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Model Systems for Spinocerebellar Ataxias: Lessons Learned About the Pathogenesis

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1. Introduction

Model systems are important tools for the investigation of pathogenic processes. Especially for diseases with a late onset of symptoms and slow progression, like most spinocerebellar ataxias (SCA), it is time-consuming or even impossible to analyze all aspects of the pathogenesis in humans. Due to the reduced lifespan of model organisms, it is possible to study disease progression in full within a reasonable timeframe and due to the shorter generation time of most model organisms more individuals can be generated and analyzed, thereby strengthening the reliability of data via an increased number of replicates. Detailed studies of the histopathology can only be performed as endpoint analyses in humans, but with the help of an animal model, multiple time points can be analyzed throughout the course of the disease. In addition, model systems allow not only for the reduction of time from idea to results but also reduce the complexity due to their smaller genome sizes, less genes, nonredundant pathways, and a simpler nervous system.

Before using a specific species to model a disease it is of interest to check whether the proteins affected in humans are conserved within the respective model organism in order to increase the probability that binding partners and other keyplayers, involved in the pathogenesis of this disease, are likewise conserved. For those SCA which are caused by polyglutamine (polyQ) expansions, the respective affected genes are conserved in most organisms used as models (Table 1). Especially the proteins affected in SCA2, SCA6 and SCA17 are conserved with high similarity down to even yeast. This is not surprising as the TATA-binding protein (affected in SCA17) or a subunit of a voltage-dependent calcium channel (affected in SCA6) are important proteins for cellular maintenance. Although polyQ repeats are comparatively frequent in drosophila (Alba et al., 2007), only the repeat region of the TATA-binding protein is conserved. For most other non-mammalian model organisms, the respective orthologues are smaller and the polyQ repeats itself or even including the whole surrounding domains are not conserved. For analyses of SCA, various model systems have been employed. From the worm (*Caenorhabditis elegans*) and the fly (*Drosophila melanogaster*) all the way to mammals, i.e. the mouse (*Mus musculus*), model systems have

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made important contributions to the understanding of disease progression and will be important tools for the first line tests of potential treatment strategies.

This review aims to sum up the model systems used for the investigation of SCA and especially focuses on the lessons learned from these models about the pathogenesis of SCA. We also compare commons and differences in the results obtained using these animal models and highlight the species-specific advantages and possible problems associated with the use of this species as a model organism.

2. Lessons learned from non-mammalian models of SCA

2.1 Lessons learned from worm models

The nematode *Caenorhabditis elegans* is frequently used as a model organism, primarily because of its anatomic and biochemical simplicity as well as its genetic tractability. The worm genome encodes orthologues for about 65% of all known human disease genes. Moreover, it allows for easy and rapid establishment of transgenic lines, thus facilitating screening and characterization of human disease-causing mutations *in vivo*. Overall it is an often used model organism to analyze pathological features of neurodegenerative diseases (Huntington's disease, Parkinson's disease or Alzheimer's disease) (reviewed in Driscoll and Gerstbrein, 2003 and Brignull et al., 2006b). Except for ataxin-7, the worm contains orthologues for all SCA caused by polyQ expansion. Interestingly, for SCA *C. elegans* strains have only been generated and characterized for SCA2 and SCA3 (Ciosk et al., 2004; Khan et al., 2006; Kiehl et al., 2000; Rodrigues et al., 2007; Teixeira-Castro et al., 2011).

In the field of polyQ diseases (e.g. HD or SCA) the formation of aggregates, and therefore, the transition of polyQ proteins to their toxic forms is not well understood. Due to its transparency, *C. elegans* is especially suitable to address this question. PolyQ proteins can be attached to a fluorescent protein (e.g. GFP, YFP, CFP) and the dynamics of aggregate formation both within individual cells and over time can be examined throughout the worm lifespan. Transgenic lines can be rapidly generated by feeding *C. elegans* wildtype strains with genetically transformed bacteria or by microinjection of manipulated DNA into the germline. The worm's life-cycle of about 2 to 3 weeks under suitable living conditions is short. This allows studying the aggregate formation of many different constructs with various polyQ lengths, with or without flanking sequences of the endogenous protein and under control of a wide range of different promoters. When expressed in the body wall muscle of *C. elegans*, even short polyQ stretches (with less than 40 Qs) without any flanking sequences from endogenous proteins tend to aggregate in old worms indicating a balance of different factors including repeat length and changes in the cellular protein-folding environment over time (Morley et al., 2002). In neurons, however, the pathogenic threshold turned out to be about 35-40 repeats, which correlates well with the human disease. This means that in comparison with muscle cells, neuronal cells have a higher aggregation threshold (Brignull et al., 2006a). By way of contrast, the analysis of aggregation in the protein context of (full-length) ataxin-3 revealed that only a highly expanded polyQ stretch (Q130) was able to induce the formation of aggregates in the cytoplasm and nucleus of neuronal cells in transgenic *C. elegans* lines. Non-expanded (Q14, Q17) and even pathological expanded polyQ stretches (Q75, Q91) were diffusely distributed within neurons

	SCA1	SCA2	SCA3/MJD	SCA6	SCA7	SCA17
Human (***Homo sapiens***)	**ATXN1** **815 aa** **(6-39 Q)**	**ATXN2** **1313 aa** **(14-32 Q)**	**ATXN3** **361 aa** **(12-40 Q)**	**CACNA1A** **2512 aa** **(4-18 Q)**	**ATXN7** **945 aa** **(7-18 Q)**	**TBP** **339 aa** **(25-43 Q)**
Mouse (*Mus musculus*)	Atxn1 791 aa (2 Q) 89 %	Atxn2 1286 aa (1 Q) 91 %	Atxn3 355 aa (6 Q) 87 %	Cacna1a 2368 aa (no polyQ) 93 %	Atxn7 867 aa (5 Q) 87 %	Tbp 316 aa (13 Q) 95 %
Zebrafish (*Danio Rerio*)	atxn1a 781 aa (no polyQ) 52 %	si:dkey-165i4.1 1112 aa (no polyQ) 66 %	atxn3 266 aa (no polyQ) 71 %	cacna1ab 2338 aa (no polyQ) 79 %	LOC100001490 918 aa (1 Q) 55 %	tbp 302 aa (6 Q) 91 %
Fly (*Drosphila melanogaster*)	Atx-1 230 aa (no polyQ)	Atx2 1084 aa (no polyQ) 30 %	n. o.	cac 1850 aa (no polyQ) 63 %	n. o.	Tbp 353 aa (8 Q) 68 %
Worm (*Caenorhabditis elegans*)	K04F10.1 299 aa (no polyQ)	ATX2 959 aa (no polyQ)	atx-3 317 aa (no polyQ) 38 %	unc-2 2087 aa (no polyQ) 55 %	n. o.	tbp-1 340 aa (no polyQ) 59 %
Yeast (*Saccharomyces cerevisiae*)	n. o.	PBP1 722 aa (no polyQ)	n. o.	CCH1 2039 aa (no polyQ)	SGF73 657 aa (no polyQ)	SPT15 240 aa (no polyQ) 79 %

Table 1. Orthologues of the affected proteins in spinocerebellar ataxias caused by polyglutamine expansions. For each protein, its name, size in aa, number of polyglutamine repeats and % of sequence identity (if specified in HomoloGene) is listed. Sizes of human proteins depend on polyglutamine repeat number. Orthologues were selected according to Rubin et al. (2000), Ciosk et al. (2004), Khurana and Lindquist (2010) and Tsuda et al. (2005) as well as using HomoloGene (Sayers et al., 2011); Repeat numbers according to Schöls et al. (2004). n. o., no orthologue; no polyQ, the polyglutamine repeat expanded in humans is not conserved in the respective orthologue

without aggregation (Khan et al., 2006; Teixeira-Castro et al., 2011). In a truncated protein of ataxin-3, however, just 63Q are sufficient for aggregation mainly in the perinuclear region but rarely in the nucleus (Khan et al., 2006). These results are in line with observations made in mouse models, where a truncated form of the polyQ expanded protein induced more aggregates and a more progressive neurological phenotype than the full-length protein (Ikeda et al., 1996).

C. elegans is also a useful organism for studying the normal distribution and function of polyQ proteins both during development and throughout the full lifespan. For example, a SCA2 transgenic model, which expressed the *C. elegans* orthologue of the human ataxin-2 gene under the control of the endogenous promoter, revealed a strong expression of ataxin-2 in the central nervous system of adult worm, but also allowed the detection of ataxin-2 even in the early embryo, beginning around the 4-cell stage (Kiehl et al., 2000). Likewise, the expression of the worm orthologue of the human ataxin-3 was strongly detected during the late embryogenesis and during all stages of postnatal development. Interestingly, ataxin-3 was not only detected in the central nervous system (in the neuronal dorsal and ventral cord as well as in neurons of the head and tail) but was also observed in the spermatheca, vulval muscle, hypoderm, coelomocytes and body muscles (Rodrigues et al., 2007).

Using knock-out strains or knocking down expression of polyQ proteins with a siRNA loaded diet has provided another method for the study of polyQ distribution and function. The knockdown of ataxin-2 by siRNA results in reduced numbers of eggs and developmental arrest whereas the knock-out of this gene was embryonically lethal (Kiehl et al., 2000). In comparison, the knock-out of ataxin-3 results in viable animals, which show no obvious morphological abnormalities as well as normal lifespan and behaviour (Rodrigues et al., 2007) but a significantly increased resistance to stress (Rodrigues et al., 2011).

Aside from protein distribution *C. elegans* has been used to study synaptic function (Khan et al., 2006) and to perform genome-wide RNAi-based genetic screens to identify modifiers (Poole et al., 2011). Such a RNAi screen identified that the aggregation of pure polyQ repeats was enhanced by factors involved in RNA metabolism and protein synthesis (leading to an increased production of misfolded proteins) as well as factors involved in protein folding, transport and degradation (leading to decreased protein clearance) (Nollen et al., 2004).

Invertebrate models, like *C. elegans*, are also particularly useful models for first-line screenings of possible therapeutic compounds, especially in late-onset neurodegenerative diseases such as SCA. The useful nature of *C. elegans* in such screenings was demonstrated in 2007 when a first drug screening for Huntington's disease was published. Voisine et al. developed a so called food clearance assay by exploiting that *C. elegans* can easily be cultured in solution. For this assay, wildtype *C. elegans* were incubated in *E. coli* liquid culture to determine the optimal drug concentration. The optical density was used to measure the consumption of *E. coli* (food source) to indicate the growth or survival of *C. elegans*. Drugs in the established concentrations were then used to treat worms with a polyQ expanded huntingtin (Htn-Q150) and analyzed using a starvation assay (by measuring the presence or absence of GFP expression in neurons). In this assay, a HDAC inhibitor (Trichostatin, TSA) was able to suppress neurodegeneration and LiCl decreased polyQ-induced neurodegeneration, while NaCl had no effect (Voisine et al., 2007).

Although no single model organism is able to recapitulate all features of a human disease, *C. elegans* models have proven to be a very good starting point. Worm models allow answering research relevant questions *in vivo* in an easy to handle and "low-cost" organism, before generating a more complex and expensive, but also more comparable model to human diseases, like mouse models.

2.2 Lessons learned from fly models

A big advantage of disease models involving *Drosophila melanogaster* is the so called GAL4-UAS system (Brand and Perrimon, 1993; Fischer et al., 1988). A specific promoter controls the expression of the transcription factor GAL4 which binds the UAS (upstream activating sequence) in the responder construct containing the gene of interest. The use of different promoter GAL4-lines, thereby, allows controlling the expression strength and/or directing the expression of the disease–causing gene to different organs or cell types. A frequently chosen promoter is the mainly eye specific gmr-GAL4 driver (Freeman, 1996) directing the transgene to the flies eyes. *Drosophila* eyes are highly organized structures thereby allowing a macroscopic observation of the degeneration of (visual) neurons without the need of even preparing and staining brain sections. The high reproducibility, the simple breeding and the ease of analyzing neurodegeneration macroscopically make *Drosophila* models the ideal tool for the screening for and analysis of factors influencing neurodegenerative events in SCA. However, not all genes causing SCA are conserved in flies, e.g. there are no natural orthologues for ataxin-3 and ataxin-7 in *Drosophila melanogaster*. However, the *CACNA1A*, the affected gene in SCA6, as well as *ataxin-1* (Tsuda et al., 2005) and *ataxin-2* seem to be conserved albeit with only reduced homology (Rubin et al., 2000) as the CAG repeat is missing in these genes. This lack of endogenous genes excludes any knock-in or knock-out approaches and at first sight questions the chance of successful generation of transgenic models for these diseases as relevant binding partners for the affected proteins may also not be conserved. Interestingly, the sole overexpression of the *Drosophila* orthologue of ataxin-1 (*dAtx-1*) induced a similar phenotype than the overexpression of human ataxin-1 (*hATXN1*) although *dAtx-1* misses more than 60 % of *hATXN1* amino acids including the polyQ repeat (Tsuda et al., 2005). Not even a polyQ expansion is required as a high level of *hATXN1* with normal repeat length (30Q) caused neuronal degeneration (Fernandez-Funez et al., 2000). This data indicates that both *Drosophila* and human ataxin-1 are "intrinsically toxic at high levels" (Lu and Vogel, 2009). Likewise, the overexpression of *dAtx2*, the *Drosophila* orthologue of human ataxin-2, caused developmental defects and degeneration of tissues (Satterfield et al., 2002). As well the loss of dAtx2 had comparable effects, stressing the importance of maintaining normal ataxin-2 activity (Satterfield et al., 2002).

Analyses using *Drosophila* connected pathogenic mechanisms in SCA1, SCA2, and SCA3 and identified ataxin-2 as a potential key player both in SCA1 and SCA3 (Al-Ramahi et al., 2007; Lessing and Bonini, 2008): In both cases, the overexpression of *dAtx2* enhanced the neurodegeneration caused by ataxin-1 and ataxin-3, respectively, and downregulation of *dAtx2* had the opposite effect. Comparable observations were made even for a non-polyQ disease, amyotrophic lateral sclerosis (ALS) (Bonini and Gitler, 2011). This influence of *dAtx2* seems to be linked to the conserved PAM2 motif (PABP-interacting motif 2) within ataxin-2 which mediates the interaction of ataxin-2 with the Poly(A)-binding protein (PABP) (Lessing and Bonini, 2008) implicating ataxin-2 in the regulation of translation of specific mRNAs (Satterfield and Pallanck, 2006). The general importance of protein domains apart from the

polyQ repeat were first addressed using pure polyQ repeats which proved to be toxic in *Drosophila* in expanded, but not in normal lengths (Marsh et al., 2000). However, adding as few as 26 additional amino acids (such as addition of a myc and a FLAG tag) and even more, adding the surrounding amino acids of a full protein is able to even neutralize the toxic effect of expanded polyQ repeats (Marsh et al., 2000).

Drosophila models were also used to assess the relevance of the intracellular localization of the affected protein: Ataxin-2 is normally a cytoplasmic protein and the occurrence of intranuclear aggregates in SCA2 patients is still controversial as both the presence and absence of nuclear aggregates have been described (Huynh et al., 2000; Koyano et al., 2000). However, the intracellular localization of dAtx2 strongly influences the phenotype in flies. While nuclear dAtx2 induces strong neurodegeneration, the phenotype of flies with cytoplasmic dAtx2 is much milder (Al-Ramahi et al., 2007).

As SCA are neurodegenerative disorders, with ubiquitous expression of the disease causing gene in humans, glial cells are usually not the main focus of interest. However the choice of different driver lines allows for the analysis of glial vs. neuronal expression of the disease-causing genes in *Drosophila*. Data suggest that the effect of glial expression of the transgene is more pronounced than of neuronal expression (Kretzschmar et al., 2005).

Another strong advantage of *Drosophila* as a model organism is the suitability for large-scale screens for modifying factors. Such screens for ataxin-1, ataxin-3 or even pure polyQ repeats identified somehow expected proteins involved in protein folding (like chaperones) and protein degradation (components of the ubiquitin-proteasome system and autophagy) (Bilen and Bonini, 2007; Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000; Latouche et al., 2007). In addition, these screens gave insight into further mechanisms relevant for polyQ disease pathogenesis like cellular detoxification, protein transport, transcriptional regulation and RNA and miRNA processing (Bilen and Bonini, 2007; Bilen et al., 2006; Fernandez-Funez et al., 2000; Latouche et al., 2007). The identification of *muscleblind* (*mbl*) as a modifier of an SCA3 fly model drew attention to the role of CAG repeat RNA in the pathogenesis of SCA3 (Li et al., 2008) and led to the conclusion that not only the expanded polyQ repeat but also the RNA coding for it has an effect on the pathogenesis of polyQ diseases at least in *Drosophila*. *Muscleblind* is known to be involved in Myotonic dystrophy caused by aberrant RNA containing massive CUG expansions (Jiang et al., 2004). The expression of an untranslated CAG repeat caused neurodegeneration in *Drosophila*. This toxicity was mitigated just by the interruption of the pure CAG repeat by replacing it with a CAACAG repeat (Li et al., 2008). These results were in line with previous data for a non-polyQ SCA, SCA8, also caused by non-coding RNA. Both a normal and an expanded CAG repeat led to neurodegeneration in a fly model of SCA8 (Mutsuddi et al., 2004). Interestingly, a screen for modifiers of this phenotype caused by non-coding RNA (containing expanded CAG repeats) pointed to several pathways which were also identified as modifiers of a phenotype caused by (translated RNA coding for) expanded polyQ repeats (Mutsuddi et al., 2004). Taken together, disease models in *Drosophila* facilitated both the identification and further analysis of multiple factors and mechanisms involved in the pathogenesis of SCA.

3. Lessons learned from mammalian models of SCA

In contrast to disease models in the worm or the fly, mouse models resemble pathogenic processes in humans much closer than their non-mammalian counterparts. For example the

brain structure of mice is much closer to that of humans than those of flies or worms and mechanisms of special importance for late-onset diseases like SCA, e.g. gene expression changes during aging (Bishop et al., 2010), are better conserved. In particular, mouse models allow analyzing aspects of the disease which cannot be analyzed in simpler organisms. Although behavioural analyses are possible in *C. elegans* and *Drosophila* models, they are rather basic compared to more sophisticated behavioural tests possible with mouse models which even allow for e.g. fear and spatial learning analyses (Huynh et al., 2009).

3.1 Lessons learned from knock-out mouse models

In mouse models, it is possible to selectively inactivate a specific gene-of-interest via gene targeting. There is a large amount of insight to be gained from generating such knock-out models and a lot of information has been uncovered about the functional roles of specific genes in mammalian biology (Capecchi, 2005). To learn about the native function of genes affected in SCA knock-out mice were generated for SCA1, 2 and 3. All mice were viable, fertile and had a normal lifespan with no severe ataxic phenotype or neurodegeneration (SCA1: Matilla et al., 1998; SCA2: Kiehl et al., 2006; Lastres-Becker et al., 2008; SCA3: Schmitt et al., 2007; Switonski et al., 2011), providing evidence that loss-of-function is not the primary cause for ataxic symptoms in these disorders. However, these mice served to give indications for normal functions of the respective knock-out genes. For *ataxin-1*, the gene affected in SCA1, a role in learning and memory was identified (Matilla et al., 1998) and its function as a transcriptional co-regulator was elucidated (Goold et al., 2007). Knocking out the *ataxin-2* gene led to adult-onset obesity and reduced fertility (Kiehl et al., 2006; Lastres-Becker et al., 2008a) as well as hyperactivity and abnormal fear-related behaviour (Huynh et al., 2009). In *ataxin-3* knock-out mice increased levels of ubiquitinated proteins were detected reflecting its function as a deubiquitinating enzyme (Schmitt et al., 2007). However, in a second SCA3 knock-out model changes in the ubiquitination level were not observed. The authors suggested compensational effects as the cause for this opposing result (Switonski et al., 2011). Other analyses on SCA3 knock-out mice were able to show a protective function of ataxin-3 in the heat shock response pathway (Reina et al., 2010).

In contrast to only mild effects observed with the deletion of genes responsible for polyQ products, the knock-out of genes affected in non-polyQ SCA resulted in severe ataxic phenotypes. The deletion of the *Klhl1* gene which is mutated in SCA8 led to the loss of motor coordination due to degeneration of Purkinje cell function (He et al., 2006). The analysis of mice showing signs of a severe autosomal recessive movement disorder revealed a deletion in the inositol 1,4,5-triphosphate receptor (ITPR1 gene) as the cause of the observed symptoms. Knowing that the gene correlated to SCA15 in humans maps to the *ITPR1* genomical region, it was possible to identify a deletion in this gene as the cause of this autosomal dominant disorder (van de Leemput et al., 2007).

Taken together, the analyses of SCA knock-out mice demonstrated a toxic gain-of-function as the cause for SCA due to polyQ expansions, whereas for non-polyQ SCA loss-of-function seems to be the primary mechanism of pathogenesis.

3.2 Lessons learned from classical transgenic mouse models for SCA

Transgenic mouse models gave insight into various pathogenic mechanisms in SCA. Here, we review three examples: Lessons learned about the cell-type specificity of neuro-

degeneration, the aggregation and localization of the affected protein as well as transcriptional dysregulation caused by expanded polyQ proteins.

3.2.1 Lessons learned about the cell-type specificity of neurodegeneration

A classical transgenic mouse model is generated by using a specific promoter typically controlling the expression of a cDNA construct of the respective gene-of-interest. The effect of expressing different transgenes in a specific subgroup of neurons can be nicely compared among several proteins affected in SCA as the Purkinje-cell-specific promoter (Pcp2/L7 promoter) (Vandaele et al., 1991) was used for the generation of transgenic mice for SCA1 (Burright et al., 1995), SCA2 (Huynh et al., 2000), SCA3 (Ikeda et al., 1996), SCA7 (Yvert et al., 2000) and SCA17 (Chang et al., 2011), respectively. In the SCA1, SCA2 and SCA17 mouse models the expanded full-length transgene causes a strong degeneration of Purkinje cells (Burright et al., 1995; Chang et al., 2011; Huynh et al., 2000). By contrast, in the SCA7 mouse model, the sole expression of full-length *ataxin-7* with 90 Q induced a behavioural phenotype, but only mild degeneration of Purkinje cells in quite old mice (Yvert et al., 2000). Ironically, the expression of full-length *ataxin-7* (92 Q) in most neurons except for Purkinje cells (Garden et al., 2002; La Spada et al., 2001) or even just in Bergmann glia cells (Custer et al., 2006), led to a strong degeneration of Purkinje cells (Custer et al., 2006). Likewise, when a full-length ataxin-3 protein with 79 Q was expressed using the same promoter, no phenotype was induced. Only a fragment containing not more than a few amino acids surrounding the expanded polyQ repeat was able to induce a phenotype (Ikeda et al., 1996). These data demonstrate that Purkinje cells in transgenic mice seem to be more vulnerable by a repeat expansion within *ataxin-1*, *ataxin-2* and *ataxin-17*, than by an expansion within *ataxin-3* and *ataxin-7*, thereby –at first sight- nicely replicating the situation in humans where Purkinje cells are strongly affected in SCA1 (Cummings et al., 1999a), SCA2 (Lastres-Becker et al., 2008b) and SCA17 (Rolfs et al., 2003), but the loss of Purkinje cells can be observed but is not so prominent in SCA3 patients (Rüb et al., 2002a; Rüb et al., 2002b). In SCA7, however, Purkinje cells are typically affected (Holmberg et al., 1998), thereby possibly indicating that the pathogenic processes leading to Purkinje cell death in SCA7 differ from those in SCA1, SCA2 and SCA17.

3.2.2 Lessons learned about the aggregation of polyQ proteins and their localization

A common feature of polyQ as well as other neurodegenerative diseases is the accumulation of insoluble proteins in neurons, a feature recapitulated by most model systems of these disorders. Despite this fact the role of these so called neuronal nuclear inclusions (NIIs) in the pathological processes of polyQ diseases is still controversially discussed but it is known that these structures are associated with pathogenesis. Analysis of a *C. elegans* model of SCA3 directly linked the formation of aggregates to neuronal dysfunction (Teixeira-Castro et al., 2011), whereas several opposing results in mouse models exist. Observations in transgenic mouse models for SCA1, SCA2, SCA3 and SCA6 (Boy et al., 2010; Cummings et al., 1999b; Huynh et al., 2000; Klement et al., 1998; Silva-Fernandes et al., 2010; Watase et al., 2008) reveal that the development of a pathological phenotype is independent of the formation of inclusions excluding large aggregates as a primary cause for neuronal dysfunction. Even more, evidence exists for a protective role of inclusion bodies (Bowman et al., 2005). Inclusions in human SCA patients and respective mouse models stain positive for

ubiquitin and other components of the ubiquitin-proteasome-system (UPS) (Bichelmeier et al., 2007; Cummings et al., 1998; Holmberg et al., 1998; Klement et al., 1998; Koyano et al., 1999; Paulson et al., 1997; Schmidt et al., 2002; Watase et al., 2002; Yvert et al., 2000) pointing to an involvement of this protein degradation system in the clearance of proteins with expanded CAG repeats. In *C. elegans* it was observed that expanded polyQ tracts impair the functions of UPS (Khan et al., 2006). In brains of SCA3 patients a marked misdistribution of proteasomal subunits was detected leaving only a subpopulation of neurons with the possibility to form functional proteasome complexes (Schmidt et al., 2002). Comparable results were obtained for SCA1 patients and transgenic mice (Cummings et al., 1998) and further studies revealed that an impairment or altered function of the ubiquitin and the proteasomal degradation system could contribute to the SCA1 pathogenesis (Cummings et al., 1999b; Hong et al., 2002). Data gained using a knock-in model, though, excluded an impairment of the ubiquitin-proteasome-system as a major neuropathological cause of SCA7 (Bowman et al., 2005).

The mechanism which leads to the formation of aggregates is not well understood. It has been proposed that proteolytic cleavage of polyQ-containing proteins is required for aggregate formation, because polyQ-containing fragments are predominantly found in NIIs. Another indication for the cleavage hypotheses is the detection of protein fragments in brains of mouse models for SCA3 (Goti et al., 2004), SCA7 (Garden et al., 2002) and SCA17 (Friedman et al., 2008) as well as human SCA patients (Garden et al., 2002; Goti et al., 2004). As possible protein cleavage enzymes, caspases or calpains are under controversial discussion. For ataxin-3, calpain (Haacke et al., 2007; Koch et al., 2011) and caspase cleavage was analyzed *in vitro* (Berke et al., 2004; Pozzi et al., 2008). It was shown that a C-terminal fragment of ataxin-3 containing the polyQ stretch leads to a more progressive phenotype (Ikeda et al., 1996), but also an N-terminal fragment without the CAG repeats can cause SCA3 symptoms (Hübener et al., 2011). In addition, mice expressing a fragment of the TATA-binding protein (affected in SCA17) exhibit a more severe phenotype (Friedman et al., 2008) than those expressing a full-length protein (Friedman et al., 2007). These studies suggest that cleavage of the affected protein is important for the pathogeneses of polyQ SCA. Although neuronal nuclear inclusions (NIIs) are a common feature of polyQ diseases, in some SCA the affected protein is normally localized in the cytoplasm. For this reason, the question arose whether the intracellular localization of the affected protein is of relevance for the pathogenesis of SCA. For an polyQ expansion within an ectopic protein context (Jackson et al., 2003), for ataxin-1 (Klement et al., 1998) and for ataxin-3 (Bichelmeier et al., 2007) it was demonstrated that the nuclear localization of the affected protein is a requirement for the manifestation of symptoms. Mice in which the respective protein was kept in the cytoplasm typically had less and smaller aggregates and milder or even almost no behavioural phenotype. For SCA1, Emamian et al. (2003) even went one step further demonstrating that although the nuclear localization of ataxin-1 is required, it is not sufficient to induce a phenotype. A serine residue close to the endogenous NLS within ataxin-1 (S776) was required additionally for the induction of a phenotype (Emamian et al., 2003).

3.2.3 Lessons learned about transcription dysregulation

Transcriptional dysregulation is a common feature of most polyQ diseases, but the underlying mechanisms which cause the differential regulation remain unknown. Many

proteins affected in polyQ diseases are functioning as transcription factors/cofactors or at least interact with transcription factors: TBP (SCA17) is a general transcription factor, ataxin-7 is a part of a transcriptional co-activator complex and both ataxin-1 and ataxin-3 interact with various transcription factors (Helmlinger et al., 2006).

Especially for SCA1, the molecular basis of transcriptional dysregulation and therefore its influence on the pathogenesis is thoroughly studied. Transcriptional dysregulation mediated by ataxin-1 has been attributed to the interaction with the polyQ binding protein 1 (PQBP1). This interaction interferes with the cellular RNA polymerase-dependent transcription (Okazawa et al., 2002). Microarray analyses of SCA1 knock-in and knock-out mice revealed differential expression of proteins involved in calcium signaling (Crespo-Barreto et al., 2010). In SCA3 and SCA7, components of the NIIs are transcriptionally dysregulated, including subunits of the proteasome and heat shock proteins (Chou et al., 2010; Chou et al., 2008). Several other transcription factors such as CREB (cAMP response element binding protein) and HDAC proteins and therefore histone deacetylation is often differential regulated in polyQ diseases (McCampbell et al., 2000; McCullough and Grant, 2010). For this reason, treatment studies using HDAC inhibitors such as sodium butyrate were performed (Chou et al., 2011; McCampbell et al., 2001). In several studies, transcriptional dysregulation is associated with the degeneration of specific neurons: for SCA17, a downregulation of TrkA (nerve growth factor receptor) is linked to Purkinje cell degeneration (Shah et al., 2009), or for SCA1 an interaction of ataxin-1 and PQBP1 and therefore transcriptional dysregulation leads to selective neuronal loss in the cerebellum (Okazawa et al., 2002).

3.3 Lessons learned from YAC, BAC and knock-In mouse models

In the process of generating classical transgenic mice it is only possible to insert cDNA randomly into the animal genome, not allowing for controlling the expression of the pathogenic gene in the native genetic environment at endogenous levels or excluding alternative splicing events. Therefore, different techniques have been developed to overcome these limitations and to generate models which more closely resemble human disease conditions. One strategy was the use of a yeast artificial chromosome (YAC) containing a large fragment of the human *MJD1* locus for the generation of a model for SCA3 thus enabling the expression of a full-length *ataxin-3* gene with the endogenous regulatory elements needed for cell specificity and endogenous levels of expression (Cemal et al., 2002). Mice with expanded CAG tracts showed mild and slowly progressing cerebellar symptoms with nuclear inclusions and cell loss in specific brain regions closely resembling main features of the SCA3 disease in humans (Cemal et al., 2002). A likewise approach was used to generate a model for SCA8. Moseley *et al.* (2006) used a bacterial artificial chromosome (BAC) to control the expression of the SCA8 locus encoding a non-expressed transgene. If they would have used just a classical transgenic construct without 116 kb of flanking sequences they may not have observed that the construct is indeed expressed in both directions encoding both a non-translated RNA containing a CTG expansion as well as a polyQ containing protein expressed from the opposite strand (Moseley et al., 2006).

A different more widely used strategy in the generation of SCA mouse models is to take advantage of homologous recombination techniques leading to knock-in models. This allows for endogenous levels of expression in proper spatio-temporal patterns (Yoo et al.,

2003). The first knock-in model generated for SCA1 targeted an expanded CAG tract of 78 repeats to the endogenous *ataxin-1* mouse locus. These mice reflected genetic repeat instability observed in human SCA1 patients, but showed only mild behavioural changes in late life with no clear neuropathological changes (Lorenzetti et al., 2000). From this first attempt the conclusion was drawn that the short lifespan of mice seems to be a limiting factor and that the longer exposure of the mutant protein in humans might be necessary for the development of neuronal dysfunctions. This drawback can be overcome by either overexpression of mutant proteins or by the use of extremely long CAG tracts to produce neurodegeneration (Yoo et al., 2003; Zoghbi and Botas, 2002). Therefore, in the next knock-in model for SCA1, more CAG repeats (154 repeats) were used and this model then indeed resembled main features of the human SCA1 disease (Watase et al., 2002). Analyzing these mice it was also shown that there is no direct relationship between the degree of somatic instability and the selective neuronal toxicity (Watase et al., 2003), but that the selective neuropathology rather arises from alterations in the function of the ataxin-1 protein (Bowman et al., 2007). Furthermore, these mice served to demonstrate that a partial loss-of-function contributes to the SCA1 pathogenesis (Bowman et al., 2007; Crespo-Barreto et al., 2010; Lim et al., 2008). SCA6 knock-in mice with up to 84 (hyperexpanded) CAG repeats in the *CACNA1A* gene (encoding for a calcium channel subunit) gave evidence against the assumption that the SCA6 pathogenesis is caused by alterations of channel properties and rather indicated that it is due to the accumulation of mutant calcium channels (Saegusa et al., 2007; Watase et al., 2008). In infantile cases of SCA7 expansions of 200-460 CAG repeats were documented (Benton et al., 1998; van de Warrenburg et al., 2001) and knock-in mice with 266 CAG repeats indeed reproduced hallmark features of the infantile disease (Yoo et al., 2003). Using this knock-in model it was shown that polyQ nuclear inclusions seem to have a protective role against neuronal dysfunction, that an impairment of the ubiquitin-proteasome-system can be excluded as a major neuropathological cause (Bowman et al., 2005) and that SUMOylation influences the aggregation process of ataxin-7 (Janer et al., 2010). A most recent publication reported on the attempt to generate the first knock-in mouse model of SCA3, but due to unexpected splicing events ended up creating another SCA3 knock-out model (Switonski et al., 2011) showing some of the difficulties which may occur generating animal models.

3.4 Lessons learned from an alternative strategy to generate mouse models

An alternative approach for the generation of animal models is the use of viral injections. By using lentiviral vectors it was possible to overexpress wildtype or polyQ expanded ataxin-3 in brain regions of adult wildtype rats. An expression of polyQ-expanded ataxin-3 in the substantia nigra, an area affected in SCA3, led to the formation of ubiquitinated ataxin-3 positive aggregates, loss of dopaminergic markers and an apomorphine-induced turning behaviour. If polyQ expanded ataxin-3 is overexpressed in the striatum or cortex, regions previously not linked to SCA3 pathogenesis, by the lentiviral-based system it results in accumulation of misfolded ataxin-3 and loss of neuronal markers especially in the striatum (Alves et al., 2008b). Using the lentiviral vector system it is also possible to co-express ataxin-3 with knock-down vectors or other proteins and to analyze direct effects in specific brain regions. For example a co-expression of expanded ataxin-3 with beclin, an autophagic protein, led to stimulation of autophagic flux, clearance of mutant ataxin-3 and neuroprotective effects (Nascimento-Ferreira et al., 2011).

3.5 Treatment approaches using mouse models

At the moment, curative treatment for SCA is not possible. Only treatments directed towards alleviating symptoms are available (Duenas et al., 2006). Therefore, one or the most important goal in the research of SCA is the development of a cure.

The basic question of whether any treatment -if available- would be able to even reverse symptoms already manifested was addressed using conditional mouse models. With these models which allow to turn off the pathogenic trangene expression using the Tet-off system it was possible to demonstrate that already developed symptoms of SCA1 and SCA3 indeed can be reversed (Boy et al., 2009; Zu et al., 2004). Inhibiting or reducing the production of pathogenic proteins could therefore be a powerful tool in the therapy of dominant neurodegenerative diseases. Using the RNA interference (RNAi) technology (Mello and Conte, 2004) to inhibit the expression of mutant ataxin-1 in a mouse model of SCA1 led to improved motor coordination, restored cerebellar morphology as well as resolved ataxin-1 inclusions demonstrating the *in vivo* potential of this strategy (Xia et al., 2004). RNAi knockdown was also successfully used for a selective allele-specific silencing of mutant ataxin-3 showing to mitigate neuropathological abnormalities in a lentiviral-mediated model of SCA3 (Alves et al., 2008a; Alves et al., 2010) and may be a possible treatment approach. As protein misfolding and impaired protein degradation is implicated in the pathogenesis of polyQ SCA and other related diseases that present with intracellular inclusion bodies, supporting the correction of these alterations might be a therapeutic strategy. In this manner it was possible to show that crossbreeding of SCA1 transgenic mice with mice overexpressing a molecular chaperone leads to the mitigation of the SCA1 phenotype (Cummings et al., 2001).

In addition to genetic approaches, some of the published mouse models have already been used to test the effect of different compounds on the movement phenotype, neuronal loss and aggregate formation: Lithium carbonate enhanced the motor performances and improved spatial learning, but had neither an effect on the distribution and formation of aggregates nor did it improve the lifespan of the SCA1 knock-in mice (Watase et al., 2007). A treatment approach using lithium chloride in a *C. elegans* model for Huntington's disease, however, was beneficial (Voisine et al., 2007). A dietary supplementation with creatine improved survival and motor performance and delays neuronal atrophy in the R6/2 transgenic mouse model of Huntington's disease. In a SCA2 transgenic mouse model, however, creatine extended the Purkinje cell survival, but was not able to improve or delay ataxic symptoms (Kaemmerer et al., 2001). Two promising studies were performed using transgenic models for SCA3: The HDAC inhibitor sodium butyrate (SB) delayed the onset of ataxic symptoms and improved the survival rate by reversing polyQ induced histone hypoacetylation and transcriptional repression (Chou et al., 2011). In addition, a rapamycin ester (also called temsirolimus or CCI-779) which inhibits the mammalian target of rapamycin and upregulates the protein degradation by autophagy, reduced the number of aggregates and improved the motor performance (Menzies et al., 2010). In a study using a SCA2 mouse model, the Ca^{2+} stabilizer dantrolene was able both to alleviate motor symptoms and to reduce the loss of Purkinje cells in this model (Liu et al., 2009). Another group used a specific mouse model, the so called rolling mouse Nagoya, which has been suggested as an animal model for some human neurological diseases such as autosomal dominant cerebellar ataxia (SCA6). This model was treated with talrelin, a synthetic

analogue of the thyrotropin-releasing hormone (TRH) which alters the metabolism of acetylcholine and dopamine and therefore activates the dopaminergic system. Talrelin significantly elevated the cerebellar dopamine and serotonin levels of mice and improved the locomotion phenotype (Nakamura et al., 2005).

Other therapeutical attempts are based on the functional restoration of affected cell populations. Expanded ataxin-1 causes the degeneration of Purkinje cells thereby also negatively effects the synthesis of the insulin-like growth factor–I (IGF-I) a factor promoting Purkinje cell development (Fukudome et al., 2003). Administering this factor to SCA1 transgenic mice (SCA1[82Q]) intranasally led to significant improvement of motor coordinative abilities as well as to partial restoration of Purkinje cell survival (Vig et al., 2006). Using the same SCA1 transgenic model, improved motor skills and a higher Purkinje cell survival rate was reached after grafting neural precursor cells into the cerebellar white matter (Chintawar et al., 2009). Although there is a long way from successful treatment approaches in animal models to clinical application, the recent results give hope that treatment of SCA will be possible in the future.

4. Commons and differences between SCA models in worms, flies and mice

It is self-evident that the data acquired in different model organisms especially those obtained in non-vertebrate compared with those from vertebrate models cannot be identical. However, if results obtained in a specific model are to be translated to the situation in humans one would expect that basic mechanisms in the pathogenesis of SCA are conserved among species. Previous studies revealed that many pathogenic mechanisms are indeed comparable among species, however, also indicated that there are some differences between model organisms (Table 2). Orthologues of ataxin-2 can be found all the way down to simple organisms and even in yeast (Table 1). However, the knock-out of ataxin-2 gave rise to contradictory results among model organisms: The knock-out of the endogenous SCA2 gene in the worm and the fly is embryonic lethal. In contrast to that, SCA2 knock-out mice are viable and showed no developmental defects. Further analyses of SCA2 knock-out worms demonstrated that ataxin-2 is functioning during development, since the knockdown by RNAi results in developmental arrest.

These results indicate that the function of homologous proteins as well as the interaction of different proteins in special pathways is not conserved in the species analyzed (Kiehl et al., 2006; Kiehl et al., 2000; Lastres-Becker et al., 2008a; Satterfield et al., 2002). Since in *C. elegans* the polyQ repeats in all orthologous genes are not conserved, one could assume that much shorter repeat expansions than e.g. in the mouse may already give rise to a phenotype. However, the exact opposite seems to be true: Within the full-length context of a protein, much higher polyQ repeat numbers are required to be toxic (Khan et al., 2006; Teixeira-Castro et al., 2011). Proteins with polyQ repeats are frequent in *Drosophila* (Alba et al., 2007), but these repeats are generally encoded by interrupted rather than pure CAG repeats and, therefore, more resistant to expansion (Alba et al., 2001). This could lead to the assumption that pure CAG repeats may behave unstable in *Drosophila* as observed in human SCA patients and mouse models (Boy et al., 2010; Kaytor et al., 1997; Lorenzetti et al., 2000). However, CAG repeats seem to be perfectly stable in *Drosophila* even within a challenging genomic context (Jackson et al., 2005) pointing to a specific protection mechanism against repeat expansion in *Drosophila*.

	Caenorhabditis elegans	*Drosophila melanogaster*	*Mus musculus*
knock-out	SCA2: lethal (1) SCA3: viable (2)	SCA2: lethal (3)	SCA1/2/3/8: viable (4-9)
overexpression of pure polyQ causes phenotype	yes (10; 11)	yes (12)	yes (13; 14)
truncated protein requires less repeats to induce phenotype	yes (15)	yes (16)	yes (13)
full-length protein causes phenotype	yes (≥ 130 Q) (15; 17)	wt: no or mild exp: strong (3; 18-22)	wt: no exp: mild to strong (23-26)
instability of repeats		no (27)	SCA1/3: yes (28-30) SCA2: no (24)
repeat numbers causing phenotype	≥ 130 Q (15; 17)	≥30 Q (18)	≥ 30 Q (18; 24; 26)
increasing repeat length intensifies phenotype	yes (15; 17)	yes (3; 18-22)	yes (23-26)
formation of aggregates	yes (15; 17)	yes (31)	yes (33; 25) no (24; 32) late (30; 34)
neurodegeneration/ neuronal loss		yes (18; 19; 35)	wt: no exp: mild to strong (13; 23; 24; 36)
switching-off led to reversal of symptom		yes (31)	yes (30; 37)
transgene leads to reduced lifespan	yes (17)	yes (31)	yes (25)

References: (1) Kiehl et al., 2000; (2) Rodrigues et al., 2007; (3) Satterfield et al., 2002; (4) Matilla et al., 1998; (5) Kiehl et al., 2006; (6) Lastres-Becker et al., 2008a; (7) Schmitt et al., 2007 ; (8) Switonski et al., 2011; (9) He et al., 2006; (10) Brignull et al., 2006a ; (11) Morley et al., 2002; (12) Marsh et al., 2000; (13) Ikeda et al., 1996; (14) Ordway et al., 1997; (15) Khan et al., 2006; (16) Lu and Vogel, 2009; (17) Teixeira-Castro et al., 2011); (18) Fernandez-Funez et al., 2000; (19) Al-Ramahi et al., 2007; (20) Warrick et al., 1998; (21) Warrick et al., 2005; (22) Moseley et al., 2006; (23) Burright et al., 1995; (24) Huynh et al., 2000; (25) Bichelmeier et al., 2007; (26) Friedman et al., 2007; (27) Jackson et al., 2005; (28) Kaytor et al., 1997; (29) Lorenzetti et al., 2000; (30) Boy et al., 2009; (31) Latouche et al., 2007; (32) Silva-Fernandes et al., 2010; (33) Cummings et al., 1999b ; (34) Watase et al., 2008; (35) Lessing and Bonini, 2008; (36) Aguiar et al., 2006; (37) Zu et al., 2004

Table 2. Exemplary phenotypical features of human SCA patients compared among model organisms. For clearness, only examples for the respective phenotypic features are listed. The table is not intended to be exhaustive. (wt, normal repeat; exp, expanded repeat).

5. Conclusion

Multiple successful attempts generating transgenic animal models for SCA were performed in different species. While each model organism has its own advantages and disadvantages, all animal models contributed to the knowledge about the pathogenesis of SCA. The transparency of *C.elegans* together with the simplicity to generate transgenic models as well as the option to study neurodegeneration even macroscopically by targeting the gene of interest to the *Drosophila* eye make smaller organisms like the worm or the fly especially suitable for the screening of compounds or genetic modifiers. Since many pathologic mechanisms in SCA are conserved in these models, there is a high probability that results obtained in worms and flies can be translated to mammals. Although unsuitable for large-

scale (genetic and compound) screening approaches, mouse models are the ideal tools for verification of screening results in mammals. Viral injections now even allow a comparatively rapid analysis without the need of breeding or even generating transgenic mice. Especially to answer questions which require brain structures closer to humans or for analyses of ataxic movement or even emotional phenotypes, mammalian models are required. Taken together, model organisms are indispensable tools for the analysis of pathogenic mechanisms important for SCA *in vivo*.

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7. References

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