University of South Bohemia in České Budějovice
Faculty of Science
Department of Molecular Biology

BACHELOR THESIS

Study of the Role of mutations L39Q, L49Q, and N78T of Casein Kinase I epsilon in Breast Cancer Using Drosophila Melanogaster as a Model Organism

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Annotation

Several somatic mutations were found in human gene encoding Casein kinase I epsilon in breast cancer tissue samples. Since there is a homologue in Drosophila genome, *discs overgrown* locus that leads when mutated to hyperplastic overgrowth of imaginal wing discs, I attempted to obtain a Drosophila, which would carry one of the observed mutations in its genome.

I hereby solemnly declare that I have written this thesis independently and without assistance, other than the specified sources and resources.

I hereby declare that, in accordance with Article 47b of Act No. 111/1998 in the valid wording, I agree with the publication of my dissertation thesis, in full / in shortened form resulting from deletion of indicated parts to be kept in the Faculty of Science archive, in electronic form in publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice accessible through its web pages.

In Prague May 21, 2010

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Anotace

Ve vzorcích nádoru prsu bylo nalezeno několik somatických mutací genu Casein kinase I epsilon. Vzhledem k tomu, že genom Drosophily obsahuje homolog tohoto genu - *discs overgrown* locus, který, pokud je mutován, vede k hyperplastickému přemnožení imaginalních disků křídla - snažil jsem se získat jedince, který by nesl jednu z pozorovaných mutací CKIε ve svém genomu.

Prohlašuji, že svoji diplomovou práci jsem vypracoval samostatně, pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č.111/1998 Sb. v platném znění souhlasím se zveřejněním své diplomové práce, a to v nezkrácené podobě elektronickou cestou a na veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách.

V Praze May 21, 2010

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Abstract

Fuja et al., 2004 has found somatic mutations in gene sequence encoding a Casein Kinase I epsilon (CKIε) in breast cancer tissue. However there is no proof of their influence on the cancer development. One way to find their role is to study the mutations on a model organism. We have chosen *Drosophila melanogaster* because in previous studies it was discovered that disc overgrown gene (dco) is a homolog of a human gene encoding for Casein Kinase I epsilon (CKIε) and has 86% of the amino acid composition identical (Zilian et al., 1999; Kloss et al., 1998).

The aim of the thesis was to produce a *Drosophila melanogaster* that would carry mutations found in breast cancer tissue by Fuja et al., 2004. We have chosen mutations that were found in cancer tissue of patient number 4 (L39Q, L49Q, N78T). The homologue of the gene including these mutations was inserted into the fly's DNA using the P element and transposase. The construct was injected into the embryo using microinjection technique. Using a designed crossing with individuals with balancers (CyO, MKRS) we would obtain a transgenic *Drosophila melanogaster,* which could be used for further investigation.

However the results we have obtained showed that the breeding of the transgenic *Drosophila melanogaster,* which carry mutations L39Q, L49Q, N78T, is very problematic. From 686 injected embryos there was no such an individual obtained during the study. The results also showed that there is a very low survival rate. Only 11.8 % of the embryos survived the injection and developed into the larva stadium. However none of these larvae developed in fly, which carried wanted mutations. Based on the results we think that the inserted mutations have a dominant effect, and because the dco gene is especially expressed during the embryonic stadium of development, it might have caused death.

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1 Introduction

1.1 Breast Cancer

Breast cancer was diagnosed at 429 900 European women in 2006. This makes it the most frequent cancer form in female population, and third in mortality when both sexes are counted. (Ferlay et al., 2007)

There is a strong predisposition in women with family history of breast cancer, however there is still very little known about the origin and progress of the disease. In 1994 Miki et al. presented the discovery of gene BRCA1 that was associated with the familial occurrence of breast cancer. BRCA1 plays a significant role in DNA repair, cell-cycle-checkpoint control, protein ubiquitination and chromatin remodeling. The foundation of BRCA1 was followed by the discovery of BRCA2 (Wooster et al, 1995). This gene is involved in process of homologous recombination. Other genes have been identified to play a role in increasing risk of developing breast cancer, however their influence is not that significant as in case of BRCA1 and BRCA2.

Although the familial predisposition has been proved by several studies, only 5 – 10 % of the cases are attributed to inheritance. However 75 % of the familial breast cancer genes have not yet been discovered (Figure 1) (Balmain et al., 2003).

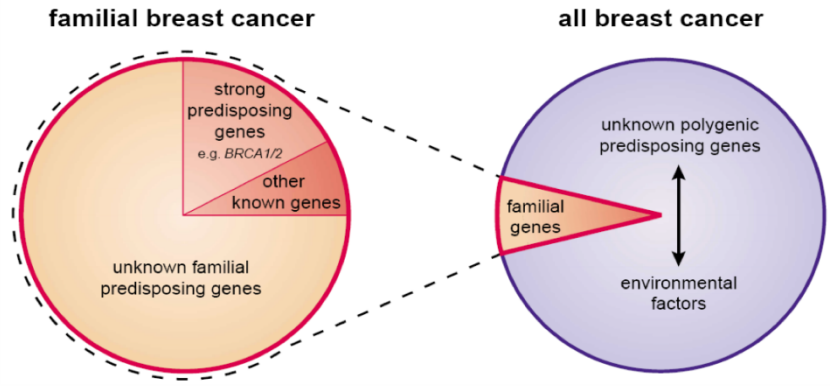

Figure 1: Proportion of known and unknown genes involved in the development of breast cancer. (Balmain et al., 2003)

1.2 Casein Kinase I epsilon (CKIε)

Casein kinase I epsilon (CKIε) belongs to the CKI family of serine/threonine specific protein kinases. The CKI family presence was already proved in yeast and stayed throughout the evolution till human. There are seven known members of CKI family: CKIα, CKIβ, CKIγ1, CKIγ2 CKIγ3, CKIδ and CKIε (Fish et al., 1995; Rowles et al., 1991; Zhai et al., 1995).

CKIε is a protein that consists of 416 amino acids with kinase domain (residues 9 to 293) of 53-98% identity to the kinase domains of other isoforms and is most closely related to the CKIδ (Fish et al., 1995).

CKIε takes part in many cellular processes. Its participation was proved in circadian rhythms, cell proliferation, cell differentiation, cell death, or cell adhesion. If the CKIε function breaks down, many human diseases such as sleep disorders, carcinogenesis or neurodegenerative diseases could develop (Knippschild et al., 2005). CKIε is also involved in several signaling pathways:

1.2.1 Canonical Wnt signaling pathway

In Canonical Wnt signaling pathway CKIε is responsible for cell proliferation, planar polarity, cell adhesion and other processes. CKIε is involved in both positive and negative regulation of the canonical pathway. As a positive regulator CKIε monitors downstream of Wnt and upstream of Dvl. The mechanism is regulated through phosphorylation of Dvl at positive regulatory sites in response to Wnt signaling, which results in its enhanced affinity to FRAT and consequently in stabilization of β-catenin (Hino et al., 2003; Klimowski et al., 2006). Its negative role consists in direct phosphorylation of β-catenin when incorporated into the degradation complex (Amit et al., 2002; Sakanaka, 2002) as well as in Axin-dependent phosphorylation of APC with subsequent down-regulation of β-catenin (Rubinfeld et al., 2001).

1.2.2 Non-canonical Wnt signaling pathway

Non-canonical Wnt pathway is characterized by the absence of β-catenin. It regulates the establishment of epithelial planar cell polarity (PCP), which is responsible for the same polarity of single cells or multicellular units in epithelium.

CKIε was first shown to act negatively in the PCP pathway using cell-culture assays. This led to the suggestion that CKIε might play a role of a switch between the canonical Wnt signaling and the PCP pathway (Cong et al., 2004). Later *in vivo* experiments positive regulation of PCP was discovered. Its kinase activity is required for its function in the canonical signaling, however its action in the PCP regulation possibly happens through a kinase-independent mechanism (Klein et al., 2006; Strutt et al., 2006).

1.3 ***Drosophila Melanogaster***

Drosophila melanogaster began its important role as a model organism in the first half of the twentieth century. The breakthrough discovery already happened in the first decade when recessive white-eye pigment mutation was discovered on the X chromosome (Morgan, 1910). This made *Drosophila melanogaster* one of the most powerful model organism used for studying almost every biological process. One of the reasons that enable studying human diseases on *Drosophila melanogaster* is the fact that the most of the signaling pathways and developmental mechanisms are well conserved between vertebrates and invertebrates.

There are many benefits that makes *Drosophila melanogaster* so powerful model organism: very short generation time, only 4 chromosomes, well known and sequenced genome, and variety of molecular tools facilitating experiments such as tissue-specific expression or directed mutagenesis. However the most important benefit of the *Drosophila melanogaster* was a discovery of balancer chromosomes. Balancer chromosome is a tool used in genetics that suppresses recombination between homologous chromosomes, and therefore simplify the analysis and makes possible to produce organisms with lethal mutations. Other very important property of balancer chromosome is the presence of dominant markers. This makes simpler to distinguish an individual carrying the balancer from other population. Homozygous balancer constitution is lethal or makes flies sterile and thus prevents the balancer from taking over the stock. Examples of balancer chromosome used today's are with dominant markers as curled wings (*Curly*, *Cy*), shortened larval/pupal body (*Tubby*, *Tb*), shorter and thicker bristles (*Stubble*, *Sb*) or wings with notched margin (*Serrate*, *Ser*).

Figure 2: Curly winged female, full genotype: w1118/w1118 ; +/CyO ; +/+ (w1118 is not a balancer, it indicates there is a mutation on each X chromosome, which gives the fly white eyes). http://arrogantscientist.files.wordpress.com/2009/01/cyo-f1.jpg?w=500&h=375

1.4 Transgenic line and P-element

For the preparation of transgenic line P element are used as a transmitter of DNA sequence. We used two plasmids to produce a transgenic Drosophila. One plasmid contained a P-element, marker and a sequence we want to insert. The second plasmid also called helper contained a transposase.

Transposase is an enzyme that binds to the ends of a transposon. It catalyzes the transport of the transposon from one to another part of the genome using "cut and paste" mechanism. The transposase inserts the P-element randomly into the genome of the embryo.

P-element is a very powerful tool used in today's genetics. It allows introducing cloned DNA sequence into a chromosome. The length of P elements is about 2.9 kb, and contains 4 exons that are necessary for well functional transposase. They constantly contain a perfect 31-bp terminal inverted repeat (IR) and an 11-bp subterminal inverted repeat, which essential for the transposition (Dolezal, 2001; Mullins et al., 1989).

There are two types of P-elements: (a) autonomous P elements and (b) non-autonomous P elements. The autonomous P elements carry a transposase gene and mobilize itself. On the other hand non-autonomous P elements do not have a coding sequence for

transposase, and therefore it must be added separately. (Rio et al., 1986). In order to enable an easy screening for transgenic flies, the construct usually carries a short version of the *white gene* that causes reddening eyes of the originally white-eyed flies after insertion (Klemenz et al., 1987).

1.5 Disc overgrown (Dco)

dco^3 is a recessive lethal allele of the *Discs overgrown* (*Dco*) gene causing an overgrowth of imaginal discs in *Drosophila melanogaster* (Jursnich et al., 1990). When *Dco* was isolated and sequenced it was found that it is identical to previously discovered double time gene (*DBT*) (Zilian et al., 1999). DBT plays a significant role in control of the circadian rhythm period. The structure of DBT (Dco) is very similar to human casein kinase I epsilon (CKIε). They have 86% of the amino acid composition identical (Kloss et al., 1998). Homozygous *dco*^{3} allele or in combination with deficiency in the *Dco* gene causes increase in larval period in which imaginal discs continue to grow and increase its size to several times greater than wild type final size (Zilian et al., 1999).

1.6 Somatic mutations of CKI tfound in breast cancer tissue

DNA from 82 breast tumor samples and corresponding normal tissue was obtained by laser capture microdissection. Each protein critical regions were amplified using PCR method and searched for somatic mutations using direct sequencing. They revealed 19 nonsynonymous and no synonymous mutations at NH_2-terminal portion of the Ser/Thr kinase domain of CKIε. Some were found repeatedly (Table 1) (Fuja et al., 2004).

Patient number	Changes predicted by mutations
1	I29T, A36G, L39Q, S101R
2	E52K, G64A, A73V, E74*
3	L39Q, S101R
4	L39Q, L49Q, N78T
5	L39Q, L49Q, G72A, A73V
6	L39Q

Table 1: Summary of the mutations in CKIε found in breast cancer samples of 6 patients out of 82 examined (Fuja et al., 2004).

This high frequency of somatic mutations in the proteins suggests that CKIε plays a role in the development of at least some cases of breast cancer. However these data don't prove an active participation of these somatic mutations on development of breast cancer. Thus studying the mutation on the model organism (e.g. *Drosophila melanogaster*) may prove their functions.

1.7 Molecular design of mutagenesis in Drosophila *Dco*.

The construct that was a part of the P-element and which was inserted into the Drosophila genome is shown in figure 3. The construct containing the mutated *Dco* gene was randomly inserted in one of the chromosomes of a fly.

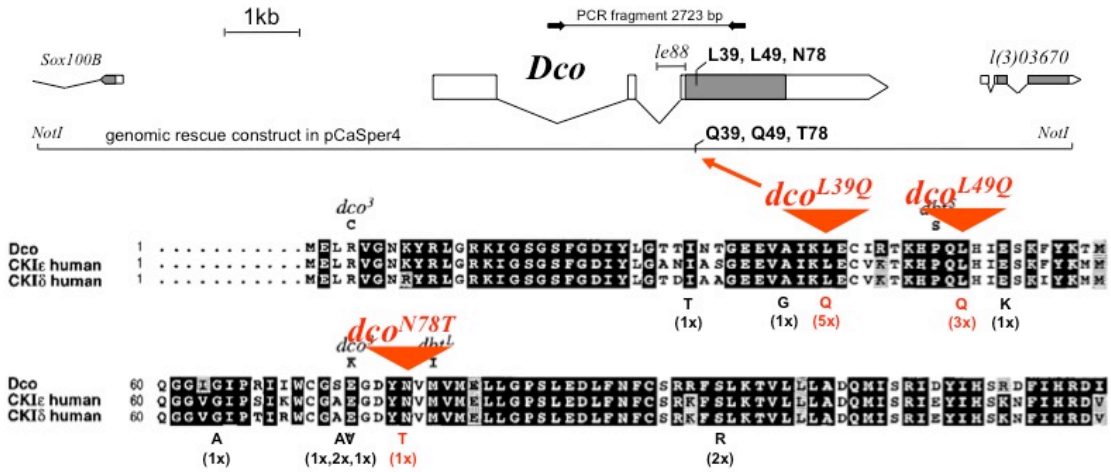

Figure 3: First 129 amino acids sequence of the Drosophila's Dco, and human CKIε, and CKIδ; exons are illustrated as grey boxes with protein-coding region. The positions of amino acids, which were mutated, are shown by orange arrow; Mutations found in human CKIε in breast cancer are shown below the alignment with the number of their occurrences in parentheses. The dco[3] (Zilian et al. 1999) and doubletime[L/S] (Kloss et al. 1998) are shown above the alignment.

1.8 Aims of the thesis

The aim of the thesis was to introduce somatic mutations L39Q, L49Q, N78T discovered in Casein kinase I epsilon (CKIε) coding sequence in the breast cancer tissue (Fuja et al., 2004), into a model organism *Drosophila melanogaster* and find the chromosome on which the sequence was inserted.

For the insertion of the mutations into the fly's genome the method of microinjection of plasmids containing P-element carrying modified *Dco* gene and transponase was used. The sequence of $Dco^{L39Q,\ L49Q,\ N78T}$ gene was randomly inserted into the fly's genome. During its development the modified dco was naturally expressed together with already present dco gene.

The chromosome of insert was searched using designed crossing with flies containing balancers (CyO and MKRS) in their genome.

2 Materials and Methods

2.1 Maintaining drosophila culture

In the experiment two cultures of *Drosophila melanogaster* were maintained. We have worked with a stock of yellow-white flies with genotype yw (573) and with stock of balanced flies with genotype yw; Xa / Cyo; MKRS (281) (table 2). Flies were raised on a standard cornmeal diet. The diet was prepared from 120 g cornmeal, 75 g sacharose, 60 g instant yeast, 15 g agar that were dissolved in 1.5 L of distilled water. The medium was then supplemented with 25 mL of 10% methylparaben in ethanol. The cultures were kept in room temperature and the diet was changed periodically.

Internal stock number	Genotype
281	yw; Xa / Cyo; MKRS
573	yw

Table 2: List of fly stocks used in this work.

2.2 Preparation of injection material

2.2.1 Generation of mutant construct for injection (prepared by Tomáš Doležal).

Mutations L39Q, L49Q and N78T were introduced into a genomic rescue construct "NotI of lambda88.9 in CasPer4#7" (described in Zilian *et al.* 1999) as follows: 6-kb fragment including *dco* coding sequence was cut out by *Xho*I and *Bam*HI and cloned into pBluescript II SK(+) (Stratagene). Mutations were introduced by QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene) using following primers: L39Q – gtggccatcaagcAggagtgcatccgc L49Q - gcaccaaacacccccagcTgcacatcgagtcaaagtt and N78T - cggcagcgagggcgactacaAtgtgatggtgatggag (mismatches capitalized). Mutant sequences were cloned back to genomic rescue construct using *Swa*I and *Bsi*WI enzymes.

2.2.2 Buffer preparation

1 mL of the buffer for the injection solution was prepared from 20 μL 10mM KCl, 20 μL 1mM Na_2PO_4, 50 μL of the color for the indication of proper injection and 910 μL distilled H_2O. The mixture was well shaked.

2.2.3 Preparation of injection medium

The actual injection material was prepared from 17 μL of the construct containing the *dco* coding sequence with mutations (L39Q, L49Q, N78T), 6 μL of the transposase - "helper" construct and 2.3 μL of sodium acetate (NaAc). These parts were mixed together and stored in -20 °C. After about 15 minutes the solution was centrifuged for another 15 minutes and 50 μL of 70% ethanol was added to the mixture, centrifuged again. The pellet was then diluted using 75 μL of previously prepared buffer and stored in -20 °C.

2.3 Preparation of microinjection needles

The microinjection needles were prepared from Capillary Glass Standard Wall Borosilicate Tubing With Filament (Sutter Instruments) using the P-97 Flaming/Brown type micropipette puller (Sutter Instruments). The technique used was followed Matulová, 2009. The setting used to prepare the microinjection needles is described in following table (Table 3.).

Step	Heat	Pull	Velocity	Time
1	539	0	10	5
2	515	0	15	5
3	515 – 560*	150	55	20

*Table 3:Program used for pulling microinjection needles. * Depends on the needle's batch*

2.4 Preparation of juice-gel plates and gel-coated slides

4 g of Bacto-Agar (Scharlau) was dissolved in about 100 ml of black currant juice. The solution was boiled in the microwave and shaked during the procedure. The boiled medium was then poured into small Petri dishes and led cool down. When cooled the dishes were stored at 4°C.

The medium for preparation of the gel-coated slides were used the same as for the petri dishes. The microscopic glasses were repeatedly submerged vertically into the medium till layer of the medium reached thickness of about 3 mm .

2.5 Injection technique

The culture of *yellow white* (*yw*) flies was kept in a column on juice-gel plates with yeast paste for few days. The plates with the paste were daily changed. One day before the injection the plates with yeast were changed to juice-gel plates with addition of only few drops of pure ethanol. The plates were changed every two hours. This method should have increased the egg laying.

When the microinjection procedure was started, juice-gel plates with addition of pure ethanol were changed every 30 min. During this period eggs from the juice-gel plate were collected and washed with 96% ethanol to prevent grow of bacteria or yeast during the incubation. Washed and collected eggs were transported on the gel-coated slides and aligned using a feather. The eggs were aligned so that the injection was done into their posterior end.

The injection of prepared DNA mixture was done using standard micromanipulator (Narishige) and prepared needles. It was essential to finish the microinjection within the 30 min after eggs collection, as until 1 hour after the fertilization. This ensured that the syncitial blastoderm did not become partitioned into separate cells (cellular blastoderm).

The injected lines of embryos were cut out of juice-gel with and were then transferred onto the standard LB agar plates supplemented with ampicillin (50-100 μg/ml). The plates were kept at 25 °C for 24 hours.

2.6 Care of hatched larvae

After 24 hours of incubation at 25 °C the LB agar plates supplemented with ampicillin were visually monitored for presence of hatched larvae. All present larvae were carefully transported into the standard cornmeal diet. The maximum of individuals present in one vial was set to be 20 larvae per vial. The vials were then stored at 25°C till first flies occurred.

2.7 Crossing and mapping

The model of the crossing (Figure 3) was designed so that we would obtain F2 generation, which carry our inserted mutations, but also two balancers: CyO on the chromosome II and MKRS on the chromosome III. The cross of F2 generation with

yellow-white (yw) flies enabled mapping the transgenic insertions and showed on which chromosome the inserted sequence is present (Figures 5-7).

2.7.1 Cross 1 (F0 generation): Crossing of injected individuals with *yellow-white (yw) flies*

After about 10 days the first injected flies has hatched (F0 generation). They were immediately separated into individual vial to ensure only virgin male and especially virgin females are present. 3 - 4 individuals of opposite sex were added from *yellow-white (yw)* stock to each member of F0 generation. This crossing ensured that the maximum members of the next F1 generation would carry the inserted mutation.

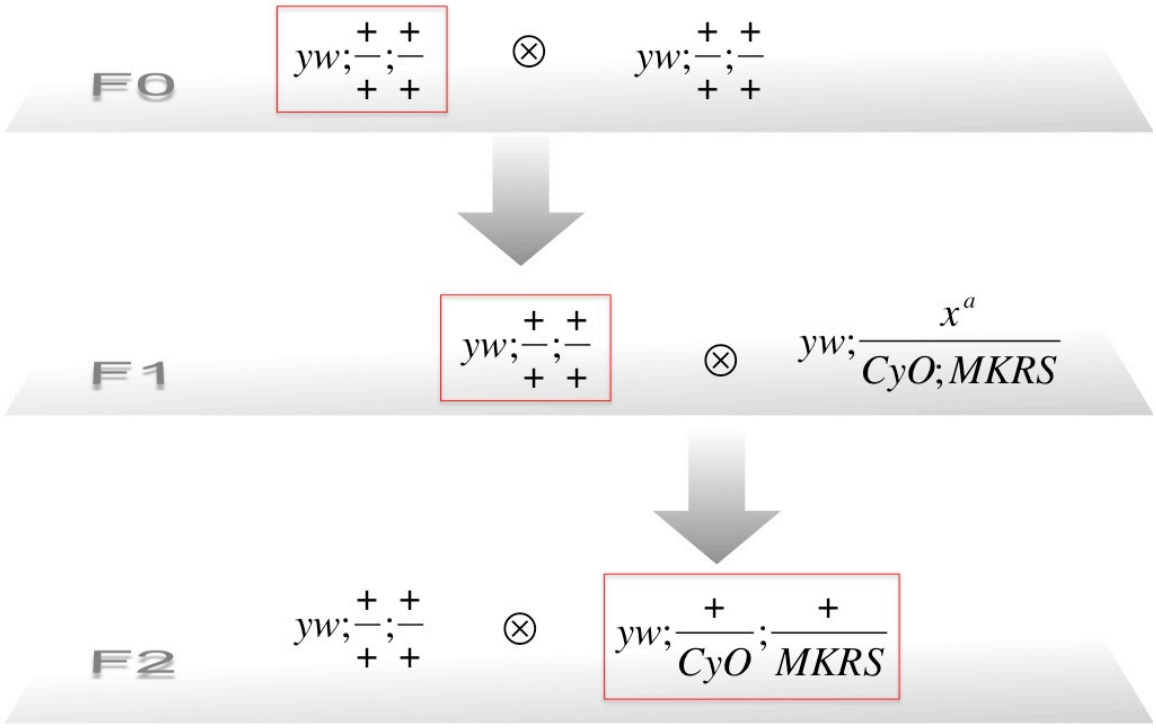

Figure 4: Crossing scheme - Cross 1-3; red rectangle indicates individuals that carry inserted mutations

2.7.2 Monitoring of F1 generation and Cross 2: Crossing of positive individuals with balanced yw; Xa / Cyo; MKRS

The members of F1 generation were visually screened for presence of the marker - orange-red eyes, which indicated the presence of the inserted sequence. All positively screened individuals were collected and individually crossed with 3 - 4 members of opposite sex from the stock of the balanced flies (yw; Xa / Cyo; MKRS).

2.7.3 Cross 3: Search for the mutation position

To find the position on which chromosome the mutation is present only flies with marker and genotype (yw; +/Cyo; +/MKRS) were collected from F2 generation. Member of F2 stock was individually crossed with 3 - 4 individuals of opposite sex from yellow-white (yw) stock. The gained F3 generation had a very typical scheme that depended on the position of the inserted sequence.

2.7.3.1 Position on chromosome X

To prove the sequence is present on chromosome X, male gained from F2 generation was crossed with 3 - 4 females of *yellow-white (yw)* stock. The indication, that the mutation was present on X chromosome, was the fact that in all females in F3 generation marker was present. On the other hand all males obtained did not have inserted mutation, and thus were white-eyed (Figure 5). The presence of the balances was not important in this case.

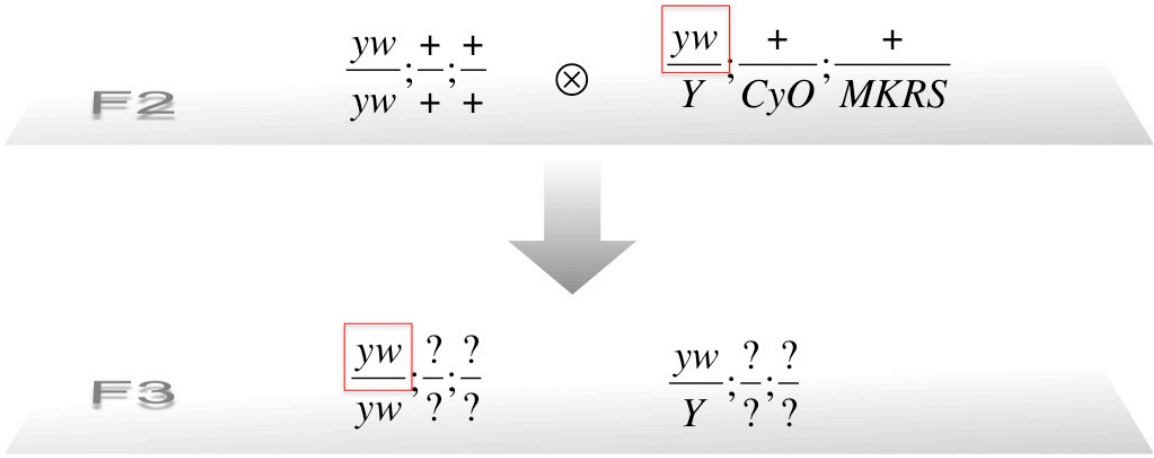

Figure 5: Cross 3 – Typical infants scheme of the cross 3 if mutation is located on the chromosome X; red rectangle indicates chromosome that carries inserted mutations; ? marks that the presence of the balancer on the chromosome was not important

2.7.3.2 Position on chromosome II

If the mutation sequence was inserted into chromosome II, we used the balancer CyO as an indicator of the presence. In this case F3 generation consisted from two groups. One group had a marker but CyO balancer was not present in its genome (yw; +*/+; ?). The other group of flies did not have the marker, but had curled wings (yw; +/CyO; ?). This typical scheme of F3 generation indicated that the mutation was present on chromosome II. The sex or presence of MKRS balances was not important in this case.

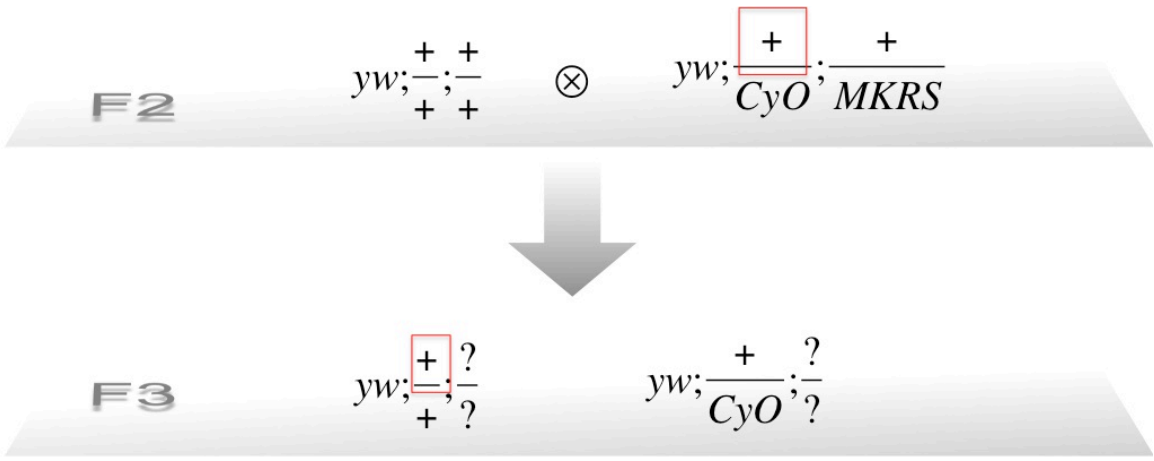

Figure 6: Cross 3 – Typical infants scheme of the cross 3 if mutation is located on the chromosome II; red rectangle indicates chromosome that carries inserted mutations; ? marks that the presence of the balancer on the chromosome was not important

2.7.3.3 Position on chromosome III

If the mutation was present on chromosome III, the balancer MKRS was used as an indicator. In this case F3 generation contained group with the marker but no MKRS in its genome (yw; ?; +*/+), and group of flies with no marker but with presence of MKRS (yw; ?; +/MKRS). This typical scheme of F3 generation indicated that the mutation was present on chromosome III. The sex or presence of CyO balances was not important in this case.

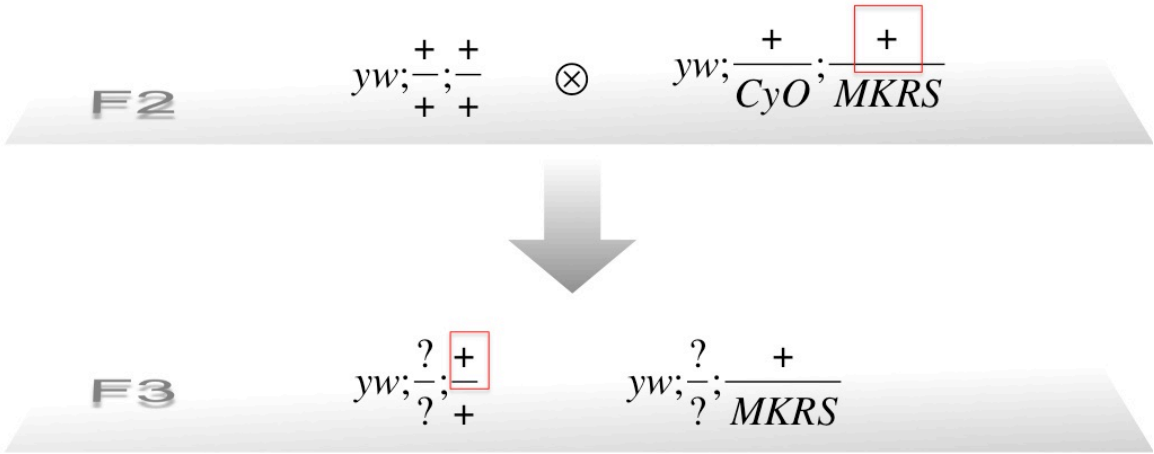

Figure 7: Cross 3 – Typical infants scheme of the cross 3 if mutation is located on the chromosome III; red rectangle indicates chromosome that carries inserted mutations; ? marks that the presence of the balancer on the chromosome was not important

2.8 Stock production

For further investigation of the mutation, stocks were prepared. The stocks were prepared using individuals form the F2 generation. Two flies of opposite sexes that were carrying the marker and both balancers (yw ; +/CyO ; +/MKRS) were crossed. The stocks are

design so that at all further generations it is ensured that the infants would have the same mutations and balancers as their ancestors. This function is enabled using the balancers, which are present on two chromosomes. The one type of infants is due to a fact that presence of heterozygous balancers is lethal.

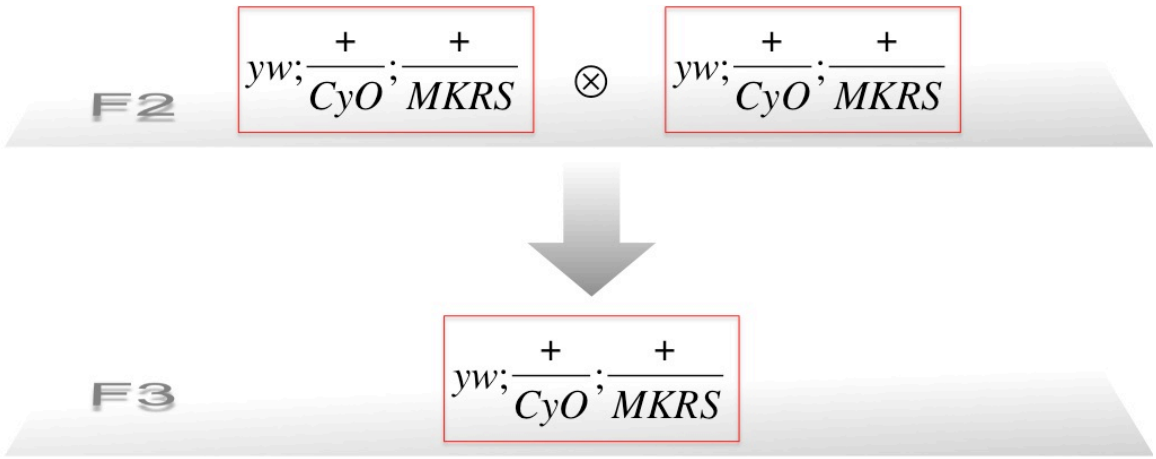

Figure 8: Crossing 4 – Production of the; red rectangle indicates individuals that carry inserted mutations

3 Results

Following the methods six individual injection sessions were carried out. The injections were marked from A to F. In the first injection (Injection A) 158 embryos were injected. Only 18 larvae were found in the LB agar plates with ampicillin and were carefully transported into the standard cornmeal diet and stored in 25°C. From these 18 larvae only 16 flies hatched. From injection A there were obtained 9 females and 7 males and were marked A1 - A16. The flies were separated in individual vials and crossed with 3-4 individuals of opposite sex from yellow-white (yw) stock. However no positive individual with marker was present in F1 generation.

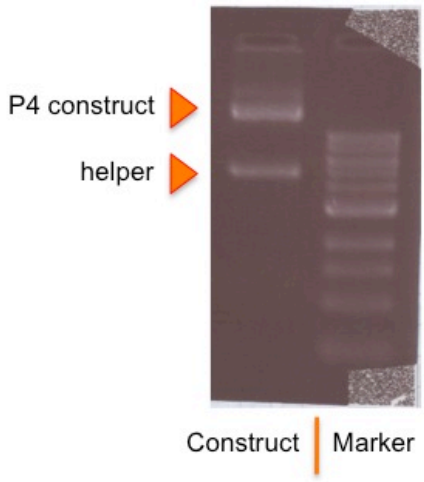

Figure 9: Result of the electrophoresis of P4 construct and helper construct

The result of the injection B followed the results of injection A. From 75 injected embryos only 8 larvae hatched. Unfortunately 3 individuals did not transfer to flies, so from 8 larvae only 5 flies hatched – all females - and were marked B1 – B5. However no positive individual with marker in F2 generation was present in this injection too.

All of the other injection (C-F) followed the results of the injection A and injection B. The detailed obtained data are showed in following table 4.

Injection	Number of injected embryos	Number of hatched larvae	Number of hatched flies	No. of males	No. fo females	Number of positive results
A	158	18	16	7	9	0
B	75	8	5	0	5	0
C	80	5	4	3	1	0
D	10	1	0	0	0	0
E	188	19	10	3	7	0
F	175	30	15	8	7	0
Total	**686**	**81**	**50**	**21**	**29**	**0**

Table 4: Results of the injection and breading in total numbers

In total view as seen in Table 4 and 5 we can see that the survival rate is very low. From total of 686 injected embryos only 81 hatched into larvae stadium. This gives a survival rate only 11.8%, however the surviving rate for the flies is even lower. From the total of 686 embryos only 50 flies survived, this gives a survival rate of 7.3%. More of the statistical data are described in following table 5.

Injection	% of injected embryos	% of hatched larvae	% of hatched flies	%. of males	%. of females	% of positive results
A	23.0%	11.4%	10.1%	43.8%	56.3%	0.0%
B	10.9%	10.7%	6.7%	0.0%	100.0%	0.0%
C	11.7%	6.3%	5.0%	75.0%	25.0%	0.0%
D	1.5%	10.0%	0.0%	0.0%	0.0%	0.0%
E	27.4%	10.1%	5.3%	30.0%	70.0%	0.0%
F	25.5%	17.1%	8.6%	53.3%	46.7%	0.0%
Total	**100.0%**	**11.8%**	**7.3%**	**42.0%**	**58.0%**	**0.0%**

Table 5: Results of the injection and breading in percentage

4 Discussion

As seen in the results there was no transgenic individual obtained, therefore we have to ask why. There were several factors that might have an influence on the results. There could have been a problem in the methods, right at the beginning during the plasmid architecture, or in the manual injection. However, even though all methods were done precisely there is still one very important factor, which might have and very significant influence on the results – lethal mutation.

4.1 Wrong technique of injection

One reason that might have had an influence on the result is a wrong technique of the injection. The injection of the DNA into the embryo is a very aggressive method and if not carried out correctly it has lethal consequences. The technique of injection was processed following the method used by Matulova, 2009 and Dolezal 2010. In the results of Dolezal, 2010 it was proved that using this method the mutation L39Q and S101R were successfully inserted into an embryo and living genetically modified organism was obtained. Using this argument we can conclude that the injection could not have such a large influence on the result.

4.2 Bad plasmid/injection-solution preparation

Another reason why no positive results have occurred could have been caused by an error during the production of the plasmids, which have carried the mutation. This problem was known before the actual injection was performed, and therefore we have tested the plasmids using the electrophoresis. This method proved that the plasmids had proper wanted size and no other fragments had occurred, which indicated that the plasmids were in proper shape. Two other constructs which are almost the same (just introduced mutations differed) and which gave the same results on gel (Dolezal 2010) as well as the injection buffer itself were not killing the injected embryos.

4.3 Dominant effect of the introduced mutation

Last reason, which might have had an influence on the negative results, is a potential dominant effect of introduced mutation on embryo/larvae survival. The injected construct ensures a natural expression of the gene, which is especially strongly expressed in early embryo (Figure 10). If the mutations would have a dominant effect causing abnormal development it might cause death of embryo/larvae.

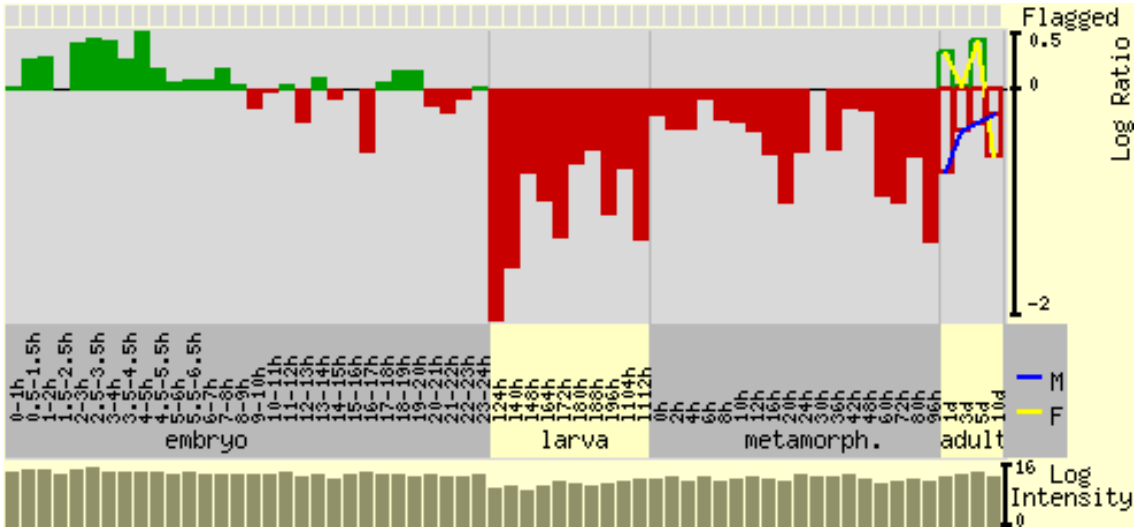

Figure 10: Normalized natural expression of dco gene throughout the development of Drosophila melanogaster (Flybase)

Introducing the mutations into a construct that does not naturally express, but enables its expression on demand (e.g. using regulatory UAS/Gal4 system) can solve this negative effect of the dominance. The gene may be then promoted in later stadium of the development, where it will not cause such damage.

5 Conclusion

Fuja et al., 2004, found several mutations in human breast cancer tissue. These mutations were in sequence of the DNA coding for Casein kinase I epsilon. The aim of the thesis was to produce a stock of genetically modified *Drosophila melanogaster*, which carry one set of these mutations. We have chosen to study specific mutations that were found in cancer tissue of patient 4 (P4): L39Q, L49Q, N78T. These mutations were inserted into the DNA of the model organism using the injection of two plasmids.

The result shows that the breeding of the mutated *Drosophila melanogaster,* which carry mutations L39Q, L49Q, N78T in its coding sequence for Casein kinase I epsilon, is very problematic. From 686 injected embryos there was no such an individual obtained during the study. The results show that there is a very low survival rate. Only 11.8 % of the embryos survived the injection and developed into larva stadium. This phenomenon might have been caused by several influences; however we think that the inserted mutations are incompatible with life and stop the development of the *Drosophila melanogaster* in the embryo stadium.

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7 List of tables and figures

7.1 Tables

7.2 Figures