Proposal for Masters degree internships (estágios)

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Starting date – September 1st 2008

Number of positions - 2

Summary of the lab's research interests
The aim of our research is to understand the molecular mechanisms that regulate specification, differentiation and degeneration of the photoreceptors, the cells that sense light in the visual system, using *Drosophila* as our biological model. Our most recent work focuses on the protective role of the Unfolded Protein Response (UPR), a cellular signaling pathway activated by the presence of unfolded proteins in the Endoplasmic Reticulum (ER), against photoreceptor degeneration in a *Drosophila* model for Autossomal Dominant Retinitis Pigmentosa. We use the tools of modern genetics, cell biology and imaging to pursue the signaling mechanisms that regulate cell death/cell protection in our biological model system.

Supervisors' Publications
1) **Domingos PM**, Steller H. (2007) Pathways regulating apoptosis during patterning and development. **Curr Opin Genet Dev**. Aug;17(4):294-9. Review.

2) Ryoo HD*, **Domingos PM***, Kang MJ, Steller H. (2007) Unfolded protein response in a Drosophila model for retinal degeneration. **EMBO J.** Jan 10;26(1):242-52. *equal contribution

3) Mollereau B, **Domingos PM**. (2005) Photoreceptor differentiation in Drosophila: from immature neurons to functional photoreceptors. **Dev Dyn** Mar;232(3):585-92. Review.

4) **Domingos PM**, Mlodzik M, Mendes CS, Brown S, Steller H, Mollereau B. (2004) Spalt transcription factors are required for R3/R4 specification and establishment of planar cell polarity in the Drosophila eye. **Development**. Nov;131(22):5695-702.

5) **Domingos PM**, Brown S, Barrio R, Ratnakumar K, Frankfort BJ, Mardon G, Steller H, Mollereau B. (2004) Regulation of R7 and R8 differentiation by the spalt genes. **Dev Biol.** Sep 1;273(1):121-33.

6) Itasaki N, Jones CM, Mercurio S, Rowe A, **Domingos PM**, Smith JC, Krumlauf R. (2003) Wise, a context-dependent activator and inhibitor of Wnt signalling.
Development. 2003 Sep;130(18):4295-305.

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Mech Dev. Aug;116(1-2):177-81.

8) **Domingos PM**, Itasaki N, Jones CM, Mercurio S, Sargent MG, Smith JC, Krumlauf R. (2001) The Wnt/beta-catenin pathway posteriorizes neural tissue in Xenopus by an indirect mechanism requiring FGF signalling. **Dev Biol.** Nov 1;239(1):148-60.

Research Proposal

Introduction and previous results

One of our lab's scientific goals is to understand the molecular mechanisms that regulate neuronal degeneration. We have begun investigating this problem, by focusing on the role of the Unfolded Protein Response (UPR) in a model of retinal degeneration in *Drosophila*. The endoplasmic reticulum (ER) is the cell organelle where secretory and membrane proteins are synthesized and folded. This process requires the recruitment of ribosomes, translocation of the nascent peptides into the lumen of the ER, and a variety of post-translational modifications and folding events. When the folding capacity of the ER is impaired, the presence of unfolded/misfolded proteins in the ER causes stress to the cell ("ER stress") and activates a cellular response, the Unfolded Protein Response (UPR), to restore homeostasis in the ER [1] [2]. The UPR is mediated by signaling pathways, which sense stress in the ER and activate a variety of cellular responses, including translational attenuation, to reduce protein synthesis and prevent further accumulation of unfolded proteins, and the transcriptional upregulation of genes encoding ER chaperones and enzymes, to increase the folding capacity of the ER. However, in situations where ER stress is severe or prolonged, or when the cellular responses induced by UPR are not sufficient to overcome the origin of ER stress, cells can undergo programmed cell death (Apoptosis). Apoptosis is a genetic determined program of cell death, which involves a variety of intracellular signaling pathways leading to the activation of members of the caspase family of cysteine proteases. Although much progress has been made in understanding the signals and mechanisms by which caspases are activated, little is known about how apoptosis is induced in the context of ER stress.

Retinitis pigmentosa (RP) is a major cause of human blindness. In this disease, the photoreceptor cells in the eye progressively degenerate over time. About 30% of autosomal dominant RP cases are caused by mutations in Rhodopsin, the light sensitive protein of photoreceptors. In *Drosophila*, equivalent mutations in *ninaE* (the gene encoding Rhodopsin 1), also cause dominant degeneration of the retina, which occurs by apoptosis and can be blocked by the baculoviral caspase inhibitor p35 [3]. In addition, most of these mutations produce misfolded forms of Rhodopsin 1, which are not properly processed and accumulate in the ER [4].

We have started to investigate the role of the UPR in the degeneration process caused by *ninaE* mutations. One of the mediators of UPR is IRE1, which is a transmembrane protein resident in the ER, with a luminal domain sensitive to ER stress and cytoplasmic domain that mediates downstream signaling. In mammals and *C. elegans*, the cytoplasmic domain of IRE1 has endonuclease activity and, in the presence of ER stress, splices the mRNA of Xbp1 to create a functional protein [5] [6] [7]. Spliced Xbp1 functions as a transcription factor that activates the expression of many target genes to increase the ER client protein processing capacity. We have cloned *Drosophila* Xbp1 and constructed a fusion protein of Xbp1with GFP, in which GFP is only in frame with Xbp1 upon ER stress-induced IRE1 mediated splicing. We used this reagent as a reporter for "ER stress" and activation of the UPR. We found Xbp1::GFP expression in the *ninaE* mutations that cause retinal degeneration. This result demonstrates that these mutations activate the UPR, in particular, IRE1/Xbp1 mediated signaling. Moreover, we found that a mutation in Xbp1 has a dominant effect accelerating the retinal degeneration in *ninaE* mutants. This result demonstrates that Xbp1 has a protective role against *ninaE* induced photoreceptor degeneration [8].

Specific Aims

1. Analysis of the molecular mechanisms required for induction of cell death in the context of ER stress

The goal of this project is to identify and characterize genes required for the activation of apoptosis in the context of ER stress. This will involve testing known candidates, such as caspases and proteins required for the regulation of caspases (Apaf, Diap, Cytochrome C, etc) and screening for novel genes. For this, we have developed an assay where *Drosophila* tissues are cultured *in vitro* in the presence of ER stress inducing agents (such as tunicamycin, thapsigargin or dithiothreitol - DTT) in order to induce apoptosis. The induction of apoptosis can be monitored by TUNEL assay or immuno-staining with an antibody against activated caspase 3. The mutations in genes with an effect in this screen will be further tested in a secondary screen, where we will test for a possible modifier effect in the *ninaE* induced retinal degeneration. Mutations with an effect in both assays will then be further characterized. The specificity of these mutations in ER-stress induced apoptosis will be assayed by testing possible effects in other paradigms, such as radiation or DNA damage induced apoptosis. Of the possible outcomes of this approach, we are especially interested in genes encoding proteins regulating the cross-talk between the ER and mitochondria in the context of ER-stress induced apoptosis.

2. The mechanism of induction of cell death by $Xbp1_{spliced}$

From our previous findings, Xbp1 has a protective role against neuronal degeneration induced by *ninaE* mutations. However, transgenic expression of the activated form of Xbp1 ($Xbp1_{spliced}$) also leads to cell death and degeneration, both in the developing eye during larval stages and in the differentiated photoreceptors of the adult organism (Figure 1). This apparent paradox led us to hypothesize that the timing of Xbp1 activation is important for the final outcome, protection or death. For example, activation of Xbp1 during brief periods could have a protective role, while the continuous activation of Xbp1 would lead to a variety of cellular responses leading to cell death and degeneration. Consistent with this idea, two reports have shown that unspliced Xbp1, although inactive as a transcription factor, functions in a negative feedback loop to downregulate $Xbp1_{spliced}$ [9] [10]. To test this hypothesis, we will perform experiments where $Xbp1_{spliced}$ will be expressed in a conditional manner using $Gal80^{ts}$, a thermo-sensitive version of the Gal4 inhibitor Gal80 [11]. In these experiments, we will activate $Xbp1_{spliced}$ during a variety of periods of time and look for the induction of markers of cell protection or death.

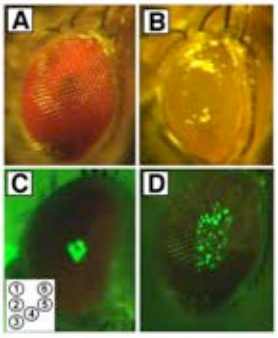

Figure 1 – Expression of $Xbp1_{spliced}$ induces cell death and degeneration in the developing eye (A-B) and in the photoreceptor cells (C-D). (A) Control eye. (B) Adult eye with "glossy" phenotype caused by expression of $Xbp1_{spliced}$ during larval stages (GMR-Gal4 driver). (C) Control eye with intact pseudopupil, a projection image resulting from expression of GFP in the outer photoreceptors (Rh1Gal4 driver). The identity of each photoreceptor (1-6) is represented in the inset. (D) Expression of $Xbp1_{spliced}$ in the photoreceptors leads to degeneration, which can be observed by the degradation of the pseudopupil image. Only dispersed GFP light is observed

Expression of $Xbp1_{spliced}$ in the developing eye causes a "glossy" eye phenotype. We will use this phenotype as an assay for a screen where we will look for suppressor genes of the $Xbp1_{spliced}$ induced "glossiness". In this screen, we expect to find mutations in genes that are required for the induction of cell death downstream of $Xbp1_{spliced}$. We will further characterize these mutations by testing for a possible modifier effect in the *ninaE* mutants induced degeneration and, to assay for the specificity, in ER-stress independent models of cell death.

Conclusions

The two specific aims described above attempt to elucidate the molecular mechanisms that regulate the induction of cell death in the context of ER stress. This is an important problem with relevance in terms of human health, as ER stress has been described in many neurodegenerative disorders and in cancer. To study this problem, we will use *Drosophila* as a model system, in which many aspects of the regulation of cell death are conserved with humans and has many experimental advantages. Hence, novel discoveries may provide good insights and strategies to fight these human diseases.

References

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