論　文　要　約

Studies on the regulatory expression of uncoupling protein 1 in bovine skeletal muscle
（ウシ骨格筋における脱共役タンパク質 1 発現調節に関する研究）

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Uncoupling protein 1 (UCP1) is a brown adipocyte-specific protein located in the internal mitochondrial membrane. Through uncoupling oxidative phosphorylation from ATP synthesis, UCP1 leads to the dissipation of chemical energy in the form of heat instead of ATP in a non-shivering thermogenesis (NST) manner. Unexpected Ucp1 expression was found in bovine skeletal muscle. Immunohistochemical analyses showed that UCP1 surrounded muscle fibers, but not adipocytes residing in skeletal muscle. In this thesis, bovine Ucp1, especially muscular Ucp1 regulatory expression was explored. In this thesis, I clarified the following points:

1. Four alternative splice Ucp1 variants were identified in bovine brown adipose tissue. mRNAs of Ucp1 variant 2/4 are less stable than those of Ucp1 variant 1/3 with longer 3′- untranslated region (UTR). Variant 1 is the main form of Ucp1 because variant 2-4 are efficiently degraded by the proteasome system. Variants differing in the 3′-UTR were previously shown in murine Ucp1, but the spliced regions in bovine Ucp1 gene are distinct from those in murine Ucp1 gene.

2. Ucp1 expression in bovine skeletal muscle tissue was analyzed. The skeletal muscle consists of two types of muscle fibers (fast and slow)- the slow muscle predominantly generates ATP through aerobic metabolism, while ATP is mainly generated through anaerobic metabolism in the fast muscle. Considering the metabolism characteristics, the relationship between Ucp1 expression and muscle fiber type was evaluated in this chapter. The results indicate that Ucp1 prefers to express in fast-twitch muscles or the requirements of muscular Ucp1 activation is similar to those of fast-twitch muscle growth.

3. I established a method to stimulated myogenesis especially fast-twitch myosin heavy chain (Myh) expression so that the relation between Ucp1 and Myhs could be

studied in this model. Myogenesis method was explored in C2C12, a mouse myogenic cell line. In this chapter, a combination treatment of capsaicin, an Endoplasmic reticulum stress (ER stress) inducer, and vitamin C synergistically stimulated C2C12 myogenesis; fast-twitch Myh1 and MYH1/2 expression was dramatically increased by this treatment. Capsaicin stimulated the ER stress marker gene expression in C2C12. After 1 day of ER stress induction by capsaicin, vitamin C stimulated myogenin (MYOG) and MYH1 expression, accompanied by increased collagen (Col)1 expression. In this experiment, capsaicin treatment amplified effects of vitamin C on collagen synthesis and myogenesis in C2C12.

4. I performed capsaicin and vitamin C treatment in primary bovine myosatellite cells. Capsaicin also stimulated the expression of ER stress marker genes. However, vitamin C could not further stimulate myogenesis in bovine myogenic cells after capsaicin treatment. In the following experiments, insulin and triiodothyronine were supplemented in differentiation medium to stimulate myogenesis and Ucp1 expression. I treated bovine myosatellite cells with 4-Phenylbutyric acid (4-PBA), a histone Deacetylase (HDAC) inhibitor, during myogenic differentiation. Vitamin C treatment was also conducted because of its condition-dependent effects on myogenesis. 4-PBA stimulated expression of fast-twitch Myhs and myogenic regulatory factors (Mrfs) except Myog. 4-PBA also stimulated the expression of Ucp1 and brown/beige adipocyte markers. The p38 mitogen-activated protein kinase seems to be one of the main signaling pathways that are involved in 4-PBA induced Ucp1 expression. Unlike the data in chapter 3, Ucp1 was not positively related to fast-twitch Myhs. This is because that vitamin C stimulated Myhs expression but had a negative effect on Ucp1 expression. Anyway, 4-PBA inhibited Myh7 expression, which indicates the requirements for activation of Ucp1 in bovine myosatellite cells may suppress slow twitch Myh7 expression. This data supports the hypothesis that bovine Ucp1 prefers to express in fast-twitch muscle fibers from another perspective.

In conclusion, these results indicate that bovine muscular Ucp1 is related to myofiber type, prone to express in fast-twitch muscle.