氏名	小　林　麻己人
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第１９号
学位授与の日付	平成４年　３月１６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４条第１項該当
学位論文題目	Sequence-strength relationship of the Escherichia coli promoter
論文審査委員	主　査　教　授　堀　内　賢　介 教　授　池　村　淑　道 教　授　五條堀　　孝 助教授　廣　瀬　　進 教　授　饗　場　弘　二（名古屋大学）

論文内容の要旨

In prokaryotes, the control of transcription initiation is a key step in the regulation of gene expression. In order to reveal the mechanism how the order of transcription is determined among thousands of genes in a cell, it is important to understand the intrinsic promoter strength for individual genes (the term "promoter strength" refers to the relative rate of synthesis of full length RNA product from a given promoter). The level of transcription initiation is basically determined by the sequence of the promoter, the start signal of RNA synthesis. DNA sequence analyses of a wide variety of prokaryotic promoters have indicated that promoters for the major form of *Escherichia coli* RNA polymerase (Eσ70) are composed of two conserved hexanucleotide sequences, TATAAT and TTGACA, which are located at 10 and 35 base-pairs, respectively, upstream of the transcription initiation site, although a considerable variation exists in the promoter sequence between genes within the same organism. From thermodynamic and kinetic studies, these two sequences are believed to determine the affinity to RNA polymerase and the rate of DNA opening, altogether affecting the promoter strength. However, little is known about the role of individual bases within these two regions with respect to RNA polymerase binding and DNA opening. In this study, I carried out a systematic analysis of the relationship between the promoter sequence and the promoter strength (Kobayashi, M. *et al.* (1990) Nucleic Acids Res., **18**, 7367-7372).

A set of 18 variant *lac*UV5 promoters was constructed, each carrying a single base substitution within the promoter -35 region (nucleotide positions from -36 to -31 relative to the

transcription start site). Using truncated DNA fragments carrying these variant promoters and purified Escherichia coli RNA polymerase holoenzyme (Eσ70), the *in vitro* mixed transcription assays were performed to determine two parameters governing the promoter strength, *i. e.*, the binding affinity to RNA polymerase (parameter I) and the rate of open complex formation (parameter II).

Parameter I was affected to various extents, while parameter II was mostly decreased except for two variant promoters, 34G and 33G (the variant promoters were named according to the position and base species of substitution). The 34G has a sequence of TTGACA, which is completely identical with the consensus sequence. The degree of change in parameter I mainly depends on the position of base substitution. Base substitutions at position -31 gave only a little effect; substitutions of C at position -32 to any other base caused significant reduction; base substitutions at position -35 also led to reduction, although the effects were somewhat smaller than those of -32 base substitutions; the effects of base substitutions at position -33, -34 and -36 were variable depending on the base introduced. Among all possible sequences, TTGACA should be the strongest promoter in terms of parameter I. The rate of open complex formation (parameter II) was slower for most variant promoters than for the reference promoter, except for the 34G (consensus) and the 33G promoters. Again the promoter with the consensus TTGACA sequence was the strongest.

In order to confirm these results, I next performed an abortive initiation assay, in which the formation of initial oligonucleotides is measured. The reaction conditions of the abortive initiation assay were made identical to those of the

mixed transcription assay, except that ApA was added as a primer, and ATP, GTP and CTP were omitted (and thus [α-32P]UTP was a sole substrate). The final level indicates the binding affinity to RNA polymerase (parameter I'), while the reciprocal of the time required for reaching plateau level represents the rate of open complex formation (parameter II'). The pattern of the promoter strength determined by the abortive initiation assay was essentially the same as that for the mixed transcription assay. The degree of change in parameter I' is due to both the position and species of base substitution. However, all variant promoters except for 34G, displayed lower values of parameter II' than the reference promoter. In the case of parameter II', TTGACA was the only exception that was stronger than the reference promoter, but all other base substitutions resulted in marked reduction to less than half the level of the reference promoter. The alteration pattern of both parameter I' and II', measured by the abortive initiation assay, was essentially identical with that of parameter I and II determined by the *in vitro* mixed transcription assay.

As an attempt to compare the promoter strength of the synthetic promoters measured by two in vitro assays with *in vivo* activities, I performed β-galactosidase assay using variant *lac*UV5 promoter collections fused to the *lac*Z structural gene. The DNA fragments containing variant *lac*UV5 promoters were inserted between the inducible ara promoter and the *lac*Z structural gene of plasmid vector pMS4342. I examined six variant promoters, which all showed unique promoter strength patterns *in vitro*. The promoter strength *in vivo* was determined simply by monitoring β-galactosidase activity in the absence of arabinose. The promoter 34G was as strong as the reference, and

the promoters 33G and 31T were intermediate while the others were weak (less than 25%). When compared with the results of two *in vitro* transcription assays, the promoter strength *in vivo* is in good agreement with parameter I measured by the productive initiation assay. The consensus sequence (34G) again exhibited the highest activity.

The following conclusions were drawn from the data presented: (1) Alteration in the promoter strength of variant promoters is dependent on both the position and base species of substitutions; (2) the consensus sequence (TTGACA) exhibits the highest values for both parameters; (3) base substitutions at nucleotide position -34 cause marked effect on both parameters; (4) cytosine at nucleotide position -32 cannot be replaced with other nucleotides without significant reduction of the promoter strength; (5) base substitution at nucleotide position -31 exerts only a little effect on parameter I; (6) the promoter strength *in vivo* is in good agreement in parameter I of *in vitro* promoter strength; and (7) the consensus sequence (TTGACA) exhibits the highest activity *in vivo* as well as *in vitro*.

This type of experiments has been done as a collaboration research for the analysis of sequence-strength relationship of the promoter -10 region.

論文の審査結果の要旨

転写開始の制御は遺伝子発現の調節において中心的役割を担う重要な問題である。大腸菌の主要転写酵素Eσ^{70}によって認識されるＤＮＡ領域、すなわちプロモーターの活性は主として -35領域および -10領域と呼ばれる部域の塩基配列によって決定される。プロモーターの活性の強度は、主として転写酵素のプロモーターへの結合親和性（変数 I）とオープンコンプレックスの形成速度（変数 II）の二要因によって規定される。本研究において小林氏は、代表的プロモーターの一つである lacUV5 から出発して、先ず -35領域、次いで -10領域の塩基を一個づつ置換したプロモーターのセットを系統的に構築した。そしてその各々について混合転写法およびアボーテイブ転写法の二法を用いて、変数 I および変数 IIを in vitro において測定した。更に、構築した変異プロモーターの代表例について、それを lacZ 遺伝子の上流に挿入したプラスミドを大腸菌に導入し、菌体内での lacZ 遺伝子の発現量とプロモーターの in vitro 活性を比較した。これらの実験結果から、1) 変異プロモーターの強度は置換塩基の位置と種類の双方に依る、2) コンセンサス配列は両変数の最高値を与える、3) -35領域も-10領域も共に両変数に影響するが、-35領域は変数 I、-10領域は変数 II への影響がより大である、4) in vitro で測定したプロモーター強度は、in vivo での遺伝子発現強度にほぼ対応する、こと等が結論された。

この論文の内容はプロモーター強度と塩基配列の関係についての基礎的データを提供するもので、博士論文としての条件を満たすものであることを、審査委員全員一致で認めた。