F-actin barriers attenuate CNP-induced process extension in HeLa S3 cells

To determine whether CNP mediates process extension in less morphological plastic cell types such as HeLa S3 cells, which contain thicker cortical actin and more abundant stress fibers (Fig. S3 A), cells that were transfected with the high expressing RcCMV-CNP construct were analyzed. In contrast with C397S (Fig. S3 B), CNP induced formation of filopodia and membrane expansion in localized areas but did not induce MT-filled processes (Fig. S3 C). In some cells, a subpopulation of cytoplasmic CNP was enriched along filamentous strands that partially colocalized with MTs (Fig. S3 C, arrowheads). In other cells, CNP was enriched in large membrane protrusions that extended from the apical cell surface (Fig. S3 C, arrow). The cytoskeletal nature of these protrusions is shown by deconvolution of single cell planes at 0.6-μm intervals, revealing continual colocalization of CNP with tubulin (Fig. S3 D) and F-actin (Fig. S3 E). Similar apical filopodia-like protrusions have been observed in HeLa cells coexpressing Rho family GTPase Rif and Cdc42 (Ellis and Mellor, 2000).

F-actin barriers may impede process formation and MT projection from the cell surface even though cortical actin and stress fibers were noticeably diminished in CNP-expressing cells (Fig. S3 E). Because cytochalasin-induced depolymerization of cortical actin was required for MAP2-induced process formation in certain nonneuronal cell types (Edson et al., 1993), we tested this possibility by depolymerizing F-actin completely with 10 μM cytochalasin. Normal HeLa S3 cells transiently formed tubulin-rich apical protrusions after 10 min, which was followed by cell shape elongation, spreading, and projection of small, thin MT spikes after 1 h (Fig. S4 A, top). After a 10-min exposure, CNP-expressing cells formed similar membranous protrusions but also began to extend numerous MT strands and processes (Fig. S4 A, middle and bottom). After 1 h, approximately half of the cells formed long, arborized processes even in the absence of de novo F-actin assembly (Fig. S4 B). Thus, CNP-mediated process extension is attenuated by F-actin barriers in less morphologically plastic HeLa S3 cells.

References

Figure S2. CNP colocalizes with tubulin/MTs in immature OLs. (A) Immature OLs double stained for CNP (red) and tubulin (green). CNP colocalizes extensively with tubulin/MTs in the cell body and in certain regions of immature OL processes. CNP is enriched in branching sites (inset A’) and in large, tubulin-rich punctate varicosities (inset A”). (B) Immature OLs double stained for CNP (red) and acetylated tubulin (green). Within the cell body, CNP is enriched in the vicinity of MTOC, where growing MTs are initially nucleated and projected into larger, growing processes. Bar, 10 μm.
Figure S3. Effect of CNP overexpression in HeLa S3 cells. (A) HeLa S3 cells stained with rhodamine-phalloidin. (B) C397S-transfected cells stained for CNP. (C) CNP-transfected cells double stained for CNP (green) and tubulin (red). Arrowheads show partial colocalization of filamentous CNP with MTs. Arrow shows apical-projected membrane protrusions. (D and E) CNP-transfected cells with apical-projected membrane protrusions were imaged by z-sectioning at 0.6-μm intervals. Cells double stained for CNP (green) and either tubulin (D, red) or F-actin (E, red) show CNP, tubulin, and F-actin enrichment in cytoskeletal protrusions. Bars, 10 μm.
Figure S4. CNP overexpression in HeLa S3 cells promotes process formation after cytochalasin treatment. (A) F-actin disruption permits CNP-mediated process extension. 10 μM cytochalasin D effect on normal HeLa S3 cells stained for tubulin (top) and CNP-transfected cells double stained for CNP (green) and either tubulin (middle, red) or F-actin (bottom, red). Images were obtained by normal epifluorescence. (B) Cytochalasin-treated CNP-transfected cells were scored for the presence or absence of MT-filled processes ($n > 100$).