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学 位 論 文 題 目	Molecular cloning of a POU domain transcription factor involved in regulation of Bombyx sericin-1 gene
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論 文 内 容 の 要 旨

The POU domain is a DNA-binding region consisting of a 75-82 amino acids POU-specific domain, a short variable linker region and a POU-homeodomain of 60 amino acids (for a review, see Ruvkin and Finny, 1991). It was originally found in three mammalian transcription factors, the pituitary-specific Pit-1/GHF-1, the ubiquitous Oct-1 and the predominantly B cell specific Oct-2, and the product of the cell lineage control gene Unc-86 of Caenorhabditis elegans (Herr et al., 1988 and references therein). By means of sequence similarity, several other mammalian POU domain genes have also been identified (He et al., 1989; Monuki et al., 1990; Okamoto et al., 1990; Rosner et al., 1990; Scholer et al., 1990; Suzuki et al., 1990). All of them were shown to interact with an octamer-like sequence and to activate transcription via an octamer motif near the TATA box. The Drosophila Cf1-a protein, which interacts with a DNA element required for expression of the dopa decarboxylase gene in selected dopaminergic neurons (Johnson and Hirsh, 1990), was also found to possess a POU domain similar to those of the mouse Oct-6 (Suzuki et al., 1990) and the human Brn-1 and Brn-2 (He et al., 1989) proteins. These POU domain genes are likely regulatory genes controlling transcription of distinct sets of genes during development. The finding that two dwarf mutations in mice are null mutations in the Pit-1/GHF-1 gene (Li et al., 1990) provide further support on the roles of POU transcription factors in development. Recently, the maternally expressed POU domain transcription factor, the Oct-3/4 protein (Okamoto et al., 1990; Scholer et al., 1990), has also been shown to be required for the first embryonic cell division in mice (Rosner et al., 1990).

Suzuki and his colleagues have been studying the developmental regulation of the silk protein genes in Bombyx mori (Suzuki et al., 1990). Among them, the sericin-1 gene is expressed exclusively in the middle silk gland while the fibroin gene is specific to the posterior silk gland. Both genes are actively expressed during the intermolt but repressed during the molting stages. Several silk gland proteins have been identified as putative regulatory factors involved in the transcriptional control of the fibroin and sericin-1 genes (Hui et al., 1990; Matsuno et al., 1989, 1990). One of these proteins, SGF-3, was found to bind with high affinity to the SC region of the sericin-1 gene and the distal upstream region of the fibroin gene (Hui et al., 1990). These regions are known to be important for an efficient transcription in the silk gland extracts. A multimer of the SC region gave transcriptional enhancement in extracts prepared from the middle silk gland where the sericin-1 gene is specifically expressed but that of a mutant SC region giving a reduced affinity for SGF-3 did not (Matsuno et al., 1990). Mobility shift assay revealed that SGF-3 is far more abundant in the silk gland of the 2-day-old fifth-instar larvae than in the posterior silk gland (Hui et al., 1990; Matsuno et al., 1990; Y. Suzuki, unpublised). These observations suggest that the SGF-3 is a key regulatory factor in the transcription control of the sericin-1 gene.

The SGF-3 was supposed as an octamer binding protein (Hui et al., 1990). Since high affinity SGF-3 binding sites, such as the SC and fibroin distal upstream regions, also contain octamer-like sequences, it has been speculated that SGF-3 might possess a POU domain similar to the mammlian octamer-binding proteins. This report presents here the isolation and characterization of a POU domain containing cDNA (POU-M1) from the middle silk gland. It encodes a protein with a POU domain identical to that of the Drosophila Cf1-a protein. By mobility shift assay and nuclease protection assay, the POU-M1 protein and the

putative silk gland factor SGF-3 were fornd to interact in an indistinguishable manner with the SC region of the sericin-1 gene, which is a key cis-acting element involved in the stimulation of sericin-1 gene transcription through the interaction with SGF-3. Antibodies raised against the synthetic oligopeptides corresponding to the two regions of putative POU-M1 protein reacted specifically to both the POU-M1 protein and the SGF-3. Northern blot hybridization and Western blotting revealed that the POU-M1 expression is regulated both temporally and spatially during the silk gland development. It is concluded that the POU-M1 protein is identical to the SGF-3 and proposed that the differential expression of the POU-M1 gene is probably involved in the transcriptional regulation of the silk protein genes.

論文の審査結果の要旨

福田君は、セリシン－１遺伝子のＳＣ領域に結合する因子ＳＧＦ－３の解析を行った。ＳＧＦ－３が結合するＳＣ領域内には‘オクタマー配列’として知られるmotif が含まれていることから、ＳＧＦ－３はオクタマー（又は POU）タンパクであろうとの予測を立て、既知のPOU-ドメインタンパク内のコンセンサス配列２ケ所を選んで、対応するオリゴヌクレオチドをプライマーとしたＰＣＲ法を行い、中部絹糸腺からPOU-ドメイン構造を検出した。この DNAをプローブとして、中部絹糸腺 cDNA バンクから全 ORFをカバーする cDNA を得て、構造解析を行って POU-M1 と名づけた。さらに、reticulocyte lysate 中で POU-M1 タンパクを合成し、SC DNAをプローブとしてゲルシフトを行い SGF-3との著しい類似性を明らかにした。ついで当研究所の松野が作製した POU-M1 に対する抗体２種を活用して、POU-M1 が SGF-3 と同一であることを明かにした。絹糸腺中における POU-M1 mRNA の発現、POU-M1 タンパクの発現などの解析も行っており、学位を授与するに充分な論文であると判断した。POU-M1が転写制御因子としての機能をもつことの証明が待たれる。