An analysis of DRONC function and its regulation of expression during *Drosophila* development

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Abstract

Correct development of multicellular organisms requires the programmed removal of supernumerary, redundant, or damaged cells, a process achieved by apoptosis. Apoptosis, or Programmed Cell Death (PCD) is executed by caspases, a highly conserved family of cysteine proteases. The removal of redundant larval tissues during metamorphosis is controlled by the steroid hormone ecdysone. Ecdysone signalling is mediated by the nuclear receptor heterodimer EcR/Usp, which in turn transcriptionally activates a host of transcription factors which then go on to regulate genes essential for PCD, like caspases. The apical caspase *dronc* is upregulated in the larval midgut and salivary glands prior to their destruction and is dependent on the BR-C and E93 transcription factors. To further understand the role of *dronc* in development, a *dronc* mutant fly was generated and a transgenic promoter-reporter strategy was employed to investigate *dronc* regulation.

Larval organs from *dronc* mutants lack dying cells and, when irradiated, fail to show a radiation-induced PCD response. The midguts from *dronc* mutants undergo apoptosis and have high caspase activity. These data indicate that a *dronc*-independent caspase activation pathway is active in the midgut. Salivary glands from *dronc* mutants failed to be removed and have reduced caspase activity. Consequently it is clear that the role of DRONC differs significantly between the midgut and salivary glands.

The employment of a transgenic *dronc* promoter-*LacZ* reporter system identified promoter regions essential for the correct temporal and spatial expression of *dronc*. A region of the *dronc* promoter between 1.1kb and 2.8kb has elements essential for *LacZ* expression in salivary glands. This region was also dependent on the BR-C and E93

transcription factors for salivary gland expression. A functional ecdysone receptor binding element (EcRBE) was identified in the *dronc* proximal promoter. The EcR-B1 isoform directly binds this EcRBE and is necessary for correct *dronc* expression in the larval salivary glands.

This work revealed some novel findings regarding the role of DRONC in development and the availability of a specific *dronc* mutant now makes it possible to explore some of the recently published non-apoptotic roles of *dronc*. This work aids in understanding how nuclear hormones control transcription and shows *dronc* to be an ideal model gene to explore these molecular and genetic processes.

Statement

This thesis contains no material which has been accepted for any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge, contains no material previously published or written by any other person, except where due reference has been made. I give my consent for this thesis to be made available for loan and photocopying.

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Publications

Daish, T. J., Mills, K., and Kumar, S. (2004). *Drosophila* Caspase DRONC Is Required for Specific Developmental Cell Death Pathways and Stress-Induced Apoptosis. Dev Cell *7*, 909-915.

Chew, S. K., Akdemir, F., Chen, P., Lu, W. J., Mills, K., **Daish, T.J.,** Kumar, S., Rodriguez, A., and Abrams, J. M. (2004). The Apical Caspase *dronc* Governs Programmed and Unprogrammed Cell Death in *Drosophila*. Dev Cell *7*, 897-907.

Cakouros, D., **Daish, T. J**., and Kumar, S. (2004a). Ecdysone receptor directly binds the promoter of the *Drosophila* caspase *dronc*, regulating its expression in specific tissues. J Cell Biol *165*, 631-640.

Cakouros, D., **Daish, T. J**., Mills, K., and Kumar, S. (2004b). An arginine-histone methyl transferase, CARMER, coordinates ecdysone-mediated apoptosis in *Drosophila* cells. J Biol Chem *279*, 18467-18471.

Quinn, L., Coombe, M., Mills, K., **Daish, T.J**., Colussi, P., Kumar, S., and Richardson, H. (2003). Buffy, a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. Embo J *22*, 3568-3579.

Daish, T. J., Cakouros, D., and Kumar, S. (2003). Distinct promoter regions regulate spatial and temporal expression of the *Drosophila* caspase *dronc*. Cell Death Differ *10*, 1348-1356.

Cakouros, D., **Daish, T.J**., Martin, D., Baehrecke, E. H., and Kumar, S. (2002). Ecdysone-induced expression of the caspase DRONC during hormone-dependent programmed cell death in Drosophila is regulated by Broad-Complex. J Cell Biol *157*, 985-995.

Harvey, N. L., **Daish, T.J**., Mills, K., Dorstyn, L., Quinn, L. M., Read, S. H., Richardson, H., and Kumar, S. (2001). Characterization of the *Drosophila* caspase, DAMM. J Biol Chem *276*, 25342-25350.