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# The Mapping and Characterization of *Cruella* (*Cru*), a Novel Allele of *Capping Protein α* (*Cpa*), Identified from a Conditional Screen for Negative Regulators of Cell Growth and Cell Division

**Ashley Cosenza[1,2], Jacob D. Kagey[1*]**

[1]Biology Department, University of Detroit Mercy, Detroit, MI, USA
[2]Honors Program, University of Detroit Mercy, Detroit, MI, USA
Email: *kageyja@udmercy.edu

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## Abstract

A Flp/FRT EMS mutagenesis screen was conducted in the eye of *Drosophila melanogaster* on chromosome 2R to identify negative regulators of cell growth and cell division. In addition to the EMS mutation in the mosaic eye, an *ark* loss of function allele ($ark^{82}$) was utilized to block apoptosis in the homozygous mutant cells, setting up a screen for conditional regulators of cell growth and cell division. In the present study, we focus on the characterization and mapping of one mutant that resulted from this screen, *Cruella* (*cru*). A cross between flies with the flippase enzyme directed to the developing eye and flies with the mutations *cru,* $ark^{82}$, revealed an unusual phenotype that resulted in the homozygous mutant tissue appearing black, in contrast to the expected red. To map the location of this mutation, complementation tests against the Bloomington deficiency kit were conducted. *Cru* failed to complement previously characterized alleles of *capping protein α* (*cpa*). Thus, $cpa^{cru}$ is a novel allele of *cpa* and displays phenotypes similar to previously characterized alleles such as *cpa* 107*E,* *cpa* 69*E,* and $cpa^{scrd}$. The human homolog, *Cap Z,* is conserved in humans and serves a similar role in act in filament regulation.

## Keywords

*Capping Protein α*, Apoptosis, Genetic Screen, *Drosophila melanogaster*

## 1. Introduction

The use of genetic screens in *Drosophila melanogaster* has been a powerful tool for

identifying genes associated with a variety of different biological phenomena, including cell growth and cell cycle regulation [1]. As a result of evolutionary conservation, information gleaned from the study of *Drosophila* can often be applied to improve both our understanding of the genetic causes of human diseases and our approaches to combating such diseases [1] [2]. One such gene example is *hippo* (*hpo*), a gene involved in regulation of apoptosis and cell division in *Drosophila*, which has two human homologs, *Mst* 1 and *Mst* 2. Mutations in *hpo* have been shown to inhibit apoptosis and promote cell division; tissue overgrowth from *hpo* mutations in *Drosophila* has been found to be conserved in humans, with *Mst* 1/2 mutations being linked to human cancer [3]. In addition to *hpo*, additional other genes first identified in *Drosophila* have been found to play a role in human cancer development [1] [3]-[6].

To study mutations that otherwise would be homozygous lethal to the entire organism, the Flp/FRT system is utilized to restrict the homozygous mutant tissue to unessential organs or tissues, thus allowing the survival of the heterozygous organism into adulthood [7]. The expression of flippase (Flp) is under regulation of the eyeless promoter (*Ey>Flp*), which restricts the expression of Flp and results in a restriction of mitotic recombination in developing eye tissue. The FRT site used here is located on chromosome 2R and when Flp is expressed, recombination is facilitated between the FRT sites of the homologous chromosomes. Using ethyl methanesulfonate (EMS) to induce mutagenesis along with the Flp/FRT system directed to the *Drosophila* eye allows for a screen in which the ratio of homozygous mutant tissue to homozygous wild type tissue can be analyzed. Previous Flp/FRT screens of this design relied on a single EMS mutation to look for regulators of cellular pathways involved in governing tissue size, cell division, cell growth, and apoptosis. These screens resulted in the discovery of a variety of genes involved in different developmental and cellular processes [3] [5] [6] [8]-[10]. In the present study, we utilized a "two-hit" Flp/FRT screen on chromosome 2R. We began the screen with the ;*FRT*42*D*, $ark^{82}$ chromosome, which would block the canonical apoptotic pathway in homozygous mutant tissue, and then induced the mutations with EMS [11]. This screen, along with several others, has identified a conditional class of regulators of cell growth and cell division that are dependent on a block in apoptosis for phenotypic changes [11]-[13]. One of the mutants identified from this $ark^{82}$ based screen was named *Cruella* (*Cru*), as a result of the abnormal black coloration of homozygous mutant tissue. The mutant *Cru* is the focus of the present study. It maps genetically to the gene *capping protein α* (*cpa*) and represents a novel allele of this gene, $cpa^{cru}$.

## 2. Methods

### 2.1. Phenotypic Characterization of Cruella through Mosaic Eye and Wing Crosses

The experiments performed stem from a conditional EMS mutagenesis screen of chromosome 2R for abnormalities in growth and development [11]. To study these mutations, the *Ey* > *Flp*/*FRT* system was employed. For the screen, male ;*FRT*42*D*, $ark^{82}$ flies

were fed EMS. Then, F1 mutants were mated to *Ey* > *Flp* ;*FRT*42*D* virgin females, and F2 progeny were screened for eyes that depicted an increase in tissue size or a change in the ratio of mutant to wild type tissue. It should be noted this in this screen, the mutant tissue is pigmented due to the *mw* + gene associated with the $ark^{82}$ allele [8].

The *Cru,* $ark^{82}$ mutant eye was characterized by imaging F1 progeny from the cross of virgin females *Ey* > *Flp* ;*FRT*42*D* mated to males ;*FRT*42*D, Cru,* $ark^{82}$. Similarly, mosaic clones were created in the wing by mating male *Cru*, $ark^{82}$ mutants to virgin females *UBX* > *Flp* ;*FRT*42*D.* To analyze the phenotype of an eye entirely comprised of *Cru,* $ark^{82}$ homozygous tissue, mutant males were mated to virgin females with a cell lethal mutation associated with the FRT chromosome *Ey* > *Flp* ;*FRT*42*D, M*(2)/*CyO.* To determine whether a block in apoptosis was necessary for the *Cru* phenotype, the wild type *ark* allele was recombined onto the ;*FRT*42*D, Cru* chromosome. This recombinant male was then mated to virgin females of the *Ey* > *Flp* ;*FRT*42*D, ubi GFP*. In this final experiment, the wild type tissue was pigmented with the *mw*+ gene, and the ;*FRT*42*D, Cru* chromosome was unpigmented. All mosaic eyes were imaged under ethanol, and mosaic wings were imaged under vegetable oil. Images were taken with Am Scope 5.0 MP digital microscope camera.

### 2.2. Genetic Mapping

To determine the locations of the mutant phenotype in *Cru*, we performed a series of complementation tests using Bloomington Stock Center Deficiency Kits for chromosome 2R. Crosses were set up between male ;*FRT*42*D*, *Cru*, $ark^{82}$/*CyO* and virgin female deficiency stocks: *Df*(*R*)/*CyO* to test for complementation. The F1 progeny were scored for complementation for each cross, and a region of non-complementation was identified. Complementation was determined by the presence of both curly and straight wing flies in the F1 generation. Non-complementation was identified when all F1 progeny had curly wings. Overlapping deficiencies were used to further narrow the region of chromosome 2R from the original failure to compliment genomic region. Finally, mutant alleles of candidate genes were mated to the *Cru* mutant to identify which gene the mutation is located. In particular, *Cru* was mated to the alleles *cpa* 107*E* and *cpa* 69*E* of the gene *cpa* [14].

## 3. Results

### 3.1. Phenotypic Characterization of Cruella

We initially identified *Cru* from the conditional screen due to the striking nature of the homozygous mutant tissue. As shown in **Figure 1(B)**, ;*FRT*42*D*, *Cru*, $ark^{82}$ resulted in a mosaic eye phenotype in which mutant tissue appeared black, as opposed to the red coloration typically seen in homozygous mutant tissue of mosaic eyes, compare tissue pigmentation of **Figure 1(B)** to that of **Figure 1(A)**. Despite the change in pigmentation, the size of the eye and the relative ratio of mutant to wild type tissue appeared similar to the control ;*FRT*42*D*, $ark^{82}$ mosaic (**Figure 1(A)**).

The entire eye was made homozygous by flipping the ;*FRT*42*D*, *Cru*, $ark^{82}$ chromo-

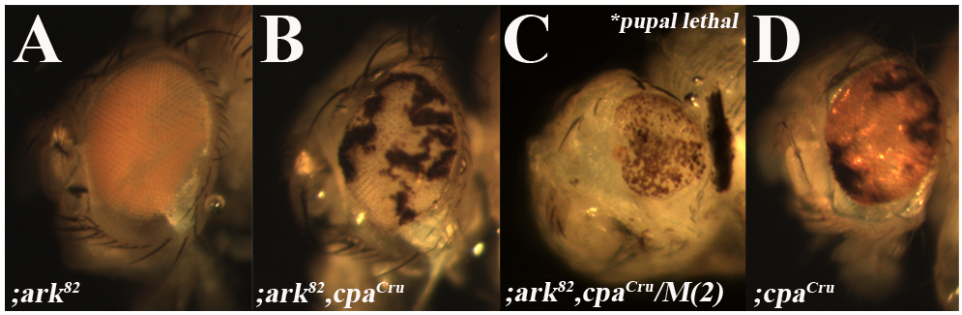


**Figure 1.** *Cru* mosaic eye depicts a homozygous mutant tissue that is black in nature. A) control mosaic ;*FRT*42*D*, $ark^{82}$ B) *FRT*42*D*, $ark^{82}$, $cpa^{cru}$, mutant tissue is pigmented C) *FRT*42*D*, $ark^{82}$, $cpa^{cru}$/*FRT*42*D*, *M* (2), mutant tissue is pigmented D) *FRT*42*D*, $cpa^{cru}$ wild type tissue is pigmented. Eyes were imaged at 40 X magnification under 70% ethanol.

some over a cell lethal mutation on 2R (;*FRT*42*D, M* (2)). The result of this experiment was a complete pupal lethality, suggesting that Cru plays a necessary role in head and eye development. The dissected pharate pupae from this cross have underdeveloped eyes, and the eyes are almost entirely comprised of darkly pigmented tissue in a similar manner to the mosaic eye (**Figure 1(C)**).

To determine whether the *Cru* phenotype was dependent on a blockage of apoptosis and to demonstrate that the alteration in pigmentation is not related to the $ark^{82}$ allele, we recombined a wild type *ark* allele onto the ;*FRT*42*D*, *Cru* chromosome. As can be seen in **Figure 1(D)**, the mutant tissue (note that the wild type tissue now has the *mw*+ gene) is still black in appearance, suggesting that the $ark^{82}$ allele is not associated with the phenotype that alters the appearance of the pigment. Without blocked apoptosis, however, the relative amount of mutant tissue is decreased, suggesting that *Cru* homozygous mutant tissue may result in apoptosis, compare ratio of black pigmented tissue in **Figure 1(B)** to **Figure 1(D)**.

To determine whether the *Cru* phenotype was exclusive to the eye or was more ubiquitous in the body, we created mosaic clones in the developing wing disc through the use of the *UBX* driver. Several notable phenotypic changes were observed in the *Cru*, $ark^{82}$ mosaic wing including disruption in wing vein patterning compare **Figure 2(B)** to **Figure 2(A)**. In addition to the alterations in wing vein patterning, the adult flies demonstrated a "wings held out" phenotype (**Figure 2(C)**) a commonly seen characteristic of alterations in wing development [15].

### 3.2. Genetic Mapping of Cruella

To map the location of the *Cru* mutation, crosses were setup between males ;*FRT*42*D*, *Cru*, $ark^{82}$ and virgin females of the deficiency stocks representing the overlapping deficiency stocks from the Bloomington Deficiency Kit for chromosome 2R. Complementation mapping identified that the lethal mutation of *Cru* resides on 2R between bases 21,056,798 and 21,088,247. A previously characterized gene in this region, capping protein $\alpha$ (*cpa*), was selected as a candidate. Matings between ;*Cru*, $ark^{82}$ and two previously identified *cpa* alleles, *cpa* 107*E* and *cpa* 69*E*, resulted in a failure to complement

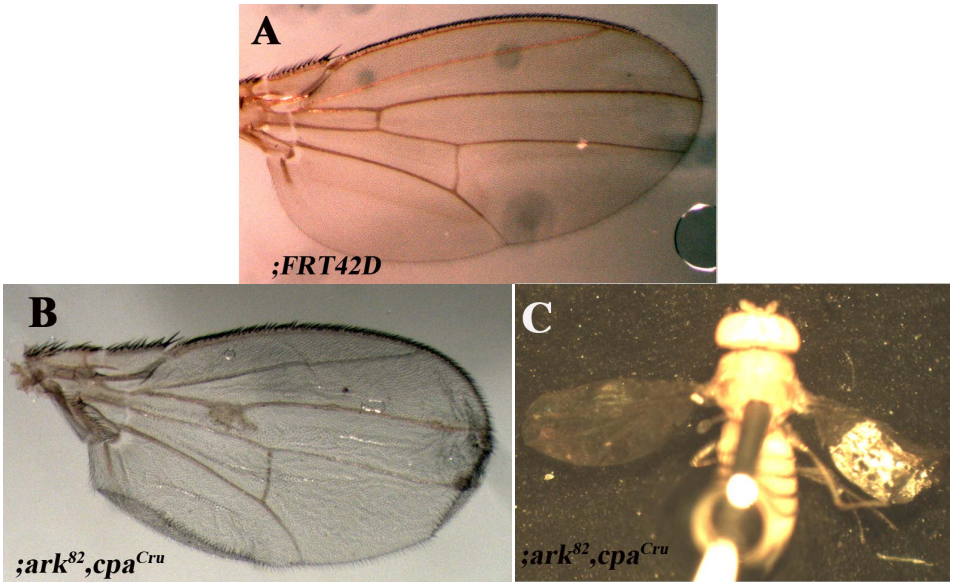


**Figure 2.** *Cru* mosaic wings result in vein alterations and a "wings held out" phenotype in adult *drosophila.* A) control mosaic adult wing, B) *FRT*42*D*, $ark^{82}$, $cpa^{cru}$ mosaic wing, C) *FRT*42*D*, $ark^{82}$, $cpa^{cru}$ adult with mosaic wings. All animals and wings are imaged from female flies. Wings were imaged at 70 X magnification under vegetable oil.

with *Cru,* suggesting that *Cru* is a novel allele of *cpa* [14]. Therefore, *Cru* will be referred to as $cpa^{cru}$ from this point forward.

## 4. Discussion

In the present study, we present the mapping and characterization of a novel allele of *cpa*, $cpa^{cru}$. The *cpa* protein functions as the $\alpha$ subunit of an actin capping protein. An $\alpha/\beta$ heterodimer is necessary for proper functionality. Previous evidence has shown that both the $\alpha$ and $\beta$ subunits are necessary for a functional CP and that both subunits play a role in the regulation of one another [16]. A mutation in either subunit can therefore lead to an inability to perform F-actin capping, resulting in an accumulation of actin [16] [17]. The phenotype resulting from $cpa^{cru}$ homozygous tissue causes a loss of F-actin capping ability in the eye tissue of *Drosophila*, resulting in improper actin polymerization. Such accumulation of actin filaments has led to what appears to be the discoloration of customarily red mutant eye tissue, causing it to appear black in *Drosophila* with $cpa^{cru}$ alleles. Our finding is in agreement with the results of a study by Delalle *et al.*, in which a similar alteration in pigmentation was observed [9]. Although the mosaic eye of the ;$cpa^{cru}$, $ark^{82}$ has a similar ratio of mutant to wild type tissue as the ;$ark^{82}$ mosaic eye, there is an essential role for *cpa* in eye development. When the ;$cpa^{cru}$, $ark^{82}$ mosaic eyes were flipped over a cell lethal mutation, the result was pupal lethality, suggesting a necessary role of *cpa* in eye development. Previous *D. melanogaster* studies in retinal *CP* mutants have suggested that F-actin accumulating resulted in alteration of the regulation of actin-binding activities throughout the cell. This, in turn, could inhibit signaling events due to its effects on cell movement and polarity [18]. It is likely that

the ;$cpa^{cru}$ mutant heads are defective in developmental signaling. Future experimentation could look into the necessary role that *cpa* plays in regulating eye development in *Drosophila*.

In addition to phenotypic alterations in the mosaic eye ;$cpa^{cru}$, $ark^{82}$ also demonstrated mosaic wing phenotypes. The accumulation of F-actin, resulting from the $cpa^{cru}$ cells, led to the flies' inability to form proper wing veins. Other studies have also observed wing alterations in *cpa* and *cpb* mutant cells in both wing imaginal discs and adult wings [9] [14]. Furthermore, *cpa* or *cpb* mutations have also been found to lead to alterations in bristles, in which transheterozygous mutants displayed significantly shorter bristles [17] [19]. This, in turn, caused disorganization and abnormal bundling of the actin cytoskeleton, resulting in the abnormal bristle phenotype shown in these studies [16] [17] [19].

Many of the genes involved in the cytoskeletal pathways are evolutionarily conserved from *Drosophila* to humans. The relationship between *cpa* and *cpb* is well conserved in humans through the gene *Cap Z*, a subunit of the alpha-beta heterodimer, which functions in F-actin regulation and polymerization [20] [21]. Because of the high levels of conservation between *cpa* and its human homolog *Cap Z*, the results of the present study have various implications for study of human diseases and disorders such as cell growth and nuerodegeneration [9]. *Cap Z* also plays a gatekeeper role in inhibiting Yap/Taz oncogenes in human cells, similarly to the way *cpa* and *cpb* inhibit Yki [9] [18] [22]. Mutations in *Cap Z* subsequently up-regulate Yap/Taz activity, which has been associated with human cancer development [4]. This type of evolutionary conservation in basic protein function, as well as in the associated cell signaling, makes *Drosophila* a powerful model for an array of human systems.

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