

2014

Pittcon '14

March 2–7, 2014
Chicago, IL
www.pittcon.org

British Society for Cell Biology

March 16–19, 2014
Warwick, UK
www.bscb.org/?url=meetings/spring2014/index

Frontiers of Structural Biology (Z2)

March 30–April 4, 2014
Snowbird, UT
www.keystonesymposia.org

Focus on Microscopy 2014

April 13–16, 2014
Sydney, Australia
www.focusonmicroscopy.org

MRS Spring Meeting

April 21–25, 2014
San Francisco, CA
www.mrs.org/spring2014

Experimental Biology

April 26–30, 2014
San Diego, CA
www.the-aps.org/mm/Conferences/EB

NANOSMAT-USA 2014

May 19–22, 2014
Houston, TX
www.nanosmat-usa.com

66th Inter/Micro

June 2–6, 2014
Chicago, IL
www.mcrl.org/home/section/101-915/inter-micro-2014

Microscopy & Microanalysis 2014

August 3–7, 2014
Hartford, CT
www.microscopy.org

2015

Microscopy & Microanalysis 2015

August 2–6, 2015
Portland, OR
www.microscopy.org

2016

Microscopy & Microanalysis 2016

July 24–28, 2016
Columbus, OH
www.microscopy.org

2017

Microscopy & Microanalysis 2017

July 23–27, 2017
St. Louis, MO
www.microscopy.org

2018

Microscopy & Microanalysis 2018

August 5–9, 2018
Baltimore, MD
www.microscopy.org

More Meetings and Courses

Check the complete calendar near the back of this magazine.

Carmichael's Concise Review

Really Cool Cells (and how they got that way!)

Stephen W. Carmichael

Mayo Clinic, Rochester, MN 55905

carmichael.stephen@mayo.edu

The ability to preserve tissues and organs for later use is of tremendous clinical importance. Whereas many types of individual cells can be frozen and later functionally restored, this has proven to be more difficult with tissues. This is due to intracellular ice formation (IIF), which for some reason is more common when cells are connected, as they are in tissues. Using the fastest high-speed video cryomicroscopy yet developed, Adam Higgins and Jens Karlsson have shown for the first time that penetration of extracellular ice into paracellular spaces at the cell-cell interface is a precursor to IIF. They termed this precursor phenomenon “paracellular ice penetration” (PIP), which is the growth of extracellular ice crystal protrusions into paracellular spaces that contain supercooled liquid (Figure 1). Because PIP is associated with cell-cell and cell-substrate interactions, and does not occur during freezing of suspended isolated cells, this mechanism may contribute to the observed increase in IIF in tissues.

It has been thought that IIF moves from cell to cell through intercellular channels known as gap junctions, which are formed by connexin proteins. To test this hypothesis, Higgins and Karlsson tested three genetic variants of a mouse insulinoma cell line (MIN6): the wild-type containing connexin-36, as well as two other types of junctional proteins, E-cadherin and occludin. One of the genetically transformed strains contained almost none of those three proteins; whereas, another had only E-cadherin, and the third expressed only connexin-36. Pairs of the various cells were placed in a cryomicroscope stage chamber and then cooled rapidly to -60°C at $130^{\circ}\text{C}/\text{min}$. Images were acquired with their high-speed video camera at a sampling rate of 3,000–4,000 Hz. This gave a temporal resolution on the order of 0.3 ms. The IIF starting locations were classified into three

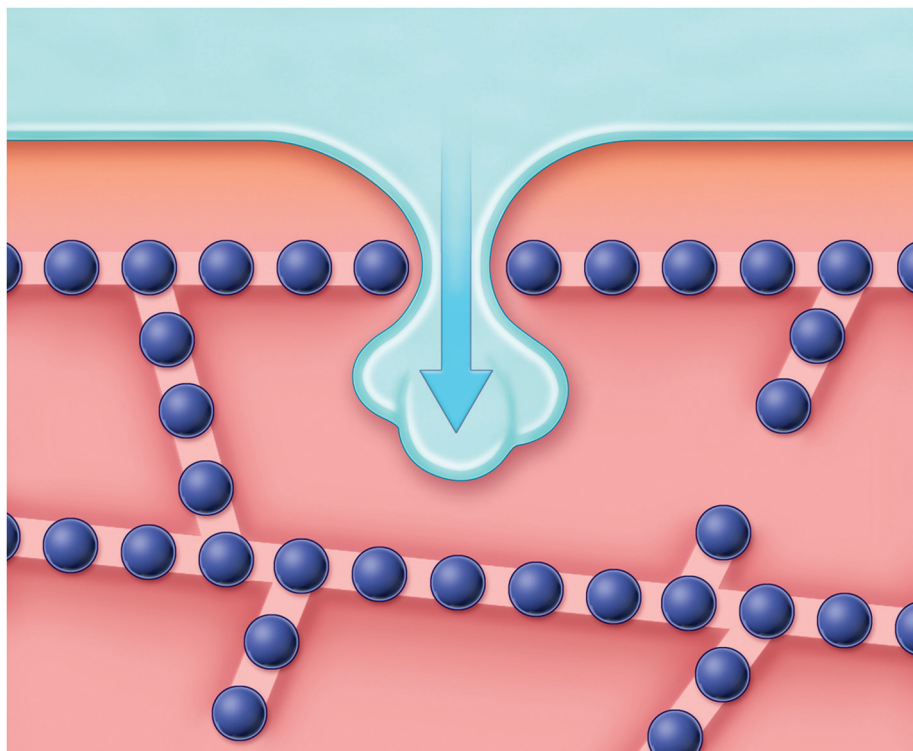


Figure 1: Paracellular ice penetration (PIP), the growth of extracellular ice crystal protrusions into paracellular spaces.



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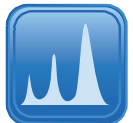


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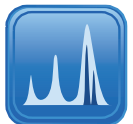
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groups: cell-cell interface, perimeter of the cell-substrate contact area, or internal. Ice formation was identified by the observation of a faint wave (the ice-liquid interface) rapidly traversing the affected cell volume. A video of some of these events can be viewed at www.eurekalert.org/multimedia/pub/63976.php.

In the wild-type cells, with all three junctional proteins present, PIP occurred in about half of the pairs observed, whereas it was observed more frequently (79% to 91%) in the modified cells. Moreover, cell pairs of the modified strains of MIN6 were significantly more susceptible to intracellular crystallization during rapid cooling than were the wild-type cells. Surprisingly, the probability of cell-to-cell propagation of intracellular ice was not decreased by the inhibition of connexin-36 proteins required for gap junction formation. The results of Higgins and Karlsson shed additional light on the mechanism of cryoinjury during freezing by identifying and characterizing an intermediate step in the IIF pathway for multicellular systems. Specifically, this intermediate step is the penetration of extracellular ice into supercooled paracellular domains at the cell-cell interface. Finding ways to minimize the deleterious effects of this phenomenon may hold the key for long-term storage of tissues for clinical use.

References

- [1] AZ Higgins and JOM Karlsson, *Biophysical Journal* 105 (2013) 2006–15.
- [2] The author gratefully acknowledges Dr. Jens Karlsson for reviewing this article.

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