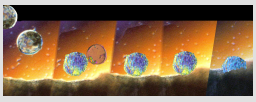
Paul Bischof UNIVERSITY OF GENEVA
Department of Obstetrics and Gynaecology

IMPLANTATION & TROPHOBLAST INVASION

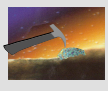
CONTENT

THE IMPLANTATION WINDOW



THE IMPLANTATION PROCESS

Apposition Adhesion Invasion



REGULATION OF INVASION

Oxygen

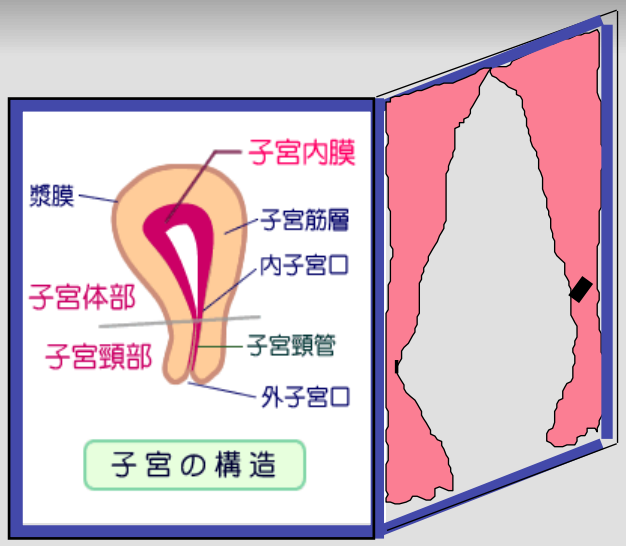
Trophoblastic factors

Endometrial factors

Oncosupressor p53

WHY STUDYING IMPLANTATION ?

THE IMPLANTATION WINDOW



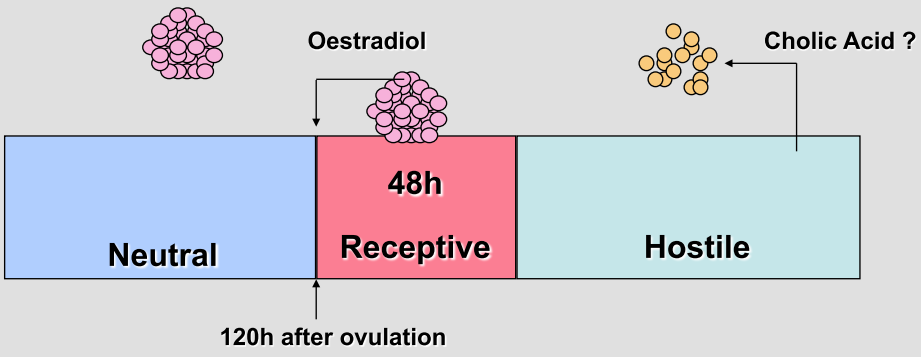
THE IMPLANTATION WINDOW

Mammalian embryos will initiate implantation-type reactions in many different non-uterine sites such as the eye (Runner 1947), the kidney (Fawcett 1950), the spleen and the testis (Kirby 1963) .

Most of the time this is not true for the endometrium because it protects itself from implantation except during a limited period known as the receptive phase.

THE MURINE IMPLANTATION WINDOW

Psychoyos 1963



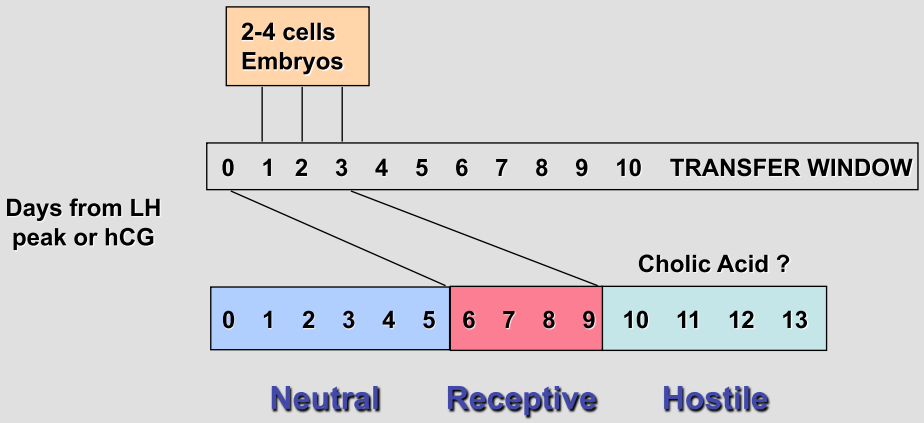
THE HUMAN IMPLANTATION WINDOW

PREGNANCY RATES PER TRANSFER AFTER IVF & ET IN STIM.,SPONT., ARTIFIC. CYCLES

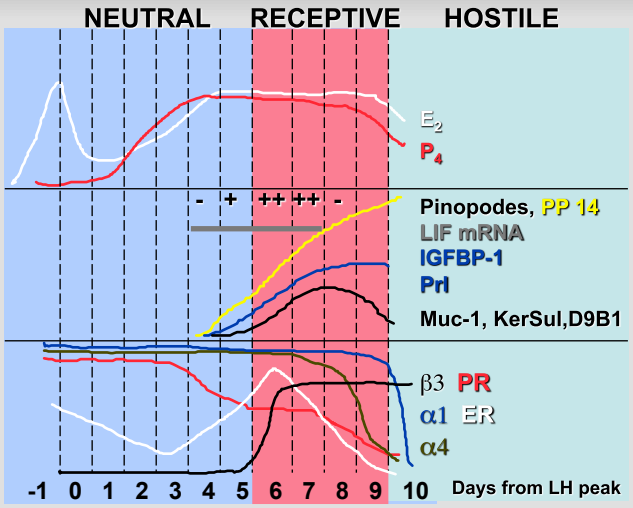
Age of the Embryo in Days

Days fom LH peak	0 Oocyte	1 Zygote	1- 2 2-4 cells	2 4-8 cells	3 8- 16 cells	4 -5 Morul.	6 -7 Blasto.
0	2 0		1 2				
1			1 7				
2		2 1	1 2	2 4			1 8
3			3 8	1 8			
4			0	1 6	3 2	3 7	
5				1 2		3 1	2 0
6				0			2 2
7							
8							
9							
10							

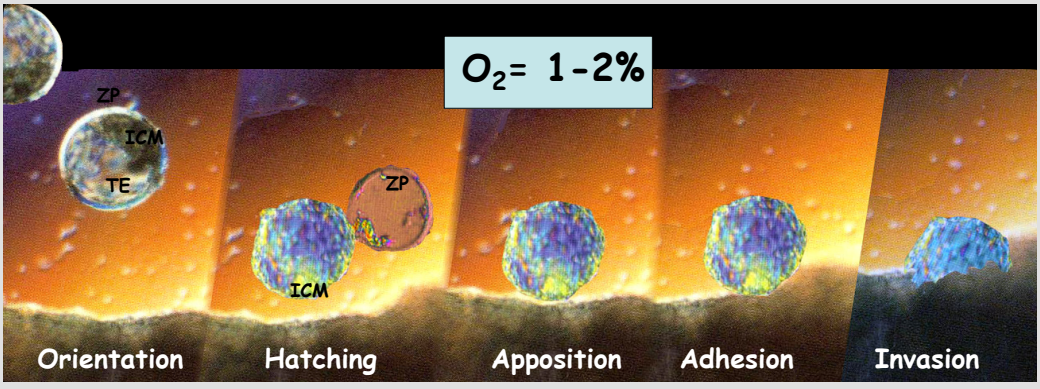
THE HUMAN IMPLANTATION WINDOW



THE HUMAN IMPLANTATION WINDOW



STEPS OF THE HUMAN IMPLANTATION PROCESS

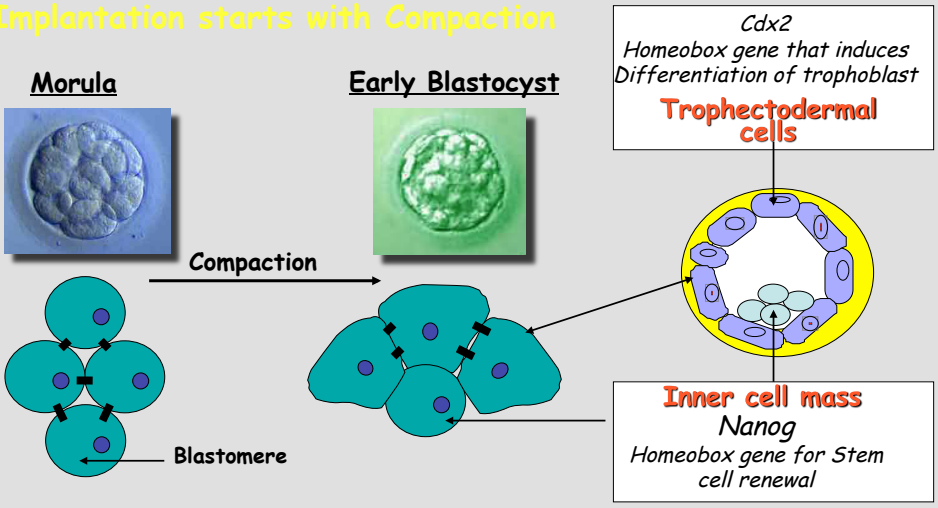


72h *132h* *180h*

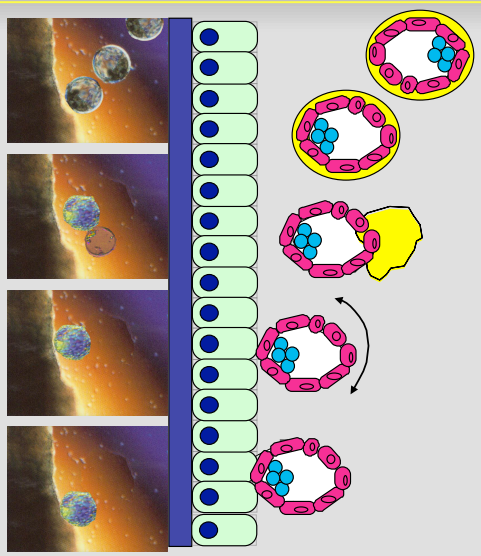
Time from ovulation

STEPS OF THE HUMAN IMPLANTATION PROCESS

Implantation starts with Compaction



STEPS OF THE HUMAN IMPLANTATION PROCESS



1. TRANSPORT The blastocyst arrives in the uterus about 72 hours after fetilisation

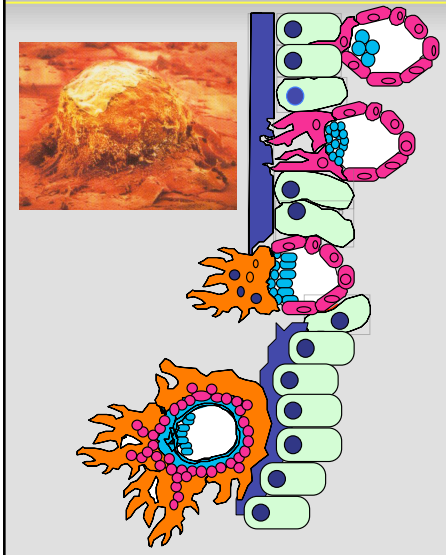
2. ORIENTATION The inner cell mass is oriented towards the endometrial epithelial lining.

3. HATCHING The zona pellucida dissolves possibly because of the secretion of proteases by trophectodermal cells.

4. APPOSITION The blastocyst is now in close contact with the endometrial lining but no connections have been established. The embryo can still be dislodged by washing.

5. ADHESION Connections of an unknown nature are established between the embryo and the endometrial epithelium. The embryo cannot be dislodged anymore.

STEPS OF THE HUMAN IMPLANTATION PROCESS



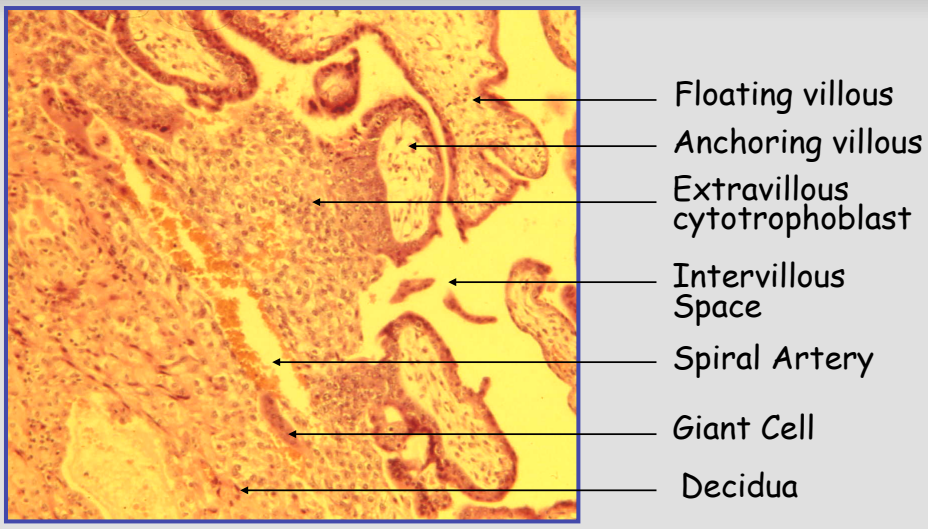
6. INVASION Thin folds of trophectodermal cells intrude between the endometrial epithelial cells.

7. DIGESTION At the tips of the invadopodia, integrins anchor the trophoblast to the basement membrane. This binding triggers the secretion of proteases which digest the basement membrane.

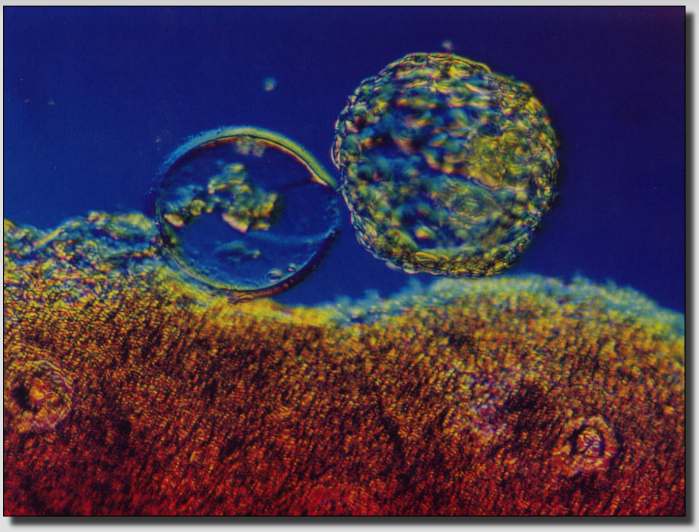
8. SYNCYTIALISATION Some trophectodermal cells fuse to form syncytia. These syncytia proliferate and invade the endometrial extracellular matrix.

9. VILLOUS FORMATION The former trophectodermal cells, now called cytotrophoblastic cells migrate between the syncytia followed by the fetal stoma. This will lead to the formation of the placental villi.

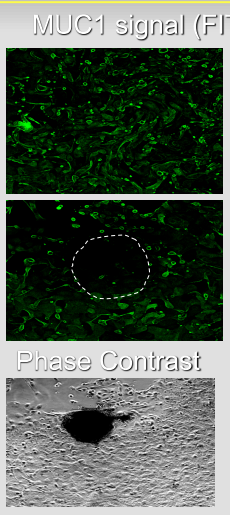
STEPS OF THE HUMAN IMPLANTATION PROCESS

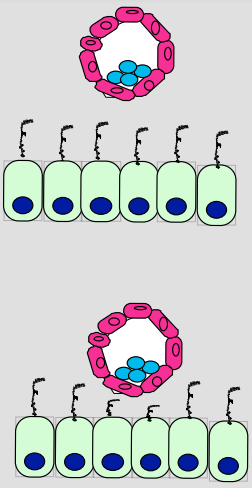


STEPS OF THE HUMAN IMPLANTATION PROCESS: Apposition



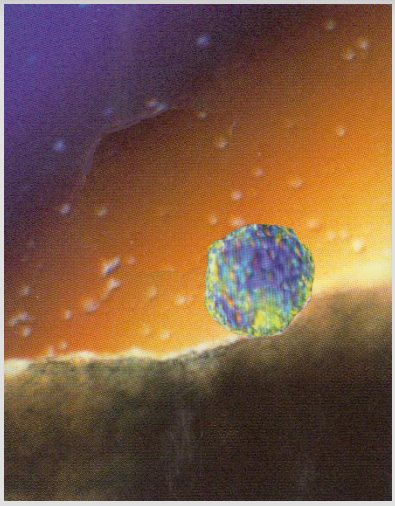
STEPS OF THE HUMAN IMPLANTATION PROCESS: Apposition





Meseguer et al. Biol Reprod, 2001

STEPS OF THE HUMAN IMPLANTATION PROCESS: Adhesion

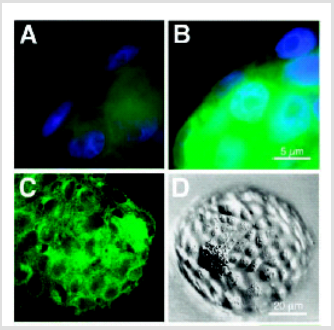


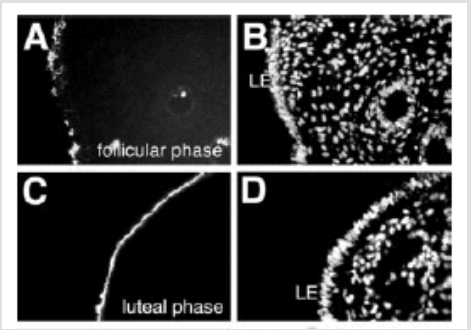
STEPS OF THE HUMAN IMPLANTATION PROCESS: Adhesion

Blastocyst **Endometrium**

L-selectin

Hoescht

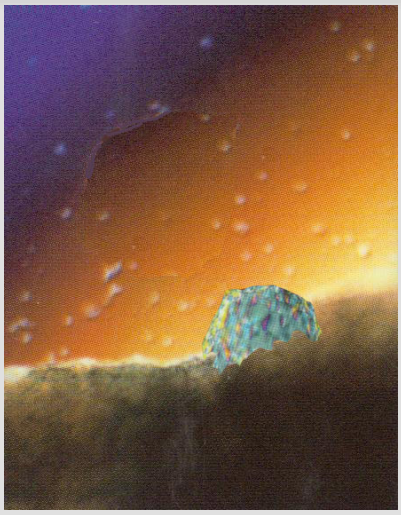




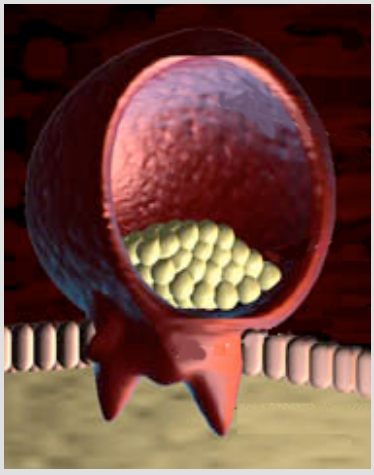
Genbacev et al, Science 2003

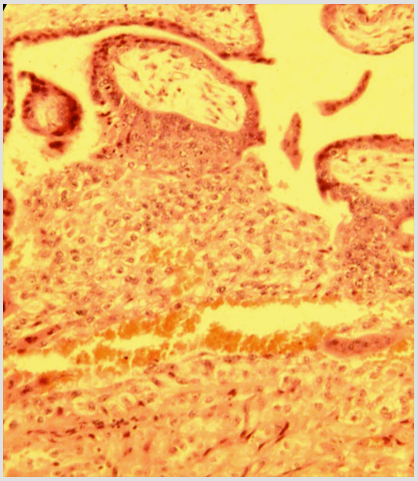
L-selectin-ligands Meca-79 Hoescht

STEPS OF THE HUMAN IMPLANTATION PROCESS: Invasion



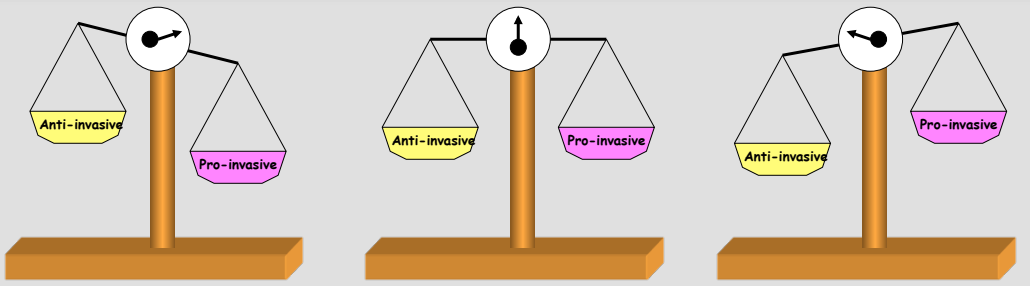
CYTOTROPHOBLAST TROPHECTODERMAL CELLS





TROPHOBLAST INVASION

TROPHOBLAST INVASION



EXCESSIVE TROPHOBLAST INVASION

Hydaditiform Moles
Choriocarcinoma

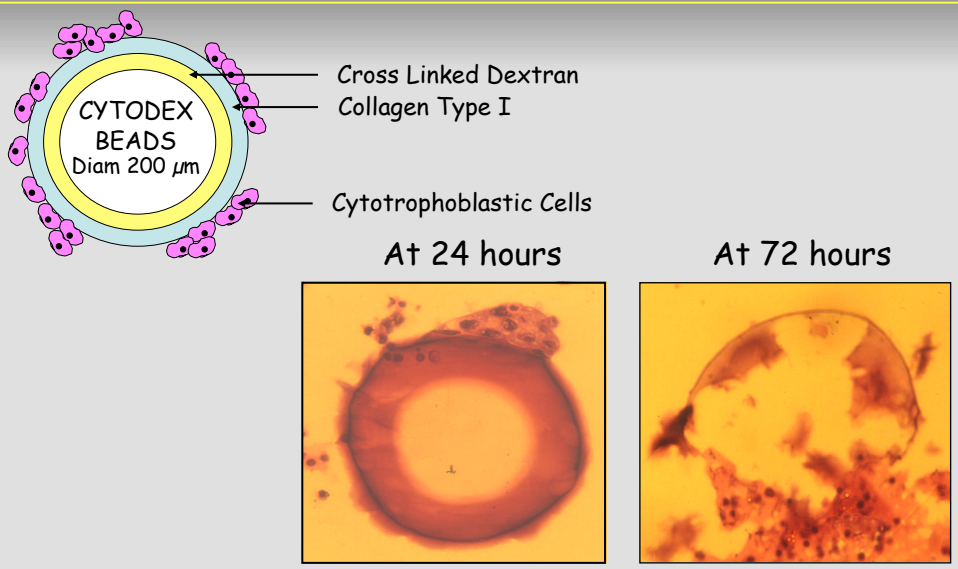
EQUILIBRATED TROPHOBLAST INVASION

Normal Pregnancy

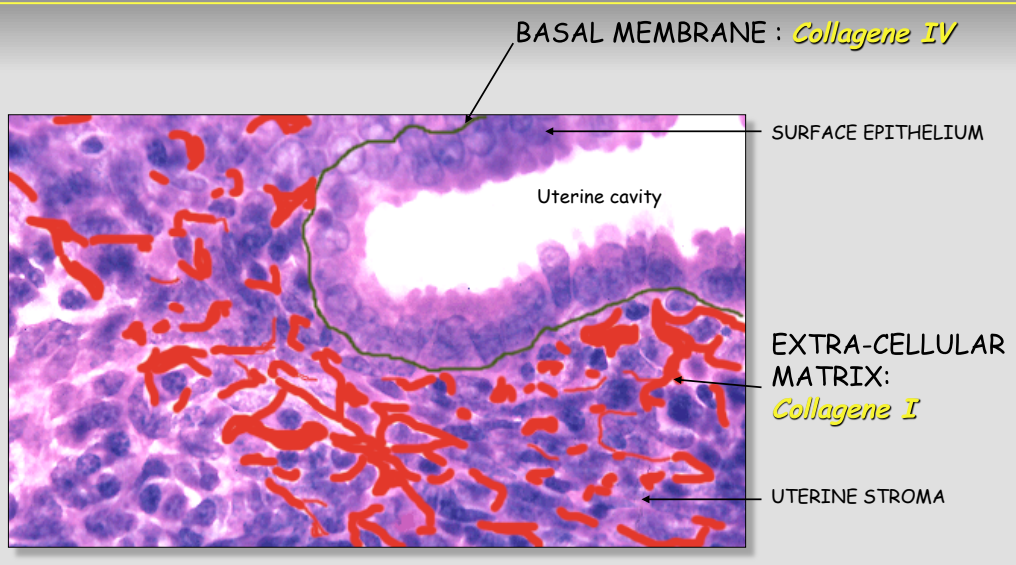
INSUFFICIENT TROPHOBLAST INVASION

Preeclampsia
Early Abortions

TROPHOBLAST INVASION



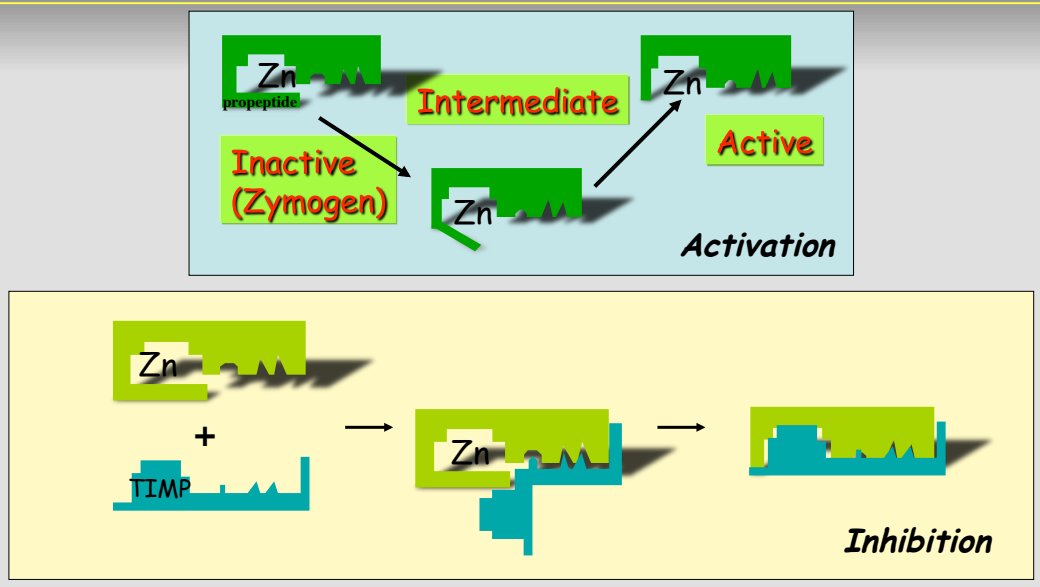
TROPHOBLAST INVASION



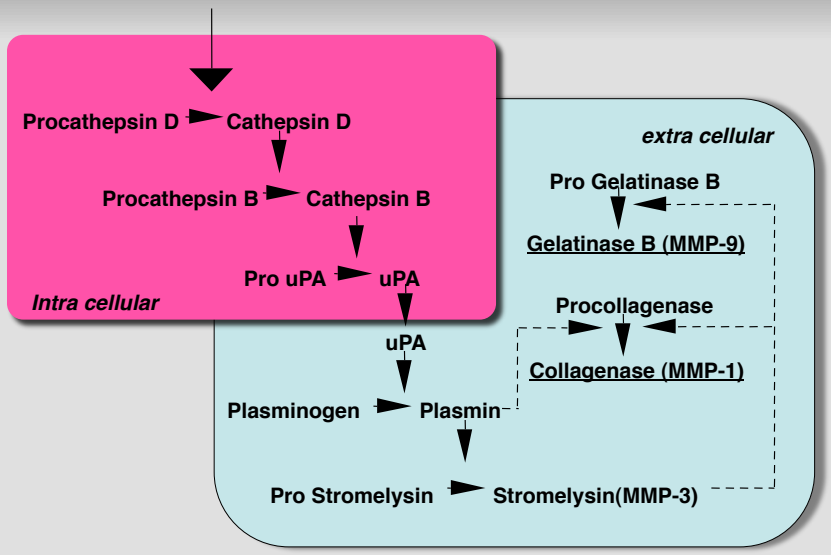
TROPHOBLAST INVASION

Subfamily	MMP	Other names	Substrates
Collage-nases	MMP-1	Interstitial Collagenase	Col I,II,III,VII,X, Gelatins,
	MMP-8		Col I, II, III,
	MMP-13	Collagenase-3	Col I, II, III
Gelatin-ases	MMP-2	Gelatinase A 72 KDa gelatinase	Col I, IV, V, VII, X, XI, Gelatins, Fibronectin, Laminin Gelatins, Col IV, V, XIV, Aggrecan.
	MMP-9	Gelatinase B 92 KDa gelatinase	Elastin, Entactin, Vitronectin
Stromely-sins	MMP-3	Stromelysin-1	Aggrecan, Col III, IV, IX, X, Gelatins,
	MMP-7	Matrilysin, PUMP-1	Fibronectin, Laminin, Tenascin-C,
		Stromelysin-2	Vitronectin, Elastin, Casein
	MMP-10	Transin-2	Aggrecan, IGFBP-1
	MMP-11	Stromelysin-3	Entactin, Small tenascin-C,
	MMP-12	Metallo-elastase	Vitronectin, Casein
Mem-brane-type MMPs	MMP-14	MT1-MMP, MMP-X1	Activates proMMP-2
	MMP-15	MT2-MMP	Col I, II, III, Fibronectin, Laminin-1,
	MMP-16	MT3-MMP	Vitronectin, Dermatan sulfate
	MMP-17	MT4-MMP	proteoglycan : activates proMMP-2,
	MMP-24	MT5-MMP	proMMP-13
	MMP-25	MT6-MMP	
Others	MMP-19	Matrix metalloprotein-ase	Aggrecan
	MMP-20	Enamelysin	amelogenin
	MMP-23	MIFR/FEMAL-YSIN	Gelatin, Beta-Casein
	MMP-26	Matrilysin-2	fibronectin

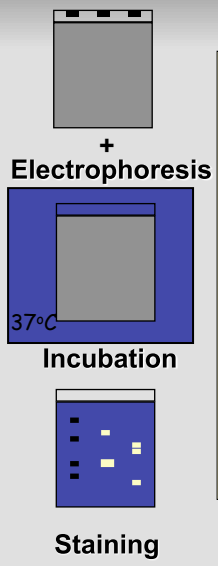
TROPHOBLAST INVASION



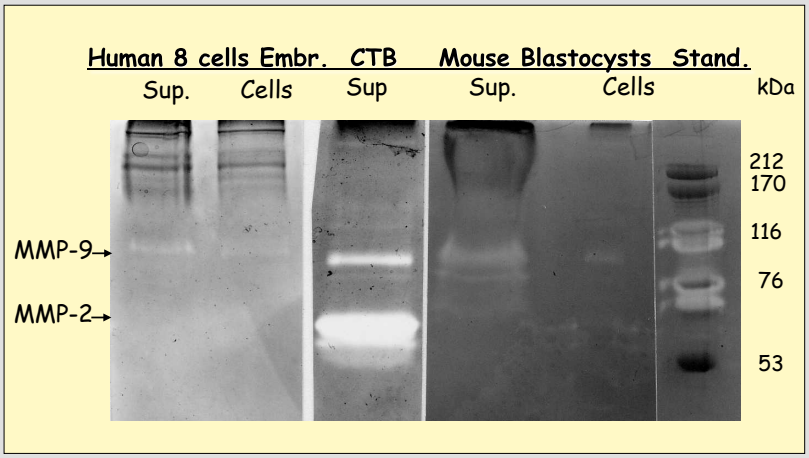
TROPHOBLAST INVASION



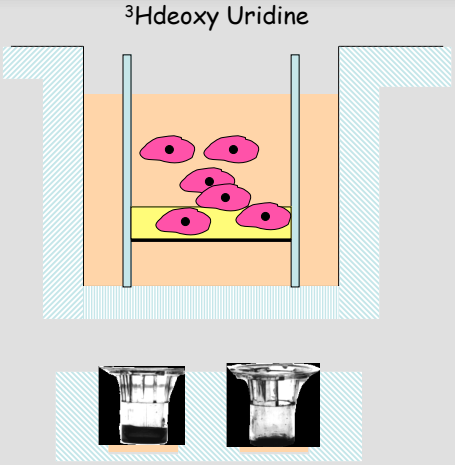
TROPHOBLAST INVASION

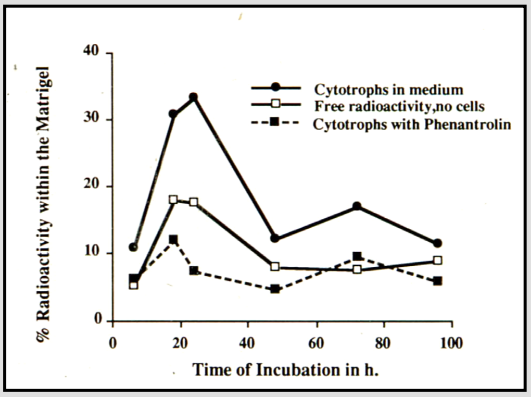


ZYMOGRAPHY

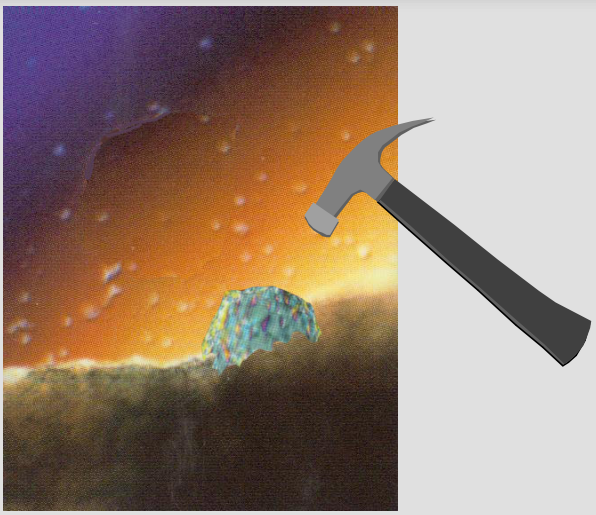


TROPHOBLAST INVASION



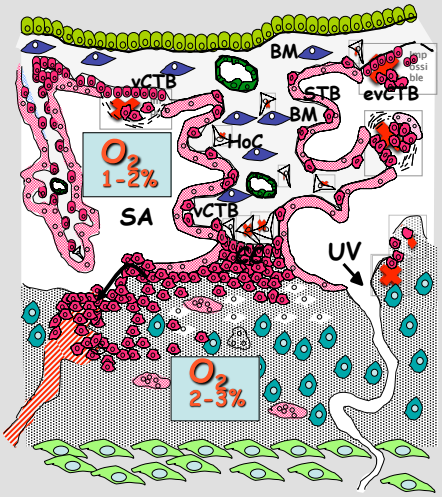


REGULATION OF TROPHOBLAST INVASION

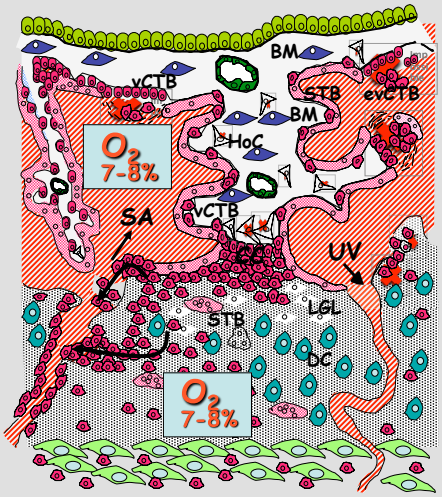


REGULATION OF TROPHOBLAST INVASION: OXYGEN

Before 10-12 weeks



After 10-12 weeks



REGULATION OF TROPHOBLAST INVASION: OXYGEN

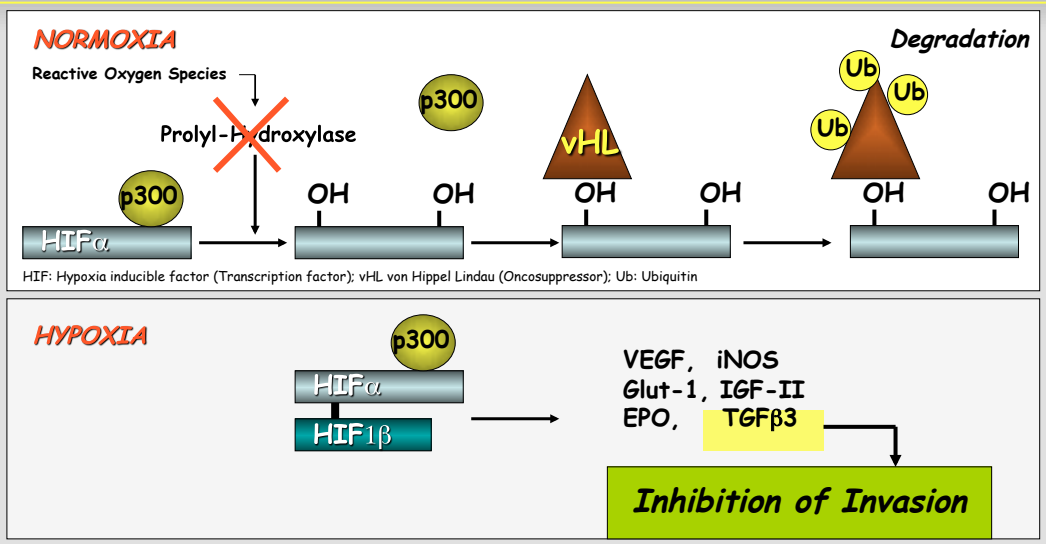
HYPOXIA

Trophoblast Proliferates
Inhibition of Invasion
Decrease in uPA activity
Increase in PAI-2

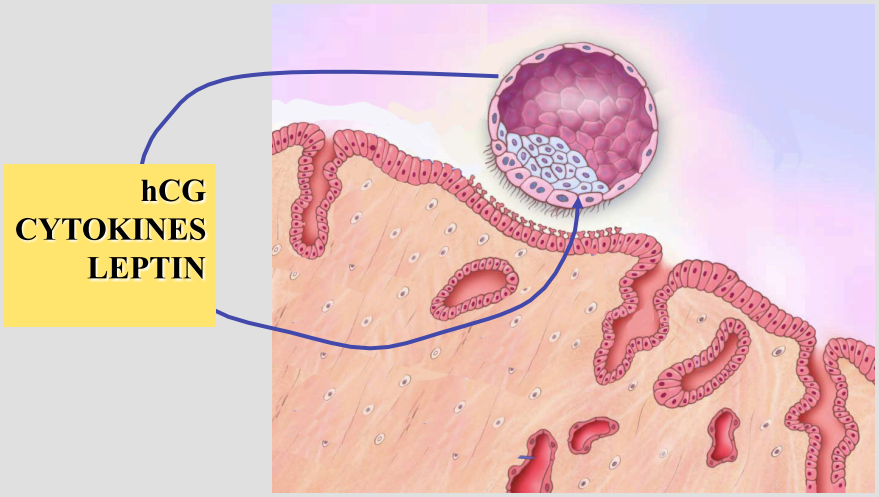
NORMOXIA

Trophoblast Invades
Inhibition of Proliferation
Increase in uPA activity
Decrease in PAI-2

REGULATION OF TROPHOBLAST INVASION: OXYGEN



REGULATION OF TROPHOBLAST INVASION: TROPHOBLASTIC FACTORS



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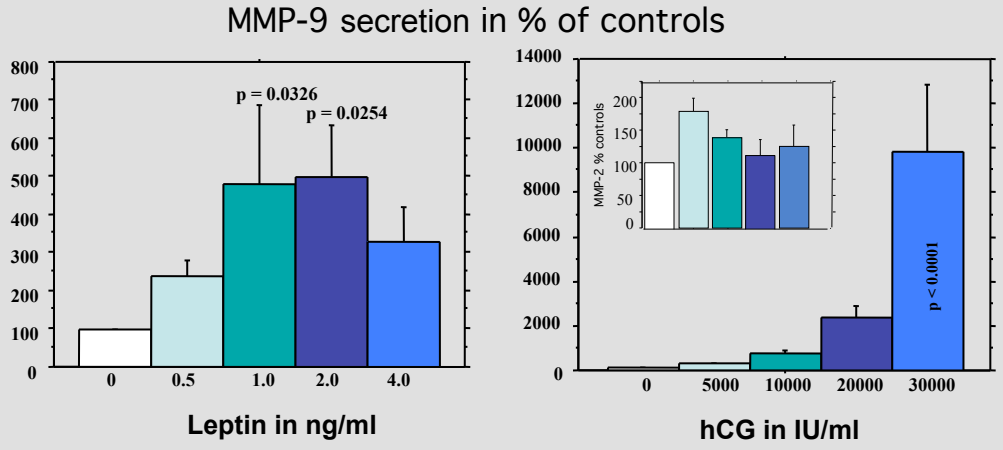
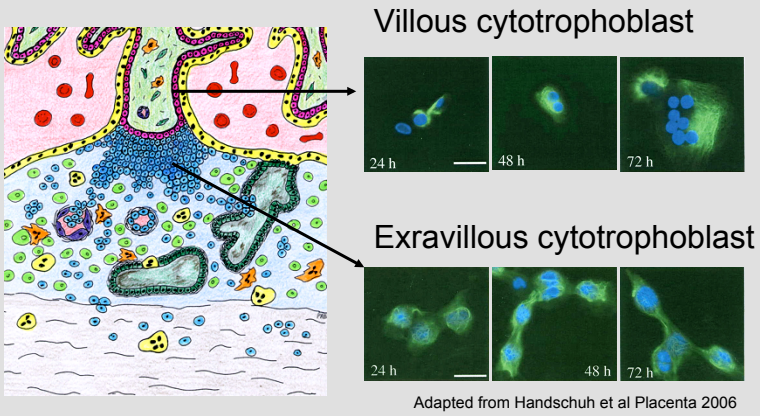


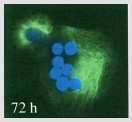
REGULATION OF TROPHOBLAST INVASION: hCG



Adapted from Handschuh et al Placenta 2006

REGULATION OF TROPHOBLAST INVASION: hCG

Villous cytotrophoblast



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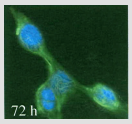
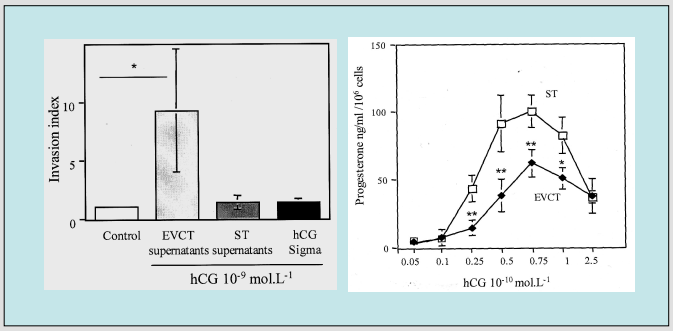


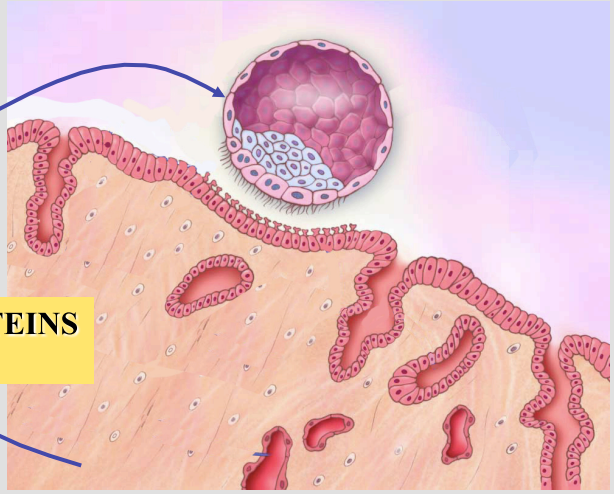
EVCT

Exravillous cytotrophoblast



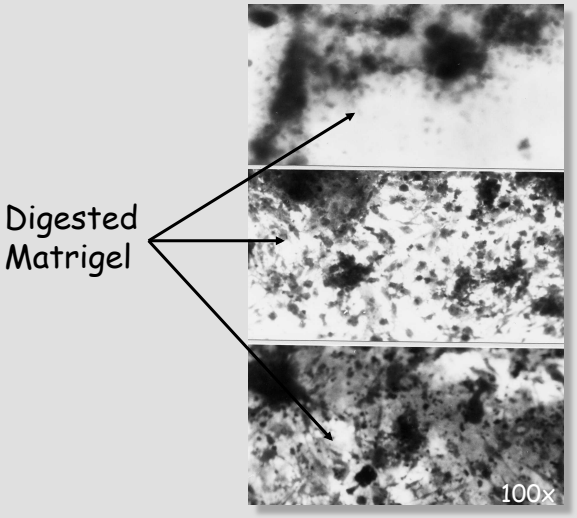
Adapted from Handschuh et al Placenta 2006

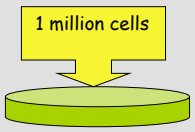
REGULATION OF TROPHOBLAST INVASION: ENDOMETRIAL ECM FACTORS



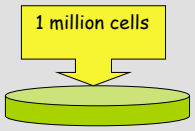
MATRIX- GLYCOPROTEINS
INTEGRINS

THE ROLE OF ECM

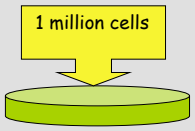




Undiluted ECM

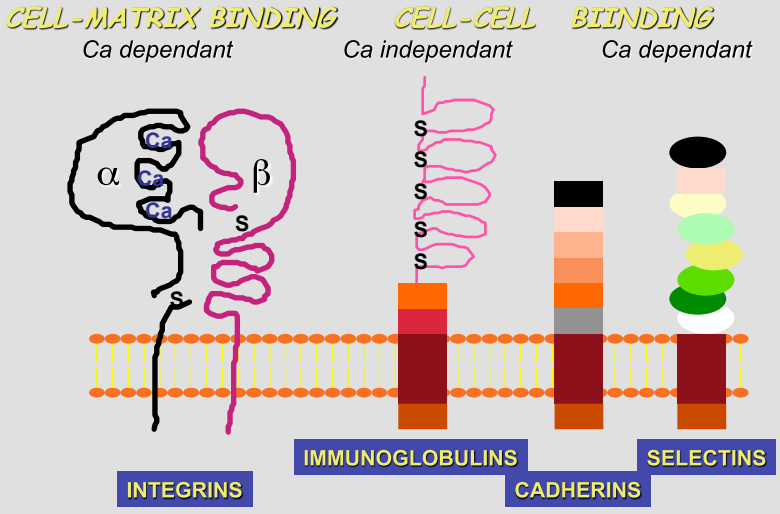


1/5 diluted ECM

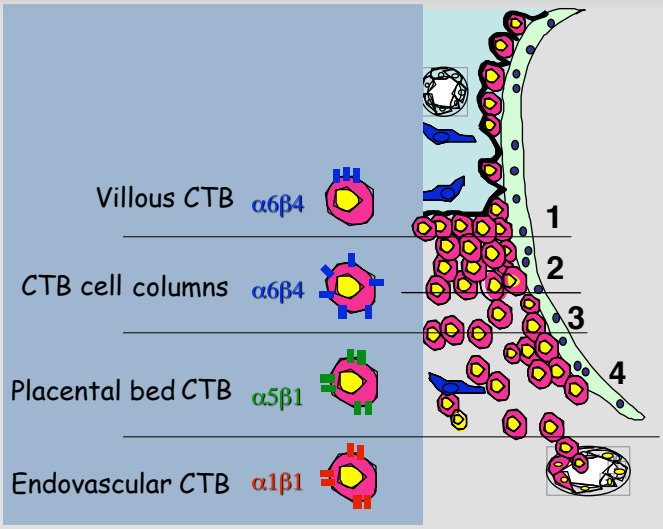


1/10 diluted ECM

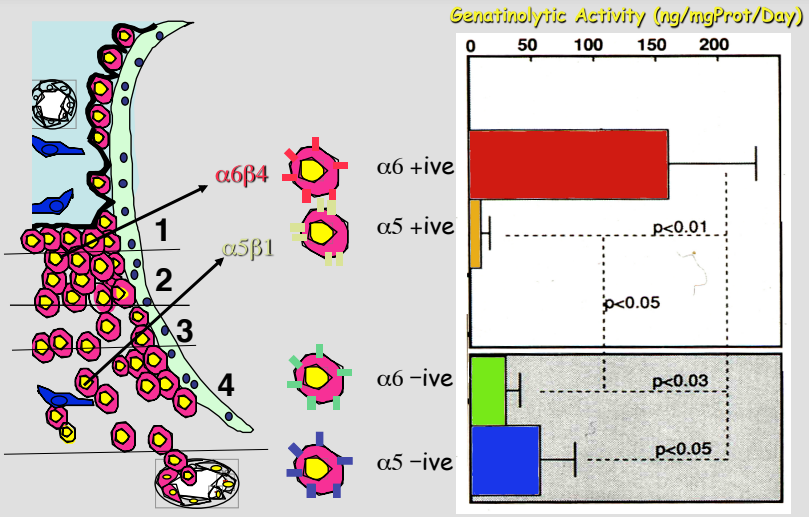
ADHESION MOLECULES



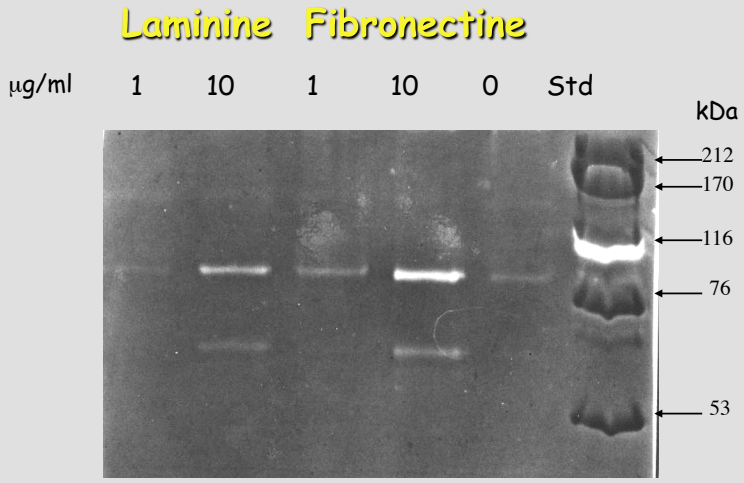
INTEGRINS OF THE TROPHOBLAST



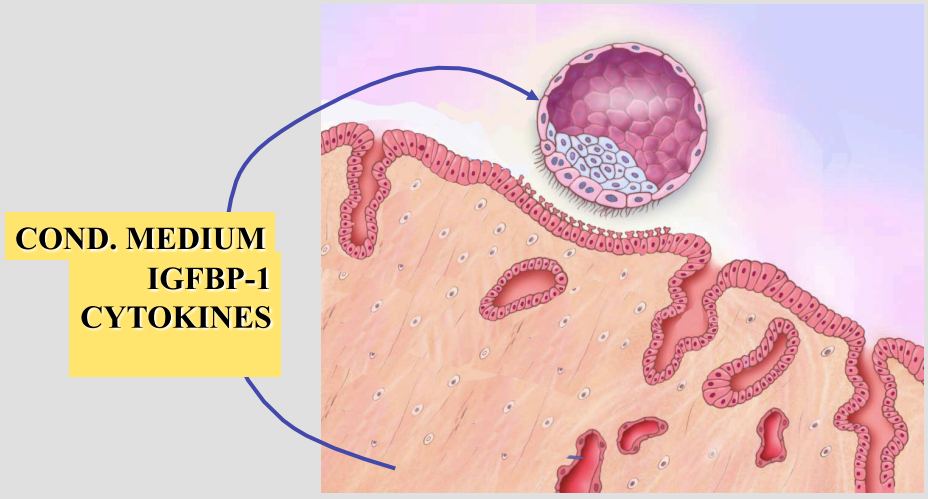
TROPHOBLASTIC INTEGRINS



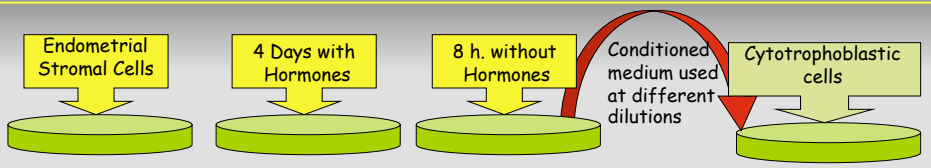
ECM GLYCOPROTEINS MODULATE MMPs

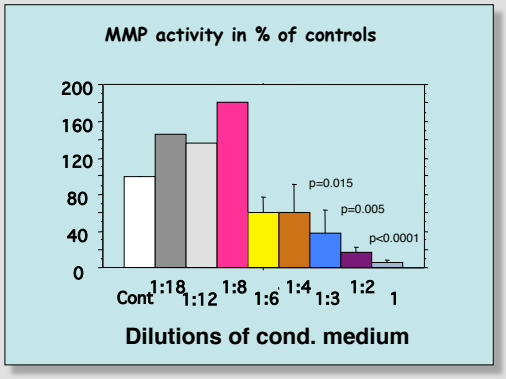


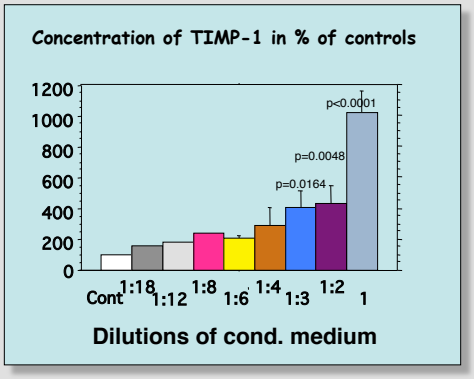
REGULATION OF TROPHOBLAST INVASION: SOLUBLE ENDOMETRIAL FACTORS



EFFECT OF ENDOMETRIAL FACTORS







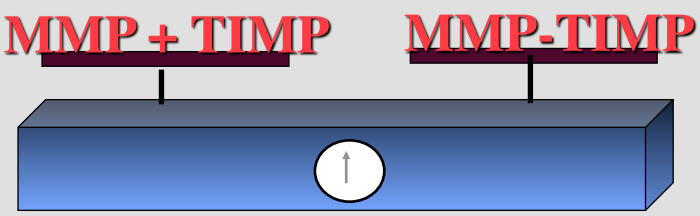
SOME ENDOMETRIAL CYTOKINES

LIF	EEC			
TNFα	EEC	ESC	Macro	
IL-1			Macro	Endoth
TGFβ	EEC	ESC	Macro	
MCSF	EEC		Macro	Endoth

REGULATORS OF MMP ACTIVITY

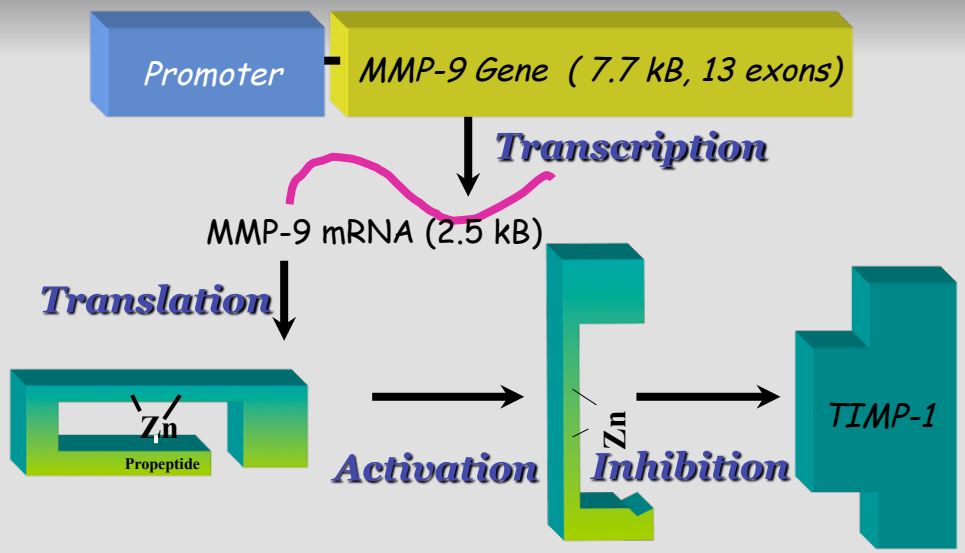
IGFBP-1
TNFα
IL-1β, IL-6
hCG
Leptin
Matrix proteins
Prostaglandins

TGFß
LIF
Steroids
Protease Inhibitors
Retinoic acid
EPO
P53



DOES P53 REGULATE MMP-9 EXPRESSION?

REGULATION OF MMP-9 EXPRESSION

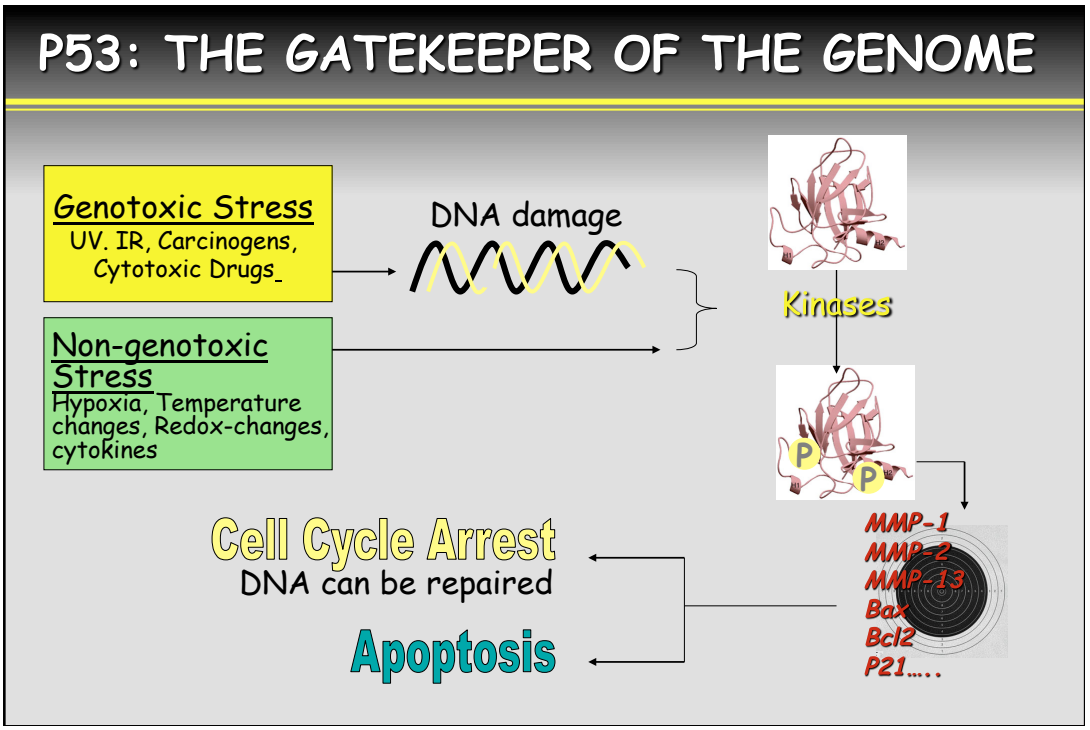


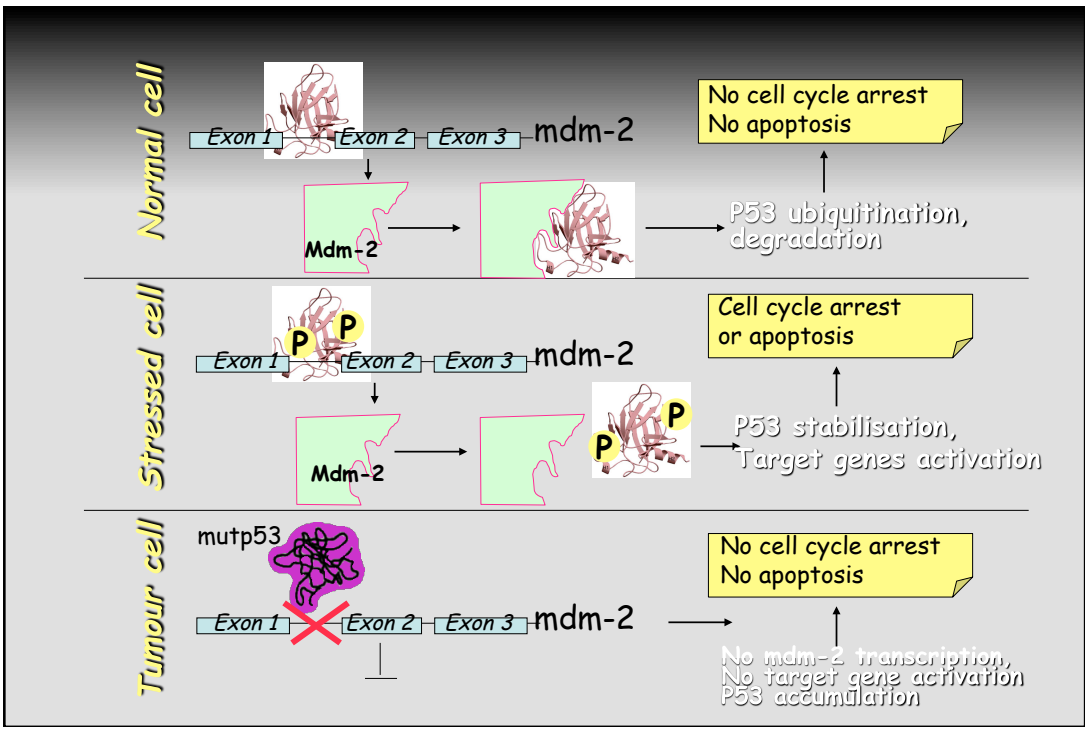
P53: THE DEFENDER OF THE GENOME

THE TUMOUR-SUPPRESSOR P53 IS DYSFUNCTIONAL IN MOST HUMAN CANCER !

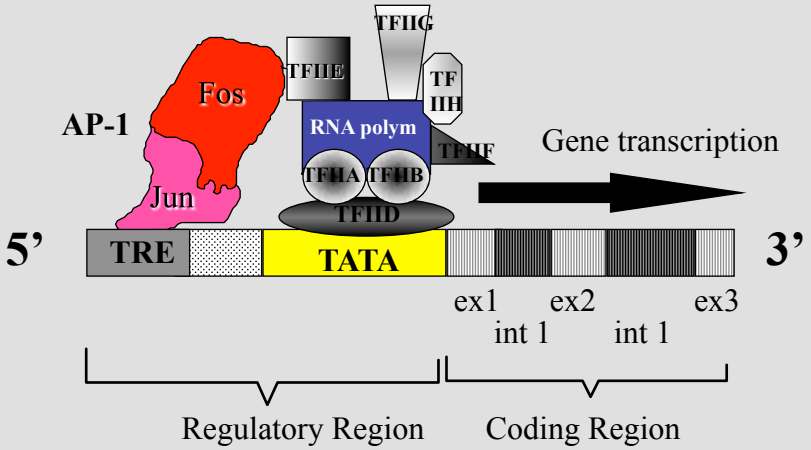
wtp53 is a transcription factor that avoids DNA damage to be carried-over to daughter cells. It induces cell cycle arrest or apoptosis. It is thus **anti-oncogenic**

mutp53 has lost this property and is **oncogenic.** Transfection of mutp53 in normal cells induces an invasive phenotype

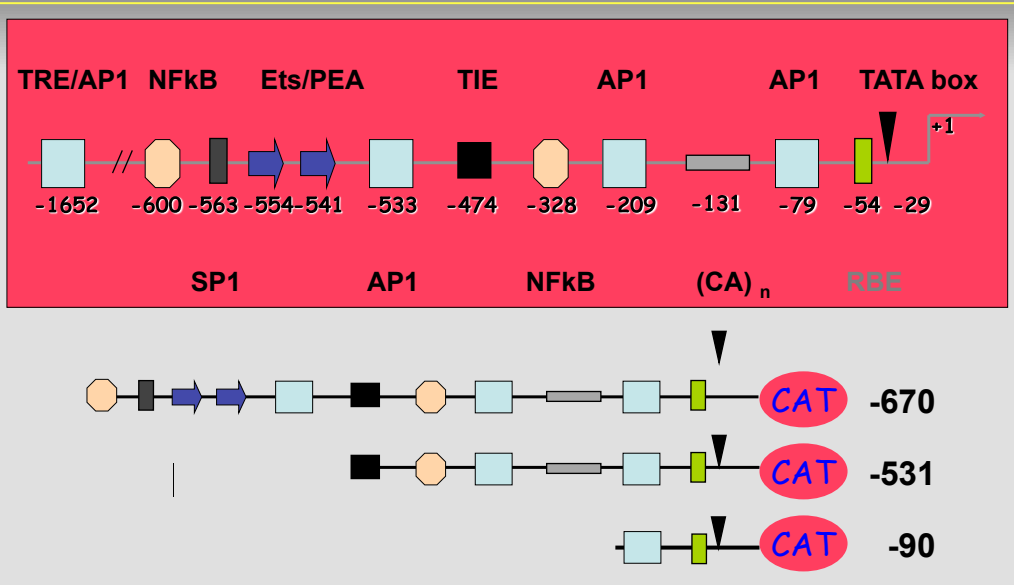

P53: THE GATEKEEPER OF THE GENOME
Genotoxic Stress
UV. IR, Carcinogens, Cytotoxic Drugs_
Non-genotoxic Stress
Hypoxia, Temperature changes, Redox-changes, cytokines
DNA damage
Kinases
P
P
MMP-1
MMP-2
MMP-13
Bax
Bcl2
P21…..
Cell Cycle Arrest
DNA can be repaired
Apoptosis


Normal cell
Exon 1
Exon 2
Exon 3
mdm-2
Mdm-2
No cell cycle arrest
No apoptosis
P53 ubiquitination, degradation
Stressed cell
P
P
Exon 1
Exon 2
Exon 3
mdm-2
Mdm-2
P
P
Cell cycle arrest or apoptosis
P53 stabilisation, Target genes activation
Tumour cell
mutp53
Exon 1
Exon 2
Exon 3
mdm-2
No cell cycle arrest
No apoptosis
No mdm-2 transcription, No target gene activation P53 accumulation

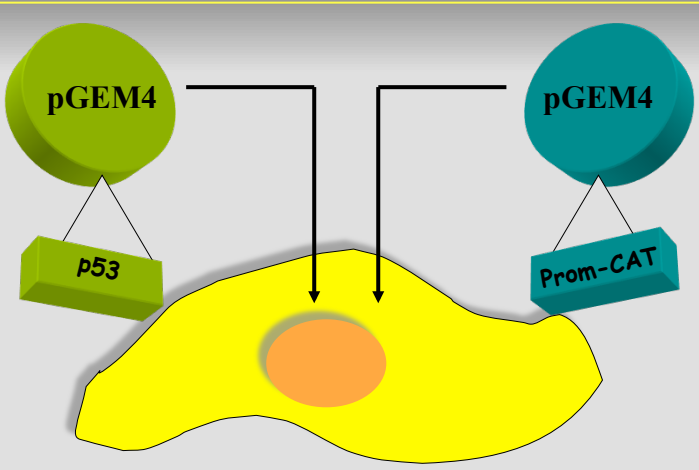
TRANS-REGULATORS OF GENE EXPRESSION



REGULATORY REGION OF MMP-9 GENE

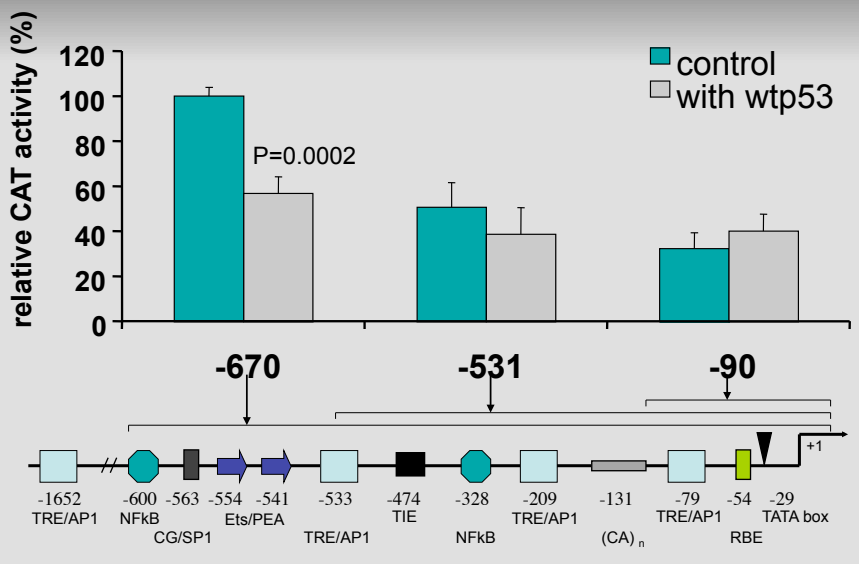


TRANSFECTION OF CELLS

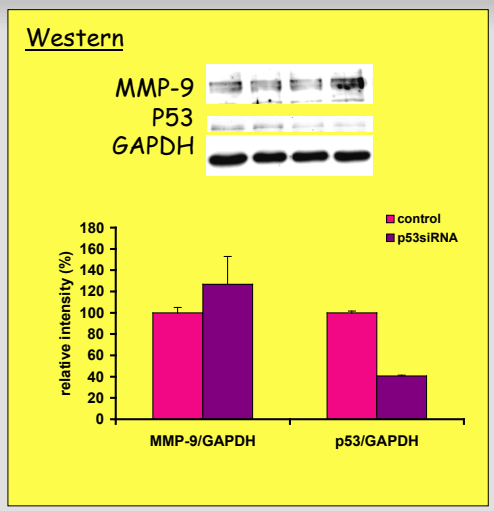


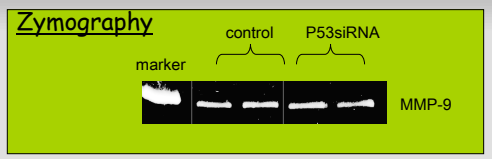
Activitiy of Chloramphenicol acetyl transferase

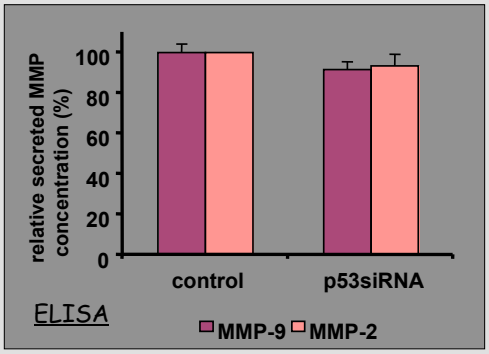
COTRANSFECTION OF -670MMP-9-CAT WITH wtP53 IN CTB



EFFECTS OF p53 siRNA







QUESTIONS

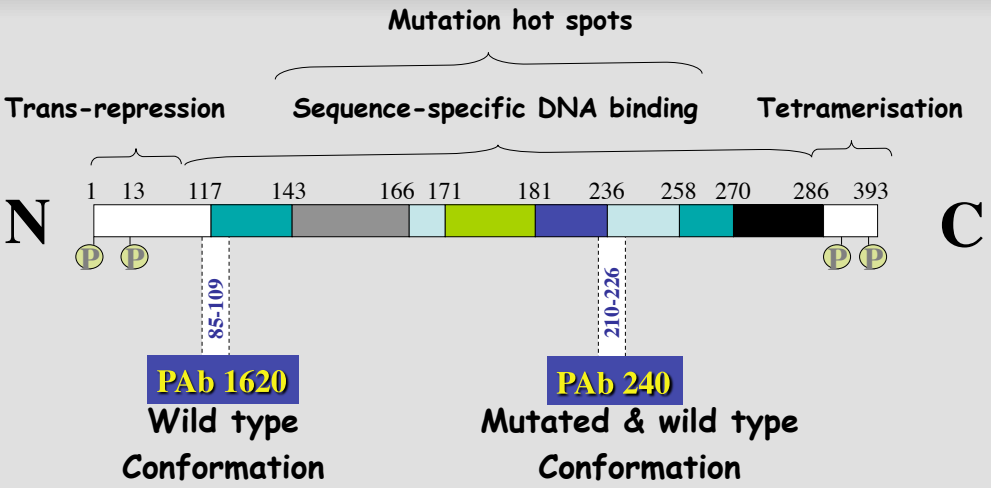
We have shown that:

- MMP-9 is a target gene for p53 trans-repression
- P53 trans-repression occurs through interaction with other TF: NFkB, ets.
- Trophoblastic p53 does not inhibit MMP-9.

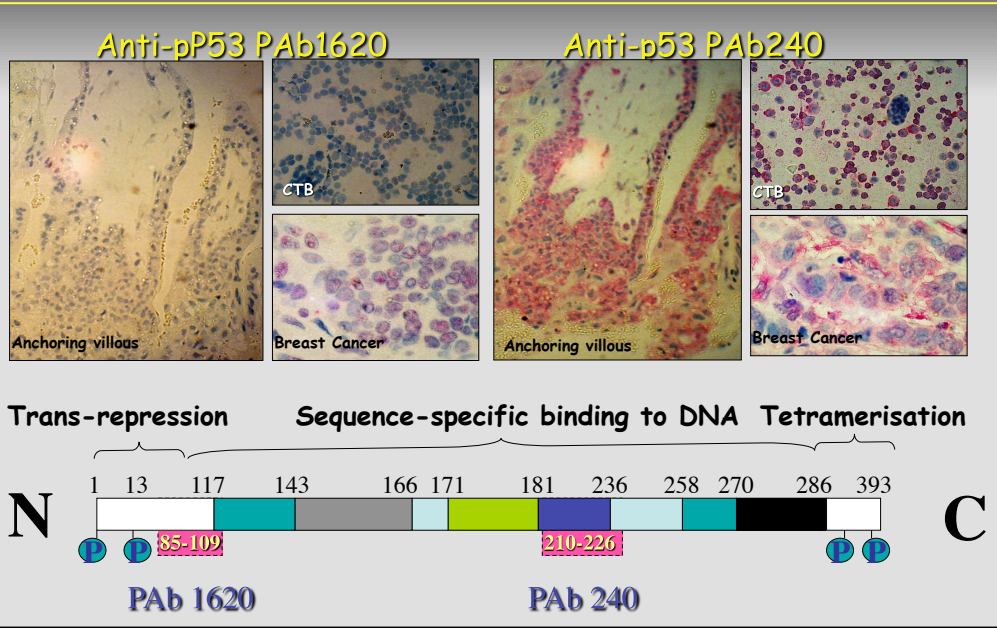
THUS:

Either p53 is not expressed in the human trophoblast
or
Trophoblastic p53 is functionally incompetent.

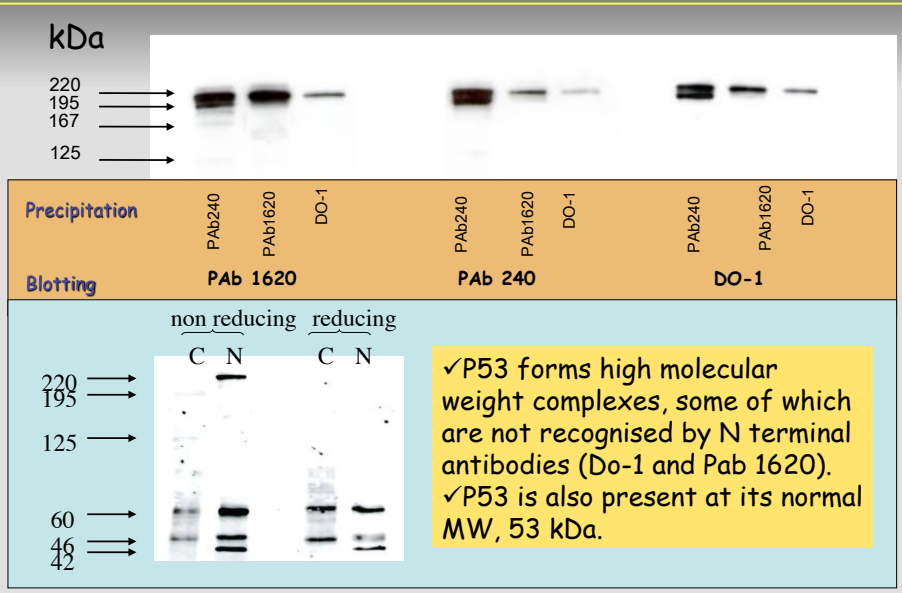
THE HUMAN P53 PROTEIN



IMMUNOLOCALISATION DE P53

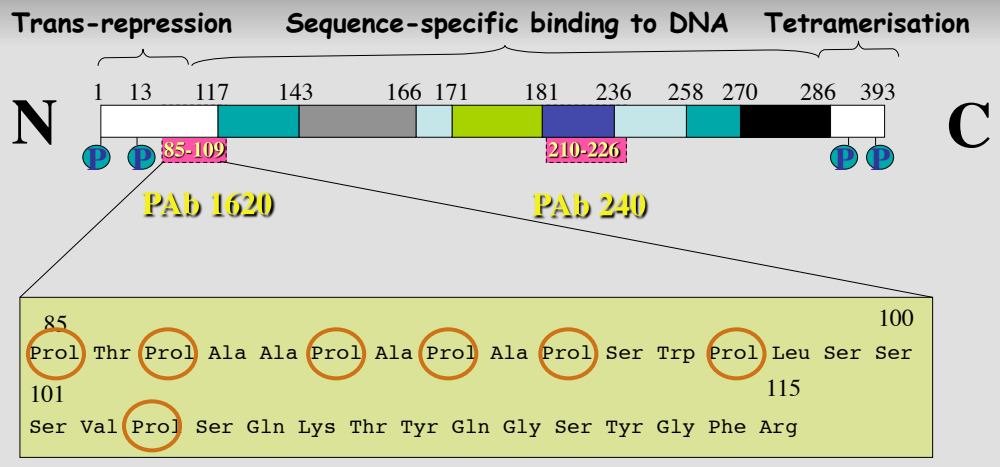


TROPHOBLASTIC P53 AS HMWC

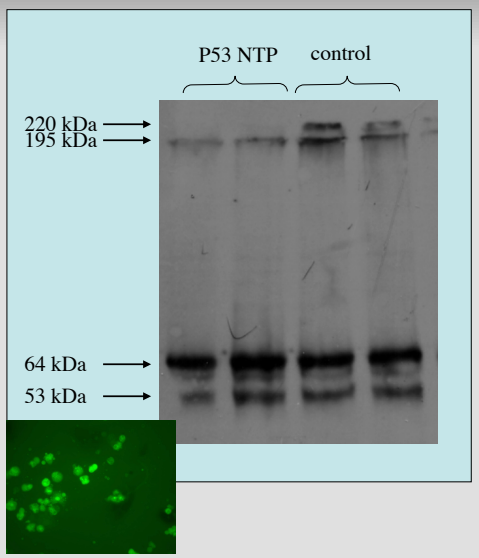


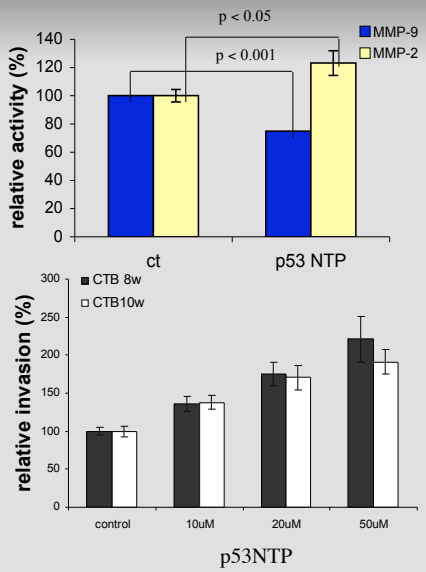
✓P53 forms high molecular weight complexes, some of which are not recognised by N terminal antibodies (Do-1 and Pab 1620).
✓P53 is also present at its normal MW, 53 kDa.

AMINO ACID SEQUENCE OF p53 N TERMINUS



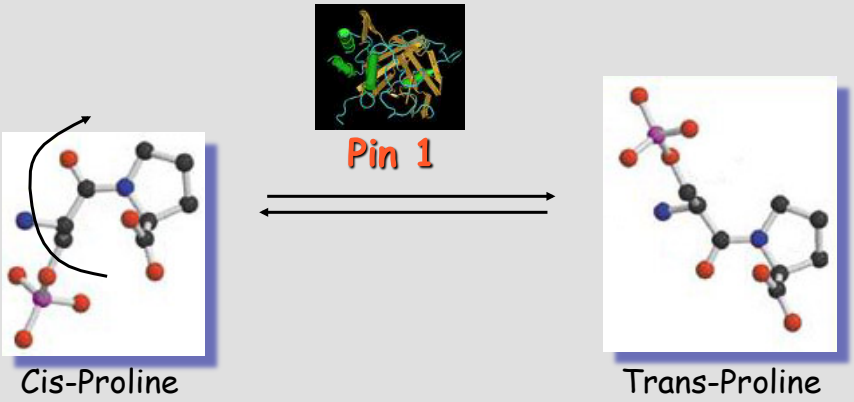
EFFECTS OF AN N TERMINAL PEPTIDE



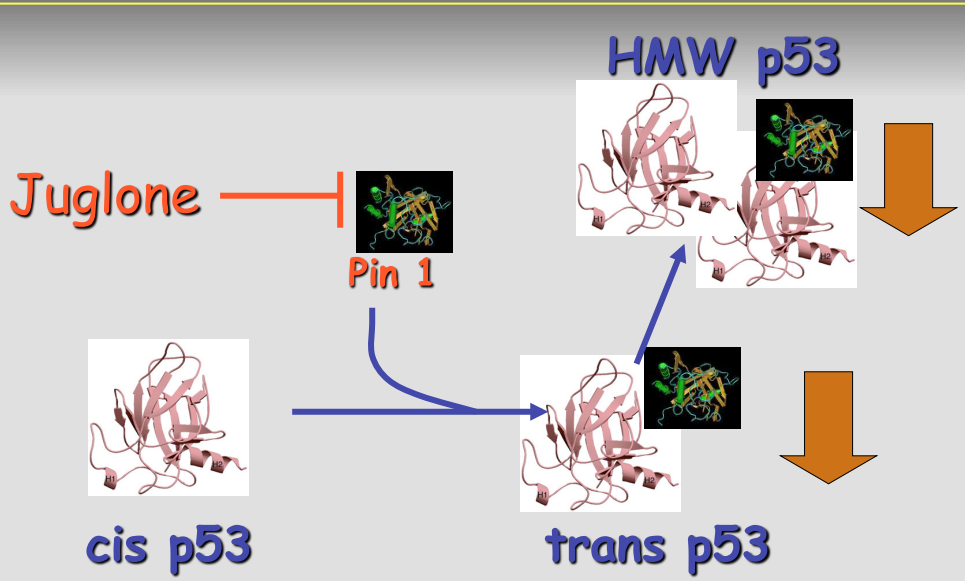


PEPTIDYL-PROLYL CIS/TRANS ISOMERASE (Pin1)

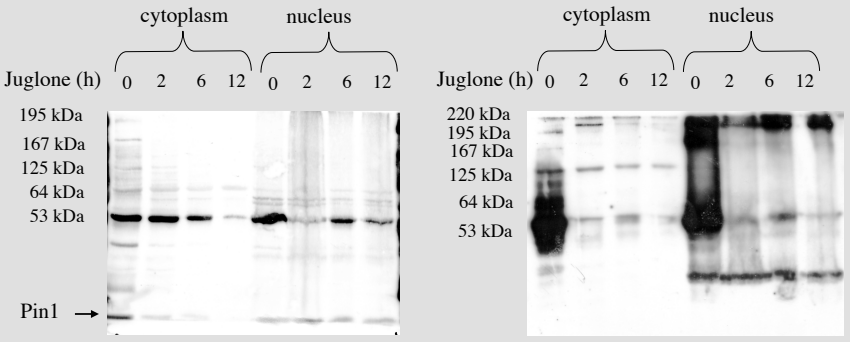
Pin1 is a human peptidyl-prolyl isomerase that recognises a specific motif of a phosphorylated Ser or Thr residue preceding a Pro residue.



PEPTIDYL-PROLYL CIS/TRANS ISOMERASE (Pin1)



PEPTIDYL-PROLYL CIS/TRANS ISOMERASE (Pin1)



blot Pin1 blot p53

CONCLUSIONS

øAn invalid p53 pathway is instrumental to the invasive behaviour of most human tumours.

øThe same seems to be true for trophoblast invasion.

øTrophoblastic p53 is functionally incompetent towards some of its target genes(MMP-2, 9, Bax).

øTrophoblastic p53 is inactive because of a pin-1 induced conformational change and the formation of HMWC