extension to these ideas. Their data indicate that cells with supernumerary centrosomes, which form as a consequence of failed cytokinesis, can propagate in a p53^{-/-} background, whereas in a p53^{+/+} background such cells arrest or die. These observations suggest that p53 activation is not restricted to mitotic failure in syncytia, but is a more general scenario following failed division. What exactly signals such failure is unclear — possibly physical restrictions are sensed when chromosomes are pulled apart by a multipolar spindle, or, alternatively, p53 might monitor centrosome status or DNA content/damage in the subsequent G1 phase.

More experiments should be done to understand the analogy between mitotic failure in syncytia, and division of 'normal'

cells with multipolar spindles. It will be very interesting to analyse spindle formation and mitotic progression in the syncytia, and to test the involvement of known mitotic-checkpoint proteins in the apoptotic pathway. HIV also encodes a protein (Vpr) that is reported to prevent mitotic entry¹¹, suggesting that the virus may have evolved ways to inhibit karyogamy, possibly to benefit viral production. Likewise, it will be instructive to assess whether mTOR is a common mediator in the apoptotic demise of cells with numerical centrosomal aberrations. In the end, HIV could teach us how normal cells prevent centrosome anomalies, and how tumour cells become aneuploid. □

Jan Paul Medema is in the Department of Clinical Oncology, Leiden University Medical Center,

Albinusdreef 2, 2333 ZA Leiden, The Netherlands and René Medema is in the Division of Molecular Biology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands e-mail: J.P.Medema@lumc.nl or r.medema@nki.nl

1. Castedo, M. *et al.* *EMBO J.* **15**, 4070–4080 (2002).
2. Brinkley, B. R. *Trends Cell Biol.* **11**, 18–21 (2001).
3. Johnson, R. T. & Rao, P. N. *Nature* **226**, 717–722 (1970).
4. Rao, P. N. & Johnson, R. T. *Nature* **225**, 159–164 (1970).
5. Castedo, M. *et al.* *J. Exp. Med.* **194**, 1097–1110 (2001).
6. Gingras, A. C., Raught, B. & Sonenberg, N. *Genes Dev.* **15**, 807–826 (2001).
7. Durocher, D. & Jackson, S. P. *Curr. Opin. Cell Biol.* **13**, 225–231 (2001).
8. Fingar, D. C., Salama, S., Tsou, C., Harlow, E. & Blenis, J. *Genes Dev.* **16**, 1472–1487 (2002).
9. Ravid, K., Lu, J., Zimmet, J. M. & Jones, M. R. *J. Cell Physiol.* **190**, 7–20 (2002).
10. Meraldi, P., Honda, R. & Nigg, E. A. *EMBO J.* **21**, 483–492 (2002).
11. Emerman, M. & Malim, M. H. *Science* **280**, 1880–1884 (1998).

'ER we go

During phagocytosis, it has generally been accepted that phagosomes form by invagination of the plasma membrane (PM). So why did a proteomics study by Michel Desjardins and colleagues show that phagosomes contain several endoplasmic reticulum (ER) proteins? Now, a paper from the Desjardins laboratory (Gagnon *et al.* *Cell* **110**, 118–131 (2002)) has answered the question by showing that fusion of the ER with the PM underneath phagocytic cups — membrane structures that surround partially engulfed particles — provides a source of membrane for phagosome formation in macrophages.

Pronase digestion of purified intact phagosomes — a method that degrades all protein moieties that are exposed on the cytoplasmic side of these organelles — allowed the authors to confirm that the ER membrane protein calnexin is a genuine component of the phagosome membrane, and that it is orientated as would be expected if the ER directly fuses with phagosomes. This result was confirmed using pre-embedding immunogold labelling at the electron microscopy (EM) level with antibodies against the cytoplasmic domain of calnexin.

Desjardins and co-workers then monitored the relative abundance of calnexin in phagosomes during phagocytosis, and found that it is present in phagocytic structures ranging from phagocytic cups to mature phagosomes. Using immunoblotting to monitor the kinetics of calnexin association with phagocytic structures, they confirmed that the ER associates with phagosomes at a very early stage of phagosome formation, and also showed that successive waves of ER are incorporated during phagosome maturation.

Phosphatidylinositol 3-OH kinase (PI(3)K) is known to be important for phagocytosis, and the authors found that inhibitors of PI(3)K decrease ER-mediated phagocytosis in macrophages. When EM was used to visualize the inhibited cells, they saw direct contacts between the ER and PM at sites of internalization, which suggests that the ER is directly recruited to the PM to form phagosomes. Using an immunocytochemical approach to localize the activity of the ER enzyme, glucose-6-phosphatase (G-6-Pase), they confirmed the recruitment of the ER to the PM.

ER-mediated phagocytosis seems to be a widely used mechanism in macrophages, as the authors found that it is used for the

internalization of inert particles through various receptors (for example, Fc and complement receptors). They also showed that it is used for the internalization of the intracellular pathogens *Leishmania donovani* and *Salmonella typhimurium*. The image shows a *Leishmania* parasite (L) inside a phagosome that displays G-6-Pase activity. G-6-Pase is normally found in the ER and the nuclear envelope (NE), as shown in the image.

The discovery of ER-mediated phagocytosis in macrophages by Desjardins and colleagues has altered our long-held view of phagosome formation, and has provided explanations for previous observations (for example, how antigens from intracellular pathogens can be presented by MHC class I molecules). As the authors found that ER-mediated phagocytosis is not the preferred mechanism in neutrophils (where pathogens are killed rapidly), they propose that intracellular pathogens have evolved to exploit ER-mediated phagocytosis to evade destruction by macrophages.

RACHEL SMALLRIDGE

Associate Editor, *Nature Reviews Molecular Cell Biology*