Potential role for histone H3K4 methylation in diapause induction in the larva of *Chymomyza costata*.

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Introduction

Epigenetic modifications are considered as potentially important players in regulation of seasonal phenotypic transitions, including insect diapause. Our earlier transcriptomic assay (Poupardin et al., 2015) revealed that genes coding for some epigenetic factors are differently regulated during the diapause induction in the larvae of drosophilid fly, *Chymomyza costata*. Specifically, we found that *dpy30* and *wds* are two genes with the strongest response (down-regulation) to a change of photoperiodic signal from long days to short days (inducing diapause). As *dpy30* and *wds* genes are coding for subunits of histone H3K4 methylases, we speculated that diapause induction might be linked to decreasing levels of H3K4 methylation in insect tissues. Low levels of H3K4 methylation in diapause would make sense, as the H3K4 tri-methylation is a strong inducer of chromatin de-condensation and active gene transcription (typical situation for direct development). We will present results of preliminary ELISA assays of H3K4 global methylation and immunocytochemical staining of cell nuclei using antibody against tri-methylated H3K4 in larval fat body and muscle tissues.

Results

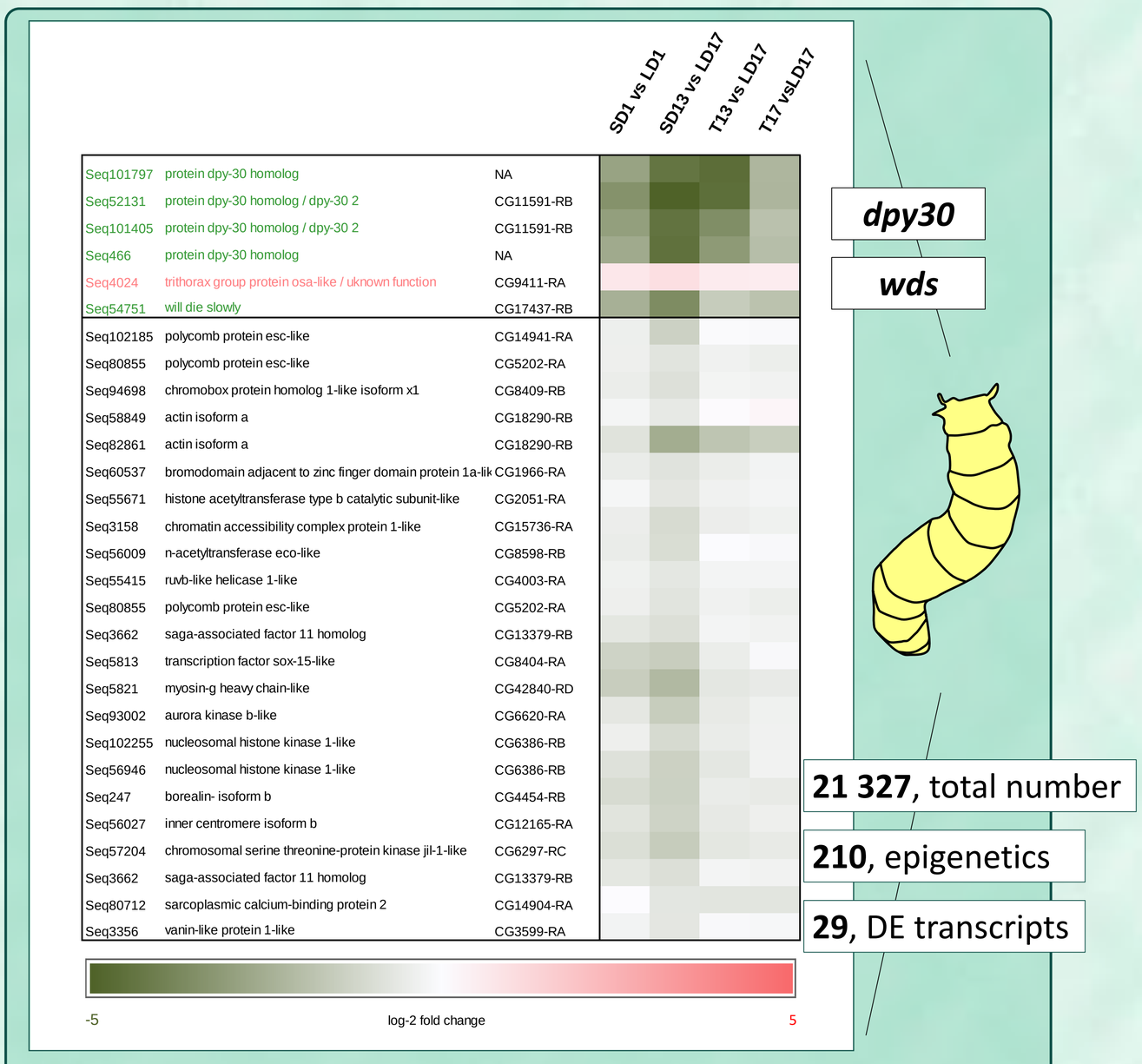


Fig. 1: A heat map based on transcriptomic study (Poupardin et, al., 2015). Of a total 21 327 transcripts, 210 were coding for various epigenetic factors with impact on histone modifications . The 29 differentially expressed transcripts are shown (criteria: - 0,55 < Log2 fold change > +0,55, and Benjamini-Hochberg *p* value < 0,05). Out of these 29 sequences, two, namely: *wds* and *dpy30,* showed the strongest response (down-regulation) to either a short photoperiod (SD) or to a transfer (T) from long days (LD) to short days (SD). The *dpy30* was down-regulated ca 25-fold. The two genes are coding for subunits of histone H3K4 methyltransferases. H3K4 di-metylation is known to de-condensate the chromatin, while tri-methylation is considered to generally activate gene transcription. That setting is typical for direct development, so we expected that H3K4 tri-methylation will be much more abundant in direct development than in diapause larvae.

Furthermore, when *dpy30* transcripts are genetically depleted in mammalian hESC cells, they stop to proliferate (Jiang et, al., 2011), which closely resembles the phenotype of diapause insects. It is important to note, that histone methyltransferases are highly conserved in evolution in all eukaryotes .

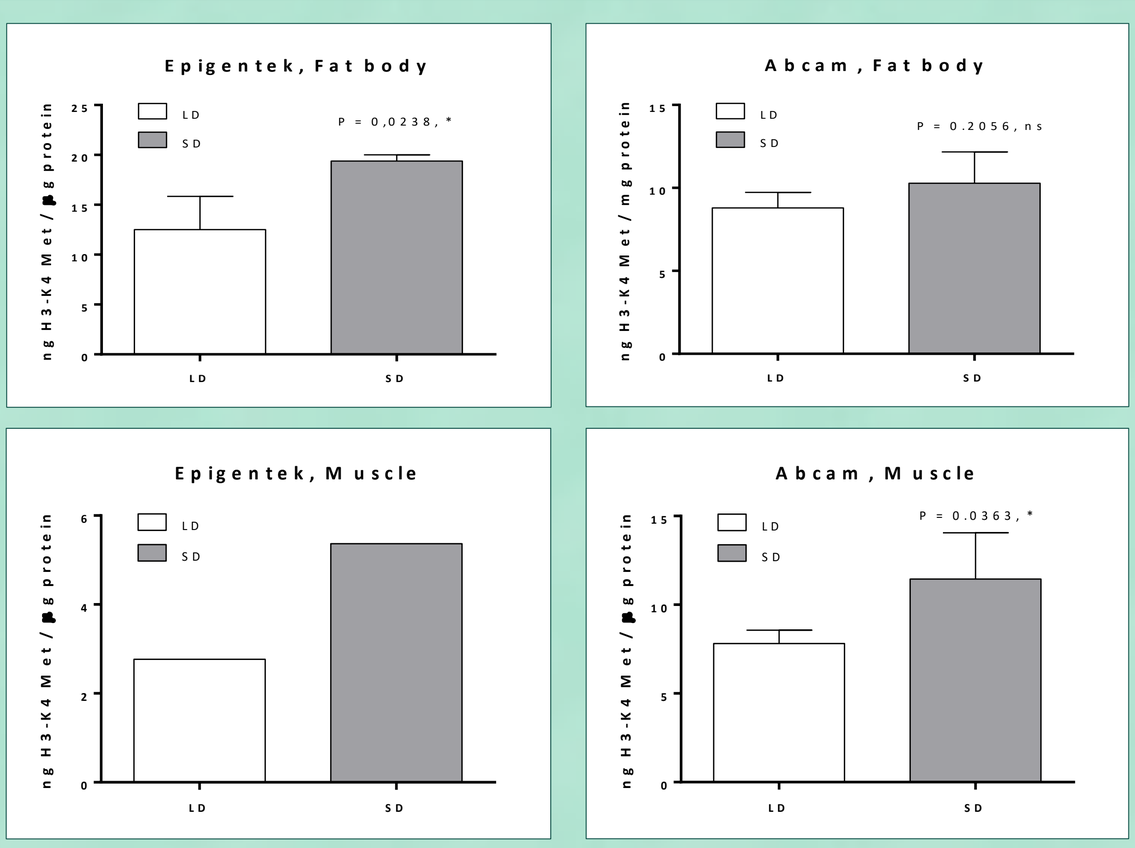
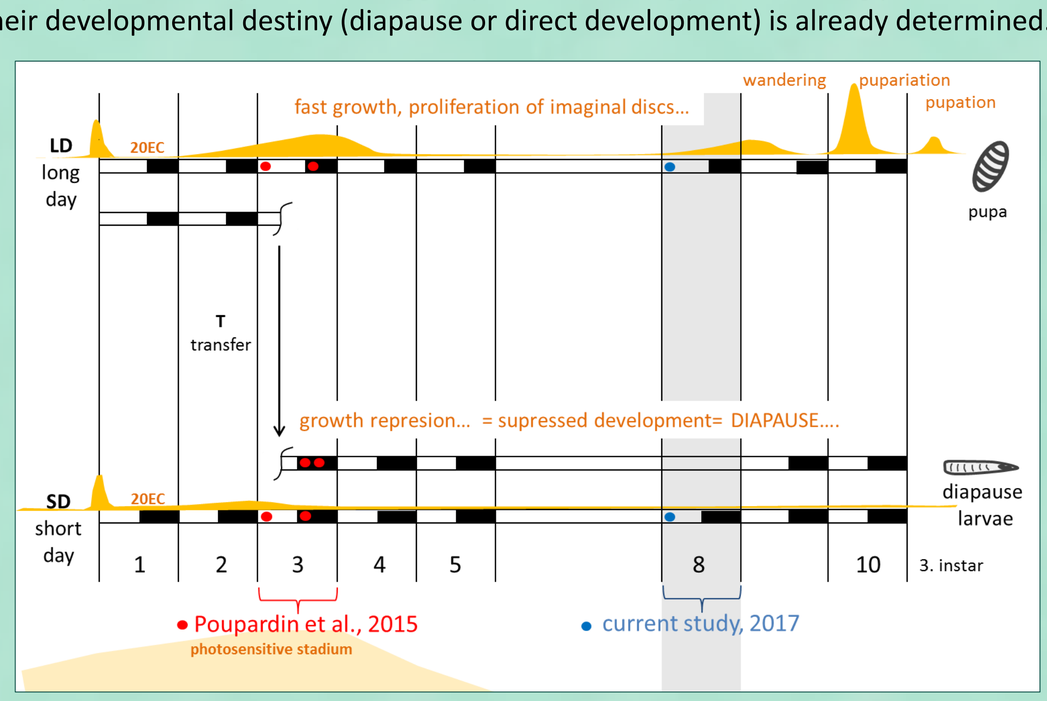


Fig. 2: Global levels of H3K4 methylation expressed per µg of nuclear protein. Two different kits were used (Epigentek, Abcam) to quantify the levels of H3K4 methylation in fat body and muscle cells dissected from larvae reared under LD (direct development) or SD (diapause) conditions . Contrary to our hypothesis, the global levels of H3K4 methylation were significantly lower in LD larvae, in both muscle and fat body tissues (t-test, P is shown).

Experimental design

Chymomyza costata larvae were cultured on artificial diet at constant temperature of 18°C and two different photoperiodic conditions – short days (SD, 16 h light / 8 h dark) inducing the larval diapause, and long days (LD, 12 h light / 12 h dark) inducing the direct development to pupa and adult. The larvae were sampled on day 8 of their 3rd instar (approx. 20 days from the egg deposition), during pre-wandering phase, when their developmental destiny (diapause or direct development) is already determined.



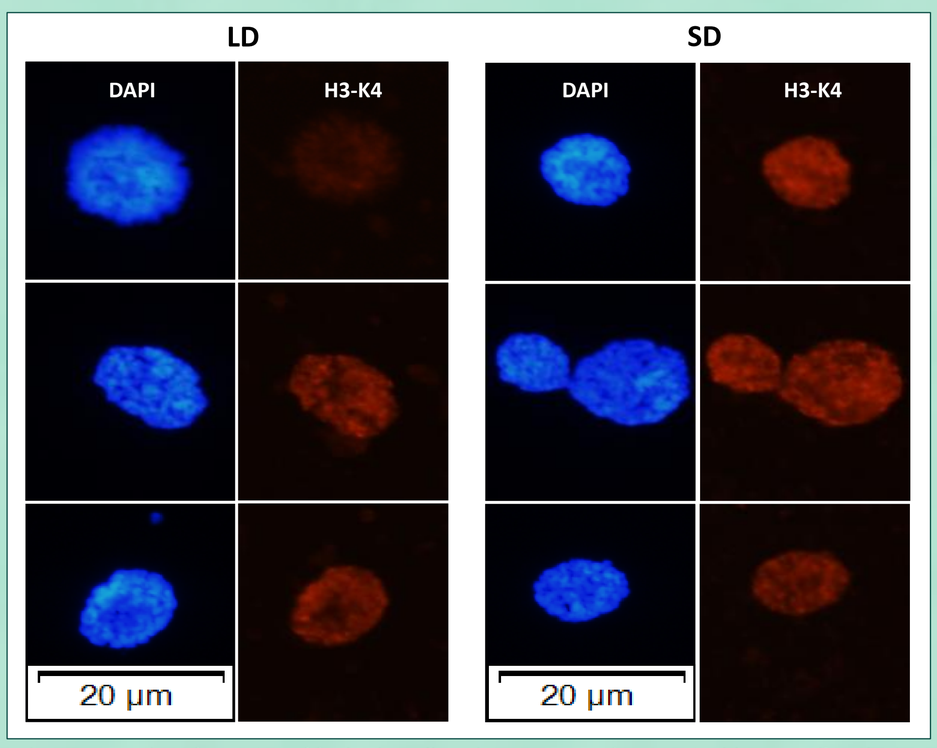


Fig. 3: Examples of staining of fat body cell nuclei using DAPI (DNA stain, blue signal] and H3K4 tri-Met antibody (Abcam, red signal). Note slightly weaker red signal in direct development larvae (LD) than in diapause larvae (SD).

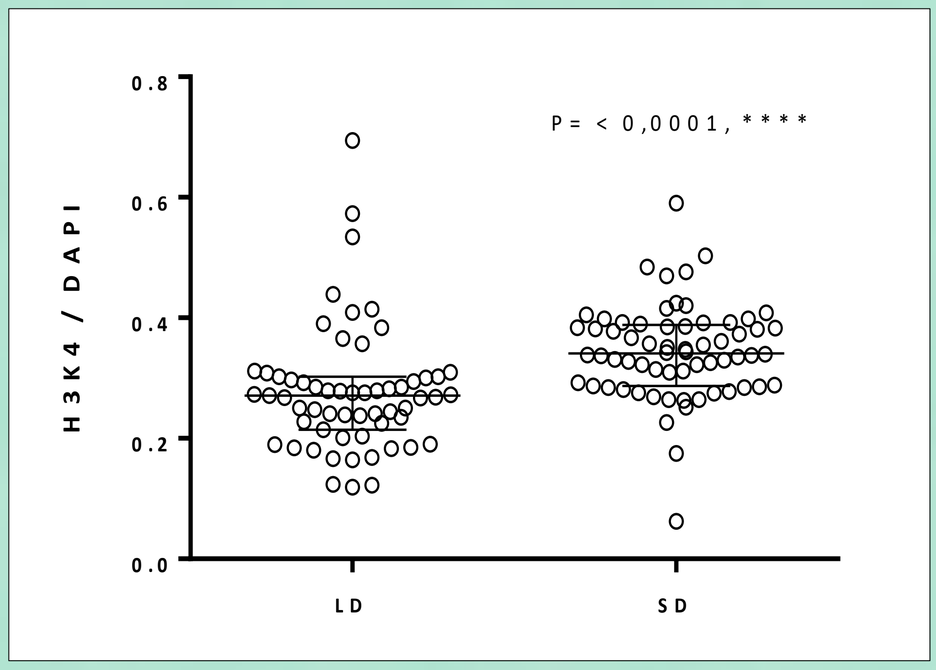


Fig. 4: Relative intensity of H3K4 methylation signal in the nuclei of fat body cells.
The difference between the SD and LD photoperiodic conditions is significant (Mann Whitney two-tailed test). Contrary to our expectation, the direct development larvae (LD) exhibited slightly lower level of H3K4 tri-methylation than the diapause larvae (SD).

Conclusions

- The levels of H3K4 tri-methylation were slightly but statistically significantly lower under long days (LD) than under short days (SD) in two tissues of *C. costata* larvae.
- Our hypothesis was not proven: we could not see any direct relationship between high expression of *dpy30* (Poupardin et al., 2015) and high levels of H3K4 tri-methylation (current study) in LD larvae.
- It appears that the investigation of a *complex* epigenetic machinery is required (*e.g.* using a Chip-seq method) in order to get first insight into the potential epigenetic regulations of insect diapause …

Literature

Jiang, Hao, et al. "Role for Dpy-30 in ES cell-fate specification by regulation of H3K4 methylation within bivalent domains." Cell 144.4 (2011): 513-525.

Poupardin, Rodolphe, et al. "Early transcriptional events linked to induction of diapause revealed by RNAseq in larvae of drosophilid fly, Chymomyza costata." *BMC genomics* 16.1 (2015): 720.

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