

## CONCISE REVIEW

# Epigenetic regulation of neural stem cells: The emerging role of nucleoporins

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## Abstract

Nucleoporins (Nups) are components of the nuclear pore complex that, besides regulating nucleus-cytoplasmic transport, emerged as a hub for chromatin interaction and gene expression modulation. Specifically, Nups act in a dynamic manner both at specific gene level and in the topological organization of chromatin domains. As such, they play a fundamental role during development and determination of stemness/differentiation balance in stem cells. An increasing number of reports indicate the implication of Nups in many central nervous system functions with great impact on neurogenesis, neurophysiology, and neurological disorders. Nevertheless, the role of Nup-mediated epigenetic regulation in embryonic and adult neural stem cells (NSCs) is a field largely unexplored and the comprehension of their mechanisms of action is only beginning to be unveiled. After a brief overview of epigenetic mechanisms, we will present and discuss the emerging role of Nups as new effectors of neuroepigenetics and as dynamic platform for chromatin function with specific reference to the biology of NSCs.

## KEYWORDS

Alzheimer's disease, epigenetics, neural stem cells, neurogenesis, nucleoporins

## Significance statement

Adult neurogenesis supports brain function by providing new neurons for tissue homeostasis and memory processes. Unfortunately, decreased neurogenesis occurs during aging and in neurodegenerative diseases, thus reducing brain repair capacity. Greater understanding of the mechanisms implicated in neural stem cell biology could lead to increasing the regenerative potential of these cells for many therapeutic purposes. The present study reviews the emerging role of nuclear pore proteins as novel key molecules that, by epigenetic mechanisms, control stemness and fate specification of neural progenitors in the developing embryo and in the adult brain.

## 1 | EPIGENETIC MECHANISMS INVOLVED IN ADULT NEUROGENESIS

Neural stem cells (NSCs) are multipotent neural progenitors that generate neurons and glial cells during embryonal development and in the neonatal and adult brain. Adult NSCs have generally less potential

than embryonic NSCs, but they still provide new neurons throughout life, although this process is limited and spatially restricted to specific neurogenic niches (the subgranular zone [SGZ] of the dentate gyrus of the hippocampus and the subventricular zone [SVZ] of the lateral ventricles).<sup>1-3</sup> Recent research gave evidence of other unconventional neurogenic niches in the adult brain, such as the circumventricular

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organs surrounding the third and fourth ventricle.<sup>4</sup> Stem cells resident in these regions showed increased proliferation and differentiation after brain injury indicating a regenerative potential outside the canonical neurogenic niches.<sup>5</sup>

Both physiological and pathological stimuli affect proliferation and fate determination of NSCs that may undergo either symmetric or asymmetric division, respectively, promoting self-renewal/maintenance of stem cell population and their differentiation into neuronal or glial phenotype. Improper activation of symmetric/asymmetric division might lead to premature depletion of the stem cell niche or altered differentiation.

Adult NSCs are important for tissue homeostasis; however, their brain repair capacity is limited and negatively affected by aging and several neurological diseases.<sup>6</sup> Thus, current research is focused on the comprehension of NSC regulatory mechanisms, and specifically the impact of neuroepigenetics<sup>7,8</sup> on their regenerative potential to either promote endogenous neurogenesis or improve exogenous stem cell-based therapies.<sup>9,10</sup> The ability of stem cells to maintain pluripotency as well as the capability to differentiate in a specific cell type requires a finely tuned regulation of certain gene programs that must be expressed in a temporal and spatial restricted manner.<sup>11</sup> This complex regulation is achieved by the interplay of epigenetic mechanisms that are dynamic, reversible and heritable changes of chromatin architecture and gene expression, which do not affect DNA sequences. The epigenetic regulation of chromatin modulates its condensation, the accessibility to transcription factors and interaction with coactivators or corepressors by different mechanisms, which include histone posttranslational modifications, DNA methylation on cytosine, short and long noncoding RNAs, further control chromatin topological changes.

Recently, nucleoporins (Nups) have emerged as novel partners/coordinators of these epigenetic mechanisms. So far, most studies focused on pluripotent embryonic stem cells (ESCs) while the characterization of Nup function to regulate multipotent NSCs is in its infancy. Nevertheless, a growing body of evidence suggests that Nup-dependent epigenetic mechanisms are involved in both embryonic and adult neurogenesis. Here, we will compare Nup-related epigenetic mechanisms occurring in ESCs and NSCs to provide a wider view of this important class of regulators and their impact on stemness and neuronal lineage acquisition.

## 1.1 | Histone modifications

A stretch of 146 bp of DNA is wrapped around nucleosomes composed of two copies of four core histones, H3, H4, H2A, and H2B, forming the basal chromatin filament, which is then subjected to further condensation on the basis of charge-dependent nucleosome-DNA interactions. Several residues within histone proteins undergo posttranslational modifications including acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation by several classes of chromatin remodeling enzymes (eg, histone acetyltransferase [HAT], histone deacetylases [HDAC], histone methyltransferase [HMT]) that reversibly modulate nucleosome charge, thus inducing either a more closed or relaxed chromatin

conformation favoring repression or activation of gene expression, respectively.

In this context, our previous studies demonstrated that changes in H3K9ac levels at the promoter of neurogenesis related genes (Hes-1, NeuroD1, and Neurogenin1) were responsible to modulate both NSC proliferation and adult neurogenesis upon dysregulated metabolic signals or electromagnetic field stimulation.<sup>12-14</sup>

While histone acetylation (eg, H3K9ac, H3K14ac) is associated with actively transcribed genes, histone methylation is associated with either activation (eg, H3K4m) or repression (eg, H3K27m) depending on the aminoacidic residue involved. Histone SUMOylation, due to the covalent attachment of the small ubiquitin-like modifier (SUMO), can have either negative or positive effects on transcription.<sup>15</sup>

Activated as well as repressed genes are often characterized by the presence of a specific set of modifications on the promoter that has been defined as histone code. Many promoters in ESCs show bivalent loci containing both active (mainly H3K4me3) and repressive (mainly H3K27me3, H3K9me3) marks, a condition that allows developmental and lineage-specific genes to be expressed rapidly because poised for activation.<sup>16-18</sup> Along this line, it has been recently reported that also different subtypes of adult NSCs in the SVZ neurogenic niche display bivalent histone marks (H3K27me3, H3K36me3, H3K4me3).<sup>19</sup>

In addition to be posttranslationally modified, histones can be replaced with noncanonical histone variants, such as H2A.z and H3.3. These variants play a role in neurogenesis, learning and memory.<sup>20</sup> In this regard incorporation of H3.3 in actively transcribed genes allows storing of epigenetic signals important for memory mechanisms occurring in a specific subset of neuronal cells.<sup>20</sup>

Experiments with H2A.z KO mice revealed that this histone variant has an important role in embryonic neurogenesis since it regulates, in complex with the histone methyltransferase Setd2, proper development and differentiation of NSCs by promoting the methylation of H3K36 on NKx2-4 promoter and thus its transcription.<sup>21</sup>

In ESCs, the response to differentiation is associated with the level of the histone variant H3.3 that modulates the pattern of H3K27me3 on developmental regulated genes by recruiting the polycomb repressor complex PCR2.<sup>22</sup> During early brain development instead, H3.3 silencing determines the reduction of proliferation of embryonic NSCs, which is paralleled by increased Map2 expression and neuronal differentiation. Mechanistically, the interaction of H3.3 with the acetylase MOF was found to be responsible for the activation of the transcriptional activator GLI1.<sup>23</sup>

## 1.2 | DNA methylation

DNA methylation on cytosine in position 5 in CG dinucleotides (also referred to as 5mC or CpG) by DNA methyltransferases (DNMT family) is another well-known epigenetic mechanism for gene silencing that is crucial for many biological processes including neurogenesis. Acquisition of a specific DNA methylation pattern is likewise important for proliferation and differentiation of NSCs and is regulated by

the interplay of the DNMT enzyme family with several methyl-CpG-binding proteins (MeCP2, MBD1, MBD2, MBD4), corepressors such as HDACs and other regulators implicated in the maintenance of the methylated status, which overall determine the activity of the promoters and the accessibility of transcription factors.<sup>24</sup> For example UHRF1, which preserves the DNA hemimethylation pattern, regulates the proliferation of the active adult NSCs and its loss induces a strong depletion of neurogenesis due to promoter demethylation and derepression of the cell cycle inhibitor p21.<sup>25</sup> TET enzymes, a family of ten-eleven translocation methylcytosine dioxygenases, demethylate DNA by oxidation of 5mC into 5-hydroxymethylcytosine (5hmC), which can be further oxidated and then removed from DNA. Recent research indicates that the TET family regulates neurogenesis and that 5hmC levels are dynamically modulated during development and parallel the acquisition of the neuronal lineage.<sup>26</sup> Accordingly, knock down of TET3 during neuronal differentiation of ESCs leads to DNA demethylation and re-expression of embryonic stem cell factors.<sup>27</sup> In adult NSCs, TET3 has a role in the maintenance of stem cell pool in the SVZ niche and prevents premature differentiation by transcriptional repression of the imprinted gene *Snrpn*.<sup>28</sup>

TET1 promotes both fetal neurogenesis<sup>29</sup> and the proliferation of the nestin-positive adult NSC pool ensuring hypomethylation in genes implicated in neurogenesis or mitochondrial function such as *Galanin*, *Ng2*, *Kctd14*, and *Atp5h*.<sup>30</sup> TET2 has similar effects on adult NSCs, and its overexpression is able to rescue age-related decline in neurogenesis.<sup>31</sup> Interestingly, the establishment of a genome-wide epigenetic status of several genes involved in either stemness or differentiation implicates the crosstalk of both DNA- and histone-modifying enzymes. Mutations in HMTs, such as *Suv39h1*, *Suv39h2* and *G9a*, reduces DNA methylation in mouse ESCs.<sup>32,33</sup> Furthermore, DNA methylation causes the reduction in the histone active mark H3K4me2 and immediately downstream the transcription start site.<sup>34</sup>

### 1.3 | Noncoding RNAs

Noncoding (nc) RNAs are functional RNA molecules, transcribed from DNA but not translated into proteins. Initially considered as “junk” RNAs, they have been later identified as important epigenetic modulators of biological functions. This class of RNAs includes short (22 nucleotides) and long ncRNAs (lnc) (longer than 200 nucleotides), which regulate gene expression at the transcriptional and post-transcriptional levels. Among short ncRNAs, which include microRNA (miRNA), siRNA and piRNA, miRNAs have been involved in neural cell identities during neural induction, neuronal differentiation, and subtype specification.<sup>35,36</sup>

Mature miRNAs are generated from longer precursors that are sequentially processed by two ribonucleases. Pri-miRNA, the primary transcript, is processed by DROSHA and its cofactor DGCR8 in the nucleus; then the precursor pre-miRNA is exported to the cytoplasm where it is further cleaved to produce the mature miRNA. These miRNAs bind to complementary target sequences, which are generally located at the 3' untranslated regions (UTRs) of the mRNA of coding

genes, that will be silenced either for transcript degradation or for translational repression.<sup>37</sup> Of note, one mRNA can be regulated by many miRNAs and one miRNA can have multiple targets. In addition to these mechanisms, miRNAs can also function as sponge for other miRNAs reducing by competing their availability and preventing their binding to target genes.

The role of specific miRNAs as direct regulators of adult NSCs has been reported for hippocampal neurogenesis, where the miR-17-92 cluster is critical for the proliferation of stem cells and for cognitive and behavioral function through the regulation of the Enigma homolog 1 (ENH1)/ID1 signaling pathway.<sup>38</sup> A specific miRNA signature associated with neurogenic commitment of progenitors during embryonal cortical development was also recently described.<sup>39</sup> MiR-146a was found to be critical for both proper differentiation of NSCs during brain development and for the regulation of postnatal hippocampal-dependent memory.<sup>40</sup> Other miRNAs were identified as regulators to switch between NSC proliferation and differentiation. For example, miR-485-3p was found to negatively regulate NSC proliferation and nestin expression and to promote differentiation via targeting TRIP6 expression.<sup>41</sup>

LncRNAs are instead a subclass of noncoding RNAs transcripts longer than 200 nucleotides that share many mRNA features.<sup>42</sup> As for short ncRNA, they have been considered for a long time as noise resulting from stochastic transcription. Nowadays it is recognized that they may regulate gene expression by multiple mechanisms including the regulation of chromatin topological organization, recruitment of epigenetic factors acting as guide, as decoy for the sequestration of many RNAs or as scaffolds favoring the formation and localization of specific protein complexes.<sup>43</sup> LncRNAs are expressed in a tissue-specific manner and about 40% are expressed specifically in the brain.<sup>44</sup> Recent research highlighted the role of these epigenetic factors in neuronal differentiation during embryogenesis and in response to damage.<sup>45,46</sup> In the developing cortex, the *Kdm2b* gene, for example, is crucial for differentiation and migration of cortical projection neurons. Its expression is regulated by the lncKdm2b that activates in cis the transcription of the *Kdm2b* gene by binding with hnRNPAB.<sup>45</sup> The lncRNA H19 is highly expressed in NSCs where it promotes adult neurogenesis by inducing genes involved in proliferation, cell cycle, and response to hypoxia as well as by regulating neurogenesis-related miRNAs.<sup>46</sup>

In addition, lncRNAs can be also regulated by stemness transcription factors such as Oct4 and Nanog indicating a bidirectional complex regulation.<sup>47</sup>

### 1.4 | Chromatin spatial organization

Spatial genome organization has emerged as a further level of epigenetic control of gene expression to establish and maintain pluripotency and lineage commitment.<sup>48</sup> Chromatin is packed in fibers within the nucleus and further organized in higher order structures including topological associated domains (TADs) and, on a larger scale, in chromosome territories.<sup>49</sup> Within TAD, DNA sequences with

similar epigenomic profile, transcription status and association with cofactors and co-regulators interact with each other allowing only specific enhancer-promoter interactions. The interaction of cis-regulatory elements is promoted by the cooperation of the architectural proteins Cohesin and CCCTC-binding factor (CTCF), which regulate the formation and maintenance of long-range chromatin loops in a dynamic manner.<sup>50</sup> At this level, chromatin is also compartmentalized in active regions, named A compartments, characterized by active histone marks and preferentially localized at nuclear interior. Repressed regions, named B compartments, represent peripheral heterochromatin marked by repressive histone modifications (eg, H3K27me3, H3K9me), which tend to be associated at the periphery with the nuclear lamina forming lamina-associated domains (LAD).<sup>51</sup> These regions are dynamically regulated during differentiation of mouse ESCs toward NSCs where activated genes, for example, the brain-specific gene *Pcdh9*, detach from lamina whereas other genes are repressed and linked to lamina (eg, *Nanog*, *Klf4*, and *Oct4*).<sup>52</sup> Furthermore, genes detached from lamina belong to a neuronal physiology category and include either activated individual genes or large cluster of multiple genes separated by intergenic regions or poised genes that are activated in a next differentiation step.<sup>52</sup>

A and B sub-compartmentalization is dynamic during development and during neural lineage commitment.<sup>53</sup> Indeed, mapping chromatin interactions at genome wide level has revealed that in human ESCs about 36% of chromosomal compartments undergo changes between active and inactive regions with a profound reorganization of the chromatin architecture.<sup>54</sup> Further studies have uncovered the key role of the CTCF architectural protein in NSC function since loss of CTCF leads to NSC apoptosis and defective neurogenesis.<sup>55</sup> During transition of ESCs to NSCs, CTCF occupancy and anchorage on genomic regions undergo changes; CTCF binding on enhancers of pluripotency genes is lost while CTCF and the zinc finger protein YY1 are recruited to coordinate the formation of enhancer-gene loops on NSC specific genes (eg, *Nes*, *Bcan*).<sup>56</sup>

## 2 | CONTRIBUTION OF NUPs TO NEUROEPIGENETICS IN STEM CELLS

### 2.1 | NPC structure, assembly and tissue specificity

Nups are components of the nuclear pore complex (NPC), a large macromolecular structure that spans the nuclear envelope and regulates nucleocytoplasmic transport.<sup>57</sup> NPCs are selectively permeable barriers that maintain the integrity of the nuclear compartment allowing trafficking of several components such as RNAs and proteins, and preventing a free diffusion of macromolecules. The NPC is formed by the assembly of about 30 different Nups, present in a copy number of 8 or multiple of 8, disposed in cylindrical structures with a central ring, the core, inserted between nuclear and cytoplasmic ring structures (Figure 1). These rings represent the scaffold Nups with a defined structure. Within the inner ring reside the FG-Nups,

intrinsically disordered proteins characterized by hydrophobic phenylalanine-glycine (FG) repeats, which constitute the permeability barrier of the NPC. From peripheral Nups on both sides of the nuclear envelope flexible filaments protrude in the cytoplasm or in the nucleoplasm where fibrils form the nuclear basket.<sup>60</sup> While core Nups embedded in the nuclear envelope are relatively stable, some of the Nups on the nucleoplasmic side are mobile and can shuttle off and on the pore.

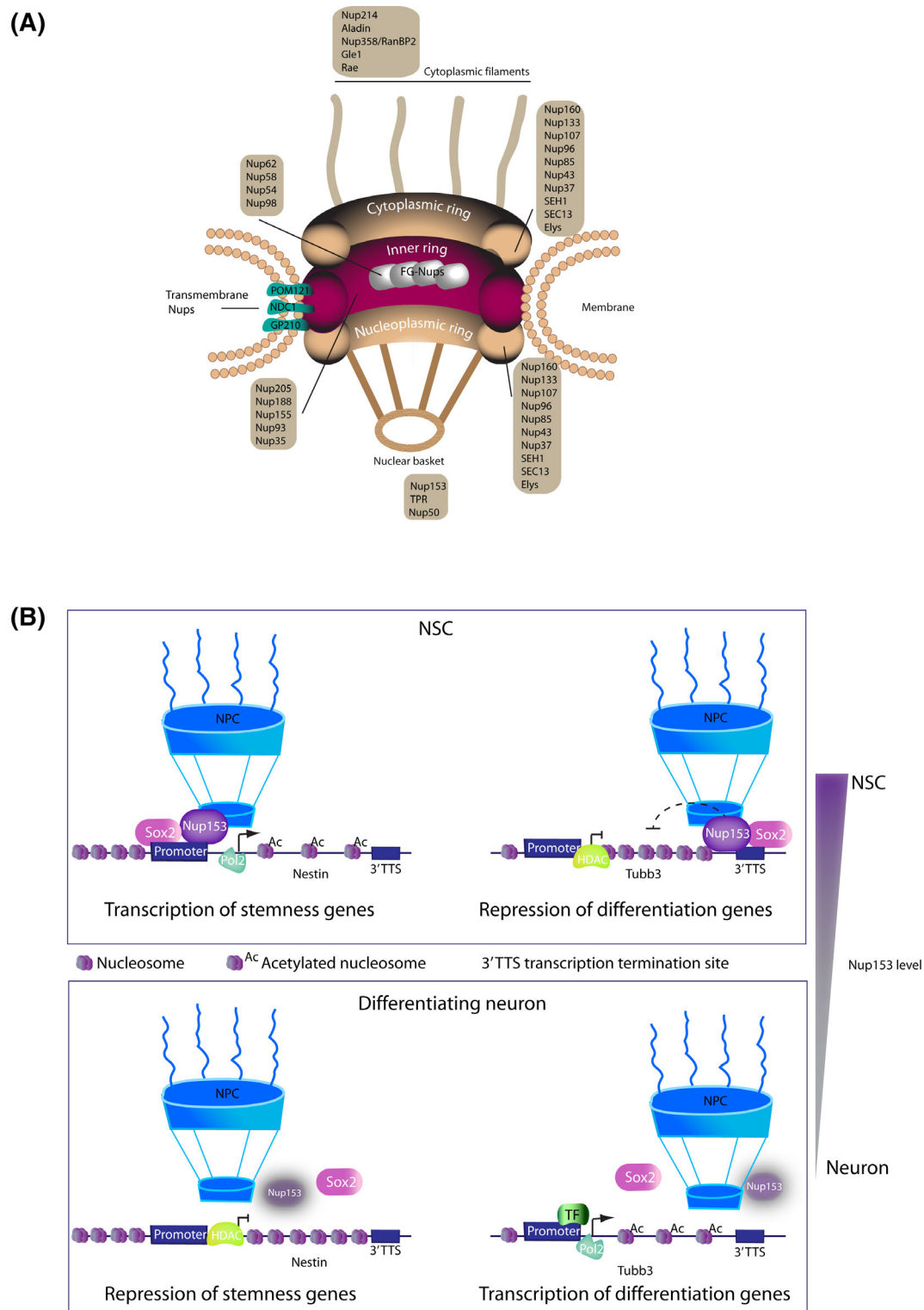
NPCs are relatively stable in postmitotic cells but undergo disassembly and reorganization in a coordinated way during the cell cycle or interphase following metabolic stimuli (for a complete review, see reference 61).

NPC formation occurs in a stepwise manner and requires membrane bending. It is thought that several mechanisms contribute to membrane bending: (a) the insertion of three Nups that contain transmembrane domain (POM121, GP210, NDC1),<sup>62</sup> (b) the action of vesicle coat proteins such as COPI, COPII and clathrin able to induce membrane bending when they polymerize into curve structures,<sup>63</sup> and (c) the Nup ELYS/Mel28, which contributes by anchoring nascent NPC on chromatin.<sup>64</sup>

It is believed that postmitotic NPC assembly is initiated by binding of chromatin with several core Nups, which is then followed by insertion in membranes possibly deriving from the endoplasmic reticulum. The formation of these pre-pores is then completed by addition of other core Nups and lastly by the peripheral Nups. Several mechanisms are hypothesized to temporally and spatially control this process such as Nups phosphorylation during mitosis to prevent their association with chromatin,<sup>65,66</sup> transport receptor importin  $\beta$  binding during mitosis to Nup153, Nup358 and the Nup107-160 complex to prevent their assembly in NPC<sup>67</sup> and Nup SUMOylation, which controls proper incorporation of Nups in nascent NPC.<sup>68</sup> As regards depletion of SENP1 and SENP2, two SUMO proteases associated with the NPC, resulted in severe mislocalization of SUN1, POM121, Nup133, Nup98, and Nup96.

It is becoming clear that NPC composition is not uniform across tissues, and the insertion of particular Nups is required for differentiation along a specific lineage or for tissue homeostasis (for a detailed overview of NPC changes in different organ development, see references 69, 70). Although the mechanisms of Nup-dependent neuronal development are still largely unexplored, recent papers described below point to the interaction of individual Nups with genes and transcription factors rather than nuclear transport regulation to determine neural cell fate. For example, incorporation of Nup210 in NPC is necessary for both myogenic and neural differentiation.<sup>71</sup> By genome-wide expression analysis the authors showed that deletion of Nup210 in myoblasts prevents myotube formation and expression of muscle specific genes (*Asb2*, *Cand2*, *Clic5*, *GDF5*, *Neu2*, *Ndr2*, *Stra13*) as well as genes involved in neuronal differentiation (*Nefl*, *Crim1*, *L1CAM*, *NOXP20*, *Wnt10a*), thus suggesting a role for this Nup also in neural development.<sup>71</sup> Accordingly, the authors found that Nup210 is incorporated in NPCs during ESC differentiation into NSCs and its depletion determines apoptosis and reduction of nestin positive cells.<sup>71</sup>

Other important changes in NPC composition occur when adult NSCs differentiate in neurons.<sup>58</sup> In this context, Nup153, highly



**FIGURE 1** A, Cartoon illustrating the structure of the nuclear pore complex (NPC). Nups are organized in three cylindrical structures (rings). Within the inner ring reside FG-Nups, the permeability barrier of the NPC. Only three Nups are integral protein membrane (POM121, NDC1, GP210). Peripheral Nups on cytoplasmic and nucleoplasmic sides are organized in fibrils forming cytoplasmic fibrils and nuclear basket, respectively. B, Proposed model for Nup153 differential gene regulation during the transition of NSCs toward neurons (based on references 58, 59). Upper panel: In NSCs Nup153/Sox2 complex bound to promoters of stemness factors and cell cycle genes (eg, Nestin, YY1, Tlx, CycD, Rest) allows histone acetylation and gene transcription; Nup153/Sox2 complex bound to the transcription termination site (3' TTS) is associated with chromatin compaction (histone deacetylation) and inhibition of proneural genes (eg, Tubb3, NeuroD1, Ascl1). Lower panel: During NSC differentiation toward neurons, Nup153 levels are downregulated determining lack of stemness/cell cycle gene activation and derepression of proneural genes

expressed in NSCs, is selectively downregulated in neurons while the number of nuclear pores remain constant. Accordingly, its depletion accelerates neural differentiation. Chromatin immunoprecipitation followed by DNA sequencing demonstrated that Nup153, in association with the transcription factors Sox2, directly acts as negative regulator of differentiation genes and positive regulator of stemness core factors (reference 58 is further discussed below).

Concerning this matter, we recently showed that an alteration in NPC composition is present in NSCs isolated from a mouse model of Alzheimer's disease (AD-NSCs).<sup>59</sup> These cells have lower Nup153 levels in comparison to WT-NSCs and, consequently, a reduced Nup153/Sox2 interaction and altered Sox2 recruitment on the promoter of the cell cycle gene *CycD1* consistent with reduced expression of *CycD1* and proliferation of AD-NSCs.<sup>59</sup>

However, the fact that mutations in many Nups that are ubiquitously expressed among tissues result in organ specific diseases suggests a more precise role for these Nups.<sup>72</sup>

Several Nups have been identified as important regulators in NSC function during embryonic development. Retinal progenitor cells in zebrafish, which lack *Elys*, a component of the Nup107-160 complex, show reduced expression of the cell cycle genes *Cdkn1c* and *Ccnd1* and of the proneural transcription factor *Ath5*. Accordingly, the authors found that NSCs cycle more slowly, undergo apoptosis and fail the transition to neuronal differentiation.<sup>73</sup> *Sec13* is another Nup important for retina development and its depletion causes reduced proliferation and differentiation of all cell types with defect of lamination, a process in which NSC migrate, differentiate, and organize into distinct layers.<sup>74</sup>

Nup133 is highly expressed in the neuroepithelium and in NSCs and its absence determines reduced multipotency and inefficient differentiation into the neural lineage.<sup>75</sup> In the developing neuroepithelium, deletion of Nup50 causes altered distribution of the cell cycle inhibitor *p27<sup>Kip1</sup>* and neural tube defects.<sup>76</sup>

Although the role of Nups in the differentiation of adult NSCs is still underinvestigated, recent research has demonstrated the essential role of Nup153 in the maintenance of NSC multipotency<sup>58</sup> and its involvement in defective neurogenesis in an animal model of Alzheimer's disease (AD).<sup>59</sup>

### 3 | NUP-BASED MECHANISMS OF CHROMATIN REGULATION

Studies during the last decade have revealed that, in addition to the canonical function in transport regulation, NPC and Nups modulate stem cell function regulating gene expression by different mechanisms<sup>77</sup> (see Figure 2 and Table 1 for a summary of mechanisms and regulated genes).

#### 3.1 | Chromatin higher order regulation

New findings highlighted that NPC is an anchorage site for chromatin, and Nups either interact with transcriptionally active regions or

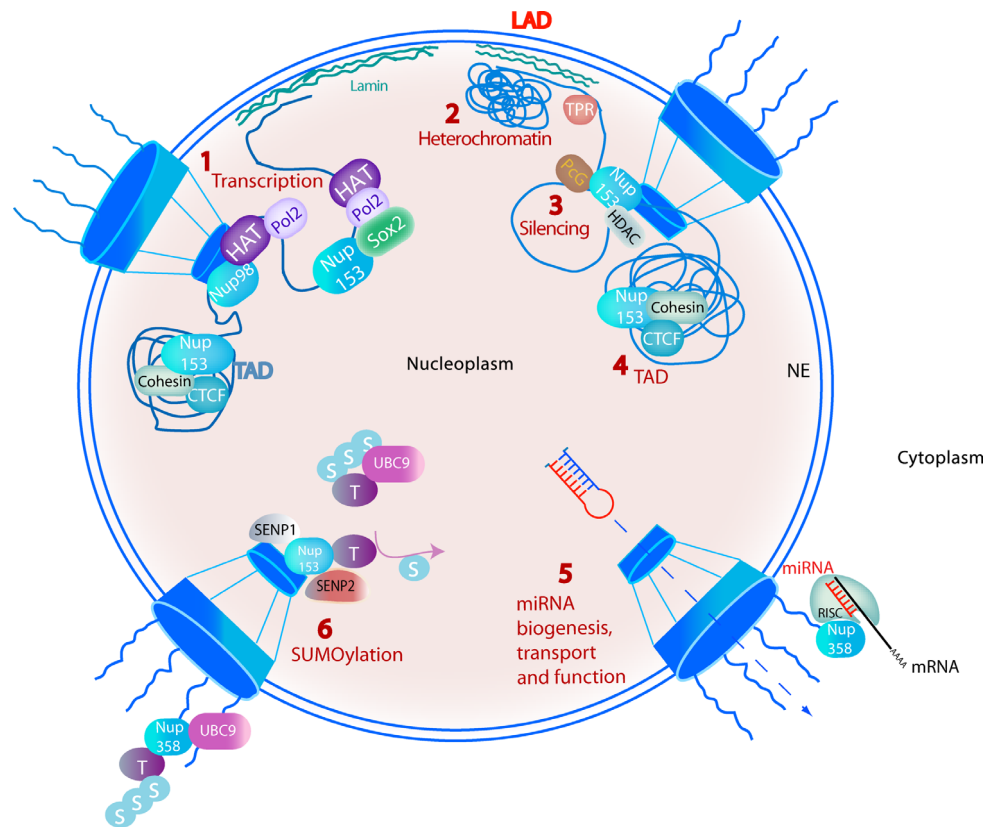
silenced heterochromatin, thus playing an active role in the formation or maintenance of these domains contributing to the spatial organization of the higher-order chromatin architecture.

The activity of genes important for stem cell identity is regulated by the cluster of enhancer formation, referred to as super enhancers (SE), which are hyper-active regulatory domains deriving from the interaction of long-range 3D superstructures that work together to control target genes. In this context, Nup93 and Nup153 were found to bind to H3K27ac enriched regions of SE, away from LAD, driving the expression of cell identity genes.<sup>78</sup> Sox2, crucial for NSC maintenance and identified as molecular partner of Nup153,<sup>58</sup> is also implicated in Pol II-mediated long-range chromatin interactions at the enhancers, thus providing a global chromatin connectivity network essential for NSC pluripotency.<sup>79</sup> Furthermore, recent research has demonstrated that Nup153 modulates genome organization through the formation of molecular complexes with the structural proteins CTCF and Cohesin, which allows the interaction of cis-regulatory elements and TAD boundaries in mouse ESCs.<sup>80</sup> In this study, Nup153 was found to be enriched at the enhancers of developmentally regulated genes containing bivalent histone marks.

Specific Nups are important in defining which chromatin regions interact with the NPC. For example, as demonstrated very recently, the interaction with different states of chromatin at the NPC is mediated by distinct core Nups, Nup107 and Nup93, which bind active regions or polycomb enriched domains for silencing, respectively.<sup>81</sup> Furthermore, the Nups TPR, interacting with Lamin B1 but not with Lamin A/C, limits the extension of LAD and defines the chromatin regions associated with NPC.<sup>82</sup> Lamin-NPC interaction is also important for adult neurogenesis. Imbalance between the Lamin B1 and SUN1 level, the NPC-associated protein, which is part of the LINC complex that connects the cytoskeleton to the nucleoskeleton, is cause of altered adult NSC proliferation and neurogenesis during aging.<sup>83</sup> Mechanistically, decreased Lamin B1 and increased SUN1 levels impair the correct factors segregation during asymmetric division of NSCs.

#### 3.2 | Gene regulation

Nups modulate stem cell pluripotency and identity, which is either directly associating with target genes or providing a nuclear architectural and functional platform for the spatial and temporal crosstalk between chromatin remodelers, transcription factors and coactivators/corepressors.<sup>84</sup> Differently from yeast where chromatin-nup interaction only occurs at the NPC in higher eukaryote Nup-genes, interplay may take place in the nucleoplasm, and is mediated by mobile Nups such as Nup153 and Nup98, which have been identified as regulators of lineage-specific genes and multipotency in NSCs.<sup>58,85</sup> For example, Nup98 interacts with developmentally regulated genes at the NPC during the early stage of embryonic stem cell differentiation, but it moves to the nuclear interior when genes are highly activated.<sup>85</sup> In addition, due to the highly dynamic nature of the Nup-chromatin enzyme interaction many inducible genes are



**FIGURE 2** Cartoon illustrating the contribution of nucleoporins to epigenetic mechanisms in stem cells. (1) Active transcription occurring either at the NPC or in the nucleoplasm with mobile Nups (Nup98, Nup153). For example, Nup98 induces neuronal differentiation by recruitment of histone acetyltransferases (HAT) and Polymerase 2 (Pol2) on target genes (*Tubb3*, *Syn1*, *Map2*, *Sema3A*). In the nucleoplasm, the complex Nup153/Sox2 activates stemness/cell cycle genes (*Nestin*, *Tlx*, *CycD1*) by binding to target promoters. (2) The nucleoporin TPR delimitates the NPC area devoid of heterochromatin and lamina-associated domains (LADs). (3) Gene repression induced by Nup-dependent recruitment of polycomb group proteins (PcG) and histone deacetylases (HDAC). Nup153-polycomb interaction, for example, prevents ESC differentiation into NSCs by repressing neural-specific genes such as *Pax6*, *Blbp*, *Nes*, and *Tubb3*. (4) Nup153 organizes higher-order chromatin architecture in association with the structural proteins Cohesin and CTCF and long-range chromatin interactions by binding to super enhancers (SE) in the topological associated domains (TADs). (5) Nups participate in the biogenesis and transport of miRNA precursors. Nup358 facilitates the association of mRNAs with the miRNA-containing RISC complex. (6) Nups regulate the balance of SUMOylation protein level: Nup358 interacts with UBC9 and promotes protein SUMOylation; Nup153 binds to SENP1 and SENP2 regulating deSUMOylation of target proteins (T). NPC, nuclear pore complex; NE, nuclear envelope; LAD, lamina-associated domains; HAT, histone acetyl transferase; HDAC, histone deacetylase; TAD, topological associated domain; S, SUMO polypeptide; T, target protein

tethered at the NPC, where chromatin loops are stabilized, and, thus can be readily activated.<sup>86</sup> This could be a way for keeping NSCs in the niche in a primed-quiescent state ready for activation following the stimuli.<sup>97</sup> In ESCs, Nup153 ensures the maintenance of pluripotency state acting as a repressor of genes involved in neuroectoderm differentiation (eg, *NeuroD1*) by recruiting polycomb-repressive complex 1 (PRC1) at the transcription start sites.<sup>88</sup> Consequently, Nup153 silencing in ESCs drives their differentiation into NSCs, thus establishing an important tissue-specific role for this Nup. RNA seq analysis indicated also that Nup153 knockdown did not affect the core pluripotent factors *Oct4*, *Nanog*, and *Klf4* but allowed the expression of neural-specific genes such as *Pax6*, *Blbp*, *Nes*, and *Tubb3*. Subsequent studies from Toda et al have expanded our knowledge on the role of Nup153 in the physiology of adult NSCs.<sup>58</sup> They showed that Nup153, in cooperation with the transcription factor

*Sox2*, binds and regulates hundreds of genes necessary for both multipotency and neural development. Specifically, Nup153 was found to activate genes related to cell cycle and stemness while repressing differentiation genes (see Table 1). Of note, Nup153 silencing determined alteration of *Sox2* genomic localization and induction of neuronal differentiation. Genome-wide analyses also revealed that Nup153 might activate or repress target genes depending on the binding position on promoters or transcriptional end sites respectively. This bimodal Nup153 distribution also correlated with a chromatin signature on regulated genes. High level of H3K4me3 and H3K27ac marks were present on active genes while a co-occupancy of H3K4me3 and H3K27me3 was found on repressed genes poised for activation. While the link between chromatin status (presence of specific histone marks), gene expression and Nup occupancy has been widely investigated so far, less known is the direct relationship of

TABLE 1 Nucleoporin regulatory functions in stem cells

Nucleoporin	Cell type	Mode of action	Genes	Biological function/species	Reference
Sec13	Embryonic NSCs	Regulation of proliferation and maturation	-	Retinal differentiation ( <i>Danio rerio</i> )	Schmidt et al, 2013
Elys	Embryonic NSCs	Gene activator; regulation of proliferation and maturation	Cdkn1c, Ccnd1, Ath5	Retinal differentiation ( <i>Danio rerio</i> )	Cervený et al, 2010
Nup98	Embryonic NSCs	Gene activator	Rg1, Erbb4, Sox5, Map2, Sema3A, Grik1, IGF1R, Tubb3, Syn 1	Induction of neuronal development ( <i>Homo sapiens</i> )	Liang et al, 2013
Nup50	Embryonic NSCs	Regulation of p27 <sup>Kip1</sup>	-	Neural tube formation ( <i>Mus musculus</i> )	Smitherman et al, 2000
Nup133	Embryonic NSCs ESCs	Gene activator; potential interaction with chromatin remodeling enzymes	Id1, Otx2, Tgf5, Oct4	Necessary for neural differentiation ( <i>Mus musculus</i> )	Lupu et al, 2008
Nup155	ESCs	Regulation of miR-SOX2/OCT4/NANOG circuit	Sox2, Oct4, Nanog	Embryonic stem cell pluripotency ( <i>Mus musculus</i> )	Preston et al, 2019
Nup210	ESCs	Gene activator	Nes, Nefl, Crim1, L1CAM, NOXP20, Wnt10a	Differentiation of ESCs into neuroprogenitors ( <i>Mus musculus</i> )	D'Angelo et al, 2012
Nup153	ESCs	Regulation of chromatin architecture	Activated: Fbln5, Comp, Ntn4, Dmp1 Repressed: Fgf1, Fgf9, Dlk1, Bmp7, Hoxb13, Wnt, Gata3, Bcl11a, ApoB, Lhx1, Pou3f2, Hox	Regulation of bivalent developmental genes ( <i>Mus musculus</i> )	Kadota et al, 2020
Nup153	ESCs Adult NSCs	Gene silencing	Repressed: Pax6, NeuroD1, Fabp7, Sox11, Nefh, Nrp1, Reln	Embryonic stem cell pluripotency; NSC differentiation ( <i>Mus musculus</i> )	Jacinto et al, 2015
Nup153	Adult NSCs	Gene activator/inhibitor	Activated: Nes, YY1, Ccnd2, Ccnd1, Fabp7, Bmi1 Repressed: Tubb3, Prox1, Ascl1, Syp, Hes5, Gfap, S100β	Stemness maintenance and differentiation control ( <i>Mus musculus</i> )	Toda et al, 2017
Nup153	Adult NSCs	Gene activator	Tlx, CycD1, Mash1, NeuroD1, Rest	Neurogenesis in AD ( <i>Mus musculus</i> )	Leone et al, 2019
SUN1/Lamin B1	Adult NSCs	Factor segregation in asymmetric division	-	Neurogenesis, proliferation ( <i>Mus musculus</i> )	Bin Imtiaz et al, 2021
Seh1	Adult oligodendrocyte stem cells	Gene activator	Mbp, Cnp, Sox10, Myrf, and Nkx2-2	Differentiation and myelination ( <i>Mus musculus</i> )	Liu et al, 2019

Abbreviations: ESCs, embryonic stem cells; NSCs, neural stem cells.



Nups with chromatin modifiers enzymes. It is less clear whether Nups bind to permissive chromatin or they recruit modifiers, inducing chromatin changes. During HDAC inhibitor treatment, which induces histone hyperacetylation, Nup98 binding on active regions was found increased together with enrichment in RNA PolIII, H2A.z, and CTCF binding.<sup>89</sup>

Instead, our previous studies have shown that Nup153 directly binds to chromatin and recruits the HAT P300 and PCAF on target genes both in cardiomyocytes<sup>90</sup> and in cancer cells.<sup>91</sup> Recently, Nup155 was found necessary for chromatin positioning and activity of HDAC4 indicating a further key role for Nups on chromatin remodelers.<sup>92</sup>

In the adult brain, the role of the Nup Seh1, as a scaffold for the assembly of transcriptional complexes important for the differentiation of oligodendrocyte progenitor cells, has been recently described.<sup>93</sup> This Nup regulates the chromatin accessibility of oligodendrocyte differentiation genes (Mbp, Cnp, Sox10, Myrf, Nkx2-2) by recruitment of transcription factors Olig2 and Brd7, a member of the SWI/SNF chromatin-remodeling complex, which is implicated in pluripotency and lineage specification of ESCs regulating the level of histone acetylation.<sup>94</sup>

An additional layer of gene expression control, provided by NPC and Nups, occurs through the regulation of miRNAs, which play key roles in modulation of both embryonic and adult neurogenesis.<sup>38,39</sup> In this context, Nups provide a platform control for miRNA precursor biogenesis and export through the NPC.<sup>95</sup> Other Nups do not control miRNA biogenesis but participate directly in miRNA-mediated silencing. For example, RanBP2/Nup358, one of the main components of the cytoplasmic filaments of NPC, promotes, through its SUMO-interacting motif, the association of target mRNA with the RISC complex,<sup>96</sup> thus participating directly to miRNA-mediated translation suppression. The investigation of the direct relation between Nups and miRNA in stem cells is only at its beginning and requires further efforts. Nevertheless, this is an important topic since many miRNAs are involved in the regulation of embryonic and adult neurogenesis. A recent study revealed that in ESCs, Nup155 disruption induces a decreased expression of pluripotency factors, alteration of a large miRNA cluster involved in pluripotency, and interruption of the miR-SOX2-NANOG-OCT4 regulatory circuit, which impaired stem cell function.<sup>97</sup>

Increasing evidence indicates that NPC dependent regulation of gene expression is also achieved by protein SUMOylation level control by Nups.<sup>98</sup> The addition of SUMO polypeptides to targets occurs by sequential SUMO-activation, transfer, and conjugation involving Aos1/Uba2 enzyme (E1), the UBC9 enzyme (E2), and a SUMO ligase (E3), respectively.<sup>99</sup> Localization at NPC of the machinery for the addition and deconjugation of SUMO polypeptides has been demonstrated: for example, the SUMO conjugating enzyme Ubc9 localizes both at cytoplasmic and nucleoplasmic filaments of the NPC<sup>100</sup> and the Sentrin-specific proteases SENP1 and SENP 2 associate with Nup153 regulating SUMO deconjugation, while one of the few known SUMO E3 ligases is the RanBP2/Nup358.<sup>101,102</sup> Importantly, SUMOylation leading to transcription repression generally negatively

modulate the activity and binding ability of transcription factors, coregulators and chromatin-modifying proteins.<sup>103,104</sup>

The relevance of the SUMOylation pathway in stem cell biology is supported by several findings. In ESCs, appropriate levels of SUMO are necessary to preserve pluripotency by maintaining the proper level of genome wide H3K9me3 and heterochromatin by recruitment of polycomb repressive complexes on target genes.<sup>105</sup> Along this line, knockdown of UBC9 is associated with decrease in stemness factors such as Nanog, Klf4, Oct4, and Sox2 and decreased pluripotency of ESCs.<sup>106</sup> In the organism, Planaria UBC9 silencing induces NSC reduced proliferation by altering repressors and activators of the Hedgehog signaling pathway.<sup>107</sup> Modification of the SUMOylation cascade also affects adult NSCs where overexpression of UBC9 increases their survival and differentiation after transplantation in a murine ischemic brain model.<sup>108</sup>

Although further characterization of the epigenetic role of Nups in adult NSCs is needed, so far, available evidence strongly suggests that Nups, as in ESCs, might function as epigenetic platform organizing chromatin structure, recruiting transcription factors, chromatin modifiers and co-regulatory molecules.

## 4 | NUPS AND AD

Neuroepigenetics play a critical role in the pathophysiology of numerous neurological disorders that also includes, though not limited to, impairment of adult neurogenesis. In depth and extensive analysis of this subject is out of scope of the present paper. However, as representative example of this topic we briefly review literature reports showing the role of Nups in AD. The latter is a neurodegenerative pathology progressively leading to an irreversible decline of cognitive functions.<sup>109</sup> While less than 5% of AD cases are of genetic origin with an early onset and caused by mutation in three genes (APP, PSEN1, PSEN2), late onset AD is sporadic with no clear etiology and represents the majority of AD patients. In both cases, accumulation of misfolded and toxic proteins, such as amyloid- $\beta$  and tau, characterizes the onset and progression of the disease.

Decreased neurogenesis has been demonstrated in a number of AD mouse models highlighting the contribution of AD hallmarks to impaired NSC function<sup>110,111</sup> and suggesting that impaired neurogenesis can contribute to the disease phenotype. Several works have confirmed decreased neurogenesis in AD patients showing reduced maturation of DCX<sup>+</sup> NSCs starting at early stages (ie, at Braak stage III)<sup>112</sup> of AD, as revealed by a decrease of double-positive DCX/PSANCAM, DCX/Prox1, DCX/NeuN, DCX/ $\beta$ III-tubulin, and DCX/CB cells.<sup>113</sup> However, other studies reported little or no changes in neurogenesis efficiency in AD patients.<sup>114-116</sup> These discrepancies might derive from suboptimal preservation and processing of human brain tissue samples or to the great variability among patients.

So far, a few studies reported on the contribution of NPC and Nups to AD highlighting the involvement of either dysfunctional nuclear-cytoplasmic transport or Nup-dependent chromatin mechanisms.

Early ultrastructural studies, performed in AD brain specimens, revealed a connection between neurofibrillary tau tangles, nuclear irregularity and alteration in the Nup Nup62 and in the transport factor NFT.<sup>117</sup> The relation between tau and NPC function has been more recently confirmed in a study showing the direct interaction of tau with Nup98 in a transgenic tau mouse model that leads to Nup98 delocalization in the cytoplasm, which in turn favors tau aggregation.<sup>118</sup> This study showed that tau induces an alteration of the NPC diffusion barrier and a consequent impairment of nucleocytoplasmic transport; however, possible effects of tau on Nup98 dependent regulation of genes was not investigated. The interaction of tau with components of NPC could be underestimated considering that tau nuclear accumulation occurs in AD patients and tau binds to chromatin.<sup>119</sup> Several hypotheses have been made regarding tau effects on nuclear function including a negative impact on nuclear and nucleolar RNA transcription and chromosome stability processes that are also regulated by Nups.<sup>120</sup> Although this topic has not been extensively investigated yet, the involvement of Nups in AD could be much larger than expected. Genome-wide studies applied to pathway analysis in AD patients revealed the implication of protein transport genes grouped in mitochondrial genes and a large Nup gene family, including Nup98, Nup88, Nup133, Nup205, Nup12, Nup160 and Nup37,<sup>121</sup> which is worth further functional studies.

The presenilin 1 protein (PS1) is a  $\gamma$  secretase that cleaves amyloid precursor protein, and its mutations are cause of familial Alzheimer's disease (FAD). How PS1 mutations lead to neurodegeneration is still debated. While the amyloid hypothesis is based on higher activity of mutated PS1 and accumulation of toxic A $\beta$ 42, new hypothesis are based on essential functions of PS1 that are lost with its mutation leading to reduced A $\beta$  clearance and neurodegeneration.<sup>122</sup> Interestingly, PS1 was found to associate with the Nup Sec13 in the complex COPII, involved in vesicle transport from endoplasmic reticulum to Golgi apparatus and misfolded protein sequestration and degradation.<sup>123</sup> According to these data, an interesting hypothesis is that the PS1/Sec13 COPII complex is involved in the control and sorting of newly synthesized transmembrane proteins. Thus, PS1 mutation could

lead to misfolded protein accumulation, which is a common pathogenic mechanism in several neurodegenerative disorders.<sup>124</sup>

A large body of literature has pointed out the contribution of impaired neurogenesis in early AD dysfunction.<sup>125</sup> In this context, we have expanded the knowledge regarding the epigenetic role of Nup153 in adult NSCs providing novel evidence that Nup153 alteration impairs the function of NSCs isolated from the 3 $\times$ Tg mouse model of AD.<sup>59</sup> Reduced Nup153 interaction with Sox2 determined impaired proliferation, differentiation and migration of AD-NSCs. Recovery of an appropriate Nup153 level was sufficient to restore expression of key genes (CycD1, TLX, Mash1, NeuroD1, Rest) and multipotency as well as neuronal maturation.

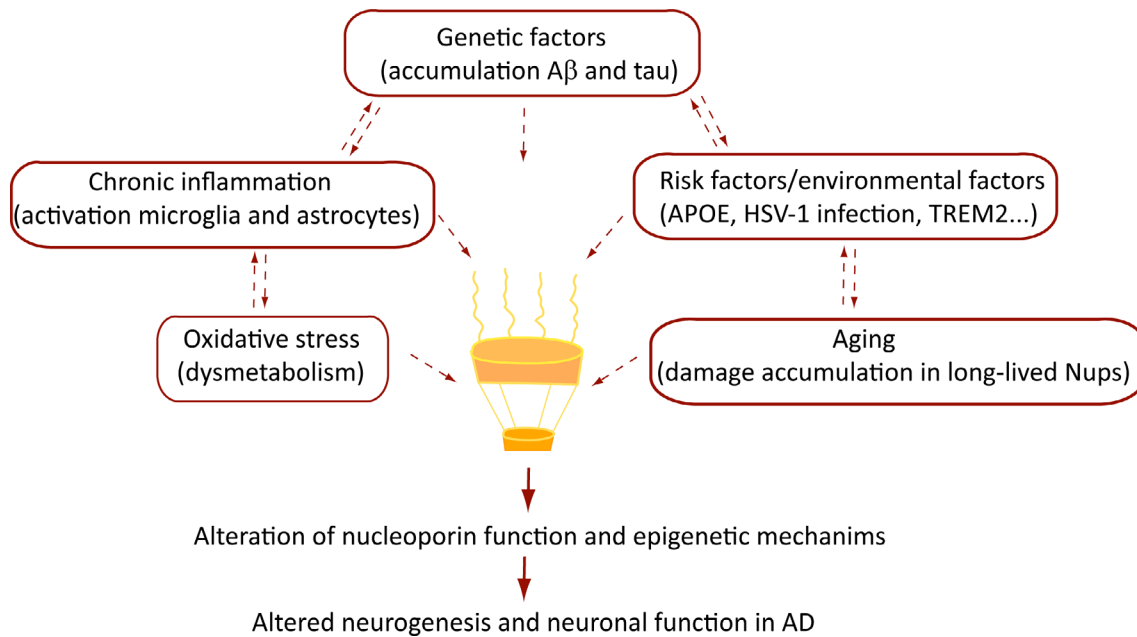
Chronic neuroinflammation, secondary to infection, injury or aging is a common feature of many neurodegenerative diseases including AD<sup>126</sup> in which microglia and astrocyte alterations are key events.<sup>127</sup> Specifically, senescence of replicant-competent glial cells leads to the development of a senescence-associated secretory phenotype (SASP) characterized by increased secretion of pro-inflammatory cytokines and consequent neuroinflammation, which significantly contribute to AD pathophysiology by affecting both differentiated neurons and NSCs.<sup>128</sup> Hence, in an experimental in vivo model of recurrent HSV-1 infection, neuroinflammation induces an AD-like phenotype, A $\beta$ /tau accumulation and neurogenesis impairment.<sup>129,130</sup>

Recent research demonstrated that the Nup TPR, inducing the loss of heterochromatin localization at the nuclear periphery and the formation of internal senescence-associated heterochromatin foci (SAHF), plays a pivotal role in the induction of SASP and the activation of inflammatory cytokine gene expression,<sup>131</sup> thus suggesting a potential direct role for this Nup in inflammatory-mediated AD neurodegeneration.

It is widely recognized that aging represents a major risk factor for late-onset AD.<sup>132</sup> While non-scaffold Nups are short lived, the core Nups embedded in the NPC are instead long-lived proteins that make them very susceptible to aging and damage accumulation. Age dependent deterioration of Nups over time might include oxidatively-damaged Nups and transport factor mislocalization, which can affect

**TABLE 2** Contribution of nucleoporins to Alzheimer's disease (AD)

Nucleoporin	Finding	Model	Biological function	Reference
Nup62/NFT	Altered protein distribution around the nucleus and association with neurofibrillary tangles	AD patient biopsies	-	Sheffield et al, 2006
Nup98, 88, 160, 205, 133, NUPL2 (e.g.)	Enrichment of genes in nuclear transport and nucleoporins	Genome-wide association studies on AD patients	-	Hong et al, 2010
Sec13	Association with presenilin I	P19 embryonic pluripotent cells	Sorting and degradation of misfolded proteins	Nielsen et al
Nup98	Cytoplasmic Nup98 accumulation and tau interaction	Tau transgenic mouse model (rTg(tauP301L)4510)	Disruption of NPC Diffusion Barrier and nuclear import/export	Eftekharzadeh et al, 2018
Nup153	Altered Nup153 protein level and association with Sox2	3xTg-AD mouse model	Impaired neurogenesis	Leone et al, 2019



**FIGURE 3** Scheme illustrating the main genetic and nongenetic risk factors for Alzheimer's disease (AD) that might affect nucleoporin function. Altered neurogenesis and neuronal function might derive from the combination of both genetic and nongenetic factors, such as oxidative stress, aging, environmental factors and neuroinflammation, which impair epigenetic mechanisms based on nucleoporin function

stem cell differentiation. For example, correct transcription factor segregation, through specific importin alpha subtypes, is fundamental for neuronal differentiation of ESCs.<sup>133</sup> Thus, with aging, NPCs may acquire increased nuclear permeability, loss of selectivity or altered transport of specific cargo.<sup>134</sup> In addition to nucleus-transport deterioration, Nup aging can potentially affect also the chromatin structure and mechanic connection between the nucleus and the cytoplasm<sup>135</sup> (see Table 2 and Figure 3 for a summary of Nup dependent epigenetic dysregulation in AD).

## 5 | CONCLUSIONS/PERSPECTIVES

Novel findings suggest that Nups likely play a critical role in epigenetic mechanisms regulating stemness and cell identity in embryonic and adult NSCs. Nups act as hub for many processes since they modify gene expression at either higher-order chromatin organization or at gene specific levels. They also recruit chromatin modifiers and regulate their association with DNA and with co-regulators, providing a platform for gene modulation, miRNA export and protein SUMOylation. Some of these Nups participate and coordinate more than one process working as master epigenetic regulators of stem cell function. Although the role of Nups in adult NSCs is still under-investigated and needs further characterization evidence accumulated so far suggests that the Nup family could represent a novel class of therapeutic targets whose modulation could enhance endogenous neurogenesis, which open new avenues for regenerative and personalized medicine approaches.

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## CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

## AUTHOR CONTRIBUTIONS

C.C.: conception and design, manuscript writing, figures and tables preparation; C.G.: conception and design, manuscript writing, final approval for submission.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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