Of wombats and whales: telomere tales in Madrid

Conference on Telomeres and Telomerase

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The CNIO/Josef Steiner Cancer Conference: Telomeres and Telomerase took place in Madrid, Spain between 13 and 15 November 2006, and was organized by M. Blasco and J. Shay.

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and Carol Greider received the 2006 Lasker Award (de Lange, 2006; Lundblad, 2006). The Josef Steiner Cancer Conference on Telomeres and Telomerase took place in the centre of Madrid, at the Centro Nacional de Investigaciones Oncológicas (CNIO) campus, with Blackburn as the guest of honour and representative of the Lasker awardees. One of the highlights was a formal dinner at the Casino de Madrid, where Titia de Lange summarized Blackburn's contributions in a moving speech. De Lange pointed out that the telomere field, with its rich variety of model organisms, approaches and nationalities, as well as its healthy tradition of equal gender representation, is a fitting reflection of Blackburn's spirit and creativity. Another highlight was that, after a year of absence, Ginger Zakian and Woody Wright attended the meeting, adding to the special nature and significance of the gathering.

Mechanisms of telomere function

The conference touched on several of the puzzles surrounding the mechanisms of telomere maintenance. Telomeres have traditionally been viewed as sites that distinguish natural chromosome ends from damage-induced DNA ends. However, it is becoming increasingly clear that at least some telomeres are transiently recognized as DNA damage during the late S-phase of each cell cycle (Verdun *et al*, 2005), and indeed several factors involved in DNA-damage surveillance localize to telomeres (d'Adda di Fagagna *et al*, 2003; Oikemus *et al*, 2004; Takai *et al*, 2003; Takata *et al*, 2004, 2005; Verdun *et al*, 2005; Zhu *et al*, 2000). Intriguingly, recent data have also implicated participants in a second nucleic-acid surveillance pathway, the non-sense-mediated mRNA decay pathway (NMD), in both DNA-damage response and telomere maintenance. Human ever-shorter telomeres 1A (EST1A)—homologous to budding and fission yeast Est1—is required for the activity of telomerase, the ribonucleoprotein reverse transcriptase complex that is engaged during late S-phase at a subset of telomeres (see below) to synthesize the most terminal telomere repeats. Furthermore, EST1A efficiently immunoprecipitates human telomerase activity (Reichenbach *et al*, 2003; Snow *et al*, 2003). At the same time, EST1A is essential for NMD (Chiu *et al*, 2003). J. Lingner (Epalinges, Switzerland) showed that this duality of function extends to Upf1, a helicase that is required for NMD. Upf1 is not only a substrate of the ATM-related (ATR) kinase during S-phase, but also interacts with both S-phase chromatin and DNA polymerase δ , and is required for S-phase completion (Azzalin &

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Introduction

In November 2006, a diverse group of telomere researchers gathered in Madrid to celebrate two high-profile recognitions. First, the 2003 Josef Steiner Cancer Research award to Maria Blasco allowed her to organize this Cancer Conference with the help of Jerry Shay. Blasco was honoured for her contributions to our understanding of the connections between telomere function and cancer development. This task was made possible by the discovery of telomerase—the ribonucleoprotein enzyme that extends the telomere repeat tracts that comprise the terminal DNA sequences of each chromosome. Second, it was for this crucial discovery that Elizabeth Blackburn, Jack Szostak

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Lingner, 2006). Strikingly, new studies from the Lingner laboratory show that Upf1 interacts with telomerase and that depletion of Upf1 leads to the abrupt loss of some telomeres. These data suggest a model in which Upf1 links S-phase completion to telomere processing and telomerase activation. Generation of a DNA-damage response at telomeres undergoing DNA replication might trigger ATR-mediated phosphorylation of Upf1, in turn leading to its interaction with EST1A and telomerase.

Another crucial puzzle surrounding S-phase events is the feature that determines whether individual telomeres are accessible to telomerase activity. Telomeres seem to exist in two states of accessibility to telomerase—extendible and non-extendible—with the shortest telomeres in the cell being preferentially in the extendible state (Hemann *et al*, 2001; Teixeira *et al*, 2004). The molecular differences between these two states are still not understood; it could be that the extendible state arises when telomeres become so short that they lose their invisibility to the DNA-damage processing activities that create a telomerase substrate. Alternatively, shorter telomeres might adopt a conformation that engages specific telomere-processing activities and/or telomerase. V. Zakian (Princeton, NJ, USA) reported on progress towards defining the molecular differences between short and long telomeres. Zakian discussed experiments that used a system developed by Marcand and colleagues (Marcand *et al*, 1999). In this system, a unique telomere can be abruptly shortened on demand by expressing the Flp site-specific recombinase to excise a section of telomeric DNA bounded by Flp recombinase target (FRT) recombination sites. Hence, a specific 'extendible' telomere can be generated and its components monitored. The Zakian group found that the budding yeast ataxia telangiectasia mutated (ATM) orthologue Tel1 binds preferentially to short telomeres with peak levels of binding during late S-phase—the period in which these telomeres are elongated by telomerase. Est1 and Est2, the budding yeast telomerase catalytic subunit, are also enriched at short rather than long telomeres, and this preferential binding depends on Tel1. Interestingly, some telomere-length regulators, such as Cdc13, Rif1 and Ku80, do not localize preferentially to short telomeres, suggesting that these factors bind constitutively to telomeres and control telomerase, which itself appears at a subset of telomeres in an ATM-controlled manner.

Telomeres and other heterochromatic regions often localize to the nuclear periphery, raising the long-standing question of whether location is important for function. S. Gasser (Basel, Switzerland) described results that indicate distinct classes of peripheral sites and their possible functional relevance. By using the HO endonuclease to control the generation of a unique irreparable non-telomeric double-stranded break (DSB) in yeast DNA (Lee *et al*, 1998), the Gasser group found that these DSBs associate with components of the nuclear pore complex (NPC) and localize to sites at the nuclear periphery that are distinct from the telomeric foci that remain there. This localization depends on an intact NPC, Mec1 (the yeast homologue of ATR) kinase and the Slx5/8 proteins (RING-finger proteins involved in DNA repair and recombination). Together with recent data showing that subtelomeric recombination is reduced in NPC mutants (Therizols *et al*, 2006) and that the Rad52-recombination pathway becomes essential in NPC mutants (Loeillet *et al*, 2005), these data suggest that certain kinds of DNA-processing pathways are promoted at the nuclear periphery. It remains unknown whether telomeres undergo regulated movements between distinct peripheral sites as the cell cycle progresses.

A great deal of recent attention has focused on protection of telomeres 1 (Pot1), a telomere protein that has a crucial but mechanistically poorly understood role in regulating telomere processing. Loss of fission yeast Pot1 results in immediate and complete telomere loss (Baumann & Cech, 2001). Reduced levels of POT1 in human cells lead to deregulation of telomere length, loss of precision in resection of the telomeric 5' end, a reduced 3'-overhang signal and the formation of telomere dysfunction-induced foci (TIFs) that comprise DNA-damage response factors such as the p53-binding protein 1 (53BP1) and γ H2AX (Hockemeyer *et al*, 2005; Loayza & de Lange, 2003; Loayza *et al*, 2004). Mice harbour two Pot1 paralogues, Pot1a and Pot1b, both of which have been studied in conditional knockout mouse embryonic fibroblasts (Hockemeyer *et al*, 2006; Wu *et al*, 2006). Confusingly, however, studies from two groups came to different conclusions about the assignment of functions to Pot1a and Pot1b. T. de Lange and colleagues found that Pot1a and Pot1b have distinct functions, as loss of Pot1a, but not Pot1b, resulted in TIF formation and a modest number of end-to-end chromosome fusions (Hockemeyer *et al*, 2006). Conversely, loss of Pot1b, but not Pot1a, led to an excessive accumulation of a telomeric 3' single-strand overhang signal. By contrast, Chang and colleagues (He *et al*, 2006; Wu *et al*, 2006) reported that loss of Pot1a alone triggered not only TIF formation, but also 3'-overhang extension and elevated sister telomere recombination, and that loss of Pot1b function triggered a similar spectrum of events. The distinction between the two conclusions is crucial, as we do not yet understand which features of dysfunctional telomeres trigger the DNA-damage response manifested by TIFs. De Lange (New York, NY, USA) described experiments that address the reason for this discrepancy. The de Lange group constructed dominant-negative alleles of both Pot1a and Pot1b in which the oligonucleotide-oligosaccharide-binding (OB) folds were removed from the respective proteins. Notably, expression of the Pot1a Δ OB protein led to extension of the telomeric 3' overhang, and expression of Pot1b Δ OB led to TIF formation and end-fusions. Hence, each Δ OB allele interferes with the function of both Pot1 variants. This mutual interference can be explained by the observation that both Pot1 variants are recruited to telomeres by binding to a third protein, TPP1 (for TIN2, PTOP and PIP1; Houghtaling *et al*, 2004; Liu *et al*, 2004; Ye *et al*, 2004). Each Δ OB construct competes with both endogenous Pot1a and Pot1b for TPP1 binding, leading to a double-knockout phenotype. De Lange suggested that as the gene deletion strategies used by Chang and colleagues had the potential to generate dominant-negative forms of Pot1, the greater range of phenotypes seen in these studies could originate from the inhibition of both Pot1 paralogues. It will be interesting to determine the primary actions of Pot1 and to what extent the spectrum of roles assumed by Pot1a and Pot1b are shared by the single Pot1 protein present in many other organisms.

Z. Songyang (Houston, TX, USA) suggested that Pot1 heterodimerizes with TPP1, and that this association has a role in telomerase-dependent telomere length regulation. Songyang also presented evidence that TPP1 contains an OB-fold and is the structural homologue of the ciliate chromosome end-binding factor TEBP- β (Wang *et al*, 2007; Xin *et al*, 2007). In a global effort to better understand telomere function, the Songyang laboratory modelled protein-protein communication between telomeric proteins and numerous interacting factors. This led Songyang to propose a 'telomeric hub' concept, which illustrates the central role of telomeres in the interplay between DNA replication, DNA-damage signalling and cell-cycle progression.

Further suggestions on how telomeres communicate with the mitotic cell-cycle regulatory machinery were made by S. Smith (New York, NY, USA). Smith described data suggesting that the SA1 subunit of cohesin interacts with telomerase. Tankyrase is a poly-ADP-ribose polymerase that acts on the mammalian telomere repeat-binding factor 1 (TRF1) as well as other cellular targets. The Smith group previously reported that inhibition of tankyrase leads to a failure to dissolve telomeric cohesion at mitosis (Dynek & Smith, 2004). New results presented by Smith raise the possibility that alterations of TRF1 activity are required for SA1 release and mitotic progression.

Compelling insights into TRF2 function were presented by E. Gilson (Lyon, France), who showed that TRF2 facilitates the invasion of single-stranded telomeric tracts into double-stranded telomeric DNA in an *in vitro* assay. This ability correlated with TRF2-catalysed DNA condensation and TRF2-dependent introduction of positive supercoils into double-stranded templates, both of which might contribute to the mechanism that underlies the ability of TRF2 to promote t-loop formation *in vitro* (Stansel *et al.*, 2001).

Cancer and ageing

Cancer and ageing were prominent topics at this Joseph Steiner Conference. E. Blackburn (San Francisco, CA, USA) expanded on her observations that inhibition of telomerase in cancer cells has rapid and profound effects. Such cells do not respond with the gradual telomere-shortening phenotype seen on telomerase inhibition in normal cells, but rather the cultures exhibit immediate growth arrest and massive amounts of cell death. Furthermore, Blackburn showed that cancer cells harbour a subset of telomeres that are markedly short, termed t-stumps. These t-stumps presumably represent telomeres on the verge of becoming dysfunctional. The inhibition of telomerase through RNA interference of human telomerase RNA (TER) might lead to rapid detection of these critically short telomeres by the cellular DNA-damage sensing machinery. This would result in an abrupt response rather than in gradual telomere shortening, potentially allowing cancer cells to be specifically targeted by telomerase inhibition.

M. Blasco (Madrid, Spain) presented data implicating the mouse telomerase reverse transcriptase component (Tert) in the mobilization of epidermal stem cells (Flores *et al.*, 2005). The Blasco group observed increased stem-cell proliferation on expression of exogenous Tert driven by the constitutively strong K5 promoter. This might be related to the telomere-length-independent cancer phenotypes seen on Tert overexpression (Artandi *et al.*, 2002; Stewart *et al.*, 2002). The Blasco group is now testing whether K5-driven telomerase could also have an effect on mouse life span. They are also developing a technique that uses telomere length as a marker for stem-cell niches. This methodology might provide a new and powerful approach to finding such compartments in various tissues.

The stem-cell theme was expanded further by S. Artandi (Stanford, CA, USA). Artandi's group has previously shown that Tert overexpression in hair follicle stem cells leads to cell proliferation and increased hair growth (Sarin *et al.*, 2005), and that these effects are independent of the telomere repeat synthesis function of Tert. However, when these transgenic animals aged, they suffered from hair loss, suggesting that the hair follicle stem-cell niches become prematurely exhausted after the overproliferation triggered by Tert overexpression. To investigate further the role of Tert in stem-cell proliferation, the Artandi group has developed a model in which

melanoblast expansion can be monitored. Overexpression of Tert in the melanoblast niche led to depletion of these cells and to premature greying of the hair of the animals. This suggests that TERT is a general regulator of stem-cell expansion, the ectopic activation of which can ultimately lead to the depletion of stem-cell niches.

L. Rudolph (Hannover, Germany) described checkpoint responses in ageing mice that lack p21 (also known as Cdkn1a, Cip1 and Waf1; Choudhury *et al.*, 2007). Deletion of p21 led to a significant elongation of lifespan in animals harbouring dysfunctional telomeres, suggesting that p21-dependent checkpoint activation is involved in monitoring critically short telomeres *in vivo*. p21 deletion improved the ability of mice with dysfunctional telomeres to repopulate haematopoietic stem-cell compartments without compromising apoptotic pathways, and without accelerating chromosomal instability and cancer formation during the course of the experiment. This suggests that inhibition of p21-induced cell-cycle arrest confers a survival advantage to mice with critically short telomeres.

Telomeres as a target for cancer therapy?

Highly proliferative cells lose telomeres rapidly in the absence of telomerase or an alternative telomere maintenance mechanism. The fact that all cancer cells must find a way to maintain telomere length to survive makes telomerase an attractive target for anti-cancer therapies. Although this field is still in its infancy, several approaches exploiting telomerase as a cancer-cell-specific molecule are being evaluated. A factor complicating such approaches is the requirement of telomerase for telomere maintenance in highly proliferating cells and stem cells. Nonetheless, an encouraging example was described by R. Vonderheide (Philadelphia, PA, USA), who has investigated the possibility of inducing an immune response against telomerase (Vonderheide *et al.*, 2004). In a phase I clinical study, women with metastatic breast cancer were treated with a peptide derived from telomerase together with adjuvant, and this was shown to generate a telomerase-specific CD8⁺ T-cell response. Furthermore, the treatment induced CD8⁺ tumour infiltrating lymphocytes and, in two cases, tumour necrosis; no autoimmune side effects were observed. This study highlights the potential of telomerase as an anti-cancer target.

Evolutionary considerations

This meeting had a decidedly 'down-under' flair, and not only because of the presence of Tasmanian-born Blackburn. W. Wright (Dallas, TX, USA) described his group's studies of telomere length among a broad cross-section of the mammalian kingdom, stretching from whales to dogs to Australian marsupials such as the wombat. As a result of this effort, it will soon be possible to group mammals according to mechanisms of telomere maintenance, or the lack thereof. Although replicative ageing is an ancient phenomenon that dates back approximately 105 million years, it is clear that not all species are subject to this process. Wright described his particularly surprising finding that several species, including the wombat, appear to rely on recombinational modes of telomere maintenance—known as alternative lengthening of telomeres (ALT)—rather than telomerase-based mechanisms (Fig 1).

R. Reddel (Sydney, Australia) pioneered molecular studies of the ALT mechanism and continues to be the leading contributor of insights into this pathway (Bryan *et al.*, 1995, 1997; Muntoni & Reddel, 2005; Varley *et al.*, 2002). Consequently, it was fitting

Fig 1 | Two models for telomere-length stabilization. On the left, telomerase activation counteracts replication-associated telomere loss. Telomerase has been suggested to act preferentially on the shortest telomeres, preventing attrition to critically short lengths and thereby preventing telomere fusion and DNA-damage signalling (see text). The grey whale, similar to humans, is an example of an animal that uses telomerase to maintain telomeres and experiences replicative cellular ageing owing to the lack of telomerase expression in most somatic tissues. On the right, in some organisms such as the Australian wombat, no telomerase activity can be detected. Telomere length is maintained through the inter-telomere recombination-dependent alternative lengthening of telomeres (ALT) pathway (see text). A significant subset of human tumours maintain their telomeres using ALT, but the ALT pathway is inhibited at functional telomeres in primary cells.

that the Australian wombat was found to rely on ALT for chromosome-end maintenance. We therefore encourage enhanced collaboration between the Wright and Reddel laboratories, not only because of the considerable lack of wombats in the USA, but also because ALT-dependent immortalization might be a significant source of cancer development.

Conclusions and perspectives

The telomere field has widened significantly since its establishment by Blackburn. Initially, it focused on the question of complete replication of a linear DNA molecule, but the field became more diverse and complicated when groups started to tackle the challenging question of what distinguishes a natural chromosome end from a DNA break on a molecular level. This led to the discovery of telomere-binding proteins in many model organisms, and it is becoming increasingly clear that these proteins have many

interacting partners and influence several pathways in the cell. Now the field is progressively becoming more microscopically detailed, asking such challenging questions as what distinguishes a short from a long telomere, what defines a protected telomere, and how telomere structure changes during development and the cell cycle. At the same time, the field is moving towards macroscopic details, exploring how to exploit telomerase and telomere function for cancer therapy and enhanced longevity. Considering the many questions that remain to be answered, this field is expected to have a bright future, yielding many surprises to come.

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