Regulation of Polyamine Transport by *Chmp1* Overexpression in *Drosophila melanogaster*

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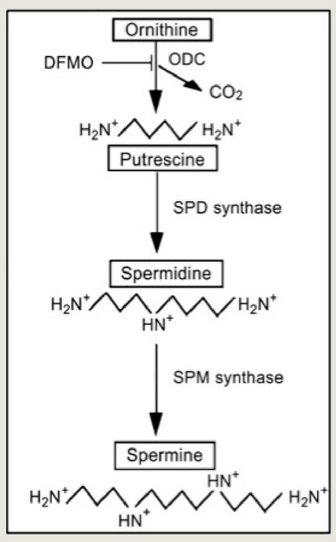
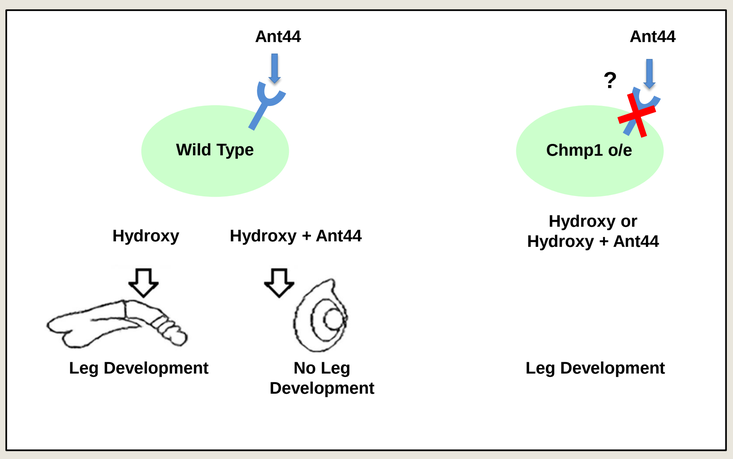
Introduction

Polyamines are small organic cations that are essential for a number of biological processes such as cell proliferation and cell cycle progression. High concentrations of polyamines are often associated with diseases like cancer (Paz *et al.*). While the metabolism of polyamines has been well studied (Schneider and Wendisch), the mechanisms by which polyamines are transported are poorly understood. Previous research has described lung carcinoma cells (H157), which are devoid of polyamine transport activity (Shao *et al.*). It has been hypothesized that these cells exhibit high expression of Chmp1. Chmp1 has been shown to be involved in vesicular trafficking, which makes Chmp1 a valid potential player in the polyamine transport system (PTS) (Howard *et al.*). *Drosophila melanogaster* larvae were used in these experiments because the overexpression of Chmp1 in tissue culture was unsuccessful. In these studies, imaginal discs from wild type (WT) and Chmp1 overexpressing (o/e) larvae were harvested and incubated for 18 hours at 25°C in the presence or absence of 1µg/mL 20-hydroxyecdysone (hydroxy) as it is known that discs develop in the presence of hydroxy and not in its absence (Wang, *et al*). In order to study whether Chmp1 overexpression regulates polyamine transport, a cytotoxic drug known to enter cells via the PTS, Ant44 (a generous gift from Dr. Otto Phanstiel) was added to the leg imaginal discs. It was proposed that if Chmp1 o/e down-regulates the PTS, then the addition of Ant44 would not inhibit leg development because it cannot enter the cell through the polyamine transporter. Polyamine rescue experiments were also performed in the presence of hydroxy and difluoromethylornithine (DFMO), an inhibitor of polyamine metabolism, to study whether the addition of different polyamines could rescue development of imaginal leg discs in Chmp1 o/e flies. It was proposed that if Chmp1 o/e down-regulates the PTS, then adding polyamines in the presence of hydroxy and DFMO would decrease leg development in Chmp1 o/e flies. If Chmp1 is found to be a down-regulator of the PTS, this may provide insight into the underlying mechanisms that are involved in polyamine transport. A better understanding of the players involved in the PTS could provide a vital target for cancer drug development (Nowotarski *et al.*).

The Polyamine Pathway



Proposed function of Chmp1 overexpression in polyamine transport



Adapted from Wang, M., et al. (2017). "Evaluation of Polyamine Transport Inhibitors in a Drosophila Epithelial Model Suggests the Existence of Multiple Transport Systems." Med Sci (Basel) **5**(4).

Leg development can be observed *in vitro*

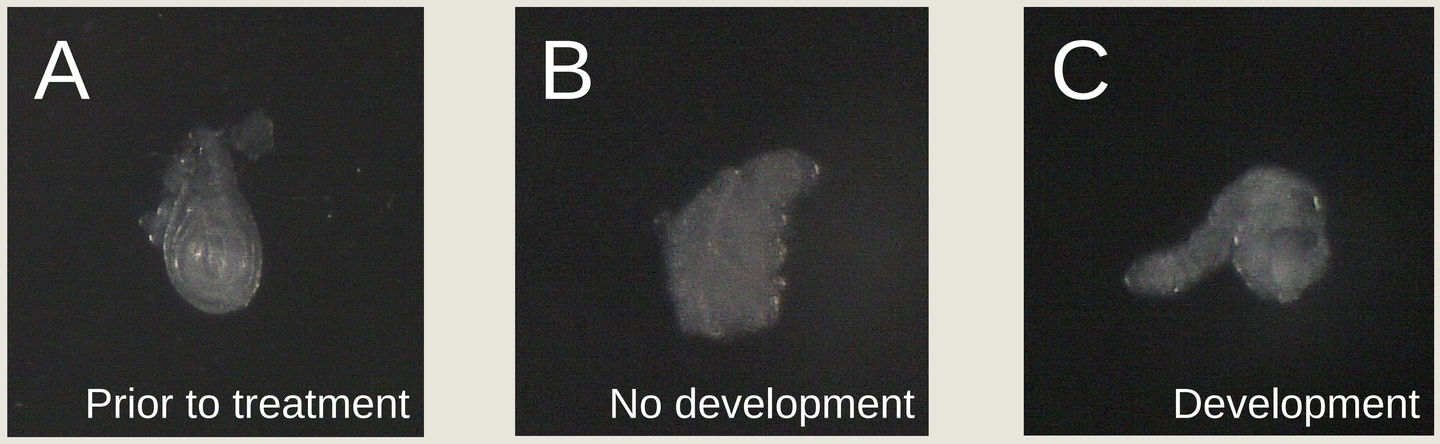


Figure 1. Leg imaginal discs dissected from *Drosophila melanogaster.* (A) Depiction of a leg imaginal disc immediately after dissection. Depictions of (B) non-developed and (C) developed leg imaginal discs after 18 hours at 25°C.

Overexpression of Chmp1 promotes development of leg imaginal discs in the presence of Ant44

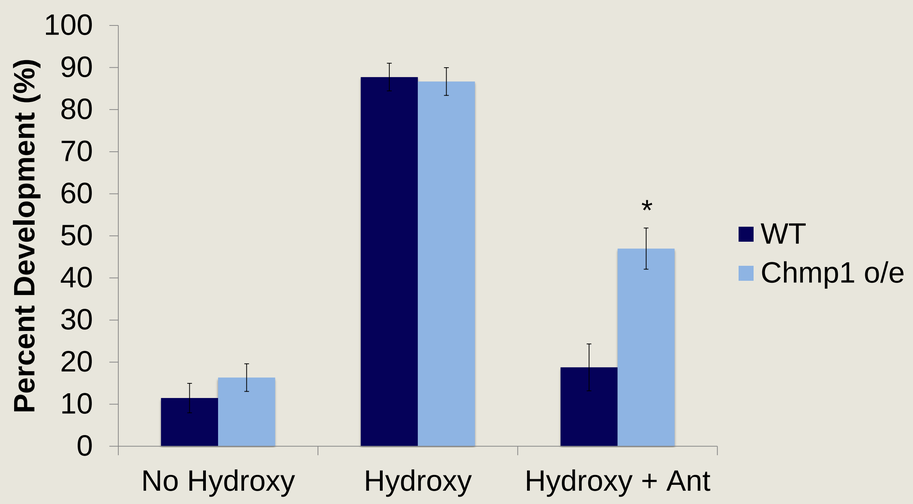


Figure 2. Chmp1 overexpression blunts the developmental effects of Ant44 on leg development. Leg imaginal discs were dissected from WT and Chmp1 overexpressing fly larvae. Discs were incubated for 18h at 25°C in the presence or absence of 1µg/mL 20-hydroxyecdysone and in the presence or absence of 50µM Ant44, which enters the cell via the polyamine transport system. The discs were scored and the data are shown as the mean +/- SEM. A Student's t-test was conducted. * $p < 0.05$ are indicated.

Overexpression of Chmp1 alters polyamine rescue of imaginal disc development in the presence of DFMO

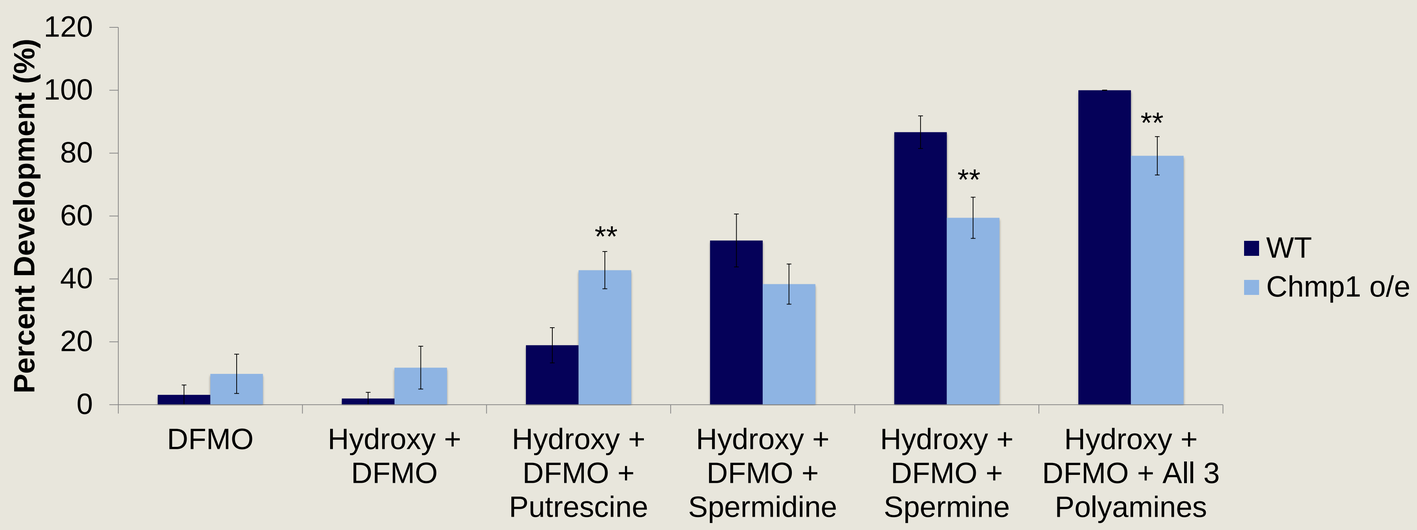


Figure 3. Chmp1 overexpression alters the ability of polyamines to rescue imaginal disc development in the presence of DFMO. Leg imaginal discs were dissected from WT and Chmp1 overexpressing fly larvae. Discs were incubated for 18h at 25°C in the presence of 1µg/mL 20-hydroxyecdysone, 10mM DFMO, and either 500µM putrescine, 200µM spermidine, 200µM spermine, or all 3 polyamines. The discs were scored and the data are shown as the mean +/- SEM. A Student's t-test was conducted. * $p < 0.05$; ** $p < 0.01$ are indicated.

Conclusions & Future Directions

- **Chmp1 overexpression has no effect on leg development**
- **The overexpression of Chmp1 appears to attenuate the negative impact of Ant44 on leg development, suggesting that Chmp1 down-regulates the PTS**
- **In the presence of DFMO, it appears that spermine or the 3 polyamines combined cannot rescue leg development, while putrescine was able to rescue development in Chmp1 overexpressing larvae, suggesting that there are unique mechanisms for individual polyamine transport**

- Validate *Chmp1* overexpression via qPCR
- Create *Chmp1* overexpressing cell lines

References

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