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# Identification and expression of the cDNA of KIN17, a zinc-finger gene located on mouse chromosome 2, encoding a new DNA-binding protein

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## ABSTRACT

**We report the cloning of *KIN17* cDNA, 1414 bp long with an ORF of 391 residues showing a zinc finger and nuclear localization signals. By recloning the cDNA into an appropriate vector, we produced kin17 protein in *E. coli*, purified it partially and shown that kin17 protein binds to double-stranded DNA. The *KIN17* gene was localized by cytogenetic mapping in mouse chromosome 2, band A. Genomic sequences homologous to *KIN17* cDNA were detected also in rat and human DNAs. *KIN17* mRNA is highly expressed in rodent transformed AtT-20 neuroendocrine cells whereas it can be detected only in the total RNA of mouse embryos and various normal adult tissues by reverse transcription and PCR amplification. The mouse nuclear kin17 protein was identified by a local small structural similarity with *E.coli* recA protein. Kin17 and recA have only 39 amino acid residues in a region that might be involved in DNA-binding.**

## INTRODUCTION

Antibodies have been used for long to detect proteins sharing structural similarities. The immunological detection of epitopes coupled to recombinant DNA technology has provided a particularly potent tool to clone new genes coding for polypeptides even poorly expressed.

Since we detected *nuclear* proteins recognized by anti-recA antibodies in *actively dividing cells* of mouse embryo (1), we set to clone the corresponding cDNAs in the hope of identifying proteins of interest.

We went through two successive steps. First, we cloned from embryonic mRNA a partial cDNA fragment called $KIN17_{601}$ (2). Second, we have used this fragment as a probe to clone *KIN17* cDNA and characterize the encoded protein. This is what we report here.

*KIN17* cDNA is 1414 bp long with an ORF of 391 residues with a zinc finger and nuclear localization signals. We have produced kin17 protein in *E. coli*, partially purified it, and demonstrated that kin17 binds double-stranded DNA.

*KIN17* gene was localized by cytogenetic mapping in mouse chromosome 2, band A. Genomic sequences homologous to *KIN17* cDNA were detected also in rat and human DNAs. *KIN17* mRNA is highly expressed in rodent transformed AtT-20 neuroendocrine cells whereas it is poorly detected in the total RNA of mouse embryos and various normal adult tissues even if we use reverse transcription and PCR amplification.

Kin17 protein is a newly identified mammalian nuclear protein as shown by computer analysis. The region of homology shared with prokaryotic recA proteins spans only 39 residues. This region is large enough to define an antigenic determinant and may be a motif that governs DNA-binding.

## MATERIALS AND METHODS

### Media, cultures, phages, plasmids, bacterial strains, and cell lines

M9 minimal medium was supplemented with 1% casamino acids, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.2% glucose and 100 μg/ml ampicillin. Bacteria were all derivatives of *E. coli* K12, including JM103 (3).

Strain GY7648, a kind gift of A. Bailone, is BL21 (4) deleted of *recA*, with the T7 RNA-polymerase gene under the control of the IPTG-inducible *lac*-UV5 promoter. The 1430 bp NcoI-BamH1 DNA fragment of plasmid pKGC101, which contains the ORF, the polyadenylation signal and 48 nucleotides of vector pcD2, was ligated with plasmid pET8c (4), digested with NcoI and BamH1. GY7648 bacteria were transformed with this mixture and selection was for an IPTG-induced kin17 protein strain called GY8544. GY8544 carries plasmid pKGC102:

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pET8c-*KIN17*. The pET8c vector was introduced into GY7648 to give GY8548 to serve as control. Plasmid pGEM3$^+$ DNA was from Promega (Coger).

Transformations and lysogenizations were as in (5, 6). AtT-20 (7) and RIN cell lines (8) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (9).

### Isolation of cDNA clones from the pcD2 library

The KIN17$_{601}$ cDNA fragment of λ-KIN17 (2) was used as a probe for the screening of the pcD2 cDNA library from the MB66 mouse cell line (kindly provided by R.Bertolotti and H.Okayama) (6).

### Affinity-purification of anti-kin17 antibodies

The fused protein β-galactosidase-kin17 carried by phages λKIN17 was induced with IPTG after infection of GY6334 bacteria and incubation for 4 hr at 42°C and absorbed onto nitrocellulose filters, and then incubated with rabbit antibodies (2). The affinity-purified antibodies immobilized on each filter were eluted (10, 11). The antibodies were also affinity-purified against non-recombinant λgt11 phages as a control.

### Detection of *KIN17* transcripts and genomic DNAs

*KIN17* transcripts were detected by northern blotting (3). RNAs were electrophoresed in 1% agarose containing 2.2 M formaldehyde, transferred to hybond N (Amersham), hybridized at 65°C in 6×SSC to the *KIN17*$_{601}$ DNA, which was randomly labeled using the multiprime Amersham kit. To amplify RNA transcripts by PCR (12), we used 5′ CTGGAACTTCTG-AGGCGACGC as forward *KIN17* specific primer and 5′ GTTGCCGACGGATGGTTTCTGG as reverse primer. The primers delimit a 229 bp fragment from nucleotide 252 to nucleotide 481 (Fig. 1). The amplified DNAs were separated by gel electrophoresis on 4% NuSieve agarose (FMC), transferred to a membrane and hybridized with the radiolabelled *KIN17*$_{601}$ probe.

Restriction digested genomic DNAs were separated on 0.8% agarose gels by electrophoresis and hybridized as above (3).

### Chromosomal location of the *KIN17* gene

**Gene mapping by** *in situ* hybridization in the mouse was performed using metaphase spreads from a WMP male mouse, in which all but 19 autosomes were in the form of metacentric robertsonian translocations.

Concanavalin-A-stimulated lymphocytes were cultured at 37°C for 72 hr with the addition of 5-bromodeoxyuridine for the final six hr of culture (60 μg/ml of medium) to ensure a chromosomal R-banding of good quality.

The pGEM-*KIN17*$_{601}$ plasmid was labelled by nick-translation with tritium ($1.6\times10^8$ dpm×μg$^{-1}$). The radiolabelled probe was hybridized to metaphase spreads at a final concentration of 25 ng per ml of hybridization solution (13).

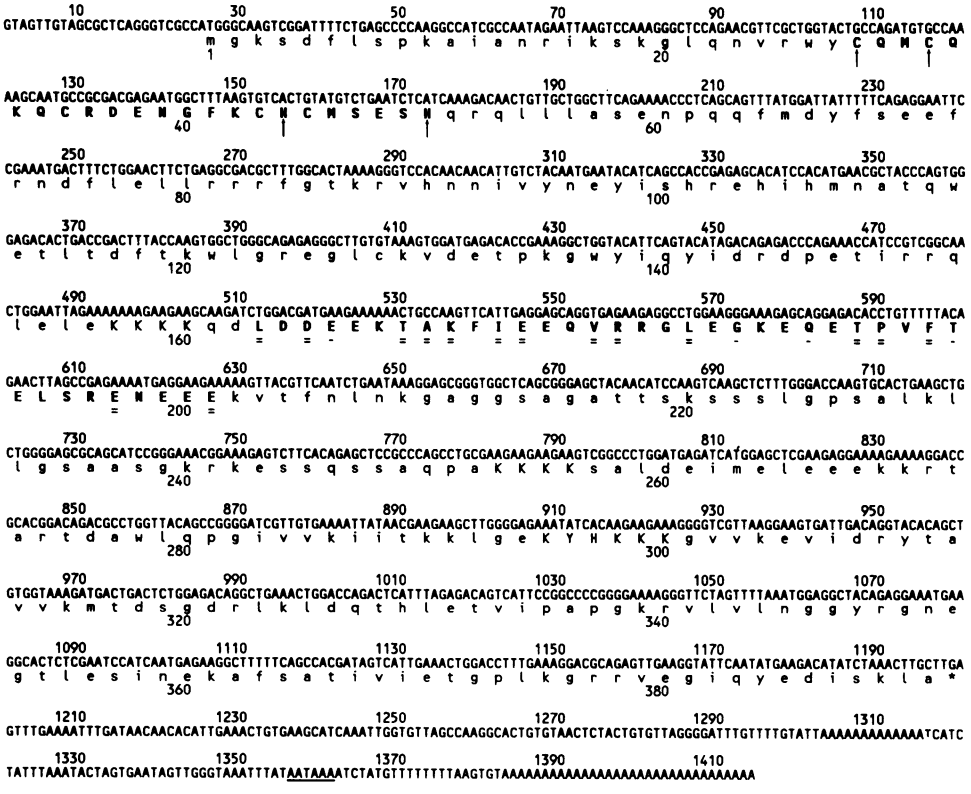

**Figure 1.** Nucleotide sequence of the insert and predicted amino acid sequence of kin17 protein. Numbers above the sequence designate nucleotides and those below amino acid residues. The *KIN17*$_{601}$ probing sequence is between nucleotides 234 and 835. The amino acid residues are designated with lower case characters and with capitals for particular motifs. The ATG at nucleotide 25 is taken as translation initiation codon. The zinc-finger is between residues 28 and 50, arrows pointing to cysteine and histidine residues. Three putative nuclear localization signals are between residues 157–160, 253–256, and 295–300. The polyadenylation signal between nucleotides 1356 and 1362 is underlined. The recA-like antigenic determinant is between amino acids 162 and 201. Residues identical to recA's are indicated by = whereas conserved substitutions by –.

After coating with nuclear track emulsion (KODAK NTB2), the slides were exposed for 15 days at + 4°C, and then developed.

Chromosome spreads were first stained with buffered giemsa solution and metaphases photographed to avoid any slipping of silver grains during the banding procedure. R-banding was then performed by the fluorochrome-photolysis-giemsa (F.P.G.) method and metaphases were re-photographed before analysis.

### Expression of *KIN17* cDNA in *E. coli*, partial purification and detection of kin17 protein-DNA interaction

GY8544 bacteria at $OD_{600}$ = 0.6 were treated with 1 mM IPTG, incubated for 3 hr at 37°C to induce T7 RNA polymerase and centrifuged at 20°C. After washing the pellets with 1/10 of the original volume of 50 mM Tris-HCl, pH 8.0, 1 g of wet cells were resuspended in 2.5 ml of 50 mM Tris-HCl, pH 7.8, 10% (w/v) sucrose, frozen in dry ice and stored at −80°C. Before lysis, the cells were thawed in a water/ice bath and supplemented with 25 µl of 4 M NaCl and 75 µl of lysozyme at 4 mg/ml. After incubation for 15 min on ice and 5 min at 37°C, 3 ml of 4 M NaCl were added and the suspension was stirred for 2 hr at 4°C and centrifuged (65.000 g) for 30 min at 4°C.

The supernatant was dialyzed against 2 l of 10 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA and 10% glycerol for 18 hr. Most of kin17 protein precipitated during dialysis and was solubilized in 5 M urea, 0.1 M NaCl, 0.1% Triton X-100. Eight ml of the solution were loaded onto a 3 ml Heparin-Ultrogel column (IBF)(14).

The column was washed first with 10 ml 5 M urea, 50 mM Tris-HCl pH 7.5, 0.1 M NaCl, 1mM DTT, 1 mM EDTA, 0.1% Triton X-100 then with 10 ml of running buffer (50 mM Tris-HCl pH 7.5, 1 mM EDTA and 1 mM DTT, 0.1% Triton X-100 and 10% v/v glycerol) + 0,1 M NaCl, with 10 ml of running buffer + 0.2 M NaCl and, finally, kin17 protein was eluted in running buffer + 0.6 M NaCl. The heparin-purified fraction was dialyzed overnight at 4°C against 2 l of D buffer (50 mM Tris-HCl pH 7.5, 20 µM $ZnCl_2$, 0.1 M NaCl, 0.1% Triton X-100 and 10% glycerol) and 1 ml was loaded onto a 1 ml column of calf thymus double-stranded (ds) DNA-cellulose (Pharmacia).

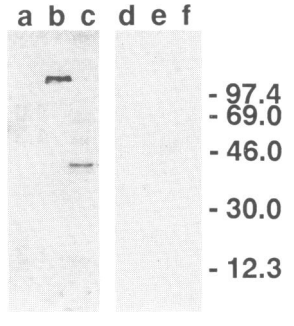


**Figure 2.** Anti-λKIN17 affinity-purified antibodies react with *E. coli* recA protein. 5 µg of total extracts of λgt11 lysogens (a and d) and of λKIN17 lysogens (b and e), and 2 ng of recA protein (c and f) were separated on an 11% SDS-polyacrylamide gel, transferred onto a nitrocellulose filter and probed with antibodies affinity-purified using the proteins present either in λKIN17 plaques (a, b, c) or in λgt11 plaques (d, e, f). After incubation with alkaline phosphatase conjugate horse anti-rabbit IgG, the reacted complexes were visualized with BCIP-NBT (41). The $M_r$ markers ($\times 10^3$) are from the top: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome C.

After washing the ds-DNA-cellulose column with 6 ml of D buffer, the kin17-containing fraction was eluted with D buffer adjusted to 1 M NaCl. In a mock experiment, a cellulose column replaced the ds-DNA cellulose column.

### Sequence analysis

Searches for homologies and protein motifs were performed mainly using the National Biomedical Research Foundation (NBRF) data base as well as the programs and facilities of the Centre InterUniversitaire de Traitement de l'Information (15).

## RESULTS

### Isolation of *KIN17* cDNA

Using $KIN17_{601}$ cDNA as a probe, we screened $3 \times 10^5$ colonies from a pcD2 cDNA library. We identified 10 positive clones among which two had inserts of about 1.4 kbp.

One insert, 1414 bp long, carried by plasmid pKGC101 is the largest, as confirmed by extensive PCR analysis. It shows a single open reading frame (from nucleotide 25 to 1198), coding for a protein of 391 amino acids with a calculated molecular ratio of 44693 (Fig. 1). This ORF has a stop codon (TGA) between nucleotides 1198 and 2000 and includes the 601 bp fragment used as probe (from nucleotide 234 to 835).

*KIN17* ORF is followed by a 3′ non-coding sequence of 213 nucleotides. A polyadenylation signal (AATAAA) is located 21 nucleotides upstream of the start of the polyA tail between nucleotides 1356 and 1361 (Fig. 1).

*KIN17* ORF displays a MET codon at positions 25 to 27, which is in a good context for translation initiation (ĀNN[ATG]G) (16). The upstream region does not contain any other in-frame MET codon and the second in-frame MET codon is located at position 112−114.

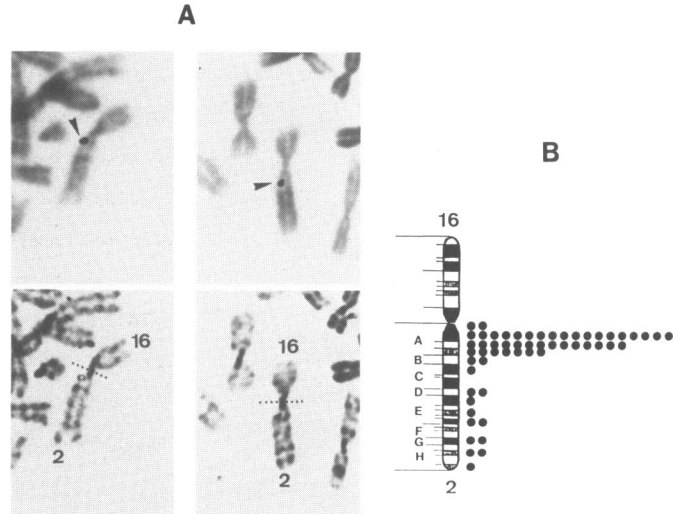


**Figure 3.** Localization of the *KIN17* gene on mouse chromosome 2 by *in situ* hybridization. A: Two partial WMP mouse metaphases, showing the specific site of hybridization to chromosome 2. Top, arrowheads indicate silver grains on Giemsa-stained chromosomes after autoradiography. Bottom, chromosomes with silver grains were subsequently identified by R-banding. B: Diagram of WMP mouse Rb (2; 16) chromosome, indicating the distribution of labelled sites.

### A zinc-finger motif in kin17 protein

By comparing *KIN17* cDNA and kin17 protein sequences to those in two data bases, Genbank (release 67) and National Biomedical Research Foundation (NBRF) (release 23), we did not find striking homologies. We observed some slight similarities between kin17 protein and several eukaryotic proteins using the FASTA alignment program (17). Kin17 displayed a small homologous region with some proteins involved in DNA transactions. For example, the kin17 motif lysine-lysine-lysine-serine (KKKS), from residues 253 to 256, is present in DNA topoisomerase II (NBRF accession no. A25630) and in the k-ras mouse transforming factor (NBRF accession no. A01369).

Kin17 protein (calculated pI = 9.1) has several short sequences of basic amino acids from 157 to 160, 253 to 256, and 295 to 300. These short sequences are similar to localization signals of nuclear proteins (18).

Kin17 has a zinc-binding domain, $CX_2CX_9FX_2HX_5H$, between residues 28 and 50 (Fig. 1), which belongs to the CC-HH class of zinc-finger motif (19, 20).

### In scripto basis for kin17 recognition by anti-recA antibodies

In spite of a strong cross-reactivity between kin17 and anti-recA antibodies, kin17 does not display any large homology with recA sequence (Fig. 1) (21, 22). Yet, kin17 has a 39 amino acid fragment ($kin17_{163-201}$) significantly homologous to a recA protein fragment ($recA_{308-347}$). Fifteen of the 39 residues are identical in the two proteins and 4 are conservative substitutions (2). This homology may account for the cross-reactivity between kin17 protein and anti-recA antibodies.

Narrowing down the range to $kin17_{169-181}$, eight residues are identical in $recA_{315-327}$; they occur singly, in two pairs and in a group of three (Fig. 1). This distribution might correspond to the tertiary structure recognized by anti-recA antibodies.

### Experimental basis for kin17 recognition by anti-recA antibodies

Kin17 and recA polypeptides share antigenic determinants as shown below:

1) Antibodies, affinity-purified against proteins in λKIN17 plaques, recognize recA protein, showing the cross-reactivity between kin17 protein and anti-recA antibodies. The epitope-purified antibodies against λKIN17 recognized the β-galactosidase-kin17 fusion protein and recA protein, but did not react with β-galactosidase (Fig. 2: lanes b, c). These three proteins did not react with epitope-purified antibodies against λgt11 (Fig. 2; lanes d, e, f). Thus, the observed cross-reactivity is due to a kin17 epitope rather than to contaminated anti-recA antibody preparation.

2) Anti-recA antibodies recognize kin17 protein produced in *E. coli*, eliminating a possible artifactual antigenic determinant at the interface kin17-β-galactosidase (Fig. 6).

3) Kin17 and recA proteins compete for binding to anti-recA antibodies, as evidenced by the suppression of the λKIN17 positive signal after pre-incubation of antibodies with pure recA protein (2).

### The mouse KIN17 gene is on chromosome 2, band A

The *KIN17* gene was localized by cytogenetic mapping. In the 150 metaphase cells examined after *in situ* hybridization, there were 312 silver grains associated with the chromosomes. Fifty-five grains (17.6%) were located on chromosome 2 in a non-random distribution: 41/55 grains (74.5%) mapped to the [A1−A3] region of chromosome 2 (Fig. 3) along with the vimentin gene (23).

The $KIN17_{601}$ probe hybridized with mouse, rat and human genomic DNAs, indicating that the sequence is widespread in mammals (Fig. 4 and 5).

### Partial purification of kin17 protein and binding to double stranded DNA

A major protein of apparent $M_r$ of 43 kDa, cross-reacting with anti-recA antibodies (Fig. 6, panel 2, lanes a and b) was synthesized only when the *KIN17* gene was in an expression

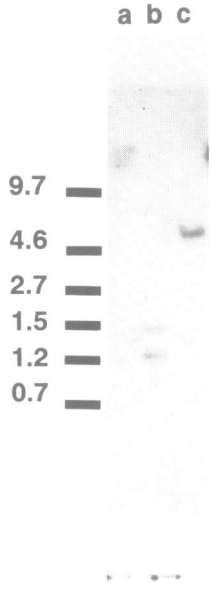


**Figure 4.** Southern blot analysis of *KIN17* sequences in mouse DNA. Mouse liver genomic DNA (20 μg), digested with BglII (lane a), or EcoR1 (lane b) or BamH1 (lane c), was separated with a 0.8% agarose gel, transferred to a membrane, hybridized with the radiolabelled $KIN17_{601}$ probe and washed at 65°C for 45 min with 0.1×SSPE, 0.1% SDS. $M_r$ markers in kbp are on the left.

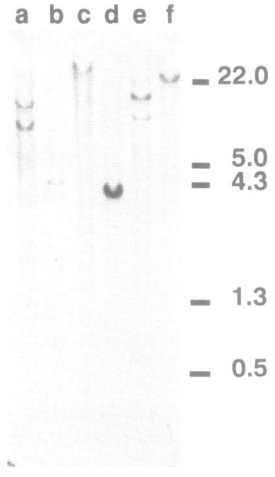


**Figure 5.** Southern blot analysis of *KIN17* sequences in rat and man. Ten μg of rat DNA (lanes a, b, and c) and human DNA (lanes d, e, and f) digested with EcoR1, or BamH1 or Sal1 were separated and hybridized as above and finally washed at 65°C for 30 min with 1×SSPE. $M_r$ markers in kbp are on the right.

vector (4), that is, in GY8544 (pKGC102 [pET8c-*KIN17*]) and not in GY8548 (pET8c) bacteria (Fig. 6, panel 1, lanes a, b, and data not shown).

A partially purified fraction eluted from a heparin column (Fig. 6, lane d) was loaded onto a double-stranded DNA-cellulose column. After extensive washing with running buffer, we observed that kin17 protein was retained on the column and eluted at 1M NaCl. This fraction contains the 43 kDa band representing about 50% of the loaded kin17 protein (lanes e and f). Binding of kin17 to the DNA-cellulose column was optimal in the presence of zinc ions. This observation suggests that the interaction kin17-DNA is zinc-dependent (Mazin, Angulo, Simonin, de Murcia and Devoret, manuscript in preparation).

About 50% of the loaded amount of kin17 protein passed through the DNA-column and was eluted in the first fractions (Fig. 6, lane e). This could be due to a denaturation of kin17 protein upon purification, decreasing the kin17 ability to recognize and bind DNA. An alternative explanation is that the shift in ionic strength may induce protein precipitation or the inactivation of kin17 protein or both.

Kin17 protein did not interact with the cellulose matrix devoid of DNA and went in the flowthrough with all other proteins (Fig. 6, panels 1 and 2, lanes h, and i).

### *KIN17* transcripts in normal and transformed cells

The level of *KIN17* mRNA in mouse embryos was too low to be detected by northern blot. *KIN17* mRNA was revealed only after amplification by PCR. A DNA fragment of the expected size (229 bp) was visualized after gel electrophoresis and ethidium bromide staining (Fig. 7: lane b). This fragment hybridized by southern blot with the $KIN17_{601}$ probe (Fig. 7: lane e), showing that it corresponds to *KIN17* transcripts.

*KIN17* mRNA was also expressed at a low level in mouse tissues (liver, muscle, kidney) as well as in MB66, the cell line used to isolate the pKGC101 clone.

We observed previously that the neural tissues were preferentially reactive with anti-recA antibodies (1). We checked the level of *KIN17* mRNA in two transformed cell lines: a neuroendocrine mouse cell line derived from an anterior pituitary tumor (AtT-20) and in RIN cells derived from a rat insulinoma, used as a control. An elevated concentration of *KIN17* mRNA was detected in both cell lines by northern blots (Fig. 8).

The size of *KIN17* mRNA, determined by northern blots of total RNA of mouse transformed cells, is about 1.8 kbp (Fig. 8).

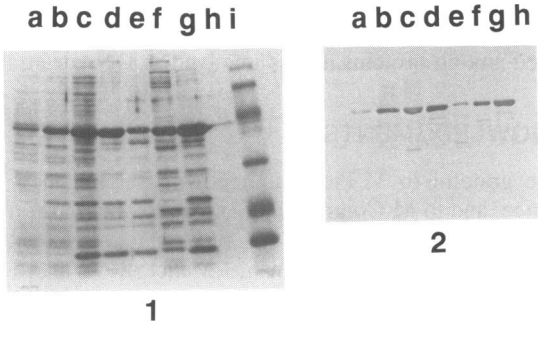


**Figure 6.** Expression in *E. coli* and DNA binding of partially purified kin17 protein. Aliquots of GY8544 bacterial extracts and semi-purified fractions were separated by electrophoresis on a 11% SDS-polyacrylamide gel and the proteins were either stained with Coomassie Blue (panel 1) or transferred onto a nitrocellulose membrane and probed with anti-recA antibodies (panel 2). Lane a: total proteins of untreated cells; lane b: the same but IPTG treated; lane c: cleared lysate fraction of IPTG treated cells; lane d: Heparin-Ultrogel eluted fraction; lane e: DNA-cellulose fraction flowthrough; lane f: 1 M NaCl eluted fraction; lane g: cellulose fraction flowthrough; lane h: eluted fraction. The $M_r$ markers ($\times 10^3$), lane i, indicated by bars are as in Fig. 2.

## DISCUSSION

*KIN17* cDNA nucleotide sequence and that of kin17 deduced polypeptide were compared with DNAs or proteins from various data banks and there were no extensive similarities. We conclude that *KIN17* represents the cDNA of a newly identified gene.

### *KIN17* gene localization

The *KIN17* gene was localized in mouse chromosome 2, band A, in a region where there are few markers. Attempts to localize the *KIN17* gene in the human genome indicate the presence of *KIN17* sequence on the short arm of chromosome 10 (Mattei M-G, data not shown). The vimentin gene is also located in mouse chromosome 2 (23) and in the short arm of the human chromosome 10 (24, 25). The data favor a synteny between the murine 2A region and the human 10p region.

Nucleotide sequences related to the *KIN17* gene are conserved in three mammalian DNAs (mouse, rat and man) and in *Schizosaccharomyces pombe*; the latter data were found by DNA hybridization under stringent conditions (Baldaci and Moussy,

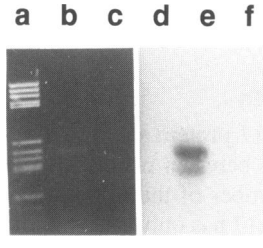


**Figure 7.** Amplification of *KIN17* transcripts from mouse embryo total RNA by RT-PCR. The RNA was reverse-transcribed and amplified by PCR using specific primers. Products were seen after ethidium bromide staining (lanes a to c) or by hybridization with the $KIN17_{601}$ probe after southern blotting (lanes d to f). Lanes a and d: $M_r$ markers given by pBR322 cut with HaeIII: 587, 540, 504, 458, 434, 267, 234, 213, 192 and 184 bp. Lanes b and e: PCR products from mouse embryo total RNA (229 bp expected size). Lanes c and f: PCR products from control samples without cDNA.

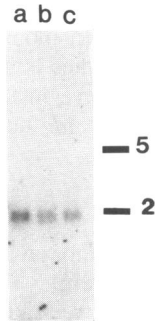


**Figure 8.** Detection of *KIN17* transcripts in rodent cell lines. Total RNAs (1 $\mu$g) of AtT-20 (a) and RIN (b and c) cell lines were separated in a 1% formaldehyde gel, blotted and probed with the randomly labelled $KIN17_{601}$ probe. $M_r$ markers in kbp are on the right.

pers. comm.). Complementation of lower eukaryotes by *KIN17* gene should shed some light on the biological function of kin17 protein in higher eukaryotes.

### Detection of *KIN17* mRNA and size of kin17 protein

The isolation of *KIN17* cDNA was rendered difficult by the fact that *KIN17* mRNA level is very low in embryo tissues. *KIN17* mRNA level is also low in all the adult mouse tissues examined (liver, muscle, kidney) and in the MB66 cell line used to isolate the pcD2-*KIN17* clone. In contrast, a high level of *KIN17* transcript was observed in two transformed cells lines. We do not know yet whether it is due to preferential expression of kin17 protein in some actively dividing cells.

The estimated size of *KIN17* RNA from northern blots of mouse transformed cells total RNA is about 1.8 kbp. The total of the nucleotide sequence of 1414 nucleotides plus a polyA tail of 200 residues (expected for a mammalian mRNA) makes about 1600 bases. *KIN17* cDNA sequence may lack about 200 nucleotides at the 5′ end. The missing nucleotides may span the regulatory region before the start of the open reading frame. This hypothesis is supported by the following arguments: (i) extensive PCR analysis confirmed that pcD2-KIN17 carries the largest *KIN17* cDNA of the screened library, (ii) an AUG codon between 25−27 is in an excellent context for translation initiation (16), (iii) a 40 kDa protein detected with the epitope-purified anti-λKIN17 antibodies in AtT-20 cells extracts or in other mammalian cell lines is compatible with our data (data not shown), (iv) the observed $M_r$ of 43 kDa for *E. coli*-produced kin17 protein agrees with the above data.

### Kin17 protein motifs

*Zinc finger.* The kin17 protein shows a zinc-binding domain of type $CX_2CX_{12}HX_5H$ between residues 28 and 50. Thus, kin17 protein is a new member of the Cys-Cys...His-His zinc-finger protein subclass. Kin17 has only one zinc finger unit as proteins from the Cys-Cys...Cys-Cys subclass. Interestingly, in the zinc-binding domain of kin17 protein, there are 2 cysteines flanking the first histidine residue at the C-terminal part of the motif that might be involved in a Cys-Cys...Cys-Cys type of coordination complex (19, 20). Other zinc finger genes are specifically transcribed in the developing mouse nervous system as well as in tumor cells (26, 27). More recently, Tanaka et al (28) have described a DNA-repair protein, called XPAC, with one zinc-finger and with an apparent $M_r$ close to kin17 (about 42 kDa) and likely implicated in excision of DNA lesions.

*Nuclear localization signals.* In mouse embryos, the material reactive with anti-recA antibodies is in the nucleus (1, 29). V. Schreiber and J. Menissier have just demonstrated that kin17 residues 234 to 264 are *bona fide* nuclear localization signals (pers. comm.).

### About kin17 cross-reactivity with anti-recA antibodies

Three lines of evidence account for an immunological relationship between kin17 and recA protein.

1) There is competition between kin17 and recA protein for binding to anti-recA antibodies, as evidenced by the suppression of the λKIN17 positive signal after pre-incubation of antibodies with pure recA protein (2).

2) Antibodies, affinity-purified against the proteins from λKIN17 plaques, recognize recA protein. This demonstrates that kin17 and recA polypeptides share antigenic determinants (Fig. 2).

3) *E. coli* produced Kin17 protein is recognized by anti-recA antibodies. This rules out that the fusion between kin17 and β-galactosidase may produce a spurious antigenic determinant (see Fig. 6).

### Motif common to kin17 and recA

The 39-residue homologous region between kin17 protein and recA protein is large enough to account for the observed cross-reactivity with anti-recA antibodies. The kin17-recA homology is located in recA carboxy terminal end, a known major antigenic determinant (30). In contrast, the ribonucleotide reductase yeast gene was cloned because the encoded protein cross-reacted with anti-recA antibodies at recA protein C-terminal end (last 5 residues)(31, 32).

Prokaryotic recA proteins have a remarkably conserved amino acid sequence (33). A residue, leucine 309, is conserved in all recA proteins and is observed in the kin17-recA homologous region. RecA protein binds to double-stranded DNA (33) as well as kin17 protein. kin17-recA homologous region may be part of a functional domain involved in DNA binding.

This idea is also supported by the demonstration that four antigenic determinants of recA protein are recognized by monoclonal antibodies, which inhibit homologous pairing, the processive DNA-unwinding, strand-exchange and protein-protein interactions between recA molecules (34, 35). These data indicate a relationship between recA motifs and antigenic determinants.

It remains to be established whether the DNA-binding activity of Kin17 is mediated by the zinc-finger, or by the antigenic motif or whether both regions are part of only one larger domain.

Cases of similarity between mammalian proteins, involved in DNA repair, and their prokaryotic counterparts have been reported. For instance, human genes *ERCC1* and *ERCC2* have amino acid sequences similar to the proteins encoded by excision repair genes such as *RAD10* and *RAD3* of *S. cerevisiae* and *uvrC* and *uvrA* of *E. coli* (36, 37, 38, 39, 40).

The molecular mechanisms of DNA-binding reactions may differ because of the structural differences between the prokaryotic chromosome and the mammalian chromatin. Further investigations will determine whether local similarities in distantly related proteins are part of an evolutionary conserved motif. An alternative possibility is that some convergence may have occurred among proteins motifs involved in DNA transactions.

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