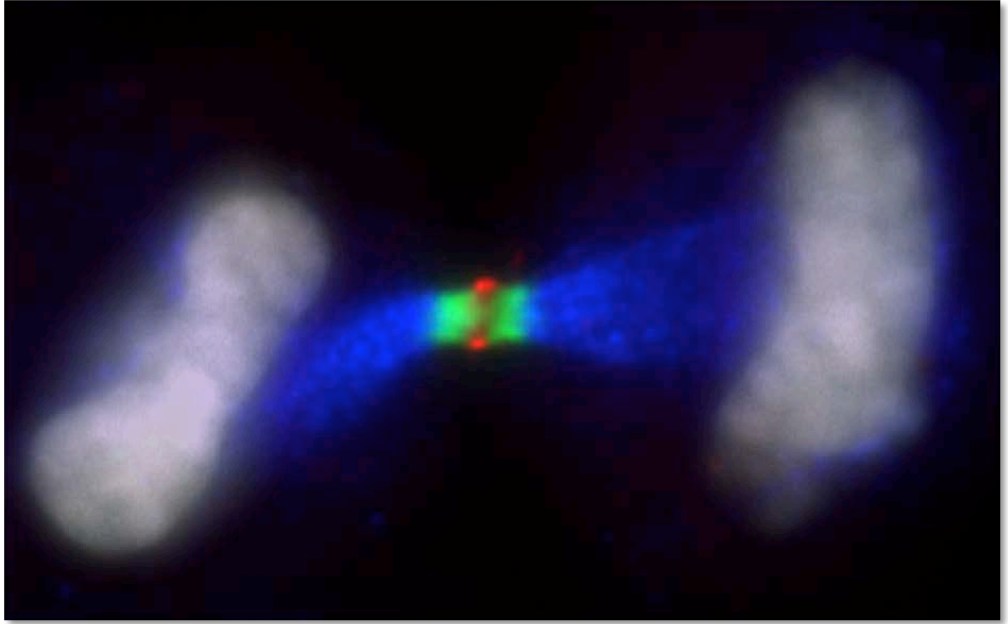


Department of Pathology fully-funded PhD studentships: project proposal form

To be completed and returned to your Head of Division by Monday 25th September 2017

Division	Molecular and Cellular Pathology
Supervisor	Paolo D'Avino
Project title	Identification of substrates of Citron kinase, a serine/threonine kinase involved in cell cycle and microcephaly
Project abstract for advert (Max 100 words)	Citron kinase (CIT-K) is a multifunctional serine/threonine kinase that plays important roles in different aspects of the cell cycle, including DNA damage control, the orientation of the mitotic spindle and cytokinesis. In addition, mutations in CIT-K have been linked to the development of human primary microcephaly. However, the molecular mechanisms by which CIT-K-mediated phosphorylation regulates the cell cycle and affects human brain development are still not understood, mainly because its substrates have not been identified. To address this, we propose to use complementary and convergent experimental strategies that employ a combination of gene editing, chemical genetics and quantitative phospho-proteomics.
Full details (Max 250 words. Will be published on Departmental website; do not include confidential information)	<p>Citron kinase (CIT-K) is a multifunctional serine/threonine kinase that plays important roles in different aspects of the cell cycle, including DNA damage control, the orientation of the mitotic spindle and cytokinesis. Mutations in CIT-K have been linked to the development of human primary microcephaly and this kinase has been identified as a potential target in cancer therapy. Compelling evidence indicates that CIT-K kinase activity is necessary for cell division and that its impairment causes human primary microcephaly. However, only one <i>bone fide</i> CIT-K substrate has been identified so far. Therefore, a more systematic and comprehensive approach is required to identify CIT-K targets to understand the molecular mechanisms by which CIT-K-mediated phosphorylation regulates the cell cycle and affects human brain development.</p> <p>To this aim, we will employ complementary and convergent experimental strategies combining gene editing, chemical genetics and quantitative phospho-proteomics. We will use CRISPR/Cas9 gene editing to generate a cell line harbouring a CIT-K 'analogue sensitive' (AS) mutant, which can accept and be selectively inhibited by 'bulky' ATP analogues. This CIT-K^{AS} cell line will then be utilised to dissect the role of CIT-K's kinase activity and identify its substrates using multiple approaches. First, we will investigate the role of CIT-K's kinase activity in mitosis by treating CIT-K^{AS} cells with a bulky inhibitor at different stages of cell division and analyse mitotic events by both time-lapse and immuno-fluorescence microscopy. Then, we will identify CIT-K substrates using two complementary methodologies. We will characterise and compare the phospho-proteomes of this CIT-K^{AS} cell line in the presence or absence of bulky ATP inhibitors in cells synchronised at different stages of the cell cycle. In parallel, we will also use this cell line to thio-phosphorylate CIT-K substrates by incubating extracts from the same cell cycle stages with bulky N⁶-substituted forms of ATP_γS. Thio-phosphorylated proteins will then be immuno-precipitated and identified by mass spectrometry.</p>

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<p>Image(s) related to project</p> <p>(For use in adverts and on Departmental website)</p>	
<p>5 recent publications</p>	<ul style="list-style-type: none">• Bassi I.Z., Audusseau, M., Riparbelli, M.G., Callaini, G. and D'Avino P.P. (2013) Citron kinase controls a molecular network required for midbody formation in cytokinesis. <i>Proceedings of the National Academy of Sciences USA</i>, 110(24):9782-9787.• McKenzie C., Bassi I.Z., Debski J., Gottardo M., Callaini, G., Dadlez M. and D'Avino P.P. (2016) Cross-regulation between Aurora B and Citron kinase controls midbody architecture in cytokinesis. <i>Open Biology</i>, 6: 160019 (doi: 10.1098/rsob.160019).• Capalbo, L., Mela, I., Abad, M.A., Jeyaprakash A.A., Edwardson, J.M. and D'Avino P.P. (2016) Coordinated regulation of the ESCRT-III component CHMP4C by the chromosomal passenger complex and centralspindlin during cytokinesis. <i>Open Biology</i>, 6: 160248 (doi: 10.1098/rsob.160248)• McKenzie, C. and D'Avino P.P. (2016) Investigating cytokinesis failure as a strategy in cancer therapy. <i>Oncotarget</i>, 7(52):87323-87341 (doi: 10.18632/oncotarget.13556)• D'Avino P.P. and Capalbo L. (2016) Regulation of midbody formation and function by mitotic kinases. <i>Seminars in Cell and Developmental Biology</i>, 53:57-63.• D'Avino P.P. (2017). Citron kinase - renaissance of a neglected mitotic kinase. <i>Journal of Cell Science</i>, 130(10): 1701-1708; doi: 10.1242/jcs.200253