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Mouse Genomic DNA Sequences Homologous to Sea Urchin TU Elements Are Genetically Stable Polydispersed Repeats Useful for Analysis of Multiple RFLPs

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Segments of the murine genome that hybridize to the inverted repeat regions of the transposable TU elements of sea urchins include tandem repeats of a sequence (CTCC) that encodes the recognition site for the restriction enzyme *Mnl*1, as do the analogous polypurine/polypyrimidine (pPu/pPy) stretches in humans. The *Mnl*1-sensitive repeats, which exist as a microsatellite sequence 200–300 bp in length, lack the terminal dyad symmetry characteristic of the TU elements and are structurally and functionally distinct from these elements. DNA fragments containing these repeat units that are isolated from different generations of isogenic (or congenic) mice or from different tissues of genetically identical individuals are indistinguishable by RFLP analysis; however, they show restriction fragment length polymorphism in different strains. This polymorphism appears to reflect DNA sequence changes occurring at sites flanking the repeats rather than variability in the number of repeats. Their genetic stability and occurrence in a wide variety of animal species make the *Mnl*1 repeats useful in studying genetic variation that has occurred over an evolutionary time scale of greater duration than can be examined conveniently by VNTR analysis.

INTRODUCTION

The TU elements are a heterogeneous family of modularly structured transposons discovered in sea urchins (Liebermann *et al.*, 1983; Hoffman-Liebermann *et al.*, 1985). These elements are characterized by large (840 bp) inverted repeat (IVR) segments that bracket a middle region variable in length and sequence (Liebermann *et al.*, 1983; Hoffman-Liebermann *et al.*, 1985, 1989). In contrast to the TU element middle region, the distally located outer domain (OD) of the IVR segment is highly conserved among separately isolated TU elements and has been found to hybridize to a family of repetitive DNA sequences present in a wide variety of eukaryotic species, including humans (Liebermann *et al.*, 1986). When probed with the TU element IVR-OD, the repetitive sequences show multiple restriction fragment length polymorphisms (RFLPs) between different human individuals (Liebermann *et al.*, 1986); however, it has not been feasible to assess experimentally whether these RFLPs are due to contemporary transposition events or, alternatively, to mutations or DNA rearrangements that have occurred over an extended period of time. To address this question, we have examined the genomic organization of TU IVR-OD hybridizing sequences in different tissues of inbred strains of mice and have followed the inheritance of these sequences during several mouse generations.

Our results indicate that, as previously reported for human DNA (Hoffman-Liebermann *et al.*, 1986), the majority of mouse genomic DNA segments hybridizing with the TU IVR-OD in the mouse genome contain multiple repeats of the sequence CTCC, the recognition site for the restriction enzyme *Mnl*1. However, we find no evidence for the organization of these sequences in inverted repeat structures, as has been observed for the TU family of elements in sea urchins, nor any indication of mobility of the CTCC repeats in the genomes of different individuals of inbred mouse strains or within different tissues of a single individual. Instead, the RFLP pattern characteristic of a particular mouse strain is found to persist over many generations, and the DNA sequences hybridizing to the IVR-OD probe showed RFLP differences only between individuals of genetically unrelated strains of mice. We conclude that the majority of TU hybridizing sequences in mouse chromosomal DNA belong to a nonmobile family of repetitive elements whose genetic stability enables their use for the simultaneous analysis of dispersed RFLPs occurring at multiple genomic locations. The RFLPs observed in this type of analysis are not due to length polymorphism within repeats as has been described previously for simple tandemly repeated sequences (Nakamura *et al.*, 1987; Jeffreys *et al.*, 1988; Litt and Luty, 1989; Weber and May, 1989), but instead are the result of genetic changes occurring at sites adjacent to the repeats and over an evolutionarily longer time period.

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MATERIALS AND METHODS

DNA isolation. Tissues (brain, liver, spleen, and tumor) were removed from individual mice, finely minced on a petri dish with a razor blade, and then homogenized in a cold solution of 0.1 *M* Na_2 EDTA, 0.2 *M* Tris (pH 8.5), 1% SDS, and 100 μg/ml proteinase K (10 vol/vol of liver, brain, and tumor tissue; 100 vol/vol of spleen tissue). The homogenized tissue was transferred to glass corex tubes and incubated at 60°C for 2 h. Ten microliters of diethyl pyrocarbonate was added for each 5 ml of buffer, and the tubes were incubated at 60°C for an additional 30 min. Tubes were cooled on ice and 5 *M* potassium acetate was added to a final concentration of 2 *M*. After the mixture was allowed to sit on ice for 30 min, the precipitate was sedimented at 13,000 rpm (27,000 *g*) in a Sorvall HB-4 rotor for 30 min. The supernatant was transferred to fresh tubes, and the DNA was precipitated with 2 vol of cold ethanol, washed with 70% ethanol, and air-dried on a glass rod. The DNA was resuspended in TE (10 m*M* Tris, 1 m*M* EDTA) containing 50 μg/ml RNaseA and incubated at 37°C 1 h. The suspension was then extracted with phenol, phenol:chloroform:isoamyl alcohol (25:24:1), and chloroform:isoamyl alcohol (24:1), ethanol precipitated as before, and resuspended overnight in 200 μl of TE. Samples were diluted 1:50 and the absorbance was determined at 260, 280, and 320 nm on a Gilford spectrophotometer.

Gel electrophoresis and preparation of filters for Southern blotting. Five micrograms of DNA was digested with the appropriate restriction enzyme and electrophoresed on 30-cm 0.7% agarose gels at 60 V for either 24 h or overnight as indicated in the figure legends. DNA was transferred to nitrocellulose as described in Maniatis *et al.* (1982). The blots shown have been trimmed at the top to remove portions of the filter that lack DNA bands.

Hybridizations and probes. The purified *Hpa*II IVR-OD fragment from TU-1 has been described (Hoffman-Liebermann *et al.*, 1985); introduction of this fragment into the *Cla*I site of pBR322 yields the plasmid pIVR27. The mouse probe, MUT5, was isolated by hybridizing the TU-IVR probe to a λ gtWES library (gift of M. Uhler) containing mouse genomic DNA. Plaques hybridizing to the TU IVR-OD probe were isolated, and a 675-bp *Bgl*II/*Pst*I fragment containing the TU-hybridizing sequence of one such isolate was cloned into the polylinker site of pHSG299 (Takeshita *et al.*, 1987), yielding the plasmid pMUT5.

Probes were uniformly labeled with ^{32}P by nick translation (Maniatis *et al.*, 1982). Nitrocellulose filters were hybridized in 5× Denhardt's solution, 50 m*M* sodium phosphate (pH 7.0), 0.1% sodium pyrophosphate, and either 5× SSC (for hybridizations at 55°C) or 2× SSC (for hybridizations at 65°C) for the TU IVR-OD and MUT5 probes, respectively. Hybridized blots were rinsed in 2× SSC (Maniatis *et al.*, 1982) and washes were performed as follows: For the TU IVR-OD probe, 15 min at room temperature in 1× SSC, 0.1% SDS; 15 min at room temperature in 0.5× SSC and 0.1% SDS; 20 min at 42°C in 0.5× SSC and 0.1% SDS; and 15 min at 42°C in 0.1× SSC and 0.1% SDS; for the MUT5 probe, 15 min at room temperature in 1× SSC and 0.1% SDS; two changes for 15 min at room temperature in 0.5× SSC and 0.1% SDS; and two changes for 40 min at 50°C in 0.1× SSC and 0.1% SDS.

RESULTS

Characterization of TU IVR-OD hybridizing sequences in the murine genome. Previously reported analysis of two separately purified human DNA sequences homologous to the TU IVR-OD indicated that they contain a series of tandem repeats of the trimer TCC punctuated at certain sites within variant trimers, which differ for the two sequences analyzed (Hoffman-Liebermann *et al.*, 1986). The repeating TCC unit creates a series of *Mnl*I restriction sites (CTCC), allowing one to infer the presence or absence of the repeat unit without chemical sequencing by determining whether the hybridization

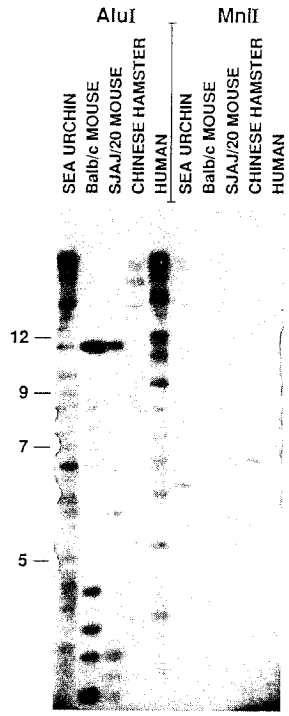

FIG. 1. Hybridization of TU IVR-OD to genomic DNA from various species. Genomic DNA isolated from sea urchin, SJAJ/20 (a congenic strain of SJL/J; Parsons *et al.*, 1986) male and Balb/c female mice, Chinese hamster fibroblasts, and human buffy coat blood cells was digested with either *Alu*I or *Mnl*1, electrophoresed for 24 h, and subjected to Southern blot analysis using the TU IVR-OD probe as described under Materials and Methods. The positions of the molecular size markers (1-kb ladder, BRL) are shown at the left.

target is destroyed (i.e., by cutting it into short segments) with *Mnl*I. To learn whether the IVR-OD hybridizing sequences in the mouse genome are similar in structure to the human sequences, we isolated DNA samples from 77 separately picked IVR-OD hybridizable bacteriophage λ clones purified from a phage library representing the complete mouse genome and then subjected them to digestion with *Mnl*I and *Hae*III endonucleases and subsequent Southern blotting to the IVR-OD probe. Sixty of these isolates gave specific hybridization bands when digested with *Hae*III, but only 1 of the 77 clones showed an IVR-OD hybridizing DNA fragment after digestion with *Mnl*I (data not shown); in a similar analysis of phage clones containing human chromosomal DNA (Hoffman-Liebermann *et al.*, 1986), 1 clone among 10 was not cleaved multiple times with *Mnl*I.

Southern blot hybridizations to genomic DNA samples from mouse, sea urchin, Chinese hamster, and human showed a series of prominent IVR-OD hybridizable bands when the DNAs were cut with the restriction enzyme *Alu*I (Fig. 1, left). In contrast, only a few IVR-OD hybridizing DNA stretches of sufficient length to be observable as gel bands survived treatment with *Mnl*I (Fig. 1, right), which as noted above also has a 4-bp recognition site and was shown previously to cause fragmentation of human DNA segments containing tandem repeats of the trimer TCC (Hoffman-Liebermann *et al.*,

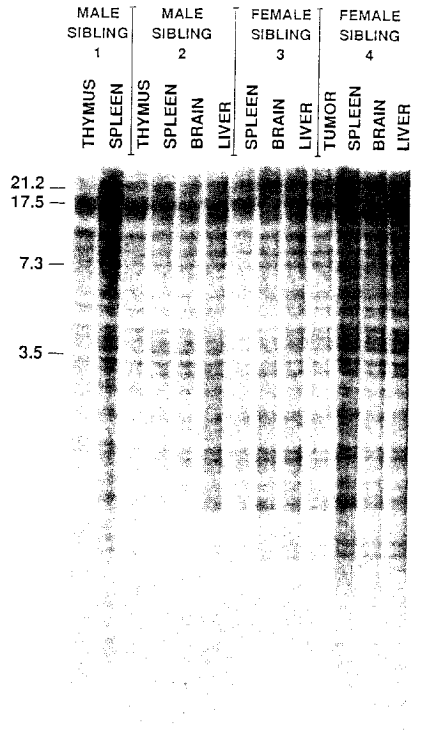

FIG. 2. Southern blots of genomic DNA isolated from different tissues of sibling SJL/J mice. Siblings from a single SJL/J × SJL/J cross were sacrificed. DNA was extracted from the indicated tissues as described under Materials and Methods and cut with *Bam*HI endonuclease. Southern blot analysis was performed using the TU IVR-OD probe, and electrophoresis was carried out overnight. The molecular size markers (in kb) are DNA fragments that have been generated by digestion of bacteriophage λ DNA by *Hin*dIII.

1986). Thus, most of the observed hybridization to the TU IVR-OD probe in all of these genomes results from a family of repeats [previously termed polypurine/polypyrimidine (pPu/pPy)] sequences (Hoffman-Liebermann *et al.*, 1986) containing tandem arrays of *Mnl*I restriction sites (i.e., CTCC).

Stability of the genomic location of pPu/pPy sequences. We determined whether RFLP pattern changes consistent with altered genomic positions of the pPu/pPy sequences occur between different tissues of individual mice, between sibling individuals, and between different strains of mice. DNA was isolated from multiple organs of individual sibling mice derived from intrastrain matings of each of two strains, BAB/25 (a congenic strain of Balb/c) and SJL/J. The DNA was then digested with either *Bam*HI or *Eco*RI and analyzed by Southern blotting using the TU IVR-OD as a hybridization probe. The analysis of DNA isolated from two male and two female siblings resulting from a single intrastrain SJL/J × SJL/J cross is shown in Fig. 2; analogous results were obtained in sibling mice from a Balb/c × Balb/c cross.

As seen in Fig. 2, no variability was observed either among DNA samples from different tissues of a single individual or between the tissues of siblings. The hybridization pattern seen for DNA prepared from a spontaneously arising SJL/J reticulum cell sarcoma tumor (Festing, 1979), which presumably is derived from a single somatic cell clone, and that seen for DNA isolated from other tissues taken from individuals of the same SJL/J strain were identical. During our analysis we did observe a single adventitious DNA band in a splenic DNA sample taken from one mouse (data not shown). However, no other instance of intrastrain variability was seen; restriction fragment length polymorphism was observed only between strains.

To assess the role of genetic drift in the restriction pattern observed with SJL/J and Balb/c, which have widely different genetic origins, we collected DNA samples from several mouse strains (BAB/25, BAB/14, Balb/c, C3H, SJL/J) differing in their relatedness, as well as from a tissue culture line of mouse cells (L-cells) derived from an individual of one strain (C3H) nearly 50 years ago (Earle, 1943). It is evident from the results (Fig. 3) that the genomically located pPu/pPy sequences detected by this experiment were stable for at least the number of generations that separate L-cells from those of present day C3H mice; polymorphism was detected only between distantly related strains (see Table 1). We conclude that the RFLPs observed between these different mouse strains reflect mutational changes or genome rearrangements that have occurred over an extended evolutionary period of time rather than more contemporary transposition events.

Use of pPu/pPy sequences in RFLP analysis. Our finding that the bands detected with the TU IVR-OD probe represent multiple restriction fragments that are constant for DNA obtained from cells of different individuals of the same strain and are genetically stable over a period of at least 50 years implies that for a given mouse strain, each restriction band represents a separate stably inherited genetic locus. Therefore, the banding patterns should follow simple rules of inheritance in the offspring of interstrain crosses. To test this notion, we examined the parents and F_1 progeny of an SJAJ/20 male × Balb/c female cross using the restriction enzymes *Alu*I and *Hae*III. These experiments employed the MUT5 probe, which had been purified as described under Materials and Methods, to allow hybridization at a stringency much greater than that used in the previous studies. The results, which are shown in Fig. 4, indicate that all of the polymorphic loci identified between the mouse strains were inherited by each of the F_1 progeny of a cross in a Mendelian fashion. Similar blots that were hybridized sequentially with sea urchin and mouse DNA probes using their respective hybridization conditions (see Materials and Methods) gave similar patterns, although some bands (less than one-third) were detected by only one of the two probes.

DISCUSSION

Previous work from our laboratory has shown that sequences hybridizing to the TU IVR-OD probe in hu-

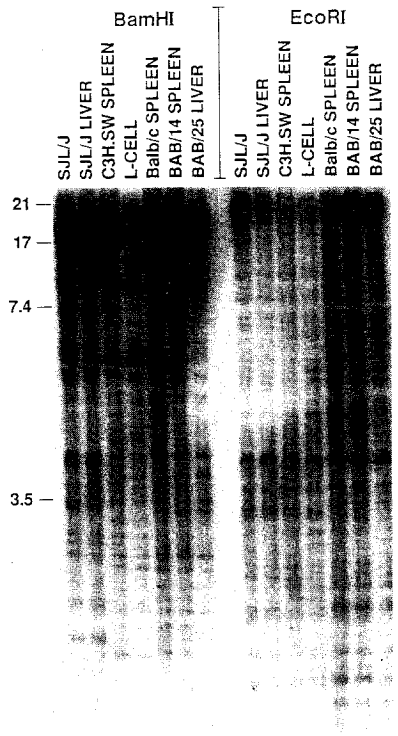

FIG. 3. Hybridization of TU IVR-OD sequences to genomic DNA isolated from different strains of mice. Genomic DNA was isolated from the spleens or livers (as indicated) of individual mice of the indicated strains and from mouse L-cell fibroblasts (a C3H derived cell line; Earle, 1943) and digested with either *Bam*HI or *Eco*RI. Electrophoresis was carried out overnight, and Southern blot analysis was performed using the TU IVR-OD probe under the conditions described under Materials and Methods. BAB/14 and BAB/25 are congenic strains of Balb/c (Black *et al.*, 1978; Weigert and Riblet, 1978; Parsons *et al.*, 1986). Each differs from Balb/c in having been once crossed to C57 black and subsequently backcrossed 14 and 25 times respectively to Balb/c, selecting each time for progeny that differ only in their MHC haplotype. BAB/14 and BAB/25 were then inbred for more than seven generations before DNA was extracted for the results presented in the figure. The two SJL/J DNA samples represent animals that were sacrificed for DNA extraction three generations (1 year) apart. While C3H and Balb/c may have been derived from the same noninbred stock about 70 years ago, SJL/J originated from a completely different geographical location (Festing, 1979). Mouse L-cell tissue culture cells were established from a C3H mouse nearly 50 years ago (Earle, 1943) representing at least 50–80 generations removed from the individual that was sacrificed for the DNA representing C3H mice in the figure. The number of somatic cell divisions that the tissue culture cells have undergone is not determinable; however, it is likely to be considerably more than the number of mouse generations. The molecular size markers are as in Fig. 2.

man cells consist of tandemly repeated (TCC) trimers termed pPu/pPy sequences (Liebermann *et al.*, 1986). We show here that at least 90% of the murine genomic DNA sequences that hybridize with the TU IVR-OD probe are similar in structure, sequence, and frequency to the human $(TCC)^n$ sequences. In cases in which genomic locations have been mapped in detail (two phage library inserts containing human genomic DNA, Hoffman-Liebermann *et al.*, 1986; and three phage library inserts containing mouse genomic DNA, Gilbert and Cohen, unpublished results), only one segment hybridizing to the IVR-OD probe was found in each cloned genomic DNA fragment. We also found that: (1) none of 77 individually cloned mouse genomic DNA fragments contains more than one hybridizing *Hae*III restriction fragment, consistent with the view that the mouse pPu/pPy sequences are not organized as inverted repeat structures separated by middle sequences; and (2) foldback DNA isolated from hydroxyapatite columns following reassociation of human placental DNA was not enriched for TU IVR-OD hybridizing sequences (Gilbert and Cohen, unpublished data). These observations contrast with those made for sea urchin DNA, in which TU hybridizing foldback segments were readily identified in genomic DNA libraries (Hoffman-Liebermann *et al.*, 1985). Taken together, our results indicate that TU hybridizing sequences in mammalian cell DNA are not organized in the inverted repeat conformation typical for the TU transposable elements of sea urchins, which also contain an analogue of the pPu/pPy (CTCC) segments of human and mouse DNA [i.e., a significant fraction of the sea urchin DNA sequences that hybridize to the IVR-OD probe are sensitive to *Mnl*I (Fig. 1)]. Thus, the pPu/pPy sequences appear to comprise a sequence element family structurally distinct from the *Mnl*I-insensitive TU element segment used to detect them.

The lack of intrastrain and intraorgan variability of mouse DNA sequences that hybridize to the TU element IVR-OD probe leads us to conclude that transposition or rearrangement of the pPu/pPy sequences does not readily occur. Thus, it appears that the *Mnl*I-sensitive sequences are also functionally distinct from the cross-hybridizing transposable TU elements of sea urchins. The simplest interpretation of the interstrain RFLPs we have observed is that they result from mutations or genome rearrangements not specifically related to the sequences themselves. Analogous polymorphisms observed in human DNA (Liebermann *et al.*, 1986) seem likely to be of similar origin since the structure, sequence, and distribution of the IVR-OD hybridizable mouse and human sequences are similar. Moreover, as some bands of hybridization of TU IVR-OD remain after digestion with *Mnl*I of both sea urchin and mammalian genomic DNA (see Fig. 1), the possibility remains that mammalian genomes also contain sequences arranged in a TU-like structure.

Probes of the type studied here contrast with the previously described variable number tandem repeat (VNTR) probes (Nakamura *et al.*, 1987) that identify genetic changes that have occurred over relatively short periods of evolutionary time. VNTRs were identified originally as long (approximately 1 to 5 kb) stretches of repeated simple sequence (minisatellites) that are present at one or a few loci and differ sufficiently in length that polymorphisms in the sequence can be identified using conventional Southern blotting. More recently, Litt and Luty (1989) reasoned that smaller satellites (microsatel-

TABLE 1

Percentage of Shared Restriction Fragments between Different DNA Sources

	SJL/J (%)	C3H/SW (%)	L-cells (%)	BAB/25 (%)	BAB/14 (%)	Balb/C (%)
SJL/J	100	78	78	70	70	70
C3H	82	100	100	85	85	85
L-cells	82	100	100	85	85	85
BAB/25	68	78	78	100	100	100
BAB/14	68	78	78	100	100	100
Balb/C	68	78	78	100	100	100

Note. 5 μg of DNA was digested with *Bam*HI and electrophoresed on a 0.6% agarose gel for 48 h at 60 V (Maniatis *et al.*, 1982). The samples were subjected to Southern blot analysis using the TU IVR-OD probe as described under Materials and Methods. Bands were counted from Kodax XAR-5 X-ray film that was exposed for 19 h using an intensifying screen. The total number of bands seen for each sample is SJL/J, 34; C3H/SW, 36; L-cells, 36; Balb/C, BAB/14, and BAB/25, 33. This table shows the percentage of similarly positioned bands between samples.

lites) might also show variable length polymorphisms that can be resolved on a sequencing gel after polymerase chain amplification. Because of their small size (200–300 bp; for review see Hoffman-Liebermann *et al.*, 1989), length polymorphism within the microsattelite pPu/pPy sequences would not be detected by the methods we have used. Instead, the multiple RFLPs that we report here appear to be due to conventional genetic variation such as restriction site mutations that have accumulated over a much greater time scale than that detected by VNTR polymorphisms, which can occur at mutation rates reaching 5% per gamete (Jeffreys *et al.*, 1988). In fact, the pattern of inheritance between SJL/J, C3H, and Balb/c of RFLPs detected by pPu/pPy sequences (Fig. 3 and Table 1) is consistent with the genetic relatedness of these strains as measured by histocompatibility antigen allotyping (Parsons *et al.*, 1986). Thus, RFLP analysis with pPu/pPy sequences can provide a measurement of genetic change over longer evolutionary periods of time, between populations, within species, or possibly between species.

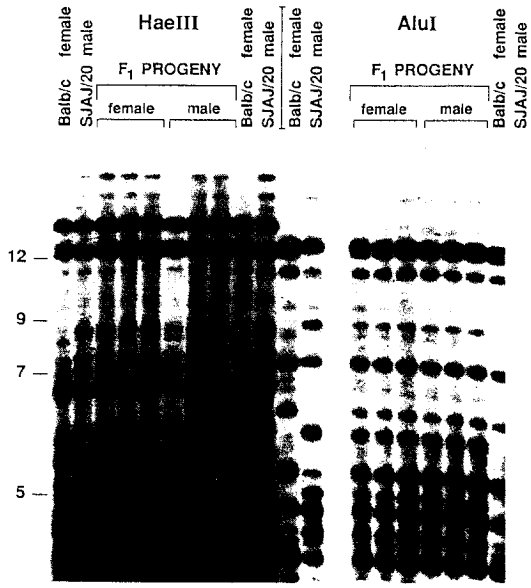

FIG. 4. Inheritance of restriction fragments hybridizing to the MUT5 probe. Southern blot analysis of genomic DNA prepared from the spleens of an SJAJ/20 male and a Balb/c female and six of their F_1 progeny, cut with either *Hae*III or *Alu*I, electrophoresed for 24 h, subject to Southern blot analysis, and hybridized with the MUT5 probe under the conditions described under Materials and Methods. The molecular size markers are as in Fig. 1.

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