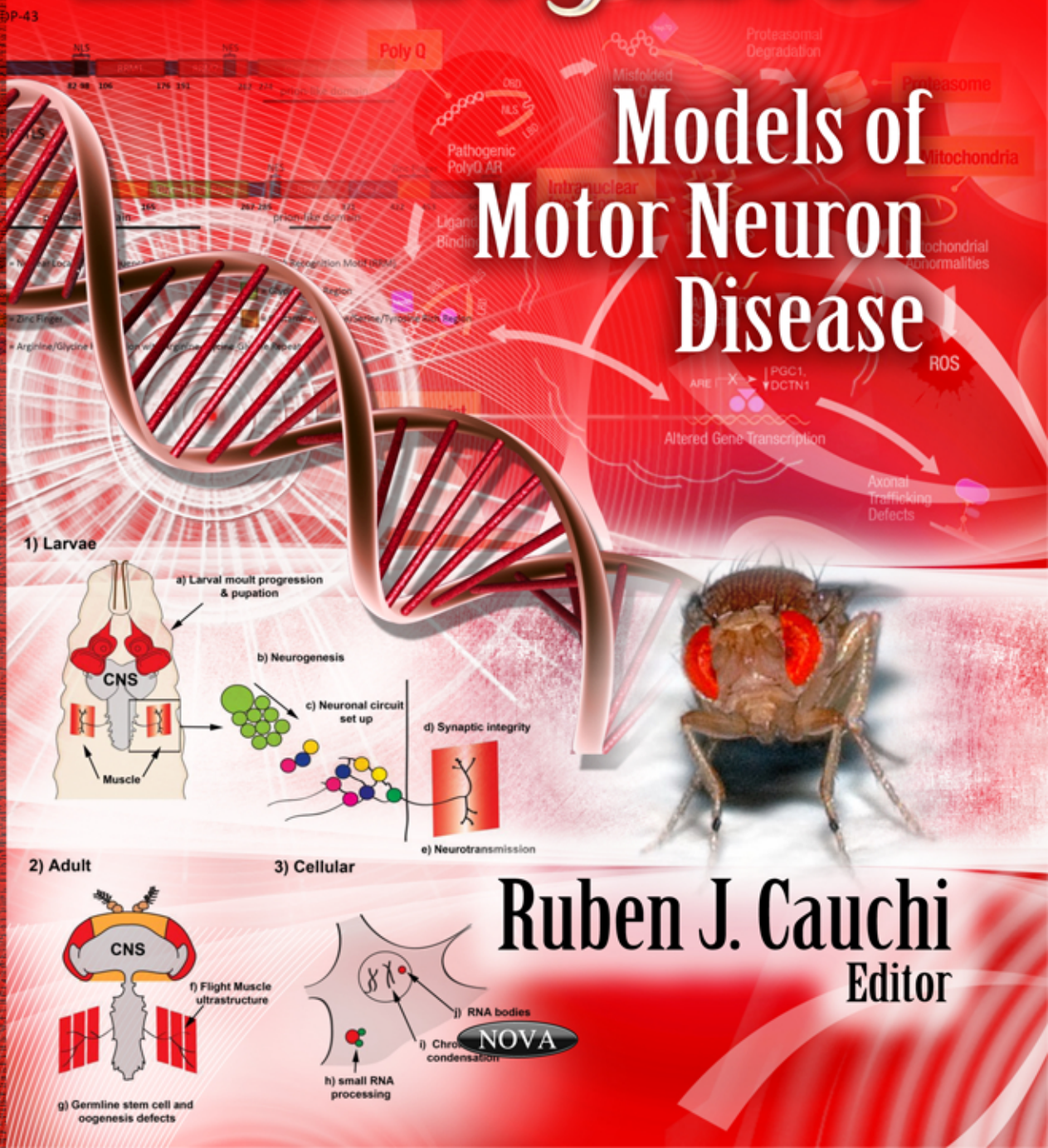
NEURODEGENERATIVE DISEASES - LABORATORY AND CLINICAL RESEARCH

Drosophila melanogaster

Models of Motor Neuron Disease

Ruben J. Cauchi
Editor

Nova Biomedical



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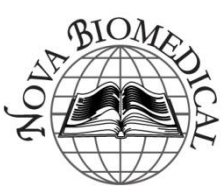

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PREFACE

A RESPONSIBLE CHOICE OF MODEL ORGANISM

Motor neuron diseases (MNDs) are the most catastrophic of neurodegenerative disorders in that cognitive function is spared yet motor neuron degeneration translates into progressive muscle weakness and paralysis that propel the afflicted patient to eventual death. Neurodegenerative disorders constitute one of the major challenges of modern medicine in view of the current lack of effective therapies.

The fruit fly, *Drosophila melanogaster*, has a distinguished history as an important model organism capable of shaping our fundamental understanding of life including neuromuscular development and physiology. Through the efforts of the *Drosophila* and human genome projects, more then a decade ago, we learned that the genetic makeup of the fruit fly is remarkably similar to humans. In this respect, it's no surprise that the vast majority of all known human disease genes have a similar fly counterpart that is devoid of the genetic complexity so typical of the human gene structure and families.

Compared to vertebrate models, the fly is small, has a rapid lifecycle and gives rise to a large numbers of offspring. Importantly, at a molecular and physiological level, the basic principles of neuromuscular function are amazing conserved. Combine this with the presence of numerous genetic tools developed over the last century allowing genes and the proteins they encode to be manipulated swiftly to decipher their *in vivo* function and you have a superb genetic animal model organism of disease. This publication singles out the past and recent accomplishments of *Drosophila* in modelling MNDs with particular emphasis on the emerging molecular pathways underpinning these diseases.

The volume opens with Chapter One where *Rebecca K. Sheean* and *Bradley J. Turner* (University of Melbourne, Australia) introduce us to the features of the two groups of neurons that are primarily affected in MND, namely upper motor neurons (also known as giant pyramidal cells or Betz cells) and lower motor neurons (also known as anterior horn cells in the spinal cord). MNDs are categorised according to which group of motor neurons is affected and in this context, the authors give us the core clinical and pathological features of upper MNDs including primary lateral sclerosis (PLS) and hereditary spastic paraplegia (HSP); lower MNDs including progressive muscular atrophy (PMA), progressive bulbar palsy (PBP), spinal muscular atrophy (SMA), spinobulbar muscular atrophy (SBMA), axonal Charcot-Marie-Tooth disease, which significantly overlaps with distal hereditary motor

neuropathy (dHMN) and lethal contracture syndrome (LCCS); and amyotrophic lateral sclerosis (ALS), in which there is combined upper and lower motor neuron loss.

Chapter One also includes a detailed account of the multitude of genes linked to motor neuron degeneration. Interestingly, most of these genes encode ubiquitously expressed and fundamental proteins that, when defective, lead to selective motor neuron injury. Based on this rich genetic evidence captured in the past decades as well as seminal contributions by mammalian models, the authors eloquently highlight the key cellular pathways that are disrupted in both motor neurons and their neighbouring cells including mitochondrial function, intracellular membrane trafficking, axonal transport, cytoskeletal dynamics, RNA processing, proteostasis, myelination and lipid metabolism. Importantly, various mentioned genes and the proteins they encode will be subject to deeper investigation in the chapters that follow.

In Chapter Two, *Hiroshi Tsuda* et al. (McGill University, Canada) kick-start a series of accounts that underline the contribution of *Drosophila* to our understanding of the pathophysiology of motor neuron degeneration. The authors focus on ALS (also known as Lou Gehrig's disease), a mercilessly fatal neurodegenerative disease, which was first described by the founder of modern neurology, the French neurologist Jean-Martin Charcot. ALS occurs in hereditary or sporadic forms and since both these forms share several features, insights into the mechanisms through which gene mutations have a negative impact on motor neuron physiology can potentially lead to novel therapeutic approaches that are effective in both forms of the disease.

Identification of the first ALS-linked gene, superoxide dismutase 1 (SOD1), 20 years ago spurred a successful hunt for additional causative genes among which was the VAMP associated protein B (VAPB). VAPB, an endoplasmic reticulum (ER) transmembrane protein, is conserved in *Drosophila* and numerous other species, including the N-terminal major sperm protein (MSP) domain, which harbours the dominantly-inherited missense mutation (proline 56 to serine) that confers motor neuron dysfunction. Human VAPB and its fly orthologue are functionally interchangeable with regards to their influence on the architecture and electrophysiological properties of *Drosophila* neuromuscular junctions (NMJs). Furthermore, *Drosophila* studies were pivotal to reveal the VAPB MSP domain cleaved from the full length protein is secreted in a cell type-specific fashion and acts as a diffusible hormone. Importantly, VAPB-MSP was identified as a ligand for Ephrine (Eph), Roundabout (Robo) and leukocyte-antigen related (LAR) family receptors, which were originally identified as mediators of axon growth cone guidance cues during nervous system development.

The downstream effects of VAPB signalling are still a work in progress though studies in the fly implicate a function in the maintenance of muscle mitochondria. The authors conclude this compelling narrative by delving into the cellular defects associated with mutant VAPB, which can potentially shed light on the pathophysiology of ALS. Based on several lines of evidence, the ALS mutation is thought to cause two different types of defects: failed secretion of mutant VAPB (loss-of-function) and accumulation of mutant VAPB as ubiquitinated ER inclusions that lead to ER stress (gain-of-function), a common defect observed in the pathology of both familial and sporadic forms of ALS.

The subject of Chapter Three remains ALS though *Daniela C. Zarnescu* and colleagues (University of Arizona, USA) focus on TAR DNA binding protein (TDP-43) and fused in sarcoma (FUS), two RNA binding proteins that not only associate with ubiquitinated

intracellular inclusions but also act as causative agents of disease in view of the recent discovery of MND-linked mutations in the respective gene. The identification of these proteins in addition to the involvement of additional RNA binding proteins such as Senataxin and Angiogenin as well as RNA itself (C90RF72 noncoding expanded repeats) in ALS, prompted an 'earth-shaking' shift in our thinking about the pathophysiology of ALS whereby RNA metabolism is presently seen as a central disrupted pathway in the presence of disease.

The authors discuss the contribution of the fruit fly to our understanding of ALS through a review of the studies on TDP-43 and FUS. At a sequence level, both these proteins share several domains including those that enable RNA binding and nucleocytoplasmic shuttling. They also have a similar job description and in this regard, they have been implicated in several RNA processing steps including transcriptional regulation, mRNA splicing, miRNA processing as well as mRNA transport and local translation. *Drosophila* was vital for demonstrating a link between FUS and TDP-43, whereby through genetic interaction approaches it was shown that FUS acts downstream of TDP-43. A flurry of studies have tried to unravel the function of these two proteins through loss-of-function of the orthologous gene or overexpression of either wild-type or mutant human protein in an otherwise wild-type background (the *Drosophila* orthologue of the disease gene is intact). Interestingly, in the case of TDP-43, overexpression studies tended to produce similar ALS-like phenotypes to those observed on loss-of-function which raise the question of whether protein overexpression mirrors the loss-of-function condition. The authors also discuss the genetic interactions reported in the fly models of TDP-43 and FUS which highlight the cellular pathways that are functionally important in ALS including protein folding, proteasome-mediated degradation, apoptosis and microtubule organisation. Differences do exist between fly models of ALS and human pathology such as the absence of ubiquitinated inclusions, which are a hallmark feature of the disease. However, the fly model could be telling us that cytoplasmic aggregates are not a prerequisite for motor neuron degeneration and most probably these pathological inclusions are a consequence rather than a cause of motor neuron injury. Rest assured that you will hear about further twists in this riveting story in the years to come.

It is worth noting that whereas *Drosophila* was heavily exploited to make great strides in deciphering the biology of ALS-linked VAMPB, TDP-43 and FUS, the same cannot be said of SOD1. In this regard, only a few scattered reports exist in the literature. Particularly, Watson et al. (*J Biol Chem* 2008) report that expression of wild-type or disease-linked mutants of human SOD1 selectively in motor neurons induced progressive climbing defect which were accompanied by defective neural circuit electrophysiology, focal accumulation of the human SOD1 protein and stress response in glia surrounding motor neurons. The utility of *Drosophila* to model SOD1 pathophysiology might have been overtaken by the mutant SOD1 mouse model of ALS. However, since therapeutic success in the mouse model has not translated into effective therapy for human ALS in clinical trials (Benatar *Neurobiol Dis* 2007; Aggarwal & Cudkowicz *Neurotherapeutics* 2008), the use of *Drosophila* for high-throughput screening to identify pharmacological and genetic modifiers of disease phenotype might eventually come of age.

The length of motor axons, which can be up to 10^5 times longer than that of cell bodies, presents great challenges to the subcellular trafficking machinery of motor neurons. Impairment of the mechanisms that maintain axonal function can lead to axon degeneration diseases, particularly in the distal regions of the axons that lie furthest from the cell body.

Chapter Four deals with one such group of diseases, namely hereditary spastic paraplegias (HSPs), which are characterised by progressive spasticity and weakness in lower extremities, caused by progressive distal axonopathy mostly in the longest upper corticospinal motor neurons. The large number of genetic loci identified as causative explains the phenotypic heterogeneity of HSPs although the gene products point at an unexpectedly limited range of disease mechanisms, including endoplasmic reticulum organisation and function, axonal microtubule-based transport and endosomal trafficking and signalling, mitochondrial function as well as the interactions of axons with the myelin sheath.

Most but not all causative human genes have orthologues in *Drosophila*. In view of the powerful genetic tools for generation of specific mutant or transgenic flies, as well as the myriad of analytic tools for understanding the cellular roles of these gene products in neurons, particularly in axons and synapses, *Drosophila* offers a compelling system to study HSP-linked genes as well as the consequences on mutation. *Belgin Yalçın* and *Cahir J. O'Kane* (University of Cambridge, UK) review the major contributions from flies so far including the dissection of the roles of several HSP proteins in ER organisation, transport of specific cargoes in axons and in pathways including bone morphogenetic protein (BMP) signalling. As additional HSP-linked proteins are identified, the fly model offers a great opportunity to understand their cellular roles and ultimately provide plausible mechanisms for these diseases.

Charcot-Marie-Tooth (CMT) disease is characterised by the degeneration of peripheral motor and sensory neurons, leading to progressive muscle weakness and wasting, and sensory loss. The disease is clinically heterogeneous although electrophysiological and pathological criteria allow the distinction between demyelinating, axonal and intermediate forms of CMT. Since more than 30 genes have been causally associated with CMT to date, the disease is also genetically heterogeneous. These genes encode proteins with often very different molecular functions suggesting that peripheral motor and sensory neuropathy can result from impairment of multiple molecular pathways including myelination and myelin maintenance, axonal transport, mitochondrial dynamics, endosomal trafficking, axon-Schawann cell interaction, transcriptional regulation and protein chaperone activity. The exact molecular underpinnings of the peripheral motor and sensory neuropathy are still poorly understand, and there is no effective drug treatment available. Chapter Five concentrates on the use of *Drosophila* as a genetic organism to model CMT in view of the possibility of studying the effect of CMT-associated mutant proteins on motor and sensory neurons in their physiological context as well as the suitability of this model system to perform genetic screens. The organisational principles of the nervous system as well as basic neurophysiological principles including but not limited to conduction of action potentials, signal transmission through release of neurotransmitters and the synaptic vesicle cycle, are remarkably conserved between flies and humans. The development and anatomy of the *Drosophila* neuromuscular system is beautifully described in this section.

Mutations in the genes encoding tyrosyl-tRNA synthetase (*YARS),* glycyl-tRNA synthetase (*GARS),* alanyl-tRNA synthetase *(AARS)* and possibly lysyl-tRNA synthetase *(KARS)* and histidyl-tRNA synthetase *(HARS)* give rise to axonal and intermediate forms of CMT. Such enzymes ligate amino acids to their cognate tRNA and therefore catalyse an important step in protein synthesis. Aimed at illustrating the usefulness of the fly as a model for CMT, *Georg Steffes* and *Erik Storkebaum* (Max Planck Institute for Molecular Biomedicine, Germany) highlight the features of the *Drosophila* model of CMT associated

with mutations in YARS, which aminoacylates tyrosyl-tRNA with tyrosine. The authors underline a series of experiments, the results of which suggest unexpectedly that loss of aminoacylation activity *per se* is not necessary to cause peripheral motor and sensory neuropathy, although the possibility that altered subcellular localization of aminoacylation-active mutants could lead to defects in local protein synthesis and terminal axonal degeneration cannot be excluded at the present moment. Current evidence suggests that the disease may be caused by a gain-of-toxic function mechanism, the molecular nature of which remains elusive. The future use of *Drosophila* CMT models in genetic screens for disease-modifying genes may be of great value to unravel the molecular mechanisms of disease, and to identify possible therapeutic targets.

Chapter Six focuses on spinal and bulbar muscular atrophy (SBMA) or Kennedy's disease, a progressive X-linked motor neuron disorder arising from the build-up of toxic aggregates due to an abnormal expansion of the glutamine tract in the androgen receptor (AR) gene as well as loss of the endogenous function of the AR. SBMA forms part of an ensemble of neurodegenerative disorders referred to as polyglutamine (polyQ) diseases which, although affecting different neuronal subtypes, share several features including an earlier disease onset and a more acute disease progression, the longer the glutamine expansion. Interestingly, polyQ-expanded AR is necessary to induce motor impairment, hence suggesting a gain-of-function by the pathogenic AR in motor neurons and in this regard, patients with loss-of-function mutations in the AR gene only show androgen insensitivity.

Fly models of SBMA were key to establish that toxicity is dependent on glutamine length as well as the ligand-dependent activation of the AR, hence, flies that ectopically express the human AR with either a non-toxic glutamine tract or with an expanded glutamine tract in the absence of dihydrotestosterone exhibit no neurodegeneration or motor defects. Activation gives the mutant AR unrestrained access to the nucleus where it is thought to alter numerous processes. In this context, restricting the mutant AR to the cytosol by genetic manipulation strategies in the fly was shown to abolish toxicity. Furthermore, *Drosophila* studies, including genetic screens for modifiers of polyQ-induced eye degeneration ('rough-eye phenotype'), revealed several cellular pathways such as gene expression, axonal trafficking and mitochondrial physiology that are affected by the disease. Considering that a myriad of cellular mechanisms sustain a negative impact from a polyQ pathology and highlighting evidence from SBMA model organisms, *Adrienne M. Wang* (University of Washington, USA) makes the case for the stimulation of the cell's innate protein quality control pathways as one of the best therapeutic approaches aimed at clearing the mutant protein upstream of its toxic effects.

Chapter Seven addresses spinal muscular atrophy (SMA) which is the most common autosomal recessive disorder in the population following cystic fibrosis. The causative gene is the *survival of motor neuron 1* (*SMN1*), where its homozygous loss in patients with SMA leads to a situation of low SMN levels resulting from a partially-functional duplicate gene, *SMN2*. *SMN2* copy number is inversely correlated with disease severity and in this regard, SMA is usually classified into three types. SMN forms a multimeric complex that participates in the cytoplasmic phase of spliceosomal Uridine-rich small nuclear ribonucleoprotein biogenesis. *Stuart J. Grice* and colleagues (University of Oxford, UK and University of North Carolina, USA) review the present fly models of SMA by giving a thorough description of the *Smn* mutant and transgenic flies that were generated so far. Importantly, the authors highlight the developmental defects observed in *Drosophila* SMA models including alteration

in P body organisation and nuclear architecture in the *Smn* knockout germline; growth defects, stem cell defects, abnormal neuromuscular junction morphology and reduced motor function at the larval stage; and, flight defects as well as muscle atrophy in the adult stage. Importantly, this chapter discusses the recent thrilling findings in *Drosophila*, which shed light on the selective vulnerability of motor systems. Interestingly, such studies introduce a concept that was first observed in ALS, specifically, the possibility that a subpopulation of neurons might be prone to degeneration as a result of alterations in the function of neuronal circuits that impinge onto these neurons.

The final chapter (Chapter Eight) of this collection revolves around the application of *Drosophila* in the conduction of genetic screens aimed at identifying novel genes that cause motor neuron degeneration or finding modifiers – enhancement or suppression – of the phenotype resulting from disruption of MND causative genes. Both approaches hold promise to decipher the molecular mechanisms underpinning both normal physiology and pathophysiology. *Patrik Verstreken* and colleagues (KU Leuven, Belgium) first highlight the key features of the screen phenotype as well as discussing some of the phenotypes that are amenable to genetic screens such as lifespan, behaviour (crawling of larvae during the larval stage and climbing or flight during the adult stage), retinal morphology, electroretinogram (ERG) recordings and NMJ architecture.

Through the use of engaging diagrams, the authors also discuss different screening strategies including classic genetic screens using either Ethyl Methane Sulphonate (EMS)-based or transposable element (TE)-based mutagenesis; clonal genetic screens, where homozygous tissue is generated in an otherwise heterozygous animal, hence allowing the investigator to assess the phenotypes of genes required for organismal viability; dominant genetic screens, which aim at identifying dominant modifiers of a phenotype; and, *UAS*/Gal4-based screens, whereby the use of the potent *UAS*/Gal4 system enables researchers to manipulate gene expression through either overexpression or RNAi-mediated knockdown in a spatially- and temporally-controlled manner. This account also includes examples of genetic screens that were successful in fly models of MND including SMA, SBMA and ALS. Furthermore, the plethora of genetic tools presently available are adequately described, and exploiting their use will undoubtedly help us to further understand the molecular mechanisms giving rise to MNDs. Importantly, the ability to conduct high-throughput genetic screens with relative ease augurs well for the future application of high-throughput pharmacological screens aimed at identifying novel therapeutics, which can be considered as the final frontier in MND research.

Whilst editing this assemblage of stimulating works, I confess that I have learned several interesting things even though I have been in the 'business' for quite some time. In this regard, I am definitely convinced that this timely collection will be welcomed not only by Drosophilists and MND aficionados alike but also by newcomers to the field. Whilst thanking wholeheartedly the authors for their expert contribution, I would like to invite the reader to enjoy this volume, an inspiring look at the indispensability of the fruit fly, and of model organisms in general, to neuroscience research.

Ruben J. Cauchi
Dept. of Physiology & Biochemistry,
Faculty of Medicine & Surgery,
University of Malta, MALTA G.C.
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