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論文内容の要旨

Escherichia coli RNA polymerase core enzyme with a subunit structure $\alpha_2\beta\beta'$, has the activity of DNA dependent RNA synthesis in non-specific manner but cannot initiate transcription from promoters. Upon binding one of the multiple species of σ subunit, the core enzyme forms a holoenzyme which is able to initiate transcription from a certain group of promoters recognized by the associated σ subunit. The enzyme complex assembles under the sequence, $\alpha \rightarrow \alpha_2 \rightarrow \alpha_2\beta \rightarrow \alpha_2\beta\beta' \rightarrow \alpha_2\beta\beta'\sigma$, where the dimerization of α subunit is the first step and, therefore, α plays an important role in the assembly. C-terminal truncated α subunit, α(1-235), lacking C-terminal 94 aa residues up to position 235 is able to assemble $\beta\beta'$ subunits into core complex. Thus, the region of aa 1-235 is called N-terminal assembly domain and this domain must contain at least three functions; *i.e.*, α-dimerization, β-assembly and β'-assembly.

A holoenzyme containing α(1-235) is as active as a wild type enzyme in transcription from factor-independent or non-class I factor-dependent promoters (including class-II factor-dependent promoters), but it cannot initiate transcription from class-I factor-dependent promoters. This suggests that the class-I factors interact with C-terminal region of α. The structure-function relationship of the C-terminal region has since been studied extensively. Contrary to the well-characterized C-terminal domain, little is known about the structure-function relationship of the N-terminal assembly domain. In this study, for detailed functional mapping of the domain, site-directed mutations were introduced into this region and a total of 52 α derivatives thus generated were characterized both *in vitro* and *in vivo*.

At first, a set of deletion mutants were constructed, including a series of successive deletions from the N-terminus, a series of internal deletions of 20 residues, two carboxy terminal deletions, and one mutant with deletions at both termini. The mutant α-subunit proteins were overexpressed in *E. coli*, purified and characterized *in vitro* for their abilities in dimerization, assembly into active core enzyme and promoter-specific transcription. Among a total of 22 α-deletion mutants tested, only three mutants, $\alpha\Delta$N10, $\alpha\Delta$N20, lacking N-terminal 10 or 20 aa, respectively, and α(21-235) lacking N-terminal 20 and C-terminal 94 aa retained activity to form active core enzymes and one mutant, $\alpha\Delta$N30, lacking N-terminal 30 aa formed a core enzyme but at a reduced efficiency. All other mutants did not assemble $\beta\beta'$ subunits into core enzymes, nor even formed α-dimers. These results indicate that the region consisting of position 21-235 is the minimum requisite for core enzyme assembly and suggest that the region is composed of a single building block which is decomposed by any deletion of 20 aa examined. Reconstituted holoenzymes containing

αΔN10, αΔN20 or αΔN30 were capable of transcribing from *lac*UV5, CRP-dependent *lac*P1 and OmpR-dependent *ompC* promoters *in vitro*, indicating that the extreme N-terminal region is dispensable for transcription activation in these systems.

Assemblies *in vivo* of αΔN10 to 40 were tested using His_6-tagged mutant α subunits by Ni^{2+}-affinity chromatography. β and β′ subunits were copurified with His_6-tagged αΔN10 and 20 from extracts of *E. coli* expressing them, consistent with the results of *in vitro* reconstitution. However, αΔN10 and αΔN20 were not fully functional *in vivo*, because neither could complement a *ts* allele of α gene, *rpoA*101, and because αΔN20 could not do another *ts* allele, *rpoA*112.

In the next approach, eleven species of α-insertion mutants having two extra amino acids, Ala and Ser, at every 20 aa interval were prepared. Among these mutants, designated like αI-N, in which N represents a position of insertion, five mutants (αI-60, 80, 120, 160 and 200) dimerized *in vitro*, one mutant (αI-20) dimerized at reduced efficiency and five mutants (αI-40, 100, 140, 180 and 220) did not form dimers. In *in vitro* reconstitution experiments, four mutants (αI-20, 60, 120 and 160) formed active core enzymes, three mutants (αI-40, 100 and 140) assembled neither β nor β′, αI-80 formed an unstable $\alpha_2\beta$ subassembly, αI-180 formed an $\alpha_2\beta$ subassembly but did not bind β′, αI-200 formed an $\alpha_2\beta$ subassembly but formed less core enzyme, and αI-220 formed an $\alpha_1\beta_1$ complex. These results suggest that the region around position 180-200 takes a part in β′ assembly; the region around 80 is involved in both β and β′ binding; and multiple regions are concerned with α-dimerization. His_6-tag was also introduced into all these α-insertion mutants and the assembly *in vivo* was examined by Ni^{2+}-affinity chromatography. αI-40, 100, 140 and 220 were totally inactive in the assembly. αI-180 assembled β but not β′. All other mutants including αI-80, which did not form a core enzyme *in vitro*, assembled β and β′ subunits. These agree with prediction that the region around position 180 participates in β′ binding. Abilities of these mutants to complement the *ts* alleles were also examined. In spite of the assembling activity *in vivo* of as many as six species of insertion mutants, only three of them (αI-20, 120 and 160) could suppress the *ts* phenotypes, indicating that other three mutants (αI-60 80 and 200) lack yet unidentified functions.

α subunit shares two homologous regions with the corresponding prokaryotic, eukaryotic and plastid subunits (aa 30-90 and 200-230). For further detailed functional mapping, Ala was substituted for 19 highly conserved residues around aa 40, 80 and 170-210. All these α-point mutants were analyzed *in vitro* for their abilities to dimerize and assemble ββ′ subunits. α-R45A (having substituted Ala for Arg at aa 45) dimerized but did not assemble β or β′

subunits, and α-L48A showed a decreased level of $\alpha_2\beta$ subassembly formation, indicating that this region (aa 45-48) is responsible for β-assembly. With α-K86A and V173A, the binding of β′ subunit was greatly decreased. This indicates the participation of the two separated regions in β′-binding, consistent with the results of the insertion analysis. Involvement of multiple regions widely distributed within the assembly domain in α dimerization was supported by the analysis of this series of mutants. These α-point mutants were analyzed *in vivo* as well. All the mutants assembled β and β′ subunits, but only α-R45A failed to complement *rpoA*112.

Taken all these results together, the functional organization of the N-terminal assembly domain of α subunit can be summarized as follows:

(1) Multiple segments widely distributed within the assembly domain are responsible for α dimerization.

(2) The β-binding site is located on the N-terminal proximal conserved region. Especially, the region around position 45 plays a key role in the binding but the region near aa 80 also participates in it.

(3) The β′-binding site is composed of two separated regions. One is the C-terminal proximal region of the assembly domain centered on aa 173-200. The other is within the N-terminal proximal conserved region at position 76-80 overlapping with the β-binding site.

論文の審査結果の要旨

大腸菌のＲＮＡポリメラーゼは$\alpha_2\beta\beta'$というサブユニット構成をもち、ＲＮＡ合成を担うコア酵素とプロモーターの認識に関与するσ因子から形成される。αサブユニットのＮ末側２３５アミノ酸残基からなり、Ｃ末側を欠く変異型αはβ、β'、σサブユニットと会合してホロ酵素を形成するが、クラスＩに属する転写因子に依存するプロモーターからの転写を開始できない。従って、αサブユニットのＮ末側はサブユニット集合に関与するドメインで、Ｃ末側はクラスＩ転写因子との相互作用に必要なドメインと考えられる。

本研究で木村　誠氏はαサブユニットのＮ末端側（サブユニット集合ドメイン）に合計５２種の部位特異的変異を導入し、それらの変異型αを用いて *in vivo* と *in vitro* のサブユニット集合能、*in vitro* のＲＮＡ合成活性、この領域にアミノ酸置換をもつため、菌の生育が温度感受性となった２種の変異株に対する相補能などについて解析した。その結果、以下のことが明らかとなった。１）コア酵素形成の最初のステップであるαのダイマー形成に必要な領域はサブユニット集合ドメイン全体に分布している。２）βサブユニットの結合部位は種間でアミノ酸配列が保存されている領域内に存在する。特にアミノ酸残基４５付近が重要な役割を果すが、残基８０付近も関与する。３）β'サブユニットの結合部位は２つの領域から成る。一方はアミノ酸残基１７３～２００の領域で、他方はβサブユニット結合部位と重なっており、残基７６～８０の領域である。

本研究で木村氏が行った徹底した変異の導入と、その誠実な解析は余人をもって行い難く、高く評価される。これらの結果の一部は国際学術誌である J. Mol. Biol. にすでに発表されており、他の部分も同誌に印刷中である。以上のように、この論文の内容はＲＮＡポリメラーゼの構造と機能に関して重要な基礎的データーを提供するもので、遺伝学専攻の博士論文としての条件を満すものであることを、審査委員全員一致で認めた。