UNDERSTANDING THE FUNCTION OF DIFFERENT 3-DIMENSIONAL GENOME ORGANIZATIONS

By

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Abstract:

Recent studies identified the condensin II subunit Chromosome-associated Protein H2 (CapH2) as a master-regulator of chromosome territory (CT) formation in *Drosophila melanogaster*. Specifically, Cap-H2 overexpression in interphase cells disrupts homologous chromosome pairing, inducing chromosome individualization and the formation of distinct CTs. By manipulating this new regulatory pathway, it is now possible to investigate the function of different 3-dimensional chromosome organizations in cells by comparing the expression patterns of cells containing paired chromosomes to those with CTs. To obtain cells containing CTs we generated stable, inducible Schneider 2 (S2) cell lines that express wild-type (WT) Cap-H2. Cells were scored for the formation of normal, weak, and strong CTs over a six day time course. We also conducted cell viability counts on CapH2-expressing cells to determine the effect of CTs on cell health. We found that strong CT formation was most prevalent after six days of Cap-H2 expression. However, cell cultures expressing WT Cap-H2 displayed a reduced number of viable cells over the six day time course. Taken together, we conclude that it requires almost a week of WT Cap-H2 induction for most stable cells to form CTs and CTs cause reduced cell health.

Introduction:

Genome organization in eukaryotic cells is not random, but highly spatially regulated (Kosak and Groudine, 2004; Misteli, 2007; Cremer and Cremer, 2010). The non-random nature of genomes is important because it maintains its integrity and influences its nuclear shape, DNA replication and repair, and gene expression patterns (Ferrai et al.,

2010; Aparicio, 2013; Agmon et al., 2013). Recent cutting-edge technologies that examine the 3-dimensional organization of genomes reveal that interphase chromosomes are arranged into subnuclear compartments know as chromosome territories (van Berkum et al., 2010; Sanyal et al., 2011). Although the function of chromosome territories is not known, it is thought that the specific arrangement of CTs within the nucleus may control genome function (Rajapakse et al., 2010; Rajapakse and Groudine, 2011).

Condensins I and II are conserved protein complexes that serve as major players in genome organization. They also play roles in shaping mitotic chromosomes. Condensin I promotes lateral chromosome compaction and condensin II promotes axial compaction. Correct mitotic condensation and chromosomal segregation require both condensins (Shintomi and Hirano, 2011). Condensin I associates with mitotic chromosomes while condensin II is present in the nucleus and binds to chromatin throughout interphase (Hirano, 2005). Condensin I and II also both share the SMC2/4 heterodimer, but differ in their chromosome-associated proteins. Condensin I contains the proteins CAP-D2, -G, and –H while condensin II includes the proteins CAP-D3, -G2, and –H2 (Hirano and Hirano, 2004; Hirano, 2005). In *Drosophila* condensin II specifically performs functions in interphase that include chromosome compaction, unpairing of homologous chromosomes, and the formation of ‘chromosome-like territories’ (Buster et al.,2013; Nguyen et al., 2015).

Recent studies identified a pathway for the regulation of condensins. Condensin II activity requires the regulatory subunit Cap-H2. The Slimb protein localizes to chromatin and targets Cap-H2 for degradation by ubiquitination. Because of this interaction, Slimb acts as a negative regulator of Cap-H2. Overexpression of Cap-H2 also

results in an increase of condensin II activity. Overexpression of Cap-H2 in cells results in interphase chromatin compaction and chromosome unpairing and is referred to as the "chromatin- gumball" phenotype. In *Drosophila* hyperactivity of condensin II through Slimb depletion or Cap-H2 stabilization also results in nuclear envelope abnormalities (Buster et al., 2013).

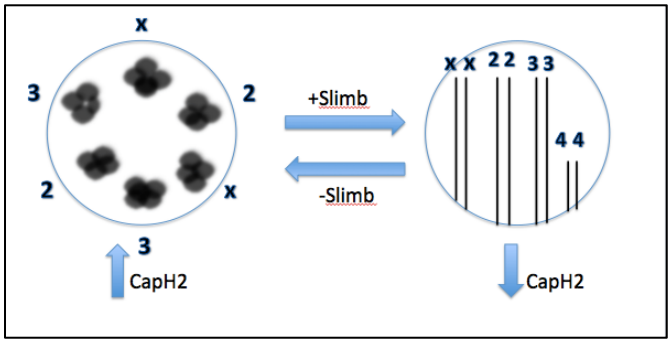

Figure 1: Chromatin regulation by CapH2

Defects in the nuclear envelope occur in many diseases, including Emery-Dreifuss muscular dystrophy and Hutchinson-Gilford progeria syndrome. In these diseases, deformation of the nuclear envelope causes altered gene expression and disorganization of the genome. The abnormal nuclear envelopes in these diseases are also similar in morphology to those observed in cells with hypercompaction of chromosomes (Scaffidi et al., 2005; Scaffidi and Misteli, 2006). Possibly condesnin II causes the abnormal nuclear envelopes observed in these diseases.

The function of chromosome territories remains unknown. With this newly discovered CapH2 regulatory pathway, it is now possible to turn chromosome territory expression on and off in cells. We are attempting to determine the function of chromosome territories by manipulating the *Drosophila* model system to induce CT expression in cells that normally would not produce this phenotype. To do this, we manipulate cultured cell systems to test the idea that CTs influence gene expression

pathways by using immunofluorescence microscopy to determine the changes in phenotype of the cells and conducting viability counts to determine the effects on cell health.

Methods:

Immunofluorescence microscopy

Immunofluorescence microscopy was used to score the gumball phenotype in two types of cell lines. Wild Type Drosophila Schneider 2 cells and stable line S2 cells expressing the CapH2-GFP construct were each plated on six wells of a tissue cultured treated six well plate (Olympus Cat #: 25-105). Confluent cells (100%; 0.5 mL) were added to each well of a six well plate with 0.5 mL of Serum Free Media Complete (Life Technologies). Copper Sulfate (1 mM) was added to each of the wells of the Cap-H2 cells in order to induce expression of the construct. Every 24h over the course of six days, 200 µl of media and four µl of $CuSO_4$ were added to each well due to evaporation overnight. Each well of the six well plate corresponded to a day (Buster, et al., 2013).

Every 24h for six days, the cells from one well of the Cap-H2 plate were also used to fix slides for microscopy. Each day, one well was resuspended to lift the cells off the bottom of the plate. The cells were then transferred to a concanavalin A-coated glass bottom dish (Sigma-Aldrich) containing 150 µl of media until the cells reached 80% confluency. The cells sat for 45 min to adhere to the bottom of the dish. After 45 min, the cells were washed with 1x PBS + 10% formaldehyde solution (Electron Microscopy Sciences Cat #: 15714-S) for 15 min. The slides were then washed three times with PBS-Triton and blocked in 150 µl of blocking buffer for 15 min. A solution of 1.5 mL of PBS

Triton and 0.5 mL of Hoechst (Invitrogen) was prepared and 200 µl was added to the slide for five min. After that, the slide was washed two times with PBS Triton and 600 µl of mounting media was added directly to the slide (Rogers, et al., 2009).

The slides were scored for the presence of gumballs in the cell nucleus using microscopy (Zeiss Axiovert 200). A 100x oil immersion objective was used to view cells in the DAP1 and FITC channels. These channels showed the cell nuclei and presence of Green Fluorescent Protein respectively. Only cells that were expressing GFP were used to score the gumballs. One hundred cells per slide were scored based on the presence of a normal, weak, or strong gumball phenotype in their nuclei. The phenotypes were scored based on the image below.

Buster, et al., 2013

A bar graph was constructed to show the results of the change in gumballs over the six-day period.

Cell Viability Counts

Cell viability counts were conducted to determine the effects on cell health between the wild type S2 cells with no gumballs and the Cap-H2 cells with gumballs. Every 24h for six days, 20 µl from one well of the Cap-H2 plate and 20 µl from one well of the S2 plate were resuspended and transferred to a 96 well plate (Olympus Cat #: 25-109). Twenty µl of Trypan Blue Solution (HyClone) was added to each well. This dye stained the dead

cells blue and allowed them to be distinguished from the living cells. Ten µl of the cells with Trypan Blue were moved to a hemacytometer with a cover slilp on it (Bright-Line). Living and dead cells were counted in each 4x4 grid of the hemacytometer using a 20x objective on a microscope (Olympus CKX41). The number of cells was divided by four, multiplied by two, and then multiplied by 10,000 to determine the number of cells per mL (Freshney, 1987). The number of living S2 cells vs. the number of living Cap-H2 cells was plotted on a line graph to determine if there was any difference in cell health.

Results:

Cap-H2 expressing cells were scored for the presence of a normal, weak, and strong chromatin gumball phenotype using immunofluorescence microscopy. We found that weak gumball formation increased from days one through five and peaked on day five with 42% of cells exhibiting a weak gumball phenotype. Strong gumball formation increased throughout the six days and was most abundant on day six with 12% of cells demonstrating a weak gumball phenotype (Fig 2).

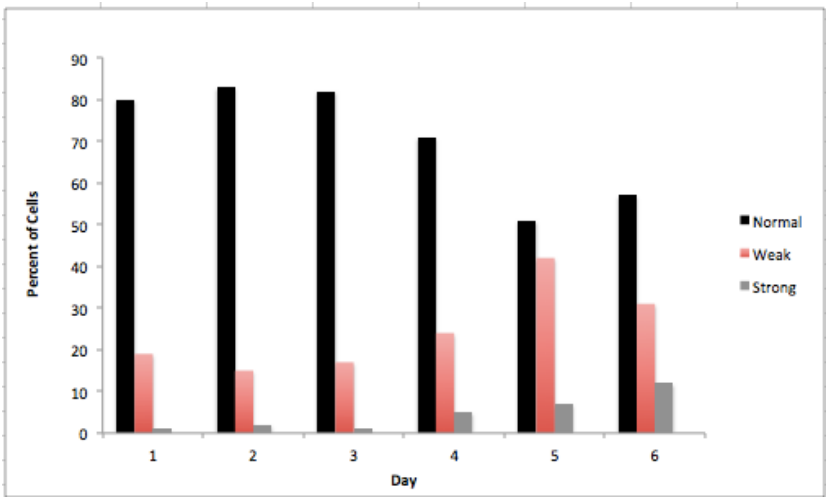

Figure 2: Gumball counts on Cap-H2 expressing cells over a six day time course

We conducted viability counts on Cap-H2 expressing cells and wild type S2 cells to determine the effect that chromosome territories had on cell health. We found that Cap-H2 expressing cells had noticeably lower cell viability than wild type S2 cells. Cap-H2 cells had an average of 1,265,833 viable cells while S2 cells had an average of 6,382,500 viable cells (Fig 3).

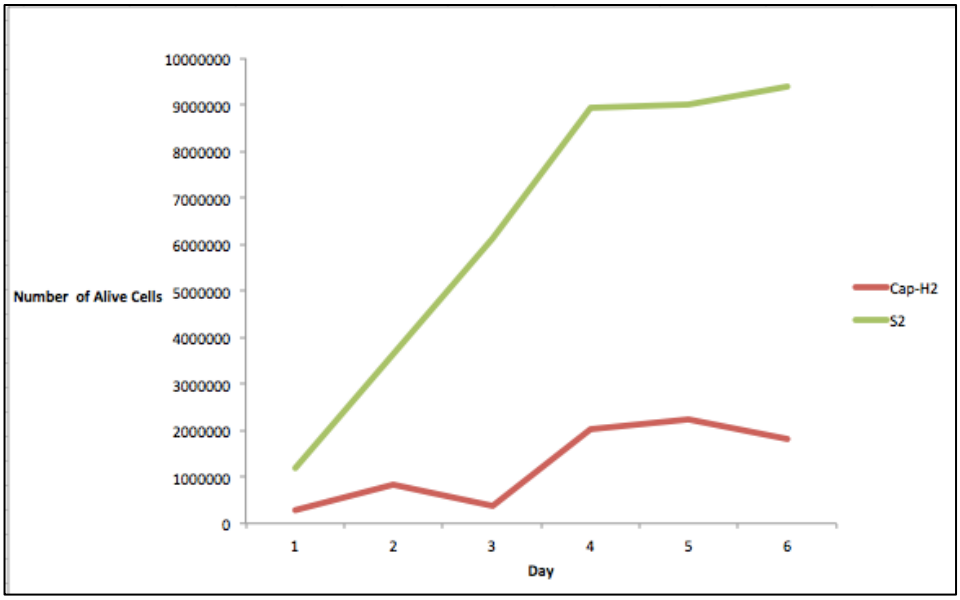

Figure 3: Number of viable Cap-H2 and S2 cells over a six day time course

Discussion:

We found that gumball formation increased over a six day period. Since gumball formation was present over the course of six days in Cap-H2 expressing cells, we conclude that Cap-H2 induction in stable S2 cells causes a chromosome territory phenotype because gumballs are a type of CT. This agrees with the previous literature that states that Cap-H2 overexpression stimulates CT formation. Since weak gumball formation increased from days one through five and strong gumball formation increased throughout the six days, we conclude that almost a week of Cap-H2 induction is required

for most stable cells to form CTs. One possible explanation for why weak gumball formation peaked on day five could be that on day six the strong gumball phenotype took over and more cells displayed the strong phenotype. Therefore, there was a decreased percentage of cells with the weak phenotype. The percentage of cells with strong gumball formation peaked on day six at 12%. Because this is not a very high percentage, we are repeating the experiment with stable line S2 cells expressing nondegradable Cap-H2. By using nondegradable Cap-H2, we expect to see faster genomic reorganization and an increased number of cells expressing strong gumball phenotypes throughout the six days.

We also found that Cap-H2 expressing cells have a reduced cell viability compared to wild type S2 cells. A lower cell viability count correlates with a greater amount of cell death. We conclude that Cap-H2 expressing cells have a reduced cell health compared to S2 cells because there was a greater amount of cell death seen in the Cap-H2 cells. This finding could possibly mean that CTs are harmful to the cell.

The conclusions from this experiment are significant because they give us insights into what role CTs might play in the cell. New insights into the role of CTs are important because they can give us new information about the function of CTs, which is currently unknown. We found that Cap-H2 induction takes six days to cause a CT phenotype. This time frame gives us insight into what gene regulation pathways might be required to stimulate CT formation. Because CTs cause a reduced cell health, this gives us insight into possible cellular mechanisms that CTs might be influencing. Lower cell viability from CT expressing cells also provides evidence for CTs playing roles in some diseases where disorganization of the genome is seen such as muscular dystrophy and progeria syndrome.

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