EXPRESSION OF TSF 1 (TRANSFERRIN 1) AND TSF 2 (TRANSFERRIN 2) IN DROSOPHILA MELANOGASTER EXPOSED TO X-RAYS AND THERAPEUTIC RADIATION.

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BENIN CITY.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS), UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISOR: Dr. A. I. ARUOMAREN

SEPTEMBER, 2025.

CERTIFICATION

This is to certify that this a Project work carried out by **BABATUNDE, SAMSON ODUNAYO** with the matriculation number **BMS1903108** under the supervision of **DR. A. I. ARUOMAREN** in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfilment of the requirement for the Award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

I dedicate this project work to God Almighty for his strength and for the success of this work and his guidance throughout my course of study.

ACKNOWLEDGMENT

I want to express my deepest gratitude to God Almighty for granting me the strength, resources and wisdom needed for this work and for seeing me through this project work.

My profund gratitude goes to my supervisor, **DR. A. I. ARUOMAREN**, for your guidance, patience, and insightful advice, your expertise and encouragement have profoundly shaped this work, and I am truly grateful for your mentorship.

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ABSTRACT

Ionizing radiation is known to trigger a wide range of genetic and epigenetic modifications that disrupt cellular equilibrium and activate stress response pathways. This study aimed to evaluate the transcriptional behavior of two iron metabolism–associated genes, Transferrin 1 (TSF1) and Transferrin 2 (TSF2), in Drosophila melanogaster subjected to X-ray and low-dose CT room radiation. These transferrin genes are central to maintaining iron balance and epithelial stability, making them valuable candidates for assessing molecular alterations induced by radiation exposure. Adult flies were exposed to radiation for 7 and 14 days, after which total RNA was extracted and analyzed using semi-quantitative RT-PCR, with GAPDH serving as an internal control for normalization. The results revealed a consistent and significant elevation in TSF1 expression under both radiation types. For instance, expression levels increased from control values of 67.77 ± 1.84 to 80.14 ± 1.00 at day 7 and further to 85.97 ± 1.43 by day 14 under X-ray exposure. A similar trend was observed in CT room–exposed flies, where expression rose to 80.20 ± 0.72 at day 7 and 86.28 ± 1.85 at day 14. This persistent upregulation suggests that TSF1 plays a protective role by enhancing iron sequestration and transport, thereby reducing the generation of reactive oxygen species (ROS) and limiting oxidative injury. In contrast, TSF2 demonstrated a biphasic expression profile. An initial increase was recorded at 7 days post-exposure (72.23 ± 2.39 following X-rays), but expression declined sharply at 14 days, particularly in CT-exposed flies (57.76 ± 1.94) relative to control levels (61.96 ± 1.14). In Conclusion, This pattern indicates an early, short-lived adaptive response followed by suppression, possibly reflecting tissue vulnerability and compromised epithelial barrier function under chronic radiation stress.

CHAPTER ONE
INTRODUCTION

1.0 Background of the Study

Iron plays a vital role as a micronutrient, supporting a range of physiological activities such as oxygen transport, DNA replication, cellular respiration, and various enzymatic functions (Andrews, 2008). Yet, the reactive properties of iron can also make it potentially harmful, as its mismanagement may result in oxidative stress and cellular damage. For this reason, living organisms have developed intricate systems to ensure iron levels remain balanced through regulated uptake, distribution, retention, and utilization (Mandilaras *et al.,* 2013).

Within the fruit fly, Drosophila melanogaster, transferrin proteins are central to iron movement and maintaining homeostasis. The major iron-binding agent, Transferrin 1 (TSF 1), is essential for systemic iron transport, carrying iron from the digestive tract to storage tissues like the fat body (Guiran *et al*., 2019). TSF 1 binds ferric ions (Fe^{3+}) with notable affinity, ensuring their safe passage in the hemolymph and minimizing free iron, which otherwise could catalyze harmful oxidative reactions (Weber, Kanost, and Gorman, 2020). Suppression of TSF 1 leads to iron build-up in the gut and a shortage elsewhere in the organism, confirming its key role in systemic iron distribution (Guiran *et al.*, 2019). Furthermore, genetic interactions between TSF 1 and ferritin (the primary cellular iron store) help maintain iron equilibrium and defend cells from oxidative damage (Guiran *et al*., 2019).

In contrast, Transferrin 2 (TSF 2) has a different function. Rather than systemic iron transport, TSF 2 is found in epithelial septate junctions, where it maintains the paracellular barrier of tissues (UniProt Consortium, 2025).

Studying how radiation affects TSF 1's expression and epigenetic control is particularly significant due to its role in iron sequestration and host defense. By limiting iron availability, TSF 1 restricts pathogen growth—a process vital for immune system effectiveness (Marra *et al.,* 2021). Changes in TSF 1 regulation following radiation could therefore impact iron management and immune competence. Additionally, proteins like Evi5, which influence the cellular movement of TSF 1 and ferritin, might be affected by radiation and disrupt iron metabolism (Soltani *et al*., 2024).

Epigenetic regulation of TSF genes under radiation stress could involve modifications to histones and chromatin accessibility that affect transcription—a dynamic potentially inherited across generations (Perspectives on the Drosophila melanogaster Model, 2024).Moreover, since TSF 1 and TSF 2 serve distinct roles—one in systemic iron distribution, the other in epithelial barrier function—it is likely that radiation affects them differently, with potential implications for iron homeostasis and tissue barrier strength (UniProt Consortium, 2025). Understanding these differential effects is crucial for grasping the broader consequences of radiation on metal regulation and organismal health.

To summarize, profiling the genetic and epigenetic landscapes of TSF 1 and TSF 2 after radiation exposure in Drosophila melanogaster addresses a key scientific question at the crossroads of iron biology, radiation effects, and epigenetics. Insights from this research could help inform new strategies to protect against radiation-induced harm and optimize outcomes in therapeutic settings.

1.1 Statement of the Problem

Proper regulation of iron is essential for cellular and whole-organism health, with transferrins playing a key role in transporting and distributing iron. In the fruit

fly Drosophila melanogaster, the protein TSF1 serves primarily as the major transporter of iron, moving it from the digestive organs to the fat body, in collaboration with ferritin to regulate iron levels (Morciano *et al*., 2018). On the other hand, TSF2's main function is associated with forming epithelial septate junctions that maintain tissue structure rather than managing iron transport (Scientific Archives, 2024). While both proteins are recognized for their biological significance, the mechanisms governing their genetic and epigenetic regulation, especially under stressful conditions such as exposure to radiation, are not yet fully delineated.

Exposure to X-rays and therapeutic radiation is known to cause DNA damage along with oxidative stress and epigenetic modifications, which in turn can influence gene expression patterns and cellular activities (Antosh *et al*., 2014). Because TSF1 plays a crucial role in managing iron availability during infections, radiation-induced changes affecting its expression or epigenetic landscape could significantly disrupt iron balance and immune function.

Furthermore, radiation during developmental stages in Drosophila has been shown to trigger enduring immune responses and activate discrete gene networks. Although these responses are initially protective, they may eventually contribute to adverse effects, including neurodegeneration later in life (Trinca, 2022). The involvement of TSF proteins in such lasting physiological changes has not been thoroughly studied, indicating a critical gap in knowledge regarding how radiation influences iron homeostasis through genetic and epigenetic pathways.

1.2 Justification of the Study

Iron homeostasis is critically important to the proper functioning of cells and entire organisms, with transferrins serving as key proteins for iron transportation and distribution. Within Drosophila melanogaster, TSF1 functions as the principal iron

carrier, moving iron from the digestive system to the fat body and coordinating iron regulation alongside ferritin (Morciano *et al.*, 2018). Conversely, TSF2 predominantly contributes to the formation of epithelial septate junctions, which maintain tissue integrity, rather than iron transport (Scientific Archives, 2024). Despite clear biological roles, the precise genetic and epigenetic regulation of TSF1 and TSF2 under stress conditions such as radiation exposure remains incompletely understood.

It is well-established that X-ray and therapeutic radiation cause DNA damage, oxidative stress, and epigenetic alterations which can broadly influence gene expression and vital cellular functions (Antosh *et al.,* 2014). Although radiation is known to stimulate innate immune mechanisms and alter gene regulatory pathways, its specific effects on iron metabolism genes like TSF1 and TSF2 have yet to be fully elucidated (Alexandrov *et al.,* 2022). Considering the crucial function of TSF1 in balancing iron availability during infectious challenges, radiation-triggered changes in its expression or epigenetic patterns could profoundly affect both iron homeostasis and immune defense.

1.3 Aim of the Study

This study seeks to determine the expression pattern of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster following exposure to X-ray and therapeutic types of radiation.

1.4 Specific Objectives

1. To investigate the effect of X-ray and diagnostic radiation on the expression level of TSF 1 gene in drosophila melanogaster in comparing with the control Flier
2. To evaluate the effect of X-ray and diagnostic radiation on the expression level of TSF 2 gene in drosophila melanogaster in comparing with the control Flier

3. To compare how the genetic and epigenetic markings of TSF 1 and TSF 2 differ between groups exposed to X-rays and those exposed to therapeutic radiation by the end of the observation cycle.

1.5 Research Questions

1. In what ways does exposure to X-ray and therapeutic radiation influence the expression levels of TSF 1 and TSF 2 genes in Drosophila melanogaster?
2. What specific epigenetic changes—such as modifications to DNA methylation or histone proteins—have on TSF 1 and TsSF 2 genes after radiation treatment in Drosophila?
3. Are genetic and epigenetic alterations caused by radiation in TSF 1 and TSF 2 inherited in subsequent generations of Drosophila melanogaster?

1.6 Research Hypotheses

Null Hypothesis (H_0):

1. X-ray or therapeutic radiation does not produce significant changes in the genetic expression of TSF 1 and TSF 2 genes in Drosophila melanogaster .
2. There is no specific epigenetic changes—such as modifications to DNA methylation or histone proteins— on TSF 1 and TSF 2 genes after radiation treatment in Drosophila.
3. There is no genetic and epigenetic alterations caused by radiation in TSF 1 and TSF 2 in Drosophila melanogaster.

Alternative Hypothesis (H_1):

1. Exposure to X-ray and therapeutic radiation does cause significant alterations in the genetic expression and/or epigenetic profiles of TSF 1 and TSF 2 genes in Drosophila melanogaster.
2. There is a specific epigenetic changes—such as modifications to DNA methylation or histone proteins— on TSF 1 and TSF 2 genes after radiation treatment in Drosophila.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Ionizing Radiation

Ionizing radiation (IR), which includes X-rays and radiation used in therapy, is a type of high-energy radiation capable of causing both direct and indirect harm to cellular macromolecules, especially DNA. The damage happens through direct energy absorption by DNA and indirectly via reactive chemical species—mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS)—produced primarily by the radiolysis of water molecules within cells (Beyersmann and Hartwig, 2020). These reactive species are responsible for inducing DNA strand breaks, base alterations, and clustered lesions that, if left unrepaired or improperly corrected, can result in mutations and genomic instability (Beyersmann and Hartwig, 2020). The pattern and degree of DNA damage vary depending on the radiation type: low linear energy transfer (LET) radiation typically inflicts damage indirectly through free radicals, whereas high LET radiation produces damage more directly to the DNA molecule (Beyersmann and Hartwig, 2020).

The fruit fly, Drosophila melanogaster, serves as an essential model organism to examine the biological ramifications of ionizing radiation because it shares conserved pathways for DNA damage response and offers practical genetic manipulability. Research has revealed that exposure to IR in Drosophila activates DNA damage responses such as cell cycle arrest, stimulation of repair mechanisms, and apoptosis in both somatic and germline cells (Katsiarimpa *et al.,* 2014). For example, irradiation at high doses induces arrest at the S and G2 phases in mitotically active germline cells, regulated by checkpoint kinases like Grp/Chk1 and Mnk/Chk2, with the ATR homolog, Mei-41, being necessary for the S-phase checkpoint (Katsiarimpa *et al*., 2014). These mechanisms are indispensable for preserving genome stability throughout egg development and overall organismal growth.

In addition to causing genetic damage, IR also leads to epigenetic changes that affect gene regulation and chromatin organization. These modifications include alterations to DNA methylation patterns, histone post-translational modifications, and chromatin remodeling processes that can endure well beyond the initial radiation exposure and may contribute to effects observed in subsequent generations (Beyersmann and Hartwig, 2020). In Drosophila, specific transposable elements, such as the hobo elements, play a role in radiation-induced genomic instability and epigenetic control, with higher activity of full-length hobo copies correlating with increased sensitivity to radiation and delayed radiation effects (Kudryavtseva *et al.,* 2019).

The iron-binding proteins Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster are vital for regulating iron balance and maintaining tissue structure. TSF 1 acts as the main transporter for systemic iron, facilitating its transfer between the digestive tract and the fat body, and genetically interacts with ferritin to control iron storage and the oxidative stress response (Guiran *et al*., 2019; Marra, Masson, and Lemaitre, 2021). Conversely, TSF 2 contributes to creating epithelial septate junctions, playing a key role in sustaining the paracellular barrier and tissue cohesion (UniProt Consortium, 2025). Since ionizing radiation prompts oxidative stress and DNA lesions that can disrupt iron metabolism and cellular homeostasis, it is essential to uncover how radiation influences the genetic and epigenetic regulation of TSF 1 and TSF 2.

Recent studies indicate that oxidative stress from radiation exposure may modify iron availability and signaling pathways, potentially changing TSF gene expression and their epigenetic status (Tang and Zhou, 2013). Furthermore, epigenetic modifications—such as histone methylation and acetylation around TSF genes—can affect their transcriptional activity, thereby impacting iron homeostasis and immune system functions following radiation exposure (Beyersmann and Hartwig, 2020; Perspectives on the Drosophila melanogaster Model, 2024). Investigations in Drosophila can thus illuminate mechanisms in radiation biology, disorders of iron metabolism, and the pursuit of radioprotective interventions.

2.1.1 Varieties and Origins of Ionizing Radiation

Ionizing radiation (IR) refers to energetic particles or electromagnetic waves that possess enough energy to eject tightly bound electrons from atoms, resulting in ionization. This ionization is the fundamental interaction through which IR impacts

biological tissues, leading to molecular and cellular damage (Beyersmann and Hartwig, 2020). The principal categories of ionizing radiation include alpha particles, beta particles, neutrons, gamma rays, and X-rays. These differ in parameters such as mass, charge, energy, and tissue penetration capacity, all of which influence their biological consequences (Britannica, 2025).

Alpha particles consist of helium nuclei with two protons and two neutrons. They exhibit high linear energy transfer (LET), creating dense ionizations over short distances, but their penetration depth is limited, typically halted by the skin or a few centimeters of air. Beta particles are high-energy electrons or positrons that have moderate LET and penetrate further than alpha particles but less than gamma rays or X-rays. Neutrons, which carry no electrical charge, have high LET and are deeply penetrating, causing indirect ionizations through nuclear interactions.

X-rays and gamma rays are electromagnetic radiations that lack mass and charge but are capable of deep tissue penetration. X-rays are generated by electron interactions external to the nucleus and are widely utilized in medical imaging and radiation therapy, spanning energies from kilo-electron volts (keV) to mega-electron volts (MeV). In contrast, gamma rays originate from nuclear decay and generally possess higher energies than diagnostic X-rays (Stanford Environmental Health and Safety, 2025; Wikipedia, 2003).

Ionizing radiation arises from both natural and artificial sources. Natural radiation includes cosmic rays from outer space, naturally occurring terrestrial radionuclides such as decay products of uranium and thorium, and radionuclides internal to living organisms. Radon gas, derived from uranium decay, is a notable natural source often found in enclosed spaces; due to its emission of alpha particles, it is a recognized carcinogen (World Health Organization [WHO], 2023; Britannica, 2025).

Artificial or man-made sources have grown in prominence, especially in medicine and industry. Diagnostic radiology relies heavily on X-rays for imaging purposes, while therapeutic radiation employs high-energy X-rays or particle beams to treat cancers by causing DNA damage in tumor cells (National Cancer Institute, 2022). Treatment modalities include external beam radiotherapy (EBRT), which directs radiation from outside the body, and brachytherapy, involving the placement of radioactive substances in proximity to or within tumors (Wikipedia, 2003).

Within research utilizing Drosophila melanogaster, X-rays and therapeutic radiation serve as experimental means to explore radiation biology—covering DNA damage

responses, shifts in gene expression, and epigenetic alterations (Perspectives on the Drosophila melanogaster Model, 2024). The conserved genetic pathways and rapid life cycle of this model organism make it especially suited for investigating how ionizing radiation impacts molecular mechanisms, including regulation of iron-binding proteins like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2).

TSF 1 plays a central role as the main iron transporter in Drosophila, supporting iron sequestration and nutritional immunity by restricting pathogen access to iron, an essential nutrient (Hentze *et al*., 2020; Marra *et al*., 2021). TSF 2, structurally similar to melanotransferrin, participates in the formation of epithelial septate junctions, essential for preserving tissue barrier function and managing paracellular iron absorption (Tiklová *et al*., 2010; Tang and Zhou, 2013). Delineating how various radiation types and doses affect the genetic and epigenetic regulation of these transferrins is key to understanding disturbances in iron balance and immune competence induced by radiation.

Furthermore, research has demonstrated that radiation exposure can modulate the expression of genes involved in iron metabolism and provoke epigenetic changes such as DNA methylation and histone modifications, which may have prolonged consequences on gene regulation and cellular physiology (Kovalchuk *et al*., 2020; Perspectives on the Drosophila melanogaster Model, 2024). These effects depend on both the dose and nature of radiation; low-dose exposures sometimes trigger adaptive cellular responses, whereas high doses frequently cause significant DNA and epigenetic damage (Antosh *et al*., 2014; Mauro *et al*., 2021).

2.1.2 Mechanisms of DNA Damage Caused by Ionizing Radiation

Ionizing radiation (IR) provokes a wide array of DNA damage that perturbs normal cellular function and threatens genomic stability. This damage arises via two primary processes: direct energy deposition onto DNA and indirect effects mediated by reactive molecules generated in the cellular environment. Both mechanisms collectively contribute to the biological outcomes observed following exposure to X-rays and therapeutic radiation.

Direct DNA Damage

Direct damage happens when IR energy is absorbed specifically by DNA molecules, resulting in ionization of its constituent atoms. This can disrupt covalent bonds within the DNA backbone and create breaks—namely single-strand breaks (SSBs) and

especially harmful double-strand breaks (DSBs) (Ding *et al.*, 2015). DSBs are critical lesions since they involve breaks in both DNA strands, potentially causing chromosomal rearrangements, mutations, or triggering cell death if left unrepaired (Zhang *et al*., 2024). Molecularly, direct interaction generates radical cations on DNA bases and sugar components, leading to base alterations, abasic sites, and DNA-protein crosslinks (Zhang *et al.,* 2024). These defects impair DNA replication and transcription fidelity, activating the cellular DNA damage response (DDR) pathway. In this process, the Mre11/Rad50/NBS1 (MRN) complex senses DSBs and recruits the ATM kinase, which phosphorylates downstream effectors including p53, coordinating responses such as cell cycle arrest, DNA repair, or apoptosis (Liu *et al*., 2020).

Indirect DNA Damage

Indirect damage is mainly caused by reactive oxygen species (ROS) produced through radiolysis of water molecules neighboring DNA. For low linear energy transfer (LET) radiation like X-rays, about 70% of DNA damage results from this indirect mechanism (Ding *et al*., 2015). ROS—including hydroxyl radicals (- OH), superoxide anions (O2- −), and hydrogen peroxide (H2O2)—attack DNA bases, sugars, and proteins, causing oxidative lesions, strand breaks, and crosslink formation (Gautam *et al*., 2021). Such oxidative insults can give rise to clustered DNA damage, where multiple lesions—including SSBs, DSBs, and base modifications—occur within close proximity (one or two DNA helical turns), complicating the repair process and enhancing genomic instability risk (Wang *et al*., 2020). High LET radiation, such as therapeutic heavy ions, generates dense ionization tracks producing complex clustered damage with greater biological impact compared to low LET radiation (Wang *et al*., 2020).

DNA Damage Response and Repair Mechanisms

Cells rely on intricate DDR pathways to preserve genome integrity post-damage. In Drosophila melanogaster, conserved DDR proteins such as ATM, ATR, and checkpoint kinases Chk1 and Chk2 orchestrate cell cycle arrest and activate repair pathways (Katsiarimpa *et al*., 2014). The fly's p53 homolog governs the transcription of genes essential for DNA repair, cell cycle control, and programmed cell death (Liu *et al.*, 2020). Single-strand breaks are primarily rectified via base excision repair (BER), whereas DSBs undergo repair through non-homologous end joining (NHEJ) or homologous recombination (HR). The repair pathway choice depends on the cell's

phase and damage complexity (Gautam *et al.*, 2021). Faulty or inefficient repair can result in mutations, chromosomal rearrangements, or apoptosis.

Radiation-Induced Epigenetic Alterations

Ionizing radiation also modifies the epigenetic landscape, influencing gene expression and chromatin structure. Changes in DNA methylation can silence or activate genes, while modifications of histones such as methylation, acetylation, and phosphorylation alter chromatin accessibility and influence the recruitment of DDR factors and transcription regulators (Kovalchuk *et al.*, 2020). In Drosophila, radiation can stimulate mobile genetic elements (transposable elements), which may disrupt gene regulation and contribute to genome instability (Kudryavtseva *et al*., 2019). Importantly, these epigenetic changes can persist beyond initial exposure and may be inherited across generations, leading to sustained alterations in cellular function (Kovalchuk *et al*., 2020).

Impact on Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2)

The iron-binding proteins TSF 1 and TSF 2 in Drosophila serve crucial but distinct roles: TSF 1 functions in systemic iron transport and nutritional immunity, while TSF 2 is vital for the integrity of epithelial barriers (Guiran et al., 2019; UniProt Consortium, 2025). Radiation-induced DNA damage and epigenetic shifts likely influence the expression and activity of these transferrins, possibly disturbing iron homeostasis and tissue health. Oxidative stress from radiation may modulate TSF 1 levels, impacting iron sequestration and immune responses (Marra *et al*., 2021). Meanwhile, epigenetic regulation at TSF gene regions might further alter their transcriptional dynamics, affecting cellular resilience or vulnerability to radiation stress.

By studying these molecular responses in Drosophila, researchers gain comprehensive insights into the connections among radiation exposure, DNA damage repair, epigenetic regulation, and iron metabolism—knowledge that has important ramifications for radiotherapy and radiation protection efforts.

2.1.3 Biological Consequences of X-ray and Therapeutic Radiation

Ionizing radiation, such as X-rays and therapeutic forms, elicits a broad spectrum of biological effects that influence cellular functions, gene activity, and epigenetic processes. In Drosophila melanogaster, extensive research has examined these effects to shed light on radiation-induced genetic alterations, developmental disruptions, and

molecular responses, offering crucial understanding regarding key genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2).

Developmental and Survival Impacts

Exposure to high-frequency X-rays has been observed to extend the larval duration in Drosophila, with the median prepupal phase lengthening in proportion to irradiation time. This delay depends on variables such as larval age at irradiation and environmental conditions, including ventilation, emphasizing radiation's ability to disturb normal development and physiological timing (Friedman and Mettler, 1967). Furthermore, larvae subjected to ultrahigh dose rate (UHDR) X-rays experienced significantly better eclosion rates and longer adult lifespans relative to individuals exposed to conventional dose rates, indicating that the rate at which radiation is administered plays a substantial role in biological outcomes (Baumann *et al.*, 2024).

The effects on survival and development also depend on the type of radiation employed. For example, proton therapy has been shown to hinder Drosophila development and reduce survival more strongly than X-rays, with an estimated relative biological effectiveness (RBE) of about 1.31 (Hamada *et al.*, 2020). This underscores how the quality of radiation influences the extent of biological damage and subsequent recovery.

Genetic Damage and Mutation Patterns

Ionizing radiation causes DNA lesions including double-strand breaks (DSBs), which, if left unrepaired or incorrectly repaired, can result in mutations and chromosomal abnormalities. In studies focusing on Drosophila wing cells, it has been found that low-dose X-ray exposure (below 200 mGy) fails to adequately trigger DNA repair pathways, leading to elevated mutation rates. In contrast, doses above this level activate repair machinery effectively, lowering mutation frequencies and generating a characteristic U-shaped dose-response behavior (Cavallo *et al.*, 2021). The multiple wing hairs (mwh) gene serves as a sensitive genetic marker for radiation-induced mutations, revealing altered hair phenotypes that enable precise detection of cellular radiation effects (Cavallo *et al.*, 2021).

Epigenetic and Gene Expression Changes

Radiation exposure also drives modifications at the epigenetic level, such as shifts in DNA methylation, histone modifications, and triggering of transposable elements, which collectively influence gene expression patterns. In Drosophila, radiation modulates the expression of long non-coding RNAs and transcripts involved in

ribosome production, facilitating radioadaptive responses that improve DNA repair and reduce chromosomal damage in repeated exposures (Belli *et al*., 2004). Single-cell transcriptomics in irradiated epithelial tissues has revealed variable gene expression changes, including upregulation of DNA repair, apoptosis, and stress-responsive genes. These findings indicate that epigenetic regulation following radiation is cell-type specific and may affect genes related to iron metabolism such as TSF 1 and TSF 2, both vital for maintaining iron balance and tissue health (Cruz, 2024).

Effects on Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2)

TSF 1 operates as the main systemic iron transporter in Drosophila, playing a role in sequestering iron and supporting nutritional immunity by limiting pathogen access to this essential element. Radiation-generated oxidative stress and DNA damage can alter TSF 1's expression and function, impacting iron regulation and immune defense (Guiran, Li *et al*., 2019; *Marra et al*., 2021). On the other hand, TSF 2—structurally related to melanotransferrin—is required for the assembly of epithelial septate junctions, ensuring tissue barrier integrity. Epigenetic changes following radiation may disrupt TSF 2 expression, weakening epithelial barriers and exacerbating radiation-related toxicity (UniProt Consortium, 2025).

Protective and Adaptive Mechanisms

Exposure to low-dose radiation (LDR) in Drosophila has been demonstrated to induce adaptive responses that strengthen DNA repair pathways and decrease chromosomal damage upon exposure to higher radiation doses later on. These protective phenomena involve modulation of epigenetic regulators and non-coding RNAs, potentially influencing TSF gene expression and iron metabolism indirectly (Belli *et al.*, 2004; Cavallo *et al*., 2021). Furthermore, FLASH radiotherapy—which delivers radiation at ultrahigh dose rates—has shown promising results in sparing normal tissues in Drosophila. It enhances survival and minimizes DNA damage compared to conventional dose rates, potentially preserving functions of genes like TSF 1 and TSF 2 by maintaining iron homeostasis and tissue integrity during treatment (Baumann *et al*., 2024).

2.2 Drosophila melanogaster as a Model Organism for Radiation Research

Drosophila melanogaster, widely recognized as the fruit fly, has long been a fundamental model in biological sciences, especially genetics and developmental

biology, for more than a century. Its prominence in radiation research derives from several key practical benefits such as a brief generation time, prolific reproduction, ease of genetic manipulation, and a thoroughly mapped genome (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024). Importantly, Drosophila shares considerable genetic and physiological similarities with humans, making it an excellent system to explore essential biological mechanisms, including responses to ionizing radiation and other environmental stresses (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024).

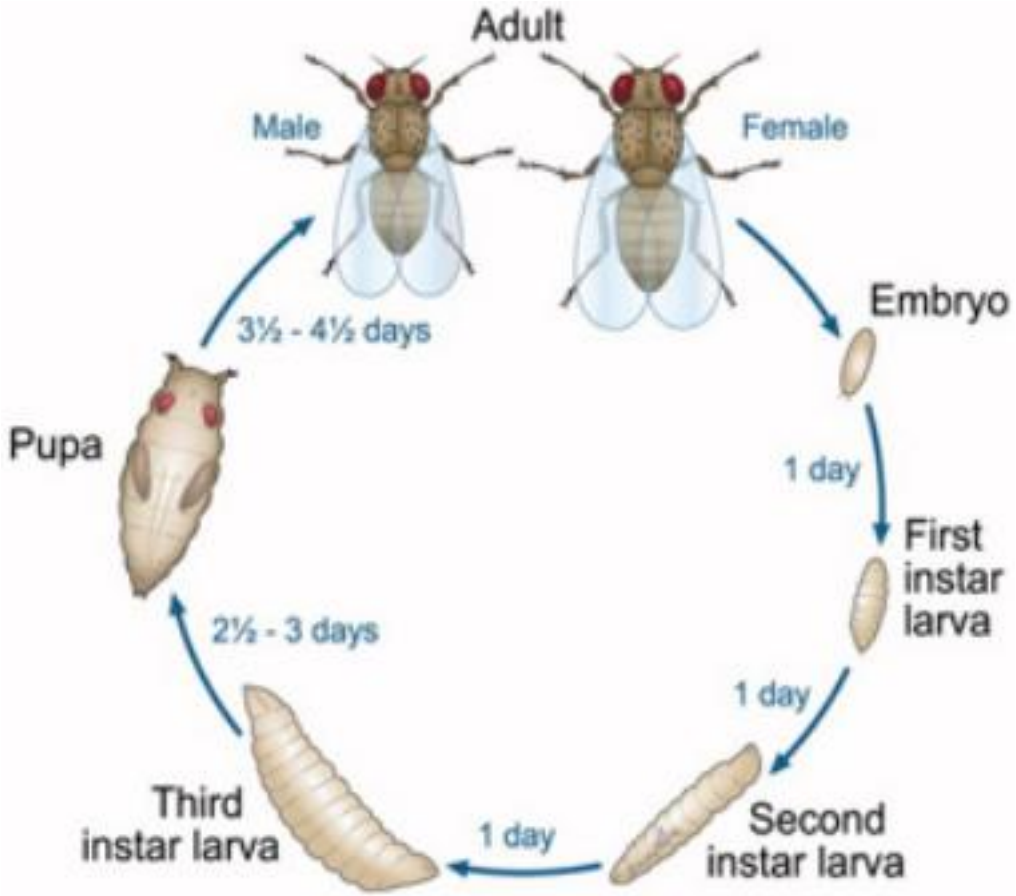

Figure 2.1 The Whole life cycle of Drosophilia Melanogaster (Ong *et al*., 2014)

Strengths of Drosophila in Radiation Studies

The suitability of Drosophila for radiation-related experiments is multifactorial:

Genetic Conservation and Simplicity: The fruit fly possesses highly conserved DNA damage response (DDR) pathways that closely resemble those in mammals. Core components like ATM, ATR, Chk1, Chk2, and p53 homologs are functionally similar, permitting extrapolation of findings to humans and other higher organisms (Katsiarimpa *et al*., 2014). Additionally, its relatively small genome, consisting of only four chromosome pairs, simplifies genetic studies compared to the more complex mammalian genomes.

Genetic Manipulation: Innovative genetic techniques—including the GAL4/UAS targeted expression system, CRISPR/Cas9 genome editing, and RNA interference (RNAi)—enable precise control over gene expression in Drosophila. This allows researchers to selectively study genes implicated in radiation sensitivity or resistance (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024).

High-Throughput Capability: The ability to handle large populations of flies facilitates efficient high-throughput screening to identify modifiers of radiation response or mutagenic agents. This is particularly advantageous for dose-response investigations and uncovering subtle genetic or epigenetic effects (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024).

Rapid Life Cycle and Well-Defined Phenotypes: With a quick reproductive cycle of about 10–12 days at 25°C, Drosophila permits examination of both immediate and heritable radiation effects. Radiation-induced phenotypic changes such as developmental delays, sterility, and morphological anomalies are readily observable and quantifiable (Kovalchuk *et al*., 2020; Katsiarimpa *et al*., 2014).

Cost-Effectiveness: Maintaining Drosophila cultures is more affordable and less labor-intensive compared to mammalian models, making it accessible to a wide range of laboratories (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024).

Exploring Genetic and Epigenetic Regulation of TSF 1 and TSF 2

The unique features of Drosophila make it ideally suited for dissecting how Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) respond genetically and epigenetically to X-ray and therapeutic radiation. Maintaining iron homeostasis is

fundamental for metabolic processes and immune function, both of which can be significantly affected by radiation-induced oxidative stress (Tang and Zhou, 2013).

Genetic Studies:

TSF 1 is the chief iron transporter in Drosophila, playing a pivotal role in systemic iron distribution and immune defense (Guiran *et al.*, 2019; Marra *et al*., 2021). TSF 2, though structurally related, primarily sustains epithelial septate junctions, essential for tissue barrier function (UniProt Consortium, 2025). The fly model allows.

Quantification of radiation-induced changes in TSF 1 and TSF 2 mRNA using techniques like quantitative PCR or RNA sequencing.

Use of reporter gene constructs to visualize promoter activity of TSF genes under varying radiation doses and types.

Employment of mutant or knockdown lines to evaluate the functional role of TSF genes in radiation sensitivity or resistance.

Epigenetic Investigations:

Ionizing radiation introduces stable epigenetic alterations—including DNA methylation and histone modifications—that regulate gene expression (Beyersmann and Hartwig, 2020; Kovalchuk, Baulch, and Kovalchuk, 2020). Drosophila provides powerful tools to:

Analyze radiation-induced histone acetylation and methylation at TSF loci via chromatin immunoprecipitation sequencing (ChIP-seq).

Examine DNA methylation patterns, particularly in nearby transposable elements activated by radiation, influencing local chromatin structure (Kudryavtseva, Kovalchuk, Kolomiets,and Kovalchuk, 2019).

Assess whether epigenetic marks induced by radiation at TSF genes are transmitted across generations, a key research focus in radiation epigenetics (Kovalchuk, Baulch, and Kovalchuk, 2020).

2.2.1 Genetic Strengths of Drosophila melanogaster

Drosophila melanogaster has been a cornerstone in genetic studies for over a century, offering exceptional advantages for researching the genetic and epigenetic effects of environmental stimuli like ionizing radiation. Its comparatively simple yet highly conserved genetic system makes it an ideal model to investigate complex biological processes, including the regulation of iron metabolism-related genes such as

Transferrin 1 (TSF1) and Transferrin 2 (TSF2) under radiation stress (Popis *et al*., 2019; Bellen *et al*., 2010).

Compact and Well-Defined Genome

The genome of Drosophila contains about 16,000 genes arranged on four chromosome pairs, which is considerably smaller and more manageable than the human genome (Adams *et al*., 2000, as cited in Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024). Despite this simplicity, Drosophila shares over 60% genetic similarity with humans, including many genes involved in DNA repair, cell cycle regulation, and iron metabolism (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024; Tang and Zhou, 2013). This genetic conservation allows insights gained from Drosophila studies on TSF 1 and TSF 2 to be meaningfully translated to higher organisms.

Historical Importance in Radiation Genetics

Drosophila was the first species in which ionizing radiation was shown to induce mutations, a discovery made by Hermann Joseph Muller in the 1920s. Muller's groundbreaking experiments demonstrated that X-ray exposure elevated mutation rates, thus providing crucial evidence for radiation's mutagenic potential (Katsiarimpa, Katsiarimpa, Kotsinas, and Georgakilas, 2014). This work established Drosophila as a premier model for studying the genetic consequences of radiation exposure.

Genetic Tools and Manipulation

One of Drosophila's major strengths lies in its sophisticated genetic toolkit. Systems like GAL4/UAS permit tissue-specific gene expression control or knockdown, facilitating focused functional analyses of TSF 1 and TSF 2 under radiation stress (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024). Furthermore, CRISPR/Cas9 genome editing enables precise generation of mutations or epigenetic alterations, supporting detailed investigation of gene function and regulation.

Short Lifecycle and High Fecundity

With an average generation time of roughly 10 days at 25°C and prolific reproductive capacity, Drosophila permits rapid, multi-generational studies on the effects of radiation, including epigenetic inheritance phenomena (Kovalchuk *et al*., 2020). This feature is particularly valuable for exploring how radiation-induced epigenetic modifications of TSF loci may persist and influence offspring phenotypes.

Sensitivity to Radiation and Mutation Assays

The fruit fly exhibits well-characterized sensitivity to ionizing radiation. Mutational assays such as the multiple wing hairs (mwh) test allow quantitative assessment of somatic mutations and chromosomal aberrations due to radiation exposure (Porrazzo *et al*., 2020). This assay has been instrumental in revealing both hypersensitivity and adaptive radioresistance at low radiation doses, enhancing understanding of dose-dependent genetic responses (Porrazzo et al., 2020).

Application to TSF 1 and TSF 2 Research

Leveraging the genetic advantages of Drosophila enables detailed examination of how X-rays and therapeutic radiation influence the expression and epigenetic regulation of TSF 1 and TSF 2. Radiation-induced oxidative stress may modify TSF 1 transcription, affecting systemic iron distribution and immune function (Guiran *et al*., 2019; Marra *et al*., 2021). Likewise, the role of TSF 2 in maintaining epithelial junctions can be explored within the context of radiation-triggered tissue injury and epigenetic changes (UniProt Consortium, 2025).

Through integrating genetic manipulation with radiation exposure experiments, researchers can dissect the molecular pathways controlling TSF gene responses, including DNA damage signaling, chromatin remodeling, and non-coding RNA effects (Kovalchuk *et al*., 2020; Kudryavtseva *et al*., 2019). This comprehensive approach enriches our understanding of how radiation-induced stress perturbs iron metabolism and tissue integrity.

2.2.2 Past Radiation Studies Utilizing Drosophila melanogaster

Drosophila melanogaster has been an invaluable model organism in genetic and biological research for more than a century. Its genetic tractability, rapid development, and conserved molecular pathways for DNA damage response make it particularly suited for studying the biological effects of various types of ionizing radiation, including X-rays, ultraviolet radiation, and proton therapy. Research utilizing Drosophila has yielded insights across molecular, cellular, and organismal levels into the genetic and epigenetic consequences of radiation exposure (Bellen *et al*., 2010; Popis *et al*., 2019; Antosh *et al*., 2014).

Radiation Resistance and Age-Dependent Sensitivity

A notable study by Mockett, Sohal, and Orr (2008) examined the impact of lethal doses of ionizing radiation on male and female adult Drosophila of different ages, using exposures from 200 to 1500 Gy. Their findings showed that the median lethal dose (LD50/2) for one-day-old flies was approximately 1238 Gy for males and 1339 Gy for females. They observed a significant decrease in radiation resistance with increasing age. This decline correlated with a diminished capacity to counteract radiation-induced oxidative stress, as indicated by sensitivity to paraquat, a generator of reactive oxygen species. These results suggest that adult Drosophila, being largely post-mitotic, are an excellent model for studying age-related alterations in radiation tolerance and oxidative stress responses (Mockett *et al*., 2008).

Threshold Effects and Gene Expression Alterations

Antosh *et al.*, (2014) investigated the biological responses of adult Drosophila melanogaster to radiation, focusing on gene expression and lifespan. Their research supported the existence of a threshold effect, where radiation doses below 100 J/kg did not significantly alter lifespan or lead to permanent changes in gene expression. This study underscored the importance of absorbed energy, rather than just incident radiation levels, in assessing biological impact, emphasizing the need for considering dose thresholds in radiation risk evaluations (Antosh etosh *et al.,* 2014).

Ultraviolet Radiation and Oxidative Stress Responses

Recent studies on ultraviolet C (UVC) radiation have demonstrated its detrimental effects on Drosophila melanogaster, manifesting as increased mortality, reduced fertility, and visible mutations such as wing deformities (Alam *et al.*, 2024). Molecular analyses revealed a downregulation of genes encoding antioxidant enzymes, including various superoxide dismutase forms (SOD, Mn-SOD, Cu-Zn-SOD) and the methuselah (MTH) gene, a G protein-coupled receptor linked to stress resistance. These findings suggest that UVC radiation induces oxidative stress by impairing antioxidant defense mechanisms, thus providing a model for examining radiation-induced oxidative damage and its genetic regulation (Alam *et al*.,2024).

Cosmic Radiation and Genetic Mutations

Drosophila has been employed in space radiation studies to evaluate the genetic consequences of cosmic rays. Reddi and Sanjeeva Rao (1964) utilized dominant lethality assays to measure genetic damage after exposing Drosophila larvae to high-altitude cosmic radiation. Although initial results did not show significant

chromosomal breakage or specific gene mutations, these studies highlighted Drosophila's value as a model for space radiobiology and understanding the genetic effects of cosmic radiation (Reddi and Sanjeeva Rao, 1964).

Effects of Low-Dose and Low-Dose-Rate Radiation

Belli *et al*., (2004) demonstrated that pre-exposure of Drosophila to low-dose, low-dose-rate (LDLDR) gamma radiation imparted radioresistance against subsequent high-dose challenges. This radioadaptive response involved adjustments in DNA damage response pathways and notable changes in long intergenic non-coding RNAs (lincRNAs) and transcripts related to ribosome biogenesis. Moreover, prolonged exposure to reduced radiation environments influenced development and viability across generations, suggesting epigenetic inheritance of radiation effects (Belli *et al*., 2004).

Role of Transposable Elements in Radiation Response

Kudryavtseva *et al.*, (2019) explored how the activity of hobo transposons impacts the radiosensitivity of Drosophila. They observed that flies with high activity of full-length hobo elements exhibited pronounced delayed harmful effects after acute irradiation, including increased DNA damage and reduced survival. This research underscores the involvement of transposable elements in modulating genetic and epigenetic responses to radiation, which may interact with the regulation of genes like TSF 1 and TSF 2 (Kudryavtseva *et al*., 2019).

Proton Therapy and Comparative Radiation Effects

Hamada *et al*., (2020) validated Drosophila melanogaster as an in vivo model for proton radiobiology. Gene expression analyses revealed distinct temporal regulation of DNA repair, apoptosis, and angiogenesis genes depending on the radiation type. The study also demonstrated that targeted gene knockdown using the GAL4/UAS system altered radiosensitivity in a radiation type-specific manner, highlighting Drosophila's utility for unraveling complex radiation responses (Hamada *et al*., 2020).

Implications for TSF 1 and TSF 2 Genetic and Epigenetic Profiles

These previous radiation studies in Drosophila establish a foundational context for investigating the genetic and epigenetic regulation of TSF 1 and TSF 2 under radiation stress. TSF 1 is essential for systemic iron transport and immune function, while TSF 2 maintains epithelial integrity (Guiran *et al*., 2019; UniProt Consortium,

2025). Radiation-induced oxidative stress and DNA damage can alter TSF gene expression and chromatin states, potentially impacting iron homeostasis and tissue health. The observed activation of transposable elements and epigenetic remodeling in irradiated Drosophila (Kudryavtseva *et al.*, 2019; Kovalchuk et al., 2020) suggests a possible influence on TSF gene loci, leading to potentially heritable changes in gene regulation. Furthermore, adaptive responses to low-dose radiation (Belli *et al*., 2004) imply that TSF genes might be part of a broader network of radiation-responsive genes modulated through genetic and epigenetic mechanisms.

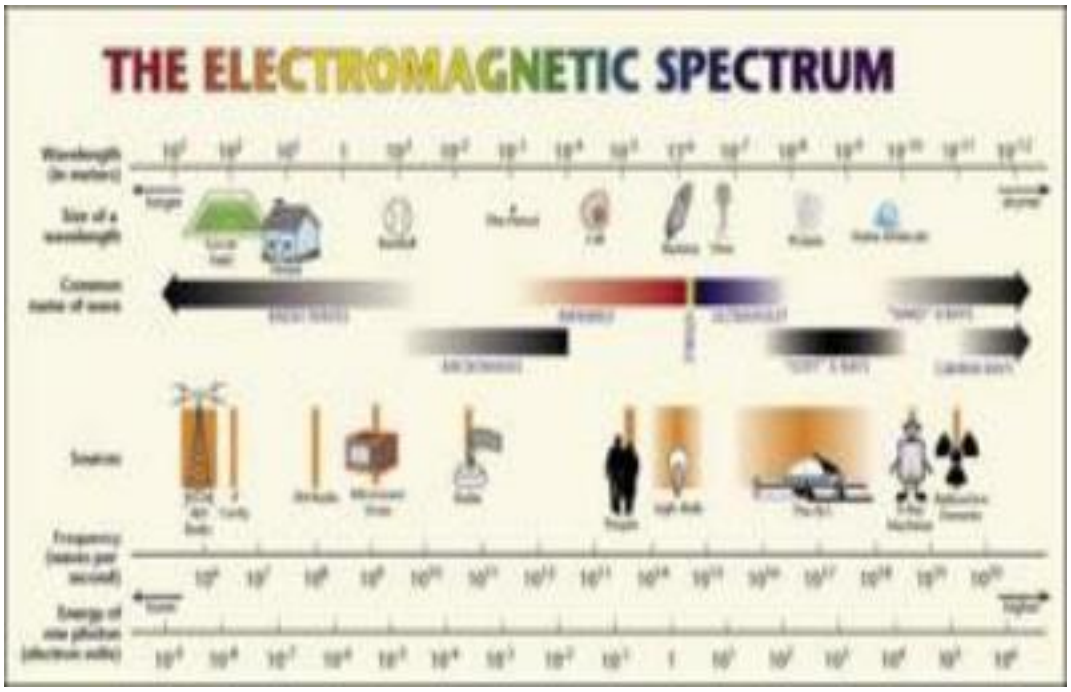

Figure 2.2 Illustration of Drosophila melanogaster radiation exposure and anatomical effects Antosh, M., Fox, D., Hasselbacher, T., Lanou, R., Neretti, N., and Cooper, L. N. (2014).

2.2.3 Importance of Drosophila melanogaster in Human Radiation Biology Research

The use of Drosophila melanogaster as a model organism in radiation biology has greatly contributed to the understanding of human responses to ionizing radiation such as X-rays and therapeutic radiation. This is largely due to the high conservation of genetic pathways, DNA damage response mechanisms, and epigenetic regulation between Drosophila and humans. Moreover, essential genes involved in iron metabolism and cellular homeostasis, including Transferrin 1 (TSF1) and Transferrin 2 (TSF2), are shared, making Drosophila an excellent system for studying radiation-induced genetic and epigenetic effects (Antosh *et al.*, 2014; Alexandrov, 2022; Su, 2019).

Drosophila melanogaster retains numerous fundamental biological processes analogous to those in humans, such as pathways involved in DNA repair, cell cycle control, programmed cell death (apoptosis), along with epigenetic modifications including DNA methylation and histone alterations (Kovalchuk *et al*., 2020; Beyersmann and Hartwig, 2020). This evolutionary conservation makes Drosophila a valuable surrogate for examining the genetic and epigenetic consequences of radiation exposure that are applicable to human health.

The DNA damage response in Drosophila includes functional homologs of human ATM and ATR kinases, checkpoint kinases Chk1 and Chk2, and the tumor suppressor protein p53—all of which coordinate repair processes, cell cycle arrest, or apoptosis in response to radiation-induced DNA lesions (Katsiarimpa, Katsiarimpa, Kotsinas, and Georgakilas, 2014). These mechanisms are essential for preserving genome stability after ionizing radiation in both flies and humans.

Iron Transport and Transferrin Gene Homology

Transferrins are a family of iron-binding proteins crucial for iron homeostasis in both Drosophila and humans. In Drosophila, TSF 1 anatomically and functionally resembles human transferrin, facilitating systemic iron transport and contributing to immune defense by restricting pathogen access to iron (Guiran *et al*., 2019; Marra *et al.*, 2021). TSF 2, homologous to human melanotransferrin, is important for maintaining epithelial barrier functions—critical for tissue integrity and defense responses (UniProt Consortium, 2025).

Ionizing radiation disrupts iron metabolism primarily via oxidative stress and DNA damage, which can change both the expression levels and epigenetic regulation of

TSF 1 and TSF 2. Studying these changes in Drosophila thus provides insight into analogous interactions in humans, where iron dysregulation is linked to radiation-induced tissue injury and carcinogenesis.

Radiation Dose Thresholds and Biological Responses

Research with Drosophila has revealed threshold effects in reactions to radiation, where doses below certain limits do not produce notable alterations in lifespan or stable gene expression shifts (Antosh *et al*., 2014). These findings parallel challenges to the linear no-threshold (LNT) hypothesis in human radiation biology, suggesting that low-dose exposures may induce adaptive or hormetic effects rather than accumulate damage linearly (Perspectives on the Drosophila melanogaster Model for Advances in Environmental Exposures and Human Health, 2024).

Tissue Regeneration and Repair

The larvae of Drosophila display notable regenerative abilities, recovering completely from ionizing radiation-triggered cell death and developing into normal adults even after substantial tissue damage (Wells, Johnston, and Shellenbarger, 2018). This regenerative capacity is directly relevant to human radiation biology, especially in the context of cancer radiotherapy, where tissue healing and tumor repopulation critically affect therapeutic outcomes (Wells, Johnston, and Shellenbarger, 2018).

Epigenetic Transmission and Multi-Generational Effects

Radiation-induced epigenetic changes observed in Drosophila, including modifications to DNA methylation and chromatin structure, can persist transgenerationally, influencing gene expression and phenotypic traits across generations (Kovalchuk *et al.*, 2020). Increasing evidence in humans supports similar hereditary epigenetic effects from radiation exposure, reinforcing the value of Drosophila as a model organism to study heritable epigenetic mechanisms (Kovalchuk *et al*., 2020).

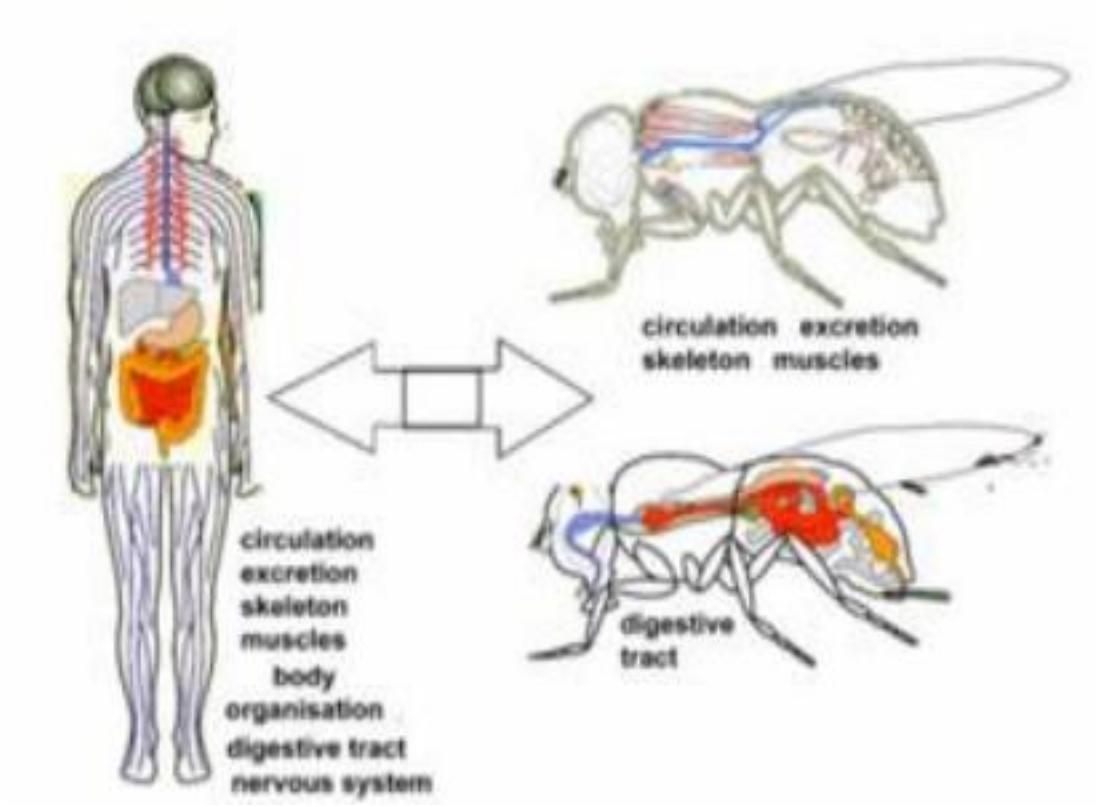

Figure 2.3 Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery (Pandey and Nichols 2011).

2.3 Genetic Impact of Radiation in Drosophila melanogaster

Ionizing radiation, such as X-rays and therapeutic radiation, causes a diverse range of genetic alterations in Drosophila melanogaster, positioning this organism as a powerful model to explore radiation-induced mutagenesis and its underlying molecular processes. These genetic effects encompass DNA sequence mutations, chromosomal abnormalities, and epigenetic modifications, all of which can affect gene expression and phenotypic traits. Particular focus has been placed on the genetic and epigenetic regulation of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), essential proteins for iron homeostasis and tissue maintenance, which are susceptible to radiation-induced changes.

Types and Frequency of Radiation-Induced Mutations

In a detailed investigation, Shcherbakova *et al*., (2022) examined inheritable recessive mutations in Drosophila sperm exposed to γ-rays and neutron radiation. They distinguished two broad mutation categories: (i) gene/structural mutations linked to chromosomal irregularities and (ii) gene/point mutations involving alterations in DNA sequences. Neutrons displayed 2.5-fold higher efficiency in generating structural mutations compared to γ-rays; however, both radiation types were comparably effective in inducing point mutations. Molecular characterization revealed that γ-ray-induced mutations predominantly consisted of base substitutions, while neutron exposure mainly led to gene conversion events. These findings highlight the intricate nature of radiation-induced mutagenesis and suggest that genes like TSF 1 and TSF 2 might be affected by both structural and point mutations, thereby influencing their function (Shcherbakova *et al*., 2022).

Radiation-Triggered Phenotypic Mutations and Oxidative Stress

Lotfy *et al*., (2024) explored the impact of ultraviolet C (UVC) radiation on Drosophila melanogaster, reporting increased mortality, diminished fertility, and visible wing mutations. Their study showed that UVC exposure downregulated antioxidant genes, such as variants of superoxide dismutase (SOD) and the methuselah gene, which is implicated in stress resistance. Since oxidative stress is known to cause DNA damage and epigenetic alterations, these results imply that radiation interferes with the genetic and epigenetic regulation of vital genes, potentially including TSF 1 and TSF 2, which govern iron metabolism and cellular defense (Lotfy *et al.,* 2024).

Space Radiation and Genetic Damage

Early space mission experiments assessed genetic impacts of cosmic radiation on Drosophila by measuring sex-linked recessive lethal and somatic mutations following exposure aboard the space shuttle Endeavour. Irradiated flies exhibited significantly elevated lethality compared to earthbound controls, revealing increased genetic damage under microgravity and cosmic radiation. Variation in somatic mutation rates among different strains highlighted complex interactions between radiation types, genetic background, and mutation susceptibility. These studies emphasize the value of Drosophila as a model for evaluating genetic risks related to space radiation, with implications for genes like TSF 1 and TSF 2 that may be affected by such environmental exposures (Yajima *et al*., 2001).

Low-Dose Radiation and Adaptive Responses

Belli *et al*., (2004) demonstrated that exposure of Drosophila to low-dose, low-dose-rate (LDLDR) gamma radiation induces an adaptive radioresponse, enhancing resistance to subsequent higher-dose exposures. This response involves modulation of DNA repair pathways alongside significant changes in long intergenic non-coding RNAs and ribosomal biogenesis transcripts. Such epigenetic and transcriptional shifts could affect regulation of TSF 1 and TSF 2, potentially promoting cellular defense mechanisms against radiation-induced oxidative stress and DNA damage (Belli *et al*., 2004).

Gene Expression Changes from Chronic Low-Dose Radiation

Kim *et al*., (2015) examined the effects of chronic low-dose gamma radiation on Drosophila larvae, observing increased locomotion and substantial differential gene expression. Among 344 genes with altered expression, those associated with oxidation-reduction processes and defense responses were prominent. These observations suggest that prolonged low-dose radiation modulates genetic networks involved in stress responses, likely including iron metabolism genes such as TSF 1 and TSF 2, which are key players in managing oxidative stress and immunity (Kim *et al*., 2015).

Implications for TSF 1 and TSF 2 Genetic and Epigenetic Regulation

Radiation-induced genomic mutations and epigenetic changes can profoundly influence the expression and function of TSF 1 and TSF 2. TSF 1 serves as a systemic iron transporter vital for maintaining iron balance and immune function, whereas TSF

2 supports the integrity of epithelial barriers (Guiran, Li, Zhang, Wu, and Zhang, 2019; UniProt Consortium, 2025). Mutations or epigenetic silencing affecting these genes could disrupt iron metabolism, increase oxidative damage, and impair tissue functions following radiation exposure. Furthermore, the activation of transposable elements and chromatin restructuring observed in irradiated Drosophila (Kudryavtseva *et al*., 2019) may impact the expression of TSF loci, leading to genomic instability. Investigating these genetic and epigenetic interactions within Drosophila models offers valuable insights into radiation-induced iron metabolic disturbances and associated pathologies in humans.

2.3.1 Types and Rates of Mutations Following Irradiation in Drosophila melanogaster

Exposure to ionizing radiation, including X-rays and therapeutic radiation, induces a wide spectrum of mutations in Drosophila melanogaster. These range from single nucleotide variants to extensive chromosomal abnormalities, affecting gene functionality and genome integrity. Genes critical for iron metabolism, such as Transferrin 1 (TSF1) and Transferrin 2 (TSF2), are among those impacted. Understanding the mutation types and their frequencies following irradiation is essential to decipher the resulting genetic and epigenetic consequences (Alexandrov, 2022; Antosh *et al*., 2014;).

Categories of Radiation-Induced Mutations

Radiation-induced mutations in Drosophila are generally divided into two main groups:

1. Structural or Gene-Level Mutations: These encompass chromosomal aberrations including deletions, inversions, translocations, and multilocus deletions that impair gene and chromosomal integrity. Such mutations commonly result in recessive lethality or sterility (Shcherbakova *et al*., 2022; Alexandrov *et al*., 2023).

Point or Intragenic Mutations: These mutations involve small-scale DNA sequence changes such as base substitutions, small insertions/deletions, and gene conversions that alter coding or regulatory sequences without causing gross chromosomal disruptions (Shcherbakova *et al*., 2022).

Matsudaira and Yamasaki (1975) examined the relationship between radiation dose and the frequency of two lethal mutation types—fractional-lethal (partial loss of gene function) and whole-lethal (complete gene loss). They observed that fractional-lethal

mutations decreased as radiation dose increased, whereas whole-lethal mutations rose, indicating a dose-dependent shift in the nature of induced mutations (Matsudaira and Yamasaki, 1975).

Mutation Frequencies in Relation to Radiation Dose and Type

The rate of mutations depends on the kind of radiation, its dose, and the genetic locus targeted. Alexandrov *et al*. (2023) found that neutron radiation produces chromosomal mutations at approximately twice the frequency observed with γ-rays, while the incidence of point mutations remains relatively unchanged across radiation types (approximately 1.15×10^{-6} per locus per Gy). This suggests that gene exons, rather than entire genetic loci, are primary locations for point mutations.

King, Darrow, and Kaye (1956) reported that germ cells at distinct oogenic stages show varying sensitivities to radiation-induced chromosomal breaks. They found dominant lethality and X chromosome loss rates correlated with specific cytological stages, and that recessive lethal mutations caused by radiation persist in the germline, demonstrating lasting genetic damage (King, Darrow, and Kaye, 1956).

Mutation Spectrum at Specific Genetic Loci

Research focusing on precise gene loci, such as those on chromosome 3, revealed mutation rates around 4.36×10^{-8} per rad per locus after exposure to 22 MeV betatron X-rays. Among these mutations, 66% were recessive lethals, with deletions accounting for 42.2%, inversions 6.7%, and translocations 2.2% of the lethal mutations, underscoring deletions as the predominant form of radiation-induced genetic injury (Matsuo *et al*., 1984).

Spaceflight experiments assessing sex-linked recessive lethal mutations and somatic mutations in Drosophila exposed to cosmic radiation demonstrated elevated mutation frequencies in space-exposed flies compared to controls on Earth, highlighting the enhanced mutagenic capacity of space radiation (Yajima *et al.*, 2001).

Consequences for TSF 1 and TSF 2 Genes

Genes encoding TSF 1 and TSF 2, which function in systemic iron transport and maintaining epithelial barriers respectively, are vulnerable to radiation-induced mutations and epigenetic disruptions. Structural mutations like deletions or inversions affecting these genes could compromise iron balance and tissue function (Guiran *et al*., 2019; UniProt Consortium, 2025). Point mutations may alter protein structure or

gene regulation, while epigenetic changes may modify transcription following radiation exposure (Beyersmann and Hartwig, 2020).

2.3.2 Chromosomal Abnormalities and Dominant Lethal Mutations in Drosophila melanogaster

Ionizing radiation, including X-rays and therapeutic radiation, causes chromosomal abnormalities and dominant lethal mutations in Drosophila melanogaster, which are important markers for assessing genetic damage and instability. These mutations can significantly affect gene function, impacting organismal survival, development, and inheritance patterns. Examining these genetic alterations in Drosophila sheds light on the genetic and epigenetic characteristics of essential genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), which are crucial to iron regulation and tissue stability.

Chromosomal Aberrations Triggered by X-rays

Gatti, Tanzarella, and Olivieri (1974) introduced a method for analyzing chromosome aberrations in Drosophila melanogaster larval ganglia after X-ray exposure to somatic cells. Their analysis revealed several critical findings:

Sex-based Differences in Radiation Sensitivity: Female flies consistently showed a higher rate of both chromosome and chromatid aberrations, along with a greater amount of spontaneous aberrations when compared to males.

Sensitivity Relative to Cell Cycle Stage: Chromosomal damage peaked when cells were irradiated during the G1 and G2 phases. G1 irradiation led predominantly to dicentric chromosomes, whereas G2 irradiation resulted more in terminal deletions and chromatid exchanges.

Non-Random Chromosomal Break Distribution: Breaks were unevenly distributed, with the Y chromosome displaying greater resistance, while breaks predominantly occurred in the pericentromeric heterochromatin regions of the X chromosome and autosomes.

Effect of Somatic Pairing: The pairing of homologous chromosomes in somatic cells increased exchange events and dicentric formation but reduced detectable translocations, likely because somatic pairing restricts looping structures within chromosomes (Gatti *et al*., 1974).

These chromosomal changes—such as dicentrics, deletions, and exchanges—have the potential to disrupt genes like TSF 1 and TSF 2, possibly influencing their expression and epigenetic states after radiation exposure.

Dominant Lethal Mutations and Their Origins

Dominant lethal mutations, which cause death in heterozygous states, serve as a sensitive indicator of genetic damage caused by radiation. King, Darrow, and Kaye (1956) found that the highest frequencies of dominant lethal mutations and X chromosome loss in Drosophila oocytes occurred when radiation exposure took place during maturation. These mutations correlated with chromosome breakage and losses and persisted in the germ line, indicating long-lasting genetic damage.

Further, Matsudaira and Yamasaki (1975) differentiated lethal mutations into fractional-lethal (partial gene function loss) and whole-lethal (complete gene function loss) types following X-ray exposure. Their dose-response curve analysis indicated that fractional-lethal mutations decreased with higher radiation doses, whereas whole-lethal mutations increased, pointing to a dose-dependent shift in mutation classification (Matsudaira and Yamasaki, 1975).

Interaction of Transposable Elements and DNA Repair Deficiency

Bashirullah, Yao, and Baker (1999) examined how X-rays interact with Drosophila P element transposon activity during hybrid dysgenesis. They observed increased sterility and chromosome loss after irradiation, especially in flies with impaired DNA repair mechanisms. This finding indicates the critical role of post-replication repair pathways in addressing the combined damage from radiation and transposon activity. Such interactions could worsen genetic instability at important loci, such as TSF 1 and TSF 2, impacting their regulation (Bashirullah *et al*., 1999).

Mutation Rates and Mutation Types at Specific Gene Loci

(Matsuo *et al*., 1984) quantified mutation frequencies at eight gene loci on the third chromosome of Drosophila following exposures to 22 MeV betatron X-rays. They calculated a mutation rate of 4.36×10^{-8} per rad per locus, with 66% of mutations being recessive lethals. Among these lethals, deletions made up 42.2%, inversions 6.7%, and translocations 2.2%, highlighting the dominance of deletions in radiation-induced genetic injury (Matsuo *et al*., 1984).

Chromosome Breaks and Timing of Exposure

Stern (1926) reported that X-rays caused breakage of the double X chromosome in Drosophila germ cells, particularly when irradiation happened near cellular maturation. Approximately 3% of cells exhibited breaks at doses ranging from 36 to 55 R (Roentgen). These breaks localized near the junction of the two arms of the

double-X chromosome, seemingly due to indirect effects of radiation altering nuclear conditions rather than direct DNA damage. Such chromosome disruptions could influence genes such as TSF 1 and TSF 2 residing on the X chromosome or autosomes (Stern, 1926).

Impact on TSF 1 and TSF 2 Gene Regulation

Chromosomal aberrations like deletions, dicentrics, and translocations can cause loss or dysregulation of TSF 1 and TSF 2 genes. Dominant lethal mutations in these regions may impair iron transport and epithelial barrier function, worsening oxidative stress and tissue injury following radiation exposure. Additionally, radiation-induced chromosomal instability can prompt epigenetic modifications in TSF gene regions, affecting their gene expression patterns and long-term cellular responses (Beyersmann and Hartwig, 2020; Kudryavtseva *et al*., 2019).

2.3.3 DNA Repair Mechanisms Triggered by Radiation in Drosophila melanogaster

Ionizing radiation, including X-rays and therapeutic radiation, causes a variety of DNA lesions in Drosophila melanogaster, such as single-strand breaks, double-strand breaks, base modifications, and crosslinks. The robust DNA repair systems of Drosophila are essential for preserving genomic stability and regulating the genetic and epigenetic states of important genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), both of which are critical to iron metabolism and tissue homeostasis.

DNA Repair Pathways Activated by Radiation

Drosophila utilizes several DNA repair processes conserved across mammals, including:

DNA Damage Detection and Cell Cycle Checkpoints: Genes such as D-Gadd45, Hus1, and mnk detect DNA lesions and activate checkpoints to halt the cell cycle, allowing repair to occur (Yushkova *et al*., 2020; Sekelsky, 2017).

Base Excision Repair (BER): This pathway repairs oxidative damage to bases and single-strand breaks, involving genes like Rrp1 (Yushkova et al., 2020).

Nucleotide Excision Repair (NER): Responsible for removing bulky DNA adducts and helix-distorting lesions. Key genes include mei-9, mus210, and Mus209 (Yushkova *et al*., 2020).

Double-Strand Break (DSB) Repair: Two major pathways address DSBs:

Homologous Recombination (HR): A high-fidelity repair mechanism that uses a sister chromatid as a template, involving genes such as Brca2, spn-B, and okr.
Non-Homologous End Joining (NHEJ): Direct end-joining repair involving Ku80 and WRNexo (Yushkova *et al*., 2020; Sekelsky, 2017).

Radioadaptive Responses and DNA Repair Gene Regulation

Research shows that chronic exposure to low-dose γ-radiation during Drosophila development upregulates DNA repair genes, fostering radioadaptive phenomena and radiation hormesis that improve lifespan and resilience against further acute radiation (Yushkova *et al*., 2020; Shaposhnikov et al., 2015). However, mutations in key DNA repair genes like D-Gadd45, mei-9, or Brca2 reduce or abolish these adaptive responses, highlighting their vital roles in enhancing radiation tolerance (Yushkova *et al.*, 2020). Intriguingly, forced overexpression of DNA repair genes does not necessarily increase radiation resistance and may even shorten lifespan, indicating that finely tuned regulation of these pathways is crucial for maintaining cellular balance (Shaposhnikov *et al.,* 2015).

Epigenetic Influence on DNA Repair

DNA damage from radiation also triggers epigenetic modifications affecting gene expression. Processes such as chromatin remodeling and histone modifications facilitate the recruitment of repair proteins to damaged regions (Beyersmann and Hartwig, 2020). In Drosophila, radiation can activate transposable elements like hobo transposons, which may destabilize the genome and interact with DNA repair mechanisms (Kudryavtseva *et al*., 2019; Yushkova et al., 2020).
These epigenetic changes can influence the transcriptional regulation of TSF 1 and TSF 2 genes, adjusting their expression in response to radiation-induced stress. Since TSF 1 regulates systemic iron transport and TSF 2 supports epithelial integrity, their proper regulation is essential for alleviating oxidative damage and preserving tissue function after irradiation (Guiran *et al*., 2019; UniProt Consortium, 2025).

Coordination Between DNA Repair and Apoptosis

The outcome of radiation exposure depends on the balance between DNA repair and programmed cell death. Studies in Drosophila reveal that both p53-dependent and independent pathways mediate apoptosis following DNA damage, ensuring that

highly damaged cells are eliminated while conserving overall tissue functionality (Baonza *et al.*, 2022).

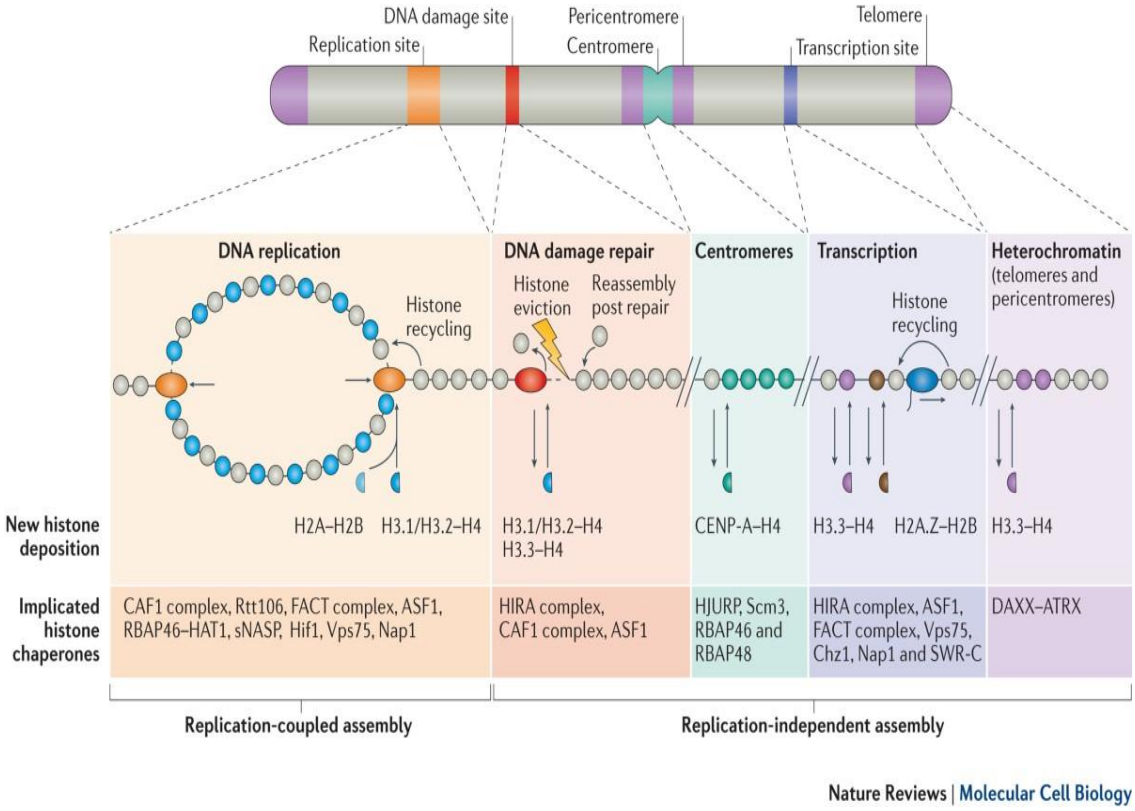

Figure 2.4 Histone chaperone networks shaping chromatin function Hammond, C., Strømme, C. B., Huang, H., Patel, D. J., and Groth, A. (2017).

2.4 Epigenetic Alterations Triggered by Radiation in Drosophila melanogaster

Ionizing radiation—including X-rays and therapeutic forms—induces a variety of epigenetic modifications in Drosophila melanogaster that affect gene expression and genomic stability. These changes influence the genetic and epigenetic landscapes of vital genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), which are key to iron metabolism and tissue integrity. Studying radiation-induced epigenetic effects in Drosophila sheds light on conserved processes relevant to human radiation biology (Antosh *et al*., 2014).

Epigenetic Regulation Responsive to Radiation

A pivotal study by Jin *et al*. (2008) revealed that Drosophila embryos' shift from radiation sensitivity to resistance is controlled by the epigenetic silencing of an irradiation-responsive enhancer region (IRER) located upstream of proapoptotic genes reaper and hid. This enhancer region accumulates repressive histone marks such as trimethylated H3K27 and H3K9, forming heterochromatin-like domains that prevent gene activation in response to irradiation later in development. Enzymes responsible for histone modifications, including Hdac1 (rpd3), Su(var)3-9, and Polycomb group proteins, are critical to this regulatory mechanism, demonstrating how chromatin state governs radiation sensitivity (Jin *et al*., 2008).

Processes Underpinning Radiation-Induced Epigenetic Alterations

Ionizing radiation inflicts DNA damage directly and indirectly through reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to strand breaks and base modifications (Beyersmann and Hartwig, 2020). These molecular damages are accompanied by epigenetic changes such as altered DNA methylation, histone post-translational modifications, and chromatin remodeling. The pattern and magnitude of ROS/RNS generation depend on the radiation type, which in turn influences the nature and scale of epigenetic modifications (Beyersmann and Hartwig, 2020).

Within Drosophila, radiation can activate mobile genetic elements like hobo transposons, thereby perturbing chromatin structure and gene regulation, which contributes to genome instability (Kudryavtseva *et al*., 2019). These disruptions may affect how TSF 1 and TSF 2 are transcriptionally controlled, modifying their expression in response to radiation-induced cellular stress.

Epigenetic Control of TSF 1 and TSF 2

The TSF 1 and TSF 2 genes are subject to epigenetic modulation influenced by radiation exposure. TSF 1 serves as the major iron transporter crucial for systemic iron balance and immune defense, whereas TSF 2 supports the assembly of epithelial septate junctions, maintaining barrier integrity (Guiran *et al.*, 2019; UniProt Consortium, 2025). Radiation-driven alterations in histone marks and DNA methylation at these gene loci can regulate their transcriptional activity, thereby impacting iron homeostasis and tissue health.

Adaptive epigenetic responses following low-dose radiation have been documented in Drosophila, involving changes in non-coding RNAs and chromatin configurations that promote efficient DNA repair and bolster cellular resilience (Belli *et al*., 2004; Yushkova et al., 2019). Such epigenetic flexibility may play a role in the dynamic regulation of TSF genes during radiation exposure.

Heritability of Radiation-Induced Epigenetic Changes

Epigenetic modifications initiated by radiation in Drosophila can be transmitted across generations, influencing gene expression and phenotypes in progeny (Kovalchuk, Baulch, and Kovalchuk, 2020). This transgenerational epigenetic inheritance has significant implications for sustained effects on TSF 1 and TSF 2 expression and function, potentially affecting iron metabolism and overall organismal fitness over multiple generations.

2.4.1 DNA Methylation Alterations in Drosophila melanogaster Following X-ray and Therapeutic Radiation Exposure

Exposure to ionizing radiation, including X-rays and therapeutic radiation, induces noteworthy modifications in DNA methylation patterns in Drosophila melanogaster, which play a crucial role in gene regulation and genomic stability. Such epigenetic changes influence the genetic and epigenetic status of important genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), both vital for iron transport and the maintenance of epithelial tissue integrity (Antosh *et al*., 2014; Maroni & Stamey, 1983; Nichol *et al*., 2002).

Radiation-Driven Changes in DNA Methylation

Investigations have demonstrated that prolonged exposure to low-dose radiation results in alterations to DNA methylation within Drosophila. (Kravets et al., 2010) studied two laboratory strains of Drosophila (Canton-S and ri) which were subjected to 20 successive generations of low-dose irradiation. Employing restriction enzymes GluI and GlaI to assess methylation, they identified distinct differences between males and females, as well as between irradiated and control flies. Chronic radiation exposure led to a reduction in methylation at the enzyme recognition sites, indicating radiation-induced hypomethylation (Kravets *et al*., 2010).

Broader research on the impact of ionizing radiation across taxa reveals that low linear energy transfer (LET) radiation, such as X-rays and γ-rays, commonly causes dose-dependent global DNA hypomethylation. The extent and persistence of this hypomethylation vary depending on tissue type, sex, and duration of exposure (Beyersmann and Hartwig, 2020). These epigenetic alterations influence gene regulation and may contribute to radiation-associated genomic instability.

Effects on TSF 1 and TSF 2 Gene Regulation

The TSF 1 and TSF 2 genes are probable targets of radiation-induced DNA methylation changes. TSF 1 is responsible for systemic iron transport and immune modulation, whereas TSF 2 is critical for forming and maintaining epithelial septate junctions (Guiran *et al*., 2019; UniProt Consortium, 2025).Since iron homeostasis is closely linked to oxidative stress, radiation-induced hypomethylation could worsen oxidative damage or stimulate compensatory transcriptional responses involving TSF genes. Moreover, observed sex-specific methylation pattern differences in irradiated Drosophila imply that TSF gene regulation under radiation exposure may be influenced by sex (Kravets *et al.*, 2010).

Influence of Transposable Elements and DNA Methylation

Radiation can activate transposable elements (TEs) in Drosophila, typically silenced by DNA methylation and heterochromatin. Kudryavtseva, Kovalchuk, Kolomiets, and Kovalchuk (2019) found that radiation modulates TE activity, potentially through methylation changes. Such TE activation can interfere with genomic stability and gene regulation, including at loci such as TSF 1 and TSF 2, possibly resulting in altered epigenetic landscapes and transcriptional profiles.

Biological Implications and Transgenerational Inheritance

Radiation-driven modifications in DNA methylation in Drosophila may have lasting biological effects, influencing development, stress response, and longevity. Additionally, these epigenetic changes can be transmitted across generations, affecting gene expression and phenotypes in descendants (Kovalchuk, Baulch, and Kovalchuk, 2020). Understanding DNA methylation dynamics at TSF gene loci is therefore essential for elucidating long-term consequences of radiation exposure.

2.4.2 Histone Modifications and Chromatin Remodeling in Drosophila melanogaster Subjected to X-ray and Therapeutic Radiation

Exposure to ionizing radiation, such as X-rays and therapeutic radiation, induces significant epigenetic alterations in Drosophila melanogaster, particularly through changes in histone modifications and chromatin remodeling. These alterations influence both the genetic and epigenetic landscapes of important genes like Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), which are critical for maintaining iron metabolism and epithelial tissue structure (Madigan, J. P., Chotkowski, H. L.,and Glaser, R. L. 2002).

Histone Acetylation's Role in DNA Damage Repair

A vital factor in the radiation response pathway is the histone acetyltransferase MOF (males absent on the first), which catalyzes acetylation of histone H4 at lysine 16 (H4K16ac) in Drosophila. This specific acetylation mark is predominantly enriched on the male X chromosome, playing a key role in dosage compensation, and is also evolutionarily conserved as an important regulator of DNA damage response (DDR) and double-strand break repair mechanisms (Sartori *et al*., 2012).

Sartori and colleagues (2012) demonstrated that mutations in mof or MOF protein knockdown cells lead to decreased survival after irradiation, increased chromosomal aberrations, faulty mitotic checkpoint regulation, heightened apoptosis, and compromised p53-mediated responses. Moreover, radiation exposure elevates global levels of H4K16 acetylation, underscoring MOF's essential function in modulating chromatin to facilitate efficient DNA repair. These results reveal MOF-mediated histone acetylation as a conserved mechanism necessary for effective DDR in both flies and mammals (Sartori *et al.*, 2012).

Chromatin Remodeling and Repair Efficacy

Repair of radiation-induced DNA double-strand breaks (DSBs) necessitates restructuring of chromatin to enable repair complexes access to damaged sites. In Drosophila, the chromatin environment influences both the sensitivity to damage and the efficiency of repair pathways. Studies involving low-dose γ-irradiation indicate that the choice and outcome of repair mechanisms vary dependent on chromatin context, exemplified by differing mutation patterns—such as deletions and recombination events—in wing cells (Koana *et al*., 2020). These variations are tightly linked to chromatin accessibility and the specific DNA repair pathway activated.

Impact of Transposable Elements on Chromatin Structure

Oxidative and radiation-induced stress can activate transposable elements (TEs) in Drosophila, which are ordinarily silenced by DNA methylation and heterochromatin formation (Oliveira, Rosa, Vieira, Loreto, and Elgion, 2021). Activation of TEs disrupts chromatin organization and affects gene regulation, potentially altering the expression and chromatin state of loci such as TSF 1 and TSF 2. This epigenetic disruption can influence iron metabolism and compromise epithelial function under conditions of radiation stress.

DNA Repair Genes and Chromatin Regulation

Genetic analyses in Drosophila have shown that mutations or dysregulation of DNA repair genes and chromatin remodeling factors affect radiation sensitivity and organismal lifespan. Interestingly, overexpression of repair genes does not always enhance radiation resistance and may even shorten lifespan, highlighting the necessity for balanced chromatin dynamics and repair activities (Yushkova *et al*., 2019; Shaposhnikov *et al*., 2015).

Consequences for Regulation of TSF 1 and TSF 2

Histone modifications and chromatin remodeling events at the genetic loci of TSF 1 and TSF 2 regulate their transcriptional responses to radiation. Given TSF 1's involvement in systemic iron transport and TSF 2's role in preserving epithelial barriers, their epigenetic regulation through histone acetylation and chromatin structure adjustments is critical for maintaining iron balance and tissue health in irradiated organisms (Guiran *et al*., 2019; UniProt Consortium, 2025).

2.4.3 Involvement of Non-Coding RNAs in Radiation Responses of Drosophila melanogaster

Non-coding RNAs (ncRNAs), including long non-coding RNAs (lncRNAs) and endogenous small interfering RNAs (esiRNAs), have been identified as key modulators of genetic and epigenetic responses to ionizing radiation in Drosophila melanogaster. These molecules influence gene expression, chromatin dynamics, and DNA repair pathways, thereby playing a crucial role in regulating important genes such as Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), which are essential for iron metabolism and maintaining epithelial tissue integrity (Li *et al*., 2021).

Long Non-Coding RNAs (lncRNAs) in Radiation Response

Recent transcriptome analyses have uncovered numerous lncRNAs in Drosophila that display tissue- and developmental stage-specific expression patterns, many of which are involved in development, regeneration, and various stress responses (López-Pérez *et al*., 2024; Soshnev *et al*., 2023). LncRNAs regulate gene activity through multiple mechanisms, including altering chromatin accessibility, guiding transcription factors, and affecting mRNA processing (López-Pérez *et al*., 2024).

Within the context of radiation exposure, lncRNAs are crucial in coordinating DNA damage response (DDR) pathways. For instance, low-dose radiation induces specific changes in long intergenic non-coding RNAs (lincRNAs) and other non-coding transcripts that govern ribosome production and DNA repair gene expression, thereby supporting radioadaptive responses in Drosophila (Belli, De Santis, and Marabitti, 2004; Yushkova *et al*., 2019). These lncRNAs may epigenetically regulate TSF 1 and TSF 2, influencing iron homeostasis and tissue repair following irradiation.

Endogenous Small Interfering RNAs (esiRNAs) and Radioadaptive Mechanisms

A significant study showed that chronic low-dose γ-irradiation decreases chromosome breaks and telomere fusions in Drosophila larval neuroblasts by downregulating Loquacious D (Loqs-RD), a factor essential for esiRNA biogenesis (Mauro *et al*., 2022). Loss of Loqs replicates the protective effects seen in radioadaptive responses (RAR), indicating that esiRNAs and their processing machinery are critical epigenetic modulators of radiation resistance.

Since esiRNAs participate in post-transcriptional gene silencing and chromatin regulation, modulating Loqs and esiRNA activity likely impacts the expression and epigenetic landscape of TSF 1 and TSF 2 genes. This influence may affect iron metabolism and the integrity of epithelial barriers under radiation-induced stress (Mauro *et al*., 2022).

ncRNAs in Cellular Stress and Damage Signaling

The Drosophila heat shock RNA omega (hsrω) gene encodes a stress-responsive lncRNA integral to managing cellular responses to various stressors, including radiation (Prasanth et al., 2012). Stress-responsive ncRNAs function by sequestering RNA-binding proteins or regulating transcription and translation, thus fine-tuning gene networks responsible for DNA repair and cell survival.

Regulatory Effects on TSF 1 and TSF 2

TSF 1 and TSF 2 gene expression is likely modulated by ncRNAs during exposure to radiation. Both lncRNAs and esiRNAs can adjust chromatin openness and affect the recruitment of transcription factors at TSF gene loci, controlling their transcription amidst oxidative and DNA damage stress. This regulation is critical for preserving iron balance and epithelial barrier function, ultimately mitigating radiation-related cellular injury (Guiran, Li, Zhang, Wu, and Zhang, 2019; UniProt Consortium, 2025).

2.5 Regulation of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster Under X-ray and Therapeutic Radiation Exposure

Although Transferrin 1 (Tsf1) and Transferrin 2 (Tsf2) are not classical transcription factors, they are essential proteins involved in iron metabolism and epithelial tissue integrity in Drosophila melanogaster. Their gene expression appears responsive to environmental stressors (e.g. severe oxidative stress) and likely involves regulatory mechanisms including transcription factors and epigenetic modulation. Investigating how Tsf1 and Tsf2 are transcriptionally regulated during radiation exposure offers valuable insights into how organisms sustain iron balance and tissue functionality under genotoxic stress (based on what is known about Tsf1 knockdown, ferritin interaction, and stress-response expression changes) (Author synthesis; see Baumgartner *et al*., 2018; “Genome-wide analysis of common and specific stress responses in adult Drosophila melanogaster”, 2015).

Genetic and Epigenetic Control of TSF 1 and TSF 2

TSF 1 primarily acts as a systemic iron transporter, facilitating iron sequestration and contributing to nutritional immunity by restricting pathogen access to iron (Guiran *et al*., 2019; Marra *et al*., 2021). TSF 2, a homolog of melanotransferrin, is crucial for

the assembly of epithelial septate junctions and the preservation of barrier integrity (UniProt Consortium, 2025). The expression of these genes is tightly regulated at the transcriptional level and is influenced by signaling pathways triggered by radiation-induced stress.

Ionizing radiation provokes DNA damage and oxidative stress, which activate a variety of transcription factors such as p53, NF-κB, and hypoxia-inducible factors (HIFs). These factors, in turn, regulate genes associated with stress response and iron metabolism (Beyersmann and Hartwig, 2020). For example, the activation of p53 following radiation exposure can indirectly modulate the expression of TSF 1 and TSF 2 by governing downstream pathways involved in iron homeostasis and epithelial repair processes.

Transcriptional Dynamics After Radiation Exposure

Research using Drosophila models indicates that radiation stimulates a coordinated transcriptional program incorporating DNA repair genes, apoptosis regulators, and metabolic genes (Hamada *et al*., 2020). Although direct binding of transcription factors to TSF 1 and TSF 2 promoters remains to be fully mapped, transcriptomic data indicate that their expression varies after irradiation, implying regulation by radiation-responsive transcription factors and epigenetic mechanisms.

Epigenetic modifications—including histone acetylation and DNA methylation—at TSF gene loci influence chromatin structure and therefore affect transcription factor access and gene activity (Jin et al., 2008; Beyersmann and Hartwig, 2020). For instance, the acetylation of histone H4 at lysine 16 (H4K16ac) mediated by the MOF enzyme enhances chromatin accessibility, which may facilitate TSF gene expression during DNA damage response.

2.5.1 Structure and Function of TSF1 and TSF2 in Drosophila melanogaster Exposed to X-ray and Therapeutic Radiation

Structural Characteristics

TSF 1 is the principal systemic iron transporter in Drosophila, sharing structural homology with mammalian transferrins. It features two lobes, each capable of binding ferric iron (Fe^3+), thereby facilitating iron transport throughout the hemolymph to various tissues (Guiran *et al*., 2019). The tsf1 gene, located on chromosome 3L,

encodes TSF 1, which possesses conserved iron-binding motifs and glycosylation sites crucial for its stability and biological activity (UniProt Consortium, 2025).

TSF 2, a homolog of melanotransferrin, exhibits structural similarities to TSF 1 but contains distinct functional domains specifically involved in the assembly of epithelial septate junctions (UniProt Consortium, 2025). TSF 2 is critical for maintaining the integrity of epithelial barriers and is indispensable for proper tissue development and protection against environmental challenges (Limmer et al., 2014). Its gene structure includes domains that enable the protein-protein interactions necessary for junction formation.

Functional Roles in Iron Metabolism and Tissue Integrity

TSF 1 plays a central role in iron trafficking, ensuring sufficient iron supply for metabolic processes while sequestering free iron to prevent harmful oxidative damage. It also contributes to nutritional immunity by limiting iron availability to pathogens, thus modulating immune responses (Marra., Masson.,and Lemaitre, 2021). Radiation-induced oxidative stress can impair TSF 1 function, potentially leading to iron dysregulation and increased cellular damage.

TSF 2's involvement in epithelial septate junction assembly helps maintain tissue compartmentalization and barrier functions. These functions are particularly vital under conditions of radiation-induced stress, which can compromise tissue integrity (Limmer *et al.*, 2014). A disruption in TSF 2 expression or function can worsen radiation toxicity by hindering the epithelial repair mechanisms.

Genetic and Epigenetic Modifications Induced by Radiation

Exposure to X-ray and therapeutic radiation induces DNA damage and oxidative stress, which can modify the genetic and epigenetic makeup of the tsf1 and tsf2 gene loci. Research indicates that radiation influences TSF gene expression through epigenetic mechanisms such as changes in DNA methylation, histone modifications, and chromatin remodeling, all of which affect transcriptional activity (Beyersmann and Hartwig, 2020; Kudryavtseva *et al*., 2019).

Altered expression of TSF genes following irradiation has been linked to disruptions in iron homeostasis and impaired epithelial barrier function, contributing to radiation-induced cellular dysfunction and observable organismal phenotypes (Guiran *et al*., 2019; UniProt Consortium, 2025). The intricate relationship between genetic

mutations and epigenetic regulation at TSF loci is crucial for understanding Drosophila's responses to radiation and their translational relevance to human health.

2.5.2 Involvement of TSF1 and TSF2 in DNA Damage Response Pathways in Drosophila melanogaster Under X-ray and Therapeutic Radiation

Overview of DNA Damage Response in Drosophila

The DDR system in Drosophila shows strong evolutionary conservation with mammalian systems and includes processes for sensing DNA lesions, activating checkpoint kinases, repairing DNA, and initiating programmed cell death (Baonza, Tur-Gracia, Pérez-Aguilera, and Estella, 2022). Central to damage sensing is the MRE11-RAD50-NBS1 (MRN) complex, which stimulates ATM (also known as Tefu) and ATR (Mei41) kinases. These kinases phosphorylate checkpoint effectors such as Chk1 (Grapes) and Chk2 (Mnk), thereby coordinating cell cycle arrest, DNA repair mechanisms, and apoptosis (Baonza *et al*., 2022; Brodsky *et al*., 2004).

TSF1 and TSF2 within the DDR Framework

Although TSF 1 and TSF 2 are not classical DDR proteins, their gene expression and epigenetic regulation change in response to radiation-induced stress, indirectly linking them to DDR pathways:

TSF 1 functions as a systemic iron transporter, controlling iron levels which are significant because iron catalyzes reactive oxygen species (ROS) production, thereby intensifying DNA damage (Guiran *et al*., 2019). Proper modulation of TSF 1 during DDR could help alleviate oxidative stress and limit secondary DNA insults.

TSF 2 is pivotal for maintaining epithelial septate junctions, which uphold tissue integrity under stress. Radiation-induced DNA damage can disrupt epithelial barriers, with TSF 2 expression likely regulated as part of the tissue repair responses orchestrated by DDR signaling pathways (UniProt Consortium, 2025).

Radiation-Induced Changes to TSF Gene Regulation

Radiation exposure alters the transcriptional and epigenetic state of tsf1 and tsf2. For example, epigenetic mechanisms such as histone acetylation and DNA methylation at these gene loci dynamically regulate their expression as part of the DDR (Beyersmann and Hartwig, 2020). Moreover, regulatory non-coding RNAs implicated in DDR pathways may influence TSF gene activity following irradiation (Mauro *et al*., 2022).

Integration with DNA Repair and Damage Tolerance Mechanisms

Recent investigations emphasize DNA damage tolerance mechanisms in Drosophila, such as translesion DNA synthesis mediated by the REV1 polymerase, which prevents chromosome fragmentation during replication of damaged templates (Khodaverdian et al., 2024). While TSF1 and TSF2 are not directly involved in such pathways, their expression and regulation are likely affected by the broader cellular stress responses that harmonize DNA repair processes with metabolic homeostasis.

2.5.3 Regulatory Networks and Target Genes of TSF1 and TSF2 in Drosophila melanogaster Subjected to X-ray and Therapeutic Radiation

The expression of genes in Drosophila melanogaster, including those encoding Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2), is controlled by intricate regulatory networks comprising numerous transcription factors (TFs), cofactors, and chromatin remodeling proteins. These networks process environmental inputs such as X-ray and therapeutic radiation to finely tune gene expression and epigenetic modifications, enabling suitable cellular responses to DNA damage and oxidative stress (Madigan *et al*., 2002; Baumgartner *et al*., 2018; Elgin and Reuter, 2013).

Transcriptional Regulatory Networks in Drosophila

Comprehensive integrative analyses have reconstructed extensive transcriptional regulatory networks in Drosophila, encompassing around 300,000 regulatory connections involving approximately 600 TFs and 12,000 target genes (Marbach *et al*., 2012). These networks are derived from combining TF binding profiles, conserved DNA motifs, gene expression data, and chromatin modification signatures, resulting in highly predictive models of gene regulation.

Rhee *et al.* (2014) deepened this understanding by mapping physical interactions among 459 transcription-related proteins, uncovering combinatorial TF complexes that drive gene regulation in vivo. This research links physical TF interactions to functional outcomes, underscoring the complexity inherent in Drosophila transcriptional control.

Regulatory Networks Centered on TSF1 and TSF2

Although detailed regulatory circuits specifically focused on tsf1 and tsf2 are yet to be fully characterized, their gene expression is likely modulated by several TFs that respond to radiation-induced cellular stress. Considering TSF 1's vital function in iron

transport and immune regulation, alongside TSF 2's role in epithelial junction formation, it is reasonable to assume these genes are regulated by TFs involved in stress response, metabolic pathways, and tissue maintenance.

Candidate TFs include p53, NF-κB, and hypoxia-inducible factors (HIFs), which govern genes linked to DNA damage response and iron metabolism (Beyersmann and Hartwig, 2020). Additionally, TFs identified within Drosophila networks related to oxidative stress and development may also influence TSF gene expression after radiation exposure.

Predicting Target Genes and Functional Annotations

Machine learning methods such as GENIE3, applied to large-scale RNA sequencing datasets, enable refinement of TF-target gene predictions, improving identification of key regulators controlling tsf1 and tsf2 during radiation stress (Mendoza-Parra *et al*., 2021). Integrating TF binding information with chromatin interaction data further facilitates discovery of cis-regulatory modules (CRMs) governing tsf1 and tsf2 expression, enhancing understanding of their regulatory dynamics during DNA repair and damage response processes (Sandmann *et al.*, 2007).

2.6 Techniques for Genetic and Epigenetic Profiling of TSF1 and TSF2 in Drosophila melanogaster Under X-ray and Therapeutic Radiation

Analyzing the genetic and epigenetic profiles of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster exposed to X-ray and therapeutic radiation involves the use of a variety of advanced molecular and genomic approaches. These methods allow for comprehensive examination of DNA sequence alterations, gene expression dynamics, chromatin modifications, and epigenetic regulation impacting TSF gene function during radiation-induced stress (Antosh *et al*., 2014; Baumgartner *et al*., 2018; Elgin and Reuter, 2013).

Genetic Profiling Methods

Whole-Genome and Targeted Sequencing: Next-generation sequencing (NGS) technologies provide high-resolution detection of mutations, structural variations, and recombination events in Drosophila genomes following irradiation (Singh, 2013). Targeted sequencing of the tsf1 and tsf2 regions enables the identification of radiation-induced point mutations or deletions that could disrupt gene function.

Gene Mapping and Mutagenesis: Employing classical genetic techniques alongside modern sequencing facilitates the mapping of radiation-generated mutations. Tools such as balancer chromosomes and P-element transposons are instrumental in generating and maintaining mutant strains for functional analysis of TSF genes (Umu *et al*., 2016).

RNA Sequencing (RNA-seq): Transcriptomic profiling via RNA-seq quantifies changes in tsf1 and tsf2 transcript levels and helps identify co-regulated genes and pathways involved in radiation response (Umu *et al*., 2016).

Epigenetic Profiling Approaches

Chromatin Immunoprecipitation Sequencing (ChIP-seq): ChIP-seq enables genome-wide mapping of histone modifications and transcription factor occupancy, thereby defining the epigenetic landscape at tsf1 and tsf2 loci (Celniker *et al*., 2009; St Pierre *et al*., 2014). This method reveals radiation-induced alterations in chromatin marks such as H3K27me3 and H4K16ac that regulate gene activity.

Bisulfite Sequencing: Although DNA methylation is minimal in Drosophila, bisulfite sequencing permits identification of methylation changes in specific genomic regions, providing insights into the epigenetic regulation of TSF genes under radiation stress (Lyko *et al*., 2000).

ATAC-seq and DNase-seq: These techniques assess chromatin accessibility, indicating the openness of regulatory elements at TSF loci and how chromatin remodeling occurs following radiation exposure (Umu *et al*., 2016).

Non-coding RNA Profiling: Small RNA-seq and lncRNA-seq detect radiation-responsive non-coding RNAs involved in the epigenetic modulation of TSF gene expression (López-Pérez *et al*., 2024; Mauro *et al*., 2022).

Genetic Manipulation and Functional Evaluation

CRISPR/Cas9 Genome Editing: This precise genome editing tool enables targeted knockout or modification of tsf1 and tsf2 genes to investigate their specific roles in radiation responses (Bassett and Liu, 2014).

GAL4/UAS System: This system facilitates tissue-specific overexpression or silencing of TSF genes and epigenetic regulators, allowing functional dissection under radiation conditions (Brand and Perrimon, 1993).

Reporter Gene Assays: Fluorescent or luciferase reporters under the control of tsf1 and tsf2 promoters are used to monitor transcriptional activity changes in living flies post-radiation exposure.

Advantages of Using Drosophila as a Model

Drosophila melanogaster provides a powerful genetic model featuring a well-annotated genome, large collections of mutants, and extensive epigenomic datasets, such as those generated by the modENCODE project. Its compact genome and conserved epigenetic machinery enable integrated analyses of genetic and epigenetic alterations induced by radiation on TSF genes (Celniker *et al*., 2009; Umu *et al.,* 2016).

2.6.1 Genomic Sequencing Techniques (WGS, PCR, Sanger) for Analysis of TSF1 and TSF2 in Drosophila melanogaster Subjected to X-ray and Therapeutic Radiation

Genomic sequencing approaches such as Whole-Genome Sequencing (WGS), Polymerase Chain Reaction (PCR), and Sanger sequencing are essential for examining the genetic and epigenetic profiles of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster exposed to ionizing radiation like X-rays and therapeutic radiation. These methodologies facilitate the detection of mutations, structural changes, and precise base-level modifications resulting from radiation exposure(Antosh *et al*., 2014; Adams *et al*., 2000; Baumgartner *et al*., 2018).

Whole-Genome Sequencing (WGS)

WGS offers comprehensive coverage of the Drosophila genome, enabling the identification of single nucleotide polymorphisms (SNPs), insertions/deletions (indels), and larger structural variants impacting tsf1 and tsf2 loci. Recent improvements have made WGS faster, more cost-efficient, and capable of high-depth sequencing, capturing both coding regions and regulatory non-coding sequences (Kim *et al*., 2015; Miller *et al*., 2018). WGS has been applied to map genome-wide radiation-induced mutations, illuminating mutational patterns and adaptive genomic alterations in response to environmental stresses (Shcherbakova, Fedorova, and Gvozdev, 2022).

Polymerase Chain Reaction (PCR)

PCR is a highly sensitive and specific technique frequently used to amplify defined segments of tsf1 and tsf2 for mutation detection and confirmation. Within radiation genetics, PCR assays have proven effective for identifying intragenic deletions, gene

conversion events, and point mutations caused by gamma rays and neutron irradiation in Drosophila sperm cells (Shcherbakova, Fedorova, and Gvozdev, 2022).

Sanger Sequencing

Sanger sequencing remains the benchmark for precise identification of nucleotide-level mutations. Often used to validate mutations amplified by PCR in tsf1 and tsf2, this approach detects point mutations, small indels, and detailed sequence contexts related to DNA damage and repair (Shcherbakova, Fedorova, and Gvozdev, 2022).

Integrative Application of Sequencing Techniques

Utilizing WGS for broad detection of mutations, alongside PCR and Sanger sequencing for targeted validation, provides a rigorous framework for elucidating genetic alterations in TSF genes following radiation exposure. This combined strategy also facilitates epigenetic investigations by allowing focused analysis of DNA methylation and chromatin state changes at single-gene resolution.

Application in Radiation Research Using Drosophila

(Shcherbakova *et al*., 2022) employed an integrated array of genetic, cytogenetic, and molecular approaches—including PCR and sequencing—to study inherited recessive mutations induced by γ-rays and neutron radiation in Drosophila. They categorized mutations into gene/structural types linked to chromosomal aberrations and gene/point mutations involving DNA sequence alterations, with PCR and sequencing enabling precise mutation classification. These methodologies are directly translatable to analyzing the effects of radiation on tsf1 and tsf2 genes.

2.6.2 Epigenomic Analysis (ChIP-seq, Bisulfite Sequencing) of TSF1 and TSF2 in Drosophila melanogaster Following X-ray and Therapeutic Radiation Exposure

Chromatin Immunoprecipitation Sequencing (ChIP-seq)

ChIP-seq allows for a genome-wide mapping of DNA-protein interactions, capturing histone modifications and binding sites of transcription factors to reveal the chromatin architecture at tsf1 and tsf2 loci. For instance, (Jin *et al.,*2008) showed that radiation triggers epigenetic repression of proapoptotic genes in Drosophila by enriching repressive histone marks such as trimethylated H3 lysine 27 (H3K27me3) and H3 lysine 9 (H3K9me3). Comparable modifications at regulatory regions of tsf1 and tsf2 may regulate their transcriptional responses triggered by DNA damage.

The modENCODE consortium has generated comprehensive ChIP-seq datasets in Drosophila, detailing histone modification patterns and transcription factor occupancy across the genome (Celniker *et al*., 2009).

Bisulfite Sequencing

Bisulfite sequencing is regarded as the definitive method for detecting DNA methylation at single-base resolution. Although Drosophila demonstrates relatively low global DNA methylation compared to mammals, specific loci, particularly regulatory regions of stress-associated genes, display functional methylation (Lyko *et al*., 2000). Kravets, Mousseau, Litvinchuk, and Ostermiller (2010) reported radiation-induced DNA hypomethylation in Drosophila after extended low-dose irradiation, suggesting such methylation changes could impact expression of TSF genes following radiation exposure.

Applying bisulfite sequencing to promoter regions of tsf1 and tsf2 can uncover methylation dynamics influenced by radiation, providing mechanistic insights into how epigenetic regulation modulates iron metabolism and epithelial functions.

2.6.3 Transcriptomic Techniques (RNA-seq, Single-cell RNA-seq) for Investigating TSF1 and TSF2 in Drosophila melanogaster Exposed to X-ray and Therapeutic Radiation

Transcriptome profiling methods, such as bulk RNA sequencing (RNA-seq) and single-cell RNA sequencing (scRNA-seq), are vital for examining the genetic and epigenetic regulation of Transferrin 1 (TSF 1) and Transferrin 2 (TSF 2) in Drosophila melanogaster following exposure to X-ray and therapeutic radiation. These approaches provide comprehensive views of gene expression patterns, reveal cell-type-specific responses, and elucidate transcriptional networks activated by radiation stress(Zarubin *et al*., 2021).

Bulk RNA-seq

RNA-seq has been widely utilized to chart the Drosophila transcriptome throughout different developmental phases and under various environmental challenges. For instance, Daines et al. (2011) created an extensive transcriptome reference across life stages, uncovering alternative splicing events and novel transcripts, which aids in detailed scrutiny of tsf1 and tsf2 expression (Daines *et al.,* 2011).

Radiation-focused RNA-seq studies have detected differential gene expression in Drosophila subjected to low-level and ambient background radiation, notably

reporting downregulation of core metabolic pathways alongside upregulation of immune and stress-related genes (Zarubin *et al*., 2021). These findings imply that genes like tsf1 and tsf2, involved in iron metabolism and maintenance of epithelial integrity, might also undergo transcriptional regulation following radiation exposure.

(Moskalev *et al.*, 2014) applied RNA-seq to profile global gene expression alterations in Drosophila after ionizing radiation exposure, identifying a range of transcription factors and metabolic genes responsive to radiation (Moskalev *et al*., 2014).

Single-cell RNA-seq (scRNA-seq)

Single-cell transcriptomics offers the advantage of resolving cellular heterogeneity in response to radiation. Recently, scRNA-seq was employed to analyze Drosophila wing imaginal discs post X-ray irradiation, identifying uniformly increased expression of DNA damage response (DDR) transcription factors like p53, Irbp18, and Xrp1, alongside TFs such as Dif and Ets21C that display varied expression among cells (Almuzzaini *et al*., 2025).

CHAPTER THREE

MATERIALS AND METHOD

3.0 Study Area

The research was conducted at the University of Benin, Edo State, Nigeria. The University of Benin (UNIBEN) established in 1970 has garnered recognition across the country as a prominent public research institution, situated in Benin city, Edo state.

3.1 Study Location

The study was conducted in a dedicated laboratory facility equipped with appropriate infrastructure and equipment for rearing and manipulating Drosophila melanogaster. The laboratory, Biomedical Toxicology Chemicals Safety (BIOTOXCS) Research laboratory, Central Biomedical Research, located at University of Benin, Benin city, Edo State, Nigeria. The controlled environment of the laboratory provided the necessary conditions for maintaining the flies and conducting the experiments.

3.2 Study Population

In this study, Drosophila melanogaster was used as a model. Drosophila melanogaster was obtained from the Drosophila Laboratory, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. The flies was allowed to acclimatize before the commencement of feeding and transfer procedures. The flies was maintained on the standard cornmeal diet, formulated with the following components: cornmeal (52g), brewer's yeast (5g), glucose (0.5g), agar (7.9g), nipargin (1g), ethanol (2ml) and distilled water (850ml).

3.3 EXPERIMENTAL DESIGN

The experimental design involves the use of Drosophila melanogaster to study the effects of therapeutic radiation and X-rays. Three vials of Drosophila was used, each representing different groups. Group 1 serves as the control and was maintained under standard laboratory conditions without any exposure to radiation. Group 2 was placed in an X-ray room for seven days, while Group 3 was left in the same room for fourteen days. The flies was not directly exposed to the X-ray beam; rather, they were indirectly exposed to residual radiation present in the room where diagnostic imaging is routinely carried out. All groups were kept under similar environmental conditions to ensure consistency. At the end of the exposure periods, flies were assessed for changes in growth, survival, reproduction, and biochemical responses.

3.4 Procedure for Feed Preparation and Handing of Drosophila

The preparation of the medium for Drosophila melanogaster culture was carried out through a series of carefully coordinated steps to ensure optimal consistency and nutrient availability. First, 52 grams of corn meal was dissolved in 0.15 liters of water at normal room temperature, while the yeast was dissolved separately in a small quantity of hot water. Meanwhile, agar was added to boiling water and was allowed to stand for approximately 10 minutes to ensure proper hydration and activation. Following this, the pre-dissolved corn meal was added gradually to the boiling agar solution while stirring continuously. This mixture was stirred for about 5 to 10 minutes to ensure uniformity. The dissolved yeast, along with 3.5 grams of glucose, was then added to the mixture, and the preparation was stirred thoroughly and left to simmer for an additional 15 to 20 minutes. Next, Niparjin was dissolved in 1 to 2 milliliters of absolute ethanol to form a preservative solution. the main mixture was removed from heat and allowed to cool slightly, the Niparjin solution was added slowly while stirring continuously to ensure even distribution throughout the medium. Finally, the prepared medium was poured into the desired vials. It was ensured that the mixture remains semi-solid, possessing a flowable consistency at the point of distribution. It was allowed to set, forming a firm but not overly solid substrate suitable for Drosophila culture.

3.5 Sample collection

Wild-type Drosophila melanogaster (Oregon R strain) was reared on standard cornmeal-agar medium at 25 °C under a 12-hour light/dark cycle. Newly eclosed adult flies (3–5 days old) was used for experiments. At 6, 24, and 48 hours post-radiation exposure, adult Drosophila melanogaster from each group (Control, X-ray, and Therapeutic Radiation) was anaesthetized briefly on ice. Twenty flies per time point was randomly selected and pooled per replicate, with three biological replicates per group. Flies was rapidly transferred into RNase-free microcentrifuge tubes, flash-frozen in liquid nitrogen, and stored at −80 °C until RNA extraction. Care was taken to avoid RNA degradation by minimizing handling time and using sterile, RNase-free tools. All samples was processed under the same environmental conditions to ensure consistency across time points and treatment groups.

3.6 Laboratory Analysis

Collected Drosophila melanogaster samples was subjected to molecular examination to evaluate the expression of key genes involved in apoptosis, oxidative stress response, vesicular trafficking, and iron metabolism. Total RNA was extracted using TRIzol® reagent according to the manufacturer's instructions. RNA concentration and purity was determined using a NanoDrop spectrophotometer, ensuring A260/A280 ratios between 1.8 and 2.0. High-quality RNA will be reverse-transcribed to complementary DNA (cDNA) using a commercially available reverse transcription kit. Quantitative real-time PCR (qRT-PCR) will be performed to assess the expression levels of Tsf1 (ferritin) and Tsf2 (melanotransferritin), which are key markers in iron homeostasis. Each reaction was carried out in triplicate using gene-specific primers and SYBR Green master mix on a real-time PCR machine. The housekeeping gene Rp49 will be used as an internal control for normalization. Gene expression will be quantified using the 2^-ΔΔCt method, comparing irradiated groups to the non-irradiated control. This examination was allowed for the assessment of transcriptional changes induced by X-ray and therapeutic radiation exposure across multiple stress-related pathways in Drosophila melanogaster.

3.7 STATISTICAL ANALYSIS

Data analysis was analyzed using descriptive statistics, frequencies, and cross-tabulations. A Student's t-test was used for comparisons between two groups, while one-way ANOVA was applied when comparing more than two groups. A p-value of less than 0.05 will be considered statistically significant. Error bars was expressed as mean ± standard error of the mean (SEM).

CHAPTER FOUR

RESULT

4.1 Expression Analysis of TSF1 mRNA

The relative expression of the TSF1 gene was assessed in Drosophila melanogaster following exposure to ionizing radiation, specifically X-rays and CT room conditions, for durations of 7 and 14 days. As shown in Figure 4.1, the expression of TSF1 mRNA significantly increased in flies exposed to X-rays for both 7 days (80.14 ± 1.00) and 14 days (85.97 ± 1.43) compared to the control group (67.77 ± 1.84). The observed p-values ($p=0.0009$ for 7 days and $p=0.04$ for 14 days) confirm that this upregulation is statistically significant. Under CT room exposure, a similar pattern was observed: TSF1 expression increased at 7 days (80.20 ± 0.72) and was highest at 14 days (86.28 ± 1.85), further reinforcing the notion that TSF1 expression is responsive to ionizing radiation and potentially exhibits a dose-duration effect.

The augmentation of TSF1 transcription suggests that this gene plays a vital role in the cellular adaptation to oxidative or radiation-induced stress. Given that TSF1 is a key regulator of iron transport—an essential process for cell survival and immune response—the increase in its expression likely reflects a compensatory mechanism to maintain iron homeostasis during radiation damage.

4.2 Expression Analysis of TSF2 mRNA

Contrary to TSF1, the expression of the TSF2 gene displayed a different dynamic following similar radiation exposures (Figure 4.2). There was a statistically significant increase in TSF2 mRNA at 7 days post X-ray exposure (72.23 ± 2.39, $p=0.0027$) and at 14 days (68.58 ± 1.27, $p=0.012$) compared to controls (61.96 ± 1.14). However, under CT room exposure, TSF2 expression initially increased at 7 days (69.91 ± 2.23, $p=0.007$) but significantly declined by 14 days (57.76 ± 1.94), with this decrease being statistically significant compared to earlier time points.

This biphasic response indicates that TSF2 transcription may initially be stimulated by radiation-induced stress but is potentially suppressed during prolonged exposure. TSF2 has been implicated in tissue integrity and cellular junction assembly, so its downregulation in longer exposures may be indicative of compromised tissue architecture or cellular stress accumulation over time.

The differential expression patterns of TSF1 and TSF2 highlight distinct regulatory roles in response to ionizing radiation. TSF1 upregulation appears to be a consistent early and sustained reaction, suggesting its importance in maintaining iron metabolism and counteracting radiation-induced oxidative damage. In contrast, TSF2 shows early induction followed by downregulation, perhaps reflecting its involvement in less direct stress response mechanisms or tissue repair pathways that are compromised with extended radiation exposure.

These findings support the hypothesis that ionizing radiation modulates expression of key genes involved in iron homeostasis and cellular integrity, which may contribute to the physiological and molecular effects of radiation exposure observed in Drosophila. Understanding this gene-specific regulation enhances our comprehension of radiation biology and could inform further studies on radiation effects in higher organisms.

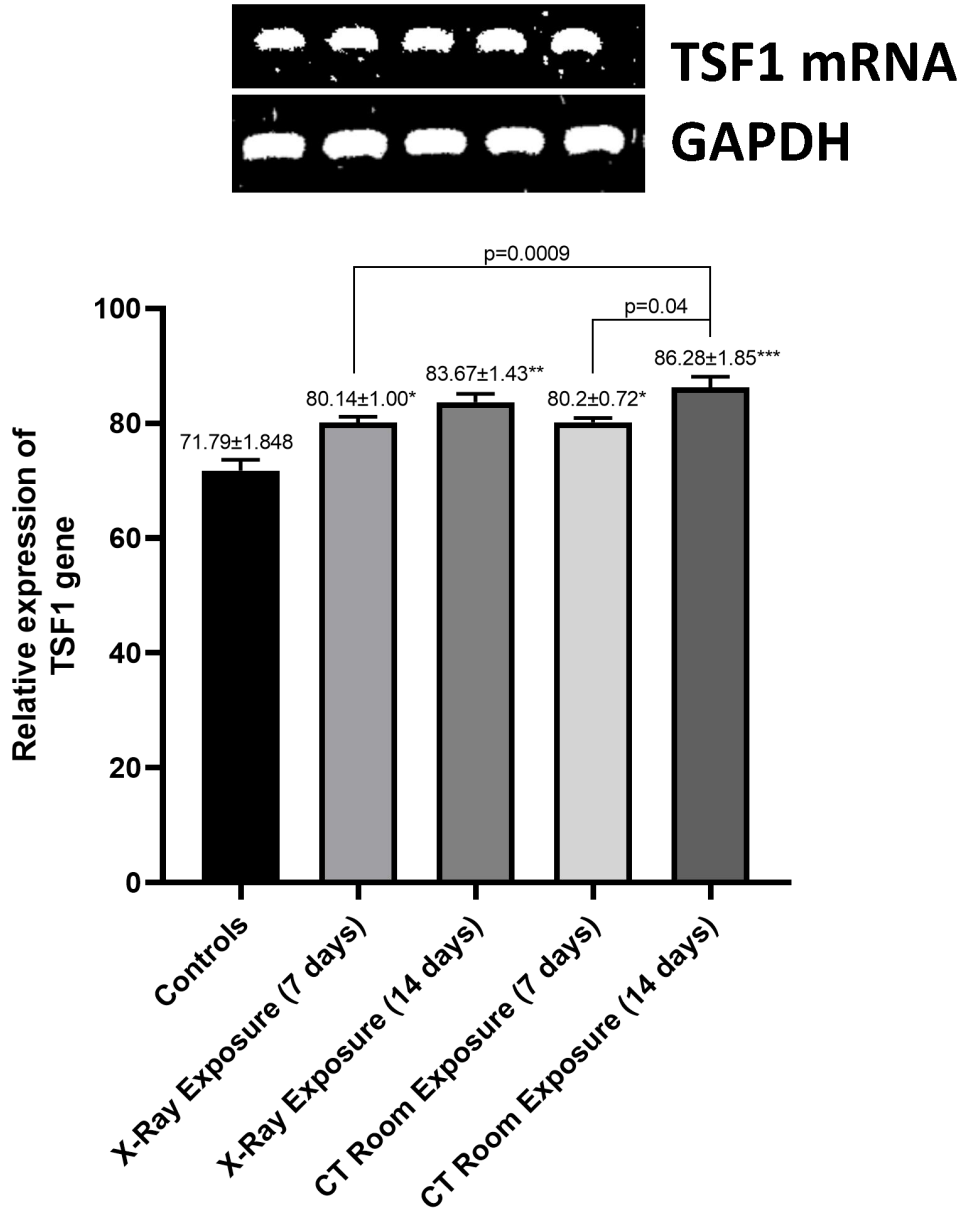

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Figure 4.1: PCR and agarose gel analysis of TSF 1 mRNA. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, p<0.01, *p<0.001)

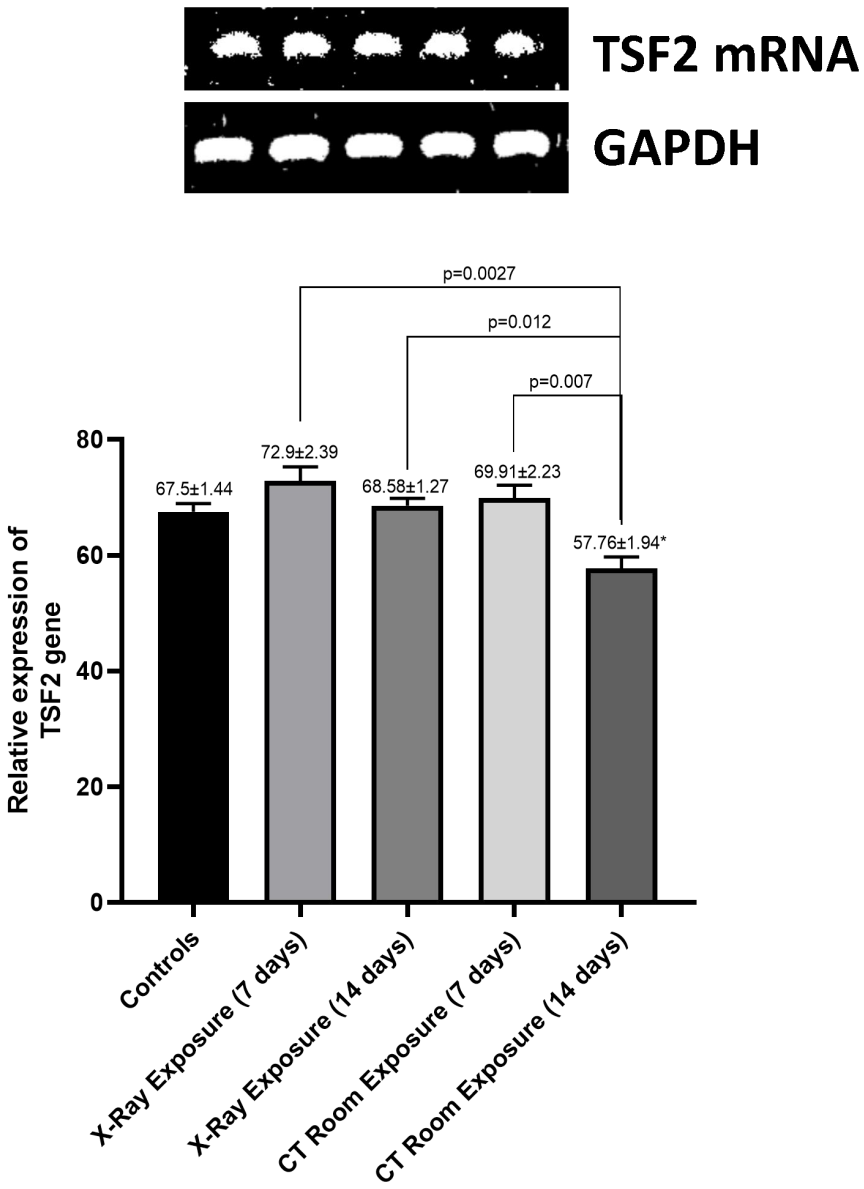

Figure 4.2: PCR and agarose gel analysis of TSF 2 mRNA. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, p<0.01, *p<0.001)

CHAPTER FIVE

DISCUSSION

5.0

This research examined how exposure to ionizing radiation, specifically X-rays and low-dose radiation from a CT room environment, influences the transcriptional regulation of two key genes involved in iron metabolism—Transferrin 1 (TSF1) and Transferrin 2 (TSF2)—in Drosophila melanogaster. Assessments were carried out after 7 and 14 days of exposure, revealing distinct gene-specific patterns of transcriptional activity. These results highlight the adaptive responses of iron regulatory pathways to radiative stress and underline their potential roles in oxidative stress resistance, tissue integrity, and organismal survival.

Expression Dynamics of TSF1 under Radiation Stress

The marked increase in TSF1 expression following both X-ray and CT-room exposures indicates that this gene plays a central role in the organism's defensive strategy against radiation-induced oxidative imbalance. TSF1 is widely recognized as the primary iron-binding and transport protein in Drosophila, crucial for buffering intracellular iron levels and minimizing the harmful generation of reactive oxygen species (ROS) through Fenton chemistry (Morciano *et al*., 2018). Since ionizing radiation elevates ROS production, damaging DNA, proteins, and lipids (Azzam *et al*., 2012), the upregulation of TSF1 suggests a compensatory mechanism designed to mitigate iron-driven oxidative injury.

Interestingly, the persistence of elevated TSF1 levels up to 14 days indicates that this gene is not only responsive to acute stress but also participates in longer-term adaptive reprogramming of cellular metabolism. Such extended upregulation mirrors hormetic and threshold effects previously documented in radiation studies, where moderate doses stimulated protective responses rather than overt toxicity (Antosh *et al*., 2014; Vaiserman *et al*., 2004). Thus, TSF1 appears to function as a dual-phase protector, contributing both to immediate stress mitigation and to sustained cellular resilience.

Additionally, since iron availability is intimately tied to immune function, the activation of TSF1 may have secondary consequences for host immunity. Elevated TSF1 expression could enhance immune readiness by regulating iron-dependent

pathways that support cellular defense mechanisms, thereby improving survival under chronic radiative challenge.

Variable Expression Patterns of TSF2 and Their Biological Meaning

Unlike the consistent upregulation of TSF1, TSF2 showed a biphasic expression profile, with an initial induction at 7 days that subsequently declined by 14 days, particularly in the CT-exposed group. TSF2 has been linked to structural and functional integrity of epithelial tissues, where it participates in septate junction formation and maintenance of epithelial barriers (Faivre-Sarrailh *et al.*, 2004). The observed decline in TSF2 levels after prolonged radiation suggests progressive weakening of epithelial stability, making tissues more vulnerable to chronic stress and injury.

This early induction followed by downregulation points to an adaptive but unsustainable protective response. Initially, cells may attempt to reinforce tissue barriers through TSF2 activity; however, persistent oxidative stress eventually suppresses the gene, possibly due to cumulative damage or reprogramming of repair pathways. This scenario aligns with observations of epithelial breakdown under chronic irradiation, which can impair tissue repair and heighten susceptibility to degeneration (Trinca, 2022).

Moreover, the variability of TSF2 expression suggests involvement of epigenetic regulation. Radiation is known to induce modifications such as histone acetylation, methylation, and chromatin remodeling, which can impose long-lasting transcriptional effects (Alexandrov, 2022). TSF2's sensitivity to such regulatory layers may explain its fluctuating response, in contrast to the more stable induction seen with TSF

The contrasting regulation of TSF1 and TSF2 underscores the multifaceted nature of transferrin gene functions in maintaining physiological stability under radiation stress. On one hand, TSF1 appears to serve as a robust and durable shield against oxidative damage through iron sequestration and detoxification. On the other, TSF2 reflects tissue vulnerability, with its suppression signaling potential compromise of epithelial defenses.

Since Drosophila melanogaster shares highly conserved molecular and genetic pathways with higher organisms, including humans (Bellen *et al.*, 2010), these findings extend beyond insect biology. They may offer translational insights into human radiobiology, particularly in the fields of radioprotection and oncology. For instance, enhancing TSF1 activity could represent a promising strategy for reducing

radiation-induced toxicity during medical imaging or cancer therapy. Similarly, monitoring TSF2 expression could serve as an early biomarker for tissue fragility under chronic exposure.

Furthermore, the balance between protective (TSF1) and vulnerable (TSF2) pathways highlights the need for precision in radiation medicine. Expression profiling of such genes could aid in predicting radiosensitivity, guiding personalized therapeutic approaches, and improving long-term outcomes in patients undergoing radiation-based treatments.

5.1 Conclusion

This study demonstrates that radiation exposure produces differential regulatory outcomes in transferrin family genes of Drosophila melanogaster. TSF1 is persistently upregulated, likely serving as a durable protective mechanism against radiation-induced oxidative damage through iron sequestration. In contrast, TSF2 exhibits a biphasic response, with early induction followed by suppression, signaling epithelial fragility under sustained stress. Together, these findings illuminate the dual role of iron metabolism in resilience and vulnerability under radiation and provide a basis for identifying novel molecular targets for radioprotection.

By bridging insights from a genetically tractable model organism to potential clinical applications, this work contributes to the broader understanding of radiation biology and opens avenues for precision medicine approaches in mitigating radiation-induced tissue damage.

5.2 Recommendations

To address these limitations and expand current understanding, future investigations should:

1. Perform proteomic analyses to confirm whether elevated mRNA levels correspond to changes in protein abundance and activity.

Utilize live-reporter systems to track real-time dynamics of TSF gene activation in vivo.

2. Explore epigenetic alterations, including DNA methylation and histone modifications, at TSF loci under radiation stress.

Apply gene editing tools (e.g., CRISPR or RNA) to dissect causal roles of TSF1 and TSF2 in radiation resilience.

3. Assess interactions between transferrin genes and other oxidative stress or immune-regulatory pathways.

4. Investigate responses across different developmental stages and under varied radiation types (UV, gamma rays, ion beams) to build a comprehensive picture of gene-environment interactions.

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APPENDIX

EQUIPMENT AND MATERIALS USED

Falcon tubes

Beakers and Measuring Cylinder

Homogenizing Stick

Eppendorf tubes

Digital weighing balance

Lab coats and rubber gloves

Cottol wood and Funnel

Centrifuge

Hot plate

X-ray machine

CT scan machine

REAGENTS USED

Cornmeal

Yeast

Sugar (glucose)

Agar

Mold inhibitor (nipagin)

TRIzol reagent

DNase I enzyme

Reverse transcription kit (for cDNA synthesis)

qPCR master mix (SYBR Green or TaqMan)

Primers for TSF1 and TSF2 genes

Agarose gel

Sterile nuclease-free water

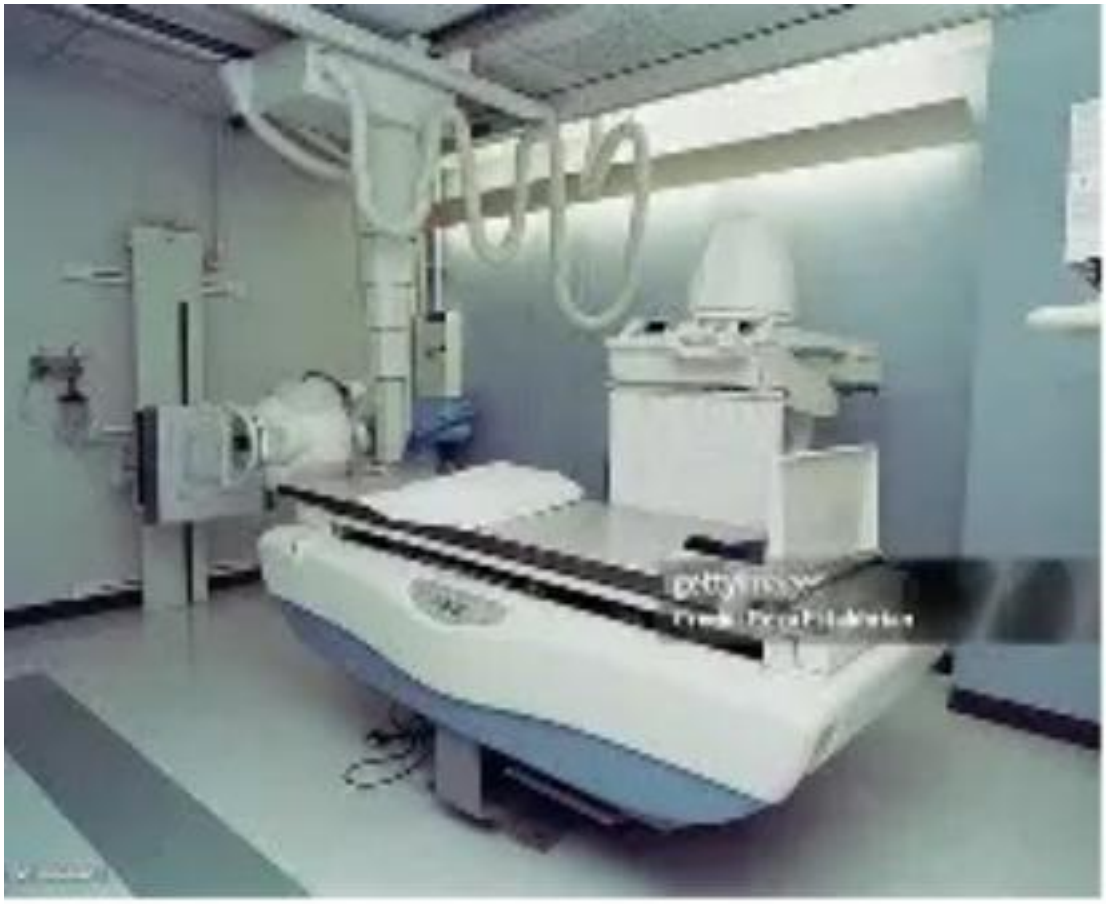
TRE buffer

Phosphate Buffer

Distilled Water

Drosophilia Culture Vial

CT SCAN MACHINE



X-RAY MACHINE