Research Roundup

A molecule with a sense of timing

Duration of kinase signaling determines how a cell responds to external cues like growth factors. But how a cell measures signaling time has been a mystery. Now, Leon Murphy, John Blenis (Harvard Medical School, Boston, MA), and colleagues have found that immediate early gene (IEG) products have a sensor mechanism that makes these distinctions. Their findings may provide a target for cancer treatment drugs.

Growth factors PDGF and EGF elicit different responses in fibroblasts, with only PDGF inducing cells to enter S-phase. The group found that EGF transiently activated ERK MAP kinases, whereas PDGF stimulated prolonged activation. The IEG product c-Fos is known to be transcribed with similar kinetics in response to either growth factor, but c-Fos was only phosphorylated when ERK activity was sustained. This not only stabilized c-Fos, but also primed it for further phosphorylation by revealing a DEF domain, an ERK-docking motif. Without secondary phosphorylation, c-Fos was less able to promote cellular transformation.

“Since several other IEGs also have DEF domains,” says Blenis, “this group of IEGs may act as molecular sensors in many processes,” including neuronal differentiation and the generation of an immune response. Blenis and Murphy hope it will be possible to antagonize these sensors to block specifically ERK-regulated proliferation, such as that seen in Ras-induced cancers, thus possibly avoiding these sensors to block specifically ERK-regulated proliferation, such as that seen in Ras-induced cancers, thus possibly avoiding toxic side effects caused by disturbing ERK’s other homeostatic cellular functions.


Active cells choose life

When macrophages perform their search-and-destroy missions, it is not simply up to apoptotic cells to flag down their destroyers. New results from Simon Brown (University of Edinburgh, Edinburgh, UK) and colleagues reveal that viable cells must actively avoid engulfment by phagocytes. A single cell adhesion molecule, CD31, can lead to either engulfment or escape.

Brown and his colleagues found CD31 by purifying proteins involved in tethering of cells to macrophages at low temperatures, when phagocytosis and cytoskeletal rearrangements were inhibited. Both viable and apoptotic leukocytes bound to macrophages through homophilic CD31 interactions. At 37°C, however, this interaction was transient for viable leukocytes. The cytoplasmic portion of CD31 imparted these cells with the ability to avoid destruction by actively promoting detachment from the macrophages. Apoptotic cells had somehow lost this capability.

“‘Eat-me signals’ are what are usually looked for, but we found ‘don’t-eat-me signals’ instead,” says Brown. “It would seem more efficient for an apoptotic cell to lose a signaling ability than gain one.” In this way, and by ligating self-recognition receptors, macrophages can determine whether a cell has lost its ability to respond to external signals and is thus ready for disposal. Whether viable cells actively escape from macrophages either by activating motility machinery (as happens upon CD31 ligation between leukocytes and endothelial cells) or by inhibiting subsequent interactions between the target and the macrophage is the focus of continuing studies.


Coordination of replication

A protein found in DNA replication complexes is also essential for ribosome biosynthesis, according to new results from Yi-Chieh Du and Bruce Stillman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). The findings imply that the biosynthesis of ribosomes is associated with the decision to enter the cell division cycle.

The authors identified proteins associated with the yeast origin recognition complex (ORC), a set of proteins that binds chromosomal origins of DNA replication. Among the proteins identified was Yph1p, a homologue of zebrafish pescadillo, mutations in which lead to cell proliferation defects. Yph1p was also found in complex with a variety of other proteins, including those involved in ribosome biogenesis, cell cycle regulation, and checkpoint control. “The first link to ribosome biosynthesis came from the finding of Erb1p in the Yph1p complex,” says Stillman. “Mutation of the mouse ERB1 orthologue leads to G1 arrest and alters ribosome biosynthesis.”

In vivo, Yph1p activity was required for proper 60S ribosome biogenesis and for normal progression during S-phase. Yph1p levels were high in proliferating cells, but declined as cells entered the quiescent phase. Newly synthesized Yph1p was required for cells to exit G0 and initiate cell division. Though Stillman admits the link between DNA replication and ribosome biosynthesis is speculative, he adds that it makes sense, as proliferation requires the replication of both the genome and the cellular machineries.

Decoding Ca\(^{2+}\) signals through cAMP

Transient changes in intracellular Ca\(^{2+}\) concentrations drive many biological processes, from gene transcription to growth cone turning. Ca\(^{2+}\) elevations can initiate cAMP oscillations, but new results suggest that only specific patterns of Ca\(^{2+}\) have this ability. Yuliya Gorbunova (University of Medicine & Dentistry of New Jersey, Piscatway, NJ) and Nicholas Spitzer (University of California, San Diego, CA) anticipate that their results may shift the focus in the field from Ca\(^{2+}\) spike frequency to spike timing and pattern.

The two examined reciprocity in Ca\(^{2+}\)/cAMP signaling in embryonic spinal neurons using fluorescent indicator dyes. In culture, increases in cAMP levels increased the frequency of Ca\(^{2+}\) spikes in neurons, whereas decreasing cAMP production had the reverse effect. Blocking Ca\(^{2+}\) spikes inhibited cAMP increases. Only specific patterns of induced Ca\(^{2+}\) transients—single Ca\(^{2+}\) spikes were ineffective at producing cAMP oscillations, but triplets of Ca\(^{2+}\) spikes in rapid succession were effective. Spitzer predicts the resulting cAMP oscillations control transcriptional regulation of genes responsive to these frequencies of Ca\(^{2+}\) transients.

They then derived a mathematical model to characterize cAMP/Ca\(^{2+}\) reciprocity. “If cells are generating this activity naturally, it’s probably important,” says Spitzer. The model will allow them to test certain predictions quickly and determine the interest of the results to pursue in biological experiments. For instance, the model predicts that coincident elevation of both cAMP and IP3 in a cell results in negative interaction between the messengers; only specific combinations of concentrations of the two produce Ca\(^{2+}\) and cAMP transients. Testing this hypothesis in cells may help reveal how neurons coordinate multiple signals to produce the appropriate result.


The ER contributes to engulfment

New results from Etienne Gagnon, Michel Desjardins (Université de Montréal, Montreal, Canada), and colleagues finally explain how a macrophage cell produces enough membrane to engulf material as large as itself—it uses large contributions of membrane from the ER. This is the first demonstration that the ER can fuse with the plasma membrane (PM).

In a previous proteomics experiment, Desjardins’ group found several ER-associated proteins in phagosome preparations. But their first thought was contamination, as phagocytosis is widely considered to be a function of the PM. “This was something from the textbooks we all did not question,” Desjardins says. “It was not our aim to challenge this idea.” However, the new immunogold and immuno-cytotoxic experiments clearly showed a distribution of ER marker proteins such as calnexin and calreticulin throughout the phagosome membrane.

Inhibition of phagocytosis revealed direct contacts between the ER and the PM at the sites of engulfment, where the two membranes apparently fused. ER contribution occurred early in the process, as the markers were also seen on the phagocytic cup, a structure formed before the phagosome fully surrounds the material to be engulfed. The ER contributed to phagocytosis mediated by various receptors, and even when only small amounts of membrane were required, indicating that its contribution is a general phenomenon in macrophages.

Neutrophils, in contrast, did not use ER membrane for phagocytosis. Desjardins believes this may reflect the different strategies of the two cell types. The antigen-presenting function of macrophages would be enhanced by the entry of pathogens directly into the ER, where they could undergo controlled trimming and antigen presentation, by both MHC class I and II molecules, in a nonlytic environment. Because neutrophils function primarily to engulf and destroy pathogens rapidly, they may not have the need for an ER-mediated phagocytic system, and may use other membrane sources, such as azurophilic granules, instead.