

P024 Quantitative analysis of class IA PI3K reveals tight regulation of subunit expression: PI3Ks are obligate heterodimers

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Class IA PI3K enzymes consist of a p85 regulatory and a p110 catalytic subunit. p85 has positive and negative roles in the regulation of p110. Indeed, under basal conditions, p85 stabilises the labile p110 protein while inhibiting its catalytic function. Receptor recruitment following cellular stimulation alleviates this inhibition of the p85/p110 complex, leading to p110 activation. Surprisingly, all p85 KO mice have *increased* PI3K signalling in insulin-sensitive tissues, despite a reduction in p85 and p110 expression levels. The existence of monomeric p85 in wild-type cells, which competes with heterodimeric p85/p110 for membrane association, has been invoked to explain this observation. Reduction of the pool of free p85 in p85 KO mice is then supposed to enhance engagement of heterodimeric PI3K. Evidence for the existence of monomeric p85 has largely been inferred from immunodepletion and immunoblotting experiments, and direct and formal determination of the p85 and p110 amounts is lacking. We have applied absolute quantification mass spectrometry to determine exact protein amounts of each class IA PI3K isoform. A strict 1:1 ratio between class IA PI3K regulatory and catalytic subunits was observed in murine cell lines. Biochemical analyses of the p85 to p110 ratio using affinity and ion exchange chromatography confirmed equimolarity between the class IA PI3K subunits. We furthermore investigated absolute mRNA levels by real-time PCR which revealed a tight regulation of PI3K protein expression at the transcriptional level.

In summary, our data do not support a role of monomeric p85 in the regulation of PI3K activity. Alternative explanations for increased PI3K signalling downstream of insulin in p85 KO mice will be discussed.