About OMICS Group

OMICS Group is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

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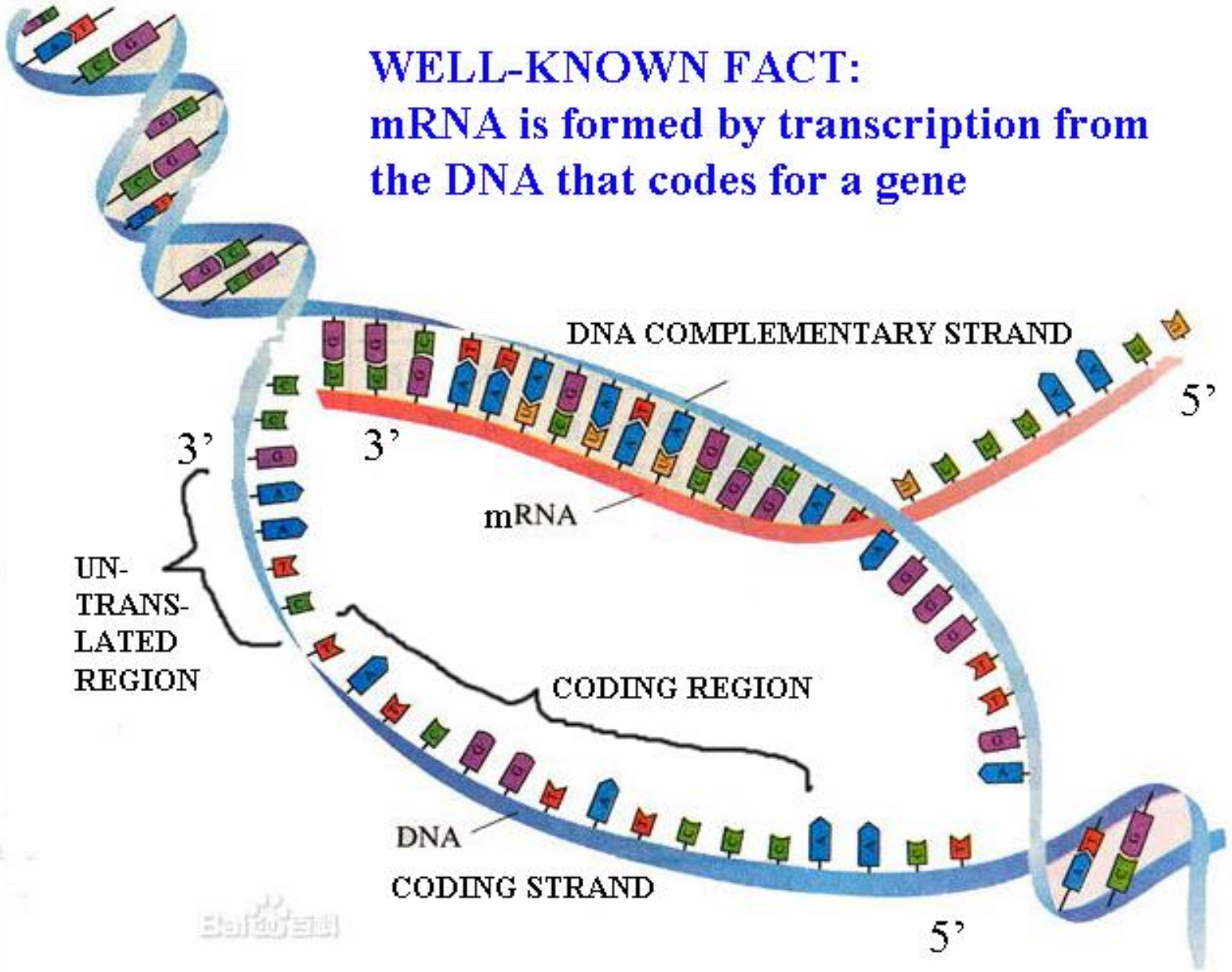
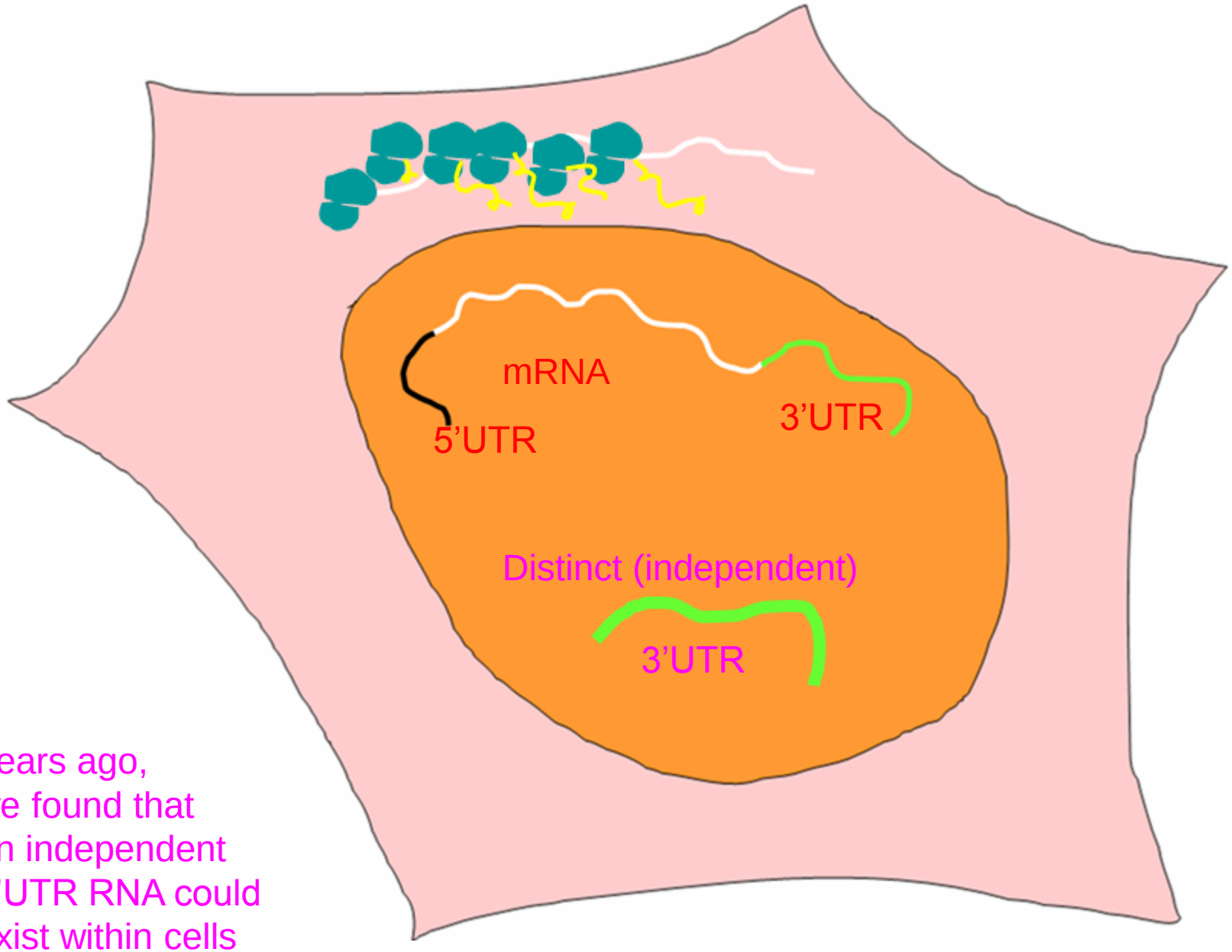
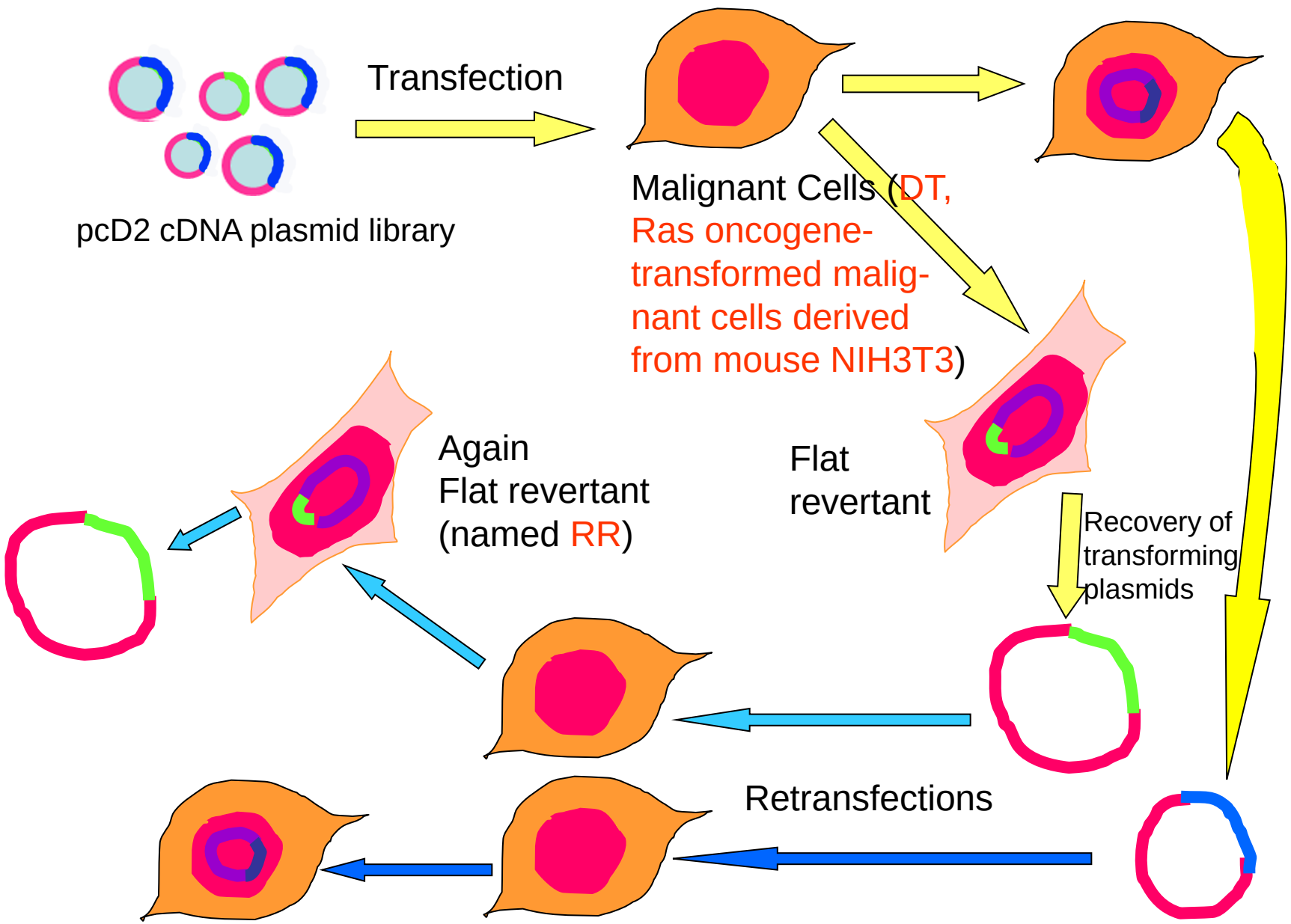
WELL-KNOWN FACT:
mRNA is formed by transcription from the DNA that codes for a gene

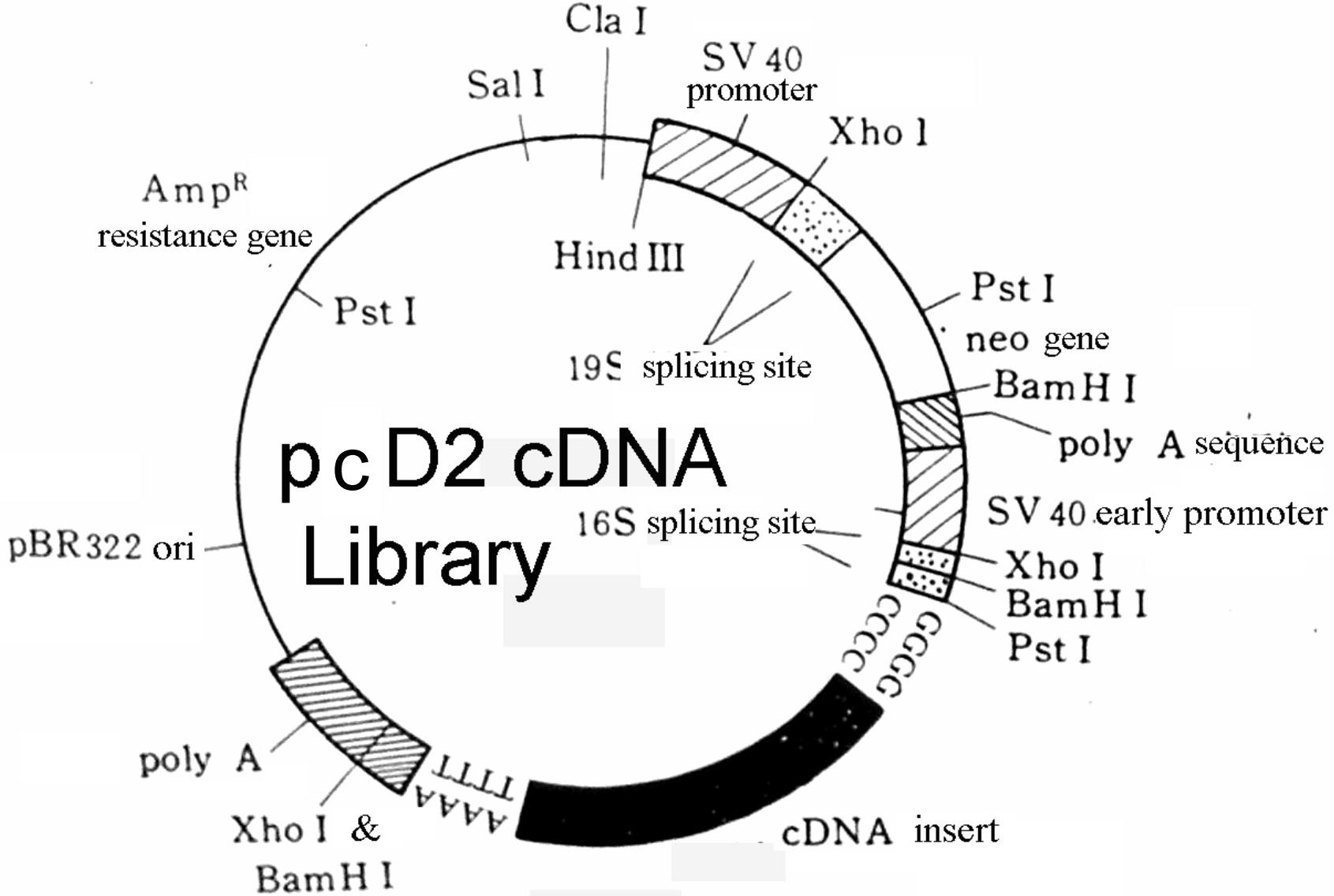




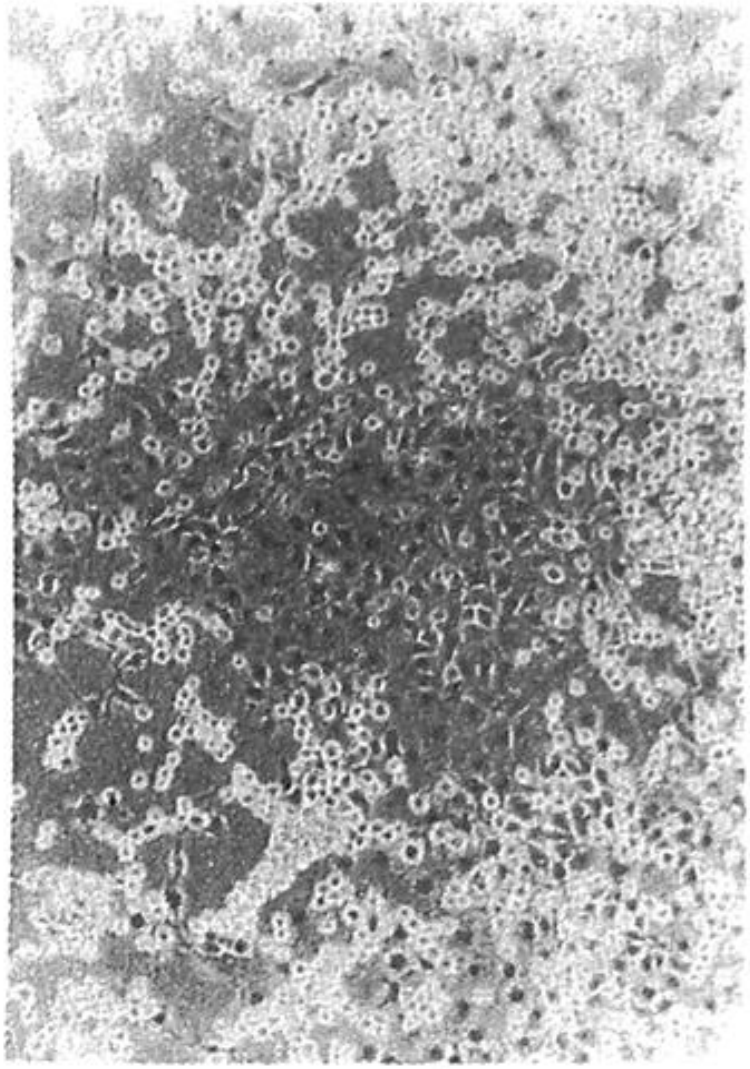
Years ago,
we found that
an independent
3’UTR RNA could
exist within cells
and exert functions.

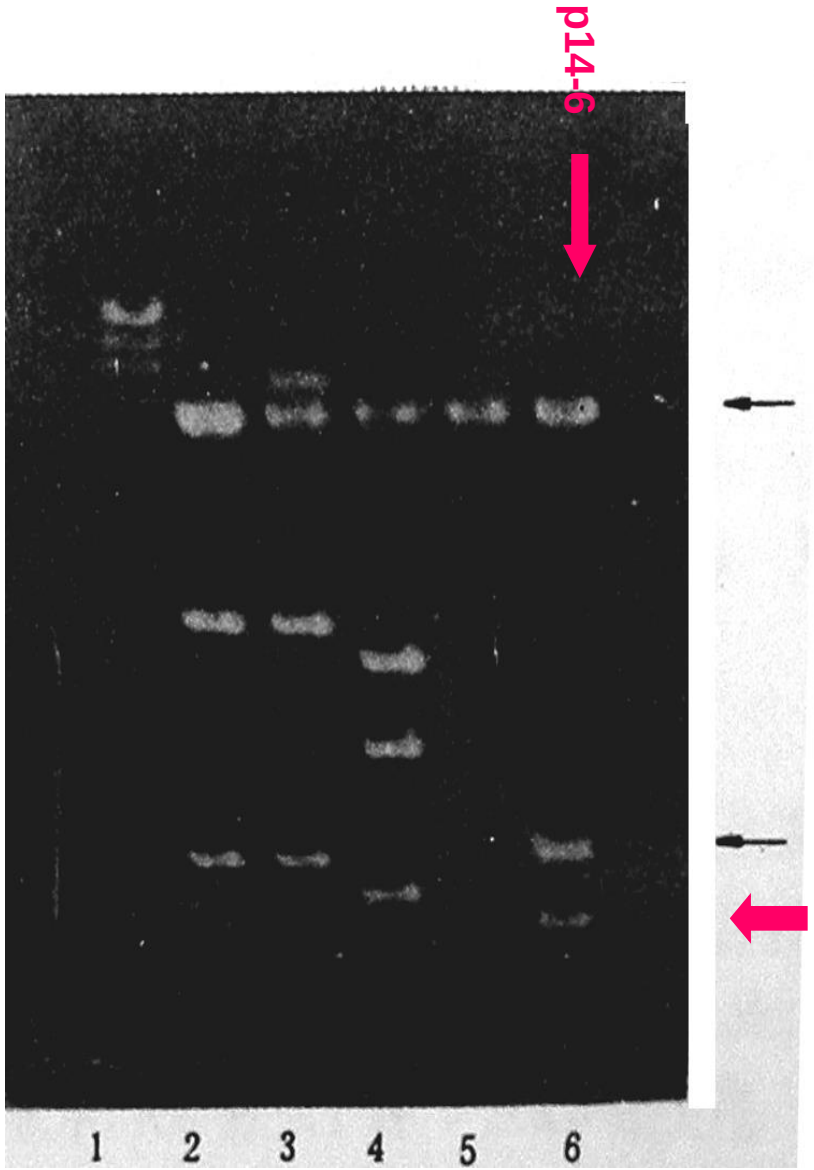
Dr.Makoto Noda's strategy to screen tumor suppressor cDNAs



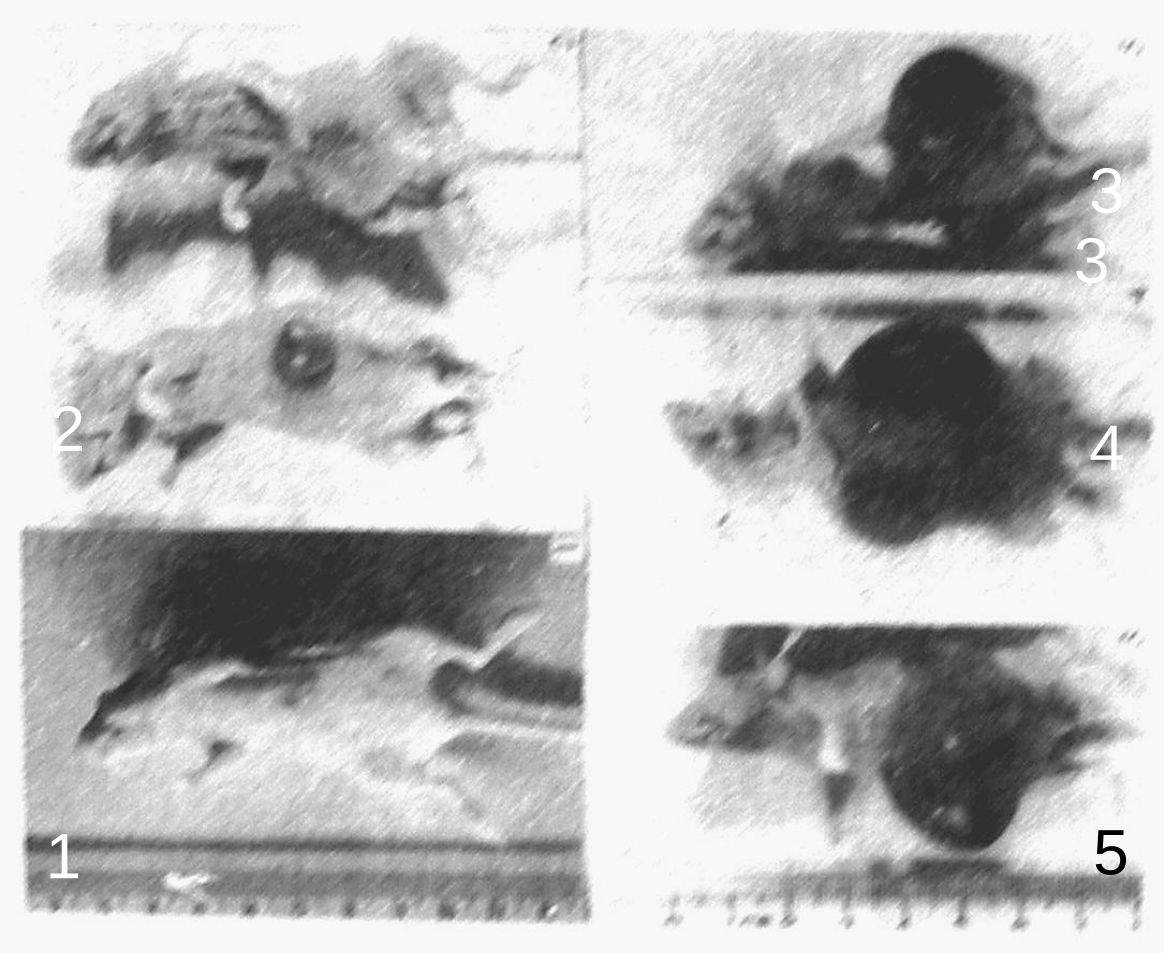


From: Okayama H. and Berg, P. Mol. Cell. Biol. 1983; 3:280-289.





Liu DG, Noda M, et al., Sci.China 1991; **7**:730-737 (Chinese)
ibid. 1992; **35**:822-833(English)



Tumorigenicity of cell strains in nude mice.

1, Normal control (NIH3T3). 2, Flat revertant (RR).
3, 4, 5, Malignant control cells: 3, DT; 4, RT (Non-reverted cells after cDNA library transfection; 5, PL (transfected by vector).

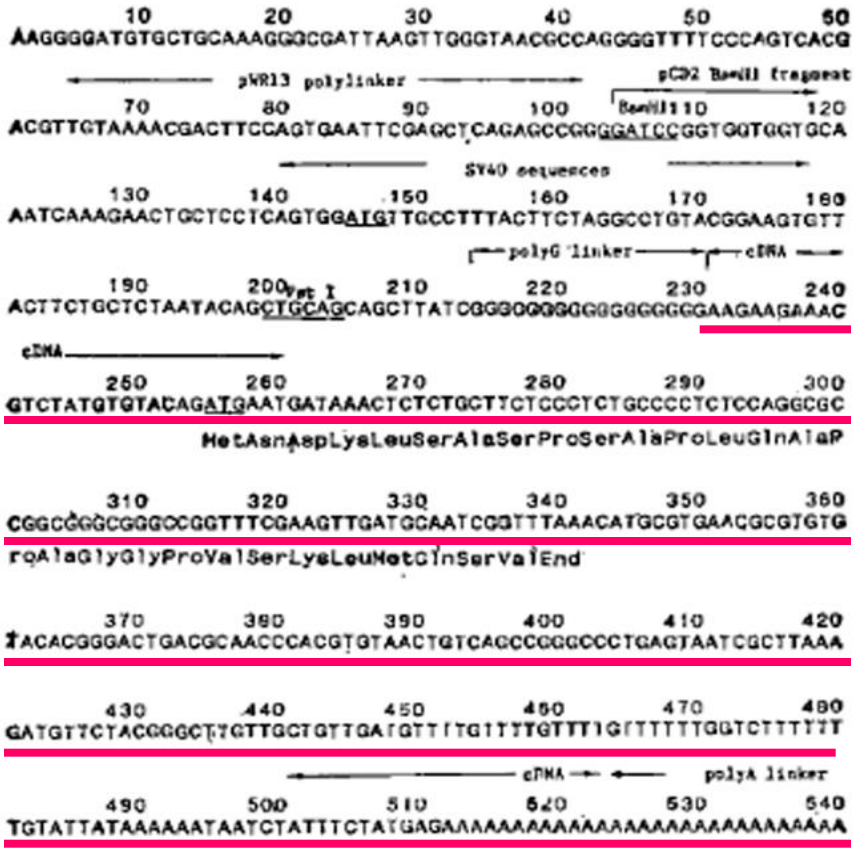


Fig. 3 The nucleotide sequence of the p14-6 cDNA, including flanking vector sequences

Liu DG et al., Acta Biochimica et Biophysica Sinica 1991; **23:**246-254.

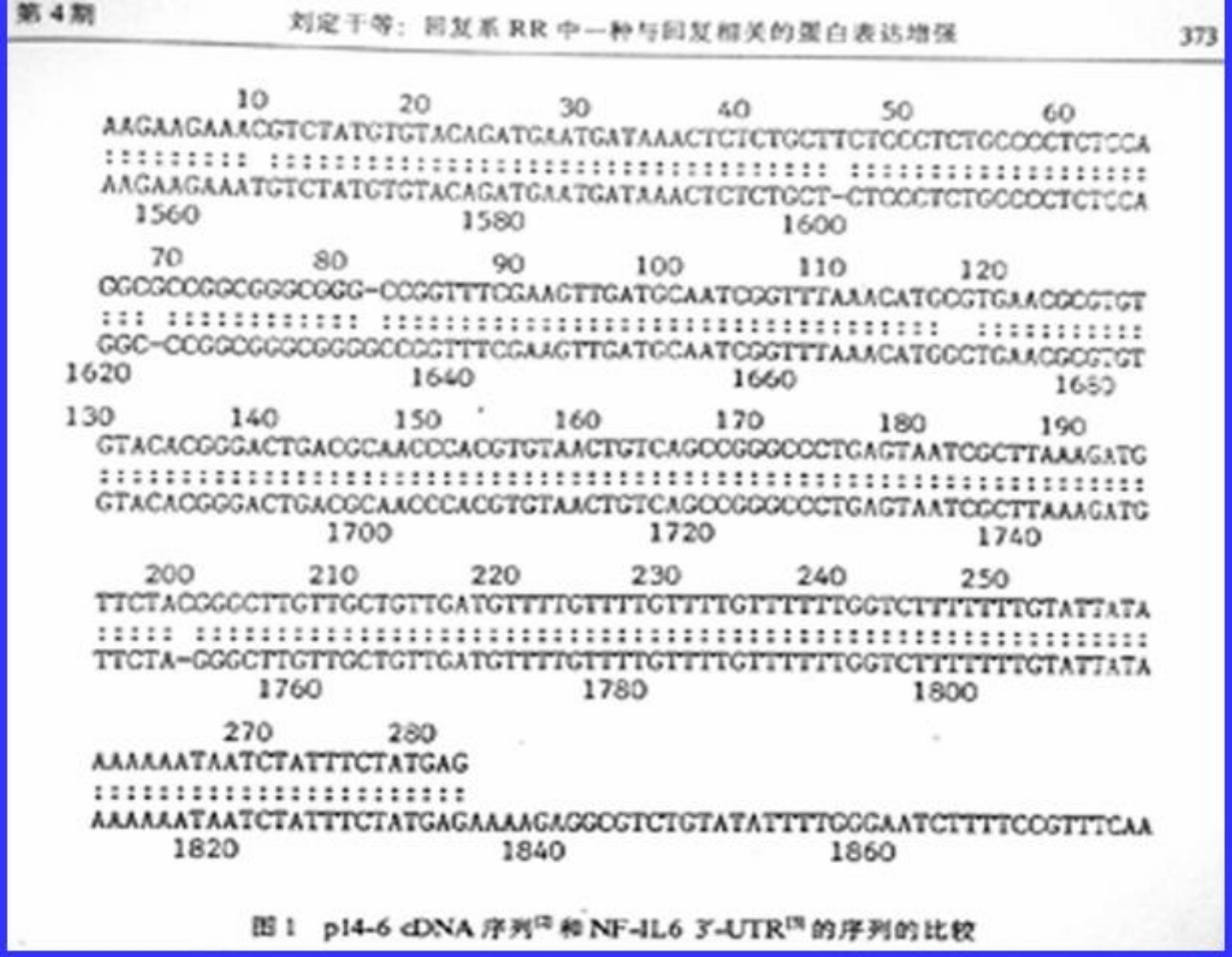


图1 p14-6 cDNA 序列[12]和 NF-IL6 3'-UTR[13]的序列的比较

Liu et al., Sci. China ser.C Life Sci., 1996, 39:300-9.

In 1992 (published in 1995-6), from a GENBANK CD, we lernt that our cDNA insert in the plasmid p14-6 was the 3'untranslated region of nuclear factor for interleukin-6 (NF-IL6, later also named C/EBPβ), which was discovered by Akira,S. et al. in 1990 (Akira et al.,EMBO J. 1990, 91897-1906). Since then we began to consider the possibility of function of independent 3'UTR as a regulator.

Since then, several 3'UTRs have been found to exert tumor suppressor effects when artificially introduced as Independent RNAs into malignant cells:

A 200 nt RNA segment in α-tropomyosin 3'UTR (1993)

Rastinejad et al., Cell, 75:1107-17

3'UTR of ribonucleotide reductase R1 and R2 messenger RNAs (1996)

Fan et al., Cancer Res 56:4366-9

3'UTR of mel-18 gene (1997)

Ishiwatari et al., Cancer lett.117:57-65.

3'UTR of prohibitin gene (2003)

Manjeshwar et al., Cancer Res. 63:5251-6.

The above facts indicate:

1, Some 3'UTRs may be active in the cells independently, *i.e*. isolated from their original mRNAs.

2, These 3'UTRs are active in regulation of cellular growth, maintaining normal phenotype of cells.

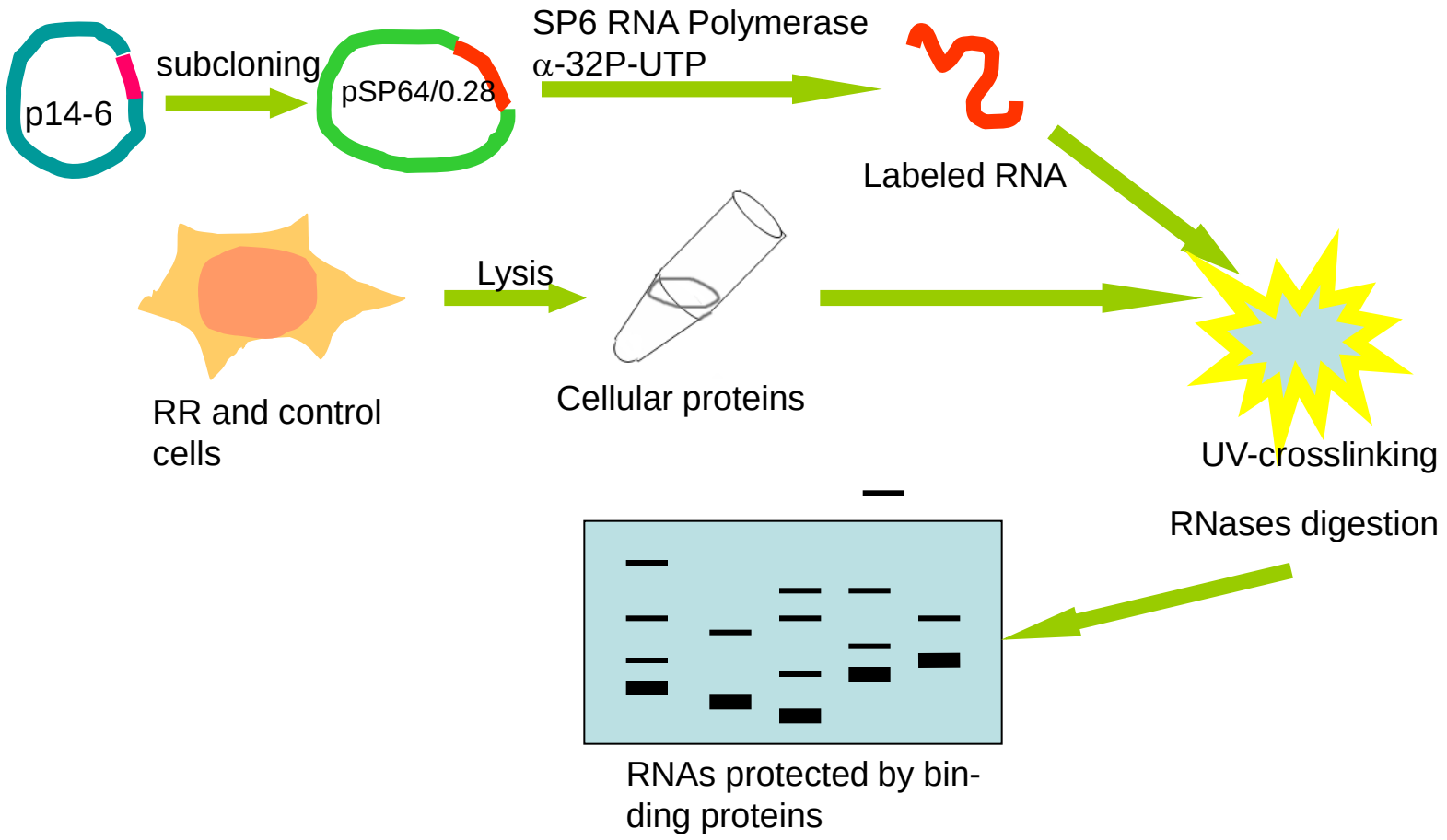
3, Therefore, independent (or distinct) 3'UTRs may be a novel type of regulators of cellular physiological activities.

4, Naturally, at that time it was unknown about the actual existence of such 3'UTR RNAs *in vivo*.

Our Earlier Attempts to Characterize Interactions between Independent 3'UTR and Cell Proteins [1]

(*Liu et al., Overexpression of a reversion-related protein in the revertant RR cells, Sci.China ser.C Life Sci, 1996;**39**:300-9*)

Strategy: RNase Protection Assay



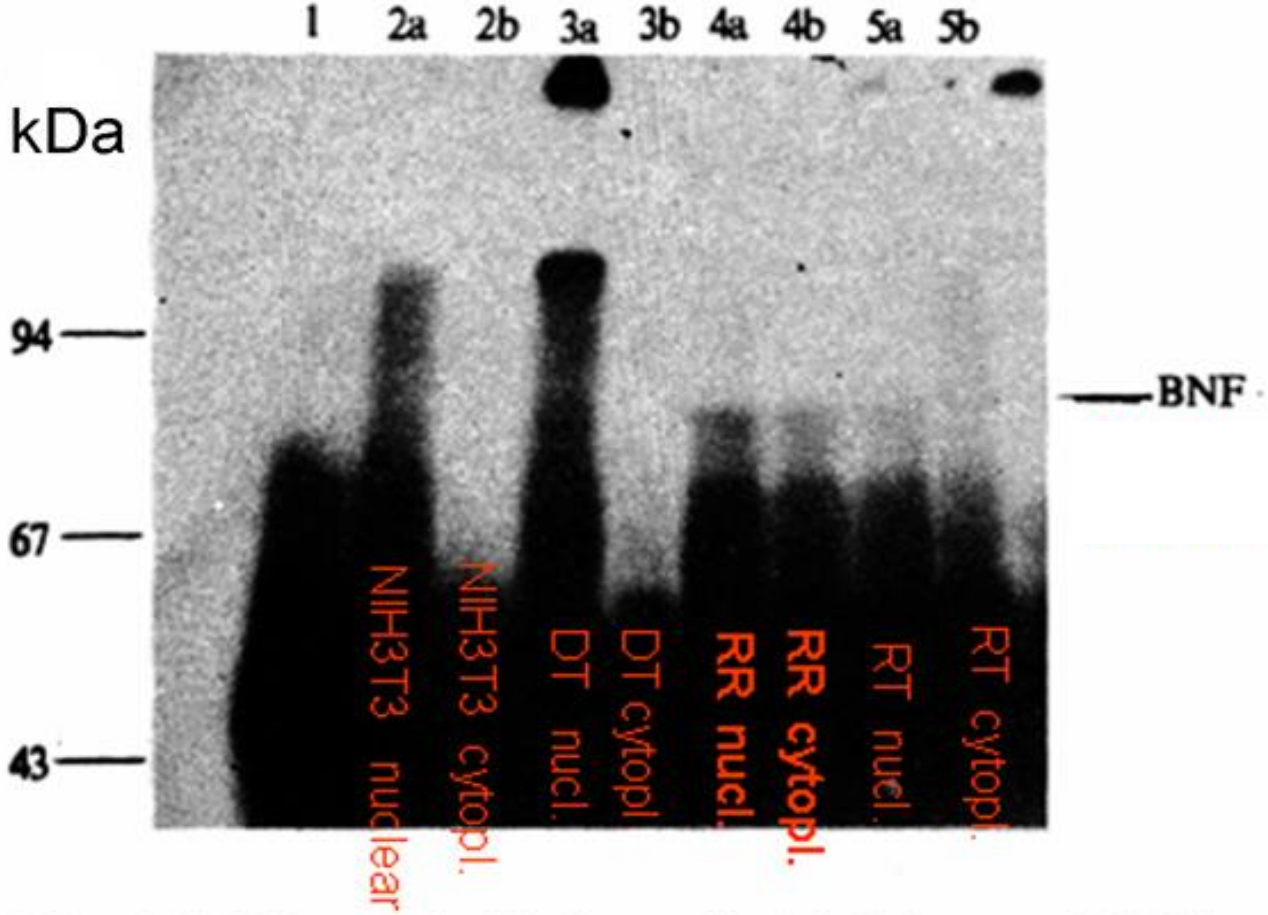


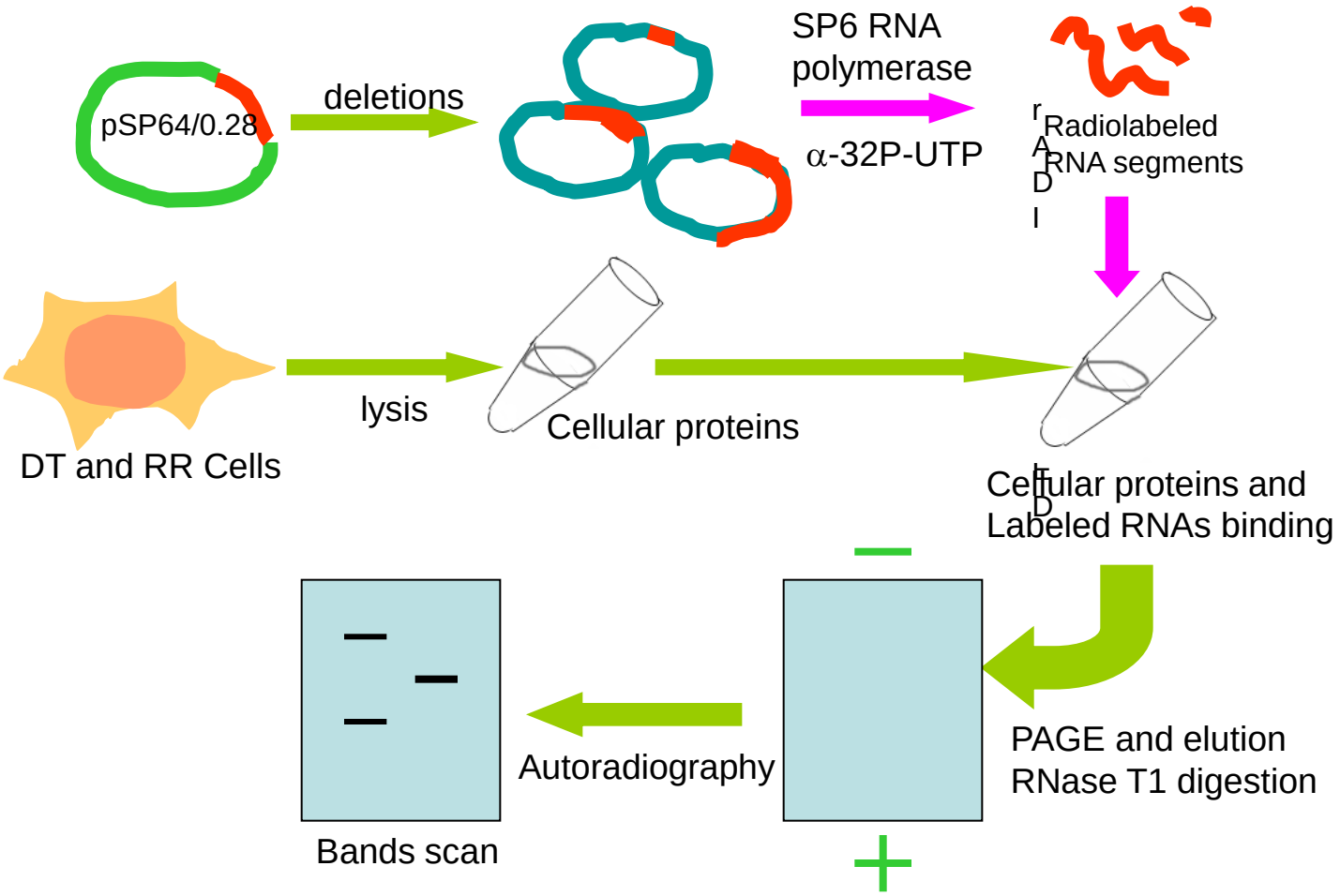
Fig. 5. RNA-protein binding with labeled sense 0.28 kb RNA. 1, Labeled RNA. In other lanes a denotes nuclei extracts, b denotes cytoplasmic extracts. 2, NIH/3T3; 3, DT; 4, RR; 5, RT.

*Liu et al., Overexpression of a reversion-related protein in the revertant RR cells, Sci.China ser.C Life Sci, 1996;**39**:300-9*

Our Earlier Attempts to Characterize Interactions between Independent 3'UTR and Cell Proteins [2]

(Li ZH, Liu DG, Li ZP. Molecular mechanism of tumor supression function of the cDNA clone p14-6. *Acta Biochimica et Biophysica Sinica*, 1996; **28**:223 - 232)

Identifying Sites on the 3'UTR that Bind Interacting Proteins



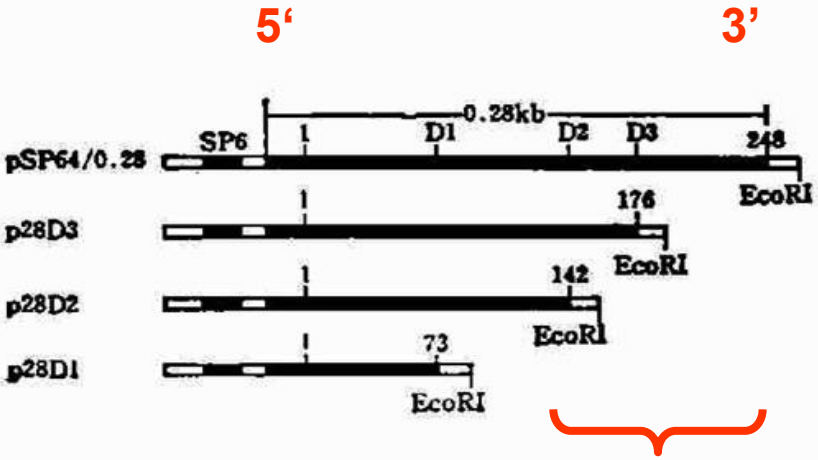


Fig. 1 Schematic diagram of the deletion mutants, p28D1, p28D2 and p28D3

The numberings are the same as literature[3].

Labeled probes

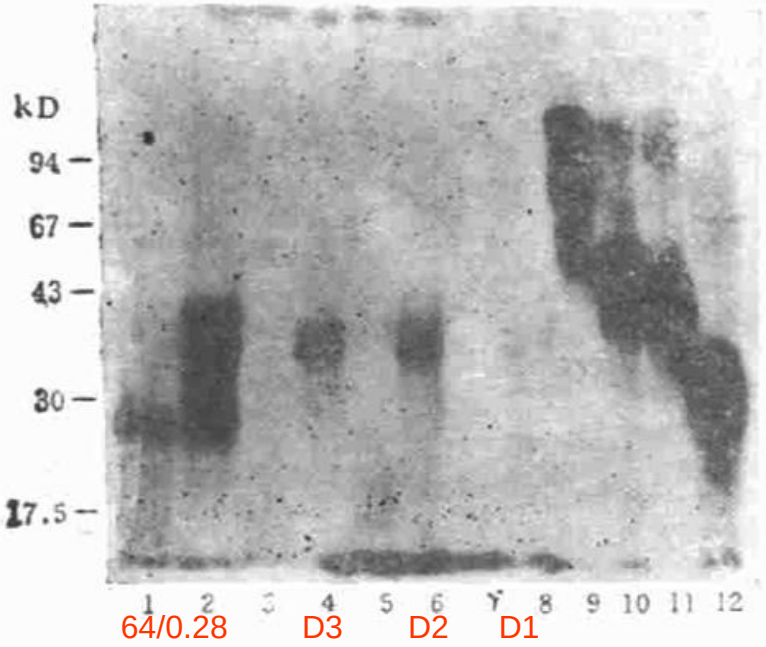


Fig. 2 Binding of RR and DT cytoplasmic proteins to labeled RNA transcripts from the deletion mutants

1, 3, 5, 7, DT proteins; 2, 4, 6, 8, RR proteins; probes are: 1, 2, pSP64/0.28; 3, 4, p28D3; 5, 6, p28D2; 7, 8, p28D1; 9~12, RNA probes of pSP64/0.28, p28D3. p28D2 and p28D1, run in parallel.

Deletions and RNase protection assays showed that interaction with cellular proteins occurred At sites of NF-IL6(C/EBPβ) 3'UTR RNA near its 3'terminus.

Li et al., *ABBS*, 1996; **28**:223-32

3'terminus of the 3'UTR
Formed by deletion

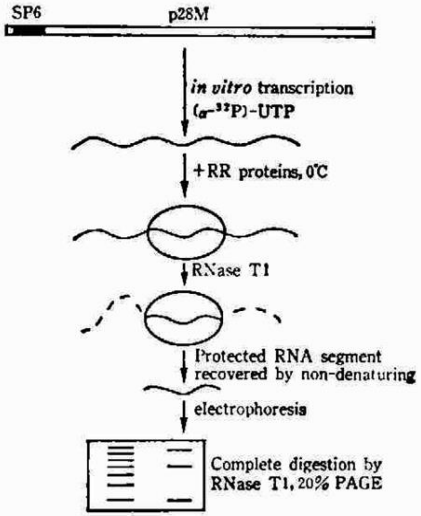


Fig. 3 Locating the RNA segment protected by proteins on the 0.28kb RNA transcript

The location of protected segment is determined by comparing with fragments with known lengths.

RNase -protected fragments

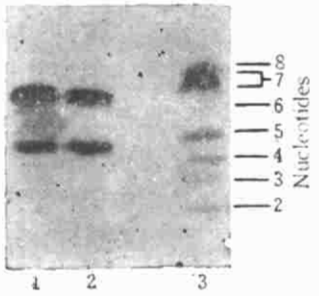
Fig.4 Non-denaturing P of RNA-protein comple

1, experiment; 2, contro (no proteins).

20% PAGE

7 nt

5 nt



(B) Scanning

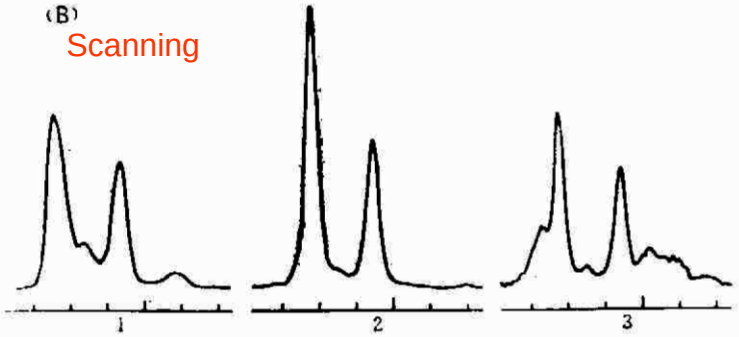


Fig.5 (A) RNase T1 mapping

1, M1 segment; 2, M2 segment; 3, p28M transcript.

(B) Band density scanning of A

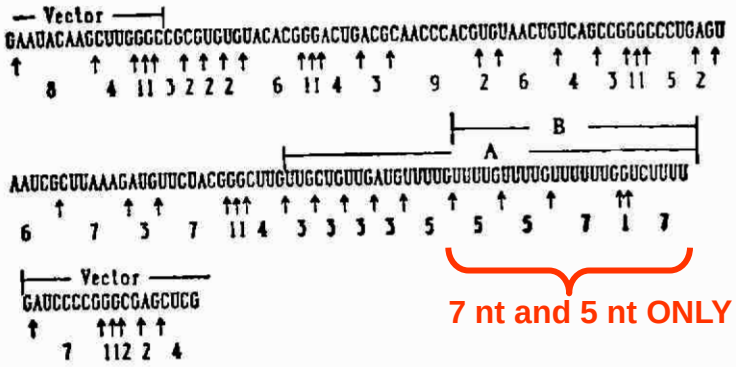


Fig.6 Nucleotide sequence of p28M

Arrows indicate sites of digestion of RNase T1. Numbers under sequence are lengths of oligonucleotides yielded by digestion.

Analysis of putative site of inter-action with cellular proteins on the independent NF-IL6(C/EBPβ) 3'UTR RNA.

(*Li et al., ABBS,1996; **28**:223-32*)

The abovementioned results showed that:

1, In the NIH3T3-derived, Ras-transformed malignant cell line DT, independent C/EBPβ 3'UTR RNA resulted in malignant phenotype suppression.

2, This tumor suppression is related to interactions between the independent C/EBPβ 3'UTR RNA and DT cellular protein(s).

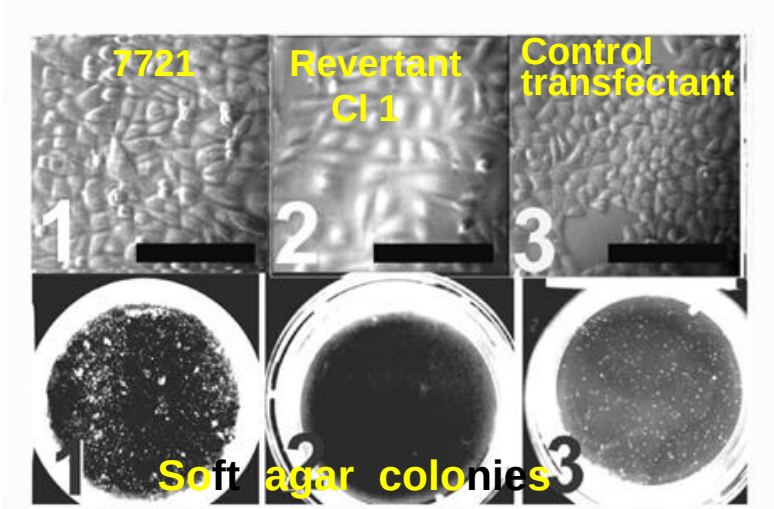
3, The site of interaction of the independent C/EBPβ 3'UTR RNA includes an AU-rich segment near 3'terminus.

Next question: is the tumor suppression of the independent C/EBPβ 3'UTR RNA only effective for a specific cell line (DT), or is it generally effective?

To answer this question, we have investigated the effects of the independent C/EBPβ 3'UTR RNA in a human hepatocarcinoma cell line, SMMC-7721. We found that this RNA also is a tumor suppressor in SMMC-7721.

Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721

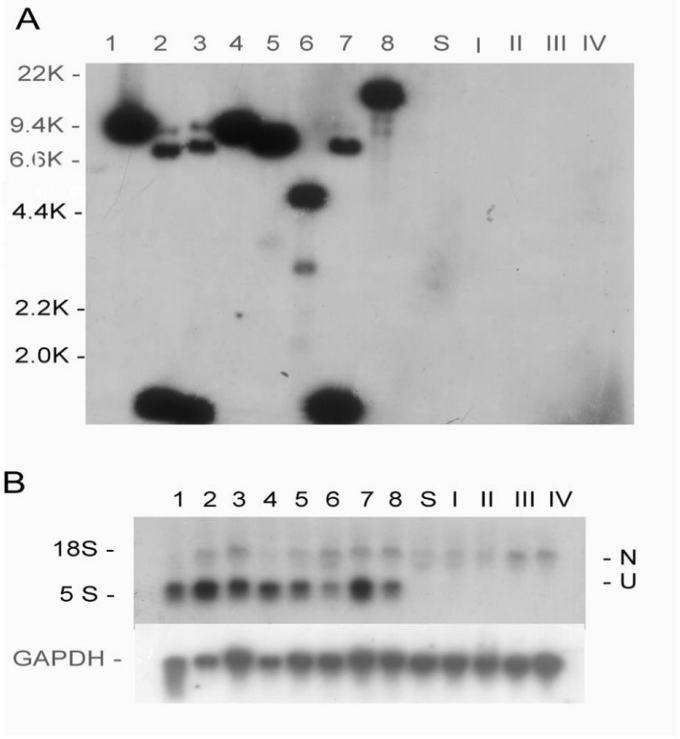
(*Liu et al., Gene expression profile favoring phenotypic reversion: a clue for mechanism of tumor suppression by NF-IL6 3'UTR. Cell Res. 2003; **13**(6):509-514*)



Nude mice tumorigenicity* of cell clones

Name	Tumorigenicity	Name	Tumorigenicity	Name	Tumorigenicity
Cl 1	1/8	Cl 6	2/4	pcDmr Cl 1	3/3
Cl 2	1/3	Cl 7	1/3	pcDmr Cl 2	3/3
Cl 3	1/6	Cl 8	3/3	pcDmr Cl 3	3/3
Cl 4	1/2			pcDmr Cl 4	3/3
Cl 5	1/2	SMMC7721	3/3		

*tumorigenicity is expressed as: Number of mice producing tumor nodule / Number of injected mice

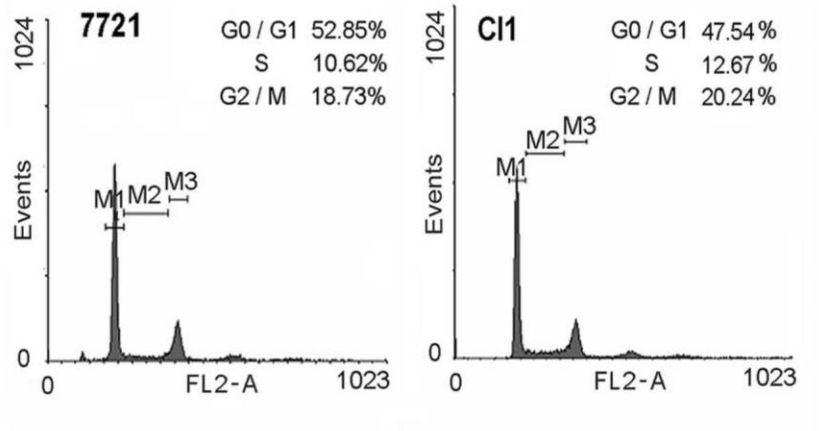


Integration status and expression of NF-IL6 3'UTR. The probe for all hybridizations was ^{32}P-labeled 3'UTR fragment. In both figures, 1-8 stand for transfectants Cl 1-8; S, SMMC7721; I - IV, pcDmr Cl 1-4. **A**. Southern hybridization of cellular genomic DNAs cut by EcoRI (which had no sites on p14-6 plasmid). **B**. Northern hybridization of cellular total RNAs. N, endogenous NF-IL6 transcript (~ 2 kb). U, the 3'UTR transcript (~ 0.5 kb). GAPDH, the same membrane re-probed by GAPDH probe.

A typical revertant clone, Cl1, was chosen for further investigation.

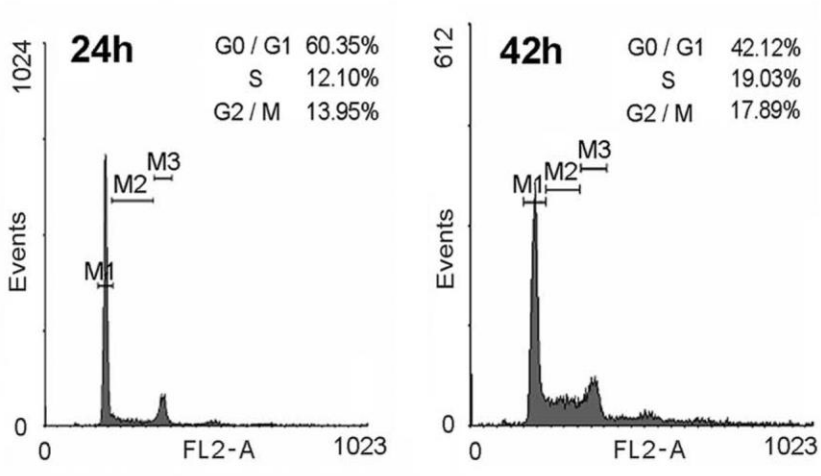
Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721

(*Wang Y et al., Tumor suppression by RNA from C/EBPβ 3'UTR through the inhibition of protein kinase Cε activity. PLoS ONE 2011; **6**(1):e16543. doi: 10.1371/journal.pone.0016543.*)



Comparison between SMMC-7721 and Cl1

C/EBPβ 3'UTR RNA causes mitosis to be delayed at S and G2/M phase



Transient transfection of SMMC-7721 by C/EBPβ 3'UTR RNA

Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721（2）

Transcriptome analysis of the NF-IL6 3'UTR revertant

The revertant Cl 1 was chosen for the cDNA array analysis to compare with SMMC7721 and with one of the negative transfectant, pcDmr Cl2.

Tab 3. Genes whose regulation is induced by NF-IL6 3'UTR, found by cDNA array analysis

GenBank Accession Number	Name	Expression ratio Cl 1/SMMC7721(average)	Known functions
1. Up-regulated genes			
AB036063	p53-induced ribonucleotide reductase small subunit 2 homolog	2.05	Tumor suppressor related to p53[9]
S59049 (NM_002922)	regulator of G protein signalling 1	2.51	Negative regulator of G protein signalling[16]
AJ133355	zinc finger protein 237	2.27	Putative regulator (DNA-binding)
U12767	nuclear receptor subfamily 4 groupA member 3	2.84	Putative regulator (DNA-binding)[14]
AL079310	high-mobility-group protein 2-like 1	2.40	Putative DNA binding protein
U87460	G-protein-coupled receptor 37 (endothelin receptor type B-like)	3.25	Function unknown
NM_020233	x 006 protein	2.55	Function unknown
BE301841	hypothetical protein (DKFZp434N1923)	11.5	Function unknown
2 *Down-regulated genes*			
AF012281	PDZ domain containing protein 1	0.45	Overexpressed in some epithelial tumors[12]
M26326	keratin 18	0.42	binds to keratin 8 to provide resistance to Fas-mediated apoptosis[13]
K00558	tubulin α, ubiquitous	0.40	Microtubule component
D31885	ADP-ribosylation factor 6-like Interacting protein	0.47	Activator of signal transduction pathways
J03592	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	0.28	ADP/ATP translocase[14]
BE222923	highly similar to spectrin β chain	0.34	Essential for TGF-β signalling[15]
AF000989	thymosin β4, Y chromosome	0.46	Related to cell differentiation; increasing expression enhances metastasis[16]
X06256	integrin α5 (fibronnectin receptor)	0.29	Cell growth regulator[17]
AL035297	human gene from PAC 747 L4	0.27	Function unknown
AK001187	hypothetical protein FLJ14642	0.37	Function unknown
AA885521	KIAA1409 protein	0.40	Function unknown

These may be changes in cells that were induced by the C/EBPβ 3'UTR RNA transfection

Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721

Sequences in C/EBPβ 3'UTR RNA Important for Tumor Suppression

(*Wang et al., Sequences near both termini of the C/EBPβ mRNA 3'untranslated region are important for its tumor suppression activity. Acta Biochimica et Biophysica Sinica 2009;**41**:459-63*)

```
1081 CAAGGCCAAG AAGACCGTGG ACAAGCACAG CGACGAGTAC AAGATCCGGC GCGAGCGCAA
1141 CAACATCGCC GTGCGCAAGA GCCGCGACAA GGCCAAGATG CGCAACCTGG AGACGCAGCA
1201 CAAGGTCCTG GAGCTCACGG CCGAGAACGA GCGGCTGCAG AAGAAGGTGG AGCAGCTGTC
1261 GCGCGAGCTC AGCACCCTGC GGAACTTGTT CAAGCAGCTG CCCGAGCCCC TGCTCGCCTC
1321 CTCCGGCCAC TGCTAGcgcg gcccccgcgg cgtccccctg gggccggccg gggctgagac
1381 tccggggagc gcccgcgccc gcgccctcgc ccccnccccc nnnnccgcaa aactttggca
1441 ctggggcact tggcagcngg ggagcccgtc ggtaatttta atattttatt atatatatat
1501 atctatattt tgccaaccaa ccgtacatgc agatggctcc cgcccgtggt gtataAAGAA
1561 GAAACGTCTA TGTGTACAGA TGAATGATAA ACTCTCTGCT TCTCCCTCTG CCCCTCTCCA
1621 GGCGCCGGCG GGCGGGCCGG TTTCGAAGTT GATGCAATCG GTTTAAACAT GCGTGAACGC
1681 GTGTGTACAC GGGACTGACG CAACCCACGT GTAACTGTCA GCCGGGCCCT GAGTAATCGC
1741 TTAAAGATGT TCCTACGGGC TTGTTGCTGT TGATGTTTTG TTTTGTTTTG TTTTTTGGTC
1801 TTTTTTTGTA TTATAAAAAA TAATCTATTT CTATGACaaa agaggcgtct gtatattttg
1861 ggaatctttt ccgtttcaag caattaagaa cattttaata aacttttttt tg
```

3'part of C/EBPβ cDNA.

5'deletion

```
5' AAGAAGAAAC GTCTATGTGT ACAGATGAAT GATAAACTCT
   CTGCTTCTCC CTCTGCCCCT CTCCAGGCGC CGGCGGGCGG
   GCCGGTTTCG AAGTTGATGC AATCGGTTTA AACATGCGTG
   AACGCGTGTG TACACGGGAC TGACGCAACC CACGTGTAAC
   TGTCAGCCGG GCCCTGAGTA ATCGCTTAAA GATGTTCCTA
   CGGGCTTGTT GCTGTTGATG TTTTGTTTTG TTTTGTTTTT
   TGGTCTTTTT TTGTATTATA AAAAATAATC TATTTCTATG AG 3'
```

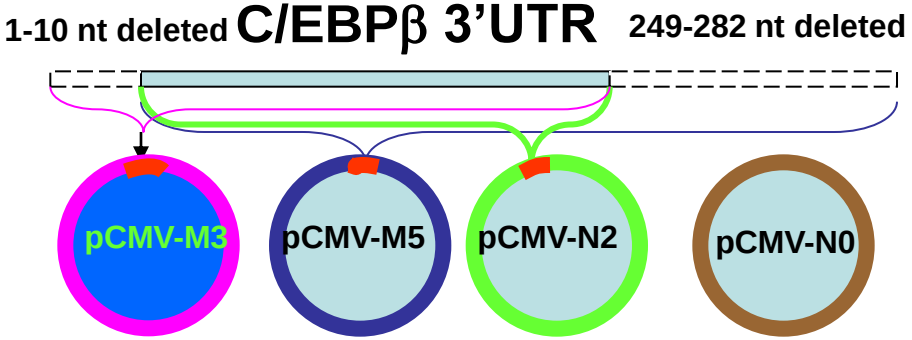
3' deletion

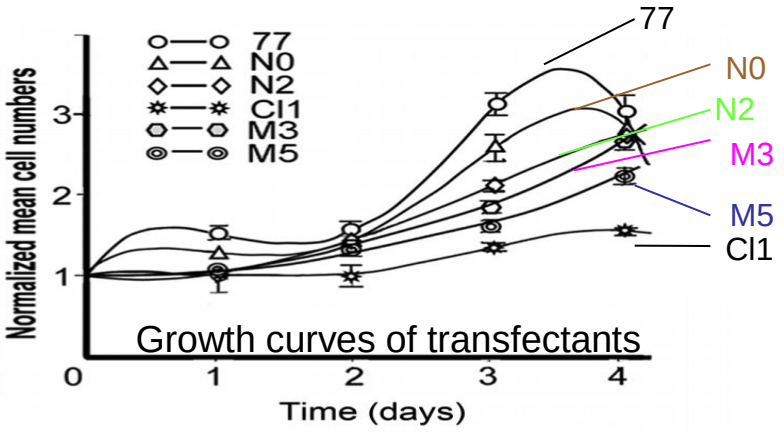
0.28kb 3'UTR and deletions.

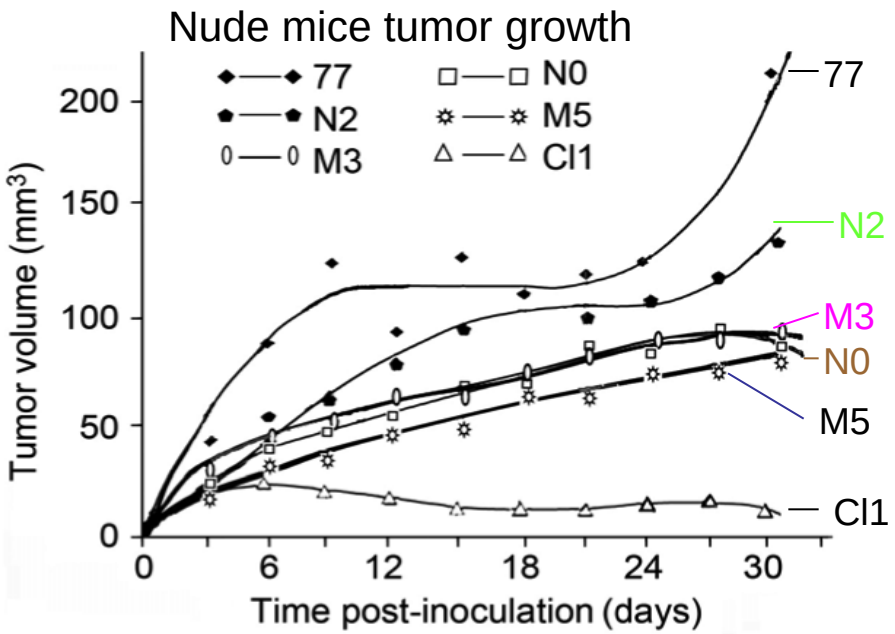
Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721

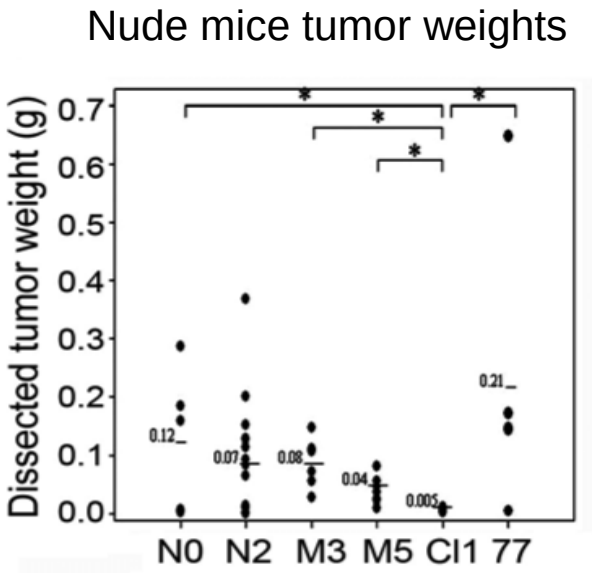
Sequences in C/EBPβ 3'UTR RNA Important for Tumor Suppression

(*Wang et al., Sequences near both termini of the C/EBPβ RNA 3'untranslated region are important for its tumor suppression activity. Acta Biochimica et Biophysica Sinica 2009;**41**:459-63*)









Tumor suppression effect of C/EBPβ 3'UTR RNA in SMMC-7721

Sequences in C/EBPβ 3'UTR RNA Important for Tumor Suppression

(Wang et al., Sequences near both termini of the C/EBPβ RNA 3'untranslated region are important for its tumor suppression activity. *Acta Biochimica et Biophysica Sinica 2009;**41**:459-63*)

5' UGUCAGCCG GCCCUGAGUA AUCGCUUAAA

GAUGUUCCUA CGGGCUUGUU GCUGUUGAUG

UUUUGUUUUG UUUUGUUUUU UGGUCUUUUU

UUGUAUUAUA AAAAAUAAUC UAUUUCUAUG AG 3'

Deleted sequence at the 3'terminus

of the C/EBPβ 3'UTR RNA(red).

Green line denotes an AU-rich element.

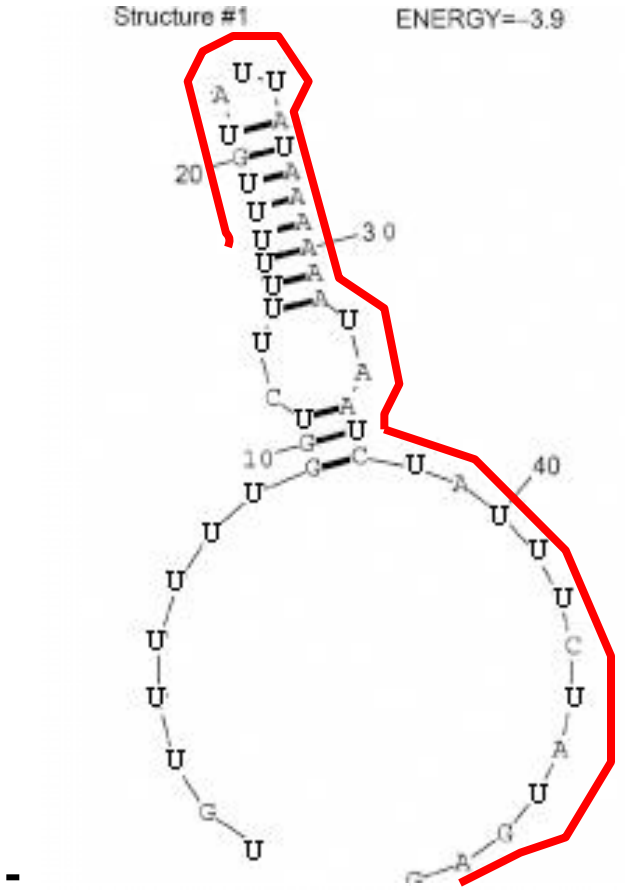


Fig. 4 The representative stem-loop structure of the sequence at 3'-terminus of the C/EBPβ 3' UTR element, including that deleted in this work It was drawn using RNA structure 4.4 software. The sequence shown is from 233rd through 282nd nucleotides of the element (233rd nucleotide is named 1st in the figure, and so on). The deletion destroys this step-loop structure.

3'terminus of the 3'UTR
Formed by deletion

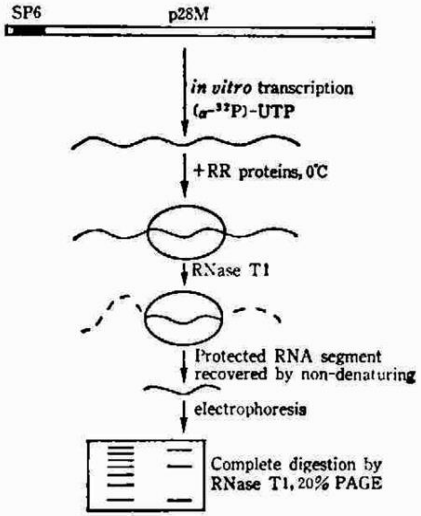


Fig. 3 Locating the RNA segment protected by proteins on the 0.28kb RNA transcript

The location of protected segment is determined by comparing with fragments with known lengths.

RNase -protected fragments

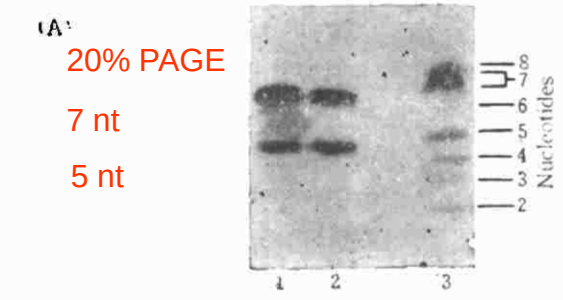
Fig.4 Non-denaturing P of RNA-protein comple

1, experiment; 2, contro (no proteins).

20% PAGE

7 nt

5 nt



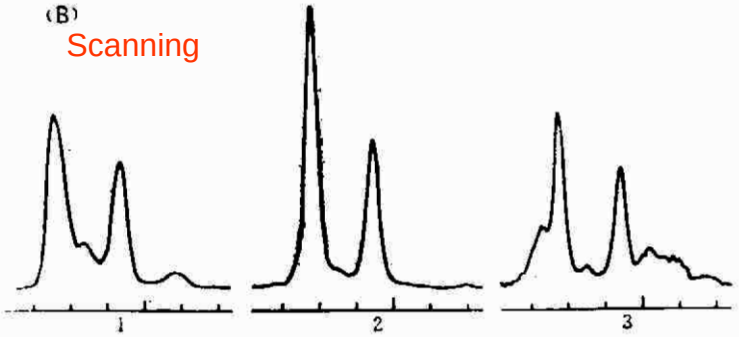


Fig.5 (A) RNase T1 mapping

1, M1 segment; 2, M2 segment; 3, p28M transcript.

(B) Band density scanning of A

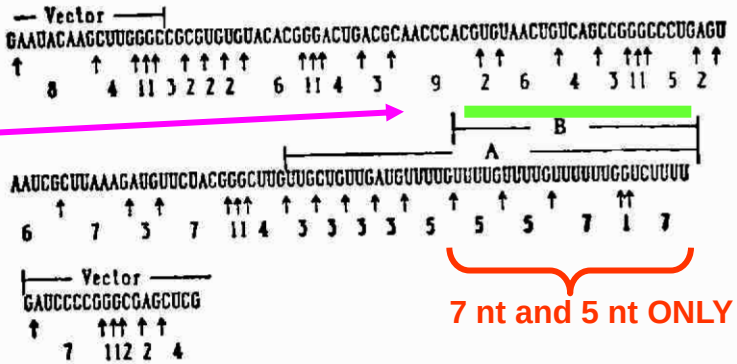


Fig.6 Nucleotide sequence of p28M

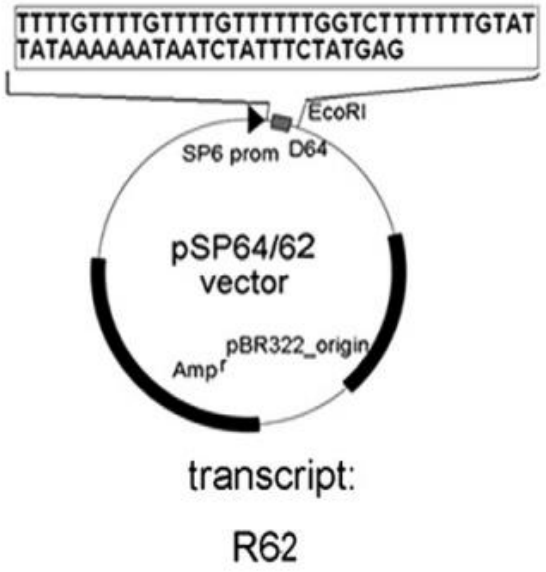
Arrows indicate sites of digestion of RNase T1. Numbers under sequence are lengths of oligonucleotides yielded by digestion.

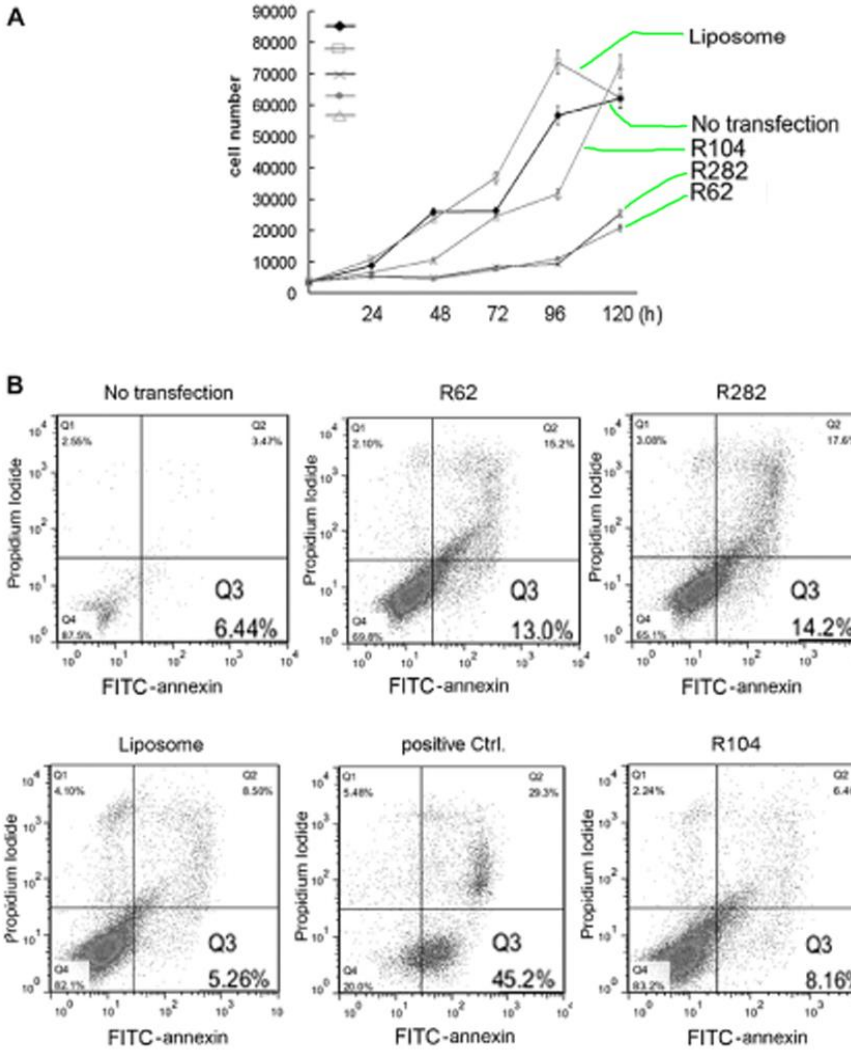
Re-display of slide 16.

Green line denotes the sequence important for tumor suppression effect as found in the *Wang et al.* (2009), which is consistent with *Li et al.*'s finding (1996).

Identification of the Cellular Protein that Interacts with the Independent C/EBPβ 3'UTR RNA and leads to Tumor Suppression

(D.-Q.Sun et al.,Cancer cell growth suppression by a 62nt AU-rich RNA from C/EBPβ 3'UTR through competitive binding with HuR, *BBRC 2012; **46**:122-128*)





Controls

R282-----Full length C/EBPβ 3'UTR RNA

R104-----Irrelevant RNA from pOTB7 vector as control

Positive apoptosis control----a proprietary compound

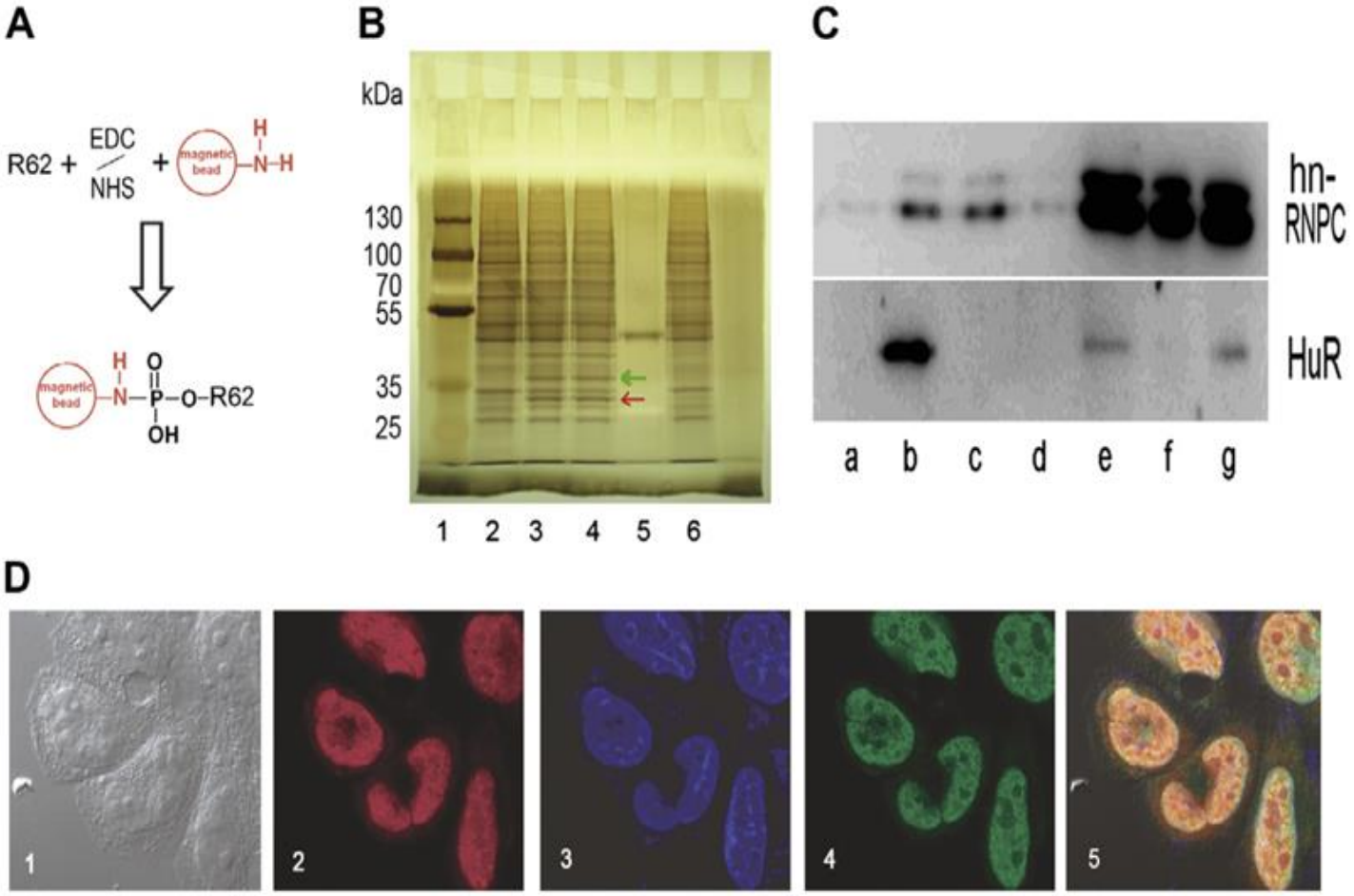


Fig. 3. R62 RNA specifically bound with HuR protein. (A) Immobilization of R62 RNA on magnetic beads through a condensation reaction using EDC and NHS. (B) Silver staining of a 10% SDS–polyacrylamide gel containing proteins eluted from R62-immobilized magnetic beads. (1) MW. (2) Beads. (3) and (4) Beads containing R62. (5) Blocking solution. (6) Beads containing control RNA (R104). Red arrow, HuR as determined by MS. Green arrow, hnRNPC as determined by MS. (C) RNA affinity chromatography and Western blots for hnRNPC and HuR, respectively. (a) Beads without RNA. (b) Beads containing R62. (c) and (d) Beads containing control RNA. (e) Cell lysate precipitate. (f) Immunoprecipitation supernatant. (g) Supernatant from cell lysate not treated with beads. (D) R62 co-localized with HuR in the nuclei and cytoplasm of hepatoma cell line SMMC-7721, as detected by confocal microscopy. (1) Phase contrast. (2) Molecular beacon for R62. (3) DAPI. (4) Anti-HuR antibody. (5) Merge. The red fluorescence arises from the molecular beacon for R62, the blue fluorescence is from DAPI, and the green fluorescence is from an HuR antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

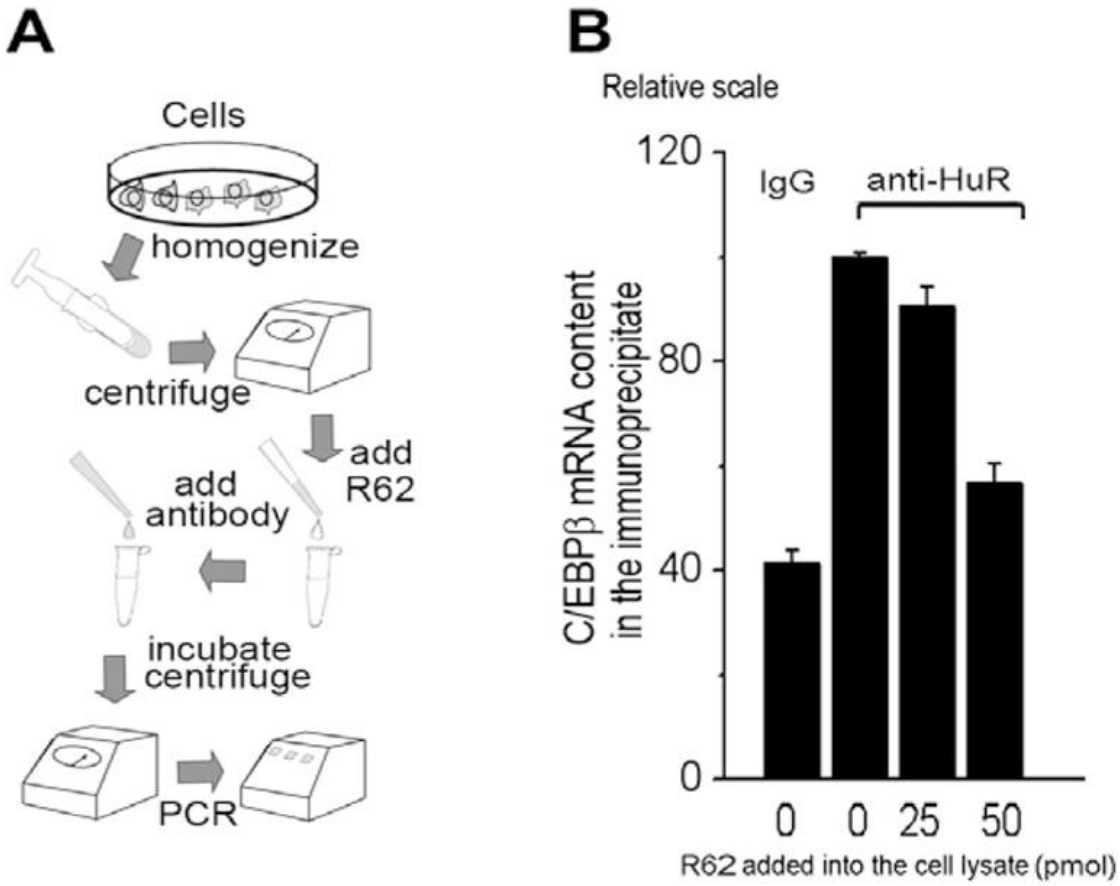


Fig. 4. R62 reduces the amount of C/EBPβ mRNA in SMMC-7721 cell lysate. (A) Experiment procedures for competitive RNA immunoprecipitation. SMMC-7721 cells were homogenized and centrifuged, the supernatant was aliquoted and increasing amounts of competitive (R62) RNA were added into each aliquot. Antibodies to HuR and to IgG were then added to the mixtures. After incubation at 4 °C, the mixtures were centrifuged and the precipitates were subjected to qPCR to determine the amounts of C/EBPβ mRNA. (B) Experimental results, showing that the contents of C/EBPβ mRNA were inversely correlated with the amounts of R62 added.

Independent C/EBPβ 3'UTR RNA interacts with cellular keratin18 and oncogene PKCε *in vivo* (Wang et al. *PLoS ONE 2011;6:16543*)

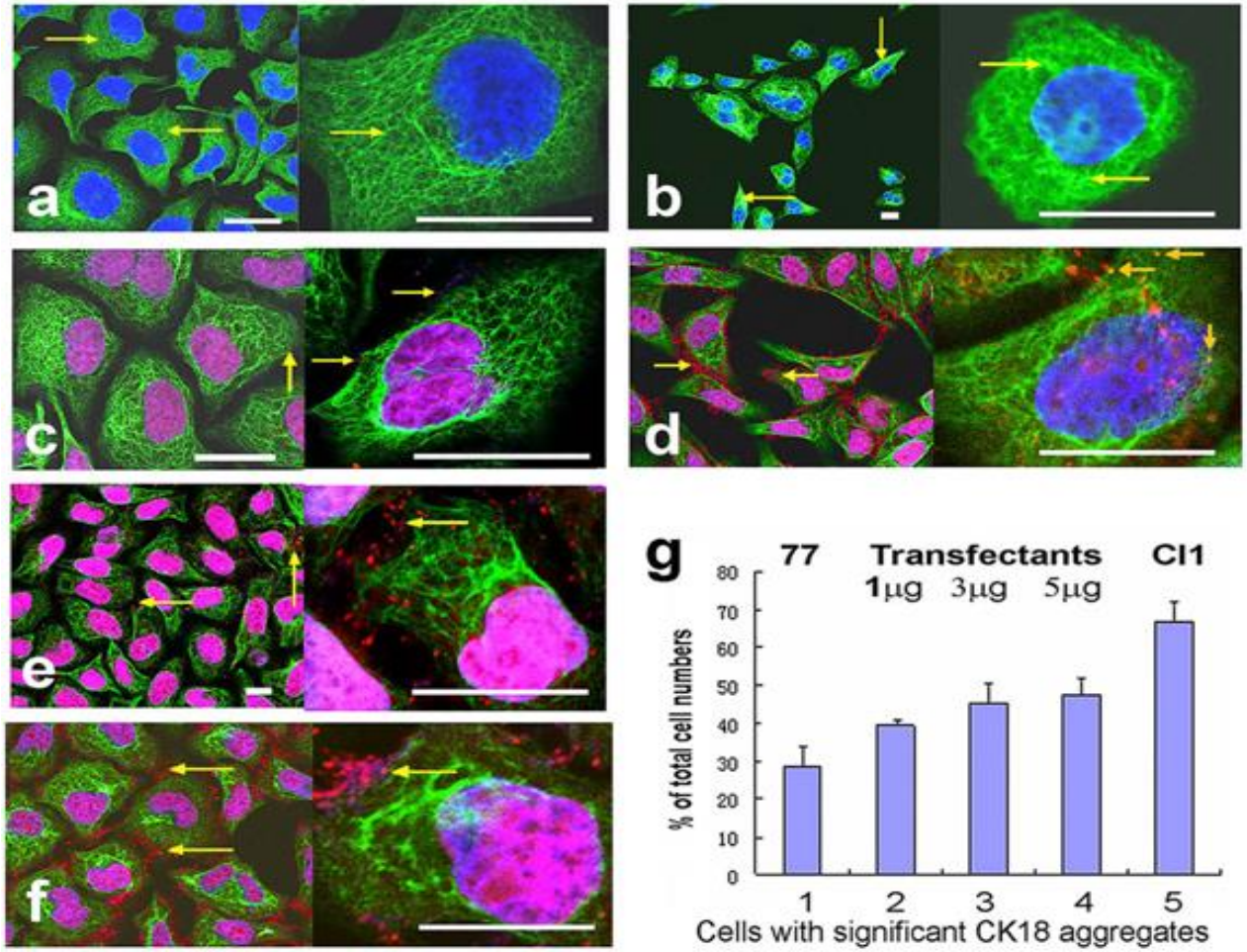


Figure 3. C/EBPβ 3′UTR RNA binds CK18 and alters its intracellular organization in SMMC-7721 and CI1 cells. Confocal micrographs of the cells immunostained with a fluorescein-labeled antibody against CK18 (green), and of the cells immunostained as above and probed with a fluorescent-labeled molecular beacon specific to C/EBPβ 3′UTR RNA (red dots). (**a**) SMMC-7721 cells. Arrows indicate the thin CK18 network in the cytoplasm. (**b**) CI1 cells. Arrows indicate the aggregates and bundles of CK18. (**c**) SMMC-7721 cells probed with the molecular beacon for C/EBPβ 3′UTR RNA. Arrows indicate the molecular beacon binding with C/EBPβ 3′UTR RNA on the CK18 filaments. (**d**) CI1 cells probed with the same molecular beacon. Note the greater amount of red fluorescence from the molecular beacon than in SMMC-7721, and the positioning of the fluorescence both around the CK18 filaments and even on the CK18 aggregates (the orange arrows in the larger amplification). (**e**) SMMC-7721 cells transfected with 1 μg/well of C/EBPβ 3′UTR RNA and probed with the same molecular beacon. Arrows indicate the fluorescence of the molecular beacon on the CK18. (**f**) SMMC-7721 cells transfected with 5 μg/well of C/EBPβ 3′UTR RNA and probed with the same molecular beacon. Arrows indicate the fluorescence of the molecular beacon on the CK18. Bars, 10 μm. (**g**) Percentage amounts of cells with significant CK18 aggregates in total cell populations of SMMC-7721, CI1 and SMMC-7721 transfected with varying amounts of C/EBP 3′UTR RNA. Cells were grown in 24-well plates. 1, SMMC-7721. 2-4, SMMC-7721 transfected with 1, 3, 5 μg/well of C/EBP 3′UTR RNA respectively. 5, CI1.
doi:10.1371/journal.pone.0016543.g003

Independent C/EBPβ 3'UTR RNA interacts with cellular keratin18 and oncogene PKCε *in vivo* (*Wang et al. PLoS ONE 2011;**6:**16543*)

Comparison of keratin 18 and phosphorylated keratin 18 in SMMC-7721 and CI1 cells

Experiments done with kinase inhibitors' library----direct adding compounds into cell culture in multiwell plates

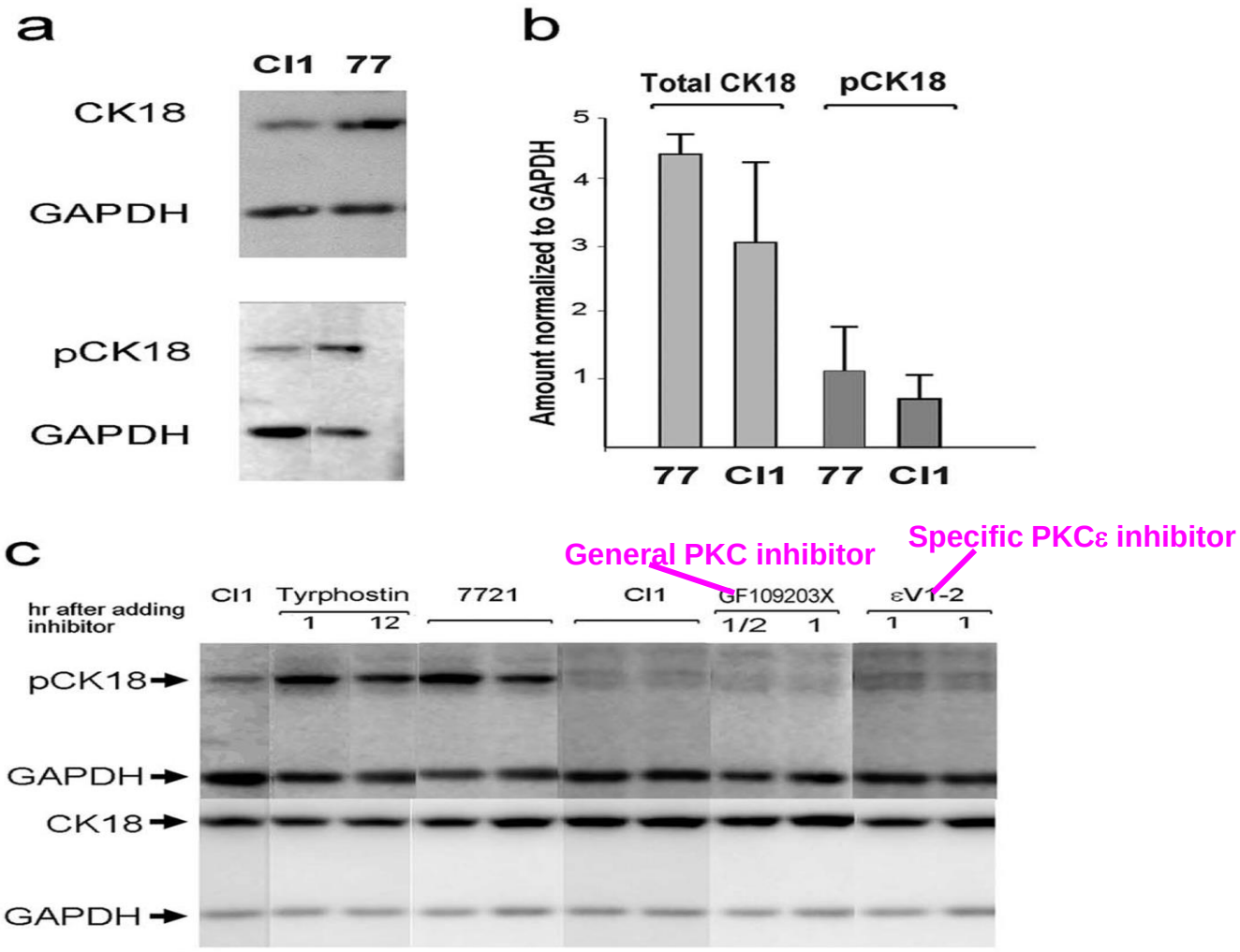


Figure 4. Amounts of pCK18 and total CK18 in CI1 cells decreased; the responsible enzyme is PKCε. (**a**) Typical examples of Western blots for pCK18 and CK18. (**b**) Histogram of data of band densities comparing the amounts of pCK18 and CK18 in the two cell lines, calculated from more than five independent Western blot experiments. (**c**) Protein kinase inhibition experiments. Individual components of a kinase inhibitor library were used to treat cultured cells for definite times. Then the cells were lysed and subjected to Western blotting for pCK18 and CK18. Typical Western blots, including a protein tyrosine kinase inhibitor (tyrphostin), a general PKC inhibitor (GF109203X), and a PKCε-specific inhibitor (εV1-2), are combined. Untreated CI1 and SMMC-7721 cells were used as controls.
doi:10.1371/journal.pone.0016543.g004

Independent C/EBPβ 3'UTR RNA interacts with cellular keratin18 and oncogene PKCε *in vivo* (*Wang et al. PLoS ONE 2011;6:16543*)

Independent C/EBPβ 3'UTR RNA directly inhibits the phosphorylation activity of PKCε and participates in formation of a triplex conjugate.

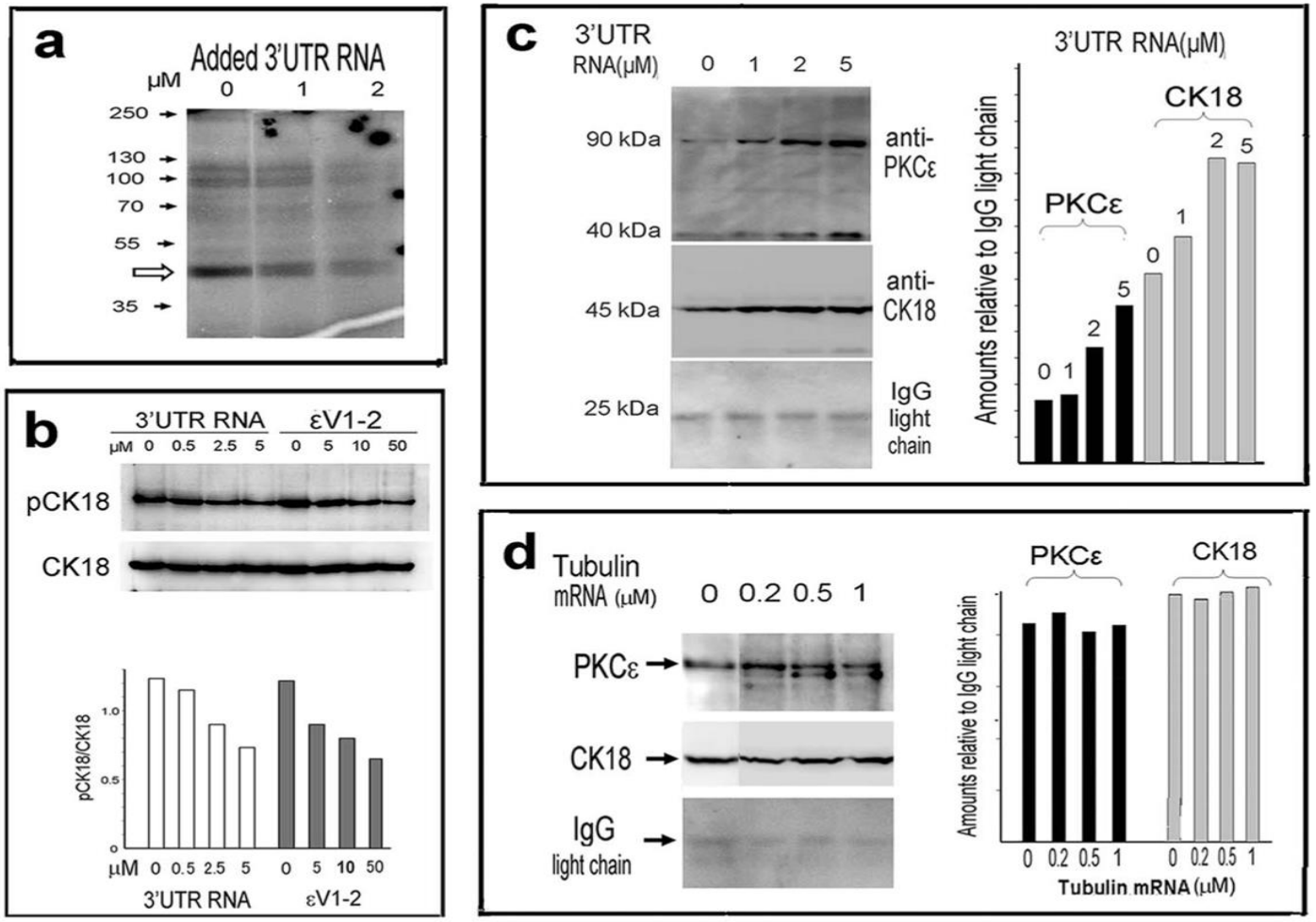


Figure 5. C/EBPβ 3′UTR directly inhibits the activity of PKCε and specifically binds CK18-PKCε conjugate. (**a**) Preliminary test of the activity of calcium-independent PKCs. The hollow arrow indicates a 45 kDa labeled band (probably CK18). (**b**) C/EBPβ 3′UTR RNA directly inhibits PKCε activity. A representative Western blot is shown, with the band densities expressed as the ratios pCK18/CK18 quantitatively displayed in the histogram. (**c**) RNA binding-coupled co-immunoprecipitation showing that C/EBPβ 3′UTR RNA forms a complex with CK18 and PKCε. The quantitative histogram indicating the amounts of CK18 and PKCε bands is included. (**d**) Control RNA binding-coupled co-precipitation, in which β-tubulin RNA (1.4 kb) was used instead of C/EBPβ 3′UTR RNA. The quantitative histogram showing the amounts of PKCε and CK18 bands is shown beside it. In this experiment the antibody against PKCε detected a smaller molecule that seems to bind tubulin RNA; but its identity is unknown.
doi:10.1371/journal.pone.0016543.g005

BRIEF CONCLUSIONS

1 The independently expressed C/EBPβ 3'UTR RNA exerts tumor suppression effect in mouse and human malignant cells.

2 This tumor suppression effect is caused by direct interactions between the 3'UTR RNA and cellular functional proteins, especially HuR and PKCε, leading to changes in cellular gene expression.

3 Therefore, our results are the first demonstration that an independent 3'UTR RNA can have gene regulation function *in trans*, as a novel type of non-coding RNAs.

Independent 3'UTR RNAs do exist in human, mouse and fly
---------- Recent finding

Published online 12 November 2010 *Nucleic Acids Research, 2011, Vol. 39, No. 6* **2393–2403**
doi:10.1093/nar/gkq1158

Expression of distinct RNAs from 3′ untranslated regions

Tim R. Mercer[1], Dagmar Wilhelm[1], Marcel E. Dinger[1], Giulia Soldà[1,2], Darren J. Korbie[1], Evgeny A. Glazov[1,3], Vy Truong[1], Maren Schwenke[1], Cas Simons[1,4], Klaus I. Matthaei[5,6], Robert Saint[7,8], Peter Koopman[1] and John S. Mattick[1,*]

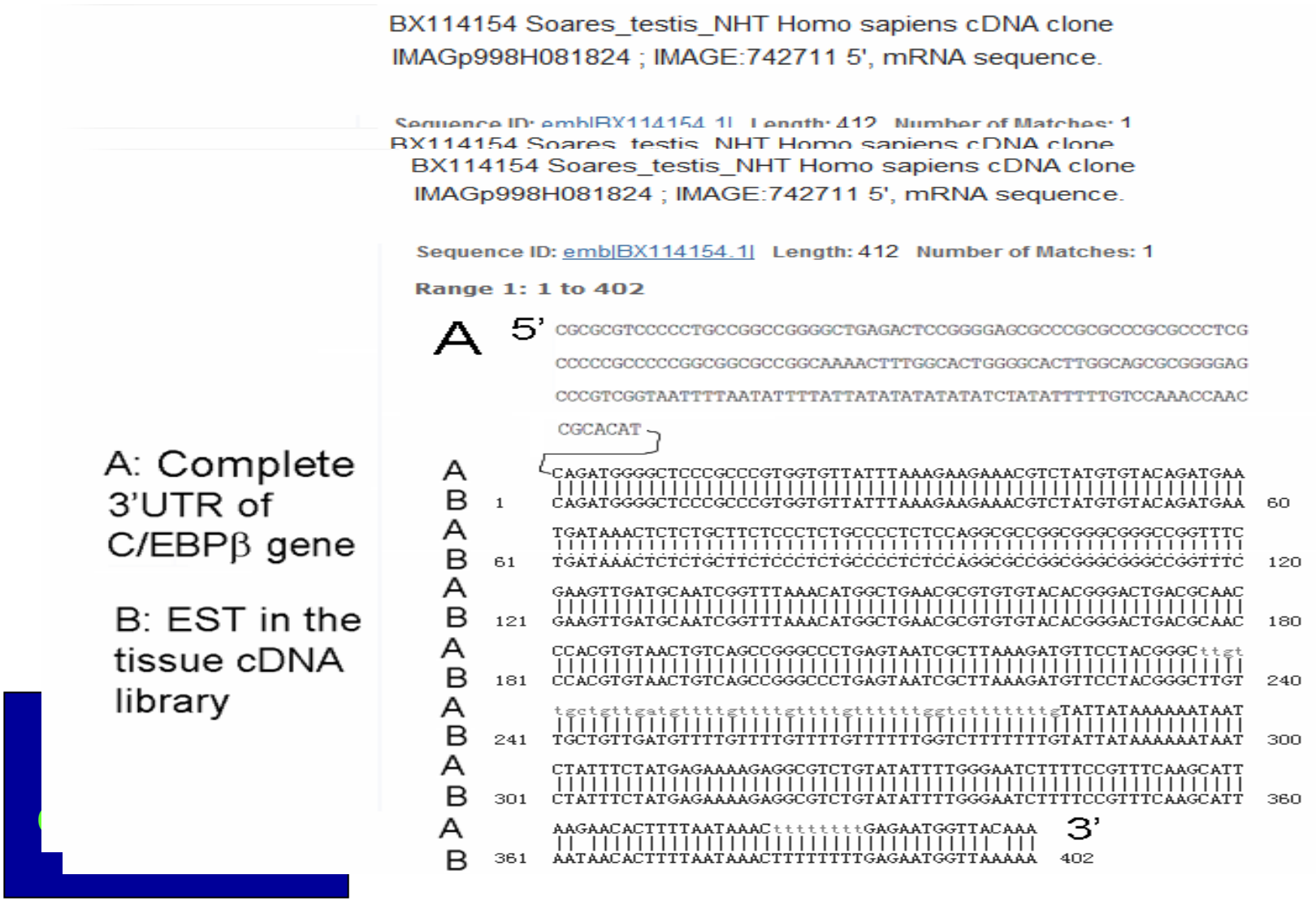
ABSTRACT

The 3′ untranslated regions (3′UTRs) of eukaryotic genes regulate mRNA stability, localization and translation. Here, we present evidence that large numbers of 3′UTRs in human, mouse and fly are also expressed separately from the associated protein-coding sequences to which they are normally linked, likely by post-transcriptional cleavage. Analysis of CAGE (capped analysis of gene expression), SAGE (serial analysis of gene expression) and cDNA libraries, as well as microarray expression profiles, demonstrate that the independent expression of 3′UTRs is a regulated and conserved genome-wide phenomenon. We characterize the expression of several 3′UTR-derived RNAs (uaRNAs) in detail in mouse embryos, showing by *in situ* hybridization that these transcripts are expressed in a cell- and subcellular-specific manner. Our results suggest that 3′UTR sequences can function not only in *cis* to regulate protein expression, but also intrinsically and independently in *trans*, likely as noncoding RNAs, a conclusion supported by a number of previous genetic studies. Our findings suggest novel functions for 3′UTRs, as well as caution in the use of 3′UTR sequence probes to analyze gene expression.

Evidence for Natural Existence of Independent C/EBPβ 3'UTR RNA in Human Tissue: an Example

A: Complete 3'UTR of C/EBPβ gene

B: EST in the tissue cDNA library



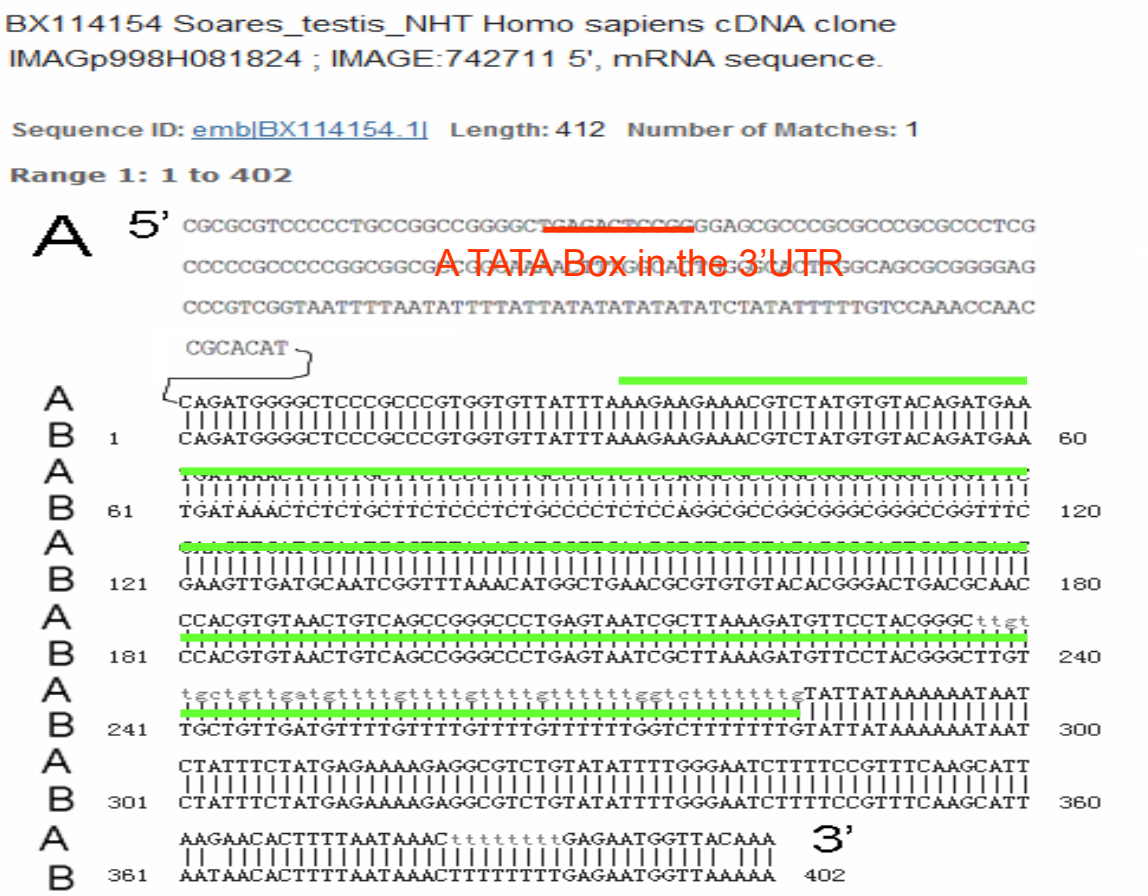
Existence of independent C/EBPβ 3'-UTR RNA in human tissues

Liu DG and Xue P, Acta Biochim Biophys Sin 2014; Jan,46(1):76-7.

A: Complete 3'UTR of C/EBPβ gene

B: EST in the tissue cDNA library

Green line: Our cDNA clone



Наука требует от человека всей его жизни. И если бы у вас было бы две жизни, то и их бы не хватило вам.

И.П.Павлов

Science requires of man his entire life. Even if you had two lives, it would not be enough for you.

I. P. Pavlov

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