Telomere-binding protein TRF2 protects the linear chromosome ends, telomeres, from being recognized as damaged DNA. TRF2 also regulates gene expression outside telomeres, but the detailed mechanism has not been fully understood. Mukherjee and colleagues have employed ChIP-Seq and biochemical analyses to identify G-quadruplexes at gene promoters across the genome as nontelomeric TRF2-binding sites. TRF2 occupancy on such target sites leads to epigenetic gene repression, implicating TRF2–G-quadruplex interaction as a sophisticated regulator of gene expression.

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2 The abbreviations used are: TRF2, telomeric repeat-binding factor 2; G4, G-quadruplex; PG4 motif, potential G4-forming motif; REST, RE1-silencing transcription factor; TPE, telomere position effect; TSS, transcription start site.

From the wings to the center stage of chromosomes

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Telomeres are the specialized nucleoprotein complexes at chromosome ends. A critical difference between a telomere and a broken DNA strand is that the former is protected by a specific protein complex called shelterin. Among the shelterin components, TRF2 directly binds to telomeric TTAGGG repeats to play an essential protective role. Accumulating evidence also indicates that TRF2 has a nontelomeric function. For example, TRF2 has been shown to interact with the gene repressor RE1-silencing transcription factor (REST), thus regulating neuronal differentiation (1). A subsequent mechanistic study explained that TRF2 binds to specific gene promoters outside telomeres and recruits REST and lysine-specific demethylase 1, which alters histone modifications to a repressive status (2). However, the precise mechanism for TRF2 binding to such promoters remained elusive. Simultaneously, there have been hints that TRF2 could associate with noncanonical nucleic acid structures called G-quadruplexes (G4s), which are definitive stacks of Hoogsteen-bonded guanine tetrads, but the broader significance of these results was unclear. Now, Mukherjee and colleagues confirm that TRF2 binds to G4s at transcription start sites (TSSs) across the genome (3). They demonstrate that this TRF2–G4 interaction alters the epigenetic status of the promoter and resultant gene expression. These observations bring TRF2 out onto the center stage of the genome and additionally change our understanding of G4 from a mere obstacle in the genome into a functional mediator of the epigenome.

The initial idea that TRF2 might have a nontelomeric function came from the finding that TRF2 can bind to TTAGGG sequences outside of telomeres, in intergenic regions (i.e. between genes) (4). For example, TRF2 was shown to bind and transactivate the platelet-derived growth factor receptor β gene, the promoter of which contains a single TTAGGG sequence (5). In another study, TRF2 occupancy at specific gene promoters has been shown to be reduced by telomere elongation, presumably because TRF2 is preferentially sequestered by long double-stranded telomeric repeats. Accordingly, shorter telomeres allow larger numbers of TRF2 to associate with nontelomeric gene promoters, which in turn increases repressive histone modifications and reduces gene expression (2). Curiously, previous research also showed that TRF2 can bind ssDNA, whether telomeric or not, with comparable affinities and can even interact with sequences that do not contain the TTAGGG motif (2). How then does TRF2 recognize these sequences, and are these interactions functionally relevant?

To identify TRF2-binding sites in a genome-wide manner, Mukherjee et al. performed ChIP-Seq for endogenous TRF2 in human fibrosarcoma cells. As expected, many of the reads corresponded to telomeric (TTAGGG)2 repeats (3). However, the majority of reads did not. Looking more carefully, the authors identified 1,956 TRF2-high-confidence (TRF2HC) peaks, which were enriched near promoters and TSSs. Among them, only 465 had at least one interstitial TTAGGG sequence, indicating that the rest of the peaks binds to TRF2 without TTAGGG. Importantly, potential G4-forming motifs (PG4 motifs) were enriched in TRF2HC peaks compared with randomly selected peaks.

The authors then focused on nine promoters that overlap with TRF2HC peaks and have at least one PG4 motif within 500 bp of the TSS. CD profiles of the same sequence oligonucleotides indicate the formation of parallel or antiparallel/hybrid G4s for all of the nine sequences in vitro. Enzyme-linked immunosorbent and fluorescence-intercalator displacement assays showed specific interaction of a recombinant TRF2 with these G4s but not with mutant oligonucleotides that would be unlikely to form G4 structures. Consistent with these observations, in fibrosarcoma cells, TRF2 binding to the relevant G4 sites was confirmed by a ChIP assay. Ectopic TRF2 overexpression in the cells increased the TRF2 occupancy on the nine promoters, resulting in epigenetically suppressed histone modifications and respective gene down-regulation, except for one gene, which was activated. On the other hand, TRF2 deletion mutants lacking either the N-terminal basic domain known to be required for G4 binding (6) or the C-terminal Myb domain known to bind double-stranded telomeric DNA did not down-
regulate the target gene expression. This stands in contrast to the telomere-capping function of TRF2, which does not require the basic domain (7). Finally, 360A, a chemical compound that binds to G4 (G4 ligand), displaced TRF2 from the promoters and rescued the TRF2-mediated gene suppression in five of nine genes. Together, this study provides new insight into the nontelomeric function of TRF2 and potential biological significance of G4 in the transcriptional regulation of the genome (Fig. 1). Because telomeres gradually shorten at each cell cycle and eventually elicit senescence in human somatic cells, telomeres are likened to a “mitotic clock,” which counts the number of allowed cell divisions. Besides such established functions for chromosome stability and cell proliferation capacity, telomere length is also implicated in gene regulation and tissue reorganization (8). Telomeres can affect gene regulation via several mechanisms: (i) the telomere position effect (TPE), in which gene expression near the long telomeres is repressed; (ii) TPE over long distances (TPE-OLD), which occurs through the chromosome looping; (iii) gene repression by telomeric non-coding RNA (TERRA); and (iv) telomere sequestering of transcriptional activator/repressor proteins, such as Rap1 and TRF2 (2). TRF2 binding to the PG4 motifs across the genome corroborates this final mechanism, in expanding the concept of nontelomeric functions of the telomere maintenance system. It would be of interest to elucidate the functional significance of TRF2-regulated gene sets in the physiological and pathological contexts.

It has been postulated that intracellular dynamics of G4 formation and resolution could affect replication, transcription, and translation. However, the biological significance of promoter-associated G4s was not fully understood. Therefore, the G4-mediated TRF2 recruitment to specific promoters and subsequent alteration of the epigenetic modification are true highlights of the present work, although when and how G4s are formed remains elusive (3). In a therapeutic aspect, G4 stabilization by chemical ligands is a promising strategy to target cancer, including intractable glioma stem cells (9). Because cancer cells often maintain telomeres shorter than normal cells, it might be possible that TRF2 occupancy on PG4 motifs increases in cancer cells and alters the gene expression. ChIP-Seq with a G4-recognizing antibody has demonstrated a genome-wide distribution of G4s, which are enriched at the promoters and 5’-untranslated regions of transcriptionally active genes, especially those related to cancer (10). Although not yet directly addressed, these gene sets might be therapeutic targets of G4 ligands. In conclusion, now we can further broaden our view of telomere biology toward more integrated life science for human health.

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**Figure 1. A model for TRF2-mediated gene regulation through interaction with G-quadruplexes.** In a cell with long telomeres, TRF2 occupancy at the nontelomeric promoters through interaction with G-quadruplexes is low, allowing their expression (top). In a cell with short telomeres, TRF2 occupancy at nontelomeric promoters is elevated, causing epigenetic repression of the genes (bottom).