Research article

Pharmacological intervention in invertebrate aging

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Abstract

Prompted by successful genetic interventions in aging we now consider the utility of pharmacological interventions. A few clear cases of lifespan extension resulting from exposure to compounds suggest that aging can be slowed and that the pharmacology of lifespan extension is a feasible area for research. Compounds that slow aging may prompt the rational design of therapeutics against age-related diseases. Here we evaluate invertebrate models for drug discovery in aging and outline how the field may develop from these simple first steps.

What is the pharmacology of aging?

Aging is the single most important risk factor in human disease in developed countries, and it is thought that an understanding of aging mechanisms would prompt design of rational therapies against age-related diseases (Hadley et al. 2005). A corollary is that a pharmacological agent that slows aging itself is likely to be effective against a wide spectrum of diseases. Oxidative stress provides a good example; if indeed aging phenotypes and limited lifespan turns out to be in part due to the actions of reactive oxygen species (ROS), then compounds that detoxify ROS or enhance repair of oxidative damage could, not only postpone aging-related changes, but also prevent pathological changes. Since invertebrates have proved enormously useful in defining the genetic landscape of aging, a number of investigators have begun to search for pharmacological agents that can extend lifespan in these model organisms. We will review these early attempts to slow aging through drug treatment and highlight some of the prospects and challenges ahead.

The plasticity of aging rate

Until genetic interventions in aging were described in the late 1980s, the prospects for pharmacological interventions in aging were slim. Genetic effects on lifespan as first described were polygenic (Johnson and Wood 1982; Rose and Charlesworth 1981) but the discovery of the *age-1* gene in the nematode model had a revolutionary effect on attitudes to the determination of lifespan (Friedman and Johnson 1988a; Johnson 2002). Mutation of the gene, now known to encode a component of an insulin signaling pathway (Morris et al. 1996), resulted in a 70% increase in mean lifespan and many hundreds of genes with similar effects have been identified in the intervening years. With the realization that single gene products had such a dramatic effect on aging rate the idea of pharmacological interventions mimicking such mutations took hold. The further realization that some ‘gerontogenes’ encoded discrete genetic pathways (Kenyon et al. 1993; Gottlieb and Ruvkun 1994) made it very likely that such compounds would exist. Although to date only a limited number of studies have clearly demonstrated

lifespan extension following pharmacological intervention, this number is likely to increase dramatically as newer screening methods are employed. As we identify such interventions we should be aware that genetic mutations that enhance lifespan also tend to exhibit a fitness cost (Walker et al. 2000; Marden et al. 2003; Jenkins et al. 2004) and we should consider such important side-effects as we go toward interventions in mammals.

The worm and the fly

Many of the advantageous characteristics of *C. elegans* as an experimental system, noted by Sydney Brenner in the 1960s, are also attractive for the development of whole animal drug screens (Brenner 1974) and high throughput screening (HTS) strategies. *C. elegans* is a microscopic (1.2 mm) self-fertilizing, free living nematode that is readily grown and maintained in the laboratory on simple liquid media and agar Petri dishes on a diet of *E. coli*. The animal has a three day life cycle (from egg through four larval stages to reproducing adult) and a 20-day natural lifespan. Each adult hermaphrodite produces over 300 progeny in a six-day reproductive period. This life history allows for rapid population expansion and bulk growth of millions of synchronously aged individuals (Riddle et al. 1997). It also offers the very important advantage that *C. elegans* is a naturally inbred system, thus populations of genetically identical individuals are readily available for HTS. An alternate life cycle occurs when food is limiting. First stage larvae develop into an alternate larval form called the 'dauer' (Riddle and Albert 1997). This is a non-reproducing, non-feeding diapause stage that is highly stress resistant. When food is introduced the dauer develops into a normal adult. This life cycle is useful for synchronizing populations of worms ('dauer arrest synchrony') and also provides convenient endpoints (dauer *versus* reproducing adults) for interesting HTS. The animal is transparent allowing individual cells and morphological features to be readily examined, and facilitates the use of various small compound fluorescent dyes and green fluorescent proteins (GFP) (Chalfie et al. 1994). There is a plethora of molecular genetic techniques that allows strains to be engineered for screening purposes. Until recently, culturing and manipulation was undertaken 'by hand', using techniques resembling traditional microbiological practice. Now automated handling has been developed

that is based on flow cytometry methods, and was recently combined with automated fluorescence measurement for screening purposes (Gill et al. 2003).

Although *C. elegans* is known as a genetic workhorse, a significant amount of work has been undertaken with drugs in this organism (Rand and Johnson 1995). Commonly researchers have combined drug treatments with genetic mutants to uncover mechanisms. For example, selection for drug resistant mutants has been used to investigate the mechanisms of antineoplastic compounds (Rand and Russell 1985; Driscoll et al. 1989). A number of bio-technology companies, such as DevGen, Exelixis and Divergence have historically incorporated *C. elegans* as a significant part of their drug discovery strategy. It is not clear how successful this strategy has been to date but it is clear that the system presents some major challenges and opportunities for HTS.

Pharmacological approaches to investigate aging have also been popular since the demonstration that worms mutant at the *age-1* locus were long-lived (Friedman and Johnson 1988a, b) and conferred resistance to the herbicide, paraquat (Vanfleteren 1993). Since paraquat is a superoxide anion generator, the interpretation was that the lifespan extension resulted from an enhanced resistance to oxidative stress. Indeed *age-1* mutants are also resistant to hydrogen peroxide (Larsen 1993) and a range of other stresses (Lithgow 2000).

Drosophila melanogaster shares many of the advantages of the worm as a system for screening drugs for effects on aging. In addition, it has a number of behavioral characters that may prove useful in aging studies such as flight, climbing, circadian behavior and sleep. In addition, the existence of cell culture lines (S2 and Kc) provides an important resource for cell based drug screening in conjunction with whole animal studies. There are some additional molecular genetic tools, such as inducible transcriptional promoters, that are an improvement over those available for *C. elegans* and the large well-defined tissues and organs (*e.g.*, flight muscle, eye, brain) may also be advantageous for downstream studies of drug action.

Pharmacological interventions that increase lifespan

A few compounds that extend invertebrate lifespan have already been identified. Since little or no truly high throughput screens (HTS) have been published

to date, there is little understanding of the 'chemical space' that influences lifespan. Rather, specific candidate compounds have been tested for their effects based on their known activities in biological systems.

Reducing oxidative stress

D. melanogaster provided one of the first clear lifespan extensions as a consequence of drug treatment (Brack et al. 1997). The antioxidant and glutathione precursor *N*-acetylcysteine (NAC) has a life-extending effect on flies. Dietary uptake of NAC results in a dose-dependent increase in median and maximum life span. Flies fed on 1 mg/ml NAC-containing food live 16.6% longer; at 10 mg/ml, life span increased by 26.6%. The mode of action has not been fully investigated but interestingly when mammalian neurons were treated with NAC, phosphorylation decreased and the activity of a forkhead transcription factor, related to the *C. elegans* DAF-16 protein, increased. DAF-16 over-expression itself results in lifespan extension, thus the effect of NAC on fly lifespan could be by an analogous mechanism (Nemoto and Finkel 2002).

Based on the stress resistance exhibited by long-lived mutants like *age-1*, we have undertaken pharmacological treatments to ask whether oxidative stress was a significant cause of aging in *C. elegans*. We demonstrated that *C. elegans* longevity could be significantly increased by treatment with synthetic, catalytic superoxide dismutase/catalase mimetics (SCMs) (Melov et al. 2000). Other groups have failed to observe such lifespan increases both in the housefly (Bayne and Sohal 2002), and in *C. elegans* under subtly different conditions (Keaney and Gems 2003), stressing the importance of further research with such compounds (Sampayo et al. 2003a). However, we have repeated the lifespan experiments with EUK-134, this time with sterile worms, and continue to observe a lifespan extension (Figure 1). In addition, we have gone on to show that SCMs protect against paraquat treatment but do not constitutively induce a cellular stress response (Sampayo et al. 2003a, b). These compounds have been shown to have beneficial effects in a number of models of human disease where oxidative stress is involved (Baker et al. 1998; Gonzalez et al. 1995, 1996; Malfroy et al. 1997; Melov et al. 2001) and illustrate how the screening for compounds in

Figure 1. EUK-134 extends the lifespan of sterile hermaphrodite *C. elegans*. Synchronous populations of hermaphrodites were cultured in liquid medium as previously described and transferred to fresh media each day of the lifespan experiment. Unlike our previously published account of lifespan extension using this compound (Melov et al. 2000), here we used a strain (TJ1060) containing two temperature sensitive mutations for self-fertility. Curves were compared by a log rank test as implemented in Prism™. Lifespan was significantly increased with both doses (20 nM, $p = 0.0023$; 200 nM, $p = 0.028$). Worms were cultured at 25 °C; during development and at 20 °C thereafter. N = number of death events recorded.

C. elegans may yield important research tools for age-related disease in mammals.

There are many published and unpublished accounts concerning the effects of antioxidant treatment on lifespan. Recently, α -lipoic acid was shown to have no effect on mouse lifespan (Lee et al. 2004), however, in a separate study, a 12% increase in lifespan was observed when female *Drosophila* were treated from day 10 past eclosion (Bauer et al. 2004). In this study, α -lipoic acid was identified in an 'accelerated screening assay' in which age-dependent enhancer-trap lines were constructed that expressed tetanus toxin light chain as a lethal reporter. Age-dependant expression of the toxin resulted in a greatly shortened lifespan with one line having a mean survival of 8 days and a maximal survival of 15 days, which is 80% shorter than normal fly lifespan. This is potentially an interesting route to speeding up drug testing in the fly (Bauer et al. 2004).

Other notable accounts involving antioxidants and their effects on invertebrate lifespan include studies on Vitamin E (Harrington and Harley 1988; Adachi and Ishii 2000; Ishii et al. 2004; Driver and Georgeou 2003), Coenzyme Q₁₀ (Ishii et al. 2004), and

Melatonin (Bonilla et al. 2002; Izmaylov and Obukhova 1999; Anisimov et al. 1997). However, with the exception of Coenzyme Q₁₀, the other antioxidants mentioned have been shown to have differing lifespan effects both in the same organism (Harrington and Harley 1988; Adachi and Ishii 2000; Ishii et al. 2004; Driver and Georgeou 2003) and between species (Driver and Georgeou 2003; Bonilla et al. 2002; Izmaylov and Obukhova 1999; Anisimov et al. 1997; Anisimov 2003).

Histone acetylation modulation

One of the first robust effects on invertebrate lifespan following a drug treatment was obtained with 4-phenylbutyrate (PBA) administration in *Drosophila* (Kang et al. 2002). Lifespan was increased without diminution of locomotor vigor, resistance to stress, or reproductive ability. The drug is an inhibitor of histone deacetylase activity and administration in *Drosophila* does result in increased histone acetylation. The compound also appears to have beneficial effects in mouse models of Parkinson's and Huntington's disease (Gardian et al. 2004, 2005) which raises the interesting question of the role of aging in each of these diseases.

Natural products

There are many advantages in studying the effects of natural products on aging that relate to the ease with which a product could be brought to market. However, unless there are well-characterized simple constituents, it is likely that unravelling the mode of action will be very challenging. Ginkgo biloba extract is readily available and has been tested in *C. elegans*. The Ginkgo biloba extract, EGb 761, has been shown to increase resistance to heat and oxidative stress and extend lifespan in the worm (Wu et al. 2002). Based on the reduced expression of a small heat shock protein report gene (HSP-16::GFP) in the face of oxidative stress, it appears that the EGb 761 extract reduces the level of stress experienced by the worms (Strayer et al. 2003).

A second interesting example of the potential for natural products is resveratrol, a compound found in many dietary plants and commonly found in red wine. There is evidence that it helps prevent a

number of human diseases such as atherosclerosis and cancer, and acts as an antioxidant, an anti-inflammatory and a cell cycle inhibitor. Resveratrol is an activator of SIR2 in yeast and homologous proteins in *Drosophila* and *C. elegans* and increases lifespan by 60% in budding yeast, 40% in the fly and a smaller effect in the worm with little or no obvious effects on fertility (Bauer et al. 2004; Howitz et al. 2003; Wood et al. 2004). In both yeast and fly, lifespan extension requires the *SIR2* gene and shows no additive effects with caloric restriction (CR), suggesting that resveratrol, along with other sirtuin-activating compounds (STACs), may act as CR mimetics without the normal costs on fertility associated with this dietary manipulation (Wood et al. 2004).

Anti-convulsants

A recent survey of 19 compounds used to treat human complaints revealed that anticonvulsants had effects on *C. elegans* lifespan (Evason et al. 2005). The drug ethosuximide has the largest effect extending mean adult lifespan by 17% under normal worm growth conditions. However, temperature proved to be an important modulator of this effect; ethosuximide extended mean life-span by 35% at 15 °C; 17% at 20 °C; and insignificantly at 25 °C respectively. At high concentrations the compound caused toxicity and shortened life-span. Importantly, other anticonvulsants also extend lifespan but similar chemical structures that do not have anticonvulsant activity do not. These anticonvulsants also affected neuromuscular activity in the worms which may be related to the mechanism of lifespan extension (Evason et al. 2005).

Considerations for the interpretation of results

One of the major limitations of invertebrate model systems is that lifespan can be affected inadvertently by a range of different environmental parameters. Thus, the mechanisms of action of a given compound on lifespan may be unrelated to the expected drug targets. For example, any drug that affects the rate of feeding is likely to also affect lifespan and some consideration should be given to experiments that shed light on this issue. Caloric restriction tends to slow development and decrease fertility, thus compounds

that have such side-effects may act by reducing feeding rates or slowing nutrient uptake. These compounds are not true caloric restriction mimetics but rather would fall into the category of dietary modulators. Relatively straightforward controls for food uptake can be performed in both flies and worms. In *Drosophila*, radiolabelled food is used to measure feeding rates and in the worm, parameters such as pumping rate (Friedman and Johnson 1988a) and fluorescent peptide uptake by the intestinal cells (Meissner et al. 2004) can be usefully employed.

A second major confound is the potential for compounds to induce 'hormesis'. Hormesis is where a sub-lethal stressful event can lead to improved survival when the organism is challenged by a lethal stress (Lithgow 2001). This becomes confounding in pharmacological interventions in aging because mild stresses also lead to increases in mean and maximum lifespan in invertebrate systems. The late evolutionary biologist John Maynard-Smith published a seminal paper in which he demonstrated a large increase in lifespan of female *Drosophila* fruit flies subjected to transient heat shock at 30.5°C (Maynard Smith 1958). Treatment of *C. elegans* with elevated temperature also resulted in significant increases in thermotolerance (so called 'acquired thermotolerance') and also in lifespan under non-stress conditions (Lithgow et al. 1995). A large range of toxins can induce the expression of heat shock proteins and affect feeding behavior in a similar fashion to a mild thermal stress (Candido and Jones 1996; Jones et al. 1996; Jones and Candido 1999). Consequently, specific concentrations of such toxins may result in beneficial effects on stress resistance and lifespan itself. There is essentially a fine balance between the degree of toxic insult and benefit derived from the response to the insult and it is likely that many pharmacological agents will induce apparently beneficial effects (such as lifespan extension) due to a toxic action. As with inadvertent caloric restriction, some experimental approaches may help resolve the issue. If lifespan extension is obtained under conditions where fertility is lowered or development slowed, then a possible toxic action could be responsible. In addition, it is relatively trivial to examine the expression of heat shock protein genes or other stress genes following treatment with the compound. In *C. elegans*, the HSP-16 transcriptional promoter fused to GFP has been used in this regard to show that antioxidant mimetics did not induce a generalized cellular stress response

under conditions which provided protection against oxidative stress and extended lifespan (Sampayo et al. 2003b). However, the picture becomes more complex when the compound of choice activates the stress response regulatory signaling pathways. In this situation, the compound is not in itself toxic but simply results in an elevated stress response. This indeed would be a highly desirable situation as potential benefits could be gained without the damage associated with toxicity.

The major limitation of invertebrate systems is the lack of good pharmacokinetic techniques. Attempts to derive specific mechanisms of action are greatly hindered by our ignorance of how much compound is taken up by invertebrates, how much makes it to the target cells, and how fast it is metabolized or modified thereafter. Such measures are routine in mammalian pharmacology and it would be highly desirable to develop such techniques in invertebrates. Some researchers have begun this process either by measuring compound directly or by measuring the proposed biochemical activity (Evason et al. 2005; Keaney et al. 2004; Davies et al. 2003). Another approach would be to feed fluorescent derivatives of specific compounds which would facilitate measures of uptake and tissue distribution.

Another major pharmacokinetic issue is that of metabolism of compounds *in vivo* resulting in dramatically altered activity. Antioxidants provide a series of simple examples that demonstrate the problems. It is possible that some antioxidant compounds act as pro-oxidants *in vivo*: ascorbate can act as a pro-oxidant by reducing iron (Fe(III) to Fe²⁺) which is then oxidized by H₂O₂ and results in OH[•] generation, and many other antioxidants including α -tocopherol, propyl gallate and plant phenolics can act likewise. Cu²⁺ can also take part in such reactions. Both metals are present in invertebrate growth media so the potential for such confounding reactions exists.

The need for pharmacogenomics

Because lifespan is a multifactorial phenotype with a strong environmental component, the interpretation of drug studies should be undertaken cautiously. This is compounded by the high variance exhibited for quantitative traits such as lifespan even in isogenic populations. In the absence of reliable pharmacokinetics for model invertebrates, microarray and other

techniques can be used to probe the effects of the compound. For example, in the mouse, α -lipoic acid treatment results in decreased expression of genes encoding molecular chaperones, the protein repair enzyme, protein-L-isoaspartate methyltransferase, and some antioxidant functions including glutathione peroxidase 4 (*Gpx4*) and a cytosolic peroxiredoxin (Lee et al. 2004). This illustrates that antioxidant treatment can result in gene expression changes likely to be detrimental for lifespan outweighing direct benefits of the antioxidant functions. This indeed may be the reason that α -lipoic acid has beneficial effects on fly (Bauer et al. 2004) and worm lifespan (Lithgow, unpublished data) but fails to extend mammalian lifespan.

Surrogates for lifespan analysis

Lifespan determination in *C. elegans* is a highly labor intensive activity which usually involves the daily examination of individual animals with a dissection microscope. As a result the number of animals that can be investigated is limited thus drug screening based on such longitudinal assays of survival is not feasible. Lifespan determination for *Drosophila* is a more straightforward process of 'tipping' live flies from one vial to another and counting the remaining dead flies. Consequently, hundreds of compounds can be tested in parallel. The use of genetically sterile strains or chemically sterilized worms allow large synchronous populations to be maintained over the course of a lifespan (Fabian and Johnson 1994). This makes possible an 'endpoint' rather than a longitudinal assay: any population of worms with a significant fraction alive after 30 days of life has potentially been subject to a lifespan extension treatment. An alternative is the use of surrogate markers for aging.

Biomarkers—There has been considerable interest in searching for biomarkers of aging in mammals and a few simple biomarkers have been determined in invertebrates (Herndon et al. 2002). For example, the accumulation of auto-fluorescent pigment is a hallmark of aging in *C. elegans* and correlates with genetically determined lifespan (Herndon et al. 2002; Hosokawa et al. 1994; Clokey and Jacobson 1986; Klass 1977; Hsu et al. 2003). Using automated methods it should be possible to screen for compounds that delay the age-related rise in auto-fluorescence signal. Aging is also associated with a

decline in motility and touch-provoked movement (Hosono et al. 1980). Methods that can quantify movement lend themselves to automation and it seems likely that screens based on these phenotypes will be forthcoming. Hertweck and Baumeister recently surveyed phenotypes associated with a couple of long-lived strains (insulin signaling and *sgk-1*) and discussed each phenotype in relation to the prospects for automated screens (Hertweck and Baumeister 2005). They described the potential for automation of behavioral and life history traits such as fertility, motility and thermal avoidance. Motility may be easily monitored in *C. elegans* by tracks made by the animals in the bacterial lawn on an agar plate. Machine recognition either of these tracks or the movement of the worm itself would facilitate an automated assay such as that recently developed to measure the sinusoidal body movements of the worm (Cronin et al. 2005). Wild type and untreated worms tend to cease unprompted movements by 16 to 18 days of age. Therefore worms that can be detected moving at 20 to 25 days of age are likely to have an altered rate of aging.

Stress response — Rather than screening on an aging characteristic, one in principle could screen for compounds that confer a common characteristic of long-lived genetic mutants. Perhaps the most robust predictor of longevity is stress resistance (Lithgow and Walker 2002). In both *C. elegans* and *Drosophila*, mutations leading to extended lifespan also result in resistance to common stresses such as heat shock (Lithgow et al. 1995; Lin et al. 1998), oxidative stress (Vanfleteren 1993; Larsen 1993; Honda and Honda 1999), UV radiation (Murakami and Johnson 1996), and heavy metals (Barsyte et al. 2001), and screening for stress resistant genetic variants has proved a useful method for identifying novel aging genes (Lin et al. 1998; Walker et al. 1998; Munoz and Riddle 2003). Similarly, compounds which conferred stress resistance on young animals (Sampayo et al. 2003b) may also extend lifespan (Melov et al. 2000). However, many factors determine organismal stress resistance, leading to potentially conflicting results as discussed above.

Automation and the prospects for high throughput screening

Although pharmacological intervention in the aging process has been demonstrated in *C. elegans* (Melov

Figure 2. General scheme of an automated assay for survival in *C. elegans*. (a) Synchronous mass cultures of adult *C. elegans* worms are prepared by growth on agar plates supplemented with concentrated *E. coli* as a food source. The worms are washed off of the plates and resuspended in media at high concentration for high throughput dispensing into microtiter plates. (b) Dispensing is achieved with a COPAS BIOSORT 'wormsorter' (Union Biometrica) in which worms are sorted on the basis of size. (c) Worms can be dispensed by the wormsorter into microtiter plates of various formats. (d) Fluorescence can be read in standard commercial microtiter plate readers. (e) Death induced by thermal stress is assessed using the dye SYTOX, which is only taken up by worms with compromised cell membranes such that dead worms display strong fluorescence. (f) Single worms dispensed into 384-well plates can be analyzed longitudinally to establish the time of death. The mean survival of a wild type strain (N2) is significantly shorter than that of an *age-1* mutant, thermo-tolerant strain (TJ1052).

et al. 2000; Harrington and Harley 1988; Wood et al. 2004; Evason et al. 2005), *Drosophila melanogaster* (Kang et al. 2002; Wood et al. 2004) and budding yeast (Howitz et al. 2003), the number of compounds that have been investigated remains small. Moderate to high-throughput screens of other compounds that elicit a similar effect in *C. elegans* have been limited by the necessity for microscopic inspection of individual worms to assess survival. Touch provoked movement is commonly used as the first indicator of death and remains the method of choice for everyone in the field. This method works well in the hands of experienced investigators, yet the score remains somewhat subjective and is highly labor intensive (a sample of 100 worms may take as long as 45 min to analyse). We have utilized a dye that fluoresces on DNA binding, yet will only enter cells with compromised plasma membranes (SYTOX green) to identify

dead worms. To increase throughput, we employed COPAS BIOSORT automated worm handling technology to dispense individual nematodes into microtiter plates containing SYTOX, and used a fluorimetric plate reader to quantify fluorescence (Figure 2). This combination promoted the development of a technique that allows for a rapid and objective score of survival in *C. elegans* (Gill et al. 2003). This assay illustrates just one of the many potential opportunities for HTS with *C. elegans* as in addition to death events, gene expression, auto-fluorescence, and behaviour can all be machine measured.

Drug screening in age-related disease models

Since the construction of the first *C. elegans* model of an age-related disease (Alzheimer's disease (AD))

(Link 1995; Fay et al. 1998; Link and Johnson 2002) the potential for drug screening has been clear. Since then transgenic animals have been engineered to express human β -amyloid peptide (A β) (Link et al. 2003), huntingtin and other polyglutamine repeat proteins (Faber et al. 1999; Satyal et al. 2000) and alpha-synuclein (Lakso et al. 2003), and generally show various forms of muscle or neuronal dysfunction. Various genetic modulators of toxicity have been identified in these systems. For example, in triplet repeat disease there is a critical threshold of repeat number (30–40) that results in aggregation (Morley et al. 2002). The *age-1* gene is a modifier such that long-lived mutants have a very significant delay in the onset of polyQ toxicity and appearance of protein aggregates. The mechanism of action could be via up-regulation of molecular chaperone genes consistent with the observation that over-expression of yeast Hsp104 causes a similar delay in aggregation. Following this, a genome-wide RNA interference screen was undertaken with 185 genes identified that altered the rate of PolyQ aggregation.

A Parkinson's disease (PD) model nematode has also been constructed (Nass et al. 2002) by expressing GFP from a presynaptic dopamine transporter (DAT) promoter resulting in expression-marking of the eight dopamine neurons. With this marker they demonstrated that dopamine neurons could be killed with the neurotoxin 6-hydroxydopamine resulting in complete absence of any fluorescent signal. Combinations of dopaminergic neuron-specific toxins and screening for compounds that prevent neuronal death could provide a cost effective *in vivo* screen for anti-Parkinson's disease agents.

Drosophila is also a good system to model AD (Finelli et al. 2004), PD (Feany and Bender 2000; Auluck et al. 2002) and triplet repeat disease (Warrick et al. 1998; Feany 2000). Just as with *C. elegans*, genetic modifiers of toxic effects have been identified but the prospects for true HTS are less certain.

Invertebrate model systems have long been touted as having many advantages over *in vitro* and cell-based assays for drug screening. Principally, there is the opportunity to examine the effects of compounds in a whole animal setting thus quickly screening out highly toxic or pleiotropic drugs. However, there have been few published accounts of successful drug discovery with these model systems. As the business of drug discovery is outsourced from the private

sector into the academic domain, a second opportunity is arising for these model systems to shine.

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