

The neuronal ceroid lipofuscinoses

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The Neuronal Ceroid Lipofuscinoses: opportunities from model systems

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Abstract

The neuronal ceroid lipofuscinoses are a group of severe and progressive neurodegenerative disorders, generally with childhood onset. Despite the fact that these diseases remain fatal, significant breakthroughs have been made in our understanding of the genetics that underpin these conditions. This understanding has allowed the development of a broad range of models to study disease processes, and to develop new therapeutic approaches. Such models have contributed significantly to our knowledge of these conditions. In this review we will focus on the advantages of each individual model, describe some of the contributions the models have made to our understanding of the broader disease biology and highlight new techniques and approaches relevant to the study and potential treatment of the neuronal ceroid lipofuscinoses.

Introduction

The neuronal ceroid lipofuscinoses (NCLs), as with all neurodegenerative diseases, represent a highly complex biological problem. With all challenging biological questions, model organisms, which allow the researcher to attune the complexity of the model to the complexity of the task at hand, represent an invaluable tool.

The NCLs are a group of lysosomal storage disorders of related clinical pathology. They are characterised by neurodegeneration, leading to a progressive decline in cognitive and motor function, blindness and epilepsy, eventually leading to premature death. NCLs have traditionally been categorised based upon their age of onset but as the underlying genetics of these diseases has become clearer, the NCLs are now subdivided based upon the causative genetic defect [1]. Individually these different forms of NCL are rare. Together however they represent the most common cause of childhood neurodegeneration, with an incidence, depending upon global variation, of between 1:12,500 and 1:100,000 [2]. Given that these conditions remain fatal with few therapeutic options available to slow disease progression or ease patient suffering, they represent a significant unmet clinical need.

All basic biology and pre-clinical therapeutic development requires relevant model systems, from larger animal models for the development of therapies and therapeutic technologies to simple single cellular systems for the study of cell biology and systems-level experimentation. The NCL field has developed a range of such models, which have now been used extensively to study the biology of NCLs and to aid in therapeutic development. Animal models used to study NCLs include classical model systems, such as the mouse, fruit fly, nematode and zebrafish, and larger animal models, including dogs, sheep and cattle. The high degree of evolutionary conservation of some of these genes has even allowed the development of yeast models [3].

The model organisms available to study the NCLs have been reviewed in significant depth previously [3]. Consequently, in this review these contributions will only be summarised. Instead, the aim is to provide a more detailed account of the advantages of each of these model systems, to highlight new research findings, and to describe new techniques and approaches likely to be of benefit to NCL researchers in the future. The availability of such a range of systems provides a unique tool kit for studying NCLs, from mammalian models for disease pathology and therapeutic development to simpler systems allowing a more detailed understanding of basic

biology. The unique features of these individual models form the main focus of this review.

Large animals

Numerous naturally occurring large animal models of the different forms of NCL have been described since the first report in two English Setter dogs in 1953 [4]. The most important to date are the sheep and the dog, but the disease has also been reported and studied in other species such as cattle, horses, pigs, goats, cats, ferrets, birds and monkeys. The study of these models has largely contributed to a better understanding of the pathophysiology of the disease. Despite the more recent development of genetically modified mouse models, these large animal models are still valuable. The anatomy of their gyrencephalic brain is closer to human brain anatomy than the lissencephalic brain of rodents. Consequently, the phenotype of these affected animals more closely mimics the clinical signs of NCL observed in humans with their longer lifespan allowing a more thorough evaluation of potential therapeutic strategies. For these reasons, large animal models are an excellent complement to laboratory animals. The following section will describe NCL pathology in these models and illustrate their importance to our understanding of the pathophysiology and biochemistry of the NCLs. For brevity, the other large animal affected by NCL will be summarised and referenced in table S1.

Modeling NCLs in large animals

The disease was first described in a flock of South Hampshire sheep in New Zealand in 1976 by Jolly and colleagues [5]. The clinical signs start at 7 months of age and are usually early signs of blindness that is initially of cerebral origin but as the disease progresses retinal degeneration become more apparent. As the animal ages, other signs such as ataxia and decreased mentation become apparent and, in later stages, partial seizures occur before death by two years of age [6, 7]. The disease is caused by a mutation in *CLN6* [8-10] and another mutation in the same gene causes similar pathology in Merino sheep [9, 11].

A similar phenotype affecting Borderdale sheep was reported in 2002 by Jolly et al. [12] and a colony was established. The causative mutation was found in *CLN5* [13], which is mainly responsible for the late infantile form of NCL in humans formerly known as the “Finnish variant” [14].

A form of NCL affecting newborn White Swedish Landrace Sheep was reported on a Swedish experimental farm [15]. The main clinical signs include tremors and weakness, an inability to suckle and marked cerebral atrophy [15]. A mutation in the cathepsin D gene was later identified [16, 17]. Interestingly, a mutation in this gene was subsequently identified in a newborn infant with severe microcephaly and was assigned *CLN10* [18].

Multiple forms of NCL have been described in numerous breeds of dogs. In most cases these have been sporadic descriptions of clinical cases and only a few colonies have been established and studied as animal models for NCL research.

The English Setter was the first described animal model of NCL and most canine studies were performed in this breed. The clinical signs start at 14-18 months and are characterised by decrease mentation, ataxia and blindness. Convulsions occurred a few months later and no animal survived to more than 26 months of age [19]. A mutation

in *CLN8* was identified as the cause of the disease in this breed [20]. The research colony is no longer active, but frozen semen has been stored [21].

At least three different forms of NCL have been reported in Dachshunds. The first one, in longhair Dachshunds, is caused by a mutation in the *TPP1* gene (*CLN2*). Progressive neurological signs include mental retardation, ataxia, blindness and general myoclonus by 9 month of age [22]. A research colony has been established [23], which to the authors' knowledge, is the only currently available canine colony. In people, mutation in *CLN2* mainly results in a classic late infantile form, with mental retardation, epilepsy and vision deficits [14, 24].

NCL has also been reported in numerous other species including cows [25] monkeys [26], ferrets [27], cats [28, 29], pigs [30], goats [31] horses [32] and birds [33, 34]. Specific mutations are listed in table S1.

Similar to humans with NCL, the main gross pathological findings in large animals consist of different degrees of cerebral and cerebellar atrophy [19, 35-37]. Histopathologically, there is neuronal loss and astrocytosis accompanied by the accumulation of autofluorescent storage material that is the hallmark of the NCLs. The South Hampshire sheep played a key role in the identification of subunit c of mitochondrial ATP synthase as a major component of the storage material in some forms of NCL [38, 39] and was also used to demonstrate that the degree of neurodegeneration is not correlated with lipopigment accumulation [40]. Due to their relatively complex cortex, sheep models of NCL have been used to study the selective and sequential degeneration of GABAergic interneurons in different regions of the brain [40]. A regional neurodegeneration in GABAergic neurons was also reported in the English Setter dog model [41]. Impaired neuronal synaptic function has also recently been reported in cell culture from sheep with *CLN6* mutation [42].

Recent studies suggest that impaired metal ion homeostasis plays an important role in the pathophysiology of different neurodegenerative diseases [43] and a similar finding has been reported in Merino and South Hampshire sheep carrying *CLN6* mutations [44]. This could be secondary to the progressive loss of the metal transporter *Zip7* observed in the affected sheep [45] and connected with the increases in levels of α -Synuclein, implicated in Parkinson's disease, and the metal transporter *ATP13A2*, which is mutated in an inherited form of early-onset Parkinsonism [45, 46]. Increased levels of superoxide dismutase were all seen in these sheep and likely reflect chronic oxidative stress. Taken together, findings from the sheep models point to chronic neural inflammation as important part of the disease process, as is thought likely for other neurodegenerative disorders.

Large animal models have greatly contributed to the understanding of the pathophysiology of blindness, most particularly the retinal changes. Multiple functional, histopathological and ultrastructural studies have been performed in different large animal models and although most animal models show retinal changes, there is variability in the severity and distribution of the histopathological changes and their associated functional consequences. Dachshunds with *CLN2* mutation [23, 47], Polish Owczarek Nizinny dogs [48, 49], Miniature Schnauzer [50], Devon cattle (*CLN5*) [51] and South Hampshire sheep (*CLN6*) [7, 52-54] show marked retinal atrophy and loss of the rod and cone layer with associated electroretinographic changes, in line with observations in humans with mutations in the same genes [55]. In contrast, English Setters (*CLN8*) [54, 56, 57], American bulldogs (*CLN10*) [58] and Dalmatians [54, 59] show less severe retinal changes and the photoreceptor layer is not affected, which again mirrors the presentations in humans with these mutations

[55]. Despite its late onset form, Tibetan terriers (*ATP13A2*) [60] also show marked retinal and photoreceptor changes at a more advanced stage of the disease.

Large animal models: A therapeutic focus

Depending on the form of NCL, different therapeutic approaches have been considered, including various forms of enzyme replacement therapy (ERT) and gene therapy, and large animal models have proved invaluable for pilot studies. Initially, haematopoietic cell transplantation was attempted in the English Setter (*CLN8*) and South Hampshire sheep (*CLN6*) but with no effect on disease progression [61, 62]. This approach was unlikely to succeed considering the transmembrane nature of the deficient protein in these forms of NCL, although a recent abstract suggests that intercellular correction occurs in chimeric sheep created by merging blastomeres from normal and *CLN6* affected embryos [63]. However, in forms of NCL resulting from deficiencies in lysosomal enzymes, such as *CLN1*, *CLN2* and *CLN10* diseases, direct supplementation can be considered. Dachshunds lacking the tripeptidyl peptidase-1 enzyme (TPP1, or *CLN2*) represent an excellent model for this type of strategy. Early studies suggest that intrathecal or intracerebroventricular administration of recombinant human TPP1 can delay the clinical signs [64].

Viral vector mediated gene therapy is another more recent therapeutic approach that has shown promising results in ovine *CLN5* neuronal cell cultures [65]. Initial studies in unaffected sheep confirmed extensive protein expression from the AAV9 vector [66] and the first results from dogs (*CLN2* Dachshunds) showed that viral transduction resulted in an increased enzymatic activity and delayed clinical progression [67]. Directed therapy to treat retinal degeneration in *CLN2* Dachshunds by implanting genetically modified autologous bone marrow stem cells into the eye is now being developed, with some positive initial results [68].

Large animal models are potentially very useful to test drug therapies. Palliative treatment with the anti-inflammatory minocycline that is currently in human clinical trials was assessed in a sheep model of NCL (South Hampshire, *CLN6*) but showed no ability to inhibit microglial activation or astrogliosis [69]. However, for interventions to be assessed quantitatively for therapeutic efficacy, more accurately, longitudinal biomarkers for disease progression are necessary. To this end, recent studies have focused on characterising the behavioural changes in these animal models more accurately [70, 71].

Mice

Historically, mouse models of neurodegenerative genetic disease have played a pivotal role in attempts to understand the neurobiology underpinning these complex disorders. They represent a logical progression from fly or zebrafish models as a more complex mammalian system with a greater genetic, anatomical and physiological similarity to human patients. They are easy to handle, have a high reproductive rate and are relatively cheap compared to other mammalian model systems. Such mouse models may arise through natural *de novo* mutation, induced random mutagenesis or by targeted molecular biology to disrupt a particular gene or genomic locus. The resolution of such disruptions can vary from deletion of multiple exons from a gene of interest to specific point mutations that accurately mimic the genetic defect in patients. There is no guarantee that the introduction of mutations to the mouse version of the gene will accurately recapitulate a phenotype similar to that observed in the

patient population. However, if they do then this represents an extremely powerful biological tool.

Mouse models that faithfully recapitulate and mimic the human form of the disease have been used to confirm the role of candidate genes in the disorder. They can be used to study the underlying mechanism and progression of the disorder and this information can be used to design urgently required novel therapies or improve on existing ones. The identification of reliable biomarkers remains a high priority area in the neurodegenerative disease field and mouse models of disease provide an ideal resource in which to search. Finally, the model itself can be used to test potential novel therapies and small molecule drugs – do these have any disease modifying effects in the model, do they ameliorate the disease symptoms and pathology and do they rescue the model from premature death? Essentially, this is a fundamental species in which pre-clinical testing of therapies can take place before moving onto a larger model of the disease (if available) or moving directly into the clinic. The NCL field has greatly benefited from investment and a strategy to generate mouse models of the various forms of the disease.

Modeling NCLs in mice

There are currently 12 mouse models of NCL: *Cln10* mouse [72], two *Cln1* mice [73, 74], *Cln2* mouse [75], *Cln5* mouse [76], *Cln6* mouse [77], *Cln8* mouse [78], four *Cln3* mice [79-82] and most recently a *Cln7* mouse [83]. The reader is referred to a comprehensive review for details of the various features of the mouse models of NCLs with the exception of the recent *Cln7* mouse [84]. In addition to these *bona fide* models of NCLs, there are other candidate mouse models that display features that resemble human NCL symptoms. These include null mutation in genes for *ctsf* [85], *Ppt2* [73, 86] and the chloride ion channels *Clcn-6* [87] and *Clcn-7* [88]. However, it should be noted that none of these candidate models can be linked directly to established human forms of NCL.

An obvious starting point of investigation in the NCL mice is the characterisation of temporal and spatial CNS pathology. All the models display the characteristic accumulation of fluorescent material and brain pathology and, where investigated, retinal involvement and premature mortality. However, there are also often both obvious and subtle differences between models of the different forms of the disease. Furthermore, there can be differences between the models of the same form of the disease. The four models of CLN3 disease provide a good example of this. The different mutations in the same gene lead to differences in the age of neurological symptom onset ranging from less than two months [80] to 16 months [82]. It is worth noting that this may be due to residual CLN3 activity due to variant mRNA transcripts [79] also reported in patient samples [89]. The *Cln3* mice also differ from the other models in that although there is subtle activation of glial cells, there is not the prominent astrogliosis-associated proliferation or macrophage formation [90, 91].

Mouse models: A therapeutic focus

The production and characterization of the models has undoubtedly furthered our understanding of the disease in its various forms and is an ongoing process. Perhaps the greatest value of the information gathered lies in the development of novel therapies. Any such endeavor requires knowledge of: (1) whether the mouse model is a reliable tool for therapeutic efficacy i.e. does the model faithfully recapitulate the

symptoms observed in patients (2) the target e.g. a particular cell type or receptor (3) when the therapeutic agent should be administered i.e. the therapeutic window of opportunity and (4) where it is required to be administered i.e. localised to one particular area or a broader anatomical distribution?. There is also the issue of selecting the most practical and suitable model for the desired experiment. For example, if a drug requires administration twice daily to a *Cln3* mouse to measure delay of neurological onset or increase in survival, then the investigator may be advised to select the model that shows symptoms at 2 months rather than 16 months. It is clear that the vast majority of novel therapies have been tested in those models of NCLs that are associated with a defective or absent soluble enzyme. This includes ERT, stem cell and gene therapies.

ERT has an established history in the lysosomal storage disorder (LSD) field and has revolutionised the treatment of patients e.g. Gaucher disease. The drawback is that intravenously administered recombinant enzyme may have a limited ability to cross the blood-brain barrier and so treating the CNS has proved difficult. However, high doses of intravenously administered recombinant Ppt1 enzyme have been shown to ameliorate pathology in the thalamic region of the brain in *Cln1* mice leading to a subsequent later onset of motor function deterioration and extended survival. The effects of this approach are enhanced if therapy is initiated in the mice from birth [92] and highlights the importance of intervening within the optimal therapeutic window of opportunity. Similarly, the mouse model of *Cln2* has also demonstrated amelioration of CNS pathology, reduced neurological symptoms and extended lifespan following intrathecal and intraventricular administration of recombinant Tpp1 enzyme [93, 94]. Encouragingly, this data in CLN2 mice has contributed to the initiation of a Biomarin Pharmaceuticals sponsored phase I/II clinical trial of recombinant Tpp1 enzyme (BMN-190) administered to patients with CLN2 disease via intracerebroventricular infusion (ClinicalTrials.gov identifier: NCT01907087).

The recent ascendancy of the gene therapy field has led to a number of successful clinical trials including paediatric neurological conditions such as X-linked adrenoleukodystrophy and metachromatic leukodystrophy [95, 96] and the mouse models of NCL have been used to evaluate similar gene therapy approaches as potential treatments. This has primarily been focused on the use of adeno-associated virus (AAV) gene delivery vectors that carry a therapeutic fully functional version of the defective gene and are administered directly into the brain. Promising results have been demonstrated in *Cln1* [97-99], *Cln2* [100-102] and *Cln10* mice [103, 104] resulting in reduction of disease pathology and extension of lifespan. It is very likely that continued improvements in AAV vector design and production will lead to further enhancements in therapeutic efficacy and knowledge of the mouse model pathology is vital for this process. For example, targeting a particular cell type may dictate which particular serotype of AAV should be used and which promoter should be included to provide optimal expression of the delivered therapeutic gene. Knowledge of the areas of the brain that are affected is also vital to ascertain where the vector should be delivered, or indeed, if multiple sites of administration are required. Finally, an understanding of the timing of disease progression will influence at what stage the vector should be administered. The critical issue of intervening early and even pre-symptomatically has been demonstrated in a *Cln2* mouse [100] and advantages of perinatal gene therapy has been recognised for some time [105, 106]. This is perhaps most critical in the context of acute paediatric neurodegenerative diseases where neurons must be rescued prior to irrevocable loss. The promising

results observed in the CLN2 mice led to a gene therapy clinical trial in patients [107].

Finally, stem cell therapies have also been tested in mouse models. Human neural stem cells have been administered to the brains of neonatal *Cln1* mice that have been crossed with immunodeficient severe combined immunodeficiency (SCID) mice to avoid rejection [108]. Beneficial effects included a reduction in autofluorescent material and delay in motor function deterioration. These findings led to a phase I clinical trial in CLN1 and CLN2 patients that showed that transplanted cells did not trigger any adverse effects and supports further investigation [109].

Comparatively less progress has been made in developing treatments in the mouse models that mimic the defects in transmembrane protein forms of NCL. They do represent a more difficult target compared to the soluble enzymatic forms - they cannot be secreted and recaptured by neighbouring cells making cross-correction void. This effectively excludes ERT as a realistic approach. Gene therapy is still a viable therapeutic option but will probably require much higher levels of transduction. However, a recent study has shown that AAV-mediated gene delivery to the brains of neonatal *Cln3* mice has therapeutic benefit leading to a reduction in storage material, gliosis and evidence of improved neuron counts [110]. Pharmacological therapies have also been tested in CLN3 mice including NMDA and AMPA receptor antagonists that lead to improved motor function [111, 112].

The contribution that the current repertoire of NCL mouse models available has had on furthering our understanding of this group of diseases is huge. This is evident from the continual investigation into new therapies, many of which have made their way to clinical trial. This is very encouraging and strongly supports continued investment of resources into the study and use of these models but also the generation of novel models.

Zebrafish

Zebrafish are small freshwater fish that have for many years been an important research tool. While previously used predominantly by developmental biologists, zebrafish are now established as a vertebrate disease model, and are increasingly being used to study neurodegenerative disease. The zebrafish genome can be easily manipulated and the availability of the latest reference assembly (GRCz10) on bioinformatics databases further supports its use. Orthologues of all NCL-causing genes exist in zebrafish and are highly conserved; in some cases more than one copy is present due to duplication of an ancestral locus during evolution [113].

Zebrafish have many useful characteristics for disease modelling: they are vertebrates and therefore more similar in their development and physiology to mammals than other model organisms such as fruit flies; a single pair can produce several hundred rapidly-developing offspring per week, meaning that statistically significant sample sizes can be reached quickly and larval development is transparent, enabling high quality imaging techniques to be used to view the developing animal [114].

Drug-target validation is a highly advantageous feature of zebrafish, enabling simultaneous toxicity and efficacy testing in a whole organism. Disease phenotypes manifest early in development and phenotypic endpoints may include behavioural testing or the monitoring of fluorescent reporters, for example [114]. Importantly, candidate compounds can be delivered by simple addition to the tank water. Illustrative of the potential of zebrafish for drug discovery is the identification of the

first bone morphogenetic protein inhibitor, a derivative of which successfully treats fibrodysplasia ossificans progressiva [115]. In addition zebrafish research led to the identification of a number of modifiers of gluconeogenesis [116] and the repurposing of leflunomide (previously a treatment for rheumatoid arthritis) to suppress melanoma [117].

Large-scale mutagenesis screens have highlighted approximately 2000 genes involved in development [118, 119] and mutations in many genes relevant to neurological disease (including NCLs) continue to be found [120]; many of these alleles are currently available through the Wellcome Trust Sanger Institute, Hinxton UK.

For quick, proof-of-principle enquiries into the function of zebrafish orthologues of human disease-causing genes, oligonucleotide-based knockdown studies are invaluable but suitable controls are essential [121]. Phenotypes are generally observable before 5 days post fertilization. Following initial analysis, knockout animals can also be generated for research in more stable systems.

Both zinc-finger nucleases (ZFNs) [122] and transcription activator like endonucleases (TALENs) [123] have successfully knocked-out zebrafish genes. The current tool of choice is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated system that uses the Cas9 nuclease to generate breaks or nicks in the chromosome [124]. This method also shows promise for the development of disease-specific knock-ins and the humanisation of zebrafish with healthy or mutant genes [125].

It should be noted that in some cases, phenotypes will either not develop or will manifest late in development. This may arise either due to redundancy in biological pathways or because physiological difference between zebrafish and man mean that pathological phenomena such as lipofuscin accumulation are unable to develop. For example, autofluorescent lipofuscin is undetectable in the zebrafish model of CLN2 disease, although many other features representative of the disease do exist [126].

Modeling NCLs in zebrafish

To date, six zebrafish models of LSDs have been published but several are undergoing development; three of these are NCL models including *CLN2*, *CLN10* and *CLN11*. These and other models for NCLs, are catalogued in table S2.

CLN2 disease is caused by mutations in *TPP1*, encoding the lysosomal hydrolase tripeptidyl peptidase (TPP1). The zebrafish model of *CLN2* disease carries a missense mutation leading to a premature termination codon and complete loss of enzyme function. The lack of functional lysosomal Tpp1 leads to a number of phenotypes relevant to human disease including early neurodegeneration, reduced neurogenesis, epileptic seizures, motor abnormalities, visual defects and premature death [126].

CLN10 disease is similarly caused by mutations in a lysosomal hydrolase, cathepsin D (*CTSD*). In humans *CLN10* disease is very severe, causing congenital defects. The zebrafish model of *CLN10* disease was generated by morpholino oligonucleotide knockdown and is characterized by small eyes due to the absence of retinal pigmented epithelial microvilli, disorganized musculature and premature death [127]. This model also exhibits lack of yolk resorption, a process reliant on autophagy and, by extension, lysosomal function. Mis-regulation of autophagy is thought to contribute to most neurodegenerative diseases.

Mutations in *GRNA*, encoding granulin, cause *CLN11* disease. Two groups have knocked-down *grna*, although neither group has validated the model with respect to

CLN11 disease. However, they observed motor neuron truncation and abnormal CNS development [128, 129]. Given that in humans, autosomal dominant *GRNA* mutations cause fronto-temporal lobar degeneration, a motor neuron disease variant, these phenotypes require further investigation.

CLN3 disease is caused by mutations in *CLN3*, encoding a lysosomal transmembrane protein of unknown function. Data presented at a number of international conferences have described a zebrafish model of *CLN3* disease generated by morpholino knockdown. This model exhibits small eyes, greatly disorganized neuronal axon pathfinding, astrogliosis, lysosomal storage of subunit c of mitochondrial ATP synthase, epileptic seizures, motor abnormalities and premature death (Wager et al., submitted).

Zebrafish models: A therapeutic focus

As highlighted above, zebrafish research has been used to directly inform therapy but as a relatively simple model, it is perhaps more ideally suited for the analysis of basic biological processes relevant to therapy. Although NCL research in zebrafish is a recently established field, zebrafish have a much longer history of use for studying neurodegeneration as a whole, and comparative analysis of related disease models can be of significant value in informing therapy in a range of conditions.

There are a range of established zebrafish models for neurodegeneration. Zebrafish models for Alzheimer's disease include amyloid precursor protein morphants [130] and amyloid beta transgenic animals [131]. Aberrant phenotypes in zebrafish modelling Alzheimer's disease include disordered movement. Transgenic models of tauopathy are also available [132, 133], which display both Tau accumulation and tangle formation, and Tau phosphorylation. There are also a wide range of models available to study Huntington's and Parkinson's diseases and amyotrophic lateral sclerosis (ALS).

Huntington's models include huntingtin transient overexpression models [134], transgenic animals [135] and morphants [136]. Studies in these models indicated protein aggregation, apoptosis, increased rhodopsin expression, developmental defects and embryonic lethality. Interventions such as inhibition of nitric oxide production and methylene blue treatment reduced aggregates in some of these studies.

ALS models include Sod1 mutant overexpression [137] and transgenic models [138]. Other models include Tardbp mutant overexpression, transgenic or morphant animals [139, 140] and Fus transgenic animals [140]. These models display motor neuron and neuromuscular junction defects. Studies have indicated a rescue of these defects by upregulation of Vegf [137] or by methylene blue treatment [140].

Parkinson's disease is probably the most broadly modelled neurodegenerative disease in zebrafish. Models include, Park2 [141], Pink1 [142-144], Park7 [145] and Lrrk2 [146] and cover morphant, transgenic and TILLING mutant models. Pharmacological models can also be obtained by MPTP exposure [147, 148]. Increased susceptibility to cellular stress and mitochondrial changes are common features of these models, and many recapitulate dopaminergic neuronal loss.

A range of other neurodegenerative diseases have also been modelled, including frontotemporal dementia [149], spinal muscular atrophy [150, 151] and hereditary spastic paraplegia [152]. Such models recapitulate many aspects of these diseases, such as spinal motor neuron defects and associated behavioural changes. The successful modelling of multiple neurodegenerative diseases in zebrafish highlights its relevance as a model for NCLs. Further, the interventions that have been used in

these models to correct defects associated with neurodegeneration could inform future NCL research.

Fruit Fly

For more than a century *Drosophila* has been at the forefront of research into genetics, inheritance and development. A huge arsenal of genetic tools has been developed over that time and new tools continue to be developed that help to maintain the status of *Drosophila* as a key model for studying fundamental mechanisms. More recently, these powerful genetic tools combined with the short generation time (10 days) and simple, cheap animal maintenance requirements have made *Drosophila* an attractive tool to model human disease. Approximately three quarters of human genes associated with disease are conserved in the *Drosophila* genome [153] and many inherited diseases have now been modelled in *Drosophila*. Although small in comparison to mammals, the *Drosophila* CNS shares many fundamental properties with the CNS of higher eukaryotes: it is isolated by a blood-brain barrier; neuron-glia interactions are critical for function and numerous neurotransmitters are used in different contexts, including glutamate, acetylcholine, dopamine, GABA and various peptidergic neurotransmitters. The adult *Drosophila* CNS also governs complex behaviours including mating routines, memory and learning, circadian rhythms, sleep, walking behaviour and flight navigation. Importantly, aspects of neuropathology are also similar, meaning that *Drosophila* has been used to investigate the cell biology of many of the common neurodegenerative diseases of humans, including both gain-of-function disorders, such as tauopathies and triplet expansion diseases, and loss-of-function diseases, such as inherited forms of Parkinson's disease (reviewed in [154-157]).

Modeling NCLs in *Drosophila*

In common with other invertebrates, *Drosophila melanogaster* has only a subset of the disease genes known to cause NCL in humans. These encode the lysosomal enzymes Ppt1 (CLN1) and Cathepsin D (CLN10), the endo-lysosomal membrane proteins, CLN3 and CLN7 (also known as MFSD8, encoded by CG8596 in *Drosophila*), and the synaptic vesicle protein cysteine string protein (CSP or CLN4). Additionally, *Drosophila* has genes encoding likely lysosomal chloride channels, including CLC-7 (CG8594), which is associated with NCL-like accumulation of lipofuscin and neurodegeneration [88]. Loss-of-function models have been generated for *Ppt1* [158], *cln3* [159], *cathD* [160] and *cln7* (Megan O'Hare, Richard Tuxworth and Guy Tear, unpublished) to study cell biology of the NCLs. In contrast, *Drosophila Csp* has been studied extensively not as an NCL disease locus but rather for its functions at the synapse [161, 162], reviewed in [163]. Recent reviews have covered many of the findings from NCL studies using *Drosophila* and the reader is referred to these (see [84, 164]). Information and annotation of the *Drosophila* NCL genes, details of mutant alleles and transgenic constructs for each gene, fly lines and reagents available from the stock centres and all papers referring to NCL genes can be found on FlyBase (www.flybase.org). Instead, this perspective will focus on some of the techniques and tools developed more recently in *Drosophila* that could be used to help understand aspects of NCL cell biology in the future, including newly developed synaptic labelling tools and CRISPR-mediated genome editing.

The *Drosophila* research community was quick to adopt the CRISPR method of genome editing. CRISPR was adapted from a defence mechanism in the bacterium *Streptococcus pyogenes* and involves a nuclease, Cas9, being directed to a specific genomic location by a short (20 bp) guide RNA where it generates double-stranded breaks (DSBs) in the chromosome. DSB repair occurs by one of two mechanisms: error prone non-homologous end joining (NHEJ), which results in insertions and deletions (indels), or homology-directed repair (HDR) by copying from a supplied template. HDR allows for insertions, base changes or other editing events to be included at the site. Simply change the guide RNA sequence and the mutations are directed elsewhere in the genome [165, 166].

More than 20 papers have been published in the last two years detailing strains, reagents and approaches to improve CRISPR mutagenesis in *Drosophila* (reviewed in [167]) and it is fast becoming a standard technique. *Drosophila* strains are available which express various forms of Cas9, including a mutated form that cannot generate DSBs so repair is biased towards HDR, and forms driven by various different promoters to improve efficiency. Numerous plasmids and other reagents have also been generated and made available via stock centres while protocols to test gRNA sites for efficiency of have been developed. CRISPR genome editing is also being combined with older genomic technologies such Recombination-Mediated Cassette Exchange (RCME) that enables rapid insertion of different cassettes into a single CRISPR-modified genomic strain, for instance to tag a gene with different fluorescent proteins or to generate conditional alleles [168].

The importance of CRISPR genome editing for research into the cell biology of the NCL disease genes in *Drosophila* is difficult to underestimate. By inserting sequences encoding fluorescent proteins or epitope tags into NCL gene loci the requirement for high affinity antibodies can be by-passed – a particular advantage for transmembrane proteins such as CLN3 and CLN7 that have proved poorly antigenic. Knock-in reporters will also circumvent a common issue encountered when vesicular proteins are fused to reporters and overexpressed: the protein sorting pathways can be swamped and the fusion protein found in multiple locations in the cell as a consequence. CRISPR could be used to knock-in molecular hooks for proteomics studies; genetically encoded reporters for cellular physiology studies; new markers for lysosomes can be generated; point mutations mirroring those found in human patients could be engineered into genes. While CRISPR technology will also provide these opportunities in other models and systems, the generation time of *Drosophila* will enable new *in vivo* reagents to be generated and combined with existing tools and reagents very rapidly. If the correct question is asked, CRISPR genome editing in *Drosophila* should speed up research into the cell biology of the NCLs dramatically.

***Drosophila* models: A therapeutic focus**

Being a relatively simple model system, *Drosophila* are ideally suited to the analysis of the pathways that influence disease. Modifier screens are an established technique in the *Drosophila* field. Briefly, candidate screens or larger scale, random screens are performed to identify genes that modify a phenotype (usually when made heterozygous or when overexpressed). The phenotype needs to be easily scorable for higher throughput so degeneration present in the adult eye is often used as it can be seen with a dissection microscope. This approach has been used successfully to identify genes modifying phenotypes relating to *Ppt1* [169] and *cln3* [170]. These screens highlighted likely functions regulating endocytosis for *Ppt1* [171], in stress

signalling for Cln3 [159] and in JNK signalling for both Ppt1 [171] and Cln3 [170]. More recently, new collections of RNAi lines have been generated for most genes in the *Drosophila* genome (www.flyrnai.org/TRiP-HOME.html). These lines complement previous collections and allow for very efficient, cell-type specific knock-down of gene expression via the UAS-GAL4 bi-partite expression system. A small candidate gene approach for the NCLs genes in *Drosophila* using some of these reagents has identified potential connections between *Ppt1*, *cln3* and *Cisd2* (also known as *Wfs2*), which is mutated in Wolfram Syndrome [172]. Wolfram patients develop childhood-onset optic atrophy and diabetes mellitus, usually followed by neurodegeneration. The cellular events that underpin the disease are perhaps better understood for Wolfram Syndrome than for the NCLs and endoplasmic reticulum stress is considered a major component [173]. Increases in ER stress markers have been reported previously in NCL studies [174, 175] and this type of genetic interaction study points to the common features in the cell biology underpinning different neurodegenerative syndromes.

Synaptopathy – synaptic dysfunction associated with pathology - is an early hallmark of many neurodegenerative diseases and usually precedes overt neuronal loss. Synaptopathy also occurs in NCL models [176]. Given the early onset of neurodegeneration in the NCLs, it is likely that some of the CLN genes play a role in the development of the nervous system and/or the stability of synapses. The neuromuscular junction (NMJ) synapse of the *Drosophila* larva is an established model used to study synaptic development, composition, function and plasticity [177]. Motor neurons in *Drosophila*, in contrast to mammals, are glutamatergic and their synapses are structurally similar to central synapses of the mammalian CNS. However, unlike mammalian CNS synapses, they can be made readily accessible for electrical recordings and high-resolution microscopy by simple dissection (reviewed in [178-180]). The functions of several conserved components of synapses were elucidated in studies on the *Drosophila* NMJ, including those of CSP [161, 162]. Recently, super-resolution microscopy studies of the larval NMJ have provided exquisitely detailed information about the structure of synapses that will likely hold true for the mammalian CNS [181].

Changes in the electrical properties of larval motor neurons have been reported in *Drosophila* lacking *Ppt1* function resulting in a locomotion deficit for the larva [182]. The phenotypes are rescued by re-expressing *Ppt1* specifically in the nervous system, demonstrating that *Ppt1* is required only pre-synaptically and consistent with a role in synaptic vesicle endocytosis. No changes were seen in the size of synapses in *Ppt1* mutant larvae but they are present in larvae lacking either *cln3* or *cln7* function along with changes in locomotion (Megan O'Hare, Richard Tuxworth and Guy Tear, King's College London, unpublished observations). The size of the synapse is closely controlled by homeostatic mechanisms that match pre-synaptic innervation with the requirements of the post-synaptic muscle so the changes in synapse size indicate possible alterations to synaptic activity in these mutants which warrant further examination. Similarly, a careful assessment of the NMJs in *cathD* mutant larvae may also reveal synaptic functions for cathepsin D.

By far the best understood of all the *Drosophila* NCL genes is *Csp* which was originally identified as a component of synapses via a monoclonal antibody screen [183]. There is only one *Csp* gene in *Drosophila* which corresponds to the CSP α form in mammals. CSP is a palmitoylated protein containing a dnaJ chaperone-like domain and, given the function of PPT1 as a depalmitoylating enzyme, CSP is potentially a substrate of Ppt1. *Csp* mutants in *Drosophila* display severe defects in

neurotransmission [161, 162, 184] and it will be of interest, therefore, to determine whether combining *Csp* and *Ppt1* mutations exacerbates either synaptic phenotype.

The larval NMJ of *Drosophila* has been used extensively to study synaptic development in part because individual synapses and even sub-synaptic structures such as active zones can be visualised and quantified [178]. Many genes involved in human neurological disease have been shown to affect synapse number at the *Drosophila* NMJ but whether similar changes occur in the CNS – and therefore might also be occurring also in human neuropathology – is much more difficult to answer. Neurons in the insect CNS are tightly packed in a neuropil and individual synapses and active zones cannot be visualised so easily. Traditionally, serial scanning electron microscopy studies have been used to look at synapse number in neurodegenerative disease post-mortem tissue but these are laborious and can cover usually only small areas of the CNS. However, the development of genetic tools to label synapses in *Drosophila* may help to address these issues in the future. A recent light microscopy technique, Synaptic Tagging with Recombination (StaR) developed for *Drosophila* by the Zipursky lab [185] allows pre-synaptic active zones or post-synaptic receptor clusters to be labelled with epitope tags or fluorescent proteins and quantified in small subsets of neurons – small enough subsets that individual synapses can be visualised. A cell-type specific “flip-out” technique governs which neurons are labelled. This type of approach fits well with the Fly Light project at HHMI Janelia Farm Research Institute that has generated thousands of lines to control transgene expression in small subsets of neurons within the *Drosophila* CNS [186] (see www.janelia.org/team-project/fly-light). Looking forward, it seems possible that we will be able to ask what happens to synapse number in *Drosophila* NCL and other neurodegeneration mutants in development and, potentially, as flies age and neurodegeneration progresses.

Yeast

Yeast are an invaluable tool for the study of eukaryotic cell biology. Yeast species have been utilized for decades to study basic cellular processes, such as cell division, protein and membrane trafficking and cell signaling. Yeast were also the first post-genomic eukaryotic model [187], highlighting its regard as a key system in basic biology, and also many of the current advantages that yeast species afford.

There are two commonly used yeast model systems, the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. Although fission yeast has been used as a model system for a shorter period of time, both have similar tools and advantages. Their status as long-standing post-genomic models has allowed an understanding of the yeast genome in exceptional detail. This detailed genomic understanding has afforded an equally detailed analysis of the yeast transcriptome and proteome, allowing truly quantitative analysis of these ‘-omic profiles [188], the characterization of these profiles under different conditions and the influence of different regulatory processes [189]. This understanding has also been coupled with *in silico* techniques to predict and identify functional protein domains [190], predict protein interaction networks [191, 192], and to expand our understanding of metabolic processes [193]. Importantly, all this information is integrated into comprehensive genome databases [194, 195], providing an exceptional

level of information about a gene of interest before a single experiment need be performed.

Another product of this advanced understanding is the presence of a wide range of functional genomic tools. Some of the most widely used and valuable of these tools are the yeast gene deletion collections [196, 197]. Importantly, these resources provide a platform for many technologies that are relevant to the study of disease. The combination of a genome wide-deletion collection, classical yeast genetic approaches and robotic technologies has facilitated the development of new functional genomic techniques. One of the most popular of these is the synthetic genetic array [198], which allows the generation of a genome-wide array of mutants, each also carrying a mutation in a query gene. Such approaches provide information about the functions with which a gene interacts, and can also highlight new potential therapeutic strategies.

Yeast also have more direct applications in the development of therapies, and have been used in numerous and varied drug discovery projects [199]. One particular advantage of yeast is the ability to use the deletion libraries to identify drug targets by analyzing the effect of a drug on the fitness profile of the library [200], greatly streamlining phenotypic drug screening.

Modeling NCLs in yeast

Both budding and fission yeast have been used extensively to study the underlying biology of NCLs. *CLN3* and *CLN10* are conserved in budding yeast, whereas fission yeast has orthologues of *CLN1*, *CLN3* and *CLN10*. In addition, budding yeast has a known orthologue of *ATP13A2* (*CLN12*), one of the most recently identified NCL genes [201].

Much work in yeast has focused on the budding and fission yeast orthologues of *CLN3* (*BTN1* and *btn1* respectively), which, like *CLN3*, are membrane proteins of unknown function. This work is reviewed in detail in [3], and will be summarized briefly here. Budding yeast *BTN1* is known to localize to the vacuole and Golgi, and has an established role in vacuolar (the yeast lysosome) and cellular pH homeostasis, basic amino acid transport and storage and nitric oxide production [202-209]. Fission yeast *btn1* displays the same localization pattern as *BTN1*, and regulates vacuolar pH and morphology, cell polarity, cell wall homeostasis, temperature sensitivity and osmotic homeostasis [210-212]. Fission yeast has also been used to highlight the different levels of function retained by different disease-causing mutations modeled in *btn1* [213] and the metabolic changes, including increased glycolytic flux and TCA cycle activity, seen upon loss of *btn1* [214]. It was also used to highlight a role for *btn1* in membrane and protein trafficking [215]. Loss of *btn1* has been shown to lead to the mis-sorting of the vacuolar protein carboxypeptidase Y, through processes both dependent on and independent of its sorting receptor Vps10. These observations were accompanied by changes in Golgi number, subcellular localization and morphology. A comparable role for *BTN1* has also been reported, with *BTN1* regulating Golgi morphology by modulating SNARE (*SED5*) phosphorylation by *YCK3* [216]. Finally, *BTN1* has been demonstrated to have a role in phospholipid homeostasis, in particular leading to a decrease in phosphatidylethanolamine levels [217].

The budding yeast orthologue of *CLN10* (*PEP4*) is also well studied. *CLN10/PEP4* encodes cathepsin D, a vacuolar hydrolase. In work discussed in [3], *PEP4* has been shown to mediate vacuolar protease maturation and vacuolar morphology [218]. *PEP4* is also important in the maintenance of cells in quiescence, with its expression

increasing with cellular age [219]. Further, it has been shown that the catalytic activity of PEP4 protects against apoptosis, through the degradation of damaged macromolecules, while the pro-peptide protects against necrosis by promoting histone hypoacetylation, and the retention of pro-necrotic factors in the nucleus [220].

In more recent work, *PEP4* has been demonstrated to be protective against acetic acid induced apoptosis [221]. During acetic acid induced stress, PEP4 translocates from the vacuolar lumen to the cytosol, where it is required for mitochondrial degradation, and the protection against oxidative damage that this affords. The catalytic activity of PEP4 is required for mitochondrial degradation, in a process that relies upon the mitochondrial AAC proteins, adenine nucleotide translocators found in the mitochondrial inner membrane [222].

The recent identification of *ATP13A2* (*CLN12*) as the disease-causing gene in a rare case of juvenile NCL has highlighted the relevance of a number of yeast studies to NCL [201]. Many studies have investigated the cell biology of the yeast orthologue of *CLN12* (*YPK9*), focusing particularly on its role in an early onset familial Parkinsonism (Kufor-Rakeb syndrome). *CLN12* is a Golgi-resident P-type ATPase, a family of proteins that includes cation transporters. Consistent with this, the loss of *YPK9* leads to sensitivity to cadmium, manganese, nickel and selenium, suggesting a role in cation transport [223].

In a genome-wide overexpression screen, *YPK9* was identified as a suppressor of α -synuclein toxicity [224], the key component of Lewy bodies in Parkinson's disease, which is also elevated in *CLN3* disease models [225]. Increased expression of *YPK9* reduced the number of intracellular α -synuclein inclusions, but not total levels of protein. This effect was linked to a rescue of vesicular trafficking defects, allowing the exit of carboxypeptidase Y from the endoplasmic reticulum. Disease causing mutations linked to Kufor-Rakeb syndrome led to mis-localisation of *YPK9* and prevented protection against toxicity. A mutation abolishing ATPase activity, by contrast, did not affect localization but also prevented protection. In this study, loss of *YPK9* was again linked to manganese sensitivity.

In a separate genome wide screen, this time looking at the interactions of *YPK9* itself, *YPK9* was shown to interact with a number of genes known to be involved in protein trafficking and manganese homeostasis [226]. A particularly well represented group were those linked to vesicle-mediated transport. This included a number of VPS genes, the t-SNARE *VAM3*, the Rab *YPT6*, vacuolar transport chaperone complex members, and *GLO3*, an Arf GAP involved in ER-Golgi transport.

Yeast models: A therapeutic focus

Pathology in the NCLs have been linked to a wide array of cellular processes but altered lipid homeostasis, particularly changes in sphingolipids and ceramides, and lysosomal dysfunction are common themes for a broad range of NCL genes [227]. Both processes have been studied extensively in yeast, providing insights with relevance to disease and processes that could be relevant to therapy.

Neurodegeneration can be thought of as a condition of impaired cellular lifespan. Cellular age in yeast is generally measured using two parameters: replicative lifespan (RLS), which is the number of divisions a cell goes through before death, and chronological lifespan (CLS), which is the amount of time a cell can survive in senescence [228]. In some of the earliest work to highlight genes involved in cellular ageing, budding yeast was used to identify *LAG1* (Longevity-Assurance Gene 1), a gene that, when deleted, caused a 50% increase in replicative lifespan. *LAG1* encodes

a component of ceramide synthase, which catalyses ceramide synthesis from dihydroceramide. Such an observation provides evidence that ceramide levels directly affect lifespan and interestingly, ceramide levels are perturbed in a number of NCLs [227]. *CLN8* itself shares homology with *LAG1*, containing a TLC (TRAM–Lag1p–*CLN8*) domain, suggesting a potential role in ceramide synthesis. Further, the human homologues of *LAG1* can complement aspects of the loss of *CLN9* [229].

The link between sphingolipids and cellular ageing extends beyond *LAG1*. *IPT1*, which catalyses the production of the terminal sphingolipid mannose-(inositol-P)₂-ceramide, increases *CLS* when deleted [230], and *ISC1*, an inositol phosphosphingolipid phospholipase C that produces ceramides, shortens *CLS* when deleted [231]. Lifespan in the latter deletion strain was greatly improved by deletion of *SIT4* that codes for a phosphatase positively regulated by ceramides [232]. This in turn suggests a link between the lifespan-altering effects of ceramides and downstream signaling processes. Interestingly, *SIT4* is involved in a broad range of processes linked to NCL including; protein trafficking [233], glycogen metabolism [234], heat-shock functions, cytoskeletal organization and ribosome biogenesis [235], and vacuolar homeostasis [236].

The link between vacuolar homeostasis and cellular lifespan is not limited to ceramide-dependent processes. A study of how amino acid deprivation increases lifespan in yeast indicated that methionine withdrawal increases vacuolar acidification in a manner dependent upon autophagy. The authors then went on to demonstrate that increasing vacuolar acidification alone was sufficient to increase lifespan [237], providing evidence for a key role of vacuolar function in longevity. As well as low pH in the vacuole being protective against ageing, increasing pH has been shown to be a part of normal ageing in budding yeast. Increasing vacuolar pH has been demonstrated to lead to a decrease in vacuolar amino acid storage and was also coupled with an increase in mitochondrial dysfunction [238]. This link between vacuolar pH and mitochondrial dysfunction provides a key link between vacuolar changes, as observed in models for NCL, and defects known to contribute cell death with cellular increasing age [239].

Yeast represent the simplest established model for eukaryotic cell biology, allowing the study of cellular processes at a depth not afforded by other systems. Further, the conservation of NCL genes in such a simple system suggests that they regulate fundamental cellular functions, and provides the opportunity to utilise the advantages of the yeast model. This approach has provided disease-relevant insights into the roles of *CLN3*, *CLN10* and the newly identified *CLN12*. In addition, the level of information available regarding metabolic, biosynthetic and signaling pathways in yeast species can provide valuable information about processes involved in disease. In this review, such information regarding sphingolipid biosynthesis and vacuolar function has also been explored.

Emerging models for NCLs

There are relatively few models available for the study of adult onset NCLs. Beyond the cathepsin F (*CLN13*) knock-out mouse model for type B Kufs disease [240], and the *DNAJC5* (*CLN4*) knock-out mouse, which provides a model for autosomal-dominant adult-onset NCL [241], there are few options for the study of these conditions.

The nematode worm *Caenorhabditis elegans* provides a very simple multicellular eukaryotic model system with a highly stereotyped and defined development and

body plan [242]. *C. elegans* has orthologues of *CLN3* and *CLN10*, however little work has been done to study these genes in the nematode (reviewed in [243]). *C. elegans* however has recently emerged as a potential model for autosomal-dominant adult-onset NCL [244]. Nematodes lacking the orthologue of *CLN4* (*dnj-14*), which encodes the molecular chaperone cysteine string protein α , exhibit reduced lifespan, locomotor defects and impaired neurotransmitter release. Underlying this phenotype, the worms exhibited an age-dependent decline in neuronal cell bodies, reduced neurite numbers and contorted neuronal processes, particularly in the head of the animal. Among the neurons lost, sensory neurons were particularly affected, leading to chemosensing defects.

The lifespan defect in these worms was exploited to allow screening for potential therapeutic compounds. Resveratrol was the only compound to rescue this defect, in addition to giving a partial rescue of the neurodegeneration and chemotaxis phenotypes. Resveratrol has been described in the past as being beneficial for lifespan and, although the mechanism-of-action is unclear, it is known to activate the Sirtuin class of histone deacetylases and may act as a phosphodiesterase (PDE) inhibitor [245]. Consistent with a role as a PDE inhibitor, the rescue elicited by Resveratrol was mimicked by a known PDE antagonist Rolipram and consistent with this being the main therapeutic mechanism of Resveratrol in this case, the deletion of Sirtuin *sir-2.1* did not affect this rescue. These observations provide key information about the biology of *CLN4* deficiency, as well as a new therapeutic mechanism.

The social amoeba *Dictyostelium discoideum* is another very well-established eukaryotic model organism. *Dictyostelium* has been successfully used in a number of studies to examine the role of genes associated with neurodegenerative disorders [246-248], however it has only recently been used in the study of NCLs. In a recent study, however, the role of the *Dictyostelium* orthologue of *CLN3* (*Cln3*) was explored [249]. *Cln3* was shown to localise to the vacuolar and endocytic compartments of *Dictyostelium*, consistent with observations in other model systems. Cells lacking *Cln3* exhibited altered trafficking and processing of autocrine proliferation repressor (*AprA*), leading to increased proliferation, suggesting changes in protein trafficking. *Dictyostelium* also has a well characterised life-cycle, involving the formation of coordinated multicellular aggregates (slugs), which was altered upon loss of *Cln3*. These cells displayed a more rapid development through their lifecycle and faster migrating slugs. All these pleiotropic effects were rescued by expression of *Cln3*, or human *CLN3*, indicating that these proteins are functional orthologues.

In a later study, *Dictyostelium* was also used to analyse the role of *CLN2* (*TPP1*) [250]. The *Dictyostelium* orthologue *DdTpp1* was demonstrated to contribute to the cleavage of *TPP1* substrate, suggesting the same role for *ddTpp1* as *TPP1*. Both *ddTpp1* and *TPP1* were also demonstrated to localise to the lysosome of *Dictyostelium*. Like cells lacking *Cln3*, cells lacking *ddTpp1* exhibited a more rapid multicellular development, possibly suggesting overlapping roles. Cells lacking *ddTpp1* displayed further developmental changes, exhibiting defects in spore formation. These cells also exhibited clear defects in autophagy and lysosomal function, with autophagic stimulation leading to a reduction in cell size and viability and lysosomal perturbation leading to impaired development. Finally, this later phenotype could be suppressed by the loss of an oxysterol-binding protein-like protein *stpA*, providing insights into the mechanism of this defect and a potential mechanism of suppressing defects associated with the loss of *TPP1*.

This work in both *C. elegans* and *Dictyostelium* provides new information about the roles of different NCL genes. Importantly this work also opens up the repertoire of

tools available to NCL research, providing valuable new models for the study of these diseases.

Concluding Remarks

Model organisms have provided a wealth of information about the processes that underlie the pathological changes observed in NCL. In addition, such models provide a means for the identification and development of much needed novel therapies. The work summarised in this review also highlights the advantages of these models, and the value of using a broad range of experimental systems in tackling complex biological problems such as neurodegenerative disease. Finally, by looking to broader related basic research in these models, we are able to gain an insight into the cellular processes which may influence disease.

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Highlights

- We discuss the developments made in model systems for the neuronal ceroid lipofuscinosis (NCL).
- We discuss the advantages of each model and new tools available in these systems to aid NCL research.
- We review the progress made in these models from the perspective of both basic biology and therapeutic development.

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