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How Cells Connect Their Plumbing

Researchers have uncovered an important aspect of how cells create the junctions that allow them to communicate directly with one another. These gap junctions are protein channels through which cells exchange molecules and charged atoms among each other, helping to coordinate processes ranging from embryonic development to the heartbeat.

According to researchers who reported the findings in the February 8, 2007, issue of the journal *Cell*, understanding how cells target the components of these channels to the appropriate location on the cell surface could reveal important insights into processes that have gone awry in heart failure and cancer.

The research was led by Howard Hughes Medical Institute investigators Lily Jan and Yuh-Nung Jan. Joint first authors on the paper were Robin Shaw and Alex Fay, who like the Jans are at the University of California at San Francisco.

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Hemichannels, structures made up of proteins called connexins, are produced inside cells and must be transported to the cell's surface, where they form gap junctions. These gap junctions cluster together in sites known as plaques. Hemichannels are transported to the cell surface along cellular highways called microtubules, but no one knew whether the microtubules locate specific plaque sites in order to deliver their cargo.

"There seemed to be a correlation that was more than chance between plaques and the microtubules ending at the plaques," Shaw said. "And that suggested to us that there was a functional connection between the

microtubules and the plaques themselves.”

Previous research had implicated a protein on the tips of microtubules called EB1 as key to tethering microtubules to their target destinations. The researchers decided to explore microtubule targeting by tracing how EB1 and Cx43, a connexin known to be the most common component of hemichannels, behaved during gap junction assembly.

In experiments with human cell cultures, Shaw and Fay introduced Cx43 tagged with a fluorescent protein into the cells, and used microscopy to follow the protein's travels to gap junction plaques. They used intense light to bleach the fluorescent proteins in the plaques and watched them rapidly reappear over periods of minutes. The experiments established that the microtubules did target gap junctions with their hemichannel cargo, said Shaw.

They also found that knocking down EB1 levels in the cells drastically reduced gap junction formation. Microtubules could also not deliver their Cx43 cargo without EB1-interacting proteins that helped glue microtubules to their targets at the cell surface.

“While there is a lot of cell biology yet to be worked out, this paper provides an important initial step for further research in showing that microtubule targeting is important—and that it involves proteins such as EB1 and its interaction with the junction proteins,” Yuh-Nung Jan said of the findings.

According to Lily Jan, the discovery of microtubule targeting will yield insights into how cancers escape the tight controls on proliferation that gap junctions enforce. “When cells lose control and become cancerous, they lose gap junction contact with normal neighboring cells,” she explained. “That loss of contact may be one mechanism by which cancer cells avoid being killed -- they escape the censorship that protects cells from going haywire. When they become invasive, they migrate on top of other cells and then may form gap junctions with other cancer cells to share molecules, once they take root in a different tissue.”

Shaw, who is a cardiologist, said that the findings would help in understanding how heart arrhythmias arise from loss of blood flow, or ischemia, due to blocked arteries. “During ischemia, there is a rapid reduction of connexin from the membrane, and that process is not well understood,” he said. “It is very important because reduced gap junction coupling leads to fatal arrhythmias. Now that we have implicated a mechanism for how connexin gets to its right place in the membrane, and we understand that the time course can be very rapid, the next step is to ask, what is fouled up in this process during ischemia. Specifically, what does ischemia do to microtubule-mediated delivery of gap junctions?”