



Characterising Ankyrin Repeat Proteins as Substrates of the Asparaginyl Hydroxylase FIH

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Thesis Summary

FIH (Factor Inhibiting HIF) is an oxygen-dependent asparaginyl hydroxylase that plays an important role in the maintenance of cellular oxygen homeostasis. It functions as an oxygen sensor, and regulates the activity of a family of transcription factors known as the Hypoxia-Inducible Factors (HIFs). The HIFs are essential mediators of the chronic response to hypoxia, and until recently, were the only published substrates of FIH. The identification of ankyrin repeat domain (ARD) proteins as an alternative class of substrate has highlighted the possibility that FIH has yet uncharacterised roles in a number of different pathways. Due to the large number of ARD proteins expressed in a cell at any given time, as well as the commonality of ARD hydroxylation, the issue of how FIH achieves specificity is key, and is a major focus of this PhD thesis.

The first section of this work identifies key differences in the binding affinity, hydroxylation efficiency and oxygen sensitivity of FIH with respect to HIF and ARD substrates. These data indicate that ARD proteins are likely to be the preferred substrate for FIH in a cellular context. Interestingly, FIH can bind to ARD proteins that are not substrates, suggesting a possible role for FIH that is mediated by binding as opposed to hydroxylation. In support of this, the robust nature of the FIH-ARD interaction enables ARD proteins to sequester FIH, and regulate hydroxylation of HIF substrates through competitive inhibition. The sensitivity of this interaction to the hydroxylation status of the ARD pool adds an additional level of complexity to this novel mechanism of HIF regulation.

The second part of this thesis presents a detailed biophysical characterisation of the molecular determinants of FIH substrate specificity. These data indicate that substrate hydroxylation is substantially influenced by the identity of amino acids directly adjacent to the target asparagine. Secondary and tertiary structure are also important determinants of both binding affinity and hydroxylation efficiency, providing an explanation for observed differences in hydroxylation of ARD proteins compared with the HIF CAD. Overall, this work reveals distinct molecular features in HIF and ARD substrates that likely enable FIH to discriminate between these two classes of substrate in a cellular context.

The final section of this thesis characterises the hydroxylation of a family of ARD proteins encoded by the poxvirus Orf. This work provides the first evidence for FIH-catalysed hydroxylation of proteins encoded by an intracellular pathogen, and reveals a novel mechanism of FIH-dependent cross-talk between viral ARD proteins and the HIF pathway, which may have important consequences for virus infection.

Overall, the work presented in this thesis explores several novel aspects of ARD hydroxylation, and contributes important insights into the role of FIH as an oxygen sensor, and its importance in normal physiology and disease.

Candidate's Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Sarah Wilkins and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Sarah Wilkins

26th February 2012

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