

Espressione di una proteina nel suo stato nativo

La **Proteomica** è oggi considerata a tutti gli effetti come una vera e propria disciplina scientifica.

Essa si occupa dello studio funzionale del genoma.

Il sequenziamento del genoma (circa 40.000 geni) ha consentito un enorme sviluppo nella conoscenza di molti processi fisiologici e patologici.

Lo studio del **Proteoma**, e cioè dell' informazione funzionale, potrà permettere di definire ancora di più i meccanismi mediante i quali tanti prodotti genici determinano ruoli e funzioni.

Espressione di una proteina nel suo stato nativo

Il termine ***Proteoma*** deriva dalla definizione :

“ ***Proteine esprese dal Genoma*** ”

Mentre il Genoma ci dice ciò che è possibile che sia espresso, il ***Proteoma***, invece, ci dice ciò che è funzionalmente presente nella cellula.

Un gene, che codifica per una proteina di trasporto, un enzima, un recettore, è sempre presente nel genoma ma potrebbe essere spento e quindi il suo prodotto proteico non funzionante.

Espressione di una proteina nel suo stato nativo

L' espressione funzionale dell'informazione genetica, il **Proteoma**, può variare in dipendenza :

- 1) del tipo di cellula
- 2) dello stadio di sviluppo
- 3) delle variazioni ambientali
- 4)

Il Proteoma è molto **dinamico e complesso** rispetto al Genoma.

Espressione di una proteina nel suo stato nativo

Lo studio del **Proteoma** implica necessariamente l' isolamento delle proteine di interesse dal resto delle macromolecole biologiche della cellula.

La purificazione di una proteina è la condizione essenziale per conoscere la sua struttura e la sua funzione, dal momento che, come dice un vecchio adagio molto in voga tra i biochimici,

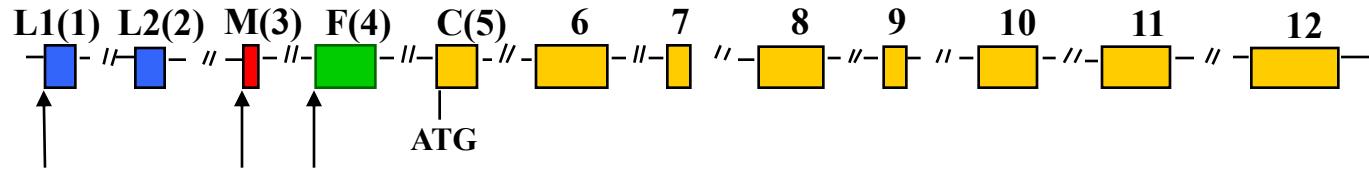
“.. non bisogna sprecare buoni propositi su una proteina non pura! ”

Espressione di una proteina nel suo stato nativo

La purificazione di una proteina non esaurisce le domande sulla sua funzione nel complesso delle attività all'interno di una cellula. Se la proteina isolata è una chinasi, per esempio, sarà utile sapere :

- 1) dove essa è localizzata**
- 2) quali altre proteine interagiscono con essa**
- 3) quali sono i bersagli della sua attività**
- 4) in quali tessuti essa è attiva**
- 5) quali vie di trasduzione del segnale ne influenzano l' attività**
- 6) qual è il suo ruolo nello sviluppo, nel differenziamento...**

Structural organization of the human Aldolase A gene



type-F mRNA



type-M mRNA

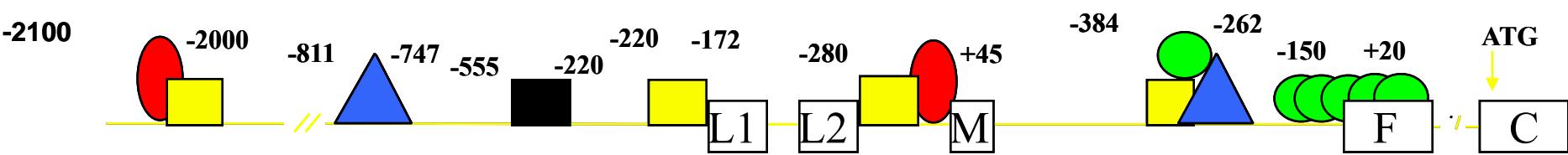


type-L mRNA



100bp

Organization of the promoter of the human Aldolase A gene



■ = negative regulatory element (AldA-NRE)

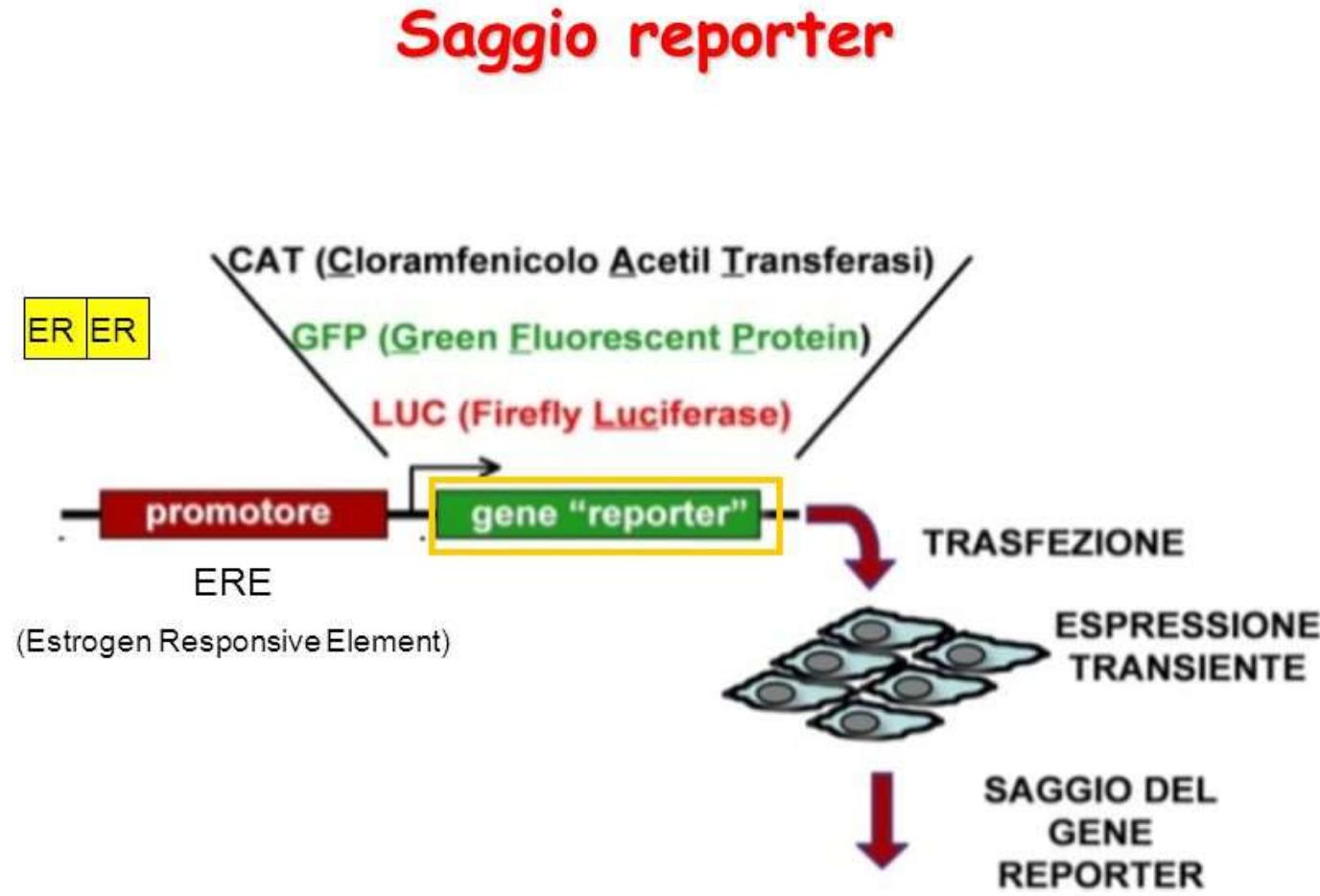
○ = Sp1 element

□ = unknown positive regulatory element

○ = muscle-specific positive regulatory element (MEF1)

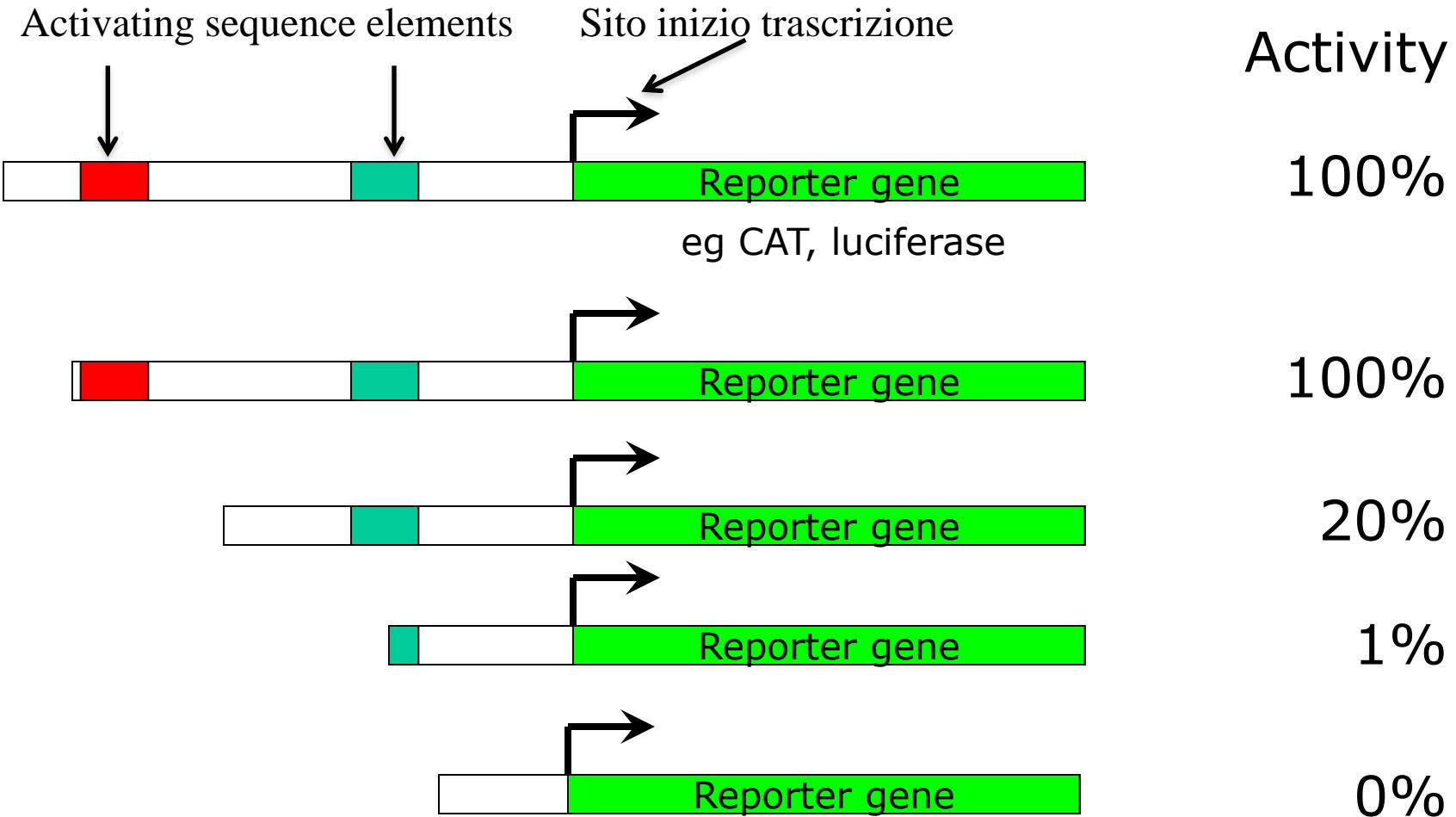
▲ = Ap1 element

Analysis of the promoter activity by transient expression of a reporter gene



Analysis of the promoter activity

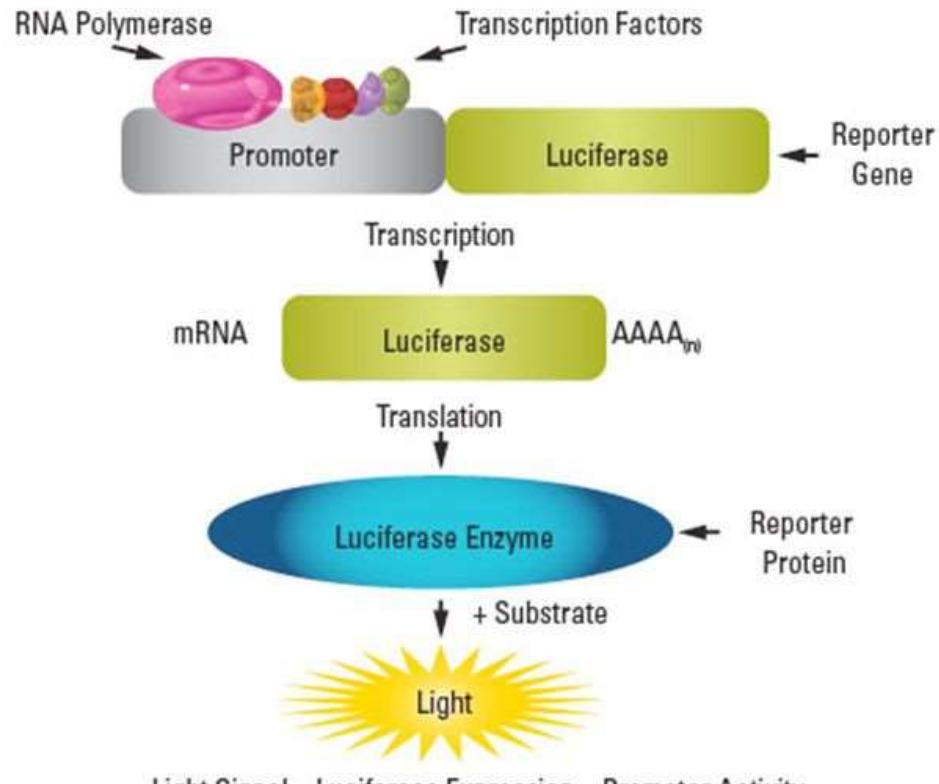
by transient expression of a reporter gene



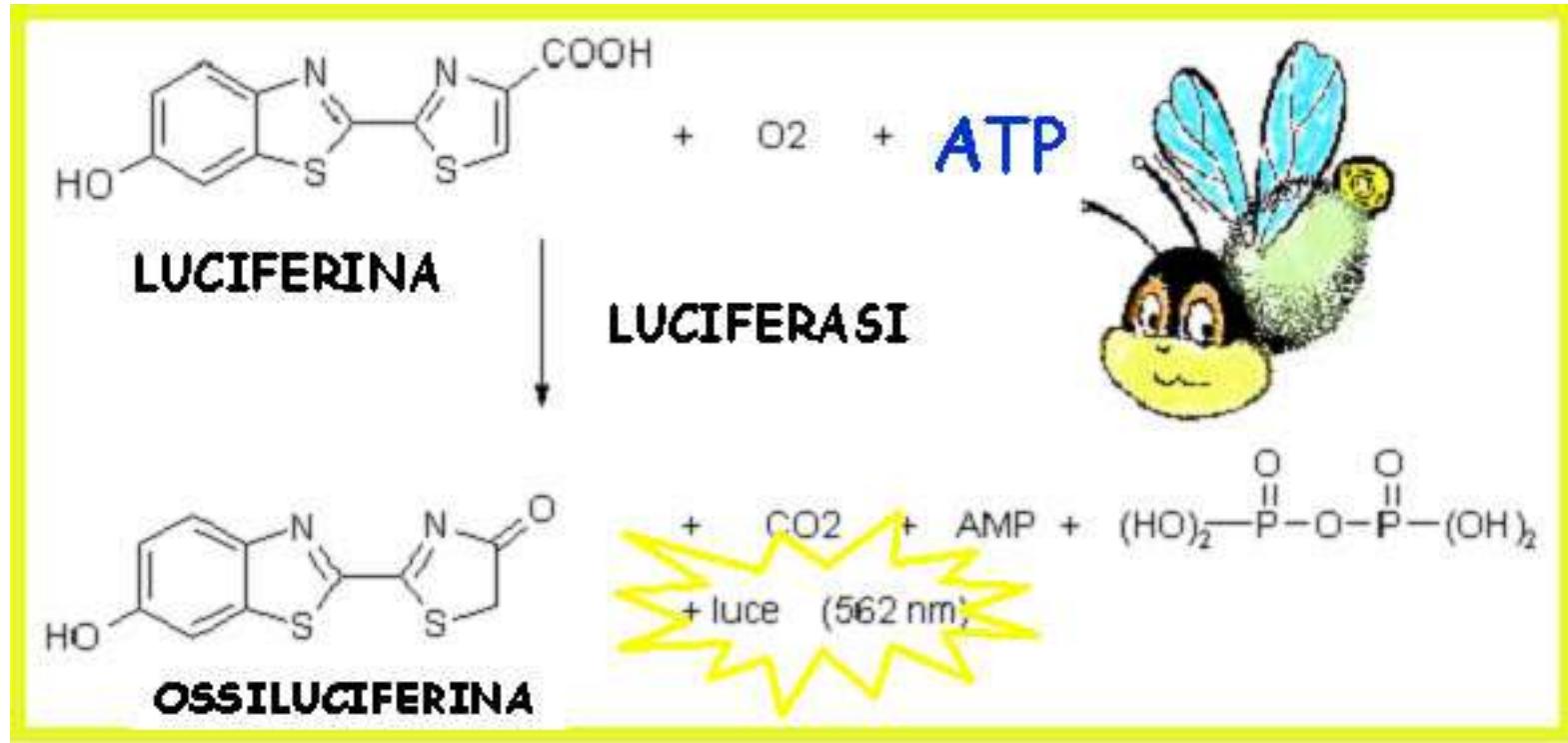
Analysis of the promoter activity by transient expression of a reporter gene

Geni reporter: **firefly luciferasi**

NB Controllo interno per efficienza di trasfezione, x es promotore forte con renilla luciferasi



Analysis of the promoter activity by transient expression of a reporter gene



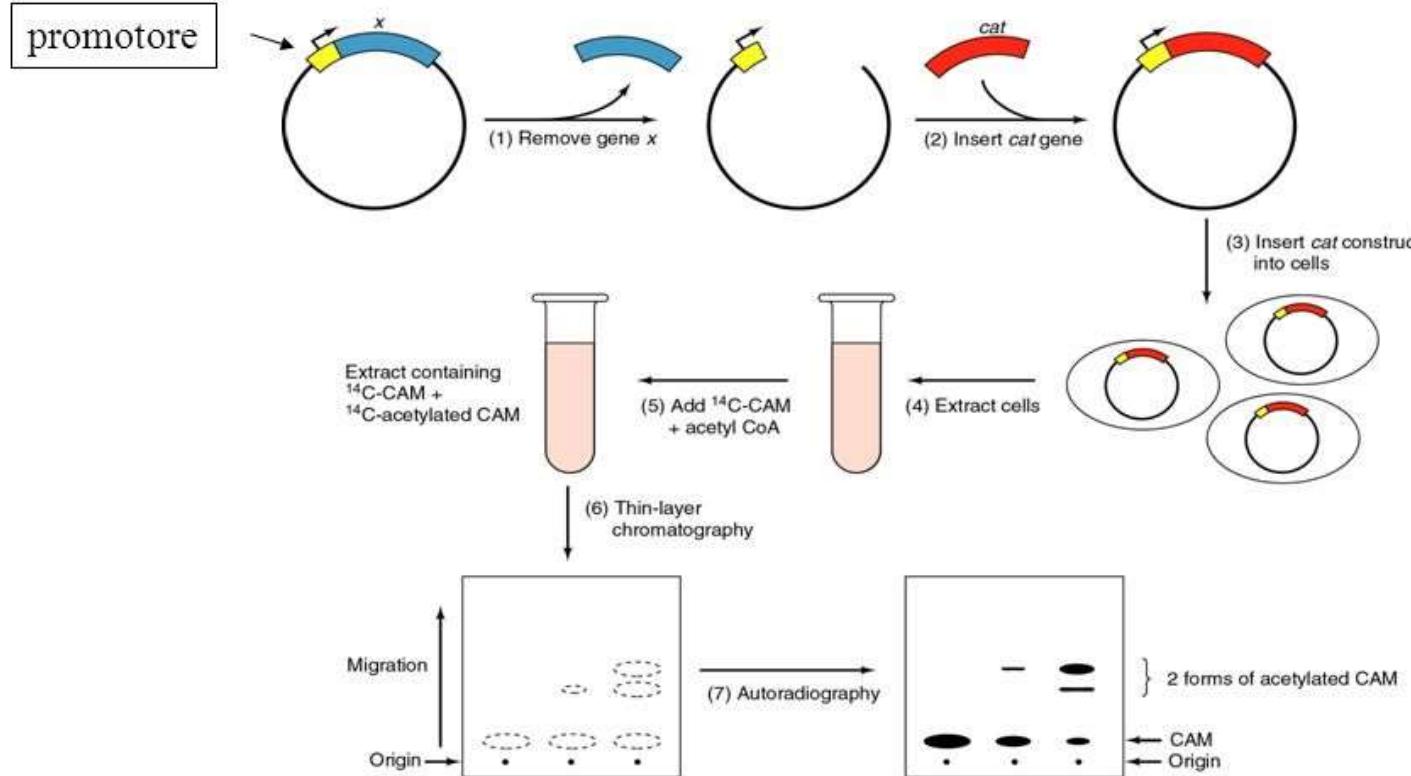
Analysis of the promoter activity

by transient expression of a reporter gene

**Analisi funzionale di sequenze regolatorie: i saggi reporter
(particolarmente utili in trasfezioni transienti)**

Geni reporter: **CAT (cloramfenicolo-acetyl-transferasi)**

NB Controllo interno per efficienza di trasfezione, x es promotore forte con luciferasi



Analysis of the promoter activity

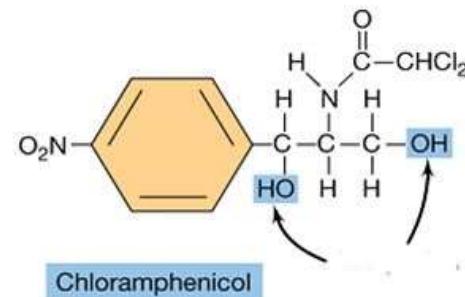
by transient expression of a reporter gene

CLORAMFENICOLO

Originariamente isolato di *Streptomyces venezuelae* è ora ottenuto per sintesi chimica.

Analagomente all'eritromicina si lega all'rRNA 23S della subunità 50S del ribosoma: inibisce la peptidil trasferasi e svolge un'azione batteriostatica

Spettro d'azione molto ampio anche se è piuttosto tossico



Analysis of the promoter activity

by transient expression of a reporter gene

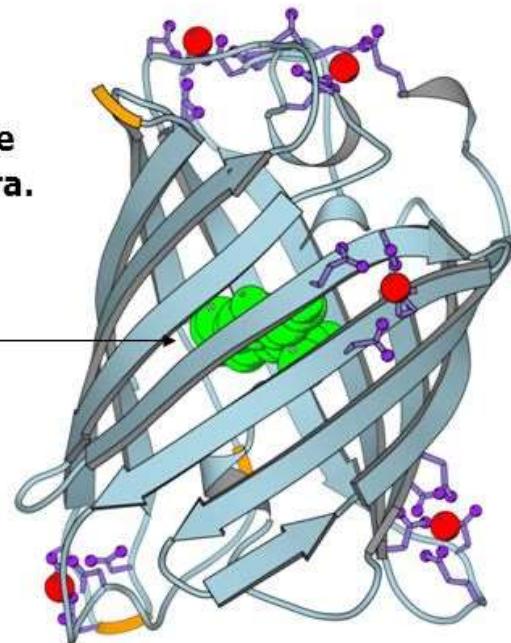
Struttura Tridimensionale Delle Proteine Verdi Fluorescenti

Questa proteina possiede una struttura tridimensionale molto particolare, che ricorda un cilindro cavo o un barilotto (in inglese, b-barrel), con un diametro di 24 Å ed una altezza di 42 Å.

Le pareti del cilindro sono formate da 11 β-foglietti intrecciati a coppie e collegati tra loro mediante delle corte a-eliche che chiudono le due basi della struttura.

All'interno di questa struttura estremamente stabile (T. stab 76 °C) è contenuta un'ulteriore a-elica che comprende il cromoforo della proteina.

Il corretto ripiegamento della proteina è necessario per ottenere un ambiente rigido e strutturato intorno al cromoforo e per schermare il cromoforo da agenti esterni in grado di deprimere la resa di fluorescenza

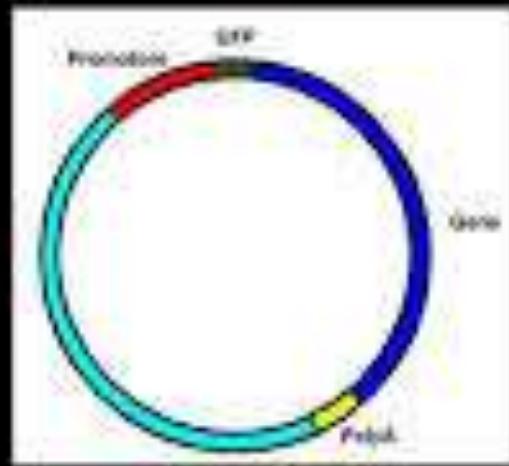
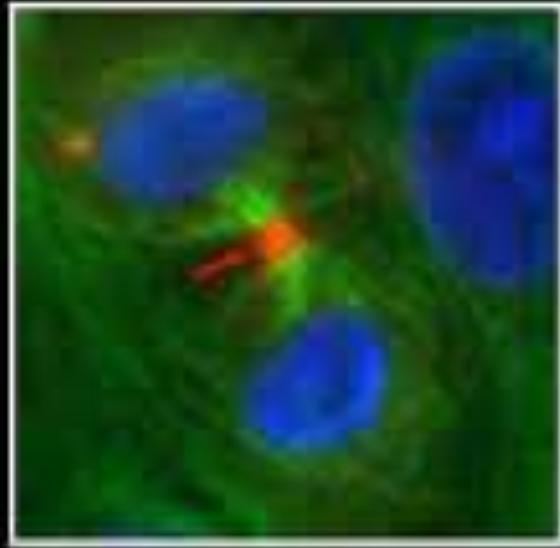


Analysis of the promoter activity

by transient expression of a reporter gene

In vitro

Localizzazione subcellulare

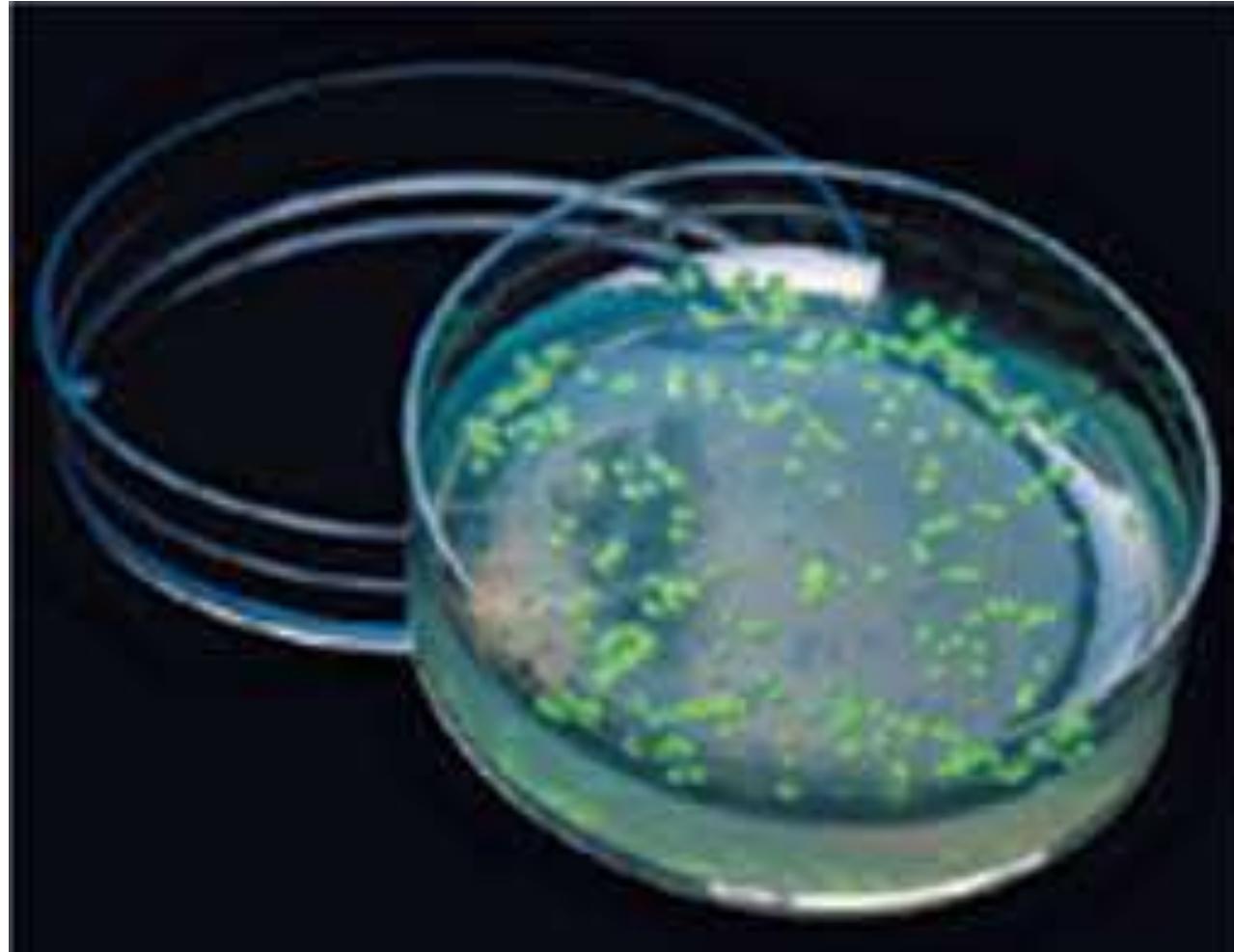


- Anticorpi (per generarli in genere bisogna produrre una proteina ricombinante)
- Costrutti di espressione in cui al cDNA della proteina viene fuso in-frame un "tag" protéico fluorescente (GFP) o che renda la proteina riconoscibile da parte di un anticorpo (epitopi HA, MYC e FLAG)

Analysis of the promoter activity

by transient expression of a reporter gene

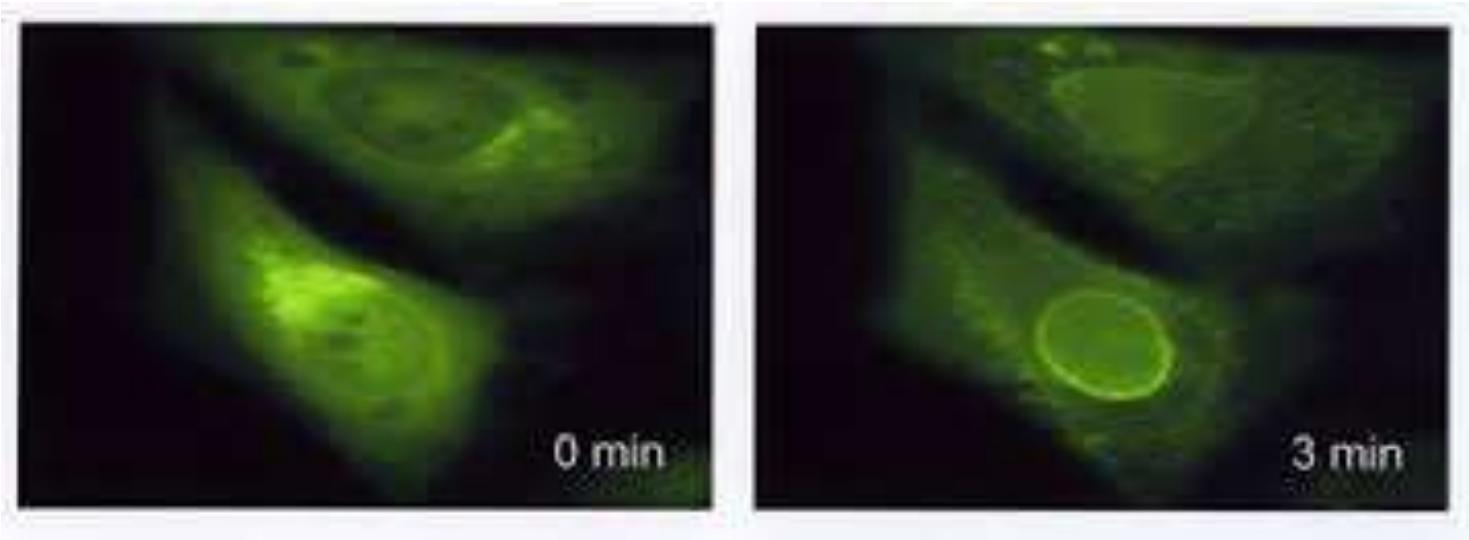
In vitro



Analysis of the promoter activity

by transient expression of a reporter gene

In vitro



Analysis of the promoter activity

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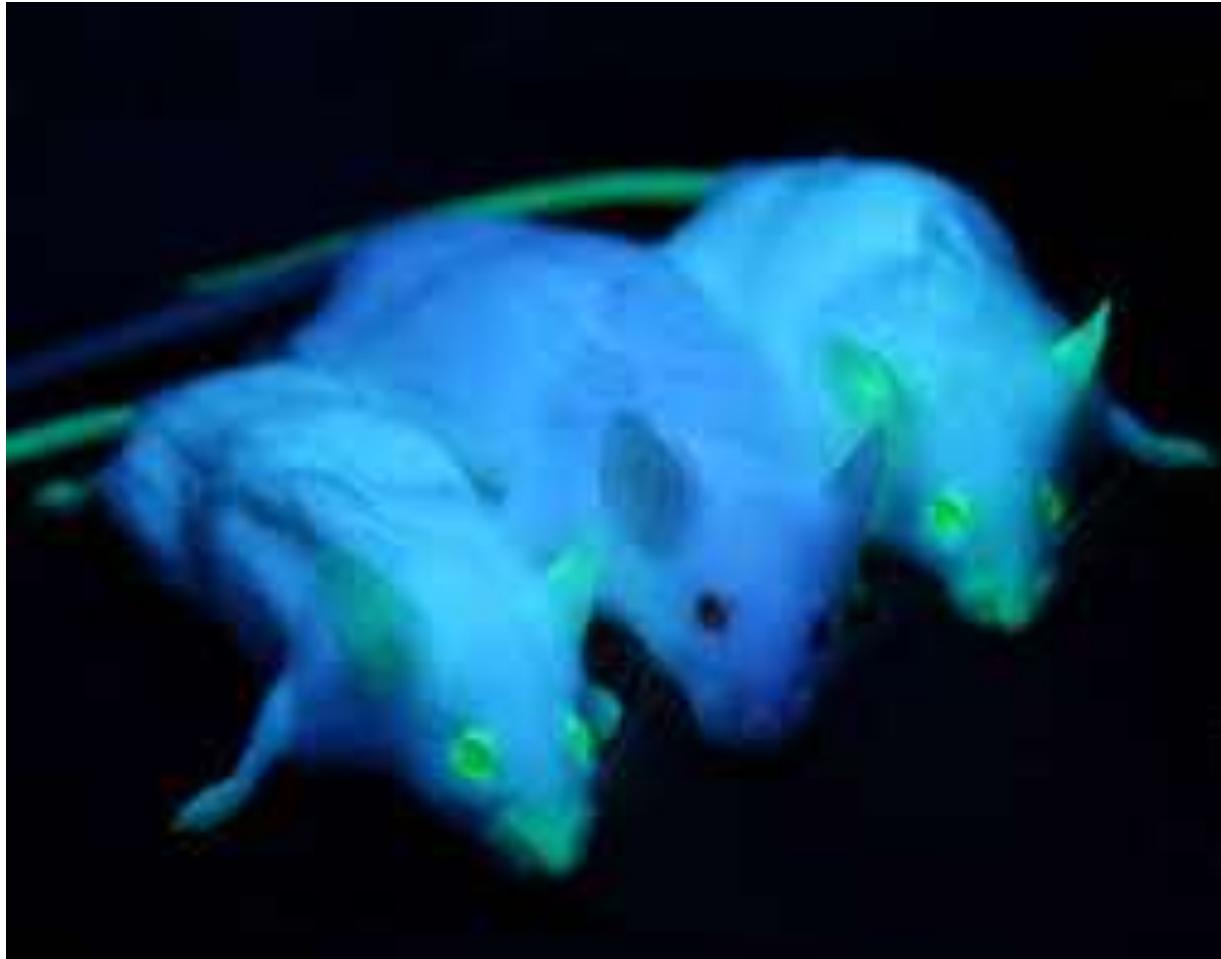
In vivo



Analysis of the promoter activity

by transient expression of a reporter gene

In vivo



Analysis of the promoter activity

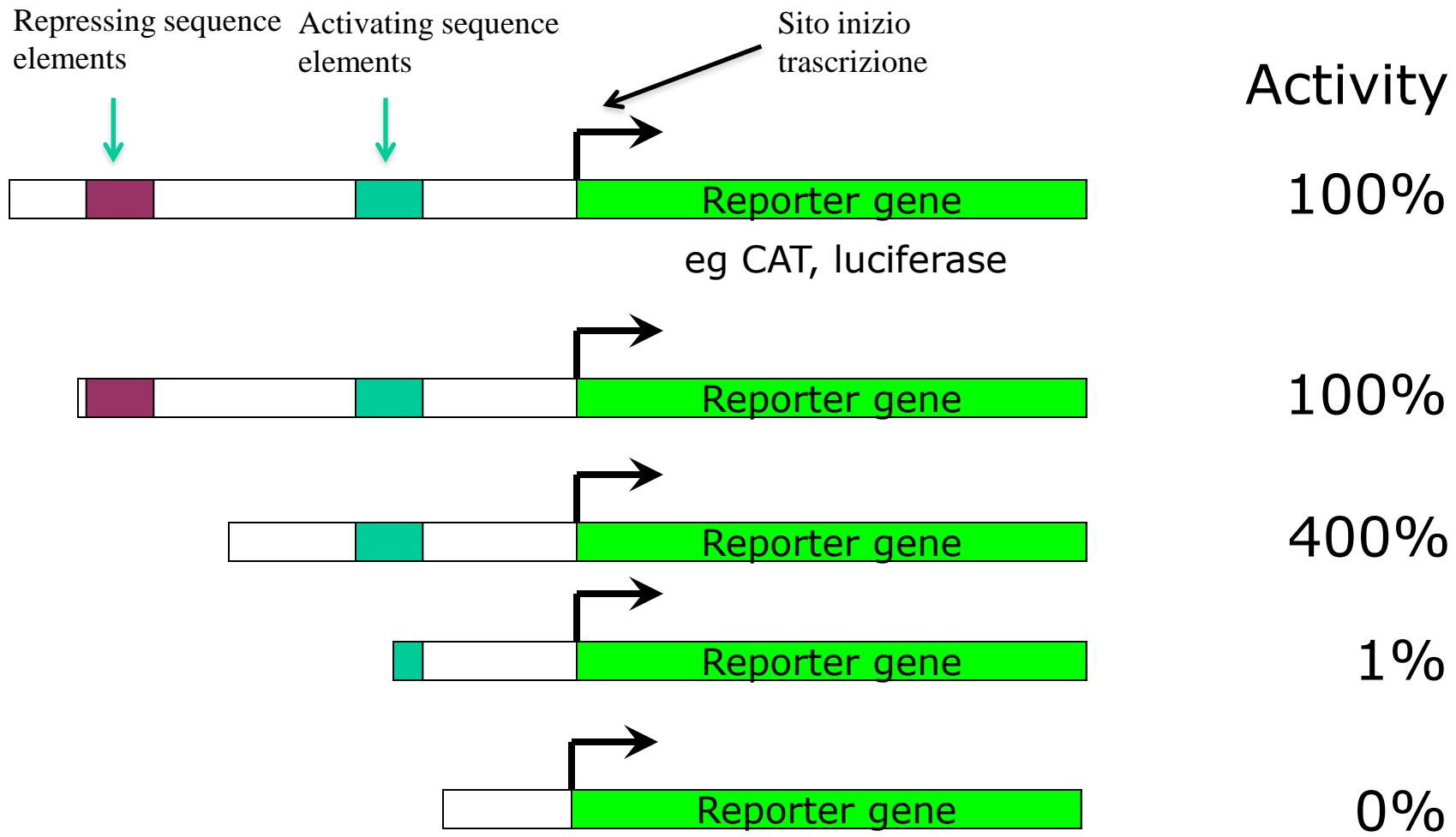
by transient expression of a reporter gene

In vivo

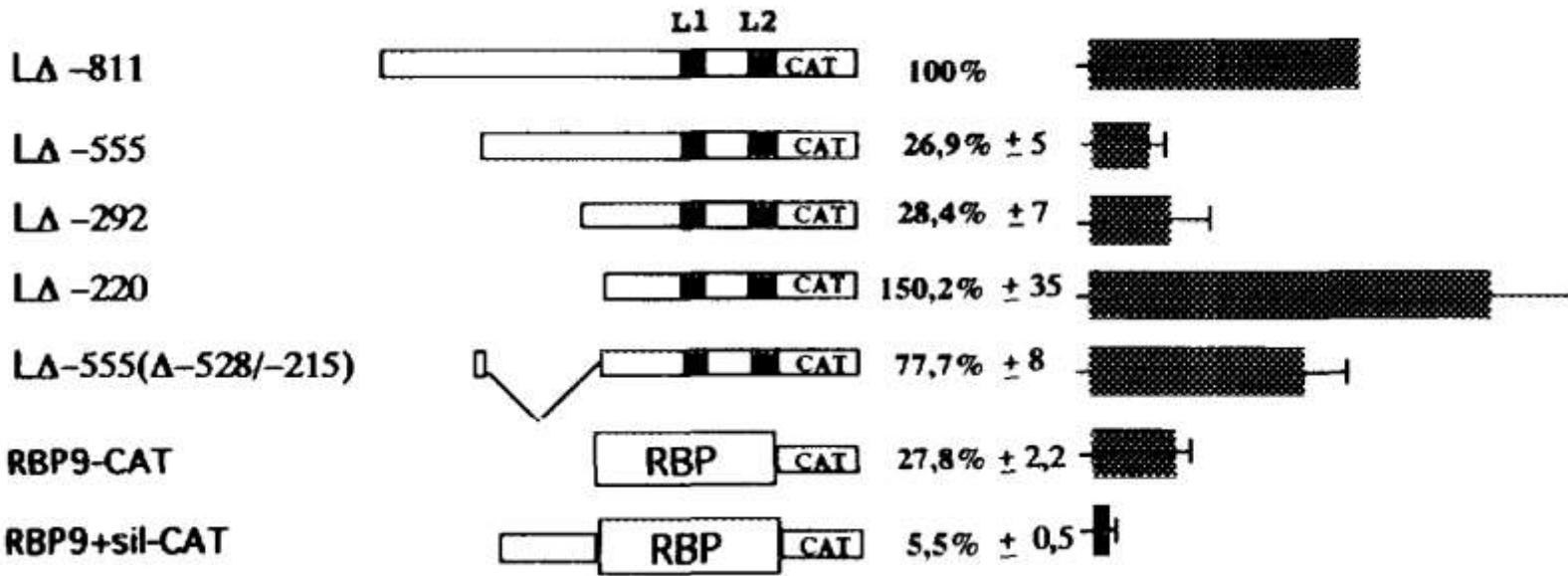


Analysis of the promoter activity

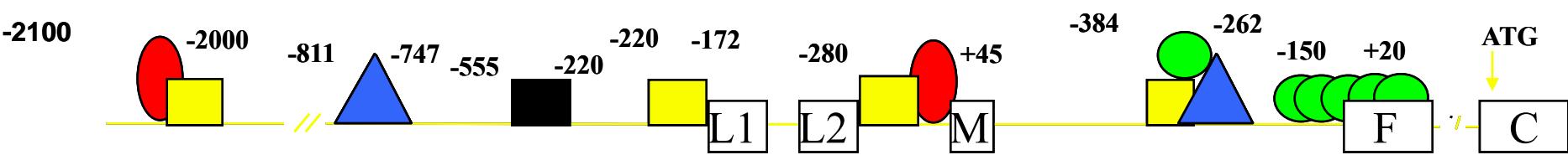
by transient expression of a reporter gene



Analysis of the promoter activity by transient expression of the aldolase A - reporter gene



Organization of the promoter of the human Aldolase A gene



■ = negative regulatory element (AldA-NRE)

● = Sp1 element

□ = unknown positive regulatory element

○ = muscle-specific positive regulatory element (MEF1)

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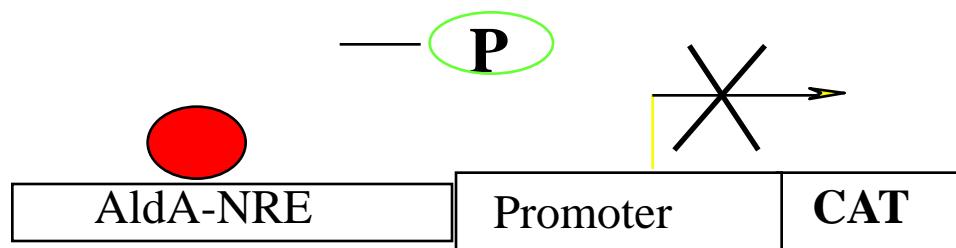
Analysis of the promoter activity by transient expression of a reporter gene

1° risultato :

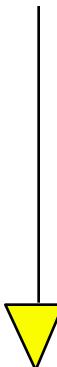
Identificazione di un'interazione specifica tra una proteina repressore e un elemento di sequenza nel promotore del gene umano codificante per l'Aldolasi A

Transcriptional control mediated by 97 kDa repressor /AldA-NRE interaction

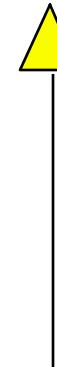
Proliferating cells



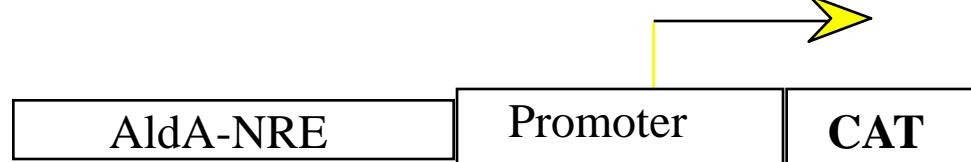
Phosphorylation of 97 kDa repressor by PKC activity



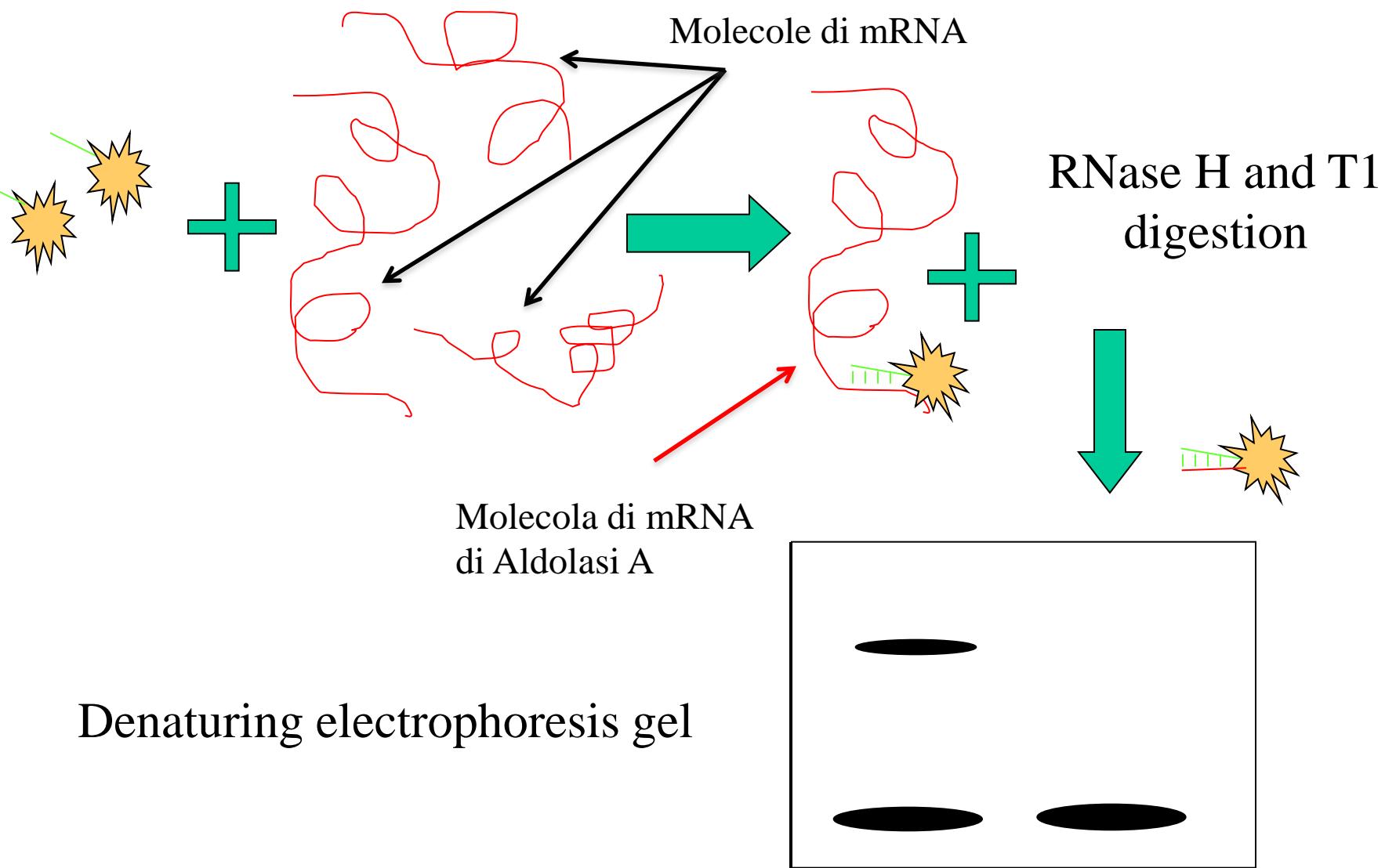
Dephosphorylation of 97 kDa repressor by inactivation of PKC



Differentiated
or
serum-starved
cells



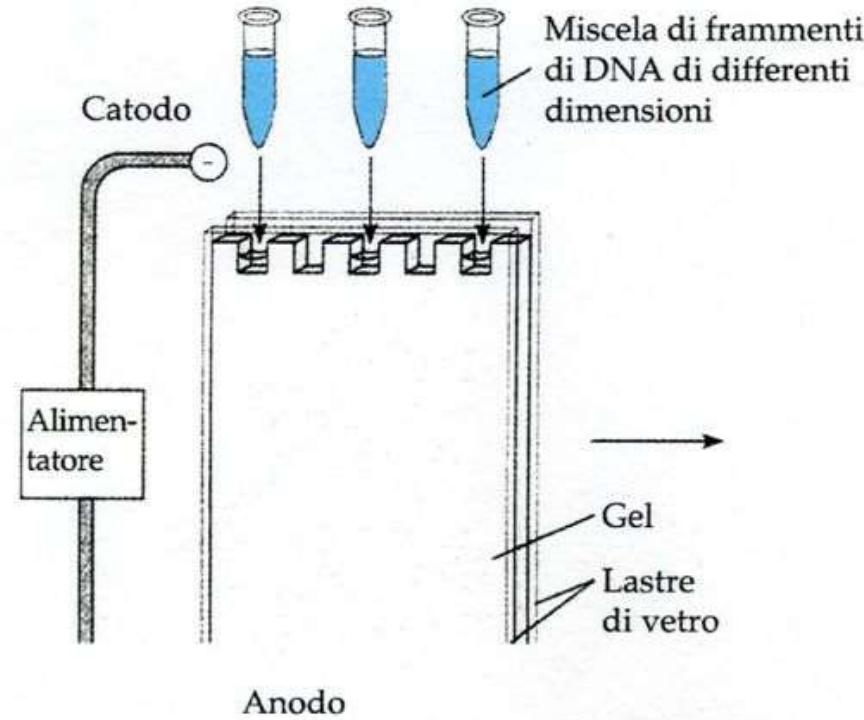
RNase Protection assay on total RNA





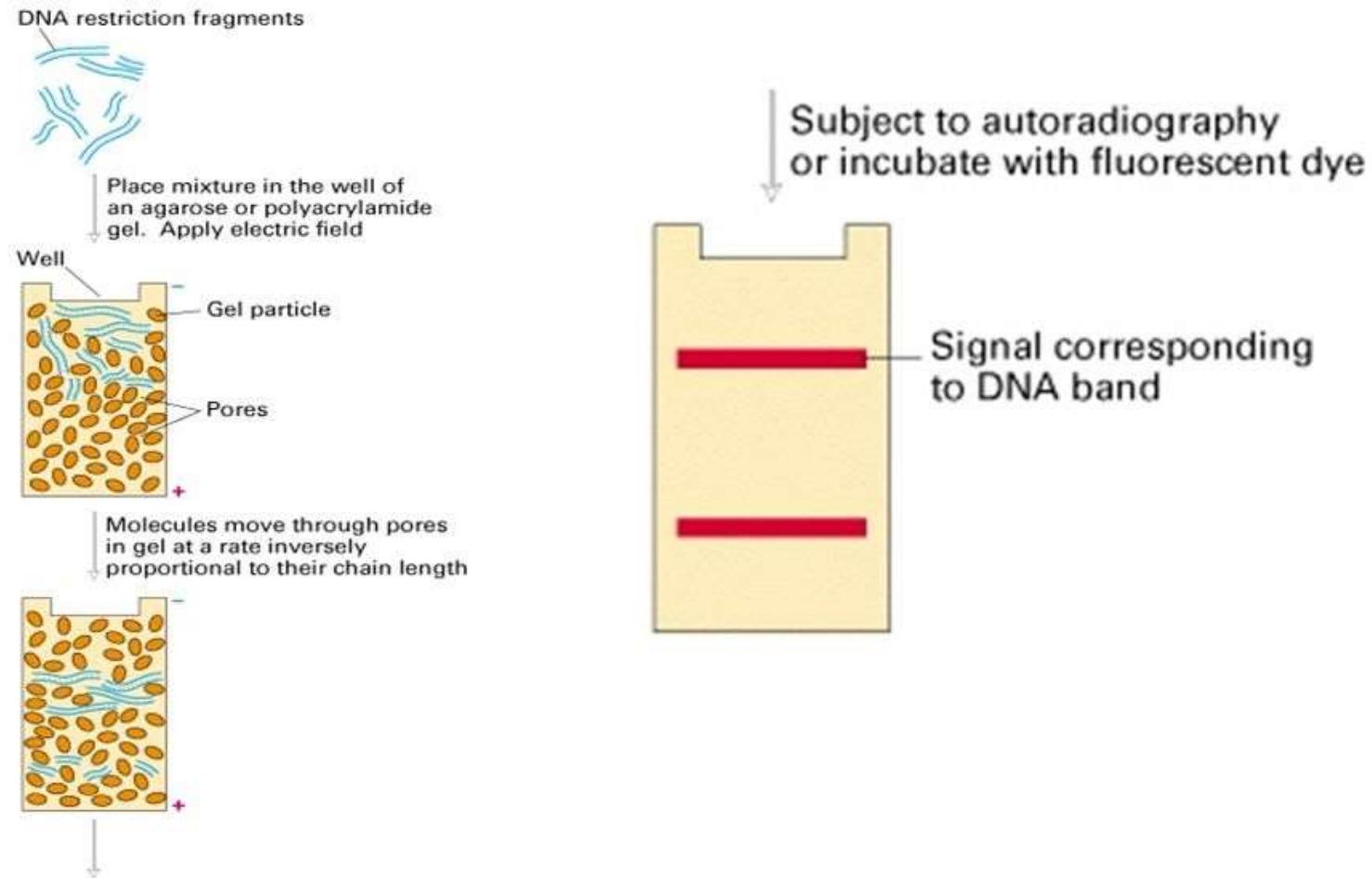
RNase Protection on total RNA

ELETTOFORESI SU GEL DI DNA

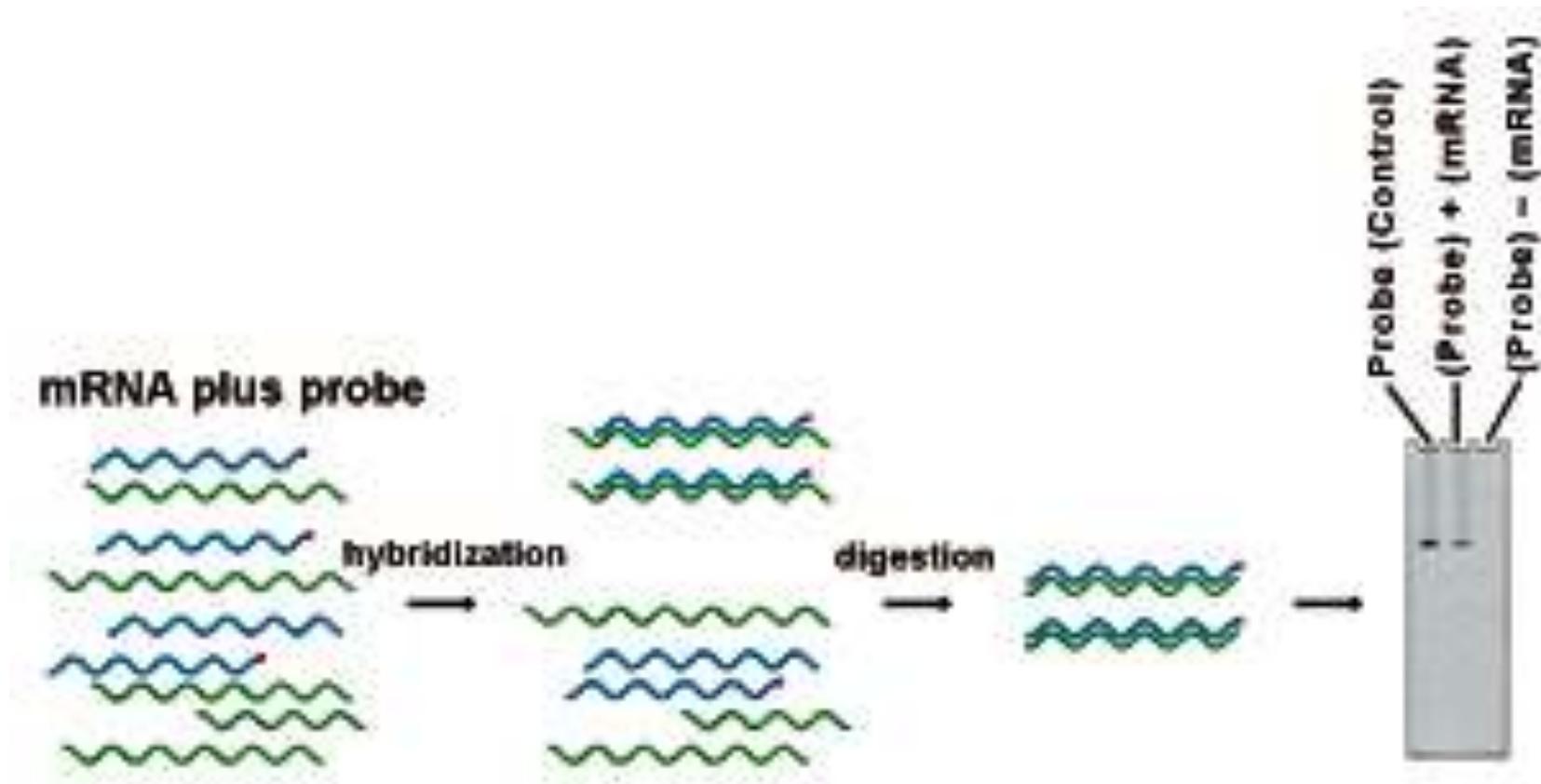


RNase Protection on total RNA

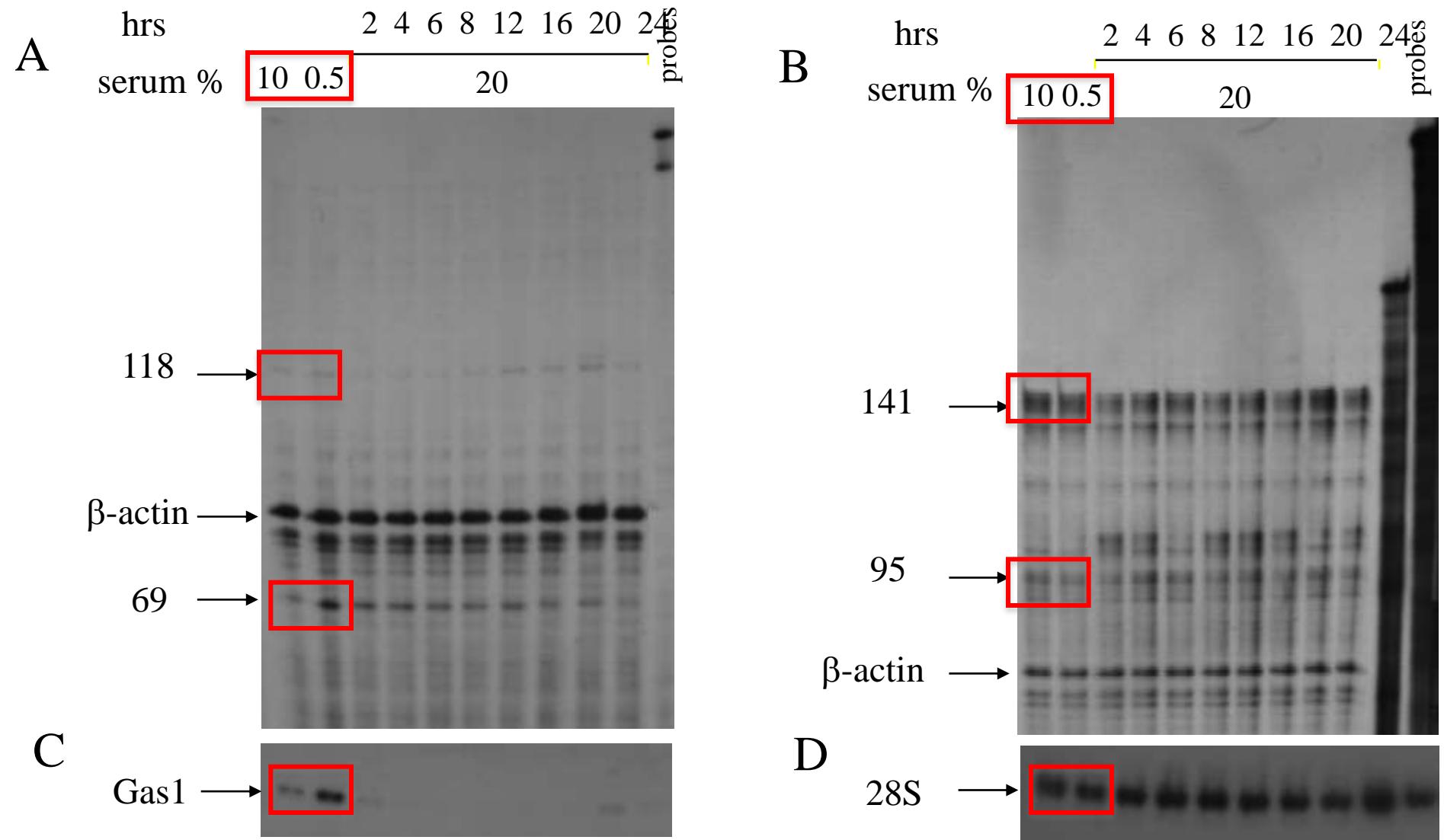
Separazione di frammenti di DNA (o RNA) di diversa lunghezza tramite elettroforesi



RNase Protection on total RNA



RNase Protection on total RNA from proliferating and starved NIH3T3 cells using the specific probes for L- and F-type mRNAs



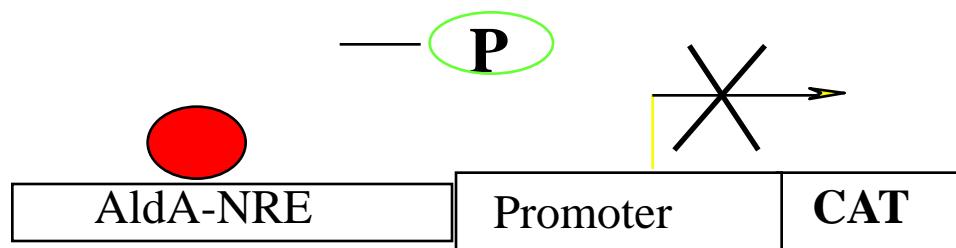
Analysis of the expression of human aldolase A gene

2° risultato:

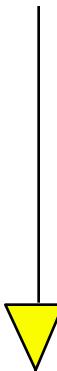
L'RNA messaggero del gene umano codificante per l'Aldolasi A è negativamente regolato nelle cellule proliferanti rispetto alle differenziate o arrestate nella crescita.

Transcriptional control mediated by 97 kDa repressor /AldA-NRE interaction

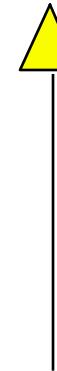
Proliferating cells



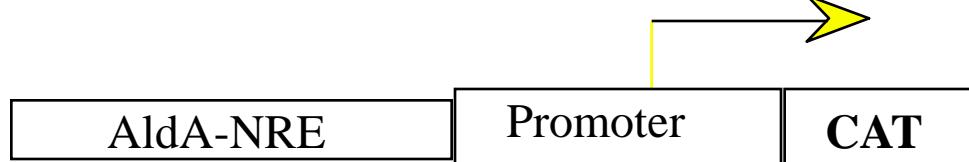
Phosphorylation of 97 kDa repressor by PKC activity



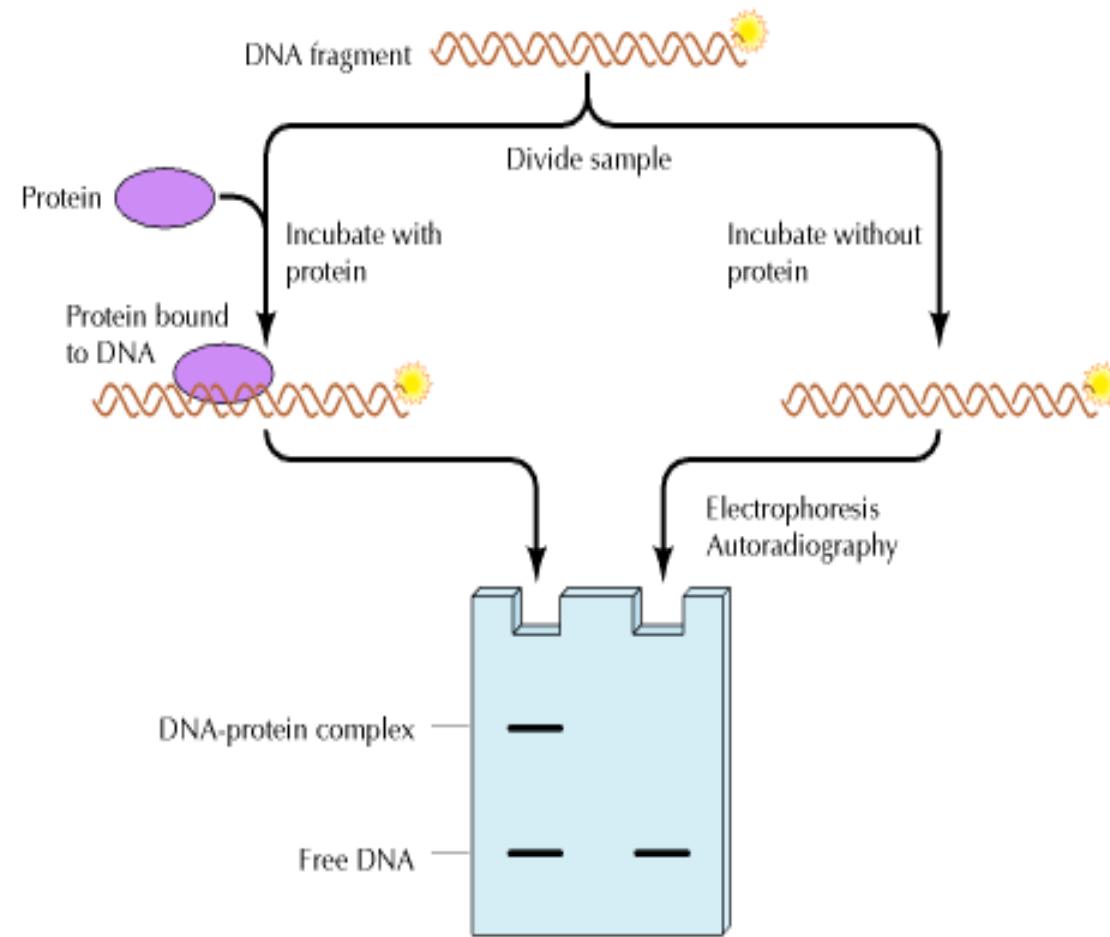
Dephosphorylation of 97 kDa repressor by inactivation of PKC



Differentiated
or
serum-starved
cells



Gel shift assay



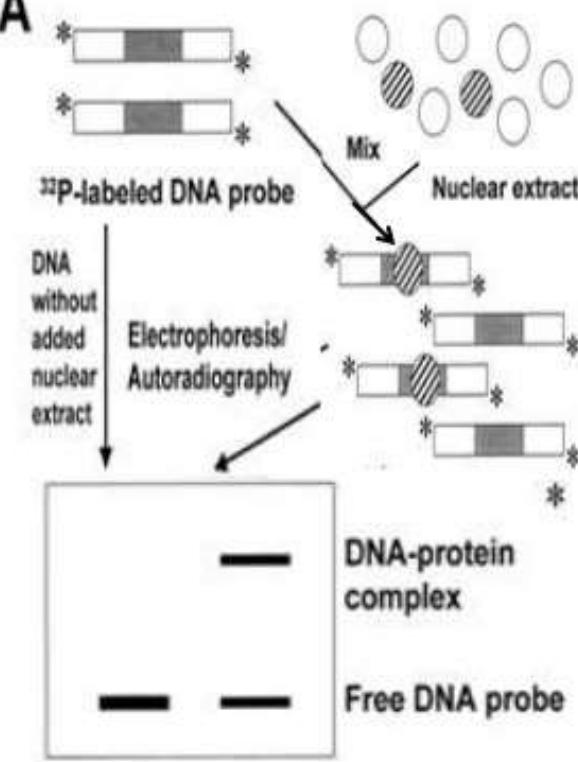
Gel shift assay

Methods

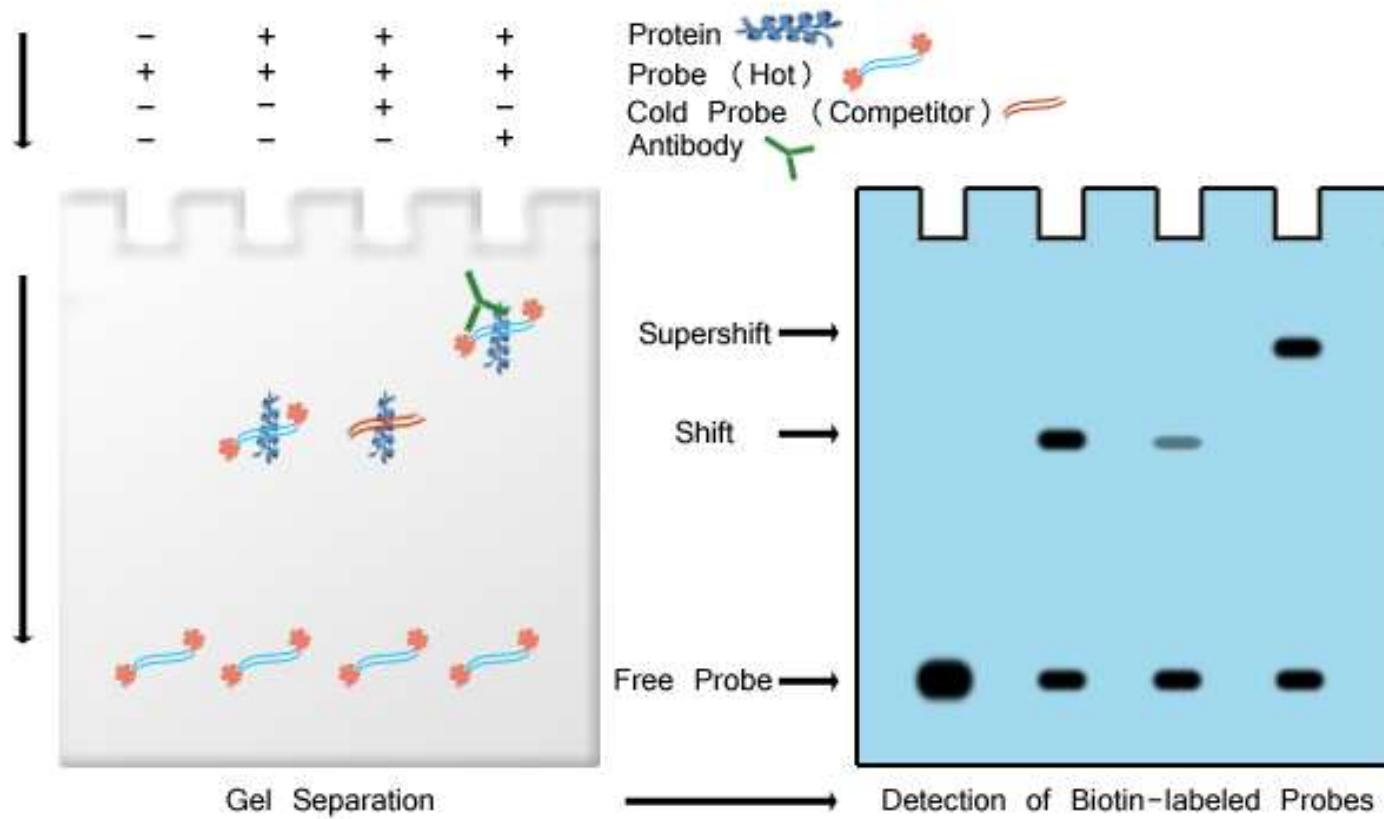
5 basic steps are in conventional EMSA protocol A

- Preparation of purified or crude protein sample
- Preparation of nucleic acid
- Binding reactions
- Non-denaturing gel electrophoresis
- Detection of the outcome

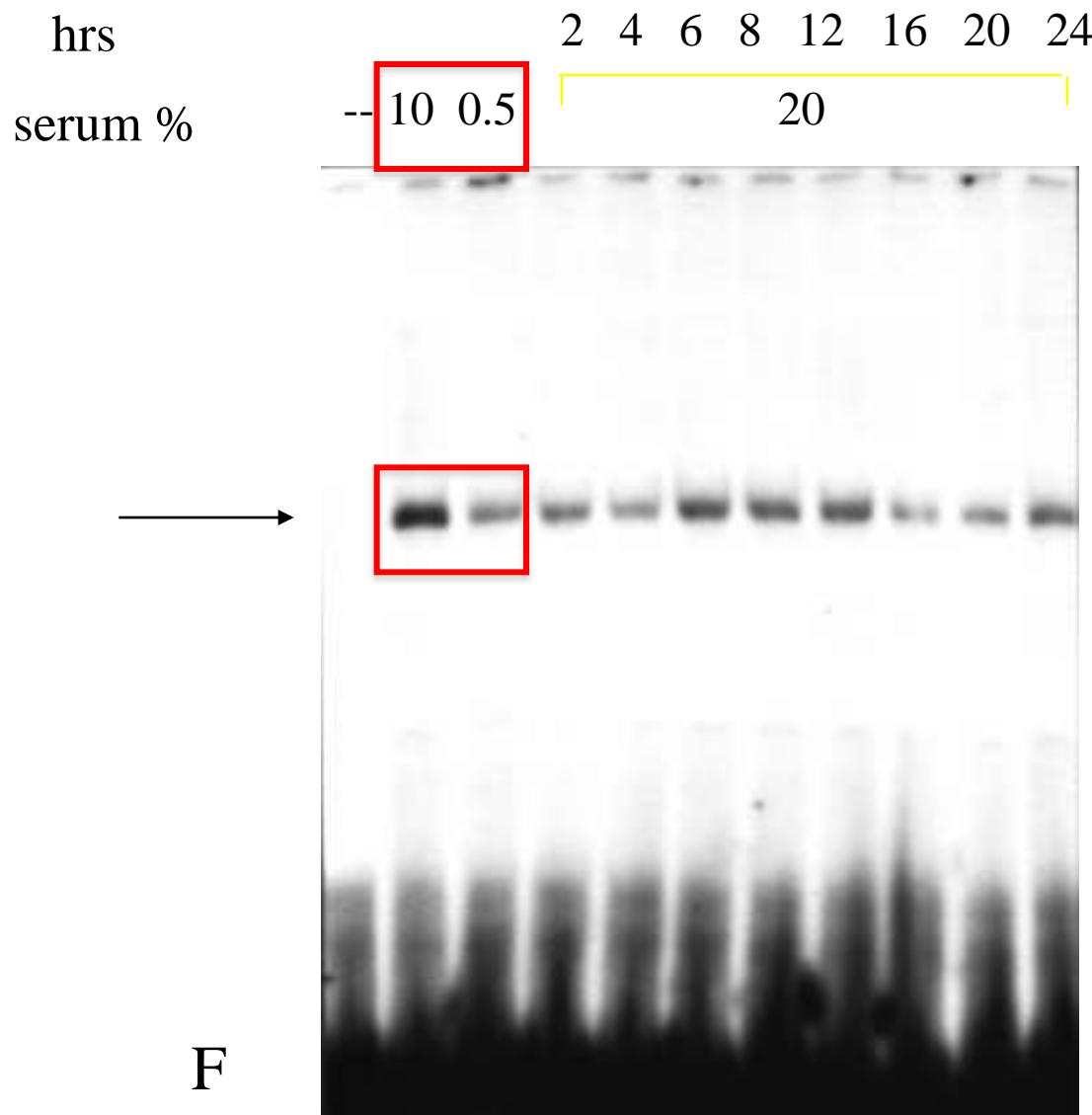
EMSA Variant: Supershift assay



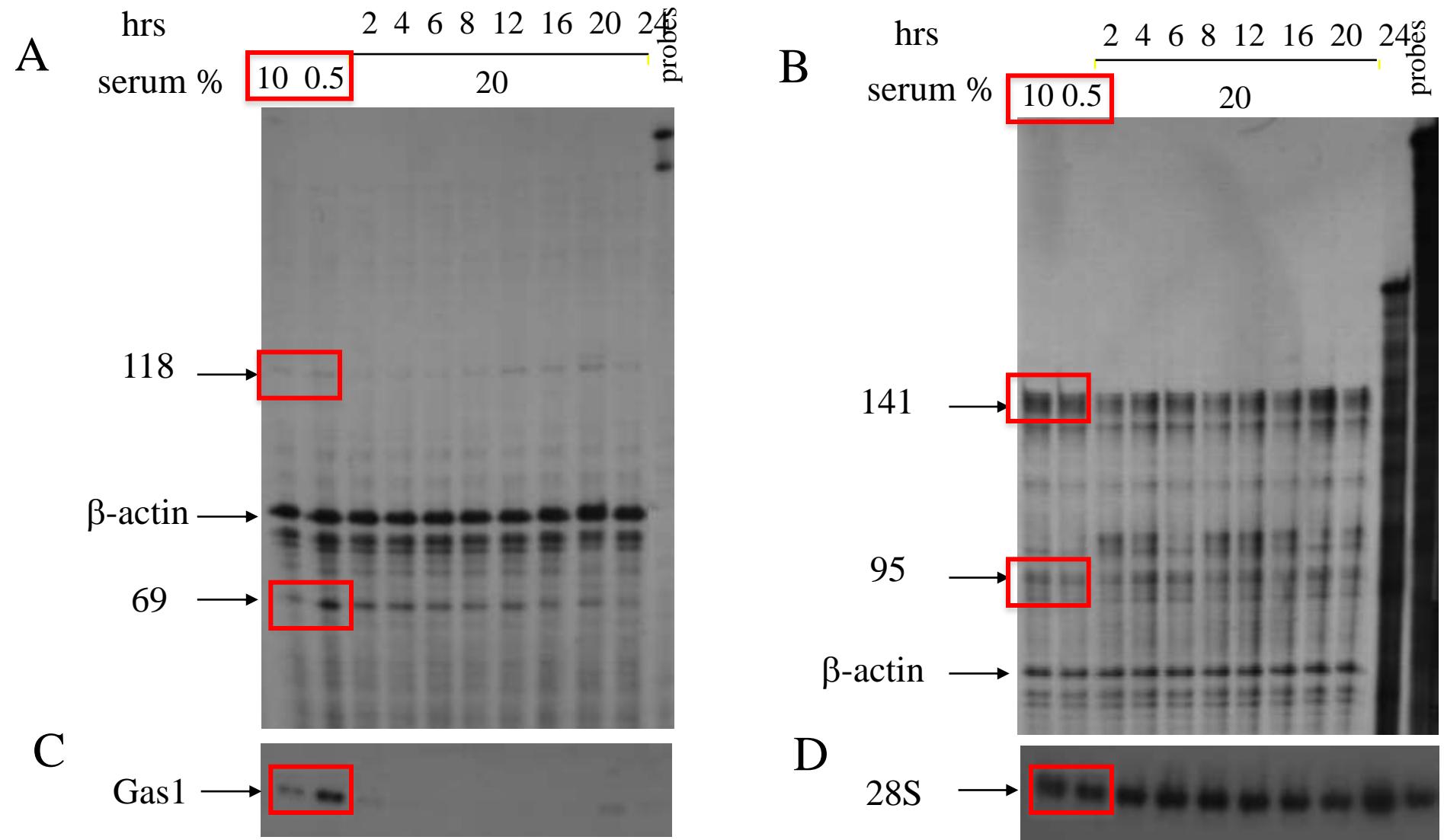
Gel shift assay



Gel-shift assay on nuclear extracts from proliferating and starved NIH3T3 cells



RNase Protection on total RNA from proliferating and starved NIH3T3 cells using the specific probes for L- and F-type mRNAs

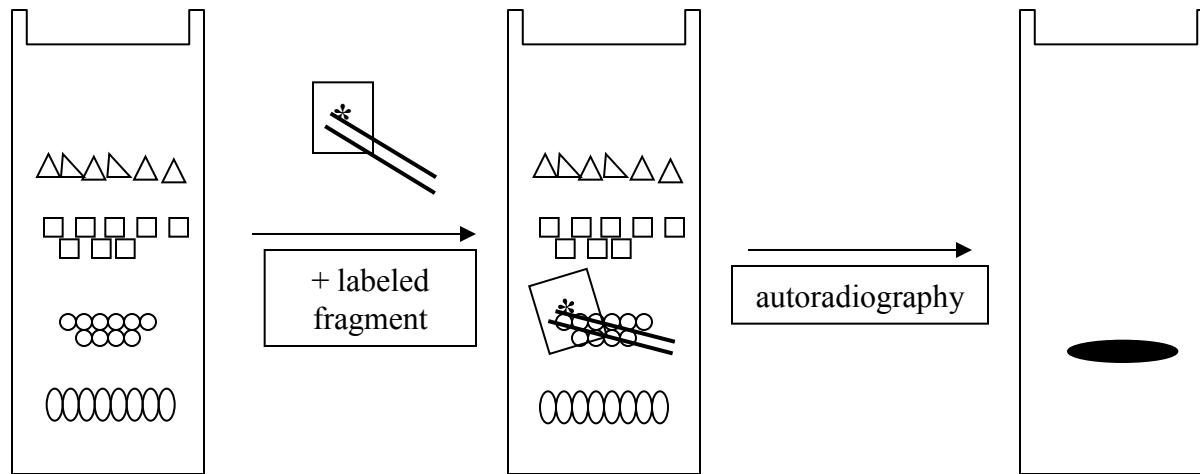


Analysis of the expression of human aldolase A gene

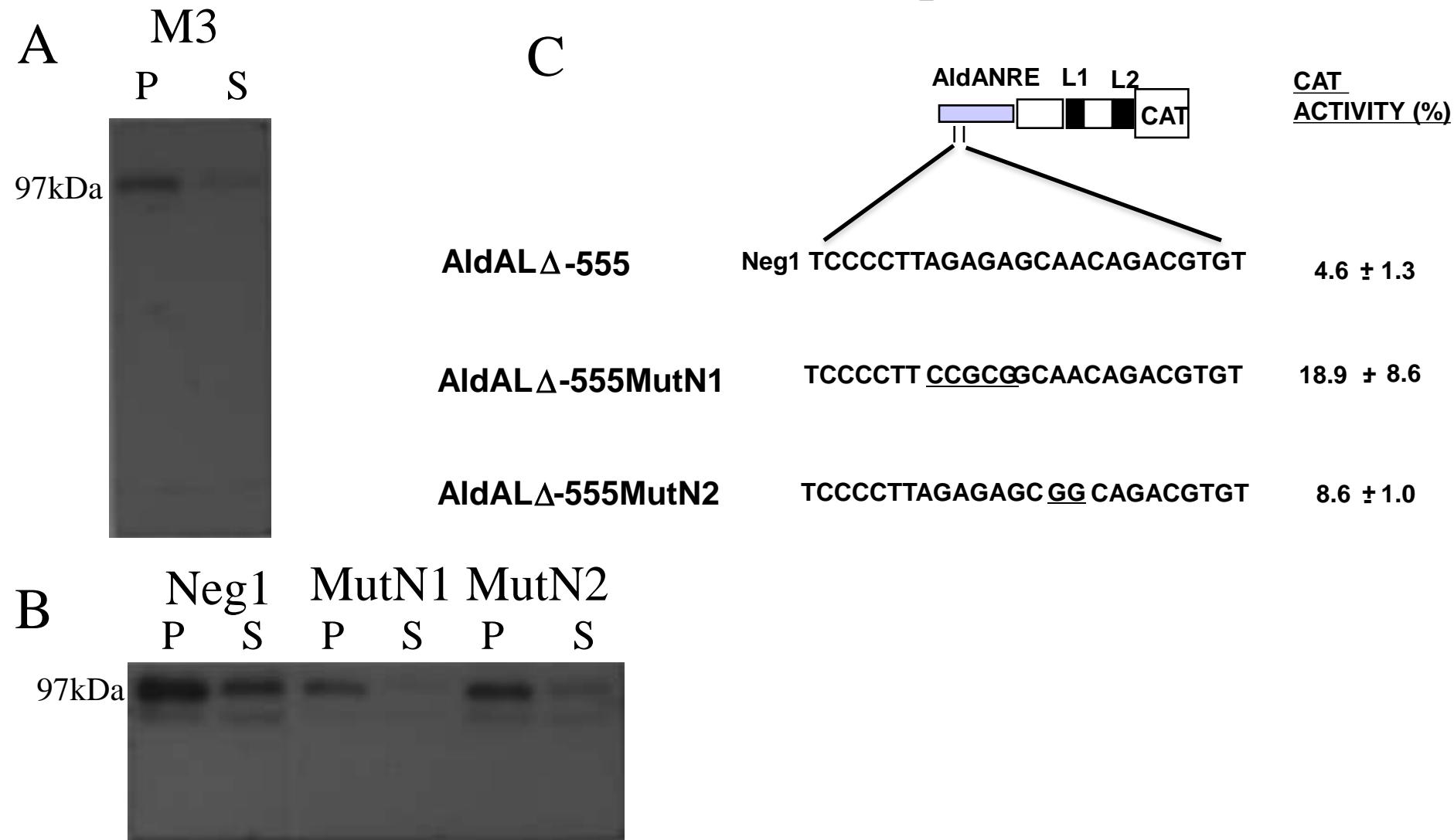
3° risultato:

L'RNA messaggero del gene umano codificante per l'Aldolasi A è negativamente regolato nelle cellule proliferanti e questa regolazione è dipendente da una forte interazione DNA-proteina.

Protein separation by SDS-electrophoresis gel and southwestern assay



Southwestern assay on NIH3T3 cell extracts with M3, Neg1, MutN1, MutN2 oligonucleotides and transient transfection experiments



Transcriptional regulation of the expression of human aldolase A gene

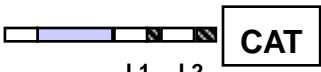
4° risultato :

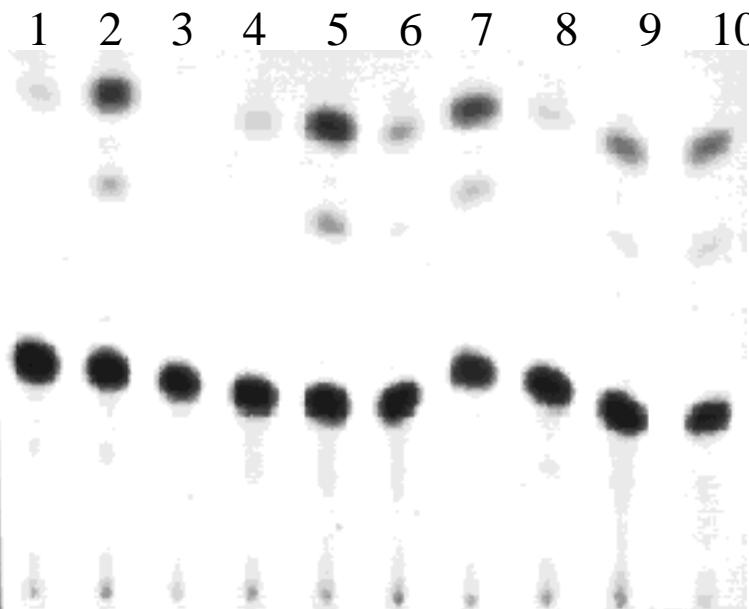
Il legame specifico del repressore trascrizionale sul promotore del gene umano codificante per l’Aldolasi A è dipendente dal tipo di sequenza (**AGAGAGCAA**).

Esperimenti di trasfezione transiente confermano la necessità del motivo di legame GA-rich.

Il peso molecolare della proteina repressore sembra aggirarsi sui 97 kDa.

Transient transfection experiment of recombinant constructs in proliferating and starved NIH3T3 cells

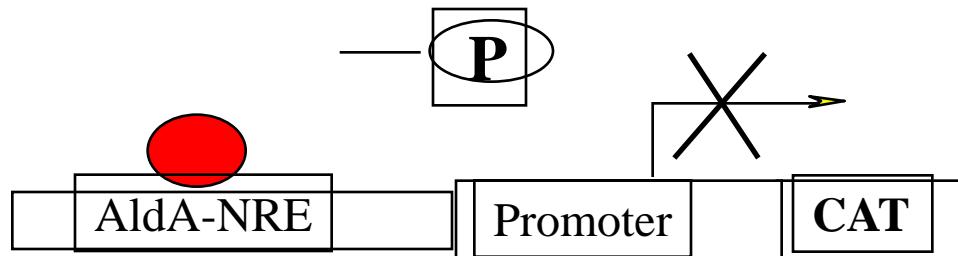
	AldA-NRE	<u>Proliferating</u>	lanes	<u>Starved</u>	lanes
AldALΔ-555		6.4 ± 1.5	(1)	12.1 ± 3.1	(6)
RBP 9-CAT		22.6 ± 6.7	(2)	21.8 ± 7.8	(7)
RBP 9-CAT+AldA-NRE1 (-218/-527)		1.5 ± 0.4	(3)	5.1 ± 2.0	(8)
RBP 9-CAT+AldA-NRE2 (-527/-218)		7.5 ± 1.2	(4)	15.5 ± 3.9	(9)



Thin layer
cromatography

Transcriptional control mediated by 97 kDa repressor /AldA-NRE interaction

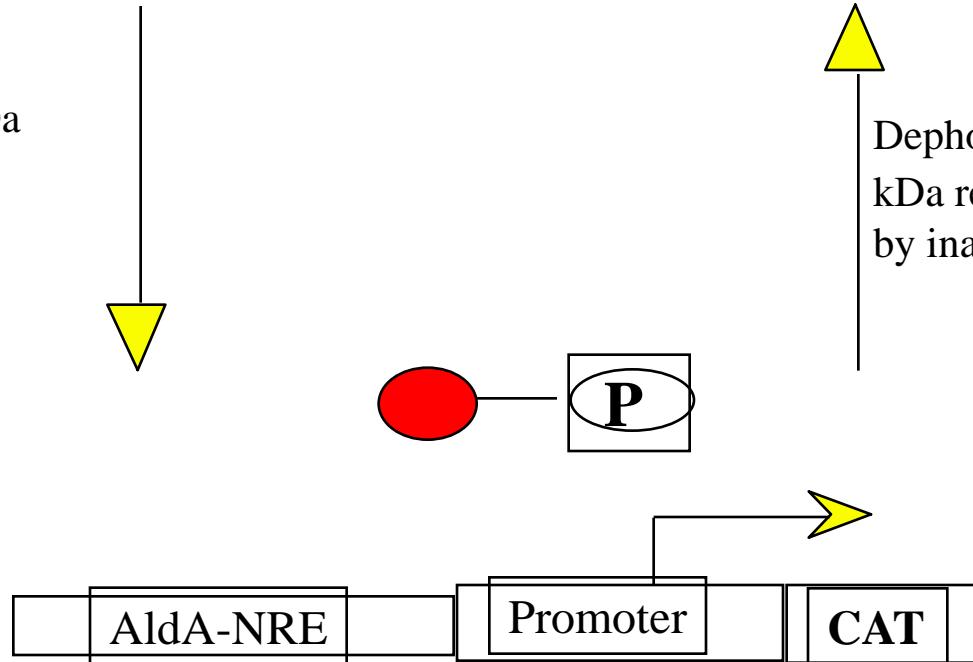
Proliferating cells



Phosphorylation of 97 kDa
repressor by PKC activity

Dephosphorylation of 97 kDa
repressor by inactivation of PKC

Differentiated
or
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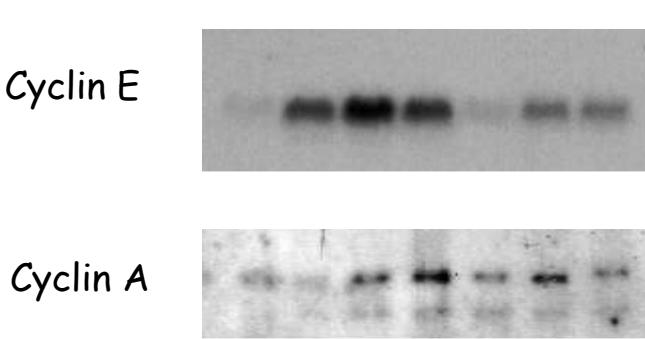
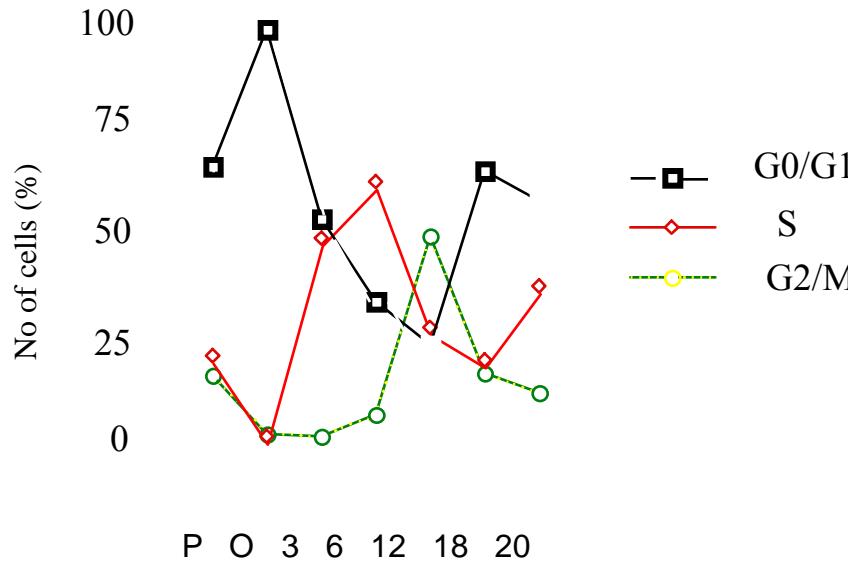


Transcriptional regulation of the expression of human aldolase A gene

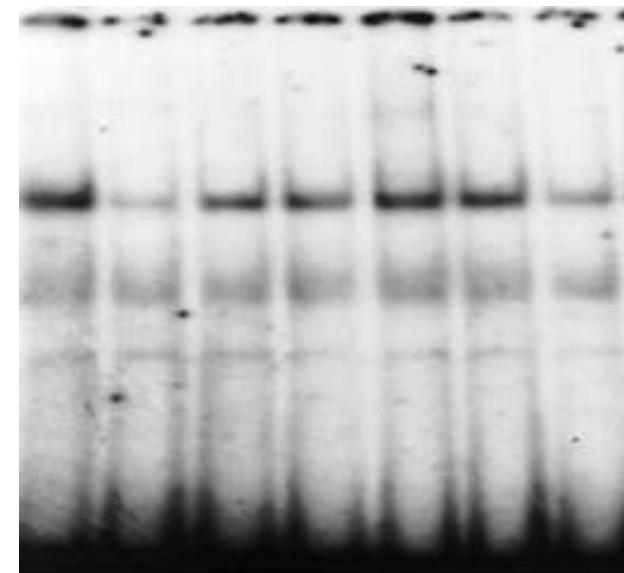
5° risultato :

La sequenza **AGAGAGCAA** che è il bersaglio del repressore trascrizionale sul promotore del gene umano codificante per l’Aldolasi A è capace di trasferire il potere di regolazione negativa anche su un promotore eterologo e svolge perciò una funzione dominante.

p97 binding activity is modulated during cell-cycle



P	0	3	6	12	18	20
64,11	95,57	52,20	33,44	23,44	63,02	56,15
20,60	2,02	47,82	60,85	27,32	19,54	36,66
16,34	5,69	2,38	7,07	48,03	16,71	12,21



Transcriptional regulation of the expression of human aldolase A gene

6° risultato :

Il legame specifico del repressore trascrizionale sul promotore del gene umano codificante per l’Aldolasi A è dipendente dal tipo di sequenza (**AGAGAGCAA**), ma anche dalla fase del ciclo cellulare in cui si trovano le cellule.

Transcriptional regulation of the expression of human aldolase A gene

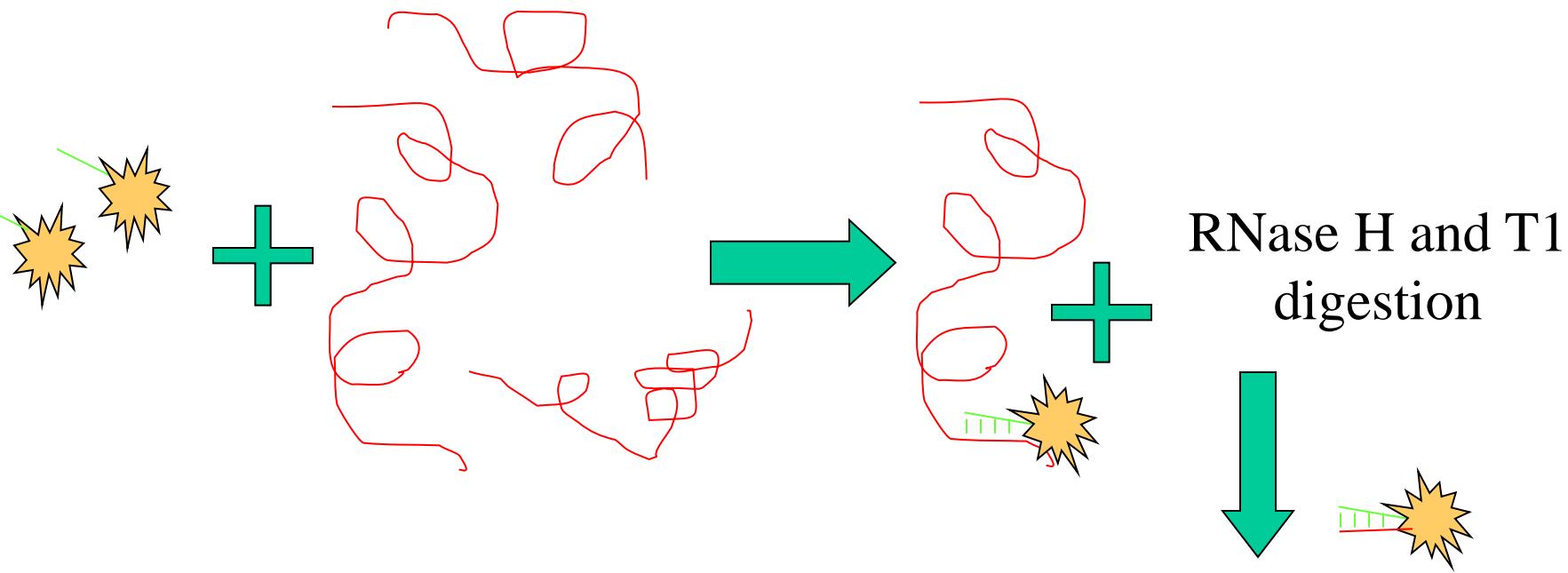
Questioni

L'interazione tra il repressore trascrizionale di 97 kDa e la sequenza **AGAGAGCAA** è presente solo nelle cellule bloccate nella crescita?

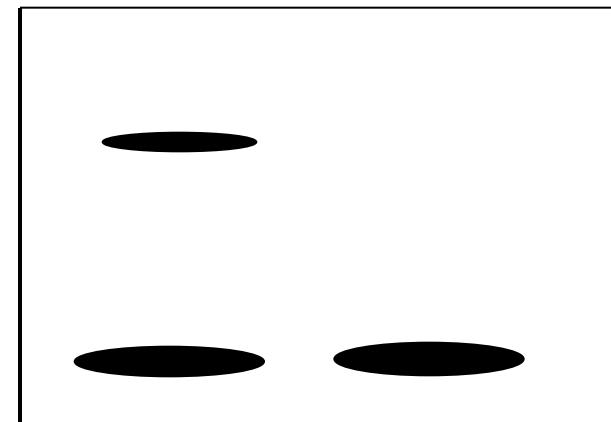
Il modello di regolazione trascrizionale è soltanto riscontrabile nelle cellule in fase di arresto di crescita (G0/G1)?

RNase Protection assay on total RNA

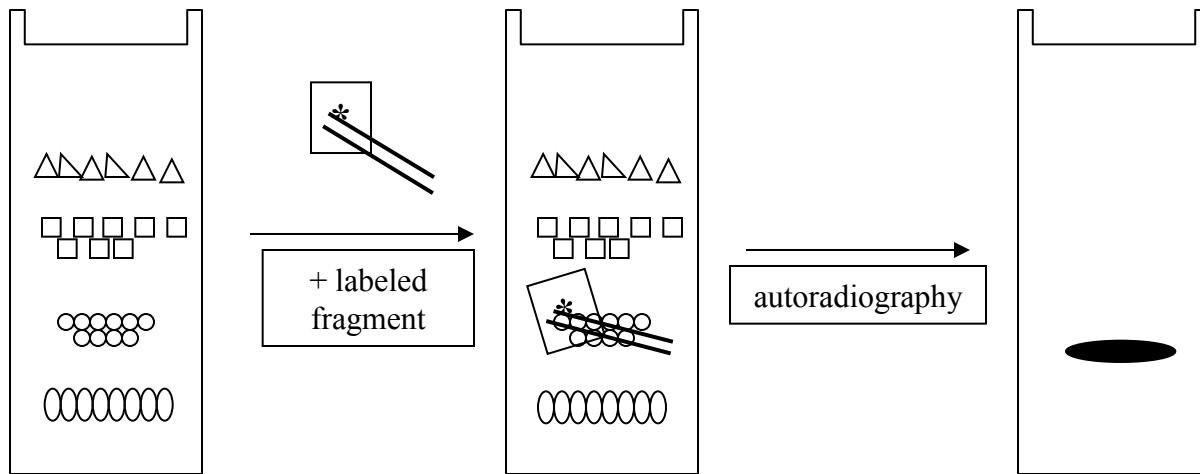
Corso di Biotecnologie Industriali-Modulo Processi



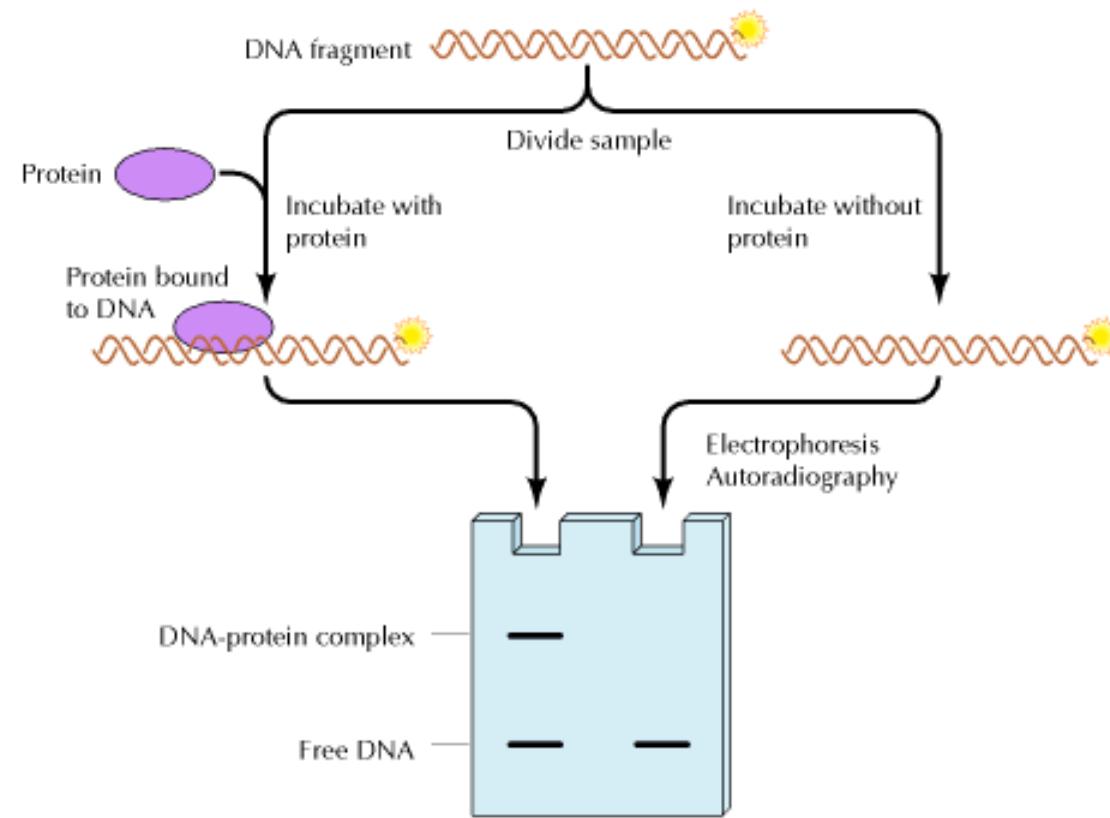
Denaturing electrophoresis gel



Protein separation by SDS-electrophoresis gel and southwestern assay

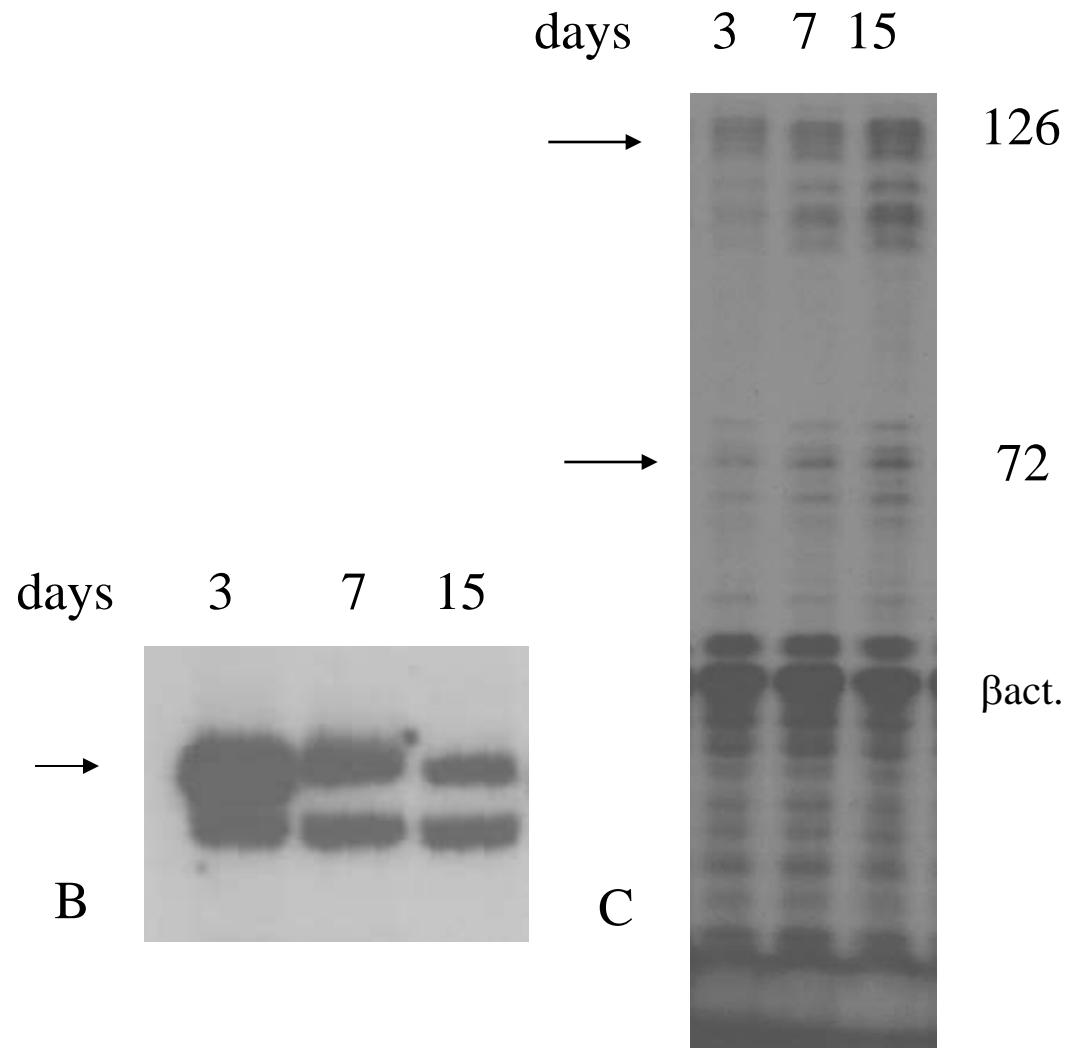
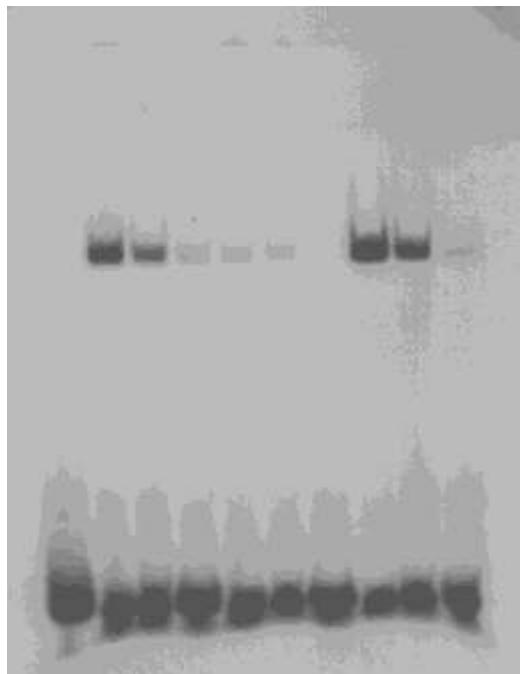


Gel shift assay



Regulation of transcription of aldolase A L-type mRNA during CaCo- 2 cells differentiation

	days			days			days		
	3	7	15	3	7	15	3	7	15
specif.	-	-	-	+	+	+	-	-	-
compet.	-	-	-	-	-	-	-	-	-
nonspecif.	-	-	-	-	-	-	+	+	+
compet.	-	-	-	-	-	-	+	+	+



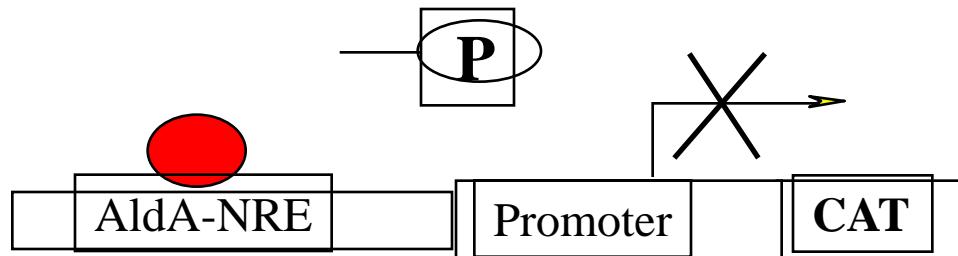
Transcriptional regulation of the expression of human aldolase A gene

7° risultato :

L'interazione DNA/proteina sul promotore del gene umano di aldolasi A e il modello di regolazione trascrizionale è dimostrato anche nel modello di cellule del colon CaCo2 che differenziano in coltura.

Transcriptional control mediated by 97 kDa repressor /AldA-NRE interaction

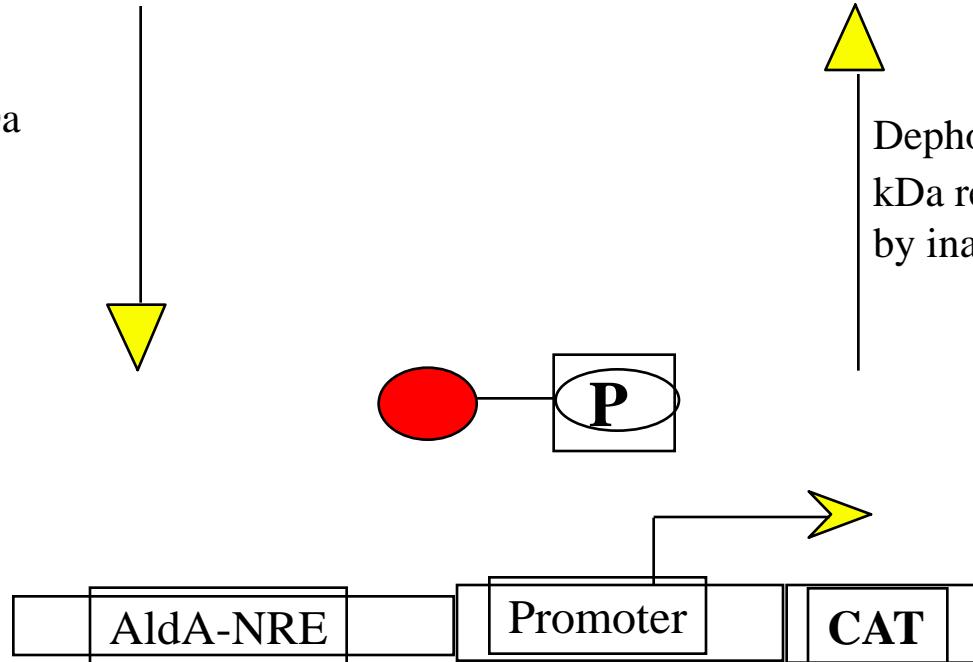
Proliferating cells



Phosphorylation of 97 kDa
repressor by PKC activity

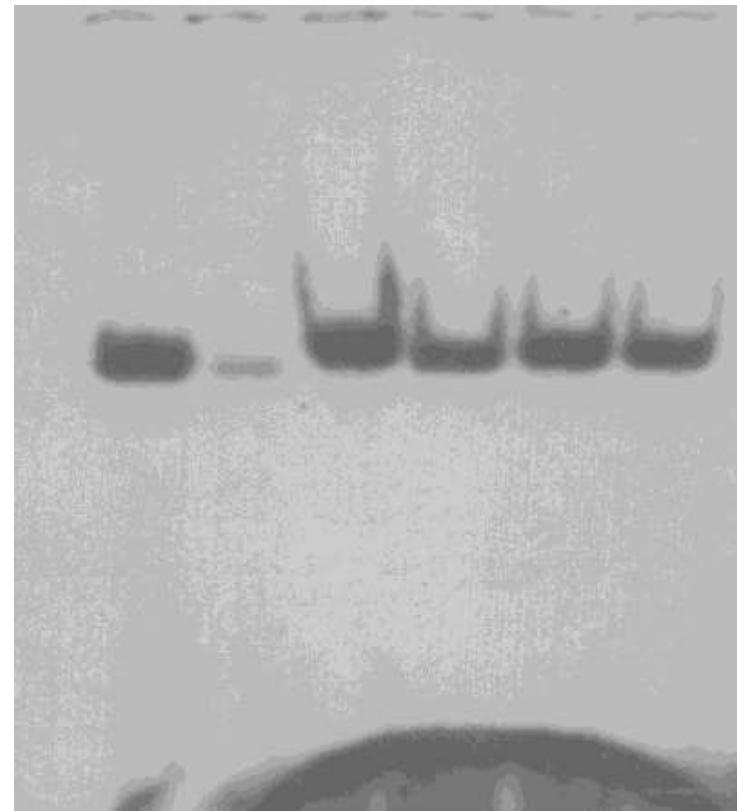
Dephosphorylation of 97
kDa repressor
by inactivation of PKC

Differentiated
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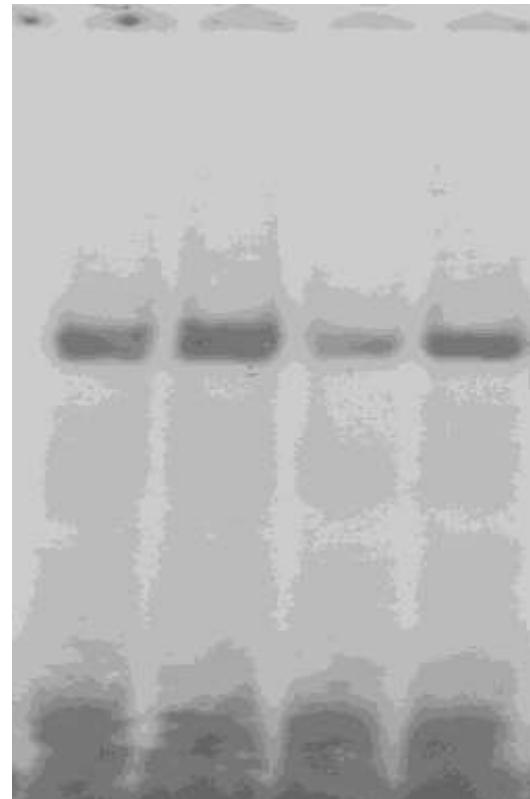
Phosphatase treatment of nuclear extracts from Caco 2 cells

	p	d	p	d	p	d
Phosphatase (0.12 U)	-	-	+	+	+	+
Times (min.)	-	-	10	10	20	20

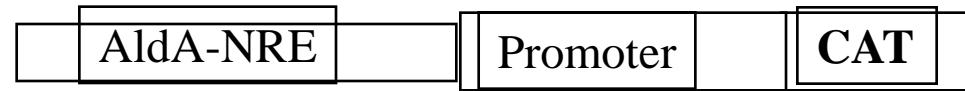


Binding activity of 97 kDa protein to AldA-NRE in TPA-induced proliferating NIH3T3 cells

TPA (200ng/ml)	-	+	+	+
times	-	24hs	10min	10min
H7 (50μM)	-	-	-	+

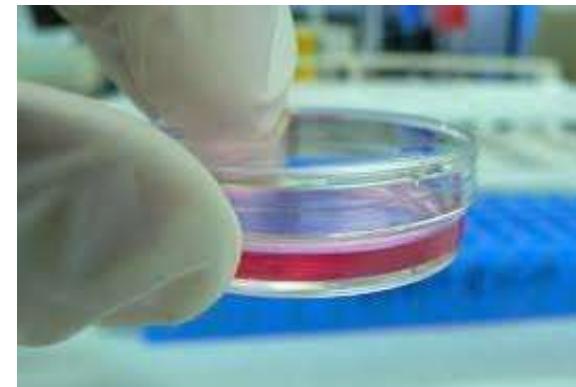


Transcriptional regulation of Aldolase-CAT constructs by p97/AldA-NRE interaction in NIH3T3



LΔ-555/220

Transient transfection



Nuclear extracts

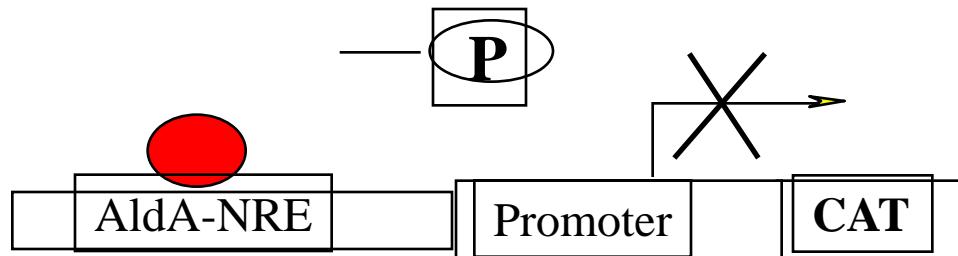
Transcriptional regulation of Aldolase-CAT constructs by p97/AldA-NRE interaction in NIH3T3

Relative CAT Activity %

	<u>Constructs</u>	
	WT	Mutant
NIH3T3 cells	LΔ-555 clone 5	LΔ-555 MN9
TPA-untreated cells	60,7 ± 8,7	100
TPA-treated cells (200ng/ml)/24hrs	22,1 ± 2,8	99,3 ± 14,9
TPA-treated cells (10ng/ml)/1hrs	108,1 ± 17,4	102,5 ± 18,6
serum-deprived cells	116,8 ± 21,1	N.D.

Transcriptional control mediated by 97 kDa repressor /AldA-NRE interaction

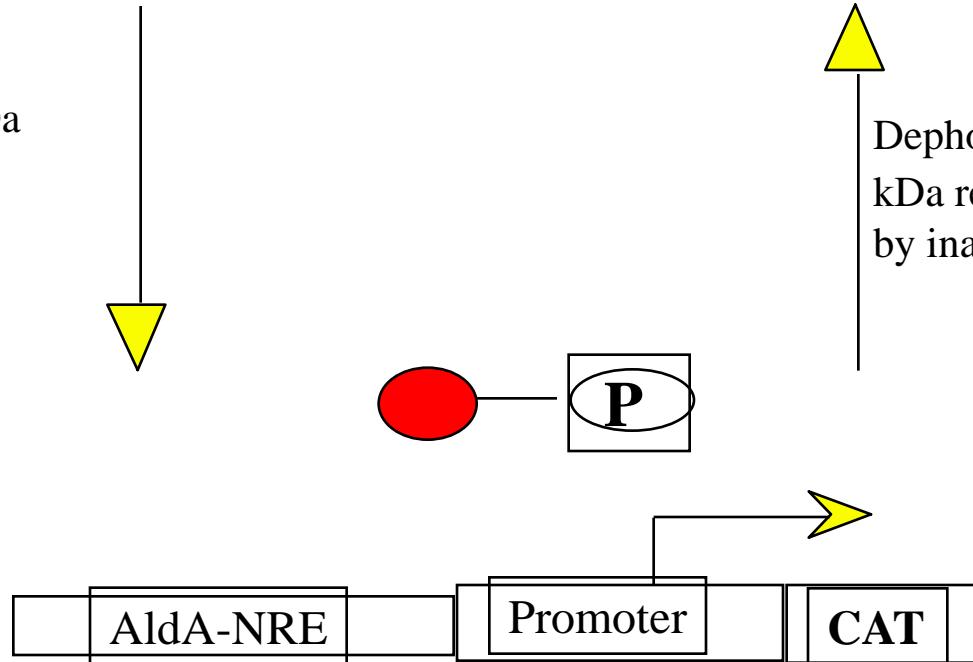
Proliferating cells



Phosphorylation of 97 kDa
repressor by PKC activity

Dephosphorylation of 97
kDa repressor
by inactivation of PKC

Differentiated
or
serum-starved
cells



Transcriptional regulation of the expression of human aldolase A gene

8° risultato :

L'interazione DNA/proteina sul promotore del gene umano di aldolasi A e la regolazione trascrizionale sono influenzati dall'equilibrio tra stato fosforilato/defosforilato del repressore trascrizionale nel modello di cellule del colon CaCo2 che differenziano in coltura.

Conclusioni preliminari

I risultati fin qui ottenuti hanno spinto verso la purificazione del fattore trascrizionale che riconosce e lega AldA-NRE :

- 1) è un forte repressore della trascrizione di un gene eucariotico
- 2) la sua attività di repressore è modulata nel ciclo cellulare
- 3) la sua stessa sintesi è probabilmente regolata durante il ciclo cellulare
- 4) la sua attività è modulata da un evento di fosforilazione mediata dalla proteina chinasi C.

Domanda fondamentale

Sulla base dei risultati ottenuti, è conveniente investire risorse per studiare il fattore trascrizionale che riconosce e lega AldA-NRE ?

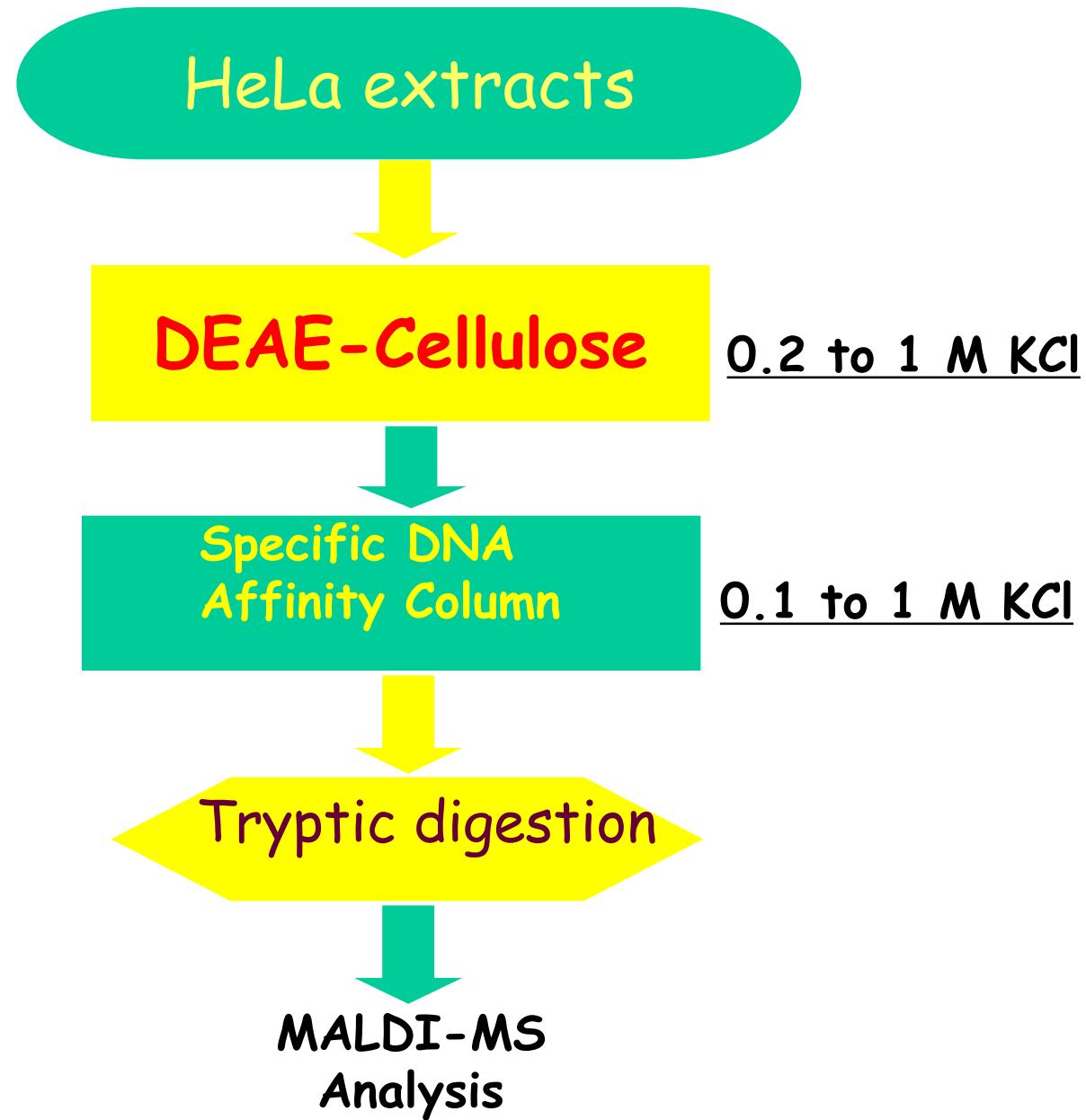
Questioni secondarie

E' la purificazione del fattore trascrizionale il passaggio cruciale ?

Quali metodologie biochimiche si possono utilizzare?

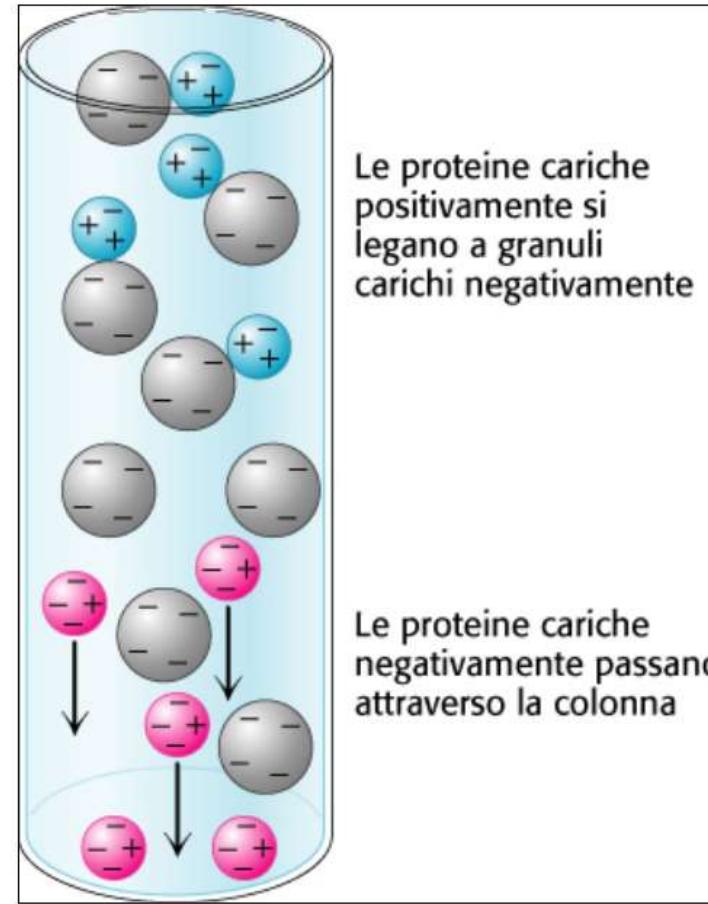
Dopo la purificazione all'omogeneità con quali tecnologie è possibile giungere all'identificazione del fattore trascrizionale?

Protocol of purification of p97 repressor-protein

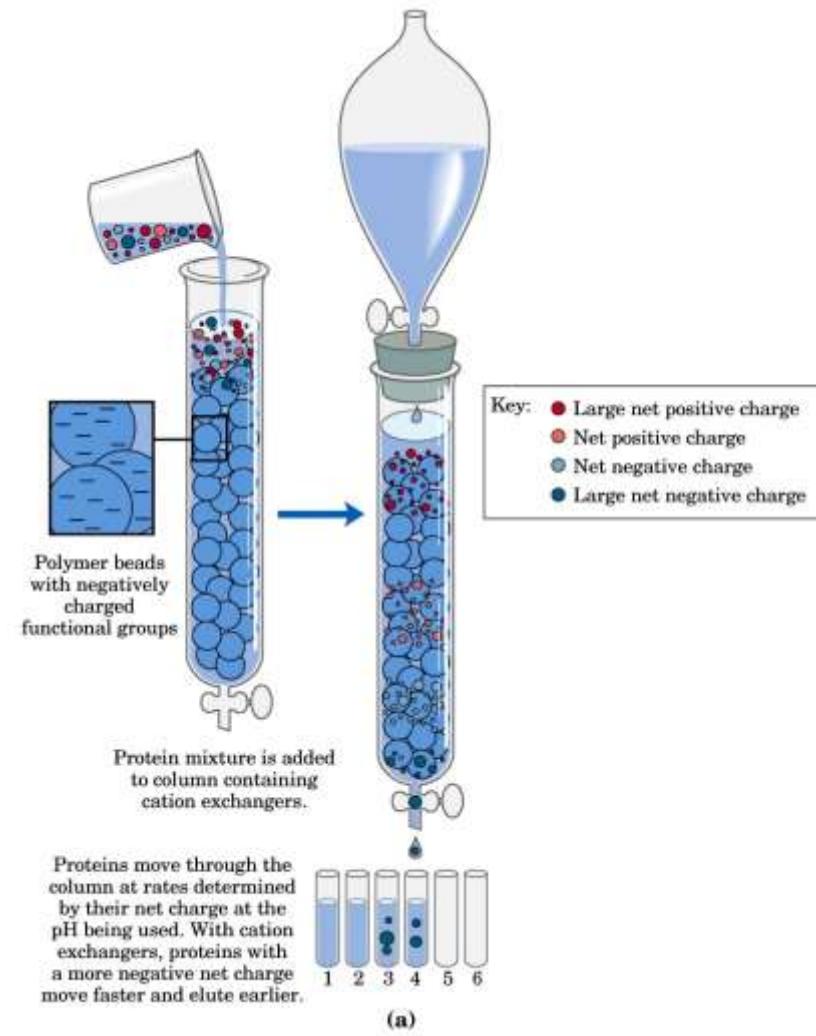


Ion Exchange Chromatography

f0404_ISBN88-08-07893-0 - Visualizzatore immagini e fax per Windows



Ion Exchange Chromatography

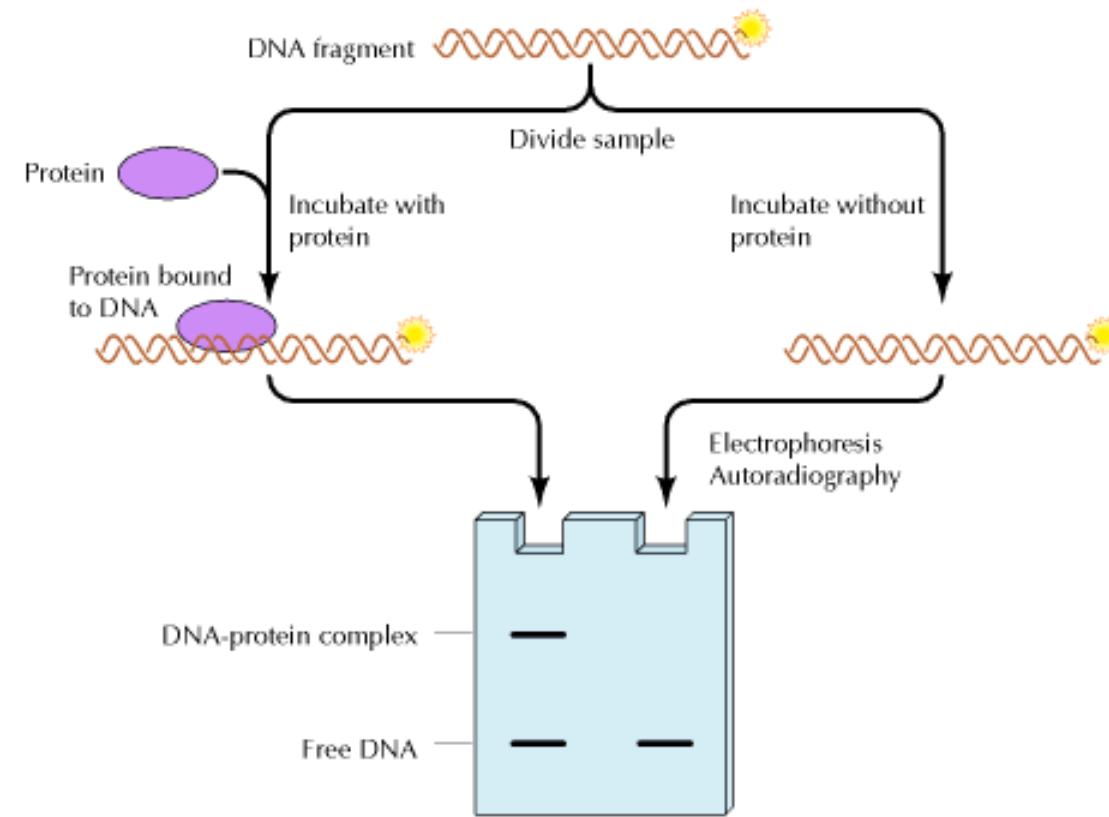




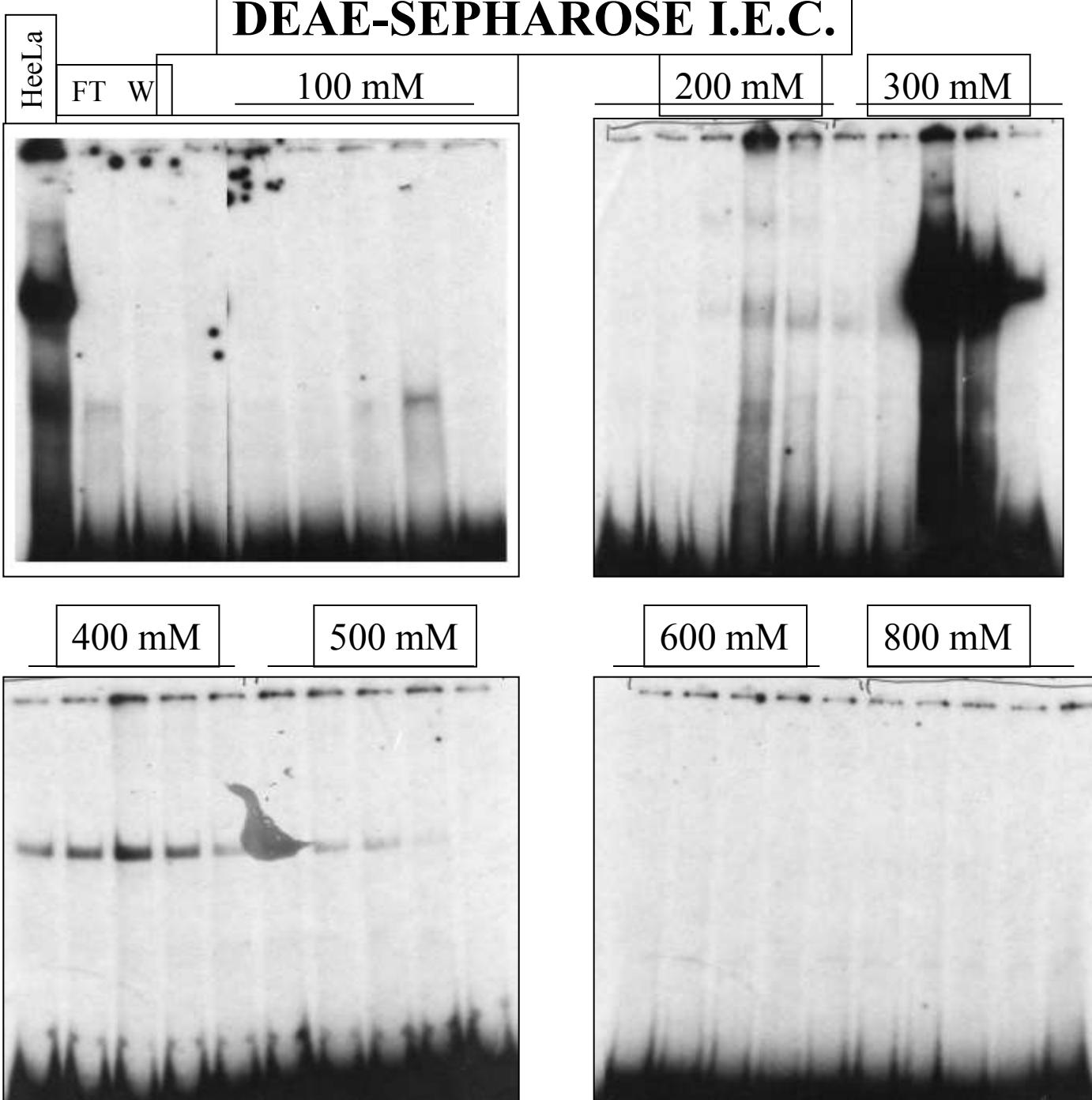
Una condizione molto importante!

Per seguire una proteina durante un protocollo di purificazione è assolutamente necessario avere a disposizione un saggio funzionale!!!

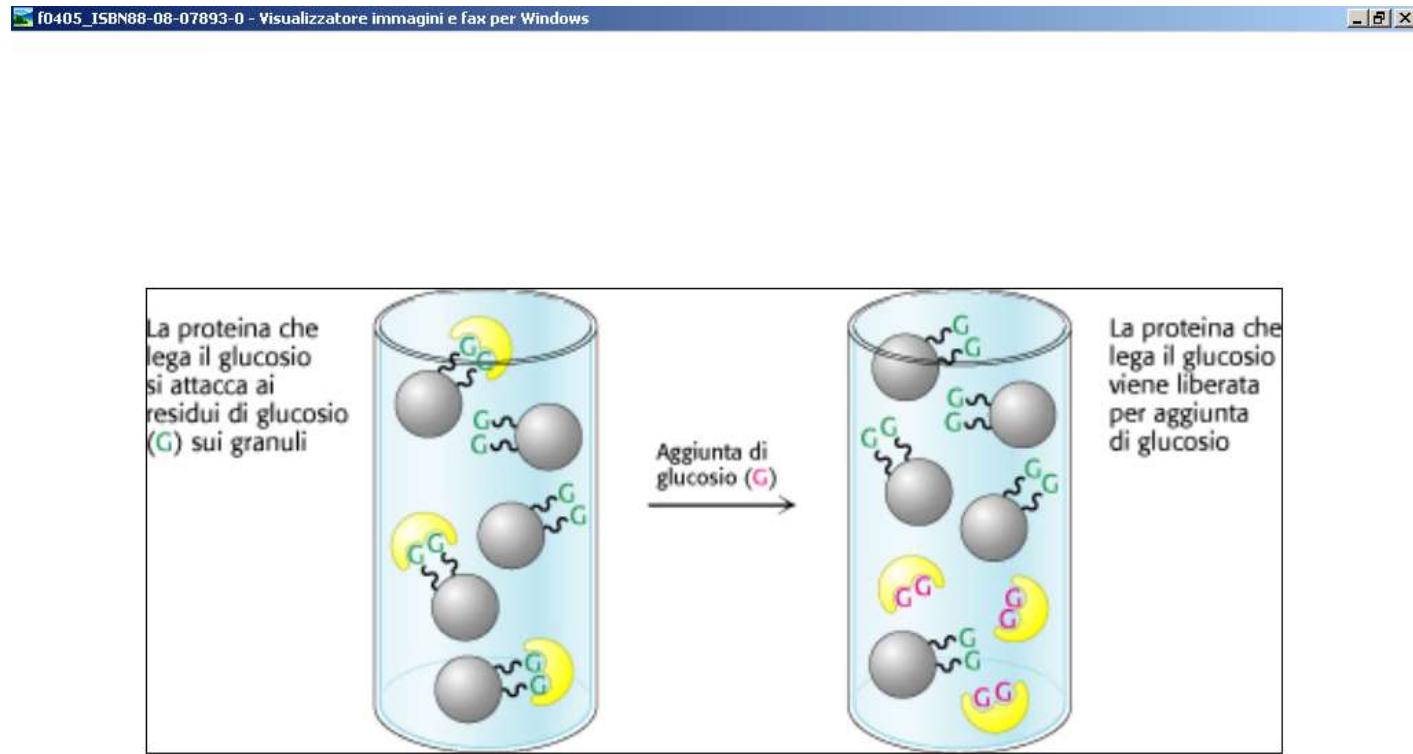
Il gel shift mobility assay può essere il nostro saggio funzionale ?



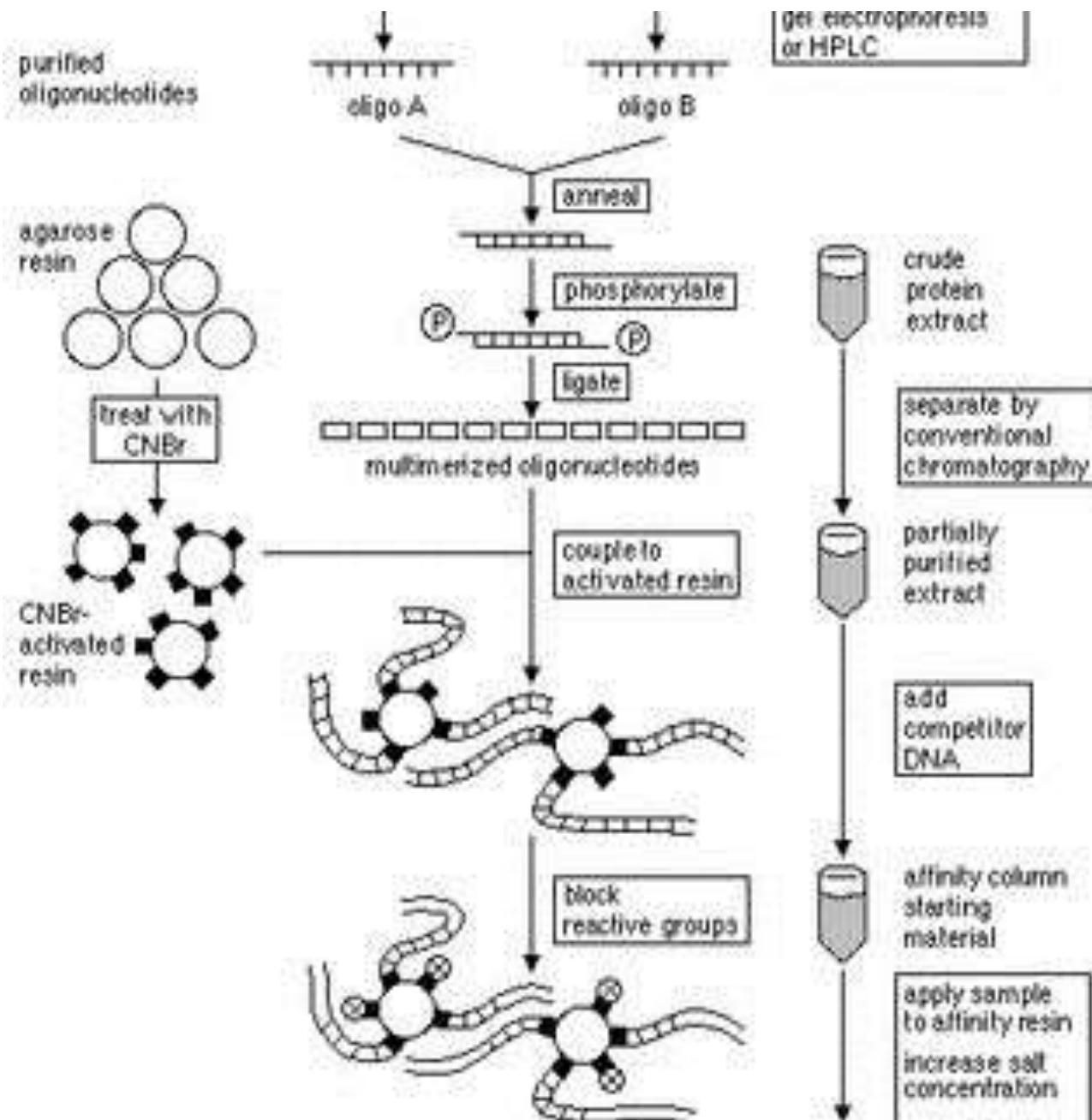
DEAE-SEPHAROSE I.E.C.



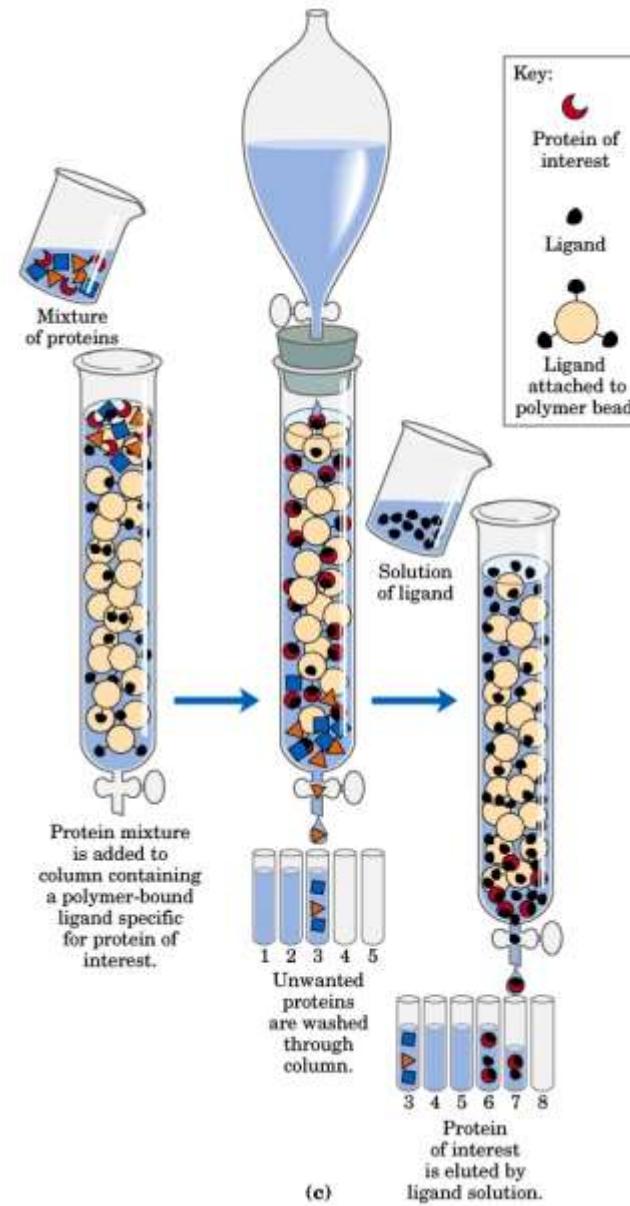
Affinity chromatography



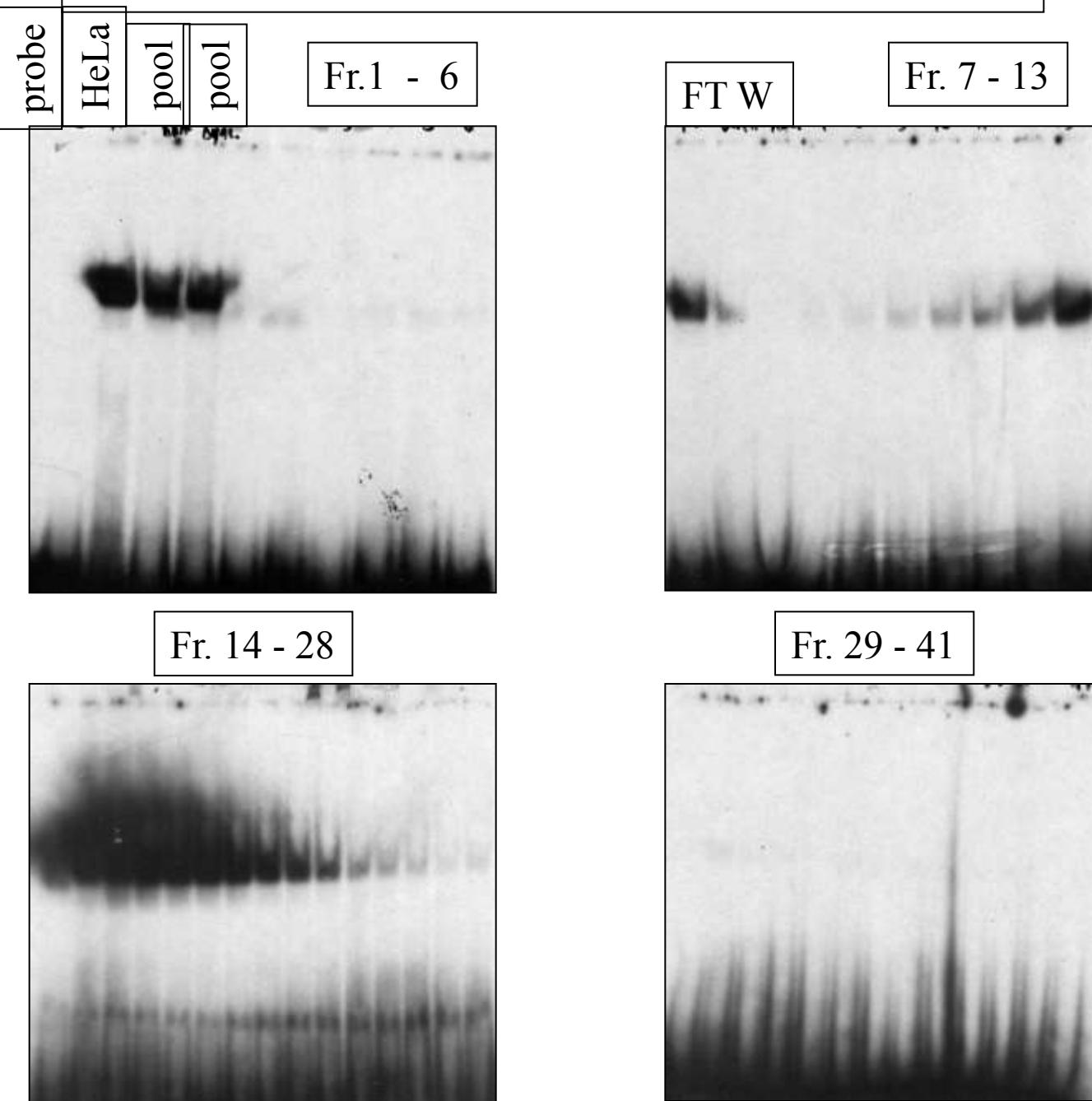
Affinity chromatography



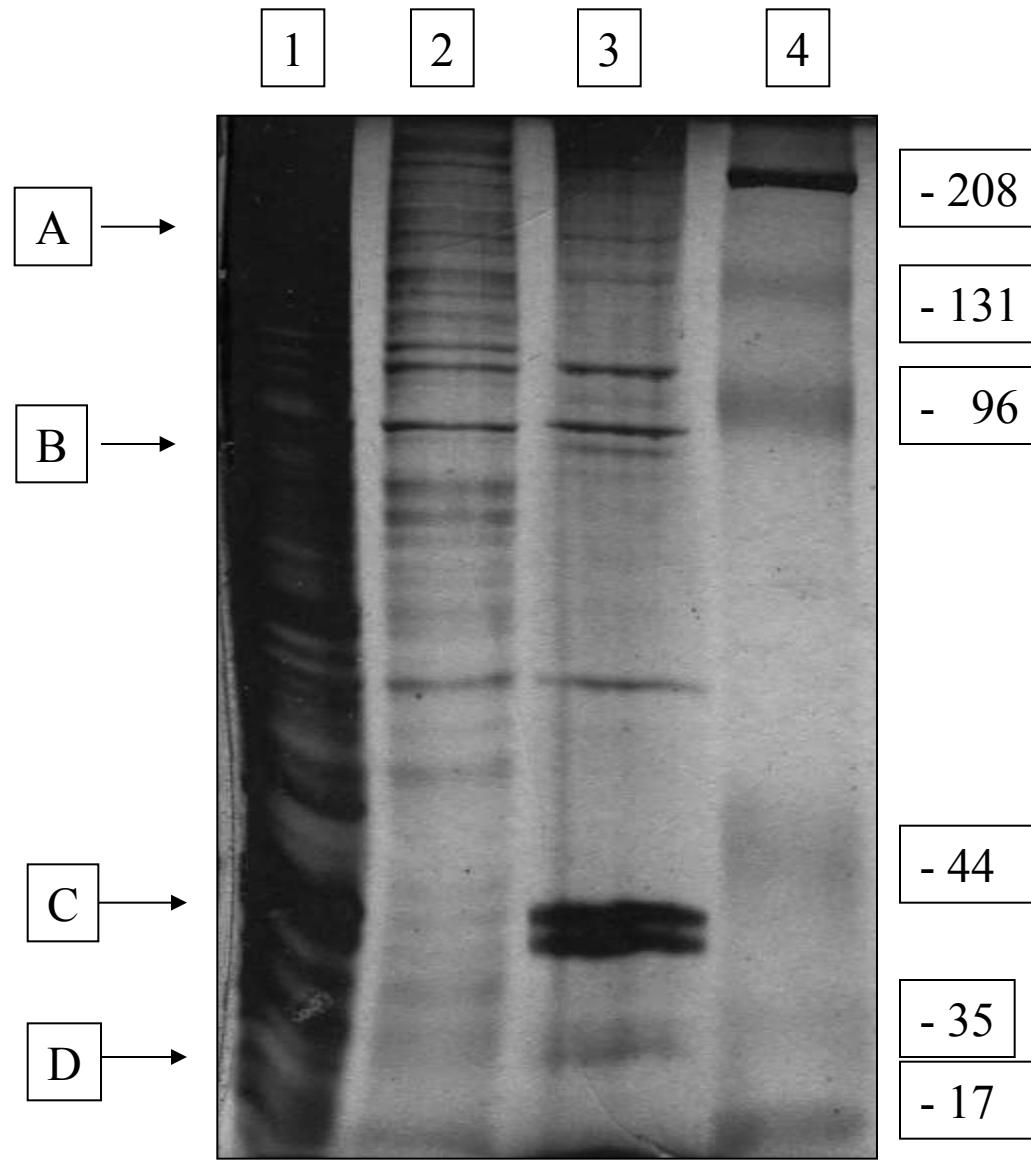
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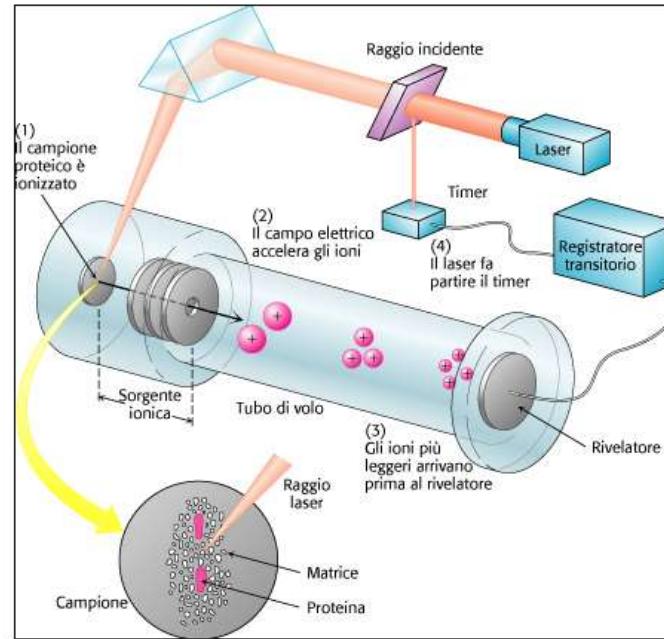
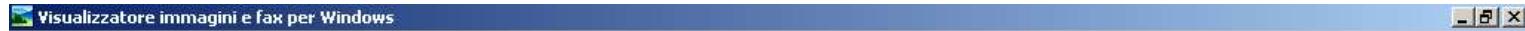
DNA-AFFINITY CHROMATOGRAPHY



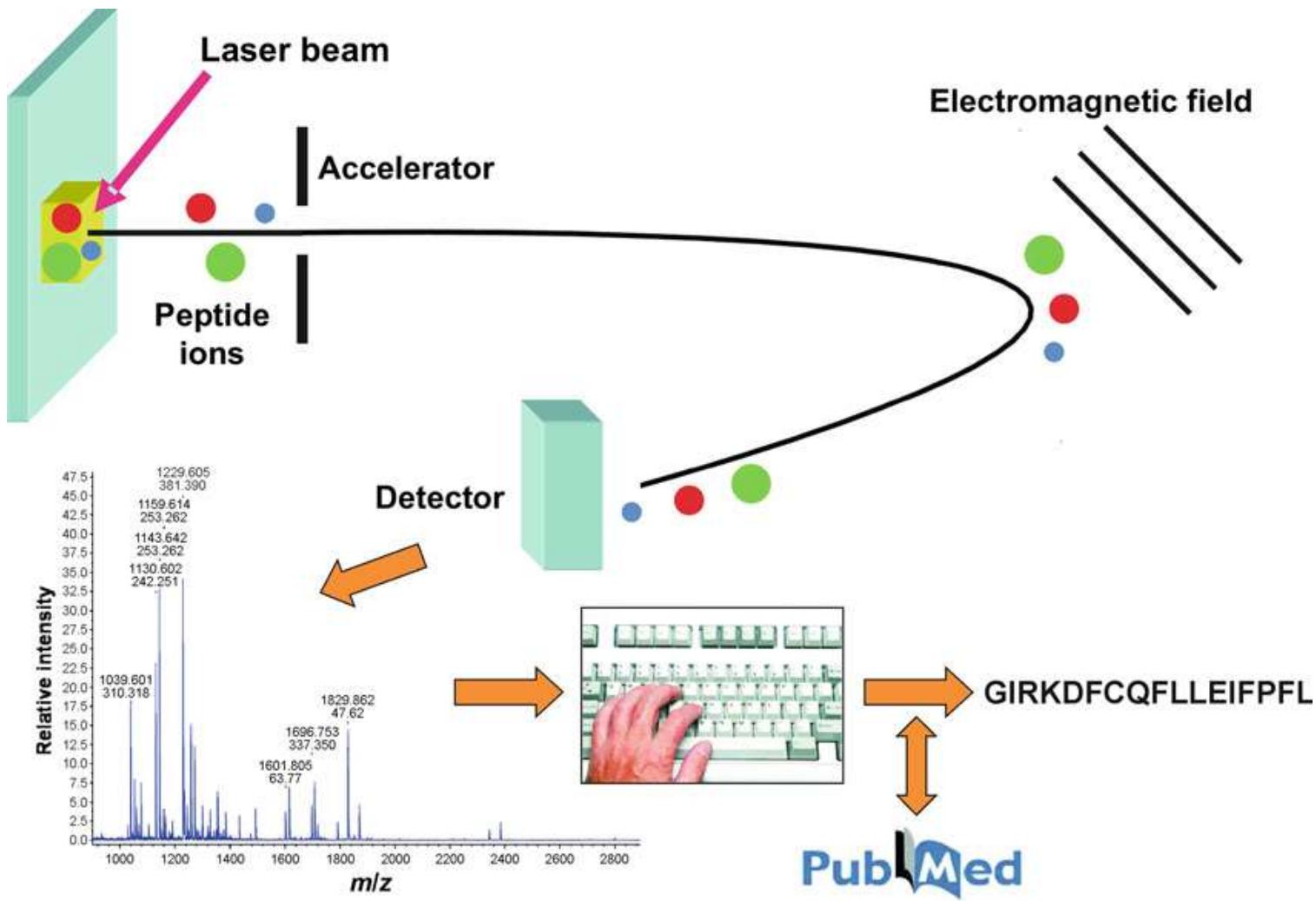
Silver stained SDS-PAGE of the chromatography fractions



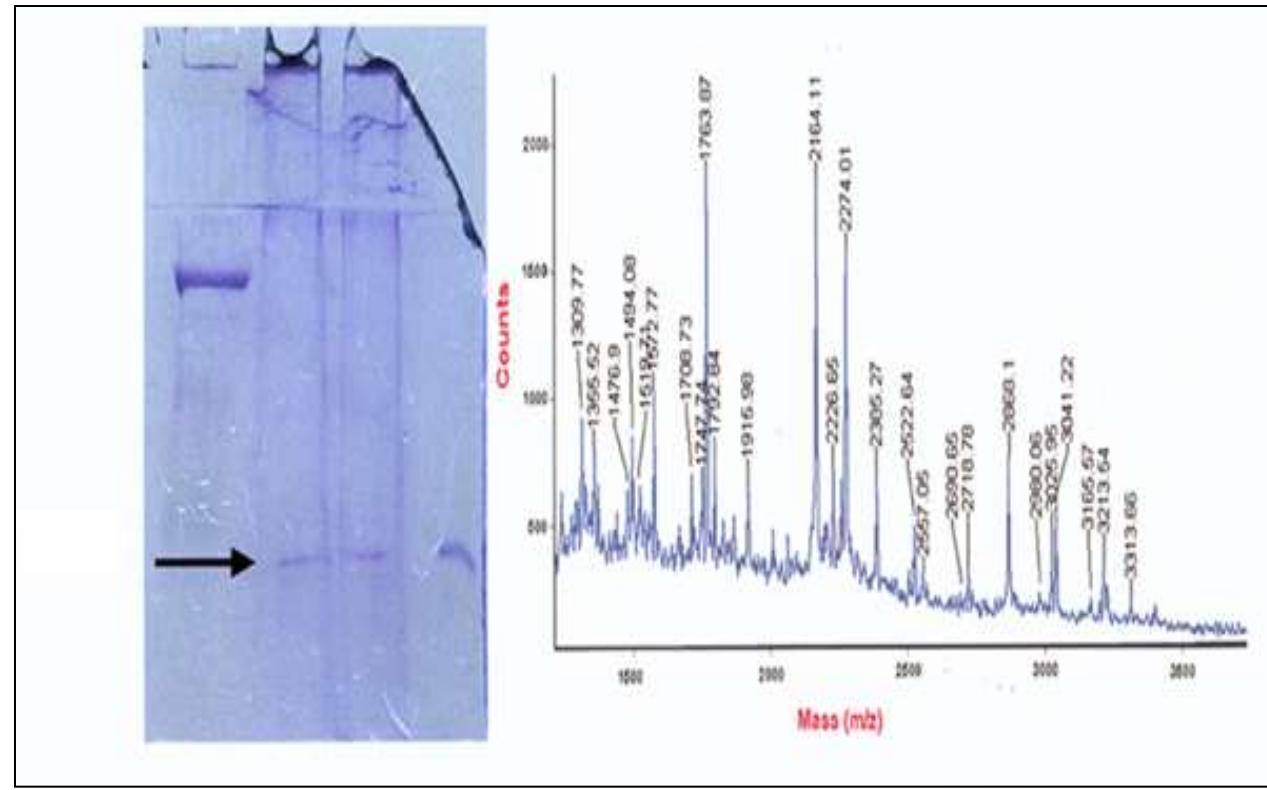
MALDI-TOF technology



MALDI-TOF technology



MALDI analysis of purified repressor



MALDI analysis of purified repressor

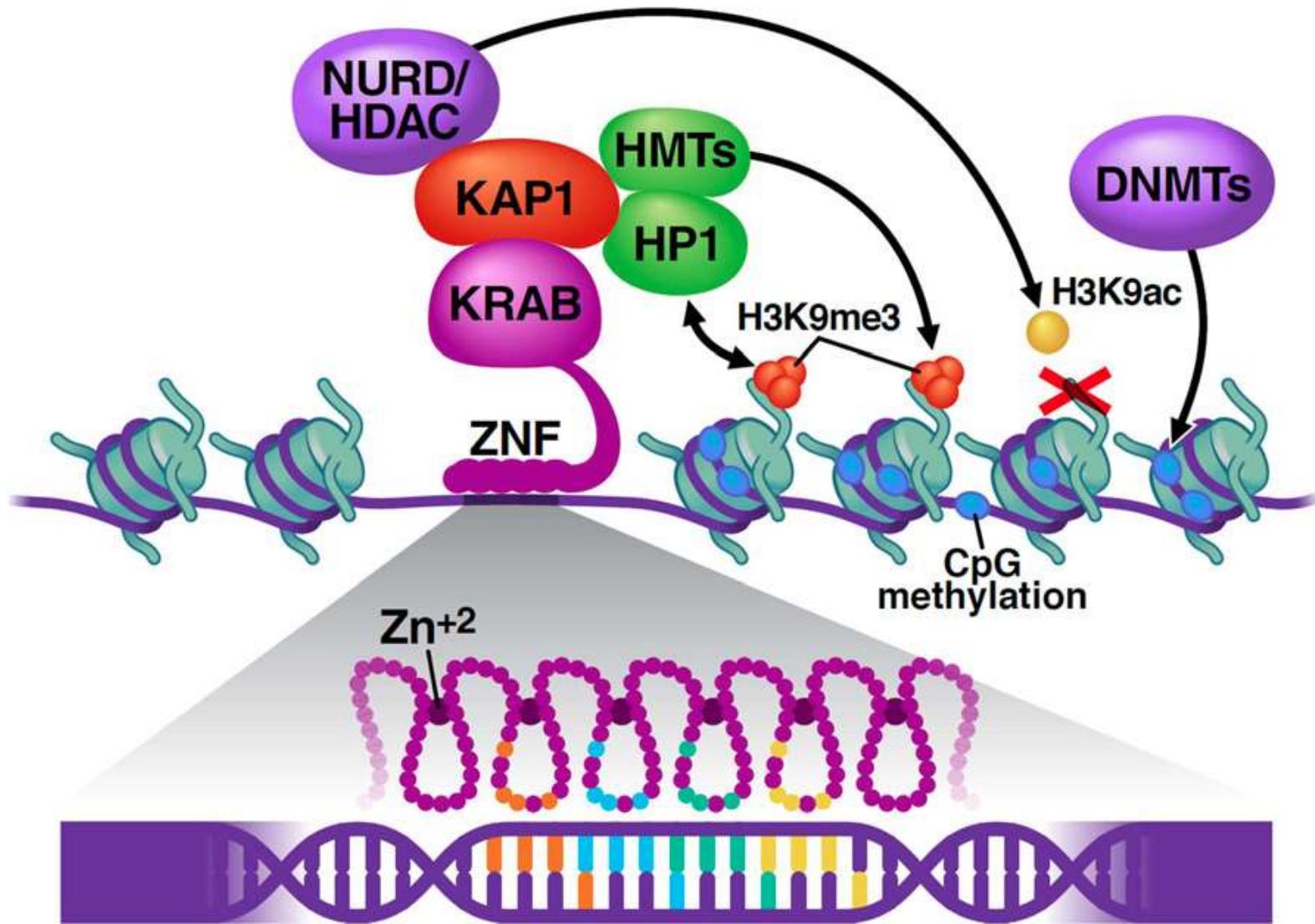
Probability	Protein Description	MW (kDa)
1.0e+00	gi 7019591 ref NP_037530.1 zinc finger protein 224	82
1.1e-04	gi 7243079 dbj BAA92587.1 (AB037770) KIAA1349 protein [Homo sapiens]	87
2.3e-05	gi 5031779 ref NP_005522.1 interferon, gamma-inducible protein 16	82
8.7e-06	gi 177960 gb AAA58358.1 (M55602) amiloride-binding protein [Homo sapiens]	81
7.4e-06	gi 6433901 emb CAA71414.2 (Y10388) Graf protein [Homo sapiens]	86
3.2e-06	gi 3417297 gb AAC31673.1 (AC002310) Unknown gene product [Homo sapiens]	88
1.3e-06	gi 7706467 ref NP_057350.1 GIOT-4 for gonadotropin inducible transcription repressor-4	75
1.0e-06	gi 4758632 ref NP_004691.1 potassium voltage-gated channel, KQT-like subfamily, member 4	77
8.0e-07	gi 479805 pir S35458 SNF2 protein homolog - human (fragment)	89
5.6e-07	gi 4455442 emb CAB36862.1 (AL022067) dJ134E15.1 (Blimp-1) [Homo sapiens]	85



ZNF224 cDNA sequence

1 GAGTCCAAACATTGGAGTCGGACACTTCCGCTCGGGACTGAGGTTGCTGCAGTTTCCGCGATAGTTG
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2401 ATGTTCATGTTGGAGAAAACCTTAGTGTGATGGTGCAATAAGTCTTCACTCAGTCTTCATG 2466
V H V G E K P *

Model of KRAB-ZNFs binding to DNA and induction of heterochromatin formation



Funzioni biologiche delle KRAB-ZNFs

Table 1. Structural and Functional Features of Some KRAB-ZNFs

Metabolism							
Protein	Species	Chromosomal Localization	KRAB	Zinc Fingers	Expression Profile	Functional Role	References
ZNF202	Human	11q23.3	KRAB+SCAN	8	ubiquitous	Lipids Metabolism	[56-58]
ZNF224	Human	19q13.2	KRAB-A+b box	19	ubiquitous	Glycolysis Oxidative metabolism	[62, 63, 65]
ZNF236	Human	18q22.3-23	KRAB-A box	25/30	ubiquitous	Glucose metabolism	[64]
Rsl1	Mouse	13B3	KRAB-A+B box	3	liver	Lipids homeostasis Sexual dimorphism	[66, 67]
Rsl2 (Zfp429)	Mouse	13B3	KRAB-A+B box	3	liver	Lipids homeostasis	[66, 67]

(Lupo et al. Current Genomics 2013)

Funzioni biologiche delle KRAB-ZNFs

Development and Differentiation							
Protein	Species	Chromosomal Localization	KRAB	Zinc Fingers	Expression Profile	Functional Role	References
ZNF268	Human	12q24.33	KRAB-A+B box	14	erythrocytes	haematopoiesis	[68, 69]
ZNF300	Human	5q33.1	KRAB-A+b box	12	promyelocytes	haematopoiesis	[70, 71]
ZNF304	Human	19q13.4	KRAB-A box	13	lymphocytes	lymphocyte activation	[72]
ZNF230	Human	19q13.31	KRAB-A box	4	testis	spermatogenesis	[73]
ZNF463	Human	19q13.42	KRAB-A+B box	12	testis	spermatogenesis	[74]
AJ18	Rat	11B1.3	KRAB-A box	11	bone	osteogenesis	[75]
NT2	Mouse	16A1	KRAB-A box	9	cartilage	development	[76]
ZNF359	Human	16q22	KRAB-A+B box	16	heart	cardiogenesis	[77]
ZFP28	Human	19q13.41	KRAB-A box	14	heart	cardiogenesis	[77]
ZFP57	Human	6p22.1	KRAB-A box	6	ovary, testis	imprinting	[40, 41]
Zfp157	Mouse	5G2	KRAB-A+B box	11	mammary gland	alveologenesis	[78]

(Lupo et al. Current Genomics 2013)

Funzioni biologiche delle KRAB-ZNFs

Apoptosis and Cancer

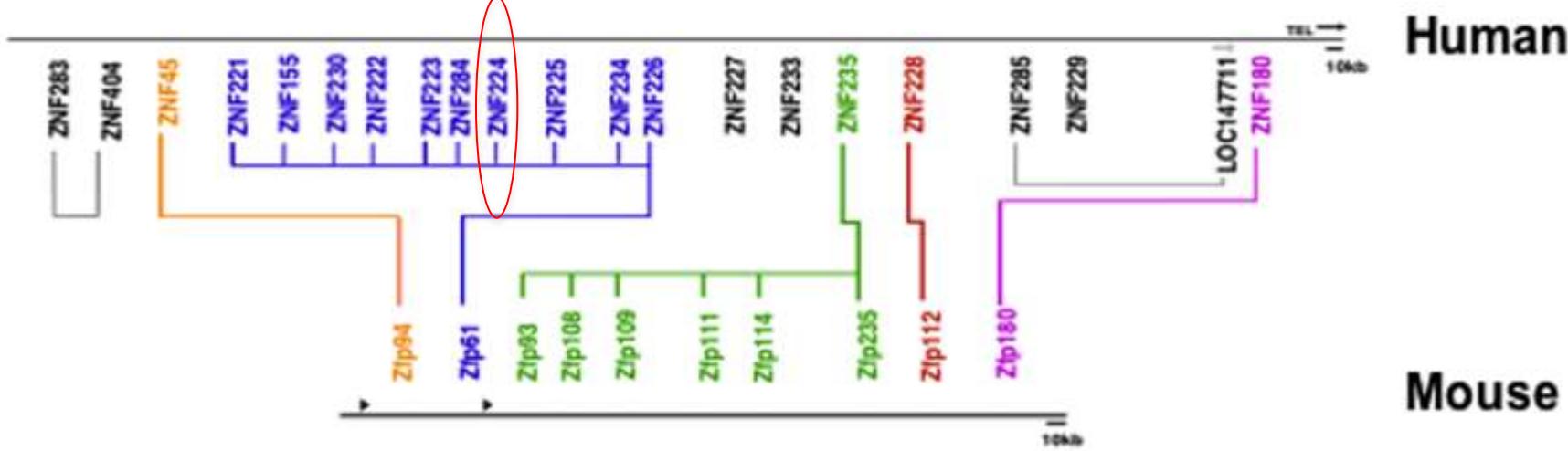
Protein	Species	Chromosomal Localization	KRAB	Zinc Fingers	Expression Profile	Functional Role	References
ZNF224	Human	19q13.2	KRAB-A+b box	19	ubiquitous	Control of apoptosis	[53, 79, 80]
ZNF382	Human	19q13.12	KRAB-A+B box	6	heart	Tumor suppressor gene	[81]
ZNF545	Human	19q13.12	KRAB-A box	8	ubiquitous	Tumor suppressor gene	[82]
ZBRK1	Human	19q13.41	KRAB-A box	8	skeletal muscle	Tumor suppressor gene	[83-86]
Apak	Human	19q13.12	KRAB-A+b box	19	ubiquitous	Regulator of apoptosis	[88, 89]
ZNF307	Human	6p21	KRAB+SCAN	7	ubiquitous	p53 degradation, apoptosis	[90]

Funzioni biologiche delle KRAB-ZNFs

Degenerative neurological diseases							
Protein	Species	Chromosomal Localization	KRAB	Zinc Fingers	Expression Profile	Functional Role	References
ZNF746	Human	7q36.1	KRAB-A box	4	ubiquitous	Parkinson's disease	[91]
ZNF224	Human	19q13.2	KRAB-A+b box	19	ubiquitous	Alzheimer's disease	[92]

(Lupo et al. Current Genomics 2013)

Ortologhi e paraloghi della proteina zinc finger ZNF224



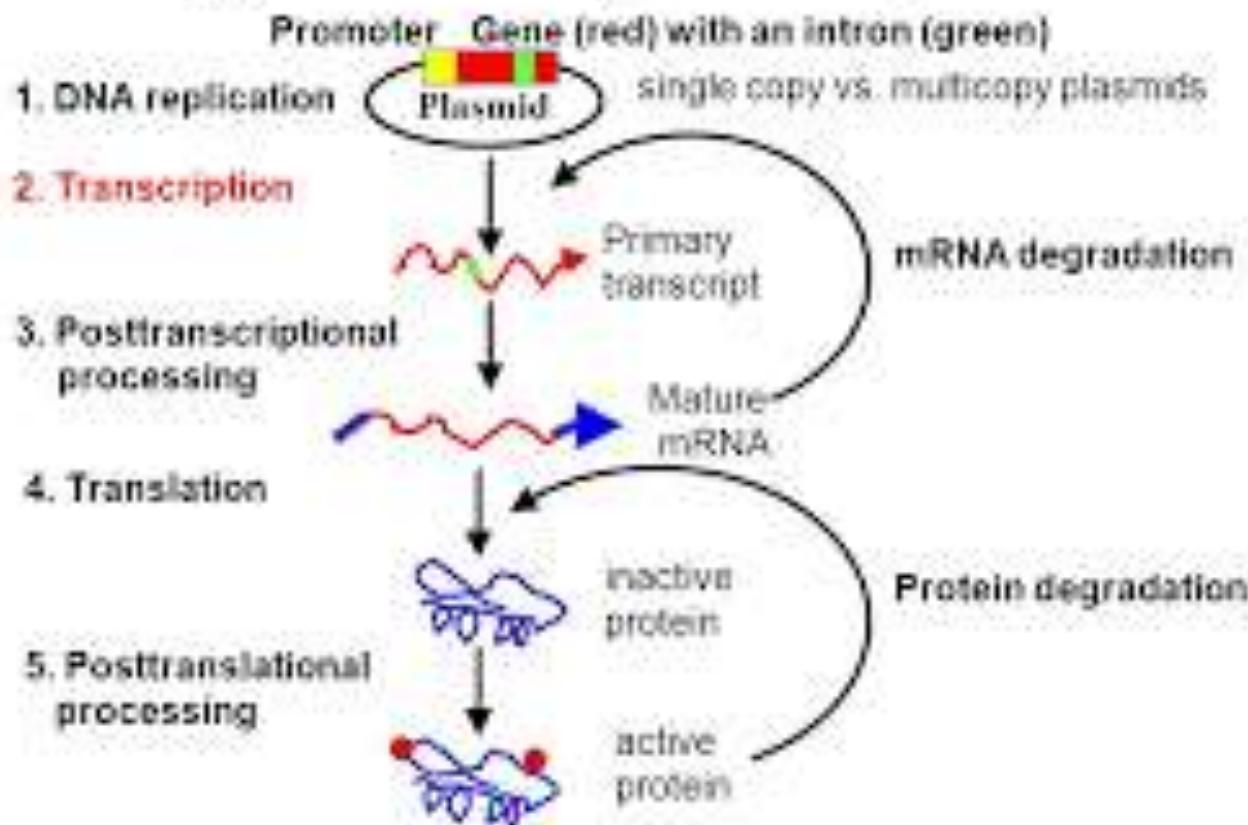
(Lupo et al. Current Genomics 2013)



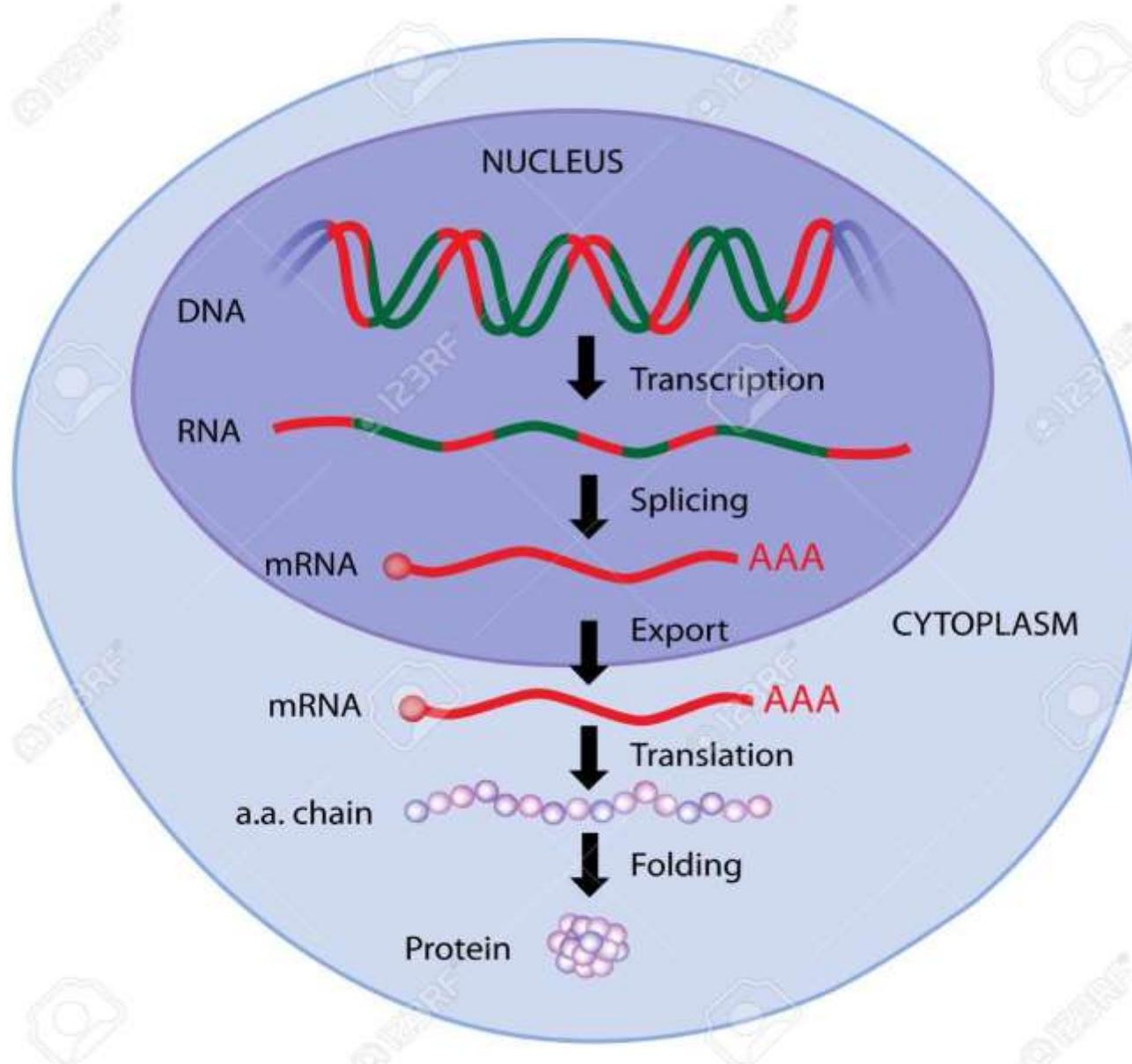
ZNF224 cDNA sequence

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H T G E R P Y N C K E C G K S F G W A S C L L K H
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C G K R G F S W S S T R L T H Q R R H S R E T P L K
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V O N S F S K V O E K V H S V E K P Y K C E D C G I
2301 CTTGATATGCATCAGAGGGTCCACATGGGAGAGAAAACATGGAAGTGTAGGGAGTGTGATATGTGCTTTAGTCAGGC
L D M H Q R V H M G E K T W K C R E C D M C F S Q A
2401 ATGTTCATGTTGGAGAAAACCTTAGTGTGATGGTGCAATAAGTCTTCACTCAGTCTTCATG 2466
V H V G E K P *

Regulation of gene expression



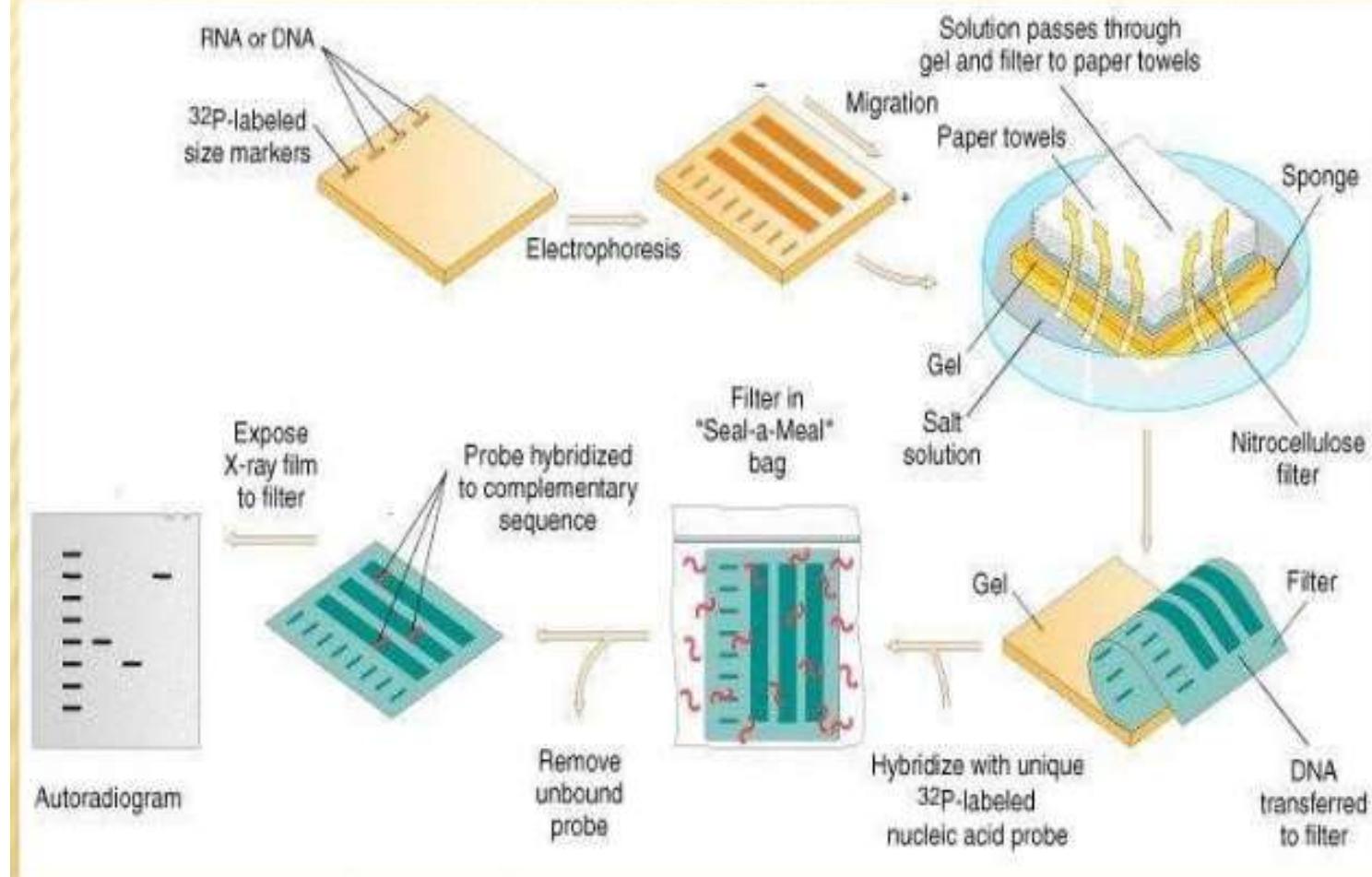
Regolazione dell'espressione genica





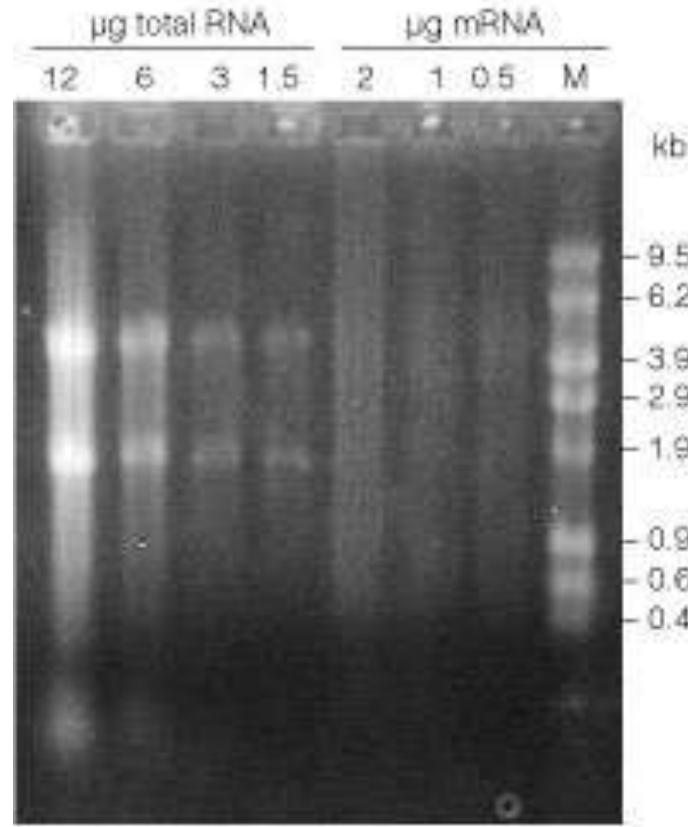
Northern Blotting analysis

NORTHERN BLOTTING

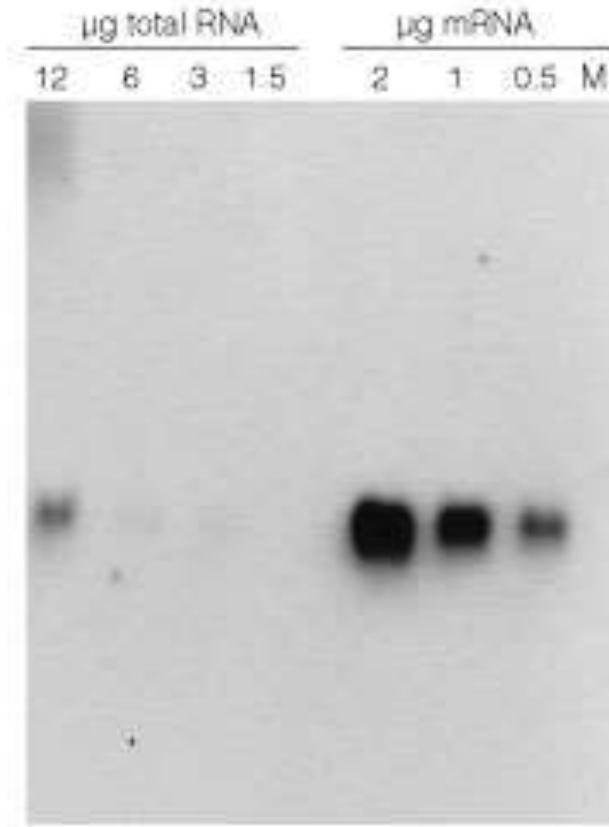


Northern Blotting analysis

A. Gel analysis of RNA

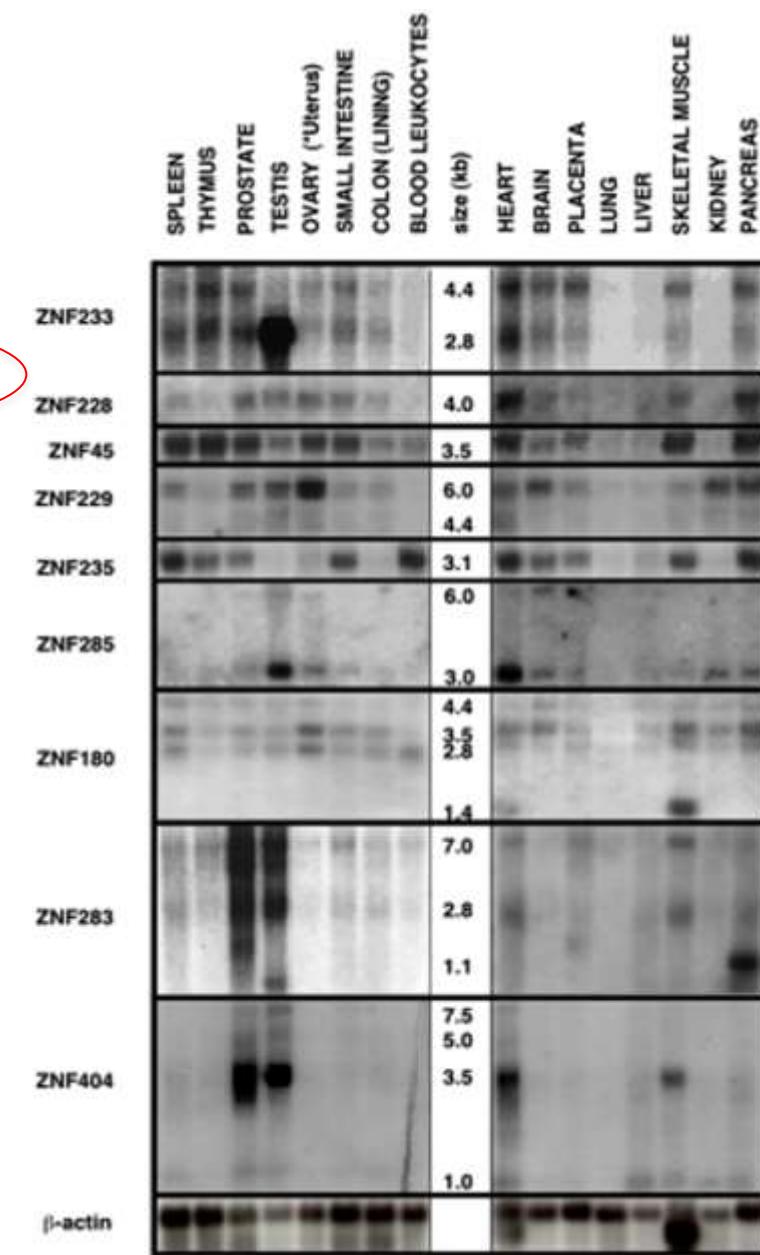
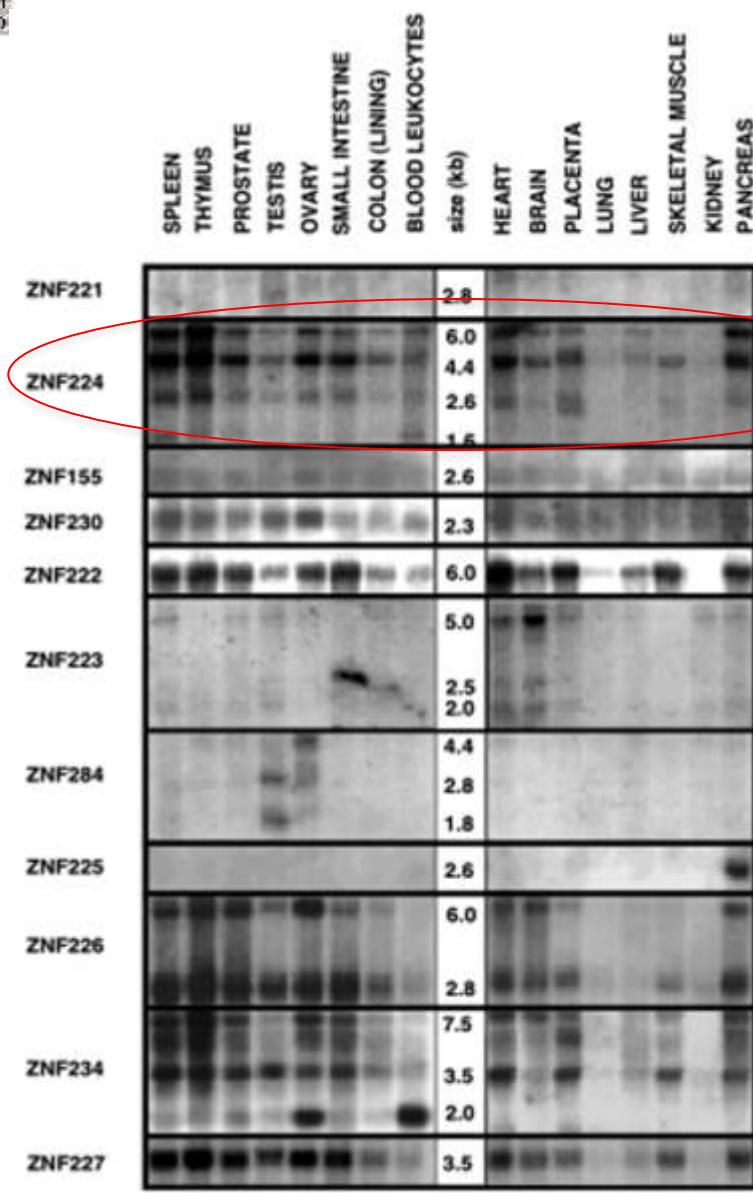


B. Northern blot

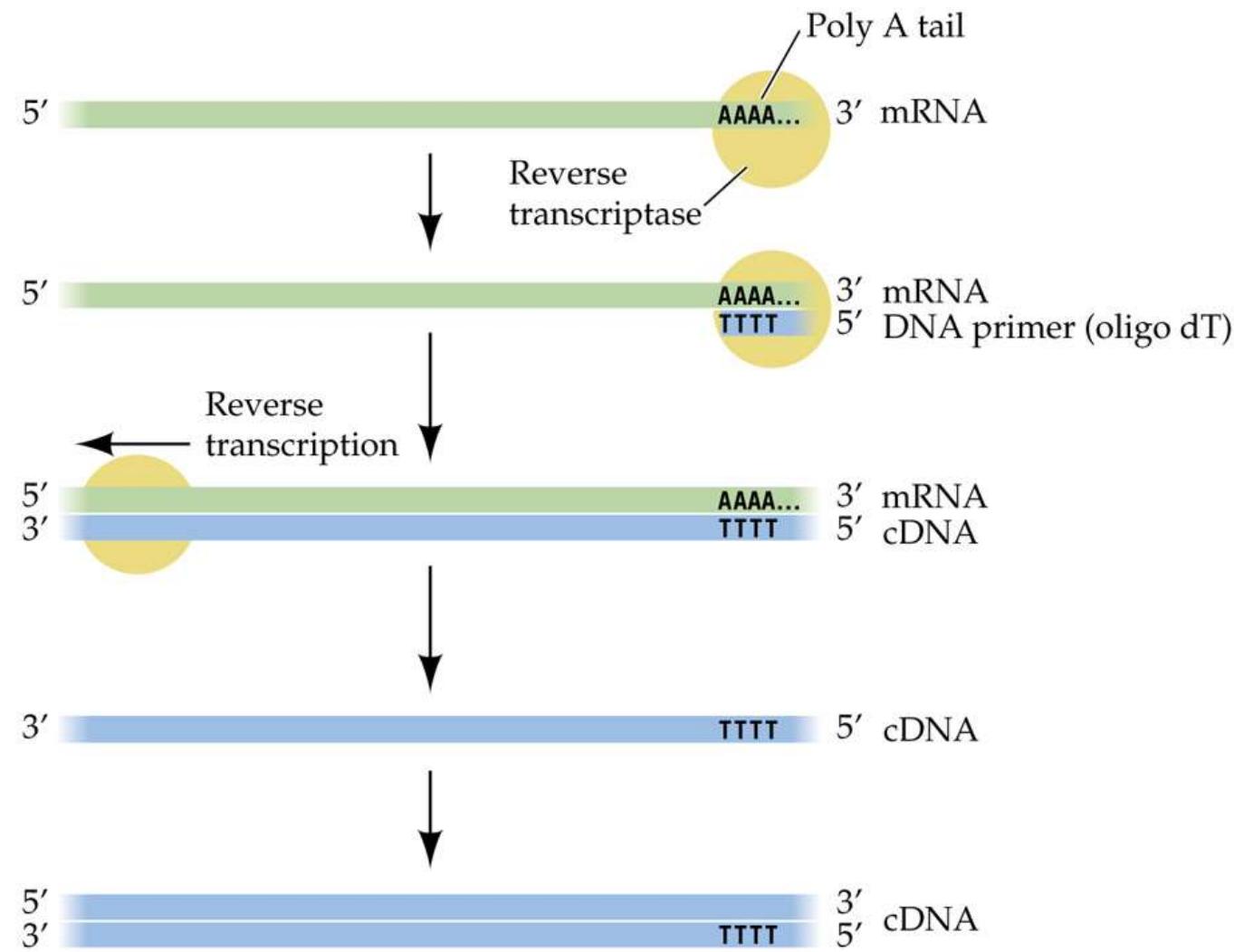


I possibili RNA messaggeri della proteina zinc finger ZNF224

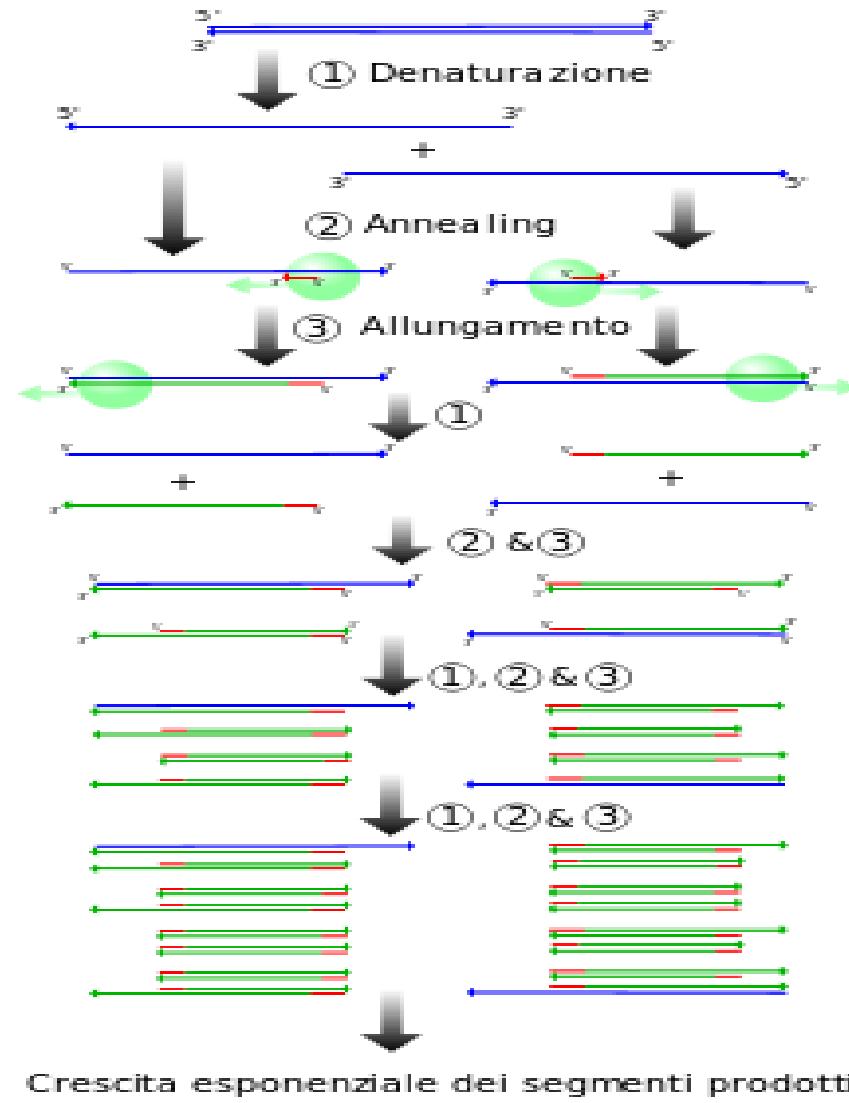
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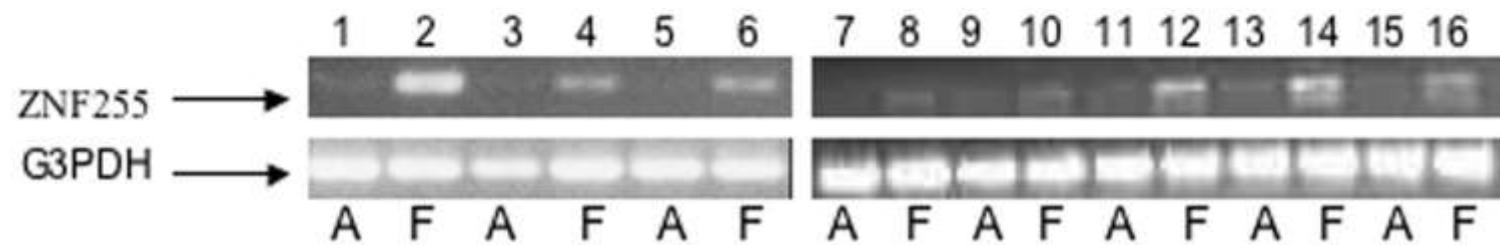
Reverse transcriptase reaction



Amplification Reaction



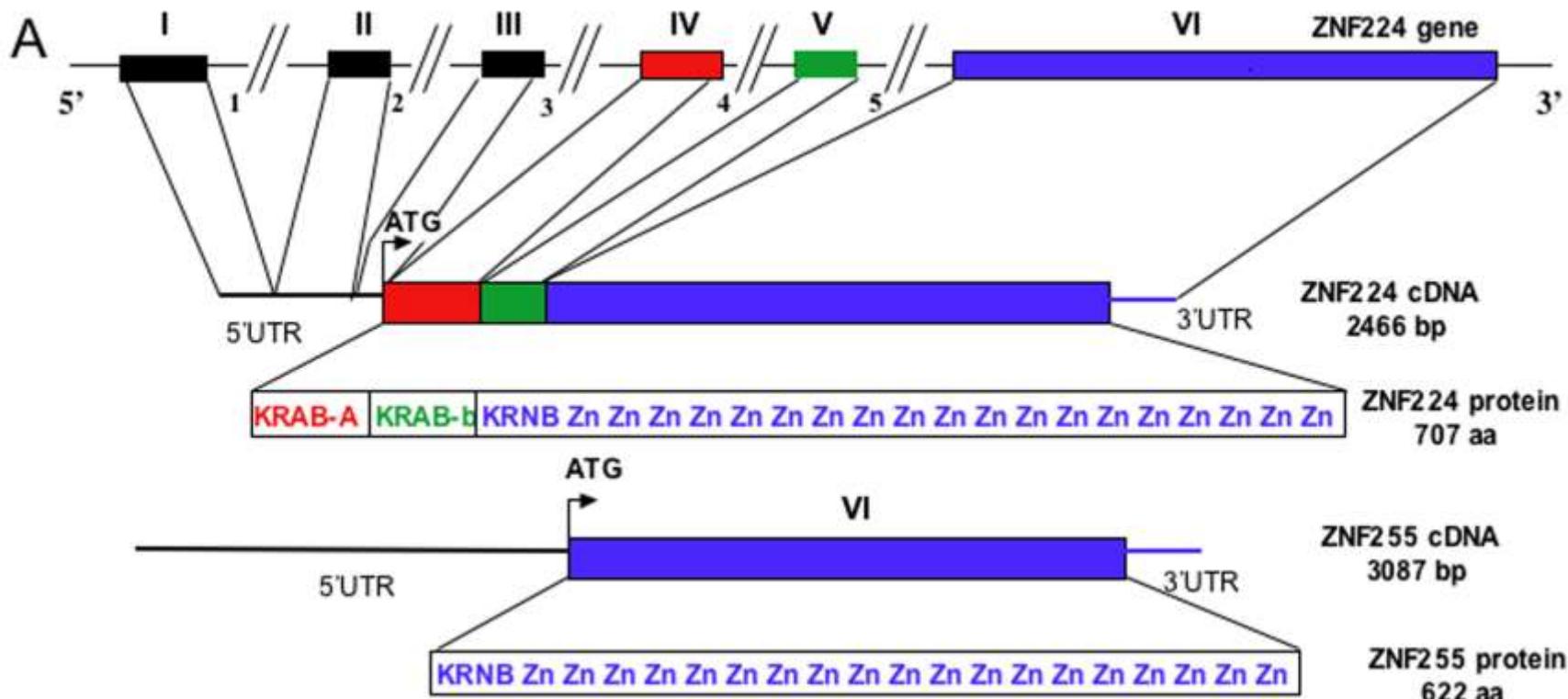
I possibili RNA messaggeri della proteina zinc finger ZNF224

A**B**

Human tissues tested

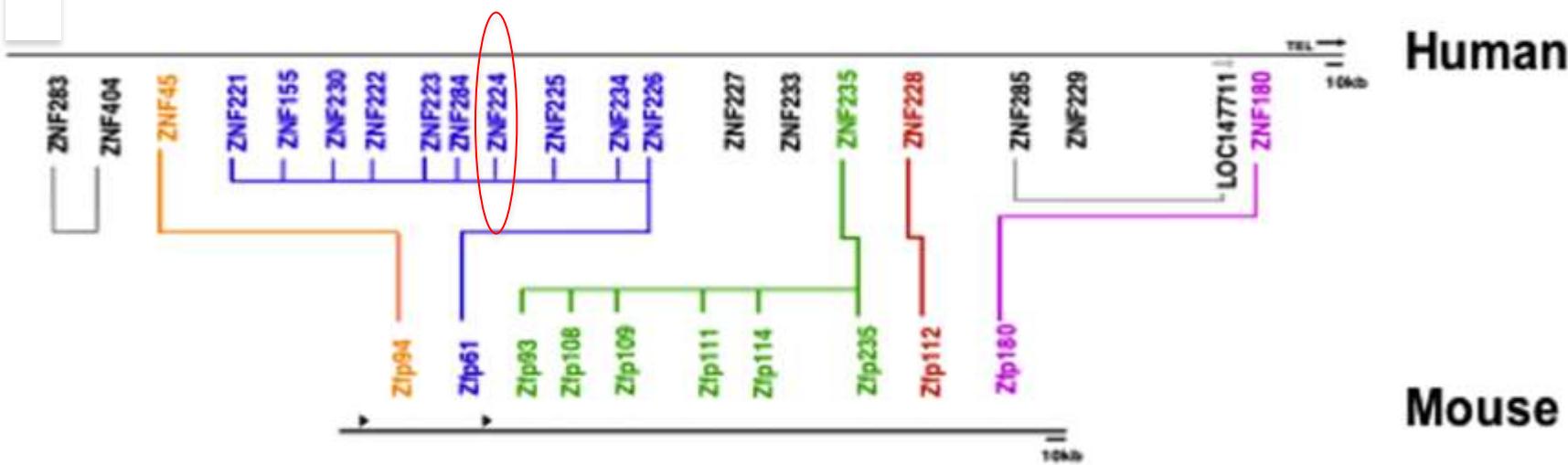
- 1) brain
- 2) heart
- 3) skeletal muscle
- 4) thymus
- 5) spleen
- 6) kidney
- 7) liver
- 8) lung

Organizzazione strutturale del gene codificante per la proteina zinc finger ZNF224



Ortologhi e paraloghi della proteina zinc finger ZNF224

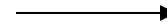
B



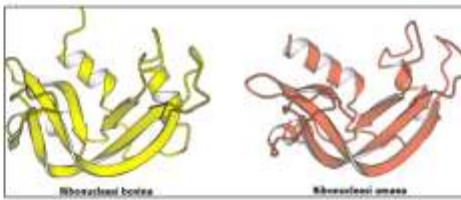
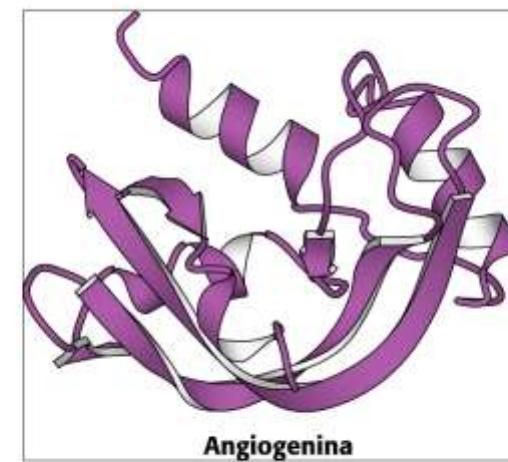
Confronto di sequenze proteiche



angiogenina



Angiogenina



ribonucleasi umana e bovina

Dall' analisi della sequenza due proteine possono essere definite:

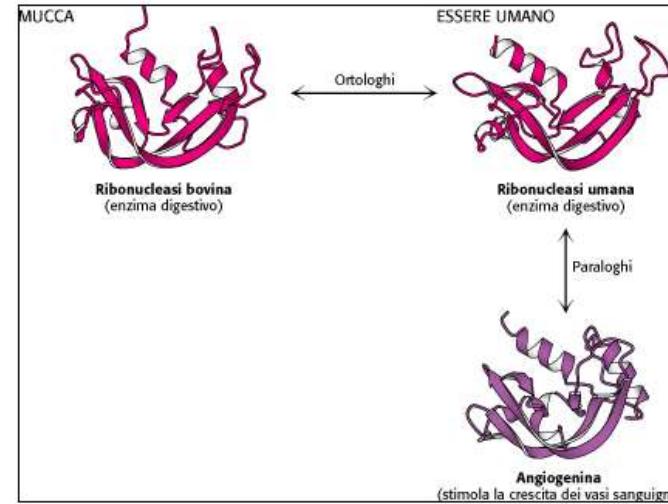
-omologhe

- { a) paraloghe
b) ortologhe

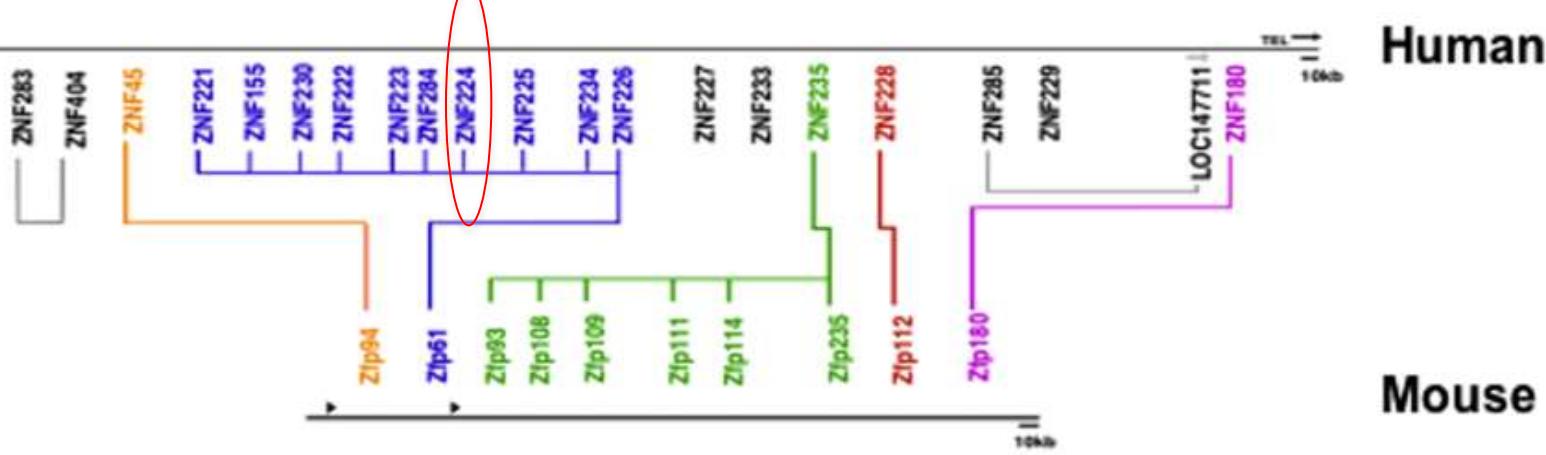
La ribonucleasi umana e quella bovina sono ortologhi, mentre
La ribonucleasi umana e l' angiogenina sono paraloghi.

Omologhi, ortologhi e paraloghi

Visualizzatore immagini e fax per Windows



Ortologhi e paraloghi della proteina zinc finger ZNF224



Un confronto tra proteine note: la mioglobina e la catena alfa dell' emoglobina



Emoglobina umana (catena α)

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHG  
SAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLS  
HCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR
```

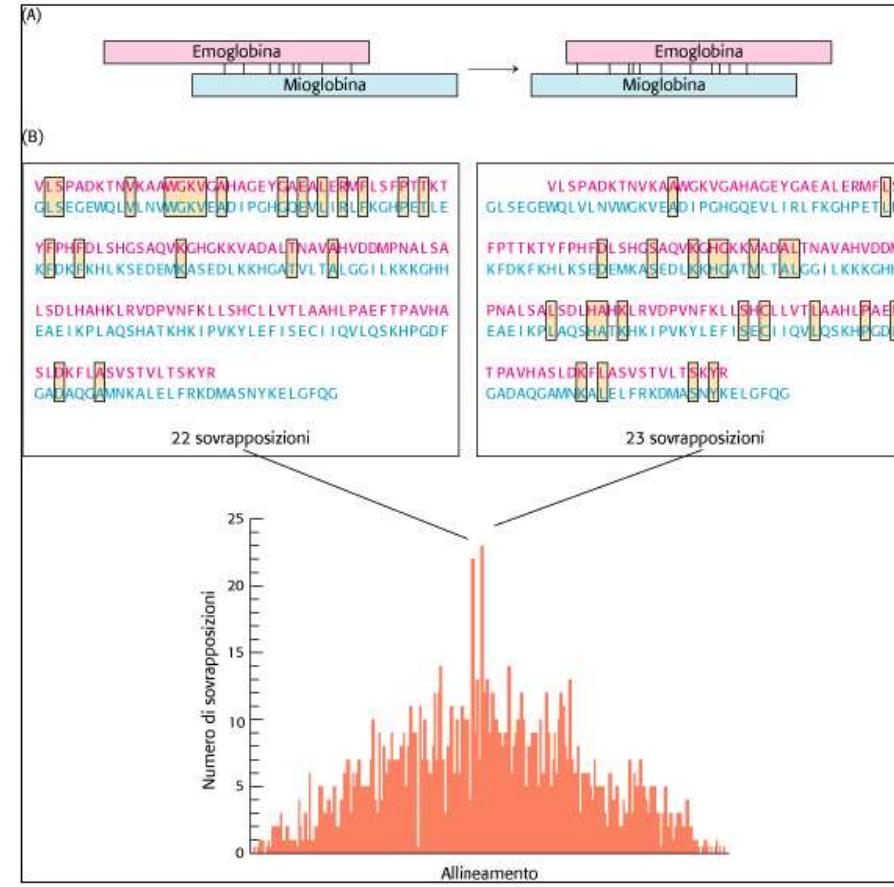
Mioglobina umana

```
GLSDGEWQLVLNVWGKVEADI PGHGQEVLIRLFKGHPETLEKFDFKFKHLKS  
EDEMKASEDLKKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPVK  
YLEFISECIIQVLQSKHPGDFGADAQGMNKALELFRKDMASNYKELGFQG
```

Un confronto tra proteine note: la mioglobina e la catena alfa dell' emoglobina

Visualizzatore immagini e fax per Windows

22 aa uguali
su 147 residui



23 aa uguali
su 147 residui

Allineamento tra sequenze con interruzioni

Visualizzatore immagini e fax per Windows

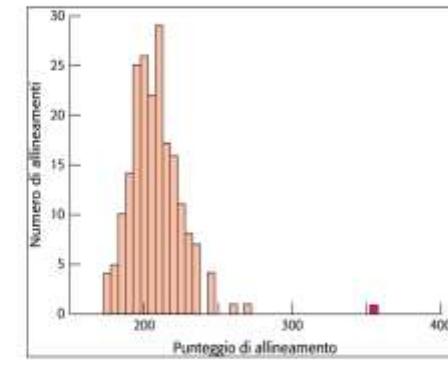
38 aa uguali su 147 residui (25,9%) !!!

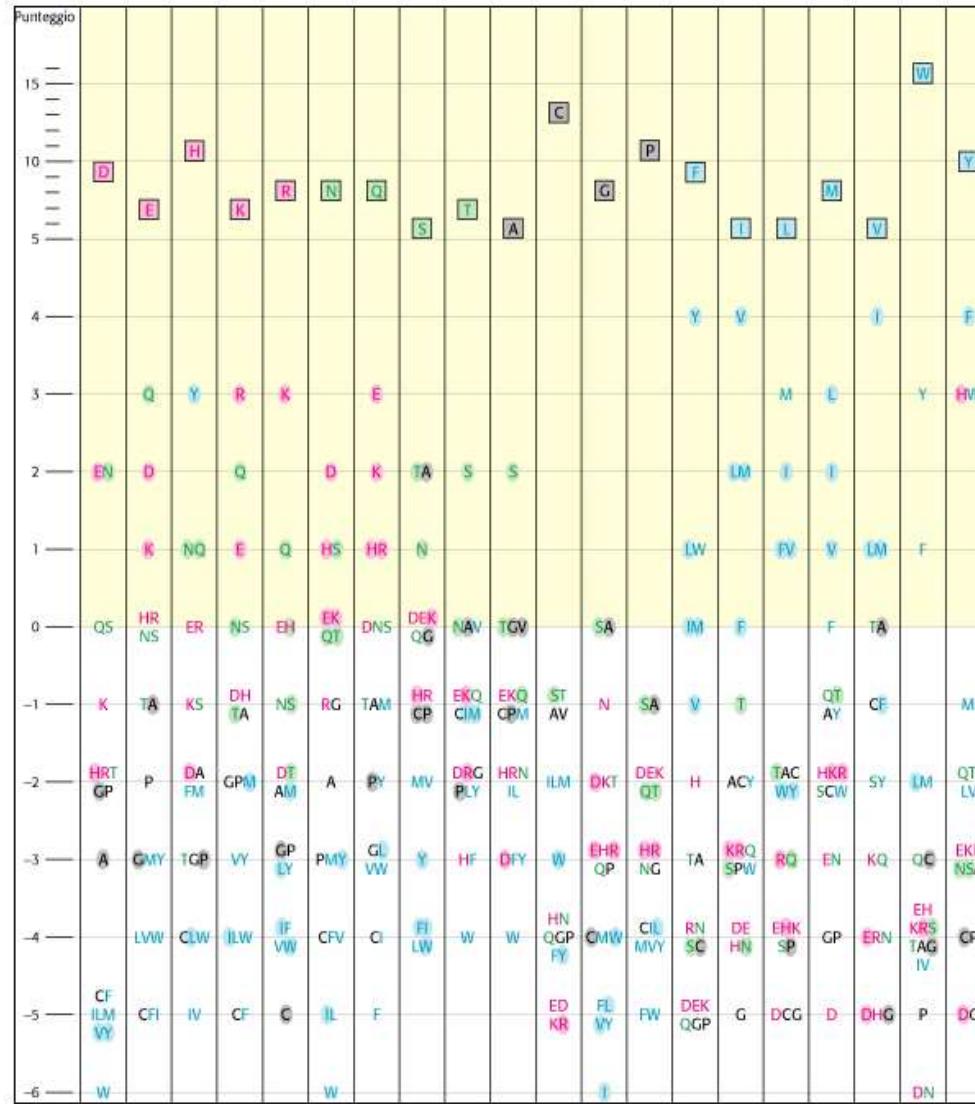


QUESTA È LA SEQUENZA AUTENTICA
↓
Rimescolamento
STA LA QUE ENZA ESEQUITE AACANTI

Allineamento per ...

...rimescolamento !



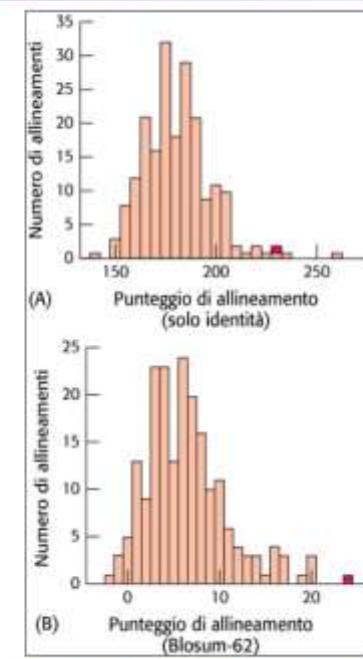
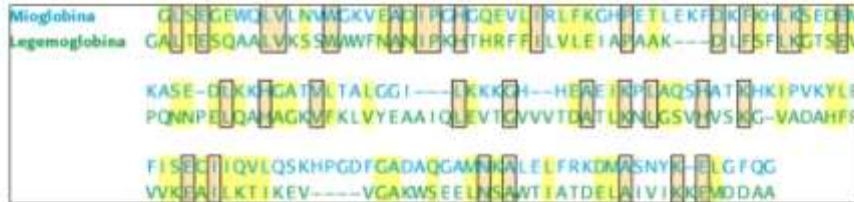


Allineamento tra mioglobina e l' alfa emoglobina

Visualizzatore immagini e fax per Windows

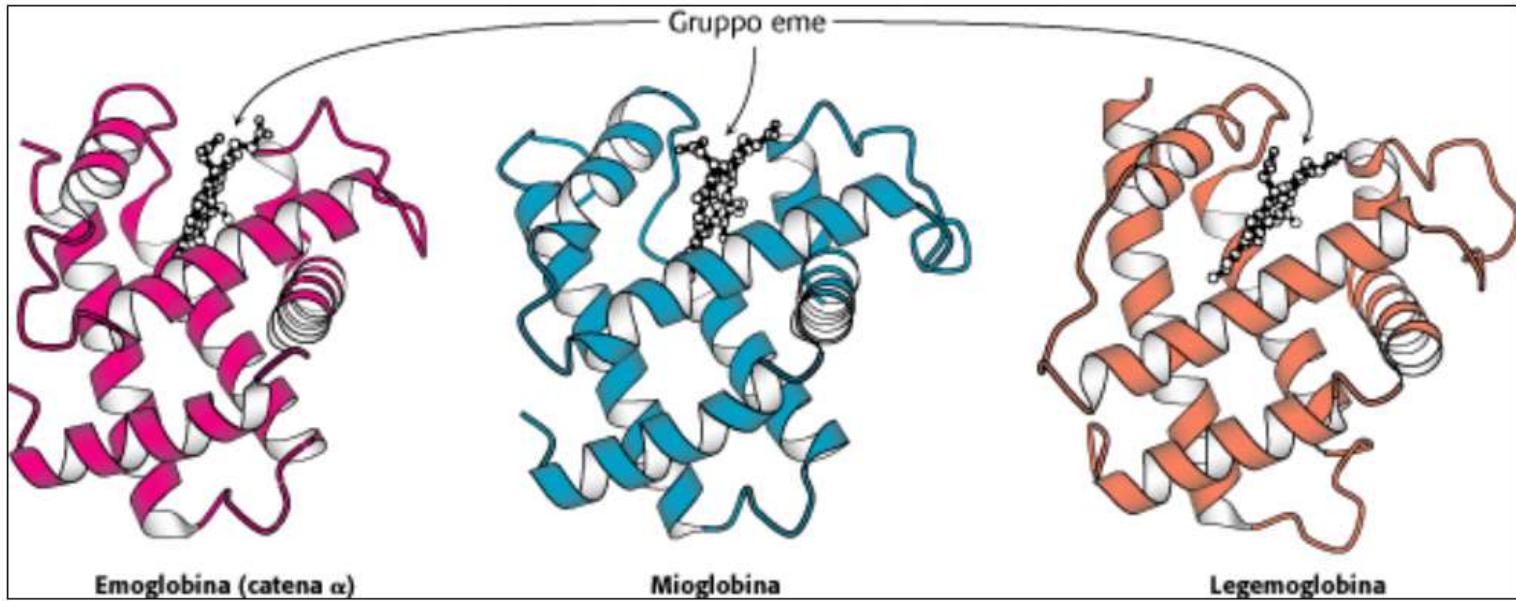


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La struttura terziaria è molto più conservata di quella primaria

Visualizzatore immagini e fax per Windows



Esempio di proteine paraloghe nella struttura tridimensionale.

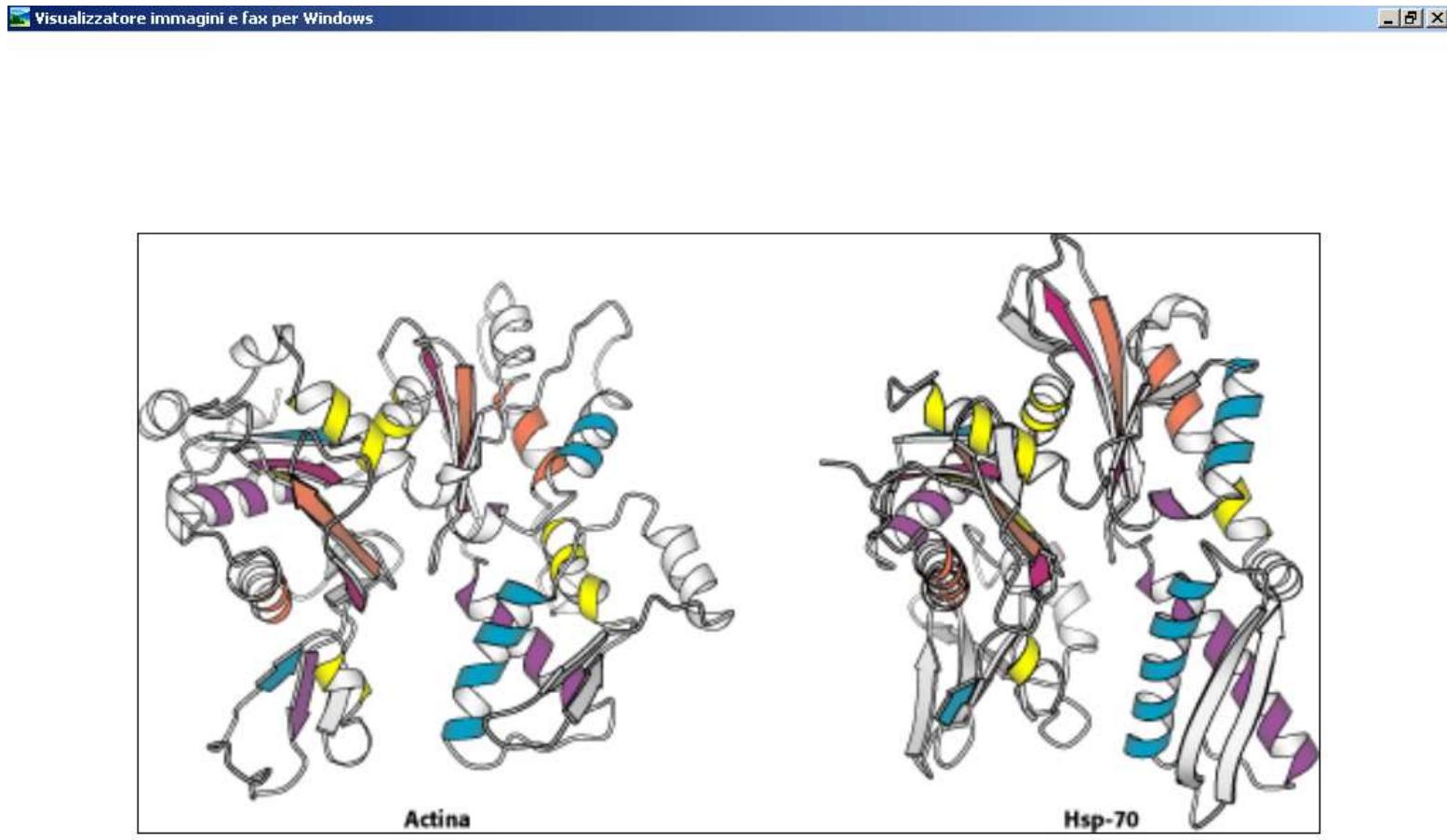
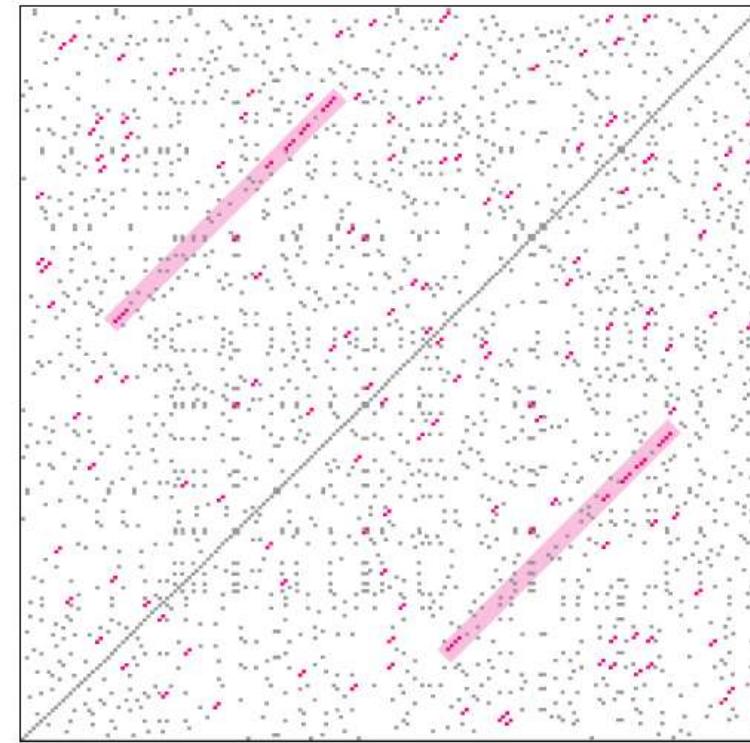




Diagramma diagonale per l' analisi di sequenze ripetute

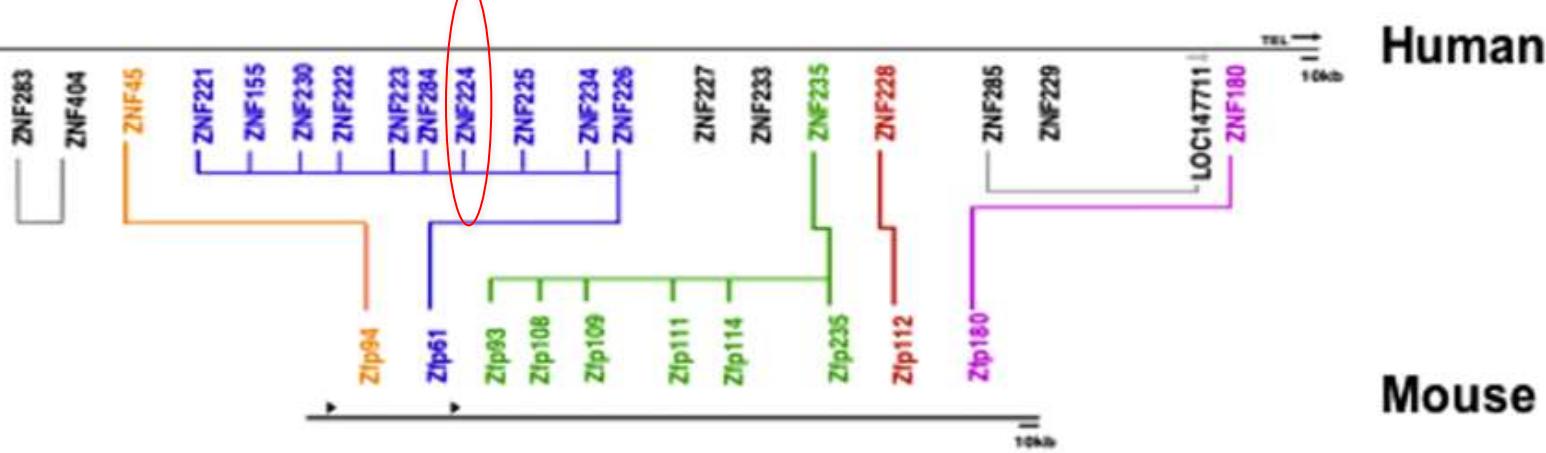




ZNF224 cDNA sequence

1 GAGTCCAAACATTGGAGTCGGACACTTCCGCTCGGGACTGAGGTTGCTGCAGTTTCCGCGATAGTTG
101 CCTTCTGAATTCCCTGGACCTACGCATTGGATCCTCAAAGAACTGCTGAATACCACTAGAAACATACTGTAACCAG
201 CAGCAAGGAAGCCCACGTACCAAGGGGCTGCTTGGCACAAATTCTGCTTCCAGGAACCTGCATCACTCAGGACTCTG
301 AAATGACCACGTTCAAGGAGGCAATGACCACTCAAGGACGCTGGCTGGTCTCACTGAGGAAGAGCTGGGCTGCTG
M T T F K E A M T F K D V A V V F T E E E L G L L
401 TCGAGATGTGATGCTGGAGAACCTCAGGAACCTGCTCTCAGTGGACATCAAGCATTCCACAGGGATACTTCCACT
R D V M L E N F R N L I S V G H Q A F H R D T F H
501 ATGATGAAGACAGCAATCCAAGGGAAAGGAATTCAAGGAGACAAGATCCAACACTGAGATGGAGACTGTTCAGAACGC
M M K T A I Q R E G N S G D K I Q T E M E T V S E A
601 TCCAGCAAATCTGGGAAAAAAATTGCAAGTGATTAAACCAGGTCTCAAGAGACTGGTGATAAATAGCTCTCAGTTCTCC
Q Q I W E K I A S D L T R S Q D L V I N S S S Q F S
701 GACTGAGGCAGGACTATCTGTAATTCACACAAGACAGAAATCTCCAGGGCAATGGATATAAACCATCCTCAGTG
T E A G L S V I H T R Q K S S Q G N G Y K P S F S
801 CAACAATTACACTCAGGAGAGAAATCTCATACGTGTGATGAGTGTGGAAAGAACCTTGTACATCTCAGCCCTTCG
Q Q L H S G E K S H T C D E C G K N F C Y I S A L R
901 GAGAGAAAATGCTATAAGTGTGACGTGTGGTAAGGAATTCAAGTCAGAGTTCACATCTGCAAACATCATCAGAGAGTC
E K C Y K C D V C G K R E F S Q S S H L Q T H Q R V
1001 ATGTGTGGAATGTGGGAAAGGCTTCAGTCGTAGATCAGCACTTAATGTTCATCACAAATTACACACAGGAGAGAAC
C V E C G K C F S R R S A L I N V H H K L H T G E K I
1101 AAGGCCTTCATTCACGATTCCAGCTCAAGAACATCAGAGAACATCCATACGGGGAGAACCCATTCAAATGTGATAT
K A P I H D S O L O E H O R T H T G E K P F K C D I
1201 GATCAAGACTTAATAGGCATTCCATGGTCACACGGCAGAGAACCATTCGGATGTGATACGTGTGATAAGAGCTTT
S R L N R H S M V H T A E K P F R C D T C D K S F
1301 TCATCGCATGATCCACACAGGAGAGAACCATACAAATGTGAGGAGTGTGGAAAAGGGCTTATTGTAGGCAGAGAC
H E M I H T G E K P Y K C E E C G K G E I C R R D J
1401 ACGGGAGAAAAGCCATATAATTGTAAGAGGTGTGGGAAGAGCTTCAGATGGCCTCGTGTCTTGAAACATCAGCG
T G E K P Y N C K E C G K S F R W A S C L L K H Q R
1501 TCAAATGTGAAGAACATGTGGGAAAGGATTTCACACAAATTCCACAAATGCTATTCCCACCAAGAGATCCCATA
K C E E C G K R G F Y T N S Q C Y S H Q R S H S G E
1601 TGGGAAGGGCTACAAAAGGAGGTTGGATCTTGACTTTCACCGCGCGTCCATACAGGAGAGAACACTGTATAATTGTA
G K G Y K R R L D L D F H Q R V H T G E K L Y N C I
1701 CGGGCCCCATGTCTTTGAAACATGAGAGACTCCACAGTGGAGAAAACCATTCAAATGTGAAGAGTGTGGGAAGAG
R A P C L L K H E B L H S G E K P F Q C E E C G K R
1801 ATTCCCACAGAGAGTTCACACTGGAGAAAAGCCATACAAATGTGAGAAGTGTGGAAAAGGGCTACAATAGTAAGTT
S H Q R V H T G E K P Y K C E R K C G K R G Y N S K F
1901 CCACACAGGAGAGAGACCATACAATTGTAAGGAATGTGGGAAGAGTTGGCTGGGCCCTCGTGTCTTTGAAACATC
H T G E R P Y N C K E C G K S F G W A S C L L K H
2001 CCTTTCAAATGTGAAGAGGTGTGGAAAAGATTACTCAGAATTCAAGCTTCATTCTCATCAAAGAGTGCACACTGG
P F K C E E C G K R F T Q N S Q L H S H Q R V H T G
2101 AGTGTGGGAAGGGCTTCAGCTGGCCCTCAACTCGTCTGACCCATCAGAGACGCCACAGCAGAGAACACCTCTCAA
C G K R G F S W S S T R L T H Q R R H S R E T P L K
2201 TGTAAGAATTCTATTCTAAAGTGCAGAGAAAAAGTTCAAGTGTAGAAAAGCCATACAAATGTGAGGACTGTGGGA
V O N S F S K V O E K V H S V E K P Y K C E D C G I
2301 CTTGATATGCATCAGAGGGTCCACATGGGAGAGAAAACATGGAAGTGTAGGGAGTGTGATATGTGCTTTAGTCAGGC
L D M H Q R V H M G E K T W K C R E C D M C F S Q A
2401 ATGTTCATGTTGGAGAAAACCTTAGTGTGATGGTGCAATAAGTCTTCACTCAGTCTTCATG 2466
V H V G E K P *

Ortologhi e paraloghi della proteina zinc finger ZNF224



L' analisi mediante l' utilizzo di softwares dedicati e grazie alle Banche dati che sono a disposizione è stato possibile definire che il repressore trascrizionale identificato come ZNF224 appartiene alla famiglia delle proteine zinc finger.

Diversi fattori trascrizionali sono compresi nella famiglia delle proteine che legano il DNA attraverso il motivo detto a dito di zinco :

- TFIIIA
- Sp1
- NGF-1A
- Kruppel
- Hunchback
- GAL4
- recettori nucleari



Dai geni alle proteine e ...viceversa

ch8f44[1] - Visualizzatore immagini e fax per Windows

