

A SUMO ligase for ALT

Roger R Reddel

A study in this issue implicates SUMOylation of telomere-binding proteins by the SMC5/6 complex in alternative lengthening of telomeres.

Most cancer cells escape from the normal barriers to unlimited proliferation by activating a telomere maintenance mechanism. The best known of these is telomerase, but in about 10% of cancers telomere lengths are maintained in the absence of telomerase, by one or more 'alternative lengthening of telomeres' (ALT) mechanisms. Although ALT cells do not have a generalized increase in recombination compared with other immortalized cells, they do have a high rate of post-replicative recombinational exchanges at their telomeres^{1–3}. It is likely that most of these exchanges involve sister chromatids, so the exchange events are often called telomere sister-chromatid exchanges (T-SCEs). ALT-positive human cell lines have a number of common features, including the presence of low-molecular weight telomeric DNA, referred to as extrachromosomal telomeric repeat (ECTR) DNA⁴. Like most other cells, their nuclei contain promyelocytic leukemia (PML) bodies—large macromolecular aggregates involved in a variety of cellular processes. Some PML bodies in ALT cells contain telomeric chromatin⁵, and because there is no definitive evidence yet for the existence of PML bodies with these contents in other types of cells, they are called ALT-associated PML bodies (APBs).

APBs also contain many DNA repair proteins⁵, and a very interesting addition to the list of proteins associated with APBs has been made by Potts and Yu in a study on

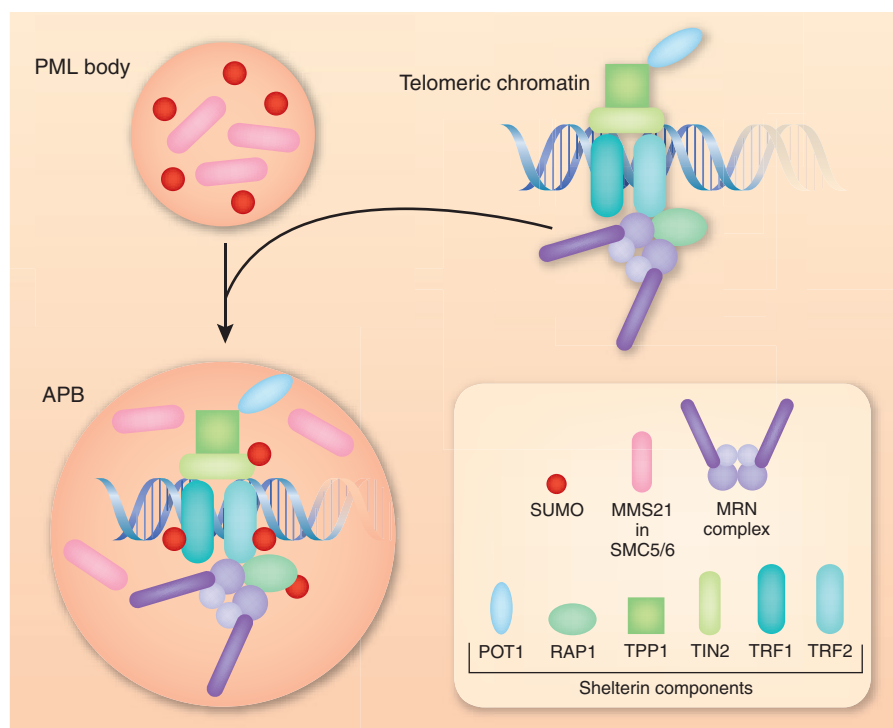


Figure 1 Involvement of SMC5/6 SUMO ligase MMS21 in APBs. Formation of APBs requires the shelterin proteins TRF1, TRF2, RAP1 and TIN2, and the MRN recombination complex. One possible role for the SMC5/6 complex (which is present in APBs) is that its MMS21 component catalyzes SUMOylation of TRF1, TRF2, RAP1 and TIN2 to integrate these proteins and the telomeric DNA (at a chromosome end or extrachromosomal) to which they are bound into PML bodies.

page 581 of this issue⁶. The authors observed that the spontaneous occurrence of nuclear foci containing the 'structural maintenance of chromosomes' (SMC) 5/6 complex is a common feature of ALT cell lines⁶. The SMC5/6 complex contains the eponymous SMC5–SMC6 heterodimer and at least six non-SMC proteins, one of which is a small ubiquitin-like modifier (SUMO) ligase called MMS21. These authors and others had

previously shown that the SMC5/6 complex is recruited to double-strand breaks (DSBs) and in turn recruits cohesin⁷, which promotes sister-chromatid homologous recombination (HR)⁸. Telomere ends are similar to DSBs, and ALT is thought to involve HR, so it was very appropriate to ask whether the SMC5/6 complex might be involved in ALT. Potts and Yu⁶ showed that SMC5/6 is present in APBs in human and mouse ALT cell lines.

Roger R. Reddel is acting director of the Children's Medical Research Institute, 214 Hawkesbury Road, Westmead, Sydney, NSW 2145, Australia.
e-mail: rreddel@cmri.usyd.edu.au

Reducing the abundance of SMC5/6 complex by short interfering RNA treatment caused a substantial decrease both in T-SCEs and in APB formation. Long-term depletion of MMS21 or SMC5 in an ALT cell line (but not in a telomerase-positive cell line) caused progressive telomere shortening and telomere dysfunction, and a progressive increase in the proportion of senescent cells. SUMOylation of proteins is important for the formation of PML bodies, and Potts and Yu⁶ went on to show that the SUMO ligase activity of MMS21 is required for APB formation, finding strong hints that this role is quite specific. MMS21 stimulated SUMOylation of four telomere-binding proteins (TRF1, TRF2, TIN2 and RAP1, of the shelterin complex) that are present in APBs. TRF1 was a substrate for MMS21 but not for three other SUMO ligases. These same four shelterin proteins shown to be substrates for MMS21-mediated SUMOylation⁶ are also known to be required for APB formation, together with PML itself and the MRE11–RAD50–NBS1 (MRN) recombination complex⁹, but not Sp100 (another core constituent of PML bodies) or two other proteins involved in DNA repair and recombination, 53BP1 (ref. 9) and RAD51 (ref. 5).

One model of how these proteins may all be involved is shown in **Figure 1**. The MRN complex interacts with the shelterin complex via RAP1 (ref. 10). MRN is a DNA damage sensor, and it is possible that some change in the state of telomeric DNA triggers a response, involving MRN and the shelterin proteins, that results in APB formation. It is not known whether SMC5/6 is present at, or recruited to, mammalian telomeres. In the latter case, SMC5/6 could be involved in translocation of telomeric chromatin into PML bodies. Alternatively, SMC5/6 could reside in PML bodies and stabilize telomeric chromatin within those structures by SUMOylation after it has translocated there (**Fig. 1**).

It is currently unknown how much of the telomeric DNA present in APBs is attached to chromosomes. Live-cell imaging analyses have shown that telomeres in ALT cells move to and from APBs in a dynamic manner¹¹. However, ALT cells contain substantial amounts of both linear and circular ECTR DNA^{4,12–14}. APBs partially purified on sucrose/Percoll gradients have been found to contain mostly low-molecular weight linear ECTR DNA, indicating that circular ECTR DNA is mostly elsewhere in the nucleoplasm¹⁴. APBs attached to chromosome ends would presumably be excluded from

this analysis, so the results do not address the question of what proportion of APBs contain ECTR DNA as opposed to chromosome ends. However, they do suggest that ALT cells may avoid many of the consequences that arise from small linear DNA molecules with unrepaired ends by sequestering these molecules within APBs¹⁴.

This raises the question of whether APBs are active participants in the ALT mechanism or whether they only serve other purposes, such as sequestering unrepaired telomeric DNA away from DNA damage signaling processes, or forming depots of DNA and HR proteins. At least one telomerase-negative cell line maintains its telomeres in the absence of APBs, but these cells have large nuclear aggregates containing many of the known components of APBs (with the exception of the PML protein), which might act as APB substitutes^{15,16}. It is difficult to prove definitively that APBs are involved directly in the ALT mechanism. Although sequestration of the MRN complex by overexpressed Sp100 suppresses both ALT activity and APB formation¹⁷, this could indicate simply that, along with its myriad other activities, MRN is required for both of these processes, without them necessarily being linked. Nevertheless, this result shows at least that the processes are associated, and other data support this association. For example, repression of ALT in somatic-cell hybrids¹⁸, or in the rare instances when ALT is repressed after induction of telomerase activity¹⁹, is accompanied by disappearance of APBs. The results of Potts and Yu⁶ showing that knockdown of the SMC5/6 complex disrupts both telomere maintenance and APB formation further tighten the association between ALT and APBs.

The presence of so many recombination proteins within APBs supports the idea that ALT activity may occur there. There are currently two models for the ALT mechanism. In one model, T-SCEs cause unequal exchanges that lengthen the telomere of one sister chromatid and shorten the other, resulting in daughter cells with proliferative capacities that are either extended or decreased². Because multiple T-SCEs occur within the same ALT cell, it has been proposed that telomere lengthening by unequal exchange can result in telomere length maintenance within the population only if there is a mechanism for nonrandom segregation of lengthened telomeres into one of the daughter cells²⁰. In another model, there is synthesis of new telomeric DNA by recombination-mediated DNA replication—

that is, because telomeres all have the same tandemly repeated hexanucleotide sequence, recombination allows a telomere to use other telomeric DNA (at a chromosome end or extrachromosomal) as a template²⁰. This fits with the observation that a DNA tag inserted into a telomere is copied to other telomeres in ALT cells²¹.

Many other questions remain. It seems likely that ALT is not a mechanism that is invented *de novo* in cell lines and cancers, but is instead the dysregulated version of an as yet unknown process that occurs in normal cells. Similarly, does the SMC5/6 complex have a role at normal mammalian telomeres? Furthermore, given that it recruits cohesin complexes to other DSBs, does it have an analogous role at telomeres? Its ability to SUMOylate TRF1 is particularly intriguing in this regard, in view of the role of TRF1 in sister-telomere cohesion²².

COMPETING INTERESTS STATEMENT

The author declares no competing financial interests.

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