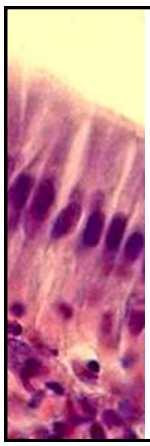


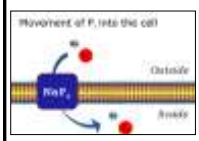
NaPi and P-responsive genes

---- Practical considerations ----

Shozo Sugiura
School of Environmental Sciences,
University of Shiga Prefecture,
Japan

NaPi transporters

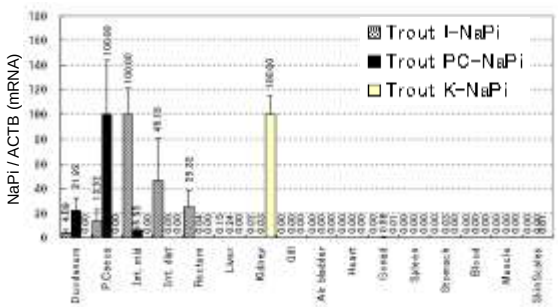
(SLC34A2, NaPi-IIb, Npt2b)



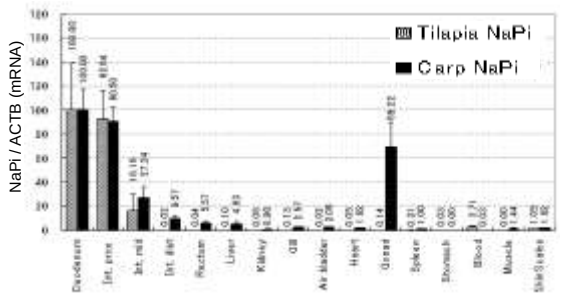
up-regulated by dietary P restriction

identified in various fishes and shellfishes

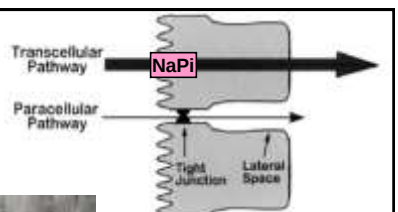
In **Trout,** NaPi is expressed in the intestine and kidney



In **Tilapia** and **Carp,** the intestinal NaPi is expressed in various other tissues



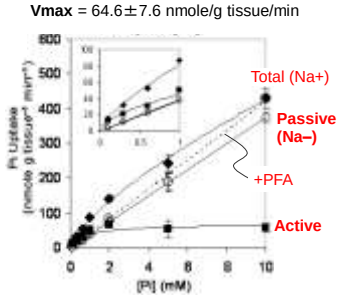
Pi transport



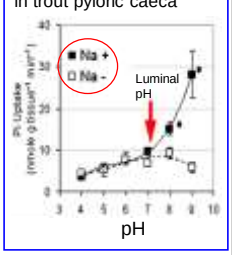
Pi-uptake assay with everted intestinal sleeves

Pi-absorption is mostly Paracellular under physiological luminal [Pi] and pH

Km = 0.474±0.199 mM;
Vmax = 64.6±7.6 nmole/g tissue/min



Effect of pH on Pi uptake in trout pyloric caeca

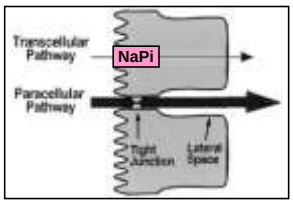


% of **active component** in total Pi transport is:

99.8% at 0.3 nM

57% at 1 mM

~5% at 20~40 mM
physiological luminal [Pi]



Not important

- QTL mapping and selection for NaPi expression
- NaPi-transgenic fish that over-express NaPi

NaPi and P-responsive genes

----- *Other possible applications* -----

⊙ Response indicator (for large fish)

Early (sensitive) response : **Diagnostic marker**

- Transgenic P-sensor fish

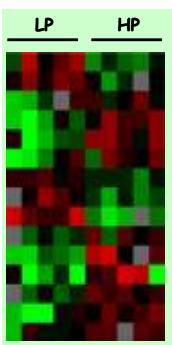
P-response gene promoter + GFP

for real-time detection of a P-deficiency

practically unfeasible

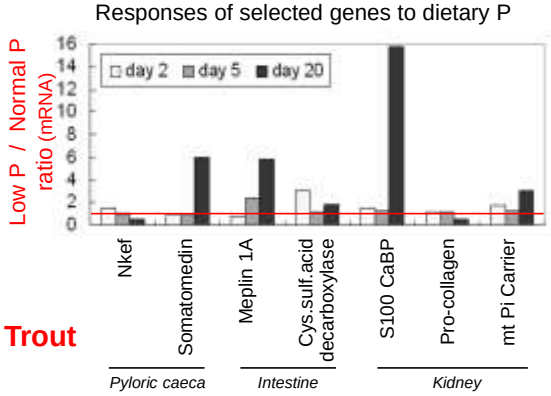
P-deficient fish

Subtractive hybridization & 16K microarray for intestinal genes responsive to changes in dietary P



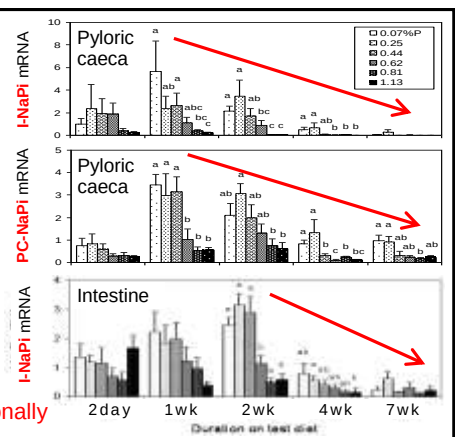
Gene name	*Response*
Type-II sodium-Pi cotransporter	++
Meprin –1-alpha	++
Carbonic Anhydrase XIII	++
Sulfotransferase 2B1	++
Mitochondrial Pi carrier	++
S100 Ca-binding protein	++
Cystein sulfinic acid decarboxylase	++
Nephrosin	- -
Trypsin II	- -
Gastrotropin	+
Vitamin D receptor	+
24-OHvitamin-D24-hydroxylase	+
Dipeptidyl peptidase IV	+
Mx1 protein	-
Ubiquitin-protein ligase E3A	-
VSHS-induced protein	-

Responses of selected genes to dietary P

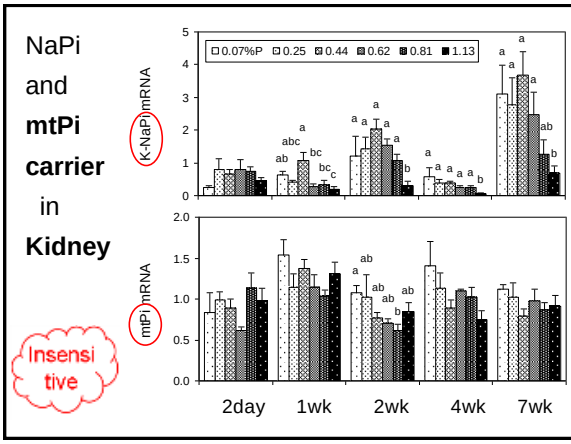


NaPi in the GI tract

NaPi is regulated post-transcriptionally

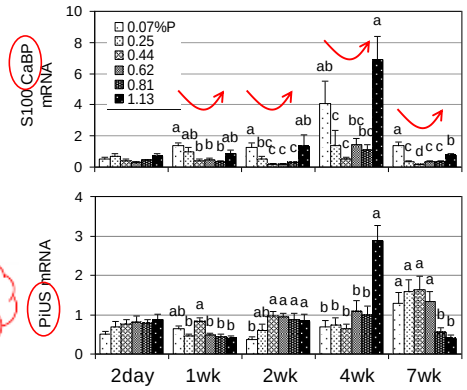


NaPi and **mtPi carrier** in **Kidney**



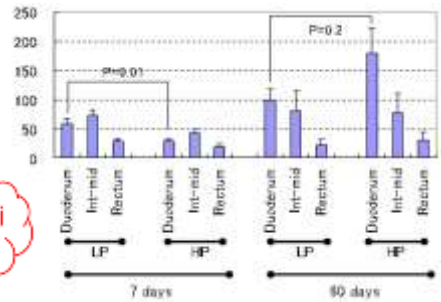
Insensitive

S100 CaBP and **PiUS** in **Kidney**



Insensitive

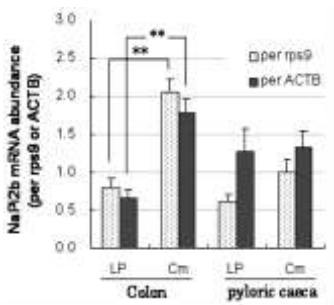
Low P diets may not increase NaPi mRNA in **Carp**



Insensitive

Low-P diet vs. Commercial diet

NaPi expression in **Salmon** fed Low-P diet (LP) or Commercial diet (Cm) for 3 months.



Oppo-site!

Other genes ?

- FGF23 - Klotho
- CYP27B1, VDR,
- miRNA
- Transcription factors, signaling cascades

---- May be responsive

But, tissue collection~ RNA extraction~ RT~ qPCR for P-response gene + housekeeping gene

--- not quick or easy, compared with existing methods

➜ not so useful

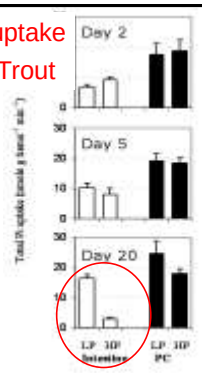
NaPi proteins ?

- NaPi protein expression could be more reliable than mRNA expression

- But, measuring protein expression is laborious and time-consuming

➜ practically infeasible

Pi uptake in Trout



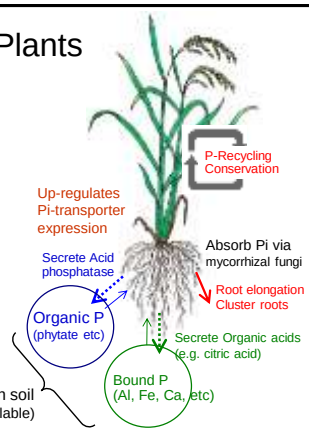
The incubation medium contained 0.1 mM Pi, where the active component represented ~75% of total Pi uptake.

P absorption in Plants

Soil [Pi] is low (< 10µM)

Thus, for plants, Pi-transporters are very important

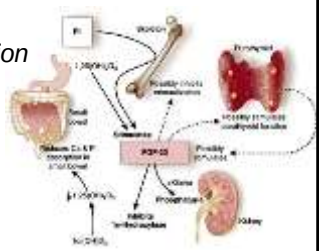
---- plants have various Pi-transporters



P studies in Humans

Goal is opposite:

to prevent P absorption in the GI tract ----
for CKD patients



1---- Low-P diets,
2---- Pi binders,
3---- Inhibitors of Pi-transporters

In fish, Pi-transporters are not functionally limiting the intestinal Pi absorption

Important considerations

To Increase Intestinal P absorption, → 1. Chemical form of P; 2. Luminal environment

To minimize P excretion, → 3. Minimize dietary P

Low pollution