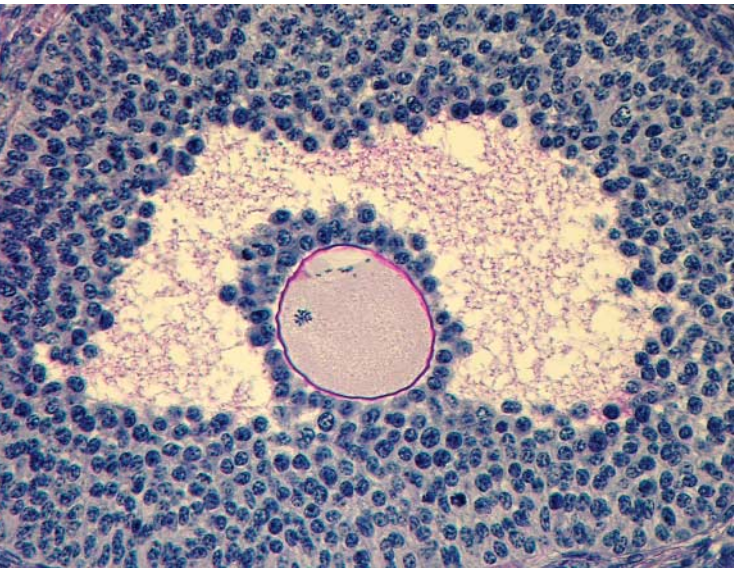
MEIOSIS

Under arrest



An oocyte with a polar body within an antral follicle of a *Gpr3*$^{-/-}$ ovary. Image kindly provided by Laurinda Jaffe, University of Connecticut Health Center, Farmington, CT, USA.

Mammalian oocytes are arrested in prophase of meiosis from a time before birth. Then, during reproductive life, luteinizing hormone (LH) induces the resumption of meiosis, which results in a haploid set of chromosomes in preparation for fertilization. Researchers reporting in *Science* have now shed light on how meiotic arrest is maintained in mouse oocytes up until the time that LH induces the resumption of meiosis and ovulation.

The heterotrimeric G protein G_s had previously been shown to be required for meiotic arrest, so Mehlmann and Jaffe, in collaboration with Eppig and colleagues, searched a database of genes that are expressed in mouse oocytes, looking for a receptor that activates G_s. The search identified the orphan G_s-linked receptor GPR3, which increases cyclic-AMP concentrations. This was an interesting result because G_s is known to keep cAMP concentrations elevated, which is important for meiotic arrest. Next, they showed that *Gpr3* mRNA was present in oocytes, and that the concentration of *Gpr3* transcripts was ~14 times higher than in the surrounding somatic cells of antral (or resting) follicles.

To probe the function of GPR3, Mehlmann, Jaffe and co-workers analysed the ovaries of *Gpr3*-knockout mice. The ovaries were normal in terms of their external morphology, growth and ovulatory activity. However, 82% of the oocytes within antral *Gpr3*$^{-/-}$ follicles had resumed meiosis, and almost half of these were in metaphase II. Only 37% of oocytes from early antral *Gpr3*$^{-/-}$ follicles resumed meiosis, whereas all oocytes in pre-antral *Gpr3*$^{-/-}$ follicles remained arrested in prophase. So, depending on the developmental stage of the follicles, oocytes that lacked GPR3 resumed meiosis.

Next, the authors showed that the resumption of meiosis in *Gpr3*$^{-/-}$ follicles was independent of LH. The action of LH would normally lead to the deposition of extracellular material around the somatic granulosa cells,

SIGNALLING

A pick-me-up

Src becomes activated in response to various extracellular stimuli, but the hows and wheres have been somewhat hazy. Thanks to the use of a Src fusion protein, Sandilands *et al.* have now found that Src becomes activated during transit from the perinuclear region to the plasma membrane. The actin cytoskeleton is required for this, as are RhoB-associated cytoplasmic endosomes.

Once the authors had established that the green fluorescent protein (GFP) tag that they engineered onto Src (to make Src–GFP) didn't interfere with the normal spatial distribution, activation or interactions of Src, they continued with the main aim of their studies to address the connection between catalytic activation and spatial localization. They saw that Src activity increased from the perinuclear region, where it was inactive, to peripheral membrane structures, where it was highly active. Artificially disrupting actin filaments inhibited both the membrane translocation and activity of Src. And when a mutant version of suppressor of cAR (SCAR)/WAVE1 was used to inhibit actin nucleation, Sandilands *et al.* showed that Src–GFP couldn't translocate to the plasma membrane in response to stimulation by platelet-derived growth factor (PDGF). This hinted that 'outside–in' signals from the PDGF receptor that induce the translocation/activation of Src required signalling to the actin-polymerization machinery.

But the results didn't preclude the idea that endosomes — actin-associated structures with which Src has previously been reported to colocalize — might be involved in this 'outside–in' signalling. Indeed, the authors showed that active Src–GFP colocalized with RhoB, an endosome-associated Rho GTPase, in the perinuclear region and in discrete intracellular structures that surround this region. In the absence of RhoB, Src failed to accumulate at the membrane or be activated in response to cell plating on fibronectin (a main Src-activating extracellular stimulus).

So, could there be a connection between these endosomal structures and the actin-polymerization machinery? Immunostaining showed that Src–GFP and fluorescently tagged forms of RhoB and SCAR/WAVE1 all localized to discrete cytoplasmic structures. Disrupting actin filaments didn't alter RhoB endosomes from cells that lacked Src and the Src-family proteins Fyn and Yes — that is, until Src was re-expressed, in which case RhoB endosomes remained in the perinuclear region. This implies that the presence of Src in RhoB endosomes somehow resulted in them being dependent on the actin cytoskeleton to move. When Src was induced to move to the peripheral membrane at the same time as actin polymerization was re-initiated (both by external means), motile Src- and RhoB-containing structures that resembled endosomal vesicles were associated with newly formed small bundles of actin — actin clouds — between the perinuclear region and the cell membrane. The actin clouds weren't seen when the mutant SCAR/WAVE1 construct was expressed, implying that Src is promoting the actin-nucleation events that are associated with the endosomes.

The authors, therefore, postulate the following model: inactive Src is usually present around the perinuclear region, from where it is 'picked up' and transported to peripheral membranes in RhoB-associated, Rab11-dependent endosomes, probably by inducing actin-nucleation events. Details of exactly how Src induces these actin-nucleation events are awaited.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Sandilands, E. *et al.* RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. *Dev. Cell* **7**, 855–869 (2004)

which results in an expanded follicle — but this was not the case in these mutants. In fact, the only detected defect in *Gpr3*$^{-/-}$ follicles was the failure to maintain meiotic arrest. This phenotype could be reversed by injecting *Gpr3* RNA into oocytes from *Gpr3*$^{-/-}$ pre-antral or very early antral follicles and letting the follicles grow in culture. Only 11% of the injected oocytes had resumed meiosis after a 4-day culture period, compared with about half of the non-injected oocytes.

Together, these data indicate that GPR3 is a negative regulator of meiotic progression in mouse oocytes. Future studies will undoubtedly look into how LH might relieve the arrest and reinitiate meiosis as well as try to identify the ligands that interact with GPR3.

Arianne Heinrichs

References and links

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DNA REPAIR

Breaking up is hard to do

Two new papers in *Current Biology* have helped to clarify the mechanism by which DNA double-strand breaks (DSBs) are held together during DNA repair.

DSB formation is a significant event for a cell as, if a DSB remains unrepaired, it could result in a chromosomal break and the potentially lethal loss of genetic information. Cell-cycle checkpoints and various DSB-repair mechanisms exist to prevent this from occurring. But how exactly are DSBs held together during DNA repair, and which factors contribute to chromosome fragmentation when it does occur?

Kaye *et al*. and Lobachev *et al*. both employed systems in which an inducible endonuclease was used to generate DSBs at an engineered recognition site in budding yeast chromosomes. Recognition sites for fluorescent fusion proteins were also engineered into the chromosome arms close to the DSB site, so that the chromosome ends could then be visualized under a microscope. Both groups found that, in wild-type cells, DSB formation did not immediately result in chromosome fragmentation. But what was holding the ends together?

Kaye *et al*. induced DSB formation in G1-arrested cells and investigated what happened to the chromosomes after passage through S phase. They found that there was an intrachromosomal association between centric and acentric fragments of the broken chromosome, which was not permanently disrupted by DNA replication. Furthermore, such intrachromosomal association depended on the homologous-recombination proteins Rad50 and Rad52, which both helped to maintain this association. If mitosis was allowed to occur, Kaye *et al*. found that, in 95% of cases, the acentric fragments co-segregated into either the mother or the daughter cell. Similarly, rejoining of the acentric and centric fragments post-mitosis occurred in the mother cell or in the daughter cell, but almost never in both, which indicates that the broken sister-chromatid fragments are passaged into the same nucleus.

The authors propose that two mechanisms contribute to the segregation of broken chromosomes. First, intrachromosomal association holds the two halves of a single, broken sister chromatid together. Second, interchromosomal association tethers the homologous fragments of broken sister chromatids to each other, which, in fact, promotes the missegregation of the chromosome fragments, particularly the acentic fragment.

Lobachev *et al*. investigated the behaviour of DSB-induced chromosome ends in cells that lacked components of the RMX complex, which comprises Rad50, Mre11 and Xrs2, and is involved in non-homologous end-joning (NHEJ)-mediated DSB repair. They showed that DNA ends moved apart in cells that lacked any of these three proteins, and that mutations in the zinc-hook domain of Rad50 also caused significant increases in the separation of DNA ends. By contrast, mutations in Ku70, Ku80 and DNA ligase IV (Dnl4), which are specifically required for DNA end-joining, did not caused separation of DNA ends. Chromosome fragmentation was also shown to require microtubule-based forces.

So, it seems that broken chromosomes are harder to split up than might be imagined. The RMX complex and Rad52 contribute to the continued association of chromosome ends; whereas inter-chromatid forces — which are probably mediated by residual cohesins — promote the continued association of chromosome fragments with their sister chromatids.

Lesley Cunliffe

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