Growth cone localization of the mRNA encoding a chromatin regulator modulates neurite outgrowth

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Abstract

Neurons exploit mRNA localization and local translation to spatio-temporally regulate gene expression during development. Local translation and retrograde transport of transcription factors regulate nuclear gene expression in response to signaling events at distal neuronal ends. Whether epigenetic factors could also be involved in such regulation is not known. We report that the mRNA encoding the high mobility group N5 (HMGN5) chromatin binding protein localizes to growth cones of both neuronal-like cells and of hippocampal neurons. We show that *Hmgn5* 3'UTR drives growth cone localization and translation of a reporter gene, and that HMGN5 can be retrogradely transported into the nucleus along neurites. Loss of HMGN5 function induces transcriptional changes and impairs neurite outgrowth while HMGN5 overexpression induces neurite outgrowth and global chromatin decompaction. Interestingly, control of both neurite outgrowth and chromatin structure is dependent on proper growth cone localization of *Hmgn5* mRNA. Our results provide the first evidence that mRNA localization and local translation might serve as a mechanism to couple the dynamic neuronal outgrowth process with chromatin regulation in the nucleus.

Keywords: mRNA localization/Hmgn5/chromatin/neurite outgrowth

Introduction

Messenger RNA (mRNA) localization coupled to local translation in axons and dendrites constitutes an efficient way for neuronal cells to control gene expression at high spatial and temporal resolution (Jung et al, 2014). High throughput technologies have facilitated the identification of broad catalogues of mRNAs localized in axonal and dendritic compartments of neuronal cells (Jung et al, 2011). For example, such mRNAs encode cytoskeletal elements that regulate axonal outgrowth and navigation, presynaptic proteins that regulate synaptic activity and metabolic enzymes that maintain axonal homeostasis (Holt & Schuman, 2013; Lin & Holt, 2008).

The recent discovery of locally translated transcription factors that are retrogradely transported to the cell nucleus to elicit cell survival/cell death transcriptional programs or specification of neuronal identity (Barrett et al, 2006; Cox et al, 2008; Ji & Jaffrey, 2012) has led to a new paradigm of neuronal gene regulation. Local synthesis coupled to retrograde transport of nuclear factors enables a constant crosstalk between the cell periphery and the nucleus, instructing transcriptional programs in response to local cues (e.g. growth factors, neurotransmitters, extracellular matrix, etc.). Moreover, local translation and retrograde transport of nuclear factors might play a key role in modulating survival of neurons after injury (Ben-Yaakov et al, 2012). In addition to transcription factor mRNAs, previous transcriptomic studies of purified neuronal processes have identified several axonal mRNAs encoding for chromatin interacting and remodeling factors (Ji & Jaffrey, 2013). However, the relevance of the axonal localization and, possibly, the local translation of such mRNAs have not been explored so far.

We previously identified ~80 mRNAs localizing to the extending neurites of neuronal-like N1E-115 cells, a mouse neuroblastoma cell line widely used as an *in vitro* system to study neuronal differentiation (Marler et al, 2005; Yakubchyk et al, 2005). This model recapitulates the extension of neurites before axon-dendrite specification. Since neurite outgrowth is the principal morphological characteristic of early neuronal differentiation (da Silva & Dotti, 2002), our results suggest that local mRNA translation might not only be a feature of axons and dendrites, but also characterizes early neuronal differentiation stages. Experimental support to this

hypothesis came from the discovery that growth cone localization and translation of the mRNA encoding the MAP kinase kinase MKK7 is essential for microtubule bundling, which is required for robust neurite outgrowth (Feltrin et al, 2012).

Among the neurite-enriched mRNAs in N1E-115 cells, we also identified transcripts encoding nuclear proteins (Feltrin et al, 2012). One of these mRNAs encodes the High mobility group N5 (HMGN5) protein, which belongs to the HMGN family of chromatin-binding architectural proteins (Rochman et al, 2010). HMGN proteins bind the nucleosome core particle and compete with linker histone H1 for chromatin binding sites, therefore affecting chromatin structure and transcriptional activity (Kugler et al, 2012). HMGN proteins have also been shown to affect the levels of histone post-translational modifications and the activity of chromatin remodeling factors (Postnikov & Bustin, 2010). Mouse HMGN proteins have tissue- and stage-specific expression patterns and non-redundant effects on the cellular transcriptome (Kugler et al, 2013). HMGN5 is the most recently characterized member of the HMGN family. Its structure comprises an N-terminal nucleosome binding domain (NBD) and a C-terminal acidic tail that is able to interact with histone H1 C-terminal tail (Rochman et al, 2010). HMGN5 knock-out *in vivo* has been shown to affect the transcriptional profile of several organs, including brain, spleen, liver and thymus (Kugler et al, 2013). Due to its recent discovery, little is known about HMGN5 physiological functions. It has been suggested that HMGN5 might play a role in controlling cellular differentiation, glutathione metabolism and tumor progression (Ciappio et al, 2014; Rochman et al, 2010).

Here, we present evidence supporting an unprecedented function of HMGN5 in controlling chromatin dynamics during neurite outgrowth in both neuroblastoma cells and mouse hippocampal neurons. We show that *Hmgn5* mRNA growth cone localization is important for neurite outgrowth and that the local synthesis coupled to retrograde transport of HMGN5 might serve as a mechanism to influence chromatin structure and function in response to signaling at distal neuronal ends.

Results

Hmgn5 mRNA localizes to growth cones of N1E-115 cells and of hippocampal neurons by virtue of a 3'UTR localization signal.

In our previous microarray analysis, we found mouse *Hmgn5* mRNA to be enriched in the neurites of differentiated N1E-115 cells (Feltrin et al, 2012). We first sought to validate the microarray data by performing quantitative real time PCR (qRT-PCR) on total RNA extracted from neurite and soma fractions of differentiated N1E-115 cells (Fig. 1A). This confirms the enrichment of *Hmgn5* mRNA in the neurite fraction, whereas a known nuclear non-coding RNA (*snord15b*) (Feltrin et al, 2012) is found to be enriched in the soma fraction (Fig. 1B). In a parallel approach, we performed fluorescent in situ hybridization (FISH) with riboprobes antisense to *Hmgn5* mRNA. *Hmgn5* mRNA localizes to bright punctate structures in the growth cones of N1E-115 cells, similarly to other known localized mRNAs (Cox et al, 2008; Feltrin et al, 2012), suggesting the association in ribonucleoprotein (RNP) particles (Kiebler & Bassell, 2006) (Fig. 1C, black arrowheads). The sense control riboprobe displays very little signal (Fig. 1C). The intracellular localization and the translation of mRNAs is usually controlled by sequences residing in mRNA 3' untranslated regions (3'UTRs) (Andreassi & Riccio, 2009). To verify whether mouse *Hmgn5* 3'UTR contains a localization signal, we fused it to a GFP reporter and performed FISH with a riboprobe antisense to *GFP* mRNA. Appending *Hmgn5* 3'UTR to the *GFP* mRNA recapitulates the growth cone localization pattern observed with endogenous *Hmgn5* (Fig. 1D, black arrowheads). On the contrary, *GFP* mRNA without *Hmgn5* 3'UTR appears as a diffuse staining throughout the growth cone (Fig. 1D), suggesting no RNP association. Finally, we confirmed growth cone *Hmgn5* mRNA localization in a primary neuronal cell culture system, mouse hippocampal neurons. FISH analysis reveals localization of *Hmgn5* mRNA in growth cones of hippocampal neurons (Fig. E1A) and shows that *Hmgn5* 3'UTR functions as a growth cone localization element also in this cell system (Fig. E1B). We conclude that *Hmgn5* mRNA localizes to growth cones of N1E-115 cells and of hippocampal neurons and that *Hmgn5* 3'UTR contains a growth cone mRNA localization signal.

Hmgn5 3’UTR drives local translation in the growth cone of N1E-115 cells.

Localized mRNAs might have the potential to be locally translated at the site of final anchoring (Andreassi & Riccio, 2009). Local mRNA translation has been shown to be controlled by RNA binding proteins (RBPs) usually binding to mRNA 3’UTRs (Hornberg & Holt, 2013; Iacoangeli & Tiedge, 2013). We therefore assessed whether *Hmgn5* 3’UTR can drive local translation of a reporter mRNA. For this purpose, we fused *Hmgn5* 3’UTR to a Dendra2 reporter (PalX2-Dendra2/*Hmgn5* 3’UTR) containing two palmitoylation signals that limit diffusion of the encoded protein in the membrane to 50 μm/h (Fivaz & Meyer, 2003). This implies that fluorescent PalX2-Dendra2 signals arising in the growth cone shortly after photobleaching reflect newly synthesized proteins rather than protein transport from the cell body (that is >50 μm away from the growth cone). After transfection of the reporter construct, N1E-115 cells were induced to differentiate. The distal neurites were bleached with intense green light and fluorescence recovery was measured over 30 minutes. A significantly higher fluorescence recovery is observed for the PalX2-Dendra2/*Hmgn5* 3’UTR construct in comparison to PalX2-Dendra2 (Fig. 2A-B, Movie E1). Further, this fluorescence recovery is dependent on local translation since it is abrogated by incubation of the translation inhibitor anisomycin (Fig. 2A-B, Movie E1). These results show that *Hmgn5* 3’UTR is able to mediate local mRNA translation in distal neurites and growth cones of N1E-115 cells.

HMGN5 can be retrogradely transported along neurites of N1E-115 cells.

The evidence that *Hmgn5* 3’UTR can drive local mRNA translation and that HMGN5 contains a nuclear localization signal (Postnikov & Bustin, 2010), led us to hypothesize that the locally translated pool of HMGN5 might be trafficked back to the cell nucleus, as previously shown for locally synthesized CREB, SMAD and STAT3 transcription factors (Ben-Yaakov et al, 2012; Cox et al, 2008; Ji & Jaffrey, 2012). To test this hypothesis, we transfected N1E-115 cells with a Dendra2-HMGN5 fusion followed by *Hmgn5* 3’UTR and allowed the cells to differentiate. We then locally photoconverted Dendra2 (from green to red) in distal neurites and growth cones (>50 μm away from the soma) with UV light and monitored the nuclear accumulation of

the red signal over two minutes after photoconversion (Fig. 3A). As previously observed (Cox et al, 2008; Ji & Jaffrey, 2012), we assumed that if HMGN5 is actively retrogradely transported along the neurites of N1E-115 cells, it should be transported to the nucleus faster than the Dendra2 protein alone by virtue of passive diffusion. Consistently, we observe an accumulation of nuclear red fluorescence when cells are transfected with Dendra2-HMGN5-3’UTR in comparison to Dendra2 alone (Fig. 3B-C). Nuclei belonging to cells that did not undergo photoconversion but are located in the same field of view as the photoconverted nuclei (white arrowheads in Fig. 3B) are used as controls for nonspecific nuclear photoconversion during imaging of Dendra2 or Dendra2-HMGN5-3’UTR (Fig. 3B-C). These data indicate that HMGN5 has the potential to be retrogradely transported along the neurites of N1E-115 cells.

Hmgn5 **KD causes transcriptional changes and impairs neurite outgrowth in N1E-115 cells.**

HMGN5 has been shown to modulate the transcriptome of different cell types, such as primary mouse fibroblasts (Rochman et al, 2011), and of whole organs, such as mouse brain, liver, thymus and spleen (Kugler et al, 2013). We therefore performed a microarray analysis of N1E-115 cells transfected with *Hmgn5* small interfering RNAs (siRNAs) and compared them to N1E-115 cells transfected with control siRNAs allowed to differentiate for 24 hours. Assessment of knock-down (KD) efficiency by qRT-PCR, Western Blot and immunofluorescence indicates an ~80% reduction of *Hmgn5* mRNA level and a ~70% reduction of HMGN5 protein level (Fig. 4A-C). Additionally, we evaluated control siRNAs-transfected cells that differentiated for 4 hours, which show few and short neurites (Movie E2). We find that 31 genes are significantly affected by *Hmgn5* KD, either up- or down-regulated (Fig. E2A, column “kd vs ctrl”), and we confirm the microarray results on nine out of ten selected candidates by qRT-PCR (Fig. E2B). Among these 31 genes, 18 are also affected by the differentiation process (column “4h vs 24h”). Interestingly, all of these genes except one show matching changes in *Hmgn5* KD and the extent of neurite outgrowth. We also noticed that several of the genes affected by *Hmgn5* KD (e.g. *Pltp*, *Adam12*, *Prkg1*, *Plxna4*) are involved in the control of glycogen synthase kinase 3 β (GSK3 β) (Dong et al, 2009; Leyme et al, 2012; Manns et al, 2012; Zhao et al,

2009), a well known regulator of axonal growth and neuronal development (Hur & Zhou, 2010). Furthermore, we show that *Hmgn5* KD leads to a small but significant increase of p- GSK3 β levels (Fig. E2C).

All these considerations led us to hypothesize that HMGN5 might control the neurite outgrowth process in N1E-115 cells. Consistently, we show that *Hmgn5* KD causes a ~40% reduction of neurite length in N1E-115 cells (Fig. 4D-E). The N1E-115 cells neurite outgrowth process is characterized by an initial stage where multiple neurites protrude and retract, before one or two neurites are established and grow continuously (Pertz et al, 2008). Phase-contrast time-lapse analysis reveals that *Hmgn5* KD cells display repeated collapse events and have difficulties in establishing continuous neurite outgrowth (Fig. 4F and Movie E2). We next evaluated whether the growth cone mRNA localization and translation of *Hmgn5* mRNA are important for the regulation of neurite outgrowth. We assessed rescue of the KD phenotype by re-expressing different siRNA-resistant versions of HMGN5. The GFP-HMGN5 constructs code only for *Hmgn5* coding sequence (CDS) fused to GFP, while the GFP-HMGN5-3'UTR constructs contain also *Hmgn5* 3'UTR. Accordingly, while the GFP-HMGN5 mRNAs will mostly localize and be translated in the cell soma, the GFP-HMGN5-3'UTR mRNAs will also localize to the growth cones of N1E-115 cells, recapitulating the endogenous *Hmgn5* mRNA localization pattern (Fig. E3A). We observe that only GFP-HMGN5-3'UTR is able to fully rescue the neurite outgrowth defect caused by *Hmgn5* KD (Fig. 4G-H), indicating that proper *Hmgn5* mRNA localization is required for the neurite outgrowth process of N1E-115 cells. To explore the existence of potential HMGN5 functions other than its chromatin-binding function, we mutated two residues in HMGN5 nucleosome binding domain (NBD) that are known to mediate chromatin binding (serines 17 and 21 mutated to glutamic acids, hence named SE mutant (Rochman et al, 2009)). GFP-HMGN5SE-3'UTR fails to rescue the KD phenotype indicating that HMGN5 function in N1E-115 cells is exerted through its binding to chromatin (Fig. 4G-H). The finding that the expression levels of the different GFP-HMGN5 constructs are approximately equal (Fig. E3B) suggests that the observed effects are exclusively due to the molecular properties of the exogenously expressed proteins. We conclude that both HMGN5 nuclear function and *Hmgn5* mRNA growth cone localization are important for the modulation of neurite outgrowth in N1E-115 cells.

Hmgn5 KD impairs neurite outgrowth in hippocampal neurons.

Next we investigated HMGN5 function in mouse hippocampal neurons. We used this neuronal type, as it constitutes a well-known system to study neuronal outgrowth and the establishment of neuronal polarity (Dotti et al, 1988). *Hmgn5* KD in hippocampal neurons (Fig. 5A-B) leads to reduced neurite length. This effect is rescued by exogenous HMGN5 expression (Fig. 5C-D). While a small rescuing effect is already observable with the GFP-HMGN5 expression construct, presence of *Hmgn5* 3'UTR is necessary for full rescue of the KD phenotype (Fig. 5C-D). No defect in axonal specification was observed, indicating that HGMN5 might function exclusively as a regulator of neurite length and not of neurite identity (Fig. E4).

HMGN5 stimulates neurite outgrowth and modulates chromatin dynamics in N1E-115 cells in a 3'UTR-dependent manner.

We next overexpressed the various HMGN5 fusion constructs in N1E-115 cells. Opposite to what we observe upon KD, we find that the overexpression of HMGN5 increased neurite outgrowth by ~ 30% and this effect is dependent both on the proper growth cone localization and on the nuclear function of the exogenously expressed mRNA and protein (Fig. 6A-B). Since we demonstrated that the chromatin binding activity of HMGN5 is important for its function in N1E-115 cells, we analyzed the effect of HMGN5 overexpression on chromatin structure and dynamics. HMGN5 overexpression in fibroblasts, U2OS, 293T and in a mouse pituitary cell line has been shown to trigger global chromatin decompaction, as revealed by DAPI and heterochromatin marker staining (Rochman et al, 2009). To evaluate chromatin structure, we used DAPI staining and quantitated the number of heterochromatic foci. We find that HMGN5-mediated chromatin decompaction (i.e. reduction or disappearance of dense heterochromatic foci) is strictly dependent on growth cone localization of *Hmgn5* mRNA (Fig. 6C-D and Fig. E5A-B). H3K9me3, a marker of constitutive heterochromatin whose localization is affected by HMGN5 overexpression (Rochman et al, 2009), was evaluated by quantification of the mean fluorescence intensity of H3K9me3 foci. We observe that overexpression of GFP-HMGN5-3'UTR, but not GFP-HMGN5, causes a reduction of H3K9me3

heterochromatic foci, as observed with DAPI staining, and a small but significant decrease in H3K9me3 foci staining intensity (Fig. 6C and E), indicating a global rearrangement of constitutive heterochromatin.

We next evaluated chromatin dynamics upon HMGN5 overexpression in N1E-115 cells. HMGN5 has been shown to interact with linker histone H1 and, by competing for nucleosome binding, to increase its mobility within chromatin (Malicet et al, 2011). This was demonstrated by measuring fluorescence recovery after photobleaching (FRAP) of histone H1-GFP fusions, whereby non nucleosome-associated H1-GFP showed faster fluorescence recovery than nucleosome-associated one. To test whether this is also the case in N1E-115 cells, we performed a FRAP analysis of cells transfected with histone H1-GFP and overexpressing HMGN5 (Fig. 7A-B, Movie E3 (Malicet et al, 2011)). We observe that only the mRuby2-HMGN5-3'UTR construct is able to influence the mobility of histone H1, while the mRuby2-HMGN5 construct does not have any effect (Fig. 7C-D). More specifically, mRuby2-HMGN5-3'UTR decreases histone H1 chromatin residency time, thus accelerating its fluorescence recovery (Fig. 7D). This implies that mRuby2-HMGN5-3'UTR is more efficient in counteracting histone H1-mediated chromatin compaction than mRuby2-HMGN5. Taken together, this data indicate that HMGN5 is able to influence chromatin structure and dynamics in N1E-115 cells and that growth localization of *Hmgn5* mRNA is instrumental for HMGN5 chromatin modulating function.

HMGN5 stimulates neurite outgrowth and controls chromatin structure in hippocampal neurons in a 3'UTR-dependent manner.

To explore whether HMGN5 is able to modify chromatin structure in hippocampal neurons, we overexpressed the different HMGN5 constructs. Only GFP-HMGN5-3'UTR is able to induce an increase in neurite length in hippocampal neurons (Fig. 8A-B). As in N1E-115 cells, this corresponded to global chromatin decompaction and reorganization, as quantified by counting the number of heterochromatic foci and the average staining intensity of H3K9me3 foci. This overexpression phenotype is strictly dependent on the presence of *Hmgn5* 3'UTR (Fig. 8C-E). We conclude that, as observed in N1E-115 cells, *Hmgn5* mRNA growth

cone localization and HMGN5 function are involved in modulating neurite length and chromatin compaction in hippocampal neurons.

Discussion

Recent transcriptomic studies have identified hundreds of mRNAs localized in neuronal processes (Cajigas et al, 2012; Gumy et al, 2011; Zivraj et al, 2010). However, little is known about the relevance of the localization of such mRNAs and of the function of the encoded proteins to neuronal physiology. mRNAs encoding transcription factors have been identified in axons and it has been proposed that their local translation and retrograde transport serves as way to couple distal signaling events, such as growth factor application, to transcriptional changes in the nucleus (Ji & Jaffrey, 2013). mRNAs encoding chromatin regulators have also been identified in synaptic terminals but whether their proper localization and function is involved in such growth cone-to-nucleus signaling program in neuronal development has not been explored. We show here that the localization of the mRNA encoding the chromatin-binding protein HMGN5 to growth cones modulates neurite outgrowth. *Hmgn5* KD impairs neurite outgrowth, while HMGN5 overexpression stimulates it in both neuroblastoma cells and primary hippocampal neurons (Fig. 4,5,6,8). Both the rescue of the KD (with a milder effect in hippocampal neurons, Fig. 5) as well as the overexpression phenotypes are strictly dependent on the presence of *Hmgn5* 3'UTR in the expression construct (Fig. 4,5,6,8), which is able to drive growth cone localization (Fig. 1D and Fig. E1B) and translation (Fig. 2). These results strengthen previous observations (Feltrin et al, 2012; Merianda et al, 2013; Yoo et al, 2013) showing the importance of proper subcellular mRNA localization for neuronal outgrowth.

How would local translation determine the biochemical properties of a protein so as to influence its cellular function is still an unresolved issue. The main obstacle in giving a definite answer to this question is the fact that locally synthesized proteins might only represent a minor fraction of the total cellular pool (Eng et al, 1999). We hypothesize that locally synthesized HMGN5 might be endowed with different post-translational modifications as compared to the protein synthesized in the soma. Although no post-translational modification of HMGN5 has been experimentally validated so far, by analogy with the other HMGN proteins, it is assumed that HMGN5 can be phosphorylated and acetylated (Pogna et al, 2010). These modifications might influence HMGN5 chromatin binding affinities and/or its

interactions within multiprotein complexes. It is worth noticing that HMGN proteins bind to DNA in a nonsequence-specific manner and their transcriptional activity is thought to be guided via interactions with defined protein partners (Kugler et al, 2012).

The existence of a complex pathway for communication between neuronal distal ends and the nucleus, that involves both localization of the *Hmgn5* mRNA to the growth cone, and the subsequent transport of its locally synthesized product to the nucleus might seem non-intuitive at first sight. However, the neuronal differentiation process is highly stochastic at the single cell level and involves successive cycles of neurite outgrowth and collapse that occur on timescales of hours (Movie E2) (da Silva & Dotti, 2002). As proposed before (Albus et al, 2013), cell-intrinsic mechanisms must exist to co-ordinate such dynamic neurite outgrowth processes with protein synthesis through regulation of transcription. The spatio-temporal regulation of HMGN5 function might provide an elegant way to couple stochastic neurite outgrowth events with the control of transcription through chromatin regulation at relevant time scales. In such a model, HMGN5 might therefore be specifically produced during episodes of neurite outgrowth when a growth cone is present, but not during collapse when the growth cone is removed, providing a mechanism that links cell morphodynamics with transcriptional control.

While indications of possible neurological functions have been ascribed to HMGN1 and HMGN3 (Abuhatzira et al, 2011; Deng et al, 2013; West et al, 2004), HMGN5 has not been implicated in brain physiology so far. Mice with impaired HMGN5 function (lacking its nucleosome-binding domain) show very mild phenotypic changes (Kugler et al, 2013). However, loss of HMGN5 function in brain induces transcriptional changes. Among the biological process gene categories mostly affected by brain loss of HMGN5 function are nervous system development and cell morphogenesis (Kugler et al, 2013). As observed for HMGN1 (Deng et al, 2013), we also detect high HMGN5 expression levels in neurogenic areas of the mouse brain (F.M. and C.R. unpublished observations). Furthermore, *Hmgn5* mRNA has been detected in preparations from the synaptic neuropil (Cajigas et al, 2012). These observations support an *in vivo* role of HMGN5 in neuronal differentiation, which

awaits further characterization. In an effort to understand HMGN5 impact on the transcriptome of neuronal-like cells, we performed a microarray analysis and find that *Hmgn5* KD in N1E-115 cells significantly affects the expression of 31 genes (Fig. E2A). Interestingly, several of the genes affected by *Hmgn5* KD are also modulated during the neurite outgrowth process (Fig. E2A). Furthermore, we notice that *Hmgn5* KD influences the expression of genes (e.g. *Pltp*, *Adam12*, *Prkg1*, *Plxna4*, *Capn6*, *Cnn2*, *septin 3*) that are known to be involved in the control of cytoskeletal dynamics (Kawaguchi et al, 2003; Rozenblum & Gimona, 2008; Tonami et al, 2011; Weirich et al, 2008; Xie et al, 2005; Yaron et al, 2005; Yuasa et al, 2012). These considerations support a role for HMGN5 in transcriptional regulation of cytoskeleton regulating genes during the neuronal outgrowth process. This would nicely correlate with the loss of capability of *Hmgn5* KD cells to extend stable neurites.

The transcriptional changes observed upon *Hmgn5* KD might be a direct consequence of HMGN5-mediated chromatin structure rearrangements. Indeed, in line with previous observations in different cell types (Malicet et al, 2011; Rochman et al, 2009), we show that HMGN5 overexpression affects global chromatin structure in both neuroblastoma cells as well as hippocampal neurons (Fig. 6,8). Interestingly, this effect is strictly dependent on the proper growth cone localization of *Hmgn5* mRNA (Fig. 6,8 and Fig. E5). The chromatin decompaction phenotype observed upon HMGN5 overexpression could be explained by HMGN5-mediated control of histone H1 dynamics in neuronal cells (Fig. 7). However, as it was recently demonstrated for human HMGN5 (Zhang et al, 2013), it is also possible that HMGN5 directly interacts with nuclear proteins thereby influencing chromatin structure and function. The regulation of higher order chromatin architecture plays a key role in controlling gene expression during all stages of neural development (Hsieh & Gage, 2005). Modifiers of chromatin structure and function, such as DNA methyltransferases, Polycomb proteins and members of the high mobility group AT-hook (HMGA) proteins, have the capacity to control the neurogenic potential of neuronal progenitor cells (NPCs) (Hu et al, 2012). Later in development, neuronal activity has been shown to influence the dynamic interaction between chromatin and the nuclear lamina, thus modulating gene expression (Walczak et al, 2013). Finally, disruption of high order chromatin structure has been linked to brain disorders such as Rett syndrome and epilepsy

(Agarwal et al, 2011; Singleton et al, 2011; Wilczynski, 2014). Several other mRNAs encoding chromatin regulators have been identified in axons of different neuronal subtypes. These include, for example, additional members of the high mobility group protein family (e.g. *Hmgb1*, *Hmgb2*, *Hmgn1*, *Hmgn2* and *Hmgn3*), members of the SWI/SNF chromatin remodeling complex (e.g. *Smarca2*, *Smarca5*, *Arid1a*) and histone modifying enzymes (e.g. *Jmjdc1a*, *Jmjdc1c*, *Ash1l*, *Fbxl10*) (Ji & Jaffrey, 2013). This suggests that modulation of chromatin structure and function via local translation and retrograde transport of epigenetic regulators might constitute a general growth cone-to-nucleus signaling mechanism during neuronal development. Our results thus pave the way for the characterization of additional chromatin-binding proteins that might be locally synthesized in neuronal processes.

Changes in global chromatin structure and dynamics in response to distal signaling at neuronal ends might affect the cellular transcriptome in a broader and more stable fashion than the effect of transcription factors. We therefore propose that, while local translation of transcription factors might constitute a way to respond to acute signals, such as growth factor application or neuronal injury (Ben-Yaakov et al, 2012; Cox et al, 2008; Ji & Jaffrey, 2012), local translation of epigenetic regulators might underlie durable changes in neuronal development, such as the ones occurring during persistent neuronal outgrowth and neuronal fate specification.

Materials and Methods

Cell culture and transfection

Mouse N1E-115 cells (American Tissue Culture Collection) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 2% L-glutamine and 1% penicillin/streptomycin (all reagents from Sigma Aldrich). For differentiation, cells were starved overnight in Neurobasal medium (Invitrogen) with 2% L-glutamine and 1% penicillin/streptomycin. After starvation, cells were replated on 10 µg/ml laminin (Millipore)- coated coverlisps. For *Hmgn5* KD, cells were transfected with 80 nM siRNA (Dharmacon siRNA smartpool Plus or a single Dharmacon siRNA (J-044143-05) for rescue experiments). For plasmid transfection, 400 ng of plasmid were used for 75000 cells. Transfections were performed with TransFectin reagent (Biorad) as previously described (Feltrin et al, 2012). Cells were starved 48 hours post-transfection, reseeded 72 hours post-transfection and analyzed between 4-24 hours post-transfection according to the experiment performed.

Neurite purification, RNA extraction and RT-qPCR analysis

Purification of total RNA from soma and neurite fractions of N1E-115 cells was performed as previously described (Feltrin et al, 2012). Total RNA from whole cell preparations was isolated with the Nucleospin RNA II kit (Macherey-Nagel). For RT-qPCR, 1 µg of total RNA was reverse transcribed with random primers using the ImProm-II Reverse Transcription System (Promega). qPCR was performed using the GoTaq qPCR Master Mix (Promega) in a Biorad instrument with the primers indicated in Table E1. *Rpl19* mRNA was used as a normalization control in all experiments.

Immunofluorescence and Western Blot

N1E-115 cells and hippocampal neurons were fixed with PBS containing 4% paraformaldehyde (Sigma Aldrich) 96 hours post-transfection or at DIV 3 or DIV 7 respectively. They were then permeabilized for two minutes in PBS- 1% Triton-X and

blocked for 30 minutes in PBS-0.1% Triton-X-2% BSA. Cells were stained with primary antibodies for one hour at room temperature in PBS-0.1% Triton-X-2% BSA, washed, and then incubated with secondary antibodies and DAPI (Invitrogen) for one hour at room temperature. Where indicated, phalloidin conjugated to different Alexa fluorophores (Invitrogen) was added to the secondary antibody mix to stain for F-actin. After extensive washes, coverslips were mounted overnight with DAKO fluorescent mounting medium (DAKO). For Western Blot analysis, cells were lysed in RIPA buffer containing protease inhibitors on ice for 30 minutes and then centrifuged at maximum speed at 4°C for 15 minutes. 10 µg of protein lysates were run on NuPAGE 4-12% Bis-Tris gels (Life Technologies) and transferred to PVDF microporous membrane (Immobilion-FL). The membrane was then incubated overnight at 4°C with primary antibodies, washed and incubated with HRP-conjugated secondary antibodies at room temperature for one hour. After washing, the signal was revealed with the Amersham ECL Prime Western Blotting Detection Reagent by autoradiography.

FISH

Template plasmids were linearized and reverse transcribed with T3 (antisense) or T7 (sense) polymerases (Roche) in the presence of digoxigenin RNA labeling mix (Roche) following the manufacturer's protocol. FISH was performed as previously described (Feltrin et al, 2012). The primers used to generate FISH probes are listed in Table E1.

Microscopy, image acquisition, and analysis

Wide field microscope experiments were performed on an inverted Eclipse Ti microscope (Nikon). Phase contrast live imaging of neurite outgrowth and Dendra2 photobleaching experiments were performed as previously described (Feltrin et al, 2012). For histone H1 FRAP experiments, bleaching was performed with the 488 nm laser from a FRAP3D module (Roper Scientific). A spot ~ 3 µm in diameter was bleached with a 400-ms bleach pulse and recovery epifluorescence images were collected in the green channel every 3 s for 200 s. Quantification of fluorescence

recovery was done as previously described (Phair & Misteli, 2000). For photoconversion experiments, distal neurites and growth cones of N1E-115 cells were photoconverted with a 1 s pulse of UV light (excitation filter: 377/50 nm, dichroic mirror: 409 nm, using Leica EL6000 fluorescence lamp) and images were taken every 10 s for 2 min in a Leica DMI 6000 B inverted microscope equipped with a temperature controlled incubation chamber using Leica Application Suite software. To analyze neurite outgrowth, automated neurite segmentation was performed using Metamorph software or the Simple Neurite Tracer plugin of ImageJ. Quantification of staining or fluorescent protein intensities was performed with either Metamorph or ImageJ. Quantification of Western Blot band intensities was performed with ImageJ. Quantification of the number of heterochromatic foci in DAPI-stained nuclei was done using the Find Maxima process of ImageJ. Where fluorescence intensities are compared, images are all equally scaled. The outcome of the first repetition of many of the experiments (Figures 4D-E, 4G-H, 6A-B) presented in the manuscript was assessed in a blinded fashion.

Statistical analysis

Data are presented as mean ± standard error of the mean (s.e.m.). Statistical analysis was performed with GraphPad Prism 6 software. Kolmogorov-Smirnov test was used to compare neurite length distributions, while paired or unpaired (according to the type of experiment) two-tailed t-test was used for parametric distributions. Normality of the distributions was assessed with the SPSS Statistics software (IBM).

Antibodies and plasmids

The following antibodies were used for Western Blot and immunofluorescence: anti-α tubulin (Sigma), anti- H3K9me3 (Abcam), anti-GFP (Roche), anti p-GSK3β (Cell Signaling), anti-doublecortin (Santa Cruz Biotechnology), anti- βIII tubulin (Abcam), anti-MAP2 (Millipore), anti-SMI312 (Covance). Secondary HRP-conjugated antibodies were from GE Healthcare while secondary Alexa fluorophore-conjugated antibodies were from Invitrogen. Anti-HMGN5 antibody was described previously (Shirakawa et al, 2000). The PalX2-Dendra2 construct (Welshhans & Bassell, 2011)

was flanked at the 3' end with *Hmgn5* 3'UTR. The Dendra2-HMGN5-3'UTR construct was generated by removing the palmitoylation sequence and inserting *Hmgn5* coding sequence 5' to Dendra2 and *Hmgn5* 3'UTR 3' to Dendra2. HMGN5 overexpression constructs were generated by cloning *Hmgn5* coding sequence into pEGFP-N1 (BD Biosciences Clontech) at the 5' end of EGFP while *Hmgn5* 3'UTR was inserted at the 3'end of EGFP. Dendra2, GFP and GFP-HMGN5 constructs bear the ~ 200 nucleotide 3'UTR derived from the pEGFP-N1 vector. All constructs bear SV40 polyadenylation signal. *Hmgn5* coding sequence was made siRNA-resistant by site-directed mutagenesis. Overexpression and rescue constructs for primary neuron experiments were obtained by subcloning HMGN5-EGFP-3'UTR sequences into the pCAG vector. For generating FISH probes, the 3'UTR sequence of *Hmgn5* and the coding sequence of EGFP were cloned into pBluescript II KS (+/-) (Agilent Technologies). The EGFP-H1 construct was described previously (Malicet et al, 2011) while the mRuby2-HMGN5 expression constructs were obtained by cloning the mRuby2 coding sequence in the place of EGFP in the GFP-HMGN5 expression constructs. All primers used for cloning are listed in Table E1. Plasmid maps are available upon request.

Primary neurons isolation, transfection and culture

Mice were maintained on a 12-h day-night cycle with adequate food and water under specific pathogen-free conditions and according to Swiss Federal regulations and under license number AF-ZH. The day of vaginal plug was considered as embryonic day 0 (E0). Primary neurons were isolated from mouse embryos at E18.5. Hippocampi were dissected, trypsinized for 20 minutes and dissociated by trituration. 5-10 * $10^{\wedge 6}$ cells were transfected with 6 µg of plasmid DNA and/or 50 pmol of siRNA using the Amaxa Nucleofector II system and protocol number 0-005. Cells were subsequently plated on coverslips coated with 100 µg/ml poly-D-lysine and cultured in Neurobasal medium supplemented with 1X B27 (Gibco), 2mM Glutamax (Invitrogen) and 1% penicillin/streptomycin. Neurons were analyzed after 3 DIV or 7 DIV (Fig. E4). Only GFP-positive cells were used for analysis.

Microarray analysis

Microarray analysis of N1E-115 cells was performed in triplicate. cRNA Target was synthesized and amplified using the WT Expression Kit (Ambion), then fragmented, biotin-labeled using the WT Terminal Labeling and Controls Kit (Affymetrix) starting from 270ng total RNA. For each sample 12 µg of cRNA was used to generate cDNA. For each sample 3.75 µg of cDNA was fragmented. All synthesis reactions were carried out in 0.2ml tubes using a PCR machine (TProfessional Trio, Biometra, Gottingen, Germany) to ensure the highest possible degree of temperature control. The hybridization cocktail containing fragmented biotin-labeled target DNA at a final concentration of 25 ng/µl was transferred into Affymetrix GeneChip Mouse Gene 2.0 ST Array (Affymetrix) and incubated at 45°C on a rotator in a hybridization oven 640 (Affymetrix) for 17 h at 60 rpm. The arrays were washed and stained on a Fluidics Station 450 (Affymetrix) by using the Hybridization Wash and Stain Kit (Affymetrix) using the Fluidics Procedure FS450_0002. The GeneChips were processed with an Affymetrix GeneChip® Scanner 3000 7G (Affymetrix). DAT image files of the microarrays were generated using Affymetrix GeneChip Command Console (Affymetrix). Data were imported into R (ver. 3.0.2, R-core team ref) and normalized with RMA (Carvalho & Irizarry, 2010). For each Entrez gene only one probeset with highest variance across the dataset was selected (using the genefilter and annotation packages from the Bioconductor repository version 2.13, (Gentleman et al, 2004)). Moderated version of t-test (limma package, (Smyth, 2005)) was used to identify the differentially expressed genes. Obtained p-values were corrected for multiple testing using the Benjamini and Hochberg method. The microarray data files are available via (… accession number).

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Author Contribution

F.M. and O.P. conceived the experiments. F.M., C.R. and M.W. performed the experiments. F.M., M.W. and O.P. analyzed the results. R.I. performed analysis of microarray experiments. M.B. and V.T. provided vital reagents and intellectual contribution to the project. F.M. and O.P. wrote the paper with input from the other co-authors.

Conflict of Interest

The authors declare they have no conflict of interest.

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Figure legends

Figure 1. *Hmgn5* mRNA localizes to growth cones of N1E-115 cells by virtue of a 3'UTR localization signal. (A) Schematics of the neurite purification technique. Serum-starved, differentiated N1E-115 cells were allowed to extend neurites on a 3 µm microporous filter coated with laminin on the bottom part (red lines). Neurites growing in the bottom filter surface were then biochemically separated from the cell bodies. (B) Total RNA was purified from neurites and cell bodies (n=3, mean ± s.e.m.), reverse transcribed and used in RT-qPCR analysis with primers specific for *Hmgn5* and *snord15b* RNA (positive control for a cell body-enriched RNA). (C) Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense (negative control) to *Hmgn5* mRNA. FISH signal is represented in inverted black and white (ibw) contrast while F-actin staining is represented in green. Black arrowheads indicate punctate structures. Scale bars: 20 µm. (D) Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense (negative control) to *GFP* mRNA and *GFP* mRNA fused to *Hmgn5* 3'UTR. FISH signal is represented in ibw contrast while F-actin staining is represented in cyan. Black arrowheads indicate punctate structures. Scale bars: 20 µm.

Figure 2. *Hmgn5* 3'UTR drives local translation in the growth cone of N1E-115 cells. (A) Representative micrographs of live N1E-115 cells transfected with PalX2-Dendra2 reporters bearing *Hmgn5* or no 3'UTR pre- and post-bleaching. The cells were plated on laminin-coated coverslips for 4-6 hours, PalX2-Dendra2 signal was bleached in distal neurites and growth cones with intense green light and fluorescence recovery kinetics were acquired using time-lapse microscopy over 30 minutes. The bleached region corresponds to the whole neurite segment present in the field of view. Images are color-coded so that warm and cold colors represent respectively high and low fluorescence intensity. Scale bar: 20 µm. (B) The increase in average fluorescence intensity with respect to the average fluorescence intensity immediately after bleaching (F-F0) is represented as percentage of the initial post-bleaching intensity (F0) for each time point. Where indicated, cells were treated with 40 µM anisomycin. n=8 cells per condition over three independent experiments, mean ± s.e.m.

Figure 3. HMGN5 can be retrogradely transported along the neurites of N1E-115 cells. (A) Schematics of the photoconversion experiment. N1E-115 cells transfected with Dendra2 or Dendra2-HMGN5-3'UTR constructs were plated on laminin-coated coverslips for 10-12 hours. The Dendra2 green signal was photoconverted to red with UV illumination in the distal part of the neurite (>50 μm away from the soma) and the accumulation of red signal in the nucleus was measured over 2 minutes. The boxed regions correspond to the field of views shown in panel B, with region 1 corresponding to the distal neurites and region 2 corresponding to proximal neurites and cell bodies. (B) Representative micrographs pre- and post-UV conversion of cells expressing Dendra2 or Dendra2-HMGN5-3'UTR. Dendra2 is shown in green while photoconverted red Dendra2 is shown in pseudocolor, with warm and cold colors representing respectively high and low fluorescence intensity. Note that the neurites in the post-UV micrographs are slightly out of focus to appropriately focus on nuclei and that neurites images of Dendra2 construct have been rotated 90° to fit the figure layout. Scale bars: 20 μm. (C) Measurement of accumulation of red nuclear signal 2 minutes post-UV photoconversion. Non photoconverted cells in the same field as the photoconverted ones were used as controls for nonspecific increase in red fluorescence (white arrowhead in panel B). Percentage increase in red fluorescence for every cell was normalized against the average percent increase of non photoconverted cells. n= 17-24 cells over three independent experiments, mean ± s.e.m. Statistical significance was evaluated by a two-tailed paired t-test (** p< 0.01, ns= not significant).

Figure 4. *Hmgn5* mRNA localization is important for neurite outgrowth in N1E-115 cells. (A-C) N1E-115 cells were transfected with control (ctrl) or *Hmgn5* siRNA and knock-down (KD) efficiency was monitored by RT-qPCR (A), Western Blot (B) and immunofluorescence staining (C). *Rpl19* mRNA serves as an internal control for RT-qPCR (A) while the upper unspecific band in panel B serves as a loading control for Western Blot. For panel A: n=3 RNA preparations and for panel C: n=8-12 cells, mean ± s.e.m. In panel C, HMGN5 staining is shown in pseudocolor, with warm and cold colors representing respectively high and low fluorescence intensity, while DAPI staining is shown in ibw contrast. Scale bar: 20 μm. (D) Representative micrographs

in ibw contrast of α-tubulin stained N1E-115 cells transfected with ctrl or *Hmgn5* siRNA. Scale bar: 50 μm. (E) Neurite length measurement of N1E-115 cells transfected with ctrl or *Hmgn5* siRNA (n=120 cells from three independent experiments, mean ± s.e.m). Statistical significance was evaluated by a Kolmogorov-Smirnov test (*** p< 0.001). (F) Neurite outgrowth dynamics of ctrl and *Hmgn5* siRNA-transfected N1E-115 cells analyzed by phase-contrast time-lapse microscopy. Arrowheads point to neurite protrusion/retraction events. Scale bars: 50 μm. Time scale is in hours:minutes. (G) Representative micrographs of α-tubulin stained N1E-115 cells transfected with siRNA and GFP rescue constructs. α-tubulin staining is shown in ibw contrast while GFP signal is shown in green. Scale bar: 50 μm. (H) Neurite length measurement of N1E-115 cells transfected with ctrl or *Hmgn5* siRNA and with rescue constructs (n=100 cells from three independent experiments, mean ± s.e.m). Statistical significance was evaluated by a Kolmogorov-Smirnov test (** p< 0.01, *** p< 0.001, ns= not significant).

Figure 5. *Hmgn5* KD impairs neurite outgrowth in hippocampal neurons. (A-B) Hippocampal neurons were transfected with ctrl or *Hmgn5* siRNA and KD efficiency was monitored in DIV3 neurons by RT-qPCR (A) and immunofluorescence staining (B). For panel A: n=2 RNA preparations and for panel B: n= 22-25 cells, mean ± s.e.m. In panel B, HMGN5 staining is shown in pseudocolor, with warm and cold colors representing respectively high and low fluorescence intensity, while GFP signal is shown in green. Arrowheads point to transfected neurons. Scale bar: 20 μm. (C) Representative confocal micrographs of doublecortin stained hippocampal neurons transfected with ctrl or *Hmgn5* siRNA and with rescue constructs. Doublecortin staining is shown in ibw contrast while GFP signal is shown in green. Scale bar: 20 μm. (D) Neurite length measurement of DIV3 hippocampal neurons transfected with ctrl or *Hmgn5* siRNA and with rescue constructs (n= 75-85 cells over three independent experiments, mean ± s.e.m.). Statistical significance was evaluated by a Kolmogorov-Smirnov test (*** p< 0.001, ns= not significant).

Figure 6. HMGN5 stimulates neurite outgrowth and chromatin decompaction in N1E-115 cells in a 3'UTR-dependent manner. (A) Representative micrographs of α-tubulin stained N1E-115 cells transfected with GFP, GFP-HMGN5, GFP-HMGN5-

3’UTR, GFP-HMGN5SE or GFP-HMGN5SE-3’UTR. α-tubulin staining is shown in ibw contrast while GFP signal is shown in green. Scale bar: 50 μm. (B) Neurite length measurement of N1E-115 cells transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3’UTR (n=100 cells from three independent experiments, mean ± s.e.m). Statistical significance was evaluated by a Kolmogorov-Smirnov test (*** p<0.001, ns= not significant). (C) Representative confocal micrographs of DAPI and H3K9me3 stained N1E-115 cells transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3’UTR. DAPI staining is shown in ibw contrast, H3K9me in red, while GFP signal is shown in green. A confocal plane in the middle of the soma was chosen to better focus on nuclei, images were collected on the same day with identical exposure settings. Scale bar: 10 μm. (D) Measurement of the number of heterochromatic foci in the DAPI staining of N1E-115 cells transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3’UTR (n= 60 cells over three independent experiments, mean ± s.e.m). Statistical significance was evaluated by a two-tailed paired t-test (* p< 0.05, ns= not significant). (E) Quantification of mean fluorescence intensity of H3K9me3 foci in N1E-115 cells transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3’UTR (n= 160-230 foci over four independent acquisitions, mean ± s.e.m). Statistical significance was evaluated by a two-tailed unpaired t-test (* p< 0.05, ns= not significant).

Figure 7. HMGN5 decreases histone H1 binding to chromatin in N1E-115 cells. (A) Representative micrographs of the FRAP experiment. The black and white panels represent histone H1-GFP signal and the pseudocolor images represent magnifications of the bleached area indicated by a dotted circle, with warm and cold colors representing respectively high and low fluorescence intensity. Scale bar: 3 μm. (B) Representative micrographs of cells expressing histone H1-GFP and either mRuby2, mRuby2-HMGN5 or mRuby2-HMGN5-3’UTR. Scale bar: 10 μm. (C) Fluorescence recovery curves of histone H1-GFP expressing cells transfected with the different Ruby constructs. The curves represent averages from n= 11 cells over three independent experiments. After quantification of absolute fluorescence recovery (see Materials and Methods), the post-bleach fluorescence intensity was normalized to 1 for every cell analyzed and relative fluorescence recovery was calculated. (D) Histogram showing the time required to recover 60% of histone H1-GFP fluorescence

intensity. Statistical significance was evaluated by a two-tailed paired t-test (** $p < 0.01$, ns= not significant).

Figure 8. HMGN5 promotes neurite outgrowth and controls chromatin structure in hippocampal neurons. (A) Representative confocal micrographs of βIII -tubulin stained hippocampal neurons transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3'UTR. βIII -tubulin staining is shown in ibw contrast while GFP signal is shown in green. Scale bar: 20 μm. (B) Neurite length measurement of DIV3 hippocampal neurons transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3'UTR (n=85-90 cells from three independent experiments, mean ± s.e.m.). Statistical significance was evaluated by a Kolmogorov-Smirnov test (*** $p < 0.001$, ns= not significant). (C) Representative confocal micrographs of DAPI and H3K9me3 stained hippocampal neurons transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3'UTR. GFP signal is shown in green, DAPI staining is shown in ibw contrast and H3K9me3 staining is shown in red. The confocal plane was chosen to better focus on nuclei, images were collected on the same day with identical exposure settings. Scale bar: 5 μm. (D) Measurement of the number of heterochromatic foci in the DAPI staining of hippocampal neurons transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3'UTR (n= 65 cells over three independent experiments, mean ± s.e.m.). Statistical significance was evaluated by a two-tailed paired t-test (* $p < 0.05$, ns= not significant). (E) Quantification of mean fluorescence intensity of H3K9me3 foci in hippocampal neurons transfected with GFP, GFP-HMGN5 or GFP-HMGN5-3'UTR (n= 20-40 foci over three independent acquisitions, mean ± s.e.m.). Statistical significance was evaluated by a two-tailed unpaired t-test (* $p < 0.05$, ns= not significant).

Expanded View Items Legends

Figure E1. (A) Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense (negative control) to *Hmgn5* mRNA on DIV3 hippocampal neurons. FISH signal is represented ibw contrast while F-actin staining is represented in green. Black arrowheads indicate punctate structures. Scale bar: 20 µm for whole cell micrographs, 5 µm for growth cone micrographs. (B) Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense (negative control) to *GFP* mRNA. Hippocampal neurons were transfected with the different rescue constructs and then subjected to FISH analysis. FISH signal is represented in ibw contrast while F-actin staining is in cyan. Black arrowheads indicate punctate structures. Scale bar: 20 µm for whole cell micrographs, 5 µm for growth cone micrographs.

Figure E2. Total RNA (n=3 preparations) was extracted from control (non-differentiated and differentiated) and *Hmgn5* KD cells and subjected to microarray analysis. (A) List of all the genes whose expression is significantly affected by *Hmgn5* KD. "kd vs ctrl" indicates the difference in expression levels between *Hmgn5* KD and differentiated control cells while "4h vs 24h" indicates the difference in expression levels between non-differentiated and differentiated control cells. Up-regulated genes are presented in shades of green, while down-regulated genes are presented in shades of red. (B) Validation of part of the microarray data by RT-qPCR (on two independent RNA preparations, mean ± s.e.m). (C) Micrographs of control and *Hmgn5* KD N1E-115 cells stained with phalloidin (shown in ibw contrast) and anti-p-GSK 3β antibodies (shown in pseudocolor, with warm and cold colors representing respectively high and low fluorescence intensity). The graph represents the quantification of p-GSK 3β staining intensity (n=70-150 cells over four independent acquisitions, mean ± s.e.m). Statistical significance was evaluated by a two-tailed unpaired t-test (* $p< 0.05$). Scale bar: 50 µm.

Figure E3. (A) Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense (negative control) to GFP mRNA. N1E-115 cells were transfected with the different rescue constructs and then subjected to FISH analysis. FISH signal is represented in ibw contrast. Black arrowheads indicate punctate structures. Scale

bars: 20 μm. (B) Western blot analysis with anti-GFP and anti- α tubulin antibodies and quantification to show that the different rescue constructs are expressed to approximately the same level in N1E-115 cells (n=3 experiments, mean ± s.e.m.).

Figure E4. Confocal fluorescence micrographs of hippocampal neurons transfected with control or *Hmgn5* siRNA and GFP and stained with anti-MAP2 and anti-SMI312 antibodies. Neurons were fixed either at 3 or 7 DIV. Scale bar: 20 μm.

Figure E5. (A) Representative micrographs of DAPI stained N1E-115 cells transfected with GFP, GFP-HMGN5, GFP-HMGN5-3'UTR, GFP-HMGN5SE or GFP-HMGN5SE-3'UTR. GFP signal is shown in green while DAPI staining is shown in ibw contrast. Scale bar: 10 μm. (D) Measurement of the number of heterochromatic foci in the DAPI staining of N1E-115 cells transfected with GFP, GFP-HMGN5, GFP-HMGN5-3'UTR, GFP-HMGN5SE or GFP-HMGN5SE-3'UTR (n= 20 cells over one experiment).

Table E1. List of all the primers used in this study.

Movie E1. Visualization of growth cone mRNA translation using PalX2-Dendra2 reporters. Time-lapse imaging of growth cones of PalX2-Dendra2 and PalX2-Dendra2/*Hmgn5* 3'UTR transfected N1E-115 cells, treated or non-treated with 40 μm anisomycin 30 minutes before bleaching. Pre-bleaching images and fluorescence recovery after bleaching time-lapses are shown. The images are color-coded so that warm and cold colors represent high and low fluorescence intensity. Timescale is in minutes:seconds. Scale bars: 20 μm.

Movie E2. Neurite outgrowth dynamics of control and *Hmgn5* KD N1E-115 cells. Phase-contrast time-lapse imaging of control and *Hmgn5* KD N1E-115 cells. Timescale is in hours:minutes. Scale bars: 50 μm. Note that control cells establish long neurites over time, while *Hmgn5* KD cells fail to do so.

Movie E3. Representative movie of the histone H1 FRAP analysis. Time-lapse green fluorescence imaging of an N1E-115 cell nucleus transfected with histone H1-GFP

and mRuby2. Pre-bleaching image, bleaching and fluorescence recovery after bleaching are shown. The images are presented in black and white contrast. Timescale is in seconds:milliseconds. Scale bar: 3 μm.