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(54) Titre : NOUVEAU FACTEUR DE REGULATION CELLULAIRE TTO 20
(54) Title: NOVEL CELLULAR REGULATION FACTOR TTO 20

(57) Abrégé/Abstract:
The invention relates to the cellular regulation factor TTO 20, to DNA that codes for the same, to the production of said cellular regulation factor TTO 20 and to its use, to antibodies that bind to TTO 20 and to the use of the DNA that codes for TTO 20 and an antibody that binds to TTO 20 in a diagnostic kit for determining inflammatory diseases, especially rheumatoid arthritis.

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(54) Title: NOVEL CELLULAR REGULATION FACTOR TTO 20

(54) Bezeichnung: NEUER ZELLULÄRER REGULATIONSFAKTOR TTO 20

(57) Abstract

The invention relates to the cellular regulation factor TTO 20, to DNA that codes for the same, to the production of said cellular regulation factor TTO 20 and to its use, to antibodies that bind to TTO 20 and to the use of the DNA that codes for TTO 20 and an antibody that binds to TTO 20 in a diagnostic kit for determining inflammatory diseases, especially rheumatoid arthritis.

(57) Zusammenfassung

Die Erfindung betrifft den zellulären Regulationsfaktor TTO 20, DNA kodierend dafür, seine Herstellung und Verwendung, Antikörper bindend an TTO 20 sowie die Verwendung der DNA kodierend für TTO 20 und einen Antikörper bindend an TTO 20 zur Verwendung in einem Diagnostik–Kit zur Festellung von entzündlichen Erkrangungen, insbesondere rheumatoider Arthritis.

Description

Novel cell regulation factor TTO 20

The present invention relates to the cell regulation factor TTO 20 and DNA therefor, and its preparation and use for screening purposes for the discovery of modulators for TTO 20 activity.

Introduction

The many biological effects of interleukin-1 (IL-1) include the action of IL-1 on the metabolism of many types of cell from the connective tissue. An example of cells of this type are articular chondrocytes. IL-1 inhibits the synthesis of proteoglycans (PG) by chondrocytes and stimulates the production of prostaglandin E_2 and metalloproteinases, which are capable of degrading molecules of the extracellular matrix.

On the basis of experimental results, and the discovery of IL-1, PG fragments and proteolytic enzymes in inflammation-modified joints, it was concluded that IL-1 plays a part in cartilage degradation in osteoarthrosis and rheumatoid arthritis (Beuton HP & Tyler JA, 1988, Biochem. Biophys. Res. Comm. 154, 421-428; Aydelotte MB et al. Comm. Tiss. Res. 28, 143-159, Wood DD et al., Arthritis Rheum. 26, 975-983; Lohmander LS et al., Trans. Orthop. Res. Soc. 17, 273). Matrix metalloproteases are potential candidates for starting points for a therapy with active compounds which interact with these enzymes, but until now no actual molecular starting points have been identified which relate to early steps in the complex process which leads to cartilage degradation. For this reason, various approaches have been chosen in order to obtain molecular starting points of this type for a medicinal therapy of osteoarthrosis and rheumatoid arthritis.

Such an approach is described in European Patent Application EP 0 705 842 A2. The question was looked into there as to whether it would be possible to obtain potential molecular starting points for a medicinal therapy of IL-1ß-induced cartilage degradation on the RNA plane in human, articular chondrocytes from osteoarthritic cartilage.

For this purpose, genes are to be identified which are expressed differentially in diseased cartilage. Total RNA from IL-1ß stimulated and nonstimulated human chondrocytes was subjected to a differential display of mRNA by reverse transcription and the polymerase chain reaction (DDRT-PCR). This method can be used for the identification and isolation of genes which are expressed differentially in two cell populations (Liang P & Gardee AB 1992, Science 257, 967-971; Liang P et al., AB 1993, Nucl. Acids Res. 21, 3269-3275; Bauer D et al. 1993, Nucl. Acids Res. 21, 4272-4280). The key element of this technology is the use of a set of oligonucleotide primers, of which one binds to the polyadenylated tail of the mRNA, the others, on the other hand, are random decamers which bind to various other sites of the mRNA. Such mRNA subpopulations, which are defined by a specific set of primers, are amplified after reverse transcription and separated on DNA sequencing gels. Band patterns are seen which are characteristic for one of each of the cell lines studied. Thus, for example, 100 different primer combinations should afford 10000 different PCR products, which represent at least approximately half of the genes expressed in a cell line at all. A comparison of the band patterns of two different cell lines indicates those bands which correspond to differentially expressed genes. On the basis of this information, it is now possible to extract, to reamplify, to subclone and to sequence bands of differentially expressed gene products from the gel.

However, this is to be qualified by saying that this method has a number of difficulties:

1. As a result of the high sensitivity of the DDRT-PCR, slightly artificial bands can result.

2. The analysis of complex gene expression patterns is difficult.

3. Only tiny amounts of RNA are available as starting material.

These difficulties cause uncertainty in the results obtained.

In European Patent Application EP 0 705 842 A2, a number of short DNA sequences are disclosed which have been identified in the manner described above. An analysis of the sequences showed that some of them had complete or very great identity with the sequences of already known

genes: thus a cDNA fragment having 100% identity with human osteopontin, another cDNA fragment having 97.2% identity with human calnexin, and a further fragment having 99.5% identity with human TNF-stimulated gene 6 (TSG-6) was found. Most of the fragments found, however, could not be allocated to any known gene from the point of view of the sequence characterizing them. This group of cDNA fragments also includes the 400 bp-long clone TTO 20/2(2), of whose DNA sequence 152 bp have been deciphered.

In the context of the present invention, the clone TTO 20/2 has now been investigated more closely. So-called antisense experiments are one means for the investigation of the functional meaning of the corresponding gene or gene product.

The expression of antisense RNA in human chondrosarcoma cells, which are regarded as model cells for cartilage differentiation, yielded indications of a role of TTO 20/2. The antisense approach is based on transforming the cultured cells with a vector which expresses antisense mRNA to TTO 20, but at the same time also an indicator protein whose activity indicates whether the antisense RNA was formed. Vectors of this type are called bicistronic or dicistronic vectors. The starting vector for the present constructs was pED4, whose construction has been described by Kaufmann et al. (Kaufmann et al., 1991, Nucl. Acids Res. 19, 4485-4490).

In antisense technology, the formation of a functional protein is restricted or even prevented via the expression of a complementary RNA (antisense RNA) which binds to the protein-encoding mRNA (sense RNA). In particular, as the present examples confirm, antisense RNA can be employed for subregions of the encoding mRNA and for the 3' or 5' untranslated region in order to prevent the formation of the target protein. With the aid of the vector, EST fragments which have no defined open reading frame can thus be used in order to work out the action of the protein which is finally encoded by the associated gene in that the "antisense-expressed" EST switches off or decreases the reading of the encoding sense mRNA. If the synthesis of the target protein which is blocked in this way plays an important role in the cell, this has direct or indirect effects on cell division, cell growth, synthesis of regulated and expressed proteins etc. If the antisense expression prevents, for example,

the formation of a factor which plays a role in signal cascades, this is disturbed.

If the factor is a transcription factor, the expression of a number of genes is disturbed. This can be recognised, for example, from morphologically visible changes which can be attributed to altered expression of secreted proteins, particularly of proteases.

The genes or products thereof identified in this way can be employed as therapeutic targets for the search for pharmacologically active substances. Likewise, cells transformed in this way can be used in screening systems in which it is attempted to block the action of the antisense RNA.

DNA chip technology allows the direct analysis of such changes in that the transcript profiles of transformed cells are compared with untransformed or mock-transformed cells. The comparison then allows conclusions to be drawn as to whether an EST plays a crucial role in the context of a clinical picture and is thus suitable as a screening target. The use of the vector is thus also suitable for the discovery of novel targets and for the profiling of novel medicaments. The vector is particularly suitable for the synthesis of HTS systems for target validation. Expediently, EST clones are cultured in HTS formats such as 96-well microtiter plates for this, the insert DNA is amplified by means of PCR using suitable PCR primers which, for example, generate a PST cleavage site on the 3' side and an Eco RI cleavage site on the 5' side for cloning in one of the pED4 derivatives described. In a second step, the PCR fragments generated in this way are cleaved using PstI and Eco RI and dicistronic EGFP vector is ligated into the vector. The screening format here is retained. The ligated vector is pipetted onto previously prepared eukaryotic cells using commercially obtainable pipetting robots with suitable transfection agents such as $CaPO_4$, Fugene 6 (manufacturer: Boehringer, Mannheim; lipofectamine, Life Technologies, Eggenstein) or others and the cells are transformed according to customary processes. Here too, the screening format is retained. The cells are incubated in the presence of CO_2 according to customary processes and tested for fluorescence emission after 24 – 72 hours under automated conditions in a fluorescence scanner. Wells with transformed, fluorescent cells are then evaluated for changes in growth and changes in cell morphology, etc. compared with transformed control cells which were transfected with the vector without PstI – Eco RI insert. If changes of this

type occur, this indicates an essential action of the expressed antisense RNA. The isolation of the cloned DNA and the subsequent sequence analysis give an indication of the nucleotide sequence and thus the gene or the coded protein involved. In this way, the first functional indications of gene activities can be found whose function cannot itself be derived by means of the EST.

The invention therefore relates to the TTO 20 protein comprising the amino acid sequence according to Table 1.

A further subject of the invention is a DNA coding for the protein mentioned, selected from the group comprising

(a) the DNA sequence as in Table 1, in particular nucleotides 1 to 1305;

(b) a DNA which is at least 80% identical with a DNA as in (a);

or

(c) a DNA which, with respect to the genetic code, is degenerate to the DNAs from (a) and (b).

The invention likewise relates to an antibody which binds to the TTO 20 protein.

Further subjects of the invention are an expression vector for the expression of the DNA described above and a host cell transfected or transformed with this expression vector.

The invention also relates to a process for the preparation of the TTO 20 protein, comprising the steps:

(a) culturing a host cell under conditions which make possible the expression of the TTO 20 protein;
and

(b) isolation of the TTO 20 protein from the host cells or the culture medium,

and a TTO 20 protein obtainable by the process described.

A further subject of the invention is a process for the identification of cell lines, cells or tissues which express the TTO 20 protein, in which a nucleic

acid probe is used which is hybridized with an RNA in a biological sample which is derived from a DNA as described above.

The invention moreover relates to the use of the DNA coding for the TTO 20 protein according to Table 1 for the completion of the DNA and protein sequence as in Table 1 by means of hybridization and/or PCR processes.

A further subject of the invention is the use of the TTO 20 protein for the determination of its three-dimensional structure and the design of inhibitors or activators of the TTO 20 protein. The invention likewise relates to the use of TTO 20 for the discovery of modulators of the TTO 20 activity.

Further subjects of the invention are a diagnostic kit for the diagnosis of inflammatory disorders, in particular rheumatoid arthritis, comprising an antibody such as described above and a diagnostic kit for the diagnosis of inflammatory disorders, in particular rheumatoid arthritis, comprising a DNA described above.

Example 1: Identification of cDNAs using the TTO20/2 sequence

The partial sequence of 152 bp designated by TTO20/2 is disclosed in European Patent Application EP 0 705 842 A2. In order to obtain information about the complete cloned sequence, the entire DNA of TTO 20/2 was sequenced with the aid of the SP 6 primer. The sequencing reactions were carried out according to the chain termination DNA sequencing method as described in Sanger et al. (Sanger, F. et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463-5467). From the sequencing, a total length for the cloned insert of 272 bp resulted. Using this sequence, a homology search was carried out in the gene bank (NCBI) and the EMBL database. To do this, the BLAST and FASTA algorithms were used, which are part of the GCG DNA analysis package of the Genetics Computer Group, Madison, USA.

The homology search showed significant similarities to two EST clones in the public database having the gene bank accession number M 51303 (sequence ID: g1192469) and the accession number H 05077 (sequence ID: g868629). The corresponding clones having the clone ID 283071 or 43508 were ordered from Genome Systems, St. Louis, USA, and completely sequenced. The clone having the clone ID 283071 showed the

largest insert of about 2580 bp, the clone having the ID 43508 is markedly smaller with an insert size of 2200 bp, but has 100% sequence homology with the clone 283071. The complete sequence of the clone 283071 is shown in Table 1 and begins at nucleotide 706.

Example 2: Identification of an open reading frame

With the aid of the Translate program from the GCG analysis package, the sequenced DNA of the clone 283071 was investigated for the occurrence of open reading frames (ORF). Accordingly, an open reading frame which codes for 200 amino acids is found at the 5‘ end of the sequence. Following the stop codon TAA, an untranslated region about 1950 bp long is found, followed by a polyadenylation signal and ending with a Poly A tail. The sequence corresponds to the sequence indicated in Table 1 from nucleotide 706 to nucleotide 3262.

A homology search was carried out in public databases using the 5‘ DNA sequence and using the protein sequence which clones the open reading frame. This showed that the open reading frame is perfectly homologous with a sequence encoding 184 amino acids, which is localized on a cDNA by the name of KIAA0282 (gene bank accession number: g87458). The homology to KIAA0282 ends abruptly after the amino acid sequence LQTSEG and is restricted to the nucleotide level at 552 bp. The reading frame in clone 283071 continues for 15 amino acids and is then ended with a TAA stop codon.

However, the reading frame in the KIAA0282 ORF continues for 123 amino acids. This region and the complete 3’ untranslated region of about 2000 bp of the KIAA0282 sequence is different to the sequence of clone 283071. For illustration, see Figure 1.

Example 3: Verification of the binding of the 3‘ region of TTO20 to the 5‘ region of KIAA0282

In order to confirm that the 5‘-coding region of the ORF of KIAA0282 is linked to the 3’ region of TTO20, further publicly accessible EST clones were sequenced. In addition to the abovementioned clone 43508, these also include the clone 47831 and the clone 36563, which are obtainable

from Genome Systems. The sequences clearly confirm the correctness of the sequence determined for TTO20.

In order to make clear the connection between the 5' region of KIAA and the 3' region of TTO20 using an independent process, the following PCR strategy was chosen. Two forward primers were derived from the 5' region of KIAA0282. Primer 1: 5'-CAACTCC TGTAT CACC-3' (SEQ ID NO.:1), Primer 2: 5'-AGTGCGATGTCTTCTACTG-3' (SEQ ID NO.: 2). The backward primers 3 and 4 were derived from the 3' region of clone 283071. Primer 3: 5'-TCTAAAGGCAG GAGAGGAAC-3' (SEQ ID NO.: 3). Primer 4: 5'-TTCATGTGTCTTGCTTACTC-3' (SEQ ID NO.: 4). In a connected region, a fragment of size 2097 bp would have to be amplifiable using the primers 1 and 4, and a fragment 1211 bp in size using the primer pair 2 and 3. These DNAs were used as matrices for the polymerase chain reaction: brain cDNA from the Multiple Tissue cDNA Panel Human 1 (order number: K1420-1, Clontech, Heidelberg, Germany) and fetal brain cDNA from the Human Fetal cDNA Panel (order number: K1425-1, Clontech, Heidelberg, Germany). 0.5-1 µl aliquots were introduced into a PCR reaction with the primer pair 1 and 4. The PCR reaction was carried out as follows using the buffer recommended by the manufacturer of the enzymes: polymerase mixture: 19 parts of AmpliTaq DNA polymerase (Perkin-Elmer, Weiterstadt, Germany) and 1 part of pfu polymerase (Stratagen, La Jolla, USA). Primer concentration: 10 pM. DNA Cycler (Perkin Elmer, Model 480). PCR conditions for one cycle: 94°C, 2 min.; 94°C, 30 sec.; 59°C, 30 sec.; 72°C, 30 sec.; 72°C, 7 min, reaction volume: 50 µl. After 25-30 cycles, the reaction was cooled to 4°C and the reaction mixture was analyzed in a 1.2% strength agarose gel. It was possible to amplify the corresponding PCR fragment of the size of about 2100 bp both from the brain cDNA and from the fetal brain cDNA. If 0.5-1 µl aliquots of these samples are employed in a further, "nested" PCR reaction using identical conditions, but with the primers 2 and 3, a PCR fragment of a size of 1200 bp results. This clearly indicates the presence of a cDNA having a homologous 5' region of KIAA0282 and the 3' region of TTO20.

For further confirmation, the DNA fragment 2100 bp in size was excized from the agarose gel and the DNA was eluted electrophoretically (Ausubel et al.). The eluted DNA was taken up in 10 µl of sterile water and cloned into the vector pCR2.1 according to the recommendations of the manufacturer (Invitrogen BV, Groningen, Holland). The cloning of the PCR

fragments was carried out with the aid of the TA cloning kit of the same manufacturer using the procedure specified by the manufacturer.

The sequencing of the cloned DNA fragment was carried out by the chain termination method according to Sanger et al. with the aid of the dye terminators and a model 377 sequencer (Perkin Elmer, Langen, Germany). The sequencing exactly confirms the finding obtained by PCR analysis. Accordingly, the sequence indicated in Table 1 (nucleotides 685 to 3262) can be extended on the 5‘ side by the DNA sequence specified by KIAA0282. The complete sequence is shown in Table 1 (nucleotides 1 to 3262).

Example 4: Indications of function

The origin of the ESTs from various tissues indicate an involvement of the expressed gene in inflammatory processes. The RNA analyses (see EP-A 0 705 842) confirm a more than four-fold induction of the expression of the gene in interleukin 1ß-stimulated chondrocytes. On the basis of the bioinformatic analyses, the discoverers of the cDNA for KIAA0282 propose a homology of the encoded protein with a zinc finger protein. Zinc finger proteins can act as transcription factors, i.e. play an essential role in the regulation of the cell process. In order to obtain immediate information about a possible function of TTO20, an antisense expression set with a subregion of the sequence (nucleotide 705 – nucleotide 3198 of Table 1) was selected. This range includes both part of the coding and the non-coding 3’ region of the cDNA.

4.1. Construction of the antisense expression vector

The starting vector chosen was the dicistronic vector pED 4 (Fig. 2; Kaufman et al., Nucleic Acids Research 19, 448 – 4490 (1991)). For this vector, whose construction is described in detail in Kaufman et al. and whose precursor plasmid pMT2 is obtainable under the No. ATCC 67122 at the ATCC, it has already been shown that antisense RNA can be expressed via the first cistron. The vector contains the following structural elements: replication origin for replication in eukaryotic cells, the SV40 expression enhancer, the adenovirus Major Late Promotor, the leader sequence of the Adenovirus Major Late Messenger RNA, a hybrid intron with a splice site, the encephalomyocarditis virus leader sequence, the

selection marker dihydrofolate reductase, the SV40 polyadenelation signal, the adenovirus V1 RNA gene, a replication origin and a selection marker for the replication of the vector in recombinant E. coli. In a first step, the selection marker dihydrofolate reductase should then be exchanged for the gene for the green fluorescent protein (GFP). The expression of the GFPs via the second cistron thus serves in transient expression experiments as a visible measure of the expression of the antisense construct.

Isolated plasmid DNA from pED4 was cleaved using the restriction endonucleases Cla1 and Xho1. For the isolation of the gene for GFP, DNA of the commercially obtainable vector pEGFP (Clontech, Heidelberg, Germany) was cleaved using the restriction enzymes Sal1 and Not1. The DNA fragments were separated by gel electrophoresis. As the singular cleavage site of Cla1 lies about 350bp after the coding dihydrofolate reductase sequence in the VA gene, a third fragment is needed for the restoration of the PolyA part and of the VA gene section. By means of PCR, a DNA fragment from the vector pED4 which has an Not cleavage site at the 5‘ end and a Cla cleavage site at the 3‘ end and which comprises the still missing sequence of the PolyA part and of the VA gene was amplified.

Primers used:

Forward primer Not L:

5‘-ATAAGAATGCGGCCGCTAAACTATCAGGAAGATGCTTTCAAGTTC-3‘ (SEQ ID NO.: 5)

Backward primer Cla R:

5‘-ACAGGCTCTCCTTTTGCAC-3‘ (SEQ ID NO.: 6)

The PCR product was digested with Not1 and Cla1, separated from nucleotides by gel electrophoresis and isolated. The three fragments were pipetted together in equimolar ratio, mixed with buffer, ATP and T4 ligase and ligated. The ligation mixture was transformed in E. coli DH5 α cells and plated out on ampicillin selection plates. After isolation of the plasmid DNA from transformants, the DNAs were checked for their fragment size by means of a BamH1 restriction digestion. The original vector pED4 has three BamH1 cleavage sites, which afford three DNA fragments of sizes of

about 2950 bp, 2190 bp and 220 bp. As a result of the gene exchange, the novel vector T782 has four BamH1 cleavage sites which lead in a BamH1 digestion of the plasmid DNA, to four DNA fragments of about 2950 bp, 1315 bp, 1075 bp and 220 bp. The correct sequence of the newly integrated indicator gene was confirmed by sequencing. The plasmid map of the vector T782 is shown in Figure 3.

For the antisense expression of the RNA of TTO20, the TTO20 sequence was amplified with the aid of PCR and appropriate primers and cloned into the GFP vector T782 via the Eco R1 cleavage site.

Primers used:

Forwards primer:
5'-GGAATTCGATCAGATCTCTCACTGCAC-3' (SEQ ID NO.: 7)

Backward primer:
5'-GGAATTCACTTCTGTGCAGTAACAGAG-3' (SEQ ID NO.: 8)

The resulting fragment of size about 2500 bp was completely digested using the restriction enzyme Eco R1, separated by gel electrophoresis and isolated. The isolated fragment was cloned into the GFP vector, which was likewise cleaved with Eco R1. In order to ensure that the fragment cloned in was cloned in the antisense direction, various recombinant clones were cleaved using the restriction enzyme Pst1 after transformation of E.coli DH5 α and the fragments were analyzed by gel electrophoresis. Owing to the fact that a singular Pst1 cleavage site is made available for the Eco R1 cloning site by means of the vector, a Pst1 fragment about 310 bp in size additionally results with the correct orientation of the DNA fragment in the vector, while a fragment about 810 bp in size is missing in the pattern of the Pst fragments. The plasmid map of the bicistronic antisense vector T814 is shown in Fig. 4.

4.2. Transfection of human chondrosarcoma cells using the antisense vector

Chondrosarcoma cells are therefore of particular interest, because they can serve as a model for joint disorders. The adherently growing cells were grown in culture flasks (75 cm^2) containing 20 ml of Dulbecco's MOD Eagle

Medium (DMEM with 4.5g of glucose/L) and the following additives: 10% fetal calf serum, 200 mM L-glutamine, 5 mM sodium pyruvate, 20 mM Hepes buffer pH 7.2, 0.02 µg/ml of hydrocortisone, 0.1 µg/ml of insulin, 0.025 mg/ml of ascorbic acid and 0.05 mg/ml of gentamycin. Every 3-4 days, the cells were detached from the culture flasks by trypsinization, taken up in 10 ml of fresh culture medium, freed from the trypsin by centrifugation, diluted in the ratio 1:5 – 1:10 and reinoculated. For trypsinization, trypsin/EDTA was used in a concentration of 0.25% trypsin and 1mM EDTA. 1 ml of this solution remained on the cells for 2-5 minutes at 37°C. The stock culture of chondrosarcoma cells was stored at -80°C in 10% DMSO and 90% of the culture medium.

For the transfection of DNA in chondrosarcoma cells, various methods were tested, among them the calcium phosphate method, the DEAE-dextran method, liposome-mediated transfection methods, use of activated dendrimers, and electroporation. The best transfection rates were achieved when the transfection agent used was Fugene 6 (Boehringer Mannheim, Mannheim, Germany).

Between 2 x 10^5 and 4 x 10^5 cells were inoculated into 1 well each of a 6-well cell culture plate (Bekton & Dickinson, Heidelberg, Germany). The cells were in about 2 ml of nutrient solution. 0.5 – 2 µg each of DNA which was isolated and purified with the aid of the Qiafilter Plasmid Midikit (Qiagen, Hilden, Germany) were employed for the transfection. For this, the cells were first incubated with 5% CO_2 for 18 – 24 h at 37°C in an incubator. The cells should be grown to 50 – 80% confluence. Best transfection rates are achieved when 4 x 10^5 cells/well are inoculated, and 2 µg of DNA and 6 µl of Fugene/100 µl of serum-free medium are used. The transfection was carried out according to the instructions of the manufacturer.

4.3. Antisense expression in chondrosarcoma cells

The transformed chondrosarcoma cells were first tested over a period of 16 – 48 hours after transfection for the appearance of the green fluorescence which takes place due to the expression of the green fluorescent protein GFP. Even after 16 hours, green fluorescent cells can be detected after fluorescence excitation, which markedly increased over a period of 20 h.

For the detection of the antisense RNA formed, cells from two wells in each case of a 6-well plate were used. For the isolation of the RNA, an RNeasy Miniprep Kit from Qiagen (Hilden, Germany) was used according to the instructions of the manufacturer. The RNA samples were taken up in 30 µl of RNase-free water and stored at –20°C. The RNA was analyzed with the aid of Northern blots (Ausubel, F.M. et al. Current Protocols in Molecularbiology, Vol. 1-3, John Wiley and Sons, New York, 1997) using digoxygenin-labeled DNA sample. The labeling of the DNA was carried out in a PCR reaction by means of the DIG Probe Synthesis Kits from Boehringer (Mannheim, Germany). The matrix used was the previously purified PCR fragment containing the TTO20 portion about 2.5 kb in size, which was freed from the nucleotides. On hybridization with this probe, the expression of the antisense RNA in the transfected chondrosarcoma cells becomes clearly visible in a time-dependent manner.

Compared with non-transformed chondrosarcoma cells, the cell form of the transformed cells is not markedly changed. However, an unequal distribution of the GFP fluorescence is visible compared with a controlled transfection which was carried out using a corresponding vector, which instead of TTO20 antisense contained the antisense RNA of cofilin, a cytoskeleton-regulating protein. The spotty fluorescence distribution allows the suspicion that the cytoskeleton of the cells is modified compared with the controls. This indicates a direct function of the expresssed antisense RNA on the suppression of the protein formed from TTO20.

Legend to Figures:

Figure 1: Schematic representation of the clones 283071, 43508, KiAA 0282, TTO20/2 and g 868629

Figure 2: Schematic representation of the plasmid pED4; abbreviations: SV40 = SV40 enhancer and origin of replication; MLP = adenovirus "Major Late" promotor; TPL = leader sequence "Adenovirus Major Late" mRNA; IVS = intron hybrid with splice site; EM C-L = encephalomyocarditis leader sequence; DHFR = selection marker dihydrofolate reductase; polyA = SV40 polyadenylation signal; VA I = adenovirus VA I RNA gene.

Figure 3: Schematic representation of the plasmid T 782; for abbreviations see Fig. 2.

Figure 4: Schematic representation of the plasmid T 814; for abbreviations see Fig. 2.

Table 1:

TTO 20-2 gene from 1 to 3262

```
1 GCCCTGGCCCCGGTGCCCCGCAACTCCTGTATCACCTGCCCCCAGTGTCACCGCAGCCTC

  1 A  L  A  P  V  P  R  N  S  C  I  T  C  P  Q  C  H  R  S  L  -

 61 ATCCTGGATGACCGGGGGCTCCGCGGCTTCCCCAAGAATCGCGTACTGGAAGGGGTAATT

 21 I  L  D  D  R  G  L  R  G  F  P  K  N  R  V  L  E  G  V  I  -

121 GACCGCTACCAGCAGAGCAAAGCCGCGGCCCTCAAGTGCCAGCTCTGCGAGAAGGCGCCC

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481 CAGCTCTCCCAGGCGCTGAACGGACTGTCAGACAGGGCCAAAGAAGCCAAGGAGTTTCTG

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Patent claims:

1. The TTO 20 protein comprising the amino acid sequence as in Table 1 (SEQ ID NO.: 10).

2. A DNA, coding for the protein as claimed in claim 1, selected from the group comprising
   (a) the DNA sequence as in Table 1 (SEQ ID NO.: 9), in particular nucleotides 1 to 1305;
   (b) a DNA which is at least 80% identical with a DNA as in (a);
   or
   (c) a DNA which, with respect to the genetic code, is degenerate to the DNAs from (a) and (b).

3. An antibody which binds to the protein as claimed in claim 1.

4. An expression vector for the expression of a DNA as claimed in claim 2.

5. A host cell transfected or transformed with an expression vector as claimed in claim 4.

6. A process for the preparation of the TTO 20 protein as claimed in claim 1, comprising the steps:

   (a) culturing a host cell under conditions which make possible the expression of the TTO 020 protein;
   and
   (b) isolation of the TTO 20 protein from the host cells or the culture medium.

7. The TTO 20 protein as claimed in claim 1, obtainable by a process as claimed in claim 6.

8. A process for the identification of cell lines, cells or tissues which express the TTO 20 protein as claimed in claim 1, in which a nucleic

acid probe is used which is hybridized with an RNA in a biological sample which is derived from a DNA as claimed in claim 2.

9. The use of the DNA as claimed in claim 2 (a) for the completion of the DNA and protein sequence as in Table 1 by means of hybridization and/or PCR processes.

10. The use of the TTO 20 protein as claimed in claim 1 for the determination of its three-dimensional structure and the design of inhibitors or activators of the TTO 20 protein.

11. The use of the TTO 20 protein as claimed in claim 1 for finding substances which influence the activity of TTO 20.

12. A diagnostic kit for the diagnosis of inflammatory disorders, in particular rheumatoid arthritis, comprising an anitbody as claimed in claim 3.

13. A diagnostic kit for the diagnosis of inflammatory disorders, in particular rheumatoid arthritis, comprising a DNA as claimed in claim 2.

HMR 1999/L026 WO

Abstract

Novel transcription factor TTO 20

The invention relates to the cell regulation factor TTO 20, DNA coding therefor, its preparation and use, antibodies binding to TTO 20 and the use of DNA coding for TTO 20 and an antibody binding to TTO 20 for use in a diagnostic kit for the detection of inflammatory disorders, in particular rheumatoid arthritis.

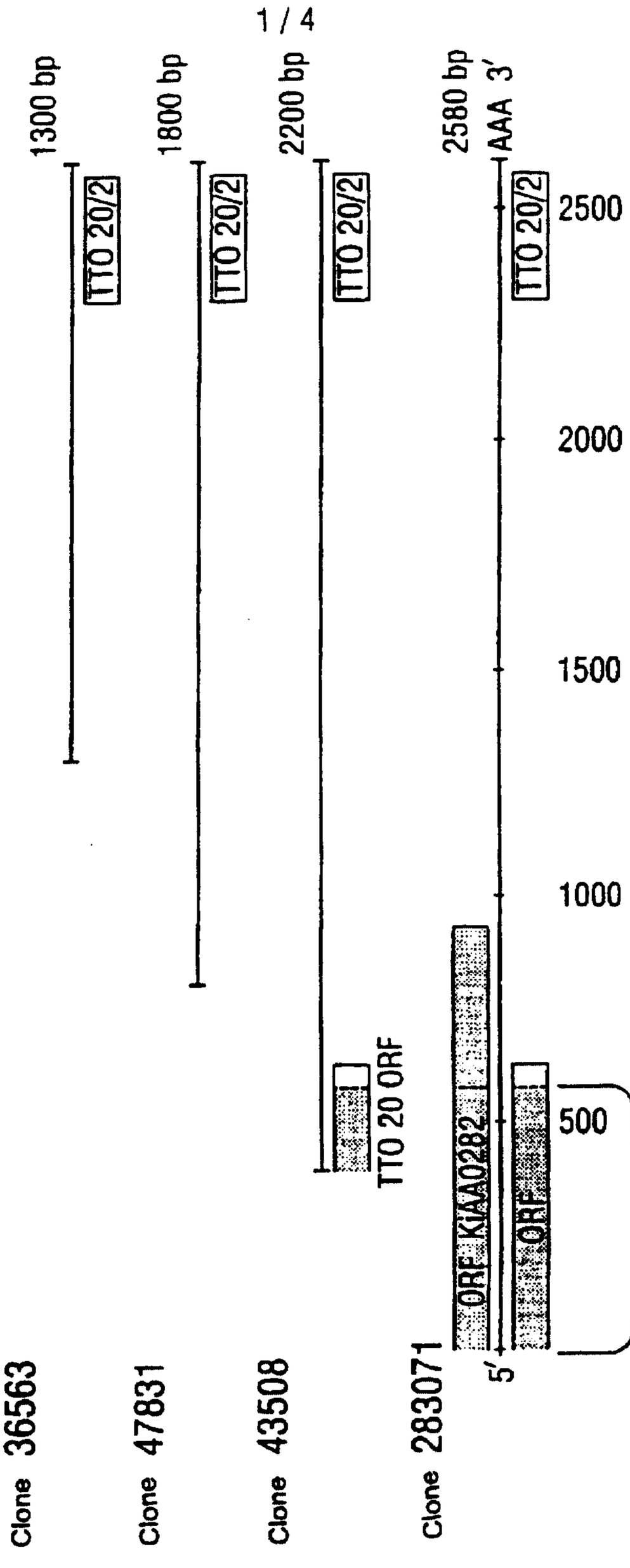
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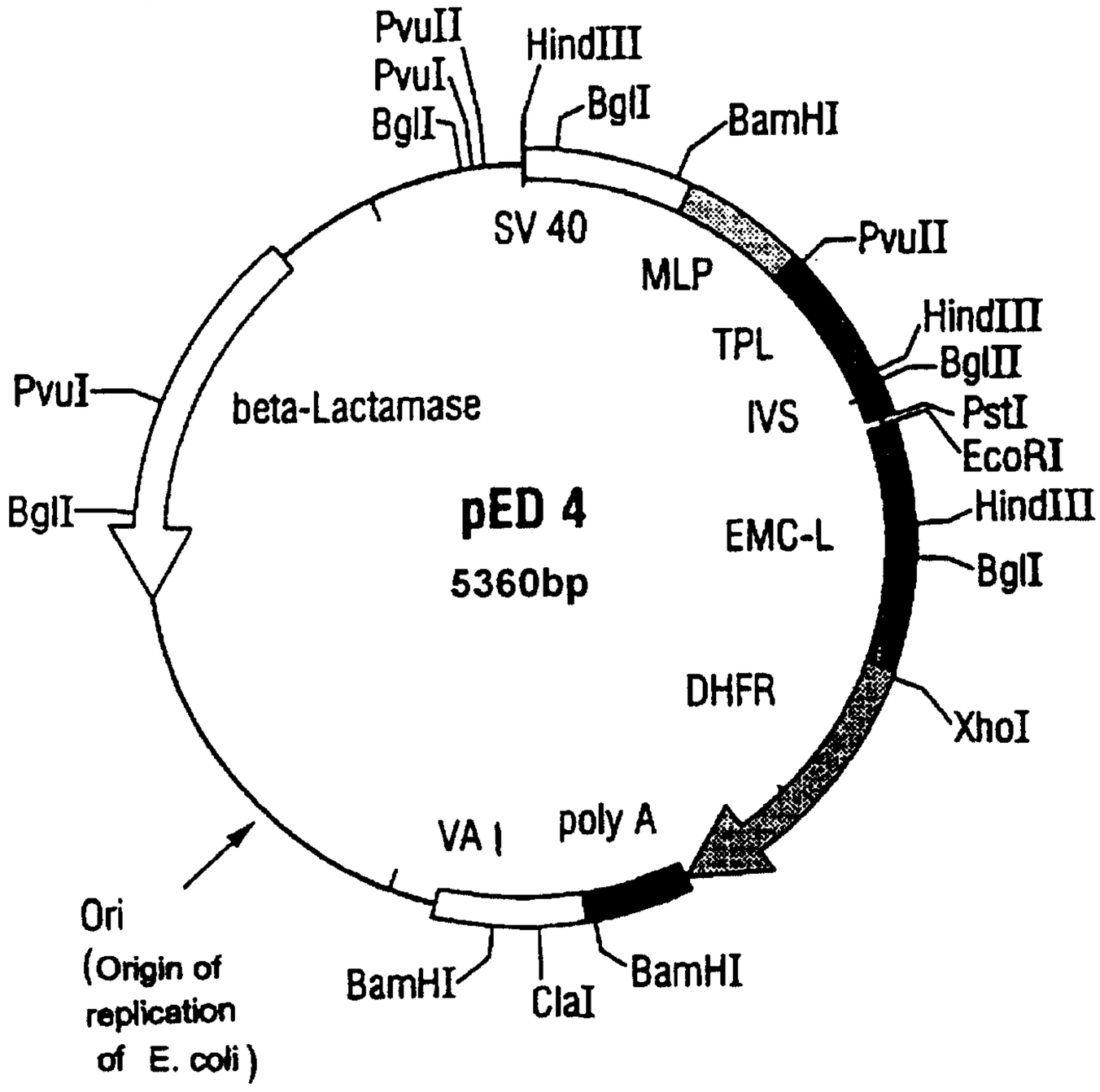
Fig. 1

Fig. 2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BglI
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XhoI
poly A
VA I
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Fig. 3

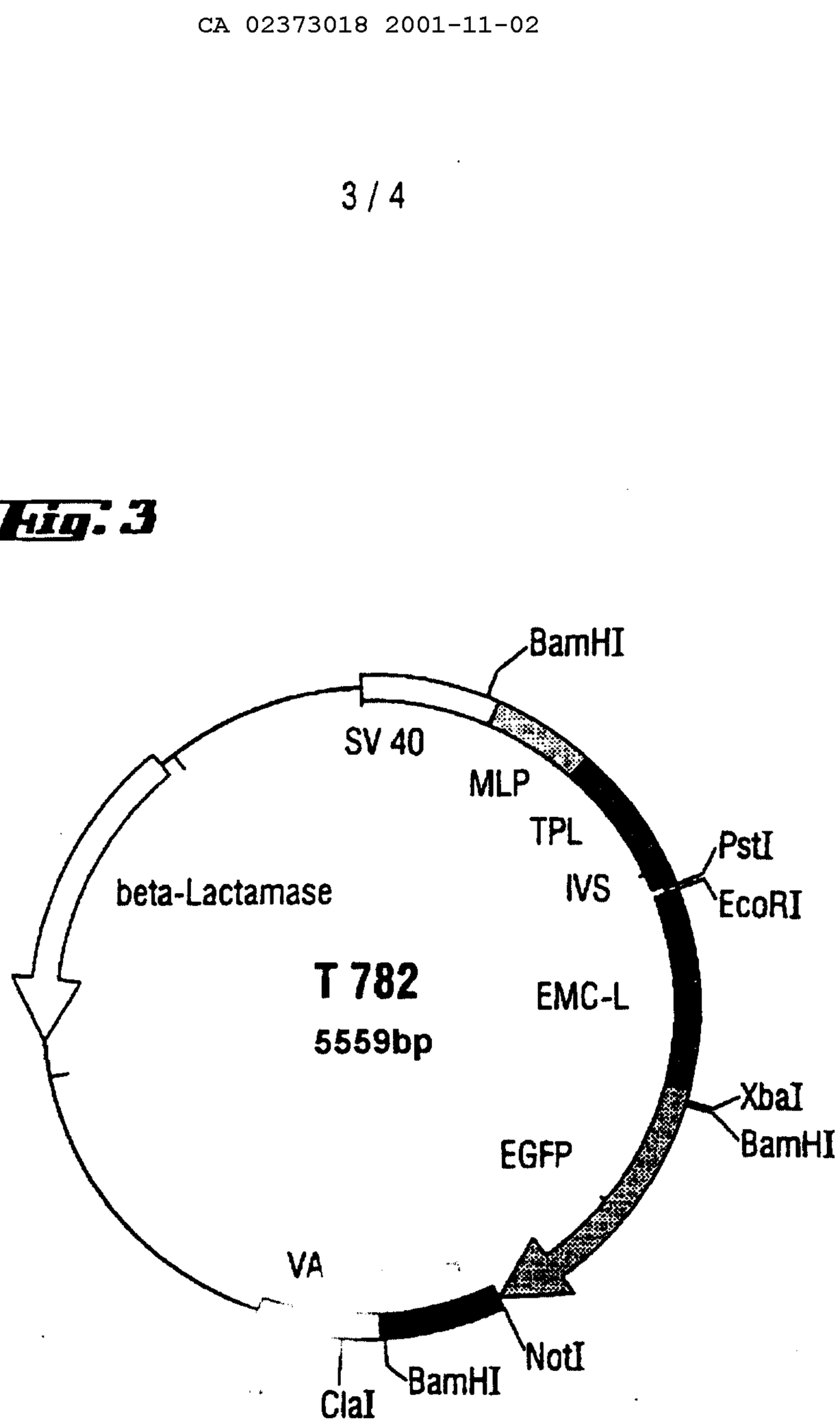
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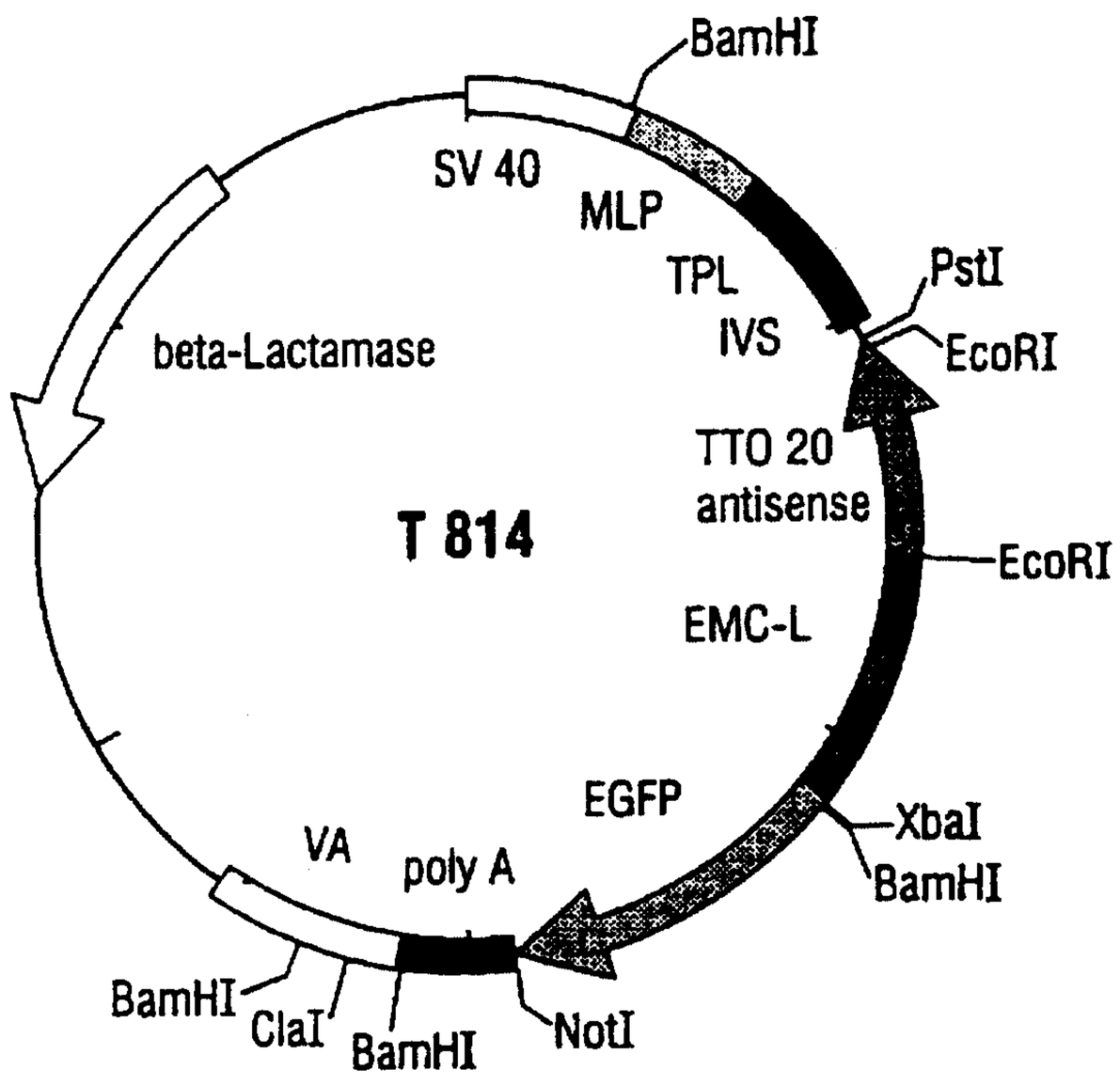
Fig. 4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