GENETIC ANALYSES OF THE IN VIVO FUNCTION AND REGULATION OF THE ONCOPROTEIN CDK8 IN DROSOPHILA

A Senior Scholars Thesis

by

LAUREN M BRIDGES & GEE YOON PARK

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

May 2012

Majors: Biology
Biomedical Sciences

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Approved by:

Research Advisor: Charles Oakes
Associate Director, Honors and Undergraduate Research : Duncan MacKenzie

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ABSTRACT

Genetic Analyses of the in Vivo Functions of the Oncoprotein CDK8 in Drosophila. (May 2012)

Lauren M Bridges and Gee Yoon Park
Department of Molecular and cellular Biology
Texas A&M University

Research Advisor: Dr. Jun-yuan Ji
Department of Molecular and Cellular Biology

Cyclin-Dependent Kinase 8 (CDK8) has recently emerged as an oncoprotein that is amplified in both colorectal and melanoma cancers. The importance of CDK8 and its regulatory partner Cyclin C (CycC) is highlighted by the clinical observations showing that CDK8 and CycC are frequently amplified, mutated, or deleted in a variety of human cancers[4]. Inhibiting CDK8 potently blocks the growth of colorectal cancer cells that harbor CDK8 amplification, suggesting that CDK8 is a promising drug target. Accordingly, several pharmaceutical companies are tremendously interested in developing CDK8-specific inhibitors[5]. However, for such drugs to be successful, it is essential to understand the regulatory network of CDK8-CycC including both the upstream regulators and the downstream effectors in normal development and tumorigenesis. To date, little is known about how CDK8-CycC activity is regulated. In addition, the downstream targets and the biological functions of CDK8 in metazoans remain poorly understood.

Importantly, both CDK8 and CycC are highly conserved in all eukaryotes. Thus we use Drosophila as an experimental system to identify both the upstream regulators and downstream effectors of CDK8. We expect to elucidate the fundamental roles of CDK8-CycC and thereby gain insight of how their deregulations contribute to various cancers. Because cdk8 or cycC homozygous mutants are lethal in the pupal stage, we set out to vary CDK8 and CycC in a tissue-specific manner by using the Gal4-UAS system. Importantly, certain phenotypes can be modified by second-site mutations, which provide us with a unique opportunity to genetically dissect the functions and regulation of CDK8-CycC in vivo.

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The authors would like to thank Dr. Jun-yuan Ji, Assistant Professor of Molecular and Cellular Medicine at the Texas A&M College of Medicine, for his assistance during this project and many other projects and for instilling in us a true enjoyment and appreciation for medical research and the biological life sciences.

NOMENCLATURE

CDK8	Cyclin-dependent kinase 8
CycC	Cyclin C
UAS	Upstream activating sequence
RNAi	RNA interference

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CHAPTER I

INTRODUCTION

Cyclin-Dependent Kinase 8 (CDK8) is a newly identified oncoprotein that is amplified in both colorectal and melanoma cancers (1-3). The importance of CDK8 and its regulatory partner Cyclin C (CycC) is highlighted by the clinical observations showing that CDK8 and CycC are frequently amplified, mutated, or deleted in a variety of human cancers (4). Inhibiting CDK8 potently blocks the growth of colorectal cancer cells that harbor CDK8 amplification, suggesting that CDK8 is a promising drug target. Accordingly, several pharmaceutical companies are developing and testing CDK8-specific inhibitors (5). However, for such drugs to be successful, it is essential to understand the regulatory network of CDK8-CycC including the upstream regulators and the downstream effectors in both normal development and tumorigenesis. To date, little is known about how CDK8-CycC activity is regulated. In addition, the downstream targets and the biological functions of CDK8 in metazoans remain poorly understood.

This thesis follows the style of *Nature*.

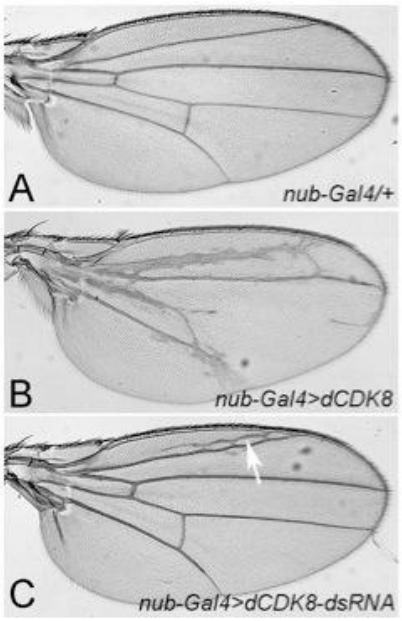

Figure 1 Varying CDK8 in fly wings generates stable and modifiable phenotypes. (A) wild-type wing; (B) over-expression of CDK8 in the wing; and (C) knocking down CDK8.

Drosophila is a well-established model system because it provides a variety of sophisticated and powerful genetic approaches. Compared to mammals, it is much simpler, yet many regulatory mechanisms are conserved at the molecular level. Importantly, both CDK8 and CycC are highly conserved in all eukaryotes. Thus we use Drosophila as an experimental system to identify both the upstream regulators and downstream effectors of CDK8. We expect to elucidate the fundamental roles of CDK8-CycC and thereby gain insight of how their deregulations contribute to various cancers.

Because cdk8 or cycC homozygous mutants are lethal in the pupal stage (6), we set out to vary CDK8 and CycC in a tissue-specific manner. For this, we applied the well-established Gal4-UAS system, which allows us to express the wild-type (or dsRNA) of cdk8/cycC, thereby over-expressing (or knocking down) CDK8/CycC in a tissue-

specific manner. As shown in Fig. 1, when driven by using a wing-specific Gal4 line nubbin-Gal4, the overexpression of cdk8 severely disrupts the vein patterns (Fig. 1B, compared to the control in Fig. 1A). Conversely, knocking down cdk8 leads to an opposite phenotype with ectopic veins in the inter-vein regions (Fig. 1C). Similar phenotypes were observed when we varied CycC levels (data not shown). Such phenotypes are highly stable with nearly 100% penetrance. Importantly, these phenotypes can be modified by second-site mutations (see below), which provide us with a unique opportunity to genetically dissect the functions and regulation of CDK8- CycC in vivo.

CHAPTER II

METHODS

Drosophila was used as an experimental system. We applied the well-established Gal4-UAS system which allowed us to express the wild type (or dsRNA) of cdk8/cycC, thereby overexpressing CDK8/CycC in a tissue-specific manner. The initial screen was based on phenotypes caused by over-expression or knockdown of CDK8. We utilized the new Bloomington Deficiency kit, which provides maximal coverage of the genome (~98% of Drosophila euchromatic genome) with the minimal number of deletions (http://flystocks.bio.indiana.edu/Browse/df/dfkit-info.htm). This deficiency collection is composed of 468 deletions and breakpoints that are molecularly mapped to single-base resolution for most of the deletions. This approach allowed us to efficiently survey through the Drosophila genome for factors that interact with CDK8 by crossing less than 500 crosses and still be able to go through 97.9% of the drosophila genes.

Specifically, we crossed each deficiency line with nub-Gal4, UAS-CDK8 (wild type or RNAi), and then scored the lines that can modify the phenotypes caused by varying CDK8. Figure 2 outlines the scheme for the genetic crosses (using a second chromosome deficiency line as an example) and shows each example of an enhancer and a suppressor. We finished the first round of the screen using the CDK8-RNAi phenotypes

and identified 53 enhancers (16 of them are strong enhancers) and 37 suppressors (6 of them are strong suppressors).

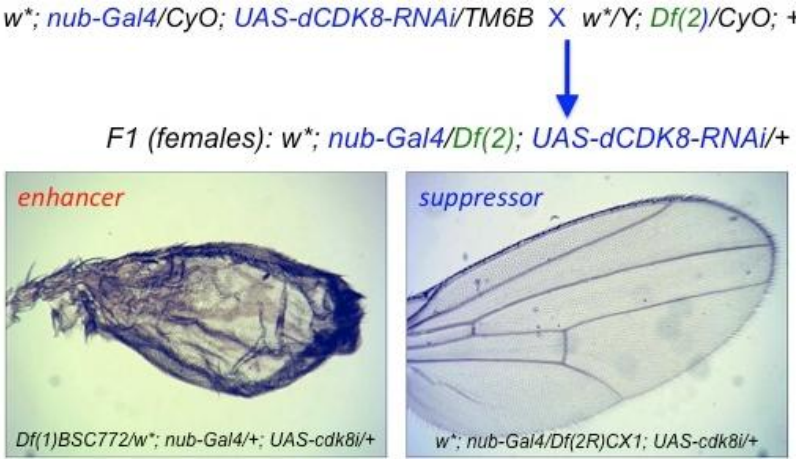

Figure 2 Phenotypes for CDK8 enhancers and suppressors.

We retested these modifier deficiency lines with the wing phenotypes caused by CDK8 overexpression and knocking down of CycC. To narrow down to specific mutant genes, we tested partially overlapping deficiency lines with the dominant modifier deficiency lines. Once we narrowed down to regions with less than ten genes, we tested all the mutants available for those genes and then performed genetic crosses to identify the candidate gene that is responsible for uncovering the deletion line.

Figure 2 exhibits a stereotypical result of a cross with an enhancer and a suppressor. In Figure 1A, we can observe the stereotypical wild-type Drosophila wing vein pattern. When we specifically over-express Cdk8 in the wing, we see loss of vein patterns, as exhibited in Figure 1B. On the other hand, when we deplete Cdk8 in the wing, we observe ectopic, or extra, veins that formed as illustrated in Figure 1C. These

phenotypes are 100% penetrant, allowing us to build stable stocks and screen for mutants that can modify these phenotypes. If a mutant worsens these phenotypes, we call it an enhancer, while if a mutant makes the phenotypes stronger, we call it a suppressor. This kind of genetic analyses allowed us to identify novel factors that regulate Cdk8 in vivo.

Because Cdk8/CycC mutants are lethal in the pupal stage, it was necessary for us to devise a new method of manipulating Cdk8 activities in Drosophila. We utilized the so-called Gal4-UAS system. Gal4 is a yeast transcription factor that specifically binds to the upstream activating sequence (UAS). On one hand, we were able to generate transgenic flies to overexpress wildtype Cdk8 using Gal4 lines that are expressed in tissue-specific manners, thereby resulting in gain of Cdk8 phenotypes in certain tissues. Alternately, we can reduce or deplete Cdk8 by expressing double-stranded RNA through a process known as RNA interference (RNAi), thereby resulting in a loss of Cdk8 phenotypes in certain tissues.

We plan to expand this same approach to the rest of the strong modifiers that we have identified. We expect to identify multiple novel factors that genetically interact with CDK8. We will select modifiers that are involved in transcription regulation and signal transduction for further characterization.

CHAPTER III

RESULTS

We crossed each deficiency line with nub-Gal4, UAS-CDK8 (wild-type or RNAi), and CycC. We then scored the lines that can modify phenotypes caused by varying CDK8. Our first round of the genetic screen identified fifty-three enhancers and thirty-seven suppressors. Sixteen of the enhancers were strong enhancers and six of the suppressors were strong suppressors. After retesting these modifier deficiency lines, we determined the regions that contained less than ten genes. We had four overlapping enhancers and one overlapping suppressor between Cdk8-RNAi and CycC-RNAi as can be seen in Figure 3.

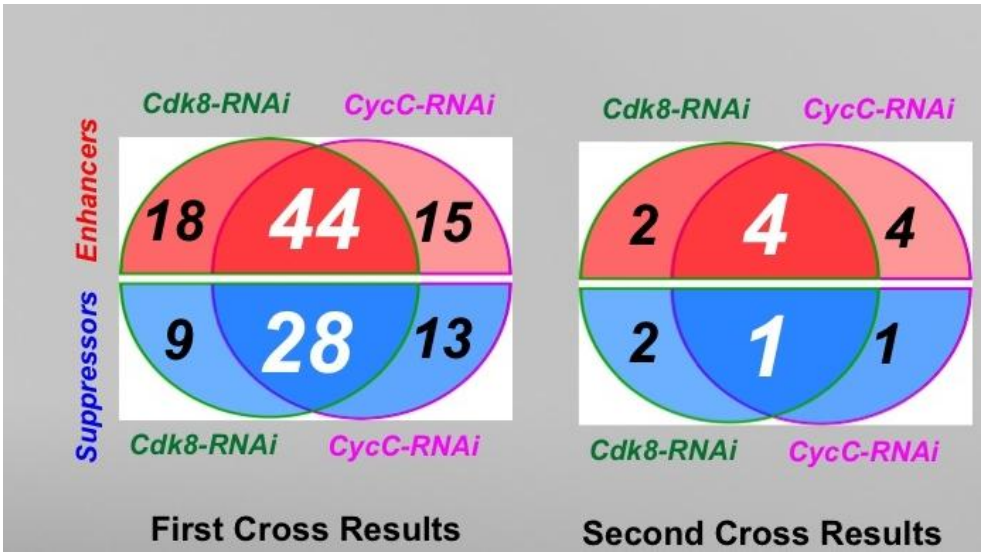

Figure 3 First and second cross results depicting modifiers identified as either enhancing or suppressing Cdk8 expression.

CHAPTER IV

SUMMARY AND CONCLUSIONS

To summarize, we started out with nearly 500 crosses of the entire Drosophila genome and narrowed down to approximately 70 different modifiers after the first round of screening by the help of other researchers in the lab. 44 modifiers for both cdk-8 and cycC enhancers and 28 for suppressors were identified on the first round of crosses. We are currently working our ways down to narrowing we were able to design a genetic screen that enabled us to identify four overlapping enhancers and one overlapping suppressor, thus far on the second round of crosses using a new set of partial deletion lines. We expect to finish the second round within the next month. Once we have identified the modifiers for both Cdk-8 and CycC, we expect to find the specific genes in each of the suppressors and enhancers that feed the phenotype accordingly. Though the function of Cdk8 is currently unknown, by narrowing down to a specific gene region we can determine its function.

In conclusion, it is important to discern the molecular development of Cdk8/CycC in the future. In the long run, this research will open up many new directions to understand the function and regulation of Cdk8/CycC in vivo. A better understanding of the function of Cdk8 will provide us with the knowledge to develop anti-cancer drugs that can target the specific gene region that is involved in tumorigenesis.

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CONTACT INFORMATION

Name: Gee Yoon Park

Professional Address:
Department of Molecular and Cellular Medicine Room
248 Reynolds Medical Building
College Station, Texas 77843

Email Address: suziepark@gmail.com

Education: B.S., Biomedical Sciences, Texas A&M University, May 2013
Cum Laude, Undergraduate Research Scholar

Name: Lauren M. Bridges

Professional Address:
Department of Molecular and Cellular Medicine
Reynolds Medical Building
College Station, Texas 77843-mailstop

Email Address: lbridges1989@gmail.com

Education: B.S., Biology, Texas A&M University, May 2012 President's
Endowed Scholar, Undergraduate Research Scholar