

Table of Contents

| | |
|---|-----------|
| Agradecimientos | iv |
| Resumo | v |
| Abstract | vi |
| Abbreviations | ix |
| | |
| CHAPTER I - INTRODUCTION..... | 1 |
| 1. Epithelium importance..... | 1 |
| 2. Epithelium remodeling and cell shape..... | 1 |
| 2.1. Apical constriction | 1 |
| 2.2. Cell Intercalation and planar cell polarity..... | 2 |
| 3. Cell Adhesion..... | 3 |
| 3.1. Vesicle trafficking and Actin regulation..... | 4 |
| 4. Apical-Basal Polarization..... | 5 |
| 4.1. Atypical Protein Kinase-C (aPKC)..... | 5 |
| 4.2. PAR proteins and the Apical aPKC-Par complex..... | 6 |
| 4.3. Apical Crumbs complex..... | 8 |
| 4.4. Basolateral determinants Scribble complex..... | 9 |
| 4.5. Basal antagonisms..... | 9 |
| 5. Epithelial Morphogenesis in <i>Drosophila melanogaster</i> | 10 |
| 5.1. Oogenesis in <i>Drosophila</i> | 10 |
| 5.2. Early Embryogenesis..... | 11 |
| 5.2.1. Cellularization of blastoderm..... | 12 |
| 5.3. Gastrulation and Segmentation..... | 13 |
| | |
| CHAPTER II - METHODS..... | 16 |
| 1. Mutants and alleles used in this work..... | 16 |
| 2. Germ Line Clones (GLC) generation..... | 16 |
| 3. Embryo fixation and staining..... | 17 |
| 3.1 Immunostaining..... | 18 |
| 4. Mosaic ovary analysis..... | 19 |
| 5. Ovaries fixation and staining..... | 19 |
| 6. Biochemistry..... | 20 |
| 6.1 Protein extraction..... | 20 |
| 6.2 Immuno-Precipitation..... | 21 |
| 6.3 Western blotting..... | 21 |

| | |
|---|-----------|
| CHARPTEr III - RESULTS | 23 |
| 1. Isolation of two aPKC alleles..... | 23 |
| 2. Maternal phenotype..... | 23 |
| 3. Aggregates formation during early Germ Band Extension..... | 25 |
| 4. <i>aPKC^{TS}</i> allele is zygotic viable at 25°C..... | 27 |
| 5. <i>aPKC^{TS}</i> is a Temperature Sensitive..... | 29 |
| 6. <i>aPKC^{TS}</i> has a mutation on kinase domain..... | 30 |
| 7. <i>aPKC^{TS}</i> mutant protein interact in vitro with Par6..... | 32 |
| 8. <i>aPKC^{TS}</i> follicle cells clones are normal at 25°C..... | 34 |
| 9. <i>aPKC^{TS}</i> follicle cells clones at 30°C and <i>aPKC^{TS}/DF6842</i> show defects in encapsulation..... | 36 |
| | |
| CHARPTEr IV – DISCUSSION | 38 |
| 1. Analysis of <i>aPKC^{TS}</i> mutants..... | 38 |
| 1.1 Hypothesis - Different epithelial tissues have differential requirements of aPKC activity..... | 38 |
| 1.2 Hypothesis - Epithelial tissue requires higher levels of aPKC activity during <i>de novo</i> formation of AJs..... | 39 |
| 1.3 Hypothesis - <i>aPKC^{TS}</i> is most likely a temperature-sensitive kinase | 40 |
| A – Qualitative scenario:..... | 41 |
| B – Quantitative scenario: | 42 |
| 2. Analysis of <i>aPKC^{PB1}</i> mutant..... | 42 |
| 2.1 Hypotheses - The apical localization of aPKC is essential for maintenance of follicular epithelium, even in the absence of kinase activity... | 42 |
| Conclusions..... | 43 |
| | |
| CHARPTEr V – FUTURE WORK | 44 |
| | |
| BIBLIOGRAPHY | 45 |
| | |
| ANNEX..... | 51 |