Sabine Louët responds:
In researching the news story, I had several interviews with Huub Schellekens, who explained to me the key findings of his laboratory's research on Eprex. At no point during these interviews did he strongly underline the fact that there was such a level of uncertainty regarding the findings of his study. However, it is clear that the activity of a therapeutic protein is likely to depend on many factors; indeed, the news article pointed out this fact: "Not only could the immunogenic reaction be triggered by a change in formulation—as in the Eprex case—but also by variations in amino acid sequence, glycosylation or even by impurities cropping up during manufacturing or administration of the drug." The adverse events associated with the manufacture, formulation and administration of Ortho Biotech's (a Johnson & Johnson affiliate) erythropoietin alpha (Eprex) exemplify the difficulties faced by companies that seek to manufacture and formulate generic biopharmaceuticals.

Chaperonins govern growth of *Escherichia coli* at low temperatures

To the editor:
Growth and multiplication of specific cells and organisms occurs within narrow physico-chemical conditions. Despite the fundamental importance of one (or at most two) cellular functions that determine the growth range of a cell or an organism, in most cases we have little idea of their identity. Here, we report the finding that chaperonins determine growth at lower temperatures of the bacterium *Escherichia coli* K-12. The finding has implications for the use of bacteria in environmental biotechnology, biochemical engineering and recombinant protein production.

E. coli is a mesophilic bacterium able to grow well in the temperature range from 21 °C to 49 °C, with an optimum at about 37 °C. The growth rate of *E. coli* strain XLOLR drops rapidly as incubation temperatures decrease from 20 °C, and the minimum for measurable growth is around 7.5 °C (ref. 1; **Fig. 1a**). Interestingly, the ability of the *E. coli* chaperonins GroEL and GroES to fold denatured proteins also rapidly decreases below 15 °C (ref. 2; **Fig. 1b**). These chaperones promote the folding and/or assembly of over 30% of cellular proteins, are required for bacteriophage morphogenesis and have a role in protein secretion[3,4]. The question thus arises of whether the vital role of chaperonins is the function that determines the lower temperature limit of *E. coli* growth.

We recently isolated a new psychrophilic bacterium, *Oleispira antarctica* strain RB-8 T (DSMZ14852 T), from Antarctic seawater[5] and characterized its chaperonin Cpn60 and co-chaperonin Cpn10 (Ferrer, M., Lünsdorf, H., Chernikova, T.N., Yakimov, M.M., Golyshin, P.N. & Timmis, K.N., unpublished data; Swiss-Prot accession numbers Q8KM30 and Q8KM31, respectively). Both chaperonins show high protein refolding activities *in vitro* at temperatures of 4–12 °C (16-fold higher than at 30 °C; **Fig. 1b**). We reasoned that if the cold-sensitive GroEL and GroES chaperonins of *E. coli* determine its lower growth temperature, and if the cold-adapted Cpn60 and Cpn10 chaperonins of *O. antarctica* can assume the roles of GroEL and GroES in *E. coli*, then introduction of the corresponding genes into, and their expression in, *E. coli* should extend its temperature range of growth by decreasing its lower temperature limit.

We therefore cloned and expressed the *O. antarctica* genes *cpn60* and *cpn10*, encoding the two chaperonins, under the control the P_{lac} promoter in *E. coli* strain XLOLR and examined the growth characteristics of the transgene after induction of expression with isopropyl-D-galactopyranoside (IPTG; **Fig. 1a**). The strain bearing the construct grows much faster than the parental strain at low temperatures: 3-fold faster than the parental strain at 15 °C, 36-fold faster at 10 °C and 141-fold faster at 8 °C (growth rate of parental *E. coli* ~0.002 h^{-1}; that of the transgenic strain ~0.282 h^{-1}). No growth of the parental *E. coli* was detected below 8 °C, whereas the transgenic strain grew at temperatures below 4 °C As determined using the square-root growth model of Ratkowsky *et al.*[6], the theoretical minimum temperatures for the parental and transgenic *E. coli* would be 7.5 °C and –13.7 °C, respectively (see **Supplementary Methods** online).

To rule out the possibility that hyperexpression of chaperones *per se* lowers the growth limit of *E. coli*, we also expressed the GroEL and GroES chaperonins to similar cellular levels—160 g GroEL/ES per milligram of protein versus 120 g Cpn60/10 per milligram of protein, using plasmids pBB528 and pBB541 (kindly provided by E. Betiku and U. Rinas (GBF)), in which the chaperonins are expressed from the same P_{lac} promoter (for details, see **Supplementary Fig. 1** online). The growth characteristics of *E. coli* at temperatures below 15 °C were not influenced by hyperexpression of the homologous chaperonins (data not shown). This demonstrates that the depression of the lower limit of growth of *E. coli* by Cpn60 and Cpn10 is due to a

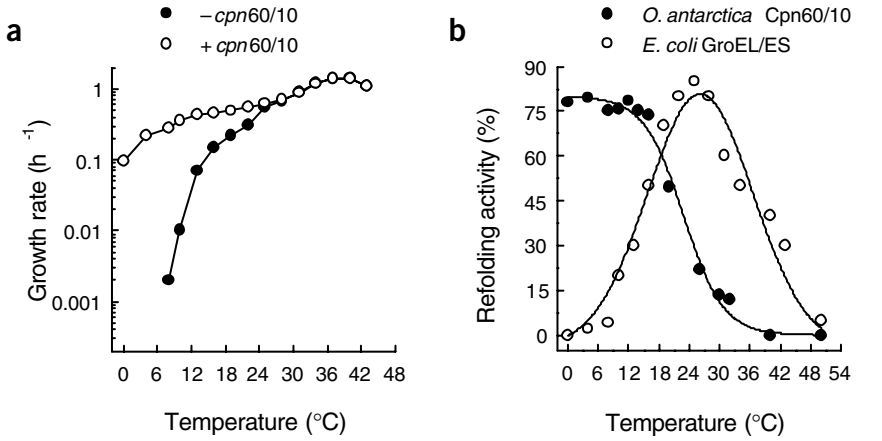

Figure 1 *In vivo* and *in vitro* properties of the chaperonins of *Oleispira antarctica*. (**a**) Effect of expression of the *O. antarctica* chaperonins on the growth of *E. coli* at different temperatures. (**b**) *In vitro* refolding activities of *O. antarctica* Cpn60/10 and *E. coli* GroEL/ES chaperonins at different temperatures. Data are not fitted to any model. For details see **Supplementary Methods** online.