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One of the essential events during the formation of the nervous system is the wiring of neural circuits, which largely consists of two steps, axon pathfinding and target recognition. During the first process, neuronal growth cones traverse long distances along stereotyped pathways to their appropriate target regions. Then during the latter process, growth cones find and synapse with their specific target(s), by searching over many neighboring cells in the target region.

Neuronal growth cones are guided by a variety of different environmental cues expressed on their pathway and in the target region, that act either as contact-mediated signals or as diffusible factors. These cues have been shown to have attractive or repulsive effects on the growth cones mediated by their corresponding receptors. However, how these molecules orchestrate to generate the precise pattern of neural connectivity *in vivo* remains largely unknown.

Capricious (CAPS) is a cell-surface protein with leucine-rich repeat (LRR) motifs which was identified as a candidate target recognition molecule in *Drosophila* neuromuscular system. During the formation of neuromuscular connectivity, CAPS is expressed on small subsets of motoneurons and muscles, including muscle 12 and the motoneurons that innervate it (muscle 12 MNs). Loss of function of the *capricious* gene (*caps*) alters the target specificity of muscle 12 MNs; the MNs often form synaptic endings on a neighboring nontarget muscle, muscle 13, in addition to its normal target. A similar and more robust phenotype was observed when CAPS is ectopically expressed on all muscles. Although these results clearly showed that CAPS on muscles can function in guiding specific motor axons, the role played by neurally expressed CAPS remained obscure. Since CAPS is expressed not only on muscles but also on the motoneurons during the targeting of muscle 12 MNs, neurally expressed CAPS is also likely to play a role in this process. However, low penetrance of the loss-of-function phenotype makes it difficult to directly address this possibility. Neurally expressed CAPS may also play a redundant role in earlier events of motoneuronal pathfinding, that was not revealed by the analysis of the loss-of-function mutants.

To assess the possible function of neurally expressed CAPS, in Chapter I, he induced ectopic and increased expression of CAPS in all neurons using the GAL4-UAS system. As expected, CAPS was ectopically expressed on all neurons, starting from embryonic stage 12, in embyos. CAPS protein was detected in all major axon tracts in the CNS and in the periphery, suggesting that ectopically expressed CAPS was properly transported to axons.

To analyze the effect of pan-neural CAPS expression on the formation of the nervous system, he first examined motoneuronal circuits in the third instar larvae by mAb 1D4 (anti-Fasciclin II) and mAb 22C10 staining. No gross morphological defects were seen

in the CNS and musculature, suggesting that their overall development proceeded normally. However, he detected a highly specific change in the trajectory of motoneurons that innervate muscle 12 (muscle 12 MNs). In wild-type, axons of muscle 12 MNs, that fasciculate to form the terminal branch of the intersegmental nerve b (ISNb), project along the internal surface of muscle 13 before reaching their final target, muscle 12. In contrast, when CAPS was overexpressed on all neurons, they passed along the exterior of muscle 13. Such a phenotype was not observed in control larvae. This finding in the larvae led us to analyze the developmental processes of axon extension of muscle 12 MNs in the embryos. In embryos that pan-neurally express CAPS, axons of muscle 12 MNs appeared to extend normally until mid-stage 16. However, from late stage 16 to early stage 17, striking defects were seen in the trajectory of the most distal part of ISNb. In addition to the misrouting phenotype as seen in the larvae, a stall phenotype, in which the terminal branch of ISNb stopped prematurely near muscle 30 was observed. These results strongly suggest that ectopic pan-neuronal expression of CAPS affects the behavior of muscle 12 MNs at a specific choice point along their pathway to the target muscle.

CAPS is a transmembrane protein with 14 leucine-rich repeat motifs in its extracellular domain. Although its intracellular domain contains no known motif, the first 28 amino acids are highly homologous to the corresponding region of Tartan, another LRR protein in *Drosophila*. To study the function of the intracellular domain of CAPS and its possible link to the cytoskeltal and/or signal transduction machineries, he performed a deletion analysis of CAPS and tried to identify molecules that interact with the intracellular domain of CAPS by using the yeast two hybrid system in Chapter II. He ectopically expressed CAPS lacking the intracellular domain in neurons and muscles, and examined if the modified CAPS could induce the pathfinding and targeting phenotypes described above. He found that the function of muscularly expressed CAPS in target recognition is intracellular domain dependent whereas that of neurally expressed CAPS in pathfinding is not, suggesting that CAPS may function in neurons and muscles in a different manner. The requirement of the intracellular domain for the function of muscularly expressed CAPS suggests the presence of a signaling event in muscles that is essential for selective synapse formation.

論文の審査結果の要旨

申請者谷口弘樹は，本学位論文において，神経回路形成の分子機構を解明するため，宍戸等によって分離されたDrosophilaのcapricious (caps)という遺伝子の機能を遺伝学的手法を用いて解析した．CAPSは，ロイシンリッチ反復モチーフを細胞膜外に持つ細胞表面蛋白質であるが，Drosophilaの神経の神経筋結合部の標的認識過程に関与すると考えられている．神経筋結合部の形成に於て，CAPSは１２番筋とそれに投射する神経を含む極く一部の運動神経とその投射先の筋組織に発現している．caps遺伝子欠損は，１２番筋に投射する運動神経の特異性を変化させ，しばしば，１２番筋以外にも，１３番筋に投射するという異常を示す．CAPSを全筋組織に於て異所発現させた場合は，より顕著な投射異常が観察される．CAPSは，運動神経に於ても発現しているが，その神経投射に果たす役割は，大部分未知であった．そこで申請者は，GAL4-UASシステムを用いて，汎神経的にCAPSを発現させ，その神経投射に及ぼす影響を調べた．先ず，第３齢の幼虫期の神経回路形成を調べたところ，全体として大きな差はみられなかった．しかし，野生型では，１２番筋に投射する運動神経が枝分かれして１３番筋の内表層に沿って伸長するものが，汎神経的にCAPSを発現させたものでは，１３番筋の外側に沿って通過する．また，同神経の先端部が３０番筋の付近を通過する際，一時停止する現象がみられた．これらの結果より，汎神経的にCAPSを発現させた場合に，特定の運動神経の標的筋への投射経路上の選択点での決定に影響を及ぼすことが明らかになった．

CAPSは，膜透過型の蛋白質である．その細胞内ドメインの最初の２８アミノ酸残基は，同じく細胞外ロイシンリッチ反復配列モチーフを有するTartanと呼ばれる蛋白質の対応する部分と相同性が非常に高いが，その細胞内ドメインの機能は不明である．申請者は，CAPSの細胞内ドメインの神経投射の選択性に与える効果を調べる為，細胞外ドメインと細胞内ドメインを各々欠落させたCAPSを筋肉又は神経に異所的に発現させた．汎神経的に発現させた場合，細胞内ドメインを欠落させたものは，完全なCAPSを異所的に発現させたものと同様，１３番筋の外側部を通過するが，細胞外ドメインを欠落させたものでは，野生型と同じ経路を走行した．一方，汎筋組織的に発現させたものでは，細胞外ドメインを欠いたもの，細胞内ドメインを欠いたものいずれも野生型と同様の投射パターンを示した．これらの結果は，神経細胞で発現するCAPSと筋肉で発現するCAPSに於ける機能が異なることを示唆する．

本学位論文は，CAPSの神経筋結合の投射選択性に関するより詳細な遺伝的解析を行ない，神経細胞に発現するCAPSの意義をより明瞭にしたのみならず，CAPSと相互作用する蛋白質がおそらく存在し，それが筋肉組織に於ける細胞内情報伝達系を活性化し運動神経の走路決定に影響を与える可能性を呈示した点で重要である．本論文の主な内容は，Jounal of Neurobiology 42, 104-116 (2000) に公表された．以上，論文審査の結果，本論文は，学位授与基準を十分に満たしていると判定された．