Superoxide and derived reactive oxygen species (ROS) have been considered for a long time to be generated as toxic byproducts of metabolic events. More recently, it has been acknowledged that ROS generated in low amounts are also able to act as signaling molecules in a variety of responses. One of the major pathways regulated by the ambient concentration of oxygen relies on the activity of hypoxia-inducible transcription factors (HIF). Originally described to be only induced and activated under hypoxia, accumulating evidence suggests that HIFs play a more general role in the response to a variety of cellular activators and stressors, many of which use ROS as signal transducers. Indeed, ROS have been found to modulate the levels of HIF not only under hypoxia, but also in response to many factors and under different stress conditions. However, the underlying regulatory mechanisms by which superoxide and derived ROS control HIF are only slowly beginning to be elucidated. We summarize here current knowledge about the mechanisms by which ROS can regulate HIF and give additional information about useful methods to determine ROS under various conditions.