

Tau Modulates VGluT1 Expression

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Abstract

Tau displacement from microtubules is the first step in the onset of tauopathies and is followed by toxic protein aggregation. However, other non-canonical functions of Tau might have a role in these pathologies. Here, we demonstrate that a small amount of Tau localizes in the nuclear compartment and accumulates in both the soluble and chromatin-bound fractions. We show that favoring Tau nuclear translocation and accumulation, by Tau overexpression or detachment from MTs, increases the expression of VGluT1, a disease-relevant gene directly involved in glutamatergic synaptic transmission. Remarkably, the P301L mutation, related to frontotemporal dementia FTDP-17, impairs this mechanism leading to a loss of function. Altogether, our results provide the demonstration of a direct physiological role of Tau on gene expression. Alterations of this mechanism may be at the basis of the onset of neurodegeneration.

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Introduction

The main known physiological function of Tau is to bind and stabilize microtubules (MTs) [1–3]. This interaction influences MTs stability, axonal transport and growth [3–6], and its alteration has a relevant role in tauopathies. During the progression of the pathology, Tau undergoes post-translational modifications, leading to detachment from MTs and an ensuing increase of the soluble pool of Tau that is considered more easily subjected to post-translational modifications and truncation. This event is thought to determine aggregation and the progression of neurodegeneration, with deficits in synaptic transmission, neuronal loss and cognitive impairment [1,2,7,8].

Despite the high and increasing incidence of these diseases, currently available drugs do not halt their progression, pointing up the need to understand the molecular mechanisms involved in early steps of tauopathies.

All current approaches to target Tau are based on its known actions on MTs and are aimed at counteracting the aggregation of soluble pool of

Tau to block its seeding and spreading. Non-canonical functions of Tau, not linked to its MT-binding properties, have been suggested [9–16], but their relevance in physiology and pathology is not known.

A small amount of Tau has been found to be localized within the nucleus, and a growing body of evidence supports the affinity of Tau for nucleic acids. *In vitro* studies demonstrated that Tau is able to bind the minor groove of dsDNA [17,18], and this interaction takes place with both AT- and GC-rich oligonucleotides [17,19]. Tau has been found, in neuroblastoma and neuronal nuclei [9,13,20], associated with heterochromatin and pericentromeric DNA [14,16,21], but Tau function in this compartment is still unclear. A putative role in nucleolar organization has been suggested [9,13,14]. Moreover, a possible role of Tau in DNA damage protection and chromosome stability has been proposed, since wild-type Tau prevents DNA damage under oxidative stress or hyperthermic conditions [10,16,18], while Tau bearing pathological mutations is associated with chromosome instability and aneuploidy [22–26].

Nevertheless, although these observations suggest a role of Tau in the nuclear compartment, this field is still largely unexplored and it is not known whether nuclear Tau could have a role in modulating gene expression or some aspects of chromatin function that might be relevant for the pathophysiology of tauopathies.

Here, we provide the first demonstration that the nuclear pool of Tau modulates the expression of the vesicular glutamate transporter VGLUT1, which is known to be strongly upregulated in early phases of tauopathies [27,28]. Moreover, we show that increasing the soluble pool of Tau favors its nuclear translocation and increases the expression of VGLUT1. The Tau^{P301L} mutation hinders this chain of events and impairs its effect on VGLUT1 expression.

Our results provide new information on the physiological role of Tau and, above all, suggest important implications for the pathogenesis of tauopathies.

Results

Tau is detectable in the soluble and chromatin-bound nuclear fractions and increases VGLUT1 mRNA and protein levels

To investigate the possible role of chromatin-bound or free Tau in the nuclear compartment, we performed a sub-cellular fractionation and isolated the soluble and chromatin-bound nuclear fractions of the hippocampal neuronal cell line HT22 and the differentiated neuroblastoma cell line SHSY5Y as established models to study the neuronal cytotoxicity in neurodegeneration. Beside the endogenous expression of Tau proteins, we overexpressed the wild-type Tau isoform 4R0N.

We verified that Tau is clearly detectable in the soluble nuclear fraction; moreover, we showed that it accumulates in the chromatin-bound fraction (Fig. 1a), confirming in a cellular context previous evidence on Tau-DNA interaction *in vitro* [17,29].

To investigate whether nuclear Tau might have a role in modulating gene expression, we checked the expression of disease-related genes in Tau-overexpressing cells. A growing body of evidence suggests that glutamate release is altered during the progression of tauopathies, causing an early synaptic hyperexcitability in the asymptomatic phase mediated by Tau [11,27,28].

Therefore, we focused on the presynaptic vesicular glutamate transporters that package glutamate into vesicles for neurotransmitter exocytosis [30].

We observed an increase of VGLUT1 protein level [HT22: 2.2 ± 0.26 SE fold change (FC); differentiated SHSY5Y: 2.24 ± 0.44 SE FC] in neuronal cell lines overexpressing Tau (Fig. 1a–b). Remarkably, we also found that Tau expression increases VGLUT1 mRNA

(HT22: 2.46 ± 0.21 SE FC; differentiated SHSY5Y: 2.69 ± 1.27 SE FC) (Fig. 1c).

To verify whether this effect is determined by the interference of the overexpressed Tau with the endogenous isoforms, we performed the same analysis on non-neuronal cells (Hela cells) that do not express endogenous Tau. We found that in this cellular background, the *de novo* expression of Tau induced the increased expression of VGLUT1 mRNA and protein as well (mRNA: 3.6 ± 0.75 SE FC; protein: 2.19 ± 0.49 SE FC) (Fig. 1). Notably, in all cell lines, we found a similar increase of VGLUT1 expression in both cytoplasmic fraction and total extract (Fig. 1a).

Taken together, these results indicate that Tau modulates VGLUT1 gene expression in a cell type-independent manner.

To further demonstrate the correlation between Tau and VGLUT1 expression, we performed Tau knock down (Tau KD) by lentiviral delivery of a pool of targeting shRNAs. A non-targeting shRNA was used as control. Seventy-two hours after transduction, cells were collected and analyzed to check the mRNA and protein expression of Tau and VGLUT1. We found that the knockdown in differentiated SHSY5Y was very efficient, causing a significant loss of Tau at both the protein and mRNA levels (mRNA: 0.35 ± 0.06 SE FC; protein: 0.49 ± 0.11 SE FC) (Fig. 2) with respect to control cells. In Tau KD cells, we observed a decrease of Tau protein also in the nuclear fractions. Concomitantly, the expression of VGLUT1 was strongly reduced (mRNA: 0.76 ± 0.06 SE FC; protein: 0.5 ± 0.08 SE FC), demonstrating that its expression at least partly depends on Tau expression.

VGLUT1 expression is increased by nuclear Tau

To investigate whether the modulation of VGLUT1 expression could be mediated by the nuclear pool of Tau or by an indirect effect of cytoplasmic Tau, we forced the localization of Tau in the nucleus or its retention in the cytoplasm by including a nuclear localization sequence (Tau-NLS) or a nuclear export signal (Tau-NES), respectively. As expected, Tau-NLS prevalently accumulated in the nuclear fraction, while Tau-NES was mainly detected in the cytoplasm (Figs. 3a, S1). The difference of the molecular weight of Tau-NLS and Tau-NES constructs in the nuclear fractions might be due to the localization signals added to Tau. However, we cannot exclude also post-translational modifications altering the apparent size of Tau nuclear monomers.

We observed that Tau-NLS induced an increase in VGLUT1 protein expression (2.0 ± 0.36 SE FC) comparable to that induced by untagged Tau. On the contrary, when the nuclear localization of Tau was prevented (Tau-NES), no increase in VGLUT1 protein could be observed (Fig. 3a–b).

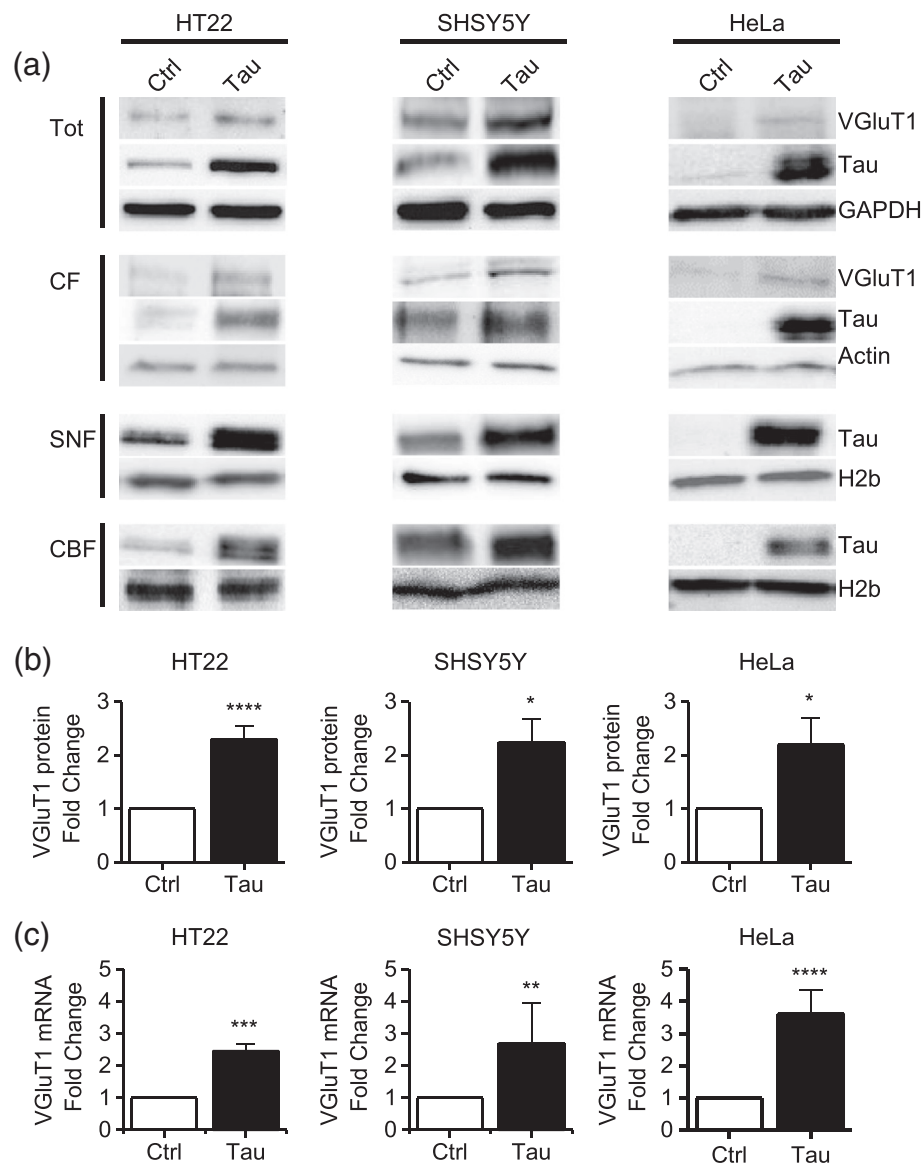


Fig. 1. VGlut1 expression is increased in Tau overexpressing cells. (a) Western blot of total extracts and subcellular fractions obtained from neuronal (HT22 and differentiated SHSY5Y) and non-neuronal (HeLa) cell lines. The ON4R overexpressed Tau is compared with the corresponding endogenous isoform. (b) Quantification of VGlut1 protein level in control and Tau-expressing cells. (c) VGlut1 mRNA quantification by qPCR. (Mann–Whitney test; **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). CF: cytoplasmic fraction; SNF: soluble nuclear fraction; CBF: chromatin-bound fraction.

Likewise, cells expressing Tau-NES showed no increase in the level of VGlut1 mRNA, while cells expressing Tau-NLS showed an increase similar to that observed with untagged Tau (Tau-NLS: $2.04 \pm 0.21\text{SE}$ FC; Tau-NES: $1.26 \pm 0.12\text{SE}$ FC) (Fig. 3c).

This set of data indicates that Tau proteins translocated into the nucleus regulate the expression of VGlut1. Accordingly, shifting the equilibrium toward cytoplasmic Tau, by forcing its nuclear export, brings VGlut1 expression back to baseline.

Displacing Tau from MTs increases the localization of endogenous Tau in the nucleus

At the onset of pathology, Tau undergoes post-translational modifications that induce its detachment from MTs. The increased availability of soluble Tau protein is currently believed to slowly favor the toxic oligomerization [1,2,31,32]. We reasoned that this event could shift the equilibrium between the Tau pools in the cytoplasmic and nuclear compartments, thus increasing Tau nuclear localization. To investigate this hypothesis, we induced the detachment of

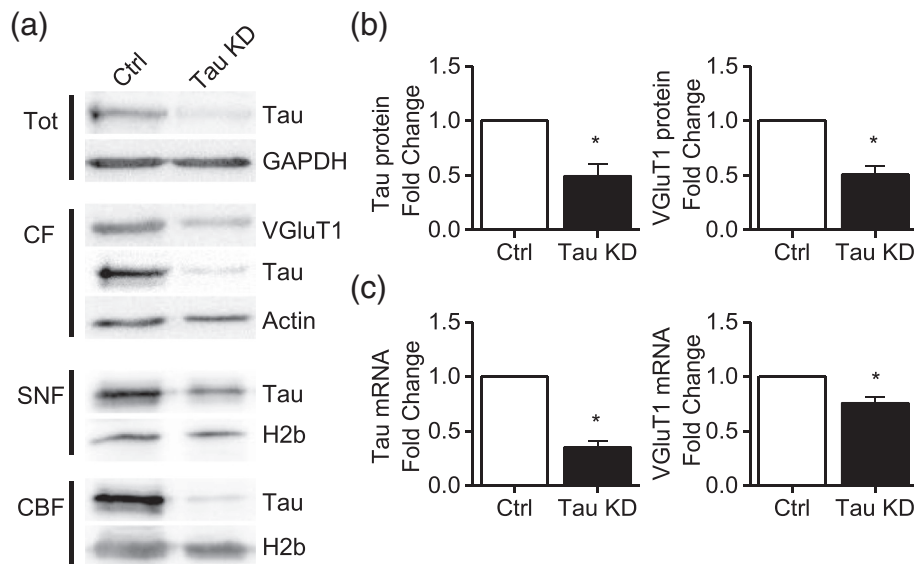


Fig. 2. Tau depletion reduces VGlut1 expression. (a) Western blot of subcellular fractions obtained from differentiated SHSY5Y cells treated with non-targeting shRNA (Ctrl) or Tau targeting shRNAs (Tau KD). (b) Quantification of Tau and VGlut1 protein levels in control and Tau KD cells. (c) VGlut1 mRNA quantification by qPCR in control and Tau KD cells. (Mann–Whitney test; * $p < 0.05$).

endogenous Tau from MTs by treating HT22 cells with paclitaxel (PTX) or nocodazole (Noc). These drugs increase soluble Tau with two different mechanisms, preserving or disrupting the MT network, respectively. Indeed, PTX competes with Tau for the same binding pocket on the tubulin dimer, causing its displacement from MTs; Noc disassembles the MT network, leading to an increase in soluble Tau [33–36]. Importantly, to avoid possible misleading results due to Tau overexpression, we treated neuron-like cells expressing the endogenous but not the exogenous Tau. We verified by Western blot that the treatments did not induce a modulation of Tau protein, and we observed a relocalization of the endogenous Tau (Fig. 4a–b). Indeed, we found an increase of Tau levels in both the nuclear soluble fraction and in chromatin-bound nuclear fraction (Fig. 4a). On the contrary, we observed a decrease of Tau in the cytoskeletal fraction, as expected (Fig. S2A). We conclude that the destabilization of the endogenous Tau from MTs favors not only an increase in the cytoplasmic soluble pool but also its translocation to the nuclear compartment.

Displacing Tau from MTs increases the expression of VGlut1 and glutamatergic synaptic transmission

Having established that the amount of nuclear Tau can be directly influenced by modulating its interaction with MTs, we investigated whether increasing the nuclear pool of endogenous Tau determines a concomitant increase in VGlut1 expression. We

observed that in PTX- and Noc-treated cells, VGlut1 expression rises significantly (PTX, $2.0 \pm 0.23\text{SE FC}$; Noc, $2.29 \pm 0.64\text{SE FC}$) (Fig. 4a–b). A strong increase in VGlut1 expression, detected by IF, was also observed in PTX- and Noc-treated primary neuronal cultures (Fig. 4c). To ask whether the increased expression of VGlut1 has a functional counterpart linked to glutamatergic transmission, patch-clamp recordings of spontaneous synaptic transmission (miniature excitatory postsynaptic currents, or mEPSCs) on primary hippocampal neuronal cultures were performed. Consistently with the increase in VGlut1 expression, we found a significant increase in mEPSC frequency after either PTX or Noc treatment (Fig. 4d). This indicates a prevalent presynaptic effect on glutamatergic transmission. In addition, both PTX and, albeit to a lesser extent, Noc also increased the mEPSC amplitude (Figs. 4d and S2B). This suggests an additional post-synaptic effect induced by modulating the nuclear Tau pool. In order to assess this possibility, the mRNA levels of AMPA and NMDA receptor subunits were measured in differentiated SHSY5Y cells overexpressing Tau (Fig. S2C). The results show that the mRNAs coding for NMDA receptor subunits GRIN2C and GRIN2D are upregulated, while the mRNAs of the AMPA receptor subunit GRIA2 and the AMPA trafficking protein MINK1 are unchanged.

Altogether, these results demonstrate that the modulation of the endogenous soluble Tau levels alters VGlut1 expression, as well as that of subunits of the NMDA receptors, ultimately impinging on glutamatergic synaptic transmission.

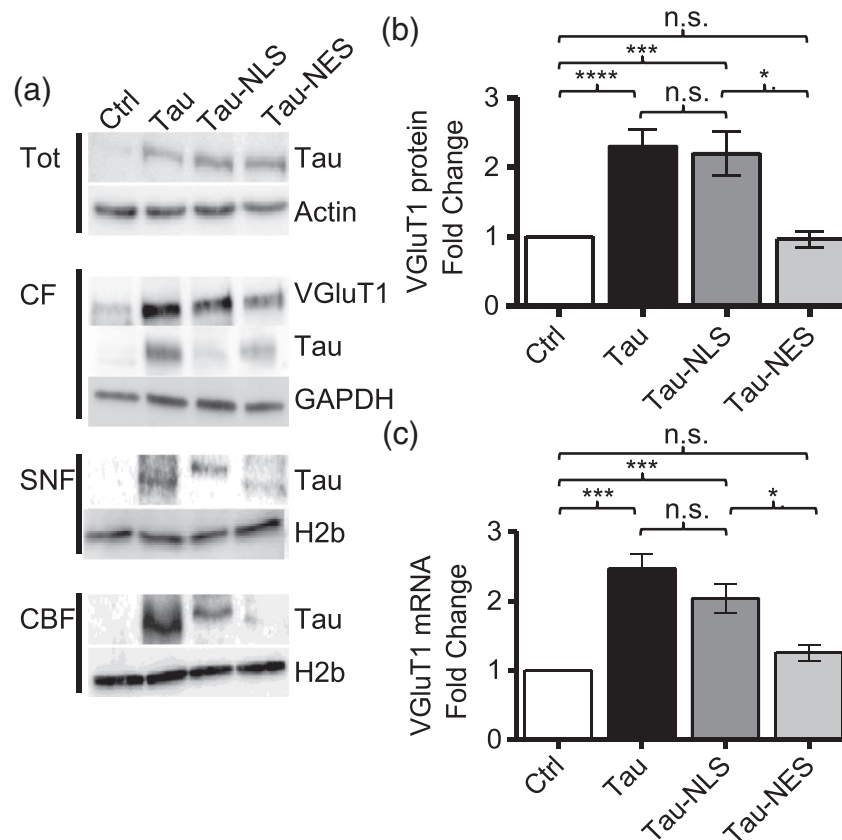


Fig. 3. VGlut1 increased expression is mainly dependent on nuclear Tau. (a) Western blot of HT22 cells expressing Tau-NLS or Tau-NES. (b) Quantification of WB experiments (Kruskal–Wallis ANOVA and Mann–Whitney test; *** $p < 0.001$, **** $p < 0.0001$, n.s. $p > 0.05$). (c) VGlut1 mRNA quantification by qPCR in HT22 cells (Kruskal–Wallis ANOVA and Mann–Whitney test; *** $p < 0.001$, n.s. $p > 0.05$; see also Fig. S1).

VGlut1 expression is not increased by P301L mutated Tau

To investigate whether pathologically mutated Tau could have an impact on its role in the nuclear compartment, we expressed Tau^{P301L} and we observed that this mutant is efficiently translocated into the nucleus as well as the wild-type Tau. Indeed, it is detectable in both the soluble and chromatin-bound fractions (Fig. 5a). We observed that Tau^{P301L} does not increase VGlut1 expression with respect to control cells. Indeed, both the protein (1.16 ± 0.13 SE FC) and mRNA levels (1.04 ± 0.23 SE FC) are not statistically different from those in control cells (Fig. 5). Taken together, these results indicate that P301L mutation does not affect the nuclear translocation but abolishes the ability of Tau to induce VGlut1 expression and thus leads to a loss of this nuclear function.

Discussion

Here we demonstrated that Tau, a well-established MT-associated protein [1,2,4,37], has a relevant role in

modulating the expression of a gene specifically involved in glutamatergic synaptic transmission. Our results suggest that this function might be specifically exerted by Tau protein localized in the nuclear compartment and that it is abolished by the tauopathy mutation Tau^{P301L}. Previous evidence identified Tau in the nuclei of neuroblastoma and neuronal cells [9,13,20]. We detected Tau in the nuclei of HeLa, HT22, and differentiated SHSY5Y cells confirming that the ability of Tau to translocate into the nucleus is retained also in non-neuronal cell lines. The mechanisms by which Tau localizes to the nucleus are still unknown, although it has been shown that the interaction of Tau with TRIM28 stabilizes and promotes Tau nuclear accumulation. In addition, both nuclear Tau and nuclear TRIM28 are increased in AD brains [38].

We observed that, in the nuclear compartment, Tau is detectable in the soluble nuclear fraction and in the chromatin-bound fraction. Our demonstration of Tau association to chromatin in a cellular context is also supported by previous *in vitro* studies demonstrating a possible interaction of Tau with DNA and by a recent genome-wide study on

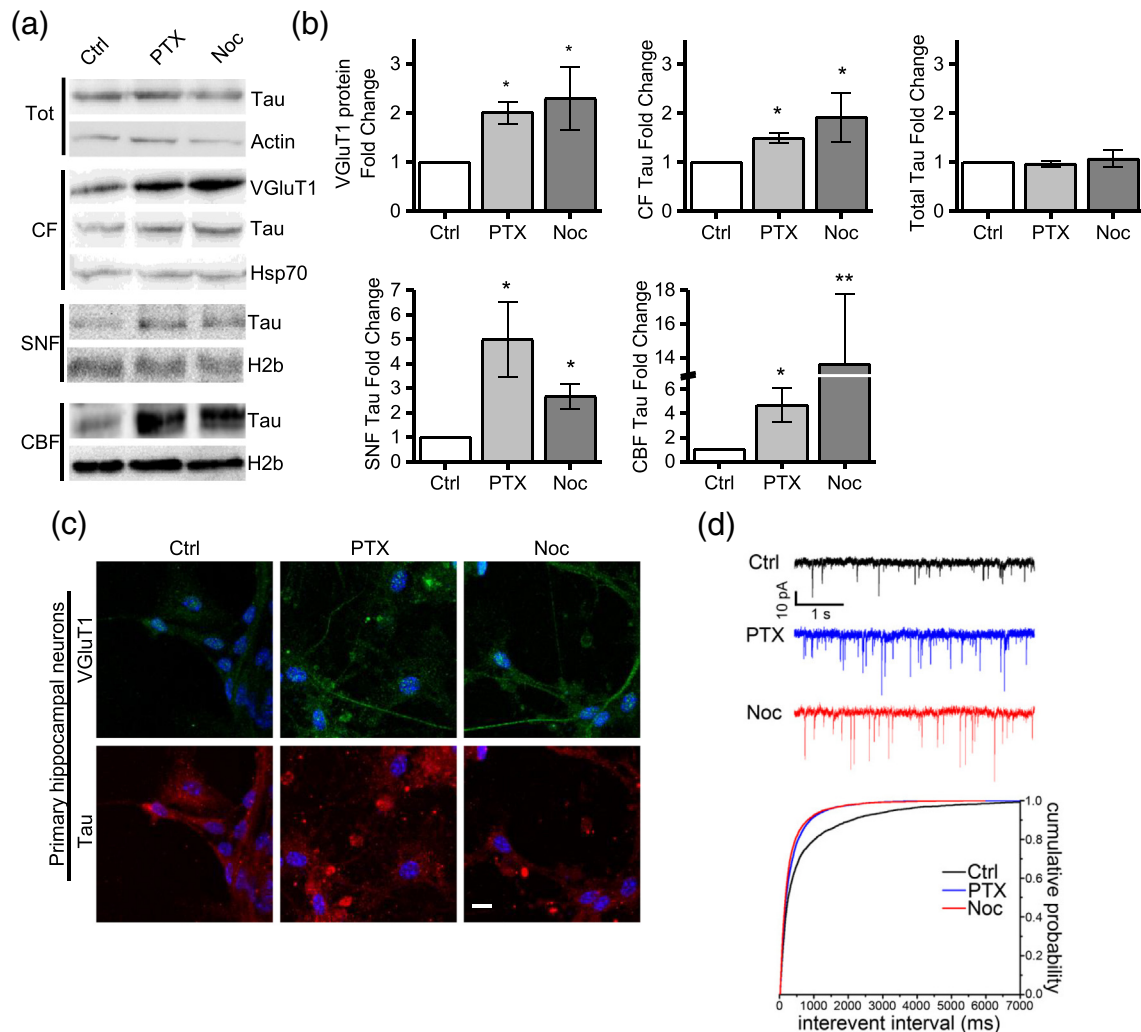


Fig. 4. Tau displacement from MTs increases Tau nuclear accumulation and VGLUT1 expression. (a) Western blot of total extracts and subcellular fractions from HT22 untreated and treated cells. UT (untreated), PTX (paclitaxel), Noc (nocodazole). (b) Quantification of Tau and VGLUT1 protein levels (Kruskal–Wallis ANOVA and Mann–Whitney test; ** $p < 0.01$; * $p < 0.05$; see also Fig. S2). (c) Representative confocal imaging of IF in primary neurons. Tau (red), VGLUT1 (green), DAPI (blue). (d) Cumulative distributions of mEPSC frequency (i.e., interevent interval) following either PTX or Noc treatment, compared to untreated controls (Ctrl, $n = 3002$ events from 12 cells; PTX, $n = 4911$ events from 10 cells; Noc, $n = 3576$ events from 6 cells; Kolmogorov–Smirnov test, Ctrl versus PTX, $p < 0.001$; Ctrl versus Noc, $p < 0.001$; PTX versus Noc, $p < 0.001$).

genic and intergenic DNA regions bound by Tau [17,29,39,40].

Up to now, the role of Tau in the nucleus is still unclear. Previous studies have suggested its involvement in nucleolar organization and in DNA protection under stress conditions [14,16]. Nuclear Tau has been found to accumulate in heterochromatin during aging [21] suggesting a role in modulating gene expression, but no evidence for this role is available.

We focused on this hypothesis and in particular on the possibility that Tau might modulate the expression of genes involved in glutamatergic synaptic transmission. Indeed, a common event identified during the progression of Tau pathology is a strong change in

synaptic transmission. In particular, in the early pathologic stage, glutamate release is increased and correlates with toxic circuit hyperexcitability; in subsequent stages, this is followed by a glutamate release impairment [27,28,41–43]. Synaptic glutamate release is mediated by several proteins, and the VGLUT family has a relevant role by mediating glutamate loading into synaptic vesicles [30]. In tauopathy mouse models, glutamate release and VGLUT1 levels are increased in the pre-symptomatic phase of the disease [27,28]. A glutamate reduction is observed in late stages of tauopathy [28,41], suggesting that alterations of VGLUT1 expression are dependent on both the stage of disease and the state of Tau protein. Thus, in early

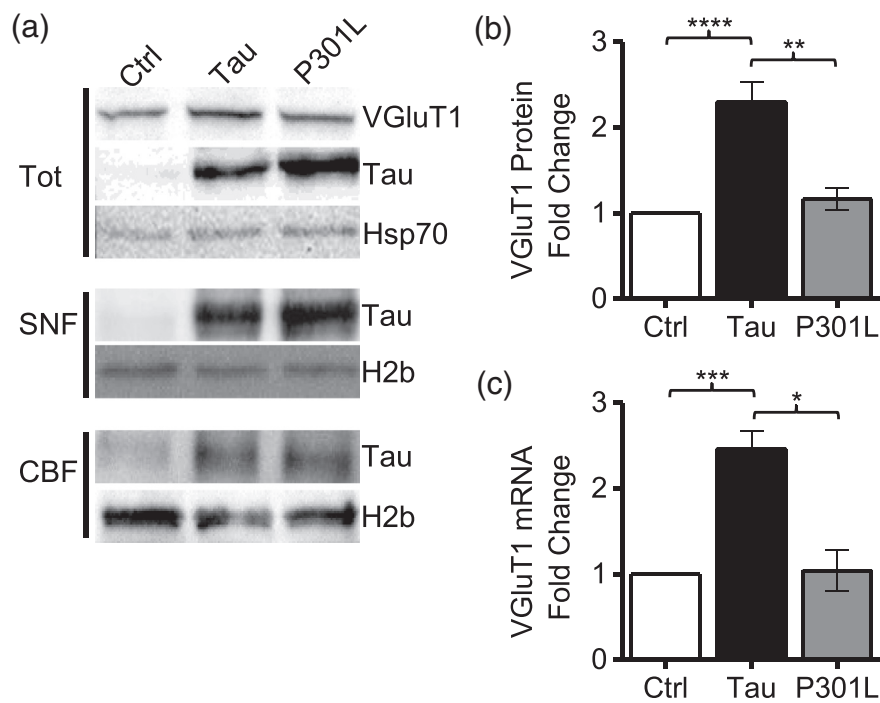


Fig. 5. P301L mutation leads to loss of nuclear function. (a) Western blot of subcellular fractions obtained from HT22 cells expressing wild-type Tau or Tau^{P301L}. (b) Quantification of WB experiments (Kruskal–Wallis ANOVA and Mann–Whitney test; **** $p < 0.0001$; ** $p < 0.01$). (c) VGlut1 mRNA quantification by qPCR in HT22 cells expressing wild-type Tau or Tau^{P301L} (Kruskal–Wallis ANOVA and Mann–Whitney test; *** $p < 0.001$; * $p < 0.05$).

phase of the disease, Tau is detached from MTs but found mostly in a soluble state, while in later stages, it is in an aggregated state. It is conceivable that the nuclear role of Tau in these two stages is distinct. We found that increasing the expression of Tau leads to a selective increase in VGlut1 expression, but not of VGlut2 or of VGlut3 (data not shown). On the other hand, Tau depletion reduced VGlut1 expression, accounting for a bidirectional correlation between the levels of Tau and the expression of VGlut1. Remarkably, forcing the nuclear localization of Tau (Tau-NLS) increases VGlut1 expression at the same level attained by overexpression of untagged Tau. This might indicate either that the amount of untagged Tau reaching the nucleus is saturating or that nuclear Tau is not responsible for that increase. We clarified this point by forcing the export of Tau from the nucleus (Tau-NES). Remarkably, we found that, in this case, the expression of VGlut1 returns to control level. We conclude that the modulation of VGlut1 expression can be triggered by Tau molecules in the nuclear compartment.

To provide further supporting evidence, we treated cells with PTX and Noc, chemicals that increase the Tau soluble pool by different mechanisms [33,36]. Both treatments increase Tau in the nucleus, highlighting an equilibrium between cytoplasmic and nuclear Tau. Concomitantly, the expression of VGlut1 protein was increased. This demonstrates that Tau detachment

from MTs not only affects MTs stability but also favors its nuclear translocation, thus affecting VGlut1 expression. Remarkably, the functional counterpart of the increased nuclear Tau and VGlut1 expression was an increased spontaneous synaptic transmission. In particular, the higher mEPSC frequency indicates a presynaptic effect that can be directly linked to higher VGlut1 levels. This electrophysiological observation represents an *in vitro* correlation of the neuronal hyperexcitability, a hallmark of early-stage tauopathy [27,28].

We also observed an increase of mEPSC amplitude following PTX and Noc treatments (Fig. S2B), which is usually attributed to a post-synaptic effect, suggesting that Tau might likewise influence the expression of glutamate receptors or of glutamate receptor interacting proteins. Consistently, we found that the mRNAs encoding for NMDA receptor subunits GRIN2C and GRIN2D are upregulated, but not for the AMPA receptor subunit GRIA2 or the AMPA trafficking protein MINK1. This shows that Tau regulates different members of the glutamatergic pathway, whose full extent will be systematically addressed by transcriptomic studies.

The modulation of VGlut1 mRNA by Tau might be exerted at the transcriptional level and Tau possible interaction with regulatory elements; that is, enhancers or silencers might not be excluded. Recently, a ChIP-on-chip high-throughput study showed that Tau directly

interacts with genome regions coding for long non-coding (lnc)RNAs, as well as with regions comprising an AG-rich motif [40].

Previous observations clearly suggested the involvement of Tau on chromatin remodeling in pathology [44], but this was not directly linked to nuclear Tau functions. In addition, it has been recently observed that Tau activates transposable elements in Alzheimer's disease [45].

A post-transcriptional effect of nuclear Tau on VGLUT1 mRNA stability is another possibility that would lead to increased levels. In addition, an effect of nuclear Tau in promoting the export of VGLUT1 mRNA to the cytoplasm could be present. It also remains to be verified whether Tau modulates the expression of other disease-relevant genes. Multi-omics approaches interrogating entire pools of genomes, transcripts, proteins and metabolites would allow understanding the flow of information that underlies Tau pathology.

This new function of wild-type Tau might have a relevant role in the onset of the sporadic pathology as it has been widely demonstrated that the glutamate release is increased and causes hippocampal hyperexcitability in the pre-symptomatic phase of tauopathies [28,41–43]. To investigate whether mutations of Tau found in human tauopathies influence the ability to modulate gene expression, we measured VGLUT1 expression in cells expressing Tau^{P301L}, a well-characterized mutant related to a group of genetic tauopathies [3,8,46,47]. Intriguingly, Tau^{P301L} nuclear translocation was not affected, but its overexpression had no significant effect on VGLUT1 level, thus indicating a loss of nuclear function and further supporting the direct involvement of nuclear Tau molecules in this modulation. This result could be due to a failure in the interaction of mutated Tau with a nuclear cofactor involved in gene expression. We previously demonstrated that P301L mutation induces an altered protein conformation in the cytoplasm [33]. We cannot exclude that this alteration could contribute in hindering the binding to a cofactor. Moreover, the P301L mutation is placed in the second repeat of the MT-binding domain, which seems to be also crucial for DNA binding [17]; therefore, an altered conformation of the repeat could have an impact on DNA binding and, consequently, on gene expression. Although we cannot explain the molecular mechanisms involved, a more extensive study on gene expression might be performed to elucidate the role of wild-type and mutated Tau on the modulation of genes involved in glutamatergic synaptic transmission. Moreover, it would be interesting to investigate whether other pathological mutations impair Tau influence on gene expression.

Altogether, these results highlight the role of Tau in gene expression. This function could have a potential therapeutic relevance, and screening assays based on

targeting this novel physiological mechanism will form the basis for new drug discovery approaches to fight neurodegeneration. To this regard, several aspects are still unclear, in particular, the mechanisms involved in this function, which will be the target of future research efforts.

It is currently accepted that, in tauopathies and in AD, Tau gains a toxic function, linked to its detachment from MTs and to the acquisition of aggregating properties. Our results open the intriguing possibility that in early phases of neuron degeneration progression, the same process that leads to Tau detachment from MTs might trigger an additional, but not necessarily alternative, gain of function, linked to modulation of pathologically relevant genes. As the neurodegeneration progresses, the increased pool of soluble Tau starts aggregating leading to a loss of the nuclear function of Tau. This would explain the observed reduction of glutamatergic transmission and VGLUT1 expression in tauopathy models and human brains. These results prompt investigations to evaluate nuclear Tau as an attractive new therapeutic target.

Experimental Procedures

Chimeric constructs cloning

The cDNA encoding Tau isoform D (383aa) has been cloned into the BglII site of pcDNA3.1. Tau-NLS has been generated by digestion and cloning of the 3xNLS in the XhoI/BamHI site. Tau-NES has been obtained by PCR amplification of NES and cloning in EcoRI/BamHI site. Tau^{P301L} construct has been obtained by site-directed mutagenesis exploiting the Q5® Site-Directed Mutagenesis Kit (NEB) and the following primers generated by the NEBaseChanger™ tool: Fwd AAACACGTCCTGGGAGGCGGC; Rev. GATATTATCCTTTGAGCCACACTTGGACTG.

Cell culture, transfections and treatments

HeLa cells and immortalized hippocampal neurons HT22 were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 10% FBS. SH-SY5Y (ATCC® CRL-2266™) cells were maintained in DMEM/F12 (GIBCO) supplemented with 10% FBS. In all the experiments exploiting the SHSY5Y cell line, cells have been differentiated as follows: 10 μM retinoic acid (Sigma-Aldrich) has been added for 5 days followed by 50 ng/ml BDNF (Alomone) in DMEM/F-12 medium not supplemented with FCS for 3 days.

The day before the experiment, cells were seeded at 10⁵ cells in six-well plates or in Willco dishes (Willcows). Lipofection was carried out with Effectene (QIAGEN) or Lipofectamine 2000 (Thermo-Fisher) according to the manufacturer's instructions.

Primary hippocampal neurons were obtained from postnatal day (P) 0 B6/129 mice. Hippocampi were dissected and triturated in cold calcium-free Hank's balanced salt solution with 100 U ml⁻¹ penicillin and 0.1 mg ml⁻¹ streptomycin, and digested in 0.1% trypsin, followed by inactivation in 10% FBS DMEM (GIBCO) with 100 U ml⁻¹ DNase. Neurons were seeded on poly-D-lysine-coated glass coverslips. For initial plating, neurons were maintained in Neurobasal-A medium (Invitrogen) supplemented with 4.5 g l⁻¹ D-glucose, 10% FBS, 2% B27 (Invitrogen), 1% Glutamax (Invitrogen), 1 mM pyruvate, 4 μM reduced glutathione and 12.5 μM glutamate. From the following day on, neurons were grown in Neurobasal-A medium (Invitrogen) supplemented with 2% B27 (Invitrogen), 1% Glutamax (Invitrogen) and 1 μg ml⁻¹ gentamicin. For IF experiments, neurons at day *in vitro* 18 have been used. HT22 cells and primary hippocampal neurons were treated with PTX 1 μM or Noc 1 μM for 4 h.

Silencing mediated by lentiviral shRNAs

MISSION® TRC shRNA bacterial glycerol stocks containing Tau shRNAs (MAPT MISSION shRNA SHCLNG-NM_010838) were purchased from Sigma. A lentiviral vector containing scramble shRNA was used as control. For the generation of viral vector stocks, HEK 293T cells were transfected with 10 μg of the packaging plasmid pCMVΔR8.91, 5 μg of VSV-G and 20 μg of the gene transfer plasmid. The cell culture supernatant was collected twice, at 48 and 72 h after transfection and filtered through a 0.45-μm pore size filter. Viral vectors were titered by quantification of p24 antigen in cell culture supernatants with an enzyme-linked immunoabsorbent assay (Innogenetics, Gent, Belgium). Differentiated SHSY5Y cells were transduced with lentiviral particles supplemented by 10 μg/ml polybrene (Millipore) for 6 h. Cells were collected and analyzed 72 h after transduction.

Western blot and immunostaining

Total protein extracts were prepared in lysis buffer supplemented with protease and phosphatase inhibitors. To guarantee the maximum reproducibility and the minimal contamination in the fractionation experiments, the Subcellular Protein Fractionation Kit for Cultured Cells (Thermo-Fisher) was used according to the manufacturer's instructions. Briefly, the protocol enables segregation and enrichment of proteins from five different cellular compartments. The first kit reagent, when added to the cell pellet, causes selective membrane permeabilization, releasing the soluble cytoplasmic contents named cytoplasmic fraction (CF). The second reagent dissolves plasma, mitochondria and ER–Golgi membranes. After recovering intact nuclei by centrifugation, a third reagent yields the

soluble nuclear extract named SNF. An additional nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear fraction named CBF. The last reagent extracts the cytoskeletal proteins. In this study the CF, SNF and CBF were considered. Proteins were quantified by BCA (Thermo-Fisher). For each sample, 20 μg of each fraction was loaded. Proteins were separated by SDS-PAGE and electro-blotted onto Hybond-C-Extra (Amersham Biosciences) nitrocellulose membranes. The total extracts and the CF were loaded on a 12% acrylamide gel; instead, SNF and CBF fractions were loaded on 8% acrylamide gel. Membranes were blocked (5% skimmed milk powder in TBS, 0.1% Tween 20).

For IF experiments, cells were fixed with ice-cold 100% methanol for 5 min. After permeabilization (PBS, 0.1% Triton-X100), samples were blocked (1% wt/vol BSA) and incubated with primary and secondary antibodies. Slides were mounted with Vectashield mounting medium (Vector Laboratories).

Primary antibodies for WB were as follows: mouse anti-tau (Tau5) 1:1000 ab80579 (AbCam), rabbit anti-actin (A300-485A; Bethyl Laboratories), goat anti-Hsp70 (sc-1060; Santa Cruz), rabbit anti-histone H2b (Santa Cruz), mouse anti-GAPDH 1:15,000 (Fitzgerald) and rabbit anti-VGLUT1 ab77822 1:500 (AbCam). Secondary antibodies for Western blot analysis were HRP-conjugated anti-mouse or anti-rabbit, purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA.

Primary antibodies for IF were as follows: mouse anti-tau (Tau-13) 1:500 (Santa Cruz), rabbit anti-VGLUT1 ab77822 1:500 (AbCam) and DAPI 1:15000 (Sigma). Secondary antibodies for IF were as follows: Alexa Fluor 633 and Alexa Fluor 488 (Life Technologies).

Western blot quantification has been performed using ImageJ software.

Real-time PCR

Total RNA was extracted by Nucleospin (Macherey-Nagel) and retro-transcribed by Reverse Transcriptase Core kit (Eurogentec) according to the manufacturer's instructions. Real-time PCR was performed using the iTaq™ Universal SYBR® Green Supermix (BioRad) and performed for 40 cycles of amplification with denaturation at 95 °C for 15 s, annealing at 60 °C for 25 s and extension at 72 °C for 20 s. The primers employed were as follows: Actin: fwd 5'-TCCAT CCTGGCCTCACTGTCCAC-3', rev 5'-GAGGGGCC GGACTCATCGTACT-3'; Tau: fwd 5'-GTGACCTC CAAGTGTGGCTCATT-3', rev 5'-CTTCGACTG GACTCTGTCCTTG-3'; VGLUT1: fwd 5'-GAGGA GTGGCAGTACGTGTTCC-3', rev 5'-TCTCCAGAAG CAAAGACCCC-3'. The modulation of GRIA2, MINK1, GRIN2C and GRIN2D genes in Fig. 2d has been extrapolated from RNAseq analysis that will be described elsewhere.

Image acquisition and analysis

A Leica TCS SP8 confocal laser-scanning microscope (Leica Microsystems, Mannheim, Germany) equipped with Leica Application Suite (LAS) X software was used. All frames were captured by means of HC PL APO CS2 40×/1.30 oil objective (N.A. = 1.30), a format size of 2048 × 2048 pixel and a sequential scan procedure. All confocal frames were taken by a suitable scanning power and speed along with gain level to achieve the greater signal definition and avoid any background noise.

Patch-clamp recordings and electrophysiological data analysis

Recordings were performed on primary neuronal cultures, by adapting the procedure described in Piacentini *et al.* [48]. Cells were continuously bathed using Tyrode's solution containing (in mM) NaCl 150, KCl 4, MgCl₂ 1, CaCl₂ 4, glucose 10 and Hepes 10 (pH 7.4) with NaOH. Borosilicate glass pipettes were pulled with a P-97 puller (Sutter, CA) to a resistance of 5–6 M Ω when filled with an internal solution containing (in mM): K-gluconate 145, MgCl₂ 2, Hepes 10, EGTA 0.1, Mg-ATP 2.5, Na-GTP 0.25 and phosphocreatine 5 (pH 7.35) with KOH. mEPSCs were recorded while holding the neuron at a command potential of –70 mV. Data were acquired using a MultiClamp 700A amplifier, connected to a Digidata 1322A digitizer (Molecular Devices, CA). Data were analyzed using Clampfit 10.7 (Molecular Devices) as described in Mainardi *et al.* [49]. An event template was constructed and used to detect mEPSCs; stringency of detection was ensured by setting the “template match threshold” to 4. After event detection, the cumulative distributions for mEPSC amplitude and frequency (the latter as interevent interval) were calculated.

Statistical analysis

For Western blot and quantitative real-time PCR, statistical significance was assessed by non-parametric Kruskal–Wallis test followed by pairwise Mann–Whitney test (one tailed). For qPCR, gene expression was calculated with Pfaffl method [50]. Each sample was run in triplicate, and at least three biological replicates were performed for each experiment. All results are shown as mean $\pm$ SEM from at least three independent experiments. Significance is indicated as * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$ and **** for $p < 0.0001$. For patch-clamp recordings, statistical significance was assessed using the Kolmogorov–Smirnov test.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmb.2019.01.023>.

CRedit authorship contribution statement

Giacomo Siano: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Martina Varisco:** Investigation. **Maria Claudia Caiazza:** Investigation. **Valentina Quercioli:** Conceptualization, Methodology, Writing - review & editing. **Marco Mainardi:** Investigation. **Chiara Ippolito:** Investigation. **Antonino Cattaneo:** Conceptualization, Writing - review & editing, Funding acquisition. **Cristina Di Primio:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Funding acquisition.

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Abbreviations used:

MTs, microtubules; Tau KD, Tau knock down; NLS, nuclear localization sequence; NES, nuclear export signal; PTX, paclitaxel; Noc, nocodazole; mEPSCs, miniature excitatory postsynaptic currents; DMEM, Dulbecco's modified Eagle's medium; CF, cytoplasmic fraction; SNF, soluble nuclear fraction; CBF, chromatin-bound fraction.

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