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

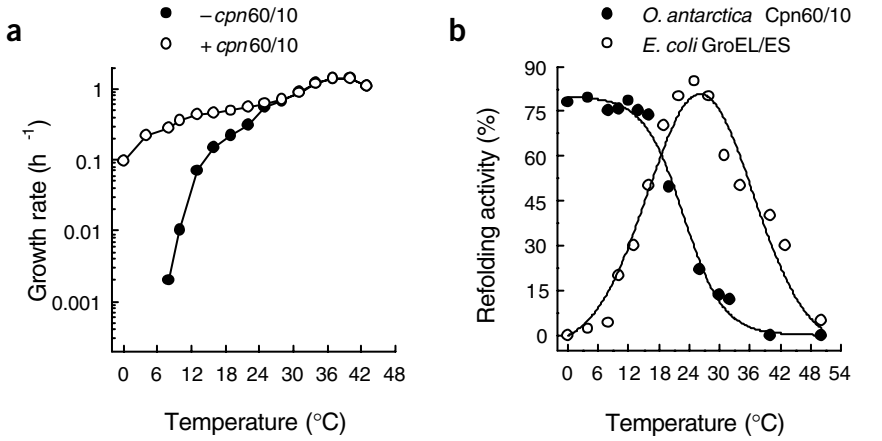

Figure 1 *In vivo* and *in vitro* properties of the chaperonins of *Oleispira antarctica*. (**a**) Effect of expression of the *O. antarctica* chaperonins on the growth of *E. coli* at different temperatures. (**b**) *In vitro* refolding activities of *O. antarctica* Cpn60/10 and *E. coli* GroEL/ES chaperonins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

qualitative change in cellular function effected by the psychrophilic chaperonins and not to a quantitative change in chaperonin level.

We have thus established causality between increased growth rates of *E. coli* at lower temperatures and depression of its minimum temperature for growth, on one hand, and recruitment of the *O. antarctica* cold-adapted chaperones, on the other. This, in turn, demonstrates that the chaperones of *E. coli* are the rate-limiting cellular determinant of growth at lower temperatures. As the principal function of chaperones is protein folding, and we have shown a correlation between protein folding ability and growth at lower temperatures, it is highly probable that the cellular function determining growth of *E. coli* at lower temperatures is protein folding. Nevertheless, we cannot presently exclude the possibility that another chaperonin-mediated cellular function is responsible for the altered growth characteristics.

Two related questions arise from this finding: how widespread is chaperone-determined growth among other organisms and under different environmental conditions, and will it be possible to extend the temperature ranges of growth of other cells by recruiting chaperones that have the required properties (*e.g.*, can the temperature ranges of growth of psychrophiles and mesophiles be extended by recruitment of chaperones from mesophiles or thermophiles)?

Whatever the case, the finding presented here has implications for biotechnology. One important strategy for developing new biocatalytic processes is to mine biodiversity by creating genomic libraries of DNA resources in *E. coli* and screening them for desired activities[7]. Enzymes from psychrophiles are particularly interesting for certain enzymatic bioconversions[8], but some cannot be produced in an active form in *E. coli* because they are denatured *in vivo* at the temperatures used in cultivating this bacterium[9]. Thus, screens for psychrophilic enzyme activities would clearly benefit from growth of such *E. coli* libraries at low temperatures, and subsequent production of identified enzymes will also require low-temperature growth of the host organism. Use of an *E. coli* host producing the *O. antarctica* chaperonins, or other cold-tolerant chaperones, will permit both lower growth temperatures and efficient folding of the psychrophilic proteins produced.

It is noteworthy that, if the temperature ranges of growth of organisms generally prove to be modifiable by recruitment of heterologous chaperones, this could become a generic means of altering their biogeography and of making them more robust either for a wide range of environmental applications that are subject to climate-related fluctuations in temperature (waste treatment, bioremediation, microbially mediated plant growth promotion and protection, retting, biomining, *etc.*) or for biotechnological processes at temperatures that are stressful for the organisms used. It may also have other interesting implications for agriculture if the cold adaptation strategy we have developed for *E. coli* is also applicable to plants and can be used to increase their robustness to weather conditions and extend their growth windows in time (length of growing season) and space (latitude growth range).

Note: Supplementary information is available on the Nature Biotechnology website.

ACKNOWLEDGMENTS

M.F. thanks the European Commission for a Marie Curie postdoctoral fellowship, and K.T. thanks the Fonds der Chemischen Industrie for generous support.

Manuel Ferrer[1], Tatyana N Chernikova[1], Michail M Yakimov[2], Peter N Golyshin[1] & Kenneth N Timmis[1]

[1]Division of Microbiology, German Research Centre for Biotechnology (GBF), Braunschweig 38124, Germany. [2]Istituto Sperimentale Talassografico, CNR, Messina, Italy.
e-mail: mfe@gbf.de

1. Ingraham, J.L. & Marr, A.G. *Escherichia coli and Salmonella: Cellular and Molecular Biology* edn. 2 (American Society for Microbiology, Washington, DC, USA, 1996).
2. Mendoza, J.A., Dulin, P. & Warren, T. *Cryobiology* **41**, 319–323 (2000).
3. Gething, M.-J. & Sambrook, J. *Nature* **355**, 33–45 (1992).
4. Walter, S. & Buchner, J. *Angew. Chem. Int. Ed. Eng.* **41**, 1098–1113 (2002).
5. Yakimov, M.M. *et al. J. Syst. Evol. Microbiol.* **53**, 779–785 (2003).
6. Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. & Chandler, R.E. *J. Bacteriol.* **154,** 1222–1226 (1983).
7. Olsen, M.J. *et al. Nat. Biotechnol.* **18**, 1071–1074 (2000).
8. Cavicchioli, R., Siddiqui, K.S., Andrews, D. & Sowers, K.R. *Curr. Opin. Biotechnol.* **13**, 253–261 (2002).
9. Feller, G., Le Bussy, O. & Gerday, C. *Appl. Environ. Microbiol.* **64**, 1163–1165 (1998).