Meeting report

Protein degradation, signaling, microRNAs and cancer

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A report on the biannual Swiss Institute for Experimental Cancer Research (ISREC) Symposium on the Cell and Molecular Biology of Cancer, Lausanne, Switzerland, 19-22 January 2005.

Every two years, biologists and oncologists gather in Lausanne, on the shores of Lake Geneva, to discuss the latest advances in research into the cell and molecular biology of cancer. Presentations at the most recent of these meetings covered a wide range of topics, a small selection of which is described here.

Several talks were devoted to the cellular protein degradation machinery and its targets in the cell cycle. The protein p27 is an inhibitor of the kinase activity of the complex of cyclin-dependent kinase Cdk2 and cyclin E, and the destruction of p27 is normally required before a cell can enter S phase of the cell cycle. An essential role in p27 degradation has been assigned to the E3 ubiquitin ligase SCF-Skp2 (named after its components skp1, cullin and F-box protein). Willy Krek (Eidgenössische Technische Hochschule, Zürich, Switzerland) confirmed the importance of Skp2 in p27 degradation but presented the audience with a new ubiquitin-ligase partner for Skp2, a RING-domain containing protein called SAR1 (Skp2-associated RBCC protein 1) that belongs to the RBCC (ring finger, B box, coiled-coil) family. Both SAR1 and Skp2 are found in a complex with Cul1 (a core subunit also found in SCF) and the whole complex has been baptized CRF-Skp2 (for Cul1-RBCC-F-box complex containing Skp2). Specific inhibition of CRF-Skp2 results in accumulation of p27, in particular of the form phosphorylated on threonine 187, and in a slowdown in G1 phase. Krek proposes that CRF-Skp2 might be the real culprit in p27 degradation, and that SCF-Skp2 plays no role in this process - a suggestion that will be bound to create a stir in the cell-cycle field.

However it happens, the degradation of p27 clears the path for entry into S phase, in which the cell is confronted with the task of replicating its DNA. Matthias Peter (Eidgenössische Technische Hochschule) stressed the role of ubiquitination in this process also. Cells of budding yeast (*Saccharomyces cerevisiae*) lacking Rtt101p, a relative of the E3 ubiquitin-ligase components Cul3 and Cul4, display a delay in the metaphase-to-anaphase transition of mitosis and show increased sensitivity to genotoxic drugs. These problems can be traced back to a defect in replicating certain regions of the genome - 'slow zones' - which include the rRNA locus. In these areas, Rtt101p appears to be required for the restart of collapsed replication forks (but not of the ones that have only stalled but are still intact), suggesting a role for ubiquitination and possibly protein degradation in this process.

Ubiquitination does not always result in protein degradation. Martin Eilers (University of Marburg, Germany) described work on the ubiquitination of the transcription factor and proto-oncogene c-Myc. In contrast to known ubiquitinations of c-Myc, Eilers reported that ubiquitination by the E3 enzyme Hect H9 does not affect c-Myc stability. Instead, ubiquitination of lysine residues within, and close to, the nuclear localization signal enhance c-Myc's affinity for the transcriptional coactivator p300. This activity of Hect H9 is inhibited by the transcriptional activator Miz1, which in turn is inhibited by binding to c-Myc. From these observations, Eilers proposed a model in which Hect H9 plays an important role in switching Myc's role from a transcriptional repressor (when in the Myc:Miz1 complex) to an activator (when Myc is bound directly to its binding sites in DNA).

Like Myc, the insulin signaling pathway plays an essential role in controlling cellular growth during development. Ernst Hafen (University of Zürich, Switzerland) reported how his group has used genetic screens in *Drosophila melanogaster* to identify novel components of this pathway. One of these, named Susi, binds to and inhibits the regulatory subunit of phosphatidylinositol 3-OH kinase (PI 3-kinase), and thus acts as a negative regulator of growth. Susi has no apparent ortholog in vertebrates, in contrast to two other cell-growth

inhibitors discovered by these screens, the paralogous proteins Scylla and Charybdis. These two function in the insulin pathway downstream of PI 3-kinase and the protein kinase (and certified proto-oncogene) Akt/PKB and upstream of the tumor suppressors TSC1 and TSC2, resulting in the inhibition of the kinase Tor (target of rapamycin) and of downstream effectors such as ribosomal protein S6 kinase, thus suppressing cell growth.

Transcription of the *Scylla* and *Charybdis* genes is induced by hypoxia via the hypoxia-inducible transcription factor HIF. The same induction by HIF and downstream effects on TSC1/TSC2 was reported for the vertebrate counterparts of Scylla and Charybdis, Redd1/Rtp801, by James Brugarolas of Bill Kaelin's group (Harvard Medical School, Boston, USA). Thus, this protein family appears to lie at the intersection of two important and evolutionarily conserved signaling pathways, the hypoxia-induced and the insulin pathway.

Turning from the control of size to the control of developmental fate, several speakers discussed molecular components of the Wnt signaling pathways, which have roles in both development and disease. Konrad Basler (University of Zürich) reported work in *Drosophila melanogaster* showing that the recently identified components Legless/Bcl-9 (Lgs) and Pygopus (Pygo) do not exclusively serve to localize the transcription factor β-catenin/Armadillo to the nucleus in response to Wnt signaling, but also themselves have roles as transcriptional coactivators. The relative importance of the two proteins in Wnt signaling appears to be different, as revealed by mouse embryos lacking either both Pygopus paralogs ($pygo1^{-/-}$ $pygo2^{-/-}$) or both Legless paralogs ($lgs1^{-/-}$ $lgs2^{-/-}$). Fabienne Murphy-Seiler, working with Michel Aguet (ISREC, Epalinges, Switzerland), reported that these mutant embryos die at different stages of embryonic development (embryonic day (E) 10.5 for $lgs1^{-/-}$ $lgs2^{-/-}$, and E14.5 for $pygo1^{-/-}$ $pygo2^{-/-}$). She also reported that only Legless is required for the regeneration of adult intestinal epithelium after damage, and that neither Legless nor Pygopus seem to be essential for intestinal epithelial homeostasis under normal conditions. As these mutant phenotypes differ dramatically from those of various Wnt mutants, it appears that non-canonical Wnt signaling, which does not involve the downstream effector TCF and its cofactors, plays an important role in many of these processes.

Wnt signaling is an important player in colon cancer, but it does not act alone. Hans Clevers (Netherlands Institute for Developmental Biology, Utrecht, The Netherlands) showed that Wnt cooperates with the cell-surface protein Notch to maintain stem cells in colonic crypts, and that a similar cooperation might also take place in adenomas. In line with these findings, inhibition of Notch signaling in such adenomas (by interference with γ-secretase, which is required for cleavage of Notch to liberate the active Notch-intracellular domain) reduced their rate of proliferation and promoted differentiation towards the goblet cell type. These results raise the exciting possibility that γ-secretase drugs that were developed for Alzheimers' disease might find a second life in the treatment of colon cancers.

A class of molecules that has attracted considerable general attention lately is the microRNAs (miRNAs), short RNAs involved in post-transcriptional regulation. Steven Cohen (European Molecular Biology Laboratory, Heidelberg, Germany) pointed to the widespread distribution of miRNA loci in metazoan genomes and suggested that they might control a substantial fraction of protein-coding genes. But how important are these molecules for development? Alex Schier (Skirball Institute of Biomolecular Medicine, New York University School of Medicine, USA) has addressed this question in zebrafish. His group created embryos that were both zygotically and maternally mutant for Dicer, an enzyme essential for the maturation of miRNAs. These embryos lacked most (if not all) mature miRNAs, although microinjection of double-stranded RNA still resulted in gene silencing, demonstrating that only the processing of the miRNAs is impaired. Despite these defects, the mutant embryos underwent a surprisingly normal development: axis formation, regionalization and the activity of all major signaling pathways appeared normal. Some major defects were found in the central nervous system (malformation of the ventricles and problems with neuronal differentiation); these could be partly overcome by injection of a specific class of miRNAs, although the partially rescued embryos were still not able to develop to term. These findings suggest that miRNAs do not control the big decisions in development, but rather function to fine-tune tissue homeostasis, or work as "micromanagers" (as Schier called them).

The audience at this meeting was treated to a diversity of presentations of high quality, and will undoubtedly be looking forward to the next ISREC symposium in 2007.