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学位論文題目	Transcriptional Regulation of the POU-M1/SGF-3 Gene Involved in the Expression of Bombyx Silk Genes
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論 文 内 容 の 要 旨

Suzuki and his colleagues have been studying the regulation of silk protein gene expression in *Bombyx mori* (for a review, see Suzuki, 1990). The sericin-1 gene is expressed exclusively in the middle silk gland (MSG) while the fibroin gene is specific to the posterior silk gland (PSG). Both genes are actively expressed during the intermolts but repressed during the molting stages. Several silk gland factors (SGF-1 to -4) involved in controlling the silk genes have been identified (Matsuno *et al.*, 1989; 1990; Hui *et al.*, 1992). Among them, the SGF-3 has been proposed to be an important factor for the regulation of silk genes (Matsuno *et al.*, 1990; Hui *et al.*, 1992). It is present abundantly in the MSG and binds to the SC region, a key element for the sericin-1 gene transcription. The SGF-3 also binds to the distal upstream region of the fibroin gene. Since the SC and fibroin distal upstream region contain octamer-like sequences, it has been proposed that SGF-3 is an octamer-binding protein (Hui *et al.*, 1992). Based on this assumption, a POU-domain-containing cDNA, POU-M1 has been cloned (Fukuta *et al.*, 1993). Its POU-domain part is identical with that of *Drosophila* Cf1-a protein (Ingham, 1988). The POU-M1 protein was shown to be identical with the SGF-3 (Fukuta *et al.*, 1993). The POU-M1 transcript and protein are subject to dramatic change during silk gland development. The transcript was observed at a high concentration in the fourth molting stage and became lower in the fifth larval instar in the MSG. In the PSG, the transcript was only detected in the fourth molting stage (Fukuta *et al.*, 1993). By Western blotting, the POU-M1 protein is estimated to have molecular weight of about 38KD and exists at a low concentration in the fourth molting stage and becomes higher in the 2-day-old fifth instar larvae in the MSG. In the PSG, the protein was only detected in the fourth molting stage (Fukuta *et al.*, 1993). This pattern suggests that the POU-M1/SGF-3 gene expression is temporally programmed and spatially restricted.

The highly conserved POU domain was initially recognized after the simultaneous cloning of three mammalian transcription factors and a *Caenorhabditis elegans* developmental regulator. Many additional POU-domain proteins have been identified subsequently and they are expressed in distinct

temporal and spatial patterns during development (for a review, see Rosenfeld, 1991). POU-domain transcription factors appear to exert critical functions in proliferation of specific cell types, as well as in the activation of specific programs of gene expression that define specific cell phenotypes within an organ (Finney and Ruvkin, 1990; Li *et al.*, 1990). Several POU-domain regulators appear in early embryogenesis and are important in the early developmental regulation of gene transcription (He *et al.*, 1989; Okamoto *et al.*, 1990; Rosner *et al.*, 1990; Scholer *et al.*, 1990a; Monuki *et al.*, 1990; Suzuki *et al.*, 1990). Since the POU domain exerts critical developmental actions, it is very important to study the roles of the POU domain in regulating organogenesis. POU-domain proteins have been described to exert either positive or nagetive transcriptional effects by binding to recognition elements as monomers or as homodimers formed as a consequence of DNA-dependent cooperative interactions (LeBowitz *et al.*, 1989; Poellinger *et al.*, 1989; Ingraham *et al.*, 1990). On the basis of precendents in *Drosophila* for other classes of developmental regulators (Akam, 1987; Scott *et al.*, 1989), it is also important to understand the mechanisms that mediate the regulated expression of POU-domain genes. Analysis of mammalian Oct-3/4, Tst-1/SCIP/Oct-6 and *pit*-1 genes suggested that the molecular mechanisms of activation and maturation of POU-domain proteins are likely to involve the actions of numerous other classes of transcriptional regulators (Jones-Villeneuve *et al.*, 1983; Strickland and Mahdavi, 1988; Chan *et al.*, 1990; McCormick *et al.*, 1990).

As a first step toward understanding the molecular basis of the regulatory hierarchy during silk gland development and probing the mechanisms of transcriptional regulation in generating the control patterns of POU-M1/SGF-3 gene expression, Xu has analyzed the expression of the POU-M1/SGF-3 promoter *in vitro*. In this work, Xu has cloned the POU-M1/SGF-3 genomic DNA fragment encompassing the whole coding region and its flanking sequences. This gene does not contain any intron. The 5'-flanking region of the gene contains several interesting motifs, such as homeodomain-binding motifs, sequences resembling the transcriptional factor Spl-binding site, and TGTTT motifs, but lacks some of typical transcriptional regulatory sequences, such as TATA and CAAT boxes. Transcriptional analysis of a series of deletion mutants of the gene in the nuclear extracts prepared from the MSG of 2-day-old fifth instar larvae revealed the presence of multiple *cis*-regulatory elements located both upstream and downstream of the start site. One of these elements, the homeodomain-binding elements, was identified to mediate negative regulation. By mobility shift assay using the POU-M1 specific antibodies, Xu found that this negative element interacts with the POU-M1/SGF-3.

Transcription analysis *in vitro* using one of the POU-M1 antibodies indicated that POU-M1/SGF-3 can negatively autoregulate its own gene expression as a consequence of binding to the homeodomain-binding element.

論文の審査結果の要旨

許　品仙は、カイコ *Bombyx mori* より POU-M1/SGF-3（以下 POU-M1と略記）ゲノム遺伝子をクローニングし、その構造と発現制御の研究を行った。

遺伝子の領域をはさんで約 6 kb の部分の塩基配列を決定した。一方、フルサイズの POU-M1 cDNA をクローン化し、配列を決定して、イントロンの無い遺伝子であることを確定した。この遺伝子は、ハプロイドゲノム当たり 1 コピー存在する。

無細胞転写系における転写実験、フットプリント法、およびゲルシフト法によって 6 つの *cis*-acting elements を同定し、これらに結合する *trans*-acting factors について解析した。そのうち 4 つは activation に関わる elements, 2 つは repression に関わる elements であった。Negative elements の 1 つ PB site には、POU-M1 遺伝子の産物であるPOU-M1 タンパクが結合して、転写を抑制することを見事に証明した。POU-M1 タンパクは、一定濃度までは結合親和性の高いセリシン－1 遺伝子の SC site に結合しておそらく転写促進に寄与し、一定濃度を超えると結合親和性の低い POU-M1 遺伝子の PB site に結合して転写を抑制し、過剰生産にならないように制御していることが示唆された。

以上の結果、当該論文は学位を授与するに値すると判断した。