CHAPTER 1.

Background and aim of the thesis
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Background of the thesis

To prevent penetration of harmful mutations in their progeny, eukaryotic cells have developed an intricate genome surveillance mechanism. Upon DNA damage cells halt their cell cycle progression and activate appropriate repair mechanisms or undergo apoptosis corresponding to the severity of the damage and the type of damage inflicted. Defects in these and associated cellular processes can cause severe alterations in cell cycle regulation and disrupt DNA damage repair, features which are associated with several diseases such as neurodegenerative disorders and cancer\textsuperscript{1-5}. Many genes that are involved in cell cycle control, sensing DNA damage or genes that are required for recombination and DNA damage repair have been identified on the basis that when mutated, the cells or organisms are hypersensitive to exogenously applied DNA damaging agents such as ultra violet light (UV), ionizing radiation (IR), cisplatin, nitrogen mustard, hydroxyurea (HU) or methyl methanesulfonate (MMS).

Over the past two decades many Drosophila genes involved in genome maintenance and repair have been cloned and characterized\textsuperscript{6-8}. Furthermore, these studies revealed that the mechanisms to prevent genomic instability in the fly resemble those observed in yeast and mammalian systems. This cross-species conservation is not only observed in DNA damage response pathways, but is also clearly demonstrated by the notion that numerous major human diseases have been successfully modeled in Drosophila and provided a wealth of information about disease pathogenesis and possible treatments. Drosophila models of human disease include several neurodegenerative disorders\textsuperscript{9}, heart and muscle disease\textsuperscript{10,11}, mitochondrial disease\textsuperscript{12,13}, diabetes\textsuperscript{14} and cancer\textsuperscript{15}.

The ease of culturing fruit flies together with the potential for genetic manipulation makes the Drosophila an attractive organism to perform large scale forward genetic screens. Using transposon based mutagenesis\textsuperscript{16} it is relatively easy to create large libraries of mutant lines and mutant loci can be easily identified after plasmid rescue analysis\textsuperscript{17}. Using P element mutagenesis many essential genes involved in a variety of processes such as meiotic recombination, synaptogenesis or central nervous system development have been isolated\textsuperscript{18-25}.

Drosophila lines that carry mutations in cell cycle checkpoint or DNA repair genes are frequently hypersensitive to DNA damaging agents and are female sterile, and this combination of mutagen sensitivity and female sterility was already recognized by Smith in the mid-seventies\textsuperscript{26}. Mutations in cell cycle checkpoint genes mei-41/dATR, mus304/dATRIP and grapes (grp/dChk1) cause sensitivity to UV, MMS, HU and IR\textsuperscript{26-32}. Likewise, flies that carry mutations in the DNA repair genes spin-A, spin-C or okra are also hypersensitive for DNA damage agents\textsuperscript{33-36}. Besides mutagen sensitivity the grp/dChk1, mus304, mei-41/dATR, spin-A, spin-C and okra mutants display a female sterile phenotype\textsuperscript{5,26,31,37}. Although many independent screens have been carried out to identify either female sterile mutations or mutagen sensitive mutations, only limited (two) screens have been performed using both characteristics as selection criteria\textsuperscript{35,38}. These two screens only uncovered new alleles of mei-41/dATR, two novel loci on the X chromosome (unknown genes) and the mus301/spindle-C gene. Because only limited screens have been performed using the combination mutagen sensitivity and female sterility as selection criteria, it is likely that these screens were not saturated and many candidate genes were missed.
Aim of the thesis
Using *Drosophila* as a model system we performed a genetic screen to identify novel genes that, when mutated, cause sensitivity to exogenously applied DNA damaging agents. In order to reduce the amount of mutants to be screened, sensitivity to DNA damaging agents was tested in collections that were previously pre-selected for female sterility. For our screen we used female sterile collections kindly provided by S. Hawley (Stowers Institute, USA) and A. Ephrussi (EMBL, Germany) and a collection of mutants generated by M. Ritsema in our laboratory. After the identification of various DNA damage hypersensitive mutants, cloning and characterization of the mutated genes was initiated. Two mutant fly lines that were recovered from the screen, E709 and MR65, are described in detail in this thesis. Both lines have a mutation in a gene that does not directly link to pathways that one would expect to find, such as genes involved in cell cycle control, signaling, DNA repair or DNA metabolism. Line E709 carries a mutation in the *Drosophila* transcriptional Mediator component 31 (*dMED31*) gene, while in MR65 the *Drosophila* (R)-4'-phospho-N-pantothenoylcysteine synthetase (*dPPCS*) gene is mutated. The *dMED31* gene has an unforeseen function during early embryonic development and especially this phenotype was analyzed in detail. For the *dPPCS* mutant, both the female sterile and the DNA damage hypersensitive phenotype were analyzed. The function of *dPPCS* in surviving DNA damage is also unexpected and interestingly, sensitivity to DNA damage is linked to neuronal dysfunction in *dPPCS* mutant flies. In the last chapter the role of *dPPCS* in female fertility and in maintaining neuronal and DNA integrity is discussed.

Outline of the thesis
CHAPTER 2. Identification of novel *Drosophila melanogaster* loci that cause mutagen sensitivity and female sterility
This chapter describes the results of a forward genetic screening procedure that was used to identify potential novel loci that are required to survive DNA stress and are essential for female fertility. A library of single P element insertion lines was generated by transposon mutagenesis and this library was expanded with existing female sterile collections. Mutant lines were screened for sensitivity to hydroxyurea and ionizing radiation. From this screen we recovered 17 potential novel loci that cause hypersensitivity to DNA damaging agents when mutated. Three of the mutant lines also exhibited female fertility defects. Because the combination mutagen sensitivity and female sterility is a characteristic of many mutant alleles of genes that are involved in checkpoint signaling and DNA damage repair, mutant lines E709 and MR65, which displayed sensitivity to DNA damaging agents and suffered from female fertility defects, were analyzed in more detail and we describe the strategies used to confirm phenotypic linkage with the mutation in these mutant lines.

CHAPTER 3. Establishment of cell fate during early *Drosophila* embryogenesis requires transcriptional Mediator subunit *dMED31*
The *dMED31* locus was recovered from a genetic screen as a gene required to survive DNA damage and for female fertility. In this chapter we analyzed the nature of the female sterile phenotype of a mutation in the *dMED31* locus. We show that a mutation in *dMED31* caused abnormal embryonic development likely due to the abnormal expression of several fate determinants during early embryogenesis. Furthermore, we discuss that *dMED31*, as part of the larger transcriptional Mediator complex, might be required for the proper initiation of zygotic expression and patterning along the anterior-posterior axis.
CHAPTER 4. *De novo* CoA biosynthesis is required to maintain DNA integrity in a *Drosophila* model of Pantothenate Kinase-Associated Neurodegeneration

In this chapter the *dPPCS* gene, which was identified as a gene required to survive DNA damage and for female fertility, is characterized. *dPPCS* encodes the second enzyme in the *de novo* CoA biosynthesis route and we demonstrated that mutations in the first (*dPANK*) and the final (*dPPAT-DPCK*) enzyme in this route also causes hypersensitivity to DNA stress and also induces fertility defects. In humans, mutations in *PANK2* are associated with the neurodegenerative disorder PKAN. We established that the *Drosophila dPANK*, *dPPCS* and *dPPAT-DPCK* mutants, which displayed neuronal dysfunction, can be used to study how impaired CoA synthesis elicits a neurological phenotype in animals. Mutations in the *de novo* CoA biosynthesis disrupted lipid metabolism and also affected DNA integrity. Interestingly, impaired DNA integrity was linked to neuronal dysfunction in CoA mutant flies. Together our findings demonstrate a novel link between disrupted CoA biosynthesis, altered lipid metabolism, impaired DNA integrity, and neuronal dysfunction in higher eukaryotes.

CHAPTER 5. *Drosophila* phosphopantothenoylcysteine synthetase is required for tissue morphogenesis during oogenesis

In this chapter we analyzed the effect of a mutation in the *dPPCS* gene on oogenesis to understand the nature of the female sterile phenotype. In *dPPCS* mutants normal tissue morphogenesis was disrupted, likely as a consequence of aberrant actin remodeling. Disrupted cell organization in turn likely induced abnormal cell specification triggered by the Notch and Gurken signaling routes. Defects in tissue organization and actin remodeling coincided with abnormal membrane levels and localization of PtdIns(4,5)P₂. Because PtdIns(4,5)P₂ is a lipid derived second messenger, essential for all processes that require actin remodeling, this indicates that the primary defect underlying the morphogenesis defects in *dPPCS* mutants is aberrant PtdIns signaling. Conversion of PtdIns(4,5)P₂ to PtdIns(3,4,5)P₃ results in phosphorylation of Akt/PKB, and consistent with a reduction in membrane levels of PtdIns(4,5)P₂, *dPPCS* mutant ovaries contained reduced levels of phosphorylated Akt/PKB. Interestingly, *dPPCS* was also required for sensory cell and vein cell patterning, indicating that *dPPCS* is required for morphogenesis of various tissues. Because *dPANK* and *dPPAT-DPCK* mutant females also exhibited abnormal egg chamber development and patterning defects, likely as a consequence of aberrant actin dynamics, impaired *de novo* CoA biosynthesis in general affects actin remodeling, possibly due to abnormal PtdIns signaling.

CHAPTER 6. General discussion and summary

**Coenzyme A: The metabolic key to many fundamental processes in eukaryotic systems**

Although CoA is well known and its essential role is widely accepted, the physiological implications of altered *de novo* CoA biosynthesis in metazoan systems remains poorly understood and research in this area has never been initiated. In this chapter we reviewed our current knowledge about the physiological implications of disrupted CoA biosynthesis in metazoans with a focus on *Drosophila*. Mutations in the *de novo* CoA biosynthesis route give rise to a plethora of phenotypic defects. CoA constitutes a major cofactor required for various cellular processes and we discuss which CoA dependent pathways might be responsible for the complex phenotypic characteristics.
Introduction and aim of the thesis

LITERATURE CITED