

# **Regulation of the BH3-only protein PUMA by growth factor signalling**

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A thesis submitted for the degree of Doctor of Philosophy of the  
University of Adelaide (Faculty of Health Sciences)

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Adelaide, South Australia

January 2011

## **Abstract**

Regulation of the BH3-only protein PUMA by growth factor signalling

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P53 Upregulated Modifier of Apoptosis (PUMA), a pro-apoptotic member of the Bcl-2 family, is transcriptionally activated by p53 and is a key effector of p53-dependent apoptosis. We show that PUMA protein is subject to rapid post-translational regulation by phosphorylation at a conserved residue, serine 10, following serum or Interleukin-3 (IL-3) stimulation. Serine 10 is not within the BH3 domain and PUMA phosphorylated at serine 10 retained the ability to co-immunoprecipitate with anti-apoptotic Bcl-2 family members. However, phosphorylated PUMA was targeted for proteasomal degradation indicating that it is less stable than unphosphorylated PUMA. Importantly, we identified NEMO/IKK1/IKK2 as the kinase complex that interacts with and phosphorylates PUMA thereby also demonstrating that IL-3 activates NF $\kappa$ B signalling. This thesis therefore identified and characterised a novel survival pathway with important implications for IL-3 signalling and haemopoietic cell development.

## **Declaration**

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Jarrod J Sandow

January 2011

## **Publications arising from this Thesis**

**Sadow, J. J.**, Jabbour, A. M., Condina, M. R., Daunt, C. P., Stomski, F. C., Riffkin, C. D., Hoffmann, P., Guthridge, M. A., Silke, J., Lopez, A. F., Ekert, P. G. (2010). Site-specific serine phosphorylation regulates PUMA apoptotic activity by signaling PUMA degradation. *Journal of Cell Biology* (under review)

Jabbour, A. M., Heraud, J. E., Daunt, C. P., Kaufmann, T., **Sadow, J.**, O'Reilly, L. A., Callus, B. A., Lopez, A., Strasser, A., Vaux, D. L., Ekert, P. G. (2009). Puma indirectly activates Bax to cause apoptosis in the absence of Bid or Bim. *Cell Death and Differentiation* **16**: 555-563.

## **Acknowledgments**

I sincerely thank my supervisor, Professor Angel Lopez, for his constant guidance, wisdom, encouragement, and support over the course of this work. It has been a great honour and privilege to undertake my PhD in your laboratory, and I thank you for taking me on as one of your students. Although difficult in the beginning, I am thankful for the freedom you gave me to follow my own ideas as much as you may not have agreed with them on occasions. I have learnt more from my mistakes than my successes, and I thank you for teaching me that the journey is equally as important as the result. You have also shown me that being a scientist is as much about managing the politics of a laboratory or institute as it is about bench work.

I am very grateful to Associate Professor Paul Ekert and Dr Anissa Jabbour for their help and support. The support, reagents and guidance they provided during my PhD was invaluable. Their intimate knowledge of my project allowed for fantastic ideas to be developed and I am thankful for the development of ideas and the polite criticisms that were provided. I can sincerely say that I would not be as confident with my findings had it not been for the input of these two fantastic people.

I must also thank Frank Stomski for his help and guidance. The practical knowledge that he shared with me from his years of experience were invaluable. I am so appreciative of all your help over the last four years and am, indeed, fortunate to have you as a friend.

I also had the privilege of studying alongside fellow PhD candidates Jennifer Young, Leila Wyatt, Daniel Thomas, and Emily Patterson who, through friendship, laughter and a significant amount of drinking, made my journey very memorable.

And of course, many thanks to all members of the Lopez and Guthridge Laboratories for their many ideas and making work a fun place to be.

Lastly, I could not have completed this PhD without my family, to whom I am most thankful. The value of the support provided to me by Ruby over the years of my PhD cannot be measured. Without her support I cannot imagine how I would have completed my PhD and I will be forever grateful.

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## **Abbreviations**

4-OHT	4-hydroxytamoxifen
CHX	Cycloheximide
CID	Collision-induced dissociation
CMV	Cytomegalovirus
ESI	Electrospray ionization
ETD	Electron-transfer dissociation
FBS	Foetal Bovine Serum
FDM	Factor dependent myeloid
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HA	Haemagglutinin
IL-3	Interleukin-3
IL-5	Interleukin-5
IP	Immunoprecipitation
LPS	Lipopolysaccharide
MOMP	Mitochondrial outer membrane permeabilisation
TNF $\alpha$	Tumour Necrosis Factor alpha
UV	Ultraviolet
Wt	Wild type