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**Changes in the post-translational modification of tubulin in response to ATP- depletion.**

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Prior research has shown that ATP depletion in many cell types causes the formation of microtubules (MTs) which are more resistant to microtubule depolymerizing agents and more heavily detyrosinated. We investigated the relationships between ATP depletion, microtubule stability, and tubulin post-translational modifications in MDCK cells during the process of polarization. ATP depletion was achieved by treatment with 20 mM sodium azide and 10 mM 2-deoxyglucose; stable microtubules were identified by their resistance to 10μM nocodazole. We found that upon ATP depletion, there was an increase in stable MTs in both 2D-polarized (subconfluent monolayers) and 3D-polarized MDCK cells, and an increase in both tubulin detyrosination and acetylation. The majority of the stable MTs showed acetylation and/or detyrosination. This was surprising in part because we had previously shown that 3D-polarized cells have little detyrosinated tubulin. The pattern of acetylation was altered as well. Instead of small, discontinuous patches of acetylated tubulin, many MTs were acetylated along their entire lengths. Immunoblotting of cell lysates showed that in both 2D- and 3D-polarized cells, detyrosinated tubulin increased 4-5 fold. Acetylation increased substantially, as well. A sudden drop in ATP is one of several stressors known to activate adenosine-monophosphate kinase (AMPK), an energy-sensing kinase thought to be one of the master regulators of metabolism. Our preliminary experiments show that inhibition of AMPK partially blocks the increase in detyrosinated and acetylated microtubules induced by ATP depletion. In summary, ATP depletion leads to a global increase in both the acetylation and detyrosination of MTs, which overrides the normal pattern of these modifications that is established as MDCK cells acquired a polarized morphology. And preliminary results suggest that activation of the AMPK pathway can modulate tubulin acetylation and detyrosination. Supported by NSF Advance Grant 0820032 (BD) & American Cancer Society RSG-10-245-01-CSM. (LAL).