

IN THE NEWS

Eternal youth

Scientists from the UK and Japan have discovered an important new player in maintaining the immortality of embryonic stem (ES) cells. This breakthrough might one day allow scientists to turn any cell into an immortal, pluripotent cell that can be used for therapeutic purposes.

Shinya Yamanaka and his colleagues, from the Nara Institute of Science and Technology, and Austin Smith's group, at the Institute for Stem Cell Research in Edinburgh, independently identified a gene that was expressed specifically in pluripotent, undifferentiated cells. They named the gene *Nanog* — after the mythological Celtic land of the ever-young, Tir nan Og.

In two papers in *Cell*, the scientists showed that overexpression of *Nanog* prevented ES cells from differentiating. What is exciting is that *Nanog* seems to be a key factor in the transcription-factor network that is known to be required for the ES cell phenotype.

"As we know more and more about pluripotency, it probably will be possible to reprogram cells to make stem cells out of any cell in the body," said James Thomson, the University of Wisconsin scientist who first isolated human ES cells in 1998 (*Washington Post*, 30th May 2003).

Although most of their experiments involved using the mouse version of *Nanog* in mouse cells, some involved the human version, which was identified thanks to its structural similarity to mouse *Nanog*. "If *Nanog* has the same effect in humans as we have found in mice, this will be a key step in developing embryonic stem cells for medical treatments," said Austin Smith (*New Scientist*, 30th May 2003).

Arianne Heinrichs

GENE REGULATION

A stimulating experience

The transcription factor nuclear factor κ B (NF- κ B) controls cytokine-regulated processes and is regulated by the I κ B kinase (IKK) complex comprising IKK α , IKK β and IKK γ . Whereas IKK β controls the degradation of inhibitors of NF- κ B (I κ Bs), resulting in rapid nuclear accumulation of NF- κ B, the role of the IKK α subunit has been more elusive. But now, reporting in *Nature*, the groups of Richard Gaynor and Albert Baldwin propose a new, nucleosomal function for IKK α .

Recent observations indicated that IKK α might have a role in cytokine-induced, NF- κ B-dependent gene regulation, independent of that of IKK β . Both groups showed that this role is likely to be nuclear, as cytokine induction resulted in the nuclear accumulation of IKK α but not IKK β . To investigate the mechanism underlying IKK α 's nuclear role, the groups

carried out chromatin immunoprecipitation (ChIP) assays and showed that, in response to tumour-necrosis factor (TNF)- α stimulation, IKK α was recruited to NF- κ B-responsive gene promoters.

As interactions between coactivator CREB-binding protein (CBP) and NF- κ B subunit p65 have been shown to be important for NF- κ B activation, Gaynor and colleagues tried to establish whether IKK α functions through complex formation with these coactivators. Indeed, IKK α interacts with CBP, but not with p65. Both groups showed that in *p65*^{-/-} cells, IKK α is not recruited to NF- κ B-regulated promoters, indicating that p65 is required for IKK α -promoter association.

So how does IKK α modulate transcriptional activation? Given that histone H3 modifications have been correlated with active gene expression,



the Gaynor and Baldwin groups carried out ChIP assays using phosphorylated histone H3 antibodies and found that the kinetics of IKK α recruitment and histone H3 serine (Ser) 10 phosphorylation correlated, and that TNF- α -induced Ser10 phosphorylation was abolished in *IKK α* ^{-/-} cells. Cytokine-induced phosphorylation of histone H3 was suggested to be due to the direct kinase activity of IKK α , as IKK α (but not IKK β) was shown to phosphorylate H3 on Ser10 using *in vitro* kinase assays.

CALCIUM



Channelling degeneration

The dystrophin-glycoprotein complex (DGC) provides structural support for the sarcolemma, the membrane that encloses myocytes, and genetic defects in the principal DGC components — dystrophin and sarcoglycan — lead to muscular dystrophy and/or cardiomyopathy in humans and animal models. Now, reporting in *The Journal of Cell Biology*, Shigekawa and colleagues begin to uncover the molecular events involved in myocyte degeneration.

Increased membrane fragility to mechanical stress and permeability to Ca²⁺ have been implicated in myocyte degeneration, and elevated Ca²⁺ concentrations ([Ca²⁺]) have been reported in dystrophic myocytes. So, the authors decided

to look for Ca²⁺-entry mechanisms that could be responsible for pathogenic myocyte degeneration. They began by searching for mammalian homologues of the *Drosophila melanogaster* Ca²⁺-permeable cation channels, which belong to the transient receptor potential channel family, because they are sensitive to physical stimuli.

Shigekawa and colleagues identified the growth-factor-regulated channel (GRC), which had previously been identified as a Ca²⁺-permeable non-selective cation channel expressed in non-muscle cells. They found that, although the total GRC content was similar to normal, GRC expression was elevated in the peripheral sarcolemma of cardiac and skeletal muscle of BIO14.6 hamsters (deficient in δ -sarcoglycan), and the skeletal muscle of *mdx* mice (a model for Duchenne muscular dystrophy) and of myopathic patients.

Next, the authors looked at the properties of BIO14.6 and *mdx* myotubes. They found that GRC

expression was increased in the sarcolemma (in normal myotubes GRC is located mostly in the interior), that it could be reduced by a Ca²⁺-influx inhibitor (Gd³⁺) and then re-established by Ca²⁺. As phosphatidylinositol 3-kinase inhibitors didn't inhibit this translocation, it was concluded that Ca²⁺ was the primary regulator of GRC translocation. In normal myotubes, GRC translocated to the sarcolemma in response to insulin-like growth factor-1 or cyclic stretch in the presence of Ca²⁺ — responses that were abolished by Gd³⁺.

In resting BIO14.6 myocytes, Shigekawa and colleagues found that there was a marked increase in Gd³⁺-sensitive Ca²⁺ uptake, and acute elevation of external [Ca²⁺] increased the intracellular [Ca²⁺] — both responses were suppressed by another Ca²⁺-influx inhibitor. Moreover, cyclic stretch in the presence of Ca²⁺ led to a high level of creatine kinase (CK) efflux, which is a marker of myocyte damage. Infection with δ -sarcoglycan cDNA corrected



It has been proposed that histone H3 phosphorylation can cause the recruitment of histone acetyltransferase (HAT) activity. Indeed, both types of histone H3 modifications were reduced in *IKK α ^{-/-}* cells, indicating that IKK α -mediated phosphorylation of Ser10 is probably important for the subsequent acetylation of lysine (Lys) 14 in histone H3. Using ChIP assays and modified histone H3 antibodies, Gaynor and colleagues showed that cytokine stimulation of wild-type

cells led to increased IKK α -promoter association and Ser10 phosphorylation and Lys14 acetylation of histone H3. CBP has HAT activity, and in *IKK β ^{-/-}* cells, association of both CBP and acetylated histone H3 was reduced, indicating that CBP might provide the HAT activity that is responsible for Lys14 acetylation. The interaction of IKK α and CBP provides an efficient way to phosphorylate, and subsequently acetylate, histone H3, resulting in cytokine-induced activation of NF- κ B-directed gene expression.

The authors concluded that their findings have identified IKK α as an essential player in NF- κ B-regulated gene expression, thereby adding a new role to the several existing regulatory functions for I κ B kinases in this process.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Yamamoto, Y. *et al.* Histone H3 phosphorylation by IKK α is critical for cytokine-induced activation of NF- κ B-regulated genes. *Nature* **423**, 655–659 (2003) | Anest, V. *et al.* A nucleosomal function for I κ B kinase α in NF- κ B-dependent gene expression. *Nature* **423**, 659–663 (2003)



these abnormalities, and treatment with GRC antisense cDNA suppressed both the intracellular [Ca²⁺] increase and the CK efflux.

By expressing GRC on the surface of CHO cells, which lack endogenous GRC, the authors also showed that Ca²⁺ influx through GRC was increased in the absence of stretch, that stretch enhanced the influx and that it caused cytoskeletal reorganization. And finally, from results obtained with cardiac-specific GRC transgenic mice, “it seems likely that elevated levels of sarcolemmal GRC result in greater Ca²⁺ influx in response to mechanical stress in cardiac

chamber walls, causing further mobilization of GRC on the cell surface, thereby exacerbating Ca²⁺ overloading and the resultant cell damage”.

So, the results obtained by Shigekawa and colleagues “...suggest that GRC is a key player in the pathogenesis of myocyte degeneration caused by dystrophin–glycoprotein complex disruption”.

Natalie Wilson

References and links

ORIGINAL RESEARCH PAPER Iwata, Y. *et al.* A novel mechanism of myocyte degeneration involving the Ca²⁺-permeable growth factor-regulated channel. *J. Cell Biol.* **161**, 957–967 (2003)

IN BRIEF

SIGNAL TRANSDUCTION

Dishevelled activates Ca²⁺ flux, PKC, and CamKII in vertebrate embryos.

Sheldahl, L. C. *et al.* *J. Cell Biol.* **161**, 769–777 (2003)

Wnt signalling through Frizzled (Fz) receptors can activate the planar cell polarity (PCP), β -catenin and Ca²⁺ pathways. Dishevelled (Dsh) functions in the β -catenin and PCP pathways, but this study now shows that Dsh also functions in the Ca²⁺ pathway — it activates Ca²⁺ flux, protein kinase C (PKC) and Ca²⁺/calmodulin-dependent kinase II. Indeed, Dsh function is required for full activation of PKC by Fz7 in *Xenopus* eggs.

CENTROSOMES

Centrosome number is controlled by a centrosome-intrinsic block to reduplication.

Wong, C. & Stearns, T. *Nature Cell Biol.* **5**, 539–544 (2003)

Centrosomes duplicate only once during the cell cycle but it has been unknown whether this is because of a decline in positive factors or the result of a specific reduplication block. Here, Wong and Stearns used cell-fusion assays to show that a centrosome-intrinsic block, rather than any cytoplasmic factors, prevents reduplication of recently duplicated G2 centrosomes, and that this block is not controlled by the centrosome:nucleus ratio.

TELOMERES

Developmentally programmed gene elimination in *Euplotes crassus* facilitates a switch in the telomerase catalytic subunit.

Karamysheva, Z. *et al.* *Cell* **113**, 565–576 (2003)

Here, Karamysheva *et al.* show that three *TERT* genes encode the catalytic subunit of telomerase in *Euplotes crassus*. Their expression requires +1 ribosomal frameshifting. *TERT-1* and *TERT-3* expression correlates with telomere maintenance, whereas *TERT-2* is expressed during *de novo* telomere formation. *TERT-2* expression is controlled by programmed DNA degradation, so that it is eliminated during vegetative growth.

SIGNALLING

A hedgehog-responsive region in the *Drosophila* wing disc is defined by Debra-mediated ubiquitination and lysosomal degradation of Ci.

Dai, P. *et al.* *Dev. Cell* **4**, 917–928 (2003)

The Hh-responsive genes *decapentaplegic* and *patched* are expressed specifically in a stripe of anterior (A)-compartment cells 9–10 cells away from the A–posterior border. The transcription factor Cubitus interruptus (Ci), which activates Hh target genes, is present at high levels in this stripe, too. Dai *et al.* have now identified Debra (Dbr) — for determiner of breaking down of Ci activator — which binds to Ci and mediates Ci polyubiquitylation. This results in Ci lysosomal degradation at the border of the Hh-responsive region.