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Characterisation of the Molecular Complexes that Regulate the $G_2/M$ Checkpoint of the Eukaryotic Cell Cycle

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A Thesis Submitted in Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

University of Auckland, 2009
Abstract

The cell cycle is one of the fundamental processes in nature, and is primarily concerned with the faithful replication of cellular contents, followed by even division to produce two identical daughter cells. It is made up of five discrete biochemical steps, comprising the interphase (G1, S and G2) and the mitotic phase (mitosis and cytokinesis), with two major regulatory checkpoints at G1 and G2. The focus of this research is the G2 checkpoint, which ensures the successful achievement of DNA replication, prior to the initiation of mitosis. Arrest or progression is principally mediated by the CDK1/cyclin B1 complex; phosphorylation of CDK1 by wee1 kinase prevents progression to mitosis, and subsequent dephosphorylation by the CDC25 phosphatases, initially the B isoform, leads to mitotic onset.

The aim of this research was the biophysical and/or biochemical characterisation of the molecular complexes that form at the G2 checkpoint to regulate entry into mitosis. CDK1 and cyclin B1 were separately expressed and purified from baculovirus-infected Sf9 cells. The wee1/14-3-3β complex was also expressed and purified, incorporating either full length wee1 or a truncated version from which the N-terminal domain of wee1 was deleted. Both exhibited wee1 kinase activity, at equivalent levels, p<0.001, with a 2.4 fold increase in kinase activity when wee1 is bound by 14-3-3β, p>0.001. Tryptic digestion of the complex indicated that its architecture was likely to be flexible and open, particularly within the N-terminal domain of wee1. CD analyses indicated that the wee1/14-3-3β complex was folded, with 30-40% α-helical content and 10-20% β-sheet content. Dissociation experiments were unsuccessful, however, indicating a high strength of interaction between wee1 and 14-3-3β. The empirical stoichiometry of the complex was determined as 1:1; subsequent native molecular weight determination suggested that the minimal functional unit is likely to be a 2:2 wee1/14-3-3β arrangement. It was proposed that the structural architecture of this complex may be similar to the serotonin N-acetyltransferase/14-3-3ζ complex. Experiments to determine the structure experimentally, using either TEM or x-ray crystallography, were unsuccessful, as the complex appeared to exhibit a high degree of flexibility in solution.
CDC25B was also expressed and purified, and was found to co-purify with a putative Sf9 14-3-3 protein. Consequently, it was re-cloned to co-express with 14-3-3β, and subsequent analysis of the resulting CDC25B/14-3-3β complex indicated that the empirical stoichiometry was 1:1, with the functional organization likely to be a 2:2 arrangement. It was proposed that the structural arrangement of this complex is most likely to be similar to that of the wee1/14-3-3β complex.
**Acknowledgements**

First and foremost, I would like to thank my supervisors, Ted Baker, James Dickson, Chris Squire, and unofficially, Ivan Ivanovich, and my mentor, Mike Walker. They helped and supported me when I asked them, contributed where improvements were required, and have contributed many hours to help me achieve clarity and direction within this endeavour. Thank you very much. Thank you to Alok Mitra, for your support and encouragement to continue, as well as all of your (and your lab’s) help with transmission electron microscopy. To Shaun Lott, and his lovely wife Michelle, thank you for listening, for supporting me and for helping me to figure out for where and what to aim. Thank you also to The Foundation for Research Science and Technology, for the Tūpapa Putaiao Māori Fellowships, the University of Auckland, for the Doctoral Scholarship, and the Maurice Wilkins Centre for Molecular Biodiscovery, for all of their combined funding and support.

Without the massive support of my friends and family, however, I would never have come so far! To Simon, my husband, Val, my mother, Liz, my sister, Bruce and Lynley, my father and step-mother, Roni and Barry, my parents-in-law, and my cousins Amiee, Zaini and Zahara, to all of the Greys and my grandparent: you all are my family, my friends and my support network, and I thank you dearly for it. You listened patiently, coaxed, prodded or bullied, as appropriate, to keep me going, and were always there to celebrate the successes and commiserate the losses. My love and thanks to you all. To Julie and Stacy, thank you for listening, for supporting me and for your advice: I finally did “just write it up and hand it in!” Thank you, Mel, for being so rock-steady, in your support of me, ngā aroha nui ki a koe! To Dave and Rachael, Dylan and Stacey, Wain, Jess and Dan, Andrea, Sarah, Jo and Victoria, thank you all for being there for me, listening, supporting and encouraging me, even if you all thought I was, or was going, crazy, from time to time! I can’t begin to describe how grateful I am to all of my friends and family, I feel constantly surrounded by wonderful people and it truly is a blessing to count myself as one of your number.

Thank you, also, to the founding members of The Royal Society for the Protection of Writers who are Maori, namely Mel, Amy, Kimiora and Jason; the time you all started reading my writing felt like a dark and stormy night, but after your many edits, improvements and re-writes, I fell there is just so much abundant deliciousness!
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<tr>
<th>Abbreviation:</th>
<th>Definition:</th>
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<td>14-3-3β</td>
<td>human 14-3-3 protein, β polypeptide, NCBI accession number NP_003395</td>
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<tr>
<td>AMP-PNP</td>
<td>5'-adenyl-β,γ-imidodiphosphate, non-hydrolysable ATP analogue</td>
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<td>amt</td>
<td>Amount</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>S phase</td>
<td>the synthesis phase of the eukaryotic cell cycle</td>
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<td>standard deviation, a measure of the variance of a dataset, calculated as the RMS deviation of the individual values from the mean value</td>
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<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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