Localization of the JanA protein during conjugation and creation of a janA (mirror-doublet) pharmacological phenocopy in *Tetrahymena thermophila*

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Abstract

Janus A (JAN-A) is a gene in Tetrahymena thermophila that has been identified as an ortholog of mammalian polo-like kinases - a family of cell cycle master regulator proteins. The knockout of JAN-A can result in a mirror-duplication of the ventral pattern of organelles on the dorsal surface (the "janus" phenotype), as well as late conjugal arrest of mating cells.
This project focuses on seeing where the JanAp gene product was localized during each stage of *Tetrahymena* conjugation, and to test the effects of two polo-like kinase (PLK) inhibitors (Volasertib and BI 2536) on wild-type *Tetrahymena* to find out if the *janA* loss-of-function phenotype can be pharmacologically reproduced. Through GFP-tagging and fluorescence microscopy, we discovered that JanAp localized to micronuclei and the mating junction through most of the conjugation process. It then ends up in small punctae within the macronuclei during MAC anlagen development. Two PLK inhibitors, Volasertib and BI 2536 caused dramatic broadening of the contractile vacuole pore domains (CVPs) in wild-type cells similar to that seen in some *janA* mutants. In cells treated with BI 2536, we occasionally saw the formation of a second oral apparatus (OP) similar to the janus mutant phenotype. In the presence of *eja* (a second site mutation that enhances the *janA* phenotype), the broadening of CVPs in the cell was more dramatic.

JanA - Ortholog of a Polo-like Kinase (PLK)

Genetic analysis has identified *JAN-A* as a homolog to CDC5 gene in yeast, which is an ortholog of the polo-like kinase family in mammals. Polo-like kinases (PLKs) are serine/threonine kinases, which are considered "master regulators of cell cycle" since they play a critical role in most mitotic and meiotic processes *(de Carcer, et al., 2011; Noatynska et al., 2013).*

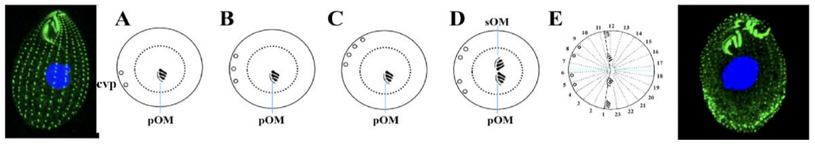


Figure 1. Emergence of the *janA* phenotype. When the JAN-A gene is silenced in a wild-type cell, one sees a gradual manifestation of the mutant phenotype over subsequent cell divisions. First, the CVP domain is broaden (A-C). Then, (and only in ~30% of the cells), a second OP forms on the dorsal side of the cell (D-E), completing a mirror-image pattern-duplication of the ventral suite of cortical organelles *(Frankel & Nelsen, 1986).*

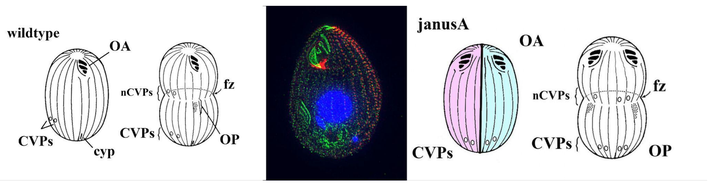


Figure 2. JanAp localization in wild-type vegetative cell. JanAp wild-type protein decorates ciliary basal bodies, but only on the dorsal hemisphere - the non-organelle side of the cell (excluding OP, CVP, cyt).

Methods

Figure 3a: JanA:GFP localization

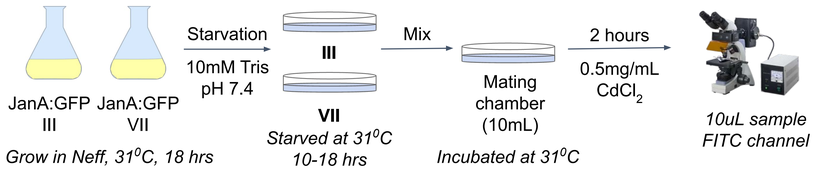


Figure 3b: PLK inhibitors

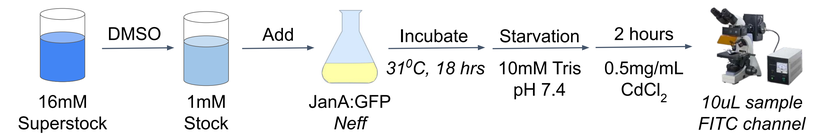
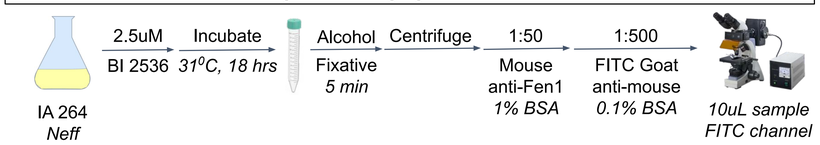
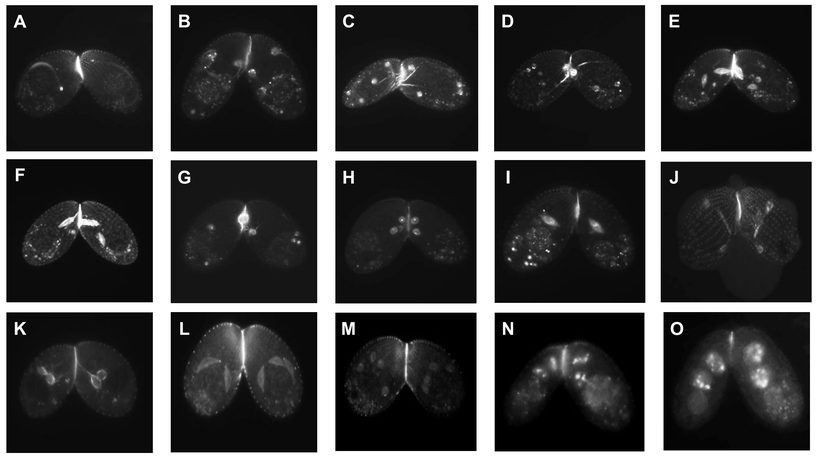


Figure 3c: Imaging CVPs in IA 264



JanA Localization During Conjugation



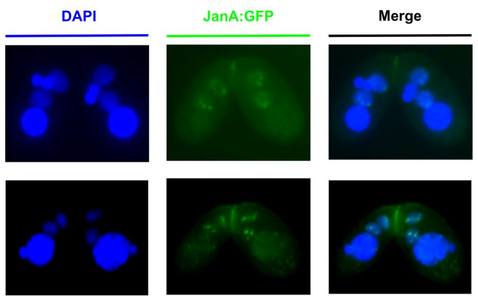


Figure 4. JanAp localization during *T. thermophila* conjugation. 15 stages from crescent state to MAC analgen (A-O). GFP-tagged mating cells are viewed between 2 hours to 10 hours after adding 0.5 mg/mL $CdCl_2$.

Figure 5. JanAp localization during MAC anlagen. A closer look into the first stage and second stage of MAC anlagen using DAPI staining and green FITC, anti-GFP labeling.

Effect of Volasertib on CVPs

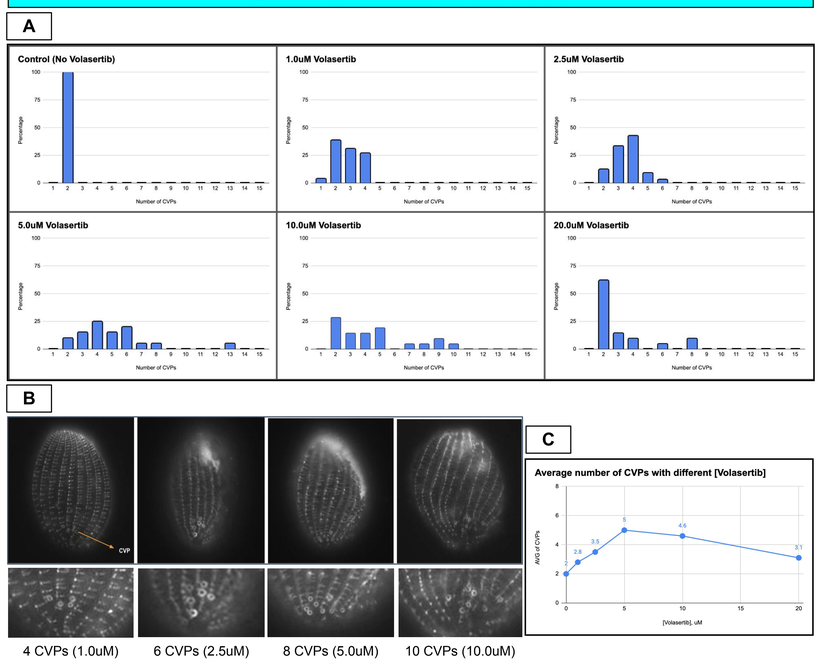


Figure 6. Effect of different concentrations of Volasertib on wild-type Tetrahymena thermophila. (A) Distribution and percentage of different numbers of CVPs for 6 different concentrations of Volasertib - Control (0 uM), 1.0 uM, 2.5 uM, 5.0 uM, 10.0 uM, 20.0 uM. The sample size is 21 cells (N = 21); (B) Examples of broadening of CVPs on 4 cell individuals of 4 different Volasertib concentrations; (C) Average CVPs for each concentration.

Effect of BI 2536 on CVPs and OA

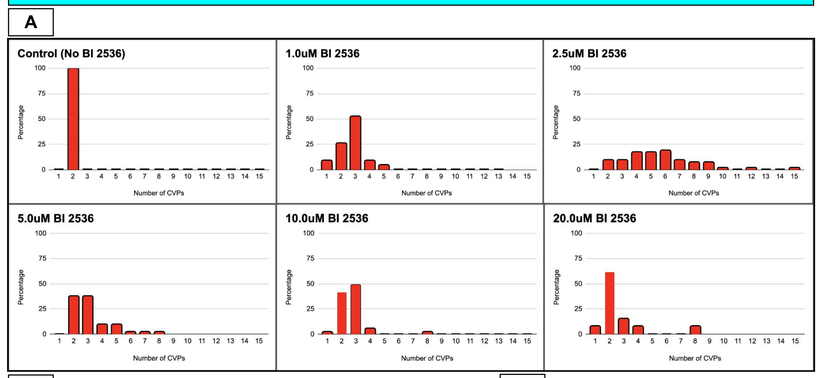


Figure 7. Effect of different concentrations of BI 2536 on wild-type cell. (A) Distribution and percentage of different numbers of CVPs for 6 different concentrations of BI 2536 - Control (0 uM), 1.0 uM, 2.5 uM, 5.0 uM, 10.0 uM, 20.0 uM. The sample size is 53 cells (N = 53); (B) 2.5 uM of BI 2536 shows possible formation of second OA; (C) Average CVPs for each concentration.

Effect of BI 2536 on *eja* cells (IA 264)

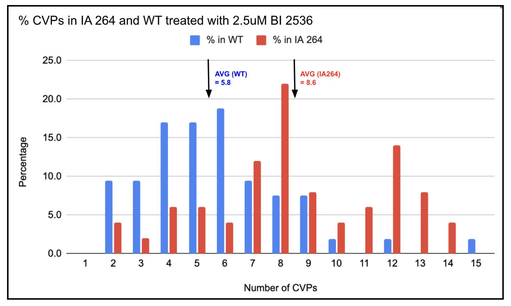


Figure 8. Difference in effect of 2.5 uM BI 2536 on number of CVPs in wild-type Tetrahymena thermophila compared to IA 264 (with *eja* enhancer background). Distribution of CVPs and average CVPs of wild-type and IA 264 treated by 2.5 uM BI 2536 is shown. The sample size is 50 cells (N = 50). The broadening of CVPs is more dramatic in IA 264 (with *eja*) than control (without *eja*).

Conclusions and Future Directions

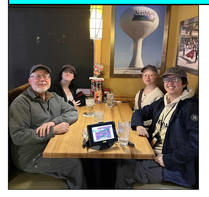
Conclusions:

- During crescent state, JanAp localizes to both ends of the crescent spindle.
- From nuclear selection moving on to fertilization and post-zygotic divisions, JanA remains localized over the **selected** MIC and the **zygotic** nucleus. The eliminated MICs lose the signal.
- During the MAC anlagen development, JanA concentrates to the two newly formed MACs and two MICs in punctae.
- Both Volasertib and BI 2536 cause dramatic broadening of CVPs of wild-type cells, especially at 2.5uM of drug. The effect is stronger in IA 264 cells.
- BI 2536 has potential to cause occasional formation of second OA.

Future directions:

- Check if JanAp and PDD1 (a DNA-eliminated foci formation protein) co-localize in MAC anlagen.
- Check for formation of second OA when IA 264 cells get treated with BI 2536.

Acknowledgements



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References