PROTEOGLYKANY

Aggrecan

Legend:
- core protein
- chondroitin sulfate chain
- keratan sulfate chain

HABR | GAG | EGF | LEC | C
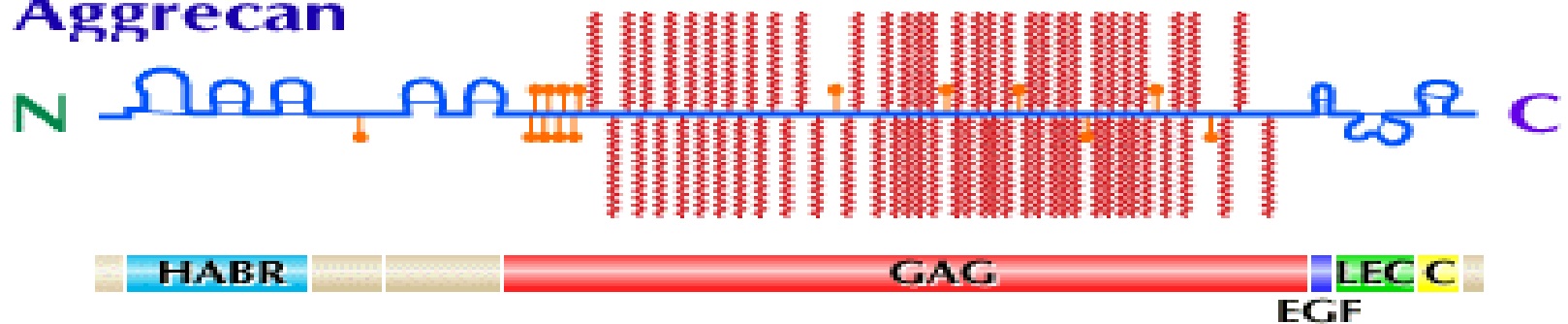
Glykokonjugaty vyskytující se volně v ECM, navázané na buněčném povrchu a v intracelulárních váčcích.

- Proteinové jádro (serin – glycin): OH skupina serinu váže xylosu
- Trisacharidová spojka: xylosa – galaktosa – galaktosa
- Glykosaminoglykanový řetězec: nevětvený, vysokomolekulární, složený z disacharidových jednotek
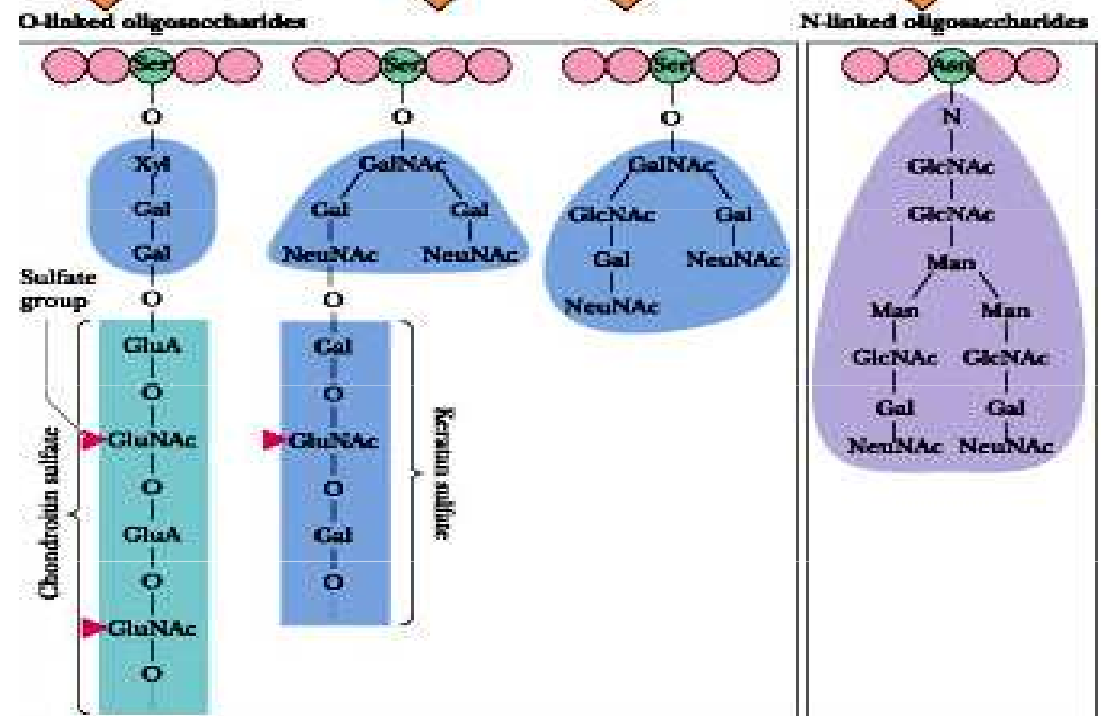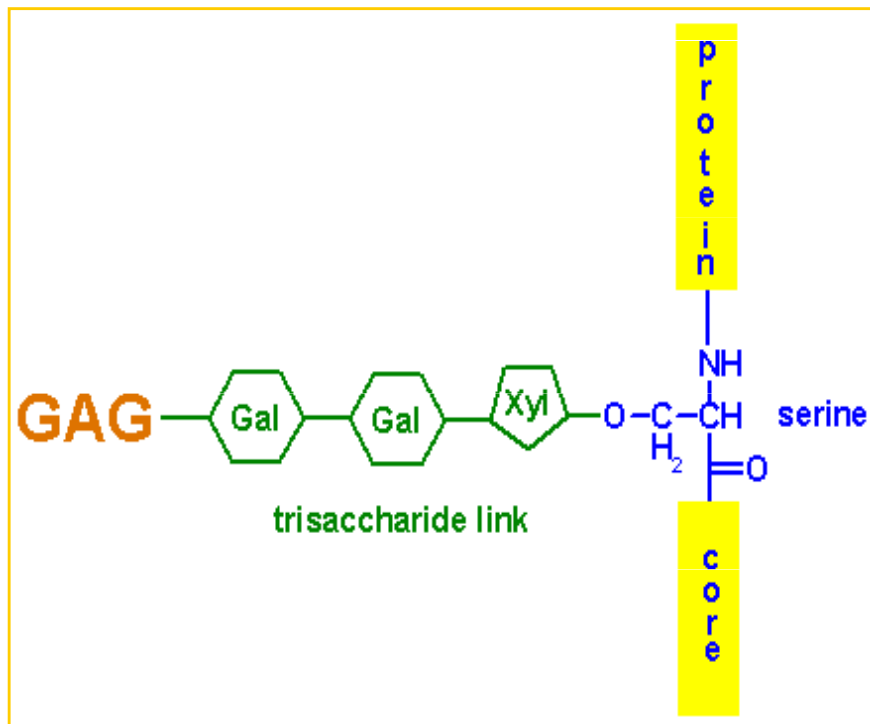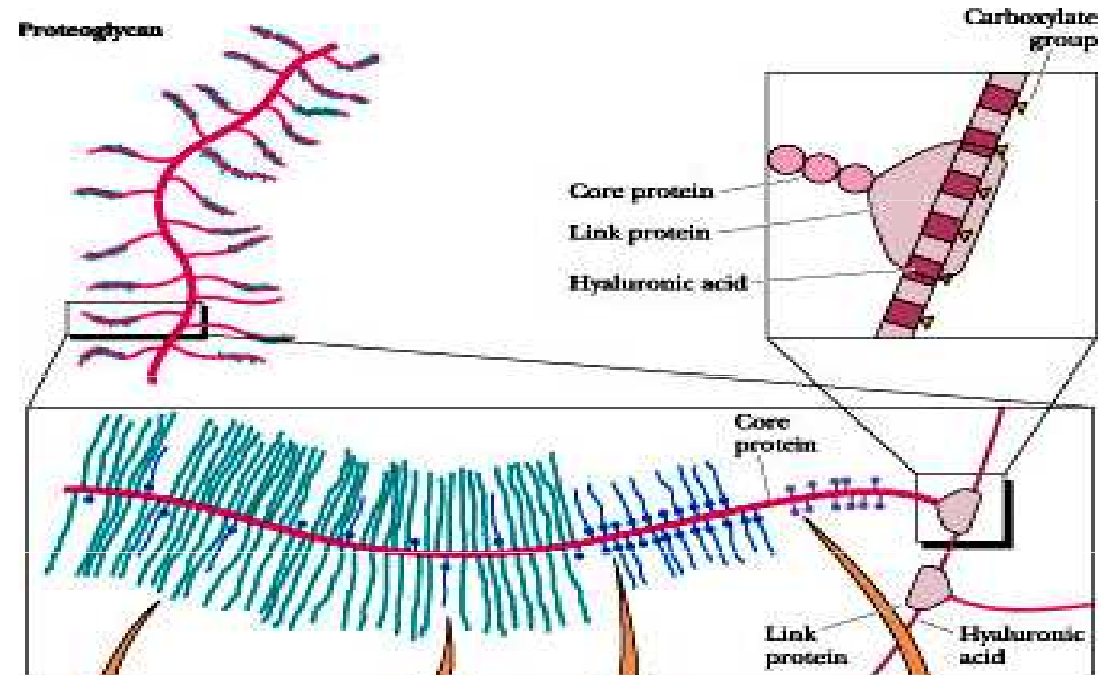
BIOSYNTÉZA HEPARAN SULFÁT PROTEOGLYKANŮ

Třístupňový proces probíhající v Golgiho aparátu:

I.   Iniciace: připojení trisacharidového linku k OH skupině serinu (Xyl transferáza, Gal transferáza I-II) a následné připojení první glukuronové kyseliny (GlcA transferáza)

II.   Polymerace: zahrnuje postupné přidávání N-acetyl glukosaminu a glukuronové kyseliny. Vzniklý řetězec obsahuje 50 – 150 disacharidových jednotek.
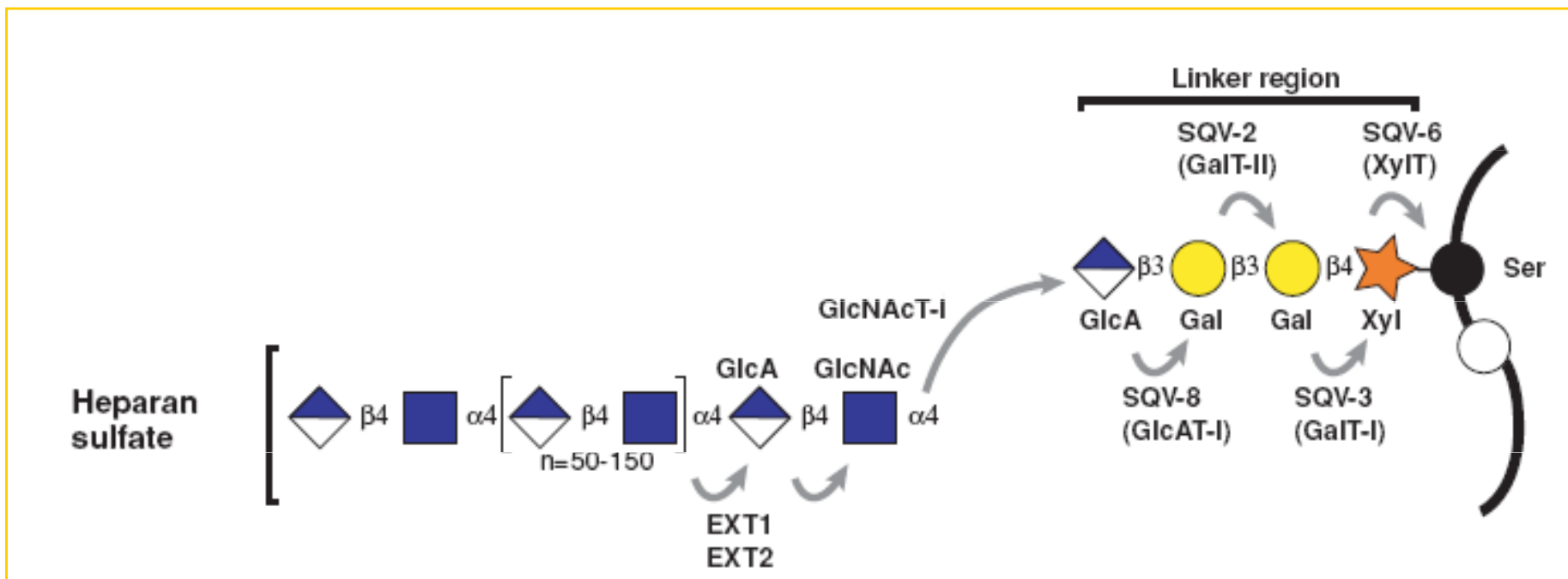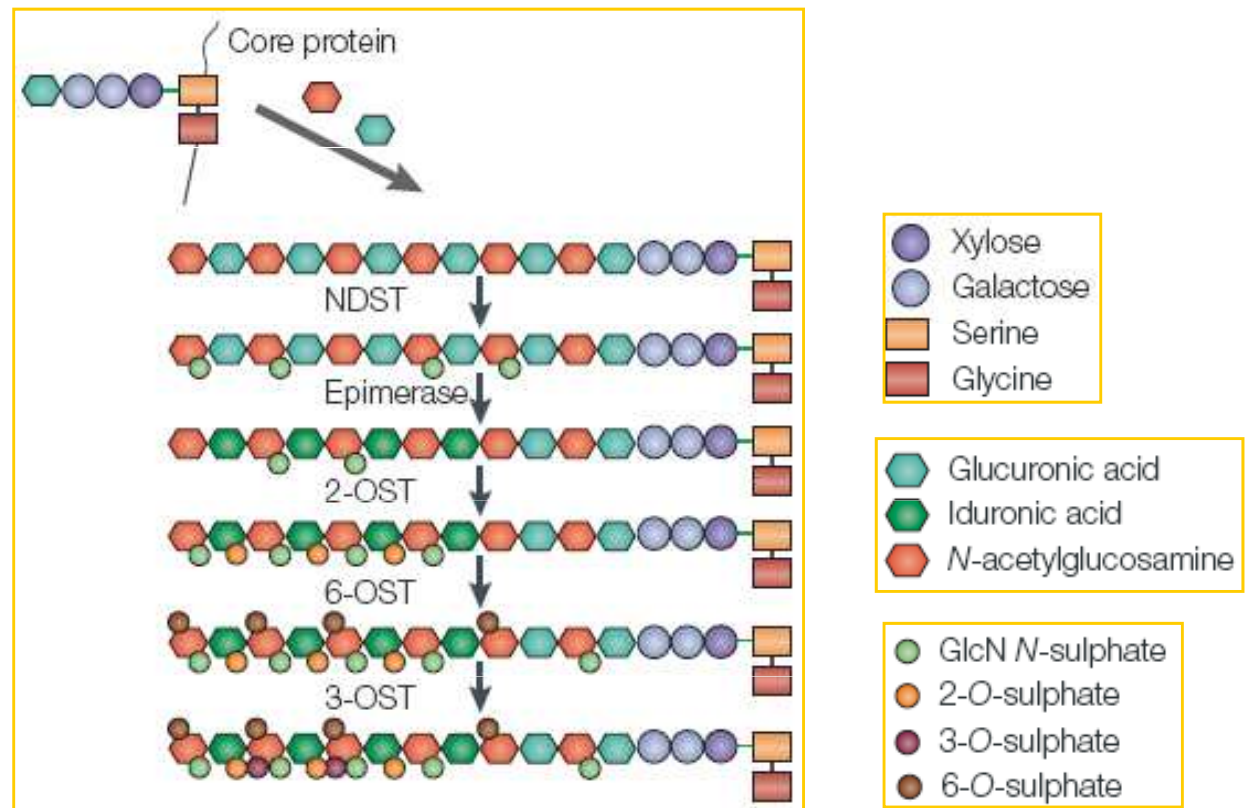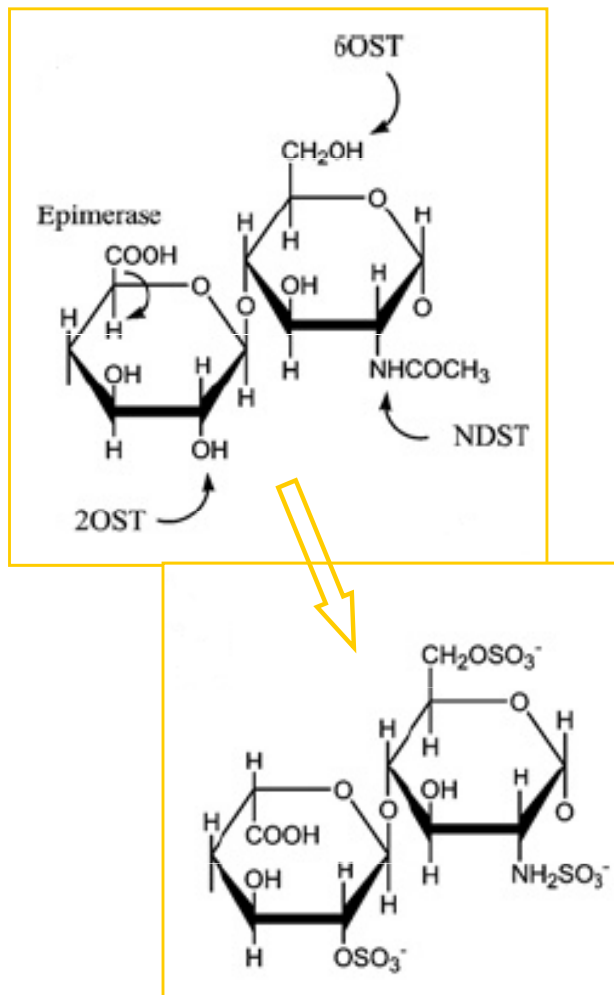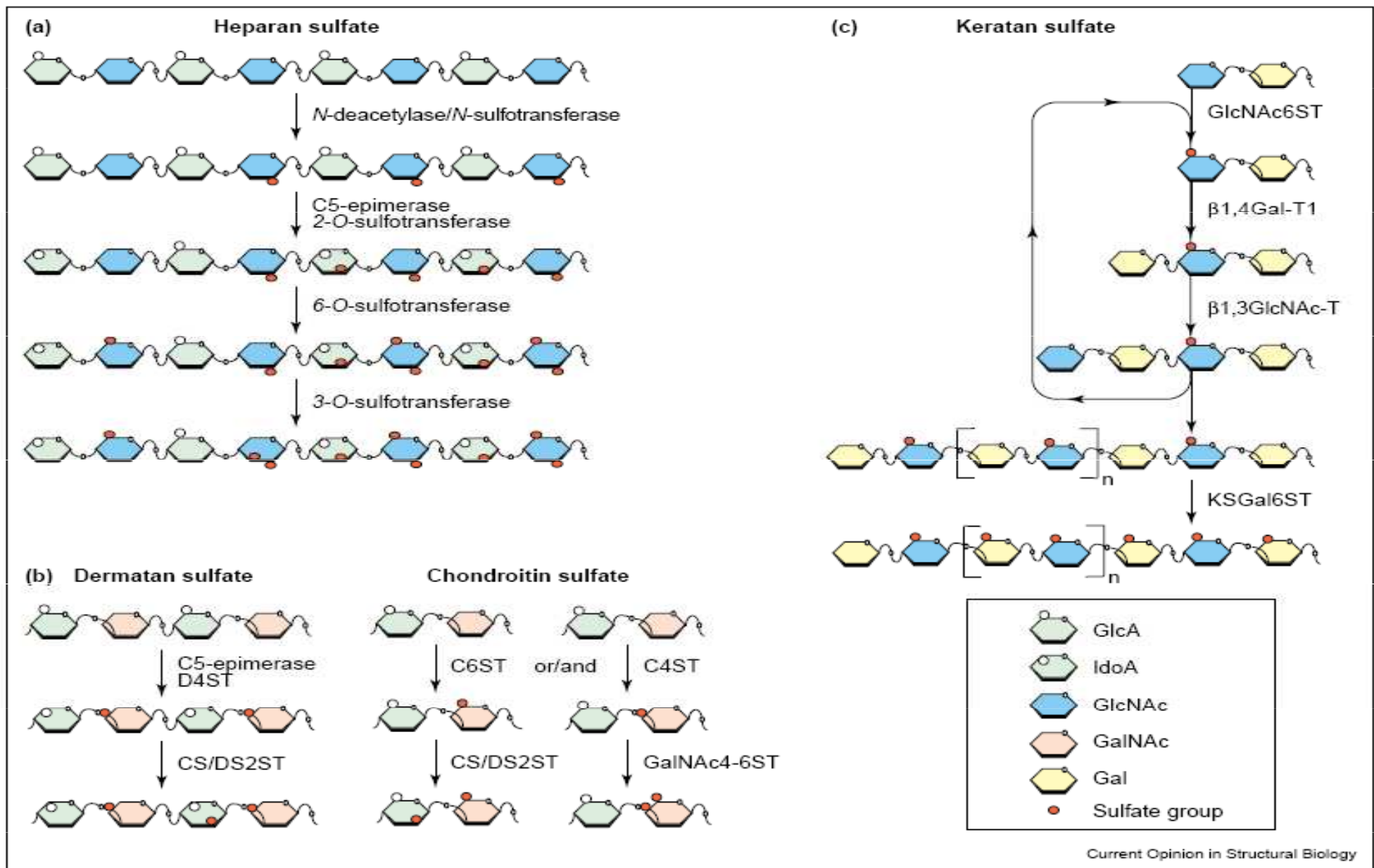
III. Modifikace: a) glukuronová kys. ⟶ induronová kys. (C-5 epimerace)

⟶ sulfonace na C-2 (+ SO_3^-)

b) N-acetylglukosamin ⟶ deacetylace aminoskup.

⟶ sulfonace na C-3, C-6

(a) Heparan sulfate

N-deacetylase/N-sulfotransferase

C5-epimerase
2-O-sulfotransferase

6-O-sulfotransferase

3-O-sulfotransferase

(b) Dermatan sulfate

C5-epimerase
D4ST

CS/DS2ST

Chondroitin sulfate

C6ST   or/and   C4ST

CS/DS2ST   GalNAc4-6ST

(c) Keratan sulfate

GlcNAc6ST

β1,4Gal-T1

β1,3GlcNAc-T

KSGal6ST

GlcA
IdoA
GlcNAc
GalNAc
Gal
Sulfate group

Current Opinion in Structural Biology

Modification reactions in GAG biosynthesis. (a) In HS biosynthesis the modification reactions are initiated by N-deacetylation/N-sulfation of selected GlcNAc residues. Subsequent modifications occur in N-sulfated regions of the polysaccharide. Note that 6-O-sulfation may occur to GlcNAc residues adjacent to N-sulfated disaccharides. (b) In DS biosynthesis, 4-O-sulfation takes place immediately after epimerization. 2-O-sulfation may then occur. In CS biosynthesis, 6-O-sulfation of GalNac and GalNAc(4S) are carried out by a separate enzymes. (c) During KS biosynthesis, 6-O-sulfation occurs to non-reducing terminal GlcNAc residues before further elongation. Sulfation of Gal residues may occur during or after chain elongation. β1,4Gal-T1, β1,4-galactosyltransferase 1; β1,3GlcNac-T, β1,3-N-acetylglucosaminyltransferase. Modified from [44•].

Table 1

GAG sulfotransferases.

Enzyme	Abbreviation	Predominant substrate (target in bold)	Predominant expression patterns* in human (left) and mouse (right) tissues		Refs
Chondroitin/dermatan sulfate					
Chondroitin 4-O-sulfotransferase-1	C4ST1	-GlcA-**GalNAc**-	Broad (hematopoetic tissues, peripherial blood leukocytes)	Broad (brain, kidney)	[25,26]
Chondroitin 4-O-sulfotransferase-2	C4ST2	-GlcA-**GalNAc**-	Broad		[26]
Chondroitin 4-O-sulfotransferase-3	C4ST3	-GlcA-**GalNAc**-	Liver		[27]
Dermatan 4-O-sulfotransferase-1	D4ST1	-IdoA-**GalNAc**-	Broad (placenta)		[28]
Chondroitin 6-O-sulfotransferase[†]	C6ST (GST0, CS6ST)	-GlcA-**GalNAc**-	Broad (aorta, testis)	Broad (spleen, lung eye)	[30,37*,59]
Chondroitin 6-O-sulfotransferase-2[‡]	C6ST2 (GST5, GlcNAc6ST-4)	-GlcA-**GalNAc**	Broad (heart, spleen, ovary)	Broad (kidney)	[34]
Chondroitin 4-sulfate 6-O-sulfotransferase	GalNAc4S-6ST	-GlcA-**GalNAc4S**-			[38]
Galactosaminyl uronyl 2-O-sulfotransferase	CS/DS2ST	-**GlcA**-GalNAc6S- -**IdoA**-GalNAc±4S	Broad		[40]
Keratan sulfate					
Keratan sulfate Gal 6-O-sulfotransferase	KSGal6ST (GST1)	-**Gal**-GlcNAc-	Brain		[42]
GlcNAc 6-O-sulfotransferase	GlcNAc6ST1 (GST2)	**GlcNAc**-Gal-	Broad	Broad	[32,60]
GlcNAc 6-O-sulfotransferase	GlcNAc6ST2 (HEC-GlcNAc6ST GST3, LSST)	**GlcNAc**-Gal-	High endothelial venules		[61,62]
Corneal GlcNAc 6-O-sulfotransferase, mouse intestinal GlcNAc 6-O-sulfotransferase[§]	GlcNAc6ST-5 (C-GlcNAc6ST, GST4β)	**GlcNAc**-Gal-	Cornea, spinal cord, trachea		[45,46]
Heparan sulfate/heparin					
N-deacetylase/N-sulfotransferase 1	NDST1	-GlcA-**GlcNAc**-	Broad	Broad	[9,63]
N-deacetylase/N-sulfotransferase 2	NDST2	-GlcA-**GlcNAc**-	Broad	Broad	[9,63]
N-deacetylase/N-sulfotransferase 3	NDST3	-GlcA-**GlcNAc**-	Broad	Adult brain, fetal tissues	[9]
N-deacetylase/N-sulfotransferase 4	NDST4	-GlcA-**GlcNAc**-		Adult brain, fetal tissues	[9]
Heparan sulfate 2-O-sulfotransferase	2-OST (HS2ST)	-**HexA**-GlcNS-		Broad	[13]
Heparan sulfate 6-O-sulfotransferase 1	6-OST1 (HS6ST-1)	-HexA±2-**GlcNS/Ac**-	Broad	Broad (liver)	[15–17]
Heparan sulfate 6-O-sulfotransferase 2	6-OST2 (HS6ST-2)	-HexA±2S-**GlcNS/Ac**-	Brain	Broad (brain, spleen)	[15–17]
Heparan sulfate 6-O-sulfotransferase 2S[#]	6-OST2S (HS6ST-2S)	-HexA±2S-**GlcNS/Ac**-	Ovary, placenta, fetal kidney		[17]
Heparan sulfate 6-O-sulfotransferase 3	6-OST3 (HS6ST-3)	-HexA±2S-**GlcNS/Ac**-		Broad	[15,16]
Heparan sulfate 3-O-sulfotransferase 1	3-OST1	-GlcA-**GlcNS±6S**-	Broad (kidney, brain)		[18]
Heparan sulfate 3-O-sulfotransferase 2	3-OST2	-HexA2S-**GlcNS**-	Brain		[18,64]
Heparan sulfate 3-O-sulfotransferase 3A	3-OST3A	-IdoA2S-**GlcNH₂±6S**-	Broad (heart, placenta)		[18,64]
Heparan sulfate 3-O-sulfotransferase 3B	3-OST3B	-IdoA2S-**GlcNH₂±6S**-	Broad (liver, placenta)		[18,64]
Heparan sulfate 3-O-sulfotransferase 4	3-OST4	Unknown	Brain		[18]
Heparan sulfate 3-O-sulfotransferase 5	3-OST5	-GlcA-**GlcNS±6S**-, -IdoA2S-**GlcNS±6S**-	Brain, spinal cord		[19,20]

*Lower levels of transcript may be present in other tissues as well. Tissues within parenthesis show the highest expression levels. [†]Has also weak KS Gal 6-O-sulftransferase activity. [‡]This enzyme has also been characterized as a GlcNAc 6-O-sulfotransferase (GlcNAc6ST4) [33,35]. [§]Mouse intestinal GlcNAc6ST has the same activity as human corneal GlcNAc6ST and is likely to produce mouse corneal KS [45]. [#]Splice forms of 6-OST2 so far only described in humans.
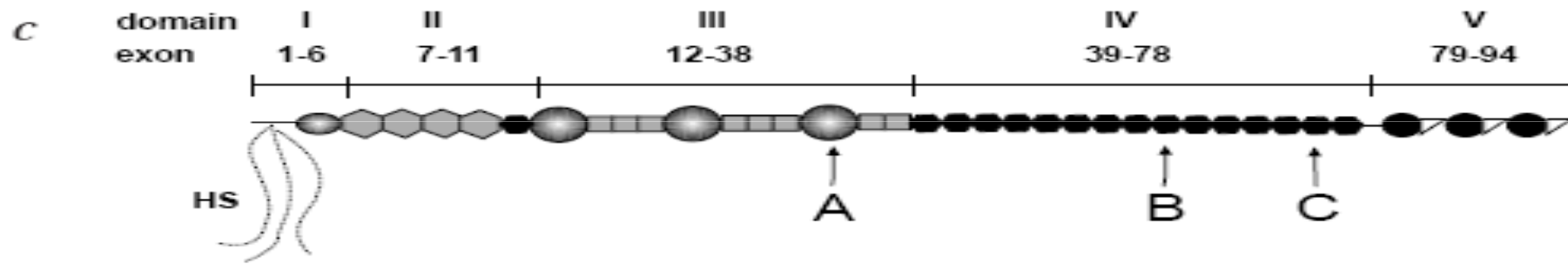
HEPARANSULFÁT PROTEOGLYKAN (HSPG)

Podle proteinové struktury rozlišujeme III. typy HSPG:

A) Syndecan: transmembránový protein, který může nést HS nebo CS řetězce

B) Glypican: protein ukotvený v membráně přes glykosylfosfatidylinositolovou kotvu, který nese HS řetězce

C) Perlecan: sekretovaný proteoglykan s HS řetězci, volně v ECM
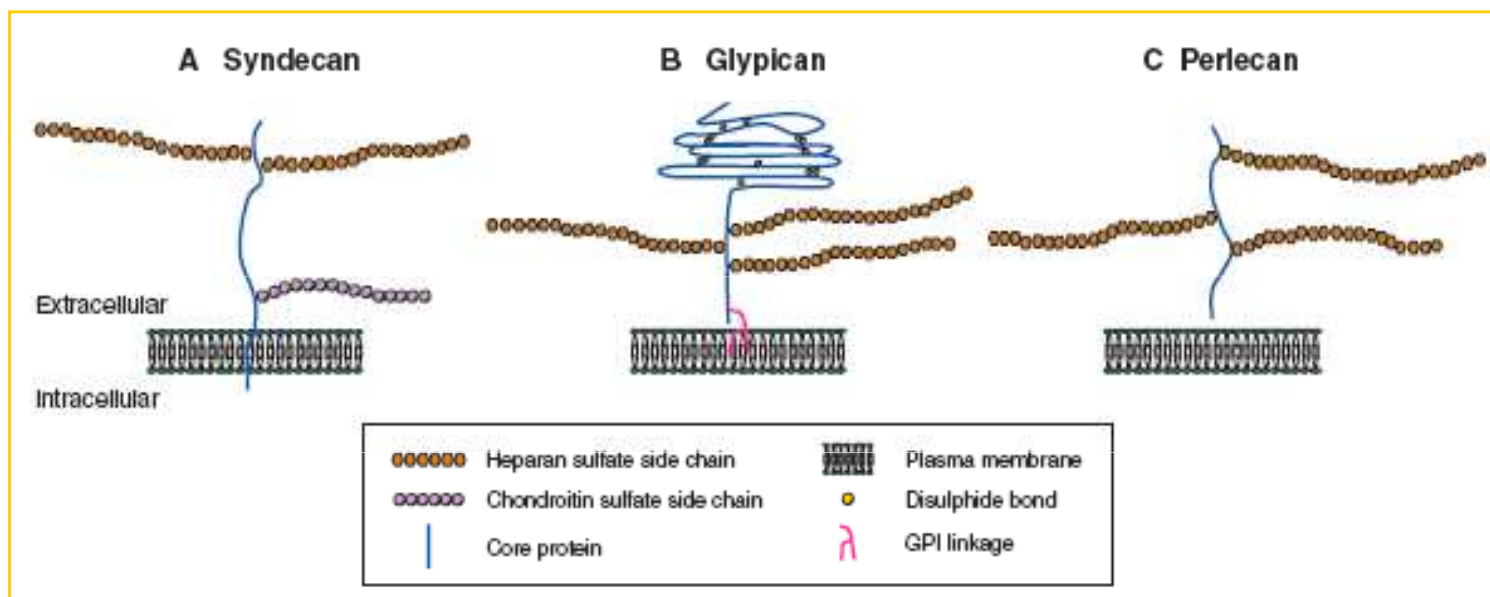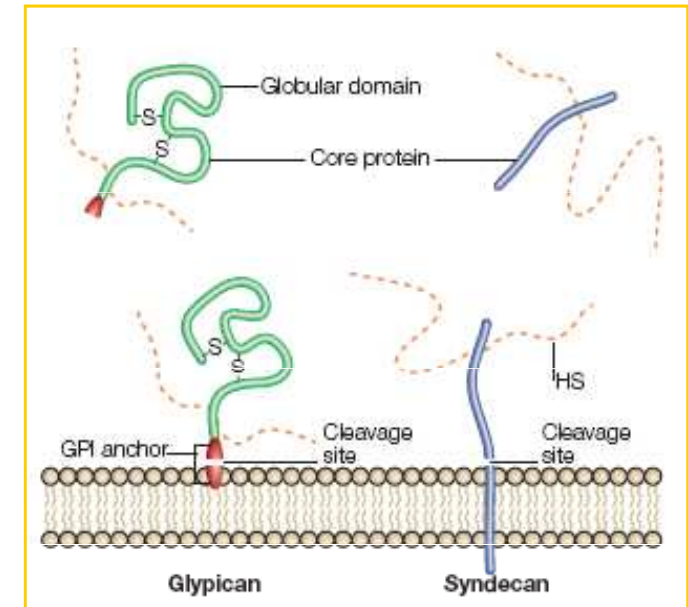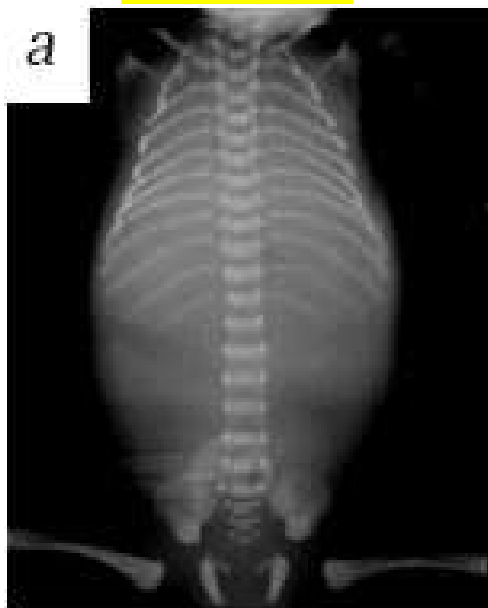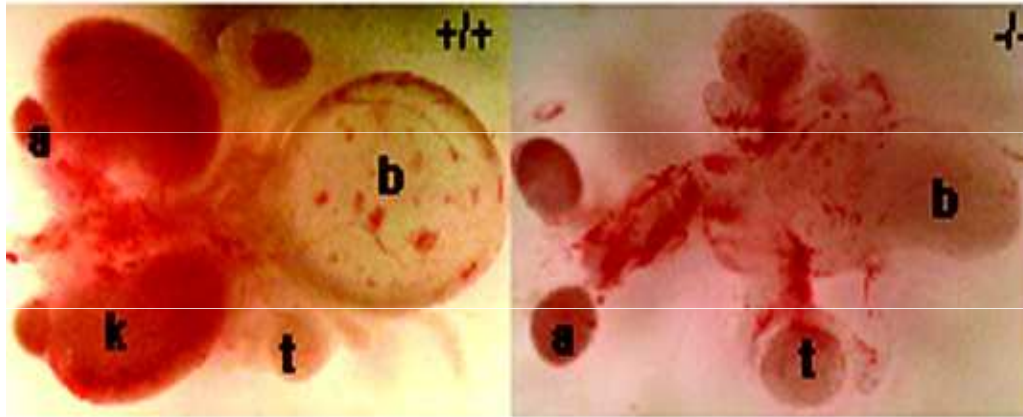
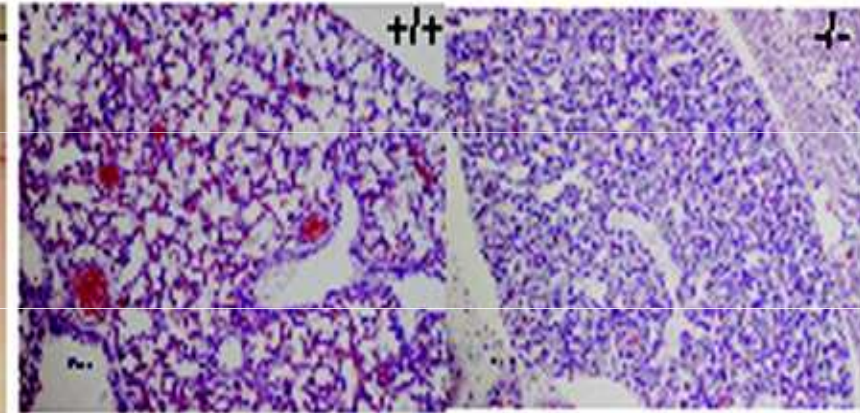# Dyssegmental dysplasia (Perlecan loss-of-function)
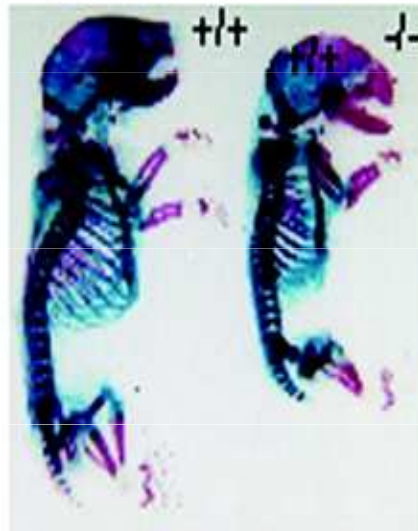
Glucuronyl C5-epimerase (HS lack Iduronic acid)
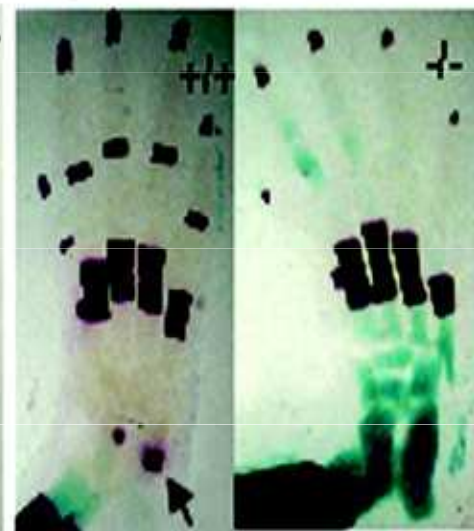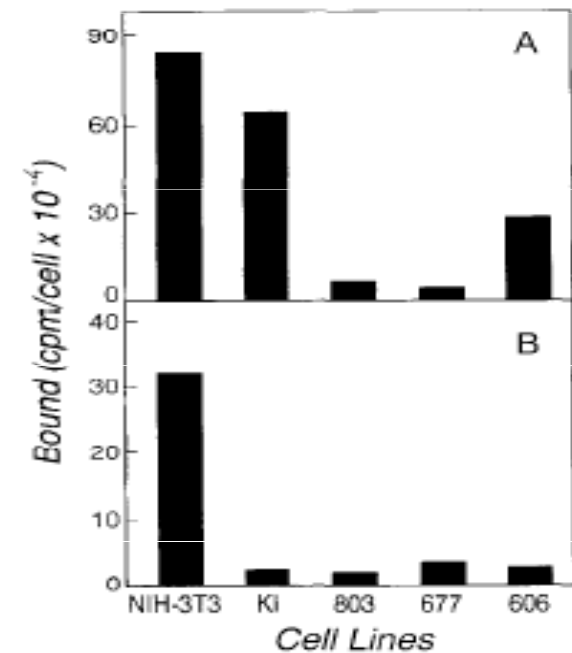

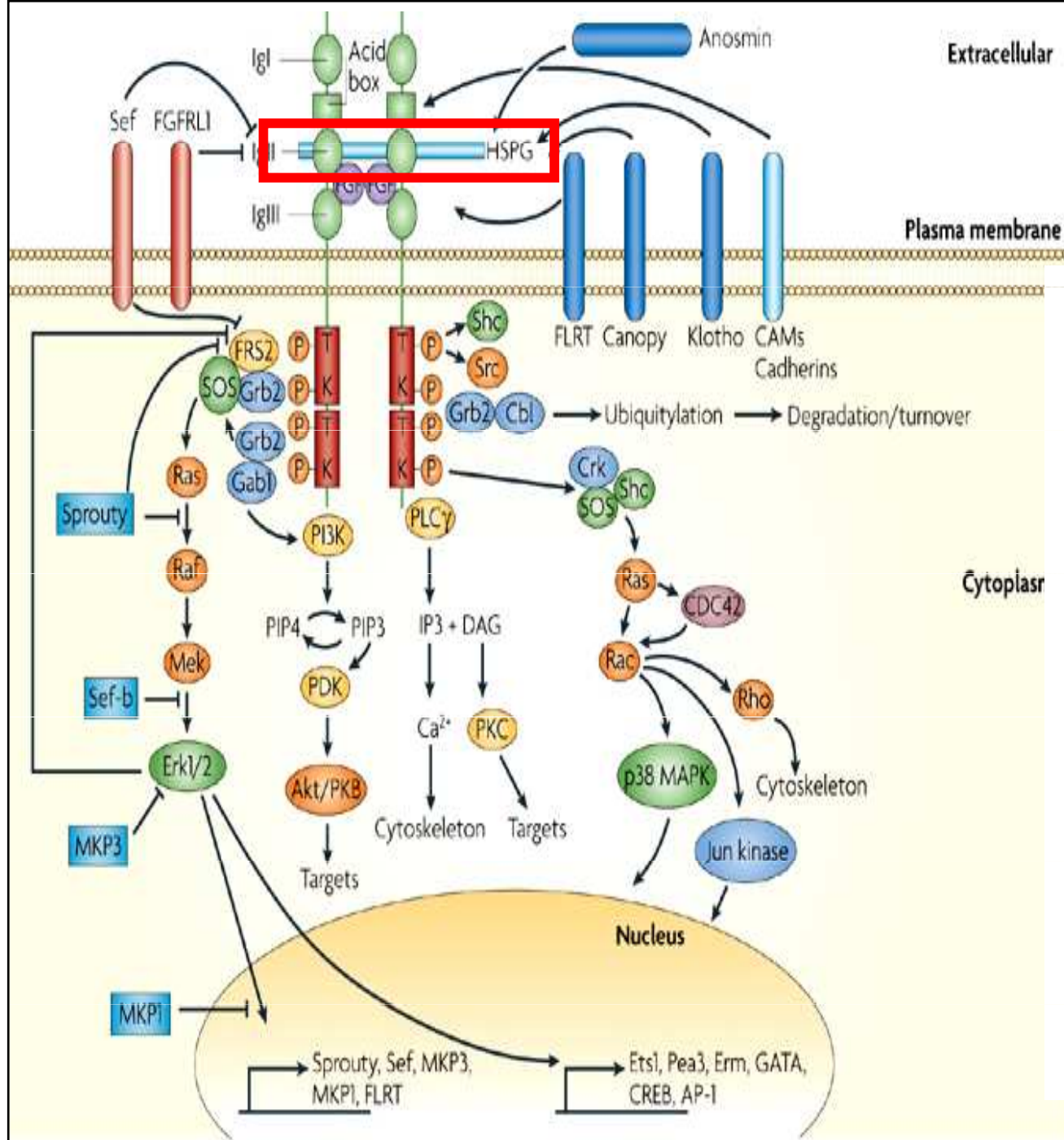**A** urogenital tract

B lung

D skeleton

E

F

Figure 1. Low and High Affinity Binding of ^{125}I-bFGF to Wild-Type and HS-Deficient Cells

Cells were incubated for 2 hr at 4°C with a saturating (10 ng/ml) concentration of ^{125}I-bFGF. (A) Low affinity binding. (B) High affinity binding. NIH-3T3 cells, mouse embryonal fibroblasts known to have both low and high affinity FGF receptors, were used as positive controls; CHO-KI, parental, wild-type CHO cells; clones 803 and 677, CHO cells that lack cell surface HSPGs. The 677 cells also overexpress chondroitin sulfate. Clone 606 is a line of HS-N-sulfotransferase-deficient cells that express undersulfated cell surface HSPGs (Bame and Esko, 1989). Results represent the mean values in one of at least two independent experiments.

TRANSCRIPTION FACTORS

-ultimate effectors of the outside-in intercellular signaling
-one of the most important molecules in shaping of the embryo
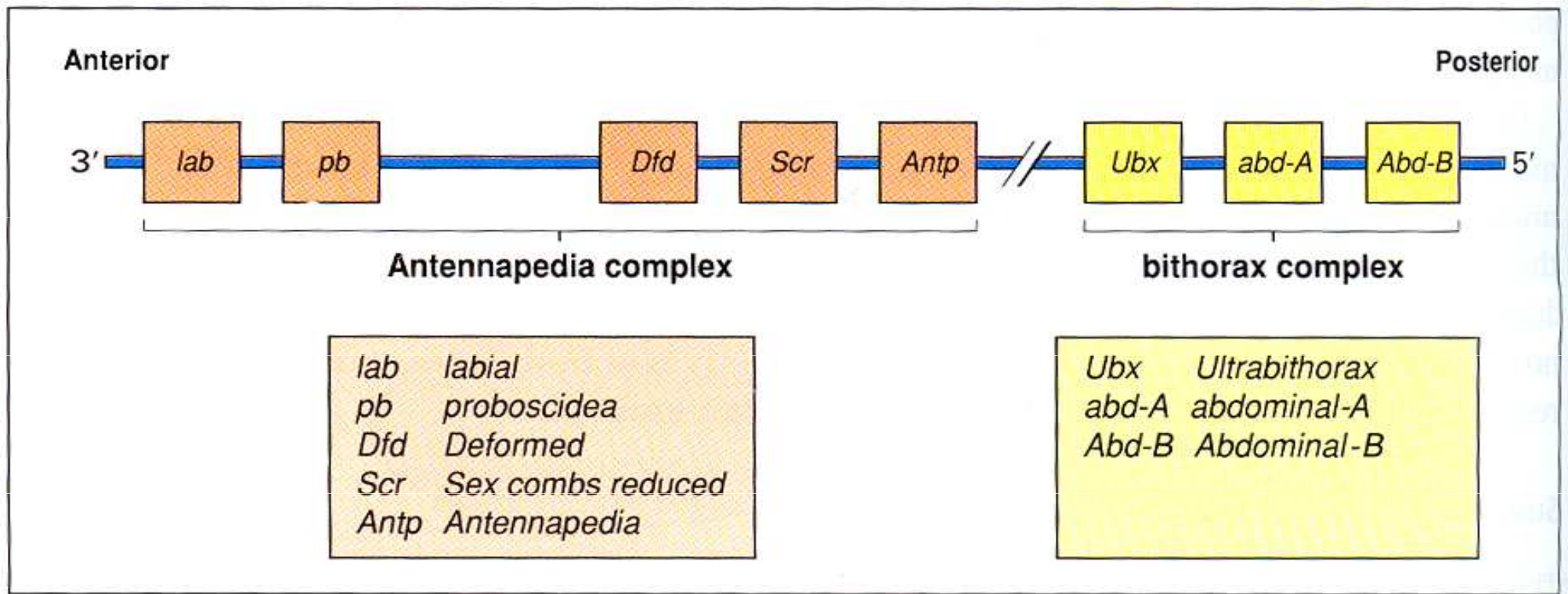-classified in families by DNA-binding domain

1. HOMEODOMAIN FACTORS

Homeodomain – 60aa sequence that interact with DNA, encoded by homeobox in the particular gene. Many homeobox genes regulate segment identity.
Homeotic genes cause **homeosis** – a transformation of one whole segment into another related one, such as antenna into leg.

Segment polarity genes – basic shape of segment, same for all segments.
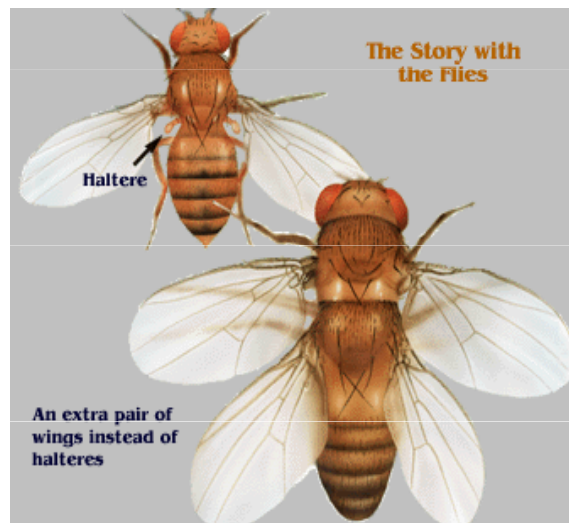Homeotic selector genes – control the differences in segment development via initiation of future developemntal pathways in each segment.
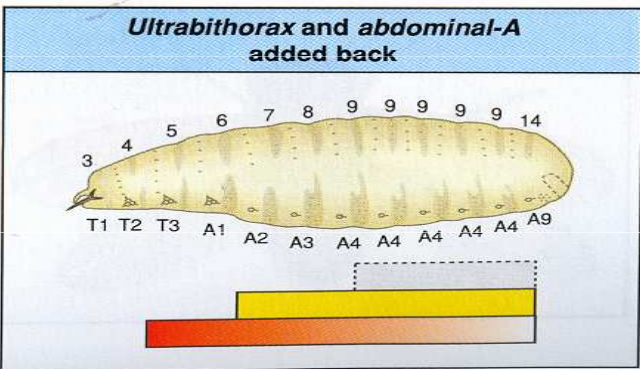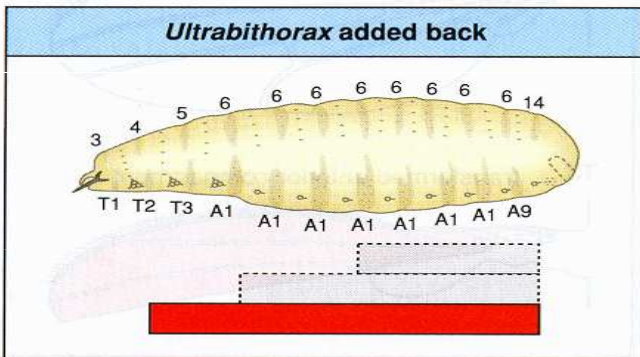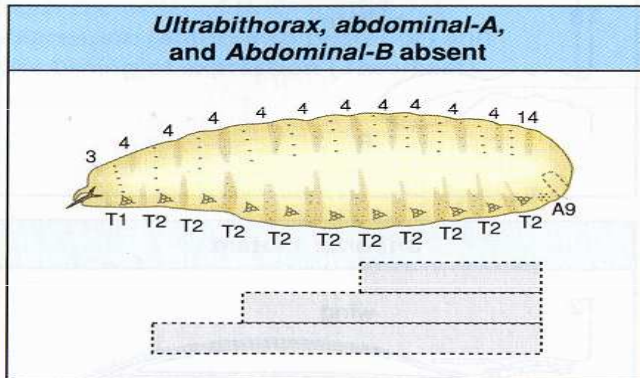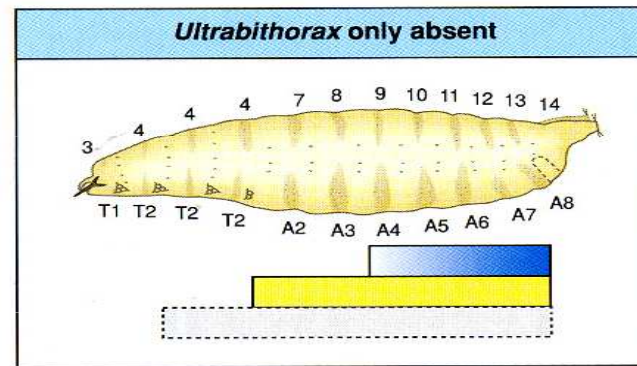
Anterior   Posterior

3′ — lab — pb — Dfd — Scr — Antp — // — Ubx — abd-A — Abd-B — 5′

Antennapedia complex   bithorax complex

lab	labial
pb	proboscidea
Dfd	Deformed
Scr	Sex combs reduced
Antp	Antennapedia

Ubx	Ultrabithorax
abd-A	abdominal-A
Abd-B	Abdominal-B

Antennapedia   *Bithorax*   *Chinook*

The Story with the Flies

Haltere

An extra pair of wings instead of halteres

Wild type

Parasegments

3 4 5 6 7 8 9 10 11 12 13 14

T1 T2 T3 A1 A2 A3 A4 A5 A6 A7 A8

Segments

Abdominal-B
abdominal-A
Ultrabithorax

Ultrabithorax only absent

3 4 4 4 7 8 9 10 11 12 13 14

T1 T2 T2 T2 A2 A3 A4 A5 A6 A7 A8

Ultrabithorax, abdominal-A, and Abdominal-B absent

3 4 4 4 4 4 4 4 4 4 14

T1 T2 T2 T2 T2 T2 T2 T2 T2 T2 A9

Ultrabithorax added back

3 4 5 6 6 6 6 6 6 6 14

T1 T2 T3 A1 A1 A1 A1 A1 A1 A1 A9

Ultrabithorax and abdominal-A added back

3 4 5 6 7 8 9 9 9 9 9 14

T1 T2 T3 A1 A2 A3 A4 A4 A4 A4 A4 A9

2. LIM-HOMEODOMAIN PROTEINS

Two LIM domains fused to DNA-binding homeodomain

Islet-1

E9.5

MLC2a (myosin-light chain)

MLC2v

sv – sinus venosus
ot – outflow tract
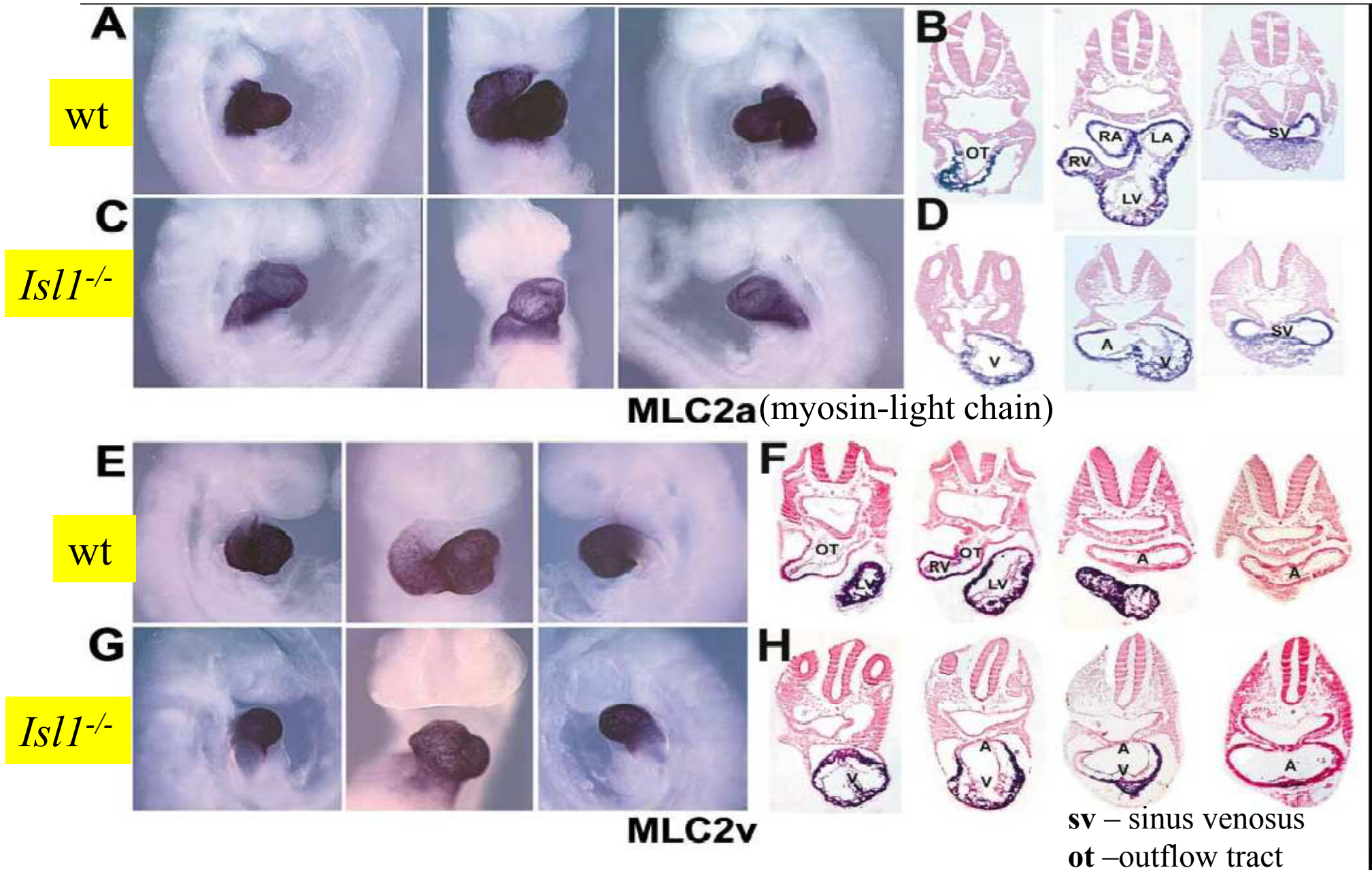
3. PAX PROTEINS
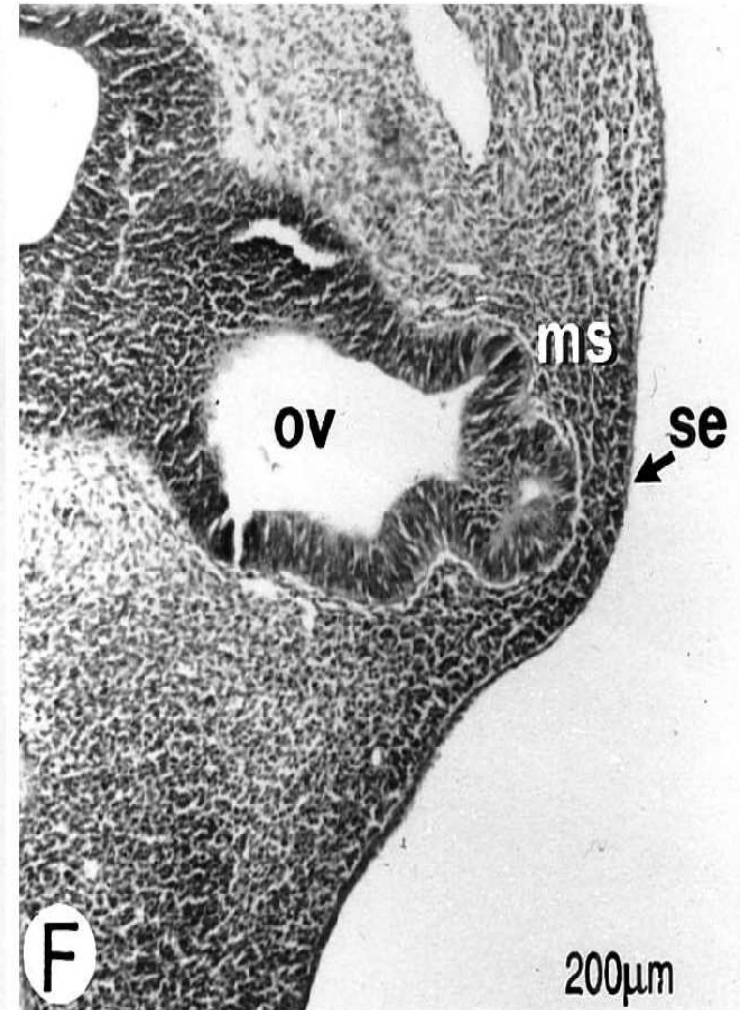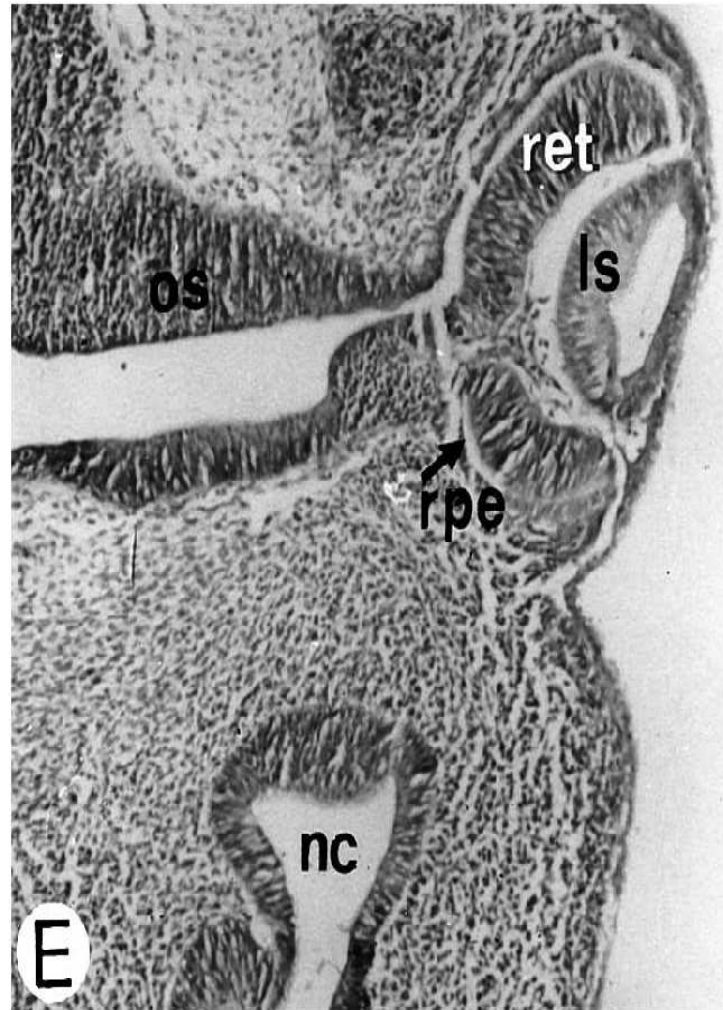
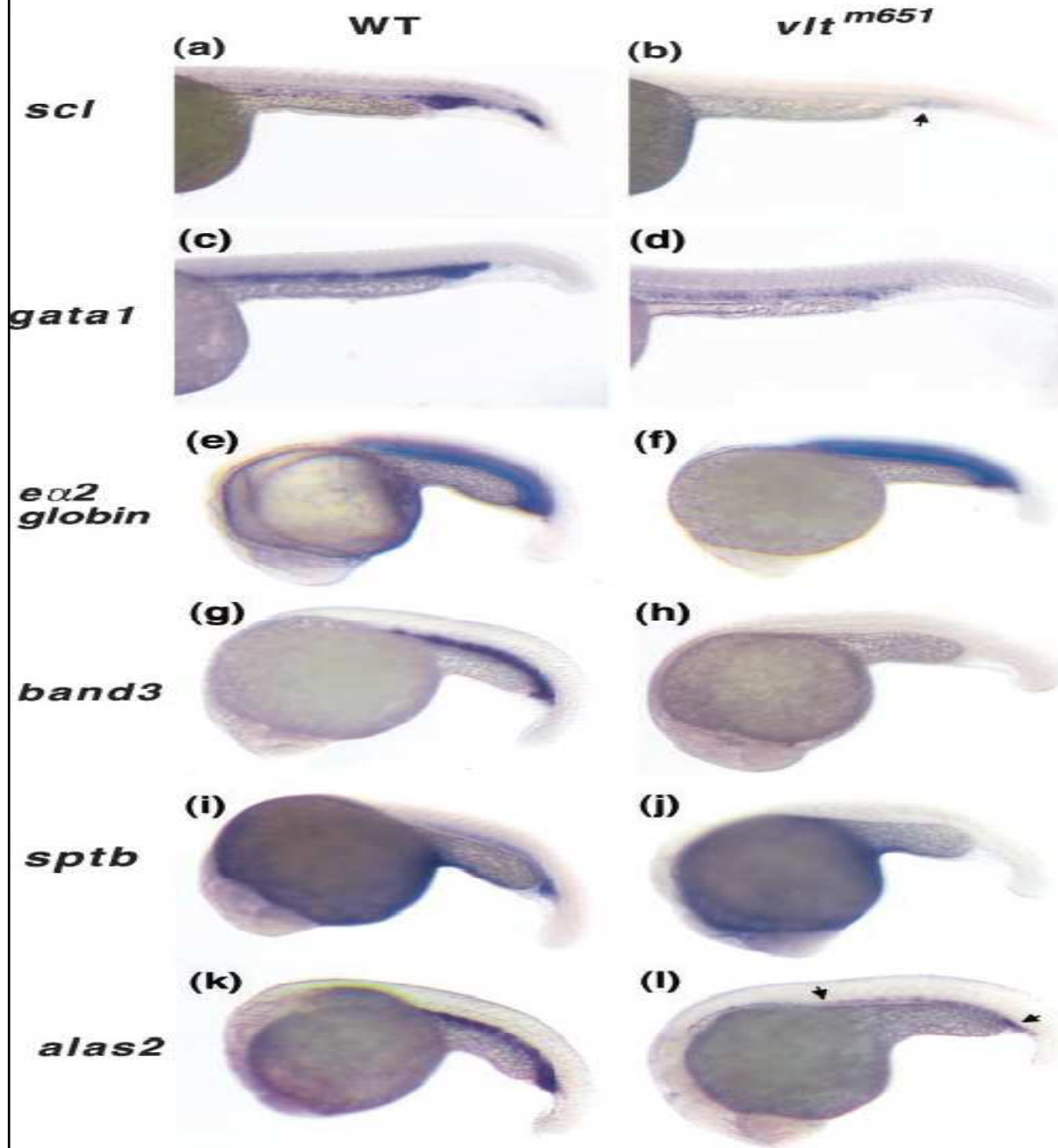DNA binding region: paired domain with 6 α-helical segments

Pax6 (paired box gene 6)

se - surface ectoderm
ms –mesenchymal-like cells
rpe – pigmented retinal
epithelium
ret – retina
os – optic stalk
ov- optic vesicle
nc – nasal cavity
ls -lens

200μm

E11.5

4. ZINC-FINGER PROTEINS

Bind DNA via zinc-finger motif

Dracula fish – loss-of-function mutation in GATA1 – impaired erythroid differentiation

5. NUCLEAR RECEPTOR SUPERFAMILY

Intracellular receptor that function as transcription factors. Lipophilic ligands: steroids, thyroid hormone, retinoid acid. Inactive – Hsp90-bound in cytoplasm, complexed with ligand, - nuclear.
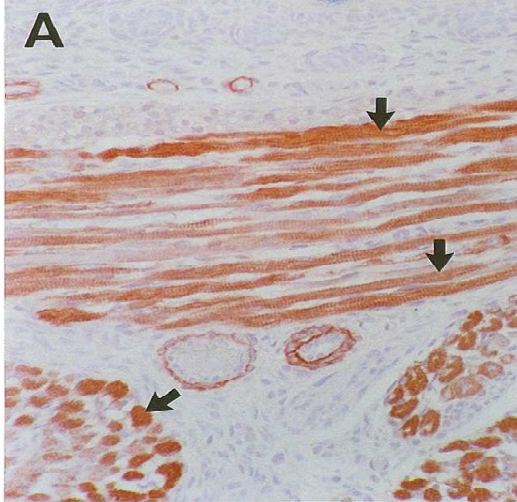Retinoid acid signaling in limiting the cardiac progenitor pool

precardiac mesoderm

cardiomyocytes
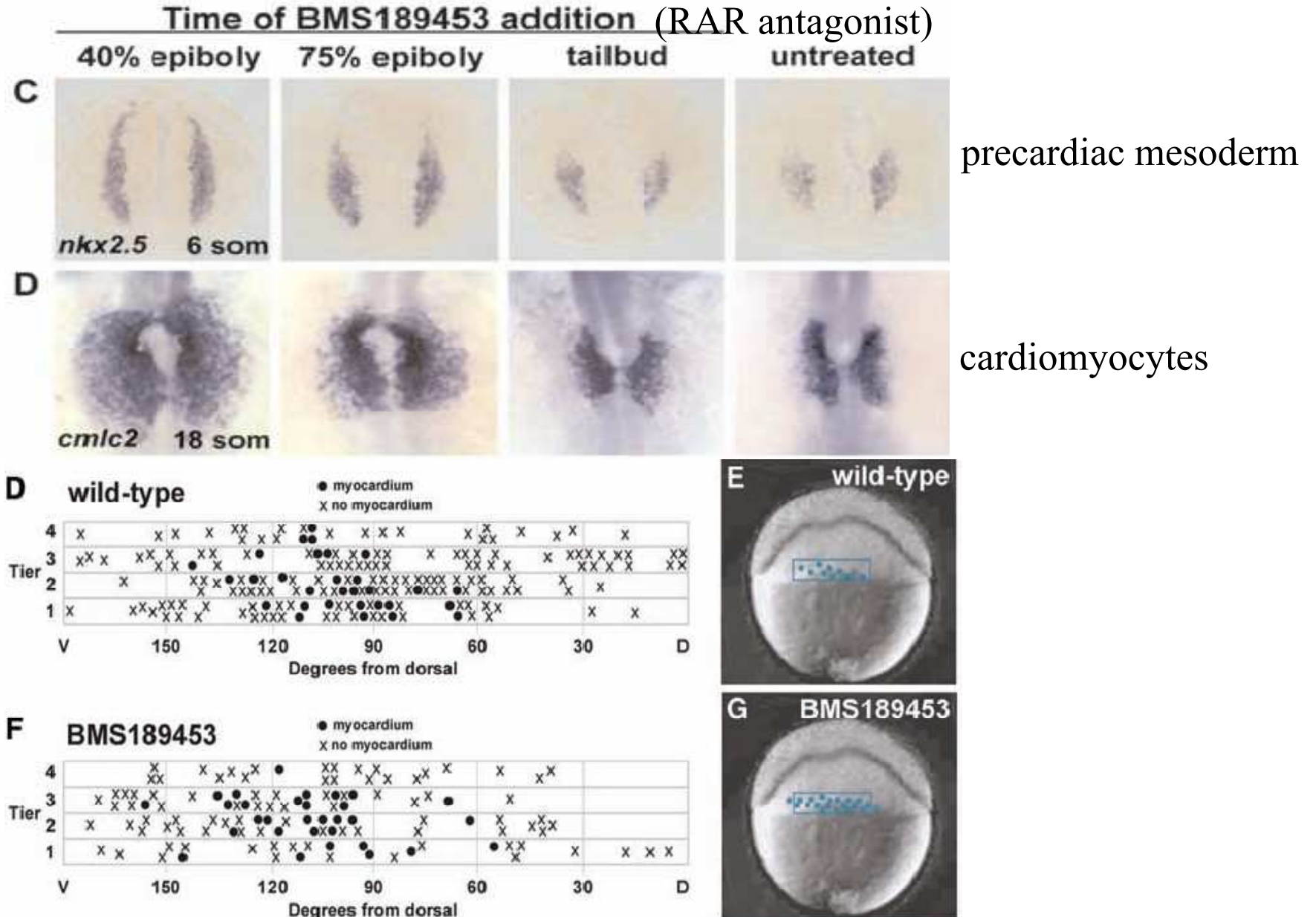
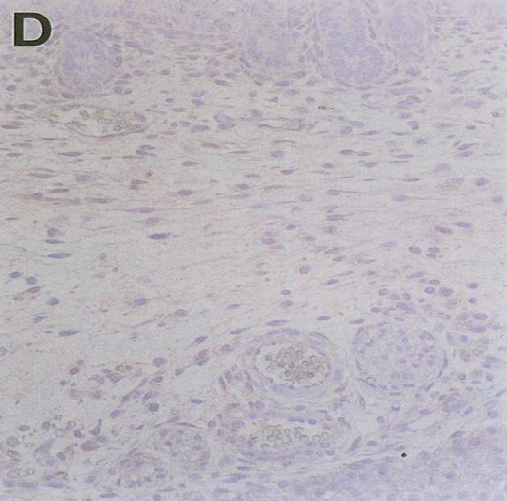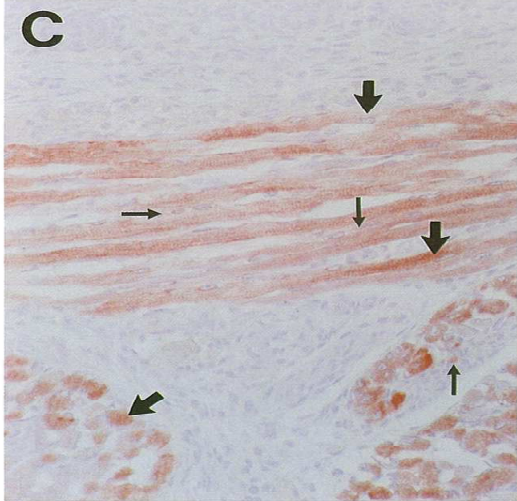# 6. BASIC HELIX-LOOP-HELIX (bHLH) FACTORS
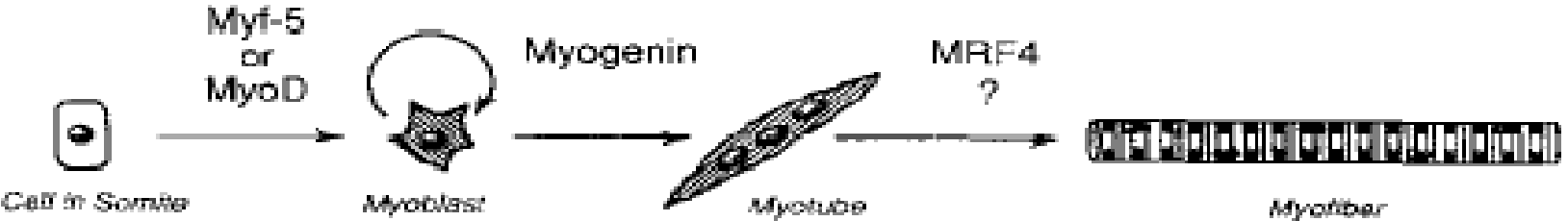
$MyoD^{+/-}\ Myf$-$5^{+/-}$

$MyoD^{-/-}\ Myf$-$5^{-/-}$

Contain basic DNA binding region and hydrophobic helix-loop-helix region responsible for dimerisation

α-actin IHC
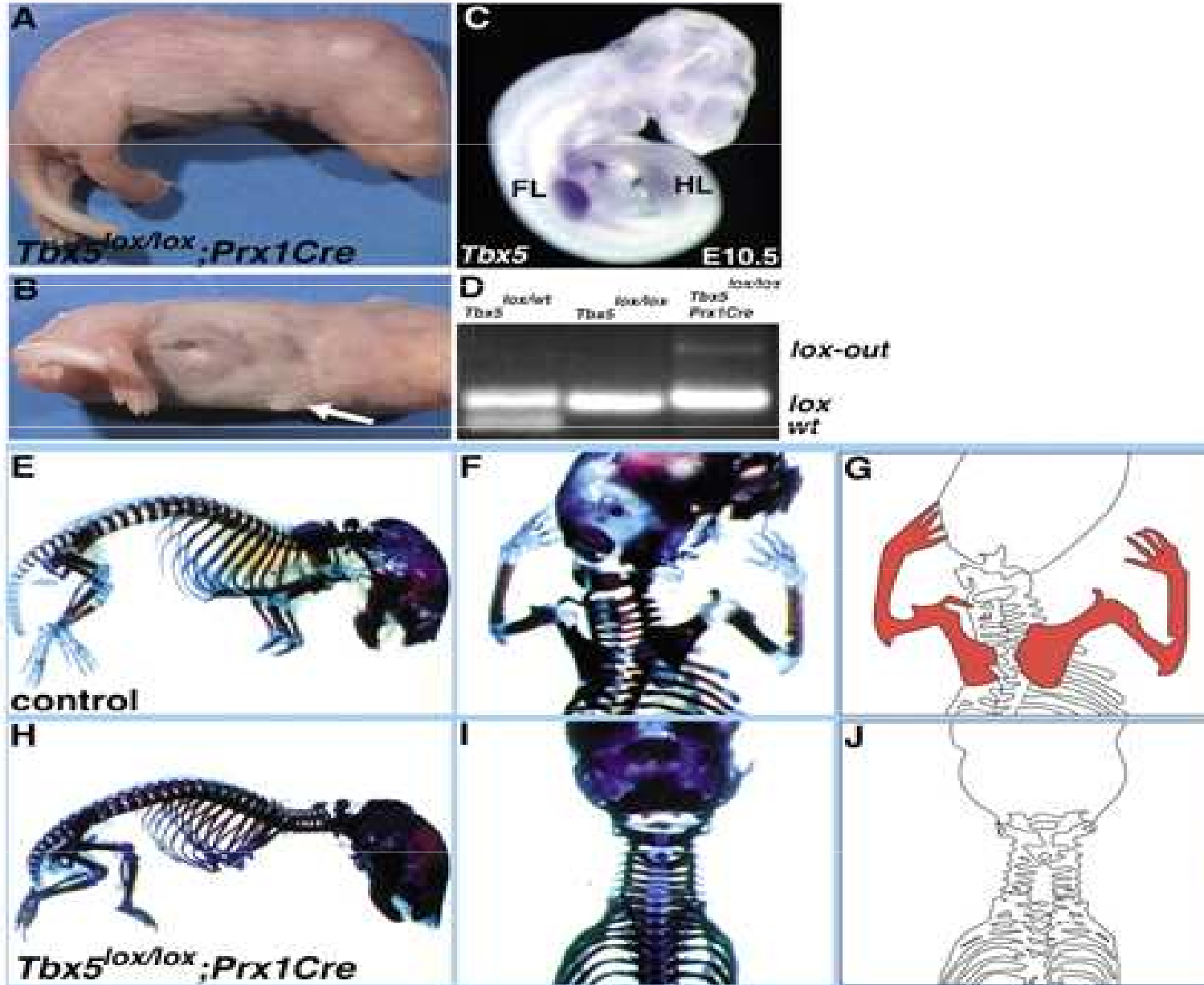(smooth and striated muscle fibers)

desmin IHC
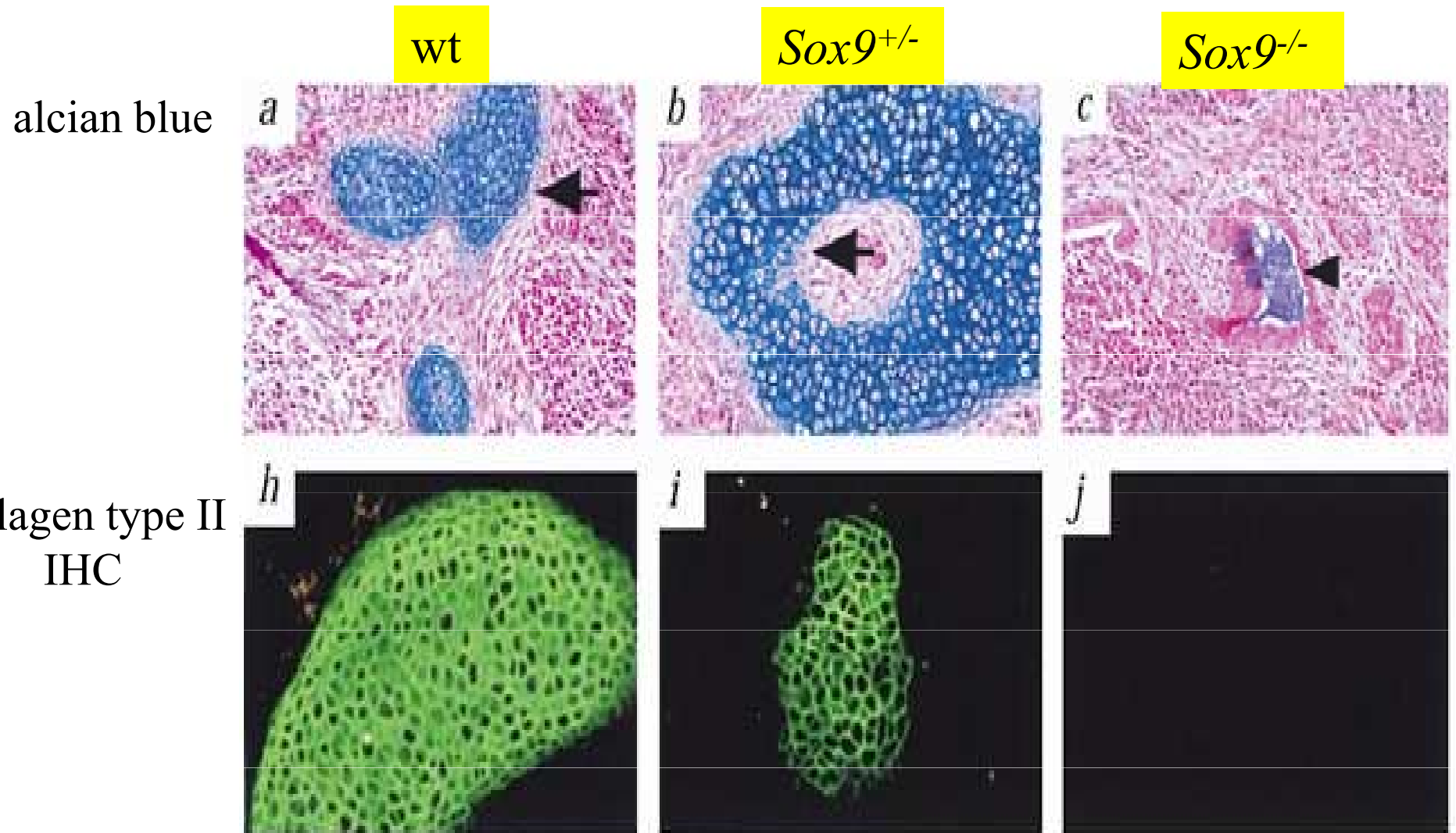(skeletal muscle fibers, myoblast-like cells)

7. T-BOX FACTORS

DNA binding domain derived from the prototype gene called transcription factor T.
Limb identity factors Tbx4 and Tbx5

8. HIGH MOBILITY GROUP (HMG)-box FACTORS

Operate via bending DNA to bring regulatory sites with transcriptional complex
Sox9 (Sry-Box9) – master inducer of cartilage

9. SRY (sex determining region Y)

Table 1 • Sex reversal observed in XX transgenic mice at 14.5 dpc

Construct	Embryos transferred	Embryos recovered	XX transgenic	Sex-reversed	% sex-reversed
L741	2,400	145	6	4	67
SryStuI	3,500	138	4	2	50
SryStop1	3,000	239	13	0	0
SryStop2	3,200	320	20	0	0
SryStop2Rev	1,000	82	4	3	75

The number of embryos transferred is approximate.
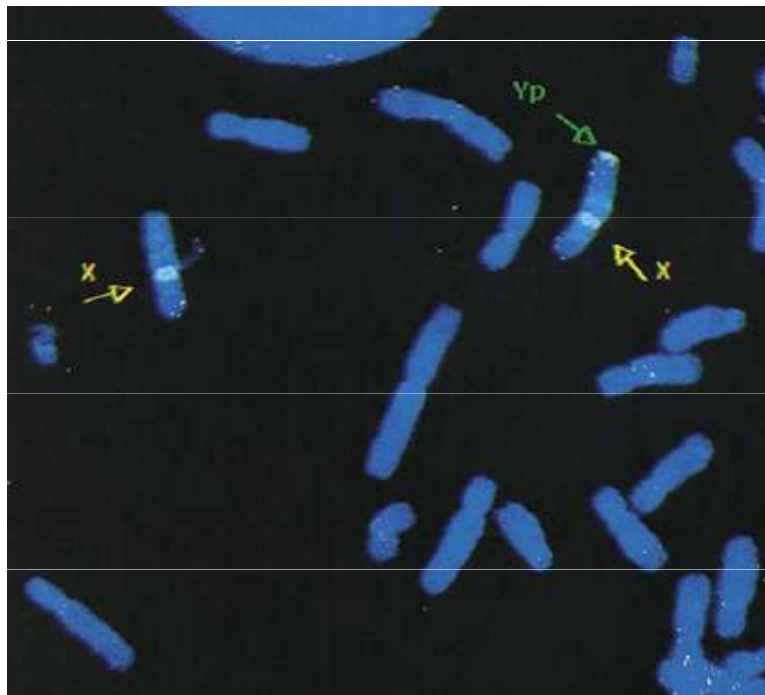
10. RUNT DOMAIN-CONTAINING FACTORS
Runx2 (Runt-related transcription factor 2)
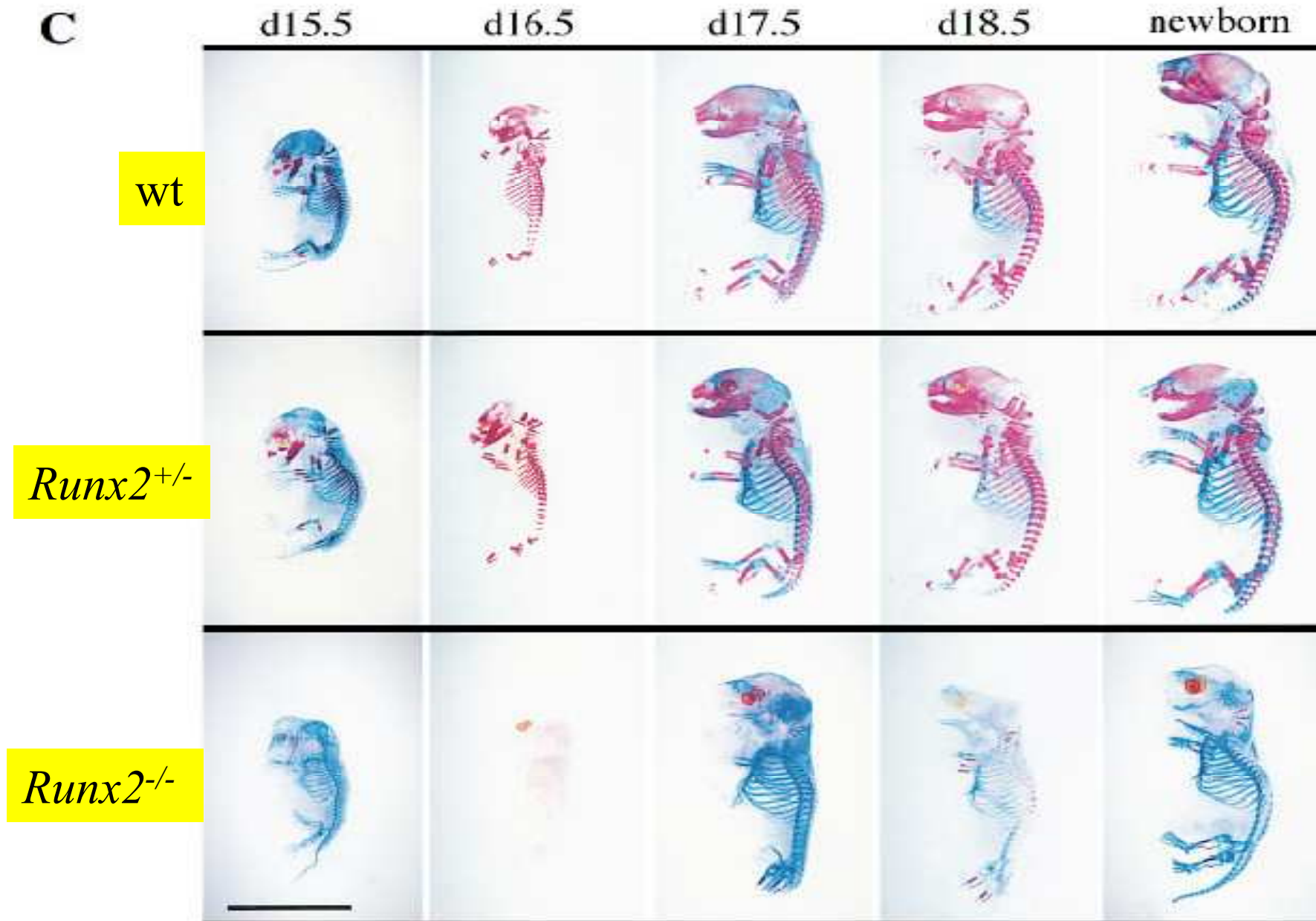
Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

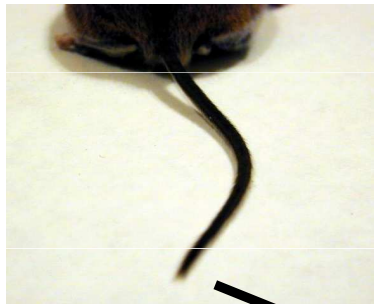[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

*Contact: yamanaka@frontier.kyoto-u.ac.jp

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we dem or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

A

B

iPS-TTFgfp4

	1	2	3	4	5	7	ES
Ecat1							
Esg1							
Nanog							
ERas							
Gdf3							
Cct3/4							
Sox2							
Fgf4							
Rex1							
Utf1							
Cripto							
Dax1							
Zfp296							
Neo (Fbx15)							
Nat1							
RT minus							

Oct3/4
Sox2
c-Myc
Klf4

C

Phase — GFP

iPS-TTFgfp 4-7

PS-TTFgfp 4-7

iPS-TTFgfp 4-3

iPS-TTFgfp 4-3

D

Heart

Gastrointestinal tract

Liver

Gonad

Neural tube

Skin
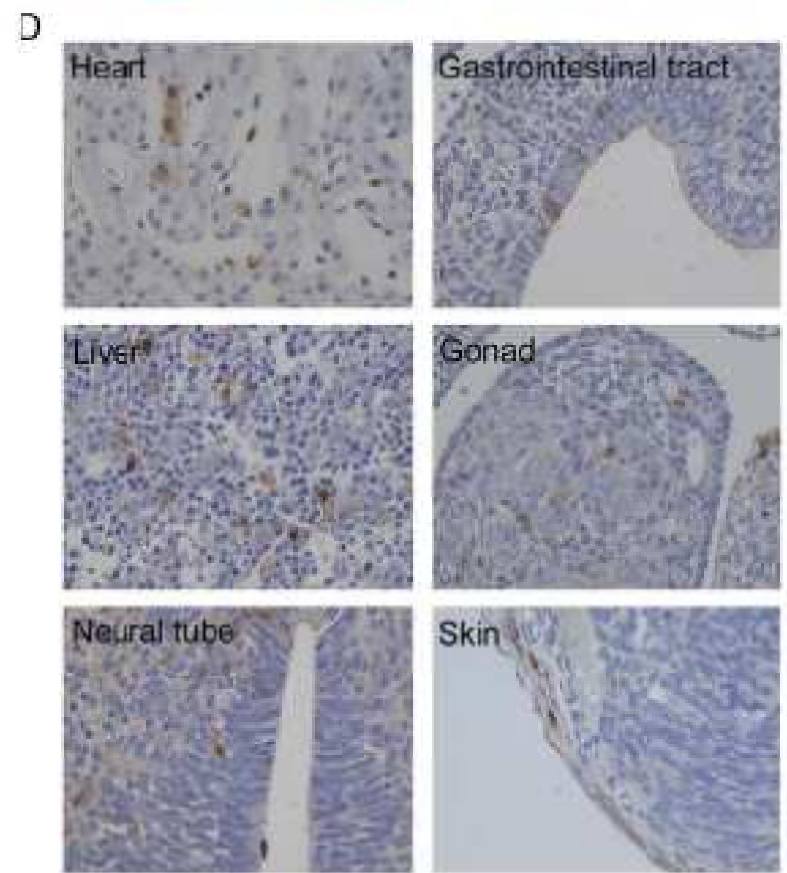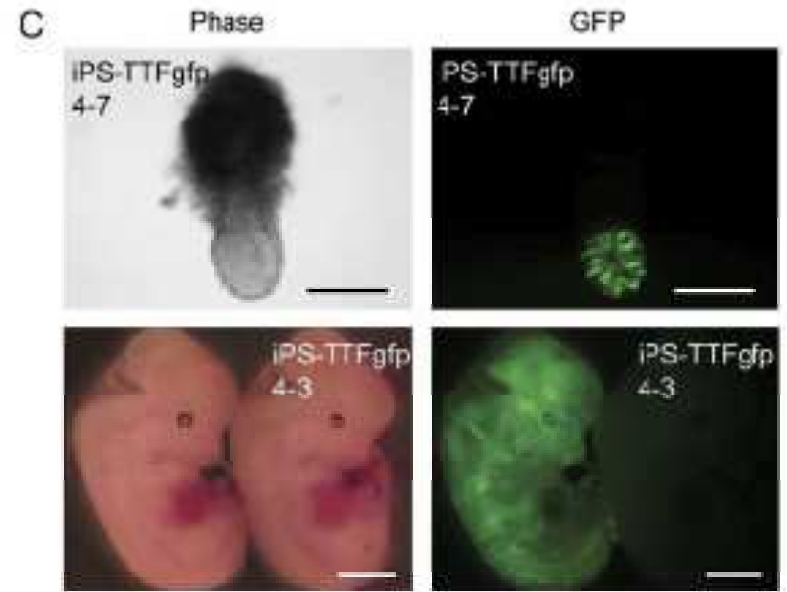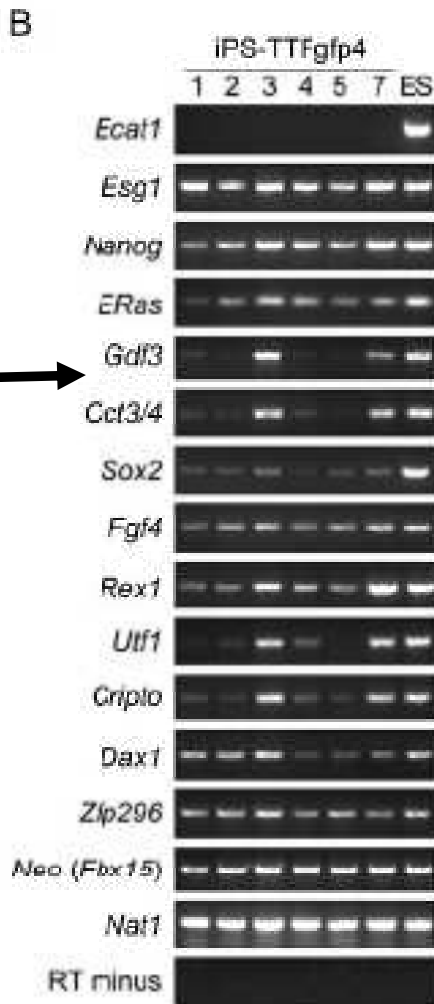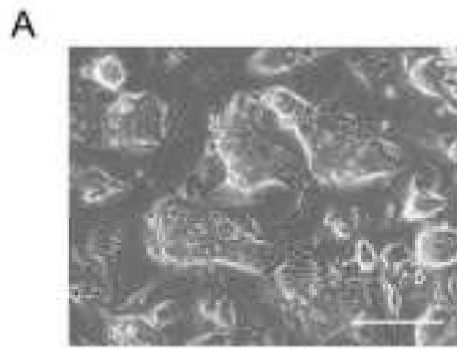
Table 1

Summary of reprogramming studies

Species	Cell type	Factors	Selection strategy	Reference study
Mouse	MEFs and tail tip fibroblasts	Oct4, Sox2, Klf4, and c-Myc	Fbx15-neo	[8••]
			Nanog-puro	[12•]
			Nanog-puro	[11•]
			Nanog- or Oct4-neo	[13•]
	MEFs		Oct4-GFP	[16]
	MEFs and tail tip fibroblasts	Oct4, Sox2, and Klf4	Nanog-puro	[45•]
	MEFs		Nanog- or Oct4-neo	[46•]
Human	HDF	Oct4, Sox2, Klf4, and c-Myc	Morphology	[17••]
	HFLS			
	BJs			
	Adult fibroblasts	Oct4, Sox2, Nanog, and LIN28	Morphology	[18••]
	Foreskin fibroblasts			
	H1F cells	Oct4, Sox2, Klf4, and c-Myc	Oct4-neo	[19••]
	Fetal fibroblasts		Morphology	
	H1F cells	Oct4, Sox2, Klf4, c-Myc, hTert, and SV40 large T	Oct4-neo	
	MSCs		Morphology	
	Adult fibroblasts			
	H1F cells	Oct4, Sox2, and Klf4	Oct4-neo	
	H1F cells	Oct4, Sox2, and c-Myc		

HDF, human dermal fibroblasts; HFLS, human fibroblast-like synoviocytes; BJ, cell line derived from neonate fibroblasts; H1F, ES cell-derived fibroblast; MSCs, mesenchymal stem cells.

ARTICLES

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