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Immunoglobulin genes of the x light chain type from two human lymphoid cell lines are closely related

H.-Gustav Klobeck, Gabriele Combriato and Hans G.Zachau

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, FRG

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ABSTRACT

As a first step in our studies of functionally rearranged K genes of man we cloned the germline J_K-C_K region from placenta DNA employing a mouse J_K clone as hybridization probe. Subclones of the human J_K-C_K region were then used to characterize and clone the rearranged K genes of the lymphoid cell lines Walker and Daudi. The Walker cell line contains one rearranged and one germline K allele (K^+,Ko; ref. 1). Only one K gene was found in Daudi cells (K^+). Restriction mapping and DNA sequencing showed, that the rearranged K genes from both cell lines are closely related. These features make the two cell lines particularly suitable for studies on the chromatin structure of K light chain genes. The 5' flanks of the two genes (388 bp) are identical while there is a 12 % divergence between the V_K gene segments themselves. This situation may reflect somatic mutation processes and/or gene conversion like events.

INTRODUCTION

Immunoglobulin genes have been studied in much detail in recent years, mostly in the mouse system (reviews e.g. refs. 2,3). During maturation of B-lymphocytes the immunoglobulin genes undergo DNA rearrangements leading to functional transcription units which, for the light chain genes, consist of L, V-J, and C gene segments. The genetic basis of the enormous size of the antibody repertoire, estimated to consist of 10^8 or more different immunoglobulin molecules (4), is an unresolved problem. In the mouse genome the number of germline V_K genes is probably in the order of several hundred (5,6) while in the human genome the number is assumed to be lower (7-11), which implies that somatic processes, e.g. point mutations, play an important role in the generation of antibody diversity in man.

The germline V_K repertoire of man has been studied in several laboratories including ours (7-13). Many germline V_K genes were

isolated in our laboratory from phage and cosmid libraries (12, 13). We recently extended these studies to functionally rearranged V genes in lymphoid cell lines. One reason for this was our search for cell lines whose configuration with respect to rearranged and non-rearranged K alleles make them suitable for chromatin studies. After cloning a rearranged V_K gene of subgroup II from the lymphoid cell line GM607 (11) we now turned to two cell lines, Walker and Daudi, which proved to be particularly useful for the intended chromatin studies (see accompanying paper; ref. 14). The rearranged K genes of both cell lines, which express K light chains of subgroup I, were cloned and sequenced. Subclones were prepared for the chromatin investigations (14).

MATERIALS AND METHODS

Cell culture

The experiments described in this paper were carried out with the Burkitt lymphoma derived cell line Daudi which was obtained from H. Wolf of Pettenkofer Institut, München. DNA of Daudi cells from the American Type Culture Collection, digested with EcoRI, BamHI, HindIII or BglII, yielded identical hybridization patterns. The Walker cell line was originally isolated from Burkitt's lymphoma tissue by I. Magrath of the National Cancer Institute and obtained from H. Rodt, Gesellschaft für Strahlen- und Umweltforschung, München.

The lymphoid cells were grown as suspensions in RPMI 1640 medium (15) supplemented with 15 % fetal calf serum and 4 mM glutamine in a 5 % CO_2 athmosphere at 37° C. The cells were inoculated at a density of $5x10^5$ cells/ml, fed every second day by removing approximately one third of the cell free supernatant, and replacing it with an equal volume of fresh medium. The cultures were grown to a density of $1.5\text{-}2x10^6$ cells/ml before they were split or harvested. RPMI 1640 and fetal calf serum were from Boehringer Mannheim.

Isolation of DNA from human placenta and cell cultures

High molecular weight DNA from human placenta was prepared according to the method of Blin and Stafford (16). Cells from cell cultures were pelleted and suspended in 0.5 M EDTA, pH 8.0, 0.5 % SDS; further steps followed the protocol of ref. 16 from

which the liquid nitrogen step was omitted.

Gel electrophoresis

Up to 50 µg DNA per track were electrophoresed on agarose slab gels (1 %, 6 mm thick; ref. 17) in a buffer similar to the one in ref. 18 (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4). The DNA was transferred to nitrocellulose (19) and hybridized with nick-translated (20) probes as described (21). Hybridization experiments were in 6xSSC, 1xDenhardt's solution, 50 µg/ml sheared and denatured salmon sperm DNA at 68° C; the filters were washed with 2xSSC, 0.5 % SDS at 68° C.

DNA fragments were isolated from low melting agarose gels (Seaplaque agarose, Marine Colloids) by melting slices at 65° C in two volumes of 1 M NaCl. The mixtures were frozen, thawed and centrifuged. The supernatants, which contain 80-90 % of the DNA, were filtered through Whatman 3MM paper (to remove residual agarose) and ethanol precipitated. DNA fragments isolated by this procedure could easily be ligated or redigested with restriction nucleases.

Cloning procedures

Phage λ arms of Charon 30 (ref. 23) or EMBL 3 (ref. 24) were isolated by gel electrophoresis. Ligation mixes were packaged in vitro with extracts prepared as described by Scalenghe et al. (25). Packaging efficiencies were in the order of $3x10^5$ plaques/µg of ligated DNA. E. coli cells LE 392 or NM 538 were infected by the recombinant phages. The infected cells were overlayed on LB agar in square 22 cm x 22 cm plates (Nunc screening plates). Screening was as described (26). Recombinant phages were grown as liquid lysates in LB medium, by infecting growing LE 392 cells at about 0.3 OD_{600} with phages using a multiplicity of infection of 0.1. Phages were isolated as described (27). Subclones from recombinant phages were prepared in pBR322 or in M13mp8,9 (ref. 28).

DNA sequencing

DNA fragments from pWVJ-1 and pDaudi-1 were subcloned in M13mp18 and mp19 (ref. 29). The DNA sequences were determined by the chain termination method (30).

RESULTS AND DISCUSSION

Characterization of the V_K-J_K rearrangements of two lymphoid cell lines

In order to find suitable cell lines for chromatin experiments we screened a number of human lymphoid cell lines with respect to production of K type light chains and DNA rearrangements in the J_K locus. The subclones from the germline J_K-C_K region which were used for this screening were derived from a 10.6 kb BamHI fragment cloned from placenta DNA. Hieter et al. isolated this fragment using a mouse C_K subclone (22). Since a mouse C_K subclone detects more than one crosshybridizing fragment in human placenta DNA (22), we used a mouse J_K subclone (1.56 kb HindIII-HindII; ref. 32) as a hybridization probe. The restriction map of the 10.6 kb BamHI fragment (Fig. 1A) is basically identical to the one of Hieter et al. (31), but includes sites for additional restriction nucleases and the location of repetitive sequences.

Two cell lines which excrete immunoglobulin molecules of the K light chain type and show at the DNA level only one rearrangement, were selected and studied further. The Walker cell line was shown by blot hybridization with the intron probe pI-1 to contain one rearranged and one germline C_K allele (K^+, K^o).

Daudi cells were found to have the configuration K^+ which means that they contain only one C_K allele and that this allele is productively rearranged (Fig. 2). A cell line which has only one C_K gene segment is of special interest for chromatin studies, because such a situation facilitates the analysis of, for instance, the DNAase I hypersensitive sites (14). Therefore the Daudi cells were investigated in more detail. The possibility that the cells contain two rearranged alleles (K^+,K^-) which are similar with respect to the location of restriction sites was tested with several restriction nucleases in addition to those used in the experiments of Fig. 2. Also with HindIII (pI-1 probe) and SstI, HindII (pJ-2 probe) only one hybridizing fragment appeared in Southern blots (not shown).

The contention that Daudi cells contain only one C_K allele which is rearranged was supported also by a semiquantitative experiment: when equal amounts of Daudi and placenta DNA digests

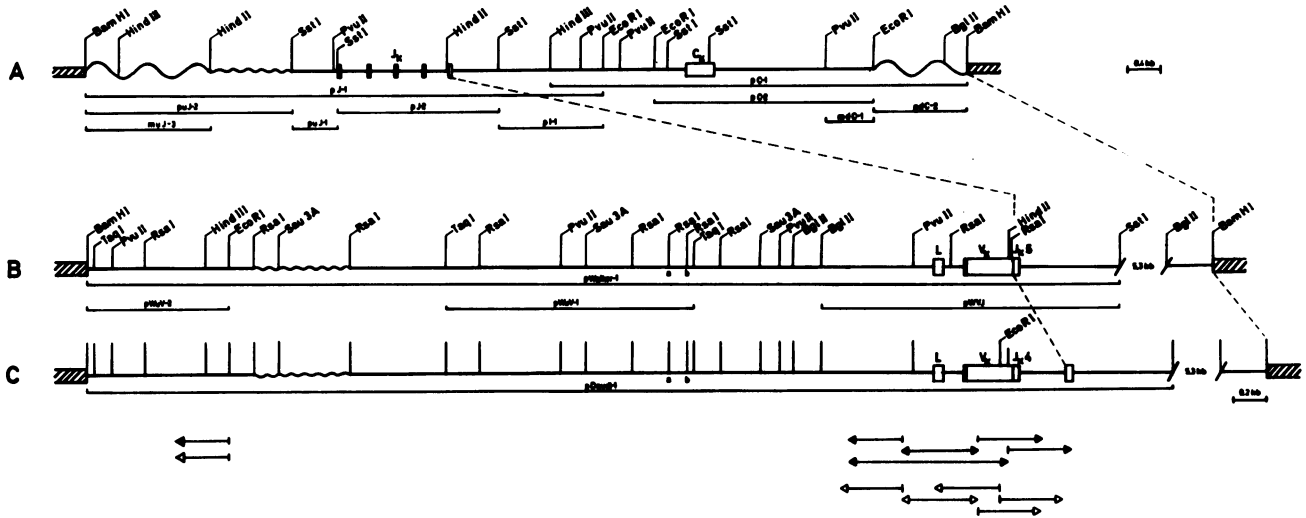

Figure 1. Restriction maps of the cloned germline J_K-C_K region from placenta DNA and the rearranged K genes from two lymphoid cell lines. Gene segments are shown as open boxes, vector arms as hatched bars. Dashed lines connect identical sites in the germline and the two rearranged gene regions. Wavy lines designate highly (~~~) repetitive sequences which were localized by blot hybridization with nick-translated placenta DNA and low (~) repetitive sequences (14).

A. The germline J_K-C_K region from human placenta DNA, cloned in Charon 30 (ref.23). Subclones were prepared in pBR322 or pCK19, a pBR322 derivative which contains a SstI site instead of the HindIII site.

B. The rearranged V_K-J_K region from Walker cells, cloned in EMBL 3 (ref. 24). Subclones were prepared in pBR322 or pCK19. The location of one RsaI site could not be determined unambiguously (a or b).

C. The rearranged V_K-J_K region from Daudi cells, cloned in Charon 30 (ref. 23). The restriction sites identical to B are indicated by vertical lines. The sequencing strategies for the V_K regions of Walker (⟶) and Daudi cells (⟶▷) is shown underneath the Daudi map.

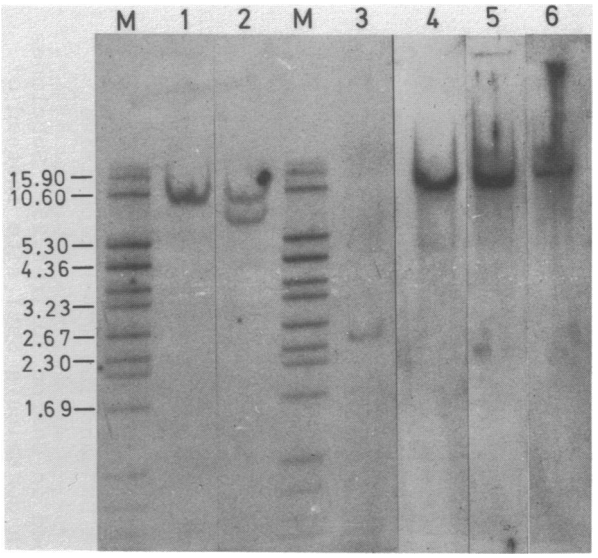

Figure 2. Rearranged K genes in Walker and Daudi cells. 50 µg each of BamHI (lanes 4-6) and EcoRI (lanes 1-3) digests of DNA from placenta (lanes 1,4), Walker (lanes 2,5), and Daudi cells (lanes 3,6) were electrophoresed on an agarose gel, blotted onto nitrocellulose and hybridized with pI-1 (Fig. 1). M, marker fragments; sizes are in kb.

were electrophoresed, blotted and hybridized with a C_K probe a ratio of about 1:2 for the radioactivity was found in the respective bands. From preliminary chromosome analyses we think that the Daudi cells we work with have two chromosomes 2 (A. Wirtz, personal communication). Also the karyotype of Daudi cells, as determined by Zech et al. (33), shows no loss of a chromosome 2. We therefore conclude that in the Daudi cell line one chromosome 2 has lost the C_K gene region.

Characterization of the rearranged K light chain genes of two lymphoid cell lines by cloning and DNA sequencing

On the basis of blot hybridization data with genomic DNA the rearranged K gene regions of Walker and Daudi cells appear to be similar. The 11.6 kb and 11.9 kb BamHI fragments were chosen for cloning and ligated into the λ phages EMBL 3 and Charon 30, respectively. Since the BamHI fragments from the germline and the rearranged alleles of Walker cells (10.6 and 11.6 kb, respec-

tively) could not be fully separated on preparative gels, the plaques hybridizing with a C_K region probe were replated and two replicas prepared on nitrocellulose filters. The filters were hybridized with a C_K and an upstream J_K probe, respectively (pC-2 and puJ-1; Fig. 1). Only those plaques hybridizing with C_K but not with the upstream J_K probe were analysed. The DNAs from recombinant phages were characterized by restriction nuclease mapping. Fig. 1B and C show the maps of the K^+ alleles of the two cell lines. While the K^+ allele of Walker cells shows a rearrangement to J_{K5}, the Daudi K^+ allele is rearranged to J_{K4}. The restriction maps 5' of the V_K genes turned out to be identical within 5.5 kb. In addition digests of the 5.5 kb BamHI-HindII fragments from the clones pWalker-1 and pDaudi-1, respectively, (Fig. 1) with HinfI, AluI, and HaeIII yielded practically identical patterns but no restriction maps were established for these nucleases. Within the V_K gene regions themselves, however, minor differences were found: the map of the Daudi gene has an additional EcoRI site, but lacks the two RsaI sites.

The very close similarity between the restriction maps of the K^+ alleles of Walker and Daudi cells appeared to be an interesting phenomenon. We therefore decided to sequence the two V_K-J_K gene regions. The sequencing strategy is outlined in Fig. 1C. Analogous regions of the Walker and Daudi genes were subcloned in M13 phages. In order to visualize sequence differences directly the corresponding sequencing mixtures were run in adjacent tracks of the sequencing gels. The DNA sequences and the deduced amino acid sequences of the coding regions are shown in Fig. 3.

Comparison of the DNA sequences of the V_K gene regions of Daudi and Walker cells

The first 442 bp of the two gene regions comprising the 5' flank and most of the leader gene segment were found to be identical (Figs. 3 and 4). The first sequence difference occurs at the 3' end of the leader. The two sequence elements dc (deca nucleotide) and pd (pentadeca nucleotide) which have been postulated to be responsible for correct initiation of transcription (34) are located within the fully conserved region. The homology of the 5' flanks of the Daudi and Walker genes is in marked contrast to a

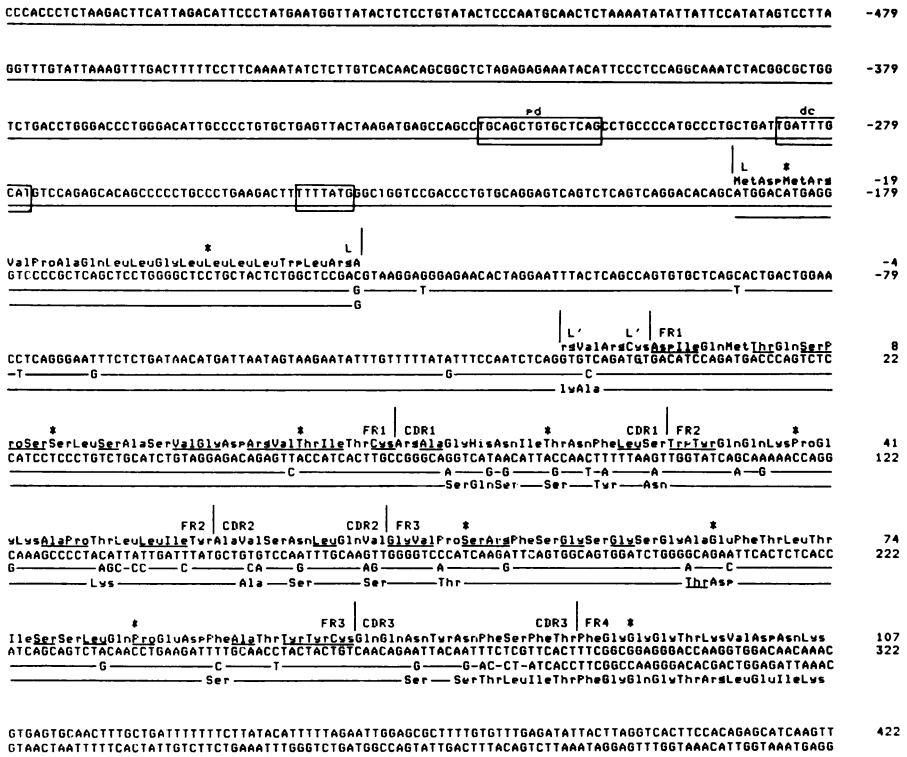

Figure 3. Nucleotide sequences of the rearranged V_K regions of Daudi and Walker cells. The Daudi sequence is presented completely together with the deduced amino acid sequence of the coding regions. For the Walker sequence (up to CDR3) only those nucleotides/amino acids which differ from the Daudi sequence are shown. Both DNA sequences were determined from position -578 to position +422. The putative regulatory elements pd and dc (34) and a TATA related sequence are boxed. Invariant amino acid residues (35) are underlined.

considerable divergence between regions coding for the mature proteins (FR1-CDR3). This divergence is in the same range as the one between the Daudi or the Walker genes and other V_{KI} genes (examples in Table 1). The J_{K4} region of the Daudi gene (codons 96-107) differs from the published germline J_{K4} gene segment (31) at two positions (315 and 317 in Fig. 3) which leads also to amino acid substitutions. The J_{K5} region of the Walker gene is identical to the corresponding germline gene segment. The sequences downstream of the J_K segments from Daudi and Walker

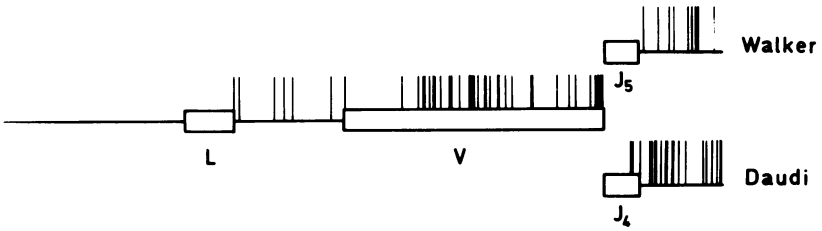

Figure 4. Sequence divergence in the rearranged K gene regions of Daudi and Walker. Sequence differences between the rearranged K regions of Daudi and Walker are indicated by vertical lines. The J segments and the 3' flanks of the two genes were compared to the J_K germline sequences of ref. 31; also here sequence differences are marked by vertical lines.

differ from the published germline sequences (31) by 21% and 13%, respectively.

The formal translation products of the two genes clearly show that the V_K genes expressed in Daudi and Walker cells belong to subgroup I. All the amino acids characteristic for a subgroup I protein (35) are present in the Walker sequence. The same holds for the Daudi sequence with one exception: the

Table 1. Homologies between DNA sequences of the rearranged V_{KI} regions of Daudi cells, Walker cells, and selected V_{KI} germline regions. The V_{KI} sequences were aligned for maximum homology as described in ref. 12. Pairwise comparisons with the computer program DNPERC (12) yielded values for homologies (in %). The V_{KI} germline regions Vd,e are described in ref. 12, the V1 sequence (13) is identical to the HK 102 sequence of ref. 7. "Total" refers to sequences from position -578 to +285 (codon 95); also in "FR1-CDR3" the comparison extends only to codon 95. The numbers in brackets behind the Walker/Daudi comparison are homologies of the respective parts of the protein sequences.

	VDa vs.				VWa vs.		
	VWa	Vd	Ve	V1	Vd	Ve	V1
5' FLANK	100	73.9	65.3	87.4	85	80.4	87.4
L	98.2 (94.7)	94.6	92.7	94.4	96.4	94.6	94.4
I	94.4	81.6	82.4	84.9	83.2	85.6	87.3
L'	91.7 (75)	91.7	91.7	83.3	100	100	91.7
FR1	98.6 (100)	95.7	87	95.7	97.1	88.4	97.1
CDR1	78.8 (54.5)	63.6	60.6	57.6	81.8	78.9	66.7
FR2	80 (93.8)	84.4	84.4	82.2	95.6	95.6	97.8
CDR2	76.2 (57.1)	76.2	76.2	66.7	95.2	95.2	85.7
FR3	92.7 (87.5)	90.6	88.5	89.6	91.7	90.6	90.6
CDR3	71.4 (42.9)	61.9	71.4	66.7	61.9	66.7	52.4
TOTAL	92.7	80.1	75.7	85.3	88.6	88.6	88.1
FR1 - CDR3	87.7 (81.1)	84.6	82.1	82.8	90.5	88.1	87.4

"invariant" Thr at position 69 has been changed to an Ala. The assignment of the Daudi and Walker proteins to subgroup I confirms a previous assignment by immuno diffusion assays (A. Solomon, personal communication).

The surprising feature of the Daudi and Walker sequences is the identity of the 5' flanks contrasted by a high divergence in the genes themselves. Also the far upstream flanks 4.5 kb 5' of the V genes are idential within a sequenced region of 300 bp (Fig.1; sequence not shown). One explanation for this observation would assume that the Daudi and Walker genes are derived from recently duplicated germline genes which have diverged, for instance by gene conversion like events, within the genes but not in the flanks. A similar situation was found recently in the analysis of human germline V_K genes (12): the neighbouring genes Va' and Vb' differ considerably within the genes but their 5' flanks are highly conserved at least within 200 bp. It appears somewhat more likely, however, that the Daudi and Walker genes are alleles which have diverged by somatic mutations (e.g. 36-38). In favour of this interpretation one may mention that the pattern of divergence (Fig. 4) resembles the patterns observed in other V_K gene systems (37,38). Also the numerous sequence differences between the 3' flanks of the Daudi and Walker genes and the respective germline sequences (Fig. 4) may be discussed in terms of somatic mutations (see also ref. 37). A decision between the two mechanisms is not possible at present.

Concluding remarks

Up to now only three rearranged human K gene sequences are known, the sequence of the KII gene of the cell line GM607 (11) and the two KI sequences reported here. It will be interesting to establish in further work which germline V_{KI} gene(s) the Daudi and Walker K^+ genes are derived from, for instance by hybridization studies with conserved 5' flanks of the genes. Such studies may also shed further light on the contribution of somatic mutations to K chain diversity.

The two cell lines Daudi and Walker are also of special interest for chromatin studies on K light chain genes, which was actually the reason for our investigation of the two genes. The loss of one K allele in Daudi cells facilitates the mapping of

DNAase I hypersensitive sites on the remaining K gene, which is rearranged. The situation in Walker cells is more complicated because of the presence of two K alleles (K^+,K^o), but the close similarity between the rearranged K genes of the two cell lines allows one to assign hypersensitive sites to the K^+ allele of Walker cells by analogy to the sites in Daudi (see accompanying paper, ref. 14).

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1. Abbreviations: L,L', leader, V, variable, J, joining, C, constant segments of the immunoglobulin K light chain genes. FR, CDR, framework and complementarity determining regions of the immunoglobulins. K^o,K^+ refer to the non-rearranged and functionally rearranged alleles of the K light chain genes. The designation of clones is the following: the letters , p, m in front of the designations of clones and subclones refer to the vectors phage , pBR322, phage M13, respectively; u, upstream (5'), d, downstream (3'); I, intron between J_{K5} and C_K; e.g. puJ-1 is the first subclone from the region upstream of J_K (uJ), prepared in pBR322 (p).
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