THE TRANSCRIPTION FACTOR SATB2 REGULATES THE DEVELOPMENT OF THE NEURONAL NETWORK OF THE CEREBRAL CORTEX THROUGH THE PROTEIN KINASES SIGNALING CASCADE

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Abstract. Using fluorescence microscopy, morphological analysis of cell growth, and PCR analysis, we have shown that deletion of the Satb2 transcription factor in mouse cortical neurons results in impaired neuronal network development *in vitro*. It was found that primary cell cultures of the cerebral cortex obtained from Satb2-null mice are not able to form a developed network of neurites during 5 days of cultivation, while cells from control mice are characterized at this time by a fully developed network of neuronal processes. Analysis of protein kinases expression involved in the processes of neuron differentiation and neurites growth showed that deletion of Satb2 leads to suppression of the expression of genes encoding protein kinase C (PKC), protein kinase B (Akt/PKB), mitogen-activated protein kinase 8 (MAPK8) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), while not affecting the expression of phosphatidylinositol 3-kinase (PI3K). Activation of neurites outgrowth and differentiation of Satb2-null neurons was achieved by the application of exogenous activators of Akt/PKB, CaMKII and PI3K, but not PKC, the expression and activity of which is probably completely suppressed by the deletion of Satb2.

Keywords: neurons, protein kinases, neurites, gene expression, neuronal differentiation, SATB2, cerebral cortex.

List of Abbreviations

SATB2 – Special AT-rich sequence-binding protein 2

PKC – Protein kinase C

CaMKII – Ca^{2+}/calmodulin-dependent protein kinase II

Akt/PKB – Protein kinase B

PI3K – Phosphoinositide 3-kinase

PMA – phorbol 12-myristate 13-acetate

Introduction

The neocortex is the main brain structure that determines cognitive abilities such as speech, fear, love, planning and performing actions, which are generally referred to as higher nervous activity. Genetic disorders affecting the brain lead not only to the development of numerous hereditary diseases, but also to the development of neurodegeneration and cognitive impairment (Bueno, 2019).

Transcription factors are proteins that control the process of mRNA synthesis on a DNA template (transcription) by binding to specific DNA regions. Transcription factors perform their function either alone or in combination with other proteins. They provide a decrease (repressors) or an increase (activators) in the binding constant of RNA polymerase to the regulatory sequences of the regulated gene, i.e., they provide reading or blocking the reading of genetic information. They bind DNA and help initiate a program to increase or decrease gene transcription (Wang *et al*., 2015; Bar-Ziv *et al*., 2020).

Genetic malformations of the brain and numerous neurodegenerative processes occur due to the mutation of various transcription factors that determine the functions of the body. The SATB2 ([MIM] 608148) gene resides on chromosome 2q32–q33, spans 191 kb, and contains 11 exons. Its open reading frame begins in exon 2, with the first stop codon in exon 11, predicting a 733-amino acid protein. The protein contains a Pfam-B_10016 domain required for di-

merization (residues 57–231), two CUT domains (352–437 and 482–560), and a homeodomain (614–677) (FitzPatrick *et al*., 2003). The transcription factor Satb2 regulates the activity of more than 1000 genes, including growth factors and signaling proteins. Satb2 mutations in the brain lead to impaired migration, proliferation, and differentiation of neurons during embryonic development, with highly specialized populations of GABAergic neurons that act as inhibitors of neuroglial networks particularly affected (Huang *et al*., 2020; Turovsky *et al.*, 2021; Leone *et al*., 2015).

Satb2 is required to initiate a specific genetic program for the development of the upper layers cells of the cerebral cortex and inactivation of genes active in neurons of the lower layers of the cerebral cortex through a change in the expression of the Bcl11B (Ctip2) gene. Satb2 stimulates cells to establish intracortical interactions (Britanova *et al*., 2008). Satb2 is also part of various signaling pathways, for example, TNF-α blocks Satb2 expression and fibroblast differentiation through activation of the MAPK-ERK signaling pathway (Zuo C *et al*., 2017). There is evidence that the Satb2 deletion is associated with the development of schizophrenia. It is suggested that many neurodegenerative diseases can be determined by this transcription factor, such as Martin-Bell syndrome, Huntington's and Alzheimer's diseases, Satb2-associated syndrome, and possibly many others (Whitton *et al*., 2018; Lee *et al*., 2016; Zarate *et al*., 2017; Cera *et al*., 2019).

At the same time, the role of Satb2 in the regulation of postnatal brain development has not been studied. There are no data on the role of the Satb2 deletion in the development of neuronal networks and their maturation. Therefore, the aim of this work was to study the disturbances in the expression of protein kinases and the intracellular mechanism of development of cerebral cortex neurons with deletion of the Satb2 transcription factor.

Materials and Methods

Ethical statement

Experimental protocols were approved by the Bioethics Committee of the Institute of Cell Biophysics. Experiments were carried out according to Act708n (23 August 2010) of the Russian Federation National Ministry of Public Health, which states the rules of laboratory practice for the care and use of laboratory animals, and the Council Directive 2010/63 EU of the European Parliament on the protection of animals used for scientific purposes.

Animals

We used conditional knockout mice carrying Satb2flox allele in which exon is flanked by loxP sites, and thereby can be excised by recombination with Cre. The Satb2 conditional allele, which can be inactivated by the expression of Cre-recombinase, was generated in R.G.'s laboratory (Srinivasan *et al*., 2012). Satb2flox-mice were taken to Charité - Universitätsmedizin from Max-Plank Institute for Experimental Medicine, Gottingen, Germany, and then they were bred to NEX-Cre mice.

Mice were kept in SPF cages 40×25×15 cm under standard laboratory conditions: a 12 hours light circuit, 22 °C. Animals had free access to food and water. For housing, individually ventilated GM500 cages manufactured by Tecniplast (Italy) with a floor area of 501 cm2 were used. In the nests, the harem type of housing (2 females + 1 male) was carried out. After weaning, on the twenty-first – thirtieth postnatal day the transplanting of the offspring into a separate cage and the taking of material for genotyping (tip of the tail) occurs. All animals were housing with littermates of the same sex in a group of 2–5 mice.

Newborn mice were used to obtain neuroglial cell cultures of the cerebral cortex. The tails of these mice were frozen and stored for subsequent genotyping. For genotyping, the tail cuts were dissolved in 0.3 ml of lysis buffer (100 mM Tris-HCl pH 8.5, 5 mM EDTA, 200 mM NaCl, 0.2% SDS, 100 μg/ml proteinase K) at 55 °C for 2–10 h. The non-lysed tissue was removed by centrifuging the samples for 10 minutes at 10.000 rpm. The DNA was precipitated by adding an equal volume of isopropanol, then mixed and centrifuged (15 min, 13.000 rpm). The precipitated DNA was washed twice in 80% ethanol, air dried, and dis-

solved in 50 μl of sterile distilled water. All PCR reactions were carried out in a volume of 20 μl.

The following primers were used to determine the amplified product. Satb2-floxed allele and wild-type allele: 5′-CAAGAGAGCCATCCAACTGC-3'; 5′-AACCATCAGGCTGCCTCAACC-3'; 5′-CCAGACCGCGCGCCTGAAGA-3'. Amplification program was as follows: 95 °C, 3 min; 95 °C, 10 s; 54 °C, 15 s; 72 °C, 30 s; 72 °C, 5 min, 33 cycles. The wild-type allele product is ~200 bp, knockout ~450 bp. Primers for amplification of the NexCre allele and the wild-type allele: 5′-CCG CATAACCAGTGAAACAG-3'; 5′-GAGTCCTGGAATCAGTCTTTTC-3'; 5′-AGAATGTGGAGTAGGGTGAC-3'. Amplification program: 95 °C, 3 min; 95 °C, 20 s; 54 °C, 30 s; 72 °C, 60 s; 72 °C, 2 min, 35 cycles. The product of the wild-type allele is ~750 bp, the NexCre allele is ~500 bp.

Cell culture preparation

To obtain cell cultures were used Control mice (Satb+/+ * NexCre/+) and Satb2-null mice (Satb2fl/fl * NexCre/+) mice. Mixed neuroglial cell cultures were prepared as described in detail previously (Turovskaya *et al*., 2018). The cortex of one mouse was used to obtain ten Petri dishes with culture to avoid the variation in the gene expression and signaling system activity between individual mice. Briefly, 0–1 day-old pups were euthanized by halothane overdose and decapitated. The mouse cerebellar cortex was excised with clippers, put in a test tube, incubated for 2 min and the supernatant was removed with a pipette. The cells were then covered with 2 ml trypsin (0.1% in Ca^{2+}- and Mg^{2+}-free Versene solution, SAFC, Cat. #59427C) and incubated for 15 min at 37 °C under constant shaking at 600 rpm. Trypsin was then inactivated by equal volume of cold embryo serum, and the preparation was centrifuged at 300 g for 5 min. The supernatant was discarded and cells were washed twice with Neurobasal A medium (ThermoFisher Scientific, USA, Cat. #10888022) before being resuspended in Neurobasal-A medium containing glutamine (0.5 mM, Sigma-Aldrich, Cat. #G7513), B-27 (2%, ThermoFisher Scientific, RRID: CVCL_A315) and gentamicin (20 μg/ml, Sigma-Aldrich, Cat. #G1397). 200 μl of the suspension was put in a glass ring (internal diameter of 6 mm) resting on a round 25 mm coverslip (VWR International, Cat. #48382-085) which has been coated with poly-L-lysine. The glass ring was removed after a 5 hour incubation period in a CO_2-incubator (37 °C).

Monitoring the cell morphology and neurite growth

The primary neuroglial culture was divided into two cortical groups 4 hours after the cells were attached. Cell cultures were loaded with a Calcein-AM probe at a final concentration of 5 μM for 40 min at 37oC in HBSS balanced with 10 mM HEPES followed by a wash three times and mounting in an experimental chamber connected to a fluorescent inverted motorized microscope Leica DMI6000B with a high-speed monochrome CCD camera HAMAMATSU C9100. For excitation and recording of Calcein fluorescence, the L5 filter set (Leica, Germany) was used containing excitation filter BP480/40, beam splitter FT-505 and emission filter BP527/30, excitation light source Leica EL6000 with a high-pressure mercury lamp HBO 103 W/2. Calcein fluorescence was registry every 24 hours after attachment for 5 days.

Extraction of RNA

Mag Jet RNA Kit (Thermo Fisher Scientific, USA) was used for the extraction of total RNA. The RNA quality was estimated by electrophoresis in the presence of 1 μg/ml ethidium bromide (2% agarose gel in Tris/Borate/EDTA buffer). The concentration of the extracted RNA was determined with NanoDrop 1000c spectrophotometer. RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used for reverse transcription of total RNA.

Table 1

Primer sequences for real-time polymerase chain reaction (RT-PCR)

Gapdh	Forward 5'- ctgagatgggagctgggactgc -3' Reverse 5'- gtgtccacgtgttagacagaacactg -3'
Pik3ca	Forward 5'-ggaggatgaggcggggacc-3' Reverse 5'-gcatgctcttcgggaggcttcaaaac-3'
Pik3cb	Forward 5'- gaggttatgagtgtgcttccgccctat -3' Reverse 5'- agtcttcgtgtttcgtcttccagttcctc -3'
Pik3cg	Forward 5'- gctgcggagttctaccaccgattg -3' Reverse 5'- caggtagtctgggagaggtttggacg -3'
Camk2a	Forward 5'- gagcagcaggcatggtttgggt -3' Reverse 5'- ggtgcttgagagcctcagcgg -3'
Mapk8	Forward 5'- ggctcaggagctcaaggaatagtgtgt -3' Reverse 5'- cagcagagtgaaggtgcttgattccac -3'
Pkca	Forward 5'- ccaacgactccacggcgtctc -3' Reverse 5'- tgcttgtgaacattcatgtcgcaggtgt -3'
Pkce	Forward 5'- tgatcatcgatctctcgggatcatcggg -3' Reverse 5'- gcccacctcgtcaggggtttc -3'
Pkcg	Forward 5'- tggttcaccgccgatgccac -3' Reverse 5'- ccgcaaagggagggcacg -3'
Src	Forward 5'- gggcagcaacaagagcaagcc -3' Reverse 5'- tctgtccgtgactcatagtcatagagggc -3'
Akt	Forward 5'- caagtactcattccagacccacgac -3' Reverse 5'- gtcctcggagaacacacgctct -3'
Dcx	Forward 5'- gcaatggggaccgttacttcaa-3' Reverse 5'- agccagcaacgcatcaaaactac-3'
Syp	Forward 5'- aggtgctgcagtgggtctttg-3' Reverse 5'- actctccgtcttgttggcacact-3'
Dlg3	Forward 5'- aggagatcacattggaaaggggtaa-3' Reverse 5'- tggtgataaagatggatgggtcgt-3'

Real-time polymerase chain reaction (RT-qPCR)

Each PCR was performed in a 25 μL mixture composed of 5 μL of qPCRmix-HS SYBR (Evrogen, Moscow, Russia), 1 μL (0.2 μM) of the primer solution, 17 μL water (RNase-free), 1 μL cDNA. Dtlite Real-Time PCR System (DNA-technology, Moscow, Russia) was used for amplification. Amplification process consisted of the initial 5 min denaturation at 95 °C, 40 cycles of 30 s denaturation at 95 °C, 20 s annealing at 60–62 °C, and 20 s extension step at 72 °C. The final extension was performed for 10 min at 72 °C. All the sequences were designed with FAST PCR 5.4, NCBI Primer-BLAST software and all the primers were synthesized by Evrogen (Moscow, Russia) (Table 1). The data were analyzed with Dtlite software (DNA-technology, Moscow, Russia). The expression of the studied genes was normalized to gene encoding Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data were analyzed using Livak`s method.

Results

Deletion of the transcription factor Satb2 suppresses the growth of neurites and the expression of protein kinases in the cortical neurons

Figure 1A (top line) shows images of a cortical cell culture obtained from a control mouse during cultivation up to 120 hours *in vitro*. It can be seen that during cultivation, the number and length of neurites increase every 24 hours of recording (Fig. 1B, black curve) and the length of neurites reaches an average of

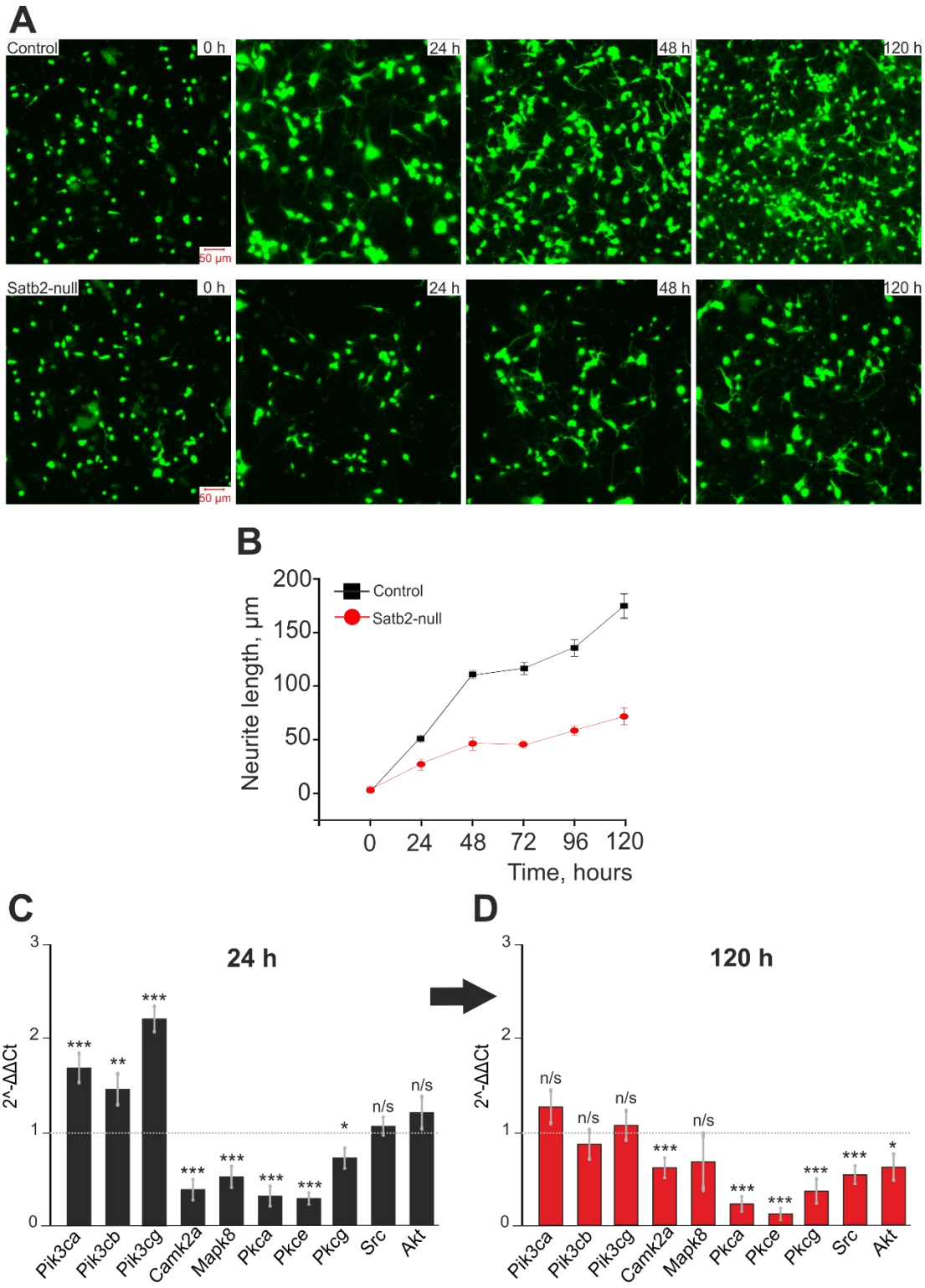

Fig. 1. Effect of Satb2 knockout on neurite outgrowth and expression of genes encoding protein kinases in mouse cortical neurons *in vitro*. A – Cortical cell culture obtained from a control mouse (Control) and a Satb2 knockout mouse (Satb2-null) during *in vitro* cultivation. Cells are loaded with a Calcein AM fluorescent probe for neurite imaging. C – analysis of neurite growth in the cortical cell culture obtained from the control and Satb2-null mice during cultivation. C, D – Expression of genes encoding protein kinases in cortical cells derived from Satb2-null mice 24 and 120 hours after attachment. 1 (dashed straight line) is the level of expression in the cortical cells from control mouse

160–170 μm after 120 hours of cultivation. Cell culture of the cerebral cortex, obtained from mice with a deletion of the transcription factor Satb2, develops quite differently. As can be seen in Figure 1A (lower line), 24 hours after attachment, the number of cells in the field of view of the microscope is approximately the same in the control and the Satb2-null group. However, during cultivation, there is no intensive development of the neuronal network and no growth of neurites, when 120 hours after cell attachment, the average length of neurites is 60–70 μm (Fig. 1B, red curve).

Cell growth and differentiation are regulated by numerous protein kinases and signaling cascades activated by them. Analysis of the expression level of key protein kinases in Satb2-null mouse cerebral cortex cells 24 hours after inoculation showed that the level of expression of PI3K subunits is 1.5–2 times higher compared to the control, and the expression of genes encoding CaMKII, MAPK8, and all three PKC isoforms was significantly suppressed (Figure 1C). After 120 hours cultivation gene expression analysis showed that the level of expression of Camk2a, Pkc, Src, and Akt in Satb2-null cells was significantly lower (Fig. 1D) compared to cells from the control mouse. The level of expression of genes encoding PI3K in cells from Satb2-null mice does not differ from the control, but when compared with the level of their expression after 24 hours (Fig. 1C); a significant decrease is seen (Fig. 1D).

Thus, the deletion of Satb2 in cortical neurons leads to a disruption in the expression of key protein kinases, which is expressed in the suppression of the development of the neuronal network and the growth of neurites during *in vitro* cultivation.

Selective activation of protein kinase B and phosphoinositide 3-kinase enhances neurite outgrowth and neuronal maturation upon deletion of the Satb2 transcription factor

To reveal the role of protein kinases in the development of the neuronal network, selective protein kinase activators were added to cells obtained from control and Satb2-null mice from the zero day of cultivation. Every 24 hours (during 5 days of cultivation) Calcein-AM probe were loaded and cell morphology and neurite growth were recorded (Fig. 2). Addition of activators for protein kinases B (SC 79.5 μM) and PI3K (740 Y-P, 5 μM) to control mouse-derived cortical cells (WT cells) resulted in a more developed neuronal network (Fig. 2A) and increased neurite outgrowth (Figure 3A) for 120 hours of cultivation. At the same time, activators of protein kinase C (PMA, 1 μM) and CaMKII (Syntide 2, 20 μM), on the contrary, suppressed the growth of neurites by 120 hours of cultivation of WT cells (Fig. 2A; Fig. 3A).

Neurons derived from Satb2-null mice grown with the addition of protein kinase B (SC 79.5 μM) and PI3K (740 Y-P, 5 μM) activators are characterized by increased neurite outgrowth after only 24 hours of culture (Fig. 2B; Fig. 3B), and the effect of activators of these protein kinases increased every day *in vitro*. At the same time, the addition of the CaMKII activator (Syntide 2, 20 μM) did not affect the development of the network of Satb2-null neurons, and the PKC activator (PMA, 1 μM), although it had a trend towards an increase in the length of neurites after 24 hours of cultivation, the differences were not significant (Fig. 3B).

Analysis of gene expression in the WT cell group after 120 hours of cultivation in the presence of PKC (PMA, 1 μM), CaMKII (Syntide 2, 20 μM), and PI3K (740 Y-P, 5 μM) activators showed that PKC activation does not significantly enhance the expression level of all three PI3K isoforms, but does not significantly affect the expression of other genes. The CaMKII activator (Syntide 2, 20 μM) leads to an increase in the expression of the gene encoding this kinase itself and the Dcx, Syp, and Dlg3 genes, which encode proteins that regulate neuronal differentiation (Fig. 3C). However, the most pronounced effect on gene expression in the WT cells group is observed when cells are grown with the addition of the PI3K activator (740 Y-P, 5 μM), when the expression of three isoforms of PI3K itself, CaMKII, Dcx, Syp, and Dlg3 is enhanced. The addition of 740 Y-P also suppresses the expression of genes encoding PKC (Fig. 3C).

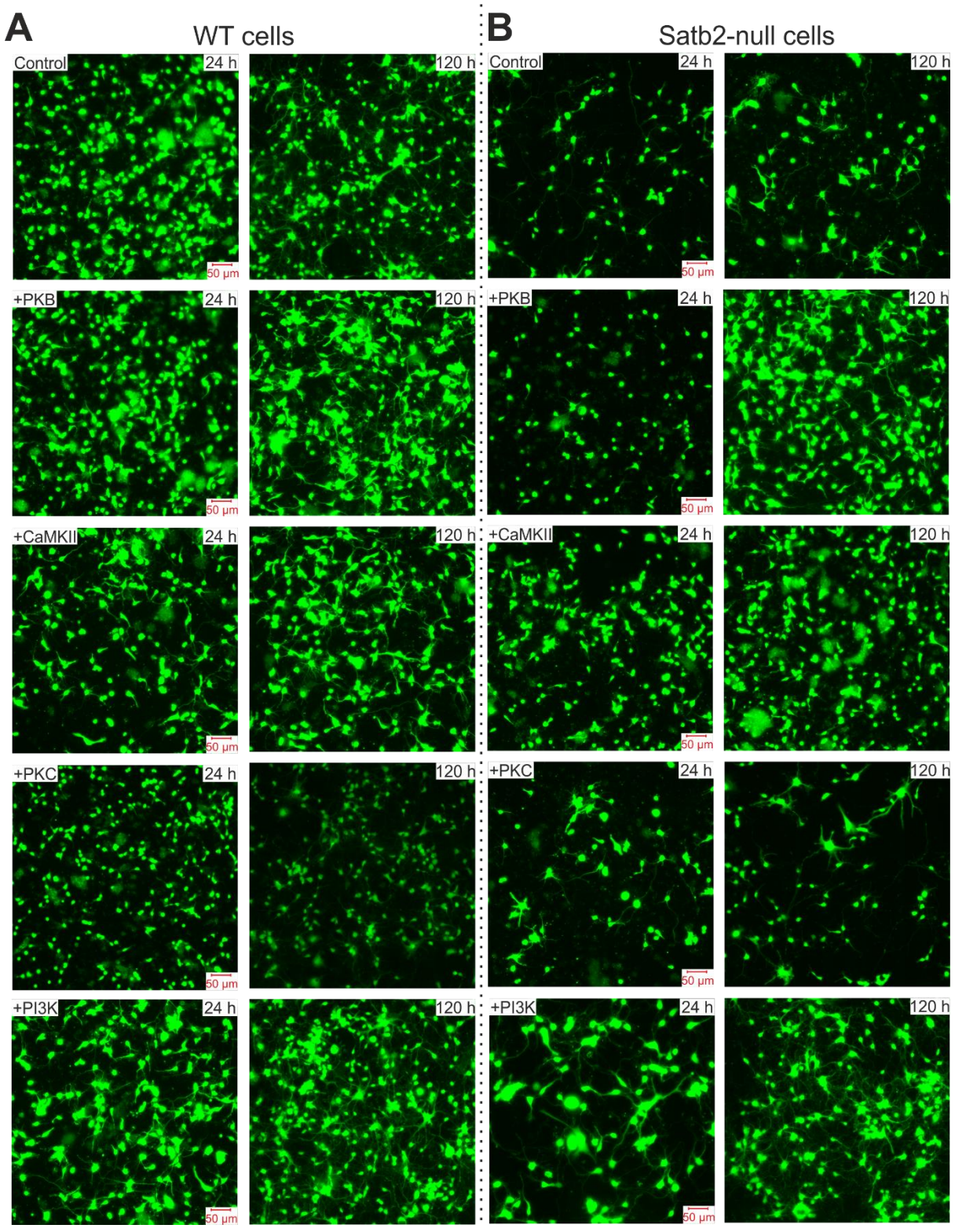

Fig. 2. Images of cortical cells derived from control mice (A) and Satb2-null mice (B) cultured in the presence of protein kinases activators: protein kinase B activators (+PKB, SC 79.5 µM), Ca2+/calmodulin-dependent protein kinase II (+CaMKII, Syntide 2, 20 µM), protein kinase C (+PKC, PMA, 1 µM) and phosphoinositide 3-Kinase (+PI3K, 740 Y-P, 5 µM) at 24 and 120 hours after attachment. Protein kinase activators were added to the culture medium from day 0 immediately after the cells were attached to the substrate. To visualize neurites, cells were stained with a Calcein AM fluorescent probe

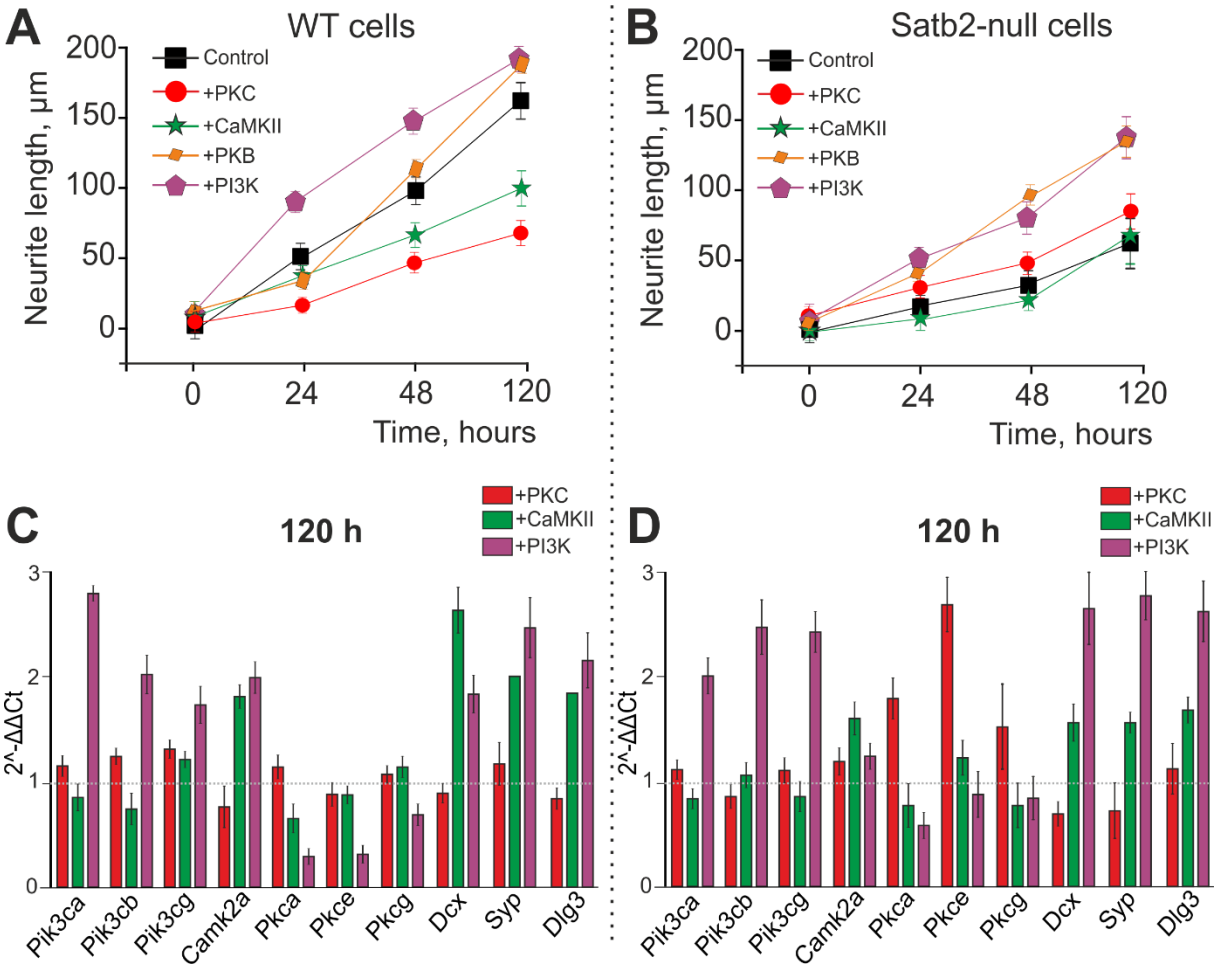

Fig. 3. Effect of protein kinase activators on neurite outgrowth and expression of genes encoding protein kinases and neuronal differentiation marker genes in neurons derived from the cerebral cortex of control (WT cells) and Satb2-null (Satb2-null cells) mice. A, B - Analysis of the growth of neurites in the cortical cell culture from control (A) and Satb2-null (B) mice during cultivation with the addition of protein kinase activators. The results in panels A and B correspond to the images of the cells in Figure 2. C, D - Expression of genes encoding protein kinases and marker genes for neuronal differentiation in cortical cells obtained from control (C) and Satb2-null (D) mice after 120 hours cultivation. 1 (dashed straight line) is the level of expression in the cortical cells without treatment with protein kinase activators. The following protein kinase activators were added to cell cultures from day 0: (+PKB, SC 79.5 µM), Ca2+/calmodulin-dependent protein kinase II (+CaMKII, Syntide 2, 20 µM), protein kinase C (+PKC, PMA, 1 µM) and phosphoinositide 3-Kinase (+PI3K, 740 Y-P, 5 µM)

Growing Satb2-null cells with the addition of a PKC activator (PMA, 1 µM) leads to an increase in the expression of genes encoding of PKC isoforms itself, without affecting the expression of other protein kinases and the Dcx, Syp, and Dlg3 genes (Fig. 3D). The CaMKII activator (Syntide 2, 20 µM) enhances the expression of Camk2a as well as Dcx, Syp, and Dlg3 (Fig. 3D). The PI3K activator (740 Y-P, 5 µM) leads to an increase in the expression of genes encoding PI3K isoforms and an almost threefold increase in the level of Dcx, Syp, and Dlg3 (Fig. 3D).

Thus, growing control neurons with the addition of PKC or CaMKII activators suppresses neurite outgrowth and network development, which, in the case of a PKC activator, correlates with the absence of changes in the expression of neuronal differentiation regulatory proteins Dcx, Syp, and Dlg3. PKC or CaMKII activators in the case of Satb2-null neurons do not significantly affect the growth of neurites and cell dif-

ferentiation. However, PKB and PI3K activators promote neurite outgrowth, network formation, and increased expression of genes encoding Doublecortin (Dcx), Synaptophysin (Syp), and PSD-95 (Dlg3) proteins responsible for neuronal differentiation.

Discussion

The continuous formation of neuronal connections is the basis of the most important organizing function of the brain in general and the neocortex in particular. Projection cortical neurons have a typical pyramidal morphology and send their axons to various regions of the central nervous system. It is important to note that the neurons of different layers of the cortex organize connections with different parts of the brain, while the neurons of one layer send axons to the same regions and have similar physiological characteristics (Briggs, 2010; Baker *et al*., 2018). Impaired neurogenesis in upper cortical neurons leads to anatomical brain defects such as agenesis of the corpus callosum and is often the cause of neurological or psychiatric diseases such as autism, schizophrenia, and intellectual disability (Britanova *et al*., 2008). The processes of neurogenesis and network formation are controlled by numerous protein kinases, the expression of which, in turn, may depend on a number of transcription factors.

Satb2 is a homologue of Satb1, but little is known about its functions and signaling in the brain. Its mutations have been shown to be associated with cleft palate and development of the cleft palate (FitzPatrick *et al*., 2003). An increase in Satb2 expression may be accompanied by activation of protein sumoylation and lead to various variants of their posttranslational modification (Dobreva *et al*., 2003). Satb2 inactivation is known to result in perinatal mortality, most likely due to multiple craniofacial developmental anomalies (Britanova *et al*., 2005; Dobreva *et al*., 2006). Satb2-associated syndrome is a recently discovered systemic disease associated with disorders in the Satb2 gene and in humans is accompanied by signs of developmental delay with significant speech impairment, facial dysmorphism, and anomalies in the development of the jaw and teeth. Although this syndrome is an extremely rare disease (less than 50 cases have been described so far), the mechanism of its induction is extremely interesting and may coincide with the mechanisms of other, much more common genetic diseases associated with neurodegeneration. Changes in the Satb2 gene can occur through contiguous deletions, intragenic deletions and duplications, translocation with secondary gene disruption, and pathogenic point mutations are also possible. Changes in the neuroradiographic characteristics of the brain have also been identified (Zarate & Fish, 2017) and signs of epilepsy (Leoyklang *et al*., 2007). At the same time, Satb2 knockout mice (Satb2 KO mice) showed no signs of epilepsy, which may be due to their high mortality rate in the postnatal period (Britanova *et al*., 2005; Dobreva *et al*., 2006). It is also known that disturbances in the structure of the gene or the level of expression of Satb2 are closely associated with the occurrence of cancer in various organs and increased metastases (Gu *et al*., 2018).

We have shown that deletion of the transcription factor Satb2 in neurons of the cerebral cortex leads to inhibition of neurite growth and network development, which correlates with the suppression of the expression of key protein kinases - PKC, CaMKII, Akt. It is known that activation of PKC through a signaling cascade involving MAP kinase leads to neurite outgrowth (Schmid *et al*., 2000; Wooten *et al*., 2000). Overexpression of PKC in the 3T3 fibroblast line stimulates cell division and anchorage-independent growth (Mischak *et al*., 1993), while in the PC12 cell line, neuronal differentiation is enhanced without effects on proliferation (Barbacid, 1987). PKC is a regulator of cytoskeletal organization. Treatment of cells with PKC activators results in neurite extension embryonic ganglia explants (Hsu *et al*., 1989), neuroblastoma cells (Fagerstrom *et al*., 1996), as well as changes in filopodial and lamellipodial protrusions in rat cerebral neurons and affect MT distribution in SH-SY5Y neuroblastoma growth cones (Rosner & Fischer, 1996). Conversely, PKC inhibition can result in reduced rates of neurite outgrowth as well as dramatic changes in growth cone structure, some-

times referred to as "GC collapse" (Bixby, 1989). However, in our experiments, there was no increase in neurite outgrowth when the PKC activator was added to cells from Satb2-null mice, which can be explained by the complete inactivity of this kinase signaling pathway upon deletion of Satb2.

The important role of CaMKII in the development of the neural network is known. CaMKII mediates Ca^{2+}-induced neurite outgrowth through activation of extracellular signal-regulated kinase or microtubule affinity-regulating kinase 2 (MARK2), and CaMKIV mediates neuritogenesis by phosphorylation of CREB and subsequent CREB-dependent transcription (Takemura *et al*., 2009). It is reported that nerve growth factor (NGF)-regulated extracellular regulated kinase (ERK) phosphorylation triggers CREB phosphorylation, which recruits the CREB binding protein (CBP) to the NGF promoter regions of cAMP-responsive genes related to neuronal morphology change, synaptic plasticity, and dendritic spine growth (Boss *et al*., 2001). At the same time, CREB can also be activated by other protein kinases, including CaMKs (Miyamoto, 2006), phosphatidylinositol-3-kinase (PI3K)/Akt (Leinninger et al., 2004), cAMP-dependent protein kinase A (PKA) (Vitolo *et al*., 2002), and protein kinase C (PKC) (Zhao & Brinton, 2003). CaMKII is one of the most critical regulators of axon regeneration and neurite regrowth (Tashima *et al*., 1996). Knockdown of CaMKIIβ suppressed BDNF-induced neuritogenesis of cortical neurons in the rat at embryonic day 18, but knockdown of CaMKIIα did not affect neuritogenesis, indicating that there was difference in expression between CaMKIIα and CaMKIIβ in the early developmental stage of cortical neurons. However, studies report that CaMKII overexpression suppresses neurite regrowth induced by dibutyryl cyclic AMP or NGF (Tashima *et al*., 1996; Masse & Kelly, 1997). In our experiments, deletion of Satb2 reduced the expression of the gene encoding CaMKII to a lesser extent compared to PKC, and the kinase itself can be active to some extent in Satb2-null neurons. This probably explains the fact that the addition of the CaMKII activator enhances the growth of neurites in the cortical cells from Satb2-null mice.

It is also known that PI3K/Akt/mTOR signaling promotes neurite growth and differentiation in cortical neurons (Jin *et al*., 2012; Salto *et al*., 2015). Deletion of Satb2 affects the expression of PI3K to a lesser extent, although the protein Akt/PKB, the previous protein in the signaling chain, is suppressed upon deletion. Akt/PKB and PI3K activators not only promoted neurite outgrowth in cultured Satb2-null mice, but also activated neuronal differentiation through increased expression of the genes encoding Doublecortin, Synaptophysin, and PSD-95 (Couillard-Despres *et al*., 2008; Verstraelen *et al*., 2018).

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Thus, in this work, we have shown that the deletion of the transcription factor Satb2 in the cortical neurons leads to impaired differentiation of neurons, inhibition of neurite growth, and arrest of the formation of the neuronal network. The mechanism of this disorder is associated with the suppression of the expression level of protein kinase C, CaMKII, and Akt/PKB. In the neurons of Satb2-null mice, PKC expression is most suppressed and the addition of selective activators of this protein does not restore neurite outgrowth *in vitro*, while CAMKII and Akt/PKB activators significantly increase neurite outgrowth and neuronal network density. With the deletion of Satb2, there is practically no suppression of the expression of genes encoding PI3K, and at the initial stage of cell development, the expression of PI3K genes is even higher compared to the control. This ef-

fect may determine the survival of cells *in vitro* during long periods of cultivation, and the addition of an exogenous activator of this kinase to the cells best enhances the growth of neurites.

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