Asexual reproduction

'Midwives' assist dividing amoebae

Asexual cells are normally able to reproduce entirely by themselves. But we have discovered that in about one-third of the dividing cells of *Entamoeba invadens* contraction of the cleavage furrow[1] may stop before separation is complete. We show here that the connected daughter cells overcome this problem by calling upon a neighbouring amoeba to help them achieve the final stage of division. The 'midwife' cell is chemotactically recruited for this mechanical intervention in what is a surprising example of primitive cooperation.

When an amoeba divides, the two daughter cells stay attached by a tubular tether which remains intact unless mechanically severed. If called upon, the neighbouring amoeba midwife (green cell in Fig. 1) travels up to 200 μm towards the dividing amoeba (see movie in supplementary information), usually advancing in a straight trajectory with an average velocity of 0.54 ± 0.08 μm s^{-1}. The midwife then proceeds to rupture the connection, after which all three amoebae move on.

Using four different samples, each at amoeba densities of about 3×10^4 cells cm^{-2}, we recorded 106 divisions, of which 32 ($30 \pm 7\%$) were involved in cooperative reproduction (statistics were averaged over samples to give a 95% confidence level). In 11 cases ($10 \pm 5\%$), the division was aborted, and the amoebae continued to live as multinucleated cells. The multinuclear cells may resume their attempt to divide, sometimes producing three or four viable daughter cells[2,3].

In most cases (63 divisions, or $60 \pm 8\%$), however, separation eventually occurred[4], with both daughter cells resuming motility and using traction force to move away from each other, tugging at their connection so that it lengthened and narrowed until it finally broke. The incidence of abortions and tripolar divisions rises significantly ($20 \pm 5\%$ aborted of 82 divisions) at low cell densities, when midwifing is rare.

To test whether the midwife is attracted chemotactically to the labouring division site, we aspirated fluid from the vicinity of the cleavage furrow of a dividing amoeba with a micropipette, then carefully discharged it near a distant amoeba. Of 41 such experiments, 15 (37%) revealed a positive chemotactic response (see movie in supplementary information; amoebae extended a directed pseudopod and followed a retracting pipette for a distance of over three times their own length); in 12 (30%), no response was seen; and in the remainder, cells moved in the general direction of the stimulus but resumed random motion before traversing 100 μm.

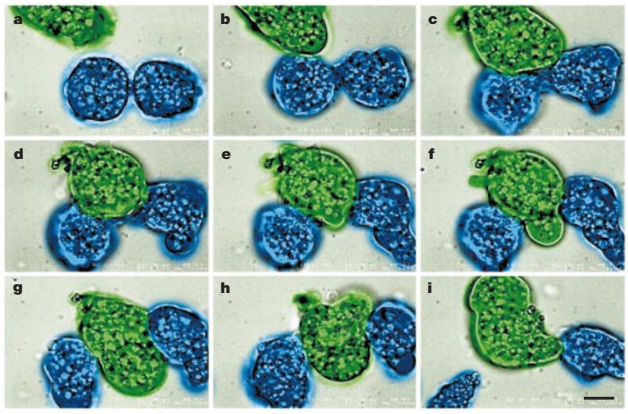

Figure 1 A typical 'midwifing' event, with the participants coloured for clarification. Frame times (**a–i**) are 0, 13, 30, 36, 41, 43, 49, 59 and 84 s, respectively. **a–c,** The midwife (green) aligns itself against the cleavage furrow; **d–f,** it puts out a pseudopod perpendicular to the furrow; **g–i,** then forcibly moves into the gap to disconnect the two daughter cells (blue). Although a few midwife amoebae may respond to the chemoattractant, only one, usually the nearest neighbour, is involved in completing the division. The midwife begins to move towards the dividing cell 2–3 min after division begins; this timing is compatible with an attractant with a diffusion coefficient of 10^{-6}–10^{-7} cm^2 s^{-1} diffusing over a distance of 100 μm. Scale bar, 10 μm.

Amoebae showing positive chemotactic responses pursued the pipette over distances of a few hundred micrometres, persistently following the tip when we changed the direction of the pipette's trajectory. Controls consisting of fluid aspirated from the vicinity of a non-dividing amoeba or of fresh medium alone elicited no response from neighbouring cells.

Identifying the chemical attractant in a volume of only 10 picolitres is difficult, so we sampled a mature culture of cells at the peak of their division cycle, reasoning that the concentration of chemoattractant would be higher than in a young culture. We found that fluid drawn from a confluent culture (3–4 days old, undergoing over a million divisions per flask per day) and transferred into a culture of only a few hours old, induced a strong chemotactic response in the younger cells (see movie). These amoebae followed retreating pipettes for over 30 min at an average linear velocity of 0.73 ± 0.09 μm s^{-1} and flocked towards a stationary pipette at an average radial velocity of 0.34 ± 0.04 μm s^{-1}. Control solutions of cell-free three-day-old medium elicited no response; to control for any possible effect of starvation, fresh medium was changed 18 hours before collecting the fluid.

As we sampled fluid from cultures before the cells started clustering, we consider it most likely that the factor responsible for attracting midwives is the one secreted by cytokinesing cells, although it could be an entirely different chemoattractant. We have established that its relative molecular mass is between 50K and 100K and that it is still active after heating for 20 min at 95 °C, but it is sensitive to oxidation by 0.24 M metaperiodate. Glycoconjugates, particularly membrane lipophosphoglycan-like molecules[5], have these properties and are therefore likely candidates for the midwife-recruiting chemoattractant molecule.

A similar phenomenon has recently been observed in dividing cells of the amoeba *Dictyostelium discoideum* (G. Gerisch and J. Köhler, personal communication). Midwifing may therefore constitute a more general adaptation of existing chemotactic mechanisms[6,7], enabling flaws in reproduction to be overcome through cooperation.

David Biron*, Pazit Libros†, Dror Sagi*, David Mirelman†, Elisha Moses*

*Departments of *Physics of Complex Systems, and †Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel*

e-mail: elisha.moses@weizmann.ac.il

1. Schroeder, T. E. *Ann. NY Acad. Sci.* **582,** 78–87 (1990).
2. Weber, I. *et al. Curr. Biol.* **10,** 501–506 (2000).
3. Makioka, A., Kumagai, M., Ohtomo, H., Kobayashi, S. & Takeuchi, T. *Parasitol. Res.* **86,** 599–602 (2000).
4. Fukui, Y. *Microscopy Res. Tech.* **49,** 183–189 (2000).
5. Moody-Haupt, S., Patterson, J. H., Mirelman, D. & McConville, M. J. *J. Mol. Biol.* **297,** 409–420 (2000).
6. Bailey, G. B., Leitch, G. J. & Day, D. B. *J. Protozool.* **32,** 341–346 (1985).
7. Mato, T. M., Losada, A., Nanjundiah, V. & Konijn, T. M. *Proc. Natl Acad. Sci. USA* **72,** 4991–4993 (1975).

Supplementary information is available on *Nature*'s website at http://www.nature.com.

Inflammatory response

Pathway across the blood–brain barrier

Inflammatory reactions against invaders in the body call upon cytokine molecules that elicit systemic responses, such as fever, fatigue, increased pain sensitivity and appetite loss, mediated by the central nervous system. But how cytokines can induce these effects has been a mystery as they are unlikely to cross the blood–brain barrier[1–3]. Here we show that cerebral vascular cells express components enabling a blood-borne cytokine to stimulate the production of prostaglandin E_2, an inflammatory mediator whose small size and lipophilic properties allow it to diffuse into the brain parenchyma. As receptors for this prostaglandin are found on responsive deep neural structures[4–6], we propose that the activated immune system controls central reactions to peripheral inflammation through a prostaglandin-dependent, cytokine-mediated pathway.

The final step in the biosynthesis of

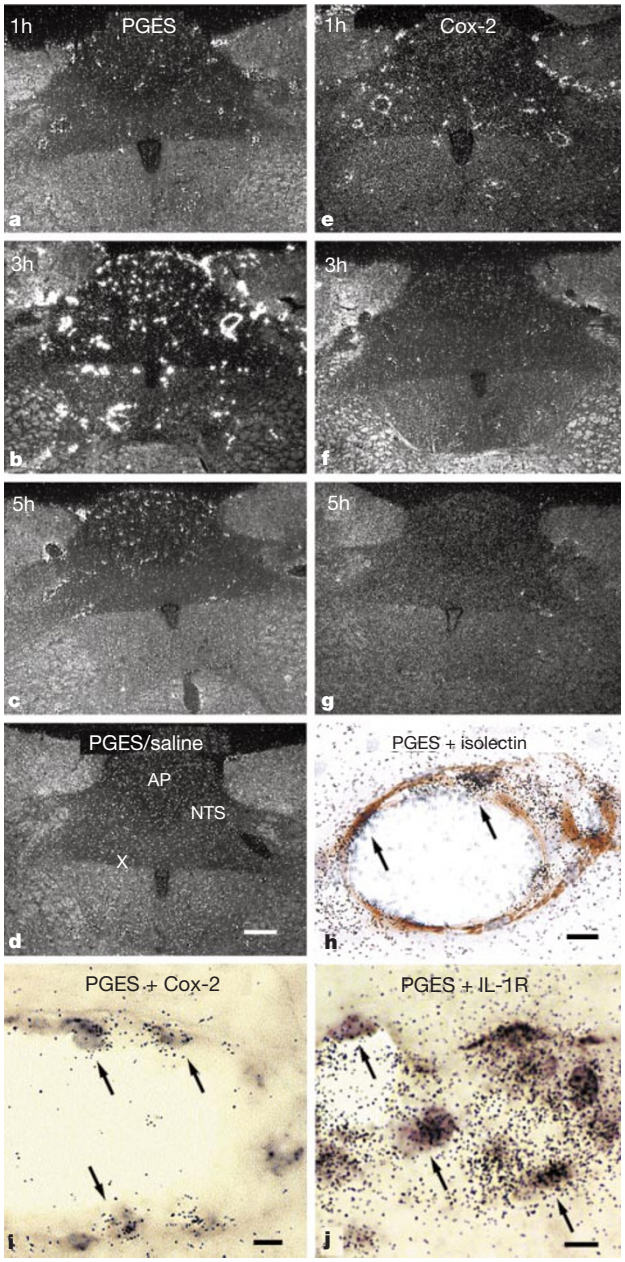

Figure 1 Kinetics and localization of cytokine-induced microsomal prostaglandin E synthase (PGES), determined by *in situ* hybridization. **a–c,** Expression of microsomal PGES messenger RNA and **e–g,** of cyclooxygenase-2 (Cox-2) messenger RNA in the lower brainstem of the rat following intravenous injection of interleukin-1β (2 μg per kg body weight). The expression of microsomal PGES messenger RNA peaks at 3 hours after injection, whereas Cox-2 messenger RNA expression is maximal at 1 hour and almost undetectable later on. **d,** No microsomal PGES messenger RNA was induced in control animals injected with saline (at 3 hours post-injection). Method specificity was confirmed by the absence of radiolabelling after hybridization with microsomal PGES and Cox-2 sense-RNA probes (data not shown). AP, area postrema; NTS, nucleus of the solitary tract; X, dorsal motor nucleus of the vagus nerve. Scale bar, 200 μm. **h,** Doubly labelled endothelial-like cells (arrows) stained for GSA B4 isolectin (brown) and microsomal PGES messenger RNA (silver grains). **i, j,** Co-localization (arrows) of microsomal PGES messenger RNA (brown) with **i,** Cox-2 messenger RNA, or **j,** type-1 interleukin-1 receptor (IL-1R) messenger RNA (silver grains), respectively. Scale bars, 15 μm.

prostaglandin E_2 involves the enzyme prostaglandin E synthase[7]. We cloned the microsomal form of this synthase (mPGES) from rat, expressed it in a bacterial system, and confirmed that the protein was of the expected size (16K–17K) by western blotting and that it was fully functional in an enzyme assay.

We used *in situ* hybridization histochemistry to investigate the temporal and spatial expression of the mPGES gene in rat brain after intravenous injection of the pro-inflammatory cytokine interleukin-1β. Expression of mPGES messenger RNA was detected in the cerebral blood vessels after one hour, peaked at 3 hours, and by 5 hours had returned almost to background levels (Fig. 1a–d). The kinetics of induction of mPGES expression in cerebral blood vessels thus matches the time course for biosynthesis of prostaglandin E_2 in the cerebral vasculature following immune stimulation[8].

This induction of mPGES was accompanied by a rapid but more transient upregulation of mRNA encoding cyclooxygenase-2 (the inducible form of the enzyme catalysing the first step in the biosynthesis pathway), which peaked one hour after injection of interleukin-1 (Fig. 1e–g). This temporal difference between the induction of mPGES mRNA and cyclooxygenase-2 mRNA expression fits with the order in which these two enzymes are engaged in prostaglandin E_2 synthesis.

We characterized the cells expressing interleukin-1-induced mPGES mRNA by combining these results from *in situ* hybridization with radioactively labelled mPGES ('antisense') RNA probes with immunohistochemical staining with GSA B4 isolectin, which labels endothelial cells, microglia and perivascular macrophages. Doubly labelled cells were abundant, with most being flat like endothelial cells (Fig. 1h) and some being more rounded like perivascular macrophages.

As cyclooxygenase-2 is associated with mPGES *in vitro*[9,10], we tested whether these enzymes co-localized inside the same cell by using a dual *in situ* hybridization technique that simultaneously detects radioactively labelled and non-radiolabelled antisense-RNA probes. We found that almost all vascular cells expressing mRNA for cyclooxygenase-2 also showed an interleukin-1-induced upregulation of mPGES mRNA (Fig. 1i), indicating that these cells have a coupled system for producing prostaglandin E_2 from arachidonic acid. Most of the cells expressing mPGES mRNA also co-expressed mRNA encoding the type-1 receptor for interleukin-1 (Fig. 1j).

We conclude that circulating interleukin-1 induces the synthesis of prostaglandin E_2 by signalling through the cell-surface interleukin-1 receptor to the interior of endothelial cells and possibly of perivascular macrophages in the brain vasculature. Our results indicate that the distinct distribution of different receptor subtypes for prostaglandin E_2 within the brain parenchyma[4–6] may account for the specificity of the cellular responses triggered by interleukin-1 in discrete brain structures[11]. Microsomal prostaglandin E_2 synthase could present a new target for developing drugs that will not only treat inflammatory conditions and their accompanying acute-phase reactions controlled by the central nervous system, but which might also be free of the systemic side effects associated with cyclooxygenase inhibitors.

Monica Ek*, David Engblom†, Sipra Saha‡, Anders Blomqvist†, Per-Johan Jakobsson‡, Anders Ericsson-Dahlstrand*

**Department of Medicine, Unit of Rheumatology, ‡Department of Medical Biochemistry and Biophysics, The Karolinska Institute, Stockholm 17177, Sweden*

†Department of Biomedicine and Surgery, Division of Cell Biology, University of Linköping, 58185 Linköping, Sweden

e-mail: andbl@mcb.liu.se

1. Rothwell, N. J. & Hopkins, S. J. *Trends Neurosci.* **18,** 130–136 (1995).
2. Elmquist, J. K., Scammell, T. E. & Saper, C. B. *Trends Neurosci.* **20**, 565–570 (1997).
3. Ericsson, A., Arias, C. & Sawchenko, P. E. *J. Neurosci.* **17**, 7166–7179 (1997).
4. Zhang, J. & Rivest, S. *Eur. J. Neurosci.* **11**, 2651–2668 (1999).
5. Ek, M., Arias, C., Sawchenko, P. & Ericsson-Dahlstrand, A. *J. Comp. Neurol.* **428**, 5–20 (2000).
6. Engblom, D., Ek, M., Hallbeck, M., Ericsson-Dahlstrand, A. & Blomqvist, A. *Neurosci. Lett.* **281**, 163–166 (2000).
7. Jakobsson, P. J., Thoren, S., Morgenstern, R. & Samuelsson, B. *Proc. Natl Acad. Sci. USA* **96**, 7220–7225 (1999).
8. Van Dam, A. M., Brouns, M., Man, A. H. W. & Berkenbosch, F. *Brain Res.* **613**, 331–336 (1993).
9. Thoren, S. & Jakobsson, P. J. *Eur. J. Biochem.* **267**, 6428–6434 (2000).
10. Murakami, M. *et al. J. Biol. Chem.* **275**, 32783–32792 (2000).
11. Ericsson, A., Kovacs, K. J. & Sawchenko, P. E. *J. Neurosci.* **14**, 897–913 (1994).