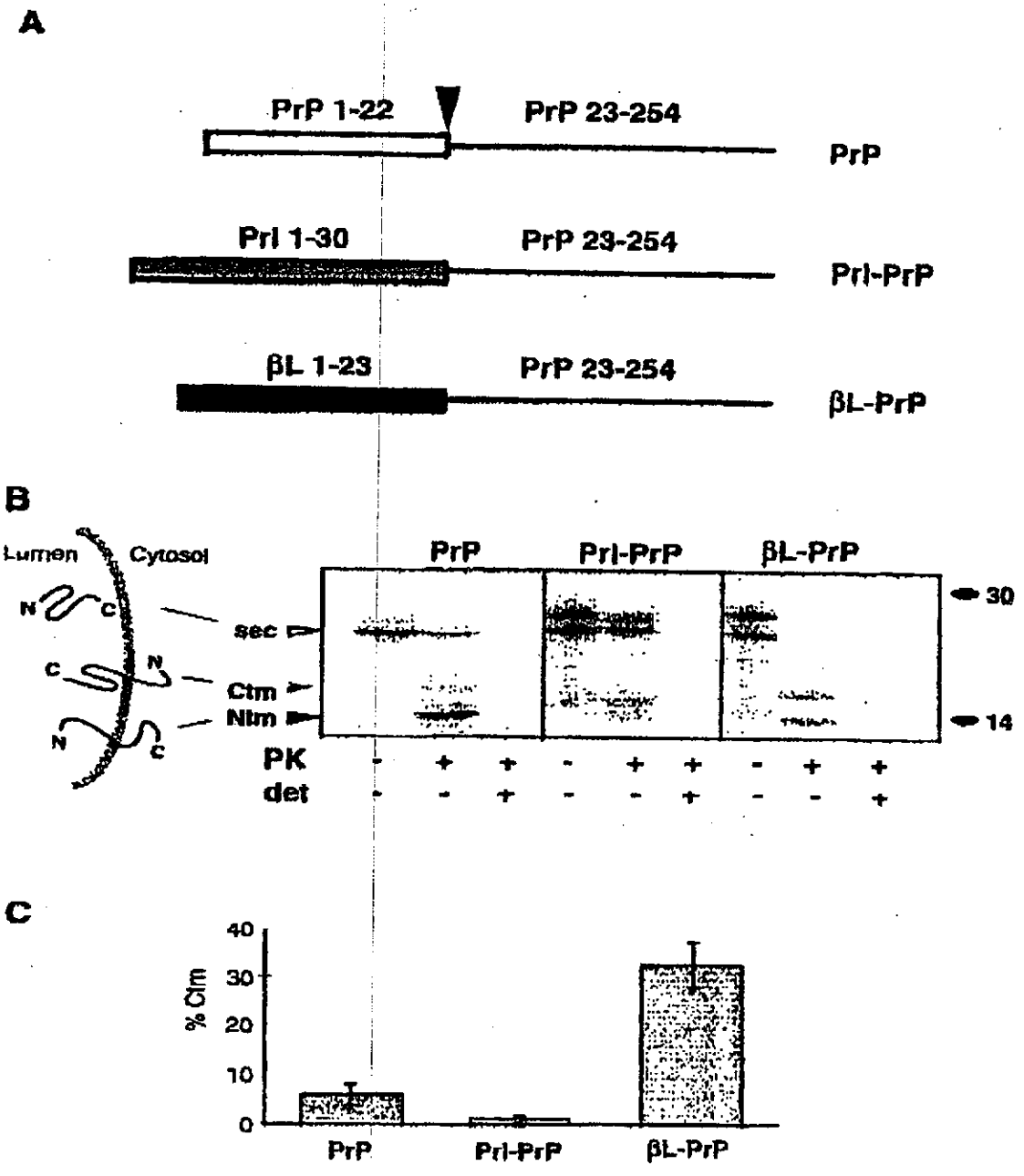

A
PrP 1-22
PrP 23-254
PrP
Prl 1-30
PrP 23-254
Prl-PrP
βL 1-23
PrP 23-254
βL-PrP
B
Lumen
Cytosol
N
C
C
N
N
C
sec
Ctm
Ntm
PrP
Prl-PrP
βL-PrP
30
14
PK
det
C
% Ctm
40
30
20
10
0
PrP
Prl-PrP
βL-PrP

FIGURE 1

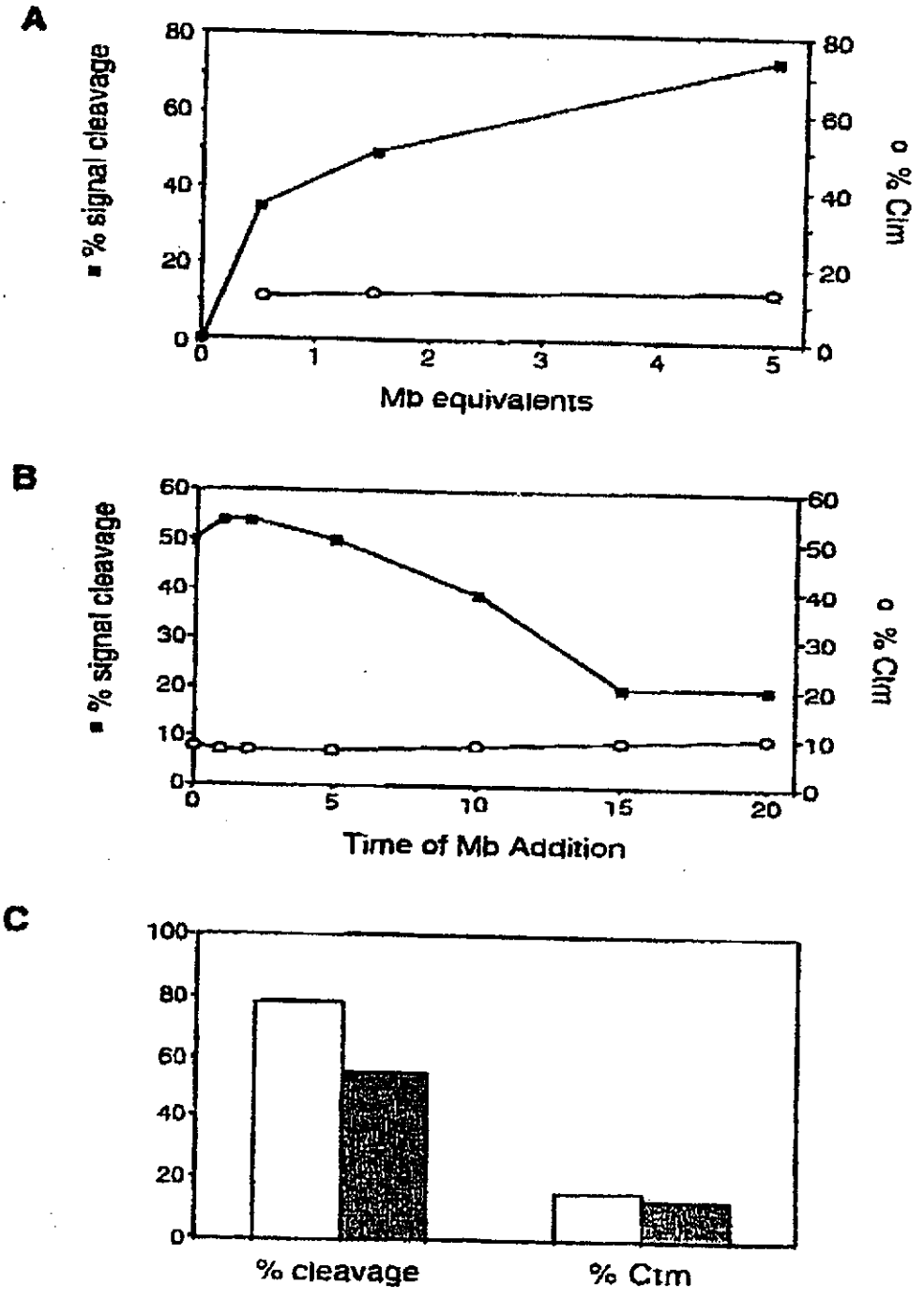
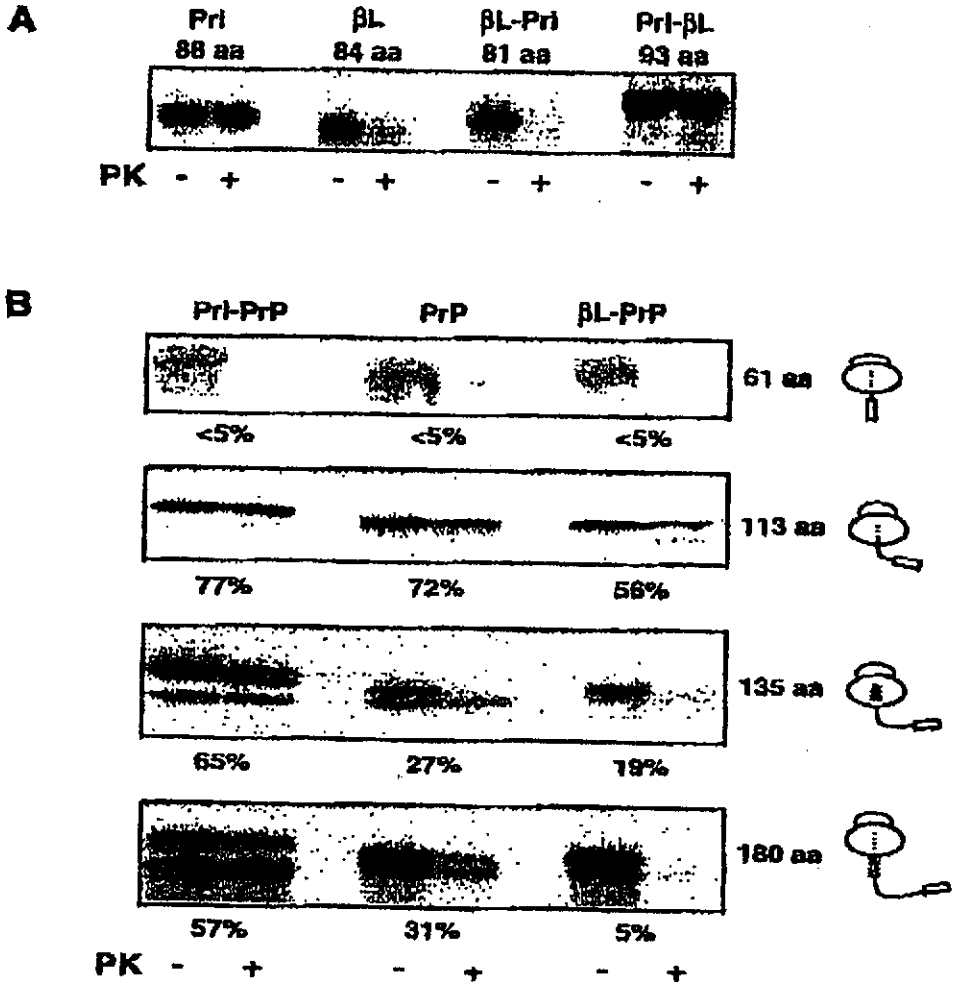

A
■ % signal cleavage
o % Ctm
Mb equivalents
B
■ % signal cleavage
o % Ctm
Time of Mb Addition
C
% cleavage
% Ctm

FIGURE 2

FIGURE 3


A
Prl
88 aa
βL
84 aa
βL-Prl
81 aa
Prl-βL
93 aa
PK - + - + - + - +
B
Prl-PrP
PrP
βL-PrP
61 aa
<5%
<5%
<5%
113 aa
77%
72%
56%
135 aa
65%
27%
19%
180 aa
57%
31%
5%
PK - + - + - +

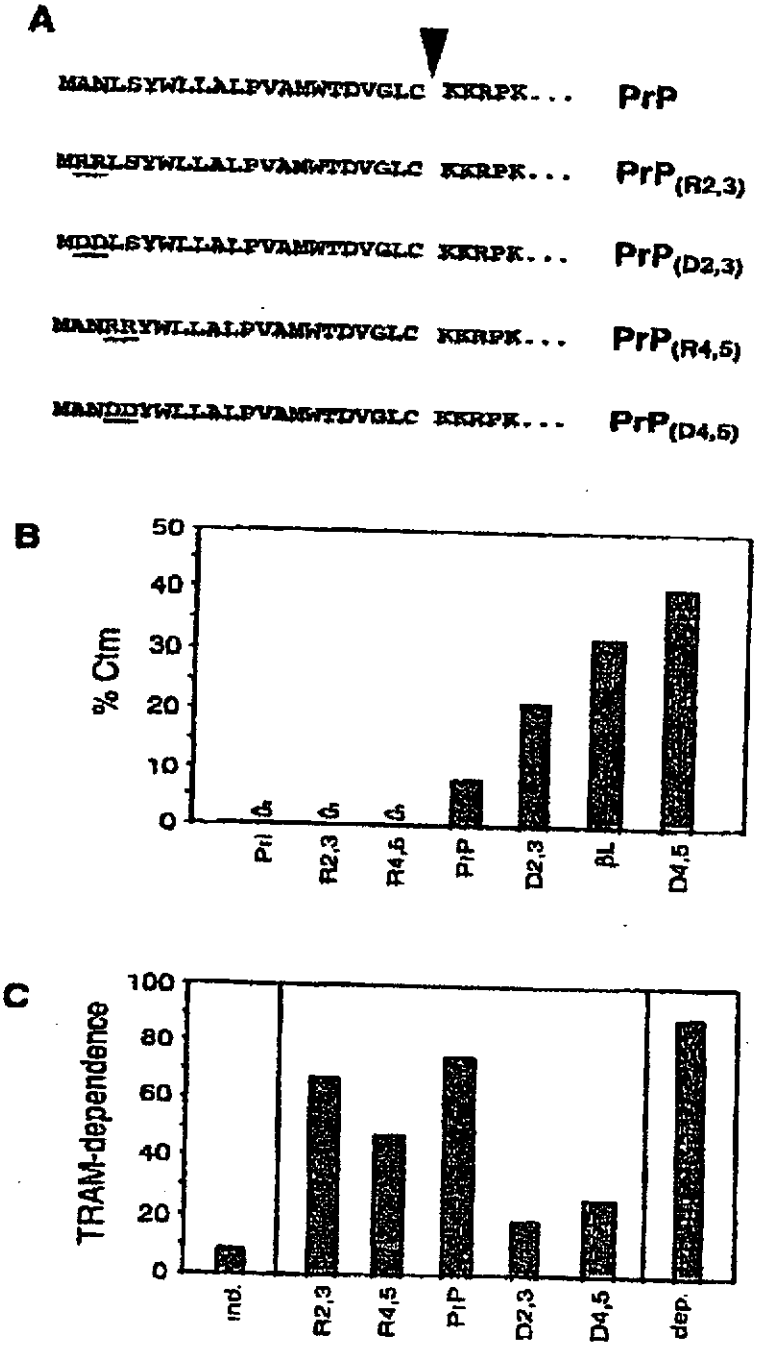

A
MANLGYWLLALFVAMWTDVGLC KKRPK...
PrP
MRRLGYWLLALFVAMWTDVGLC KKRPK...
PrP(R2,3)
MDDLGYWLLALFVAMWTDVGLC KKRPK...
PrP(D2,3)
MANRRYWLLALFVAMWTDVGLC KKRPK...
PrP(R4,5)
MANDDYWLLALFVAMWTDVGLC KKRPK...
PrP(D4,5)
B
% Ctm
50
40
30
20
10
0
Δ
Δ
Δ
PrP
R2,3
R4,5
PrP
D2,3
βL
D4,5
C
TRAM-dependence
100
80
60
40
20
0
ind.
R2,3
R4,5
PrP
D2,3
D4,5
dep.

FIGURE 4

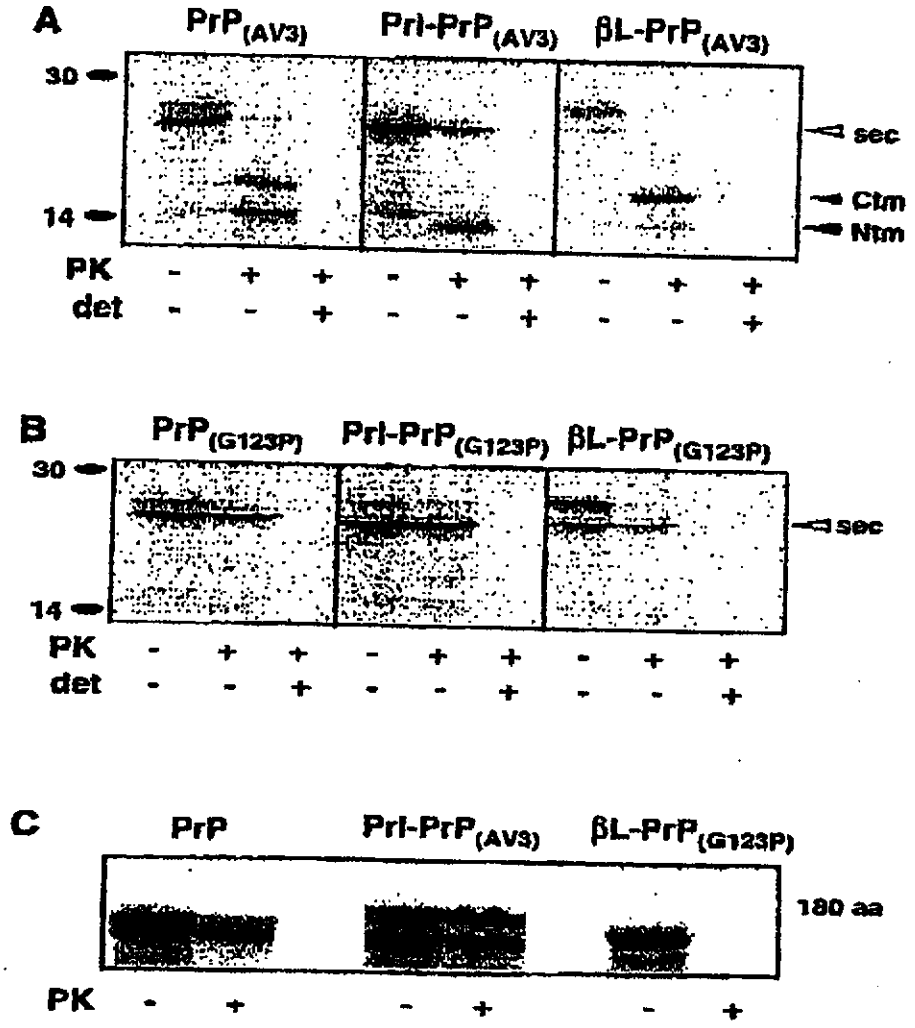

A
PrP(AV3)
PrI-PrP(AV3)
βL-PrP(AV3)
30
14
sec
Ctm
Ntm
PK
det
B
PrP(G123P)
PrI-PrP(G123P)
βL-PrP(G123P)
30
14
sec
PK
det
C
PrP
PrI-PrP(AV3)
βL-PrP(G123P)
180 aa
PK

FIGURE 5

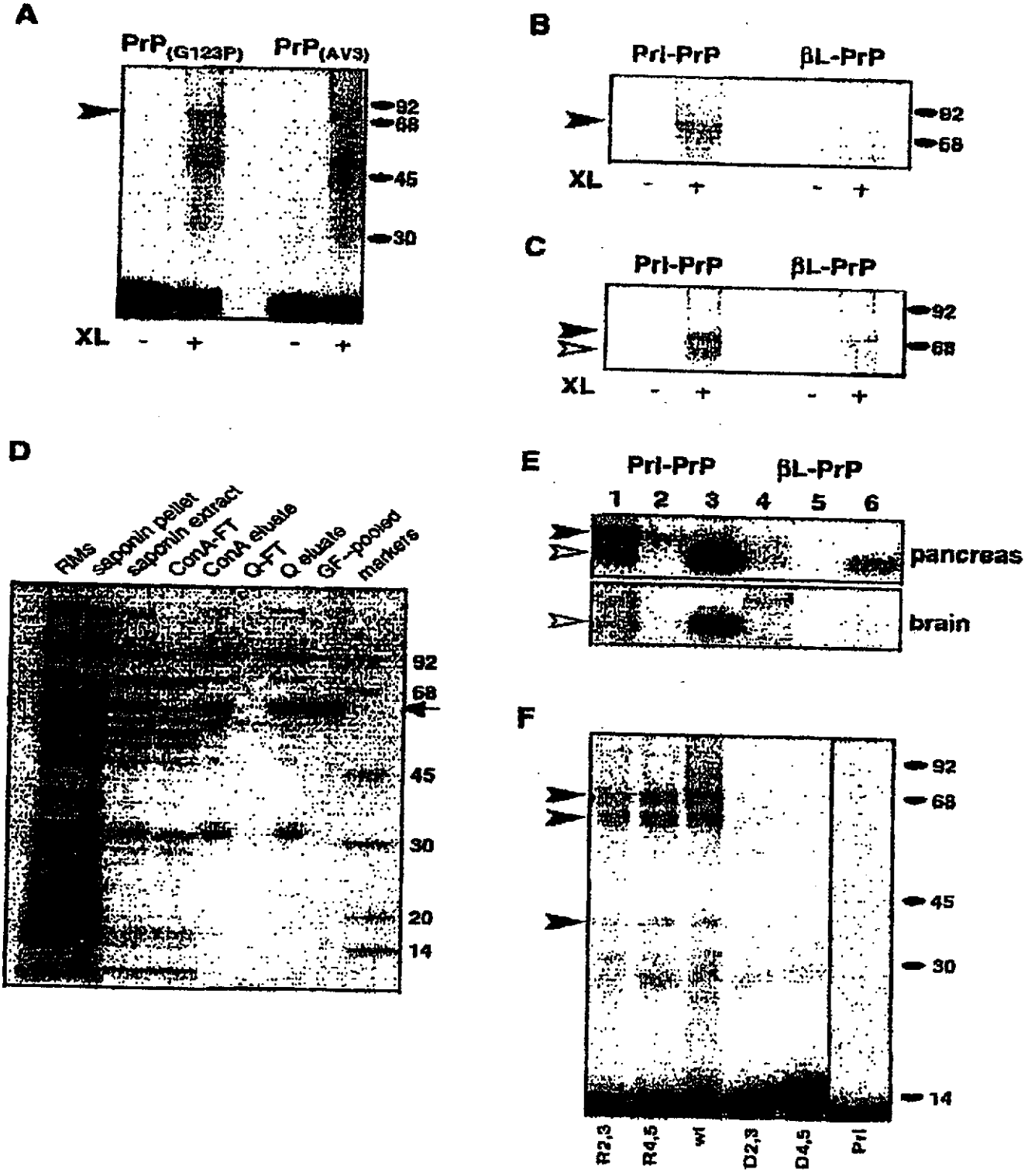

A
PrP(G123P)
PrP(AV3)
92
68
45
30
XL - + - +
B
Prl-PrP
βL-PrP
92
68
XL - + - +
C
Prl-PrP
βL-PrP
92
68
XL - + - +
D
FIMs
saponin pellet
saponin extract
ConA-FT
ConA eluate
Q-FT
Q eluate
GF-pooled
markers
92
68
45
30
20
14
E
Prl-PrP
βL-PrP
1 2 3 4 5 6
pancreas
brain
F
92
68
45
30
14
R2,3
R4,5
wt
D2,3
D4,5
Prl

FIGURE 6

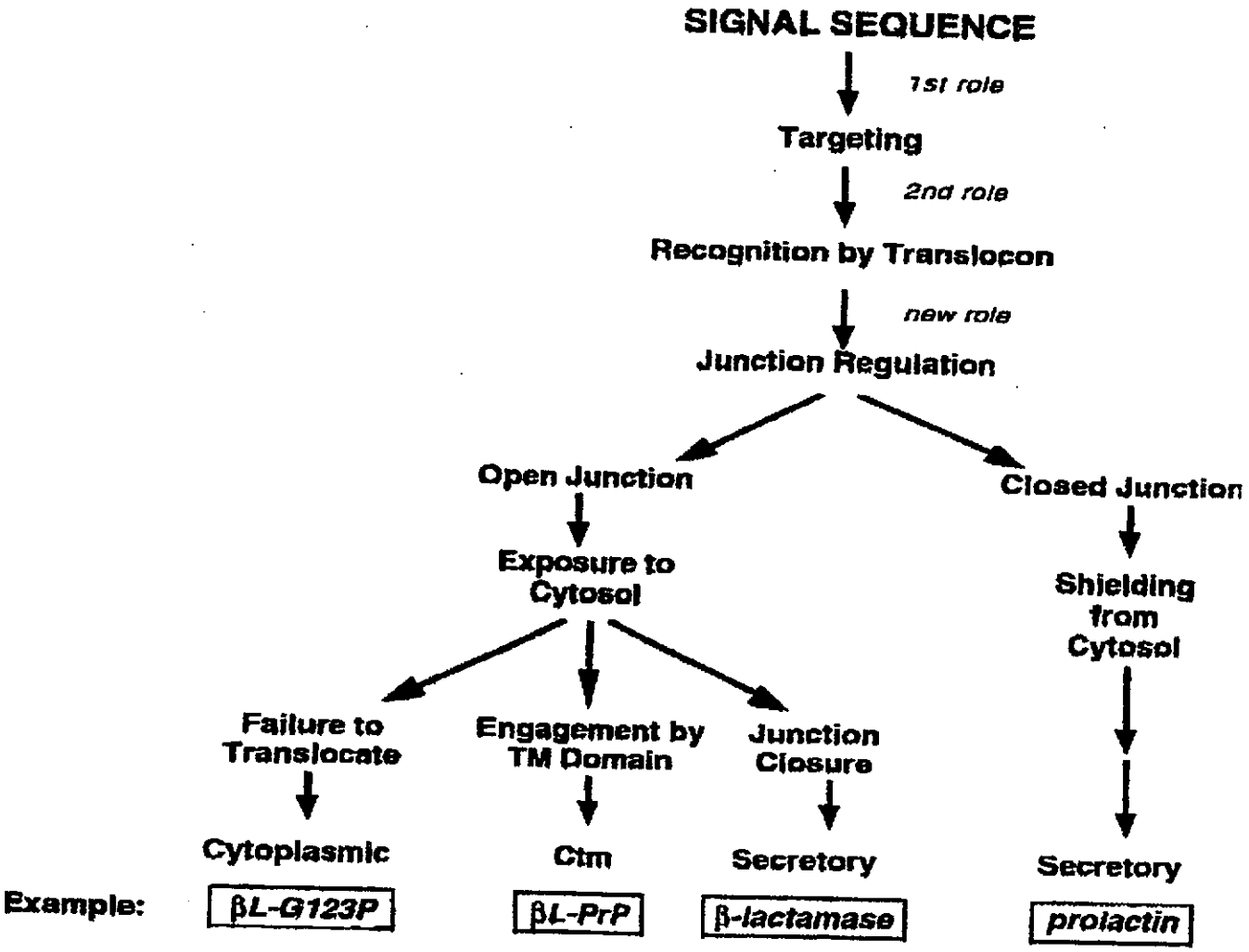

SIGNAL SEQUENCE
1st role
Targeting
2nd role
Recognition by Translocon
new role
Junction Regulation
Open Junction
Closed Junction
Exposure to Cytosol
Shielding from Cytosol
Failure to Translocate
Engagement by TM Domain
Junction Closure
Cytoplasmic
Ctm
Secretory
Secretory
Example:
βL-G123P
βL-PrP
β-lactamase
prolactin

FIGURE 7

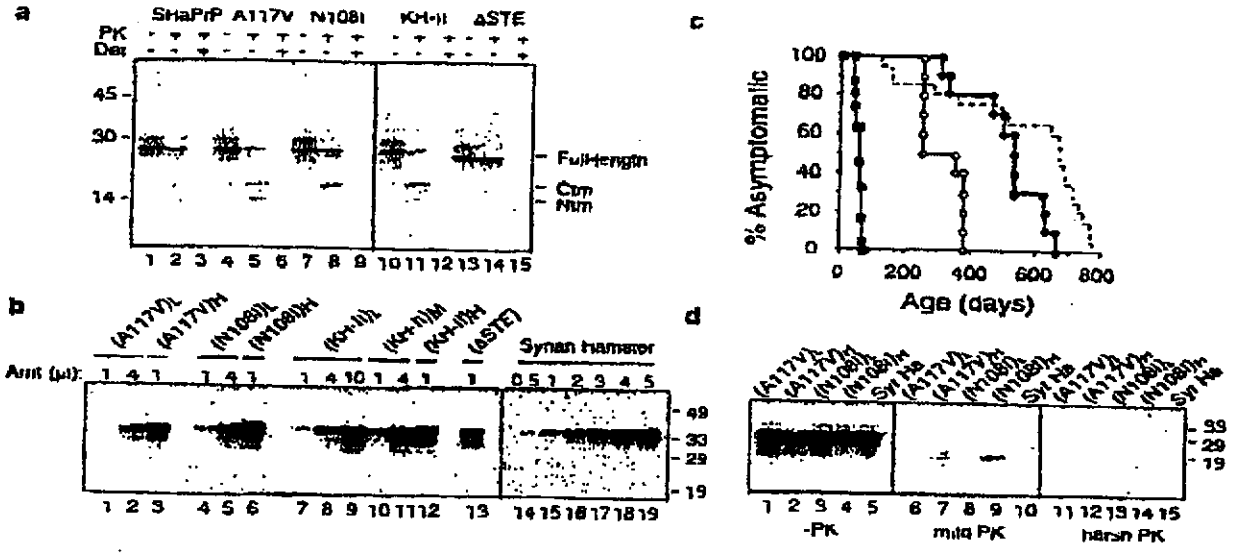

a
SHaPrP A117V N108I KH-II ΔSTE
PK
Det
45
30
14
FullHength
Ctm
Ntm
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
b
(A117V)L
(A117V)H
(N108I)L
(N108I)H
(KH-II)L
(KH-II)M
(KH-II)H
(ΔSTE)
Synan hamster
Anti (μl):
1 4 1 1 4 1 1 4 10 1 4 1 1 0 5 1 2 3 4 5
49
33
29
19
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
c
% Asymptomatic
100
80
60
40
20
0
0 200 400 600 800
Age (days)
d
(A117V)L
(A117V)M
(N108I)L
(N108I)H
Syr Ha
(A117V)L
(A117V)M
(N108I)L
(N108I)H
Syr Ha
(A117V)L
(A117V)H
(N108I)L
(N108I)H
Syr Ha
33
29
19
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
-PK
mild PK
harsh PK

FIGURE 8

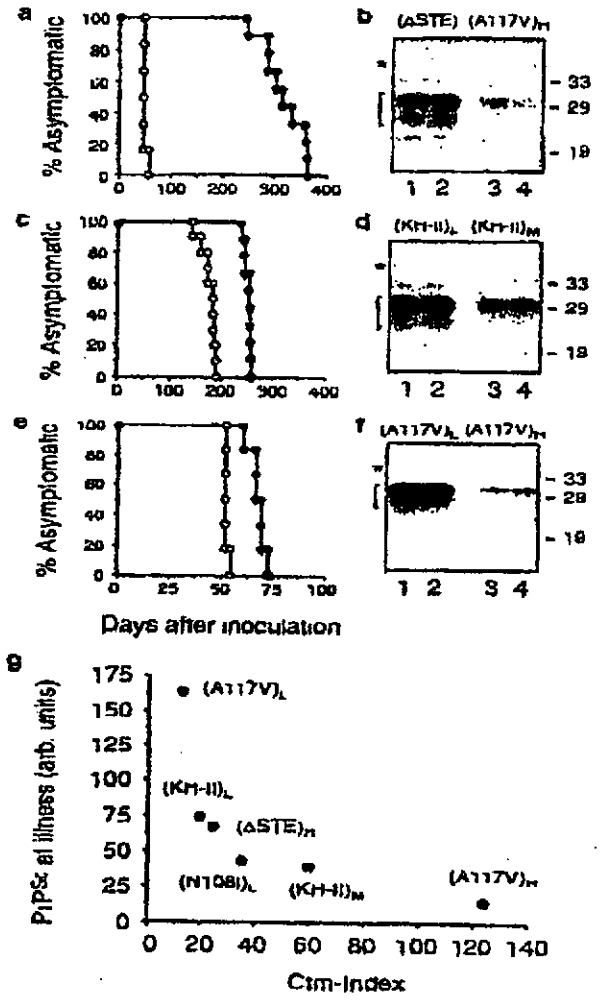

a
% Asymptomatic
b
(ΔSTE) (A117V)H
33
29
19
1 2 3 4
c
% Asymptomatic
d
(KH-II)L (KH-II)M
33
29
19
1 2 3 4
e
% Asymptomatic
f
(A117V)L (A117V)H
33
29
19
1 2 3 4
Days after inoculation
g
PrPSc at illness (arb. units)
(A117V)L
(KH-II)L
(ΔSTE)H
(N108I)L
(KH-II)M
(A117V)H
Ctm-Index

FIGURE 9

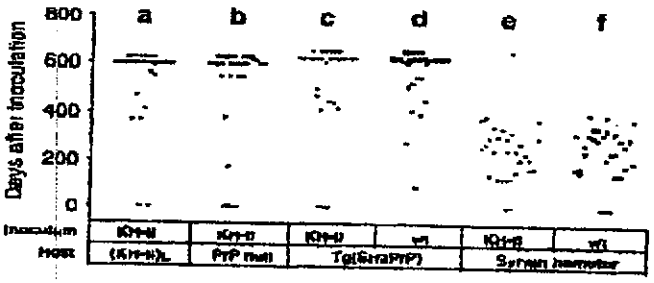

a
b
c
d
e
f
800
600
400
200
0
Days after inoculation
Host
PrP null
Tg(SHaPrP)
Serpin transgene

FIGURE 10

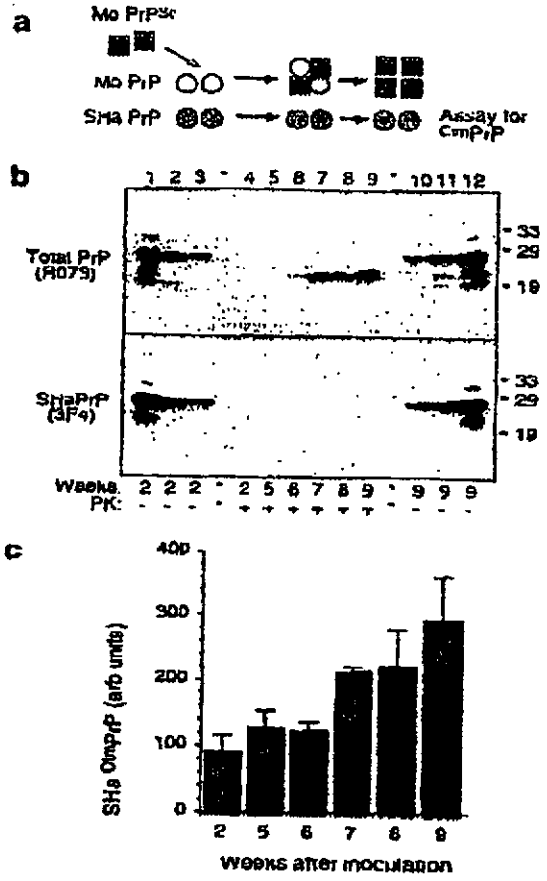

a
Mo PrPSc
Mo PrP
SHa PrP
Assay for CmPrP
b
1 2 3 4 5 6 7 8 9 10 11 12
Total PrP (R073)
SHaPrP (3F4)
33
29
19
Weeks
PK:
2 2 2 2 5 6 7 8 9 9 9 9
c
SHa CmPrP (arb units)
400
300
200
100
0
2 5 6 7 8 9
Weeks after inoculation

FIGURE 11

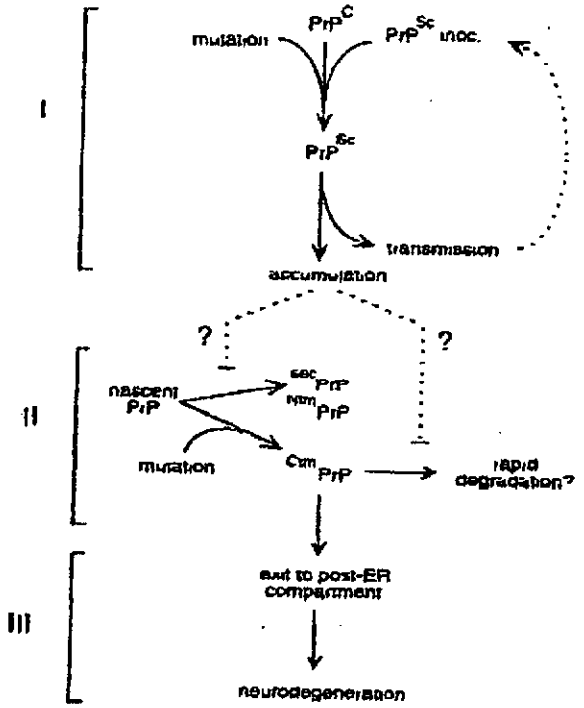

I
II
III
mutation
PrP^C
PrP^{Sc} inoc.
PrP^{Sc}
transmission
accumulation
?
?
nascent PrP
^{sec}PrP
^{ntm}PrP
mutation
^{Ctm}PrP
rapid degradation?
exit to post-ER compartment
neurodegeneration

FIGURE 12

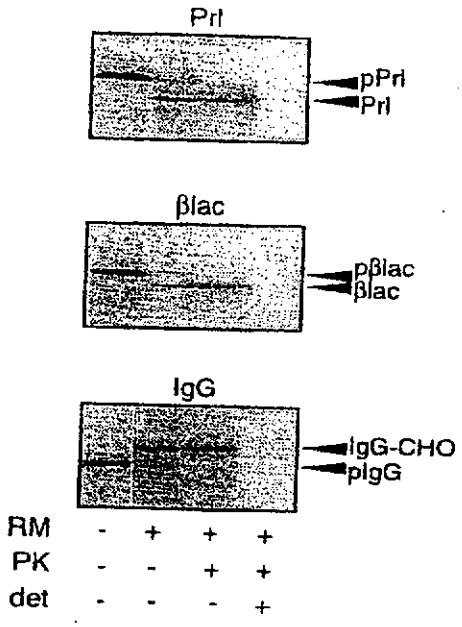


FIGURE 13

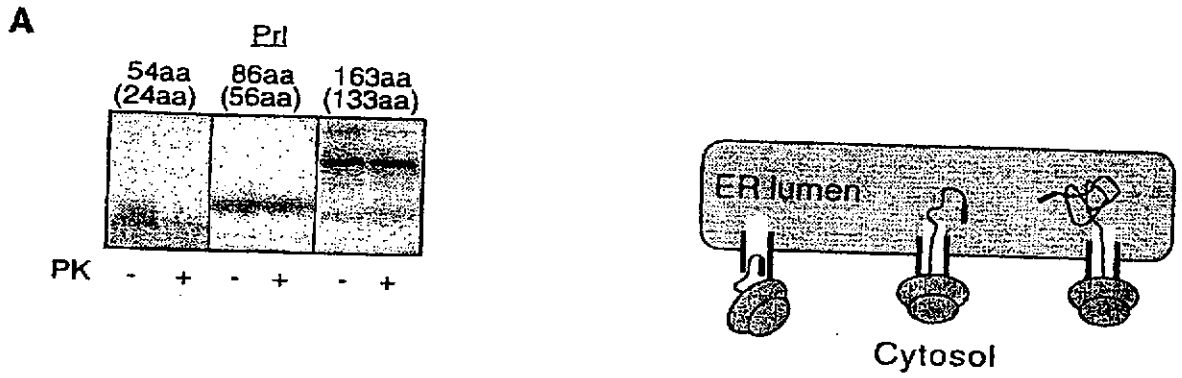

A
Prl
54aa (24aa)
86aa (56aa)
163aa (133aa)
PK - + - + - +
ER lumen
Cytosol
B
βlac
45aa (22aa)
84aa (61aa)
132aa (109aa)
PK - + - + - +
C
IgG
69aa (54aa)
101aa (86aa)
122aa (107aa)
PK - + - + - +

FIGURE 14

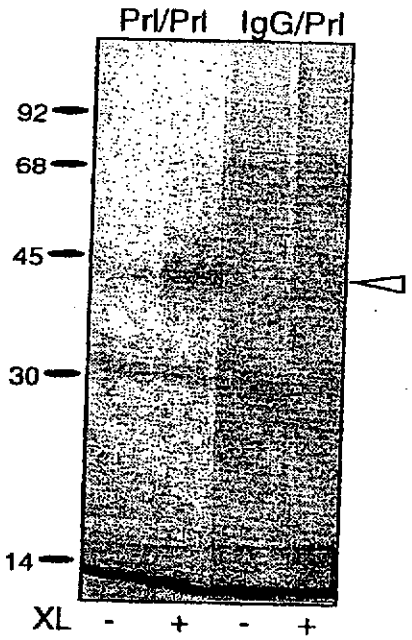


FIGURE 15

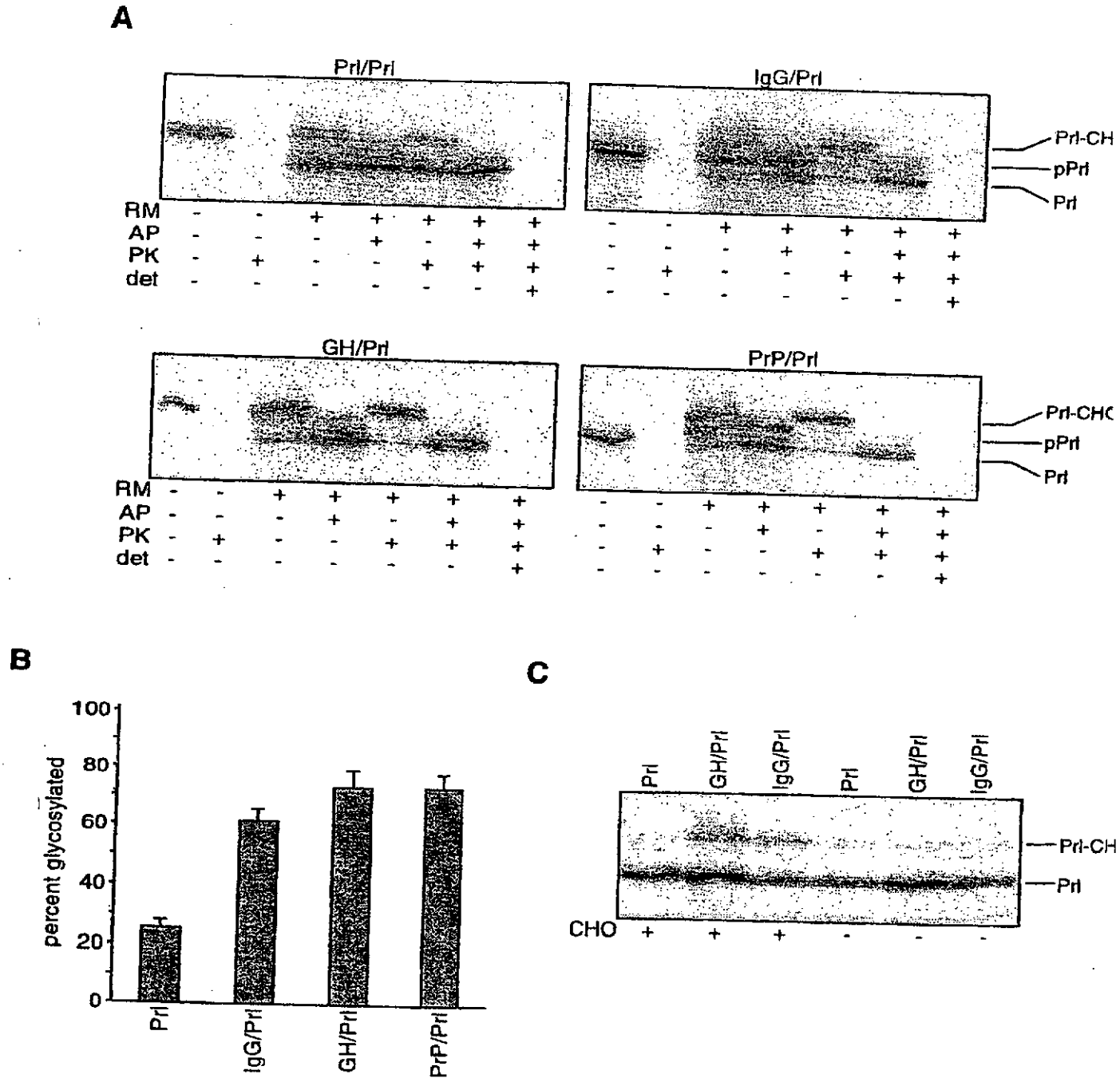

A
Prl/Prl
IgG/Prl
Prl-CH
pPrl
Prl
RM
AP
PK
det
GH/Prl
PrP/Prl
Prl-CHO
B
percent glycosylated
100
80
60
40
20
0
Prl
IgG/Prl
GH/Prl
PrP/Prl
C
Prl
GH/Prl
IgG/Prl
Prl
GH/Prl
IgG/Prl
Prl-CH
Prl
CHO

FIGURE 16

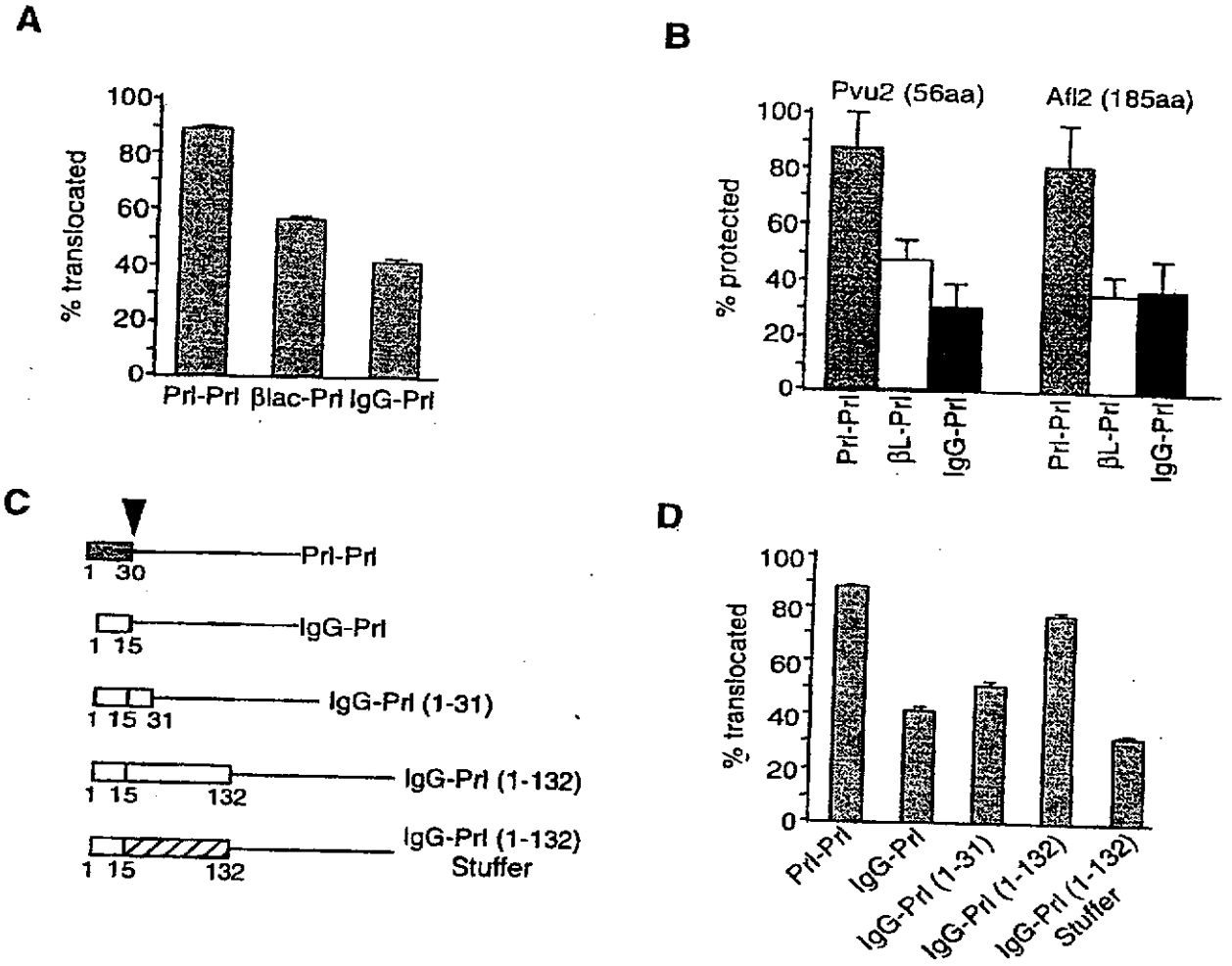

A
% translocated
100
80
60
40
20
0
Prl-Prl
βlac-Prl
IgG-Prl
B
% protected
Pvu2 (56aa)
Afl2 (185aa)
Prl-Prl
βL-Prl
IgG-Prl
C
Prl-Prl
1 30
IgG-Prl
1 15
IgG-Prl (1-31)
1 15 31
IgG-Prl (1-132)
1 15 132
IgG-Prl (1-132)
Stuffer
1 15 132
D
% translocated
IgG-Prl (1-31)
IgG-Prl (1-132)
IgG-Prl (1-132)
Stuffer

FIGURE 17

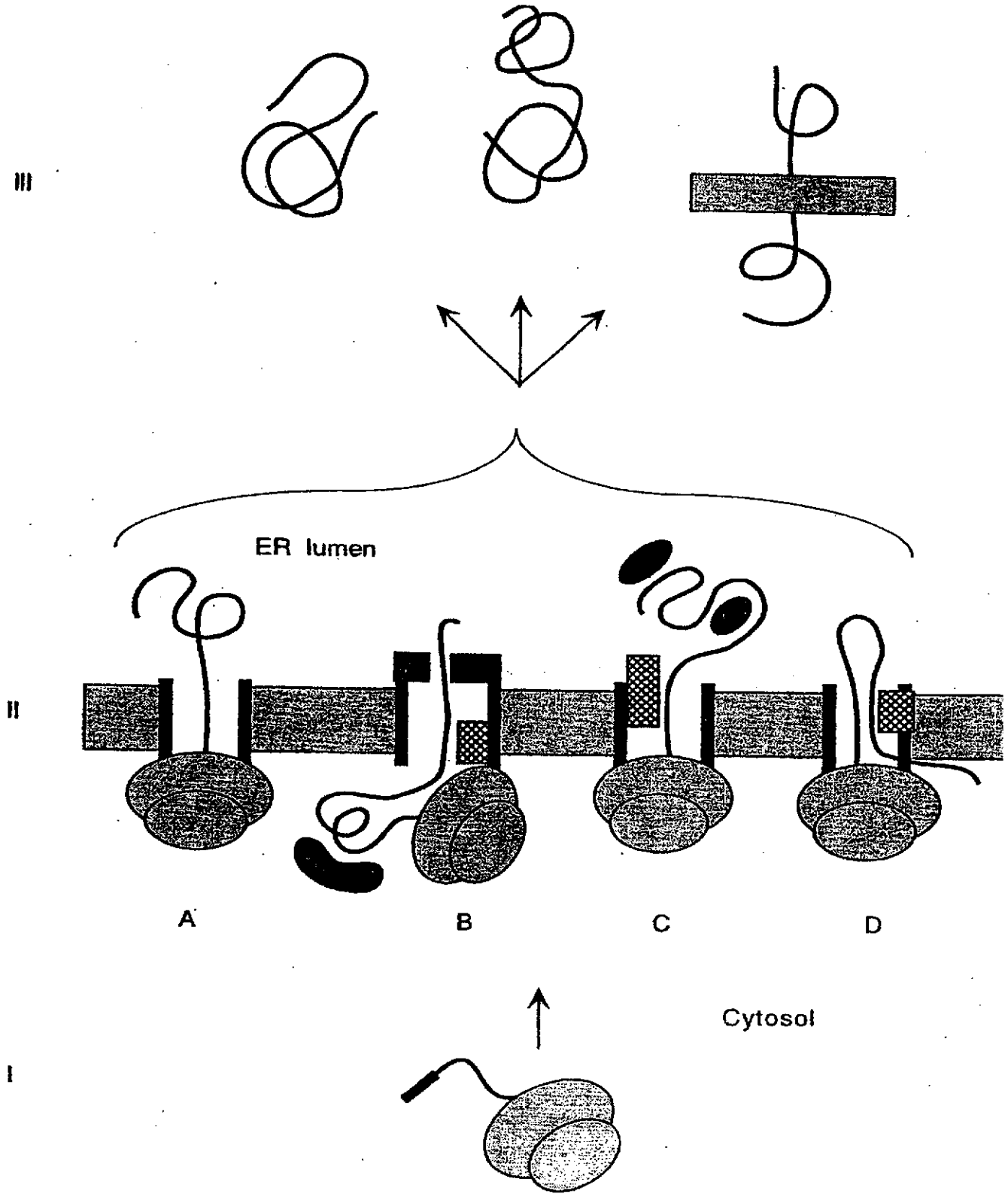


FIGURE 18

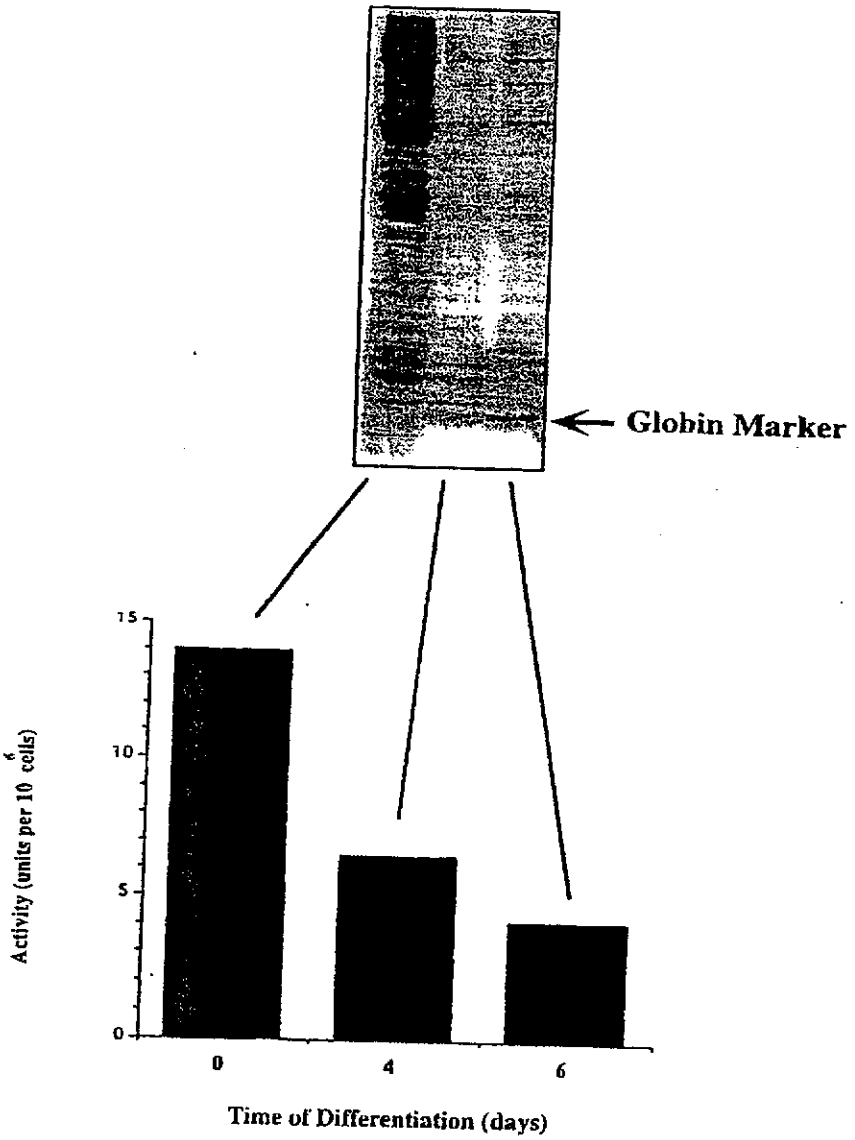

Globin Marker
15
10
5
0
Activity (units per 10 cells)
0
4
6
Time of Differentiation (days)

FIGURE 19

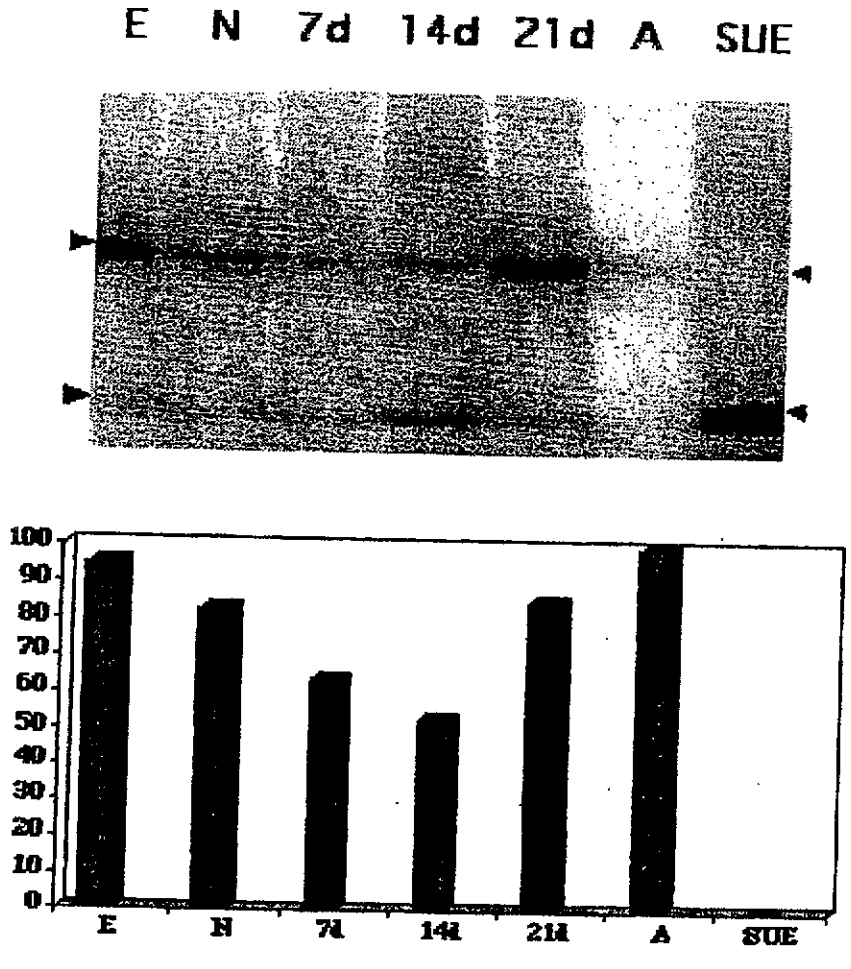

E N 7d 14d 21d A SUE
100
90
80
70
60
50
40
30
20
10
0
E
N
7d
14d
21d
A
SUE

FIGURE 20