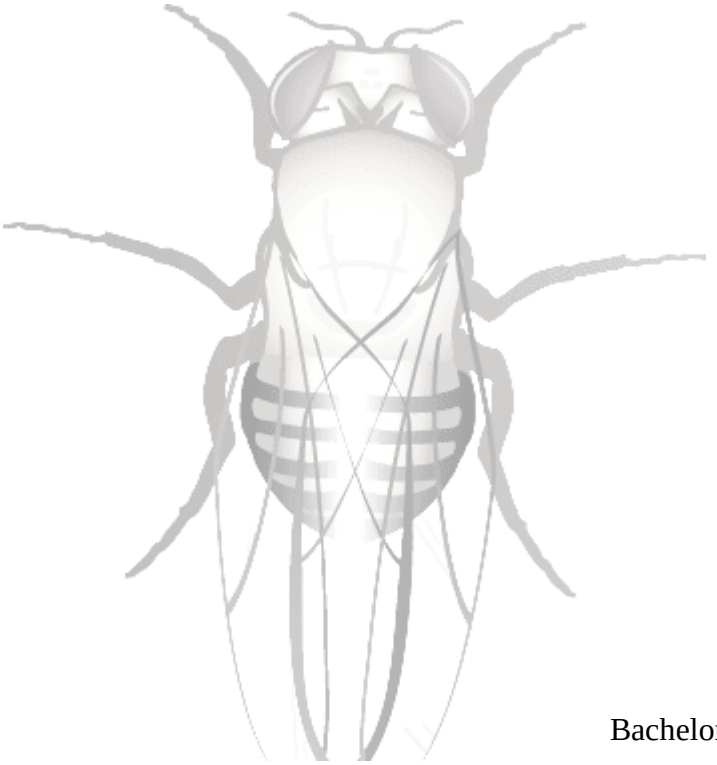
Södertörn College/University
Molecular Cell Biology

Transvection

in *Drosophila Melanogaster*

zeste dependent transvection

in loss-of-function *lamin* mutants



Bachelor thesis, 15 ECTS
Spring term 2008
Author: Anneli Pasanen
Supervisor: Per Kylsten

Abstract

Transvection is a widespread phenomenon affecting chromosomal and gene function. There are many examples of epigenetic machineries controlling gene regulation. Nuclear Lamin proteins could have this function. This project shows *zeste* dependent transvection in loss-of-function *lamin* mutants in *Drosophila melanogaster*. The *zeste* locus encodes a regulatory gene product affecting the expression of other loci, e.g. *white*. No transvection effect in loss-of-function *lamin* mutants has so far been shown. The effect of homozygosity versus heterozygosity of *lamin* on *zeste*-dependent transvection at paired *white* loci was analysed by crossing fruit flies to get homozygous z^1; lam^{D395} individuals. Whether or not the *zeste* (z^1) transvection effect on *white* was affected by lam^{D395} loss-of-function mutation was determined by comparing the eye colour phenotypes of double mutant z^1; lam^{D395} females to that of z^1/Y; lam^{D395} males, which were used as an internal negative control since they are hemizygous for *zeste* that is located on the X chromosome. Females homozygous for z^1 and lam^{D395} displayed the z^1-characteristic yellow eye colour. The conclusion is that *zeste*-dependent transvection effect at *white* also occurs in *lamin* mutants. Future research on transvection is needed in order to understand the exact mechanisms of gene regulation. Even gene therapies for some human diseases can take advantage of *trans*-acting sequences to correct gene expression.

(Keywords: transvection, *zeste*, *lamin*, nuclear lamina)

Index

1 Introduction

Transvection is an epigenetic phenomenon resulting from an interaction between an allele on one chromosome and the corresponding allele on the homologous chromosome. Transvection can cause a gene to be activated or suppressed. In transvection the heterozygous combination of two alleles with mutations in different parts of the gene complement each other and a wild type phenotype is rescued. Transvection can also occur between nonallelic regions of the genome as well as regions of the genome that are not transcribed [1].

Transvection was first discovered by Edward B. Lewis at the Drosophila bithorax complex in 1954 [1]. Since then transvection has been found for many different loci in *Drosophila,* e.g. *white, decapentaplegic, eyes absent, vestigial* and *yellow*. Most recently transvection has been found at the *Drosophila apterous* locus. [2] The function of a gene showing transvection effects can be altered by homologue pairing. [1] Lewis' definition was, "Operationally, transvection is occurring if the phenotype of a given genotype can be altered solely by disruption of somatic (or meiotic) pairing. Such disruption can generally be accomplished by introduction of a heterozygous rearrangement that disrupts pairing in the relevant region but has no position effect of its own on the phenotype". [1] In some cases, transvection between two alleles causes complementation within one locus while interference of transvection disrupts the complementation (See fig. 1.).

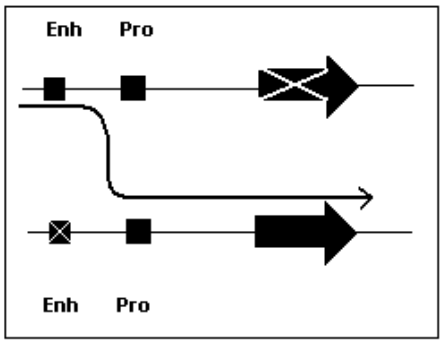


Fig. 1 Complementation by transvection. Schematic drawing of a hypothetical situation where there is a mutation on an enhancer element on one chromosome and a mutation on a transcribed part of the target gene on the other chromosome. Complementation occurs by an enhancer element (acting in *trans*) activating transcription of the promoter on the homologous chromosome.

Phenomena that are dependent upon allele pairing have been found in species other than *Drosophila*, including mice, humans, plants, nematodes, insects, and fungi[3,4,5,6]. This makes studying transvection interesting since it may be involved in many types of gene regulation mechanisms together with other homology effects. Homology effects are a class of regulatory mechanisms that rely on sequence homology. Transvection is not merely an allelic process of transcribed genes but can just as well be applied to nonallelic interactions and to loci that are not transcribed. The effect of a gene or a chromatin structure changing location has been applied to *trans*-inactivation of the *brown* and *white* genes, *trans*-silencing of *P transposable*

elements and to *trans*-suppression of position-effect variegation. [5] Position effect is a change in phenotype that occurs when the position of a gene is changed from one chromosomal site to a different location. [7] The gene can end up close to regulatory sequences like silencers or enhancers, which can affect the gene's expression. The gene can move from a euchromatic position where the expression of the gene is high to a more condensed or heterochromatic position and this in turn lowers the expression of the gene.

There are several different mechanisms proposed as models for transvection. The first mechanism was proposed by E. B. Lewis and is based on the enhancers of one allele activating the promoter of a paired second allele. Other mechanisms include pairing-sensitive silencing and enhancer bypass of a chromatin insulator through pairing-mediated changes in gene structure. One model is based on ability of RNA to activate or repress genes; for example the *zeste*^{1} mutation represses paired *white* loci causing yellow eye colour phenotype in *D. melanogaster* females while hemizygous males have the wild type eye colour. This has been proposed to be caused by a *white* transcript acting as a co-repressor when homologue pairing raises its concentration to a critical point. More recent studies are considering other explanations at *white* but the involvement of RNA in this process has not been excluded (See references in [5]).

The nuclear lamina is a fibrillous network composed of intermediate filaments that are made up of **Lamin** lining the inner surface of the nuclear envelope in animal cells. Nuclear lamins are currently being studied by analysing mutations and their phenotypes. It is commonly believed that the Lamins are important for epigenetic organisation of the genome as they contribute to shaping the nucleus, to distributing nuclear pores into the nuclear membrane and to binding DNA. Lamins could have a role in directly affecting transvection, since they are known to influence among other things the positioning of DNA in the nucleus. They could bring certain DNA sequences close to each other and contribute in this way to homology effects like transvection. On the other hand they could move homologous sequences apart and in this way disrupt transvection. No transvection effect in loss-of-function *lamin* mutants has so far been shown [8].

Fruit fly *lam* loss-of-function mutations are practically lethal, already at the larvae stage. However, some flies do survive. Loss of *lam* expression results in limited ability of these so called escaper flies to move and maybe even their premature aging. This is similar to effects of mutations leading to human laminopathies. It is not known in which tissues loss of *lamin* protein function leads to *lam* phenotypes. ***lam*** $^{\mathbf{D395}}$ is a null allele generated by excision of the P element from *lam*^{P} (See fig.2). It is a null allele because the entire exon II containing the translation start codon has been removed. [8]

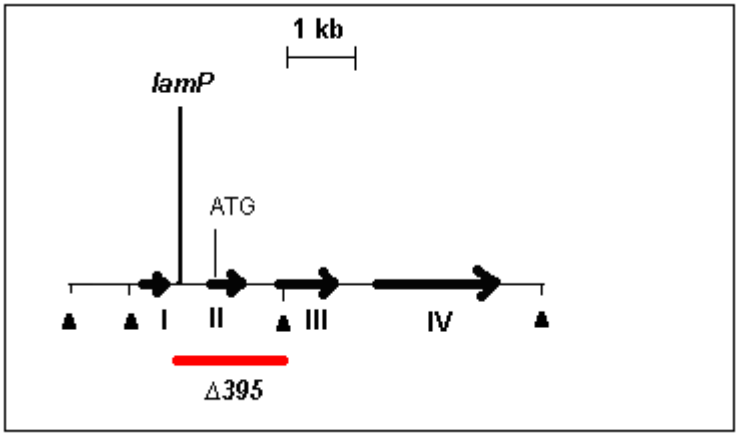


Fig. 2 Null allele *lam*^{D395}.
Schematic drawing of the lam locus. Exons are presented as black arrows, and the translation start site is shown. The site of insertion of the P element responsible for the *lam*^{P} mutation is shown, and the red bar below indicates the excision of the *lam*^{D395}. The small triangles show restriction sites by HinDIII. *Lam* locus is situated on chromosome two in *D. melanogaster*.

Zeste is not required for survival of the flies. It is a DNA binding transcriptional activator forming multimeric complexes *in vitro*. These characteristics have lead to the conclusion that these proteins might bind to certain sequences in enhancer elements and promoters. Aggregation of the proteins would help the enhancers and promoters to get close to each other. Transvection would occur if the enhancers and promoters were on homologous chromosomes (See references in [12]).

The *zeste* locus encodes a regulatory protein affecting the expression of other loci on transcription level. The loci that have been identified are *white*, *Ultrabithorax* (*Ubx*) and *decapentaplegic* (*dpp*) (See references in [9]). There are two ways to study *zeste* interactions with other genes; the first is based on transvection between mutant alleles (See fig. 1.). This pairing-dependent allelic complementation is likely to be a very wide-spread phenomenon. One of the alleles that are involved in transvection has a regulatory mutation while the other has a mutation that affects the transcribed part of the target gene, so that an intact regulatory region on one copy of the target gene compensates for the mutated non-functional regulatory region on the other copy of the target gene. This explains why the two copies need to be close to each other by means of homologous pairing in order to be able to complement each other but it does not explain exactly how zeste protein participates in transvection since this protein is not required in chromosome pairing.

The second way to reveal *zeste* interaction with other genes is studying the (z^1), gain-of-function *zeste* mutation effect on *white*. [9] Expression of *white* is down regulated by z^1 only when *white* is present in two paired copies. Transvection occurs with z^1 at *Ubx* but not *dpp*, and the *white* expression is down regulated only in certain tissue like the eye. This implies that the function of *zeste* is coupled to many different components and may have different mechanisms at different locations (See [9] and references therein). Hyperaggregation of z^1 appears to be essential in its repression of *white*. [10] Zeste binds to two sites in an eye-specific enhancer between 1 and 2 kb upstream of the white promoter. Zeste aggregation is likely caused by three hydrophobic heptad repeats in the C-terminal 75 amino acid region of the protein. z^1 mutation causes hyperaggregation of zeste. Zeste aggregates bind to the eye enhan-

cer causing the *white* repression. z^{op6} is a derivate of z^{1} that causes extreme hyperaggregation of zeste proteins leading to visible transvection effect even in the hemizygous male flies. [10]

Studies on transvection and the z^{1} effect on *white* have revealed participation of *zeste* but in order to tell how zeste proteins normally function in the cell, the structure of zeste aggregates needs to be determined. Not enough is known about the structure of zeste proteins and the mechanisms which they use in DNA binding. More research in this area is needed since the role of *zeste* in many cases of transvection has not been defined and in some cases the conclusions are not that clear.

Pirrotta et *al*. [9] have proposed that the *zeste* transcription start resembles closely RNA cap sequences of many *Drosophila* genes. This can be of regulatory significance. It is noteworthy that runs of glutamine have been found in many eukaryotic regulatory proteins, for example rat glucocorticoid receptor and the human c-myc oncogene. The predicted *zeste* protein has a long Gln and Ala bridge as well which is likely to cross-hybridize with many different sites in the genome. Much more research on the exact DNA binding mechanism of zeste protein is needed but Pirrotta et *al*. [9, 11] propose that *zeste* product binds to other DNA binding proteins by its hydrophobic domain. These proteins could be structural components of chromosomes or nucleus, other trans-acting factors or other *zeste* monomers.

Since Lamins have a role as well in organizing the genome and shaping the nucleus, it is interesting to turn our attention to the Lamin proteins in this study. The aim of the project is to quantify *Drosophila melanogaster zeste* dependent transvection in loss-of-function *lamin* mutants and to compare their eye colour to wild type eye colour. (See fig. 3)

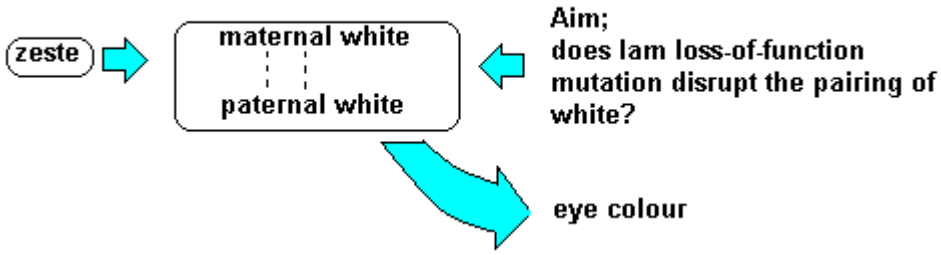


Fig. 3 The aim of the project.

2 Materials and Methods

The first fly stock used is homozygous for *scute*[1], *zeste*[1], *echinus*[1] and *cut*[6] (*sc*[1], *z*[1], ec[1], ct[6]). These loci are situated on chromosome one (chromosome X in *D. melanogaster*) while the remaining chromosomes are wild type. Females homozygous for *zeste*[1] mutation show transvection at *white* locus and have yellow eyes while hemizygous males have red eyes. Reduction of *white* expression causes the yellow eye colour of the females, but this occurs only when the *white* genes can pair. *scute* and *echinus* flanking *zeste* ensured that *z*[1] had not been lost by recombination (See fig. 4).

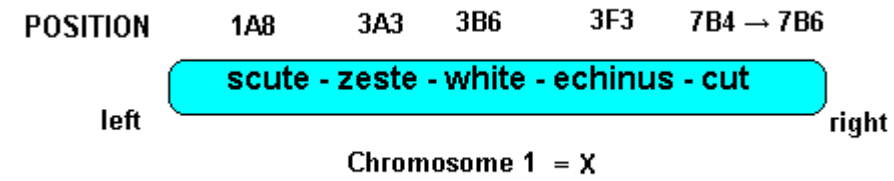


Fig. 4 Positions of the loci of interest in stock number one. The presence of *scute* and *echinus*, which flank *zeste* ensured that *z*[1] had not been lost by recombination.

The second fly stock is homozygous for *white* [1118] (*w*[1118]) on chromosome one and heterozygous for *lam* [D395] on chromosome two. The ***lam*** [**D395**] is a null allele generated by excision of the P element from *lam*[P] where the exon II containing the translation start codon also has been removed (See fig.2). A balancer for chromosome number two is used since *lam* [D395] is not fertile homozygously and is practically lethal. The balancer chromosome makes the stock stable. The balancer chromosome also makes recombination impossible while it itself is infertile or lethal in homozygous form. It also carries dominant genetic markers, CyO (curly of Oster) and GFP (green fluorescent protein) making it simple to track. Alternative marker used was Sco which is also situated on chromosome two. In the final cross, the GFP marker needs to be taken into account because it is carried on a P-element with a mini white gene which could interfere with the results. Chromosome three is not of interest for this study. The flies were grown on mashed potato agar medium.

The products of the final cross that were analyzed are homozygous for *zeste* and homozygous or heterozygous for *lam* [D395] respectively. Whether or not *zeste* (*z*[1]) transvection at the *white* locus is affected by *lam* [D395] was determined by comparing the eye colour phenotypes of these

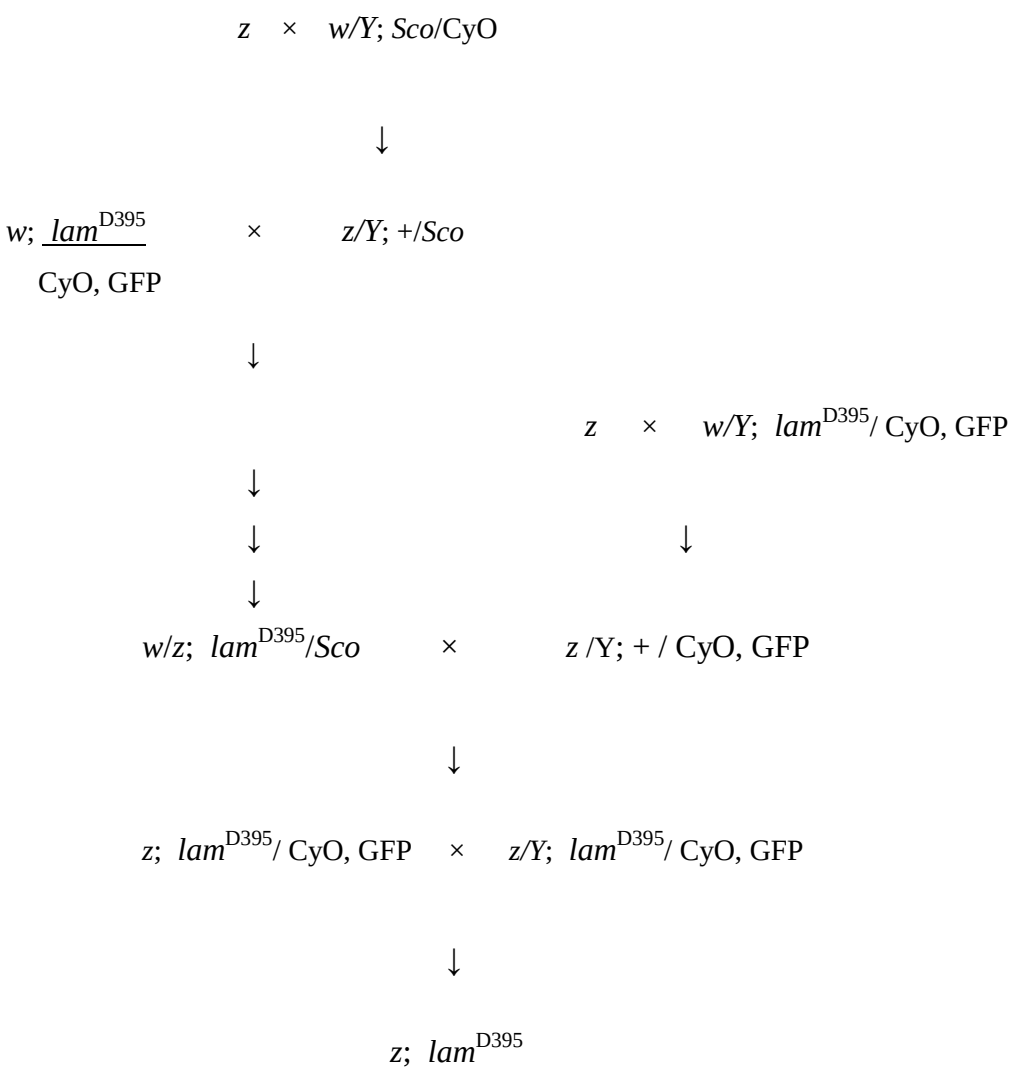
genotypes to those of *wt*, and *lam* and *zeste* single mutants represented by previous studies (see reference [8]) and stock number one. Single mutant means that in an individual there is only one or the other of *lam* or *zeste* mutations respectively. Individuals that are homozygous for *zeste* and homozygous or heterozygous for *lam* ^{D395} are called double mutants since they have both *lam* and *zeste* mutations (See table 1).

Table 1. Comparisons of eye colour phenotypes were made between the following genotypes. The table only shows chromosomes one and two.

A) Homozygous for *zeste* and homozygous for *lam* ^{D395}
z/z ; *lam* ^{D395} / *lam* ^{D395}
B) Homozygous for *zeste* and heterozygous for *lam* ^{D395}
z/z ; *lam* ^{D395} / CyO,GFP
C) Hemizygous for *zeste* and homozygous for *lam* ^{D395}
z/Y ; *lam* ^{D395} / *lam* ^{D395}
D) Hemizygous for *zeste* and heterozygous for *lam* ^{D395}
z/Y ; *lam* ^{D395} / CyO,GFP
E) Wildtype (*wt*) without either of the mutations
+/+ ; +/+
F) *lam* ^{D395} single mutants showing no transvection effect (previous studies, see reference [8])
+/+ ; *lam* ^{D395} /+
+/+ ; *lam* ^{D395} / *lam* ^{D395}
G) *zeste* single mutants showing transvection on paired *white* loci (the stock number one)
z/z ; +/+

The crossing scheme was carried out as follows (virgin females on the left, males on the right):

Crossing scheme



Progeny: *z*; *lam*^{D395} progeny was obtained and analysed.

3 Results

***A) z* × w; *Sco*/CyO**	
♂ \ ♀	z ; +
w ; Sco	z/w ; +/Sco
w ; CyO	z/w ; +/CyO
Y ; Sco	z/Y ; +/Sco
Y ; CyO	z/Y ; +/CyO

B) w; *lam*^{D395}/ CyO, GFP × *z*; +/*Sco*		
♂ \ ♀	w ; lam	w ; CyO, GFP
z ; +	z/w ; +/lam	w/z ; +/ CyO,GFP
z ; Sco	z/w ; Sco/lam	w/z ; Sco/ CyO,GFP
Y ; +	w/Y ; +/lam	w/Y ; +/ CyO,GFP
Y ; Sco	w/Y ; Sco/lam	w/Y ; Sco/ CyO,GFP

TABLE 2: Progenies obtained for the first four crosses in the crossing scheme. A) The first crossing. Males selected for the next crossing, B, are shaded with yellow. B) Females selected for the crossing D are shaded with yellow. C) Males selected for crossing, D are shaded with yellow. D) Males and females selected for the last crossing (see table 3) are shaded with yellow.

***C) z* × w; *lam*^{D395}/ CyO, GFP**	
♂ \ ♀	z ; +
w ; lam	z/w ; +/lam
w ; CyO, GFP	z/w ; +/CyO,GFP
Y ; lam	z/Y ; +/lam
Y ; CyO, GFP	z/Y ; +/CyO,GFP

D) w/z; *lam*^{D395}/*Sco* × *z*; + /CyO, GFP				
♂ \ ♀	w ; lam	w ; Sco	z ; lam	z ; Sco
z ; +	z/w ; +/lam	z/w ; +/Sco	z ; +/lam	z ; +/Sco
z ; CyO,GFP	z/w ; lam/ CyO,GFP	z/w ; Sco/ CyO,GFP	z ; lam/ CyO,GFP	z ; Sco/ CyO,GFP
Y ; +	w/Y ; +/lam	w/Y; +/Sco	z/Y ; +/lam	z/Y ; +/Sco
Y ; CyO,GFP	w/Y ; lam/ CyO,GFP	w/Y ; Sco/ CyO,GFP	z/Y ; lam/ CyO,GFP	z/Y ;Sco/ CyO,GFP

The z/Y; +/Sco offspring selected from the *z* × w; *Sco*/CyO cross (see table 2A) had red eyes and *Sco* wings. The z/w; Sco/lam offspring selected from the w; *lam*^{D395}/ CyO, GFP × *z*; +/*Sco* cross (see table 2B) had *Sco* wings. The z/Y; +/CyO, GFP offspring selected from the *z* × w; *lam*^{D395}/ CyO, GFP cross (see table 2C) had curly wings. Choosing the flies for the first four crosses was straightforward. In contrast choosing the right kind of flies for the final cross was more complicated. The w/*z*; *lam*^{D395}/*Sco* × *z*; + /CyO, ActGFP cross (see table 2D) gives offspring with phenotypes that look the same. Both *z*; *lam*^{D395}/ CyO, ActGFP- and *z*; *Sco*/ CyO, ActGFP- individuals had curly and *cut* wings, and red eye colour.

The parents for the final cross *z*; *lam*^{D395}/ CyO, ActGFP × *z*; *lam*^{D395}/ CyO, ActGFP (see table 3) were selected from this offspring, but the presence of *z*; *Sco*/ CyO, ActGFP among these parent flies in the final cross had to be ruled out first. This was done by putting only one pair of parents with curly, *cut* wings, *scute* bristles and red, *echinus*, *zeste* eyes into each tube for the final cross (see table 3). Red *zeste* eyes of *z*; *lam*^{D395}/ CyO, ActGFP individuals are due to GFP-allele introduced with a *w+* P-element. The presence of *scute* and *echinus*, which flank *zeste* ensured that z^1 had not been lost by recombination (See fig. 4).

TABLE 3. Final offspring to be analyzed;

***z*; *lam*^{D395}/ CyO, GFP × *z*; *lam*^{D395}/ CyO,GFP**		
♂ \ ♀	z ; lam	z ; Cyo,GFP
z ; lam	z ; lam	z ; lam/ Cyo,GFP
z ; Cyo, GFP	z ; lam/ Cyo,GFP	z ; Cyo,GFP
Y ; lam	z/Y ; lam	z/Y ; lam/ Cyo,GFP
Y ; Cyo, GFP	z/Y ; lam/ Cyo,GFP	z/Y ; Cyo,GFP

In the final offspring, all females are expected to be homozygous for *zeste*, one fourth of females homozygous for *lamin*, half of females heterozygous for *lamin* and one fourth of females die because they are homozygous for CyO ActGFP; while all males are expected to be hemizygous for *zeste*, one fourth of males homozygous for *lamin*, half of males heterozygous for *lamin* and one fourth of males dies because they are homozygous for CyO ActGFP. Flies

that are homozygous for *lamin* survive only when they are separated at the larvae stage into their own tubes. This is done because when the weaker individuals that are homozygous for *lamin* have to compete for space and nutrition with the stronger individuals that are only heterozygous for *lamin*, the weaker ones get competed out. The effect of homozygosity versus heterozygosity of *lamin* on *zeste*-dependent transvection was analysed by this cross.

To further rule out presence of *Sco*–individuals in the final cross the offspring of these individual crosses were carefully investigated. *Sco*-heterozygous individuals lack all scutellar bristles [13a]. One or both postscutellar bristles are sometimes present, but they are shorter and thinner than normal. The ocellar (head) and humeral (leg) bristles are often absent. *Sco*-heterozygotes have good viability and fertility. Since none of these kinds of individuals could be seen in the crosses made between the final CyO ActGFP progeny it was safe to say that the final non-GFP-bearing progeny was indeed *z*; lam^{D395} and did not carry *Sco* on the second chromosome.

A total of 21 crosses with single male and single female parent in the tube were carried out (See table 4). 14 of these crosses did not succeed to produce offspring. The offspring of two of the crosses could not have the right kind (*z*; lam^{D395}/ CyO,GFP) parents. This conclusion was drawn because about 9-15 non-CyO, ActGFP individuals (with very high motility) to about 16-19 CyO, ActGFP individuals were counted in these two crosses. Very few non-CyO, ActGFP individuals were expected in the final progeny since these *z*; *lam*-individuals have a low survival rate. From five of the crosses the offspring were separated at the larvae stage. Only two of these crosses succeeded to produce adult non- CyO, ActGFP individuals for analysis. Two *z*; lam^{D395} females and three *z*; lam^{D395} males were produced.

TABLE 4: Results of the final crosses.

Number of crosses	Status
21	total number of crosses
7	produced offspring
5	right kind parents, the offspring were separated at the larvae stage
2	produced adult non- CyO, ActGFP individuals for analysis (2 females, 3 males)

Fig. 5 *z*; *lam*^{D395} phenotypes.
The observed phenotypes of the off-spring from the final cross were the same as for z^1 single mutants (stock one), with which the whole crossing scheme was started off with. Male on the left has non-CyO, *cut* wings and *red* eye colour and the female on the right has non-CyO, *cut* wings and *yellow* eye colour.

The *z*; *lam*^{D395} females had non-CyO, *cut* wings and *yellow* eye colour. The males had non-CyO, *cut* wings and *red* eye colour since they were hemizygous to *zeste*^{1}. In conclusion the female yellow eye colour was the same for *z; lam* double mutants as for z^1 single mutants, with which the whole crossing scheme was started off with (See figure 5).

4 Discussion

The *z*; *lam*^{D395} females had non-CyO, *cut* wings and *yellow* eye color. The males were used as a negative control since they are hemizygous to *zeste*^{1}. Female yellow eye colour was the same for *z; lam* double mutants as for *z*^{1} single mutants. Transvection at paired *white* loci does occur in *lam*^{D395} mutants in the presence of the *zeste*^{1} gain-of-function mutation.

Females homozygous for *zeste*^{1} mutation have yellow eyes and hemizygous males have red eyes. [13b] Reduction of *white* expression causes the yellow eye colour of the females, but this occurs only when the *white* genes can pair, in other words transvection. No effect on transvection in loss-of-function *lamin* mutants alone has been shown. [8]

The advantage of using the *zeste* stock selected here is that the other mutations that are flanking the *zeste* gene give visible phenotypes in the adult fly so that the chromosome with the *zeste* gene can be followed even though transvection is not visible. If recombination occurs in the chromosome, *sc* (*scute*) and/or *ec* (*echinus*) markers will disappear because they are flanking *zeste* (See fig. 4). *white* gene is situated at position 3B6, *scute* at 1A8, *zeste* at 3A3, *echinus* at 3F3 and *cut* at 7B4 to 7B6 on chromosome number one. [13]

A total of 21 crosses with single male and single female parent in the tube were carried out. The final offspring were separated at the larvae stage, since flies that are homozygous for *lamin* survive only when they are separated at this stage into their own tubes. The low motility of the larvae and their slow development as well as low pupation height were indicative to presence of *lamin* double mutants, as described by Muñoz-Alarcón, A. et *al*. [8]

The future prospects of studying transvection are of interest because of new examples of transvection and other homology effects caused by homologous nucleic acid sequences affecting chromosome and gene function. Homology effects are a class of regulatory mechanisms that are caused by sequence homology, e.g. dsRNA mediates transcriptional or post-transcriptional silencing. Other examples of homology effects are parental imprinting, allelic exclusion, and many forms of dosage compensation.

Examples of parental imprinting are human epigenetic diseases like Prader-Willi syndrome (PWS; OMIM 176270) and Angelman syndrome (AS; OMIM 105830) primarily caused by the same 5- to 6-Mb deletion in 15q11-q13, but they result in very different phenotypes. Deletion of the PWS/AS cluster of imprinted genes in chromosome 15 results in PWS when paternally inherited, but in AS when maternally inherited. Characteristics for PWS are reduced motor function, obesity and mental deficiencies, while AS patients are thin and hyperactive and have unusual seizures and repetitive symmetrical muscle movements, as well as mental deficiency. [7] Uniparental disomy (UPD) causes 25% of PWS and 2-5% of AS cases [14]. PWS is caused by maternal UPD (maternally inherited alleles are doubled) whereas AS is caused by paternal UPD (paternally inherited alleles are doubled).

Transvection could provide means of reactivating normal function of the genes involved in these diseases. Diseases caused by defective homology effects could be corrected, and *trans*-gene mediated therapies could benefit from further research. But these gene replacement therapies can be used only when the disease causing defects are distinct and isolated. The mechanisms controlling homologous sequence alignment need to be known for more effective gene targeting in these therapies. The importance of methods derived from transvection models becomes evident when the disease causing defects are large and more complex. In these cases it would be more convenient to use *trans*-acting sequences to correct gene expression. ([5] and references therein)

5 Conclusion

***zeste* dependent transvection** does occur in loss-of-function *lamin* mutants. Nevertheless the amount of individuals that were obtained for analysis was so small that no statistically relevant conclusions can be drawn. More research on mechanisms used by *zeste* proteins in DNA binding as well as the role of Lamins in the cell nucleus is needed. Transvection and other homology effects appear to be valuable tools for understanding epigenetic diseases and ultimately finding treatments for human disease.

6 References

1. **Lewis, E. B.**, The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. 1954, The American Naturalist 88: 225-239.
2. **Gohl, D. et *al.***, Enhancer blocking and transvection at the Drosophila apterous locus, Genetics. 2008 Jan; 178(1):127-43, doi:10.1534/genetics.107.077768, PMID: 9724774
3. **Rassoulzadegan, M. et *al.***, Transvection effects involving DNA methylation during meiosis in the mouse, *The EMBO Journal* (2002) **21,** 440–450, doi:10.1093/emboj/21.3.440.
4. **McKee, B. D.,** 2004 Homologous pairing and chromosome dynamics in meiosis and mitosis. Biochim Biophys Acta 1677: 165-80.
5. **Wu, C.-t. et *al.***, 1999 Transvection and other homology effects. Curr Opin Genet Dev 9: 237-246.
6. **Stevens, N. M.**, 1908 A study of the germ cells of certain Diptera with reference to the heterochromosomes and the phenomena of synapsis, J Exp Zool 5: 359-374.
7. **Brooker, R. J.,** Genetics- Analysis & Principles, Third edition, pp.161-174, 430.
8. **Muñoz-Alarcón, A. et *al.***, 2007 Characterization of *lamin* Mutation Phenotypes in *Drosophila* and Comparison to Human Laminopathies, PLoS ONE. 2007; 2(6): e532. doi: 10.1371/journal.pone.0000532.
9. **Pirrotta, V. et *al.***, Structure and sequence of the Drosophila *zeste* gene, EMBO J. 1987 March; 6(3): 791–799, PMCID: PMC553464.
10. **Duncan I.W**., Transvection effects in Drosophila, Annu Rev Genet. 2002; 36:521-56, doi: 10.1146/annurev.genet.36.060402.100441.
11. **Pirrotta V.,** 1999 Transvection and chromosomal trans-interaction effects, Biochim Biophys Acta 1424: M1-8.
12. **Kennison, J. A. et *al.***, 2002 Transvection in Drosophila, Adv Genet 46: 399-420.
13. **www.flybase.org**
    a) http://www.flybase.org/reports/FBal0013044.html "Sco-phenotype"
    b) http://flybase.org/reports/FBal0018822.html "zeste-phenotype"
14. **Allis, D. C. et *al.*,** Epigenetics, 2007, pp. 435-441.

7 Acknowledgements

I wish to thank my supervisor Per Kylsten, Maja Pavlovic for supplying stocks and Fuad Ali for helping with some of the practical matters.