Molecular and Cellular Neuroscience 101 (2019) 103411

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

CBP and p300 coactivators contribute to the maintenance of *Isl1* expression by the Onecut transcription factors in embryonic spinal motor neurons

Mathilde Toch, Frédéric Clotman*

Université catholique de Louvain, Institute of Neuroscience, Laboratory of Neural Differentiation, avenue Hippocrate 55, box B1.55.11, 1200 Brussels, Belgium

ARTICLE INFO

Keywords:
Onecut transcription factors
Isl1
CBP
p300
Motor neurons
Spinal cord

ABSTRACT

Onecut transcription factors are required to maintain *Islet1* (*Isl1*) expression in developing spinal motor neurons (MNs), and this process is critical for proper MN differentiation. However, the mechanisms whereby OC stimulate *Isl1* expression remain unknown. CREB-binding protein (CBP) and p300 paralogs are transcriptional coactivators that interact with OC proteins in hepatic cells. In the embryonic spinal cord, CBP and p300 play key roles in neurogenesis and MN differentiation. Here, using chromatin immunoprecipitation and *in ovo* electroporation in chicken spinal cord, we provide evidence that CBP and p300 contribute to the regulation of *Isl1* expression by the OC factors in embryonic spinal MNs. CBP and p300 are detected on the CREST2 enhancer of *Isl1* where OC factors are also bound. Inhibition of CBP and p300 activity inhibits activation of the CREST2 enhancer and prevents the stimulation of *Isl1* expression by the OC factors. These observations suggest that CBP and p300 coactivators cooperate with OC factors to maintain *Isl1* expression in postmitotic MNs.

1. Introduction

In the developing nervous system, genetic programs controlled by transcription factors regulate the acquisition of specific neuronal identities (Dasen, 2017; Francius and Clotman, 2014; Lai et al., 2016; Lu et al., 2015). Transcription factors recruit cofactors that provide intrinsic histone acetyltransferase (HAT) or histone deacetylase activity to remodel chromatin towards the appropriate activation state and thereby enable proper regulation of gene expression. In the spinal motor neurons (MNs), the CREB-binding protein (CBP) and p300 paralog coactivators have been shown to regulate neurogenesis and subsequent steps of neuronal differentiation (Lee et al., 2009; Partanen et al., 1999). However, CBP and p300 lack DNA-binding activity, and the proteins that recruit these coactivators to regulate MN differentiation remain unknown.

The LIM-homeodomain transcriptional repressor Islet1 (Isl1) is present in spinal MNs and dI3 dorsal interneurons and is required for proper MN development (Francius and Clotman, 2014; Lu et al., 2015). Constitutive or conditional inactivation of *Isl1* results in loss of MNs early during development (Pfaff et al., 1996; Song et al., 2009). Initial activation of *Isl1* expression depends on the early MN determinant Hb9 (Arber et al., 1999; Tanabe et al., 1998; Thaler et al., 1999; William et al., 2003). However, maintenance of *Isl1* expression at later stages of differentiation relies on direct stimulation by transcription factors of the Onecut (OC) family (Rhee et al., 2016; Roy et al., 2012). OC factors comprise three members in mammals, namely OC-1 (also called HNF-6 for Hepatocyte Nuclear Factor-6), OC-2 and OC-3. They are described as transcriptional activators characterized by two DNA binding domains: a cut domain and a divergent homeodomain (Lemaigre et al., 1996). In the CNS, they are detected early during neuronal differentiation in multiple cell populations including spinal MNs (Audouard et al., 2013; Chakrabarty et al., 2012; Espana and Clotman, 2012a, 2012b; Francius and Clotman, 2010; Francius et al., 2013; Hodge et al., 2007; Kabayiza et al., 2017; Stam et al., 2012; Wu et al., 2012). In the absence of OC proteins, *Isl1* expression is initiated properly but is not maintained in maturing MNs, resulting in the partial conversion of somatic into visceral MNs and in the complete conversion of medial MNs of the lateral motor column (LMCm), which innervate the ventral muscles of the limbs, into the identity of their lateral counterparts (LMCl), which innervate the dorsal limb muscles (Francius and Clotman, 2014; Roy et al., 2012). OC factors maintain MN *Isl1* expression through direct binding to the CREST2 enhancer and by stimulating CREST2 activity (Kim et al., 2015; Rhee et al., 2016; Roy et al., 2012). However, the molecular partners that OC proteins interact with to stimulate *Isl1* expression remain unknown.

In hepatic cells, OC-1 has been shown to recruit cofactors carrying HAT activity to stimulate expression of target genes including *FoxA2* and *ttr*. On *FoxA2* regulating sequences, OC-1 binds to DNA using the

* Corresponding author.
E-mail address: frederic.clotman@uclouvain.be (F. Clotman).

https://doi.org/10.1016/j.mcn.2019.103411
Received 11 February 2019; Received in revised form 14 August 2019; Accepted 11 September 2019
Available online 21 October 2019

cut domain, and the cut and the homeodomain contribute to recruit the coactivator CBP. In contrast, on target sequences of *ttr* types, OC-1 binds using the cut and the homeodomain and instead recruits the coactivator p300 (Iyaguchi et al., 2007; Lannoy et al., 2000; Rausa et al., 2003). Interestingly, conditional inactivation of CBP and p300 in the CNS results in alterations of MN differentiation (Lee et al., 2009). The number of MN was reduced in CBP/p300 compound mutant embryos, suggesting that these coactivators act redundantly in neural progenitors to promote MN specification. In addition, MN cell body position was aberrant, with a significant proportion of cells emigrating outside of the spinal cord along the ventral root, indicating that CBP and p300 act also in postmitotic MNs to regulate later aspects of development (Lee et al., 2009). This phenotype is reminiscent of abnormal emigration of spinal MNs along the ventral root observed upon down-regulation of *Isl1* in MNs (Lee et al., 2015), suggesting that postmitotic MN activity of CBP and p300 may depend on Isl1.

Therefore, we asked whether CBP and p300 coactivators might contribute to the regulation of *Isl1* expression by the OC transcription factors. Here, we show that both coactivators are bound to the CREST2 enhancer of *Isl1* where OC factors are also found. We demonstrate that the activation of the CREST2 enhancer depends on CBP and p300 activity. Finally, we show that CBP and p300 activity is required for the stimulation of *Isl1* expression by the OC factors. Taken together, these observations suggest that CBP and p300 coactivators contribute to the maintenance of *Isl1* expression by the OC transcription factors in embryonic spinal MNs.

2. Materials and methods

2.1. Chromatin immunoprecipitation (ChIP) assay

Neural tubes were dissected from Hamburger-Hamilton (HH) 19 stage chicken embryos and dissociated in DMEM/F12 using a Dounce homogenizer, as previously described (Roy et al., 2012). Cell homogenates were treated with 1% formaldehyde at room temperature for 10 min. Chromatin was fragmented to 200–1000 bp by sonication (high power, 30 cycles of 30 s with 1 min pulse interval) and incubated overnight at 4 °C under rotation with an antibody specific of the factor of interest or mouse IgG as a negative control. Immunoprecipitation was performed using a ChIP Kit (Millipore 17–371) according to the manufacturer's instructions. Antibodies were rabbit anti-OC1 at 1:50 (Santa Cruz sc-13,050), rabbit anti-CBP at 1:50 (Santa Cruz sc-369), rabbit anti-p300 at 1:25 (Santa Cruz sc-584) and rabbit anti-acetyl-histone H3 that recognizes acetylated N-terminal regions of Histone H3 at 1:25 (Merck 06–599). Fold enrichment was calculated over IgG using $2^{(-ddCt)}$, where $ddCt = (Ct_{ip}\text{-}Ct_{input}) - (Ct_{IgG}\text{-}Ct_{input})$ on CREST1, CREST2, CREST3 and noggin sequences (Roy et al., 2012).

2.2. Chicken embryonic spinal cord electroporations

In ovo electroporation was performed at stage HH10-12, as previously described (Roy et al., 2012). *pCMV-Oc1-flag* (0.5 μg/μl) with or without the *pcS2-E1A-HA* (1 μg/μl) was co-electroporated with a *pCMV-eGFP* plasmid to visualize electroporated cells at concentrations required to standardize the final DNA concentration (2 μg/μl). Embryos were collected 6 h after electroporation (HH12-13) and fixed in PBS1x/4% paraformaldehyde (PFA) for 10 min. The *zCREST2-hsp70:GFP* reporter vector (3 μg/μl) (kindly provided by H. Okamoto (Uemura et al., 2005)) with or without the *pcS2-E1A-HA* (1 μg/μl) was co-electroporated with a *pCAG-DsRed2* plasmid to visualize electroporated cells at concentrations required to standardize the final DNA concentration (4.25 μg/μl). Embryos were collected 48 h after electroporation (HH20-22) and fixed in PBS/4%PFA for 25 min.

2.3. Immunofluorescence labelings and quantifications

After fixation, chicken embryos were washed thrice in PBS1x for 10 min and incubated in PBS1x/30% sucrose overnight at 4 °C. They were embedded and frozen in PBS1x/15% sucrose/7.5% gelatin before cryosectioning (14 μm). Immunofluorescent labelings were performed as described (Francius and Clotman, 2010). Primary antibodies were chicken anti-GFP at 1:2000 (Aves Lab GFP-1020) or rat anti-GFP at 1:3000 (kindly provided from S. Malas), rabbit anti-HA at 1:1000 (Cell signaling C29F4), mouse anti-Hb9/MNR2 at 1:1000 (DSHB 81.5C10), goat anti-Isl1 at 1:3000 (Neuromics GT15051), rabbit anti-OC1 at 1:100 (Santa Cruz sc-13050) or guinea pig anti-OC1 at 1:1000 (Novo Nordisk 4079C), rabbit anti-RFP at 1:1000 (Rockland 600-401-379) and mouse anti-β_{III}-tubulin (Tuj1 antibody) at 1:6000 (Covance MMS-435P-0100). Secondary antibodies were donkey anti-chicken/AlexaFluor 488, donkey anti-sheep/AlexaFluor 594 or 647, donkey anti-mouse/AlexaFluor 488 or 647, donkey anti-guinea pig/AlexaFluor 647, donkey anti-rat/AlexaFluor 488 and donkey anti-rabbit/AlexaFluor 594 or 647 purchased from Jackson Laboratories or Thermo Fisher used at 1:1000 or 1:2000 respectively. Immunofluorescence images of cryosections were acquired on a Confocal laser Scanning biological microscope FV1000 Fluoview with the FV10-ASW 01.02 software (Olympus). The images were treated with Adobe Photoshop CS5 software to match brightness and contrast with the observations. For quantifications, cells were counted on five sections of spinal cord in at least five independent embryos ($n \geq 5$) using the count analysis tool of Adobe Photoshop CS5 software. SigmatPlot v11 was used to perform statistical analyses. Standard Student's *t*-tests or Mann-Whitney *U* tests were applied based on the number of comparisons and on the variance in each group. Quantitative analyses were considered significant at $p \leq 0.05$.

3. Results

3.1. CBP and p300 coactivators are bound to the CREST2 enhancer of Isl1

CBP and p300 coactivators are initially detected at embryonic day (E)8.5 in the mouse throughout the developing neural tube. At later stages, they are produced in MNs and play key role during MN generation (Lee et al., 2009). *OC* factor expression is initiated around E9.0 in newborn MNs (Francius and Clotman, 2010). OC contribute to maintain MN *Isl1* expression through direct binding to the CREST2 enhancer (Kim et al., 2015; Rhee et al., 2016; Roy et al., 2012). Indeed, the regulation of *Isl1* is under the control of three conserved enhancers called CREST1-3 (Uemura et al., 2005). Our laboratory previously showed that CREST1 contains one potential OC binding site (5′-(A/T/G)(A/T)(A/G) TC(A/C)AT N (A/T/G)-3′) (Jacquemin et al., 2000; Lannoy et al., 1998), that CREST2 contains two such sequences while CREST3 has none (Roy et al., 2012) (Fig. 1A, red boxes). Furthermore, we and others previously showed by ChIP assay that OC-1 binds preferentially to the CREST2 enhancer of *Isl1* (Kim et al., 2015; Roy et al., 2012). We hypothesized that CBP and p300 cofactors may contribute to the regulation of *Isl1* expression by OC proteins. To address this hypothesis, we first assessed the binding of CBP and p300, which are ubiquitously detected in the spinal neuroepithelium (Lee et al., 2009; Fig. S1), to the *Isl1* enhancers in the chicken embryonic spinal cord at comparable developmental stages. The Noggin promoter served as a negative control as it is not bound by OC factors (Roy et al., 2012). ChIP assay indicated that, as observed for the single chicken OC protein (cOC-1) (Francius and Clotman, 2010), endogenous CBP was highly enriched on CREST2. This coactivator is also mildly enriched on CREST3 and on Noggin promoter, and in lower amounts on CREST1 (Fig. 1B). In comparison, p300 was enriched on CREST2 and less on CREST3 and on the Noggin promoter but not on CREST1. This indicates that CBP and p300 are found on CREST2 enhancer as observed for OC factors. As CBP and p300 coactivators contribute to transcriptional activity through their intrinsic HAT activity, histone H3 acetylation was similarly

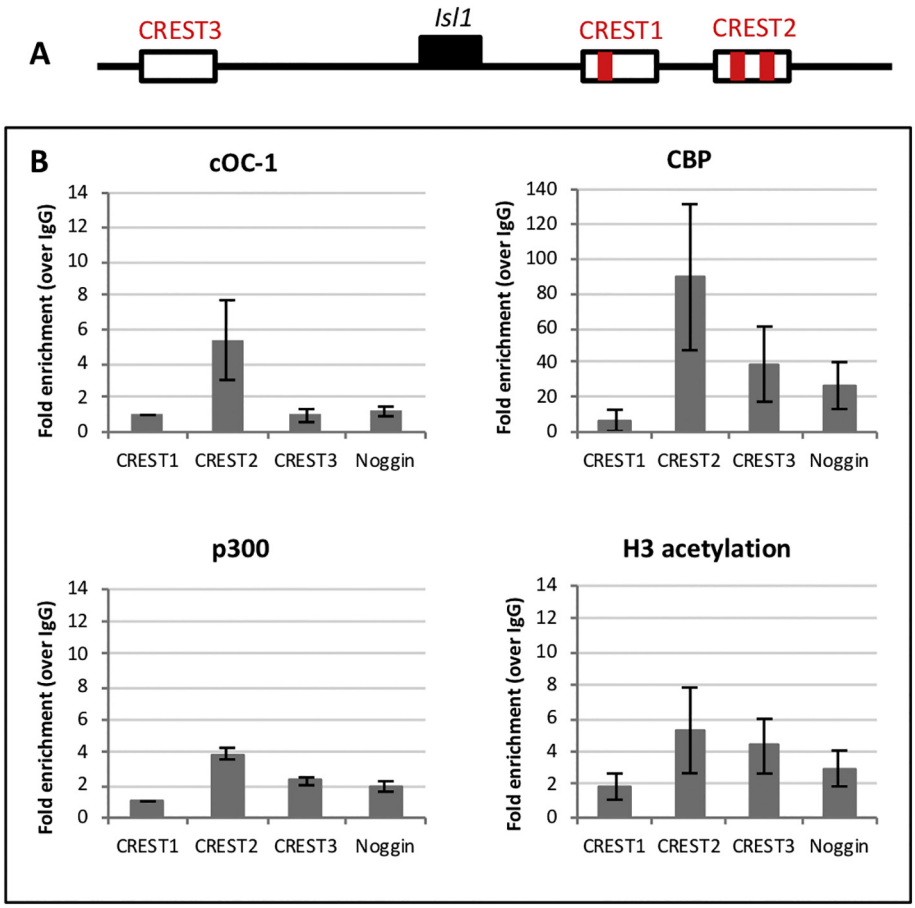

Fig. 1. CBP and p300 coactivators are bound to the CREST2 enhancer of *Isl1*. (A) Schematic representation of the *Isl1* enhancers. The coding sequence of *Isl1* is represented by the black box with the upstream CREST3 enhancer and downstream CREST1 and CREST2 enhancers shown as white boxes. The red boxes in enhancers correspond to potential OC binding sequences. (B) Binding of endogenous chicken cOC-1, CBP and p300 on *Isl1* enhancers assessed by ChIP. CBP and p300 coactivators are preferential bound on the CREST2 enhancer, like cOC-1, although they are also detected on CREST3 and to a lesser extent on the Noggin promoter. Furthermore, the CREST2 enhancer is acetylated and transcriptionally active as evaluated by the acetylation of histone H3. $n \geq 3$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assessed by ChIP assay. Consistently, CREST2 and CREST3 enhancers, as well as the Noggin promoter, were associated with enriched histone H3 acetylation and thereby increased chromatin accessibility, with an enrichment profile similar to the binding of the coactivators, i.e., CREST2 > CREST3 > Noggin > CREST1 (Fig. 1B). These data indicate that CBP and p300 are physically recruited to a cis-regulatory enhancer of *Isl1* known to be active in motor neurons and bound by OC proteins.

3.2. CBP and p300 contribute to the activity of the CREST2 enhancer of Isl1

During embryonic development, Isl1 is produced in differentiating MNs, in dorsal dI3 interneurons and in the sensory neurons located in the dorsal root ganglia. The CREST2 enhancer stimulates reporter gene expression (zCREST2-hsp:GFP plasmid) in sensory neurons and in part of MNs 24 h after chicken embryonic spinal cord electroporation (Kim et al., 2015). To assess the contribution of CBP and p300 to CREST2 activity, zCREST2-hsp:GFP was electroporated with (Fig. 2F–J) or without (Fig. 2A–E) the pcS2-E1A-HA plasmid, which encodes the adenovirus E1A oncoprotein that directly and specifically inhibits the HAT activity of the CBP and p300 cofactors (Arany et al., 1995; Lundblad et al., 1995). Production of E1A was detectable as early as 6 h after electroporation and was still visible after 48 h (Figs. 2G', 3E' and S2). Labeling for β_{III}-tubulin demonstrated that ectopic E1A production did not interfere with neuronal maturation (Fig. S3). In the absence of E1A, CREST2 was strongly stimulated in a majority of differentiating MNs labeled by Isl1 (Fig. 2C). However, in the presence of E1A, the proportion of MNs showing detectable CREST2 activity strongly decreased (Figs. 2H,K and S4). Furthermore, the presence of E1A significantly reduced the number of Isl1-positive MNs compared to the control side (Fig. 2D,I,L). Consistently, the number of MNs containing Hb9, the expression of which is stimulated by Isl1 and supports MN differentiation (Lee et al., 2008; Tanabe et al., 1998), was also significantly decreased when the HAT activity of CBP and p300 was inhibited (Fig. 2E,J,M). These results indicate that CBP and p300 contribute to the activity of the CREST2 enhancer of *Isl1* and confirm that these cofactors favor MN differentiation (Lee et al., 2009).

3.3. CBP and p300 are required for stimulation of Isl1 expression by OC factors

To assess whether CBP and p300 serve as key partners for OC factors to stimulate *Isl1* expression, we co-electroporated *Oc1* expression vector with or without the pcS2-E1A-HA plasmid in chicken embryonic spinal cord. As we previously reported, misexpression of *Oc1* induced ectopic production of *Isl1* as early as 6 h after electroporation (Fig. 3A–D), indicating that OC factors can stimulate *Isl1* expression independently from the cellular context (Roy et al., 2012). In contrast, the presence of E1A along with Oc1 (Fig. S2) completely prevented ectopic expression of *Isl1* despite similar overproduction of OC-1 (Fig. 3E–H). These observations demonstrate that the HAT activity of CBP and p300 coactivators contributes to the ectopic stimulation of *Isl1* expression by OC factors.

4. Discussion

Although multiple studies on *Isl1* expression and on its importance for MN production and differentiation in the embryonic spinal cord have been reported (Lee et al., 2008; Pfaff et al., 1996; Roy et al., 2012; Thaler et al., 2004; Thaler et al., 2002; Tsuchida et al., 1994), fewer is known about the molecular mechanisms that control the transcriptional regulation of this gene. Using chromatin immunoprecipitation and *in*

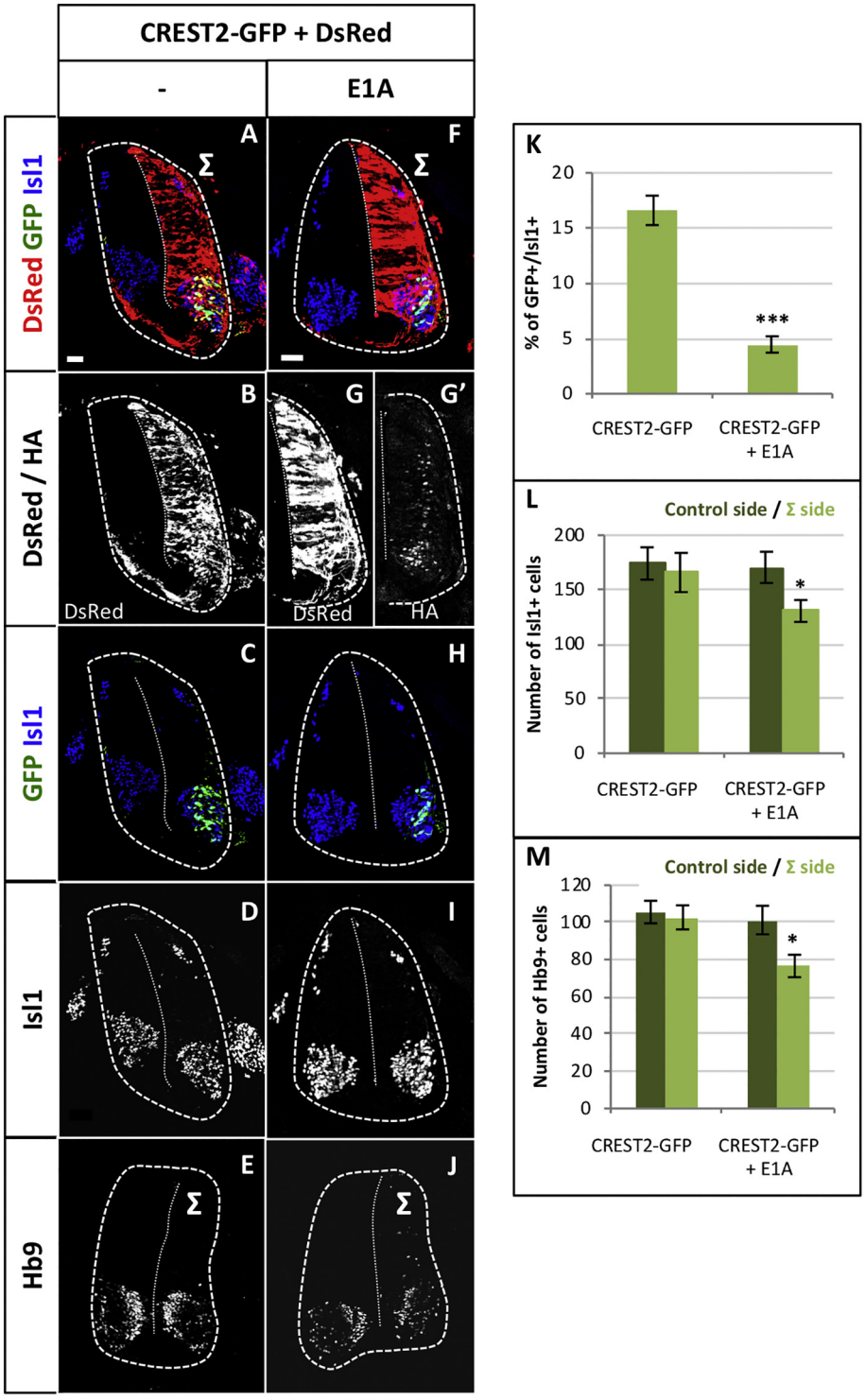

Fig. 2. CBP and p300 contribute to the activity of the CREST2 enhancer of *Isl1*. Immunolabelings for DsRed2, HA, Isl1 and Hb9 48 h after co-electroporation of the *CREST2-GFP* reporter construct with (F–J) or without (A–E) the *pcS2-E1A-HA* in chicken embryonic spinal cord at HH10-12. The *pCAG-DsRed2* is added as an electroporation control (B,G). (A–C) A reporter vector in which *GFP* expression is regulated by the CREST2 enhancer of *Isl1* is activated in most of the differentiating motor neurons. (F–H) In the presence of E1A (G'), which inhibits the histone acetyltransferase activity of CBP and p300, CREST2 activation is strongly decreased and GFP is detected in a lower proportion of motor neurons (H) although the electroporation efficacy is similar to the control (G). (K) Quantification of the proportion of motor neurons expressing GFP in the absence or in the presence of E1A (*t*-test; $p = 0.008$). (L,M) Quantification of the number of Isl1-positive (*t*-test; $p = 0.041$) or Hb9-positive (*t*-test; $p = 0.034$) motor neurons in the control or electroporated side of the spinal cord in the absence or in the presence of E1A. A,B,C,D and F,G,H,I are different images of the same sections, G' shows a section adjacent to that shown in F,G,H,I. Σ = electroporated side. $n = 7$. Scale bar = 50 μm.

ovo electroporation in chicken spinal cord, we obtained results that, in combination with previous reports (Lee et al., 2009), support the hypothesis that CBP and p300 coactivators cooperate with OC factors to regulate *Isl1* expression in postmitotics MNs.

Isl1 is critical for proper spinal MN development. Although not necessary to produce newly-born MNs, it is required for maintenance of MN identity. Indeed, constitutive inactivation of *Isl1* results in a loss of MNs (Pfaff et al., 1996). In contrast, hypomorphic *Isl1* allele or conditional inactivation of *Isl1* in spinal neurons results in the differentiation of hybrid cells coactivating MN and V2 interneuron differentiation programs (Song et al., 2009). *Isl1* expression is initiated early during the differentiation of newly-born MNs through the indirect action of Hb9 (Tanabe et al., 1998), and is later maintained in most MN subtypes (Tsuchida et al., 1994), notably through the activity of the OC factors (Kim et al., 2015; Roy et al., 2012). Subtype-specific expression of *Isl1* is controlled by three regulatory elements termed CREST1-3. CREST1, located downstream of the *Isl1* coding sequence, ensures *Isl1* expression in MNs that regulate the contraction of axial muscles of the back (MMC) and in visceral MNs of the preganglionic column (PGC) that innervate the ventral sympathetic chain and thereby control the contraction of visceral smooth muscles. CREST2, further downstream of CREST1, stimulates Isl1 production in MNs innervating intercostal muscles (HMC),

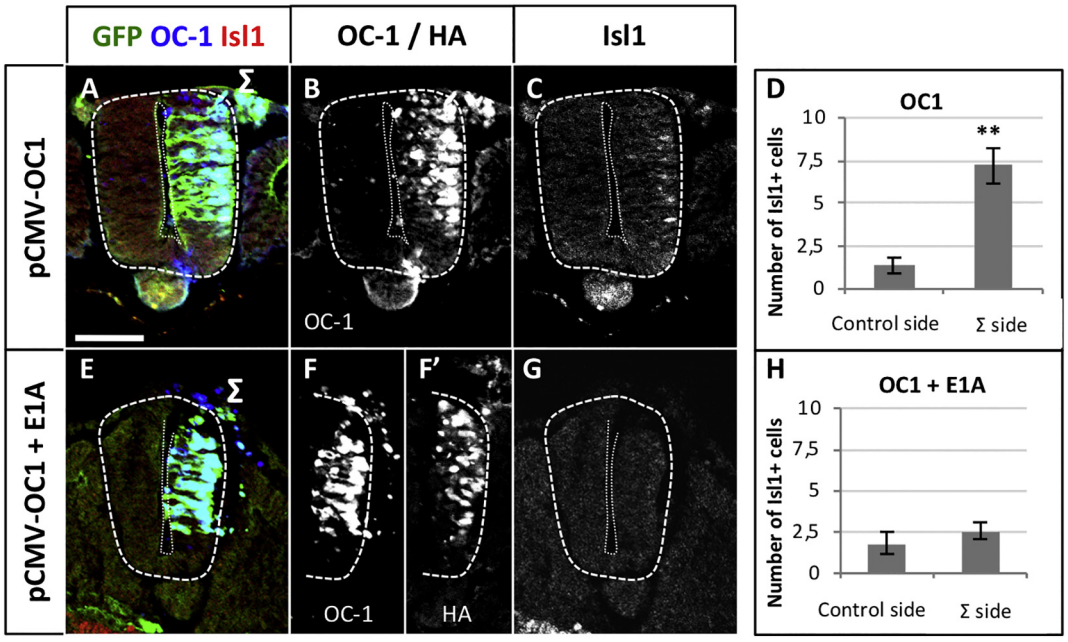

Fig. 3. CBP and p300 are required for stimulation of *Isl1* expression by OC factors. Immunolabelings for OC-1 and Isl1 6 h after co-electroporation of *pCMV-Oc1-flag* with or without *pcS2-E1A-HA* in chicken embryonic spinal cord at HH10-12. The *pCMV-GFP* is added as an electroporation control. (A–C) After 6 h, *Oc1* mis-expression results in an ectopic production of Isl1, as previously reported (Kim et al., 2015; Roy et al., 2012). (D) Quantification of the number of Isl1-positive motor neurons in the control or electroporated side of the spinal cord (Mann-Whitney; $p = 0.008$). (E–H) In contrast, inhibition of CBP and p300 activity by E1A (F') prevents ectopic Isl1 induction (*t*-test; $p = 0.41$) despite robust production of OC-1 (F). A,B,C and D,E,F are different images of the same sections, E' shows a section adjacent to that shown in D,E,F. Σ = electroporated side. $n = 5$; Scale bar = 50 µm.

PGC and LMCm (ventral limb muscles) MNs. Finally, CREST3, which is located upstream of the *Isl1* coding region, is rather active in sensory neurons (Kim et al., 2015; Uemura et al., 2005). Potential OC binding sites have been detected in CREST1 and CREST2, however OC factors only bind to CREST2 and activate it through 2 distinct conserved binding motifs (Kim et al., 2015; Roy et al., 2012). Here, as previously observed with rat proteins, we confirmed that cOC-1 is bound to the chicken CREST2 but not to CREST1 or CREST3. CREST1 is bound and stimulated by Phox2 factors in hindbrain branchiomotor neurons. In the spinal cord, CREST1 is activated by Lhx3 alone and by the MN-specific Isl1-NLI-Lhx3 hexameric complex, resulting in a feed-forward mechanism that contributes to maintain *Isl1* expression. CREST1 activation in V2a interneurons, wherein Lhx3 is also present, is prevented by the V2a-specific Chx10 repressor whereas induction in MN or V2 progenitors is repressed by Olig2 and Sox1, respectively (Kim et al., 2015). However, *Lhx3* expression in MNs is rapidly restricted to the MMC MNs (Sharma et al., 1998), raising the question of the maintenance of Isl1 production in other motor columns. As previously reported, OC factors take over to maintain *Isl1* expression in HMC, PGC and LMCm neurons through the CREST2 enhancer (Roy et al., 2012). Interestingly, OC proteins have been shown to recruit Isl1 on enhancers that ensure stable gene expression in differentiating MNs (Rhee et al., 2016), suggesting that OC also promote a feed-forward mechanism that may contribute to maintain Isl1 production. Recruitment of Isl1 to these late enhancers correlates with an enrichment in p300 (Rhee et al., 2016).

OC factors have been shown to interact with CBP and p300 in hepatic cells (Lannoy et al., 2000; Rausa et al., 2003). Our data suggest that OC proteins can also interact with these cofactors in spinal MNs and that this interaction contributes to the maintenance of *Isl1* expression by the OC factors. CBP and p300 are highly enriched on CREST2, as observed for OC proteins. CBP is also detected on CREST3 and on the Noggin promoter, and to a lesser extent on CREST1. This indicates that CBP and p300 may interact with OC proteins bound to the CREST2 enhancer, whereas these cofactors likely interact with other DNA-bound proteins or complexes on CREST3 and on the Noggin promoter, as shown in other cell types. For example, Isl1 is proposed to recruit p300 and to promote histone H3 acetylation on the *Mefc2* promoter during cardiac development (Yu et al., 2013). Consistently, Isl1 binding to MN-specific enhancers, as observed on CREST1, correlates with p300 enrichment and acetylation of histone H3 (Rhee et al., 2016). Similarly, Phox2 factors bind the CREST1 enhancer in branchiomotor neurons (Kim et al., 2015) and Phox2b has been shown to interact with CBP (Wu et al., 2009). However, CBP and p300 were not found and histone H3 acetylation was not detected on the CREST1 enhancer in our experiments, suggesting that other cofactors that remain to be identified are recruited to, and activate, CREST1 in the spinal MNs. Thus, recruitment of CBP or p300 cofactors to CREST2, which likely involves OC factors, could be critical to maintain *Isl1* expression during MN differentiation. This hypothesis was confirmed by our co-electroporation experiments in chicken embryonic spinal cord, which demonstrate that CBP and p300 activity contributes to the activation of the CREST2 enhancer and participates in the stimulation of *Isl1* expression by the OC factors. In contrast, despite the presence of OC factors in sensory neurons (Landry et al., 1997), OC are not required for Isl1 production in this neuronal type as *Isl1* expression is maintained in the dorsal root and cranial ganglia of OC mutant mice (F.C., unpublished data). Accordingly, although Cut domain proteins are predicted to bind the conserved CDP CR1 recognition motifs present in the CREST3 sequence (Uemura et al., 2005), potential OC binding site are not detected in CREST3 and OC are not bound to the CREST3 enhancer in the developing spinal cord (Kim et al., 2015; Roy et al., 2012) (and the present study). Therefore, recruitment of CBP and p300 to CREST3 involves other transcription factors that remain to be identified.

CBP and p300 have been demonstrated not only to acetylate histones but also to acetylate lysine residues of different proteins and thereby modulate their activity (Bedford and Brindle, 2012). Interestingly, stability of the OC-1 protein has been shown to depend on acetylation of the lysine 339 located in the DNA-binding cut domain (Rausa 3rd et al., 2004). Thus, recruitment of CBP and p300 to the CREST2 enhancer may result, in addition to histone acetylation, to the

acetylation of OC-1 that would stabilize this protein and promote long-term maintenance of *Isl1* expression in HMC, PGC and LMC during later steps of MN development. This hypothesis was supported by our results showing a significant decrease in Isl1 when the HAT activity of CBP and p300 is inactivated. This correlated with a reduction in the number of MNs, as evidenced by the decrease in Hb9-positive cells, likely due to a weakening of the Isl1-Hb9 feedforward loop that promotes MN differentiation (Lee et al., 2009). Furthermore, Isl1 is not only necessary for the production and for proper differentiation of the MNs but also contributes, in cooperation with Isl2, to confine MN cell bodies inside the spinal cord. Combination of hypomorph *Isl1* and null *Isl2* alleles results in massive emigration of MNs from the spinal cord along the ventral root, whereas single mutants only display minor defects (Lee et al., 2015). LMC MNs are more affected than MMC cells (Lee et al., 2015), indicating that maintenance of *Isl1* expression through the CREST2 enhancer may be of particular importance in this process. Consistently, the same MN somata emigration phenotype was reported in the single *CBP* and in the double *CBP/p300* mutants (Lee et al., 2009), supporting the possibility that later aspects of MN development may additionally implicate the regulation of *Isl* genes by CBP/p300 cofactors.

Acknowledgements

We thank members of the NEDI lab for material, technical support and discussions. We are grateful to H. Okamoto for the *zCREST2-hsp70:GFP* reporter vector and to S. Malas for the rat anti-GFP antibody.

This work was supported by grants from the “Fonds spéciaux de recherche” (FSR) of the Université Catholique de Louvain, by a “Projet de recherche (PDR)” #T.0117.13 and an “Equipement (EQP)” funding #U.N027.14 of the Fonds de la Recherche Scientifique (F.R.S.-FNRS) and by the Association Belge contre les Maladies Neuro-Musculaires (ABMM, Belgium). F.C. is Senior Research Associate of the F.R.S.-FNRS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcn.2019.103411.

References

Arany, Z., Newsome, D., Oldread, E., Livingston, D.M., Eckner, R., 1995. A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. Nature 374, 81–84.
Arber, S., Han, B., Mendelsohn, M., Smith, M., Jessell, T.M., Sockanathan, S., 1999. Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. Neuron 23, 659–674.
Audouard, E., Schakman, O., Ginion, A., Bertrand, L., Gailly, P., Clotman, F., 2013. The Onecut transcription factor HNF-6 contributes to proper reorganization of Purkinje cells during postnatal cerebellum development. Mol. Cell. Neurosci. 56, 159–168.
Bedford, D.C., Brindle, P.K., 2012. Is histone acetylation the most important physiological function for CBP and p300? Aging (Albany NY) 4, 247–255.
Chakrabarty, K., Von Oerthel, L., Hellemons, A., Clotman, F., Espana, A., Groot Koerkamp, M., Holstege, F.C., Pasterkamp, R.J., Smidt, M.P., 2012. Genome wide expression profiling of the mesodiencephalic region identifies novel factors involved in early and late dopaminergic development. Biol Open 1, 693–704.
Dasen, J.S., 2017. Master or servant? Emerging roles for motor neuron subtypes in the construction and evolution of locomotor circuits. Curr. Opin. Neurobiol. 42, 25–32.
Espana, A., Clotman, F., 2012a. Onecut factors control development of the Locus coeruleus and of the mesencephalic trigeminal nucleus. Mol. Cell. Neurosci. 50, 93–102.
Espana, A., Clotman, F., 2012b. Onecut transcription factors are required for the second phase of development of the A13 dopaminergic nucleus in the mouse. J. Comp. Neurol. 520, 1424–1441.
Francius, C., Clotman, F., 2010. Dynamic expression of the Onecut transcription factors HNF-6, OC-2 and OC-3 during spinal motor neuron development. Neuroscience 165, 116–129.
Francius, C., Clotman, F., 2014. Generating spinal motor neuron diversity: a long quest for neuronal identity. Cell. Mol. Life Sci. 71, 813–829.
Francius, C., Harris, A., Rucchin, V., Hendricks, T.J., Stam, F.J., Barber, M., Kurek, D., Grosveld, F.G., Pierani, A., Goulding, M., Clotman, F., 2013. Identification of multiple subsets of ventral interneurons and differential distribution along the rostrocaudal axis of the developing spinal cord. PLoS One 8, e70325.
Hodge, L.K., Klassen, M.P., Han, B.X., Yiu, G., Hurrell, J., Howell, A., Rousseau, G., Lemaigre, F., Tessier-Lavigne, M., Wang, F., 2007. Retrograde BMP signaling regulates trigeminal sensory neuron identities and the formation of precise face maps. Neuron 55, 572–586.
Iyaguchi, D., Yao, M., Watanabe, N., Nishihira, J., Tanaka, I., 2007. DNA recognition mechanism of the ONECUT homeodomain of transcription factor HNF-6. Structure 15, 75–83.
Jacquemin, P., Durviaux, S.M., Jensen, J., Godfraind, C., Gradwohl, G., Guillemot, F., Madsen, O.D., Carmeliet, P., Dewerchin, M., Collen, D., Rousseau, G.G., Lemaigre, F.P., 2000. Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene ngn3. Mol. Cell. Biol. 20, 4445–4454.
Kabayiza, K.U., Masgutova, G., Harris, A., Rucchin, V., Jacob, B., Clotman, F., 2017. The Onecut transcription factors regulate differentiation and distribution of dorsal interneurons during spinal cord development. Front. Mol. Neurosci. 10, 157.
Kim, N., Park, C., Jeong, Y., Song, M.R., 2015. Functional diversification of motor neuron-specific Isl1 enhancers during evolution. PLoS Genet. 11, e1005560.
Lai, H.C., Seal, R.P., Johnson, J.E., 2016. Making sense out of spinal cord somatosensory development. Development 143, 3434–3448.
Landry, C., Clotman, F., Hioki, T., Oda, H., Picard, J.J., Lemaigre, F.P., Rousseau, G.G., 1997. HNF-6 is expressed in endoderm derivatives and nervous system of the mouse embryo and participates to the cross-regulatory network of liver-enriched transcription factors. Dev. Biol. 192, 247–257.
Lannoy, V.J., Burglin, T.R., Rousseau, G.G., Lemaigre, F.P., 1998. Isoforms of hepatocyte nuclear factor-6 differ in DNA-binding properties, contain a bifunctional homeodomain, and define the new ONECUT class of homeodomain proteins. J. Biol. Chem. 273, 13552–13562.
Lannoy, V.J., Rodolosse, A., Pierreux, C.E., Rousseau, G.G., Lemaigre, F.P., 2000. Transcriptional stimulation by hepatocyte nuclear factor-6. Target-specific recruitment of either CREB-binding protein (CBP) or p300/CBP-associated factor (p/CAF). J. Biol. Chem. 275, 22098–22103.
Lee, S., Lee, B., Joshi, K., Pfaff, S.L., Lee, J.W., Lee, S.K., 2008. A regulatory network to segregate the identity of neuronal subtypes. Dev. Cell 14, 877–889.
Lee, S., Lee, B., Lee, J.W., Lee, S.K., 2009. Retinoid signaling and neurogenin 2 function are coupled for the specification of spinal motor neurons through a chromatin modifier CBP. Neuron 62, 641–654.
Lee, H., Kim, M., Kim, N., Macfarlan, T., Pfaff, S.L., Mastick, G.S., Song, M.R., 2015. Slit and Semaphorin signaling governed by islet transcription factors positions motor neuron somata within the neural tube. Exp. Neurol. 269, 17–27.
Lemaigre, F.P., Durviaux, S.M., Truong, O., Lannoy, V.J., Hsuan, J.J., Rousseau, G.G., 1996. Hepatocyte nuclear factor 6, a transcription factor that contains a novel type of homeodomain and a single cut domain. Proc. Natl. Acad. Sci. U. S. A. 93, 9460–9464.
Lu, D.C., Niu, T., Alaynick, W.A., 2015. Molecular and cellular development of spinal cord locomotor circuitry. Front. Mol. Neurosci. 8, 25.
Lundblad, J.R., Kwok, R.P., Laurance, M.E., Harter, M.L., Goodman, R.H., 1995. Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. Nature 374, 85–88.
Partanen, A., Motoyama, J., Hui, C.C., 1999. Developmentally regulated expression of the transcriptional cofactors/histone acetyltransferases CBP and p300 during mouse embryogenesis. Int J Dev Biol 43, 487–494.
Pfaff, S.L., Mendelsohn, M., Stewart, C.L., Edlund, T., Jessell, T.M., 1996. Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. Cell 84, 309–320.
Rausa 3rd, F.M., Hughes, D.E., Costa, R.H., 2004. Stability of the hepatocyte nuclear factor 6 transcription factor requires acetylation by the CREB-binding protein coactivator. J. Biol. Chem. 279, 43070–43076.
Rausa, F.M., Tan, Y., Costa, R.H., 2003. Association between hepatocyte nuclear factor 6 (HNF-6) and FoxA2 DNA binding domains stimulates FoxA2 transcriptional activity but inhibits HNF-6 DNA binding. Mol. Cell. Biol. 23, 437–449.
Rhee, H.S., Closser, M., Guo, Y., Bashkirova, E.V., Tan, G.C., Gifford, D.K., Wichterle, H., 2016. Expression of terminal effector genes in mammalian neurons is maintained by a dynamic relay of transient enhancers. Neuron 92, 1252–1265.
Roy, A., Francius, C., Rousso, D.L., Seuntjens, E., Debruyn, J., Luxenhofer, G., Huber, A.B., Huylebroeck, D., Novitch, B.G., Clotman, F., 2012. Onecut transcription factors act upstream of Isl1 to regulate spinal motoneuron diversification. Development 139, 3109–3119.
Sharma, K., Sheng, H.Z., Lettieri, K., Li, H., Karavanov, A., Potter, S., Westphal, H., Pfaff, S.L., 1998. LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. Cell 95, 817–828.
Song, M.R., Sun, Y., Bryson, A., Gill, G.N., Evans, S.M., Pfaff, S.L., 2009. Islet-to-LMO stoichiometries control the function of transcription complexes that specify motor neuron and V2a interneuron identity. Development 136, 2923–2932.
Stam, F.J., Hendricks, T.J., Zhang, J., Geiman, E.J., Francius, C., Labosky, P.A., Clotman, F., Goulding, M., 2012. Renshaw cell interneuron specialization is controlled by a temporally restricted transcription factor program. Development 139, 179–190.
Tanabe, Y., William, C., Jessell, T.M., 1998. Specification of motor neuron identity by the MNR2 homeodomain protein. Cell 95, 67–80.
Thaler, J., Harrison, K., Sharma, K., Lettieri, K., Kehrl, J., Pfaff, S.L., 1999. Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor HB9. Neuron 23, 675–687.
Thaler, J.P., Lee, S.K., Jurata, L.W., Gill, G.N., Pfaff, S.L., 2002. LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. Cell 110, 237–249.
Thaler, J.P., Koo, S.J., Kania, A., Lettieri, K., Andrews, S., Cox, C., Jessell, T.M., Pfaff, S.L., 2004. A postmitotic role for Isl-class LIM homeodomain proteins in the assignment of visceral spinal motor neuron identity. Neuron 41, 337–350.
Tsuchida, T., Ensini, M., Morton, S.B., Baldassare, M., Edlund, T., Jessell, T.M., Pfaff, S.L., 1994. Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. Cell 79, 957–970.

Uemura, O., Okada, Y., Ando, H., Guedj, M., Higashijima, S., Shimazaki, T., Chino, N., Okano, H., Okamoto, H., 2005. Comparative functional genomics revealed conservation and diversification of three enhancers of the isl1 gene for motor and sensory neuron-specific expression. Dev. Biol. 278, 587–606.

William, C.M., Tanabe, Y., Jessell, T.M., 2003. Regulation of motor neuron subtype identity by repressor activity of Mnx class homeodomain proteins. Development 130, 1523–1536.

Wu, H.T., Su, Y.N., Hung, C.C., Hsieh, W.S., Wu, K.J., 2009. Interaction between PHOX2B and CREBBP mediates synergistic activation: mechanistic implications of PHOX2B mutants. Hum. Mutat. 30, 655–660.

Wu, F., Sapkota, D., Li, R., Mu, X., 2012. Onecut 1 and Onecut 2 are potential regulators of mouse retinal development. J. Comp. Neurol. 520, 952–969.

Yu, Z., Kong, J., Pan, B., Sun, H., Lv, T., Zhu, J., Huang, G., Tian, J., 2013. Islet-1 may function as an assistant factor for histone acetylation and regulation of cardiac development-related transcription factor Mef2c expression. PLoS One 8, e77690.