Metabolic studies on Hif-p4h-2 hypomorphic mice

The three Rs in metabolism research

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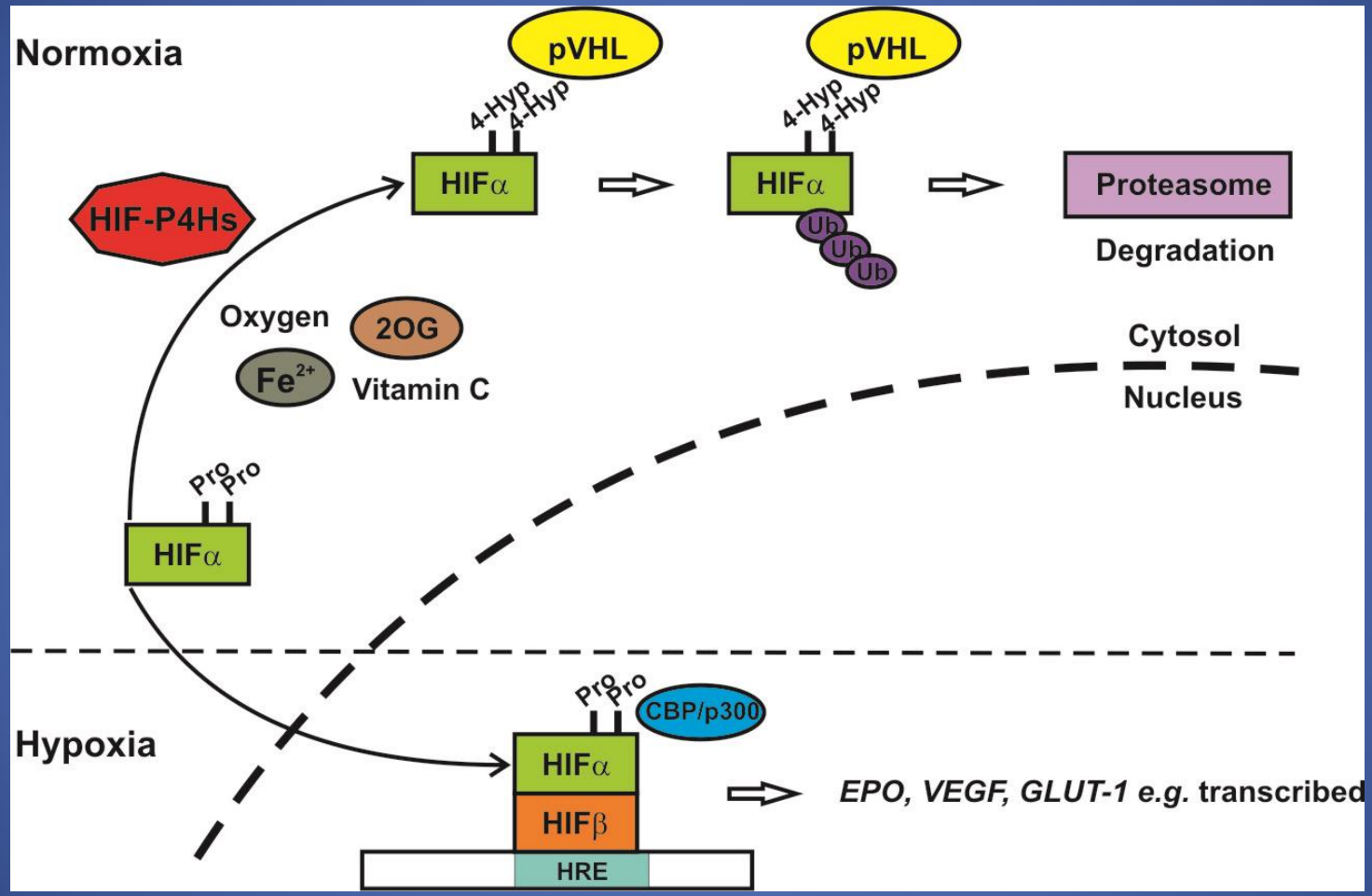
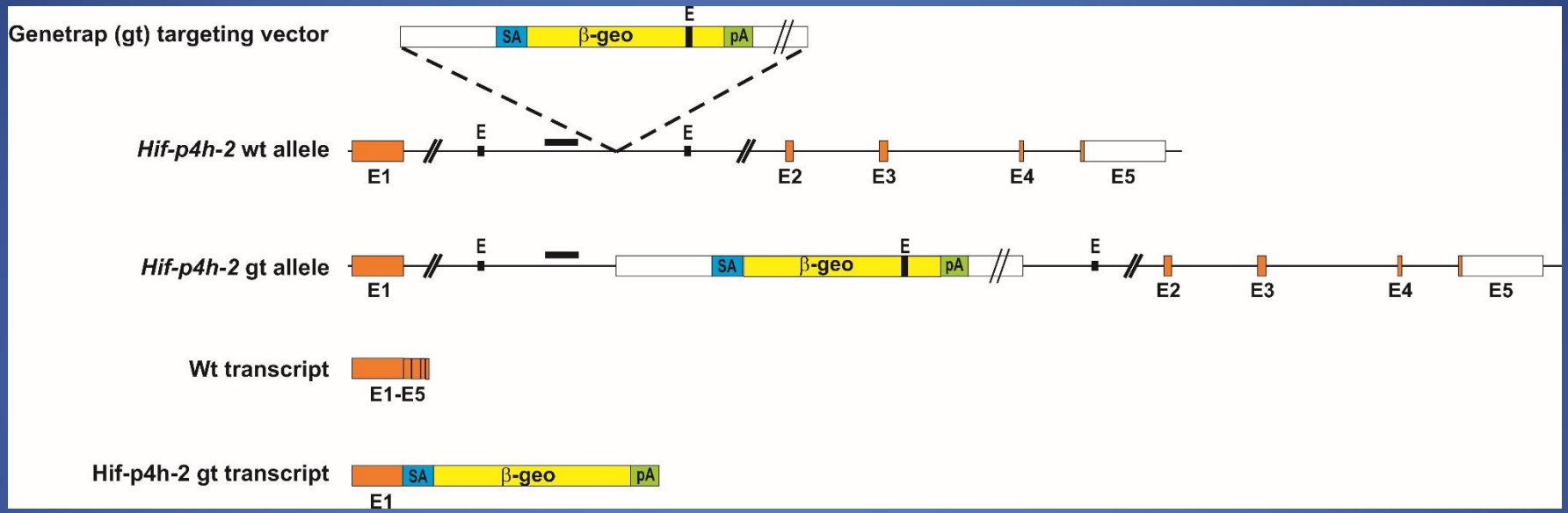
Hypoxia response



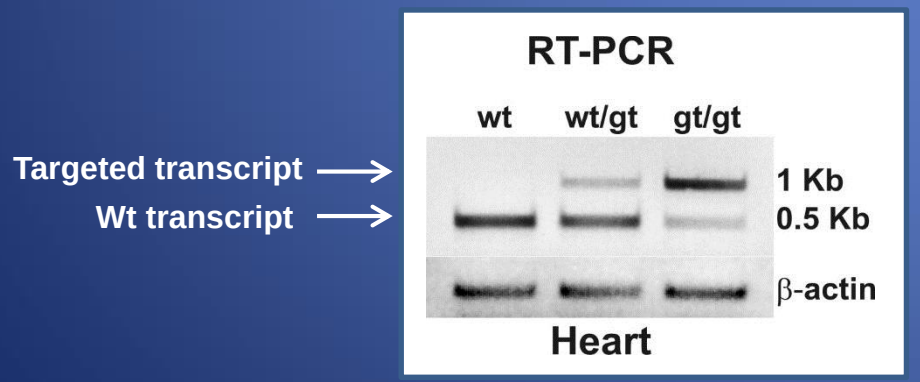
Hypoxia inducible factor prolyl 4-hydroxylases (HIF-P4Hs) act as cellular oxygen sensors

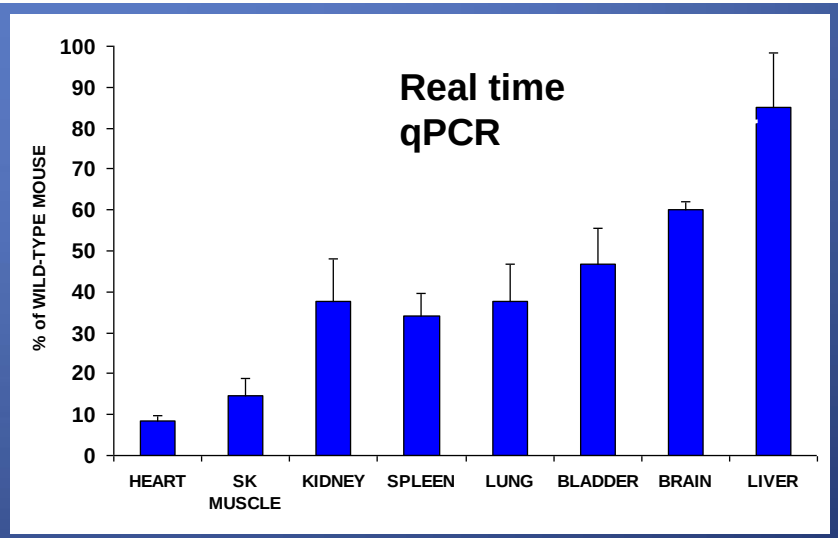
Hypomorphic *Hif-p4h-2*$^{gt/gt}$ mice express different Hif-p4h-2 levels in different tissues



E1- 5: exon 1- 5

E: *Eco*RI





Hyvärinen et al. 2010 JBC

Statistics of *Hif-p4h-2*$^{gt/gt}$ mice

453 born
between June – December 2015

90 mice
(20%) gt/gt

129 mice
(28%) wt

234 mice
(52%) gt/wt

All gt/gt and wt used for experiments

20 females, 15 males
used for breeding

Experiments

High fat diet

- To study changes in glucose metabolism
- mice are fed a standard rodent diet (18% kcal fat) or an high-fat diet (42% kcal fat) for 6 weeks

FG-4497 treatment

- Small molecule compounds that inhibit HIF-P4Hs are being developed for the treatment of e.g. anemias
- FG-4497, 60 mg/kg, is administered orally three times a week

Glucose tolerance test

- Mice that had fasted for 12 h are anesthetized with fentanyl/fluanisone and midazolam
- Fasting blood glucose measured
- The mice are then injected i.p. 1 mg/kg glucose and blood withdrawn at 15, 30, 60 and 120 min post injection for blood glucose

Alcoholic fatty liver disease -study

- Protection against steatosis and fatty liver disease with HIF-P4H-2 inhibition?
- Genetic model (*Hif-p4h-2*$^{gt/gt}$ mice and wt littermates)
  - three weeks on 5% ethanol diet
- Pharmacologic model (FG-4497) with wild-type mice
  - four weeks on 5% ethanol diet
  - Simultaneous treatment with FG-4497 or vehicle

Metabolic cage measurement

- Drinking and feeding behavior
- Metabolic performance (O_2 consumption/CO_2 production, respiratory exchange ratio and heat production)

For more information please visit our web page at:
http://cc.oulu.fi/~fysiowww/KHHmpr.html

Animal welfare

Analgesia

- Depends on the experiment

Constant monitoring (daily, several times per day if needed)

Higher room temperature post-operation (24-25°C)

Housing in single cages/with other animals

Humane endpoints

- Overall appearance (dehydration, abnormal posture, condition of fur), movement, behaviour, eating, weight loss
- If problems arise, animal is treated (hydration, antibiotics)
- If no improvement, animal is sacrificed

The 3Rs

Replacement

- We always use cell culture experiments when feasible
- E.g. silencing of HIF2α with siRNA to splenocytes in cell culture compared to AAV to mice

Reduction

- Collection of all tissues instead of one when ever we sacrifice animals
- Refinement of methodology – smaller variation, less animals needed to reach statistical significance in results
- Starting with 4+4 or 6+6, adding more animals if needed

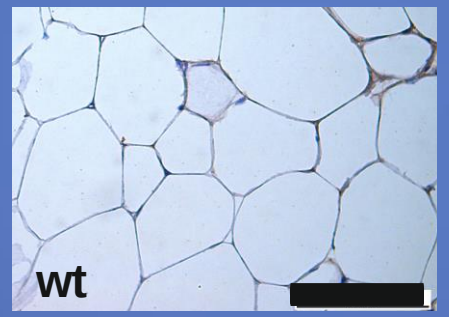
Refinement

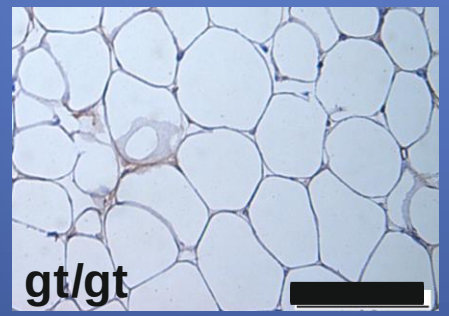
- Pain medication
- Experienced personnel: not everybody does everything but the experienced one does

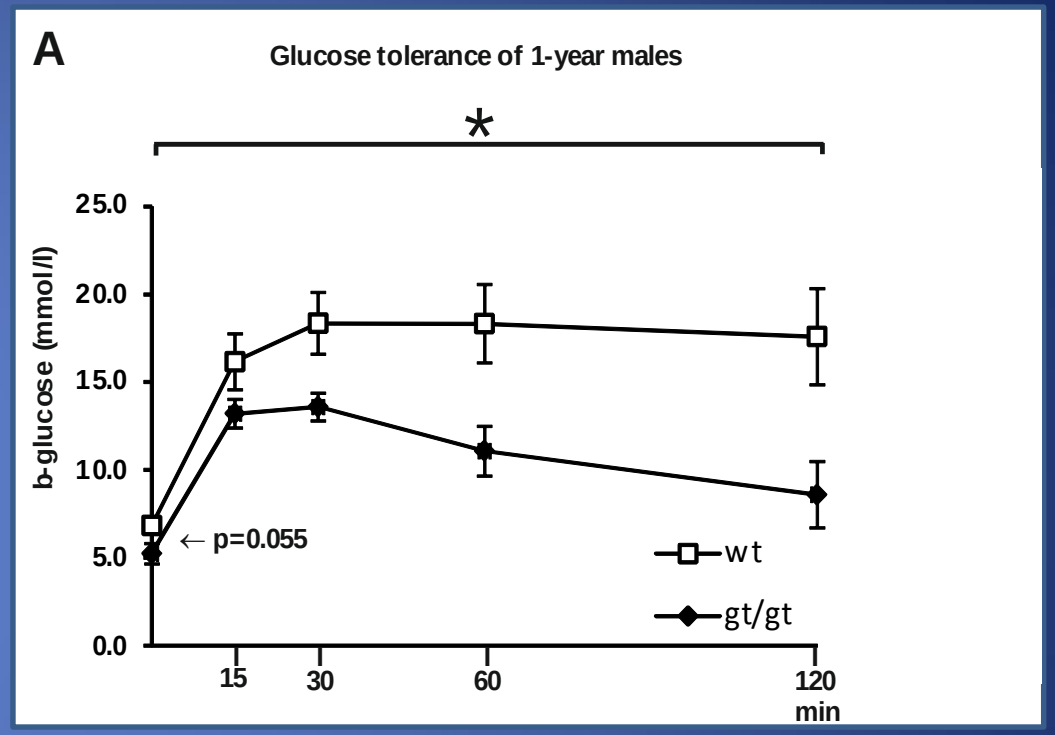
Importance of constant environment – an example when something went wrong

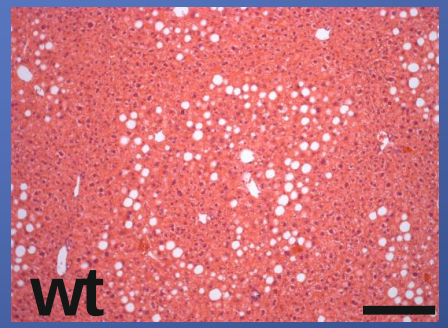
Protection against obesity and metabolic dysfunction

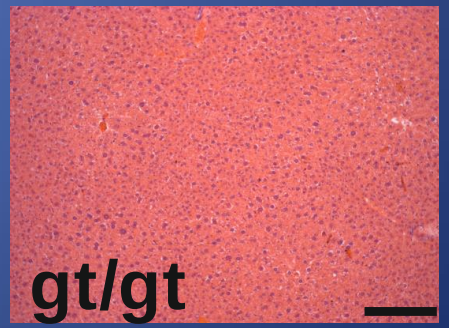
- Can HIF-P4H-2 inhibition be used to treat obesity and its consequences?
- *Hif-p4h-2*$^{gt/gt}$ mice were leaner and had less adipose tissue and smaller adipocytes
- They also had improved glucose tolerance
- Mice were protected against hepatic steatosis
- ⇒ HIF-P4H-2 inhibition may not only protect against the development of obesity and its consequences but also reverse these conditions











Rahtu-Korpela et al. 2015 Diabetes