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学位論文題目　MRP-1,a member of the ABC transporter superfamily, participates in the decision of the transition to the diapause stage, called dauer larvae, in C.elegans.

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論文内容の要旨

When living organisms encounter inappropriate environment for their survival and reproduction, they either escape from the environment (e.g., bird migration) or adapt to the environment by physiological changes. Dormancy or diapause is a representative example of such adaptation and observed in various organisms. Studies on dormancy have been performed mostly in the fields of physiology, ecology and biochemistry. However, the molecular mechanisms of the regulation of dormancy are not well known.

The nematode *C. elegans* also has a diapause stage, which is called dauer larva. The dauer larva has common features of dormant animals, i.e., low metabolism, no feeding, accumulation of fat, and resistance to stress. Since many molecular biological and genetic techniques are available, *C. elegans* is a good model organism for studying the regulation of transition into the diapause stage at molecular and cellular levels. During the life cycle, *C. elegans* grows up to adults through 4 larval stages (L1-L4) in 2-3 days at 25°C. But under inadequate conditions for growth, that is, under reduced food availability, crowding, and high temperature, animals arrest development and form dauer larvae corresponding to the 3rd larval stage. Dauer larvae can live for several months without feeding, while the life span in normal development is about 2-3 weeks. When the environmental conditions are improved, dauer larvae molt to normal L4 larvae and resume the life cycle. Like insects, the nervous system is involved in the diapause of *C. elegans*; some neurons in a pair of head sensory organs called amphids have been shown to control dauer larva formation.

Genes that regulate dauer larva formation have been studied by isolation and characterization of mutants that show abnormality in this function. These mutants consist of two groups: dauer-constitutive (*daf-c*) mutants, which form dauer larvae even under the conditions of abundant food availability and no crowding, and dauer-defective (*daf-d*) mutants, which do not form dauer larvae even under the conditions of extreme crowding and starvation. The genetic pathways of dauer formation have been revealed by epistasis tests of these mutations and molecular cloning of the mutated genes. At least four signal transduction pathways control dauer larva formation: cGMP related signaling pathway, TGF-ß signaling pathway, insulin signaling pathway and recently suggested steroid hormone signaling pathway.

In addition to these mutations that show abnormal phenotypes in dauer larva formation by themselves, mutations that show the dauer-constitutive phenotype only in the background of another mutation have been discovered and called synthetic dauer-constitutive mutations. A great majority of them were isolated by other phenotypes and later found to show this phenotype when double mutants of these mutations were constructed. *unc-31* mutation is one of such mutations, while *unc-31* gene encodes a homologue of CAPS (calcium activated protein for secretion),

which is required for the exocytosis of dense core vesicles, which contain neuropeptides and biogenic amines. Mutants in this gene show many phenotypes: slow locomotion, defective egg-laying, and constitutive pharyngeal pumping, etc., but essentially the wild type phenotype concerning dauer larva formation except at very high temperature (27°C), at which *C. elegans* cannot reproduce.

To identify new genes regulating dauer formation and to discover new mechanisms, 44 synthetic dauer-constitutive mutants were isolated in the *unc-31(e169)* background, mapped and named *sdf* (**s**ynthetic abnormality in **d**auer **f**ormation) mutants in our laboratory.

In this study, I cloned one of the mutant genes, *sdf-14* gene, by positional cloning, and analyzed its function on dauer larva formation. *sdf-14* gene encoded MRP-1 (multidrug resistance-associated protein-1), a member of the ABC transporter superfamily. ABC transporters export or import a wide variety of substrates by directly coupling these functions with the energy of ATP hydrolysis. Human MRP1 has been reported to export unnecessary compounds (conjugates, xenobiotics and detoxification products) from inside cells to outside. Since *C. elegans* MRP-1/SDF-14 had homology to human MRP1 throughout the amino acid sequence, it was predicted that, like human MRP1, *C. elegans* MRP-1/SDF-14 consists of 3 membrane spanning domains (MSDs) and 2 nucleotide binding domains (NBDs). The two mutant alleles of *sdf-14*, *ut151* and *ut155*, had missense mutations in NBD1, while another allele, *ut153*, had a mutation at the splice acceptor site of the 4th intron. In addition to the four MRP-1 isoforms that have been reported already, I found a new isoform, e-type. These isoforms seemed to differ in their functions, because the b- and c- type isoforms rescued the dauer formation abnormality of the *unc-31(e169);sdf-14(ut153)* double mutant, but the e-type isoform did not. These isoforms had variant copies of exon 13, suggesting that exon 13 may code for amino acid sequences that contribute to substrate specificity. The wild type human *MRP1* cDNA driven by the *sdf-14* promoter, but not the *dmL0* mutant *MRP1* cDNA, rescued the dauer-constitutive phenotype of the *unc-31(e169);sdf-14(ut153)* double mutant. Furthermore, the rescue was partially canceled by the addition of a human MRP1 inhibitor. Those results strongly suggested that *C. elegans* MRP-1/SDF-14 acts as an exporter like human MRP1 in the regulation of dauer larva formation. A functional *sdf-14*::GFP fusion gene was expressed in many cells, i. e., pharyngeal cells, pharynx-intestinal valve cells, intestinal cells, intestinal-rectum valve cells, vulval epithelial cells, some neurons, and hypodermal seam cells. Moreover, expression in at least two of the three types of cells (neurons, intestinal cells and pharyngeal muscle cells) was needed for the rescue of the dauer formation abnormality of the *unc-31(e169);sdf-14(ut153)* mutant. Epistasis analysis revealed that MRP-1/SDF-14 acts neither in the cGMP related signaling pathway nor in the TGF-ß signaling pathway. MRP-1/SDF-14 may act in the insulin or steroid hormone signal pathway. Alternatively, it may act in an unknown pathway, or has indirect influence on many

pathways. Sodium arsenite, which is a substrate of human MRP1, induced dauer formation of the *unc-31(e169)* mutant, and *sdf-14* mutations enhanced this effect, while *sdf-14* single mutants did not form dauer larvae even in the presence of sodium arsenite at 27°C. These results suggest that wild type MRP-1/SDF-14 molecules seemed to suppress dauer larva formation by exporting sodium arsenite or unidentified intrinsic substance of which accumulation may cause of dauer formation due to *sdf-14* mutations.

論文の審査結果の要旨

国立遺伝学研究所哺乳動物研究室で確立されたマウス突然変異系統 Rim3 は表皮の過 ケラチン化と毛嚢の退化という表現型を持ち、その原因遺伝子が新規遺伝子群に属する GasderminA-3(GsdmA-3)の一塩基置換であり優性遺伝することが知られていた。
田中君は、この遺伝子の機能を明らかにし、皮膚および毛根の形成維持に関わる調節機構を明らかにすることを目的として研究をおこなった。
まず、Rim3 の表現型を組織化学的方法で詳細に解析することにより、Rim3 において皮膚の過増殖がおこること、月齢を追うごとに毛根が失われていくことがわかった。さらにその間に正常マウスでは表皮のみで観察されるケラチンが毛嚢部において異所的に発現すること、変異体の毛嚢は皮脂腺を消失することがわかった。一般に皮膚や毛嚢、皮脂腺を形成する細胞は毛根のバルジと呼ばれる部位に存在する幹細胞に由来することがわかっている。幹細胞が毛嚢に沿って表皮側に移動して皮脂腺および基底層を形成し、毛根に移動した細胞は毛乳頭にはいり毛細胞となる。田中君は、GsdmA-3 は細胞の増殖を負に制御するとともに分化にも関与すると考えた。この分子機構を明らかにするために、GsdmA-3 に対する抗体を作成し、その蛋白質の局在を解析した。GasderminA (GsdmA)には A3 と非常によく似た A1 が存在し、抗体はこれら 2 者を区別することはできなかったが、野生型と Rim3 変異体を比較することにより、正常細胞でも変異体でも GsdmA は主にケラチン層の細胞膜に局在し、基底層では発現しないことがわかった。さらに BrdU との共染色を行った結果、分裂細胞における発現は認められず、分化した細胞に存在することがわかった。また免疫電顕でその局在を詳細に観察した結果、細胞膜以外にも細胞内の膜状の構造や細胞間隙に存在することが明らかになり、GsdmA は分泌される可能性も示唆された。田中君は、細胞の過増殖が見られるにもかかわらず分裂細胞に GsdmA が見られないことから、細胞非自立的に変異型 GsdmA3 が機能することにより、細胞の増殖や分化に異常をきたすのではないかというモデルを提唱するにいたった。
さらに、田中君は、GsdmA3 が癌抑制機能をもつのではないかという考えを検証するために、前癌状態を引き起こすことが知られている Trp53 ノックアウトマウスとのダブルヘテロ接合体を作成した。その結果、単独ではみられない皮膚癌が非常に高頻度で引き起こされる事実を発見した。また前癌細胞でみられた GsdmA の発現が癌化した細胞では消失していること、また正常な GsdmA3 やヒトの相同遺伝子 GSDMA が培養がん細胞の増殖抑制をおこすことや変異体の GsdmA3 ではその抑制効果が低くなることから考えて、GsdmA3 は増殖抑制能を持つと結論した。
以上のように、田中君は新規遺伝子 GsdmA3 が、分化した皮膚細胞で発現し、その増殖を抑制すること、そしてそれが細胞非自立的に幹細胞に機能し、細胞の増殖分化を調節している可能性を示した。このように、Rim3 変異マウスの解析を通して、癌抑制活性を持つ新規遺伝子を発見したことは高く評価でき、遺伝学専攻の博士論文としての条件を満たすと審査委員全員が認めた。