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They should be addressed to:
Correspondence
Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
or sent by e-mail to biotech@natureny.com
Please include your telephone and fax numbers.

Anhydrobiotic engineering

To the editor:

Guo et al.[1] claim desiccation tolerance in human cells producing the disaccharide trehalose. Trehalose is well known to be associated with desiccation tolerance (anhydrobiosis) in many organisms, including baker's yeast and some resurrection plants. In yeast, trehalose is thought to be necessary for anhydrobiosis (e.g., ref. 2).

The demonstration that trehalose alone is sufficient to confer anhydrobiosis would be a major step forward in the understanding of this remarkable phenomenon. This might be achieved using the approach of Guo et al., whereby a desiccation-sensitive cell (in this case, a human fibroblast) is made desiccation-tolerant by enabling it to synthesize trehalose. The strategy could be generally applicable to desiccation-sensitive cell types and might be termed "anhydrobiotic engineering."

However, the techniques used by Guo et al.—for drying cells, measuring water content of the dried cells, and demonstrating the viability of the rehydrated cells—leave their conclusions open to question.

Human fibroblasts containing trehalose were dried after removal of medium by incubating the tissue culture plate sealed with parafilm at ambient temperature for one to three days. Surprisingly, the authors claim this method results in dried cells containing no detectable residual water, comparable with cells baked at 80°C overnight. However, the Fourier transform infrared spectroscopy (FTIR) technique used was not calibrated, nor were the limits of detection determined. Also, no distinction was made between extracellular and intracellular water. It is therefore not clear how dry the fibroblasts were, or indeed whether they were dry at all.

The key attribute of anhydrobiotic organisms is the ability to continue to grow after rehydration—viability, in other words. However, Guo et al. measure viability with a "live/dead" stain, a combination of calcein AM and ethidium homodimer. Calcein AM is membrane-permeable and is converted to fluorescent calcein by active intracellular esterases, whereas ethidium is excluded by intact membranes, but taken up through the damaged plasma membrane of a dead cell, when it intercalates into DNA and exhibits enhanced fluorescence. Live cells fluoresce green, while dead cells fluoresce orange.

This was the only viability assay used by Guo et al., but we feel it is not sufficiently reliable in this context—as amply demonstrated by the accompanying paper on the cryopreservation of trehalose-containing cells[3]. In Figure 2 of that paper ("no poration" and "WT" data sets), a large disparity was seen by Eroglu et al. between viability as measured by the same live/dead stain and viability as measured by either plating efficiency or growth of cryopreserved mouse fibroblasts. Cells apparently alive according to the staining technique do not attach to a surface or grow and can be considered dead.

What the live/dead stain is actually measuring is the activity of cytoplasmic esterases and the integrity of the plasma membrane. Trehalose is known to protect proteins and membranes (e.g., ref. 4) from desiccation damage, and it may be that some stabilization of these cell components was provided by the trehalose in the cells described by Guo et al. What we feel has not been demonstrated is true viability, i.e., growth.

Given these doubts about the validity of the data of Guo et al., it is difficult to agree with the authors that they have engineered mammalian cells that retain viability in the absence of water.

Arcadio Garcia de Castro
Jens Lapinski
Alan Tunnacliffe
Institute of Biotechnology
University of Cambridge
Tennis Court Road
Cambridge CB2 1QT, UK

1. Guo, N., Puhlev, I, Brown, D.R., Mansbridge, J & Levine, F. *Nat. Biotechnol.* **18**, 168 (2000).
2. Coutinho, C. et al., *J. Biotechnol*. **7**, 23 (1988).
3. Eroglu, A. et al., *Nat. Biotechnol.* **18**, 163 (2000).
4. Crowe, J.H. et al., *Biochem. J.* **242**, 1 (1987).

Fred Levine replies:

We thank Drs. Garcia de Castro, Lapinski, and Tunnacliffe for their comments on our manuscript. They raise two points:

Limitations of our experimental design make it impossible to conclude that there was no water present in the dried cells. We agree that one cannot conclude that there is a complete absence of water in our samples due to limitations of the methodology used in the study. However, even anhydrobiotic organisms do not exist in the complete absence of water, but rather have a greatly reduced water content. As discussed in the paper, there is a small peak in the relevant area in the baked as well as dried samples that could be water or absorbance from another cellular component (Fig. 4B). Furthermore, because performing the FTIR required the samples to be exposed to atmospheric moisture, we cannot rule out the possibility that the baked samples take up a very small amount of water from the atmosphere prior to analysis. Regardless, cells that were baked overnight had water peaks indistinguishable from the dried cells. Therefore, the essential point that trehalose-expressing cells remain viable while existing in a state of very low water content is unaffected. Hopefully, our paper will encourage additional experimentation to more precisely measure the exact level of water in dried samples. This will require facilities and equipment not available to us for our studies.

The live/dead stain method of determining cellular viability is criticized as not necessarily proving that the cells remain viable. We have used a number of different methods of measuring cellular viability, including growing the cells following drying. The live/dead stain was chosen for the paper because it provides a dramatic visual depiction of the effect of trehalose on cellular viability. While it is true that the live/dead stain is an overestimate of the number of cells able to grow following rehydration, we are consistently able to grow out cells from trehalose-expressing cells that have been dried and rehydrated. Therefore, the essential point of our manuscript, i.e., that trehalose confers desiccation tolerance on human cells, remains true. ///

Errata

On p. 143 of the February issue, Eric Niiler's story, "FDA, researchers consider first transgenic fish," erroneously stated that a study to assess the effect of transgenic fish on wild populations was funded by the FDA. This study was actually funded by the US Department of Agriculture.

On p. 261 of the March issue, an incorrect title, "Combinational chemistry gives cell biology some muscle," was printed for the News and Views by Dennis Hall. The title should have read "Combinatorial chemistry gives cell biology some muscle." *Nature Biotechnology* apologizes to Dr. Hall for introducing this error.

On p. 390 of the April issue, the brain scan appears courtesy of David Borsook and Leno Becerra of the Center for Functional Pain Neuroimaging and Therapy Research, Department of Radiology, Massachusetts General Hospital (Cambridge, MA).