CENPJ REGULATES AXONAL GROWTH IN CORTICAL NEURONS THROUGH MICROTUBULE DESTABILIZATION

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Introduction

Neocortex development depends on complex series of time- and space-specific processes that culminate in the typical interconnected six-layered architecture of adult mammals. Axon growth is a fundamental process for the proper formation of the neural network. Malformations in axonal growth and pathfinding might lead to severe neuropathologies, such as the Corpus Callosum dysgenesis. Cenpj, a microcephaly gene, encodes a scaffold protein that regulates centrosome biogenesis and microtubule stabilization. During cortical development, Cenpj regulates progenitor division and neuronal migration during corticogenesis. As microtubule stabilization is crucial for axon extension, we ought to investigate the role of Cenpj in axon extension during the cortical development of callosal neurons. We performed loss- and gain-of-function assays through ex vivo and in utero electroporation of E14 mice. All procedures were approved by the local ethics committee (043/17). This study was funded by CAPES, CNPq and FAPERJ

Results

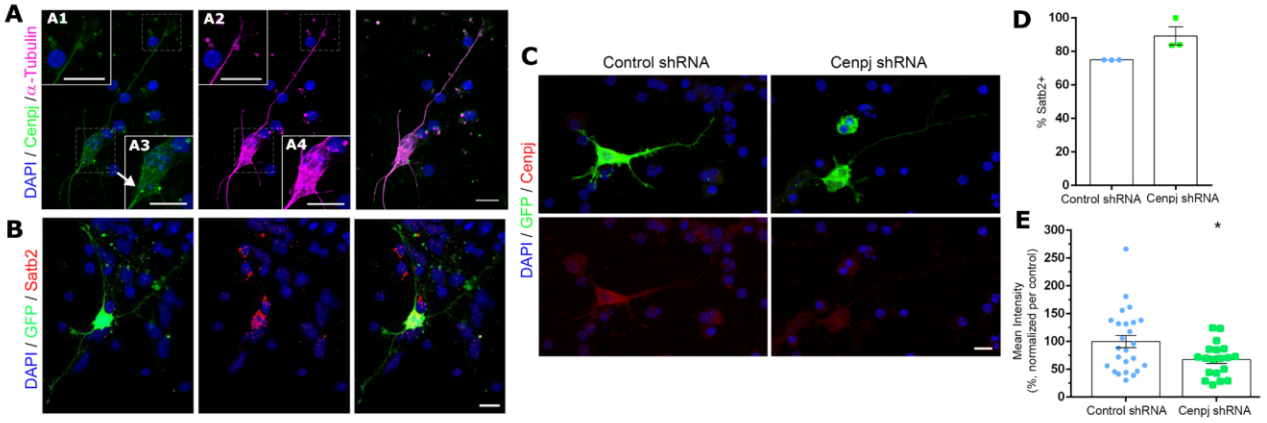
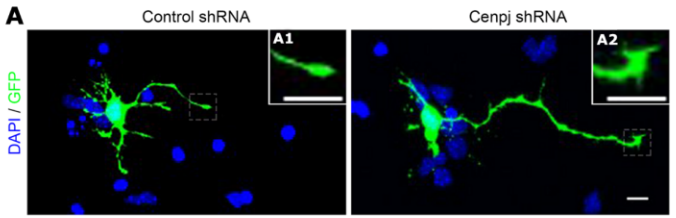
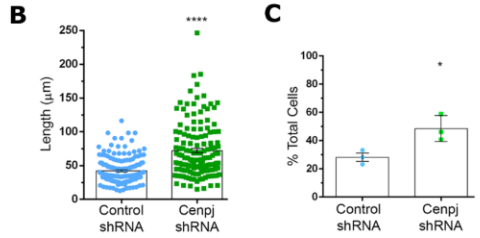


Figure 1. Cenpj is expressed in callosal dissociated and cultured neurons and Cenpj shRNA efficiently knocks the protein expression down. (A) Cenpj (green) is present in dissociated neurons from the dorsolateral cortex cultivated for 2DIV and appears to be colocalized with a-Tubulin expression (magenta). Insets show the soma (A3, A4) and the growth cone (A1, A2) magnified. Arrow indicates centrosome. (B) On average, 88% of cultivated neurons present the callosal marker Satb2 (red) (n=6 2 DIV cultures from the dorsolateral cortex). (C) Cenpj expression levels in control shRNA and Cenpj shRNA groups. (D) Comparison between control shRNA and Cenpj shRNA for Satb2+ neurons (ns, p=0.0578; unpaired t-test, parametric; SEM; n=3 independent cultures for each group). (E) Cenpj shRNA efficiently knocks down protein expression levels (red) as assessed with mean intensity in the soma (30% in average, p=0.0268; unpaired t-test, parametric; bars represent SEM; n=25 [control shRNA] and 19 [Cenpj shRNA] neurons). All scale bars represent 10 micrometers





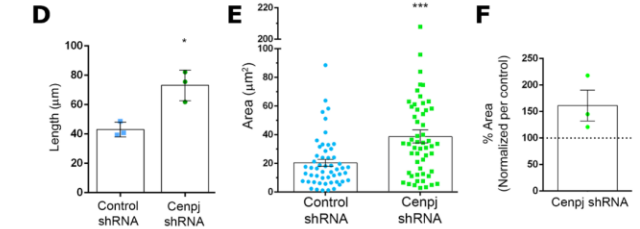


Figure 2. (A) Cenpj knockdown induces morphological changes in dissociated cortical neurons. (B) Comparison of the percentage of analyzed neurons that presented at least one primary branch from the axon shaft (p=0.0291; unpaired t-test, parametric; SEM; n=3 independent cultures for each group). (C) Cenpj shRNA neurons present longer axons compared to controls (p<0.0001; unpaired t-test, parametric; SEM; n=165 [control shRNA] and 146 [Cenpj shRNA] axons). (D) Average per experiment shows that Cenpj shRNA neurons present longer axons consistently (p=0.0106, unpaired t-test, parametric, SEM; n=3 independent cultures per group). Growth cone area (A1, A2, E) is larger in Cenpj shRNA neurons (p=0.0003; unpaired t-test, parametric; SEM; n=53 [control shRNA] and 54 [Cenpj shRNA] growth cones). (F) Average per experiment shows that Cenpj shRNA neurons do not present larger growth cones in a consistent fashion (ns, p=0.1702, paired t-test, parametric, SEM; n=3 independent cultures per group). All scale bars represent 10 micrometers

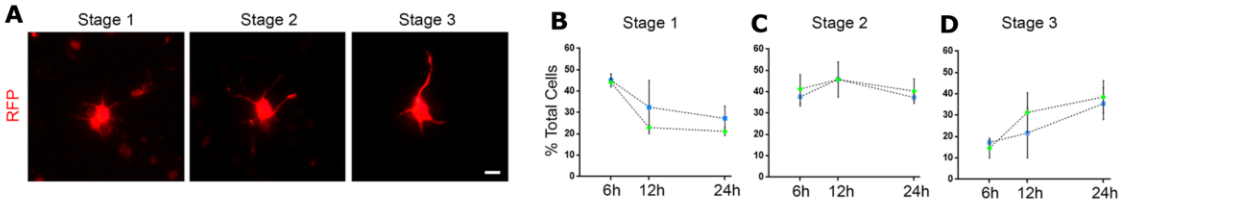


Figure 3. Cenpj does not influence neurite differentiation significatively. (B-D) Comparison of neurite differentiation stages (A) in control and Cenpj shRNA neurons over time (ns; Two-Way ANOVA, Sidak's multiple comparisons test; SEM; n=9 each for control and Cenpj shRNA groups, divided in 3 for each temporal point). All scale bars represent 10 micrometers

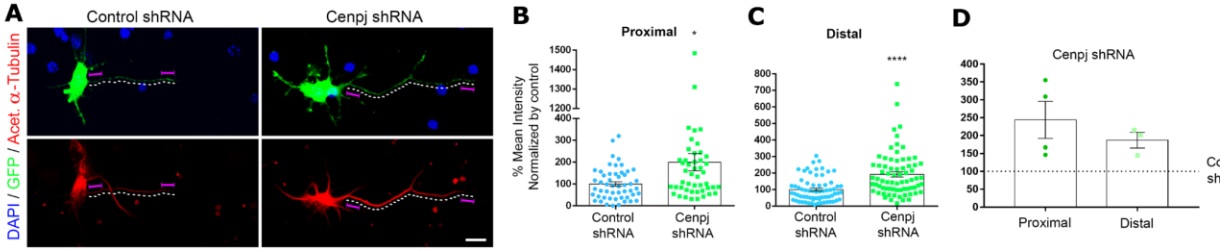
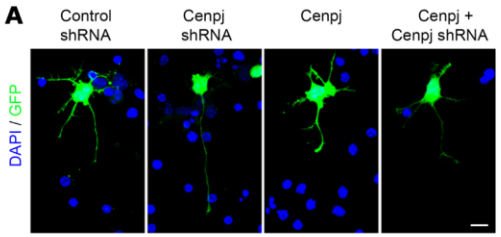


Figure 4. Cenpj knockdown neurons present more stable microtubules. (A) Cenpj shRNA neurons present more stable microtubules than control shRNA neurons. Dotted line represents axon pathway. Magenta bars indicate proximal (near soma) and distal (tip of axon) axon sites (B) Comparison for mean intensity of acetylated a-tubulin (red) in the proximal site between groups (p=0.0131, unpaired t-test, parametric; SEM; n=51 [control shRNA] and 47 [Cenpj shRNA] axons). (C) Comparison of mean intensity of acetylated a-tubulin in the distal site between groups (p<0.0001; unpaired t-test, parametric; SEM; n=68 [control shRNA] and 72 [Cenpj shRNA] axons). (D) Average per experiment shows that Cenpj shRNA neurons do not present more stable microtubules in a consistent fashion neither in proximal or distal sites (ns, p=0.1683 [proximal] and p=0.0584 [distal], paired t-tests, parametric, SEM; n=4 independent cultures for each group [Cenpj shRNA and control shRNA] for proximal comparison; n=3 independent cultures for each group [Cenpj shRNA and control shRNA] for distal comparison. All control groups present a 100% value of mean intensity, dotted line represents them). All scale bars represent 10 micrometers



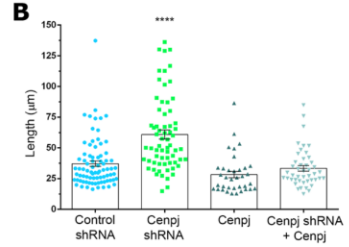
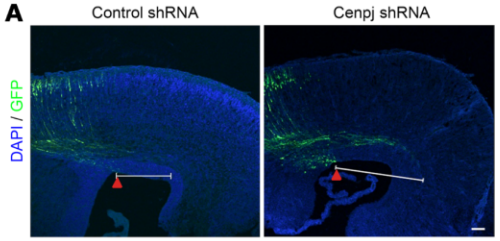


Figure 5. Cenpj expression rescues the phenotype observed in Cenpj knockdown neurons (A). (B) Comparison of control shRNA, Cenpj shRNA, Cenpj and Cenpj+Cenpj shRNA groups (p<0.0001; One-Way ANOVA, Sidak's multiple comparisons test; SEM; n=66 [control shRNA], 80 [Cenpj shRNA], 36 [Cenpj] and 45 [Cenpj+Cenpj shRNA]). All scale bars represent 10 micrometers



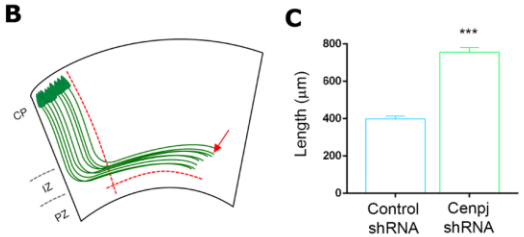


Figure 6. Cenpj in utero knockdown neurons present longer axons than control neurons in E15 (A). (B) Schematic representation of the measurement of the longest axons. A Paralel line is traced by the axon segments directed towards the ventricules. A perpendicular line is traced by the parallel one and is extended to follow the axon bundles to their most medial visible point. (C) Comparison of control shRNA and Cenpj shRNA groups (p<0.001; unpaired t-test parametric; SD). Scale bar represents 100 micrometers

Reduction in Cenpj expression levels in cortical neurons lead to higher microtubule stability and changes in neuronal morphology, the most evident one being axonal length. These alterations occur despite the effects of Cenpj in neurogenesis and neuronal migration. We hypothesise that Cenpj regulates axon growth by destabilizing microtubules during cortical development. Our findings also suggest that Cenpj might be a novel target for axonal regeneration