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学位（専攻分野）	博士（理学）
学位記番号	総研大甲第1520号
学位授与の日付	平成２４年３月２３日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第６条第１項該当
学位論文題目	Studies on maternal *mex-3* mRNA localization mechanisms in *C. elegans* embryos
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Asymmetry of distribution of mRNAs and proteins is critical for cell fate determination in the developing embryos. Especially, in very early stage of embryogenesis, mRNA asymmetric distribution, or "localization", is important for temporal/spatial regulation of maternal mRNA expression because transcription is silenced in this period. There are various mechanisms such as mRNA stabilization, degradation, diffusion, anchoring and transportation to regulate the mRNAs localization. In the regulation of mRNA localization, two elements, namely *cis*- and *trans*-acting elements, are major player. *Cis*-acting elements exist within the mRNA and act as localizing signals. *Trans*-acting elements exist in distinct molecules such as RNA binding proteins or miRNAs that recognize *cis*-acting elements to regulate mRNA localization.

In *C. elegans*, although this is a good system for studying the relationship between maternal mRNA distribution and cell fate determination, the molecular mechanism of intracellular mRNA localization remains largely unknown. Previous studies in *C. elegans* demonstrate that UTR is important for mRNA localization as known in other organisms. For example, the maternal transgene mRNAs possessing both of *pie-1* 5' untranslated regions (UTR) and *nos-2* 3' UTR are localized to germline cells after the four-cell stage, as well as endogenous *pie-1* and *nos-2* mRNAs. To explore the regulation of maternal mRNA localization, the laboratory that I belong has developed assay systems and investigated the localization mechanism for maternal *pos-1* mRNA. All of the mRNAs that have been studied on localization mechanisms in *C. elegans* are localized to germ cells. Thus, study of the mRNAs that localize to somatic cells will promote better understanding of the molecular mechanisms that distinguish germ cells from somatic cells, in addition to the mRNA localization mechanisms in *C. elegans*. Thus far, *mex-3* gene is only gene whose maternal mRNA is localized to somatic cells in very early stage of embryo among the genes whose function is well characterized. The *mex-3* mRNA is uniformly distributed in oocytes. After pronuclear formation of 1-cell stage, the mRNA is gradually localized to the anterior half of embryo, and is predominantly localized in the anterior somatic AB cell and its daughters at the 2-cell and 4-cell stages, respectively. After 4-cell stage, it is rapidly disappeared from the somatic cells.

Firstly, I evaluated whether localization of the *mex-3* mRNA can be mimicked in a reporter assay system. I made a construct that includes the *pos-1* germline promoter, the *mex-3* coding sequence fused in-frame to the reporter VENUS sequence and the *mex-3* 3' UTR. VENUS is a variant of YFP. The construct was introduced into worms by biolistic transformation. mRNAs derived from the construct were detected by *in situ* hybridization with a probe of the VENUS antisense sequence. This reporter assay system reproduced the localization of endogenous *mex-3* mRNA and I found that *mex-3* 3' UTR is sufficient for the localization.

To narrow down the *cis*-acting element, I performed deletion analysis of the *mex-3* 3' UTR. For this analysis, I generated deletion variants that lack some regions of the 3' UTR. As a result, I found that a 179 nt-sequence of the *mex-3* 3' UTR is required and sufficient for the mRNA localization to anterior half of embryo. Out of the 179 nt-sequence, I also found that a 35 nt-sequence, which is conserved among *Caenorhabditis* species, is critical for the mRNA localization to anterior blastomeres.

Next, I searched for a *trans*-acting element that binds to the identified *cis*-acting element and contributes to *mex-3* mRNA localization. Mutations in such *trans*-acting elements should abolish the *mex-3* mRNA localization. Using candidate approach, I found that the asymmetry of *mex-3* mRNA distribution was partially impaired in *mex-5* mutant. At 2-cell stage, the levels of *mex-3* mRNA of somatic AB cell were markedly decreased in *mex-5* mutant. *mex-5* gene encodes a CCCH-type zinc finger RNA binding protein. MEX-5 protein is expressed from oocyte and its localization pattern is very similar to that of *mex-3* mRNA. After pronuclear formation of 1-cell stage, MEX-5 protein starts to localize to anterior half of embryo. MEX-5 protein is more abundant in the somatic AB cell at 2-cell stages. At 4-cell stage, MEX-5 protein is predominantly expressed in AB daughter cells (ABa and ABp). After 4-cell stage, it is rapidly disappeared from somatic cells.

mex-5 has a paralogous gene, *mex-6*. The MEX-6 expression pattern is similar to that of MEX-5. MEX-5 and MEX-6 are known to function in a partially-redundant manner. In *mex-5; mex-6* double-mutant, the localization of the endogenous *mex-3* mRNA was abolished: The mRNA became low level and distributed uniformly. I examined endogenous *mex-3* mRNA of these mutant gonad by *in situ* hybridization. As a result, I found the levels of *mex-3* mRNA were decreased in oocytes of these mutants. These results suggest that MEX-5 protein functions to stabilize *mex-3* mRNA during oogenesis.

To examine whether MEX-5 protein also functions to stabilize *mex-3* mRNA after oogenesis, *mex-5* temperature sensitive (Ts) mutant was employed. The upshift experiment in which MEX-5 is inactivated just after fertilization caused the decrease of *mex-3* mRNA of anterior somatic blastomere, supporting that MEX-5 protein also stabilize the *mex-3* mRNA in early stage of embryo.

The specificity and affinity of the interaction between MEX-5 and linear RNA sequence were biochemically tested, and MEX-5 protein is reported to bind tract of six or more uridines within a 9-13-nucleotide window. The 35 nt-sequence essential for the *mex-3* mRNA localization is U rich and fulfills the features of binding site of MEX-5, thus I tried to test *in vitro* binding of MEX-5 recombinant protein to the 35 nt RNA. In electrophoresis mobility shift assay, the band shifts were observed in MEX-5 recombinant protein with the *mex-3* 35 nt-sequence. Competition analysis using cold negative control RNA confirmed that this band shifts were specific. Thus I conclude that MEX-5 protein is a *trans*-acting factor that directly binds to the 35 nt RNA

sequence and stabilizes *mex-3* mRNA.

Based on the observations, I propose a model that MEX-5 protein protects *mex-3* mRNA from degradation at anterior blastomeres through binding 35 nt-sequence of *mex-3* 3' UTR.

多細胞生物において一つの受精卵から多様な細胞種が生じるしくみの理解は、発生生物学における重要な課題である。新たな遺伝子発現が不活発な動物の初期胚においては、母性のタンパク質やメッセンジャーRNA(mRNA)を細胞間で不均等に局在化させることにより、異なる細胞運命を獲得している。金野宏之君は線虫 *C. elegans* の初期胚において *mex-3* 遺伝子の mRNA が前側細胞において選択的に局在化する機構に興味をもち、研究を進めた。*mex-3* 遺伝子の変異体は過剰な筋肉を作ることが知られており、*mex-3* mRNA の局在化は適切な細胞運命の決定に重要であると考えられる。金野君は、「*mex-3* mRNA の局在化に関与するシスエレメント(*mRNA* 内の領域)の同定」および「シスエレメントに作用するトランス因子の同定」を通じて、*mex-3* mRNA の局在化機構を解明した。

「シスエレメントの同定」については、*mex-3* mRNA の様々な領域をレポーター遺伝子につないで線虫内の局在を検討することにより、3'-UTR(3'側非翻訳領域)に存在する 179nt の領域が *mex-3* mRNA の前方側での発現に必要かつ十分であることを示した。この配列が MEX-3 蛋白質の局在化にも必要であることを示した。さらに、この 179nt の領域の中で近縁種でも保存性の高い 35nt の領域が、*mex-3* mRNA の局在化に必要であることを示した。一方で、この 35nt だけでは局在には不十分であった。

「トランス因子の同定」については、線虫初期胚で重要な働きをすることが知られている 7 種の RNA 結合タンパク質を疑い、このうち MEX-5 タンパク質の変異体で *mex-3* mRNA の前方での強い発現が見られなくなることを見いだした。MEX-5 タンパク質は *mex-3* mRNA と同様に胚の前方側に局在化するため、金野君は MEX-5 タンパク質が *mex-3* mRNA を分解から保護することによって、胚前方側でのみ強い発現が見られるとする作業仮説をたてた。その後の実験でこの仮説を支持する以下の知見を得た。(i) MEX-5 タンパク質が胚の全体に存在するようになる *par-1* 変異体では、*mex-3* の局在も胚全体で見られるようになる。(ii) MEX-5 の温度感受性変異株を用いて、MEX-5 を不活性化すると速やかに *mex-3* mRNA の発現も低下する。さらに、(iii) 先に同定した *mex-3* mRNA の局在化に必要な *mex-3* mRNA 3'-UTR 内の 35-nt の RNA に MEX-5 タンパク質が試験管内で結合する。

以上の結果から金野君は *mex-3* mRNA の局在化機構について、「MEX-5 蛋白質が *mex-3* mRNA の 3'-UTR の中の同定した 35nt 領域に結合することにより、*mex-3* mRNA を胚の前方側で選択的に安定化させ、胚内でも前方側の細胞でのみ *mex-3* mRNA およびタンパク質が強く発現する」とする機構を提案した。MEX-5 タンパク質が属する CCCH 型 Zn finger タンパク質グループは、これまで RNA の分解を促進する働きがあることが知られていたが、本研究により mRNA の安定化と分解の両方を制御する可能性が示唆された。単一のトランス因子がどのように安定化と分解を引き起こすのか、本研究をきっかけに mRNA 局在化研究が新たな方向に展開することが期待できる。本研究は、丁寧な mRNA のドメインマッピングを中心に、近縁種での配列比較、遺伝子変異体の活用、試験管内での結合アッセイなど、多種類の解析を組み合わせて、mRNA 局在化のシスおよびトランスエレメントを同定したもので、mRNA 局在化機構研究に貢献する優れた研究成果である。以上のことから、本論文は博士号授与の要件を満たすと審査員全員一致で判断した。