TELOMERASE

ALTernative pathways?

Are telomerase and ALT two equivalent pathways to immortality? This is the question that Robert Weinberg and colleagues have addressed in a report in the 1 October issue of *Proceedings of the National Academy of Sciences*.

Both pathways result in the addition of telomeric sequence onto the ends of chromosomes, preventing crisis and allowing cells to proliferate indefinitely. But ALT seems to occur less frequently than might be expected.

Cells that have been immortalized with TERT — the catalytic component of telomerase — can be converted from a non-tumorigenic to a tumorigenic state by the addition of the HRAS-V12 oncoprotein; is the same true for cells that have been immortalized by ALT? The GM847 cell line lacks telomerase activity, and maintains its long telomeres using ALT; however, the addition of HRAS-V12 did not produce cells that were able to induce tumour formation when injected into immunodeficient mice. This ability was restored when TERT was also introduced.

So does telomerase replace ALT as the mechanism by which cells maintain their telomeres? GM847 cells that have been transfected with HRAS-V12 and TERT still have AA-PML bodies — a marker for ALT cells — that contain the characteristic proteins TRF2 and PML, indicating that ALT remains active even when TERT is expressed.

Interestingly, the ability of telomerase to elongate telomeres is not even required for the tumorigenic phenotype, as TERT that is tagged at its carboxyl terminus with HA — which has *in vitro* telomerase activity, but can not elongate telomeres *in vivo* — is still able to confer tumorigenic potential when transfected into GM847 cells with HRAS-V12.

So what additional role does TERT perform in these cells? In normal tissue-culture conditions, the growth

TUMORIGENESIS

Clockwork

The daily oscillations of biological processes, known as circadian rhythms, are regulated by genetically controlled endogenous clocks. Disruptions in circadian rhythms have been associated with cancer — people and animals with irregular sleep–wake cycles are more susceptible to certain cancers, and the efficacy and toxicity of some chemotherapy agents correlate with the timing of drug delivery. A recent study by Fu *et al*. reported that mice with targeted disruption in a circadian control gene develop tumours, providing the opportunity to study the molecular basis of this relationship.

So far, eight core circadian genes have been identified. Among these are the *Per1*, *2* and *3* genes, which encode non-DNA-binding nuclear factors. The expression of these genes oscillates over the 24-hour circadian period. Mice with a homozygous mutation in *Per2* (*mPer2*$^{m/m}$) have been previously reported to be deficient in circadian clock function. In the 4 October issue of *Cell,* Fu *et al*. report that these mice have neoplastic phenotypes, such as salivary-gland hyperplasia and teratoma formation, reduced levels of thymic apoptosis in response to γ-radiation and increased susceptibility to cancer.

So Per2 seems to have a role in regulating cell proliferation and apoptosis, but what is its function? The authors examined mRNA levels of genes that are associated with cell-cycle regulation and tumour suppression in the *mPer2*$^{m/m}$ mice, to see if their expression patterns were disrupted. The first gene they investigated was the transcription factor c-Myc — an oncogene that regulates both apoptosis and proliferation, and is also known to control the expression of several circadian genes. The authors found that the expression pattern of c-*Myc* is itself regulated by circadian genes, and that its expression pattern was disrupted in *mPer2*$^{m/m}$ mice. Expression of several c-Myc-target genes, including *Ccnd1* (which encodes cyclin D1) and *Gadd45a*, were also found to be under circadian control, so were also altered in the mutant mice. Mutations in *Per2* had no effect on the expression of c-Myc-target genes that were not regulated by the circadian cycle, such as *Cdk4* and *Trp53* (which encodes p53).

Fu *et al*. propose that loss of mPer2 function leads to disrupted regulation of a number of circadian rhythm genes, such as c-*Myc*, which are involved in growth regulation. Deregulation of c-*Myc* has been associated with DNA damage, defective cell-cycle arrest and tumour development, and is likely to be a culprit in the tumours that form in *mPer2*$^{m/m}$ mice. Aberrant control of circadian rhythms might prove to be another hallmark of tumour development, but it will be important to determine whether mutations in circadian genes also contribute to cancer development in humans.

Kristine Novak

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WEB SITE
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http://imgen.bcm.tmc.edu/molgen/facultyaz/clee.html

rates of GM847 cells that have been transfected with HRAS-V12 were similar, regardless of whether TERT was also expressed; however, when the growth conditions were changed to those of limiting nutrient and oxygen levels, the expression of TERT resulted in an increased growth rate.

ALT and telomerase are therefore not functionally equivalent — despite the fact that ALT can elongate telomeres to lengths beyond those achieved with telomerase. Perhaps the reason that ALT occurs at lower levels in cancer than expected is because an additional mutation is required — compared with reactivation of telomerase — before cells can obtain a comparable growth advantage and become tumorigenic.

Emma Greenwood

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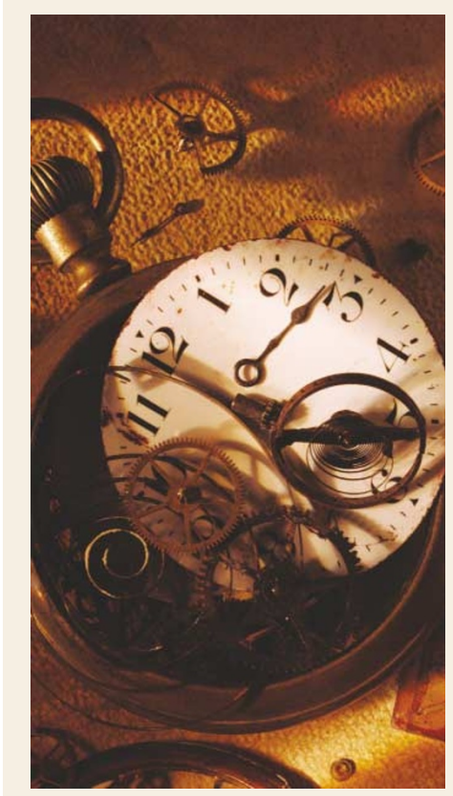
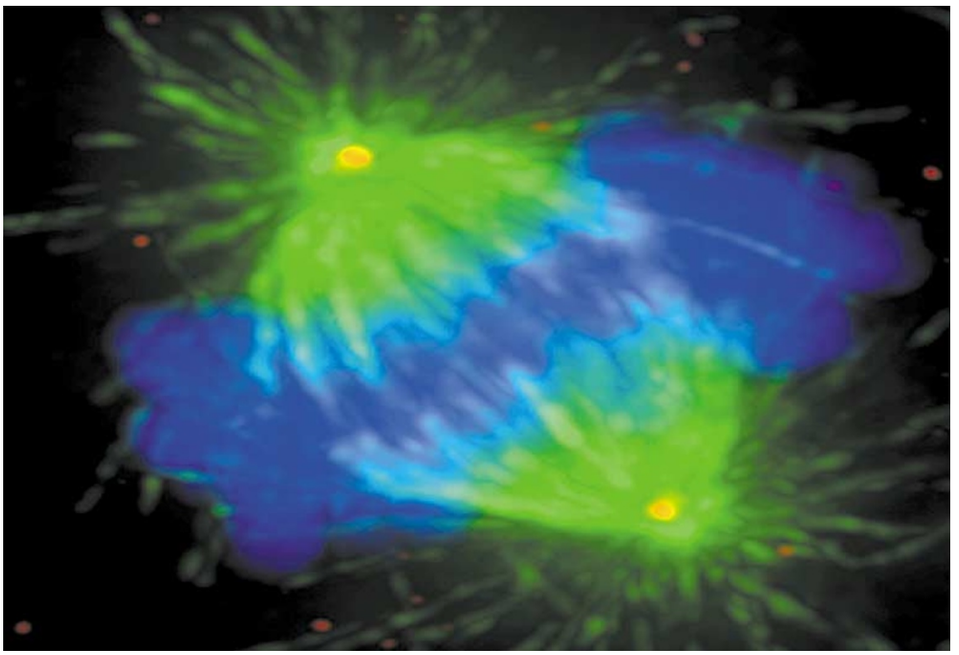
Robert Weinberg's lab: http://web.mit.edu/biology/www/facultyareas/facresearch/weinberg.shtml





CELL CYCLE

Divide and rule

As centrosome duplication and separation are linked to the cell cycle, and as cyclin-dependent kinases (CDKs) are crucial for cell-cycle progression, researchers believe that centrosome duplication is linked to the activation of CDKs. Two cyclin–CDK complexes — cyclin-E–CDK2 and cyclin-A–CDK2 — have been implicated in the centrosome cell cycle, and several CDK2 substrates have been identified. Brian Dynlacht and colleagues now report, in *Developmental Cell*, the identification of a new CDK substrate — a protein they call CP110 — that has a function in controlling the centrosome cell cycle.

With the goal of identifying new CDK targets, Dynlacht and co-workers screened a human cDNA expression library with a radiolabelled, dominant-negative form of cyclin-E–CDK2. One of the positive clones encoded a previously uncharacterized protein, which they called 'centrosomal protein of 110 kDa'. This protein does not resemble any known proteins, but among its recognizable motifs are two cyclin-binding domains and ten putative CDK phosphorylation sites.

So, is CP110 a CDK substrate? GST-tagged CP110 was phosphorylated *in vitro* by cyclin-E–CDK2, cyclin-A–CDK2 and also by cyclin-B–CDC2, which are all active from S through to M phase — but not by a G1-phase kinase. CP110 was also phosphorylated *in vivo*, and the pattern of phosphopeptides following tryptic digestion resembled that of the *in vitro* phosphorylations. This indicates that CP110 is an authentic *in vivo* substrate for cyclin–CDK complexes.

Using northern and western blot analysis of extracts prepared from synchronized cells, Dynlacht and colleagues showed that CP110 expression increased as cells progressed through the G1–S transition, and peaked during S phase, after which CP110 levels diminished. So, the expression of CP110 coincides with the activation of CDK2 and centrosome duplication and separation.

Next, Dynlacht and colleagues determined the subcellular localization of CP110 by immunofluorescence, and found that CP110 antibody staining overlaps with that of γ-tubulin as well as centrin, both of which are centrosomal markers. They confirmed the centrosome localization of CP110 by taking a biochemical approach — centrosomes were purified by means of a sucrose gradient that contained CP110.

To test the function of CP110, Dynlacht and co-workers downregulated CP110 levels by RNA interference (RNAi). They then arrested the cells in S phase — an event that would normally provoke centrosome amplification but which, instead, caused blockage of centrosome duplication. Stable expression of a non-phosphorylated mutant version of CP110 resulted in polyploidy. Equally, depletion of CP110 levels by RNAi or loss of CP110 phosphorylation increased unscheduled centrosome separation.

The authors concluded that CP110 might function in two ways — by positively regulating centrosome duplication, and by suppressing premature centrosome separation. The next step will be to elucidate the actual mechanism through which CP110 functions. The finding that polyploidy results from mutating CP110 implies a direct link between centrosome function and genomic stability. CP110 might also reveal new insight into a possible connection between centrosome defects and cancer. So, it will be a priority to investigate whether the *CP110* gene is a target of mutations in human cancers.

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Nature Reviews Molecular Cell Biology

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