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論文内容の要旨

Structural studies of Schizosaccharomyces pombe RNA polymerase II

The molecular composition of DNA-dependent RNA polymerase II from Schizosaccharomyces pombe was studied. First, RNA polymerase II was purified from the wild type S. pombe, in five steps: precipitation with Polymin P; elution from the Polymin P precipitates; separation of RNA polymerase II from RNA polymerases I and III by DEAE-Sephadex column chromatography; purification by Q Sepharose FF; and by Superose 6 gel filtration column chromatography. The highly purified S. pombe RNA polymerase II contained more than eleven polypeptides as analyzed by SDS-polyacrylamide gel electrophoresis. The molar ratio of the three large subunit, RPB1, 2 and 3, was 1:1:2, which is identical with that of the E. coli and S. cerevisiae enzymes.

In order to establish in vitro reconstitution system of RNA polymerase II and to decide the subunit composition of RNA polymerase II, preliminary attempts were made to find conditions for reversible denaturation of RNA polymerase II by treatment with urea. RNA polymerase II was not inactivated up to 0.5 M urea. RNA polymerase II lost activity from 1.0 M to 2.5 M urea, but the activity was recovered perfectly simply by dilution of urea. Above 3.0 M urea, however, RNA polymerase II activity was irreversibly inactivated. From the result of gel filtration, multi-subunit complexes of RNA polymerase II were observed but several small components were missing from the complexes after denaturation in 4 M urea.

The S. pombe gene coding for the largest subunit (subunit 1) of RNA polymerase II was cloned using the S. cerevisiae corresponding gene, RPB1, as a probe in cross-hybridization. The sequence determination of both the entire genomic DNA and parts of cDNA indicated that this rpb1 gene has six introns in the N-terminal region and encodes the subunit 1 of 1,752 amino acid residues with the molecular mass of 194 kDa. From Southern analysis and gene disruption experiments, it was found that this rpb1 gene exists as a single copy in the S. pombe genome and is essential for cell viability. Northern analysis and sequence determination of 3'- and 5'-terminal regions of rpb1 transcript indicated that the size of the rpb1 transcript is about 5.6 kb in length.

Among the subunit 1 of S. pombe RNA polymerase II and other b' homologues, nine structurally conserved domains (domain A to H and CTD) were identified: domain A, a putative zinc-binding site with the consensus sequence of CX2CX9HX2H; domains C and D, the conserved sequences within E. coli DNA polymerase I and T7 DNA polymerase; Domain C, a single two-helix motif for putative DNA binding; domain F, the putative a-amanitin binding site; CTD, highly conserved unique repetition with the unit sequence of YSPTSPS among the largest subunits of RNA polymerase II. In the subunit 1 of S. pombe RNA polymerase II, 29 repeats exist in CTD.

The cDNA fragment coding for the third largest subunit (subunit 3) of S. pombe RNA polymerase II was cloned by RT-PCR using primers designed from the amino acid sequences of V8 fragments of subunit 3. A genomic DNA fragment carrying the entire subunit 3 gene (rpb3) was isolated by hybridization using this cDNA fragment as a probe. The sequence determination indicated that the coding frame of rpb3 is interrupted by two introns and this gene encodes subunit 3 of 297 amino acids in length. Southern and Northern analyses indicated that the rpb3 gene is present as a single copy in haploid S. pombe cells and the size of rpb3 transcript is about 1.2 kb in length.

Among the RNA polymerase subunit 3 from various organisms, four structural conserved domains (domains A to D) were found: domains A and D exist even in the a subunit of E. coli RNA polymerase; and domains B and C are conserved only in eukaryotic RNA polymerases. Domain A may play a role in subunit-subunit contact of RNA polymerase. Domain B with a putative metal-binding sequence, CXCX3CX2C, exists only in RNA polymerase II, but not in RNA polymerase I nor III. Domain D with a leucine zipper-like motif may be required for the formation and/or stability of RNA polymerase. Subunit 3 of eukaryotic RNA polymerases lacks the sequence corresponding to the C-terminal region of E. coli RNA polymerase a subunit carrying the contact site I for some transcription activators.

To identify the function(s) of subunit 3, the rpb3 gene was mutagenized by lower fidelity PCR and transferred into S. pombe using either spheroplast method or electroporation method. Total 178 temperature-sensitive ts mutants were isolated from about nine thousand transformants. PCR analysis and Southern analysis were carried out for 68 stable ts mutants. Most of the mutants tested carried a single copy of full length DNA fragment integrated in the genomic DNA. With nine mutants, the mutation sites were determined after cloning and sequencing. All the mutant rpb3 genes carried multiple mutations, but many mutations were clustered in the N-terminal region of RPB3 polypeptides. Upon temperature shift from permissive temperature, 25°C to non-permissive temperature, 37°C, some of the mutants stop growing immediately, while other mutants stop growing slowly, implying that the assembly of RNA polymerase II is defective in these mutants. Some mutants show abnormal cell shapes (for example, 7 times longer in cell size) at the non-permissive temperature. None of the sixteen mutants survived after one day incubation at non-permissive temperature. Even in these non-viable cells, the nuclei still appeared intact.

論文の審査結果の要旨

東慶直君は、石浜明教授の指導のもとに真核細胞の転写装置の仕組を明かにする研究の一端として、その基本装置であるＲＮＡポリメラーゼ II（Pol II）を選び、分裂酵母（*S. pombe*）のPol II holoenzymeの構成蛋白質の中、まだ報告の無かった分子量の最も大きいsubunit（194kDa）と３番目に大きいsubunit（34kDa）について、その遺伝子（*rpb1*および*rpb3*）のクローン化を行い、構造決定によって既報の哺乳類や出芽酵母をはじめとする真核細胞のPol II構成蛋白質との対応づけを行った。分裂酵母の転写開始機構および転写産物のスプライシングは出芽酵母にくらべて哺乳動物のそれにより近く、遺伝解析が可能な分裂酵母での東君の研究成果の関連分野への貢献は大きいと評価できる。

rpb1 遺伝子については出芽酵母の相当遺伝子クローンとの塩基配列の相同性を期待して、その断片をプローブにDNA-DNA hybridization 法で、また、cDNAはmRNAからRT-PCR 法によって作成し、共にクローン化できた。予期どうり、その構造はすでに明かになっている他の真核細胞の相当蛋白質に共通に存在する８つの構造ドメインおよびC-末端ＣＴＤ配列をもち、大腸菌のb' 蛋白質のもつ機能との対応を推察した。

rpb3 遺伝子については、hybridization 法によるクローン化は成功せず、蛋白質の細胞からの分離精製から出発する方法を採用した。本遺伝子は予期どうり他の真核細胞の相当蛋白質に共通に存在する４つの構造ドメインをもち、大腸菌のa 蛋白質成分がもつb'成分とb成分の接着機能や転写因子認識機能が推察された。また、*rpb3* 遺伝子の in vitro mutagenesis を行い、細胞への変異遺伝子導入によりいくつかの温度感受性変異株を樹立した。各変異株の*rpb3* 遺伝子の変異点を４つの構造ドメインとの対応で決定した。しかし、変異株の示すPol II 活性との対応づけは技術的な困難さから成功していない。また、変異株細胞は種々の形態異常を示したが、*rpb3* 変異との対応の説明はできなかった。

東君の着実な成果によって、in vitro 再構成系を用いた真核細胞 Pol II の機能解析などに遺伝学が導入可能になった。また、東君の研究は、近年、労力と時間、それにしばしば職人的な経験が要求されることで敬遠されがちな蛋白質の生化学的な分離精製を実行して所期の目的を達成し、成果をあげたことで評価できよう。研究者として今後一人立ちできる経験と実行力発揮のための自己訓練を積み重ねたと判断し、博士論文の学問的内容の評価と合わせて、審査委員全員一致で合格とした。