Identifying proteins required for chromatin organization using a GFP protein-trap screen in *Drosophila melanogaster*

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author

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abstract

The correct compaction and organization of DNA into chromatin is imperative for maintaining proper gene expression within eukaryotic cells. This organization has been defined at the more basic levels, such as the arrangement of nucleosomes but the higher-order structures needed for arranging the chromatin fiber within the nucleus remain unknown. Although some proteins have been characterized that are required for ordering the chromatin fiber within the nucleus, it is hypothesized that there are still other proteins involved that have yet to be defined. To identify these proteins, a Green Fluorescent Protein (GFP) protein-trap screen using Drosophila melanogaster was employed. Flies obtained from a GFP protein-trap database known as Flytrap were dissected and the location of the GFP was classified using fluorescent microscopy. Fly lines that express GFP in distinct nuclear patterns that could relate to chromatin organization were kept for further analysis. From the screen, several interesting lines were found including CA06844. Further imaging was done on CA06844 in order to showcase the details of the arrangement of GFP within the nucleus by employing methods such as imaginal tissue antibody staining. Continued characterization of this line and its affected proteins can help to better define the mechanisms used to organize chromatin within the nucleus.

faculty mentor:

Dr. Victor Corces is the chairman of the biology department at Emory University, and is a Howard Hughes Medical Institute Professor, and an Arts and Sciences Distinguished Professor as well. He earned his B.S. and Ph.D. in Madrid, Spain and later did his postdoctorate at Harvard. His current focus of research concentrates on epigenetic mechanisms that affect the expression of genes.

key terms

Chromatin - the structure that DNA is compacted into with the use of various proteins
GFP - a protein that fluoresces green when exposed to blue light
Protein-trap screen - a method in which the GFP gene sequence is randomly inserted into the DNA of Drosophia, tagging random proteins. These lines are then screened for nuclear GFP

Introduction

Proper gene expression is essential for eukaryotic cell function. Misexpression of certain classes of genes can have drastic consequences for the cell. For example, incorrect regulation of cyclin genes can lead to inappropriate progression through the cell cycle. Such progression can cause prolific cell division allowing cells to bypass cellular checkpoints and divide uncontrollably. Gene expression can be affected by a number of factors such as chromatin organization. When the chromatin organization is disrupted in the nucleus, this distorts the ability of DNA regulatory elements and transcriptional machinery from interacting with target genes ultimately affecting their expression.

The correct organization of chromatin depends on a variety of proteins. Some of these proteins, such as histones, have been identified and characterized, but others remain unknown. Many proteins that help with the organization tend to bind to the DNA in order to open up and release sections for transcription while others help maintain the heterochromatin. When compacting the chromatin fiber, it is hypothesized that there are proteins used to organize the genes into related clusters. These clusters would allow coordinated regulation of genes that are related in function. One class of proteins that may be affiliated with this clustering is insulator proteins. It is believed that insulator proteins form complexes within the nucleus that cause portions of the chromatin fiber to be distinguished from other highly condensed regions allowing the clustered genes encoded within this chromatin to be available for transcription (Byrd 2003). Within these complexes, it is hypothesized that other proteins are needed in order to aid the insulator proteins. The purpose of this work is to identify these additional proteins as well as other proteins that may regulate chromatin fiber organization.

To address this question, a protein-trap screen was employed in order to identify novel proteins involved in chromatin organization based on their unique patterning within the nucleus. Fly lines were created by inserting GFP sequences randomly into the genome by using a P-element transposon. These insertions were designed such that when the GFP DNA is incorporated into the intron of a given gene it will create a false exon within that gene allowing GFP to be part of the final translated product. All lines analyzed were obtained through the Flytrap database. In the past, lines have been analyzed by other labs, and protein expression was minimally affected by the presence of GFP within its sequence (Morin 2001).

One of the lines that were identified was CA06844, which showed a spotted nuclear pattern within diploids and had specific stripes of concentrated protein within proteins. The gene associated with CA06844 was identified as CG11138 (Kelso et al. 2006), which has an amino acid sequence with homology to the Interferon Regulatory Factor 2 Binding Protein 1 (Childs and Goodbourn 2003), which affects transcription within humans. This gene is uncharacterized, and consequently, we are trying to identify the protein product produced and specify its role in chromatin organization.

Materials and methods

Screening methods were used in order to assess the autofluorescence of the GFP. third instar larvae were dissected to obtain imaginal discs and salivary glands. The DNA was stained by DAPI to show the location of the nucleus. A fluorescent microscope was then used to visualize the DAPI and the autofluorescence of the GFP. In order to obtain more detailed information about the protein patterns, antibody staining methods were used. Third instar larvae were dissected in order to obtain imaginal discs and fixed. The sample was then blocked and incubated with two primary antibodies—one that binds to GFP and another that binds to Lamin Dm0, a protein in the inner nuclear membrane of the cell. The sample was washed then a secondary antibody was added—the antibody that bound to the GFP primary antibody fluoresced green while the antibody that bound to the Lamin Dm0 primary antibody fluoresced red. DAPI was then applied to the sample, causing the DNA to fluoresce blue. The sample was washed to remove all the unbound antibodies. The sample was then visualized using a fluorescent microscope.

Results

Screening:

Nuclear	Not Nuclear
CA06922	CA06888
CA07000	CA06890
CA06997	CA06937
CA06844	CA06983
CA06588	CA07005
CA06821	CA07020
	CA07017
	CA07427
	CA07397
	CA07336
	CA06811

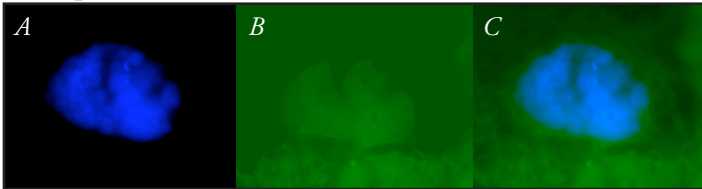| Nuclear	Not Nuclear
	CA06836
	CA06850
	CA06882
	CA06886
	CA06887

Examples:

Figure 1. Screening of CA06588
The dissected salivary glands were stained with DAPI and viewed under the fluorescent microscope. (A) is the DAPI fluorescence, (B) is the GFP fluorescence, and (C) is both (A) and (B) combined.
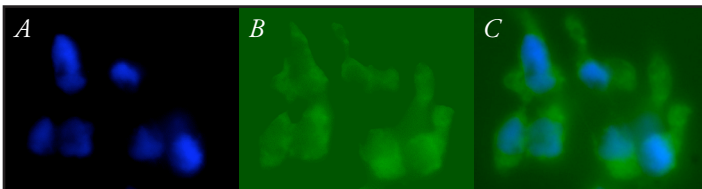

Figure 2. Screening of CA06588
The dissected discs were stained with DAPI and viewed under the fluorescent microscope. (A) is the DAPI fluorescence, (B) is the GFP fluorescence, and (C) is both (A) and (B) combined.
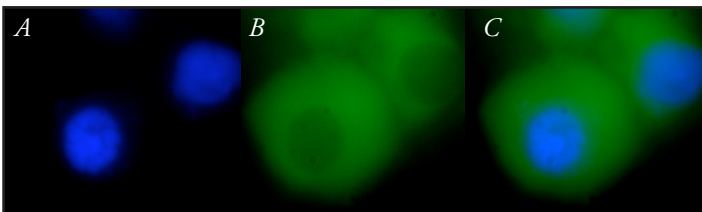

Figure 3. Screening of CA06887
The dissected salivary glands were stained with DAPI and viewed under the fluorescent microscope. (A) is the DAPI fluorescence, (B) is the GFP fluorescence, and (C) is both (A) and (B) combined.

Figure 4. Screening of CA06887
The dissected discs were stained with DAPI and viewed under the fluorescenet microscope. (A) is the DAPI fluorescence, (B) is the GFP fluorescence, and (C) is both (A) and (B) combined
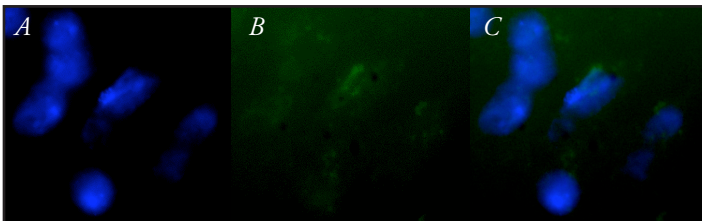
In figures 1-4, the GFP within CA06588 is classified as nuclear in the polytene (Figure 1) but shows no significant patterning. However, in the diploid cells (Figure 2), the GFP looks concentrated around the nucleus as opposed to within the nucleus. The GFP within CA06887 is not nuclear (Figure 3 and 4) and there is actually less GFP in the nucleus than the surrounding cell in the polytene. For the diploid, the GFP is extremely generalized and not found concentrated within the nucleus. Therefore, it is not likely that either protein tagged aids with chromatin organization.
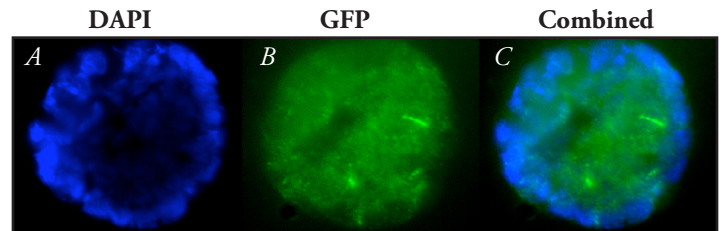
CA06844:

Screening:

Figure 5. Screening of CA06844
The dissected salivary glands were stained with DAPI and viewed under the fluorescent microscope. (A) is the DAPI fluorescence, (B) is the GFP fluorescence, and (C) is both (A) and (B) combined.

The GFP was found in the nucleus of the polyploid cells in a distinct pattern, that may bind directly to the DNA. In addition, there was a punctate pattern observed in the diploid cells. (Data not shown) This combined expression pattern merited CA06844 for further investigation.
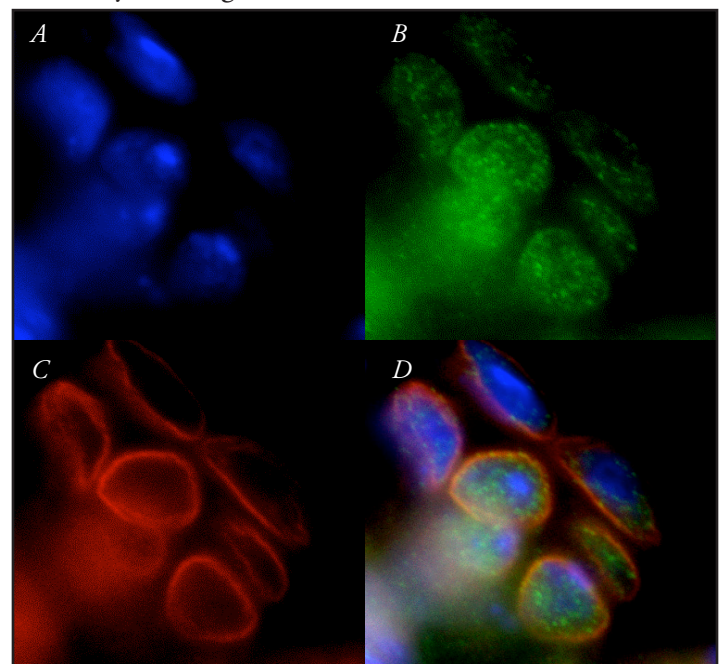
Antibody staining:

Figure 6. Antibody staining of CA06844
The CA06844 discs were stained with rabbit anti-GFP and mouse anti-Lamin Dm0 primaries as well as anti-mouse Alexa Fluor (AF) 546, anti-rabbit AF 488 secondaries and DAPI. (A) is a picture of the DAPI staining of CA06844, while (B) is the antibody tagged GFP. (C) portrays Lamin Dm0 which is an inner nuclear membrane protein, and finally, (D) is the combined picture.

The Lamin Dm0 (Figure 6C) clearly differentiates the nucleus from the cell, which helped in identifying the location of the GFP. The GFP was found in a spotted pattern within the nucleus and displayed co-localization with the inner nuclear membrane.

Conclusions

Most lines screened were found to be not nuclear; however, a select few were found to merit further study. The GFP in the CA06588 polytene was nuclear but it seemed generalized. The overall green in the picture could have been due to overlapping cells on the slide or perhaps the GFP was present in the cell body. With the CA06588 diploid, it was obviously not nuclear but was associated with the cytoplasmic side of the nucleus. Therefore, it is hypothesized that the protein created could interact with the nuclear membrane and the cellular matrix, or even the endoplasmic reticulum. Another cell line found to be interesting while screening was CA06844. The GFP looked to be in a somewhat organized pattern, with some distinct bands. The bands signify that the protein may bind directly to the DNA. Further studies of antibody staining revealed a spotted pattern clearly within the nucleus. This reveals that the protein may be present in clumps, which then implies that the protein could cluster on the DNA in order to regulate chromatin organization.

Further studies

In the future, we plan to continue studies on the lines with nuclear GFP by using a polytene spread staining method so we can clearly see the expression of GFP (and therefore the location of the protein). Employing spread out polytene chromosomes allows us to focus on the DNA and using antibody staining aids in the clear expression of GFP. The GFP may be closely interacting with the DNA and comparing the GFP expression to that of the DAPI stained DNA may help identify whether the tagged protein binds primarily to heterochromatin or euchromatin. In addition, mutants of CG11138 have been found, and phenotypes of these mutants will be examined in order to help identify the effect of the protein. This may advance the connection between the DNA and this unknown protein as well as further our knowledge of how DNA is manipulated in order to maintain correct gene expression.

In addition to furthering research on this and other such selected lines, we hope to continue screening new Flytrap lines to find and analyze more proteins that affect chromatin organization.

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