Stuttering against marginotomy

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Stuttering by telomerase contributes to the natural heterogeneity of fission yeast telomeric repeat sequences.

In the early 1970s it was realized that practically every bit of DNA synthesis of any informational importance occurs in a primer- and template-dependent fashion. These requirements left the very ends of any linear DNA without an obvious means for replication. This became known to a few as marginotomy¹, a widely underused word that defines the 'end-replication problem'. Now we know that evolution has found numerous solutions to the endreplication problem that serve linear DNA molecules from tiny viral genomes to footlong eukaryotic chromosomes. A successful solution is provided by the RNA-protein enzyme telomerase. In this issue of Nature Structural & Molecular Biology, two groups have identified the RNA component of the telomerase in fission yeast^{2,3}. Their studies begin to answer the question of why the telomere repeats of this yeast are so heterogeneous.

Telomerase solves the end-replication problem in many eukaryotes by adding a short repeated sequence of DNA to the 3' ends of chromosomes (for review, see ref. 4). This does not violate the template dependence of DNA synthesis because telomerase carries its own template in a specialized region of its RNA subunit. Each organism has a characteristic DNA repeat sequence at its telomeres, and the nature of this repeat is dictated by the particular features of the telomerase enzyme. In addition, the precise sequences created by telomerase are important for the binding of telomere proteins that have essential roles in telomere function. Without enough telomerase, chromosome ends get shorter with each replication cycle, leading to senescence. With too much telomerase, growth control and programmed cell mortality may become unhinged⁴.

In many organisms it is easy to see how the telomerase RNA template directs the synthesis of the repeat, because the template region is perfectly complementary to a uniform telomeric repeat. For example, both *Tetrahymena thermophila* and human telomerases have permuted template sequences corresponding to one and a half repeats, allowing a completed repeat to be translocated to a pairing site on the template for the next repeat addition⁴.

For other organisms, in particular the fission yeast Schizosaccharomyces pombe, it has been less obvious what sort of telomerase RNA template sequence would give rise to the different classes of repeats found at the telomeres^{5,6}. To find the S. pombe telomerase RNA, Webb and Zakian³ and Leonardi et al.² have used similar tactics. Each group immunoaffinity purified the RNA in association with a tagged telomerase protein and then reverse transcribed and cloned the RNA using primers designed to exploit the presence of the expected template core sequence 5'-GUAACC-3'. These efforts led to the identification by both groups of an ~1,200-nucleotide RNA with features in common with other telomerase RNAs. Both groups proved that the RNA, now called TER1, is the templating subunit of telomerase on the basis of the way substitutions in and near the putative template sequence became reflected in the telomeric sequences^{2,3}.

Webb and Zakian³ designed mutations to test the prediction that a stem loop just 5' of the template region behaves as a template boundary element (TBE)⁴. This shows that





the base of a predicted stem just at the 5' side of the template region (where the 3' end of the chromosome would lie after elongation) acts as a barrier to continued elongation, as has been shown for similar structures in other telomerase RNAs⁴. An intriguing complication is that the last base pair of this stem is predicted to be an A-U pair (shown in blue with a filled circle in **Fig. 1**), in which the U is positioned to template an additional A in the telomere (shown in gray) should the TBE open at this base pair. Some telomeric repeats have an additional C, which is consistent with this idea given that the next base pair in the TBE

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is a C-G pair (**Fig. 1**). Webb and Zakian³ propose that the heterogeneity at the 3' ends of the core telomeric repeat 5'-GGTTAC-3' in *S. pombe* is due to an impaired ability of the TBE to prevent its own use as a template, a hypothesis consistent with their experimental test. But what of the heterogeneity at the 5' end of the core repeat?

One of the most fascinating steps in the telomerase mechanism is translocation, the process by which the 3' end of a completely elongated repeat is moved from the 5' to the 3' end of the template for another round of reverse transcription⁴. Because telomerase activity is processive, the complex must favor this movement without risking dissociation of the telomere. This rebinding event requires melting of the completed telomere end from the template and stabilizing of the repositioned primer end at the 3' end of the template. In addition to other sequence-specific and nonspecific interactions that may exist, base pairing between the last few bases of the finished repeat and the 3' end of the template are key to this rebinding event, and would most directly lead to the next cycle of DNA synthesis⁴. Here is where things appear to get dicey for the *S. pombe* telomerase (**Fig. 1**).

If reverse transcription stops at the TBE, then the telomere ends with 5'-TAC-3'. Primer rebinding could occur perfectly at the 3'-AUG-5' sequence at the 3' end of the template region, just as in other telomerases with permuted template sequences (note how the absence of the gray A in Fig. 1 would eliminate the mismatch). Unfortunately, the A-U pair at the base of the TBE seems inadequate for its task, opening its 3' U to be copied (adding the gray A in Fig. 1) some 80% of the time². What happens next leads to heterogeneity at the 5' end of the repeats. Both groups propose that primer rebinding leads to the presence of an A-C apposition in the active site, leaving a mismatched primer end poised to add a G as the next nucleotide^{2,3}.

It is envisioned that after templating of this G by the 5'-most (and aligned) C, the primer slips back one nucleotide in a kind of stutter step that now pairs the newly added G with the (formerly mismatched) C (**Fig. 1**), allowing the addition of a second G, followed by efficient elongation through a two-base priming event^{2,3}. Alternatively, the

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Sequence	Frequency	Interpretation		
None	0.14	$GGTTAC \rightarrow GGTTAC$, no stutter		
A 0.29		$GGTTACA \to GGTTAC, stutter once$		
AG	0.09	GGTTACA ightarrow GGGTTAC, stutter twice		
AGG	0.13	GGTTACA \rightarrow GGGGTTAC, stutter 3 times		
AGGG	0.03	GGTTACA \rightarrow GGGGGTTAC, stutter 4 times		
AGGGG	0.06	GGTTACA \rightarrow GGGGGGTTAC, stutter 5 times		
C 0.07		$GGTTACAC \to GGTTAC, no stutter$		
Totals by TBE read in class	Frequency	Interpretation		
0, leads to GGTTAC	0.14 (0.19)	No requirement for stutter, none observed		
1, leads to GGTTACA	0.60 (0.73)	Must stutter, always observed		
2, leads to GGTTACAC	0.07 (0.07)	No requirement for stutter, none observed		
Other events	0.19 (NA)	Other nontemplated nucleotides		

Data are taken from Figure 3b of ref. 6. Data in parentheses refer to the class of repeat, rather than the sequence between repeats, and are from Supplementary Figure 4 of ref. 2. NA, not available.

stuttering process can be repeated multiple times, incorporating additional G residues^{2,3} before the completion of elongation. How interactions with the rest of the primer change during stuttering is unclear, but shifting the primer-template interactions in register would eliminate base pairing, suggesting that the extra residues are looped out or that other contacts are required. Alternative events in which primers ending with the extra A can lead directly to repeat synthesis without stuttering are also possible, as pointed out by Leonardi *et al.*².

What is the evidence that invasion of the TBE at the 3' end of the repeat leads to stuttering at the 5' end of the next repeat? This idea predicts that multiple G residues should be observed only after repeats that have the extra A residue. An exception might be when two bases of the TBE are copied, as that leads to repeats ending in AC, which could be correctly aligned. We reviewed the data of Trujillo et al.⁶, in which the frequencies of spacer sequences between perfect core repeats were determined from cloned telomeres. It seems clear that multiple Gs arise only when the A is included and not when no 'extra' A is found in the upstream repeat (Table 1). The frequencies of these classes and the molecular events that might lead to them argue for a strong correlation between the addition of the extra A and the stuttering that produces multiple G residues.

The discovery of TER1 suggests that S. pombe telomerase may be an evolutionary work in progress. Its weak template boundary element allows an extra A residue to be added that would appear to pose a problem for telomerase when it is time to realign the primer. This extra A is compensated for by a mysterious stuttering reaction not unlike that observed in mutant telomerases of other organisms *in vitro*⁷. But there is danger in having simple biochemical expectations for an enzyme with as many constraints as telomerase. Although it seems far from optimized for making a perfect array of core repeats, there is no evidence that such a product would function well as a telomere. Given the facility with which S. pombe can be engineered, we may soon find out whether the blind watchmaker is finished with this piece or not.

ACKNOWLEDGMENTS

We thank P. Baumann, T. Cech, K. Collins and V. Zakian for comments.

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