NOBEL PRIZE WINNER
SIR PETER J. RATCLIFFE

BAKER RUSKINN

"Research into the molecular cell biology of responses to hypoxia carries the (apparent) advantage of allowing the representation of an important disease complication in a tissue culture dish; hypoxia complicates most human diseases, and oxygen levels can be reduced in tissue culture to those that simulate disease and induce adaptive responses.

This apparent simplicity belies a number of traps for the unwary researcher. Oxygen diffuses rapidly across plastic ware, and into buffers and any materials that are used to make biochemical preparations from hypoxia tissue culture cells. So any attempt to study the hypoxic status of the cell must take account of this.

I remember Andrew Skinn visiting the laboratory (I think in late 1999 or early 2000). He was showing some very nice data from Darren Richards using the new Baker-Ruskinn InvivO2 controlled environment chamber. To be honest I wasn't absolutely convinced on the necessity straightaway; just a gut feeling that if we were working on the biochemistry of hypoxia signalling, surely we might need to control oxygen through all phases of the experiment.

And indeed that was correct. Critically, it enabled us to correct a small mistake in our work connecting HIF to VHL. We were somewhat surprised that although the interaction (between HIF and VHL) was necessary for degradation of HIF and the interaction could readily be suppressed by iron chelators and cobalt, we apparently could not see suppression in hypoxic cells.

That work also apparently explained a paradox in the field. When HIF was induced by hypoxia, then displayed by electrophoretic mobility shift assay, it generally appeared as a double band, whereas when HIF was induced by cobalt or iron chelators, it generally appeared as a single band. These results were widely observed but unexplained. We were very pleased to sort this out; the double band contained HIF complexed to VHL, as well as HIF alone, (hence two distinct mobilities) which we proudly showed with super-shift assays.

But we were always worried by this result. Despite the rapid harvest, might oxygen have got into the cells? This is where the BR chamber came in – enabling Panu Jaakkola and David Mole to revisit the position using IVTT proteins and IP-IB from human cells, respectively. This was actually at the time a real 'tour de force'. By excluding oxygen from all the buffers and performing the whole 'pull-down' procedures in the InvivO2 chamber, they were able to show that hypoxia did indeed suppress formation of the complex – a very important result.

This type of apparatus is very important for work in hypoxia, aside from the issue of control (of the oxygen level) it is all too easy to make a mistake. Unlike for pH we don't use a visible oxygen indicator, so it's easy to allow inadvertent re-oxygenation to confound your experimental results. Using a controlled environment work station greatly reduces that risk."

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