

Molecular Analysis of KP Elements Derived from Korean Populations of *Drosophila melanogaster*

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KP elements derived from Korean populations (Seoul, Cheonan and Taegu) of *Drosophila melanogaster* were examined for their molecular structure. The entire 1.15 kb sequence of the three KP elements KC-137 (Cheonan), KS-95 (Seoul) and KT-99 (Taegu) have been obtained by PCR amplification using inverted repeat primers and DNA sequencing. The 1.15 kb fragments of KP elements were cloned into pCRTMII vector plasmids, and subsequently sequenced. The sequence of the three KP elements in these populations suggested that there might have been derived from the complete P element by a 1753 bp internal deletion between positions 808 and 2560. Therefore these KP elements were confirmed to be identical to that isolated from M'/Q strains widely distributed in most Eurasian populations of *D. melanogaster*. Sequence comparison with the 2.9 kb complete P element in p π 25.1 revealed that KC-137 has only shown to be two base substitutions of A to G and G to A at positions 62 and 242, respectively. The retained sequence of the two KP elements KS-95 and KT-99 shows complete homology to the P factor in p π 25.1. Based on this result, the two base substitutions in KC-137 might be due to Taq DNA polymerase errors. Finally, it is suggested that the high copy numbers of KP elements provides an explanation for the suppression of P-mediated hybrid dysgenesis in Korean population of *D. melanogaster*.

KEY WORDS: *D. melanogaster*, Hybrid Dysgenesis, KP Element, PCR, DNA Sequence

The P element family in *Drosophila melanogaster* has been extensively studied for their significance in evolutionary biology and usefulness in a wide variety of genetic techniques (Rubin and Spradling, 1982; Kidwell, 1985; Engels, 1989). The autonomous 2907 bp element has 31 bp perfect, inverted, terminal repeats and encodes a protein of 87 kDa, transposase, which is required for P element transposition (O'Hare and Rubin, 1983). The cis-acting sites required for transposition are found within the terminal 150

bp of both P element ends (Mullins *et al.*, 1989). Defective elements are generally smaller and are nonautonomous, being derived from the 2.9 kb complete element by heterogeneous internal deletions. Most defective P elements can be mobilized when supplied with transposase from a 2.9 kb element.

It is likely that transposition of P elements is mainly regulated on at least in two types. First, the regulation of elements could be explained with two models for polypeptide-independent repression: titration of P transposase in the presence of many P elements ends (Simmons and Bucholz, 1985)

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and antisense RNA production (Rasmusson, *et al.*, 1993). The second type of regulation is known to be more than one level by a P-encoded polypeptide: transcriptional regulation, binding site competition, and multimer poisoning (Engels, 1989; Rio, 1991; Ronsseray *et al.*, 1993).

A phenomenon called 'hybrid dysgenesis' arises in F1 hybrid progenies from matings between P strain (♂) and M strain (♀) (Kidwell *et al.*, 1977). The characteristic features of the syndrome include temperature-dependent gonadal dysgenic (GD) sterility, male recombination, chromosome rearrangements, segregation distortion and high rates of mutation. In the P-M system, hybrid dysgenesis is determined by the interaction between chromosomally linked P factor activity and a cytoplasmic susceptibility referred to as M cytotype. On the basis of these properties, *D. melanogaster* strains may be classified into four broad types (P, Q, M' and M strains), depending on their phenotypic characteristics in diagnostic test crosses and their genomic distribution of P elements. P strains possess the P activity potential from low to high levels and they also have extremely low level of P susceptibility. A strong P strain has 25-60 copies of P elements per haploid genome. Approximately one-third of these are the 2.9 kb complete P element and the others are defective sequences (Bingham *et al.*, 1982). Most Q strains are not capable of inducing significant levels of hybrid dysgenesis, but they have P cytotype (Kidwell *et al.*, 1983). Some Q strains possess a similar number of genomic elements, but most of them are defective (Black *et al.*, 1987). True M and M' (pseudo-M) strains do not induce any significant levels of hybrid dysgenesis. Molecular analysis has shown that true M strains completely lack P elements, and they have extremely high susceptibility to P strains. M' strains carry P elements in their genomes, and many of them may show from low to high levels of P susceptibility (Black *et al.*, 1987; Boussy and Kidwell, 1987). True M strains have not been recently detected in nature, but many of these strains are found among collections of long-established laboratory strains (Bingham *et al.*, 1982). M' strains are currently common in nature and predominate in Asia, Europe, north Africa,

and south-east Australia (Anxolabéhère, *et al.*, 1984, 1985; Kidwell, 1983; Boussy *et al.*, 1988). Many of these strains have P elements, most or all of which are defective (Anxolabéhère *et al.*, 1985; Black *et al.*, 1987). Certain naturally-occurring M' strains have been shown to carry up to 30 copies per genome of a particular deletion derivative termed the KP element (Black *et al.*, 1987). These KP elements could be responsible for the repression of GD sterility in M' strains (Black *et al.*, 1987; Rasmusson *et al.*, 1993; Andrews and Gloor, 1995).

So far, Korean populations have been found to be mainly Q and M' strains, P strains being almost absent (Choo *et al.*, 1986; Paik *et al.*, 1991a; Sung and Kim, 1993; Kim *et al.*, 1995a). P factor activity was also found to be extremely low in these populations. Therefore, it needs to investigate the reasons for the repression of P-mediated hybrid dysgenesis in Korean population of *D. melanogaster*. We report results bearing on the presence of KP elements using DNA sequencing in this population. One of the explanations for the repression of P element mobility, KP repression is also discussed.

Materials and methods

Drosophila strains

A total of 70 lines of *D. melanogaster* were studied for the assay of KP elements using polymerase chain reaction (PCR). Isofemale lines collected from Seoul (1988), Cheonan (1993) and Taegu (1994) were used in this study. All lines were maintained under standard laboratory conditions. Two control strains were used to compare the band pattern for PCR product: Harwich^w, a strong P strain, and the true M strain, Canton-S (Kidwell *et al.*, 1977).

Polymerase chain reaction (PCR)

Genomic DNA was extracted from 100 adults from each line by method of Daniels and Strausbaugh (1986). Amplification of P element sequences was performed on the genomic DNA using PCR by the method of Kim and Kidwell (1994). Inverted repeat (IR) primers, (12/2896)

Table 1. P element primers used in DNA sequencing of the 1.15 kb KP element.

primer	#location	sequence (5' to 3')
Sp6*	97-81	ATTTAGGTGACACTATA
2683	236-257	GTGGGAGCAGAGCCTTGGGTGC
2684	703-725	GCTATTGTCTCCACCCGCAGG
830	757-776	CGACTGGGCAAAGGAAATCC
2767	2748-2767	CCTTAGCATGTCCGTGGGGT
T7*	68-87	TAATACGACTCACTATAGGG

*The inserted PCR product of the 1.15-kb P element in the pCRTM II vector is flanked on each side by Sp6 and T7 promoter sites.

AACATAAGGTGGTCCCGTTCG (31/2877), were designed to specifically hybridize to the P element terminal sequence. PCR was carried out in 50 μ l volume using *Taq* DNA polymerase and a DNA Thermal Cycler (Perkin-Elmer Cetus) according to the following protocol: 5 μ l Cetus 10 \times PCR buffer; 4 μ l 25 mM MgCl₂; 4 μ l 10 \times concentrated dNTP labeling mixture; 4 μ l (66 ng) IR primer; 1 μ l (100 ng) genomic DNA; 1 μ l (2.5 unit) Ampli Taq DNA polymerase; 31 μ l sterile deionized water. The reaction mixture was overlaid with one drop of mineral oil, and denatured at 95 $^{\circ}$ C for 2 min before going 35 cycles. The amplification was performed for 35 cycles with 1 min denaturation at 95 $^{\circ}$ C and 45 sec annealing at 52 $^{\circ}$ C, followed by a 2 min extension at 72 $^{\circ}$ C. The results of amplification were confirmed by electrophoresis using 5 μ l of PCR products on 1% agarose gel.

DNA sequencing

The 1.15 kb sequence of three KP elements (Seoul, KS-95 Q strain; Cheonan, KC-137 Q strain; Taegu, KT-99 M' strain) selected at random, was determined by the dideoxy-chain-termination method (Sanger *et al.*, 1977) using the Sequenase kit (U.S. Biochemical, Cleveland) according to supplier's protocols. This 1.15 kb sequence was generated from a fragment amplified with PCR. The PCR products were then electrophoresed on 1% agarose gel, and the fragments were isolated using glass milk (GeneClean; Bio 101, La Jolla, California). The fragments were cloned by ligating into pCRTMII using the TA cloning kit (Invitrogen, San Diego) and subsequently were sequenced by the method

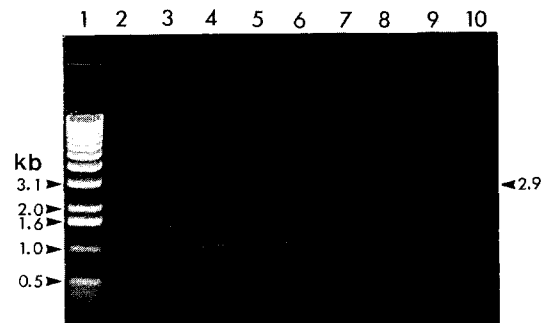


Fig. 1. PCR amplification of P sequences using inverted repeat primers. The presence of P element sequences amplified from Q and M' strains in a Korean (Cheonan) population. Lanes are as follows: (1) DNA size markers, (2-4) M' strains, (5-8) Q strains, (9) Canton-S, (10) Harwich^w.

of Clark *et al.* (1994). Oligonucleotides for PCR and sequencing of P element DNA were provided by Margaret G. Kidwell (University of Arizona, Tucson). These primers used for DNA sequencing are listed in Table 1.

Results

The presence of the 1.15 kb KP element was confirmed by PCR amplification using inverted repeat primers. Figure 1 shows the result of PCR amplification using the entire genomic DNA isolated from wild-caught Q and M' strains. Except for the control Canton-S M strain and Harwich^w strong P strain (Kidwell *et al.*, 1977), each lane of the gel indicates that a 1.15 kb element (KP) was preferentially amplified in this

genomic DNA with a minor band of 0.5 kb element which seems to be a SP-like element (Rasmusson *et al.*, 1993). Both of the 1.15 kb and 0.5 kb SP-like elements are clearly prevalent in PCR products derived from Q/M' strains. This result provides further evidence that all of the Q and M' strains carried a high copy number of KP elements in their genomes. As expected, the 2.9 kb P element was amplified in the Harwich^w strong P strain (Fig. 1, lane 10). And it was not detected P element sequences in Canton-S strain (Fig. 1, lane 9). This result indicates that Harwich^w P strain has a high copy number of the 2.9 kb complete P element than those of Q/M' strains in this population.

All of three KP elements tested were found to be derived from the complete 2.9 kb P element by internal deletion of 1753 bp from positions 808 to 2560 (Fig. 2). Consequently, these KP element appeared to be nonautonomous being composed of 1154 nucleotides. Therefore the isolated 1.15 kb P element was confirmed to be the same type of KP element that is widely distributed in most Eurasian M' /Q populations of *D. melanogaster*. Nucleotide sequence comparison with the 2.9 kb

P element in p π 25.1 revealed that KC-137 has only shown to be two base substitutions of A to G and G to A at positions 62 (Fig. 3, A-1) and 242 (Fig. 3, A-2), respectively. The retained sequence of the two KP elements KS-95 and KT-99 (Fig. 3, B-1 and B-2) shows complete homology to the P factor in p π 25.1. These results suggest that the two base substitutions in KC-137 could be solely due to PCR amplification errors by *Taq* DNA polymerase. However, a KP element from Krasnodar in Russia was known to be a single base substitution of A to T at position 32 (Black *et al.*, 1987). Therefore, the sequence of KP element in this study appeared to be complete homology to the Krasnodar KP element, with the exception of a single replacement at position 32 in Krasnodar KP.

Discussion

On the basis of earlier reports for the P-M phenotypes, Korean population of *D. melanogaster* appeared to be composed of approximately equal proportions M' and Q types, but none of the P type (Choo *et al.*, 1986; Paik *et al.*, 1991a; Sung and Kim, 1993; Kim *et al.*, 1995a). P factor activity was also known to be extremely low in this population. Combining the data obtained by Southern blot, *in situ* hybridization and PCR experiments of P elements reported earlier in Korean populations revealed that almost of lines tested (Q/M') contained multiple copies of P elements in which most of them were internally deleted, together with only a very few copy number of complete P elements (Paik *et al.*, 1991b; Sung and Kim, 1993; Kim *et al.*, 1995; Kim *et al.*, 1995a, b).

PCR analysis of P elements in this paper revealed that all of the lines tested appeared to be preferentially amplified a 1.15 kb element (KP) and a minor band of 0.5 kb element (SP-like element). This result suggests that Korean population of *D. melanogaster* contains a high copy number of KP elements and 0.5 kb SP-like elements in their genomes. These results are also generally in accordance with those found in certain populations from the coastal areas of

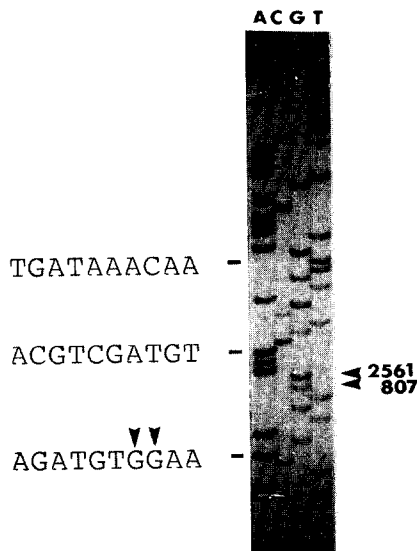


Fig. 2. Sequencing gel autoradiograph of a segment of the 1.15 KP element from a Q strain (KC-137). The sequence shown is that of a breakpoint (807/2561) of the KP element derived from a complete 2907 bp P element.

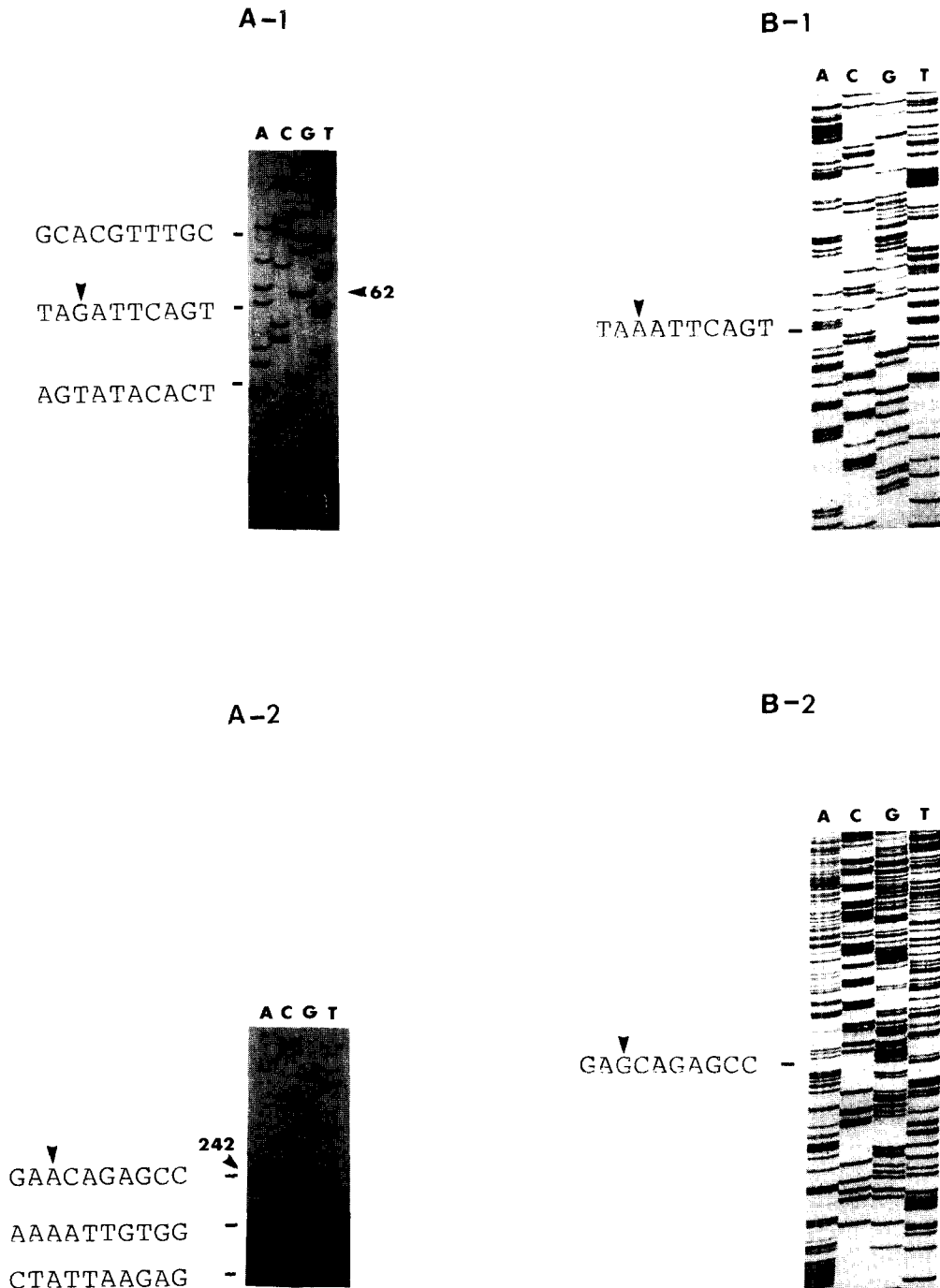


Fig. 3. Sequencing gel autoradiograph of segments of the 1.15 KP elements from a Q strain (KC-137) and M' strain (KT-99). The sequence of KC-137 is identical to that of the 2.9 kb complete P element in $p\pi 25.1$ with the exception of two base substitutions of A to G (panel A-1) and G to A (panel A-2) at positions 62 and 242, respectively. On the other hand, the sequence of the KT-99 (panels B-1 and B-2) KP element shows complete homology to the P factor of p 25.1.

China (Anxolabéhère *et al.*, 1990).

The sequencing data in this study indicate that Korean KP element is of a similar type to that which was isolated from a Krasnodar M' strain in Russia and was initially sequenced by Black *et al.* (1987). A KP element from Krasnodar carried a single replacement of an A with a T nucleotide at position 32 in comparison with $\pi\alpha 25.1$ (Black *et al.*, 1987). The two base substitutions in KC-137 could be due to PCR amplification errors by *Taq* DNA polymerase. This means that one nucleotide difference exist in between the Krasnodar KP element and the Korean KP elements.

The KP element contains the sequence required for transcription and for transcript polyadenylation (Rio *et al.*, 1986; Black *et al.*, 1987). The KP element potentially encodes a 207 amino acid protein, containing the first 199 amino acids of the transposase amino terminus and an additional unrelated 8 amino acids at the carboxyl terminus due to a frameshift caused by internal deletion (Rio, 1990). Even if the two base substitutions of the KC-137 is not PCR amplification errors, neither substitution detected in the KC-137 KP element caused any changes in the KP protein because position 62 is located in the non-coding region and position 242 occupies a degenerate position in the glutamic acid codon GAG (GAG→GAA). Therefore the KC-137 KP element might also retain the same function as the Krasnodar KP element.

P element transposition in *Drosophila* is controlled at least partially by cytotypic regulatory state. P cytotypic is determined by a combination of maternally inherited factors and chromosomal P elements in zygote (Engels, 1979; Kidwell *et al.*, 1981; Misra *et al.*, 1993). Most American P/Q strains, which do not have KP elements, have developed P cytotypic (Kidwell and Novy, 1985; Anxolabéhère *et al.*, 1988; Periquet *et al.*, 1989). On the other hand, KP-mediated repression is transmitted through both sexes which differs from the mechanism for maternal inheritance of P cytotypic (Black *et al.*, 1987; Jackson *et al.*, 1988). Most naturally occurring M' strains widely distributed in Eurasian populations are known to be regulated by one particular P element deletion-derivative termed the KP element.

The KP protein carries a heptad leucine repeat motif called the leucine zipper structure, which could mediate specific protein-protein interactions as DNA binding proteins (Jackson *et al.*, 1988; Landshultz *et al.*, 1988). This finding suggests that the KP protein might function to repress transposition by forming non-functional heteropolymers with functional transposases (Black *et al.*, 1987; Rio, 1991). Andrews and Gloor (1995) reported that the leucine zipper of KP polypeptide is important for P element regulation. They also demonstrated the multimer-poisoning model of P element repression, because leucine zipper motifs are involved in protein-protein interactions. Furthermore, Rasmusson *et al.* (1993) recently reported that the 1.15 kb KP element as well as a very small (517 bp long) P element, called the SP element, could function as strong repressors of GD sterility. They isolated these internally deleted P elements from a wild-type M' strain, called Sexi, which was derived from a natural population in Spain (Kidwell, 1985). They postulate that repression by SP is mediated by an antisense RNA that interferes with the processing or translation of complete P element transcripts. The possibility of additional repression potential of GD sterility by the SP-like elements detected by PCR in this study cannot be ruled out.

Therefore, high copy number of KP and other incomplete SP-like elements appear to provide adequate explanation for the repression of P-mediated hybrid dysgenesis in Korean populations of *D. melanogaster*.

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Drosophila melanogaster 한국집단에서 분리한 KP Elements의 DNA 염기서열 분석
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Drosophila melanogaster 한국집단(천안, 서울, 대구)에서 KP elements의 존재 여부와 이들의 분자 생물학적 특징을 PCR 방법과 DNA 염기서열 분석을 통하여 조사했다. 본 집단에서 확인된 KP elements(KC-137, KS-95, KT-99)는 모두 2907 bp의 완전한 염기서열을 가진 자율성 P element로 부터 내부 결실(808 번째 nucleotide 부터 2560 까지)에 의해 이루어진 비자율성 P element(1154 bp)로 분석되었다. 특히 본 연구에서 조사된 KP element는 자율성 P element(p π 25.1 plasmid)와 비교했을 때, KC-137 KP element 만이 62번과 242번 nucleotide 상에서 각각 A→G 그리고 G→A로 transition mutation된 것으로 나타났으며, 여타 KS-95와 KT-99 KP elements의 경우는 p π 25.1 상의 염기서열과 동일했다. 따라서 이러한 결과로 보아 KC-137 KP element의 두 염기치환은 아마도 PCR 중에 *Taq* DNA polymerase의 error에 의한 것으로 판단된다. 비록 KC-137의 염기치환이 PCR error가 아니라 할지라도 62 번째 염기치환의 경우는 transposase 효소의 non-coding 부위에 위치하며, 또한 242 번째 염기치환은 ORF-1 내에서 같은 glutamic acid(GAG→GAA)로 degenerate 됨으로써 P elements의 transposition을 repression하는 KP protein과 동일한 repressor를 합성할 수 있는 것으로 조사되었다. 따라서 지금까지 보고된 *Drosophila melanogaster* 한국집단내의 지극히 낮은 P factor activity 및 hybrid dysgenesis 현상의 억제 요인에 관하여 본 집단내에 존재하는 KP elements에 의해 일부 설명이 가능하다고 본다.