University of South Bohemia
Faculty of Science
Department of Molecular Biology and Biochemistry
Laboratory of Developmental Genetics

Bianca Spitzbart

Bachelor Thesis

Study on effect of human *Casein Kinase I epsilon* inhibitor IC261 on *disc overgrown* in *Drosophila melanogaster*

Supervisor:

Mgr. Tomáš Doležal, PhD.

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Annotation

The aim of this work was to observe the effect of the human *Casein Kinase I epsilon (CKIε)* inhibitor IC261 on *disc overgrown* (*dco*) in *Drosophila melanogaster*, where *dco* in *Drosophila melanogaster* is the homologue of the human *CKIε*.

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České Budějovice, 16.05.2012

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Abstract

The aim of this work was to observe an effect of *CKIε* inhibitor on disc overgrown (*dco*) in *Drosophila melanogaster*. Somatic mutations were detected in breast cancer tissue in the human gene *Casein Kinase I epsilon* (*CKIε*), which is a homologue of *dco* .This special point mutation leads to imaginal disc overgrowth in *Drosophila*. In this study it was tested, whether *dco* could be defeated using a *CKIε* inhibitor named IC261, which was administered to *Drosophila* in larval state in different concentrations. The *dco* mutant causes an extended larval development associated with a striking overgrowth phenotype in larval imaginal discs. Whereas the wild-type discs stop growing when they reach an appropriate size, the mutant discs were smaller or totally missing during the extended period and the pupae died without any signs of adult development.

Table of Contents

1. Introduction

1.1. Aim of the thesis 5

1.2. *Drosophila melanogaster* in general 5

1.2.1. Handling flies 6

1.3. Genetics 7

1.3.1. Chromosomes 7

1.3.2. Balancers and Markers 7

1.4. Developmental Biology 7

1.4.1. Life cycle 7

1.4.2. Body plan 8

1.4.3. Imaginal discs 9

1.5. Disc overgrown gene 11

1.5.1. Casein Kinase I epsilon (CKIε) 11

1.5.2. Disc overgrown (*dco*) gene 13

1.5.3. IC 261 inhibitor 15

2. Materials and Methods 17

2.1. Culturing larvae 17

2.1.1. Preparation of media 17

2.1.2. Preparation of experimental food 17

2.2. Feeding experiments 18

2.3. Dissection of *Drosophila melanogaster* larvae 18

2.3.1. Dissection method 18

3. Results 19

3.1. Growth problems of imaginal discs 20

4. Discussion 21

5. Conclusion 22

6. References 23

1. Introduction

1.1. Aim of the thesis

The connection between cell growth/proliferation and cell death and pattern formation has been extensively studied in the *Drosophila* wing imaginal discs (Edgar, 1999; Serrano and O'Farrell, 1997; Tapon *et al.,* 2001). The Drosophila homologue of casein kinase Iε/δ (CKIε/δ), known as discs overgrown/double time (*dco*), was shown to be essential for imaginal tissue development (Jursnich et al., 1990; Zilian et al., 1999). Weak *dco* mutants exhibit a lag in disc growth, while stronger alleles have small or severely degenerated discs. Both overexpression and loss of function studies with *dco* suggest that it encodes an anti-apoptotic factor in Drosophila imaginal tissues (J. Guan *et al*., 2007). In this work IC261, a novel inhibitor of CKIε/δ, was used to observe whether it is able to inhibit growth of imaginal discs in *Drosophila melanogaster.*

1.2. *Drosophila melanogaster* in general

It is a model organism that is widely used for biological research in studies of genetics, developmental biology, physiology, microbial pathogenesis and life history evolution. It is mainly used because it is a species that has advantages such as the large numbers of offspring, short generation time, 4 chromosomes, well known and sequenced genome, cheap food, and the possibility for easy manipulation. *Drosophila melanogaster* was among the first organisms used for genetic analysis, and today it is one of the most widely used and genetically best-known of all eukaryotic organisms. Thomas Hunt Morgan, an embryologist, began using fruit flies in experimental studies of heredity at Columbia University in 1908. He began to grow *Drosophila* in large quantities with a special interest in exploring the existence of macromutations. In 1910 T.H. Morgan came across a fly with white eyes which determined his work for the next few years because it was the first allele of white, and its linkage to the sex chromosome triggered a revolution in understanding of heredity which led to the establishment of genetics as a subject with experimental methods. Furthermore, Morgan and his team showed the universality of Mendel`s factors, to create them in linear order and use them as genetic maps. They demonstrated

that genes lie on chromosomes, that alleles are able to mutate in forward and reverse manner and the function of alleles depends on their position within the chromosome. In addition, H. Muller, a student of Morgan, demonstrated that mutations could be induced by X-ray. (Hugo Stocker and Peter Gallant, in Dahmann 2008, p. 27-44)

1.2.1. Handling flies

Flies are usually kept in glass-or plastic-vials, containing special food, which are sealed with either paper or cotton plugs to provide fresh air. The special food consists of water, sugar, agar, corn-meal, yeast and fungicides. To get a closer look at the flies under the microscope, they are anaesthetized with CO_2 using a special pad called fly pad which consists of a porous plate surrounded by a metal or plastic rim. The CO_2 passes through the porous plate and forms a cloud of gas. Thus, flies lying on the pad will be anesthetized by the lack of oxygen and can be readily inspected and handled. However, exposure to CO_2 for more than 20 minutes will result in death of the flies; a further unwanted consequence can be dehydration and loss of fertility. Flies can be moved using paintbrushes for sex determination. To bring the flies back to the vial, a simple air bulb can be used. The vial is kept in a lateral position to avoid that the flies stick to the food. Labeling the vials immediately after adding the flies to the vials using appropriate genotype of the females and males is very important.

Five virgins and two-to five males per vial will give a reasonable number of progeny. The use of virgin-flies is essential. In general, flies start mating eight hours after eclosion. Due to their pale pigmentation and a dark spot called *meconium* in the abdomen, newly emerged flies can be distinguished from others quite easily. Transferring the flies into new vials is necessary after about three days. Cleanliness is the key to healthy fly culture.

1.3. Genetics

1.3.1. Chromosomes

The fly's genome is distributed onto four chromosome pairs consisting of sex chromosomes and 3 autosomes. These chromosomes differ substantially in their sizes, whereby the third chromosome is largest. The second, as well as the sex chromosomes are much larger than the fourth chromosome. In contrast to females, which always have two X-chromosomes, males have either both an X-chromosome and a Y-chromosome or only an X-chromosome, because the Y-chromosome carries just a few genes that are mainly required for male fertility.
(Hugo Stocker and Peter Gallant, in Dahmann 2008, p. 27-44)

1.3.2. Balancers and Markers

An important feature is the total absence of recombination in males, recombination in females exists. Its control is achieved by balancer chromosomes, which are the biggest advantage of *Drosophila*. Balancer chromosomes contain multiple inversions to suppress meiotic recombination with an un-rearranged chromosome. Furthermore, balancers carry dominant mutations, thus, the presence in flies is easily recognizable by a dominant marker mutation. Consequently, their transmission to progeny can be explicitly ensured. Marker mutations are a key to decoding the genotypes; they are used to mark the chromosomes of interest. (Hugo Stocker and Peter Gallant, in Dahmann 2008, p. 27-44)

1.4. Developmental Biology

1.4.1. Life cycle

Drosophila undergoes embryonic development inside an egg and hatches from the egg as a larva. This then goes through two more larval stages, growing bigger each time and eventually becomes a pupa, in which metamorphosis into the adult occurs. Under standard laboratory conditions, which are 25°C and 70% humidity, the whole life cycle does not take longer than 10

days. Egg production can reach up to 100-400 eggs per life, which is a big advantage since a pair of flies can give rise to a remarkable number of offspring. After five days the end of the larval instar is reached and the larva stops feeding and leaves food in order to find a dry place, suitable for pupation. During the following four days metamorphosis takes place, after about nine to ten days the first flies eclose. (L. Wolpert *et al.,* 2011; Hugo Stocker and Peter Gallant, in Dahmann 2008, p. 27-44)

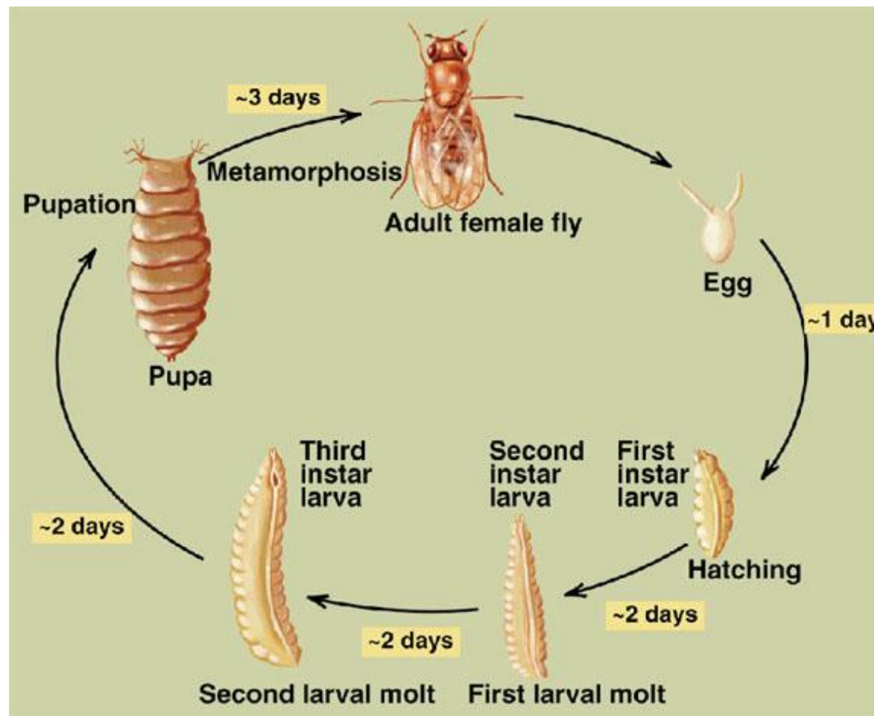

Fig. 1: source: www.zoology.ubc.ca; Embryology of Drosophila Oogenesis. 2.3.2012

1.4.2. Body plan

The insect body is bilaterally symmetrical and has two distinct and largely independent axes. The antero-posterior and dorso-ventral axis are at right angle to each other. These axes are already partly set in the Drosophila egg, and become fully established and patterned in the very early embryo. Along the antero-posterior axis the embryo gets divided into several broad regions, which will become the head, thorax, and abdomen of the larva. The dorso-ventral axis of the embryo gets divided up into four regions early in embryogenesis: from ventral to dorsal the mesoderm, which will form muscles and other internal connective tissues; the neuroectoderm,

which gives rise to the larval nervous system; the dorsal ectoderm, which gives rise to the larval epidermis, and the amnioserosa, which gives rise to an extra-embryonic membrane on the dorsal side of the embryo. The adult fly is about three millimeters in length; males are slightly smaller than females. The weight of females is about 1.4 mg; males weigh about 0.8 mg. Males can be recognized by the chitinous structure at the ventral side or their abdomen, by their continuous pigmentation at their posterior end, and by the round shape of the abdomen. Environmental conditions and genetic makeup have an influence on size and weight of the flies. (L. Wolpert *et al.,* 2011; Hugo Stocker and Peter Gallant, in Dahmann 2008, p. 27-44)

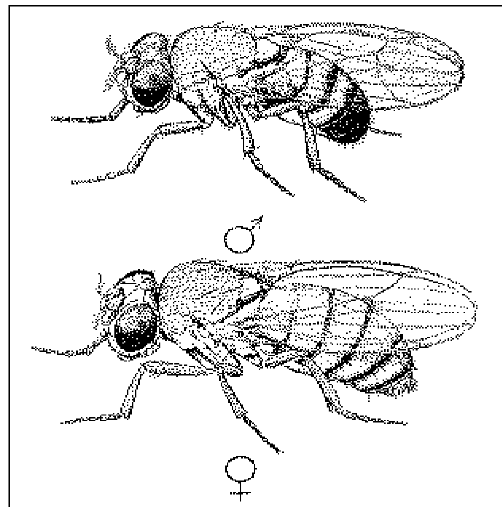

Fig. 2: Differences between male and female; Kha D. Dang, Previn B. Dutt, and Donald R. Forsdyke (1998); Biochemistry and Cell Biology 76, 129-137.

1.4.3. Imaginal discs

Drosophila melanogaster imaginal discs are a widely used model system to study signal transduction, developmental, and cell biological processes. The epidermal structure of the adult head, thorax, and external genitalia are derived from sac-like clusters of primordial cells known as imaginal discs during the process of metamorphosis. For instance, the leg imaginal discs are responsible for legs and ventral thorax, whereas the wing imaginal discs make wings and dorsal thorax (S. M. Cohen in Arias Volume 2; page 748-749). Imaginal discs, consisting of diploid

cells, are determined during the embryonic development and proliferate during the three larval stages. After the larval development, the imaginal discs consist of many cells. To analyze the question of how cell proliferation is regulated in imaginal discs, Zilian *et al.,* (1999) searched for mutants defective in growth control; they searched for recessive mutants in which mutant mitotic clones displayed excessive growth. Identification of one such mutation resulted in the isolation of the *warts* (*wts*) gene. Loss of this gene results not only in overproliferation but also in apical hypertrophy of epithelial cells, suggesting that mitosis is at least partially decoupled from cellular growth in *wts* mutants (Justice et al., 1995).

What makes them interesting to researchers is the fact that imaginal discs have no further function in larvae; which means that it is possible to manipulate them without any consequences for the larvae (Thomas Klein, in Dahmann 2008, p. 253-263).

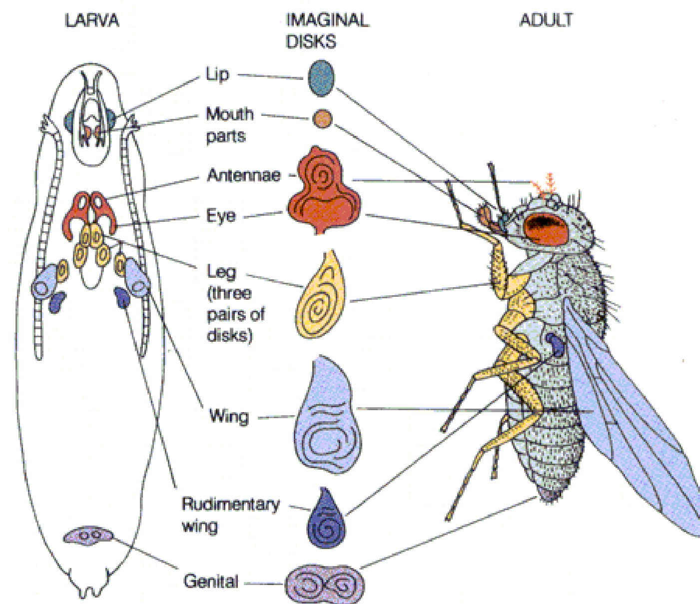

Fig. 3: Imaginal discs in the development of Drosophila; Mathews van Holde, Ahern3rd edition; www.pearsonhighered.com: 2.3.2012

1.5. Casein Kinase I epsilon (CKIε) and disc overgrown gene (*dco*)

1.5.1. Casein Kinase I epsilon (CKIε)

Casein kinase I epsilon comes from the CKI family which plays an essential role in cell regulation and disease pathogenesis. The *Drosophila* homologue of CKIε is called disc overgrown gene (*dco*). Unlike most protein kinases, CKI appears to function as constitutively active enzymes. CKI of serine/threonine specific protein kinases consists of multiple isoforms: α, β, γ1, γ2, γ3, δ and ε. Family members contain a highly conserved 290-residue N-terminal catalytic domain coupled to a variable C-terminal region that ranges in size from 40 to 180 amino acids. The C-terminal region promotes differential subcellular localization of individual isoforms and modulates enzyme activity. (Gross, S. D., and Anderson, R. A. (1998) *Cell. Signal.* 10, 699–711). CKIε phosphorylates several regulators of crucial processes, such as cell proliferation, differentiation, migration, and circadian rhythms. The key known targets of CKIε involve p53, key components of the circadian clock, the Wnt signaling pathway, and cell division machinery.

Genetic studies in *Drosophila* have led to the identification of tumor suppressor genes (TSGs) in which recessive mutations lead to neoplastic or hyperplastic overgrowth of larval imaginal discs and other tissues, mainly in the larva (C.J. Potter *et al.,* 2000 and P.J. Bryant *et al.,* 1990). Using laser microdissection, Fuja *et al.,* (2004) confirmed that the human homologues of three of these TSGs (*CSNK1*ε, *DLG1*, and *EDD/hHYD*) are expressed in normal human breast tissue. These genes were suspected of playing a role in regulating mammary cell growth because loss-of-function mutations in their *Drosophila* homologues cause excess tissue growth. Overexpression of *CSNK1*ε, the gene encoding CKIε, mimics Wnt signaling by stabilizing β –catenin and thereby increasing expression of β –catenin dependent genes. Fuja *et al.,* (2004) showed that for *CSNK1*ε, 19 nonsynonymous mutations and no synonymous mutations were found, most of the mutations affected highly conserved residues, somewhere found repetitively in different patients, indicating that the observed mutations were selected in tumors and may be functionally significant. Immunohistochemical reactivity of each protein was reduced in poorly differentiated tumors, and there was a positive association between altered protein reactivity, loss

of heterozygosity, and somatic mutations. Fuja *et al.,* (2004) demonstrated that the high frequency of somatic mutations was discovered in *CSNK1*ε, together with the assumption that CKIε activates the Wnt pathway, suggests that Wnt pathway activity changes may contribute to at least some cases of breast cancer.

Several components of the Wnt signaling pathway, including Wnt-1, β -catenin, and the target genes c-myc and cyclin D1, have been reported as showing elevated expression in breast cancer (S.C. Wong *et al.,* 2002 and S.Y. Lin *et al.,* 2000), and high β -catenin activity is associated with poor prognosis in breast cancer (S.Y. Lin *et al.,* 2000). It has therefore been suggested that Wnt pathway activation in breast cancer may result from somatic mutations in other regulators of the pathway that have yet to be discovered or analyzed (A.M. Brown; 2001). Fuja´s results suggest that CKIε may be such a regulator of the pathway but to understand the effects of mutations of *CSNK1*ε on the Wnt pathway and tumorigenesis, it will be necessary to determine whether the mutations cause loss or gain of function.

C. Modak and P. Bryant (2008) showed that the expression of CKIε causes up-regulation of the Akt pathway, which is critical for several developmental processes including cell metabolism, protein synthesis, cell cycle control, and cell survival (J. Engelmann *et al.,* 2006). While phosphorylation is critical for activation of the pathway, dephosphorylation is the major mechanism of Akt pathway inhibition. CKIε can inhibit Akt phosphorylation at both Thr308 and Ser473 and drastically reduce phosphorylation of the Akt target Glycogen Synthase Kinase 3β. The Akt pathway plays a role in many different cellular processes, which, if misregulated, can lead to both cancer progression and resistance to chemotherapy. C. Modak and P. Bryant (2008) provided new insight into Akt regulation, identifying CKIε as a new positive regulator of the pathway.

The D*rosophila* homologue of CKIε, called discs overgrown/double time (*Dbt)* can be tested using similar techniques and that may also be shown to play a significant role in cancer.

1.3.3. Disc overgrown gen (*dco)*

Zilian *et al.,* (1999) analyzed mutants showing excess cell proliferation in imaginal discs, and this resulted in the identification of the *discs overgrown* (*dco*) gene whose phenotype showed, at least for one allele, hyperplastic growth of imaginal discs. These discs showed defects in gap-junctional communication as evident from a dramatic reduction in dye coupling of imaginal disc cells (Jursnich et al., 1990). Additionally, they found that *dco* encodes a homolog of human casein kinase I δ/ ε (CKI δ/ε) and is identical to the previously cloned *double-time* (*dbt*) gene (Kloss et al., 1998) and suggested that *dco* is required for inhibition of apoptosis during cell proliferation as well as for growth arrest in imaginal discs. Since the first description of *dco* (Jursnich et al., 1990) many new alleles of the gene have been produced by various mutagenic procedures. Zilian *et al.,* (1999) therefore analyzed the phenotypes of these new alleles as well as of some new deficiencies and of the few alleles described earlier (Jursnich et al., 1990), in homozygotes and heteroallelic combinations. The study showed effects of *dco* mutations on imaginal discs are seen most clearly in the wing disc, since this disc is flat and any morphological or growth abnormalities are easily detectable. Zilian *et al.,* (1999) demonstrates that cells that lack a functional *dco* gene are unable to undergo continued growth and cell proliferation and die after only two or three divisions. The results further demonstrate that the growth inhibition and apoptosis of dco^{-} cells is cell-autonomous, which suggests that dco^{-} cells are unable to respond to a signal required for growth or survival. *Dco* kinase appears to play a role in growth arrest of imaginal discs and thus controls the size and shape of the adult structures to which they give rise. Zilian *et al.,* (1999) demonstrated that the analysis of *dco* mutant phenotypes shows that complete loss of *dco* function in heteroallelic combinations of null alleles is lethal during larval stages. Growth of imaginal discs during larval stages is strongly inhibited in *dco* null mutants. Additionally, the results of Zilian *et al.,* (1999) showed that despite this strong inhibition of growth and survival of imaginal discs, many larvae survive to the third instar, which is often prolonged by more than two weeks at 25°C before the larvae die. Weaker *dco* alleles (dco^{2}, dco^{P1396} and dco^{P915}) appear to result in reduced growth rates of discs and death during late larval or early pupal stages, while heteroallelic combinations with weak alleles (dco^{P103} and dco^{P1447}) die as pharate adults or survive to viable adults. Surprisingly, one allele, dco^{3}, hardly inhibits

growth, if at all, but rather fails to arrest growth of discs when they have reached their normal size. Finally, Zilian *et al.,* (1999) concluded that this property of *dco*^{3} is further cell-autonomous in clones and that the ability of *dco*^{3} cells to transduce a signal, required to stop growth at the end of larval life, is significantly reduced while the generation of the signal is not affected.

Building on this work of Zilian *et al.,* (1999), Guan *et al.,* (2007) also demonstrated that *dco* is required for cell proliferation and/or survival through certain mechanism. In his report it was shown that *dco* is an essential cell survival factor in the wing imaginal disc. J. Guan *et al.,* (2007) initially identified *dco* as a potent suppressor of Hid induced cell death in the eye. This Hid antagonism requires *dco* kinase activity. Loss of *dco* in the wing results in massive apoptosis or programmed cell death (PCD), which is a major cause of the small disc phenotype seen in *dco* mutants. When apoptosis is inhibited, *dco* mutant wings are normally patterned, suggesting that *dco's* effect on cell survival is not an indirect consequence of abnormal cell specification. Furthermore, J. Guan *et al.,* (2007) demonstrate that *dco* mutant cells have a dramatic post-transcriptional reduction of DIAP1 protein levels, which is sufficient to explain the increase in PCD. DIAP1 is required for blocking apoptosis-inducing caspase activity. In *Drosophila*, *dco* has been shown to act as a positive regulator of planar cell polarity in the larval eye and pupal wing (Strutt et al., 2006; Klein et al., 2006). Furthermore, in fly cell culture and the wing imaginal disc, *dco* has been shown to be required for Wnt/Wingless (Wg) signaling (Cong et al., 2004; Klein et al., 2006; Zhang et al., 2006). Wg signaling is known to promote cell survival in the wing imaginal discs (Giraldez and Cohen, 2003; Johnston and Sanders, 2003). J. Guan *et al.,* (2007) found out that in wing disc, the loss of *dco* resulted in a dramatic reduction in DIAP1 expression. The loss of DIAP1 in either fly embryos or the wing imaginal discs leads to massive activation of caspases and cell death (Ryoo *et al.,* 2004; Wang *et al.,* 1999; Yoo *et al.,* 2002). Therefore, the reduction in DIAP1 levels observed in *dco* mutants is sufficient to explain the elevated caspase activation and apoptosis in these cells. Due to these data J. Guan *et al.,* (2007) concluded that *dco* activation of DIAP1 is important to suppress apoptosis, the possibility existed that this regulation was due to non-specific effects of mispatterning in *dco* mutants. In mammalian cell lines, CKIε plays a protective role against apoptosis induced by extrinsic death signals (Desagher et al., 2001; Izeradjene et al., 2004). Also inhibition of CKIδ induces apoptosis in trophoblast cells (Stoter et

al., 2005). Further studies will be required to determine whether there are some similarities between these different systems.

Due to the high structural and functional conservation, results obtained for *dco* in *Drosophila* are likely to be equally significant for higher organisms, including man (Zilian *et al.,* 1999). Furthermore, the ability of *dco* to inhibit apoptosis and possibly promote cell proliferation makes it a very attractive candidate for functioning as an oncogene in tumorigenesis.

1.3.4. IC261 inhibitor

IC261 (Icos Corp., USA) selectively inhibits CKI compared to other protein kinases by an ATP-competitive mechanism; it is uncharged at physiological pH and can diffuse across cell membranes (N. Mashhoon *et al.,* 2000). Moreover, IC261 shows an order of magnitude higher selectivity for CKIδ and CKIε over other CKI isoforms. The basis of CKI specificity has been established at 2.8 A resolution by X-ray crystallography (Mashhoon *et al.,* 2000).

L. Behrend *et al., (*2000) showed that IC261 triggers the mitotic checkpoint control. At low micromolar concentrations of IC261 it inhibits cytokinesis causing a transient mitotic arrest. Immunofluorescence images show that at low concentrations IC261 leads to centrosome amplification causing multipolar mitosis (L. Behrend *et al.,* 2000). After p53-independent mitotic delay, IC261 induces a p53- dependent G1 arrest. L. Behrend *et al.,* (2000) concluded that CKIε/δ activity is indispensable for an ordered mitotic progression probably by regulating centrosome and/or spindle functions. Furthermore, L.Behrend *et al.*, (2000) indicates that IC261 can produce effects on cell cycle progression that are indistinguishable from an established spindle poison and are dependent on the p53 status of the cells.

The structure of IC261, 3-[(2,4,6-trimethoxyphenyl)methylidenyl]-indolin-2-one, is shown in Fig.4. It is a 3-substituted indolin-2-one derivative. Molecules of this family are commonly prepared by base-catalyzed condensation of aldehydes and oxindole and therefore typically consist of mixtures of E and Z geometric isomers. When subjected to thin layer chromatography, IC261 resolved into two principal species in 2:1 ratio, suggesting that it was a mixture of geometric isomers.

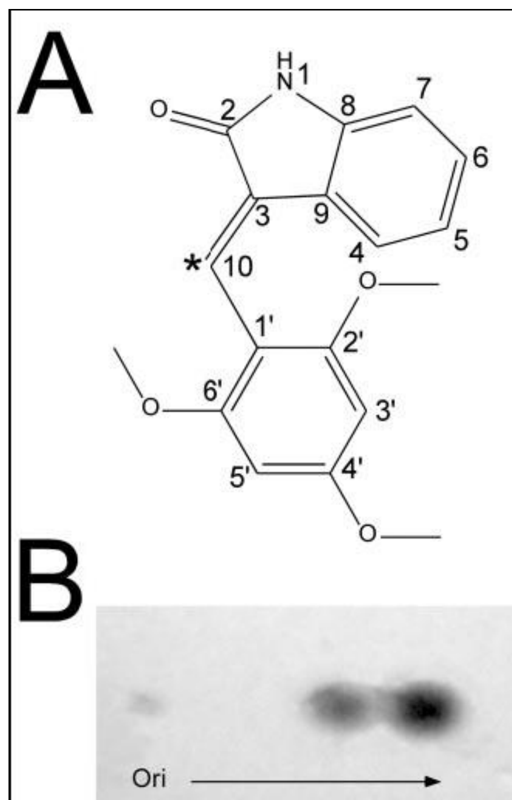

Fig. 4A: IC261 structure

Fig. 4B: Thin layer chromatography of IC261; resolved into two principal species 2:1; *Ori*, origin. *Arrow*, direction of solvent migration. N. Mashhoon *et al.,* (2000) Crystal Structure of a Conformation-selective Casein Kinase-1 Inhibitor, 20052

The selectivity of IC261 for CKI isoforms comes from an induced fit mechanism. It binds a subset of the substrate-binding pharmacophores lying in the nucleotide-binding cleft resulting in stabilization of CKI in a conformation that is midway between the unliganded and nucleotide-bound forms of the enzyme. This conformation is stabilized by additional movement of the glycine-rich loop, which makes contact with IC261 and simultaneously participates in a novel hydrogen and electrostatic bond network involving aromatic, charged, and polar amino acid residues spanning both domains. The stability of this network of delocalized interactions decreases the dissociation rate of the inhibitor, resulting in a measurable decrease in apparent IC_{50} for members of the CKI family relative to other protein kinases (N. Mashhoon *et al.,* 2000).

2. Material and Methods

2.1. Culturing larvae

For this experiment Wild-type *Oregor-R* flies, provided by Tomáš Doležal, were reared on standard corn-meal medium. The flies were kept in glass vials at 25° C. For the IC261 experiment, flies were let to lay eggs for 4 hours in small plastic cages on 6-cm Petri dishes with yeast/sucrose medium without IC261. Embryos were left on the dishes to develop for another 16 hours and then transferred to dishes with experimental food.

2.1.1. Preparation of media

<u>Standard corn-meal medium</u>: Prepared from 120 g cornmeal, 60 g instant yeast, 75 g sucrose, 15 g agar, 1,5 L water and 25 mL of 10% methylparaben in ethanol.

<u>Yeast/sucrose medium for IC261 experiment</u>: Dry yeast and agar (Scharlau) were dissolved in distilled water to get 8% and 1.2% w/v, respectively. The solution was boiled for 15 minutes maintaining the total volume. Finally, sucrose was added to make 5% w/v and the solution was boiled again. The suspension was cooled down to approx. 60°C, methylparaben (Sigma) was added to 0.16% and the medium was aliquoted by 7 mL in 15-mL falcon tubes and stored at 4°C. Before the experiment, the medium was re-heated in microwave oven and additionally cooled-down to ~50°C.The experimental food was prepared as described in 2.1.2.

2.1.2. Preparation of experimental food

The yeast/sucrose medium was enhanced with 28 µL of IC261 (Sigma-Aldrich cat. num. I0658-5MG) in DMSO, mixed and poured into 6-cm petri dishes.

The following media were used:

- 100 μM IC261
- 10 μM IC261
- 1 μM IC261
- Control (DMSO only)

2.2. Feeding experiments

Two variants of feeding experiments were used :

1) Fresh IC261 medium every day throughout larval development; larvae were transferred to freshly prepared food every day.
2) Fresh IC261 added once in the beginning, when embryos were transferred, and larvae were left on the same food throughout the rest of the larval development.

2.3. Dissection of *Drosophila melanogaster* larvae

Late third instar larvae (at certain point of time after laying eggs as depicted in Tab 1.) grown on selected media mentioned above were dissected and the morphology of dissected imaginal discs were checked and documented using stereomicroscope with digital camera.

2.3.1 Dissection Method

Carefully, a larva was taken out of its petri-dish and placed on a spot plate. Using a needle the spot plate was filled with phosphate buffered saline (abbreviated PBS) buffer, which is isotonic and non-toxic to cells. Next, the larva was fixed using forceps and the tail was cut off carefully. This made it possible to invert the larva inside out by using forceps in order to fix the larva right behind its head and trying to push the head inside the body using another pair of forceps. The larva was then upended and the imaginal discs were barred from fat tissues in order to have a closer look at them.

3. Results

The dissection was performed in order to examine whether all larvae developed their imaginal discs equally or in different way. The larvae from different media and feeding experiments were dissected under a microscope. When dissecting larvae from DMSO control both left and right wing discs could be found which showed normal size and shape.

It could be observed that some larvae, coming from IC261 media, showed totally missing discs, smaller or misshaped discs where some parts were missing (shown in Fig. 5 and summarized in Table 1.). In general, the larval period took longer for IC261 treated larvae than usual and most larvae did not metamorphose to adult state at all but died in pupa state without showing any signs of adult development.

3.1. Growth problems of imaginal discs

Table 1: Growth problems of imaginal discs detected:

	120 hours	**140 hours**	**164 hours**
DMSO control	all larvae normal; discs of late third instar larvae normal	all animals pupated	normal development
1 µM IC261	Not inspected	0/10 with defects, pupae present	Not inspected
10 µM IC261	3/6 larvae with growth defect; most of them were late third instar	6/12 larvae with growth defects; some already pupated *	1/4 remaining larvae with growth defects
100 µM IC261	larvae were slower in development; they were early-mid third instars	5/14 with growth defects, no pupae	2/4 with growth defects, pupae present

*total percentage of larvae with growth defects is possibly lower, because some larvae with no defects already pupated.

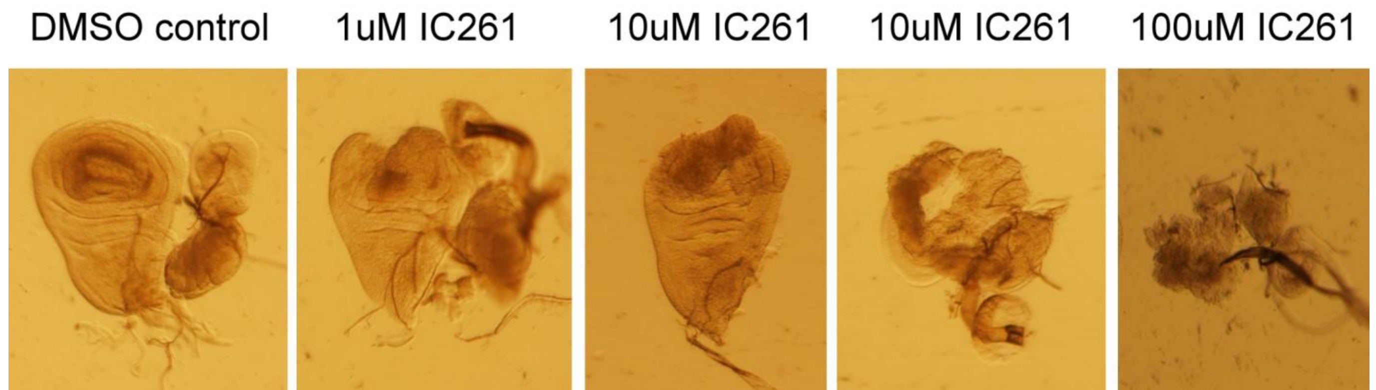

Fig. 5: Example of normal wing, leg and halterer disc (DMSO control) and discs with growth problems of larvae from different media (IC261 treated larvae).

DMSO control: Normal shape of wing disc (the biggest one) as well as leg and halterer discs.

IC261 treated larvae: Growth defects are detected: Smaller wing discs with missing parts and abnormal shapes

4. Discussion

The experiment shows that IC261 inhibitor for human casein kinase Iε/δ, which was administered to *Drosophila* in larval state in different concentrations, causes at least minor effects in development of larval imaginal discs. Some larvae, containing the IC261 inhibitor, showed missing, misshaped or smaller wing discs with great parts missing. Furthermore, these larvae had an extended developmental period and died in pupa state without any metamorphosis to adult state. On the other hand, some larvae exhibit normal wing discs which make it hard to find a conclusion. Larvae containing the DMSO control showed normal but slower development of imaginal discs compared to wild type larvae. In general, the discs are not necessary for the larval development; it may slow down, because the animal is waiting for proper discs before it pupates. However, proper discs are necessary for final metamorphosis because adult tissues are formed from them. No abnormalities in larval tissues were observed in any case suggesting that IC261 has a specific effect on *dco* protein only (*dco* null mutation does not affect larval tissue either).

5. Conclusion

These results show that knocking down disc growth in *Drosophila melanogaster* using IC261 casein kinase Iε/δ inhibitor is problematic. As some of the larvae showed an unchanged development of imaginal discs when treated with IC261, the result is ambiguous and the phenomenon of suppressing *dco* in *Drosophila melanogaster* might have been caused by several influences. Further studies for searching methods using IC261 to strike *dco* in *Drosophila melanogaster* might help to get significant results in future.

6. References

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