

HME: Insights Into Pathogenesis (Houston, Nov 2005)

Structure and Biosynthesis of Mouse Brain Heparan Sulfates

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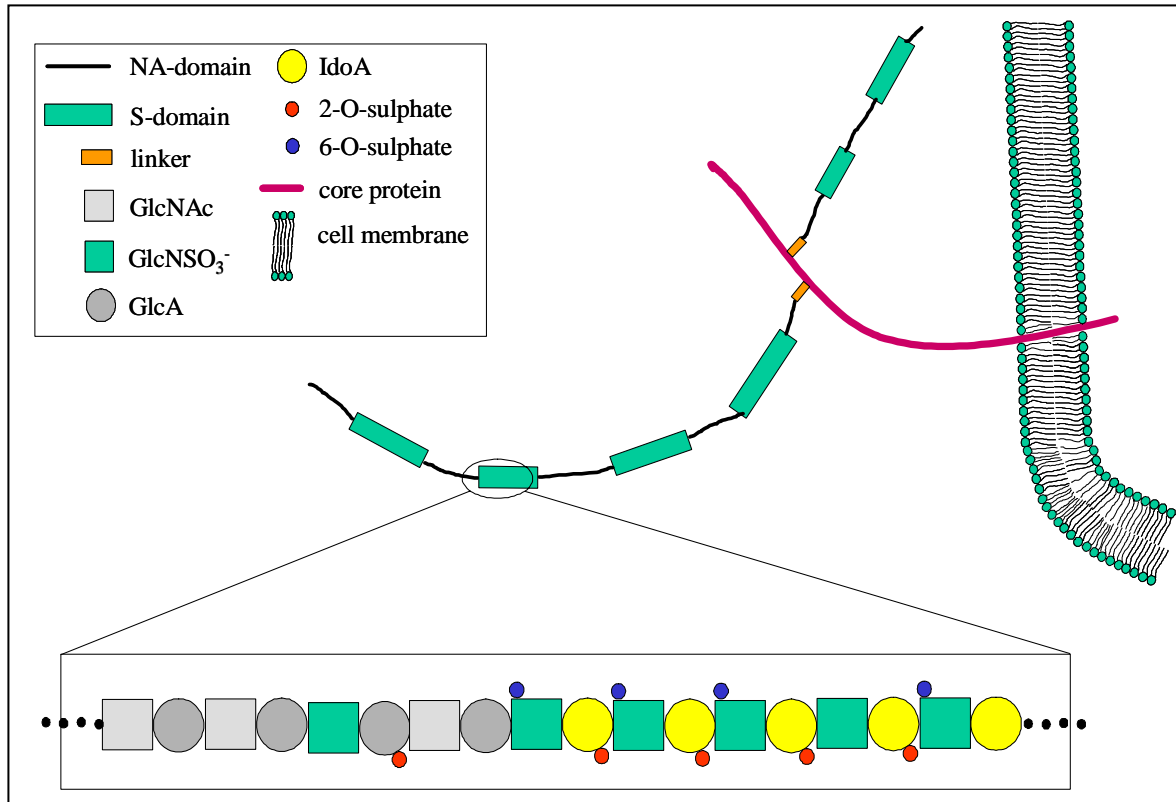
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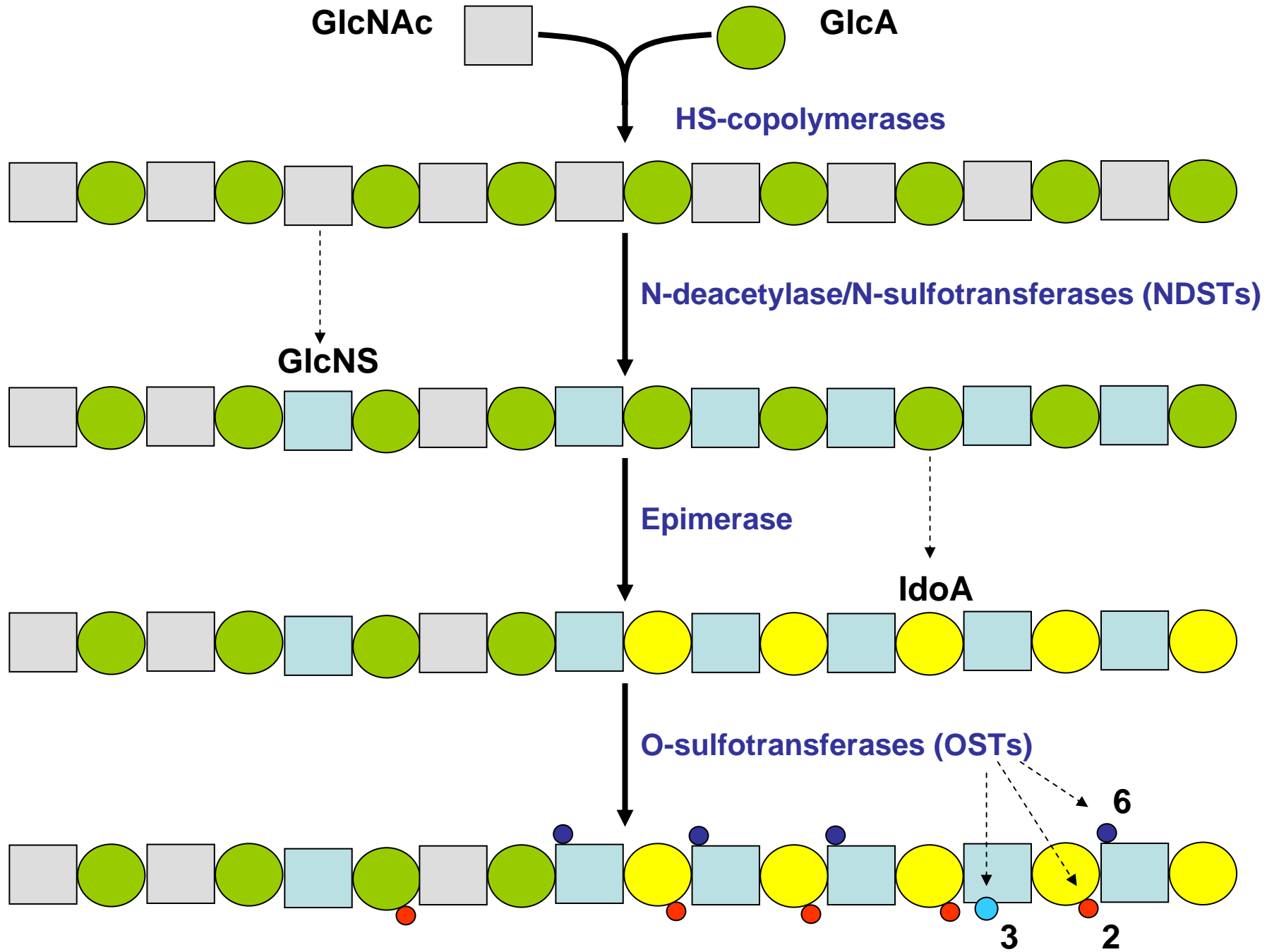
Heparan Sulphate Proteoglycans

- Family of complex sulphated polysaccharides
- Strategic expression on cell surfaces, extracellular matrix
- Multifunctional cell regulators

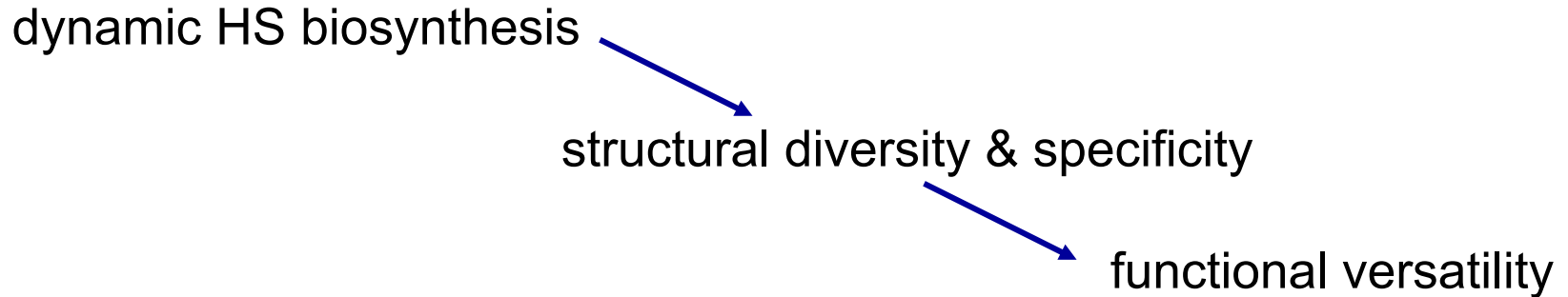


Regulates functions of many proteins

- specific sugar sequences – sulfation/acetylation/epimerisation patterns
- confer selective protein-binding properties.



Studying HS structure-function: the challenge



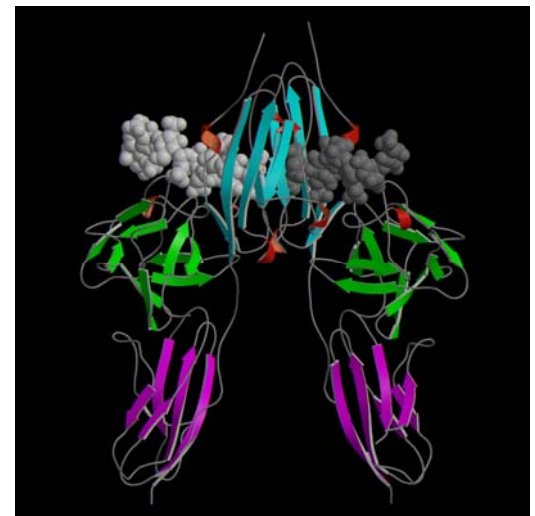
- how is biosynthesis (~20 glycosyltransferase & sulfotransferase genes) regulated to produce particular HS structures ?
- what degree of specificity is involved in protein interactions ?
- how does all this operate in functional contexts (in cells and *in vivo* in animal tissues) ?

HS structure-activity relationships in FGF-FGFR signalling

**HS is an essential co-receptor for activation of
FGFR signalling by FGF ligands**

- ligand & receptor specific saccharides regulate signalling
- positive and negative regulators

Guimond & Turnbull (1999) *Current Biology* **9**, 1343-1346.

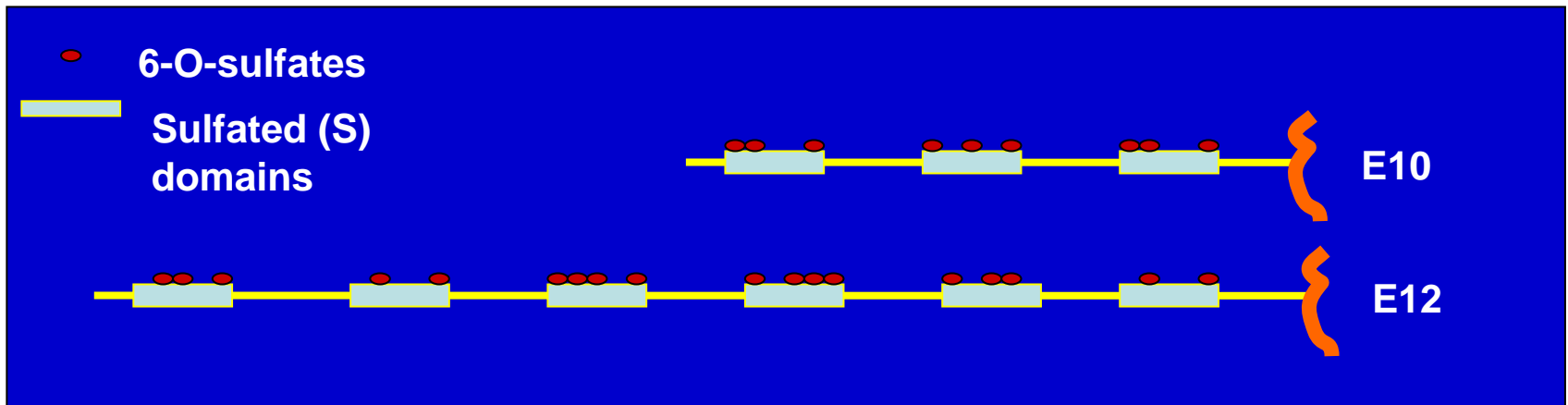


Mouse brain development

- mouse CNS develops from the neuroepithelium of the neural tube.
- FGFs (1, 2, 8, 15 & 17) involved in differentiation & expressed in specific spatio-temporal patterns
- FGFR isoform expression is regulated
- expression of core proteins of HS proteoglycans (HSPGs) is also regulated (Ford-Perriss et al, Dev Dynamics 2003)
- **developing neuroepithelium is a relevant *in vivo* system for studying the functional roles of HS as a regulator of FGFs**

Changes in HS structure during early mouse neural development

HS from neural precursor cells *in vitro*

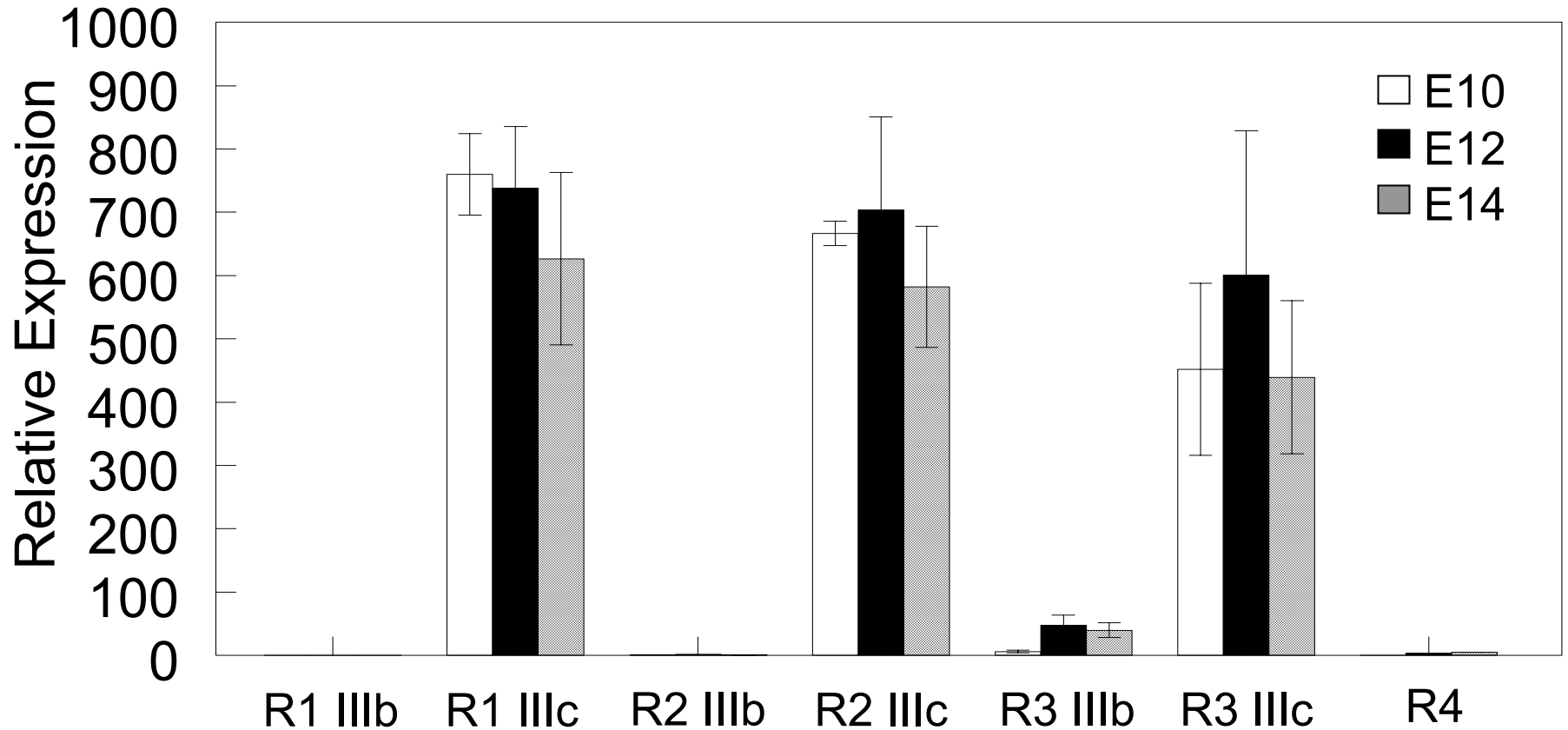


Brickman et al (1998) J Biol Chem 273, 4350-4359.

Hypotheses:

- synthesis of specific HS sequences is regulated *in vivo*, through controlled expression of sulfotransferases
- plays a role in development through regulatory interactions with specific growth factors

FGF & FGFR expression in neuroepithelium



Altered functional activity of neural precursor HS ?

FGFRs: 1, 2, and 3 (IIIc splice forms)

- transfected into BaF (lymphoid) cells lacking endogenous HS

with

FGFs: 1, 2 and 8

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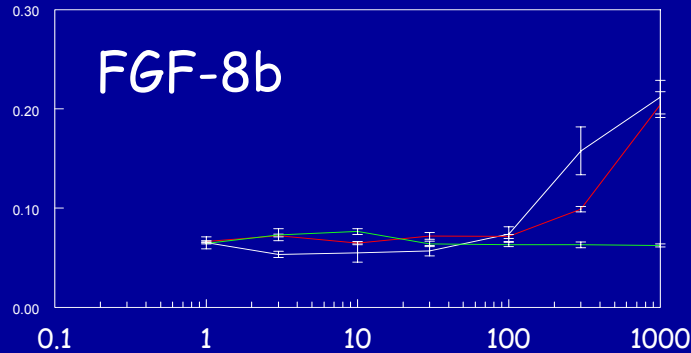
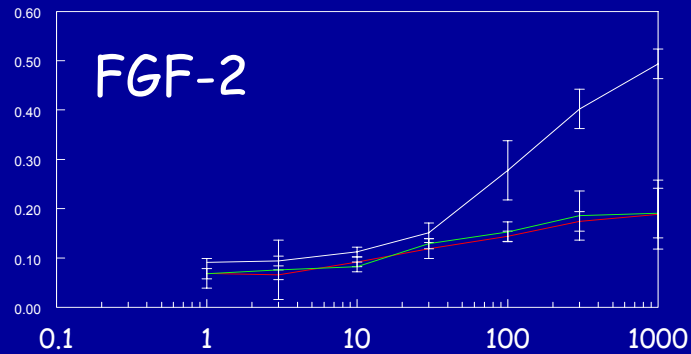
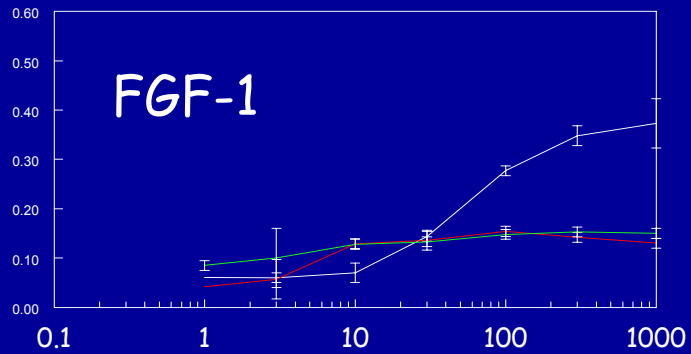
- transfected into BaF (lymphoid) cells lacking endogenous HS with

FGFs: 1, 2 and 8

- both E10 and 12 HS support FGF1 and FGF2 signalling via FGFR1 and 2
- no differences in activity

FGFR3 signalling

MTT Assay (A^{570})



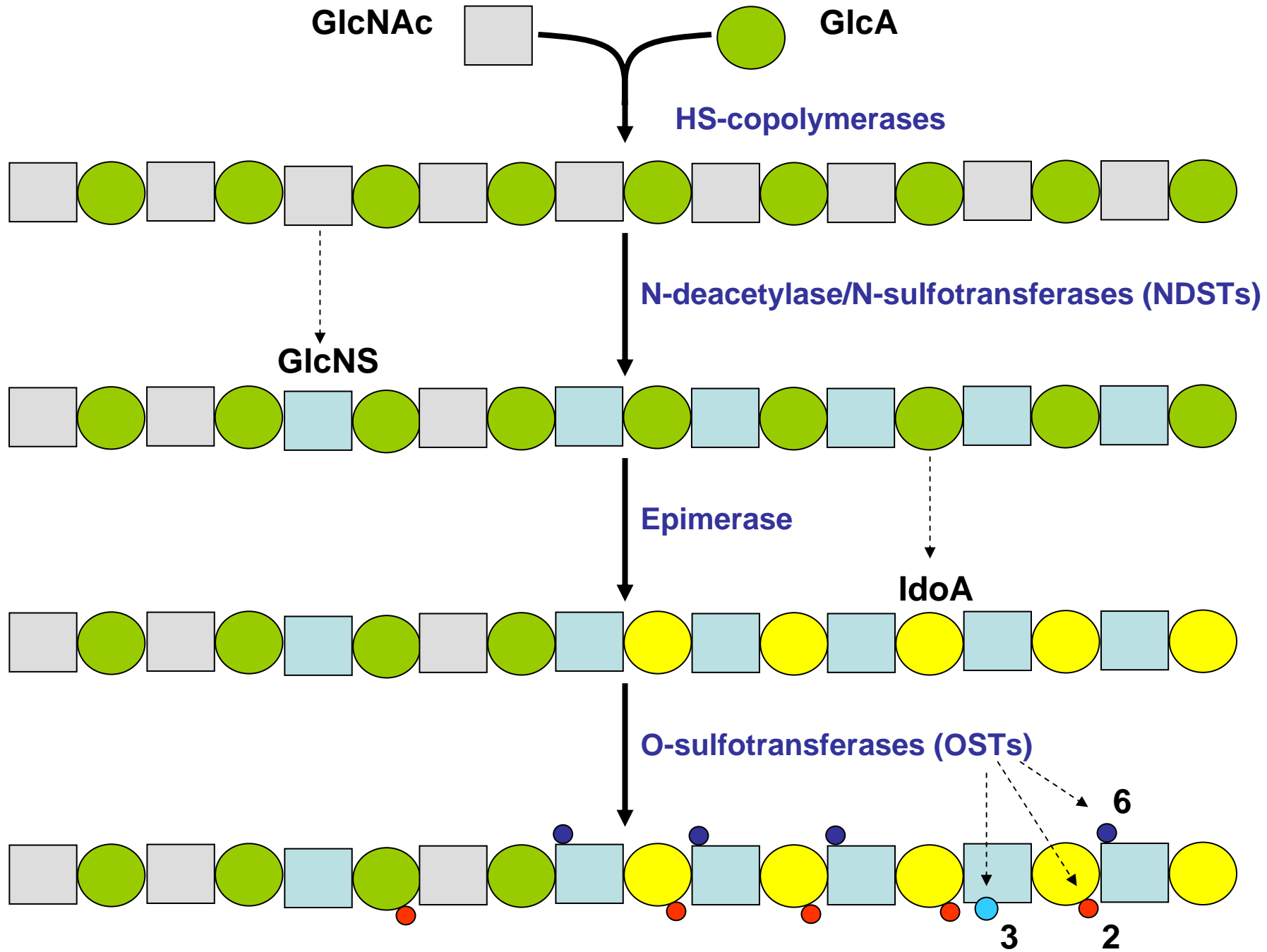
[Saccharide] (ng/ml)

.....but with **FGFR3**

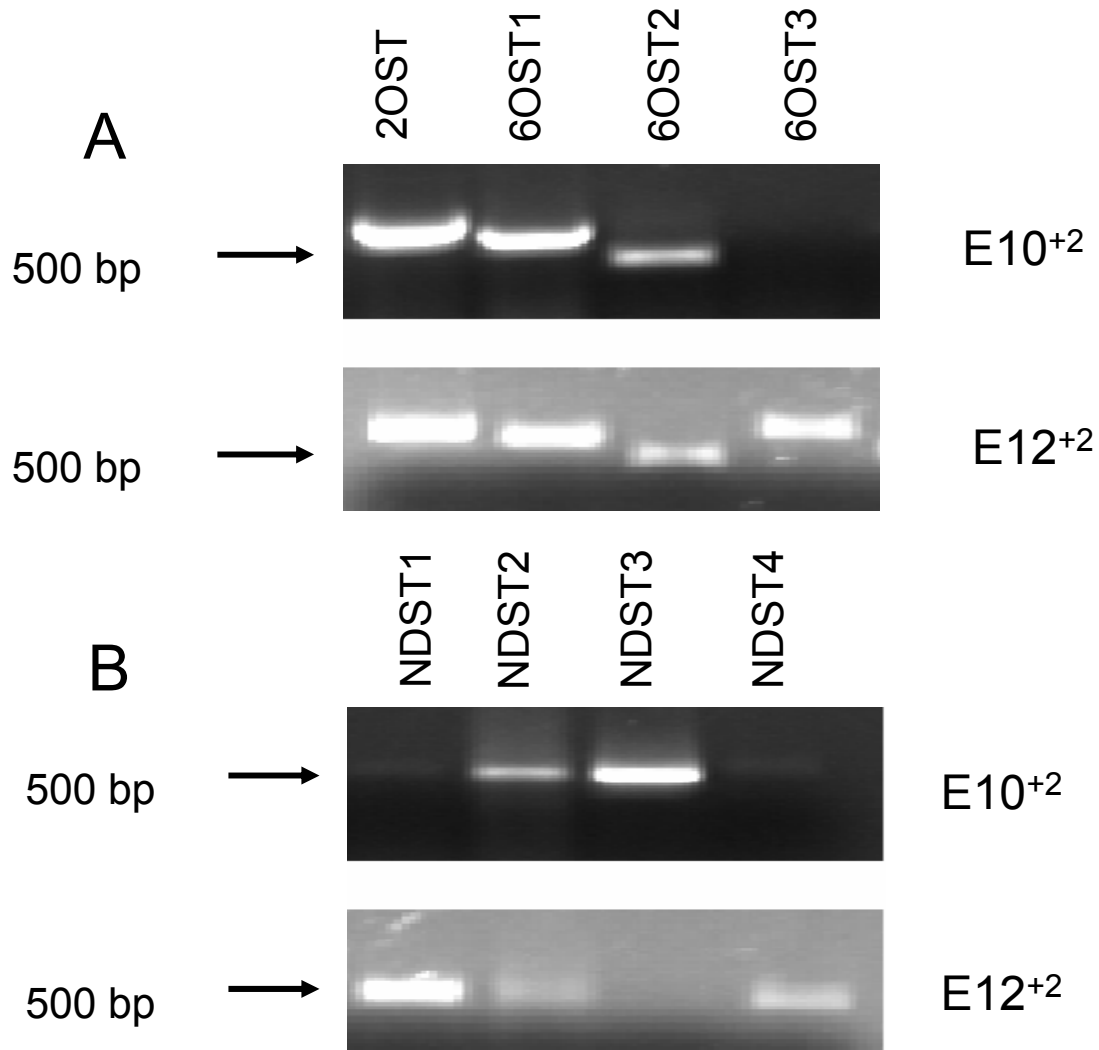
- only E10 HS activates signalling via FGF8

FGFR3c

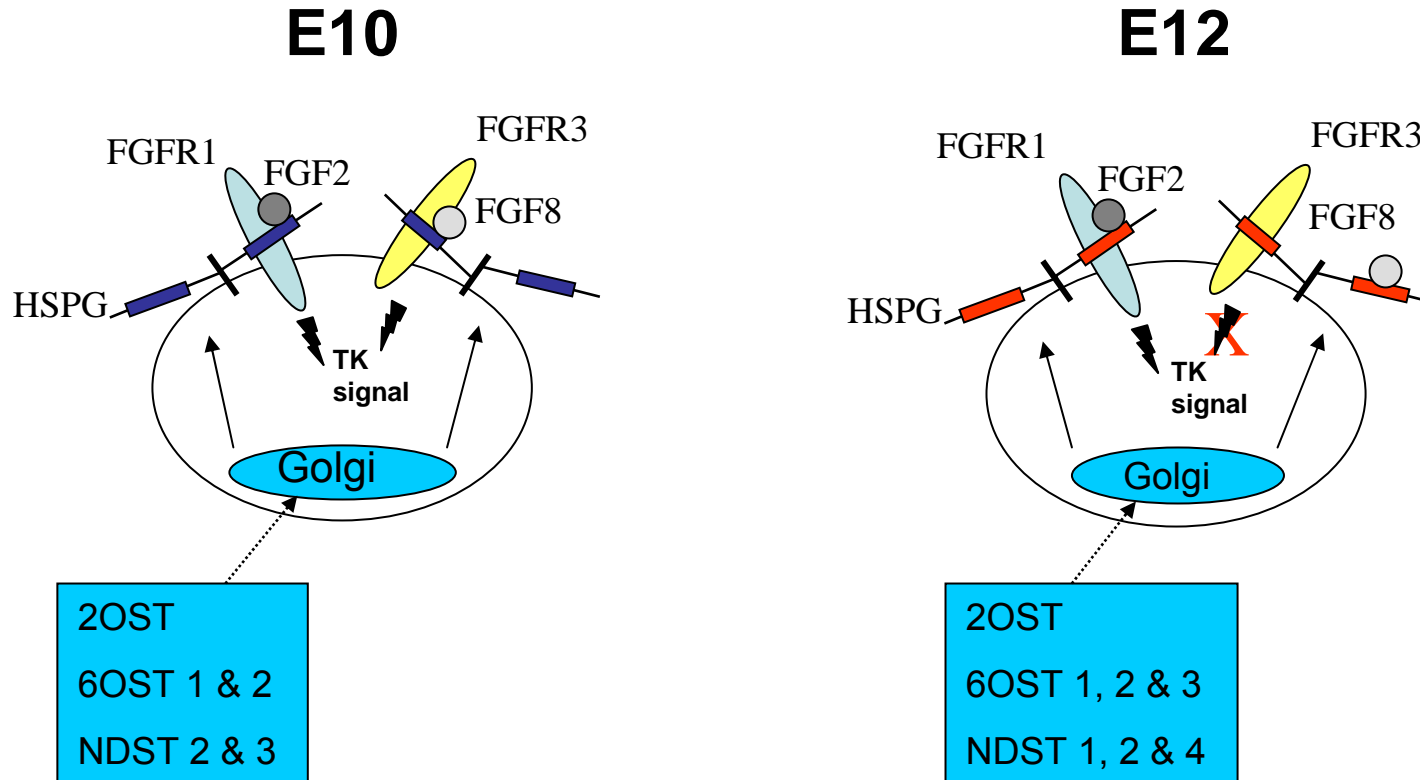
— Heparin
— E10
— E12



RT-PCR *in vitro* neural precursor cells



HS species expressed by neural precursor cells



- **dynamic HS biosynthesis produces structural variants with altered functional specificities**

A cautionary note.....

- Profiles of expression of HS biosynthetic enzymes are altered in cell culture vs in vitro
- Emphasises the need to study in vivo tissue HS for the real story !

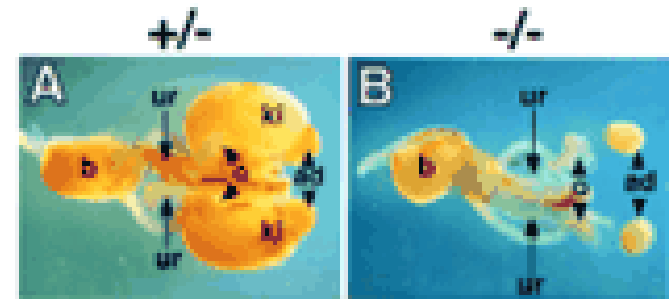
HS in mouse neurogenesis

- HS sulfotransferase isoforms are differentially expressed during early brain development
- 6OSTs have distinct spatiotemporal expression patterns & are strongly expressed in regions of active neuronal differentiation
- **dynamic expression profiles of HS sulfotransferases probably contribute to controlled changes in HS structure *in vivo***
- **dynamically altered HS structures could serve to actively regulate the responses of neuroepithelial cells to endogenous growth factors during neurogenesis**

What happens when HS biosynthesis is altered ?

Mouse brain HS: structure-activity studies in 2OST null mice

- examining structure, biosynthesis and activity of brain HS
- Evidence now being obtained that there are alterations in:
 - HS structure
 - HSST expression profiles
 - HS activity (eg in FGF signalling)
- **HS biosynthetic enzyme mutations – can produce both profound and subtle phenotypic effects**
- **selective and differential effects on signalling pathway regulation**



+ skeletal, eye and neural defects

Bullock et al 1999

C. elegans: a model organism for studying HS structure-function

adult hermaphrodite has 959 somatic cells (302 neurons)

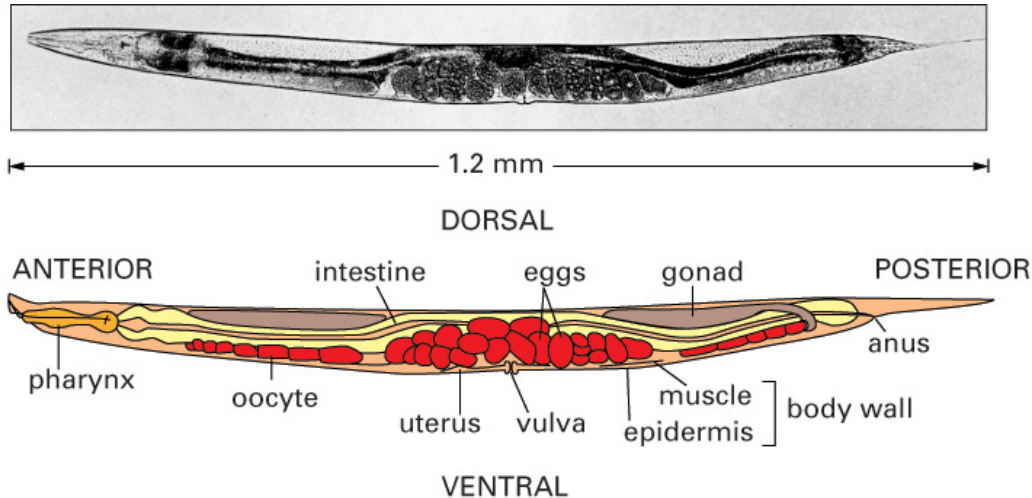
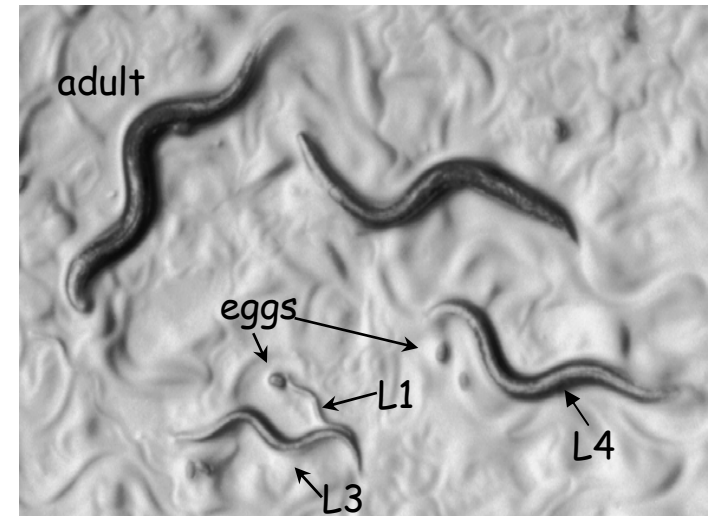
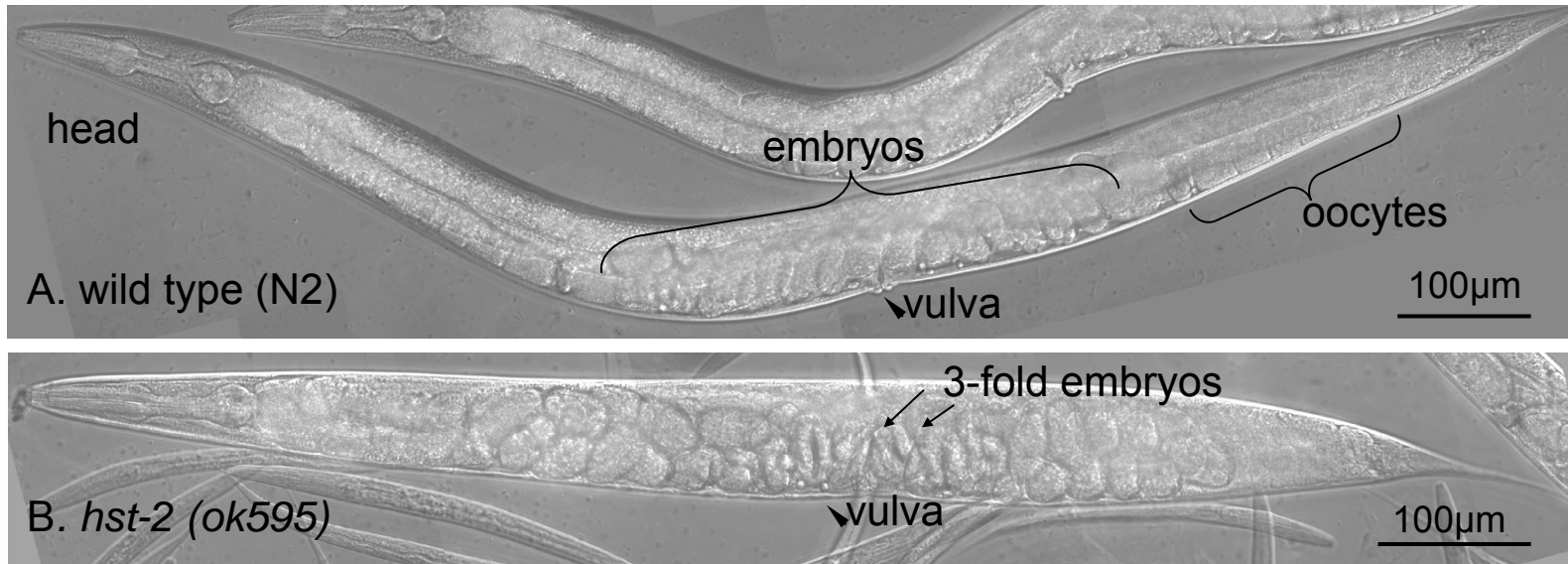


Figure 21-16. Molecular Biology of the Cell, 4th Edition.



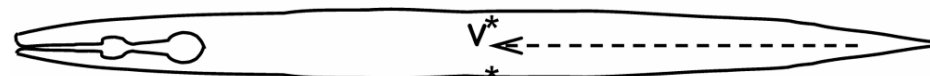
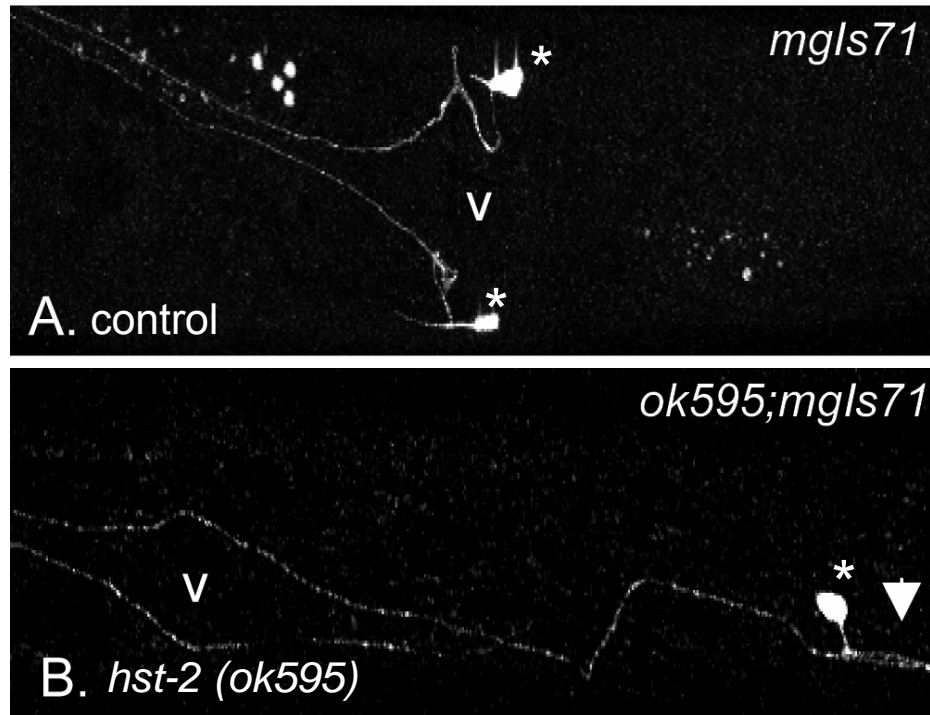
- complete genome data
- highly invariant cell-lineage
- life cycle 3 days at 25°C
- powerful genetic methods to study gene function *in vivo*
- single HS sulfotransferase and PG core protein orthologs

Lack of heparan 2-O-sulphotransferase activity in *C.elegans hst-2* mutant *ok595*, leads to egg laying defects (*egl*)



“Bag-of-worms”

Heparan 2-O-sulphation is required for the migration of the hermaphrodite specific neurons, HSNs



<i>mgl71</i>	50	2			
%	96	4			
<i>ok595;mgl71</i>	155	31	16	6	2
%	74	15	8	3	1

Mouse brain HS from EXT1 mutants

Question: Is the structure or activity of HS from heterozygous mice altered ??

- initial samples: gene trap EXT1LacZ +/- vs wt
- hypomorphic allele (hets have ~60% wild type EXT1 activity)
- HS purified from individual brains using rapid one-pot method
- initial data.....

Preliminary data

- Levels of HS – no apparent differences
- Possible smaller size of HS chains and differences in HS fine are currently under investigation

Future work

- Further detailed analysis of HS structure
- Activity studies (eg in FGF signalling)

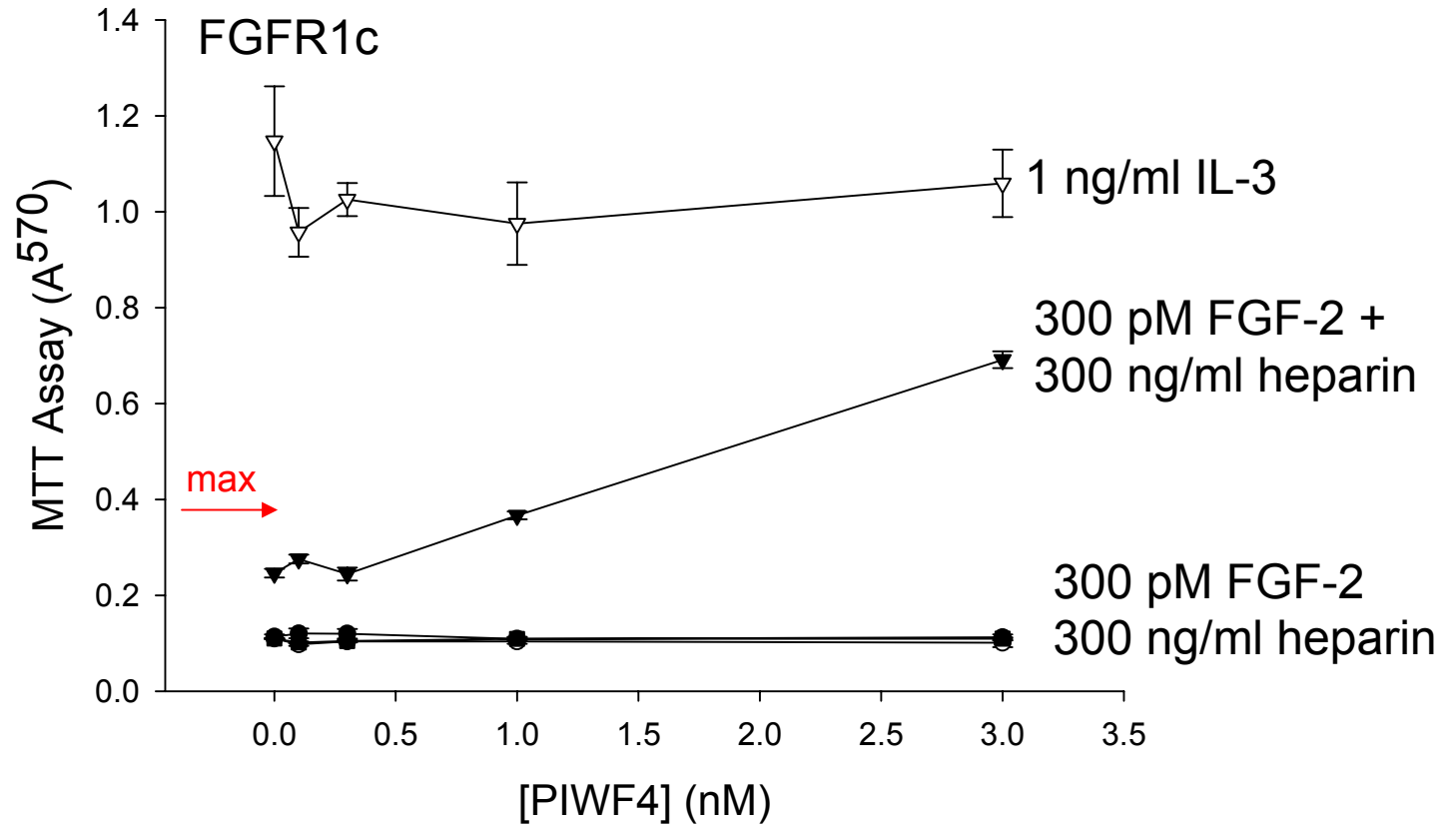
Comparison with HS from

- conditional brain EXT1 KO +/- mice (Yamaguchi)
- EXT1 +/- mice brain HS (Esko)

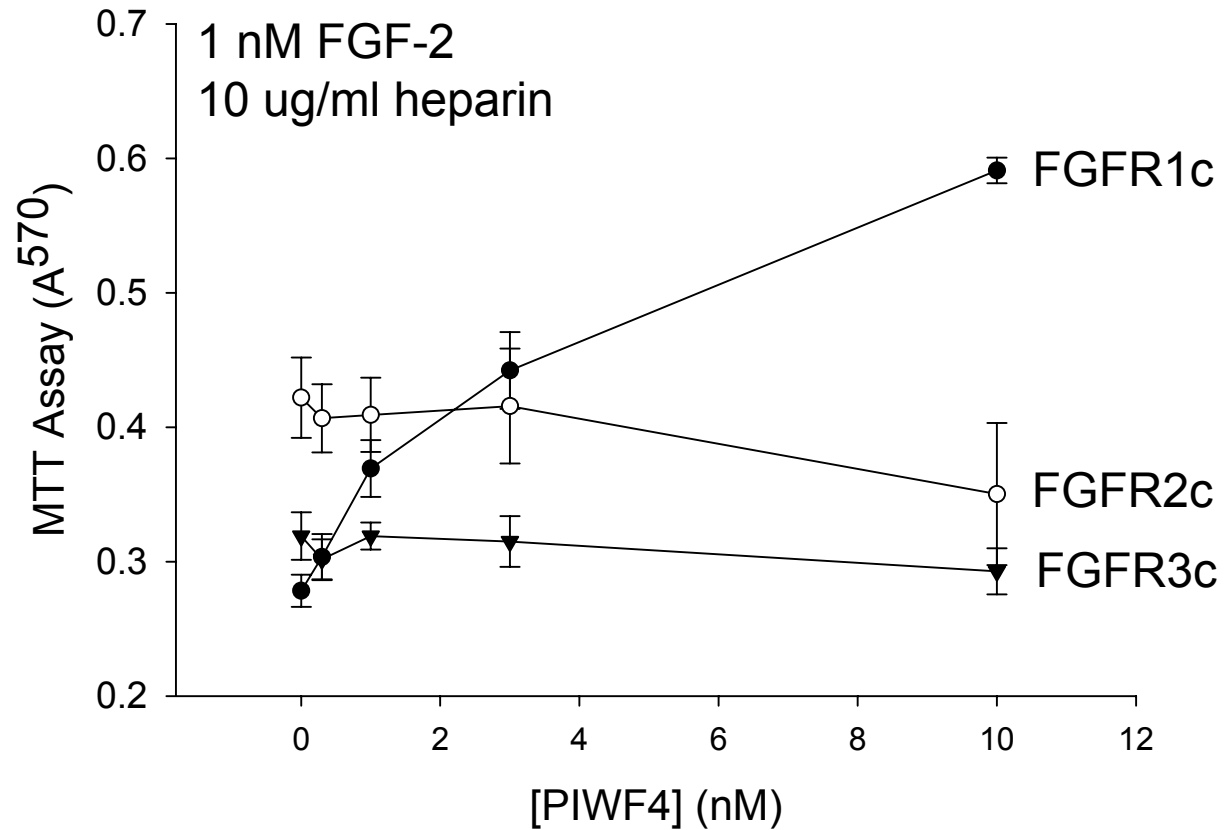
Kallmann syndrome

- hereditary KS - a human disorder due to abnormalities of development of olfactory and gonadotropin-releasing hormone-1 (GnRH-1) neurons
- Clinical phenotype – hypogonadism, anosmia (lack of sense of smell), mirror image movements
- Genetic defects
 - X-linked (KAL-1): anosmin-1 secreted ECM glycoprotein
 - Autosomal (KAL-2) – recently discovered to be the FGFR1 gene

Anosmin amplifies FGFR1 signalling response

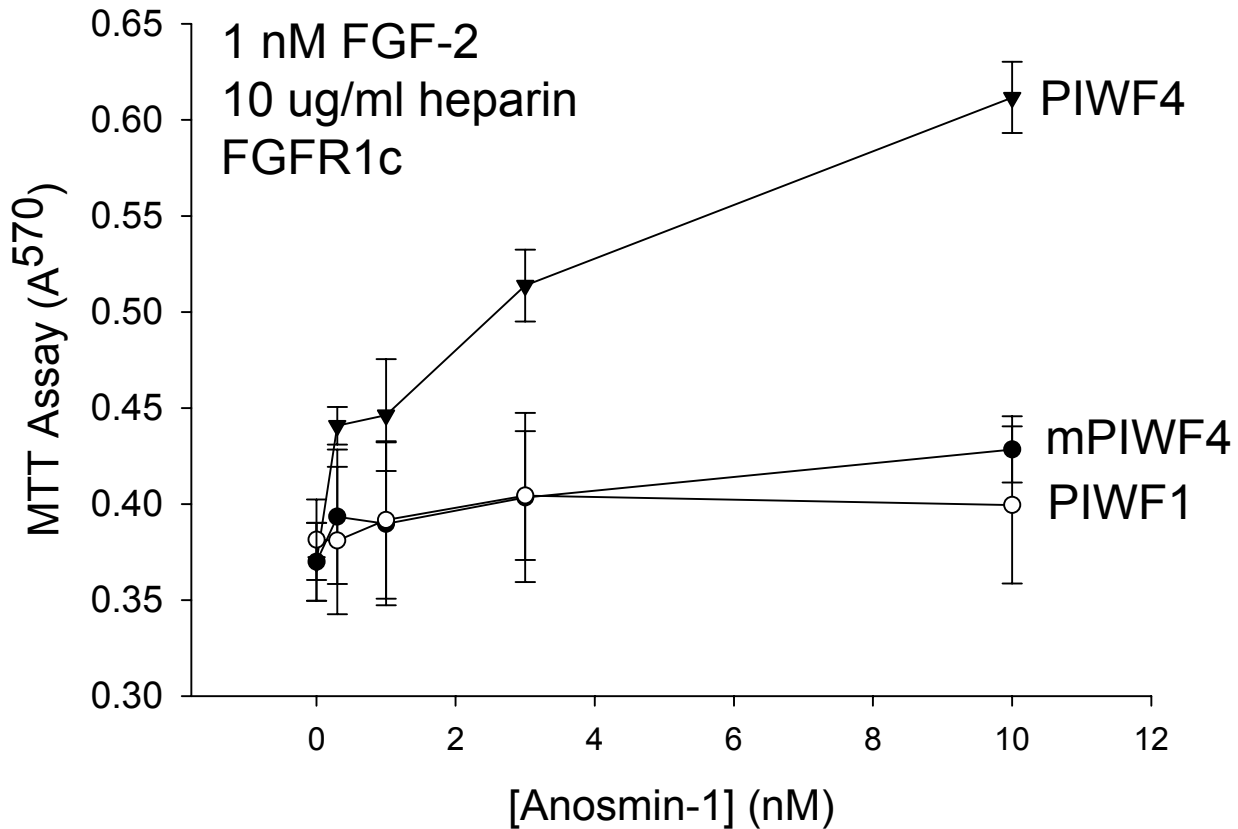


Amplification is specific for FGFR1 signalling



Mutant anosmin has greatly reduced activity

C172R mutation seen in X-KS disrupts disulfide core motif in WAP domain



Summary – Anosmin & HS in Kallmann syndrome

- uncovered a functional interaction between anosmin-1 and the FGFR1/FGF2/heparan sulphate (HS) complex
- anosmin mediates amplified responses in FGFR1 signalling in a HS-dependent manner
- these data provide the first evidence for anosmin-1 acting as an isoform-specific co-ligand modulator of FGFR1
- both amplifies and specifies FGFR1 signalling
- **reveal a defined molecular mechanism linking the autosomal and X-linked forms of KS**

Gonzalez-Martinez et al J Neuroscience (2004) 24, 10384-10392

Conclusions

- HS biosynthesis is a dynamic process that produces functionally variant HS species
 - these are used by organisms to create robust mechanisms for regulating many cellular processes
- mutations in HS biosynthetic enzymes (even in the heterozygous state) can result in significant changes in the molecular phenotype of HS
 - producing sometimes profound, but quite often subtle, effects on the organism phenotype
- its very complex ! simple answers are rare !
 - interplay of multiple functions of HS with multiple HS-dependent factors
- HME is a human condition that provides opportunities for fundamental insights into the critical need for properly orchestrated HS structure-function relationships
- hopefully improved understanding of HS function will lead to opportunities in the future for therapeutic intervention in HME



Imagine all the people.....acknowledgements

Turnbull lab

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