The Effects of Climate Change on a Basic Animal-Cell Function

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Abstract:

Increasing global temperatures during the 21st century may have detrimental effects on basic cell functions within ectothermal animals. This project aims to systematically examine the effects of climate change on secretory cargo localization through the analysis of Rab proteins found in the model organism *Drosophila melanogaster*. Rab proteins play an essential role in vesicular transport within the cell and can be genetically manipulated to monitor the biological consequences of global warming.

Introduction:

Although an increase in climate temperature will have significant ecological effects, it is important not to overlook the physiological consequences at the cellular level as well. The fruit fly, *Drosophila melanogaster*, is an excellent model organism for monitoring the physiological consequences of climate change. These ectothermic creatures have well characterized genomes that can be experimentally manipulated.

This research aims to investigate the impact of climate change on a basic characteristic of all eukaryotic cells: vesicular trafficking mediated by small Rab GTPases. These proteins function as molecular zip codes needed to target molecules to specific cells for components such as lysosomes, mitochondria, and cell membranes in eukaryotic cells that are highly compartmentalized[1]. Using the *Gal4/UAS* molecular toolkit, the Rab genes can be manipulated—to overexpress wildtype proteins, overexpress dominant-negative proteins, overexpress constitutively active proteins, or silence the proteins with RNA interference—at two different temperatures to mimic the projected increase in climate temperature. RNAi is a major tool used by organisms to protect against viral and transposon infections. This mechanism may be severely affected at elevated temperatures in ectothermic animals, which may lead to unforeseen stressors that the animal may not be able to cope with.

Materials and Methods:

The methodology used in these experiments is outlined in Figures 3 and 5. It requires the production of an assay stock and uses the *Gal4/UAS* Binary System to allow for the spatial and temporal control of *Rab* genes in the larval salivary gland of the fruit fly. The system requires the expression of a tissue specific *Gal4* driver and *UAS* responder stocks[2]. For this analysis, the driver stock produces GAL4 under the salivary gland promoter (*glueGal4*); it also contains a *glueRED* transgene to produce a fluorescently labeled red protein that localizes to secretory granules[3]. This assay stock was crossed to a collection of *UAS-Rab* stocks generated in the laboratory of Matthew Scott[4]. The *UAS-Rab*s were produced in three versions in which a wildtype open reading frame, a dominant-negative open reading frame, and a constitutively-active open reading frame were all under *UAS* control and tagged with yellow fluorescent protein (YFP). For the experiment outlined here, we focused on the *Rab-DN* constructs by crossing them to the assay stock (*glueGal4,glueRED*) and monitoring the secretion of red cargo protein in live animals as listed in Figure 4.

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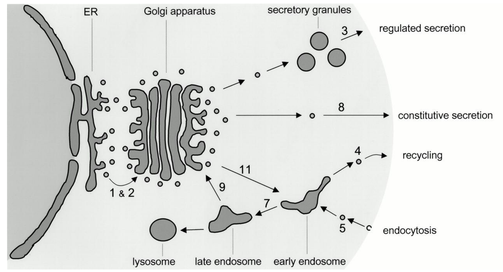

Figure 1: Summary of Rab Signaling in a Eukaryotic Cell.

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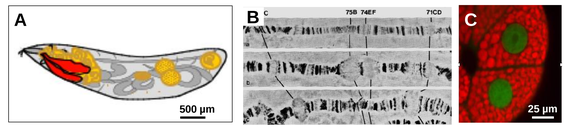

Figure 2: Why Study the *Drosophila* Salivary Gland?

A). Cartoon of third-instar *Drosophila* larva showing position of salivary glands (in red).
B). Polytene chromosomes from larval salivary gland showing position of ecdysone-responsive puffs[5].
C). Live cell of salivary gland expressing *glueRED* transgene (Fig 3)[3].

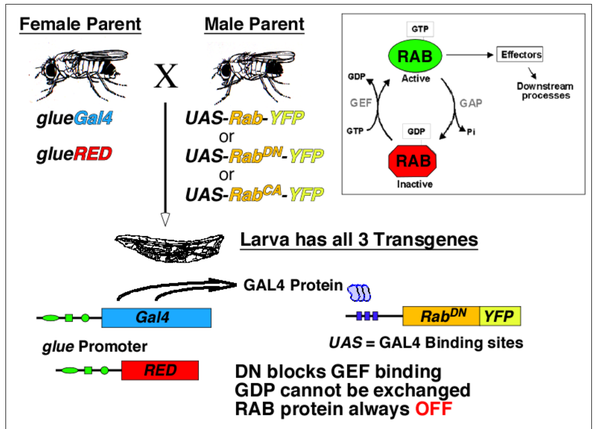

Figure 3: Overexpression of Rabs in the Salivary Gland.

The overall method used to drive expression of dominant-negative constructs of a Rab is illustrated. The *glueGal4* driver produces GAL4 proteins only in late-larval salivary glands. *glueRED* produces a cargo protein that is fluorescently tagged and used for the secretion assay. The *UAS-Rab^{DN}-YFP* construct will produce a Rab protein that has been engineered to disrupt the GEF binding site so that GDP cannot be exchanged[4]. This causes the Rab^{DN} protein to always be in an "OFF" position as indicated in the inset box[1].

Genotype	Stock	Investigator	Secretion Assay 0	+	++	+++	N
Control	101196	AA	0	3	10	45	58
Rab1-DN-a	9757	GK	60	56	0	0	116
Rab1-DN-b	23236	GK	74	16	1	0	91
Rab2-DN	9759	GK	0	0	15	35	50
Rab3-DN	9766	GK	0	2	15	35	52
Rab4-DN-a	9768	GK	1	0	4	45	50
Rab4-DN-b	9769	GK	1	0	4	56	61
Rab5-DN-a	9771	MG	4	3	0	53	60
Rab5-DN-b	9772	MG	1	3	0	41	45
Rab6-DN-a	23249	MG	5	2	0	44	51
Rab6-DN-b	23250	MG	3	0	3	45	51
Rab7-DN-a	9778	MG	3	0	5	42	50
Rab7-DN-b	23235	AA	0	5	27	31	63
Rab8-DN-a	23235	MG	4	0	1	45	50
Rab8-DN-b	23271	MG	10	0	1	52	62
Rab9-DN-a	23642	MG	0	1	0	50	51
Rab9-DN-b	23643	MG	9	4	0	36	49
Rab10-DN-b	9788	MF	0	0	29	22	51
Rab11-DN-a	9792	MF	12	35	10	0	57
Rab11-DN-b	23261	MF	44	13	0	0	57
Rab14-DN-a	23263	MF	0	0	20	30	50
Rab14-DN-b	23264	MF	2	3	7	38	50
Rab18-DN-a	23237	MF	0	0	27	23	50
Rab19-DN-a	9799	GK	0	2	9	59	70
Rab19-DN-b	23239	GK	4	2	33	80	119
Rab21-DN-a	23240	GK	0	0	4	48	52
Rab21-DN-b	23241	GK	0	3	10	57	70
Rab23-DN-a	9804	GK	0	0	0	55	55
Rab23-DN-b	9805	GK	0	0	6	105	111
Rab26-DN-a	9807	GK	0	0	4	57	61
Rab26-DN-b	9808	GK	0	1	7	107	115
Rab27-DN	23267	GK	0	0	3	57	60
Rab30-DN	9813	SC	6	21	39	31	97
Rab35-DN-a	9819	SC	2	8	27	26	63
Rab35-DN-b	9820	SC	1	6	50	17	74
Rab39-DN-a	9824	SC	1	5	31	59	96
Rab39-DN-b	23247	SC	5	12	30	37	84
Rab40-DN-a	23247	SC	7	8	18	37	70
Rab40-DN-b	9829	SC	7	11	42	38	99
RabX1-DN-a	9838	AC	0	11	30	53	94
RabX1-DN-b	23252	AC	11	9	28	41	89
RabX2-DN-a	9843	AC	0	10	15	29	54
RabX2-DN-b	23644	AC	0	3	1	41	45
RabX3-DN-a	9845	AC	6	4	15	35	60
RabX3-DN-b	9846	AC	5	6	21	35	67
RabX4-DN-a	9849	AC	0	5	17	40	62
RabX4-DN-b	9850	AC	0	0	10	45	55
RabX5-DN-a	9853	AC	19	45	0	0	64
RabX5-DN-b	9855	AC	1	2	11	45	59
RabX6-DN	23253	AC	2	2	12	54	70

Figure 4: Secretion Assay of *Rab-DN* Constructs.

An extensive collection of *Rab-DN* constructs was assayed based on the amount of cargo that remained in the larval salivary gland after secretion. Those stocks that exhibit a phenotype different from that of the wildtype are highlighted in yellow.

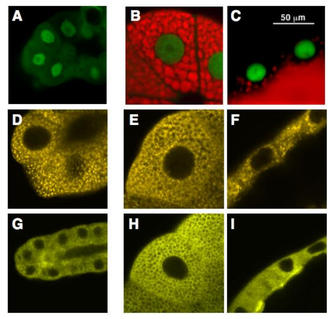

Figure 5: Localization of Rab1 and Rab 11 in Glands.

Images from confocal microscopy of single cells from live salivary glands expressing fluorescent tags (DsRED or YFP).
A-C): Progression of *glueRED* synthesis and secretion during L3.
D-F): Progression of *Rab1-YFP* during synthesis and secretion.
G-I): Progression of *Rab11-YFP* during synthesis and secretion.

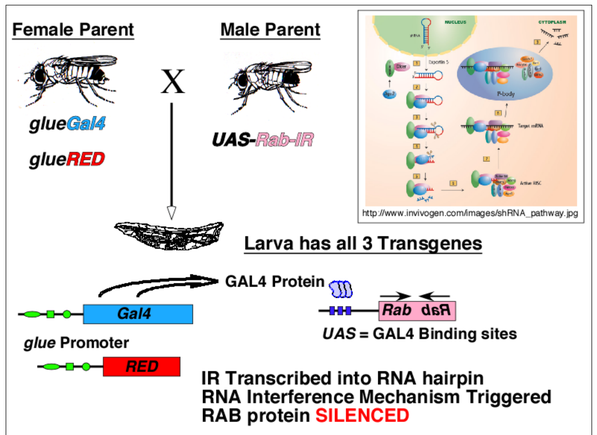

Figure 6: Silencing Rabs Using RNA Interference.

Strategy for expressing RNA hairpins for RNA interference is illustrated[6]. Insert is an overview of RNA silencing in eukaryotic cells.

Conclusions/Future Directions:

It is apparent from the data highlighted in yellow that secretion of cargo was blocked in Rab 1, Rab 11, and maybe Rab X5 when a dominant-negative construct of these Rabs were overexpressed in the salivary gland of *Drosophila*. Future experiments aim to repeat this experiment with the above Rabs using an RNAi strategy with stocks that we now have on hand or have ordered. We believe that silencing these Rabs should block secretion of cargo within the salivary gland just as the dominant-negative mutation did, and we will test if this silencing is affected by increased temperatures. Because RNA interference pathways are also used to block viral replication and transposable element transposition, this may have important implications as to what may happen in animals physiologically as a result of global warming.

References:

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[2]Brand and Perrimon (1993). *Development* **118**: 401-415.
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