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What Fruit Flies Can Tell Us About Human Birth Defects

by Dale Dorsett, PhD

Fruit flies can provide relatively rapid and inexpensive methods to understand the molecular basis diseases and to develop potential therapies, particularly for rare conditions for which standard methods for drug development are not economically feasible.



Dale Dorsett, PhD, is a Professor in the Edward A. Doisy Biochemistry and Molecular Biology Department at Saint Louis University School of Medicine.
Contact: dorsettd@slu.edu

Abstract

Many times, when a human genetic disease is mapped to mutations in a specific gene, little is known about the biological functions of the affected gene. Development of new therapeutic methods is facilitated by understanding the gene's biological roles. Such information can often be obtained in animal models, such as the fruit fly. Here we describe how understanding a gene's function in fruit flies has illuminated the etiology of Cornelia de Lange syndrome.

Introduction: Cornelia de Lange Syndrome

A few years ago, on a sweltering summer day in a park not far from Saint Louis, I met a 15-year-old boy and his mother. He only came up to his mother's waist. As I gazed down at his shining face, the features characteristic of Cornelia de Lange Syndrome (CdLS), a rare genetic disorder, were obvious even to me, trained as a fruit fly, not a human, dysmorphologist. Talking with his mother, I was surprised to learn that he had actually just been diagnosed only the week before by his aunt, who had read about CdLS online. The boy, with verbal skills that were unusually good for someone with

this syndrome, was quite excited to explain why he was different. Although CdLS can be easily suspected based on highly characteristic facial features, including arched eyebrows, thin lips, and long philtrum, there's more to it. Individuals with this syndrome suffer a raft of more serious problems, including intellectual impairment, autism, slow growth, and limb and multiple organ abnormalities (See Table 1).¹

It is not always recognized initially by physicians or other caretakers. In fact, it is not uncommon, particularly in more rural areas, for CdLS to go undiagnosed, as it was in this 15-year-old. Cornelia de Lange first described this condition, which is also called Brachmann de Lange Syndrome, in the 1930s based on two unrelated pediatric patients she saw in Amsterdam.² Today, a CdLS-likely diagnosis is made relatively easily by a physician who has seen a few cases and recognizes the pattern of abnormalities. Early diagnosis makes a difference. It can lead to substantial improvement in management, as many of the diverse health risks can be anticipated.¹

Feeding is a very common problem. On the summer day I met this patient, I also met many caring family members of other patients, including a grandfather who had used

Table 1
Common features of Cornelia de Lange Syndrome

Feature	Percentage of individuals affected ^a
Development and behavior	
Growth retardation	100
Intellectual disability	mean IQ 53, range <30 to 102
Autism spectrum disorder	65
Speech disorders	+
Self injurious behavior	70
Physical aggression	40
Facies	
Microcephaly	93
Low posterior hairline	92
Bushy eyebrows, synophrys	98
Ocular abnormalities (ptosis, myopia, nystagmus)	57
Long curly eyelashes	99
Depressed, wide nasal bridge	83
Anteverted nares	85
Long philtrum, thin upper, downturned mouth	94
High arched palate	86
Widely spaced teeth, late eruption	86
Micrognathia	84
Skin	
Hirsutism	78
Cutis marmorata	56
Hypoplastic nipples, navel	50
Extremities	
Micromelia	93
Reduction defects, oligodactyly	27
Clinodactyly (5th finger)	74
Simian crease	51
Proximally placed thumbs	72
Elbow flexion contractures	64
Syndactyly (toes 2 and 3)	86
Genitalia	
Hypoplasia	57
Undescended testes	73
Hypospadias	33
Gastrointestinal	
GE reflux	+
Gut duplication, malrotation	+
Volvulus	+
Other	
Hearing loss	60
Structural heart defects	33
Kidney, urinary tract defects	36
Thrombocytopenia	+

^aData from references 21-25. ^b "+" indicates reported in multiple individuals.

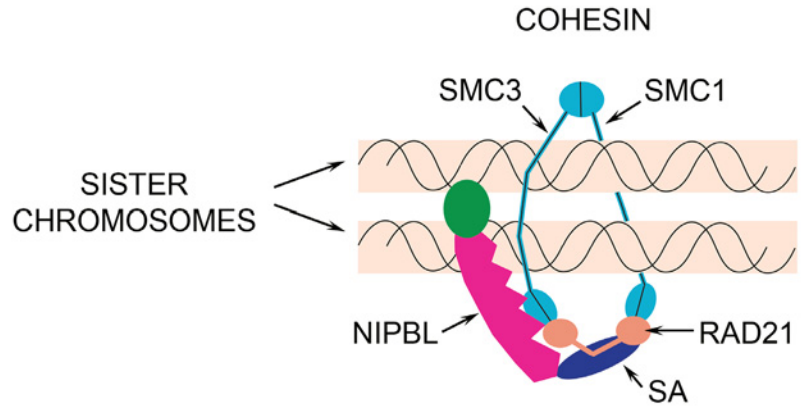
techniques similar to those he used to handle young livestock on his farm to develop a diet that was palatable to his granddaughter. I also met a young mother who refused to accept being told by her pediatrician that poor mothering was responsible for the failure of her beautiful daughter to thrive, and who ferociously sought out other opinions until the correct diagnosis was made.

Six Degrees of Separation: Making the Connection

You might wonder why a fruit fly molecular geneticist was attending a CdLS family picnic? There is a connection! The answer goes back to the mid-1990s, when our laboratory was investigating how genes are turned on and off during the development of a fruit fly called *Drosophila melanogaster*. This insect is a “model organism” that offers powerful genetic and molecular tools. It has long been a tenet of the *Drosophila* research community that for most biological functions, ranging from metabolism and endocrinology to learning and memory, the fruit fly is equivalent to a simple small model of a human, but with a more compact genome and wings. We were looking for genes that control limb development and discovered one that we named Nipped-B, based on the moth-eaten appearance of the mutant wings.³ Nipped-B, it turned out, controls development of several tissues.

Figure 1

The NIPBL and cohesin protein complexes. The cohesin protein ring topologically encircles DNA, thereby holding replicated chromosomes together until cell division, to ensure proper chromosome segregation²⁰. The cohesin complex has four subunits: Smc1, Smc3, Rad21, and SA. Smc1 and Smc3 have long arms, and Rad21 bridges the enzymatic head domains of Smc1 and Smc3. The cohesin ring is opened by NIPBL to snap it around chromosomes, and Rad21 is either removed or destroyed to reopen the ring and allow chromosomes to separate when a cell divides. As described in the article, cohesin and NIPBL bind to many genes on chromosomes and control their activities. Changes in expression of these genes are the underlying cause of the many physical and intellectual deficits in Cornelia de Lange syndrome.



It also soon became apparent, first from genetic studies with the equivalent genes in yeasts, that Nipped-B is also important in ensuring that chromosomes are dealt out equally to the two daughter cells when a cell divides. Nipped-B protein helps a protein ring called cohesin snap like a bracelet around chromosomes and hold sister chromosomes together, so they can be separated and sent in opposite directions at the moment of division (See Figure 1). It actually doesn't take much Nipped-B or cohesin, less than 20% of normal, to ensure proper chromosome segregation. Even a small reduction on the order of 30% or less can dramatically alter growth and development. This is because many other genes that are controlled by Nipped-B and cohesin produce either too little or too much of their products when Nipped-B is reduced.

Making the Human Connection

The connection to human development, which we were sure would happen sooner or later (we could see the same gene in the DNA sequence of the human genome), came earlier than expected. In 2004, I received separate phone calls from Ian Krantz at the Children's Hospital of Philadelphia and Tom Strachan at the University of Newcastle, who had both recently discovered that mutations in the human Nipped-B gene, which they named Nipped-B-Like (NIPBL), caused most cases of CdLS.^{4,5} Both were seeking any insights we might have into how NIPBL might be involved in the diverse deficits associated with CdLS. Since that time, Ian and others have found that mutations affecting portions of the cohesin ring can cause milder forms of CdLS, or a similar syndrome.⁶⁻⁸ Most recently, mutations in a gene encoding an enzyme that chemically modifies the cohesin ring were also found to cause CdLS.⁹

Mapping the first CdLS gene was not for the faint of

heart. The NIPBL mutations that cause CdLS are genetically dominant and it is extremely rare for the disorder to be inherited – virtually all cases are caused by sporadic mutations. As a consequence, the usual method of following family lineages could not be used. The key was that beginning in the 1970's, Laird Jackson of Drexel University, responding to some questions from some concerned CdLS mothers, began organizing CdLS family picnics in the US, and traveling around the world to meet other CdLS families. Laird had the foresight to collect blood samples from the CdLS children, parents, and siblings, so that a large number had been collected by the time the human genome sequence became available. This collection allowed linkage exclusion analysis to narrow things down to five regions that might harbor the critical gene. Coupled with some inspired guesswork, and a balanced *de novo* chromosomal translocation affecting one of the five suspect regions, this analysis led to NIPBL.

Identification of NIPBL led to the first CdLS Scientific Symposium in June 2004, held in Lincolnshire, Illinois, in conjunction of the biennial family meeting organized by the CdLS Foundation that had grown out of the picnics organized by Laird many years before. I was invited to talk about our work with fruit flies, which began my association with the CdLS Foundation, as a member of the Board of Directors from 2004 to 2010, and currently as Chair of their Research Council.

One of the responsibilities that I have enjoyed is meeting CdLS families at occasional family picnics along the I-70 corridor from western Illinois to eastern Kansas. It is gratifying to reassure parents that it is nothing they did wrong that caused their child's condition. They find it a relief to have the genetics and causes of CdLS explained to them, and also to learn that there are dedicated physicians and scientists who care about this rare condition, and are

working hard to improve the methods of diagnosis and treatment. They are excited to learn that members of the Clinical Advisory Board of the CdLS Foundation, who have diverse expertise, can be approached to address their many medical questions. They are also thrilled to learn that teams of scientists are exploring the mechanism of the disease in search for clues to treatments that could improve their children's lives.

So, why do we think there is hope for developing effective treatments for CdLS? The problems with growth and development begin *in utero*, and CdLS has been diagnosed by ultrasound (e.g., reference 10). It is not beyond imagining that in the near future, CdLS, like many other rare disorders, can be diagnosed early in pregnancy with a combination of ultrasound, DNA sequencing, and molecular markers. As a rare, non-inherited genetic disorder, however, it would not become routine to check for CdLS until a safe method that can detect many genetic problems with early development is available.

If *in utero* diagnoses can be made, it would then be feasible to think about very early therapeutic intervention that could ameliorate many of the physical deformities. I also believe that therapeutic intervention that begins within a few weeks of birth could be very helpful. Surgery can be used to correct the occasional severe congenital heart defects, which are a leading cause of early death.¹¹ We can even envision the possibility that we can improve growth and intellectual development.

Mental retardation, poor speech, and autism are major impediments that keep most individuals with CdLS reliant on constant care and from becoming independent. There is good reason to believe that brain development is highly plastic, and that early intervention may greatly improve intellectual development of children with CdLS. For instance, using a mouse model of Rett syndrome, a leading cause of mental retardation in girls, Adrian Bird's laboratory in Edinburgh showed that turning the affected gene back on even in young adults led to substantial improvements in learning, memory, and behavior.¹² Similarly, flies with Nipped-B and cohesin deficits show delays in axon pruning and problems with dendrite branching,¹³ two key processes in neural development, which have the potential to be corrected later in development. That's the long-range hope.

Research 'Flies' By

Where do we start, and what strategies might lead to therapeutic discoveries? First we need to know how NIPBL and cohesin control genes, what genes they control, and when they control them, in order delineate what is possible.

Our laboratory has exploited the fruit fly to gain some of this knowledge. Using techniques that allow us to examine the entire genome, we mapped where the Nipped-B and cohesin proteins bind to chromosomes in multiple types of cells and tissues, and which genes produce too little or too much product when the amount of Nipped-B or cohesin is reduced. These studies have provided important clues.

We discovered that Nipped-B and cohesin bind selectively to hundreds of active genes that control growth and development, and that the products of many of these genes and the genes they control are often altered when there is too little Nipped-B or cohesion.¹⁴⁻¹⁸ Strikingly, one of the key genes that binds Nipped-B and cohesin and whose output decreases when there is too little Nipped-B or cohesion, is *myc*, a key growth promoter in both flies and humans. The *myc* gene is perhaps best known for causing cancer when it is too active, leading to unchecked growth. In *Drosophila*, *myc* is called *diminutive*, because the first mutation, isolated back in the 1930's, only partially reduces gene function, giving rise to tiny flies.¹⁹ It turns out that the zebrafish, mouse, and human *myc* genes are also down-regulated when the *NIPBL* activity is reduced, providing one potential explanation for why CdLS was once known as "Amsterdam dwarfism."

The overriding message of our *Drosophila* studies, and parallel work by other laboratories in cultured mammalian cells, zebrafish, and *NIPBL* mutant mice, is that NIPBL and cohesin directly control the activity of hundreds of genes important for growth and development.²⁰ Many of the physical defects and other medical problems that are apparent upon birth can be handled with existing medical procedures. The diversity of the effects on gene function argue, however, that we will likely need to increase the activity of the remaining good *NIPBL* gene if we are to improve future development. It is easier to control a river at its source than at the delta.

The reality is that drug companies would go out of business if they poured buckets of money into developing treatments for disorders that occur on the order of once per ten thousand births. Different strategies have to be developed for identifying and developing effective drugs for orphan diseases. A model that we are testing for CdLS is to use fruit flies to screen libraries of chemical compounds that have already been approved for human use for the ability to enhance expression of Nipped-B. The idea is that this is a rich source of bioactive compounds, and it is not nearly as expensive to repurpose a drug as it is to develop a new one.

In collaboration with the laboratory of Justin Fay at Washington University, we screened a library of FDA-

approved drugs for those that improve the growth of yeasts that have modest defects in their *NIPBL* and cohesin genes, and then tested the dozen or so that had the desired effect for their ability to improve some of the mutant phenotypes displayed by *Nipped-B* mutant flies. This analysis identified a few chemically-related compounds that were able to normalize the levels of *NIPBL* RNA in cell lines derived from individuals with CdLS. One of these compounds is used to treat other pediatric conditions. That's hopeful! We are now seeking funding to test these compounds in a mouse model of CdLS, and consulting with physicians, allied health professionals, and CdLS families to decide what biomarkers can be used to conduct a meaningful clinical study in CdLS patients.

Conclusion

My take-home lesson is fruit flies and other non-vertebrate organisms can prove to be useful models for human maladies. They can provide relatively rapid and inexpensive methods, both to understanding the molecular basis of the disease and to developing potential therapies, particularly for rare conditions for which standard methods for drug development are not economically feasible.

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Disclosure

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