Together, these results are indicative of a mechanism that links ageing with late-onset AD. As aggregates develop, HSF-1 activity mediates their disaggregation. DAF-16 activity supports an alternative pathway (which perhaps functions as a back-up pathway) that mediates the formation of low-toxicity, high-molecular-weight aggregates from high-toxicity small aggregates. Because both detoxification pathways are mediated by the ageing-related insulin-signalling pathway, both can become compromised with ageing, leading to aggregate build-up.

Interestingly, as the insulin-signalling pathway is also associated with the formation of other toxic aggregates, such as those responsible for Huntington's disease, further research into this pathway could yield therapeutic targets for the general prevention of late-onset aggregation-linked neurodegenerative diseases.

Asher Mullard

ORIGINAL RESEARCH PAPER Cohen, E. *et al.* Opposing activities against age onset proteotoxicity. *Science* 10 Aug 2006 (doi:10.1126/science.1124646)

of Kiz is necessary for spindle-pole stabilization. As the phosphorylation of Kiz increases its association with a subset of PCM components, the authors propose that, by stabilizing interactions between centrioles and the PCM, Plk1-mediated phosphorylation of Kiz protects centrosomes from collapse and ensures bipolar spindle formation.

Unlike Plk1 depletion, which causes prometaphase arrest, cells that lack Kiz exit mitosis with multiple or malformed nuclei. The additional effects of Plk1 on chromosome segregation and cytokinesis are likely to be mediated by other substrates; however, the identification of Kiz is the first step towards unravelling the mechanism of action of Plk1.

Monica Hoyos Flight, Editor,
Cell Migration Update

ORIGINAL RESEARCH PAPER Oshimori, N. *et al.* Kizuna, a new Plk1 target, stabilizes mitotic centrosomes to ensure spindle bipolarity. *Nature Cell Biol.* 17 Sep 2006 (doi:10.1038/ncb1474)

IMAGING

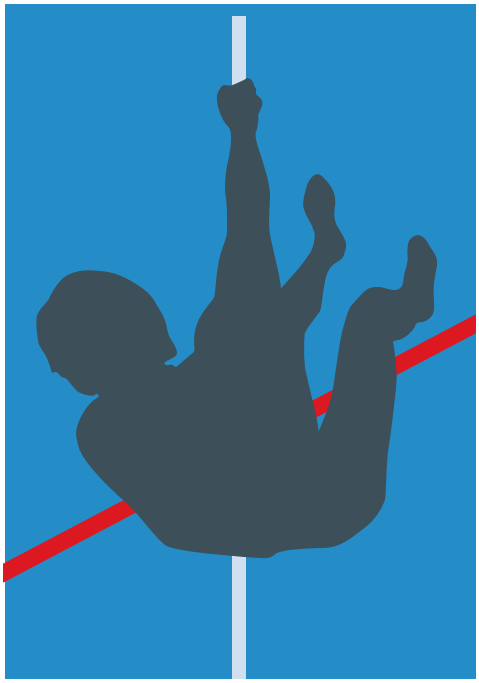
Overcoming the barrier

In the past decade or so, great progress has been made in developing new imaging techniques that can overcome the 'diffraction barrier', which limits the resolution in light-microscopy techniques to the micrometer range. Two groups have now independently developed fluorescence-microscopy techniques that allow nanometer resolution.

Stefan Hell and colleagues invented stimulated emission depletion (STED) microscopy more than a decade ago, and subsequently showed that this technique can be used to study biological samples. STED microscopy uses a scanning excitation spot that overlaps with a doughnut-shaped counterpart for the de-excitation of fluorophores by light. Oversaturating the de-excitation reduces the fluorescence spot to sub-diffraction-barrier dimensions, resulting in super-resolved images. This technique has now been tweaked so that a major source of photobleaching — known as molecular triplet-state excitation — of fluorescent markers is eliminated. This has resulted in a ~30-fold increase in total fluorescence signal and enabled a significant reduction in the focal spot area. So-called triplet relaxation (T-Rex) STED microscopy can achieve a 15–20-nm resolution in the focal plane.

This resolution was demonstrated by various applications of T-Rex STED microscopy in immunofluorescence imaging. Fluorescently labelled transmembrane synaptic vesicle proteins, such as synaptotagmin I and synaptophysin, could be identified as distinct 25–40-nm spots on purified endosomes. And, nuclear 'speckles', which are enriched in pre-mRNA splicing factors, could be separated into distinct particles — this technique might therefore be useful for studying other aspects of nuclear organization. Hell and colleagues have already started to explore the possibility of improving the axial resolution by combining STED with 4Pi confocal microscopy.

Taking a different approach, Eric Betzig, Harald Hess and co-workers developed a method for the isolation of single molecules at high density. The technique, which they called photoactivated localization microscopy (PALM), is based on the idea that the photoactivation of one fluorescent molecule at a time allows for a more precise molecular separation than when all molecules glow at once. Cultured cells that express a photoactivatable fluorescent protein that is attached to the protein of interest can be fixed and imaged as whole cells or processed in thin sections. The sample is photoactivated and



subsequently photobleached, and this process is repeated many times until sparse fields of individually resolvable molecules are obtained. The location of each molecule is determined, and a super-resolution PALM image is acquired by adding up the locations of the other molecules in the image.

Using PALM in thin sections, Betzig, Hess and colleagues visualized specific target proteins in lysosomes and mitochondria with 10–20-nm resolution. PALM images of whole fixed cells revealed the partial assembly of a vinculin network at focal adhesion regions, an increased amount of actin at the leading edge of lamellipodia and the heterogeneous distribution of the retroviral protein Gag at the plasma membrane.

T-Rex STED microscopy and PALM provide better resolution than total internal reflection microscopy and confocal imaging, as well as comparable resolution to transmission electron microscopy, without the disadvantage of cryo-preparation. These sophisticated techniques open the door to near-molecular-resolution imaging of fluorescent targets in thin sections and whole fixed cells.

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ORIGINAL RESEARCH PAPERS Donnert, G. *et al.* Macromolecular-scale resolution in biological fluorescence microscopy. *Proc. Natl Acad. Sci. USA* **103**, 11440–11445 (2006) | Betzig, E. *et al.* Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 10 Aug 2006 (doi:10.1126/science.1127344)