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LETTER

Commentary: FISHing for the light at the ends of chromosomes

The discovery of the structure of telomeres having short DNA sequences $[T_2AG_3]_n$ of tandem repeats and their role in chromosome stability has become the subject of scrutiny and debate.[1–7] It has been known that telomeres serve as a buffer against end to end chromosome fusion and permit the complete replication of the terminal regions retaining chromosomal integrity. Chromosomes without telomeres are highly unstable and frequently lost during cell kinetics.[2–8] There are numerous examples in wheat and barley, where terminally deleted chromosomes are maintained due to the addition of telomeres,[9] a concept verified in human chromosomes as well.[10,11] The addition of $[T_2AG_3]_n$ by telomerase and acquiring a new telomere through recombination (telomere capture), are the two proposed healing pathways for terminally deleted human chromosomes.[12] Knowledge concerning maintenance and regulation of telomere length is rapidly unfolding and it has become quite obvious that a chromosome will not be maintained without a functioning telomere.[2,3,13,14] All of those cases with reported terminal deletions, have at least the minimum $[T_2AG_3]_n$ sequences attached.[10] However, in *Drosophila* another possible mechanism has been proposed where telomeric structures have been formed by large repeated sequences of the non-long terminal repeat retroposon without the presence of telomeric sequences.[15,16]

Brkanac and colleagues[17] performed molecular analysis on those patients with a number of chromosome 18 specific probes including two human 18q telomeric probes; one contained sequences within the distal 270 Kb of 18q. Their results suggest that of the 35 cases with 18q 'terminal deletions' five were more complex and three had 'terminal 18q sequences present.' Telomeric sequences homologous to their probe were not detected in the remaining cases. In our opinion, those remaining cases have at least the $[T_2AG_3]_n$ 'cap' attached to them, possibly with the chromosome 18 specific telomere associated sequences (TAS) missing. Flint *et al*[11] characterized patients with terminal deletions on 16p with alpha thalassemia truncations who were stabilized with the addition of telomeric repeats but were missing any TAS sequences. Vermeesh and colleagues[18] characterized telomeres in four patients with Cri du chat syndrome [del(5)(p13.3;pter)] and a patient with Wolf-Hirschhorn syndrome [del(4)(p15.1;pter)]. They too emphasized that the main mechanism of chromosome healing was due to *de novo* telomerase synthesis via telomerase.[16] This appears to be the case and more and more data are supporting this scenario.[10,19] A $[T_2AG_3]_n$ probe can identify the 'newly acquired' telomeres. If indeed the entire terminal area including the telomere is deleted but a new telomere is added for stability, would this still be considered terminal? And should it be classified with an interstitial deletion that retains just the TAS and telomeric sequences of the original chromosome? Since the ends of chromosomes have been found to be rich in transcribed sequences,[20] it is necessary to determine the nature of deletions, specifically since the second proposed nature of acquiring new telomeres is 'telomeric capture'. Chromosomes with terminal deletions are very frequent in human neoplasia and they are both stable and retained. A number of mechanisms have been proposed.[12,18,21–27]

In recent years, exhaustive literature has been generated to emphasize the importance of FISH technique using whole chromosome and loci specific probes.[28–34] Nevertheless, like any other staining procedures, there are many pitfalls associated with this technology,[35] and one can easily be misled while characterizing a segment of DNA containing highly repetitive sequences. For example, the alphoid sequences of centromeres do not always stain positively by FISH probes.[35,36] Should we assume that because a targeted region is below detection levels by FISH techniques, it does not have a centromere? Likewise, can a chromosome exist without having a centromere? Obviously, the resolution of certain probes is not high enough to detect the presence of all centromeres, which can equally be true for highly polymorphic TAS and telomere sequences.[37,38] Recent advances in peptide–nucleic acid (PNA) based technology can be used to produce FISH probes for telomerase sequences. Hopefully, PNA probes will be able to detect the unique $(T_2AG_3)_n$ sequences and may also address the above

problem.[39] Numerous articles suggesting there are no telomeres present on deleted chromosomes have clearly challenged a very fundamental concept of chromosome stability! Nevertheless, in our experience, terminally deleted chromosomes have retained/healed the terminal ends with telomeres. It is the telomeres which retain chromosomal integrity; as both Muller[40] and McClintock[41] knew long ago, all cannot be well unless chromosomes end well.

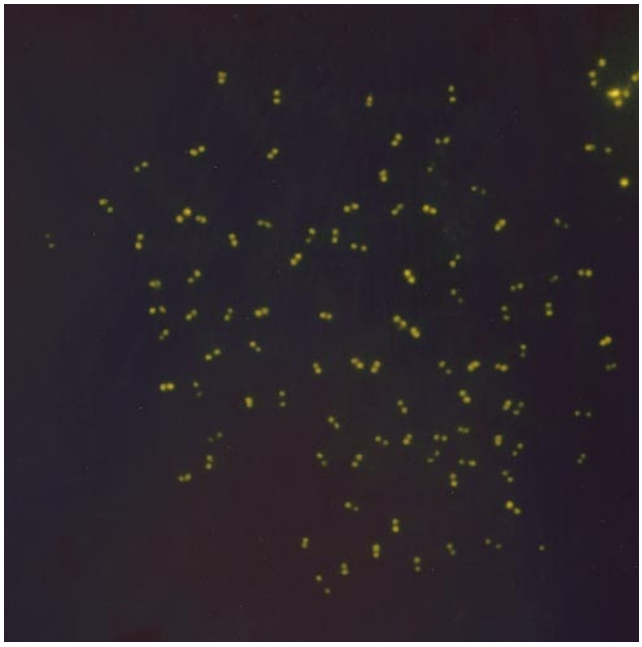

Figure 1 *Human telomeres detected with PNA (peptide nucleir acid) probe for the telomere repeat sequence (courtesy of E. de Pauw, Department of Molecular Cell Biology, Leiden University Medical Centre, The Netherlands).*

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References

1 Giulotto E, Mondello C: In: Verma RS (ed.). *Advances in Genome Biology: Genes and Genome*, JAI Press: Greenwich, CT, 1998, vol 5B, pp 323–361.
2 Kipling D (ed.): *The Telomere.* Oxford University Press: Oxford, 1995.
3 Brenner S: *Telomeres and Telomerase*, Wiley and Sons: New York, 1998.
4 Wellinger RJ, Ethier K, Labrexque P, Zakian VA: Evidence for a new step in telomere maintenance. *Cell* 1996; **85**: 423–433.
5 Biessman H, Mason JM: Telomere maintenance without telomerase. *Chromosoma* 1997; **106**: 63–69.
6 Slijepcevic P: Telomeres and mechanisms of Robertsonian fusion. *Chromosoma* 1998; **107**: 136–140.
7 Zakian VA: Telomeres: beginning to understand the end. *Science* 1995; **270**: 1601–1607.
8 Zakin VA: Life and cancer without telomerase. *Cell* 1997; **91**: 1–3.
9 Werner JE, Kato RS, Gill BS: Distribution of telomeric repeats and their role in the healing of broken chromosome ends in wheat. *Genome* 1992; **35**: 844–848.
10 Guo W-J, Callif-Daley F, Zapata MC, Miller ME: Clinical and cytogenetic findings in seven cases of inverted duplication of 8p with evidence of a telomeric deletion using fluorescence *in situ* hybridization. *Am J Hum Genet* 1995; **58**: 230–236.
11 Flint J, Craddock CF, Villegas A *et al*: Healing of broken human chromosomes by addition of telomeric repeats. *Am J Hum Genet* 1994; **55**: 505–512.
12 Meltzer PS, Guan X-Y, Trent JM: Telomere capture stabilizes chromosome breakage. *Nat Genet* 1993; **4**: 252–255.
13 Slijepcevic P, Bryand PE: Chromosome healing, telomere capture and mechanisms of radiation-induced chromosome breakage. *Int J Radiat Biol* 1998; **73**: 1–13.
14 Linger J, Cech TR: Telomerase and chromosome end maintenance. *Curr Opin Genet Devel* 1998; **8**: 226–232.
15 Melek M, Shippen DE: Chromosome healing: spontaneous and programmed *de novo* telomere formation by telomerase. *Bioassays* 1996; **18**: 301.
16 Leuis RW, Ganesan R, Houtchpns K, Tolar LA, Sheen F: Transposons in place of telomeric repeats at a *Drosophila telomere* . *Cell* 1993; **75**: 1083–1093.
17 Brkanac Z, Cody JD, Leach RJ, DuPont BR: Identification of cryptic rearrangements in patients with 18q- deletion syndrome. *Am J Hum Genet* 1998; **62**: 1500–1506.
18 Vermeesch JR, Falzetti D, Van Buggenhout G, Fryns JP, Maryanen P: Chromosome healing of constitutional chromosome deletions studied by microdissection. *Cytogenet Cell Genet* 1998; **81**: 68–72.
19 Lamb J, Harris PC, Wilkie AOM, Wood WG, Dauwerse JHG, Higgs DR: *De novo* truncation of chromosome 16p and healing with $(TTAGGG)_n$ in the alpha thalassemia/mental retardation syndrome (ATR-16). *Am J Hum Genet* 1993; **52**: 668–676.
20 Saccone S, De Sario A, Della Valle G, Bernardi G: The highest gene concentrations in the human genome are in telomeric bands of metaphase chromosomes. *Proc Natl Acad Sci USA* 1992; **89**: 4913–4917.

21 Wang S-S, Zakian VA: Telomere–telomere recombination provides an express pathway for telomere acquisition. *Nature* 1990; **345**: 456–458.
22 Morin GB: Telomere integrity and cancer. *J Natl Cancer Inst* 1996; **88**: 1095–1096.
23 Ning Y, Liang JC, Nagarajan L, Schrock E, Reid T: Characterization of 5q deletions by subtelomeric probes and spectral karyotyping. *Cancer Genet Cytogenet* 1998; **103**: 170–172.
24 Nugent CI, Bosco G, Ross LO, Evans SK, Haber JE, Lindblad V: Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr Biol* 1998; **8**: 657–661.
25 Rowley PT: Telomerase: Putting an end to DNA. *Cancer Invest* 1998; **16**: 170–174.
26 Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR: Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat Med* 1997; **3**: 1271–1274.
27 De Lange T: Telomere dynamics and genome instability in human cancer. In: Blackburn IH, Greider CW (eds). *Telomeres.* Cold Spring Harbor Laboratory Press: New York, 1995, pp 265–293.
28 Verma RS, Babu A: *Human Chromosome: Principles and Techniques.* McGraw-Hill: New York, 1995.
29 Verma RS, Batish SD, Ramesh KH, Gogineni SK: Are we FISHing in troubled waters? *Cell Mol Biol Res* 1995; **41**: 81–84.
30 Neslinger NJ, Gorski JL, Kurczynski TW, *et al.*: Clinical, cytogenic, and molecular characterization of seven patients with deletions of chromosome 22q13.3. *Am J Hum Genet* 1994; **54**: 464–472.
31 Precht KS, Lese CM, Spiro RP, *et al.*: Two 22q telomere deletions serendipitously detected by FISH. *J Med Genet* 1998; **35**: 939–942.
32 Kurenova EV, Mason JM: Telomere functions: A review. *Biochemistry (Mosc)* 1997; **62**: 1242–1253.
33 Urquidi V, Tarin D, Goodison S: Telomerase in cancer: clinical applications. *Ann Med* 1998; **30**: 419–430.
34 Bertuch A, Lundblad V: Telomeres and double-strand breaks: trying to make ends meet. *Trends Cell Biol* 1998; **8**: 339–342.
35 Verma RS, Luke S: Variation of alphoid DNA sequences escape detection of aneuploidy at interphase. *Genomics* 1992; **14**: 113–11.
36 Verma RS, Batish SD, Gogineni SK, Kleyman SM, Stetka DG: Centromeric alphoid DNA heteromorphism of chromosome 21 revealed by FISH-technique. *Clin Genet* 1997; **51**: 91–93.
37 Burr B, Burr FA, Matz EC, Albertsen MC, Stuber CW: Pinning down loose ends: mapping telomeres and factors affecting their length. *Plant Cell* 1992; **4**: 953–960.
38 Biessmann H, Manson JM: Telomeric repeat sequences. *Chromosoma* 1994; **103**: 154–161.
39 Senior K: Going FISHing with peptide nucleic acid. *Mol Med Today* 1998; **4**: 231.
40 Muller HJ: The remaking of chromosomes. *Coll Net* 1938; **8**: 182–195.
41 McClintock B: The stability of broken ends of chromosomes of *Zea mays. Genetics* 1941; **26**: 234–282.