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STOP Proteins†

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ABSTRACT: Microtubules assembled from purified tubulin *in vitro* are labile, rapidly disassembling when exposed to a variety of depolymerizing conditions such as cold temperature. In contrast, in many cell types, microtubules seem to be unaffected when the cell is exposed to the cold. This resistance of microtubules to the cold has been intriguing because the earliest and by far most studied microtubule-associated proteins such as MAP2 and tau are devoid of microtubule cold stabilizing activity. Over the past several years, it has been shown that resistance of microtubules to the cold is largely due to polymer association with a class of microtubule-associated proteins called STOPs. STOPs are calmodulin-binding and calmodulin-regulated proteins which, in mammals, are encoded by a single gene but exhibit substantial cell specific variability due to mRNA splicing and alternative promoter use. STOP microtubule stabilizing activity has been ascribed to two classes of new bifunctional calmodulin- and microtubule-binding motifs, with distinct microtubule binding properties *in vivo*. STOPs seem to be restricted to vertebrates and are composed of a conserved domain split by the apparent insertion of variable sequences that are completely unrelated among species. Recently, STOP suppression in mice has been found to induce synaptic defects associated with neuroleptic-sensitive behavioral disorders. Thus, STOPs are important for synaptic plasticity. Additionally, STOP-deficient mice may yield a pertinent model for the study of neuroleptics in illnesses such as schizophrenia, currently thought to result from defects in synapse function.

Microtubules are fibrous elements in the cytoplasm of eukaryotic cells, where they play a pivotal role in many vital cell functions, including cell division, morphogenesis, and vesicle trafficking (*1*). Microtubules are particularly abundant in neurons where they are thought to be central to cell morphogenesis and maintenance (*2*−*4*). Microtubules assembled *in vitro* from pure tubulin preparations are dynamic, exhibiting both large spontaneous length fluctuations and treadmill-type behavior (*5*, *6*). Such dynamic microtubules are strongly sensitive to variations in their physicochemical environment. For instance, exposure of microtubules to the cold (<15 °C) or to a variety of depolymerizing drugs induces rapid polymer disassembly.

Most cellular microtubules also exhibit length fluctuations and treadmill-type behavior, and microtubule dynamics seem to be important for microtubule-dependent cell functions (*7*). Yet many cell types, including neurons, glial cells, and fibroblasts, contain microtubule-stabilizing factors that can block microtubule dynamics and induce resistance of polymers to the cold and to depolymerizing drugs (*8*−*13*). For instance, when neuronal or fibroblastic cells are exposed to the cold, most microtubules do not depolymerize (Figure 1). Early studies assumed that this microtubule stabilization was the result of polymer association with microtubule-stabilizing

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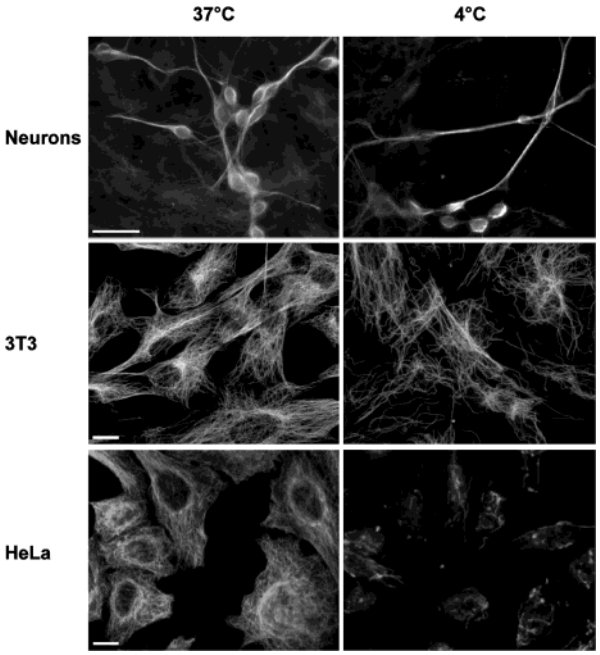

FIGURE 1: Cold stability of microtubules in cells. Mouse hippocampal neurons, mouse NIH3T3 fibroblasts, and human HeLa epithelial cells were either kept at 37 °C (left) or exposed to the cold for 45 min (right). Following extraction of free tubulin by cell permeabilization, the microtubules were stained by indirect immunofluorescence with mAb Tub2.1 tubulin antibody and a Cy3-labeled secondary antibody. In neuronal and NIH3T3 cells, most microtubules are resistant to the cold and remain intact following exposure to the cold. By comparison, microtubules in HeLa cells are depolymerized after they are exposed to the cold. The bar is 10 μm.

proteins [microtubule-associated proteins (MAPs)].[1] The search for these proteins has followed "a contorted logic" (*14*) since, for technical reasons, the early and by far most studied MAPs such as MAP2 and tau were isolated from recycled cold-labile brain microtubule preparations (*15*, *16*). In subsequent studies, these MAPs were found to be unable to reconstitute microtubule cold stability as observed in cells (*17*, *18*), and their suppression in mice had no detectable effect on microtubule stability (*19*).

The search for MAPs associated with cold-stable microtubules, which are much harder to isolate than recycled cold-labile polymers, was undertaken by only a handful of groups. However, over the past decade, it has been proven that microtubule cold stabilization is principally due to microtubule association with a family of proteins known as STOPs (for stable tubule only polypeptides). The functional domains of these proteins have been characterized, as well as the main physiological consequences of STOP suppression *in vivo*. This review covers the principal biochemical and functional properties of STOPs, their apparently curious evolutionary history, and the disorders induced by their suppression in whole animals.

N-STOP

Early work on cold-stable microtubules suggested that the putative MAPs responsible for microtubule stabilization in adult mammalian brain were calmodulin-binding and calmodulin-regulated proteins (*20*, *21*). On the basis of such work, N-STOP (for neuronal adult STOP) was initially isolated as a calmodulin-binding protein associated with purified rat brain cold-stable microtubules (*22*). N-STOP was later shown to have intrinsic microtubule cold stabilizing activity *in vitro*, and this activity proved to be inhibited in the presence of Ca^{2+}-bound calmodulin (*23*). Rat N-STOP has an apparent molecular mass of ca. 125−45 kDa depending on SDS−PAGE conditions. Cloning of N-STOP cDNA showed that the protein contained 952 aa with a calculated molecular mass of 100 484 Da (*24*). The N-STOP amino acid sequence is unrelated to that of classical MAPs such as tau, MAP2, or MAP1B. N-STOP contains two repeat domains (Figure 2A). The central repeat domain is composed of five repeated sequences of 46 aa. These sequences are almost completely identical, exhibiting an unusual degree of conservation of the repeat motif, compared to repeated sequences in other microtubule-associated proteins. The carboxy-terminal repeat domain is composed of 28 imperfect repeats of an 11 aa consensus sequence. Upstream of the carboxy-terminal repeat domain, rat N-STOP contains a highly basic sequence (called the "KR domain" after its high content in lysine and arginine residues) and a so-called "linker domain" located between the central repeat domain and the KR domain. N-STOP includes four consensus sites for phosphorylation by Cam

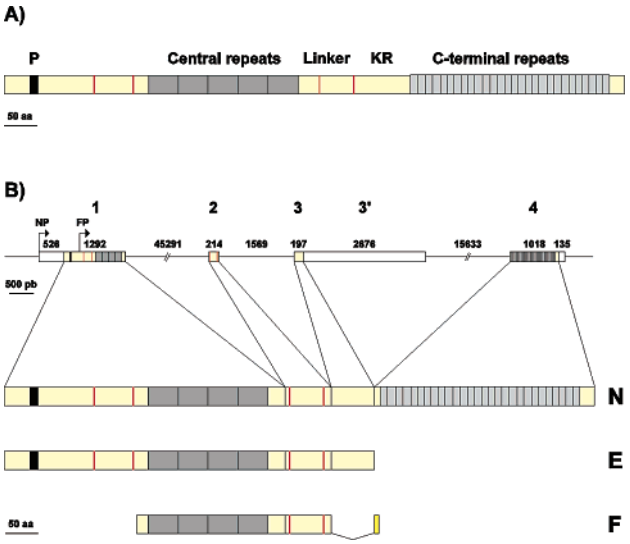

FIGURE 2: STOP proteins and gene structure. (A) Schematic representation showing the domain structure of the N-STOP protein. Sequence analysis reveals five highly conserved tandem repeats of 46 aa (central repeat, dark gray boxes), 28 imperfect tandem repeats of 11 aa (C-terminal repeat, light gray boxes), two tandem polyproline putative SH3-binding sites (P, black box), and four putative Cam kinase II phosphorylation sites (red bars). The lysine- and arginine-rich domain (KR) and the linker domain are also indicated. (B) Organization of the mouse STOP gene (Mtap6) showing STOP exons and schematic representation of three characterized STOP variants. Exon and intron lengths are indicated in base pairs. Exonic untranslated sequences are boxed. NP is the promoter of neuronal isoforms N-STOP and E-STOP. FP is the promoter of fibroblastic isoform F-STOP. N-STOP is encoded by exons 1−4. E-STOP is encoded by exons 1−3. In the E-STOP mRNA, exon 3 is elongated by a 2.7 kb intronic sequence (designated 3′) which begins with a stop codon. F-STOP is encoded by part of exon 1 and by exon 2. In the F-STOP mRNA, splicing of exon 2 with exon 4 introduces a frame shift and the termination of the ORF 21 bp downstream (dark yellow). Domain colors are as they are in panel A.

[1] Abbreviations: aa, amino acid(s); E-STOP, early STOP; F-STOP, fibroblastic STOP; MAP, microtubule-associated protein; N-STOP, neuronal adult STOP; STOP, stable tubule only polypeptide.

kinase II, and this may be the basis for the observed modulation of microtubule cold stability by calmodulin-dependent phosphorylation (ref *21* and unpublished observations of J. Baratier). Other putative consensus sequences include two tandem polyproline putative SH3-binding sites, located upstream of the central repeat sequences. N-STOP has hitherto been found only in neurons, with maximal expression in adult brain compared to embryonic or newborn brain.

The STOP Gene, E-STOP, and F-STOP

Mapping experiments and analysis of genomic data show that in rodents and humans the STOP gene is unique, being localized on chromosomes 1q32, 7 E2-F1, and 11q14 in rats, mice, and humans, respectively (*25*). The STOP gene includes four exon sequences encoding N-STOP (Figure 2B). Each exon sequence corresponds almost precisely to one of the four protein domains identified on the protein. Exon 1 encodes the N-terminal domain of N-STOP and includes the central repeat domain. Exons 2−4 encode the linker domain, the KR domain, and the carboxy-terminal repeat domain of N-STOP, respectively. So far, two splicing variants of STOP, E-STOP and F-STOP, have been characterized in rodents (Figure 2B). As with N-STOP, both variants exhibit calmodulin binding and microtubule stabilizing activity (refs *26* and *27* and unpublished data of E. Denarier). E-STOP (for early STOP) is a neuron specific variant of STOP with an apparent molecular mass of 84 kDa in rat (*26*). E-STOP is the major STOP variant in embryonic rodent brain and persists in adult brain. In the E-STOP mRNA, exon 3 sequences are fused with the downstream intronic sequences. This fusion induces the termination of the ORF precisely at the junction between exon 3 and intronic sequences. As a consequence, E-STOP is encoded by exons 1−3 and lacks the carboxy-terminal repeat domain present in N-STOP. F-STOP (for fibroblastic STOP) is a 42 kDa variant of STOP, initially characterized in NIH3T3 fibroblastic mouse cells (*27*). Recent work indicates that F-STOP is widespread in mouse tissues (*28*). In the mouse F-STOP mRNA, exon 3 sequences are missing and exon 2 and 4 sequences are fused. This fusion introduces a frame shift and a termination of the ORF 21 bp downstream of exon 2. The transcription initiation site for F-STOP mRNA is located in exon 1, 851 bp downstream of the initiation site for the N-STOP mRNA (*27*). As a result, F-STOP lacks a large part of the N-terminal aa sequences present in N-STOP, being mainly composed of the central repeat and linker STOP domains.

STOPs in Cells

HeLa cells are devoid of immunodetectable STOPs (*24*, *27*) and display rapid microtubule disassembly upon being exposed to the cold (Figure 1). Expression of either of the STOP variants described above in HeLa cells induces a complete resistance of microtubules to the cold. Thus, STOPs have microtubule cold stabilizing activity *in vivo* (*24*, *26*, *27*). Additionally, injection of STOP blocking antibodies in neuronal cell lines, which express N- and E-STOP, suppresses the resistance of microtubules to the cold (*26*). Injection of similar blocking antibodies in NIH3T3 cells, which express F-STOP, also suppresses microtubule stabilization to the cold (*27*). Thus, STOPs are the main factors

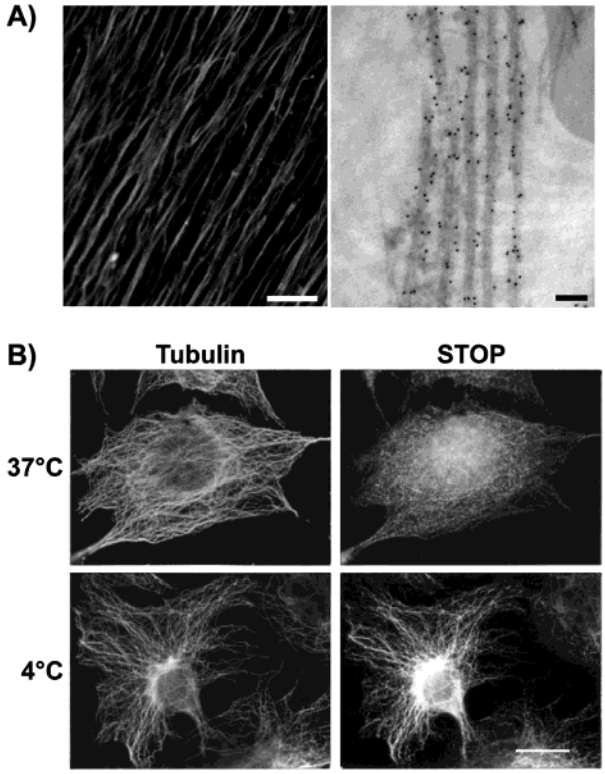

FIGURE 3: Distribution of STOP proteins in cells. (A) Immunolocalization of STOP proteins in the axons of DRG cells kept at 37 °C. Staining of DRG cells, being cultured for 10 days, was performed with affinity-purified 23C STOP antibody and a Cy3-labeled (left) or gold-labeled (right) secondary antibody. STOP proteins are associated with axonal microtubules at 37 °C. The bars are 25 μm (left) and 50 nm (right). (B) Immunofluorescence analysis of the distribution of STOP in NIH3T3 cells. Cells were either kept at 37 °C (top row) or exposed to the cold for 45 min (bottom row). Microtubules and STOP proteins were double-stained by indirect immunofluorescence with mAb Tub2.1 tubulin antibody and 23C STOP antibody. The bar is 10 μm. STOP proteins, poorly associated with microtubules at 37 °C, are recruited on microtubules during exposure to the cold.

responsible for microtubule resistance to the cold in both neuronal and non-neuronal cells.

STOP proteins block microtubule dynamics when associated with microtubules at physiological temperature (*24*). However, the different STOP variants interact with microtubules differently under physiological conditions. In intact neuronal cells, where microtubule turnover is slow (*29*), the bulk of N-STOP and E-STOP is associated with microtubules. Thus, in lysed neuronal cells, both proteins are associated with Triton-resistant cell fractions and are undetectable in soluble cell fractions. Additionally, STOP antibodies intensely decorate axonal microtubules, in immunoelectron microscopy (*26*) (Figure 3A). In contrast, in interphase 3T3 cells kept at 37 °C, where microtubule turnover is rapid, the bulk of F-STOP is found in the soluble cell fractions (*27*) and there is only a very faint microtubule decoration with STOP antibodies (*27*) (Figure 3B). Strikingly, within seconds of cells being exposed to the cold, F-STOP associates with cytoplasmic microtubules, thus inducing resistance of microtubules to the cold (*27*) (Figure 3B). We do not know precisely the mechanisms involved in the temperature-dependent association of F-STOP with microtubules. Probably, association of F-STOP with interphase microtubules in 3T3 cells is modulated by regulatory

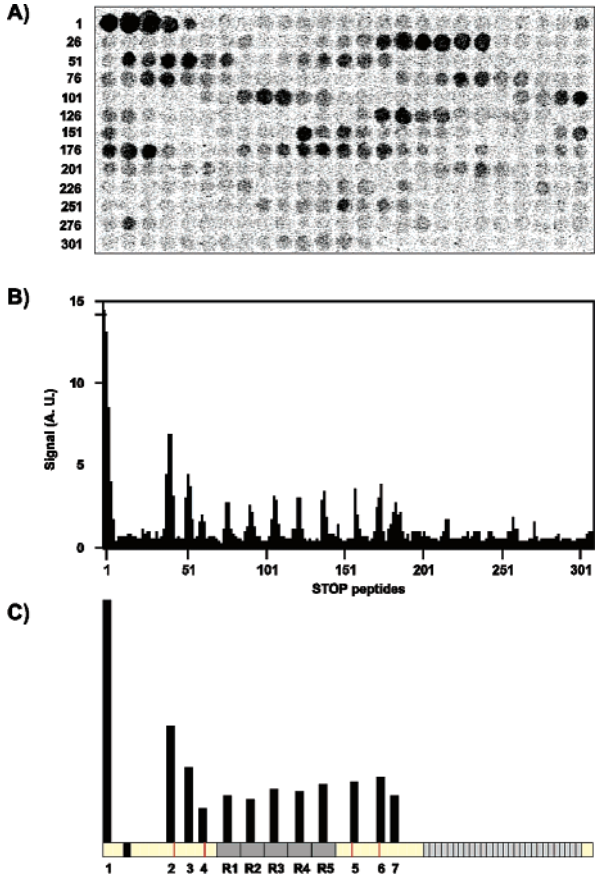

FIGURE 4: Identification of calmodulin-binding peptides using immobilized peptide arrays. (A) ^{35}S-labeled calmodulin overlay of a membrane containing immobilized rat N-STOP peptides. The 15 aa peptides were numbered from the amino-terminal to the carboxy-terminal residues of N-STOP, each peptide overlapping 12 aa with the next peptide. Numbers correspond to the first peptide of each line. Twelve peptide clusters interacting with ^{35}S-labeled calmodulin were detected on autoradiography. (B) Quantitative analysis of binding of [^{35}S]calmodulin to STOP peptides. The radioactive signals observed in panel A were quantified, and results were plotted. Peptides are numbered as in panel A, and signal values are in arbitrary units. (C) Mapping of the radioactivity peak peptides observed in panel B on a schematic representation of rat N-STOP. Five calmodulin-binding sites (R1−R5) were located in the central repeats. Other calmodulin-binding sites (1−7) are numbered from the amino terminus to the carboxyl terminus of the protein. Coloring is as in Figure 2.

mechanisms that are rapidly inhibited when the temperature drops.

STOP Modules

The characterization of the domain composition of STOPs has been essential for an understanding of both the biochemical properties and the cellular effects of STOPs. This characterization has been guided by the mapping of STOP calmodulin-binding sequences, using immobilized peptide arrays (*30*). This technique revealed what looked at first sight like a bewildering number of calmodulin-binding peptides on STOPs. At least twelve distinct 15 aa peptide sequences are elucidated when rat N-STOP peptide arrays are incubated with radiolabeled calmodulin (Figure 4A,B). The exon 1 domain of N-STOP contains a total of nine calmodulin-binding sequences, one located at the extreme N-terminus of N-STOP, three located upstream of the central repeat domain, and one per central repeat. Three additional calmodulin-binding peptides are located between the central and C-terminal repeats. The central repeat calmodulin-binding peptides are termed CamR1−CamR5 peptides, and the other calmodulin-binding sequences are named Cam1−Cam7 according to their order of occurrence in the protein sequence (Figure 4C). In addition to binding calmodulin on peptide arrays, peptides Cam1−Cam3, Cam5, Cam6, and CamR1−CamR5 have the capacity to mediate protein binding on immobilized calmodulin columns and/or calmodulin in a Scatchard assay (*30*). Biochemical measurements indicate a K_d for calmodulin binding by STOP peptides that is in the micromolar range.

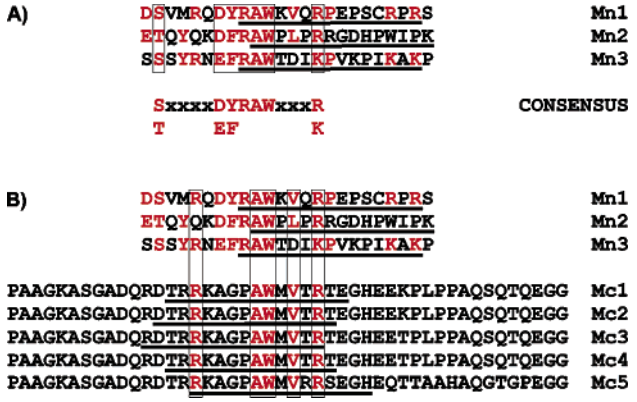

FIGURE 5: STOP modules. (A) Sequence alignment of rat STOP Mn1−Mn3 modules. Amino acids composing the calmodulin-binding peptides of these modules (Cam2, Cam3, and Cam5, respectively) are underlined. Amino acids conserved in at least two modules are shown in red. Amino acids conserved among the three modules are boxed. These aa define a consensus sequence indicated below the alignment. (B) Sequence alignment of rat STOP Mn1−Mn3 modules and Mc modules (Mc1−Mc5). Amino acids comprising the calmodulin-binding peptides of these modules (Cam2, Cam3, Cam5, and CamR1−CamR5) are underlined. Conserved aa identified in panel A are shown in red. Among these aa, those that are shared by Mn and Mc modules are boxed.

The observed inhibition of STOP microtubule stabilizing activity by Ca^{2+}-bound calmodulin (*20*, *21*) suggested that the microtubule-stabilizing domains and the calmodulin-binding peptides of STOP may be overlapping. The microtubule-stabilizing domains of STOP have been mapped on the basis of this hypothesis. The microtubule stabilizing activity of STOP deletion mutants, lacking one or several of the 15 aa calmodulin-binding sequences identified by SPOT analysis, was assessed in HeLa cells transfected with the corresponding cDNAs. Peptides Cam2, Cam3, Cam5, and CamR1−CamR5 were apparently essential, although not sufficient, for microtubule stabilization (*30*). Interestingly, peptides Cam2, Cam3, and Cam 5 were located downstream of and partially overlapped conserved peptide sequences of 15 aa (Figure 5A). The conserved sequences and their associated overlapping calmodulin-binding peptides were called Mn modules. Analogously, the 46 aa central repeats containing calmodulin-binding peptides CamR1−CamR5 were considered modules (Mc modules), as each of them is comprised of a 46 aa consensus sequence overlapping a calmodulin-binding peptide (Figure 5B). In further analysis, both kinds of modules induced microtubule cold stability when expressed in HeLa cells, but had different effects on microtubule sensitivity to nocodazole. Mn modules induced resistance of microtubules to nocodazole-induced disassembly when expressed in HeLa cells, whereas Mc modules

lacked such effects, indicating that Mc modules do not bind to cytoplasmic microtubules at 37 °C. As described above, N-STOP, which contains three Mn modules in addition to the five Mc modules, induces both the cold stability of microtubules and the resistance of microtubules to nocodazole. F-STOP has two fewer Mn modules than N-STOP, and this corresponds with a weakened ability to induce resistance of microtubules to nocodazole. Such parallelism strongly suggests that the differences in behavior and activity between neuronal STOPs and F-STOP are due, at least in part, to their different module composition.

Mn and Mc modules represent new bifunctional calmodulin-binding and microtubule cold stabilizing motifs. The Mn and Mc modules show some homology, involving 5 aa (Figure 5B). Further structural studies will tell us whether these aa are of particular importance for microtubule stabilization.

STOPs in Evolution

STOPs are apparently restricted to vertebrates (ref *30* and unpublished observations). Protein, cDNA, and genomic data are available for mammals, fishes, and birds. The following sections contain previously published information (*30*) completed by screens in recent databases.

Mammalian STOPs. In mammals, biochemical evidence and DNA sequences indicate the presence of STOP in humans, mice, rats, cows, pigs, sheep, and chimpanzees. Extensive data are available in the case of human, mouse, and rat STOPs. The human, mouse, and rat N-STOPs share a high degree of homology (79−94%) (Figure 6). All are apparently encoded by a single gene. The genomic structure of the gene is conserved, with four exons and two large intervening introns (Figure 2B). However, there is variability in the composition of the central repeat domain of N-STOP (Mc modules). The number of central repeats within a given rodent species shows allelic variability (four to five in mice and four to six in rats). The human N-STOP contains a single central repeat (Figure 6). Repeat number variability in microtubule-stabilizing domains has been observed in tau, MAP2, and MAP4. In the case of these MAPs, the variability originates from alternative splicing of exons specifically encoding one of the repeats (*31*, *32*). In the case of STOPs, central or C-terminal repeats are internal to exons 1 and 4, respectively, and their variability does not result from alternative splicing.

As described above, the translation of the mouse F-STOP mRNA uses a start site different from that of N-STOP. The corresponding ATG is not conserved in other mammals, implying interspecies variability in the non-neuronal STOP N-terminus. Actually, whereas non-neuronal STOPs have also been detected in rats (*26*, *27*), their existence remains unproven in nonrodent species.

We have detected several putative STOP variants in addition to N-STOP, E-STOP, or F-STOP using Northern and Western blot analysis of various tissues and cells and screens of sequences in database (*26*−*28*). The corresponding cDNAs are still uncharacterized. In some cases, STOP variants may involve additional exons and splicing variants. For instance, a 151 pb exon, located in intron 3, is fused with exon 4 in a retinal EST (GenBank entry AA317764). This exon is also present in the rat genome, and absent in the mouse genome.

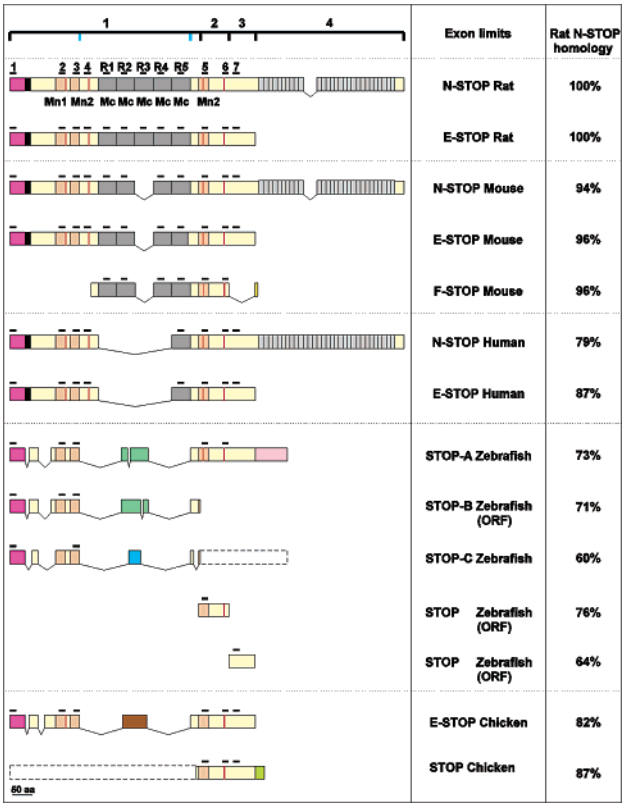

FIGURE 6: Domain structure of STOP proteins from various species. The figure shows schematic representations of mammalian, fish, and avian STOP proteins as indicated. Protein structures and exon limits were deduced from cDNA and genomic sequences. ORF indicates sequences deduced from genomic data only. Regions shown in the same color are homologous between species. Modules Mn and Mc are shown in orange and dark gray, respectively. The N-terminal module common to STOPs and SL21 is in purple. Calmodulin-binding sites are indicated with black horizontal bars. The black box is the putative binding site for SH3 domains. Light gray boxes are C-terminal repeats. Red bars are putative Cam kinase II phosphorylation sites. Fish and avian variable STOP domains are shown as dark green, blue, or brown boxes. The limits between the conserved and variable STOP domains are indicated by two blue bars (top, exon limits). Fish and avian C-terminal STOP domains are shown in pink and light green, respectively. Dashed boxes are domains of unknown sequence. The percents of homology with rat N-STOP are indicated. For mammals, the calculation excludes the additional central or C-terminal repeats. For fish and avian STOP proteins, the calculation excludes the indicated amino-terminal deletions, the variable inserted domain, and the variable C-terminal domain. Some of the accession numbers are given in ref *30*. Additional data concern the human E-STOP cDNA (GenBank entry XM_166256), the fish STOP-A (GenBank entries BG306376, BG308187, and AL921111; WGS Traces 143686104, 128641747, 119884986, 131401243, 100156129, and 102786197 at the NCBI zebrafish genome Blast program, http://www.ncbi.nlm.nih.gov/genome/seq/DrBlast.html), STOP-B (zebrafish WGS Traces 30610698 and 25633661), and STOP-C (GenBank entry CA370003; zebrafish WGS Traces 42492373 and 133224039) proteins, the additional zebrafish exon 2 and exon 3 (zebrafish WGS Traces 99997172 and 90554653, respectively), and the splicing variant of chicken E-STOP (GenBank entries BU353890 and BU357727).

Avian and Fish STOPs. Database searches show the presence of ortholog STOP DNA in chicken, zebrafish, fugu, tetraodon, and trout. In these species, the central repeat domain is replaced by shorter and nonrepetitive sequences that are completely unrelated to the mammal central repeats and that are divergent between fish and birds (*30*) (Figure 6). Strikingly, the divergent sequences are inserted in the

middle of STOP exon 1, at a strictly defined location, starting at the first amino acid following module Mn2 (aa 175, according to the rat N-STOP sequence) and terminating at the last amino acid of the last central repeat (aa 451, in the rat N-STOP sequence). The exon 1 sequences flanking the insertion, as well as the sequences of exons 2 and 3 of birds and fish, are highly conserved, being 60−87% homologous with that of rat STOP (Figure 6).

Fish and bird STOPs also lack the C-terminal repeats of mammalian N-STOPs. In these organisms, the STOP C-terminus is composed of short nonrepetitive sequences (Figure 6, pink and light green boxes). These sequences are unrelated in fish and birds. In chicken, the 22 aa C-terminus can be alternatively spliced to produce the equivalent of mammalian E-STOP, initially described as cNau (*33*).

The fish genome contains multiple copies of STOP exons 1−3. A fish STOP-A protein can be deduced from EST sequences (Figure 6). Exon 1 sequences corresponding to two additional STOPs can be deduced by genome or EST analysis (STOP-B and STOP-C, Figure 6). Genome analysis also shows additional copies of exons 2 and 3. More EST data will be necessary to ascribe these exons to either STOP-B or STOP-C and to determine the complete structure of these proteins.

Dual Nature of STOPs

Comparison between species indicates a dual-domain composition of STOP exon 1 with a highly conserved domain split by the insertion of a variable domain at a precise location. Such a variable domain can hardly arise from mutations in a common ancestral gene. Instead, it may correspond to the insertion in an ancestral N-STOP exon 1 of sequences originating from other genes, although plausible mechanisms for such an insertion remain to be defined. The insertion of foreign sequences in a conserved gene could be related to another unique feature of STOP which concerns promoters. Different promoters are used for N-STOP and F-STOP, and remarkably coding sequences of N-STOP exon 1 are part of the F-STOP promoter (*28*). Thus, the same STOP sequence is used either as a promoter sequence or as a coding sequence in the same exon (Figure 2B). There is to our knowledge only one other example of such a situation, in the Golli/myelin basic protein (MBP) gene, where an MBP promoter lies in a coding sequence for the Golli protein (*34*). In the STOP gene, this particular feature may be a signature of the "cannibalization" of a common ancestral STOP gene by inserted sequences.

During evolution, there is a strict segregation of STOP modules among the conserved and variable domains of N-STOP, which contain Mn and Mc modules, respectively. Mn modules may be essential for the microtubule stabilizing activity of a conserved ancestral brain STOP. Mc modules are apparently dispensable for N-STOP functions, at least in some species. Within the "insertion" hypothesis, Mc modules may be primarily involved in cell functions distinct from those involving Mn modules, despite intriguing convergence in the biochemical properties of Mn and Mc modules. In this view, F-STOP, which mainly contains Mc modules, could be a protein distinct from N-STOP, not merely a STOP variant. Alternatively, despite sequence divergence, the insertions observed in fish and birds may have calmodulin binding and microtubule stabilizing activity, indicating a requirement for such activities for STOP function.

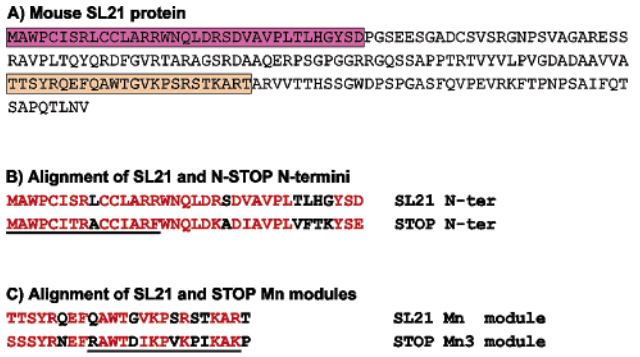

FIGURE 7: SL21 sequence and modules conserved with STOP proteins. (A) Protein sequence of mouse SL21, deduced from a cDNA sequence (GenBank entry BY727771). The SL21 N-terminal sequence and the Mn module in common with STOP proteins are boxed in purple and orange, respectively. Both SL21 sequences are aligned with the corresponding mouse N-STOP sequences in panels B and C. Conserved amino acids are shown in red. The N-STOP calmodulin-binding peptides Cam1 (N-terminal) and Cam5 (module Mn3) are underlined.

STOP-like Proteins

We have searched for proteins sharing at least one conserved module with STOPs. This resulted in the identification of a mammalian protein distinct from STOPs and containing an Mn3 module (Figure 7). In addition, the protein contained another highly conserved domain of STOP, corresponding to the N-terminus of N-STOP and comprising the calmodulin-binding peptide Cam1 (Figure 7). Besides these two shared sequences, the protein was not at all homologous with STOP. We have called this protein SL21, for 21 kDa STOP-like protein (*30*). Recent work in our laboratory indicates that SL21 is indeed a calmodulin-binding and calmodulin-regulated MAP (S. Gory-Fauré, unpublished observations). The sharing of a common N-terminal domain, containing a calmodulin-binding peptide (Cam1), by N-STOP and SL21, indicates that this domain has functional individuality and that it may be considered an additional STOP module.

STOP-Deficient Mice

We have recently produced STOP-deficient mice (STOP −/− mice) (*10*). In these mice, microtubules are cold-labile in both neuronal (Figure 8) and non-neuronal cells. This dramatic modification of microtubule stability has no catastrophic consequences for mouse organogenesis or viability. To this day, no clear phenotype has been detected in non-neuronal tissues following STOP suppression. We expected stable microtubules to be a key support for neuronal differentiation. It came as a surprise to us that STOP −/− mice, while devoid of microtubules that are resistant to the cold, actually lack detectable anomalies in brain anatomy. But STOP −/− mice display multiple deficits in synaptic function that affect both long- and short-term synaptic plasticity (*10*). These synaptic defects are associated with multiple behavioral disorders, including a disorganized activity with disruption of normal behavioral sequences and episodes of hyperlocomotion or apparent prostration, anxiety,

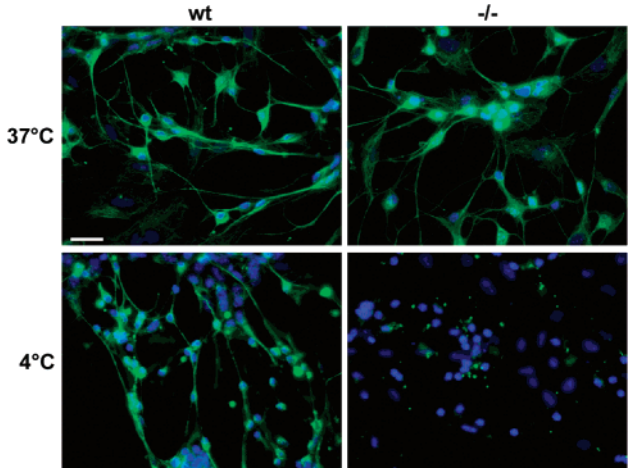

FIGURE 8: Analysis of microtubule stability and STOP content in primary cultures of neuronal cells from wild-type or STOP −/− embryos. Cells were either kept at 37 °C or exposed to the cold for 45 min. Following extraction of free tubulin by cell permeabilization, microtubules were stained with tubulin antibody (green) and nuclei with Hoescht 33258 (blue). The bar is 20 μm.

severe social withdrawal, and complete nurturing defects. Most interestingly, behavioral defects in STOP −/− mice are alleviated by long-term treatment with neuroleptics. These drugs are potent antipsychotic agents used for schizophrenia. This severe mental disease is currently thought to result from a dysfunction of synapses (*35*, *36*). STOP −/− mice, which exhibit behavioral disorders linked to synaptic dysfunction and reactive to antipsychotics, provide an interesting model for the study of the origin and treatment of schizophrenia-related disorders. These mice are currently under evaluation in two major pharmaceutical companies for potential screening of new antipsychotic agents. Interestingly, the STOP gene is located in a region of the human genome that has been linked to schizoid disorders (*37*−*39*).

Conclusion

STOP proteins are responsible for the high degree of microtubule stabilization observed in many mammalian cell types, including neuronal cells. STOP activity has been ascribed to distinct modular sequences along the protein chain, and these sequences define novel bifunctional-calmodulin and microtubule-stabilizing motifs. How STOP modules stabilize microtubules is unknown, and structural studies are clearly needed to understand the molecular basis of STOP stabilizing activity.

The synaptic defects observed in STOP −/− mice raise important questions about the relationship of microtubule dynamics with synaptic plasticity. However, some of the synaptic defects that we observe involve synaptic compartments in which microtubules have not been observed but where STOP is present (*10*). STOP proteins may therefore have other functions in cells, in addition to stabilizing microtubules. STOPs may be involved in signaling cascades, independent of their role as microtubule-stabilizing proteins. Such multifunctionality is a common occurrence for cytoskeletal proteins, microtubules themselves being both structural components of cells and involved in many signal pathways.

In current models, mental diseases such as schizophrenia are thought to be related to synaptic defects. STOP-deficient mice provide experimental evidence that perturbation of the cytoskeleton can be a cause of synaptic defects associated with severe behavioral disorders, indicating a possible involvement of the cytoskeleton in psychiatric illnesses. This conclusion has received support from a very recent paper showing that the gene involved in a major schizophrenia locus in humans (DISC1) encodes a protein that interacts with microtubule organizing centers and MAPs (*40*).

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