Human β Interferon Gene Localization and Expression in Somatic Cell Hybrids

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The human fibroblast interferon gene β_1 was mapped to human chromosome 9. Sequence homology with a β_1 cDNA clone was detected in both genomic DNA and induced mRNA of human/mouse or human/hamster somatic cell hybrids containing human chromosome 9, but not in lines lacking this chromosome or those retaining a complex translocation involving chromosomes 9 and 11. Interferon mRNA that did not share sequence homology with the β_1 cDNA clone was detected in lines containing human chromosomes 2 and 5 but lacking chromosome 9, suggesting the presence of other unlinked interferon sequences in the human genome.

Interferons comprise a multiple-gene family whose expression in animal cells can be induced by virus infection or other stimuli, such as double-stranded RNA, mitogens, or antigens. Interferons released from producing cells can trigger a number of biological responses in target cells, including the development of resistance to infection by a broad range of viruses, inhibition of cell proliferation, and activation of lymphocytes.

Human interferons have been classified into three antigenically distinct types: α , produced primarily by peripheral blood leukocytes and established lymphoblastoid cell lines; β , produced by cultured fibroblasts; and γ , produced by mitogen- or antigen-stimulated T cells. The α and β human interferon genes were cloned with recombinant DNA techniques, and it was found that α interferons represent a multiple-gene family (1, 4, 17). The nucleotide sequences of several α interferon genes showed high degrees of homology (4). However, only two β interferon genes have been cloned as yet; their nucleotide sequences are not related, and they also showed little homology with α interferons (2, 21, 25).

Somatic cell genetic studies with human/rodent hybrid cells segregating human chromosomes have resulted in assignment of β interferon loci to chromosomes 2, 5, and 9 (7, 8, 15, 19). These studies, based on the antiviral assay of human interferons produced in induced (with virus or double-stranded RNA) human/mouse hybrid cells, were able to localize β but not α interferon loci, since interferons synthesized in human/mouse hybrid cells were identified as β

type (by a neutralization test with appropriate polyclonal antibodies) regardless of the type of human cell used for fusion (7, 8, 15, 19).

In this study, we used several molecular approaches to localize the human β_1 interferon gene. First, we determined the chromosomal location of the β_1 interferon structural gene by hybridization of DNA from human/mouse and human/hamster cell hybrids with a radioactive cDNA clone of the abundant (β_1) fibroblast interferon mRNA (2, 10, 21). Second, we examined the expression of the β interferon gene in these cells, assaying both for translatable β mRNA by translation in *Xenopus* oocytes and for β_1 mRNA sequences by hybridization with the radioactive β_1 cDNA clone. Our data indicate that the functioning β_1 interferon gene is located on chromosome 9. However, our results based on the translation of polyadenylated [poly (A)] RNA in *Xenopus* oocytes indicate that an additional interferon gene(s) not cross-hybridizing with β_1 cDNA probes is present on chromosome 2, chromosome 5, or both. Our analysis, which combines gene localization by molecular hybridization and analysis of interferon gene expression on the mRNA and protein level, reveals the complexity of the β interferon gene. While this work was in progress, Owerbach et al. assigned α and β_1 interferon genes to human chromosome 9 (9) by the hybridization of genomic DNA with specific probes.

MATERIALS AND METHODS

Cell lines. Somatic cell hybrids between human and mouse cells or human and Chinese hamster cells were formed and characterized as previously described (15). Hybrid cells were grown in monolayer culture in Dulbecco modified essential medium with a high glu-

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cose content and supplemented with 10% fetal bovine serum.

Human fibroblast cells trisomic for chromosome 21 (GM2504) were grown in Eagle minimal medium supplemented with 10% fetal bovine serum.

Interferon assay. Interferon was assayed by the cytopathic assay of Finter (3) on human fibroblast cells trisomic for chromosome 21. The titers are given in international reference units (1 U of reference standard 69/17 interferon titrated as 1 U in our assay).

Plasmid. The properties of the cloned β_1 interferon cDNA in pBR322 have been described previously (10). This β_1 cDNA clone is identical to that described by others (2, 21), but does not contain the *TaqI* restriction site on the 5'-terminal end. The *HincII/BglII* fragment of the β_1 cDNA clone was isolated by electrophoresis on 1.4% agarose gels and labeled to high specific activity (10^6 cpm per μ g of DNA) as described by Rigby et al. (12).

DNA isolation and genomic blot hybridization. High-molecular-weight DNA was prepared by digestion of cell pellets with proteinase K (100 μ g/ml) in 1% sodium dodecyl sulfate in TE buffer (10 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA) for 24 h at 37°C, extraction with phenol and chloroform, RNase treatment (100 μ g/ml), and precipitation of DNA with ethanol. Aliquots of DNA were taken for endonuclease restriction. The enzymes used were *EcoRI* and *TaqI* in buffers recommended by Bethesda Research Laboratories. Restriction was carried out overnight at either 37°C (*EcoRI*) or 65°C (*TaqI*) with 2 U of enzyme for each 1 μ g of DNA, and the reaction was stopped by the addition of EDTA to a final concentration of 25 mM. The fragments of DNA were electrophoretically separated on an 0.8% agarose gel at 15 V for 24 h; gels were treated with 0.2 M HCl for 10 min, denatured in 1.0 M NaOH–0.5 M NaCl for 60 min, and neutralized with 1.0 M Tris-hydrochloride (pH 5.5) and 3 M NaCl for 120 min. DNA was transferred to nitrocellulose filters with 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 24 to 36 h (16). After transfer, filters were baked at 80°C in a vacuum oven for 2 h. Filters were prehybridized for 24 h in a solution containing 50% formamide, 5 $\times$ SSC, 5 $\times$ Denhardt solution (16), 50 mM sodium phosphate buffer (pH 6.5), 10 μ g of poly(A) per ml, and 250 μ g of denatured herring sperm DNA per ml. Hybridization was done for 24 h at 37°C in a solution containing 50% formamide, 5 $\times$ SSC, 1 $\times$ Denhardt solution, 20 mM sodium phosphate buffer (pH 6.5), 100 μ g of denatured herring sperm DNA per ml, and 10% sodium dextran sulfate (24) with 1×10^6 to 10×10^6 cpm of the 32 P-labeled *HincII/BglII* fragment of the β_1 cDNA clone per ml. After hybridization, the filters were washed three times in 0.5 M NaCl–25 mM sodium phosphate (pH 6.5)–0.5% sodium dodecyl sulfate and 0.5 M NaCl–25 mM sodium phosphate (pH 8.5)–2 mM EDTA for 1 h at 37°C for each wash and then in 0.1 $\times$ SSC for 30 min at 60°C.

For the dot hybridization assay, DNA (10 to 30 μ g) was denatured in 0.3 M NaOH, neutralized, heated in boiling water for 5 min, and cooled on ice. DNA was dotted on nitrocellulose paper soaked in 20 $\times$ SSC and dried in air (23). Filters were then baked and hybridized as described above.

Induction and mRNA preparation. The human/mouse hybrid cells were induced with polyriboninosinate and polyribocytidylate complex [poly(rI·rC)](100

μ g) in the presence of DEAE-dextran (50 μ g/ml) and cycloheximide (50 μ g/ml) as described previously (15). Poly(A) RNA was isolated from the cells 5 h after induction by the guanidine hydrochloride method (18) and purified from total RNA by two cycles of oligodeoxythymidylate-cellulose chromatography. For interferon production, cells were treated with actinomycin D (1 μ g/ml) between 4 and 5 h after induction, and medium with interferon was collected 24 h after induction.

Identification of human interferon mRNA. Poly(A) RNA (1 μ g/ μ l) isolated from induced cells and controls was injected into 10 *Xenopus laevis* oocytes; the oocytes were incubated at 22°C for 40 h, homogenized, and assayed for antiviral activity as described previously (11). For the hybridization assay, 0.2, 1, 5, and 50 μ g of poly(A) RNA was dotted on nitrocellulose filters as described for DNA, but the RNA was not denatured. Prehybridization and hybridization were done as described for DNA, in the absence of formamide, at 65°C. Filters were washed as described for DNA.

For the Northern hybridization assay, poly(A) RNA (2 or 20 μ g as indicated) was denatured with glyoxal and dimethyl sulfoxide at 50°C for 45 min, electrophoresed in 1.2% agarose gels in 10 mM sodium phosphate buffer (pH 7.0) at 90 V for 3.5 h, and transferred to nitrocellulose paper as recently described (9). Filters were prehybridized with 5 $\times$ SSC and 1 $\times$ Denhardt solution (23) overnight at 65°C, and hybridization was carried out with 10^6 cpm of 32 P-labeled probe per ml in a solution containing 5 $\times$ SSC, 1 $\times$ Denhardt solution, 50 mM sodium phosphate buffer (pH 6.5), 100 μ g of denatured herring sperm DNA per ml, and 10 μ g of poly(A) per ml at 65°C for 48 h. Filters were then washed with 2 $\times$ SSC–0.1% sodium dodecyl sulfate at room temperature for 2 h and 0.1 $\times$ SSC–0.1% sodium dodecyl sulfate at 50°C for 30 min and exposed to X-ray film (Kodak X-Omat) with an intensifying screen at –70°C.

Antisera. The rabbit anti- α interferon serum was prepared by K. Berg (University of Aarhus, Aarhus, Denmark) by immunization with electrophoretically purified human leukocyte interferon. The rabbit anti- β_1 interferon serum was prepared by immunization with purified fibroblast interferon (A. R. Neurath, N. Strick, N. B. K. Raj, and P. M. Pitha, J. Interferon Res., in press). This antiserum selectively immunoprecipitates the 22,000-dalton protein of pI 6.8 from the media of human fibroblast cells labeled with [35 S]methionine; the protein comigrates with the biological activity (10). A 10^{-3} dilution by either antiserum neutralized 10 U of corresponding interferon.

RESULTS

Detection of human β_1 interferon sequences in high-molecular-weight DNA. To test for the presence of β_1 interferon sequences in the DNA isolated from the human, mouse, hamster, and human/mouse and human/hamster hybrid cells, denatured DNA was dotted on nitrocellulose filters and hybridized with the 32 P-labeled *HincII/BglII* fragment of our β_1 cDNA clone (Table 1). Under stringent conditions (0.1 $\times$ SSC wash at 60°C), we detected signals from only

TABLE 1. Human chromosome complement of hybrid cells

Cell line	Human chromosomes	Hybridization ^a
AIM 15aA3	2, 4, 5, 7, 11, 12, 13, 14, 15, 17, 18, 21, X	—
AIM 15a/15	1, 2, 5, 6, 7, 11, 12, 14, 15, 16, 17, 18, 20, 21, X	—
AIM 15aHAT	1, 2, 5, 6, 7, 11, 12, 14, 15, 16, 17, 18, 20, 21, X	—
AIM 15aA1	1, 2, 5, 6, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	—
AIM 15aA1/Dpt	1, 2, 11, 12, 13, 14, 15, 17, 20, 21, X	—
AIM 15aB1	1, 2, 5, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	—
AIM 15aB1/Dpt	1, 2, 7, 11, 12, 13, 14, 15, 17, 20, 21, X	—
BDA 14b25	1, 4, 6, 7, 12, 14, 18, X	—
BDA 17b17	1, 2, 3, 4, 5, 6, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, X	+
BDA 17b17/Dpt	1, 2, 3, 4, 6, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, X	+
BDA 10a3a	2, 3, 4, 5, 6, 8, 10, 11, 12, 13, 16, 18, 20, X	—
BDA 10a3a/Dpt	2, 6, 8, 10, 11, 12, 13, 16, 17, 20, X	—
BDXE36-4a	2, 6, 9, 14, 20, 21, X	++
BDA 10a4a	2, 3, 9, 10, 11, 12, 13, 20, X	+
21-30b	Translocation involving 9, 11, X	—
E36 (hamster)		—
A9 (mouse)		—
FS-4 (human)	All	+++

^a High-molecular-weight DNA (25, 5, and 1 μ g) was denatured, dotted on nitrocellulose paper, and hybridized with 10^7 cpm of the 32 P-labeled *HincII/BglII* fragment of the β_1 cDNA clone per ml as described in the text. +++, Hybridization detected with 25, 5, and 1 μ g of DNA; ++, hybridization detected with 25 and 5 μ g of DNA; +, hybridization detected with 25 μ g of DNA; —, no hybridization.

four hybrid cell lines: BDA 17b17, BDA 1717/Dpt, BDXE36-4a, and BDA 10a4a. The common feature of the positive lines was the presence of human chromosome 9, whereas the lines which did not show a positive signal with this probe did not contain this chromosome. The 21-30b line, which contains a complex human translocation involving parts of chromosomes 9q, 11q, and Xq (Kamarck, Macyko, Cunningham, and Ruddle, manuscript in preparation), did not show any sequence homology with the β_1 cDNA probe. In mouse and hamster DNA, no homology with the β_1 cDNA probe was detected, suggesting divergence of the human β_1 interferon gene from the rodent β interferon genes.

The high-molecular-weight DNAs from the BDXE36-4a, 21-30b, AIM 15aA3, and FS-4 (human fibroblast) lines were digested with *EcoRI* and *TaqI* restriction endonucleases. Restriction fragments were separated on an agarose gel, transferred to nitrocellulose filters, and probed with the 32 P-labeled *HincII/BglII* fragment of the β_1 cDNA clone to determine the presence of human β_1 interferon DNA sequences (Fig. 1). It was previously shown (2, 20) that the β_1 gene is not cleaved by *EcoRI* endonuclease, and the restriction site for *TaqI* is close to the 5' terminus of mature β_1 mRNA. Since the β_1 interferon gene is not spliced and is present in human DNA in a single copy (6, 9, 22), only one radioactive band, 2.2 kilobases or 0.89 kilobases, was detected with the β_1 cDNA probe after restriction of FS-4 and BDXE36-4a DNAs with *EcoRI* and *TaqI* endonucleases, respectively. This indicates that BDXE36-4a hybrid cells contain the

full complement of β_1 interferon sequences. In 21-30b cells and AIM 15aA3 cells, no homology with the β_1 cDNA probe was detected, suggesting the absence of the β_1 interferon gene in the chromosome 9, 11, X translocation and in chromosomes 2 and 5.

Induction of human interferon mRNA in human/mouse hybrid cells. It was shown previously that human/mouse hybrid cell lines containing human chromosome 2, 5, or 9 produced interferon upon induction with poly(rI·rC) or virus and that the interferon produced was neutralized by anti-fibroblast (β) interferon serum (7, 8, 15, 19). Our results, as well as recent findings of Owerbach et al. (9), indicated that the β_1 interferon gene is located only on chromosome 9. To determine whether other active interferon genes, not related by DNA nucleotide sequences to the β_1 cDNA probe, are located on chromosomes 2 and 5, we assayed the relative levels of interferon mRNA from poly(rI·rC)-induced human/mouse cells containing either chromosomes 2 and 5 (AIM series) or chromosomes 2, 5, and 9 (BDA series) by translation in *Xenopus* oocytes. The products of translation were analyzed for antiviral activity on human and mouse cells and for their reactivity with human anti- α and anti- β interferon serum. Table 2 compares the relative levels of translatable interferon mRNA present in total poly(A) RNA isolated from the induced AIM 15aA3, BDA 17b17, and 21-30b cell lines with the amount of human interferon present in the media of these cells 24 h after poly(rI·rC) induction. It can be seen that interferon mRNA activity and interferon production were detected

FIG. 1. Presence of β_1 interferon sequences in human/mouse hybrid cells. High-molecular-weight DNA (25 μ g) was restricted with *Taq*I (tracks 1 through 3 and 7) and *Eco*RI (tracks 4 through 6 and 8) restriction enzymes. Fragments were subjected to electrophoresis in an 0.8% agarose gel, transferred to nitrocellulose filters, and hybridized to the nick-translated *Hinc*II/*Bgl*III fragment of the β_1 cDNA in pBR322 as described in the text. DNA from the BDXE36-4a cells (tracks 1 and 6) contained fragments of the same size as DNA from FS-4 cells (tracks 7 and 8); no hybridization was detected with DNA from the 21-30b (tracks 2 and 4) and AIM 15aA3 (tracks 3 and 5) cells. The fragments of the phage λ DNA digested with *Hind*III are shown as references on the right-hand margin.

both in the BDA 17b17 cell line, which contains human chromosomes 2, 5, and 9, and in the AIM 15aA3 cell line, which contains chromosomes 2 and 5 but not chromosome 9 and which shows a lack of DNA sequence homology with the β_1 cDNA clone. Both the parental mouse A-9 line and 21-30b hybrid line containing the 9,11,X translocation did not produce human interferon and did not show detectable levels of translatable interferon mRNA. Neither the oocyte homogenate nor the media from induced cells showed any detectable activity on mouse cells (data not shown).

The antiviral activity of interferon synthesized in oocytes upon injection of poly(A) RNA from induced BDA 17b17 and AIM 15aA3 cells could be neutralized by preincubation with rabbit anti- β interferon serum, whereas preincubation with rabbit anti- α interferon serum effected no neutralization (Table 3). Thus, the antiserum to β interferon seems to neutralize the interferon coded by genes present on chromosomes 2 and 5, which correlates with the data observed by

TABLE 2. Translation of interferon mRNA in *Xenopus* oocytes^a

Cell line	Interferon induced (U/ml)	Interferon produced by oocytes (U/ml)
AIM 15aA3	150	160
AIM 15aA3/Dpt	75	90
AIM 15aHAT	150	160
BDA 17b17	240	240
BDA 17b17/Dpt	150	240
BDA 17b17HAT	240	240
21-30b	<10	<40
A9	<10	<40
FS-4	10,000	1,000

^a Interferon was induced by poly(rI-rC) superinduction (16). Poly(A) RNA (1 μ g/ μ l) isolated from induced cells 5 h after induction was injected into 10 oocytes; oocytes were incubated for 40 h and homogenized, and interferon activity was assayed on human fibroblasts as described in the text. The oocyte homogenate at dilutions lower than 1/20 to 1/40 showed a cytopathic effect on the indicator cell used.

others (14). We have shown previously (10) that the antiserum used selectively precipitated only one species of [³⁵S]methionine-labeled interferon (β_1) from the media of induced human fibroblast cells; it is possible, however, that the other β interferons have much lower affinity for this anti- β serum or are present in the medium at very low levels and therefore are not detectable by immunoprecipitation.

TABLE 3. Neutralization of interferon synthesized in oocytes by antisera against human α and β interferons^a

Cell line	Residual interferon activity (U/ml) after incubation with antiserum:		
	No antibody	Anti- α interferon	Anti- β interferon
BDA 17b17	50	50	<20
BDA 17b17/Dpt	50	50	<20
AIM 15aA3	100	160	<20
AIM 15aA3/Dpt	100	160	<20
FS-4	200	200	<10
Namalva	200	<10	180
Human α interferon	250	<10	240
Human β interferon	250	240	<10

^a Poly(A) RNA (1 μ g/ μ l) isolated from induced cells was injected into 10 *Xenopus* oocytes, and interferon in the oocyte homogenate was assayed on human fibroblast cells before and after incubation (30 min at 37°C) with anti- α and anti- β interferon sera. The amounts of antisera used were able to neutralize 500 U of α or β interferon. Purified human α interferon and human β interferon were used as standards.

Finally, we examined the sequence homology between the interferon mRNAs detected and the β_1 cDNA probe. We have shown previously that no β_1 mRNA sequences can be detected in uninduced human fibroblast cells (10). Poly(A) RNA isolated from the induced AIM 15aA3, BDA 17b17, FS-4, and 21-30b cell lines was spotted on nitrocellulose filters and hybridized with the 32 P-labeled *HincII/BglIII* fragment of the β_1 cDNA clone (Fig. 2). It can be seen that mRNA sequences related to the β_1 cDNA clone can be identified in a poly(A) population from the induced BDA 17b17 and FS-4 cells, but not in a poly(A) population from the AIM 15aA3 and 21-30b cells. No hybridization was detected between the β_1 cDNA probe and poly(A) RNA isolated from the uninduced cells (data not shown). The relative levels of β_1 mRNA present in the BDA 17b17 hybrid cells were estimated by hybridization to be 50-fold lower than the levels of β_1 mRNA present in poly(rI·rC)-induced human fibroblast cells; however, the size of the β_1 mRNA in the BDA 17b17 hybrid cells was similar to that detected in induced human fibroblast cells (Fig. 3).

DISCUSSION

Our data indicate that the β_1 interferon structural gene resides on human chromosome 9 and that the β_1 sequences located on chromosome 9 are expressed in somatic cell hybrids after poly(rI·rC) induction. The β_1 sequences in high-molecular-weight DNA and β_1 mRNA were detected in cells containing human chromosome 9 but not in cells that lack this chromosome or retain a complex translocation involving chromosomes 9, 11, and X.

It is not clear how much genetic material from

chromosome 9 is involved in the 21-30b translocation. Adenosine kinase-1, a long-arm marker, is present in 21-30b cells, but adenosine kinase-3, a short-arm marker, is not (Kamarck et al., in preparation). The data also suggest the presence of other unlinked interferon sequences in the human genome which can be expressed after poly(rI·rC) induction. Human interferon mRNA that does not share sequence homology with the β_1 interferon gene was detected by translation in oocytes in poly(rI·rC)-induced human/mouse hybrid cells that lack human chromosome 9 but contain human chromosomes 2 and 5. The existence of multiple β interferon mRNA species has been previously reported by two different groups (14, 25); however, the inducibility of these species seems to be affected both by the cell line and by the method of induction.

The results of somatic cell hybrid studies, which required the expression of human interferon genes in cells, produced apparently conflicting gene assignments (7, 8, 15, 19). The use of somatic cell hybrids for the chromosomal assignment of genes of interest relies on the accurate karyotype and isoenzyme analysis of the cell hybrids, and the sensitivity of detection depends on the percentage of the cells containing the given chromosome in the total cell population. The human/mouse cells, which tend to lose human chromosomes preferentially, segregated human chromosome 9 very rapidly (D. Slate and F. H. Ruddle, unpublished data), and thus, this chromosome is often difficult to study in these hybrids. For these reasons, all the map assignments are considered tentative until confirmed in another laboratory. While this work was in progress, Owerbach et al. (9) assigned α and β_1 interferon genes to human chromosome 9,

FIG. 2. Presence of β_1 interferon sequences in poly(A) RNA isolated from human/mouse hybrid cells induced with poly(rI·rC). Poly(A) RNA isolated from induced human/mouse hybrid cells was spotted on nitrocellulose filters and hybridized with the nick-translated *HincII/BglIII* fragment of the β_1 cDNA in pBR322 as described in the text. The amounts of spotted poly(A) RNA were 50 μ g (a), 5 μ g (b), 1 μ g (c), and 0.5 μ g (d).

FIG. 3. Sizing of β_1 mRNA detected in induced cells. Poly(A) RNA from induced AIM 15aA3 (20 μ g) (track 1), FS-4 (2 μ g) (track 2), and BDA 17b17 (20 μ g) (track 3) cells was denatured with glyoxal, sized on a 1.2% agarose gel, transferred to nitrocellulose filters, and hybridized with the nick-translated *HincII/BglIII* fragment of the β_1 cDNA in pBR322 clone as described in the text. The fragments of the ϕ X174 DNA digested with *HaeIII*, denatured with glyoxal, and analyzed under the same conditions are shown as references on the right-hand margin.

which correlates with our conclusions. The previously observed discrepancy may be resolved in the near future by the discovery of multiple β interferon proteins and gene sequences.

It is interesting that although both α and β_1 interferon genes are localized on chromosome 9, they are probably not closely linked, since a 36-kilobase fragment of human genomic DNA containing the β_1 interferon gene which was recently cloned in cosmid does not contain any α interferon genes (5). The chromosomal assignment and refined localization of α and β interferon genes should facilitate the study of interferon gene polymorphism and permit the determination of whether disorders involving interferon genes may have serious consequences to the host. It is known that human chromosome 9 is the least stable human chromosome, and its breakage or translocation has been observed with high frequency both in normal individuals (J. Smith-Fergusson, personal communication) and in patients with chronic myelogenous leukemia, for which a reciprocal translocation involving chromosomes 9 and 20 has been observed with a frequency of 92% (13).

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