● INVITED REVIEW

DNA plasticity and damage in amyotrophic lateral sclerosis

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Abstract
The pathophysiology of amyotrophic lateral sclerosis (ALS) is particularly challenging due to the heterogeneity of its clinical presentation and the diversity of cellular, molecular and genetic peculiarities involved. Molecular insights unveiled several novel genetic factors to be inherent in both familial and sporadic disease entities, whose characterizations in terms of phenotype prediction, pathophysiological impact and putative prognostic value are a topic of current researches. However, apart from genetically well-defined high-confidence and other susceptibility loci, the role of DNA damage and repair strategies of the genome as a whole, either elicited as a direct consequence of the underlying genetic mutation or seen as an autonomous parameter, in the initiation and progression of ALS, and the different cues involved in either process are still incompletely understood. This mini review summarizes current knowledge on DNA alterations and counteracting DNA repair strategies in ALS pathology and discusses the putative role of unconventional DNA entities including transposable elements and extrachromosomal circular DNA in the disease process. Focus is set on *SOD1*-related pathophysiology, with extension to *FUS*, *TDP*-43 and *C9ORF72* mutations. Advancing our knowledge in the field will contribute to an improved understanding of this relentless disease, for which therapeutic options others than symptomatic approaches are almost unavailable.

Key Words: amyotrophic lateral sclerosis; DNA damage and repair; extrachromosomal circular DNA; microDNA; nuclear pore complex; SOD1 mutations; TDP-43 pathology; transposable elements

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's or motor neuron disease, is a heterogeneous relentless disorder characterized by a progressive loss of upper motor neurons (MNs) of the cerebral motor cortex and lower MNs located to the caudal brain stem nuclei and the ventral segmental horns of the spinal cord, each of which degenerate alongside with their specific axonal projections. According to these neuro-anatomic predilections, the clinical picture manifests with spastic paresis (upper MNs), dysarthria and dysphagia (cranial nerve nuclei and supranuclear lesion) and atrophic paresis affecting respiration and voluntary motor limb actions (lower MNs). Apart from neuro-muscular dysfunction and degeneration, non-motor deficits including cognitive and emotional alterations are found. Current treatment options only marginally modify the spontaneous disease course and are broadly restricted to symptomatic approaches. According to the lack of curable treatment strategies irrespective of the underlying sub-phenotype, survival period is regularly limited to 3–5 years after disease onset. European population-based incidence rates vary between 2–3 cases per 100,000 and correlate log-linearly with the age of onset, which peaks between 50 and 75 years (Al-Chalabi et al., 2014; van Es et al., 2017). Although the disease is classified into familial (fALS) and sporadic (sALS) forms, these categories share genetic similarities. To date, a multitude of ALS-associated genetic factors have been identified, with the impact being best characterized for

C9ORF72, *TARDBP* (TDP-43), *SOD1* and *FUS* genes, which make up to 70% of all fALS cases. These genes are expanded by the description of numerous susceptibility loci such as *ANG*, *SETX* and *ATXN2*, which may co-exist as oligogenic traits (Hardiman et al., 2017). Apart from the difficult assessment of genotype-phenotype correlations and penetrance rates, environmental multipliers interacting with the overall genetic stability appear underscored in their contribution to the ALS heterogeneity. Molecularly, the prominent consequences linked to these diverse predisposition factors comprise, *e.g.*, the loss of protein homoeostasis, disturbed RNA metabolism, altered endosomal trafficking, induction of oxidative stress and mitochondrial malfunctions (Gao et al., 2017), which represent only a few out of manifold pathways involved. Though linked to genomic instability in several studies (Pelegrini et al., 2010; Wang et al., 2013; Sama et al., 2014), the impact of certain mutations in these susceptibility genes on the integrity of the genomic DNA as a whole, and the induction and efficiency of counteracting repair strategies is still not sufficiently explored. The importance of defective DNA restoration in age-related ALS pathology is emphasized by several lines of evidence supporting that the loss of DNA integrity is a molecular driver and age-of-onset modifier of neurodegeneration and neurosenescence (Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium, 2015).

In this mini review, we compile present knowledge on DNA alterations in ALS elicited either by disease-associated

genetic alterations, or unrelated to such heritage factors. Alongside with DNA repair pathways, we will discuss the putative role of unconventional DNA species such as transposable elements and extrachromosomal circular DNA in the context of ALS pathophysiology.

General Features of DNA Damage and Repair in ALS

Role of base excision repair (BER), nucleotide excision repair (NER) and non-homologous end joining (NHEJ) in neuronal DNA repair

A parameter crucially involved in ALS and in neurodegeneration itself is DNA damage (Coppedè and Migliore, 2015). Neurons are particularly vulnerable to DNA damage due to their high metabolic turnover and a transcription-associated continuously open chromatin state paired with the inability to perform replication-coupled DNA repair such as homologous recombination (HR). To overcome this problem, neurons have evolved a plethora of DNA repair strategies, with partially overlapping mechanisms (Madabhushi et al., 2014; Hegde et al., 2017). In the BER path, a DNA polymerase replaces modified DNA bases that have been subjected to deamination, alkylation or oxidation (Coppedè, 2011) after excision by a DNA glycosylase (Hegde et al., 2012; Madabhushi et al., 2014). The NER also follows such a pattern but rather recognizes structural alterations of the DNA helix evoked by, e.g., DNA crosslinks, ultraviolet (UV) photoproducts or other DNA adducts (Coppedè, 2011; Madabhushi et al., 2014). As another kind of DNA damage DNA single-strand breaks (SSBs) are generated as intermediates during the BER process but can also be evoked directly, e.g., by reactive oxygen species (ROS). The SSB repair shares commonalities with BER though additionally facilitates strategies for the preparation of a precise ligation of the loose DNA ends (Madabhushi et al., 2014). The most severe kind of DNA damage are DNA double-strand breaks (DSBs) that are generated by genotoxic agents or by SSBs in close contiguity and unrepaired SSBs that are converted to DSBs by the transcription or replication machineries. If unrepaired, DSBs can lead to chromosome rearrangements and cell death. The major DSB repair mechanism in postmitotic neurons that directly ligates the broken DNA ends is the error-prone NHEJ, since the error-free HR is restricted to replicative activity (Madabhushi et al., 2014; Rulten and Grundy, 2017).

SOD1 malfunction and DNA damage

Particularly oxidative DNA damage has been extensively studied and was found to contribute to the pathogenesis of fALS and sALS cases (Coppedè and Migliore, 2015). The oxidized DNA nucleoside 8-hydroxy-2′-deoxyguanosine (8-OHdG) is used as the most prominent marker for oxidative DNA damage caused by, e.g., augmented ROS levels and is elevated in the blood, cerebrospinal fluid (CSF), urine and neurons of ALS patients (Ferrante et al., 1997; Murata et al., 2008; Coppedè and Migliore, 2015). Mutations in the *SOD1*

gene, which encodes for a free radical scavenging enzyme, make up to 20% of fALS cases and are a causative source of such oxidative stress and related DNA oxidations (Aguirre et al., 2005). In contrast, the importance of SOD1 enzyme malfunctions in severe DNA damage formation, *i.e.*, DNA SSBs and DSBs has been controversially debated (Sau et al., 2007; Barbosa et al., 2010; Carroll et al., 2015). Recent work suggests that at least the *SOD1-p.G93A* mutation has no profound impact on DNA strand integrity (Penndorf et al., 2017). Thus, in accordance with other findings (Carroll et al., 2015), a mutated SOD1 gene alone seems not sufficient to elicit pathological levels of SSBs and DSBs but requires additional stressors. This conclusion is in line with the notion that multiple and overlapping pathways are involved in the neuronal DNA damage response (DDR) (Hegde et al., 2017), whose summative action might effectively prevent severe DNA damage.

Other frequent ALS-associated mutations and DNA integrity

Interestingly, plenty others of the ALS-associated loci identified as of yet are involved in DNA damage and DNA repair pathways, including *FUS*, *NEK1*, *TARDBP* and *C9ORF72*-associated hexanucleotide repeat expansions (Pelegrini et al., 2010; Wang et al., 2013; Hill et al., 2016; Farg et al., 2017). Towards a comprehensive view, the DDR system in ALS appears to lose adaptive capacity in dependency of the underlying genetic mutation (Coppedè, 2011). Apart from *SOD1*-dependent base oxidation, SSBs and DSBs associated with other ALS-related mutations, *e.g.*, of the *C9ORF72* gene gain mounting importance for disease pathogenesis. Accordingly, in ALS patients with an expanded *C9ORF72* locus lumbar MNs showed increased DSB and DDR markers including γH2AX, 53BP1, ATM and cleaved PARP-1 (Farg et al., 2017). Moreover, genomic instability reflected by the accumulation of R-loops and DSBs alongside with defective ATM-related DNA repair are found in direct association with the *C9ORF72* mutation (Walker et al., 2017). Furthermore, para-nucleolar *C9ORF72*-derived dipeptide repeat (DPR) inclusions were shown to co-localize with the histone methylation marker H3K9me2 in the frontal cortex of patients suffering from a clinical ALS phenotype thus suggesting an impact on transcriptional suppression by epigenetic modulation (Schludi et al., 2015), which is known to participate in genomic integrity. Notably, the epigenetic regulator SIRT1 is involved in the maintenance of the genomic integrity specifically of neurons by its interaction with and activation of ATM and HDAC1 that are involved in the repair of DSB by NHEJ (Dobbin et al., 2013).

Additionally, FUS shares a well-established role in DSB restitution by operating on HR- and NHEJ-related repair strategies. In postmitotic neurons, which primarily use NHEJ for DSBs repair, *FUS* inactivation caused a drastic, approximately 65–80% decline in repair efficiency (Wang et al., 2013). Accordingly, ALS patients carrying a *FUS* mutation exhibited an increase in the DSB marker γH2AX in cortical

MNs (Wang et al., 2013). Similarly, MNs derived from induced human pluripotent stem cells harboring endogenous *FUS* mutations showed signs of DNA damage (Higelin et al., 2016). *FUS* was further speculated to regulate chromosomal stability on the telomere level, a factor critically linked to organ aging and pathology (Takahama et al., 2013). The findings that *FUS* was localized to sites of laser-evoked oxidative DNA damage in a PARP-1-dependent manner (Rulten et al., 2014) and its interaction together with the chromatin-modifying enzyme HDAC1 at the DNA damage loci (Wang et al., 2013) provide first mechanistic approaches to explain these observations. Taken together, alterations of *FUS* seem to trigger genomic instabilities and impairments in the DDR *via* mechanisms that still have to be explored.

Cytoplasmic TDP-43 inclusions are one pathological hallmark of ALS present in almost all sALS and fALS entities, with the exception of *FUS*- and *SOD1*-dependent cases (Mackenzie et al., 2010). These cytoplasmic aggregations, prone to be accompanied by a nuclear loss of TDP-43, contribute to transcriptional malfunctions, proteasome impairments and the generation of oxidative stress (van Es et al., 2017). Apart from a physiological role in RNA processing, *i.e.*, in splicing, transport and translation, TDP-43 has been shown to co-localize, together with *FUS*, with RNA polymerase II and BRCA1 at sites of DNA damage and is thus assumed to participate in the repair or prevention of transcription-evoked DNA damage (Hill et al., 2016). Hence, such data provide a direct link between TDP-43 function and DNA repair.

Transposable Element Activity in ALS

Although already discovered more than 60 years ago, the first report on transposable elements (TEs) in the brain was published in 2005, thus opening up a new view on neuronal genome regulations and their possible impact on the understanding of neurodegenerative diseases (Muotri et al., 2005; Reilly et al., 2013). Meanwhile, TE activity has been brought into context with several neurodegenerative diseases including ALS (Jeong et al., 2010; Coufal et al., 2011; Douville et al., 2011) as well as with the destabilization of DNA integrity in general (Gasior et al., 2006).

TE entities in the nervous system

Molecularly, TEs are repetitive DNA sequences that are mobile within the genome and thus can integrate into other loci, an event which can cause gene interruptions. Recent data have indicated that not only germline cells are affected but potentially any gene in somatic cells proximal to a TE insertion. Hence, unsurprisingly, TEs are thought to comprise up to 50% of the human genome (Lapp and Hunter, 2016). Two main TE families can be differentiated by their structure and mechanism of genome insertion. Whereas DNA transposons can directly migrate within the genome *via* a 'cut-and-paste' process, retrotransposons need an RNA-protein intermediate for their transposition according to the 'copy-and-paste' principle. DNA transposons are

considered to be inactive in the mammalian genome and therefore are not discussed hereafter. Retrotransposons are categorized into TEs with long terminal repeats (LTRs) similar to endogenous retroviruses (ERVs), which are remainders from precedent germline viral infections, and non-LTRs with long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) comprising the largest entities (Reilly et al., 2013) (**Figure 1**). LINEs are the only known autonomously mobile TEs in the mammalian genome and thus exhibit the highest frequency of approximately 17% in humans (Lapp and Hunter, 2016). In addition to the well-described TE insertion within the germline, somatic TE integration appears to be specifically cell type-dependent. Several studies clearly demonstrate the somatic retrotransposition of TEs in the brain, with neurons displaying the highest level of transposition of all somatic cells. Insertion rates range from less than 0.6 up to 800 integrations per neuron in the cortex and hippocampus, respectively (Erwin et al., 2014). The reasons for this high TE frequency in neurons are currently unresolved but may, at least partly, be linked to the large size, the open chromatin state of neuron-specific genes and their high transcriptional readout, features also prevailing in ALS-susceptible MNs. In support of these characteristics, TEs are a major source of transcription factor binding sites and promoter regions (Lapp and Hunter, 2016). Such a genomic mosaicism may, in addition to maturation, contribute to adaptive strength and susceptibility thresholds of individual neuronal populations towards stress factors, as it is the case for MNs in the ALS environment.

ALS mutations and TE control

So far, there are several leads alluding to a contribution of TE activity in ALS. One link is the prevailing TDP-43 pathology found in many ALS entities, which may impair TE silencing due to the loss of physiological TDP-43 function to bind TE-derived RNA (Li et al., 2012; Erwin et al., 2014). TE suppression is pivotally linked to the maintenance of DNA integrity, as uncontrolled TE integration can cause deletions in the host DNA or induce DSBs apart from or along with DNA repair processes (Gasior et al., 2006).

The presence of a conserved genomic LTR element, the human endogenous retrovirus HERV-K, including transcripts of its polymerase (*pol*), were found enriched particularly in the motor cortex and spinal cord of ALS patients (Douville et al., 2011; Li et al., 2015) and correlated with increased TDP-43 mRNA and protein presence (Douville et al., 2011). In support of this, HERV-K overexpressing mice exhibited a phenotype comparable to ALS in terms of MN dysfunction and degeneration including increased DNA damage levels (Li et al., 2015). Whereas physiological levels of TDP-43 appear to silence TE-derived RNA (Li et al., 2012), overexpressed TDP-43 was shown to bind the LTR DNA sequence of HERV-K thus leading to its transactivation (Li et al., 2015). Additionally, toxic overexpression of human TDP-43 in *Drosophila* results in the de-repression of

different TE entities, which is accompanied by DNA damage-evoked cell death resembling the degenerative phenotype of ALS (Krug et al., 2017).

Only little is known about the contribution of other ALS-related mutations to a cytotoxic TE deregulation. There are first data indicating that a mutated *SOD1* gene might not be involved in an aberrant TE activation at least in a murine environment (Penndorf et al., 2017). This finding is supported by the absence of a TDP-43 pathology, with an intact TDP-43 seemingly being capable to overtake DNA stabilizing functions (Penndorf et al., 2017). Most recently, increased transcript levels of different TE classes including LINEs and ERVs were found in the frontal cortex of ALS patients with a *C9ORF72* mutation when compared to sALS cases and healthy controls, which was associated with an elevated RNA polymerase II activity (Prudencio et al., 2017). Moreover, as mentioned above, DPRs translated from *C9ORF72* microsatellites are assumed to function as transcriptional modifiers in association with H3K9me2 and thus might be involved in the deregulation of epigenetic TE silencing (Schludi et al., 2015; Prudencio et al., 2017). Of note, epigenetic mechanisms like DNA and histone methylations are among the main processes of TE silencing (Lapp and Hunter, 2016).

Although there are estimates of TE integrations for cortical and hippocampal neurons, data on MNs and a detailed mapping of insertions for individual brain and spinal cord regions are not yet available but are desirable to further understand the contribution of mobile elements to nervous system pathologies and aging.

Environmental factors and TE activation

TE activity is also influenced by lifestyle habits and daily stressors, which might function as sensors of environmental stress and thus have an impact on the locus and frequency of TE copy integration (Erwin et al., 2014). Likewise, several epidemiological studies link the exposure to prevalent pollutants with changes in the epigenetic configuration and activation state of TEs, with the strongest evidence appearing for the L1 and *Alu* elements (Miousse et al., 2015). Related factors comprise atmospheric pollution, organic compounds resistant to environmental degradation, heavy metal contaminations, exposure to UV-radiation and others (Miousse et al., 2015). Moreover, as a well-established driver of genetic and epigenetic changes tobacco smoke exposure has been shown to counteract DNA methylation in L1 elements in several tissues (Miousse et al., 2015), and application of cigarette smoke extract to lung fibroblasts induced L1-ORF2 expression, which was considered as a marker for TE activation (Miglino et al., 2014). In general, putatively de-repressive hypomethylation of TE species appears as the most frequent epigenetic consequence elicited by the exposure to such toxicants (Miousse et al., 2015). Further comprehensive analyses are required to systematically assess the impact of pollutants, life habits and other environmental factors on the epigenetic control of TEs and their potential to induce retrotransposition events, and to infer conclusions for health

and diseases including neurodegenerative ALS and other pathologies.

The Nuclear Pore Complex in DNA Stability and Repair

Nuclear DNA compartmentalization for alternative DSB repair

Mounting evidence suggests that DNA damage and repair processes are structurally and functionally related to the nuclear envelope. DNA segments which harbor conventionally irreparable DSBs including those of subtelomeric regions, and also eroded telomeres are recruited to the inner nuclear margin and sequestered at the nuclear envelope (Therizols et al., 2006; Nagai et al., 2008; Khadaroo et al., 2009). Particularly involved in this DNA relocation and tethering steps are constituents of the nuclear pore complexes (NPC) - transport channels assembled of several copies of approximately thirty nucleoporins (Nups) that span the inner and outer nuclear membrane - as evidenced for the scaffold Nup84-associated subcomplex and the nuclear basket protein Nup60 in yeast (Therizols et al., 2006; Palancade et al., 2007; Nagai et al., 2008). Interestingly, the metazoan orthologues *Nup107* and *Nup153*, respectively, are among those genes identified to be modifiers of *C9orf72*-related neurotoxicity recently characterized in *Drosophila melanogaster* (Freibaum et al., 2015). Such relocation of irreparable DNA damage sites to the nuclear membrane appears as an alternative strategy crucial for the rescue of DNA integrity at instances where HR and NHEJ are ineffective. The molecular cues mediating recognition and recruitment of broken DNA strands to a subnuclear localization are a topic of intense research and were discovered to implement, at least in yeast, the *Nup84* interacting SUMO-targeted ubiquitin ligase complex Slx5/8 and Rad52 protein (Palancade et al., 2007; Khadaroo et al., 2009; Su et al., 2015). Strongest evidence for unorthodox NPC-related DNA repair choices originate currently from yeast, whereas DSB mobility in mammals is still a question of debate (Jakob et al., 2011; Lemaître and Soutoglou, 2015). However, since NPC structure and function are evolutionarily conserved from yeast to mammalians, it is tempting to speculate that such repair strategies will indeed play a role in DNA restitution of compulsively non-replicative cell entities like G_0-arrested mature neurons of higher organisms, given that NHEJ is incapable to accomplish the repair process. In support of this notion, the choice of DSB repair mechanisms in mammals comply a domain-specific organization (Lemaître and Soutoglou, 2015) and scale-limited DSB relocation was recently described in mouse embryonic fibroblasts following break induction by linear ion tracks (Jakob et al., 2011). Therefore, further studies would merit reveal the relevance and efficiency of alternative repair tools in a mammalian neuronal context, where the lack of HR restricts versatility of genome restitution mechanisms.

Subcellular molecule compartmentalization

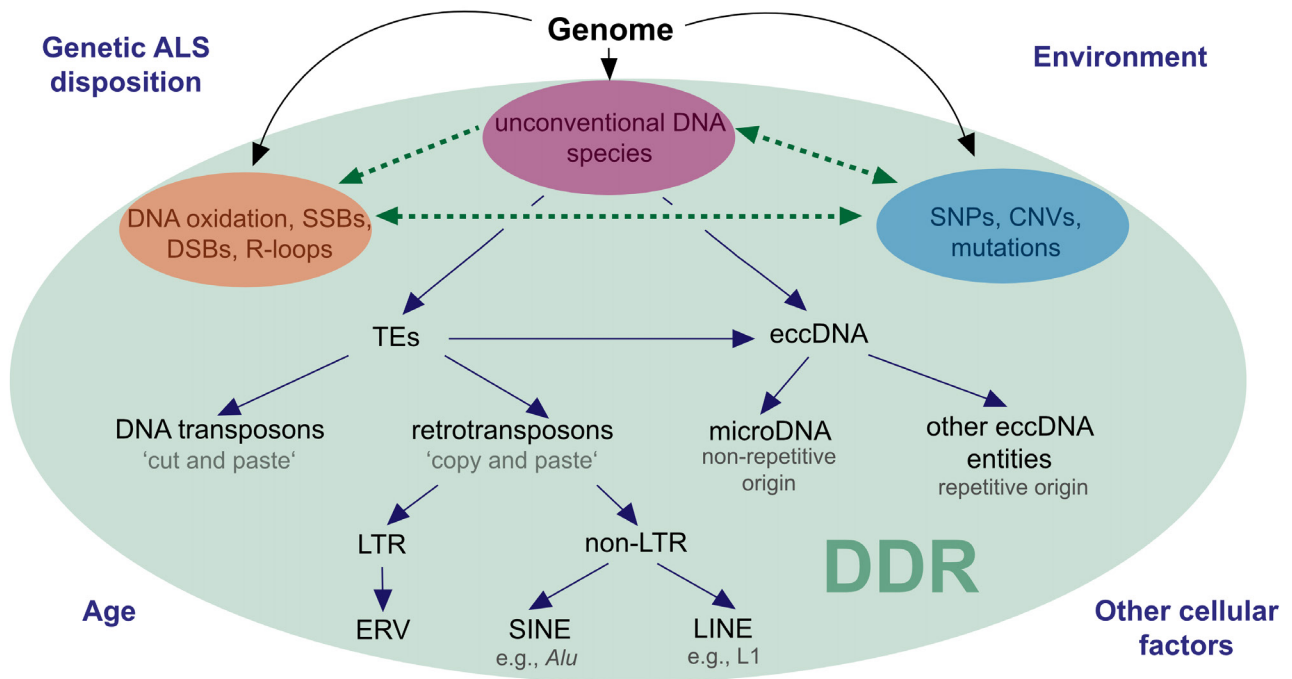Apart from such a putative side function in mammals, the

Figure 1 DNA species involved in genomic plasticity and damage.
Apart from DNA oxidation, strand breaks, mutations and repetitive DNA expansions, less characterized DNA entities have now been discovered to interfere with genomic plasticity and damage. Among them, mobile transposable elements (TEs) can integrate randomly into genomic loci *via* different mechanisms. In the mammalian brain, members of the L1 class of large interspersed nuclear element (LINE) retrotransposons in particular show a widespread somatic insertion, which may influence neurodegeneration under predisposing conditions, *e.g.*, ALS-associated genetic, environmental and cellular factors as well as age. Additionally, extrachromosomal circular DNA (eccDNA) originating from unique and repetitive sequences by distinct processes comprising DNA repair and transcription has been characterized in the context of aging and pathology including the neural environment. All of these DNA entities mutually interact and can induce the complex DNA damage response (DDR). ALS: Amyotrophic lateral sclerosis; CNVs: copy number variations; ERV: endogenous retrovirus; LTR: long terminal repeats; SINE: short interspersed nuclear elements; SNPs: single nucleotide polymorphisms.

genuine conserved task of the NPC resides in nucleo-cytoplasmic shuttling of RNA and proteins with higher molecular weight. Disturbed NPC-associated molecule transport and subcellular partitioning has been coupled to *C9orf72*-related pathology in several studies (Freibaum et al., 2015; Jovičić et al., 2015; Zhang et al., 2015). Likewise, in a fly model of *C9orf72*-associated neurodegeneration the modulated expression of numerous NPC components and Nup interacting transport effectors such as RanGTPases and karyopherin family members strongly modified the arising pathological phenotype (Freibaum et al., 2015). An obviously comprehensive explanation for the neurotoxic consequences observed in association with *C9orf72*-related NPC dysfunction arises from a recent study. Shi and colleagues discovered that the most toxic arginine-containing poly-dipeptides generated by non-AUG translation from *C9orf72* microsatellite-derived repeat RNAs are prone to plug the central channel of the NPC, through interaction with low-complexity FG Nups, and to interplay with ALS-associated *FUS* and TDP-43 proteins (Shi et al., 2017). Whether DPR adjunction to NPC components is capable to impair the aforementioned non-classical DNA damage repair strategies is unexplored and deserves further investigations.

Thus, apart from a crucial role in molecule compartmen-

talization, subtle intranuclear reorganization of damaged DNA through association with NPC components may contribute to modulate neurotoxicity in a genetically mutated ALS environment. Noteworthy, fragile trinucleotide repeats, of which the DSB frequency increases with length (Freudenreich et al., 1998), are more prone to associate with the nuclear periphery than unexpanded repeats and are found destabilized in yeast *nup84* mutants (Su et al., 2015). Therefore, stability of repetitive genomic sequences, a criterion essential for the penetrance and clinical course of many neurodegenerative disorders such as Huntington's disease, spinocerebellar ataxia, spinobulbar muscle atrophy and, as discussed, possibly also *C9ORF72*-associated ALS (Van Mossevelde et al., 2017), might require interaction with the nuclear envelope to prevent repeat length alterations also on the somatic level (Su et al., 2015).

In summary, it appears tempting to speculate that cytotoxicity of G4C2 hexanucleotide repeats originating from the first intron or the promoter region of the *C9orf72* gene might also depend on NPC-associated repair processes and be dually linked to NPC dysfunction - *via* potentiated genomic instability in terms of DSBs and microsatellite expansions, and the induction of loss-of-function and gain-of-function effects by defective compartmentalization of RNA

and proteins. Future insights will reveal whether sub-nuclear DNA redistribution is indeed relevant for the penetration of ALS characteristic symptoms in mice and humans and define the role of proper subcellular molecule compartmentalization in other ALS entities.

Decay in nuclear envelope barrier function in terms of NPC selectivity and specificity may also facilitate nuclear entrance, accumulation and genomic integration of retrotransposable elements. Likewise, for retrotransposition the LINE entity L1 requires a complex formation of RNA and proteins and its re-translocation into the nucleus (Lapp and Hunter, 2016). Whether the high rate of retrotransposition found specifically in neurons is a result of nuclear barrier dysfunction of aged or diseased neurons (D'Angelo et al., 2009) awaits further investigations.

Extrachromosomal Circular DNA as Source of Unconventional DNA in ALS
Short and long eccDNA
Apart from conventional genetic alterations as delineated above, a recently discovered DNA entity is now discussed in the amplification of genetic mosaicism across somatic cells along with DNA destabilization. Discovered to be universally expressed in eukaryotes, so-called extrachromosomal circular DNA (eccDNA) consists of single- or double-stranded loops of chromosomal repeats. Structurally, two classes of eccDNA are currently differentiated, separating short microDNA sequences (< 400 bp), which originate from non-repetitive exon-enriched genomic regions, and long segments (hundreds to millions bp), which also include repetitive chromosomal matrices, derivatives from viral elements and endogenous mobile TEs (Cohen and Segal, 2009; Dillon et al., 2015). Generally, eccDNA is thought to derive from coding as well as non-coding genomic regions. Both, microDNA and other eccDNA entities occur apart from germ line heritage and affect all tissues including skeletal muscle and brain, with a low correlation between replicative activity and abundance (Dillon et al., 2015). Longer eccDNA is known to arise from gene replication strategies including intra-strand HR and genomic repair mechanisms such as NHEJ. As a consequence of DNA loop excision and DNA circle formation, chromosomal microdeletions are generated. The corresponding deletions directly map to susceptible, tandemly organized genomic loci (Cohen and Segal, 2009). In contrast, microDNA can form independently of HR and NHEJ repair processes but originate from a replication-coupled 'copy-and-cut' strategy, excising excess DNA without leaving deletions in the genome. Furthermore, microDNA is generated by mismatch repair strategies in association with mRNA processing and replication-independent DNA transcription (Dillon et al., 2015).

MicroDNA and ALS
Structurally, such microDNA species share similarities with R-loops, which are composed of triple-stranded RNA:DNA hybrids that destabilize genomic structure along with transcription. Thus, the existing high transcriptional activity and mRNA processing in MNs might propagate microDNA formation and explain the ambivalence in DNA strand break detection, at least in a *SOD1*-related context (Penndorf et al., 2017). Indeed, the hexanucleotide repeat expansions derived from the mutated ALS-associated *C9ORF72* locus are discovered to be a source of DSBs and R-loops (Walker et al., 2017) and, as such, are conceivably an origin of microDNA that putatively also contributes to mutation-related cytotoxicity. Therefore, since the brain acts as a network of partly replicative and non-replicative cell populations, several of these mechanisms may be relevant for the genetic architecture and function of the brain and spinal cord. The sequence patterns that are prone to give rise to eccDNA formations, such as TE insertion sites and their impact on ALS pathology, remain to be characterized.

Apart from single nucleotide polymorphisms and copy number variations, transcription from eccDNA appears as a feasible mechanism to achieve genomic rearrangement and genetic over-representation by sequelae of selective gene excision and amplification autonomously from the conventional chromosome set. The resulting higher degree of genetic mosaicism putatively contributes to the genetic plasticity required for the establishment of cell type-specific functions and thus infers distinct vulnerability to different cell entities towards age- or disease-related degeneration. In this context, the open chromatin state and the high metabolic turnover rate of many neurons, including MNs, may emerge as a predisposing factor for neurodegeneration. Moreover, such eccDNA might influence or 'infect' distant cells by mediating inter-cellular long-distance communication *via* still unknown mechanisms (Kumar et al., 2017). Thus, understanding eccDNA bears the potential to reveal novel ways to explain the spread kinetics discussed for many neurodegenerative pathologies, including those being characteristic of ALS. The high variability in the cellular load of eccDNA loops should be taken into consideration to explain the enormous inter-individual variations in the clinical course between patients with identical underlying genetic mutations. On the other hand, amplification of unconventional DNA species may also represent a commonality explaining why many neurodegenerative disorders that evolve from separate hereditary factors overlap in their pathobiological characteristics.

In general, further characterization of longer eccDNA entities, microDNA, mobile TEs and R-loops as well as strategies of sub-nuclear DNA reorganization will improve our understanding of how non-inherited DNA variability can impact health and disease. Since disturbances in DNA and RNA metabolism comprise a fundamental pathogenic feature, explorations from such processes particularly in ALS may convey novel knowledge to many neurodegenerative pathologies.

Biomarkers and Therapeutic Approaches
Due to the relentless and incurable character of ALS, these new molecular achievements merit consideration in their

suitability as novel diagnostic and therapeutic options. Likewise, the detection of eccDNA in peripheral blood or CSF samples will elucidate a correlation between disease onset and progression and its validity as a biomarker. With regard to TE suppression of HERV-K specifically, a current phase I clinical trial (NCT02437110) will unravel the efficacy of several antiretroviral drugs in volunteers with ALS. Additionally, clinical trials on XPO-1 inhibitors, which are assumed to mediate neuroprotective and anti-oxidative functions along with their interference in subcellular transport properties, might also provide an innovative approach for ALS therapy. The development of a pharmacological agent, which resolves the NPC plugging through toxic DPRs appears particularly promising for *C9ORF72*-related ALS treatment. A better characterization of these factors will improve our understanding of how unconventional DNA alterations may influence ALS pathology and thereby open novel therapeutic avenues.

References

Aguirre N, Beal MF, Matson WR, Bogdanov MB (2005) Increased oxidative damage to DNA in an animal model of amyotrophic lateral sclerosis. Free Radic Res 39:383-388.

Al-Chalabi A, Calvo A, Chio A, Colville S, Ellis CM, Hardiman O, Heverin M, Howard RS, Huisman MHB, Keren N, Leigh PN, Mazzini L, Mora G, Orrell RW, Rooney J, Scott KM, Scotton WJ, Seelen M, Shaw CE, Sidle KS, et al. (2014) Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol 13:1108-1113.

Barbosa LF, Cerqueira FM, Macedo AF, Garcia CC, Angeli JP, Schumacher RI, Sogayar MC, Augusto O, Carrì MT, Di Mascio P, Medeiros MH (2010) Increased SOD1 association with chromatin, DNA damage, p53 activation, and apoptosis in a cellular model of SOD1-linked ALS. Biochim Biophys Acta 1802:462-471.

Carroll J, Page TK, Chiang SC, Kalmar B, Bode D, Greensmith L, McKinnon PJ, Thorpe JR, Hafezparast M, El-Khamisy SF (2015) Expression of a pathogenic mutation of SOD1 sensitizes aprataxin-deficient cells and mice to oxidative stress and triggers hallmarks of premature ageing. Hum Mol Genet 24:828-840.

Cohen S, Segal D (2009) Extrachromosomal circular DNA in eukaryotes: possible involvement in the plasticity of tandem repeats. Cytogenet Genome Res 124:327-338.

Coppedè F (2011) An overview of DNA repair in amyotrophic lateral sclerosis. ScientificWorldJournal 11:1679-1691.

Coppedè F, Migliore L (2015) DNA damage in neurodegenerative diseases. Mutat Res 776:84-97.

Coufal NG, Garcia-Perez JL, Peng GE, Marchetto MC, Muotri AR, Mu Y, Carson CT, Macia A, Moran JV, Gage FH (2011) Ataxia telangiectasia mutated (ATM) modulates long interspersed element-1 (L1) retrotransposition in human neural stem cells. Proc Natl Acad Sci U S A 108:20382-20387.

D'Angelo MA, Raices M, Panowski SH, Hetzer MW (2009) Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. Cell 136:284-295.

Dillon LW, Kumar P, Shibata Y, Wang YH, Willcox S, Griffith JD, Pommier Y, Takeda S, Dutta A (2015) Production of extrachromosomal microDNAs is linked to mismatch repair pathways and transcriptional activity. Cell Rep 11:1749-1759.

Dobbin MM, Madabhushi R, Pan L, Chen Y, Kim D, Gao J, Ahanonu B, Pao PC, Qiu Y, Zhao Y, Tsai LH (2013) SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. Nat Neurosci 16:1008-1015.

Douville R, Liu J, Rothstein J, Nath A (2011) Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Ann Neurol 69:141-151.

Erwin JA, Marchetto MC, Gage FH (2014) Mobile DNA elements in the generation of diversity and complexity in the brain. Nat Rev Neurosci 15:497-506.

Farg MA, Konopka A, Soo KY, Ito D, Atkin JD (2017) The DNA damage response (DDR) is induced by the C9orf72 repeat expansion in amyotrophic lateral sclerosis. Hum Mol Genet 26:2882-2896.

Ferrante RJ, Browne SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U, Kowall NW, Brown RH, Jr., Beal MF (1997) Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J Neurochem 69:2064-2074.

Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH, Badders N, Valentine M, Miller BL, Wong PC, Petrucelli L, Kim HJ, Gao FB, Taylor JP (2015) GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. Nature 525:129-133.

Freudenreich CH, Kantrow SM, Zakian VA (1998) Expansion and length-dependent fragility of CTG repeats in yeast. Science 279:853-856.

Gao FB, Almeida S, Lopez-Gonzalez R (2017) Dysregulated molecular pathways in amyotrophic lateral sclerosis-frontotemporal dementia spectrum disorder. EMBO J 36:2931-2950.

Gasior SL, Wakeman TP, Xu B, Deininger PL (2006) The human LINE-1 retrotransposon creates DNA double-strand breaks. J Mol Biol 357:1383-1393.

Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium (2015) Identification of genetic factors that modify clinical onset of Huntington's disease. Cell 162:516-526.

Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, Shaw PJ, Simmons Z, van den Berg LH (2017) Amyotrophic lateral sclerosis. Nat Rev Dis Primers 3:17071.

Hegde ML, Bohr VA, Mitra S (2017) DNA damage responses in central nervous system and age-associated neurodegeneration. Mech Ageing Dev 161:1-3.

Hegde ML, Mantha AK, Hazra TK, Bhakat KK, Mitra S, Szczesny B (2012) Oxidative genome damage and its repair: implications in aging and neurodegenerative diseases. Mech Ageing Dev 133:157-168.

Higelin J, Demestre M, Putz S, Delling JP, Jacob C, Lutz AK, Bausinger J, Huber AK, Klingenstein M, Barbi G, Speit G, Huebers A, Weishaupt JH, Hermann A, Liebau S, Ludolph AC, Boeckers TM (2016) FUS mislocalization and vulnerability to DNA damage in ALS patients derived hiPSCs and aging motoneurons. Front Cell Neurosci 10:290.

Hill SJ, Mordes DA, Cameron LA, Neuberg DS, Landini S, Eggan K, Livingston DM (2016) Two familial ALS proteins function in prevention/repair of transcription-associated DNA damage. Proc Natl Acad Sci U S A 113:E7701-E7709.

Jakob B, Splinter J, Conrad S, Voss KO, Zink D, Durante M, Lobrich M, Taucher-Scholz G (2011) DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. Nucleic Acids Res 39:6489-6499.

Jeong BH, Lee YJ, Carp RI, Kim YS (2010) The prevalence of human endogenous retroviruses in cerebrospinal fluids from patients with sporadic Creutzfeldt-Jakob disease. J Clin Virol 47:136-142.

Jovičić A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, Paul JW 3rd, Sun S, Herdy JR, Bieri G, Kramer NJ, Gage FH, Van Den Bosch L, Robberecht W, Gitler AD (2015) Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. Nat Neurosci 18:1226-1229.

Khadaroo B, Teixeira MT, Luciano P, Eckert-Boulet N, Germann SM, Simon MN, Gallina I, Abdallah P, Gilson E, Géli V, Lisby M (2009) The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat Cell Biol 11:980-987.

Krug L, Chatterjee N, Borges-Monroy R, Hearn S, Liao WW, Morrill K, Prazak L, Rozhkov N, Theodorou D, Hammell M, Dubnau J (2017) Retrotransposon activation contributes to neurodegeneration in a Drosophila TDP-43 model of ALS. PLoS Genet 13:e1006635.

Kumar P, Dillon LW, Shibata Y, Jazaeri AA, Jones DR, Dutta A (2017) Normal and cancerous tissues release extrachromosomal circular DNA (eccDNA) into the circulation. Mol Cancer Res 15:1197-1205.

Lapp HE, Hunter RG (2016) The dynamic genome: transposons and environmental adaptation in the nervous system. Epigenomics 8:237-249.

Lemaître C, Soutoglou E (2015) DSB (Im)mobility and DNA repair compartmentalization in mammalian cells. J Mol Biol 427:652-658.

Li W, Jin Y, Prazak L, Hammell M, Dubnau J (2012) Transposable elements in TDP-43-mediated neurodegenerative disorders. PLoS One 7:e44099.

Li W, Lee MH, Henderson L, Tyagi R, Bachani M, Steiner J, Campanac E, Hoffman DA, von Geldern G, Johnson K, Maric D, Morris HD, Lentz M, Pak K, Mammen A, Ostrow L, Rothstein J, Nath A (2015) Human endogenous retrovirus-K contributes to motor neuron disease. Sci Transl Med 7:307ra153.

Mackenzie IR, Rademakers R, Neumann M (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 9:995-1007.

Madabhushi R, Pan L, Tsai LH (2014) DNA damage and its links to neurodegeneration. Neuron 83:266-282.

Miglino N, Tamm M, Borger P (2014) Transposable element LINE1 is activated after exposure to cigarette smoke in primary human lung fibroblasts. Eur Respir J 44:S2023.

Miousse IR, Chalbot MC, Lumen A, Ferguson A, Kavouras IG, Koturbash I (2015) Response of transposable elements to environmental stressors. Mutat Res Rev Mutat Res 765:19-39.

Muotri AR, Chu VT, Marchetto MC, Deng W, Moran JV, Gage FH (2005) Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. Nature 435:903-910.

Murata T, Ohtsuka C, Terayama Y (2008) Increased mitochondrial oxidative damage and oxidative DNA damage contributes to the neurodegenerative process in sporadic amyotrophic lateral sclerosis. Free Radic Res 42:221-225.

Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, Varela E, Hediger F, Gasser SM, Krogan NJ (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322:597-602.

Palancade B, Liu X, Garcia-Rubio M, Aguilera A, Zhao X, Doye V (2007) Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Mol Biol Cell 18:2912-2923.

Pelegrini AL, Moura DJ, Brenner BL, Ledur PF, Maques GP, Henriques JA, Saffi J, Lenz G (2010) Nek1 silencing slows down DNA repair and blocks DNA damage-induced cell cycle arrest. Mutagenesis 25:447-454.

Penndorf D, Tadic V, Witte OW, Grosskreutz J, Kretz A (2017) DNA strand breaks and TDP-43 mislocation are absent in the murine hSOD1G93A model of amyotrophic lateral sclerosis in vivo and in vitro. PLoS One 12:e0183684.

Prudencio M, Gonzales PK, Cook CN, Gendron TF, Daughrity LM, Song Y, Ebbert MTW, van Blitterswijk M, Zhang YJ, Jansen-West K, Baker MC, DeTure M, Rademakers R, Boylan KB, Dickson DW, Petrucelli L, Link CD (2017) Repetitive element transcripts are elevated in the brain of C9orf72 ALS/FTLD patients. Hum Mol Genet 26:3421-3431.

Reilly MT, Faulkner GJ, Dubnau J, Ponomarev I, Gage FH (2013) The role of transposable elements in health and diseases of the central nervous system. J Neurosci 33:17577-17586.

Rulten SL, Grundy GJ (2017) Non-homologous end joining: Common interaction sites and exchange of multiple factors in the DNA repair process. Bioessays doi: 10.1002/bies.201600209.

Rulten SL, Rotheray A, Green RL, Grundy GJ, Moore DA, Gomez-Herreros F, Hafezparast M, Caldecott KW (2014) PARP-1 dependent recruitment of the amyotrophic lateral sclerosis-associated protein FUS/TLS to sites of oxidative DNA damage. Nucleic Acids Res 42:307-314.

Sama RR, Ward CL, Bosco DA (2014) Functions of FUS/TLS from DNA repair to stress response: implications for ALS. ASN Neuro 6:1759091414544472.

Sau D, De Biasi S, Vitellaro-Zuccarello L, Riso P, Guarnieri S, Porrini M, Simeoni S, Crippa V, Onesto E, Palazzolo I, Rusmini P, Bolzoni E, Bendotti C, Poletti A (2007) Mutation of SOD1 in ALS: a gain of a loss of function. Hum Mol Genet 16:1604-1618.

Schludi MH, May S, Grasser FA, Rentzsch K, Kremmer E, Kupper C, Klopstock T, German Consortium for Frontotemporal Lobar Degeneration, Bavarian Brain Banking Alliance, Arzberger T, Edbauer D (2015) Distribution of dipeptide repeat proteins in cellular models and C9orf72 mutation cases suggests link to transcriptional silencing. Acta Neuropathol 130:537-555.

Shi KY, Mori E, Nizami ZF, Lin Y, Kato M, Xiang S, Wu LC, Ding M, Yu Y, Gall JG, McKnight SL (2017) Toxic PRn poly-dipeptides encoded by the C9orf72 repeat expansion block nuclear import and export. Proc Natl Acad Sci U S A 114:E1111-e1117.

Su XA, Dion V, Gasser SM, Freudenreich CH (2015) Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability. Genes Dev 29:1006-1017.

Takahama K, Takada A, Tada S, Shimizu M, Sayama K, Kurokawa R, Oyoshi T (2013) Regulation of telomere length by G-quadruplex telomere DNA- and TERRA-binding protein TLS/FUS. Chem Biol 20:341-350.

Therizols P, Fairhead C, Cabal GG, Genovesio A, Olivo-Marin JC, Dujon B, Fabre E (2006) Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J Cell Biol 172:189-199.

van Es MA, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, van den Berg LH (2017) Amyotrophic lateral sclerosis. Lancet 390:2084-2098.

Van Mossevelde S, van der Zee J, Cruts M, Van Broeckhoven C (2017) Relationship between C9orf72 repeat size and clinical phenotype. Curr Opin Genet Dev 44:117-124.

Walker C, Herranz-Martin S, Karyka E, Liao C, Lewis K, Elsayed W, Lukashchuk V, Chiang SC, Ray S, Mulcahy PJ, Jurga M, Tsagakis I, Iannitti T, Chandran J, Coldicott I, De Vos KJ, Hassan MK, Higginbottom A, Shaw PJ, Hautbergue GM, et al. (2017) C9orf72 expansion disrupts ATM-mediated chromosomal break repair. Nat Neurosci 20:1225-1235.

Wang WY, Pan L, Su SC, Quinn EJ, Sasaki M, Jimenez JC, Mackenzie IR, Huang EJ, Tsai LH (2013) Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. Nat Neurosci 16:1383-1391.

Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, Daley EL, Miller SJ, Cunningham KM, Vidensky S, Gupta S, Thomas MA, Hong I, Chiu SL, Huganir RL, Ostrow LW, Matunis MJ, Wang J, Sattler R, Lloyd TE, et al. (2015) The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature 525:56-61.