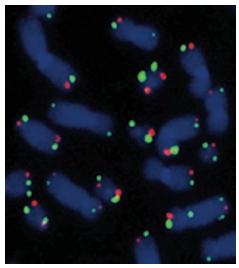
How ALT cells do it



As somatic cells replicate, their telomeres grow progressively shorter, until they enter senescence and stop dividing. In cells immortalized in culture or cancer cells, the maintenance of telomeres, and hence of cellular proliferation capacity, can be achieved by activation of telomerase or by one or more alternative mechanisms, called ALT (alternative lengthening of telomeres). ALT cells seem to maintain their telomeres through recombination, but the exact mechanisms are not clear. In ALT cells, telomeric DNA recombination is thought to take place within the so-called ALT-associated promyelocytic leukaemia (PML) bodies, or APBs. In addition to PML proteins, APBs contain telomeric DNA and proteins, and homologous recombination factors. Recent work from Yang and colleagues shows that the structure-specific endonuclease MUS81 also localizes to APBs and has a role in ALT. MUS81 was known to be involved in the processing of branched DNA intermediates formed during recombination or the restart of stalled replication forks. MUS81 is now seen to associate with telomeric DNA within APBs in different ALT cell lines but not in non-ALT cells. Knocking down MUS81 in ALT cells resulted in telomere loss and cell growth arrest; a nuclease-deficient mutant of MUS81 could not complement the knocked-down cells, showing that its enzymatic activity is required to support telomere-length maintenance. Interestingly, the telomeric protein TRF2 was found to associate with MUS81 and to regulate its activity on DNA. Although it remains to be established what exactly MUS81 does in APBs, its newly identified role in ALT opens new ways to investigate how these cells manage to keep their telomeres without telomerase. (*Nat. Cell Biol.*, advance online publication, doi:10.1038/ncb1867, 12 April 2009) *IC*

Tails of ubiquitin

Ubiquitin modification of substrates controls many cellular processes, and all seven lysine residues in ubiquitin can contribute to this process by linking together ubiquitin into chains. Conventional linkage is through Lys48, which mediates proteasomal degradation; another common and well-characterized link is through Lys63, which is involved in nonproteolytic events. The roles of the five other lysines in polyubiquitin chain formation are not well understood. Xu *et al.* profiled the entire yeast proteome to uncover the abundance of each of the lysine linkages and discovered an unexpectedly high level of unconventional non–Lys63-linked polyubiquitin chains *in vivo*, especially the Lys11 linkage. All of these noncanonical polyubiquitin linkages are recognized and disassembled by the 26S proteasome. Yet, despite the abundance of these linkages, only Lys48 is essential, although it is not sufficient by itself to sustain yeast growth. To rescue the viability of the Lys48 mutant, only Lys29 and Lys33 need to be added, although growth is severely retarded. This result confirms the physiological importance of non-Lys48 lysine residues. Xu *et al.* characterized the abundant Lys11 ubiquitin–ubiquitin isopeptide bond and established that the E2 ubiquitin–conjugating enzyme Ubc6 is a substrate of the Lys11 linkage, as well as being a major E2 contributing to the synthesis of Lys11 linkages *in vivo*. Ubc6 is a component of the endoplasmic reticulum–associated degradation (ERAD) pathway, a quality control pathway in which misfolded or improperly assembled proteins of the ER are degraded by the proteasome. Xu *et al.* show that Lys11-linked polyubiquitination has an important role in this ER stress pathway. (*Cell* **137**, 133–145, 2009) *MH*

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Regulating licensing

Genome integrity depends on DNA replication being limited to once every cell cycle. Replication is therefore tightly regulated by temporally separating the formation of the pre-replicative complex (pre-RC) from the initiation of DNA replication. The replication-licensing factors Cdt1 and Cdc6 recruit the MCM2–7 proteins to replication origins in late M or G1 phase to form pre-RCs. MCM proteins act as the replicative helicase. To prevent reassembly of pre-RCs, or licensing at inappropriate times, Cdt1 is inactivated by two separate processes. First, geminin prevents recruitment of MCM2–7 by Cdt1 via steric hindrance. Second, Cdt1 is degraded at the start of S phase by ubiquitin-mediated proteolysis. Ubiquitination occurs primarily on the ε-amino group of lysine residues. This residue can also be acetylated and deacetylated. Glozak and Seto now find that Cdt1 interacts with a histone deacetylase, HDAC11. Cdt1 also associates with and is modified by two lysine acetyltransferases, KAT2B and KAT3B, and this modification protects Cdt1 from proteasomal degradation. These studies add Cdt1 to a growing list of regulators of genomic integrity that are modulated by acetylation and deacetylation (such as p53). Likewise, they add another substrate to the list of non–histone-acetylated proteins. (*J. Biol. Chem.* **284**, 11446–11453, 2009) *BK*

Tuning β-arrestin signaling

G protein–coupled receptors (GPCRs) activate heterotrimeric G proteins but are quickly phosphorylated at their C termini by GPCR kinases. This signals recruitment of β-arrestins, which leads to desensitization of G protein signaling. β-arrestins can have transient (class A) or tight (class B) interactions with GPCRs. Prolonged receptor-arrestin interactions can lead to trafficking to endocytic vesicles. The stability of the interaction can be modulated by the ubiquitination status of β-arrestin, and deubiquitination of β-arrestins has been correlated with their dissociation from activated receptors. Lefkowitz and colleagues have now determined that the deubiquitinase USP33 is capable of deubiquitinating β-arrestins. Overexpression of USP33 destabilizes the complex formed between vasopressin type 2 receptor (V2R), a class B receptor, and β-arrestin, leading to loss of β-arrestin's endosomal recruitment and diminished β-arrestin–dependent ERK signaling. Expression of exogenous Mdm2, which binds and ubiquitinates β-arrestin upon β2-adrenergic receptor (β2AR) activation, led to localization of activated β2AR–β-arrestin complexes to the endosome, suggesting that excess Mdm2 stabilizes the receptor-arrestin complexes. Further, Mdm2-mediated ubiquitination is shown to be necessary for β2AR-dependent β-arrestin stimulation of the ERK pathway, and USP33 can antagonize this pathway. Finally, they found that phosphorylation of activated GPCRs leads to distinct conformations of the recruited β-arrestins. For class A receptors such as β2AR, the induced conformational change facilitates USP33 binding to β-arrestin, promoting its dissociation from the receptor and producing a transient ERK signal. For class B receptors, the induced β-arrestin conformation does not promote USP33 binding, allowing prolonged downstream signaling and endocytosis of the receptor-arrestin complex. These findings suggest a tuning or code for β-arrestin signaling that may be further refined, for example, by specific ubiquitin linkages on β-arrestin. (*Proc. Natl. Acad. Sci. USA* **106**, 6650–6655, 2009) *MM*