes, including heterogeneous telomere lengths and the production of C-circles. The induction of ALT activity was dependent on the interaction of BLM with the 5′ flap endonuclease DNA2, but was independent of BLM function in the dissolvasome complex (BLM/TOP3A/RMI). These data suggest a role for BLM in long-range DNA resection at ALT telomeres. However, this increase in DNA resection caused cells containing both the scaffolding proteins and BLM to undergo mitotic arrest, creating optimal conditions for DNA synthesis exclusively in mitosis (MiDAS) [7]. Mechanistically, the MiDAS observed at ALT telomeres was consistent with the MiDAS observed in genomic DNA, demonstrating a general requirement for RAD52. In summary, the authors propose that telomere clustering, combined with BLM-mediated DNA resection, catalyzes RAD52-dependent MiDAS that drives ALT activity at telomeric DNA.

Almost 30 years ago, using *Saccharomyces cerevisiae* as a model system, two different ALT mechanisms emerged from telomerase-deficient yeast survivors – type I and type II [8]. Type I survivors are characterized by tandem amplification of the Y′ element followed by a very short stretch of homogeneous telomeric repeats. By contrast, type II survivors are characterized by very heterogeneous telomeric repeats that are overall much longer than the telomeric repeats in type I survivors. In yeast, both type I and type II mechanisms are dependent on DNA polymerase POL32, a subunit of the Polδ complex, suggesting that both yeast ALT mechanisms rely on BIR. Moreover, although both mechanisms are dependent on RAD52, type I survivors maintain telomeres via Rad51-dependent recombination, whereas type II survivors maintain telomeres via a Rad51-independent mechanism. In human cancer cells, the model

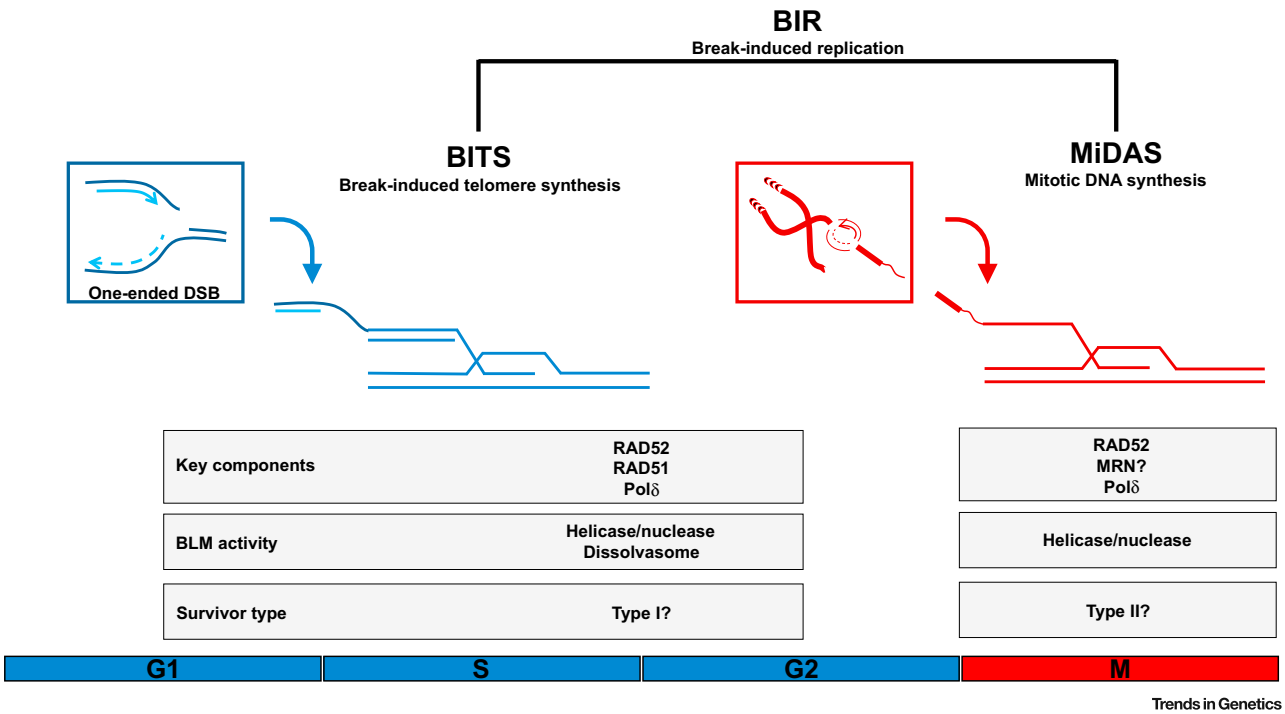

Figure 1. Proposed Different ALT Mechanisms in Human Cancer Cells Initiated by Break-Induced Replication (BIR) That Mimic Yeast Mechanisms (Type I and Type II) in Telomerase-Deficient Survivors.

Type I is at least partially dependent on RAD51 to promote break-induced telomere synthesis (BITS) outside of mitosis. By contrast, type II represents RAD51-independent RAD52-dependent MiDAS. Abbreviation: DSB, double-strand break.

beginning to emerge is one where, as in yeast, BIR drives ALT telomere elongation via Polδ, and this telomere DNA synthesis, in part, relies on RAD51-independent RAD52-dependent MiDAS [9]. This ALT mechanism resembles type II survivors in yeast, raising the possibility that mammalian cells also encompass analogous type I and type II ALT mechanisms (Figure 1). However, some open questions remain to be fully addressed. For example, if MiDAS resembles type II survivors, does BIR outside of mitosis accurately mimic the mechanism used by type I survivors? Alternatively, as recently demonstrated [6], perhaps telomere crossover events in the absence of true telomere elongation more accurately phenocopy the type I survivors?

The model system created by Min *et al.* [4] represents a step forward in further defining ALT, and may even help to identify potential ALT-specific vulnerabilities. Subsequent improvements of this model that allow continuous proliferation of cells to more accurately reflect ALT in human cancer will undoubtedly expand the available tools within the field. In addition, it will be interesting to move this model system into different cellular backgrounds because ALT can be activated in some cellular and genetic contexts, but not in others. For example, tumor types enriched in ALT include gliomas, sarcomas, and pancreatic neuroendocrine tumors [10]; however, each tumor type has a specific genetic context in which ALT occurs. Therefore, defining the requirements to induce ALT in these contexts may also shed light on the initiation and progression of ALT-positive cancers. Finally, given that APBs constitute major sites for telomeric synthesis, and may be an essential step in ALT, targeting APB formation and/or disassembly may prove therapeutically tractable in patients with ALT-positive cancers.

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Spotlight

De novo DNA Methylation: Who's Your DADdy?

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DNA methylation regulates the organization and function of the genome. Yamanaka *et al.* now report that *de novo* methylation of male germ cells of mice involves the transient opening of heterochromatin at megabase-size differentially accessible domains (DADs). This chromatin remodeling likely facilitates *de novo* methylation of the germ cell genome.

Throughout a man's lifetime, the odds of any individual sperm cell he produces influencing the next generation is worse than winning the lottery. However, each of his gametes, fruitful or wasted, represents an epic feat of chromatin reorganization and proper spatiotemporal gene expression. From the start, primordial germ cells (PGCs) are given the colossal task of epigenetic reprogramming involving genome-wide erasure of repressive 5-methylcytosine marks, removing parental imprints, and creating a naïve potency state to prime sex-specific cell differentiation [1]. Following their migration to the embryonic testis, male PGCs (now termed 'gonocytes') undergo mitotic arrest and rapidly re-establish DNA methylation *de novo*, an essential task for differentiation into spermatogonia, the source of sperm. Indeed, absence of *de novo* methylation in mouse male germ cells, such as in DNA methyltransferase (DNMT) 3 family mutants *Dnmt3a*$^{-/-}$ *and Dnmt3l*$^{-/-}$, results in failure to methylate paternal imprinting control regions, activation of transposable elements (TEs), and impaired spermatogenesis [2,3].

Two waves of *de novo* DNA methylation have been reported in mouse male gonocytes between embryonic day (E) 13.5 and postnatal day (P) 2, transitioning around E16.5 [4]. The first wave encompasses *de novo* methylation of most of the genome, while the second uses small Piwi-interacting (pi) RNAs to guide methylation of TEs [4]. One plausible feature distinguishing these waves is the accessibility of DNMTs to the genome to deposit DNA methylation marks. In dividing cells, DNMT recruitment is tightly coupled to DNA replication, taking advantage of locally unwound DNA at replication forks during S phase to gain occupancy of the genome in both euchromatic and heterochromatic regions [5]. However, in cell cycle-arrested gonocytes, the players and strategies for DNMT accessibility and subsequent *de novo* methylation, especially at inaccessible heterochromatic regions, become ambiguous.

Transient Accessibility of Gonocyte Chromatin Reveals DADs

To understand where and when *de novo* DNMTs might be recruited to the genome, Yamanaka *et al.* used pure populations of germ cells to probe the dynamics of genome-wide chromatin accessibility [6]. By performing assay for transposase-accessible chromatin using sequencing (ATAC-seq) to probe chromatin accessibility and NanoCAGE-seq to determine transcriptional start site and transcript abundance, the authors identified specific chromatin regions that acquire greater accessibility between E13.5 and E17.5, and subsequently close by P2. Such timing implicates these gonocyte-specific DADs in the second wave of *de novo* DNA methylation. Using the stringent 1-Mb minimal size cutoff, Yamanaka *et al.* identified a total of 143 DADs on all chromosomes, except the Y chromosome, accounting for ~14% of the mouse genome.

Genomic Features of DADs Highlight TEs

The authors next interrogated the defining features of DADs. Ultimately, 110 of the 143 DADs were found in gene-poor regions of the genome that encode fewer than ten genes per megabase and are instead enriched in TE sequences (70% of E17.5-specific peaks on TEs). Interestingly, a few DADs also included gene clusters, such as those belonging to olfactory receptor or