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Cloning and Characterization of Replication Origins from *Misgurnus mizolepis**

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미꾸라지로부터의 복제원점 클로닝 및 그 특성에 관한 연구*

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ABSTRACT

The nuclear matrix was isolated from *Misgurnus mizolepis* liver nuclei by low salt extraction and restriction enzyme treatment. The structure was digested with proteinase K. After centrifugation, matrix attachment regions (MARs) were obtained by RNase treatment and phenol-chloroform extraction. The result leads to the appearance of smeared bands in the range of about 0.3−15 kb.

pURY19 vector was constructed by inserting 2.13 kb Eco47 III fragment of the yeast uracil 3 gene into the unique Ssp I site of pUC19 plasmid vector as a selection marker. This vector is unable to be maintained in *Saccharomyces cerevisiae* by itself since it cannot replicate as an extrachromosomal element. Using this system, we attempted cloning the ARS (autonomously replicating sequence) from *M. mizolepis* to develop an efficient expression vector for the transgenic fish. pURY19N_{1-62} were constructed by inserting MARs in pURY19 plasmid vector and transformation of *E. coli* DH5α.

Replication origins (ARS) of *M. mizolepis* were isolated, which enabled the vector to replicate autonomously in *S. cerevisiae*. The cloned DNA fragments were sequenced by Sanger's dideoxy-chain termination method. All clones were AT-rich. pURY19N_6, one of the clones, expecially contained ARS consensus sequence, Topoisomerase II consensus, near A-box and T-box.

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INTRODUCTION

One of the most intensively studied areas in eukaryotic DNA replication is to discover the DNA sequences that specify its initiation sites. The term "origins of replication" is used to describe the DNA sequences at which replication initiates as well as cis-acting sequences that promote initiation.

Evidences are now showing that eukaryotic DNA replication normally initiates at specific sites. However, with the exception of *S. cerevisiae*, it is difficult to decide whether patterns of initiation are specified directly by sequence-specific interaction with replication proteins or indirectly as a consequence of structural features of the chromosome (Dawn Coverley and Ronald A. Laskey 1994).

The term nuclear matrix was first introduced by Berezney & Coffey (1974) to denote a highly structured residual framework obtained from rat liver nuclei by sequential salt extractions, detergent, and nuclease treatments. A variety of nomenclature has been used by different investigators to refer to the nuclear matrix ; nuclear matrix, nuclear scaffold, nuclear ghost, nuclear cage, and chromatin-depleted nucleus. All these terms refer to very similar residual nuclear structures. There have also been numerous modifications of the original procedure described for isolating the nuclear matrix, and it is important to recognize that many factors can affect of the nature of the final product.

Over the past several years evidences have accumulated that the nuclear matrix is not simply a static structure, but is rather a dynamic scaffolding system that is intimately associated with such fundamental nuclear processes as DNA organization, DNA replication, heterogeneous nuclear RNA (hnRNA) synthesis and processing, and hormone action.

Specific regions of cellular DNA are attached to the nuclear matrix ; these loci are called matrix attachment regions (MARs). MARs are at least 250 bp long, AT-rich ( ) =65 %), often contain the topoisomerase II consensus sequence of Sander and Hsieh (1985) and other AT-rich sequence motifs sometimes reside near cis-acting regulatory sequences, and are evolutionarily conserved (Cockerill and Garrard 1986 a & b ; Amati and Gasser 1988 ; Izaurralde et al. 1988). Beside the topoisomerase II consensus, MARs often contain other multiple DNA sequence motifs, including A-boxes and T-boxes (Gasser and Laemmli 1986a), and the ATATTT and ATATTTTT motifs (Cockerill and Garrard 1986a).

Autonomously replicating sequence (ARS) elements are DNA sequences which allow co-linear DNA to transform yeast at a high frequency (Williamson 1985). Seven ARS elements of *Drosophila* and the origin of the 2-μm DNA circle of yeast have been shown to be scaffold-bound. Reports show the binding of *Drosophila* MAR to rat liver, HeLa, mouse, and chick nuclear scaffolds, as well as the binding of the mouse Ig MAR to matrices from yeast and scaffolds from chicken cells. Such results suggest that the association with the scaffold be a feature of replication origins conserved from yeast to human.

But the studies on the MAR from fish and the effect of replication origins for the production of transgenic fish are rare. Replication origins of prokaryotes, their plasmids, or virus have been used as the replication origins of the vector for transgenic fish. Because, in eukaryote, all nuclear functions such as DNA replication, transcription, and recombination are carried out in the nuclear matrix, the use of the vector with prokaryotic or viral origin causes several problems in the vector copy number in the early embryo stage, integration rate of target genes and subsequent expression frequency in the transgenic fish.

To produce the effective transgenic fish, several conditions are required as follows : First, transferred vector should be highly stable and/or the integration ratio into host chromosome should be high. Second, the vector should contain strong promoter and enhancer. Third, optimal microinjection conditions for each fish species be established.

In the present study we attempted to clone the replication origins (ARSs) from MAR of *M. mizolepis*, to increase stability, copy number, and integration rate of the vector.

MATERIALS AND METHODS

Strains and plasmids

The bacterial, yeast strains, and plasmids used in this study are presented in Table 1. *E. coli* DH5α was used as host cell for the amplification of all plasmid vectors. *S. cerevisiae* was used as host cells for screening ARS phenotypes of plasmid vector pURY19Ns constructs. Plasmid pUC19 and YIP5 were used for the construction of bacterial-yeast shuttle vector. Plasmid vector pURY19 derived from pUC19 was used as a bacterial-yeast shuttle vector for cloning of ARS sequence from MARs of *M. mizolepis*. pBluescript II $KS^{(+)}$ vector was used as sequencing analysis vector.

Table 1. Strains and Plasmids used in this work

Strains/Plasmids	Description
A. Strains	
S. cerevisiae DBY 747	Mat a, his 3, leu 2−3, leu 2−112, ura 3−52, trp 1−289
E. coli DH5α	supE 44 △lac U169 (Φ80 lac Z △M15) hsd R 17 rec A1 end A1 gyr A 96 thi-1 relA1
B. Plasmids	
pBluescript II KS^+	3.0 Kb, amp^r, f1 origin, colE1 ori, lac I, lac Z, T7 and T3 promoter
pUC19	2.7 Kb, amp^r, lac I, lac Z, colE1 ori
YIP5	5.5 Kb, amp^r, ter^r, Ura^+, colE1 ori
pURY19	4.8 Kb, amp^r, Ura^+, lac I, lac Z, colE1 ori

Cell culture condition

E. coli was cultured in Luria-Bertani (LB ; 1% Bacto-tryptone, 1% NaCl, 0.5% yeast extract) medium at 37℃ with vigorous shaking. When necessary, ampicillin was added in the LB with the final concentration of 50 μg/ml.

S. cerevisiae was cultured in YPD (1% yeast extract, 2% Bacto-peptone, 2% glucose) or supplemented SD media containing 6.7 g of yeast nitrogen base without amino acids, 20 mg of adenine, 20 mg of tryptophan, 20 mg of histidine, 30 mg of leucine, 20 g of dextrose per liter at 30℃ with vigorous shaking. For the plate culture, media was solidified by adding 2% Bacto-agar.

Enzymes and reagents

Restriction enzymes, Cla I, Hind III, Pst I, and EcoR I and intestinal alkaline phosphatase were obtained from Kosco Biotech. T4 DNA ligase, restriction enzyme Eco47 III and Ssp I were obtained from Boehringer Mannheim Biochemicals. The sequenase DNA sequencing Kit was obtained from U.S. Biochemical Corp. All chemicals were purchased from Sigma.

Isolation of the nuclear matrix and MAR

Nuclear Matrix and MAR isolation were carried out by the method described by Mirkovitch et al. (2984).

Isolation of fish liver nuclei

Fishes were killed by decapitation and the livers were immediately removed and placed in 30 ml of ice-cold isolation buffer A (IB A ; 0.25 M sucrose, 50 mM Tris-HCl, 20 mM KCl, 1% (v/v) thiodiglycol, 0.125 mM spermidine, 0.05 mM spermine, 0.5 mM EDTA/KOH, pH7.4, 0.5 mM phenylmethyl-spermidine (PMSF), and 1 mM dithiothreitol (DTT)). The tissue was minced and cells were disrupted wi th 15 strokes in a teflon-glass homogenizer in IB B (0.1% digitonin added to IB A). After being filtered through 3-4 layers of cheesecloth, the homogenate was sedimented at 800 × g for 15 min and the resultant was washed twice in the same baffer. The nuclei was pelleted at 330 × g through a cushion of IS buffer containing 0.5 M sucrose. The nuclei were washed twice in the IB C (IB B without EDTA) without sucrose and the final pellets were resuspended in a small volume of IB C.

Isolation of nuclear matrix of fish liver

Ten OD250 units of nuclei in 100 μℓ of IB C were heated at 37℃ for 20 min. After heating, 60 ml of low salt extraction buffer (5 mM HEPES-NaCl, pH 7.4, 0.25 mM spermine,

2 mM EDTA/KOH, pH 7.4, 2mM KCl, 0.1% digitonin and 25 mM 3, 5-diiodosalicylic acid, lithium salt (LIS)) were slowly added at room temperature. After 5 min, histone-depleted nuclei were recovered by centrifuged at 2400 × g for 20 min at room temperature. The pellet was washed 4 times with 20 ml of digestion buffer and spin for 20 min at 2400 × g. The pellet (halo structure) were digested with EcoR I (400 unit/ml) for 3 hour at 37℃ with gentle agitation. After the digestion, the digested nuclei were fractionated into soluble supernatant and insoluble part by the centrifugation for 10 min at 6000 × g.

Isolation of matrix-attached region (MAR)

The nuclear matrices were washed twice with ice-cold wash buffer (15 mM Tris-HCl, pH 7.4, 0.125 mM spermidine, 50 mM spermine, 20 mM KCl, 1% thiodiglycol, 0.5% trasylol, 10 mM $MgCl_2$, 0.1% digitonin and 70 mM NaCl). The samples were subjected to disintegration in a lysis buffer of 1% SDS and 100μg/ml Proteinase K. Proteinase K digestion were carried out for 2 hours at 50℃. The MAR was recovered through phenol extraction followed by ethanol precipitation. Electrophoresis in agarose slab gel was carried out with TAE buffer (40 mM Tris-Acetate, pH 7.6, 2 mM EDTA). Gel was runned at low voltage (below 2 v/cm) until DNA bands reached to appropriate positions.

Preparation of plasmid DNA

The plasmid DNA was prepared from E. coli according to USBio Kits protocols. The plasmid DNA from yeast for transformation of *E. coli* was prepared as described by Charles S. Hoffman and Fred Winston (1987).

Transformation of *E. coli* and Yeast

Transformation of *E. coli* was carried out based on $CaCl_2$ method described by Mandel and Higa (1970). Transformation of yeast was carried out by Lithium acetate method (Current protocols in Molecular Biology 13 ; 7. 1−5, 1989).

Construction of the cloning vector

Cloning vector pURY19 was constructed by attaching 2.13 kb Eco47 III fragment carrying the yeast URA 3 gene for selection in yeast at a Ssp I site of pUC 19.

Sequence analysis

DNA sequence analysis was carried out as described in the protocol of DNA sequenase Kit from USB.

RESULTS

Isolation of MAR

Halo structure was isolated from *M. mizolepis* liver nuclei by modified LIS method. The structures were separated from supernatant DNA with nuclear matrix by the treatment with restriction enzyme EcoR I (400 unit/ml) for 3 hours at 37℃ and centrifugation for 10 min at 6000 × g. The pellet was digested with protease K (100 μg/ml) for 2 hours at 50℃. MARs were recovered by phenol extraction followed by ethanol precipitation. The DNA solution was treated with RNase. Agarose gel electrophoresis leads to the appearance of discrete bands in the range of 0.3−15 Kb (Fig. 1).

Construction of MAR library (pURY19N_{1-62}) for budding yeast transformation

pURY19 plasmid vector, for bacteria-yeast shuttle vector, was constructed by attaching a 2.13-kb Eco47 III fragment carries yeast URA 3 gene for the selection in yeast at the Ssp I site of the vector pUC19. The reaction mixture was transferred into *E. coli* and colonies were selected on LB plate containing ampicillin. The vector was prepared and identified using restriction enzymes Cla I, EcoR I, and Hind III (Fig. 2). Treatment with restriction enzyme Cla I which does not cut the pURY19 vector (Fig. 2 ; lane 7), however, with EcoR I and Hind III which do cut a single site in the vector (Fig. 2 ; lane 8, 9). The results of restriction enzymes analysis mean that the pURY19 vector contains the bacterial origin, the Ap^R bacterial marker, and the yeast URA3 gene for selection in yeast. Construction of pURY19N_{1-62} was carried out by ligating MARs at the EcoR I site of the vector pURY19 (Fig. 3). These ligation mixtures were transferred into *E. coli* DH5α. White colonies were selected on the LB plate containing ampicillin, X-gal, and IPTG and then 62 recombinants were obtained.

Screening of ARS from MAR library

After pURY19N_{1-62} were transferred into Ura^- *S. cerevisiae*, 20 different transformants were obtained on SC-ura plate. The transformants were incubated for 24 hour at 30℃ in the SC-ura minimal media. The plasmid vectors were prepared from the cultures and transferred into DH5α based on $CaCl_2$ method by Mandel and Higa (1970). We obtained three different transformants on the LB plate containing ampicillin. It means that the plasmid vectors act as bacteria-yeast shuttle vector and they are episomal state. Therefore, the plasmid vectors should contain eukaryotic replication origin (ARS). pURY19$N_{6.\ 10.\ 28}$ were prepared from three transformants according to USBio Kit method.

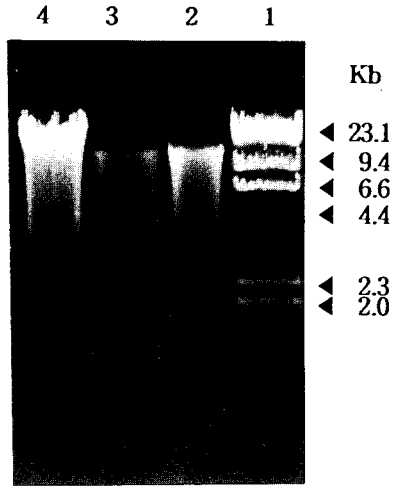

Figure 1. Size distribution of MAR. The MAR of *M. mizolepis* were isolated as followed in materials and methods and electrophoresed onto 0.7% agarose gel.
Lane 1. λDNA digested with Hind III.
Lane 2−3. MAR.
Lane 4. Supernatant DNA of halo structure digested with EcoR I.

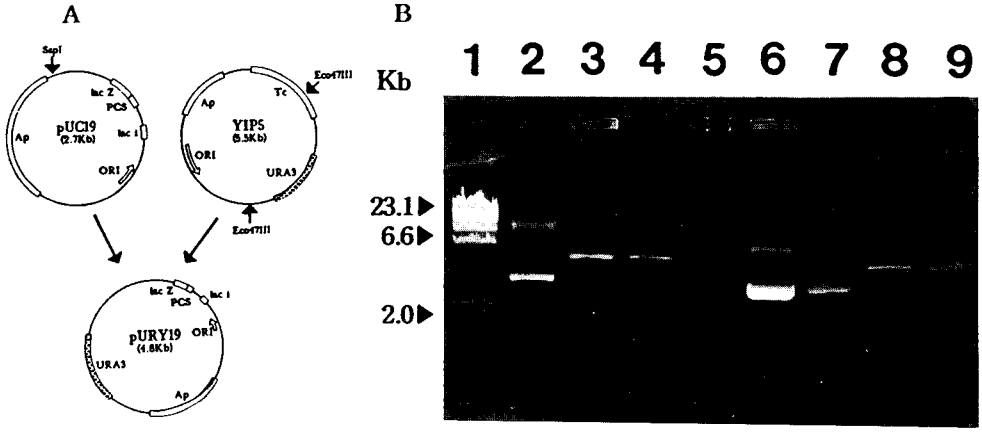

Figure 2. Construction of pURY19 vector. Plasmid YIP5 was digested with Eco47 III and isolated Eco47 III fragment carries yeast URA3 gene. The fragment was inserted into Ssp I digested pUC19 with T4 DNA ligase(A). YIP5 and pUPY19 vector were digested with Cla I, Hind III, or Pst I restriction enzymes and then subjected to 0.7% agarose gel (B).
Symbols : Lanes 1, Marker (λDNA/HindIII) 2, Intact YIP5 3, Pst I digest of YIP5 4, EcoRI digest of YIP5 5, HindIII digest of YIP5 6, Intact pURY19 7, Cla I digest of pURY19 8, EcoRI digest of pURY19 9, HindIII digest of pURY19

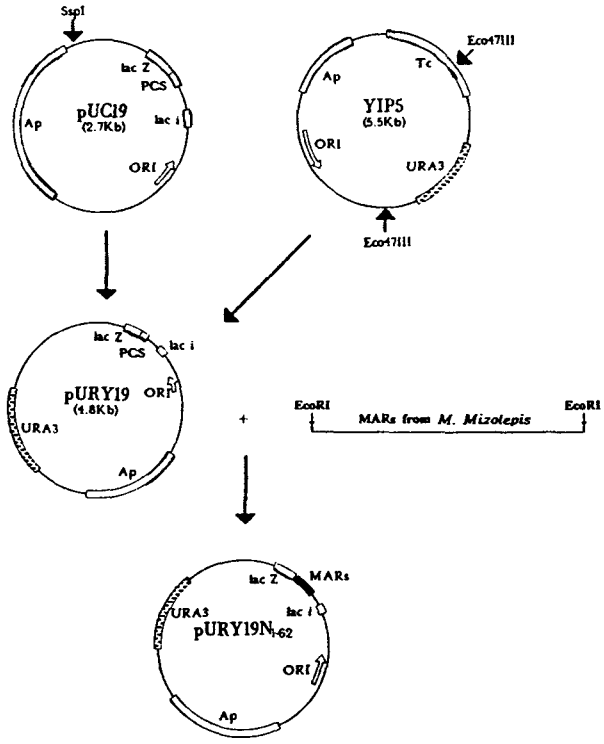

Figure 3. Construction of pURY19N_{1-62}. pURY19 vector was digested with EcoR I. The isolated MARs from *M. mizolepis* were introduced into the linearized vector by adding T_4 DNA ligase and incubating at 16℃ for 12−14 hour.

Analysis of sequence

Insert fragments of pURY19$N_{6,10}$ were isolated by agarose gel electrophoresis after digesting with EcoRI. EcoR I fragments of pURY19$N_{6,10}$ with the size of 0.5−0.6 kb were inserted into the same site of plasmid SK (+) for sequencing analysis (Fig. 4). Both vectors were analyzed by dideoxy sequencing method. Both partially and fully sequenced clones were AT-rich. pURY19N_6, one of the clones, especially contains ARS consensus sequence, Topoisomerase II consensus, near A-box and T-box, and its AT content was 66% (Fig. 5).

DISCUSSION

Previously, very few fish genes were available for gene transfer to fish and the efficiency of transgenesis was unknown. Comparison of the DNA sequences of the sockeye salmon

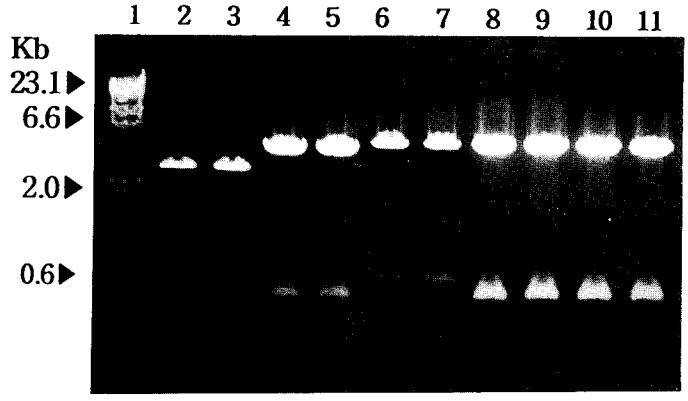

Figure 4. Identification of inserts of pURY19$N_{6,10}$. pURY19$N_{6,10}$ were digested with EcoRI. The digested mixtures were subjected to 0.7% agarose gel. λDNA was digested with HindIII (Lane 1), $SK^{(+)}$ was digested with EcoRI (Lanes 2, 3), pURY19N_6 was digested with EcoRI (Lanes 4−9), and pURY19N_{10} was digested with EcoRI (Lanes 10−11).

5' --- GAA TTC TTC AAT TCA TGC AAT TTG AGA GAT GTC GTC ATT GAA TGC
ATT TTG TGC CAA AGC AAT GAT AAT TGA TCC TCA GTT TAG CCC ACA
TAG ACT TCT GTG GTG CTC ACT GTG AAA GTT TTG CAA GTT ACC TAA
GTA TTG AGA AAT GTG TCT AGC ATC TGT AAA AAT AGC TGT AAT TTT
AAA GTG TGA TAC TAG TTG ACT GTA TGT CAG TAG TTG TCT TGG TTT
TAA TTT TAT ATT TAA ATC ATA AGC AAT TTA AAC TGA GGT TAA GAA
CAC AGA CTC TAT AGC TCT TGG AAC AAC CTA GCA --- AGG AAA AAC
ACC TGA GAG CAC AAA CTG GGN NCA AAG AAA ACA ACC AAG ACC GCA
GAT TAT GTC TTC TGT GAA AAC NNG GGA CAA CAT CTG AGC AAA TAT
TGT TTA TCT GTT TAA TTA AAT AAA ATG TTA TAA ATA TAG AGT TTT
TCA CAA TTG TTT TAT TGT TTC AAA GCA GCA TAA GCA CGA GAA TTC ---3'

Figure 5. DNA sequence of pURY19N_6. DNA sequence analysis was carried out as described in the protocol of DNA sequenase Kit from USB.
DNA sequence composition : 166 A ; 73 C ; 89 G ; 157 T ; 7 OTHER ; N
Content of (A+T)=323/492×100=66%
======: ARS consensus sequence, _____ : Topoisomerase II consensus
· · · · · · : A-box or T-box

to the rainbow trout histone H3, metallothionein-B (MT-B), and protamine (PT) promoters revealed that their DNA sequences and putative transcriptional cis-elements were remarkably conserved. Promoter activity of the sockeye salmon H3, MT-B, and PT promoters

were examined by transfection studies using cell lines from fish and human. The H3 and MT-B promoters were shown to be active in all fish cell lines but were only weakly active in HeLa and GM637 cells. The MT-B promoter was also inducible by zinc and cadmium in RTH-149 cells. In contrast, the PT promoter were nactive in all fish cell lines (Chan W. K. and Devlin R. H. 1993). Non-fish gene transcriptional regulatory elements (TRE) can be used to express foreign genes in fish cells or transgenic fish ; however, in most cases they are relatively inefficient (Monique B. et al. 1992). These data suggest that fish gene promoter be more effective than non-fish gene for the construction of expression vectors for transgenic and gene transfer studies in fishes.

In recent years many ARSs from yeast or *Drosopila* have been isolated, but not fish. Also it has yet to study on fish DNA replication system which is the most important among fish cell genetics. The study of development of expression vector about fish DNA replication origin for transgenic fish is rare. Considering DNA replication (Foster K. A. et al. 1985 ; Neri L. M. et al. 1992), transcription (Getzenberg R. H. 1994), and recombination in eukaryote are carried out in the nuclear matrix, the use of the vector with prokaryotic or viral origin cause the several problems in the copy number, expression frequency, and integration rate of target genes.

In this study, cloning of fish replication origin (ARS) from nuclear matrix-bound fish DNA fragment was carried out for construction of effective transgenic fish vector. After the microinjection of target gene into fish egg, the production of effective transgenic fish with continuous expression of target gene requires that the vector be stable and the expression of the integrated target gene into host chromosome be expressed ; Hence the region being able to attach nuclear matrix of fish is required for transgenic fish production. Finally, the effective vector for transgenic fish should contain DNA replication origin (ARS), enhancer, and adequate promoter of fish.

The vector containing the same fish species replication origin (ARS) is able to increase copy number in each of the cell cycle, binding frequency to nuclear matrix like previously existing replication origin, frequency of recombination because of containing topoisomerase II binding site, and frequency of expression because the integration site is located near nuclear matrix after microinjection into embryo.

It is suggested that pURY19N$_6$ be multifunctional and useful when it is injected into the egg of *M. mizolepis* because vector pURY19N$_6$ contains ARS consensus sequence, topoisomerase II concensus, near A-box and T-box. ARS consensus, A-box and T-box may play key roles in the replication initiation. Topoisomerase II consensus may participate in the integration of microinjected vector into host chromosome by recombination process. The target gene contained in the pURY19N$_6$ vector may be expressed effectively because the clones were originated from MAR and the highly expressing genes are generally located near nuclear matrix attachment regions.

In the further work, the full sequence of ARS in pURY19N6 will be evaluated and assay of replication intermediates will be established. Also the enhancer from MARs of *M. mizolepis* will be isolated and complete expression vector for transgenic fish will be constructed.

요 약

미꾸라지의 간으로부터 핵을 분리하여, 저농도 염추출 및 제한효소 처리로 핵기질(nuclear matrix)을 분리하였다. 분리된 핵기질을 Proteinase K로 분해한 후, phenol-chloroform 추출로 크기가 약 0.3 kb－15 kb의 분포를 나타내는 핵기질 부착 DNA (nuclear matrix attachment regions ; MARs)를 얻었다. 효모 URA 3 유전자를 가진 2.13 kb Eco47 III 단편을 제한효소 Ssp I 으로 절단된 pUC19 플라즈미드 벡타에 결합시켜, ARS (autonomously replication sequence) 클로닝을 위한 pURY19 플라즈미드 벡타를 만들었다. 이 pURY19 벡타는 *Saccharomyces cerevisiae*내에서 독립적으로 복제할 수 없기 때문에, 물고기의 효율적인 발현 벡타 개발을 위해, 이 system을 이용하여, *S. cerevisiae*내에서 독립적으로 복제 가능한 미꾸라지의 ARS를 클로닝하고자 하였다.

분리된 MARs를 pURY19 벡타에 결합시킨 다음, *E. coli* DH5α에 형질전환시켜 pURY19N_{1-62}를 얻었다. MAR Libraries (pURY19N_{1-62})를 각각 Ura$^-$ *S. cerevisiae*에 형질전환시켜, *S. cerevisiae*내에서 독립적으로 복제 가능한 *M. mizolepis* 유래의 복제원점들 (ARSs)을 분리하여, Sanger's dideoxy-chain termination method로 염기서열을 분석 하였다. 염기서열 분석결과 모든 clones들은 AT-rich하였으며, 특히 pURY19N_6에는 ARS concensus sequence, Topoisomerase II consensus, near A-box, 그리고 T-box들이 존재하였다.

REFERENCES

Amati, B.V. and S. M. Gasser, 1988. Chromosomal ARS and CEN elements bind specifically to the yeast nuclear scaffold. Cell 54 : 967－978.

Berezney, R., and D. S. Coffey, 1974. Identification of a nuclear protein matrix. Biophy. Res. Commum. 60 : 1410－1417.

Chan, W. K. and R. H. Devlin, 1993. Polymerase chain reaction amplification and functional characterization of sockeye salmon histone H3, metallothionein-B, and protamine promoters Mol. Mar. Biol. Biotechnol. 2 : 308－318.

Charles, S. Hoffman and F. Winston, 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmid for transformation of *E. coli.* Gene 57 : 267－272.

Cockerill, P. N. and W. T. Garrard, 1986a. Chromosomal loop anchorage of the kappa immunoglobluin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44 : 273－282.

Cockerill, P. N. and W. T. Garrard, 1986b. Chromosomal loop anchorage sites appear to be evolutionally conserved. REBS Lett. 204 : 5－7.

Dawn, C. and R. A. Laskey 1994. Regulation of eukaryotic DNA replication. Annul. Rev. Biochem. 63 : 745−776.

Foster, K. A., and J. M. Collins, 1985. The relation between DNA synthesis and DNA polymerases bound to the nuclear matrix in synchronized HeLa cells. J. Biol. Chem. 260 : 4229−4235.

Gasser, S. M. and U. K. Laemmli, 1986a. Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster.* Cell 46 : 521−530.

Getzenberg, R. H., 1994. Nuclear matrix and the regulation of gene-expression-tissue-specificity. J. Cell. Biochem. 55 : 22−31.

Neri, L. M., G. Mazzoggi., S. Capitani., N. M. Maraldi., C. Cinti, N. Baldini., R. Rana., and A. M. Martelli., 1992. Nuclear matrix-bound replicational sites detected in situ by 5-bromodeoxyuridine. Histochemistry 98 : 19−32.

Izaurralde, E., J. Mirkovitch, and U. K Laemmli., 1988. Interaction of DNA with nuclear scaffolds in vitro. J. Mol. Biol. 200 : 111−125.

Mandel, M. and A. Higa., 1970. Calciumdependent bacteriophage DNA infection. J. Mol. Biol., 53 : 159−162.

Mirkovitch, J., M. E. Mirault, and U. K. Laemmli, 1984. Organization of the higher-order chromatin loop : specific DNA attachment sites on nuclear scaffold. Cell 39 : 223−232.

Monique, B., P. Elisabeth., M. V. Christine., J. Genevieve., A. Joe'., C. T. Marie., P. Claudine., D. Michel., J. J. Kophchick, P. Richard., C. Franck., M. H. Louis, and C. Daniel, 1992 Gene expression following transfection of fish cells. J. Biotechnol. 26 : 315−325.

Sander, M. and T. S. Hsieh, 1985. *Drosophila* topoisomerase II double-strand DNA cleavage : analysis of DNA sequence homology at the cleavage site. Nucleic Acids Res. 13 : 1057−1072.

Williamson, D. H., 1985. The yeast ARS element, six years on : A progress report. Yeast 1 : 1−14.