IN THE NEWS

Age discrimination

Many of the body's tissues are constantly renewed. In fact, the average age of cells in a human adult body might be only 7–10 years, according to stem cell biologists from the Karolinska Institute in Stockholm, Sweden.

Jonas Frisén and colleagues have developed a new technique for measuring the age of cells, and published their first findings in *Cell*.

The Frisén team hypothesized that the dramatic increase in atmospheric radioactive carbon 14 (^{14}C) as a result of nuclear testing during the Cold War in the early 1960s and the exponential decline after 1963 might provide a modern-day 'carbon dating' equivalent.

As DNA does not exchange carbon after it is generated, the amount of ^{14}C in a cell's DNA reflects the amount of atmospheric ^{14}C at the time of its birth.

Looking at tissue samples from deceased adult subjects that had been exposed to a changing ^{14}C environment, Frisén and colleagues concluded that cells from the cerebral cortex were as old as the subjects themselves. "The reason these cells live so long is probably that they need to be wired in a very stable way," said Frisén (news@nature.com, 14 July 2005).

However, all other tissues they studied were considerably younger — the average age of cells in the intestine is ~10 years and in skeletal muscle ~15 years.

So, if the body is capable of regenerating most of its tissues, why do we age? "The notion that stem cells themselves age and become less capable of generating progeny is gaining increasing support," according to Frisén (*The New York Times*, 2 August 2005). And he is hopeful that the new carbon dating technique might shed light on this theory.

Arianne Heinrichs

CELL CYCLE

Checking the damage

The transcriptional activation of target genes after cellular stress by the tumour suppressor p53 can cause cell-cycle arrest or apoptosis. But the regulation of this process is proving increasingly complex. Now, Irena Dornreiter and colleagues have identified an isoform of human p53 that has an essential role in the intra-S-phase checkpoint that ensures accurate DNA replication.

The Dornreiter team identified an alternatively spliced p53 transcript in human and monkey (but not rodent) cells that lacked 198 nucleotides between exons 7, 8 and 9, which they named Δp53 . At the protein level, immunoblot analysis and mass spectrometry confirmed that a truncated form of p53 correlated with the alternatively spliced Δp53 cDNA and lacked the core domain of the normal protein. This Δp53 isoform could form homodimers and homotetramers but could not heterodimerize with full-length p53, suggesting that it might have an alternative function.

Full-length p53 induces the transcription of cell-cycle regulatory genes such as *p21*, *14-3-3 σ* and *GADD45*, as well as pro-apoptotic genes such as *MDM2*, *BAX* and *PIG3*. However, overexpression studies and chromatin immunoprecipitation (ChIP) analysis showed that the Δp53 isoform only regulates transcription of the cell-cycle genes and not the pro-apoptotic genes. Damage-induced p53 activation in S-phase cells causes cell-cycle arrest, but is thought to avoid the premature activation of the p53-mediated apoptotic pathway by an unknown cell-cycle-specific mechanism. So, as Δp53 does not activate the pro-apoptotic genes, the authors proposed that it might be responsible for the transcriptional activation of p53 targets during S phase.

Subsequent experiments showed that, after irradiation, the *p21* promoter binds to full-length p53 exclusively during the G1 and G2 phases of the cell cycle, and that it

specifically associates with Δp53 only during S phase. This suggests that the transcriptional activities of p53 and Δp53 are independent of each other. This was confirmed by studying irradiation-induced p21 production. For the first 10 hours after irradiation, p21 production was mediated by Δp53 , but full-length p53 did not begin to regulate p21 production until 8 hours had passed. So the transcriptional activities of p53 and Δp53 are independent and temporally separated.

The intra-S-phase checkpoint is controlled by the activation of ataxia telangiectasia and RAD3-related protein (ATR), which signals through checkpoint kinase-1 (CHK1), CDC25A and cyclin-E-cyclin-dependent-kinase-2 (CDK2) to transiently inhibit DNA replication. The CDC25-cyclin-E-CDK2 pathway is initiated rapidly, but only prevents DNA replication for ~2 hours. However, after ultraviolet light (UV) damage, S phase is delayed for ~6 hours. So could Δp53 be responsible for this continued inhibition of DNA replication?

The authors suggest that this is indeed the case: Δp53 is activated

ORGANELLE BIOGENESIS

Where did I come from?

How do peroxisomes form in eukaryotic cells? A definitive answer to this question has been elusive, with some believing that, similar to the Golgi,

peroxisomes are derived from the endoplasmic reticulum (ER) and others believing that, similar to mitochondria, they are autonomous entities. However,

Tabak and colleagues now resolve this issue in *Cell* by showing that peroxisomes come from the ER.

The authors worked in *Saccharomyces cerevisiae* and created a system that allowed the real-time imaging of peroxisome biogenesis. They focused on peroxin-3 (Pex3), an integral membrane protein that is essential for the biogenesis of these organelles. They made a yeast strain that could be induced to express endogenous levels of fluorescently labelled Pex3 (Pex3-YFP) by putting the gene encoding this construct under the control of a galactose-inducible promoter and exposing cells to galactose for a limited time. In the absence of galactose, Pex3 and peroxisomes were absent from these cells.

by ATR after UV damage and causes the p21-mediated downregulation of cyclin-A-CDK2 activity (which is required for S-phase progression). Moreover, cells that are deficient in $\Delta p53$ fail to downregulate cyclin-A-CDK2 and therefore cannot uncouple the processes of DNA repair and replication, which results in the accumulation of DNA damage.

The authors therefore propose that the intra-S-phase checkpoint requires both the CHK1–CDC25A–cyclin-E–CDK2 and the

$\Delta p53$ –p21–cyclin-A–CDK2 pathways to function properly and allow the repair of damaged DNA before replication is initiated. However, the regulatory network that controls the cell-cycle-dependent transcription of p53 and $\Delta p53$ awaits clarification.

Lesley Cunliffe

References and links

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FURTHER READING

Bartek, J. *et al.* Checking on damage in S phase. *Nature Rev. Mol. Cell Biol.* **5**, 792–804 (2004)

Using this strain, Tabak and co-workers created two further strains. The first constitutively expressed a fluorescent marker of the ER, whereas the second constitutively expressed a fluorescent marker of peroxisomes. They then used galactose to induce peroxisome reformation in both strains and monitored Pex3–YFP until peroxisomes had reformed. They saw that when Pex3–YFP first appeared, it colocalized with labelled ER structures. It subsequently moved to mature, labelled peroxisomes through dot-like structures, and these observations were confirmed using a biochemical approach.

Next, the authors monitored the movement of Pex3–YFP in wild-type cells, and showed that it again accumulated in the ER before reaching peroxisomes. The targeting of Pex3 to the ER before it arrives in peroxisomes therefore seems to be the normal procedure

for a wild-type cell.

In the final part of their study, Tabak and colleagues focused on Pex19 — a mainly cytosolic protein that associates with Pex3 and is also essential for peroxisome biogenesis. They found that Pex3 cannot leave the ER in the absence of Pex19, and that Pex3 and Pex19 colocalize on the ER during the initial stages of peroxisome biogenesis. They conclude that “...ER-localized Pex3 attracts Pex19 and marks the site for insertion of additional peroxisomal-membrane proteins, allowing the next steps in peroxisome development to take place”.

Rachel Smallridge

References and links

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FURTHER READING

Schekman, R. Peroxisomes: another branch of the secretory pathway? *Cell* **122**, 1–2 (2005)

WEB SITE

Henk Tabak's laboratory: <http://www.bijvoet-center.nl/cpc>

STRUCTURE WATCH

Moving to mammals

In *Science*, MacKinnon and colleagues now present two papers on the structure of a mammalian voltage-dependent K^+ (Kv) channel, Kv1.2, of the *Shaker* family. Kv channels conduct K^+ ions across membranes in response to changes in membrane voltage.

Previous structural information on K^+ channels has come from studies on prokaryotic channels, because they can be easily expressed at high levels. However, these new papers describe the 2.9-Å-resolution crystal structure of the rat brain Kv1.2 channel in complex with its $\beta 2$ subunit (β subunits can regulate mammalian Kv channels *in vivo*).

The tetrameric complex, which has fourfold symmetry, is composed of a transmembrane region that contains the pore and the four voltage sensors, an intracellular (T1) domain that is formed by the N termini of the four Kv1.2 subunits, and four $\beta 2$ subunits that are bound to the T1 domain. The pore, which seems to be open, is similar to those of prokaryotic K^+ channels, although the curvature of the inner helices of the Kv1.2 channel is the result of a conserved Pro-X-Pro sequence rather than a Gly residue. The pore is linked to the cytoplasm by large side portals that are located between the pore and T1 domain. The location and properties of the N termini of the T1 domain and β subunits and the electrostatic properties of the portals indicate that these termini might function as inactivation peptides that plug the portals. The structure has also indicated how β subunits might regulate channel function in other ways. Finally, as the voltage sensors seem to be in their native conformation, it has provided a simple view of how membrane voltage affects the probability of the channel being open.

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Forming fibres

In eukaryotic chromosomes, DNA is arranged in chains of nucleosomes that are compacted into chromatin fibres. Although a high-resolution structure of a nucleosome is available, how nucleosomes are organized into fibres is a controversial subject. However, Richmond and colleagues now provide insights by describing the 9-Å-resolution crystal structure of a tetranucleosome in *Nature*.

There are two main models for the organization of chromatin fibres. In the one-start solenoid helix, a linear array of nucleosomes is coiled to form a single stack of nucleosomes; in the two-start helix, nucleosomes are arranged in two stacks that have linkers between them and are twisted or supercoiled. The tetranucleosome structure supports the latter model — DNA zigzags between two stacks of nucleosome cores. The authors modelled chromatin fibres by stacking tetranucleosomes, and the resulting model contains 6.6 nucleosomes per 110 Å of fibre, which is consistent with measured values for chromatin fibres.

This work has therefore shown that the two-start helix is the molecular structure that underlies the complexity of chromatin fibres.

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