

TRAPping telomerase within the intestinal stem cell niche

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Adult intestinal stem cells (ISCs) reside at the crypt base, where they continuously proliferate to maintain homeostasis of the intestinal epithelium. As a result of a lifetime of cell division, these LGR5 + ISCs face the combined risk of genomic mutations and telomere attrition. In this issue of EMBOJ, Schepers *et al* (2011) examine whether ISCs employ telomerase expression and asymmetric chromosome segregation to protect their genome.

The intestinal epithelium sheds hundreds of millions of cells every day, placing considerable replicative strain on the stem cells located at the crypt base. Elegant lineage-tracing experiments have established the ability of LGR5-expressing cells to stably contribute to all epithelial lineages over long chase periods, thus firmly establishing them as ISCs (Barker *et al*, 2007). These LGR5 + ISCs receive self-renewing signals from Paneth cells, a terminally differentiated epithelial cell found intercalated between the ISCs (Sato *et al*, 2011). In contrast to most other adult stem cells, LGR5 + cells are in a chronically activated state, cycling once a day on average. This puts ISCs at risk of accumulating genetic lesions and suffering from telomere-induced senescence.

Telomeres are nucleoprotein structures that protect chromosome ends from recombination and degradation.

Telomeres shorten with cell division due to the end-replication problem, but this shortening is countered by telomerase, an enzyme consisting minimally of a catalytic subunit, TERT and an RNA-template component, TERC. Telomere attrition causes replicative senescence or apoptosis in cultured human cells, and in telomerase knockout mouse tissues telomere shortening promotes cell death of progenitor cells and impairs self-renewal of stem cells. Telomerase is regulated in part at the level of TERT transcription, and there is increasing evidence that telomerase is restricted in its expression pattern to a subset of stem cells or progenitor cells. A longstanding goal in the field has been to determine precisely the identity of telomerase-positive cells in normal adult tissues. To address this question, Schepers *et al* (2011) FACS-isolated LGR5 + ISCs, and directly measured telomerase activity by the telomere repeat amplification protocol (TRAP) assay. ISCs expressed the highest level of telomerase activity, whereas telomerase expression was reduced in the transit-amplifying cells of the crypt and was absent in the differentiated epithelial cells of the villi (Figure 1). A recent study using a transgenic TERT-GFP reporter also localized TERT expression to the crypts, but found that TERT was expressed only in very rare crypt cells, perhaps reflecting the relative weakness of

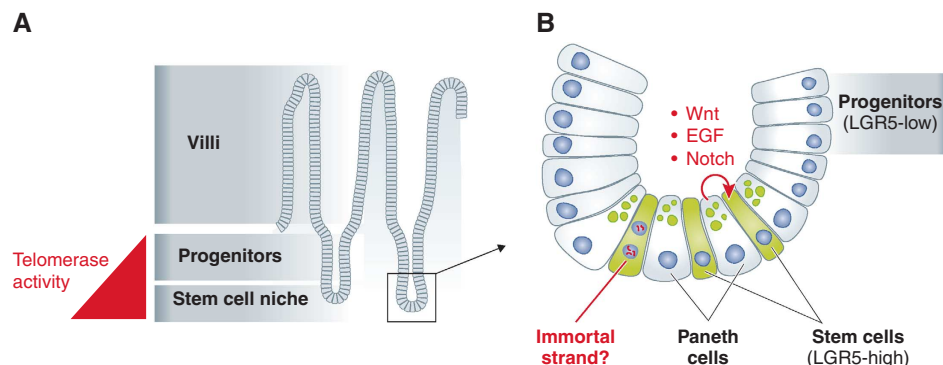


Figure 1 (A) The epithelium of the small intestine consists of a series of crypts and villi. Stem and progenitor cells proliferate in the crypts, with their differentiated progeny moving upwards into the villi, where they are eventually shed into the intestinal lumen. In this issue of EMBOJ, Schepers *et al* (2011) find that LGR5 + stem and progenitor cells express telomerase, whereas their differentiated progeny in the villi do not. (B) The stem cell niche is found at the base of the crypt. Stem cells express high levels of LGR5, and are found intercalated between Paneth cells, a specialized epithelial cell. Paneth cells are a source of self-renewal signals for the neighbouring stem cells, helping to promote their daily proliferation. During these cell divisions, Schepers *et al* (2011) find no evidence that ISCs asymmetrically distribute their chromosomes, a strategy hypothesized to minimize DNA mutation accumulation in stem cells.

the TERT promoter compared with the sensitivity of the TRAP assay (Montgomery *et al*, 2011). The pathways that lead to specific telomerase expression in LGR5+ ISCs and the progenitor cells they give rise to it are unknown, and it will be important to understand this regulation in depth. Additionally, it remains unclear whether the reduced telomerase expression in progenitor cells reflects downregulation on a per-cell basis, and/or a restriction of expression to a subset of committed progenitor subtypes, as in the haematopoietic system (Morrison *et al*, 1996).

Despite telomerase expression, Schepers *et al* (2011) observed telomere shortening with age in ISCs, suggesting that telomerase levels were insufficient to fully prevent telomere shortening during division of these stem cells. Telomerase knockout experiments have supported the idea that the enzyme serves a critical function in the intestinal crypt. Intercrossing telomerase knockout mice (to deplete the very long telomere reserves of laboratory mice) results in high rates of apoptosis in crypt progenitor cells, as dysfunctional telomeres initiate a cell death response (Wong *et al*, 2000). An intriguing possibility is that TERT is expressed in ISCs, not only for its role in maintaining telomere reserves, but also for its non-telomeric functions, such as in supporting Wnt signalling activation (Park *et al*, 2009). Regardless, these experiments highlight LGR5+ ISCs as a powerful system for studying telomerase biology in an endogenous *in vivo* setting.

In a second set of experiments, Schepers *et al* (2011) re-examined the crypt base for signs of asymmetric segrega-

tion of all chromosomes (ASAC) during cell division. Such events would be consistent with the ‘immortal strand hypothesis’, the idea that stem cells shunt newly synthesized DNA strands to non-stem daughter cells to avoid DNA mutations caused by replication errors (Cairns, 1975). Within the limited number of events examined, Schepers *et al* (2011) never saw ASAC at the crypt base. This is consistent with a recent report by Falconer *et al* (2010), who used a sophisticated DNA-labelling method to answer the same question in the mouse colon. They also never observed ASAC, but did measure a pattern of sister chromatid segregation that was non-random, implying that there may indeed be regulation controlling how sister chromatids are apportioned as ISCs divide. Both of these reports contrast with the findings of Quyn *et al* (2010), who did observe ASAC in roughly half of the cell divisions at the crypt base. However, these cells had recently recovered from irradiation-induced stem cell depletion, a context that could change the homeostatic patterns of cell division and DNA segregation. Especially useful would be a means of tracking the fates of cells receiving new and old chromosomes, to impart biological significance to the patterns observed. A definitive resolution to the immortal strand controversy awaits further experimentation, as does the discovery of the full repertoire of tools that ISCs use to protect their genome.

Conflict of interest

The authors declare that they have no conflict of interest.

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