Hypoxic response and directed migration towards oxygen in amoebae

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In many organisms, oxygen is one of the main growth limiting factors. As the level of available oxygen can fall rapidly, cells have mechanisms to adapt to this hypoxic situation. The only well-described adaptation system is the metazoans PHD (prolyl hydroxylase)/HIF-1 (Hypoxia-Inducible factor) pathway, which regulates the transcription of around a hundred genes. This regulation contributes, for example, to the decrease of the oxygen consumption or the induction of angiogenesis. An alternative strategy employed by some cells is to move towards an optimal oxygen concentration, a phenomenon known as aerotaxis. This phenomenon was first observed and studied in bacteria, but recent studies showed that it is also involved in numerous physiological processes, such as morphogenesis and cancer cells spreading. Although aerotaxis has been highlighted as a major phenomenon, it remains very poorly characterised in eukaryotes. In that context, our work focuses on the characterisation of the hypoxic response and the determination of genes involved in aerotaxis in eukaryotic organisms. For that, we use the social amoeba *Dictyostelium discoideum* that presents the interesting ability to switch from an unicellular to a multicellular phase. Moreover, this ancestral organism has no known HIF homolog and possesses aerotactic capacities. To answer our questions, we developed an oxygen migration assay that consists of placing a drop of cells and then covering it with a hermetically coverslip. The cells then consume the available oxygen, which generates an oxygen gradient. This gradient induces the coordinated migration of cells towards the outside of the drop, resulting in the formation of a ring of cells. This assay was used to produce various models and simulations to monitor cell movement which showed that the ring only forms in presence of aerotaxis. Using this assay with null mutants, we determined that several genes of the oxygen recognition pathway, such the PHD homolog *phyA*, are not involved in aerotaxis. Moreover, using different drugs that inhibit the electron transport chain, we reported that mitochondrial activity is not needed for cells directed migration towards oxygen. We also used chemicals to mimic oxidative and nitrosative stresses released during hypoxia to determine that those stresses are not involved in aerotaxis. In a second phase, we focused on the cells genetic response to hypoxia. For that, we realised a RNA-seq time course with cells placed at 1% oxygen and then reoxygenated. This experiment was coupled with proteomic analyses performed by the Pr. West team. By analysing the transcriptomic assay, we showed a huge induction of genes related to ribosome biogenesis after 1 hour in hypoxia. After 24 hours in hypoxia, we determined that genes involved in cell motility, proteolysis, and induction of the multicellular stage are overexpressed. Finally, we also showed that the reoxygenation induces huge transcriptional changes within cells that differ from the normoxic control condition. Further analysis of this RNA-seq is currently underway, as well as the comparison with the proteomic data.