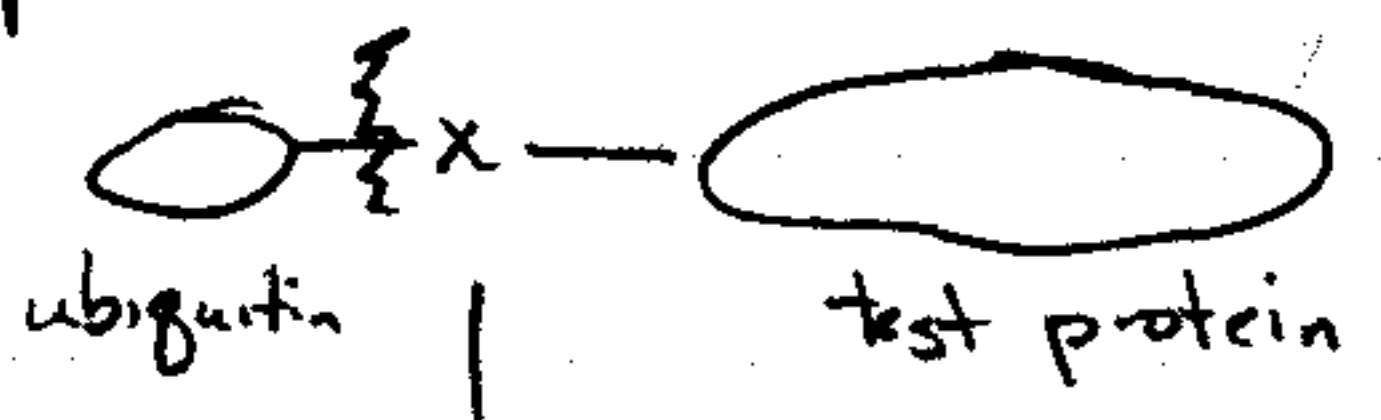


10/18/93

# Alex V. - Ubiquitin

## Ubiquitin fusions

UBIQUITIN - only in eukaryotes



↓ Ubiquitin-specific protease  
- v. fast



↓ degradation

1/2 life varies depending on X

- |     |     |     |
|-----|-----|-----|
| Arg | Leu | Ile |
| Lys | Trp | Asp |
| Phe | Tyr | Glu |
|     | His | Asn |
|     |     | Gln |

} 0-30'

These have short 1/2 life bec. of spec. proteins that recognize them.

- |     |     |
|-----|-----|
| Cys | Thr |
| Ala | Gly |
| Ser | Val |
| Met | Pro |

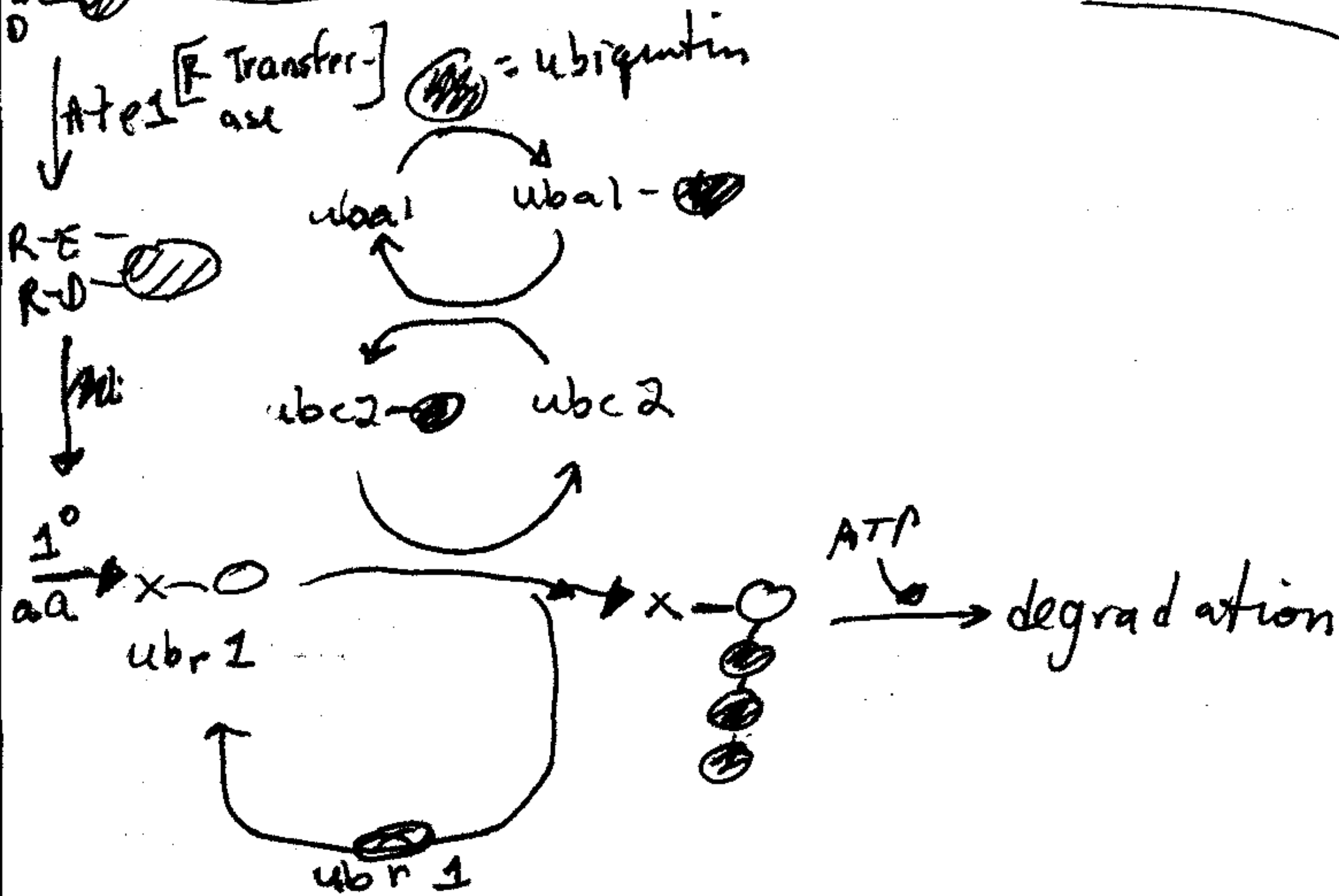
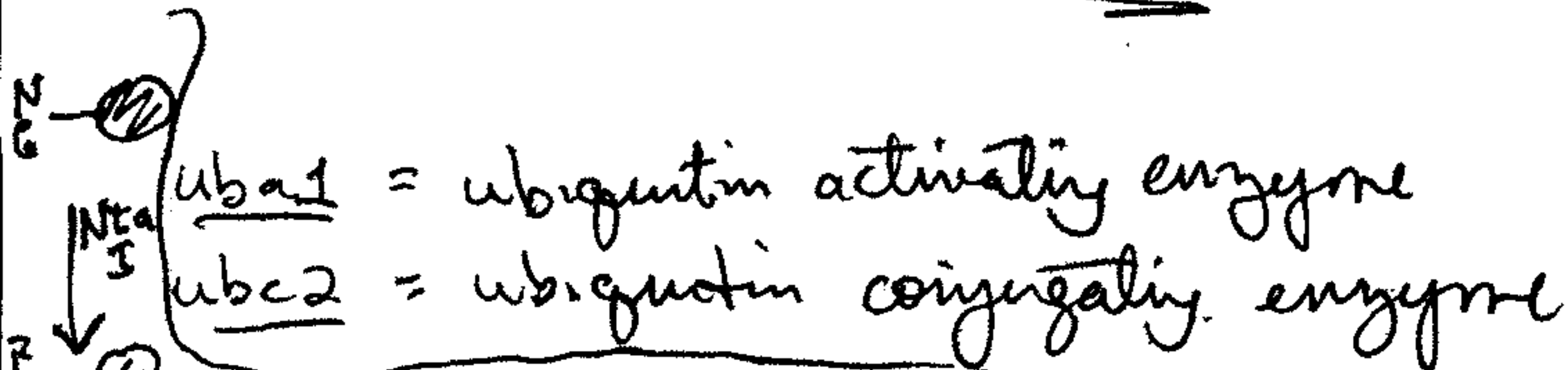
} > 20 hours

NtAI = N-terminal amidase

= N D  
6 → E

Ubr1 N-recognize

- binds to N-terminal 1<sup>o</sup> a.a.



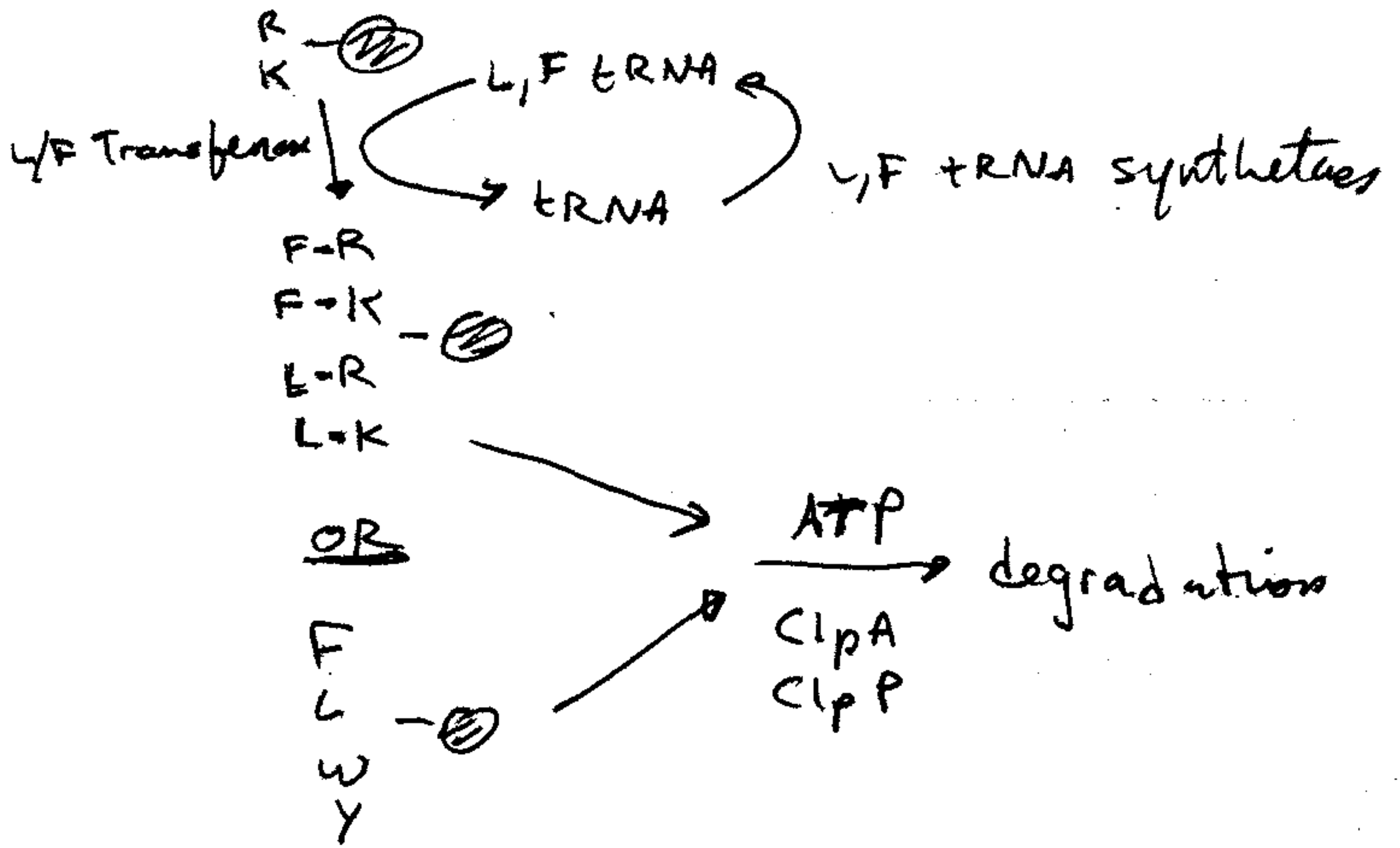
ubc2 = RAD6

Nend rule in bacteria

2ary

katH

1ary



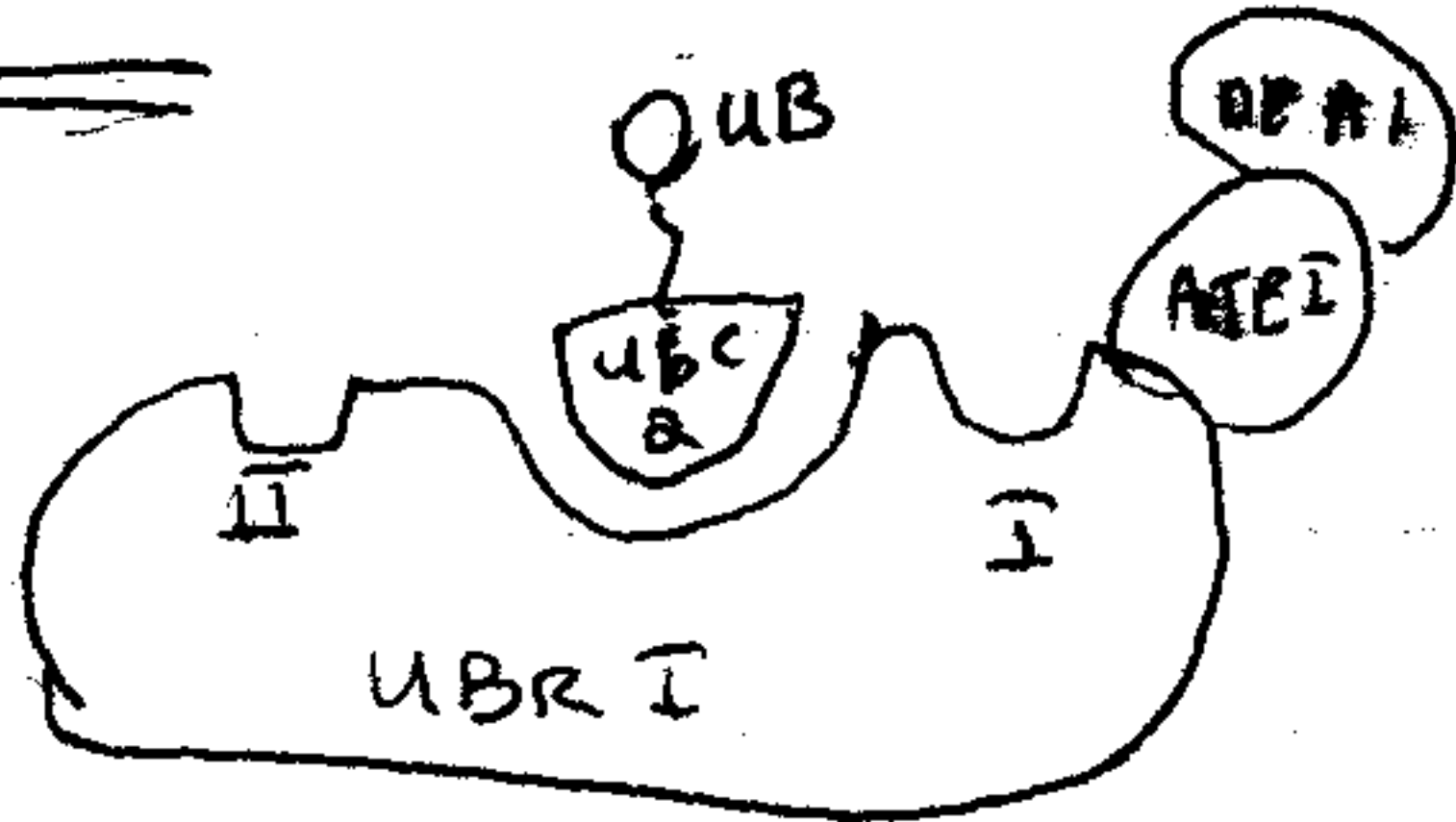
cat = near clpA gene

1<sup>o</sup>  
destab  
1<sup>o</sup>

2<sup>o</sup>

3<sup>o</sup>

Bacteria	FLWY	RK	
Yeast	FLWY I R K H	DE	
Mammals	FLWY I R K H	DEC	-



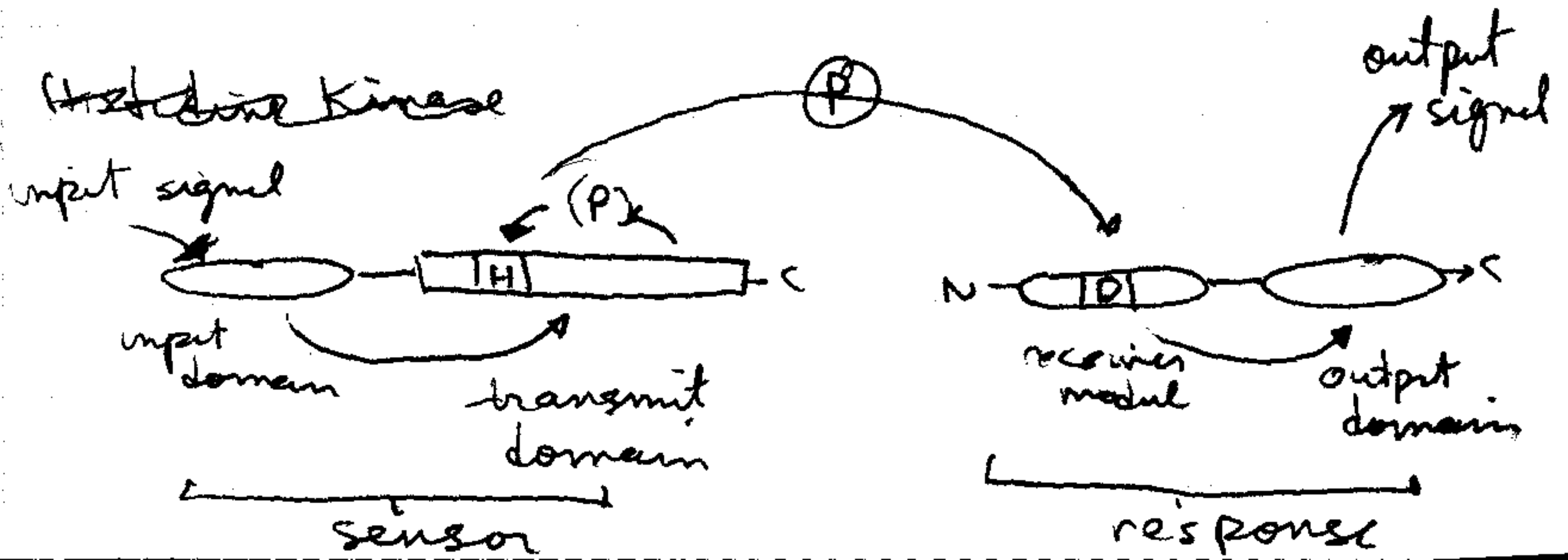
I, II = binding sites  
I = basic sites  
II =

Yeast

- synthetic lethal search - are there any mutants which are lethal when N-end rule pathway is gone

Slr gene

- two component regulatory systems



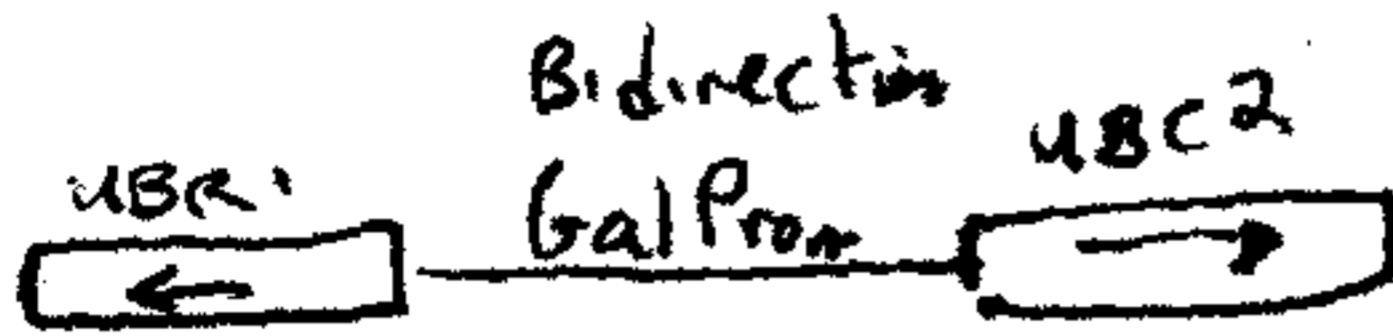
output signal ... usually change in DNA  
binding activity

another 2 component = ethylene receptor (Meyerowitz)

also Sln gene = two component system

KO = dead on rich media  
= weak on poor media

Overexpression of the pathway



+  
Pcup<sub>2</sub> vector

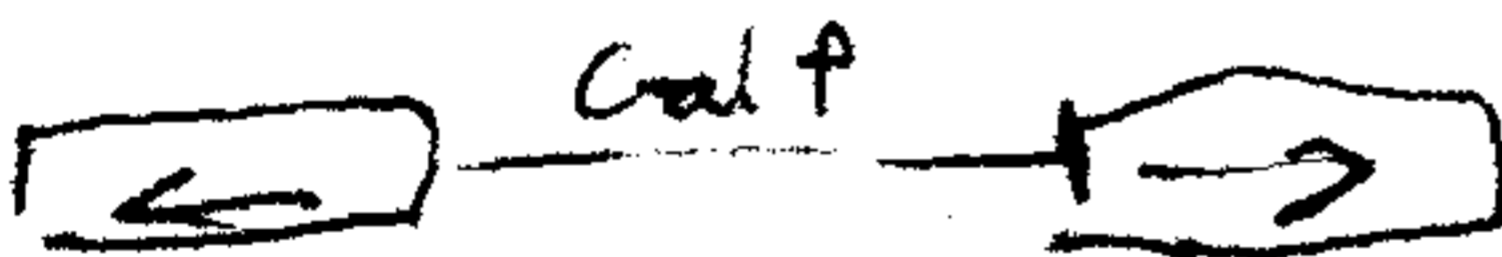
Media  
Gluc + Gal -

Also - only happens in haploids \*



+  
Pcup<sub>2</sub> vector

+ +  
∴ for lethality both genes needed

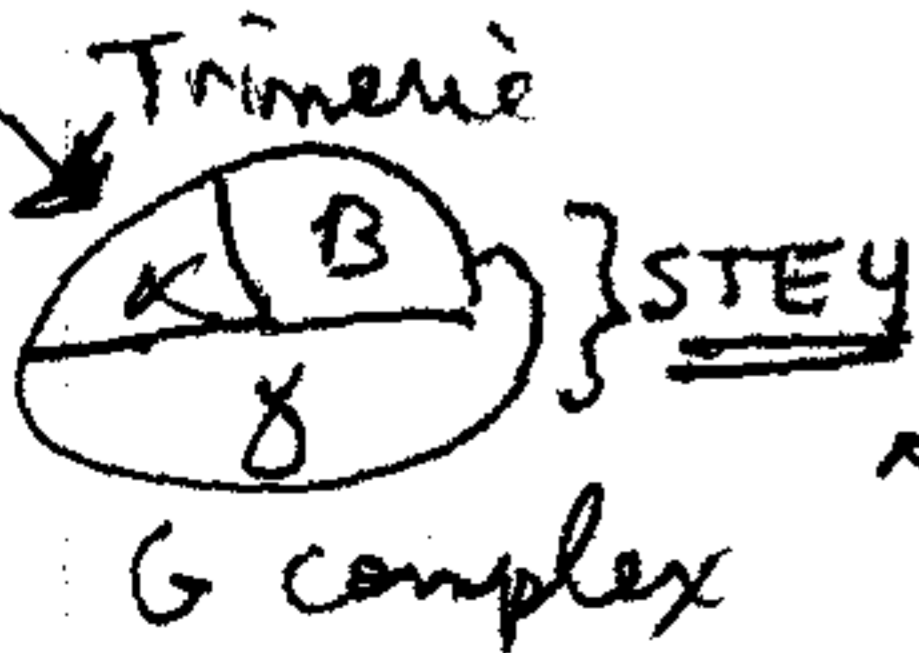


+  
Pcup<sub>2</sub> vector

+ +/-  
- not wt but OR

∴ GPAS suppresses

lethality ... probably through dec. in inhibitors or cell-proliferation



↑  
Mating factor

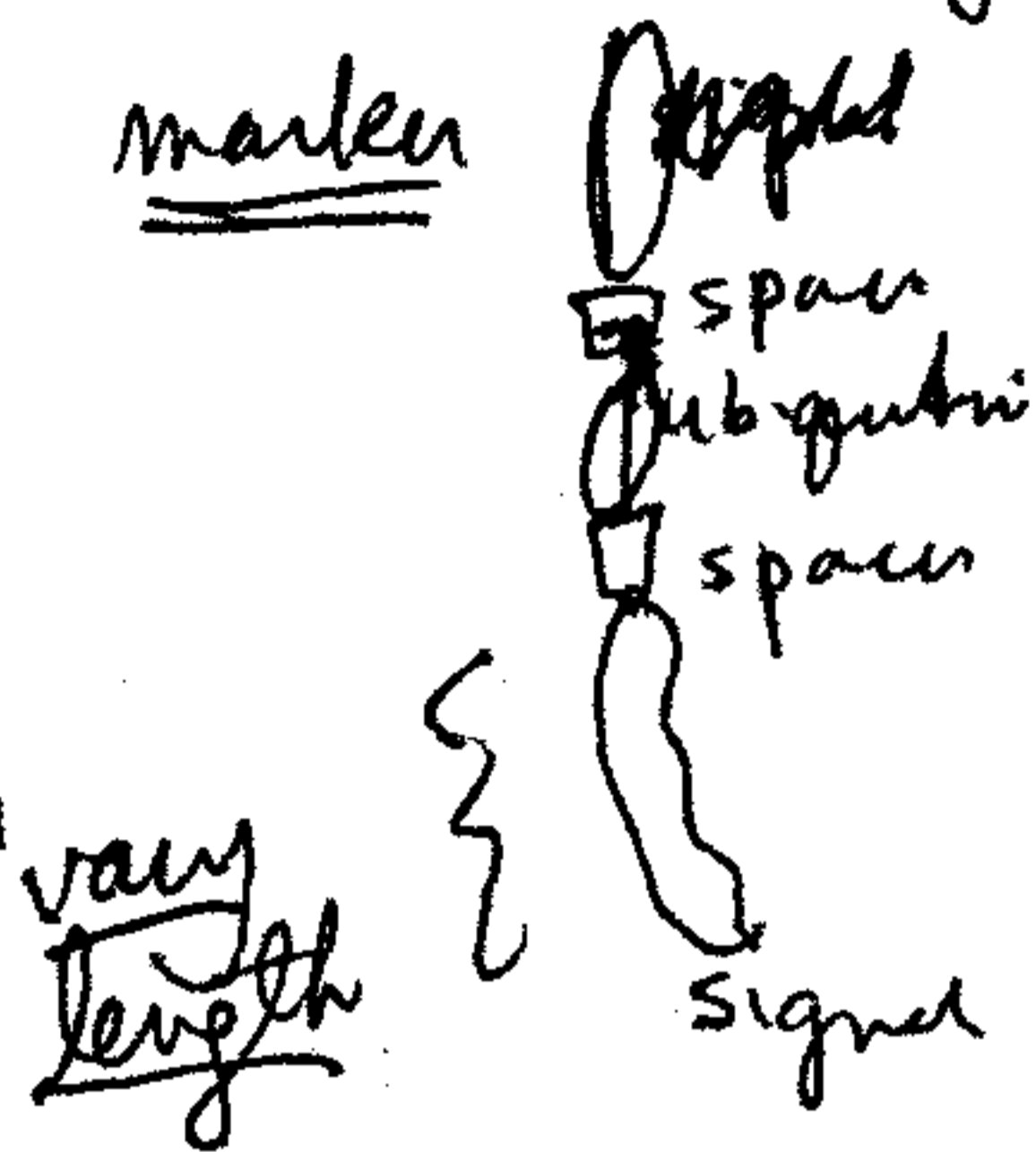
overexpress = more seen in growth

\* ∴ maybe haploids make proteins that when expressed N-term turns lethal.

# N-term v. processive

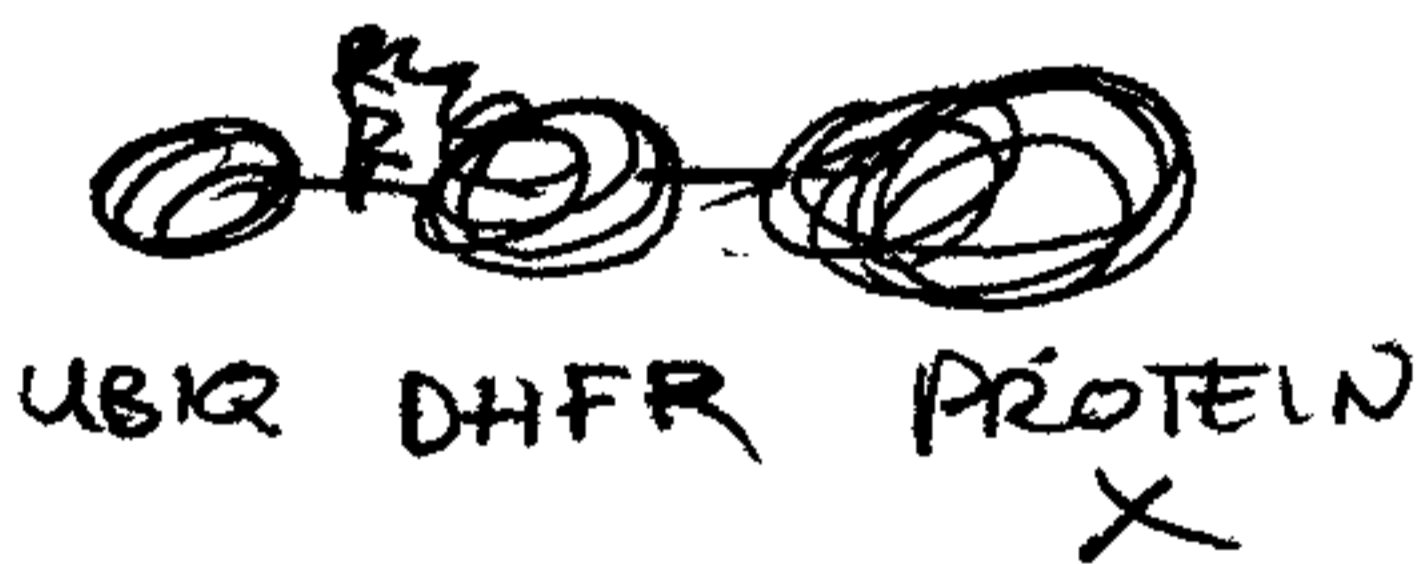
ubiquitin recognizing enzymes

- recognize folded form not specific peptides



① if ubiquitin folded then it gets cleaved & marker never gets moved to where signal says.

## Is mutants



can use ability of N-term to recog. DHFR to change  $t_{1/2}$  life of protein