Nuclear actin shaping the transcriptional response to hypoxia parallel to the HIF pathway

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Abstract

Actin, one of the most abundant and evolutionarily conserved proteins in eukaryotes, serves a well-established role as part of the cytoskeleton. However, it is also found in the nucleus, where it plays crucial roles in fundamental nuclear processes such as chromatin remodeling and transcriptional regulation. Nuclear actin dynamics have been proposed as a mechanism by which cells respond to environmental cues and organize their cellular response. This notion aligns with the finely regulated levels of nuclear actin and its shuttling between the nucleus and cytoplasm, a process facilitated by interactions with the importin-9/cofilin or exportin 6/profilin complexes. Our findings indicate a significant decrease in the nuclear actin pool under hypoxic conditions (1% O_2) across various cell types, including primary murine fibroblasts. Importantly, this decrease occurs independently of HIF-1α and is accompanied by reduced nuclear localization of actin-binding proteins such as cofilin and profilin. Fluorescence recovery after photobleaching (FRAP) experiments reveal alterations in the nucleo-cytoplasmic shuttling dynamics of actin under hypoxia, characterized by slowed import and increased export. Furthermore, we observed disruption of the nucleo-cytoplasmic Ran gradient, which is critical for actin nuclear import and is disrupted under hypoxic conditions. ATP availability, essential for Ran-dependent transport, is also decreased in hypoxic cells, consistent with literature reports of decreased mitochondrial ATP concentration under hypoxia. Importantly, decreasing cellular ATP levels under normoxic conditions led to a reduction in nuclear actin levels comparable to that observed in hypoxia, suggesting that decreased energy availability may contribute to the observed decrease in nuclear actin. Moreover, our data reveal partial co-localization of nuclear actin with active RNA polymerase II, indicating a potential role for nuclear actin in modulating gene expression under hypoxia. Preliminary RNAseq results support this hypothesis, showing changes in the hypoxic transcriptional response when nuclear actin levels are maintained.
In summary, our results demonstrate altered nucleocytoplasmic shuttling of actin, cofilin, and profilin in hypoxia, coinciding with decreased ATP availability and disruption of the Ran gradient. We propose that, alongside the classical HIF pathway, nuclear actin dynamics may contribute to the fine-tuning of the hypoxic transcriptional response.