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学 位 論 文 題 目 Molecular Characterization of CRMP5, a Novel  
Member of the Collapsin Response Mediator Protein  
Family

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## 論文内容の要旨

The CRMP (collapsin response mediator protein) family, a family of cytoplasmic proteins predominantly expressed in the developing nervous system is thought to play key roles in the growth cone guidance. CRMP was first isolated as a factor required for collapsin-1 (Semaphorin3A)-mediated signaling. Four members (CRMP1-4) of the family have been demonstrated to form hetero-multimeric structures through mutual associations. In this study, a novel member of this family, CRMP5, was cloned by the yeast two-hybrid method, and characterized from various aspects.

A search for molecules topographically expressed in the embryonic chick retina using Restriction Landmark cDNA Scanning (RLCS) was performed to identify molecules implicated in regional specificity in the retina and in the topographic retinotectal projection. Among a number of molecules thus isolated, CRMP3 was identified as being asymmetrically expressed along the nasotemporal (anteroposterior) axis, although this expression pattern was transient during retinal development.

To identify CRMP3-interacting molecules, he performed a yeast two-hybrid screen of a mouse brain cDNA library using chick CRMP3 as bait. In this screening, sixty-one clones, mainly containing CRMP1, 3, 4 and dihydropyrimidinase (DHPase), were isolated. Among them, he found a novel clone which is homologous to the CRMP isoforms already known. He termed this molecule CRMP5. This protein consists of 564 amino acids and has a calculated molecular mass of 61,516 Da. When poly (A)<sup>+</sup> RNA was analyzed, two bands of 4.8 and 5.2 kb, probably derived from a difference in poly (A) addition sites, were detected. CRMP5 bears several consensus sequences for phosphorylation sites that are conserved among the family, indicating that CRMP5 is also a phosphoprotein as are the other CRMP isoforms. CRMP5 shares relatively low amino acid identity with the other CRMPs (49-50%) and also with DHPase (51%), though CRMP1-4 exhibit higher identity with each other (68-75%). An unrooted phylogenetic tree of CRMP isoforms suggested that CRMP5 might be classified to a subfamily distinct from the four other CRMP members. This notion was supported by the genomic structure of CRMP5 because the exon-intron organization is not completely conserved with that of CRMP1 and CRMP2, or DHPase either. To determine the chromosomal location of the mouse CRMP5 gene, fluorescence *in situ* hybridization was performed using a mouse CRMP5 genomic DNA fragment as a probe. The CRMP5 gene was mapped to chromosome 5 B1, a region that shares homology with human chromosome 7q. Recently, chromosomal locations of the human CRMP family (CRMP1-4) and DHPase genes were determined. These results indicate that CRMPs loci are widely dispersed throughout the human and mouse genome.

To reveal the expression profile of mouse CRMP5, he performed Northern blot analysis and *in situ* hybridization. Northern blot analysis of various mouse tissues indicated that CRMP5 mRNA is expressed in the central nervous system but not in non-neural tissues. The CRMP5 expression profile during development resembled that of both CRMP1 and CRMP4, in that the expression level peaked in the first postnatal week and markedly decreased in adulthood. To

reveal in more detail the expression patterns in mice, *in situ* hybridization analysis was performed on developing embryonic sections. CRMP5 was expressed specifically in the nervous system, and signals were detected from the retina, olfactory epithelium, spinal cord, dorsal root ganglion, sympathetic ganglion, intestinal nerve, and brain with especially strong signals in the neocortex. This expression pattern is almost identical to that of CRMP4.

In contrast to the rapid progress in identification and characterization of the axon guidance molecules and their receptors, much remains to be explored about the intracellular mechanism by which signals are transduced into the eventual response of the growth cone. It has been reported that CRMPs form hetero-tetrameric structures. Consistently, he has identified other CRMPs by a two-hybrid screen using CRMP3 as mentioned above. Therefore, he tested the associations between CRMP5 and all CRMP isoforms. In the yeast two-hybrid system, CRMP5 interacted with each CRMP member with high affinity, except for CRMP1 and CRMP5. The same result was obtained from immunoprecipitation assays using an epitope-tagged expression system in COS-7 cells. Next, he tested the interaction between the CRMP isoforms in all combinations by immunoprecipitation. CRMP3 showed very low association with CRMP1, CRMP3 and CRMP4, similar to that between CRMP5 itself. This means that endogenous CRMP tetramers are composed in combinations of 1/2/4, 2/3/5 and 2/4/5. CRMP5 mRNA was upregulated as was CRMP4 mRNA in PC12h cells treated with NGF, suggesting that CRMP4 and CRMP5 are responsible for the neurite extension. Consistent with this observation, CRMP5 expression increases when the neural network forms during development. CRMP complexes with different isoforms may exert distinct intracellular signalings from the extracellular signal, and explain the variegated responses of axons from different origins.

## 論文の審査結果の要旨

CRMP (collapsin response mediator protein) は、神経軸索の伸長制御因子として重要な役割りを果たすセマフォリンの細胞内シグナル伝達分子として分離されたものである。すでに、CRMP ファミリーとして、CRMP1,2,3,4 が報告されているが、申請者は、酵母の two-hybrid 法を用いて、CRMP-3 と相互作用をする分子をスクリーニングし、CRMP1,3,4, dihydropyrimidinase (DHPase)等を含む幾つかのクローンを分離した。その中に、CRMP1~4と50%程の相同性を有する新規遺伝子を見出し、CRMP5 と命名しその性質を調べた。

まず、申請者は、CRMP5 の遺伝子解析から、

- 1) CRMP5は 564 アミノ酸残基からなり、61,516 ダルトンの分子量を有する、
- 2) 他の CRMP ファミリーと49-50%のアミノ酸レベルでの相同性を有し、そのいくつかのリン酸化部位の配列は、ファミリー間で共通である、
- 3) マウスの染色体の 5B1 (ヒトでは 7q 染色体に相当)に位置し、他の CRMP ファミリーとは異なる染色体上にあることを明らかにした。

次に、申請者は、Northern blot や in situ hybridization 法を用いて、CRMP5 が中枢神経系に特異的に発現し、そのマウス発生における発現パターンは CRMP1 と CRMP4に類似し、生後1週間で発現が最大に達し、以下次第に減少することを明らかにした。

更に、申請者は、前述の CRMP3 を用いた two-hybrid スクリーニングにおいて、CRMP3 と相互作用する他の CRMP ファミリーメンバーを見出したところから、CRMP5 と他の CRMP ファミリーとの相互作用を酵母の two-hybrid 法を用いて調べた。その結果、CRMP5 は、CRMP1,5 を除く、CRMP2,3,4 の異種の CRMP 間で強い相互作用を有することが明らかになった。この結果は、免疫沈降法や epitope-tag 法によっても確認された。そこで、申請者は、CRMP ファミリー間の全ての組み合わせによる相互作用を調べ、4量体を形成すると考えられる CRMP ファミリー間の相互作用として、CRMP1/2/4, 2/3/5, 2/4/5 の組み合わせが可能であることを明らかにした。神経栄養因子 NGF に反応して神経突起を伸長する褐色細胞腫 PC12 株では、NGF 処理によって、CRMP4 と CRMP5 の転写が促進され、こうした、CRMP ファミリー間の相互作用の異なる組み合わせによって、細胞外シグナルが神経細胞内における特異的シグナルとして転換される可能性を示唆している。

以上を要するに、本申請論文は、先ず CRMP ファミリーの新しいメンバーである CRMP5 を同定し、次に、発生過程での神経系特異的発現、更に CRMP ファミリー間における相互作用を明らかにした。よって、神経細胞の情報伝達に重要な役割りを果たすこのファミリーの機能を解明する上で重要な貢献をなすものであり、学位論文にふさわしいものである。

本申請に対する学位審査会を上記日程で行った。先ず、30分間申請者に学位論文の内容について発表してもらい、その後、質疑応答を行ったが、質問に対する応答は的確であった。申請者は、既に、筆頭著者を含め、水準の高い国際誌に論文を公表しており、また、学位論文も英語で書かれている。審査の結果、本学位申請論文は、学位授与にふさわしい水準に達していると判定した。