Uncovering a link between H2Av and the cell cycle during early Drosophila embryonic development

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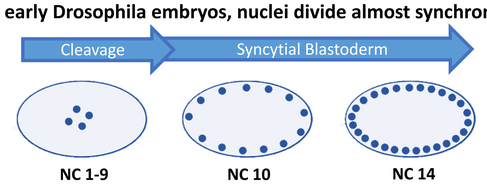
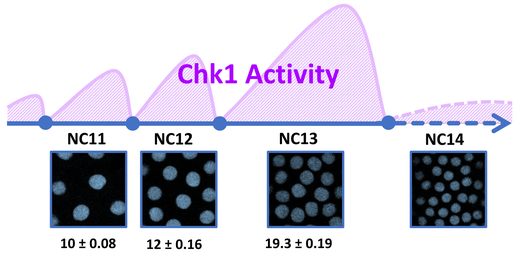
Introduction

In early Drosophila embryos, nuclei divide almost synchronously



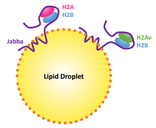
Drosophila embryos undergo 13 near-synchronous nuclear divisions to generate 6000 nuclei within just 2.5 hrs. These syncytial divisions occur extremely rapidly, consisting of DNA synthesis and mitotic phases in the absence of G1, G2 and cytokinesis.

Nuclear cycles (NC) are fast and regulated



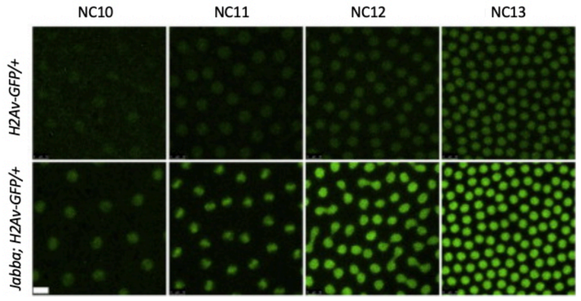
NCs are highly stereotyped. In NC12 and even more so in NC13, some origins of replication fire late instead of early, delaying overall replication. Single-stranded DNA intermediates produced during replication activate the ATR/Chk1 pathway, and that pathway prevents entry into mitosis. Thus, NC length increases progressively.

Jabba sequesters histones on lipid droplets



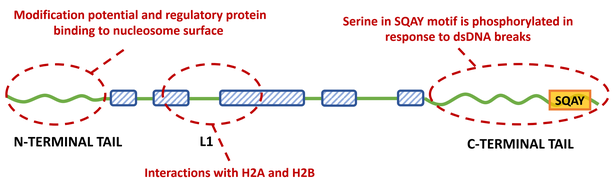
Histones H2A, H2B, and H2Av are anchored onto lipid droplets via Jabba. In *Jabba* mutants, histones are absent from lipid droplets and present only in the nucleus. (Li et al., 2012)

Jabba mutants show increased accumulation of H2Av in nuclei



Live imaging of wild-type and *Jabba* embryos containing fluorescently tagged H2Av. *Jabba* embryos show overaccumulation of H2Av in cycles 10-13 (quantified in Li et al. 2014). This over-accumulation is due to the absence of H2Av buffering by lipid droplets (Johnson et al., 2018).

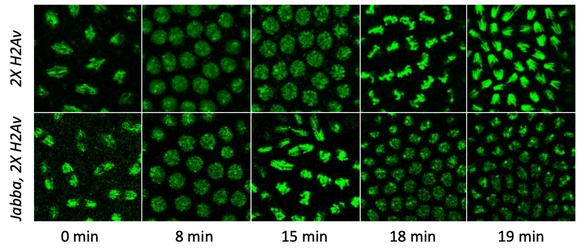
H2Av combines H2A.X and H2A.Z function

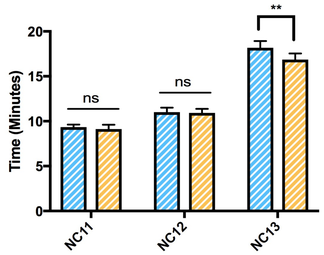


H2A.X is phosphorylated in response to DNA double-strand breaks and participates in DNA repair. H2A.Z alters nucleosome stability and is involved in transcriptional control. In Drosophila, H2A.X and H2A.Z functions are mediated by a single histone, H2Av.

Results

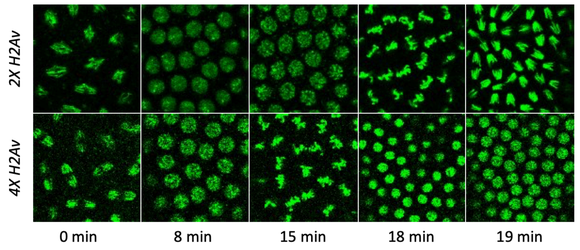
Jabba mutants display an NC13 specific speed-up

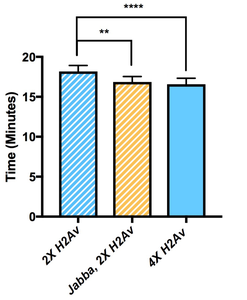




Jabba mutants show a speed-up in NC13 compared to wild-type embryos. There is no significant difference in the length of earlier cycles.

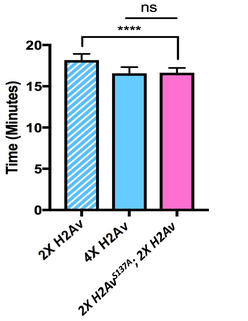
Increasing H2Av is sufficient for speed-up



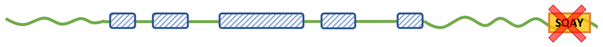


The speed-up seen in *Jabba* mutants can be recapitulated by increasing *H2Av* copy number to 4X. The two genetic means of increasing nuclear H2Av level generated a similar speed-up phenotype.

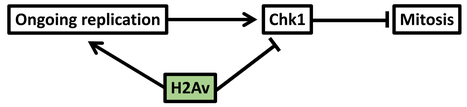
H2A.X function is not required for speed-up



We generated embryos in which the two additional copies of *H2Av* carry a mutation that prevents phosphorylation. These embryos still exhibit NC13 speed-up. Thus, speed-up is not due to the H2A.X function, but the H2A.Z function of H2Av.

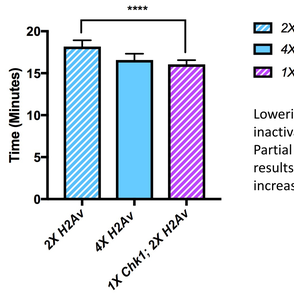


How might H2Av cause NC13 speed-up?



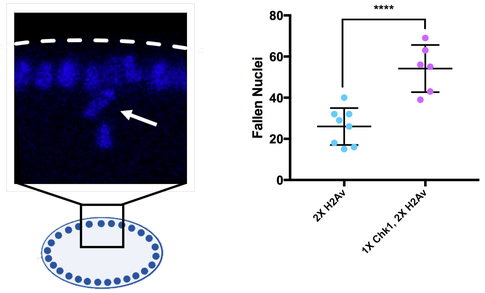
Nuclear H2Av overaccumulation in *4X H2Av* speeds up NC13. Ongoing replication triggers Chk1 activation and thereby prevents premature mitosis by lengthening the cell cycle. Inactivation of this checkpoint by H2Av would therefore decrease NC13 duration. Faster replication due to H2Av would also reduce NC13 length via progressing through this checkpoint more quickly.

Chk1 heterozygous embryos also display NC13 speed-up



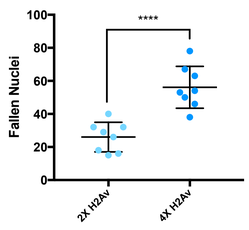
Lowering *Chk1* dosage partially inactivates checkpoint activity. Partial elimination of the checkpoint results in a comparable speed-up to increased H2Av dosage.

1X Chk1 embryos show increased nuclear falling



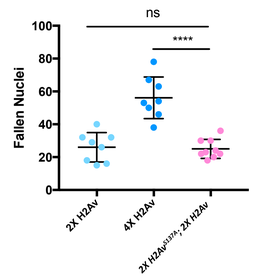
Nuclear falling is a response to DNA damage and eliminates damaged nuclei (arrow) from the embryo periphery (dashed line = edge of embryo). To quantify nuclear falling, we stained nuclei with Hoechst. In *1X Chk1* embryos, nuclear falling was significantly increased compared to wild type. We propose that reduction in Chk1 activity allows premature mitosis before replication is finished; the resulting DNA damage would trigger more nuclear falling, via activating Chk2.

4X H2Av embryos also show increased nuclear falling



4X H2Av embryos also displayed increased nuclear falling. This phenotype might result from partial inactivation of the Chk1 pathway, and thus pre-mature mitosis, by excess H2Av. Alternatively, increased H2Av may inappropriately trigger Chk2 and the nuclear falling pathway, via an as-yet unidentified mechanism.

2X H2Av^S137A; 2X H2Av embryos show wild-type levels of nuclear falling

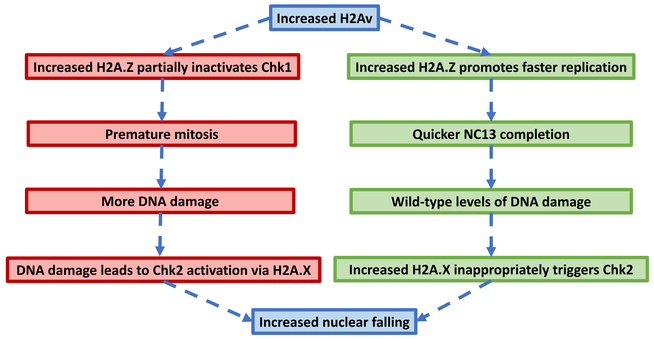


We examined embryos with two extra copies of H2Av that cannot be phosphorylated. Even though NC13 is sped up in these embryos (see above), nuclear falling returns to wild-type levels. This result reveals that H2Av's H2A.X function contributes to nuclear falling.

Conclusions

- Increased H2Av causes NC13 speed-up
- Speed-up is not a function of H2A.X
- Increased H2Av increases nuclear falling
- Decreased H2A.X function returns nuclear falling to wild-type levels
- H2Av contributes to NC13 speed-up and nuclear falling via two different functions

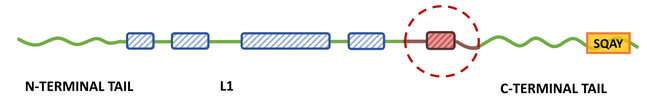
Models



Future Directions

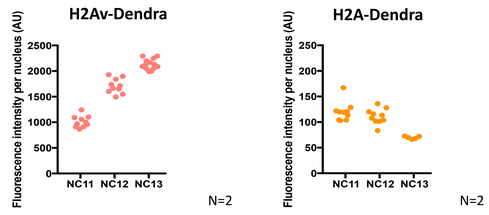
- We will directly measure Chk1 activity using a Chk1 biosensor (Deneke et al., 2016).
- Chk2-GFP accumulates on centrosomes and chromosomes upon DNA damage (Takada et al., 2015). We will use this assay to compare Chk2 activity across our genotypes.

How might H2Av affect replication?



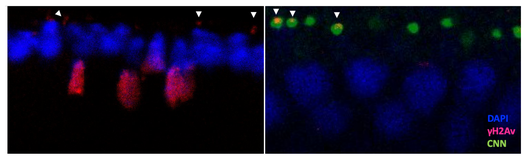
In mammalian H2A.Z, an acidic patch (circle) has been proposed to interact with the methyltransferase SUV420H1 and promote replication by leading to the recruitment of the origin licensing factor ORC1. (Long et al., 2019) Increased H2Av incorporation might similarly result in more replication origins and therefore faster replication. This region will be mutagenized and NC length will be measured via live imaging. Alternatively, replication might be altered indirectly since transcription hinders replication. RNA-Seq is being performed to examine differences in gene expression between wild-type and *4X H2Av* embryos during NC13 and 14.

H2Av levels increase as H2A levels decrease from NC11-NC13



Preliminary results from live imaging of *H2Av-Dendra* and *H2A-Dendra* embryos reveal that as nuclear H2Av increases over development, nuclear H2A decreases. Increased nuclear H2Av levels in *4x H2Av* embryos might therefore further decrease nuclear H2A levels. We will increase *H2Av* dosage in an H2A-Dendra background and directly quantify effects on nuclear H2A levels.

γH2Av signal may colocalize with centrosome staining



γH2Av immunostaining revealed γH2Av in falling nuclei and in foci above nuclei at the cortex (right, arrowheads). Preliminary immunostaining of γH2Av and CNN results show colocalization of γH2Av foci and centrosomes (left, arrowheads). We have yet to determine whether centrosomal signal is due to γH2Av or cross-reactivity of the antibody. If γH2Av is indeed at the centrosome, it might be directly involved in centrosome inactivation and the nuclear falling pathway. We will examine embryos expressing both H2Av-Dendra and Chk2-GFP to visualize and quantify the localization and signal of each protein.