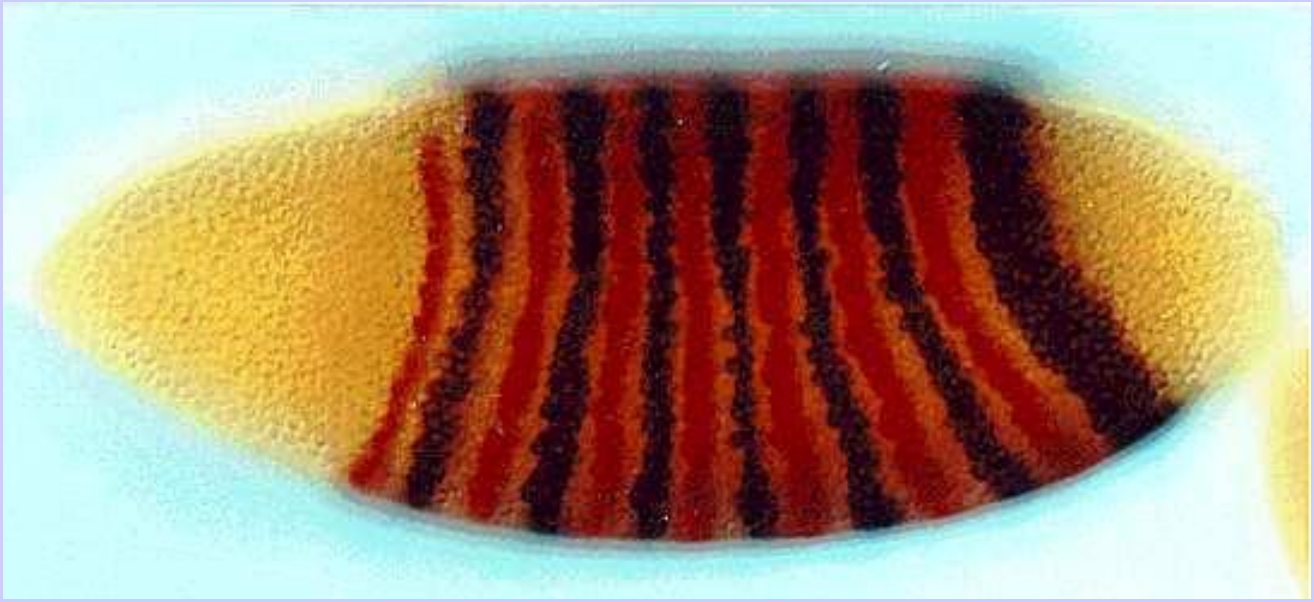
Diferenciace heterocyst

Martin Tichý

Institute of Microbiology, Czech Academy of Sciences, Třeboň
Institute of Physical Biology, University of South Bohemia, Nové Hrady
Czech Republic

Anabaena sp. PCC 7120 je Nostoc

heterocysty nejsou cysty

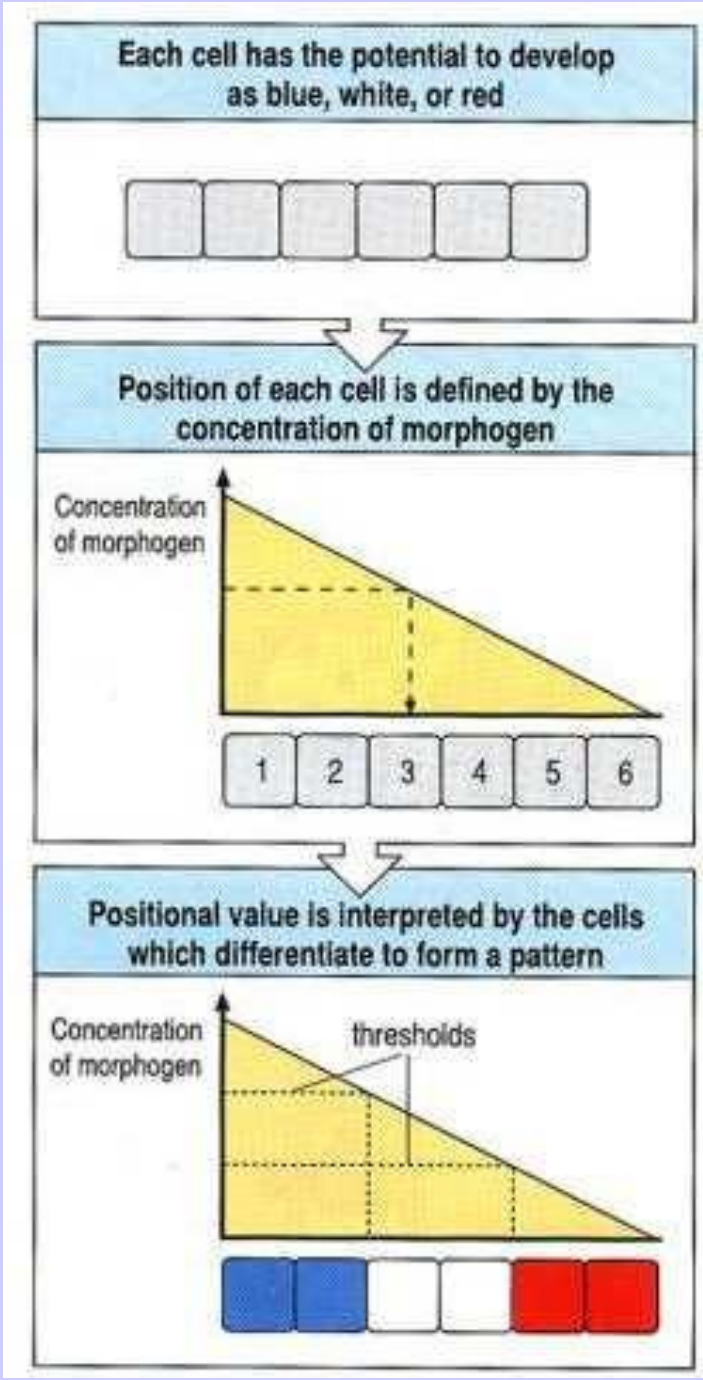


Paradox of developmental biology

How is it that a single cell gives rise to a multicellular organism composed of 100s of different cell types – yet all the cell types have the same genes?

Patterning can involve the interpretation of positional information.

French flag analogy



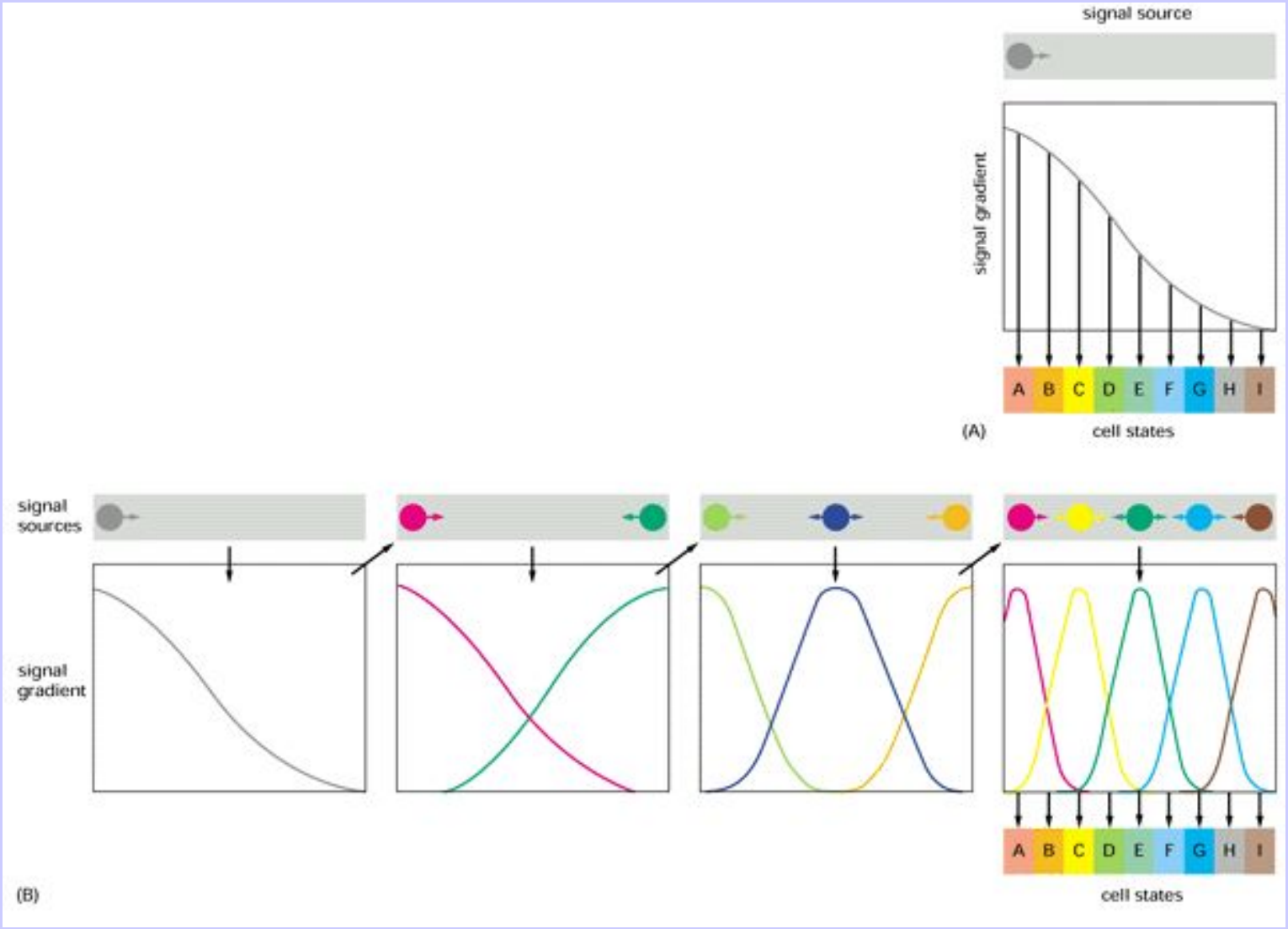
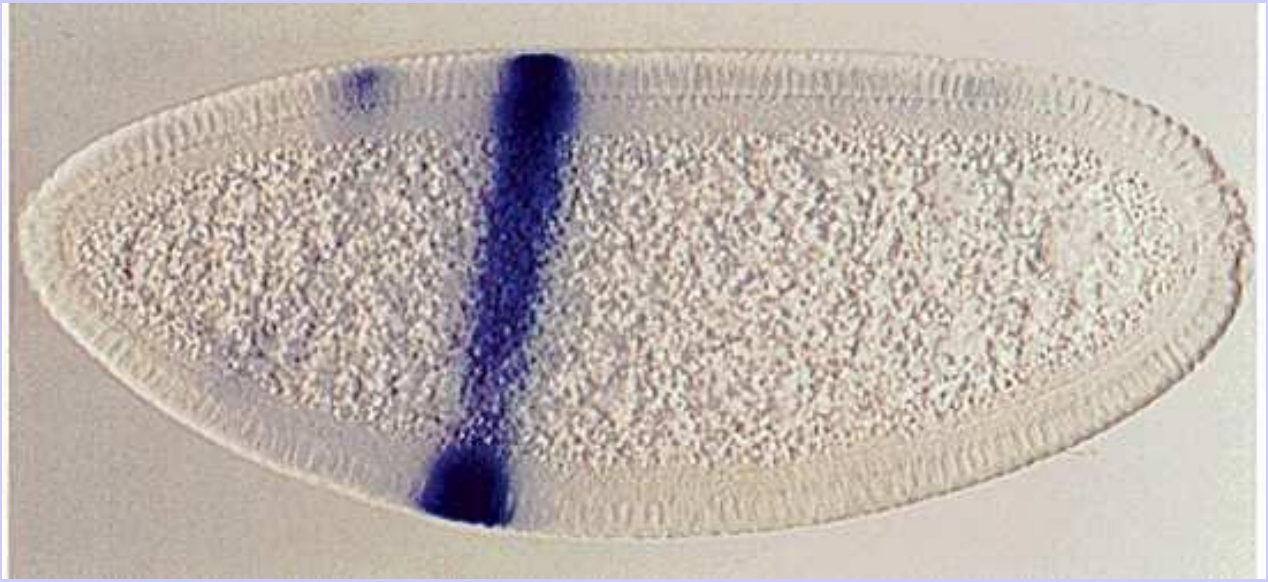


Figure 21-63. Two strategies for using signal concentration gradients to specify a fine-grained pattern of cells in different states. In (A) there is only one signal gradient, and cells select their states by responding accurately to small changes of signal concentration. In (B) the initial signal gradient controls establishment of a small number of more local signals, which control establishment of other still more narrowly local signals, and so on. Because there are multiple local signals, the cells do not have to respond very precisely to any single signal in order to create the correct spatial array of cell states. Case B corresponds more closely to the strategy of the real embryo.

Expression of *eve* Stripe 2



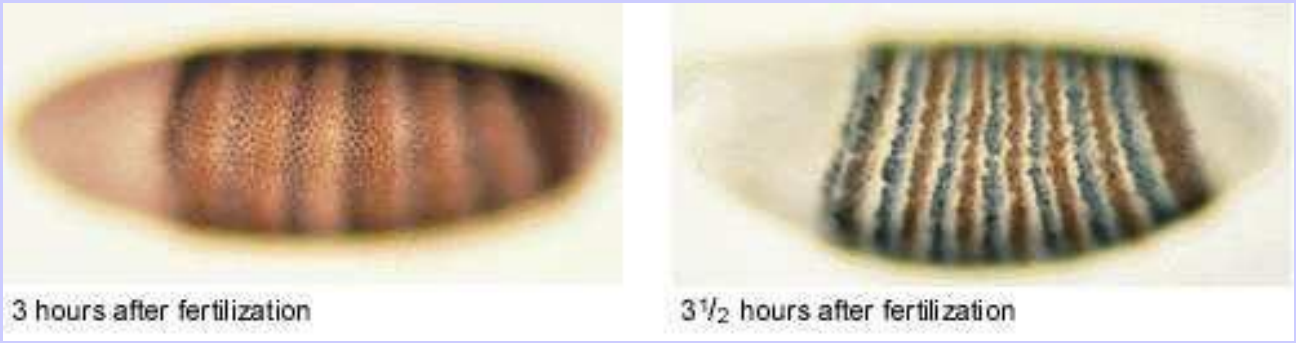
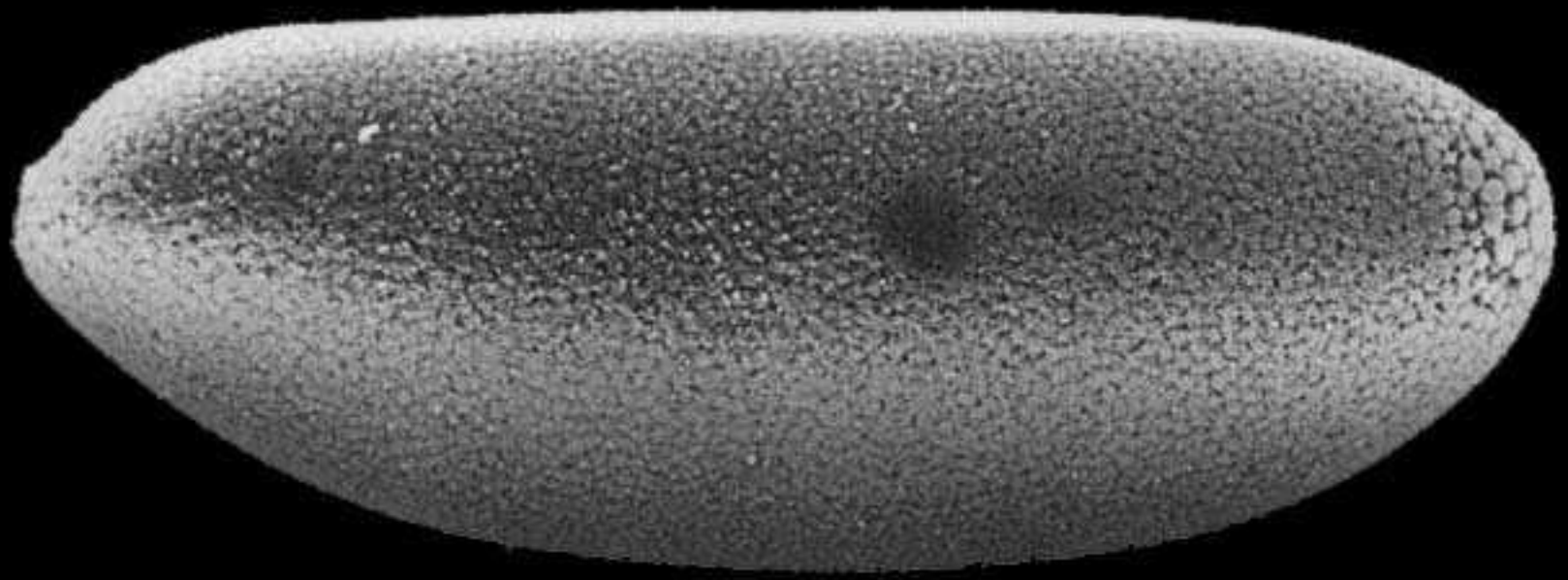


Figure 21-65. The formation of *ftz* and *eve* stripes in the *Drosophila* blastoderm. Genes *ftz* and *eve* are both pair-rule genes. Their expression patterns (shown in *brown* for *ftz* and in *gray* for *eve*) are at first blurred but rapidly resolve into sharply defined stripes. (From P.A. Lawrence, The Making of a Fly. Oxford, UK: Blackwell, 1992.)

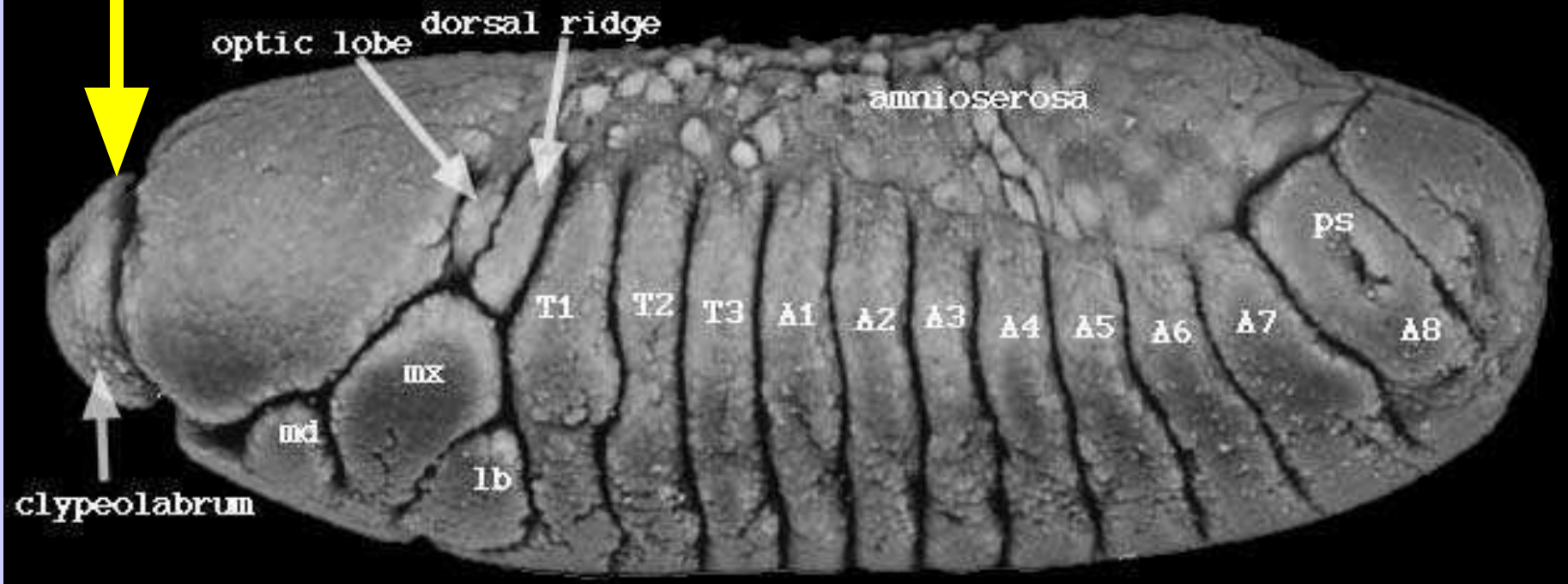
The Course of Development



The Course of Development

Time

Complicated

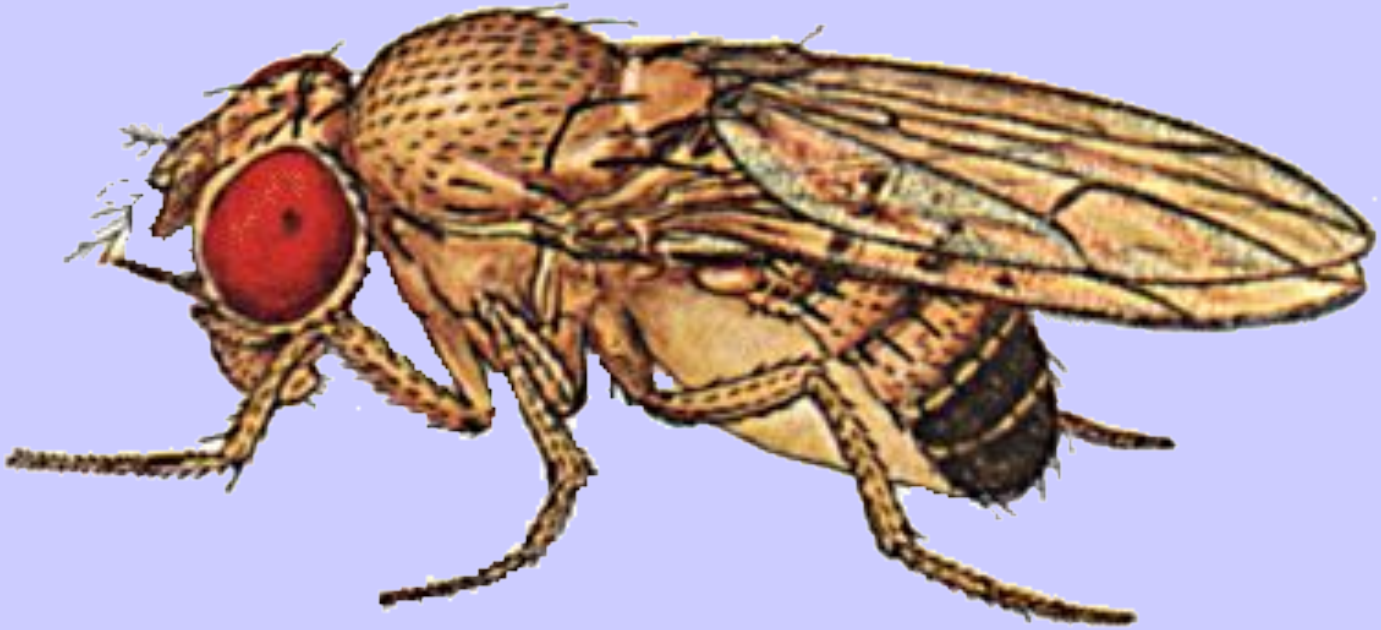


Events in time and space . . .

The Course of Development

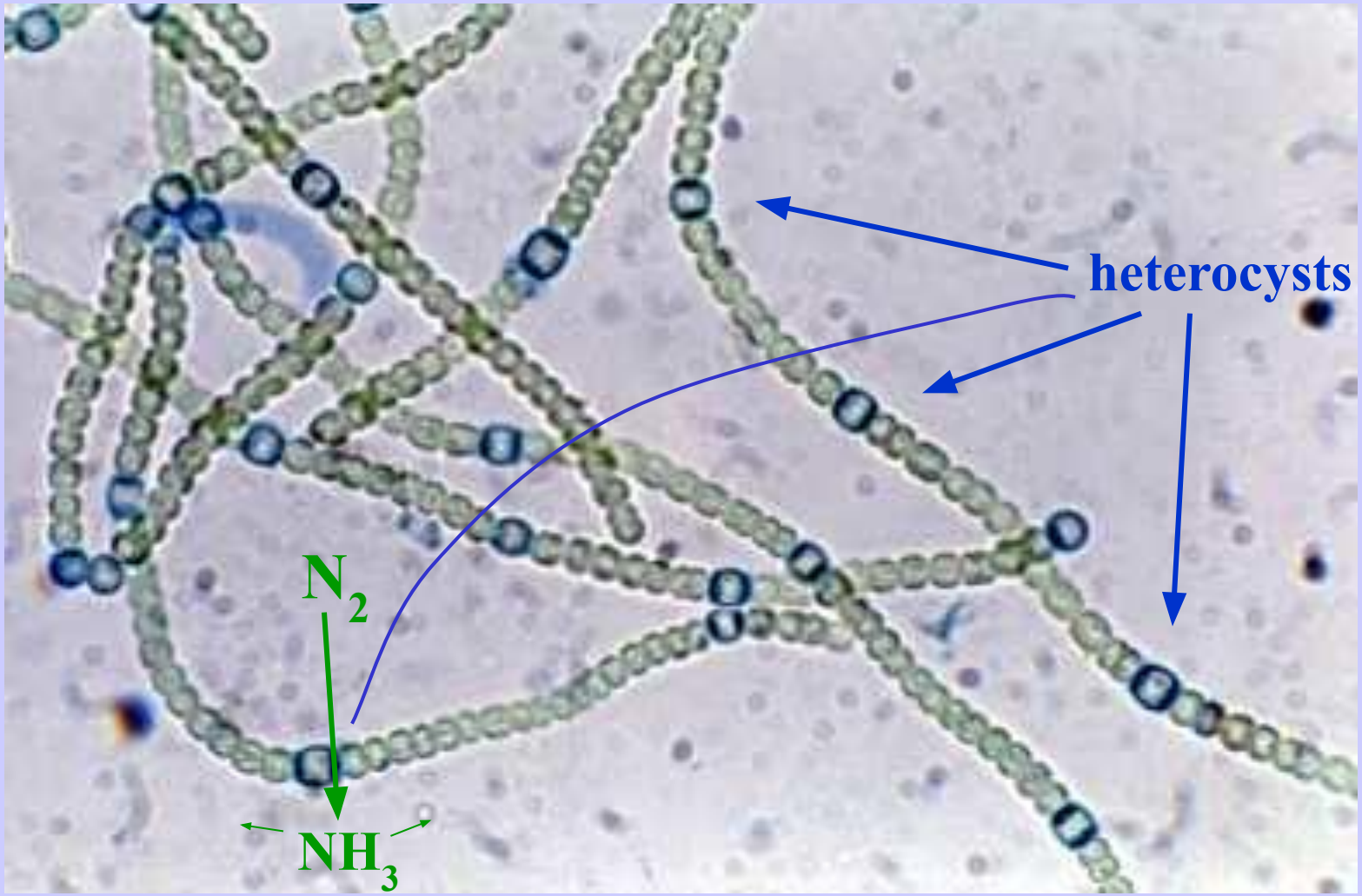
Time

Really Complicated



Cyanobacteria

Anabaena grown without fixed nitrogen



Matveyev and Elhai (unpublished)

Paradox of developmental biology

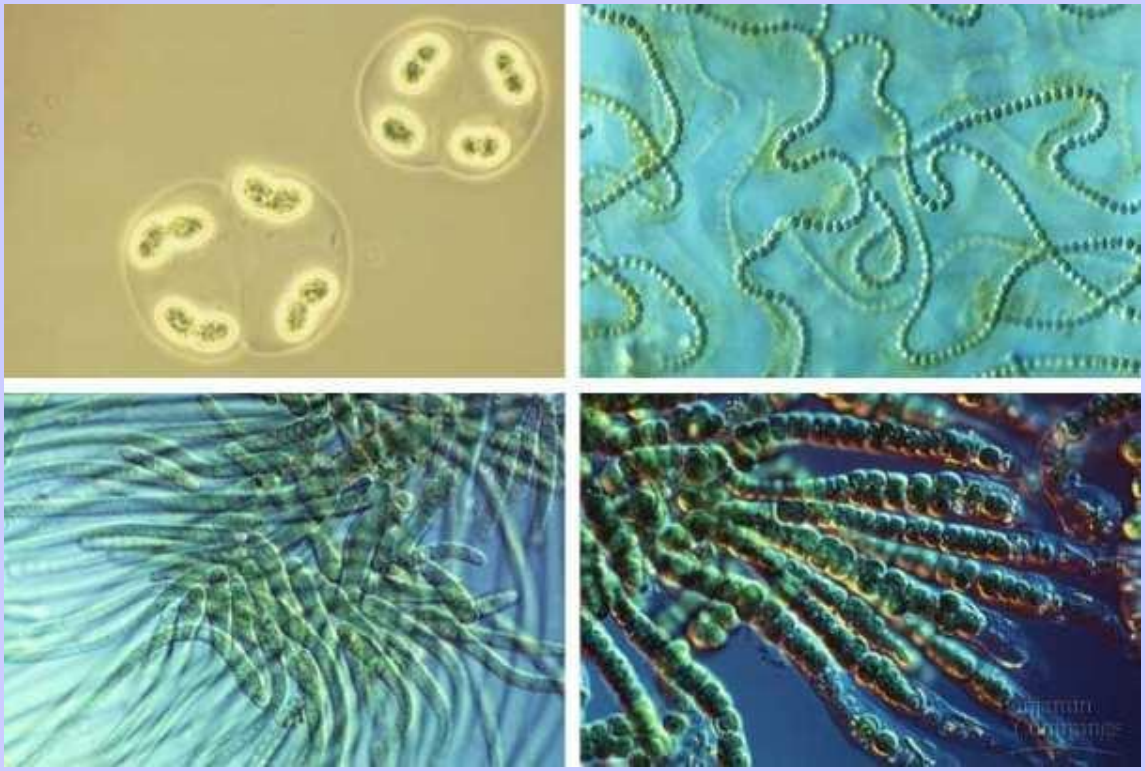
How is it that a single cell gives rise to a multicellular organism composed of 100s of different cell types – yet all the cell types have the same genes?

How Cyanobacteria Count to 10

Robert Haselkorn

Jak každá desátá buňka ví že má být heterocystou

Fixace dusíku



Biochemistry of N_2 fixation

$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$

Note: ***Very*** **expensive**

Reason why N_2 fixation by heterotrophic microbes is probably low

Key enzyme: **nitrogenase** (nif)

Ancient enzyme: highly conserved in very diverse microbes, from archaea to cyanobacteria

What is another problem with nitrogenase?

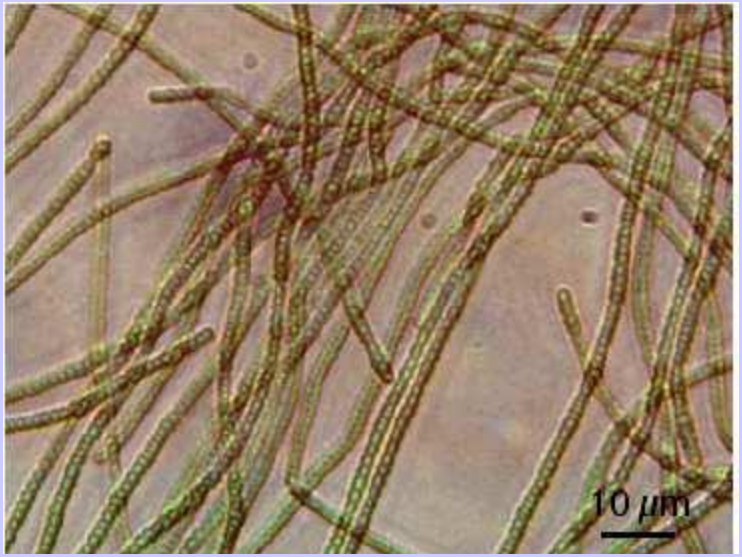
Nitrogenase is killed dead by O_2

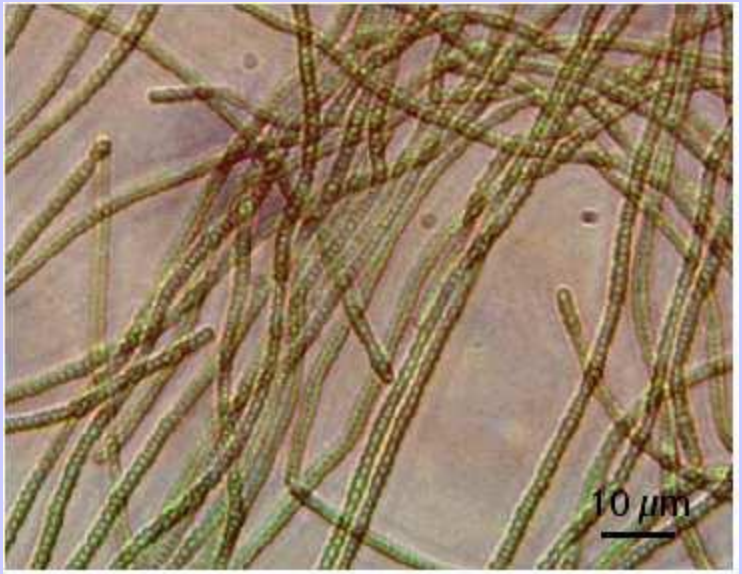
Protects nitrogenase (N_2 fixing enzyme) from O_2

Outside sources of O_2

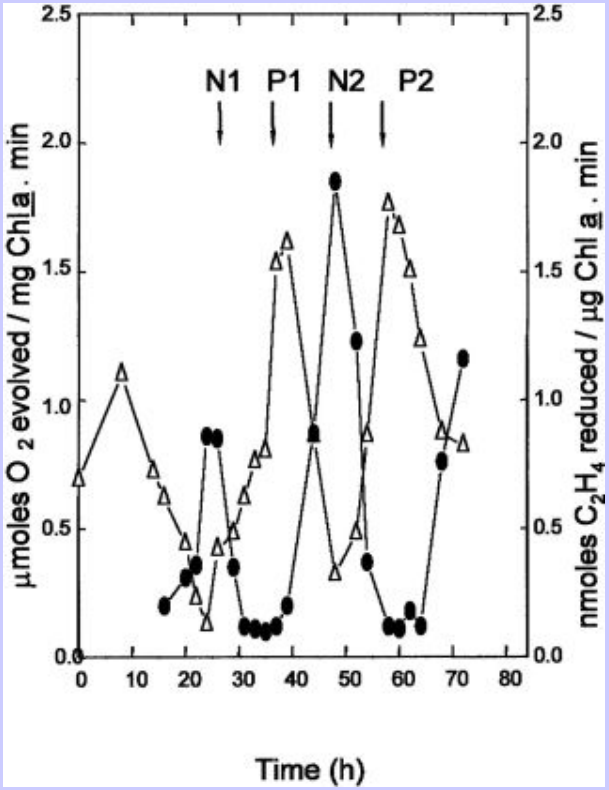
O_2 produced by cyanobacteria

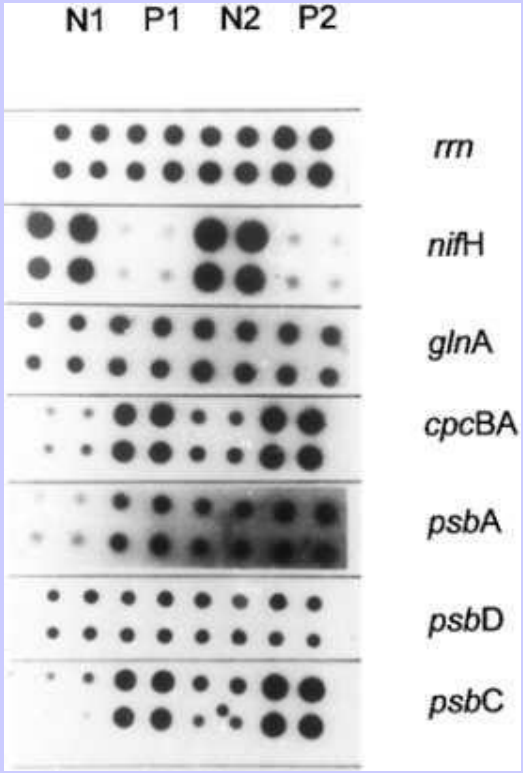
Ne všechny sinice schopné fixovat dusík tvoří heterocysty

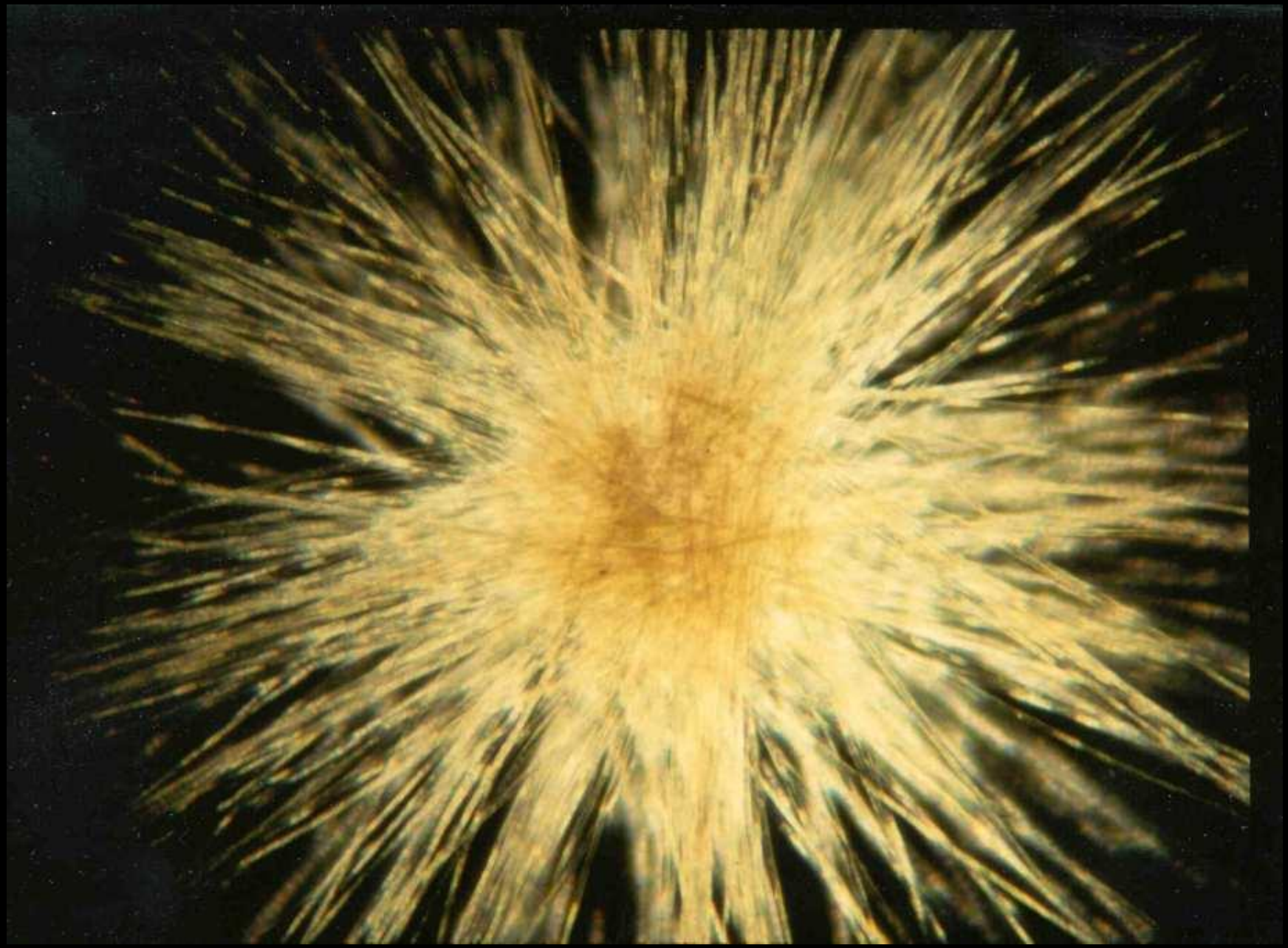




Plectonema boryanum IAM-M101


N1
P1
N2
P2
µmoles O 2 evolved / mg Chl a . min
nmoles C2H4 reduced / µg Chl a . min
Time (h)
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40
50
60
70
80


N1
P1
N2
P2
rrn
nifH
glnA
cpcBA
psbA
psbD
psbC



How does *Trichodesmium* (and single cell cyano's) fix N_2 without heterocysts?

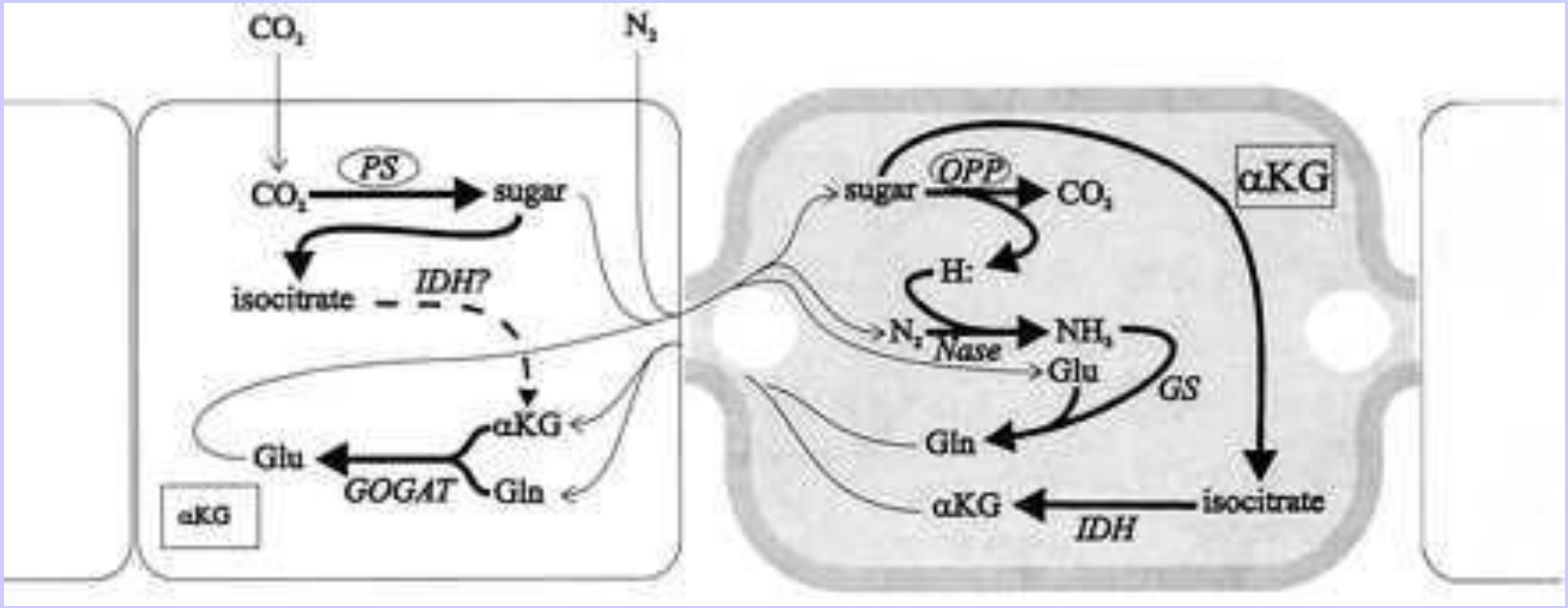
Partial answer: doesn't fix N_2 and do photosynthesis at the same time

See Berman-Frank et al. Science (2001) 294: 1534-1537.

What are heterocysts?

1. Site of N_2 fixation in many cyanobacteria.
2. Specialized thick wall cells in chain of cyanobacterial vegetative cells
3. No PS II of photosynthesis --> no O_2 evolution
4. No carbon fixation
5. Respiration

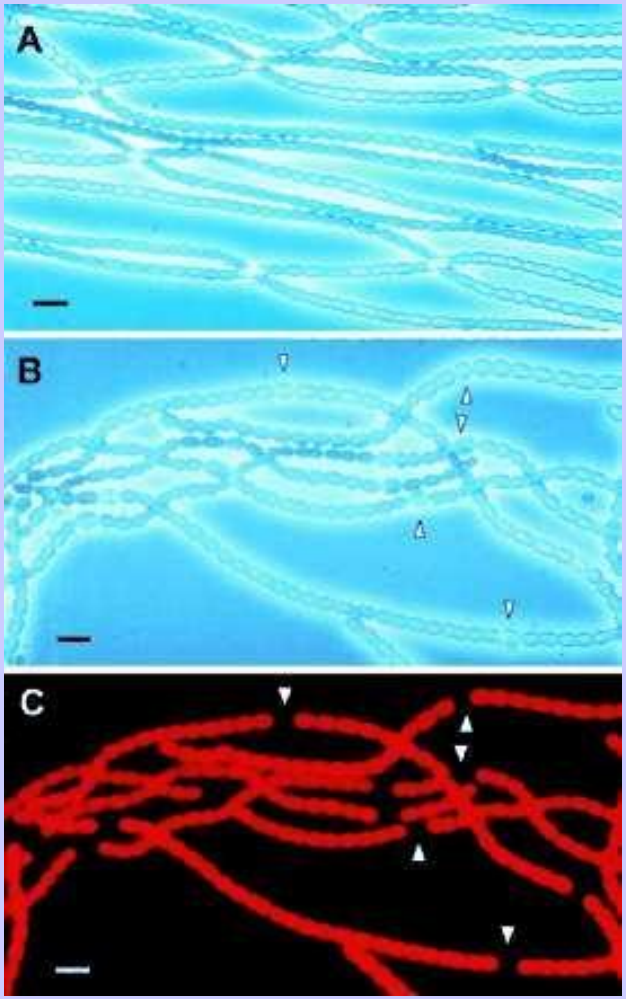
The heterocyst achieves a near anoxic state by at least three means. First, photosystem II, the O_2-producing end of the photosynthetic electron transport chain, is dismantled during heterocyst differentiation, so that the heterocyst need contend only against O_2 produced by neighboring vegetative cells and that dissolved in the environment. Second, heterocysts are invested with a specialized envelope that limits the influx of gases. Two layers within the envelope have been implicated in O_2 protection: an inner layer composed of a hydroxylated glycolipid and an outer layer of polysaccharide. Neither layer is found in vegetative cells. Third, much of the O_2 that overcomes these barriers is consumed by the high oxidase activity associated with heterocysts.


CO2
N2
PS
CO2
sugar
isocitrate
IDH?
αKG
Glu
GOGAT
Gln
αKG
sugar
OPP
CO2
αKG
H:
N2
Nase
NH3
Glu
GS
Gln
αKG
isocitrate
IDH

Dělba práce

A médium s dusíkem

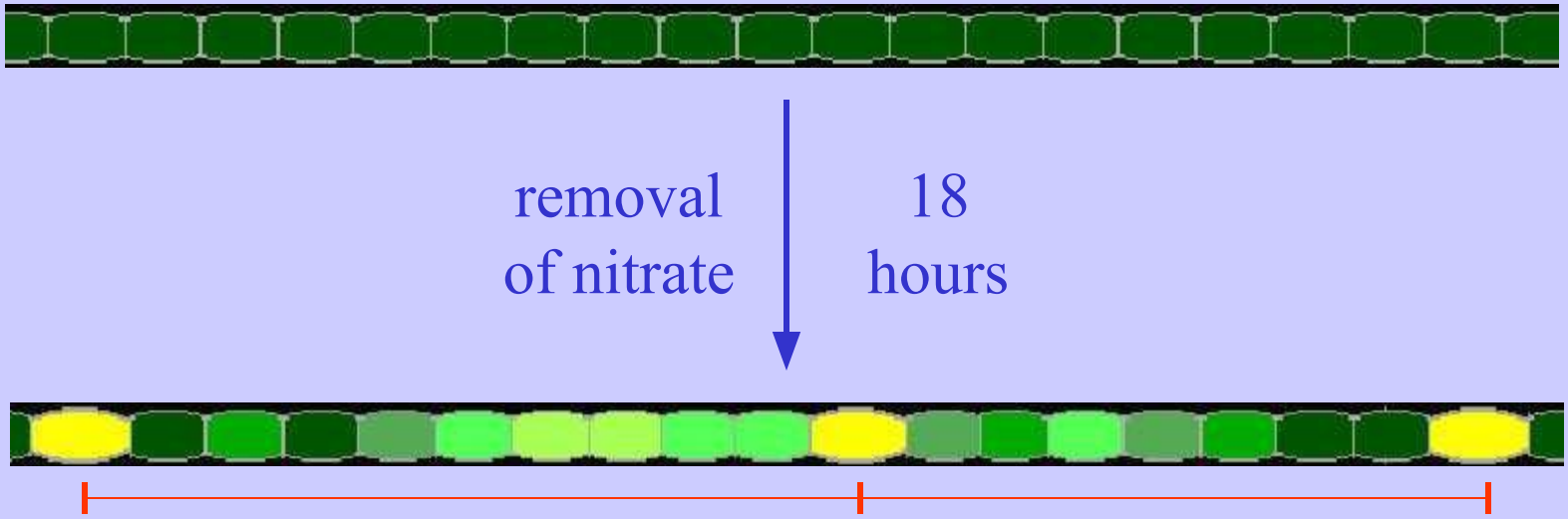
B médium bez dusíku



Excitation was at 510 to 560 nm (green), exciting phycoerythrin, and emission was greater than 600 nm. Heterocysts have negligible fluorescence, while vegetative cells have intense combined fluorescence from phycobiliproteins and chlorophyll *a*. Bar, 10 μm.

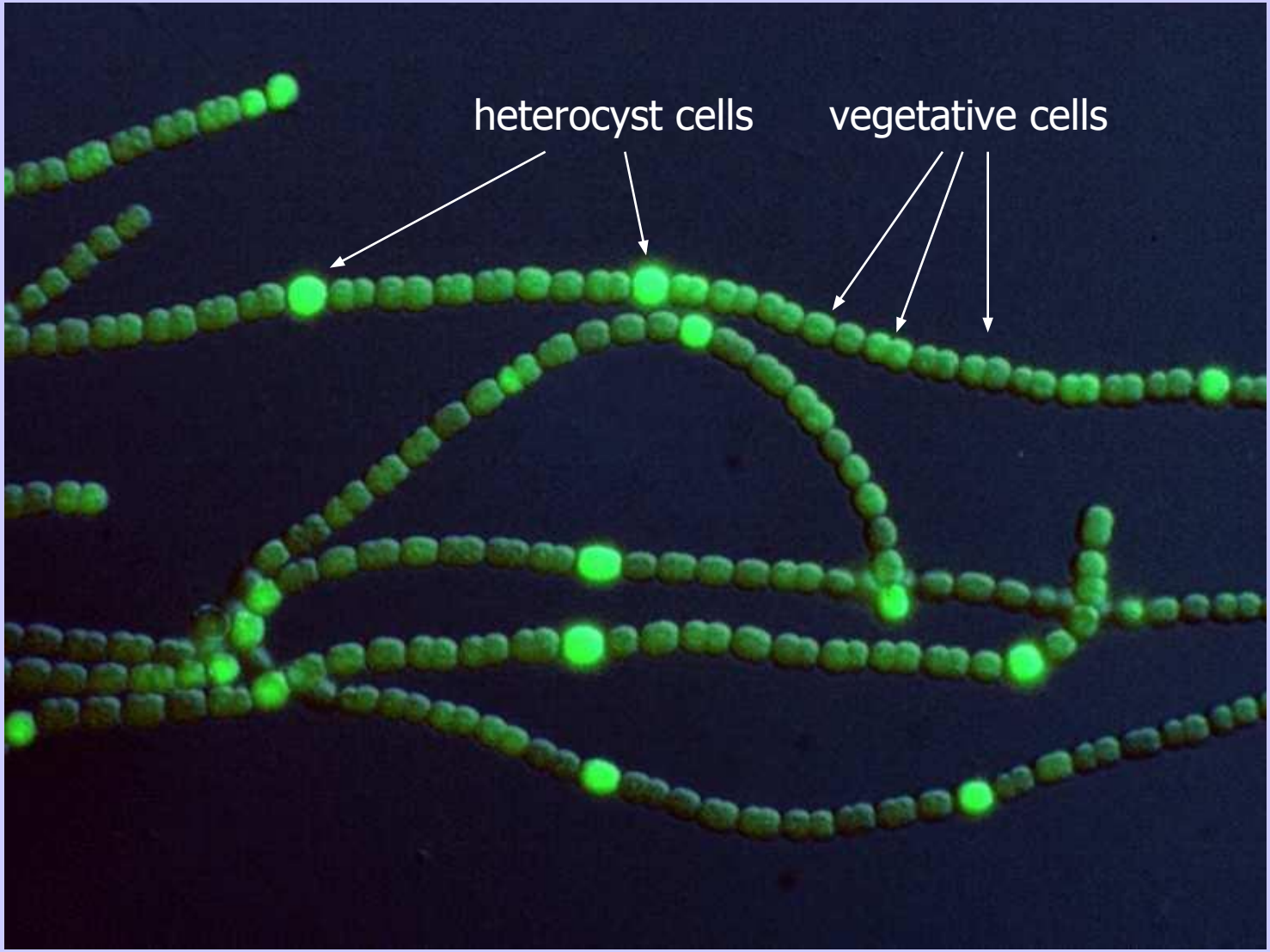
Heterocysts only when needed

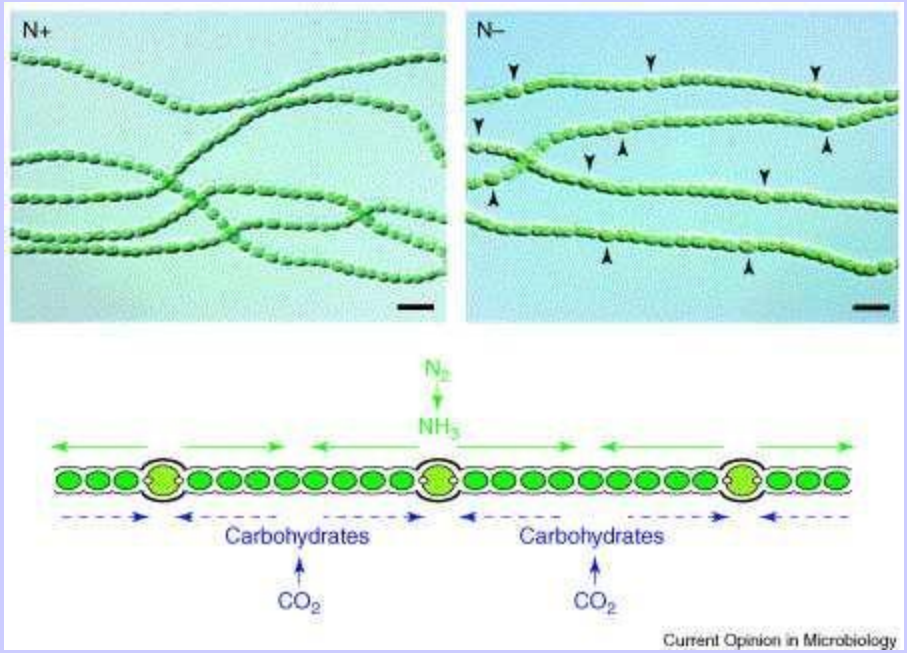
Anabaena filament growing on nitrate



Anabaena filament growing on N_2

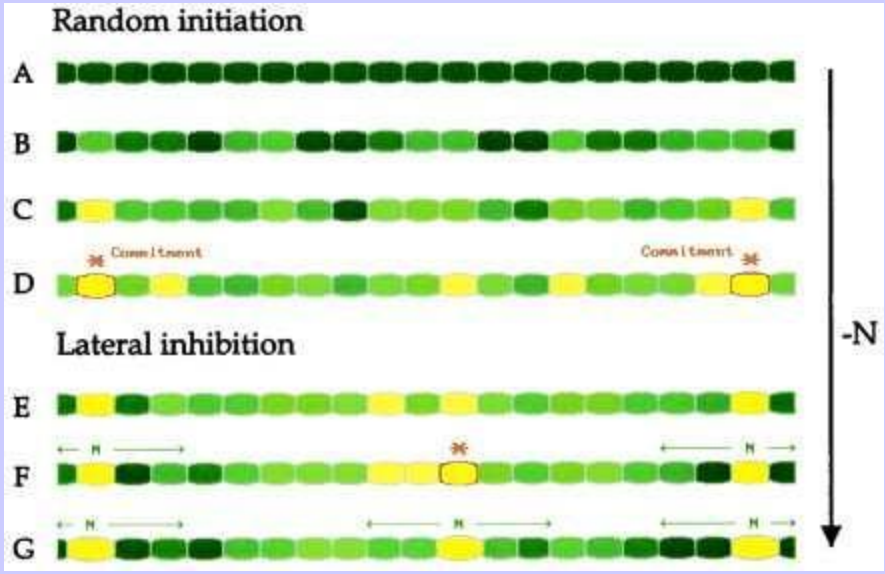
Anabaena




N+
N–
N_2
NH_3
Carbohydrates
Carbohydrates
CO_2
CO_2
Current Opinion in Microbiology

Anabaena model

- Heterocyst spacing relatively constant
  - Heterocyst cells
    - produce compound
  - Vegetative cells
    - divide
    - differentiate
    - consume compound
    - diffuse compound



First, they assumed that any cell is competent to differentiate at the moment when nitrogen is removed from the environment and that the choice of cells that initiate differentiation is random. Second, they postulated the existence of a diffusible inhibitor made by heterocysts and differentiating cells and consumed by nondifferentiating cells, as predicted by experimental data.

Anabaena – continuous model

axiom: $F_h(s_{max},c_{max})\ F_v(s_{max},c_{max})\ F_h(s_{max},c_{max})$

$F(s_l,c_l) < F_v(s,c) > F(s_r,c_r)$:

if $s < s_{max}$ & $c > c_{min}$

solve $dc/dt = D\cdot(c_l+c_r-2c)-\mu\cdot c$

$ds/dt = r \cdot s$

if $s = s_{max}$ & $c > c_{min}$

produce $F_v(k \cdot s_{max},c)F_v((1-k) \cdot s_{max},c)$

if $c = c_{min}$

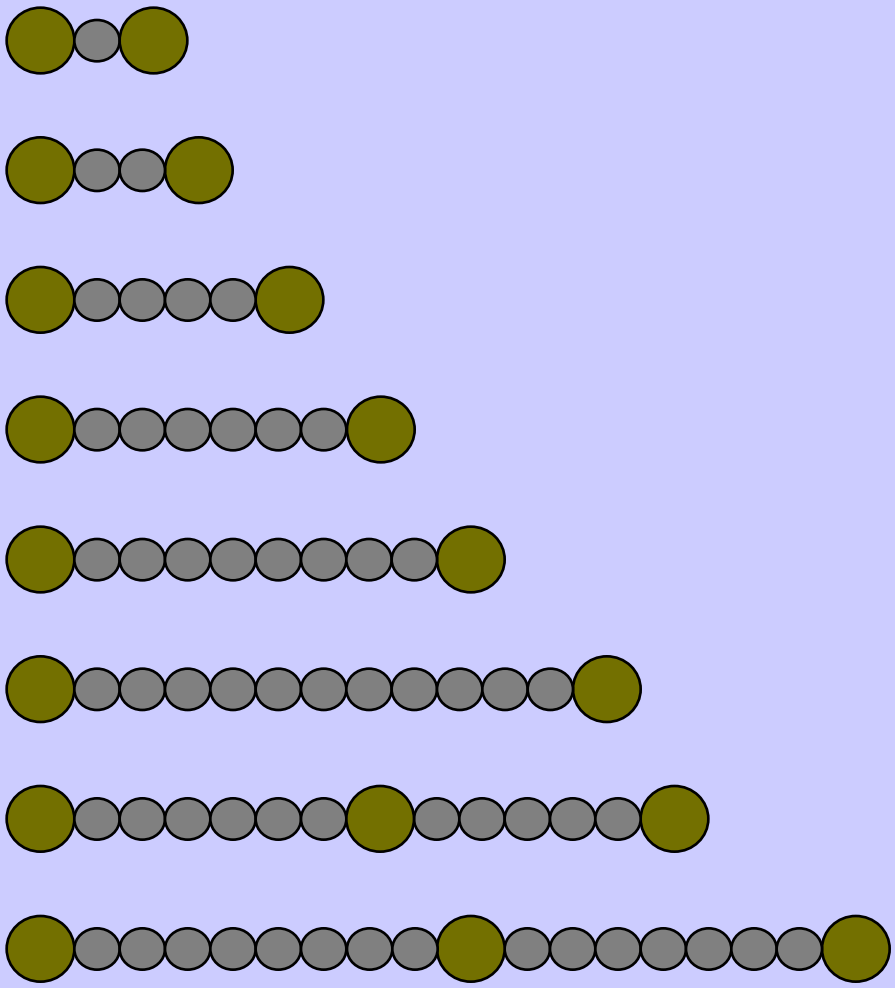
produce $F_h(s,c)$

$F_h(s,c)$:

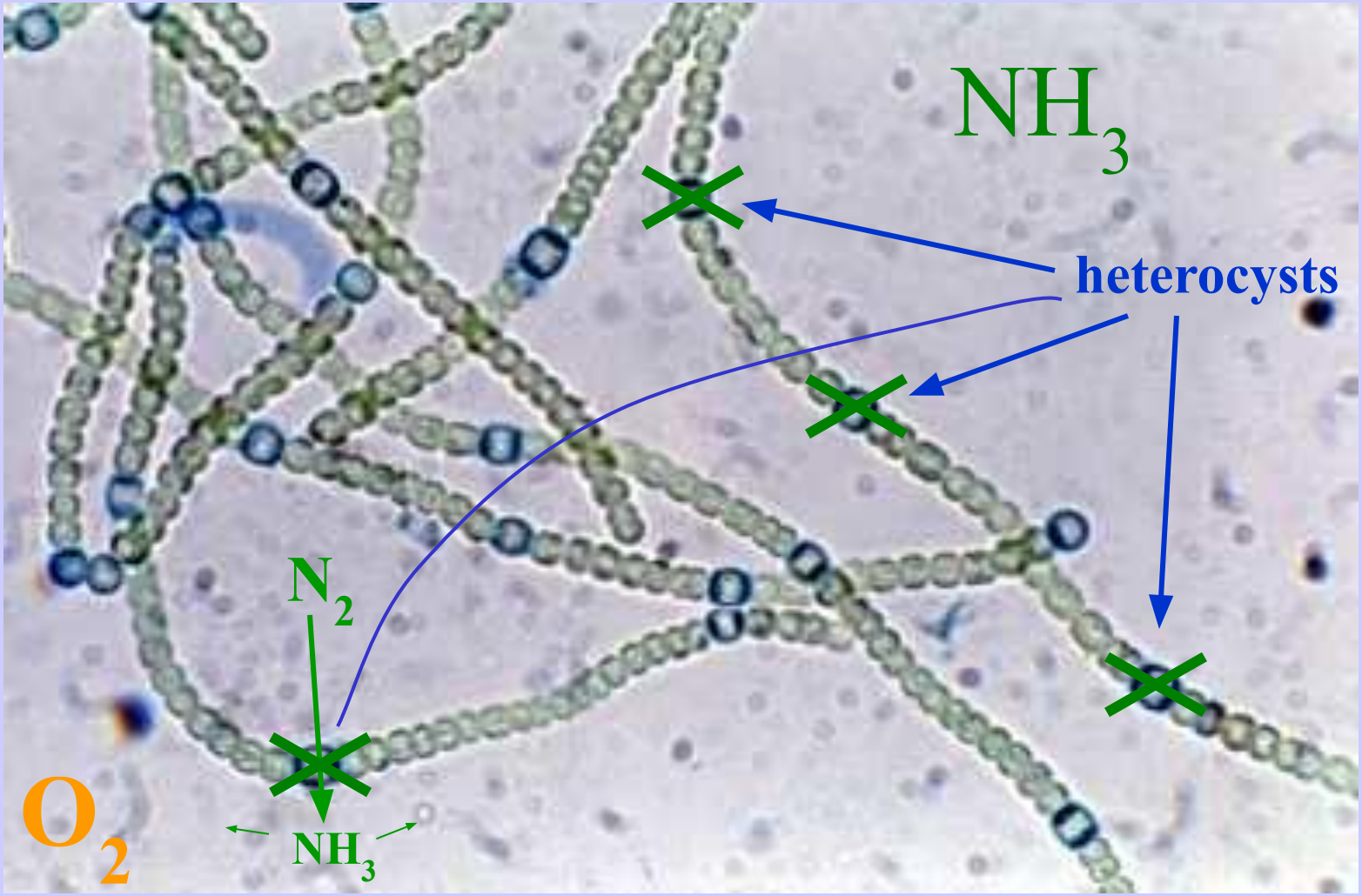
solve $ds/dt = r_s \cdot (s_{max}-s)$

$dc/dt = r_c \cdot (c_{max}-c)$

Anabaena – continuous model



Case of the Hidden Heterocyst

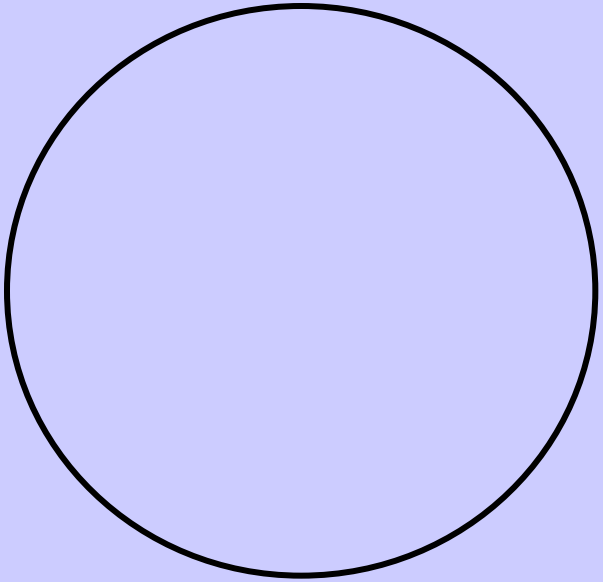


Matveyev and Elhai (unpublished)

Case of the Hidden Heterocyst

Strategy to find heterocyst differentiation genes

Nostoc genome



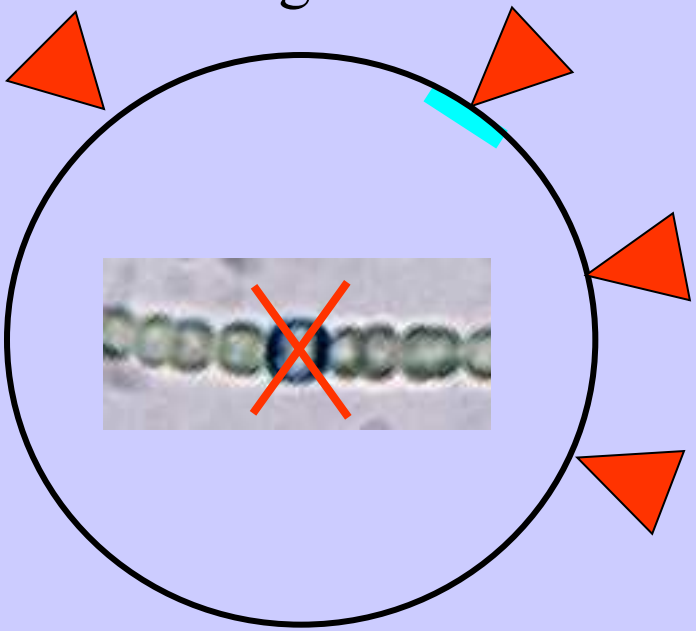
Transposon

1. Use transposon mutagenesis

Case of the Hidden Heterocyst

Strategy to find heterocyst differentiation genes

Nostoc genome



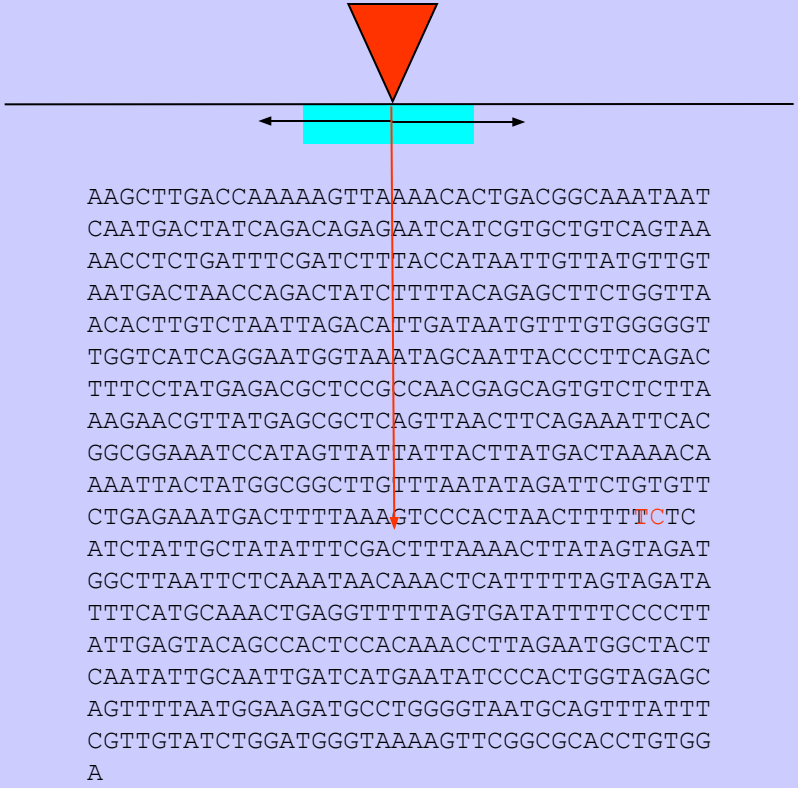
Transposon

1. Use transposon mutagenesis to find a mutant defective in heterocyst differentiation

Case of the Hidden Heterocyst

Strategy to find heterocyst differentiation genes

Nostoc genome

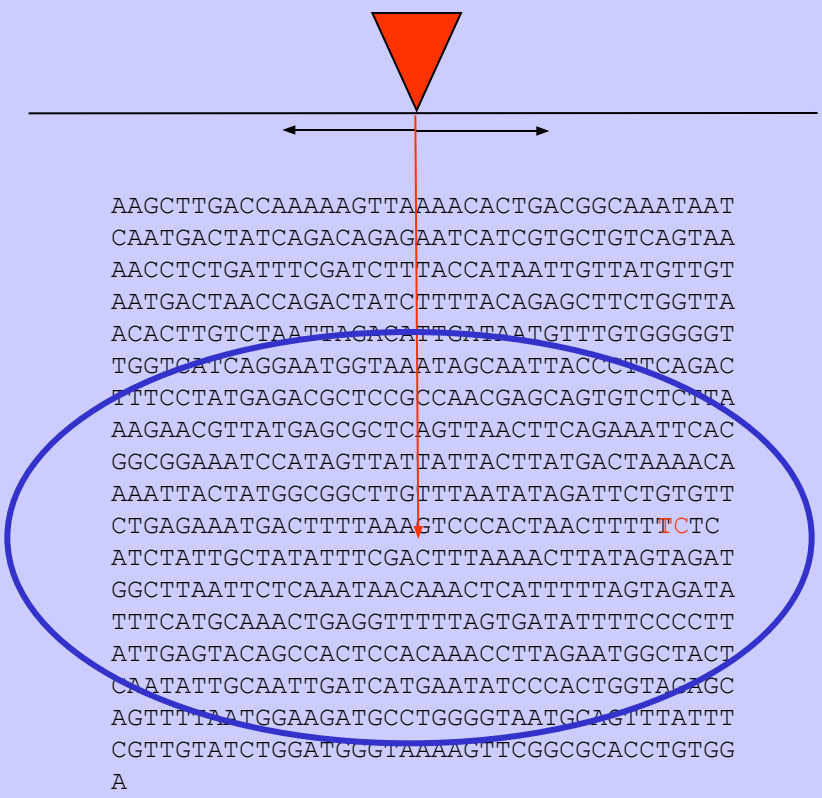


1. Use transposon mutagenesis to find a mutant defective in heterocyst differentiation
2. Sequence out from transposon

Case of the Hidden Heterocyst

Strategy to find heterocyst differentiation genes

Nostoc genome



Do it

1. Use transposon mutagenesis to find a mutant defective in heterocyst differentiation
2. Sequence out from transposon
3. Find gene boundaries
4. Identify gene

HetR

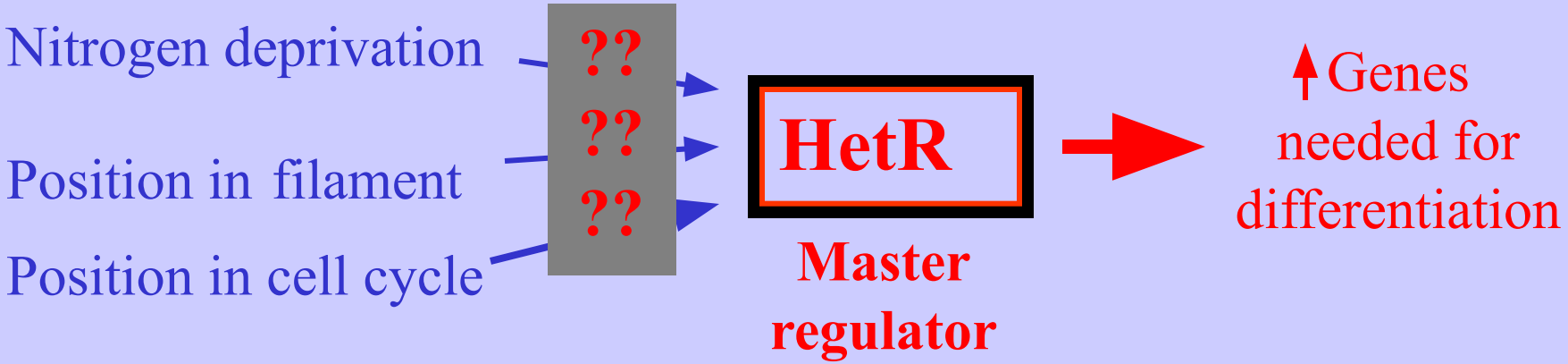
mutant - unable to make heterocysts

The spatially patterned differentiation of heterocysts in the filamentous cyanobacterium Anabaena requires a functional hetR gene

low level of transcript when Anabaena is grown with combined nitrogen
induction begins within 2 h following nitrogen deprivation
by 3.5 h, induction is localized to spaced foci
by 6 h, 20-fold increase within spatially separated cells

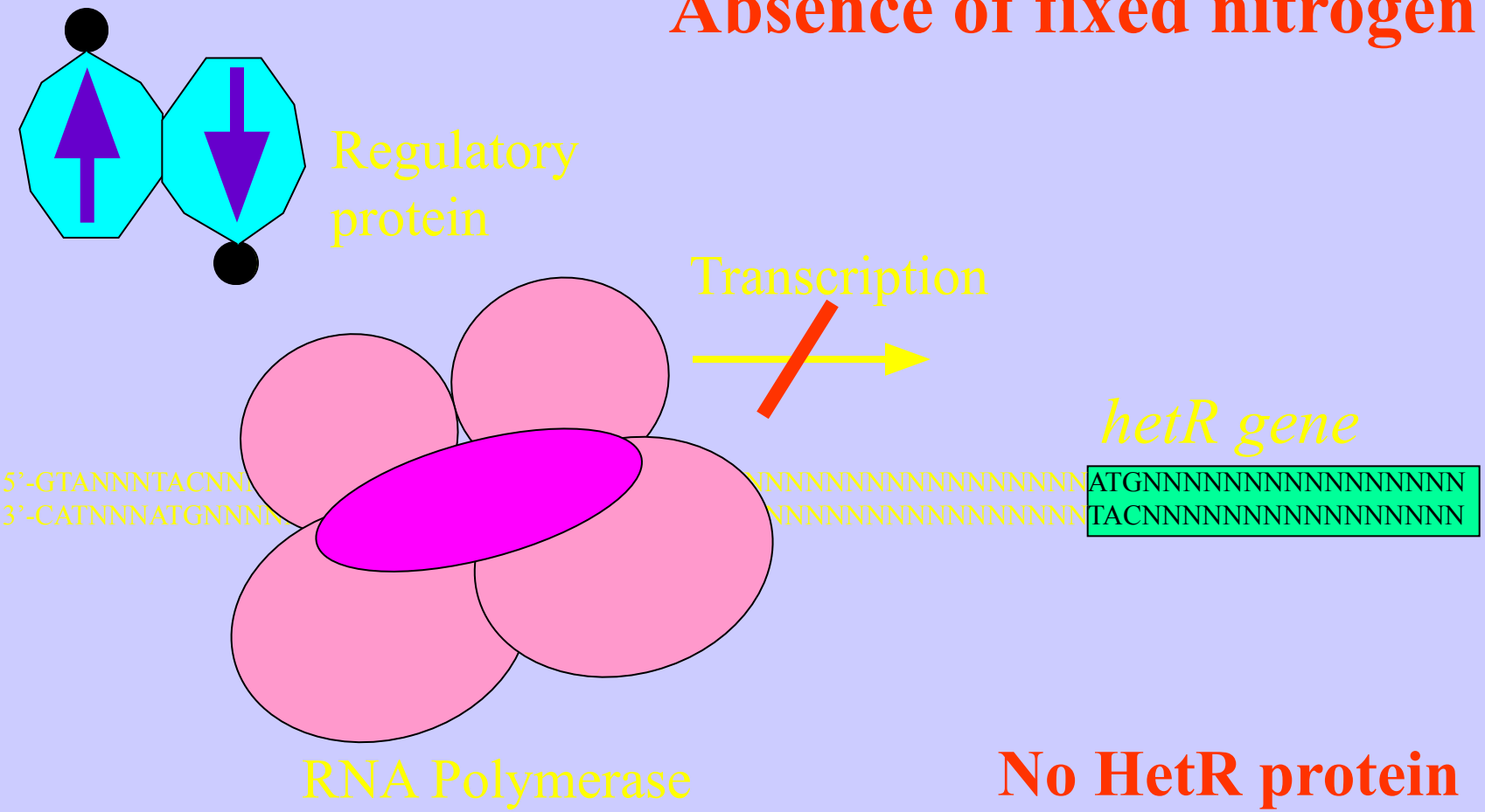
positive autoregulation

Differentiation in cyanobacteria
Integration of signals through HetR



How might *hetR* be controlled?

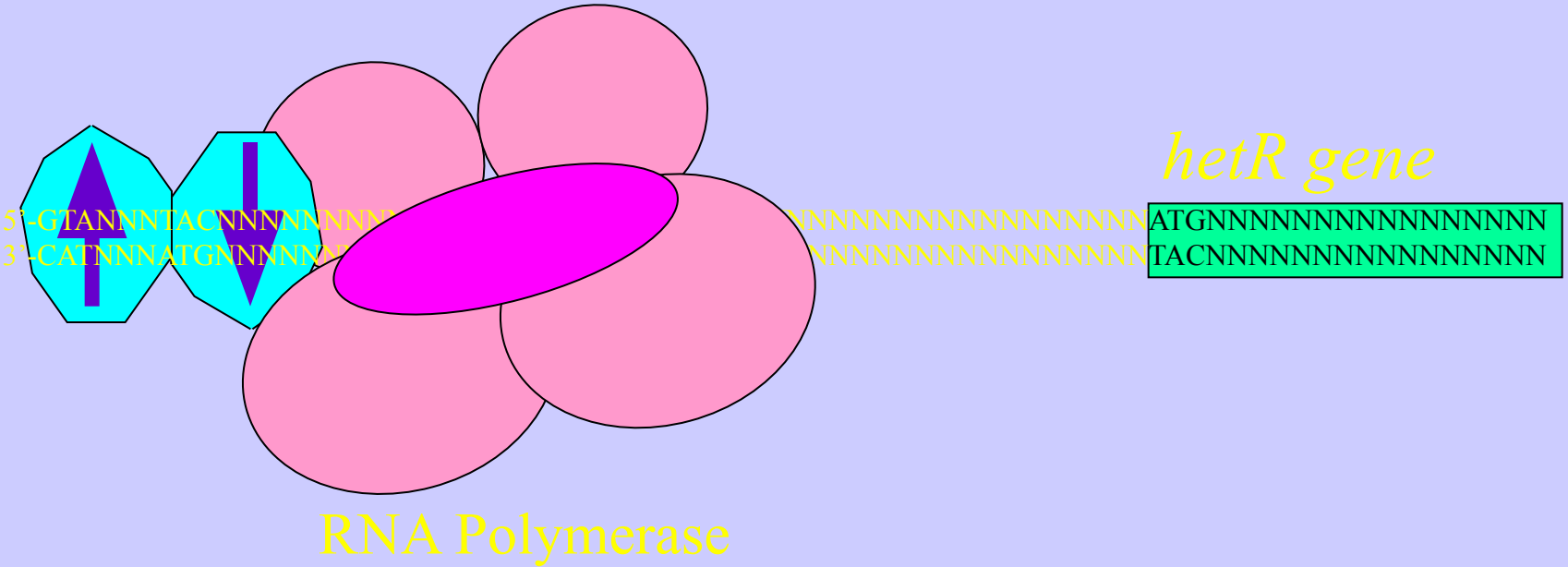
Absence of fixed nitrogen



No HetR protein

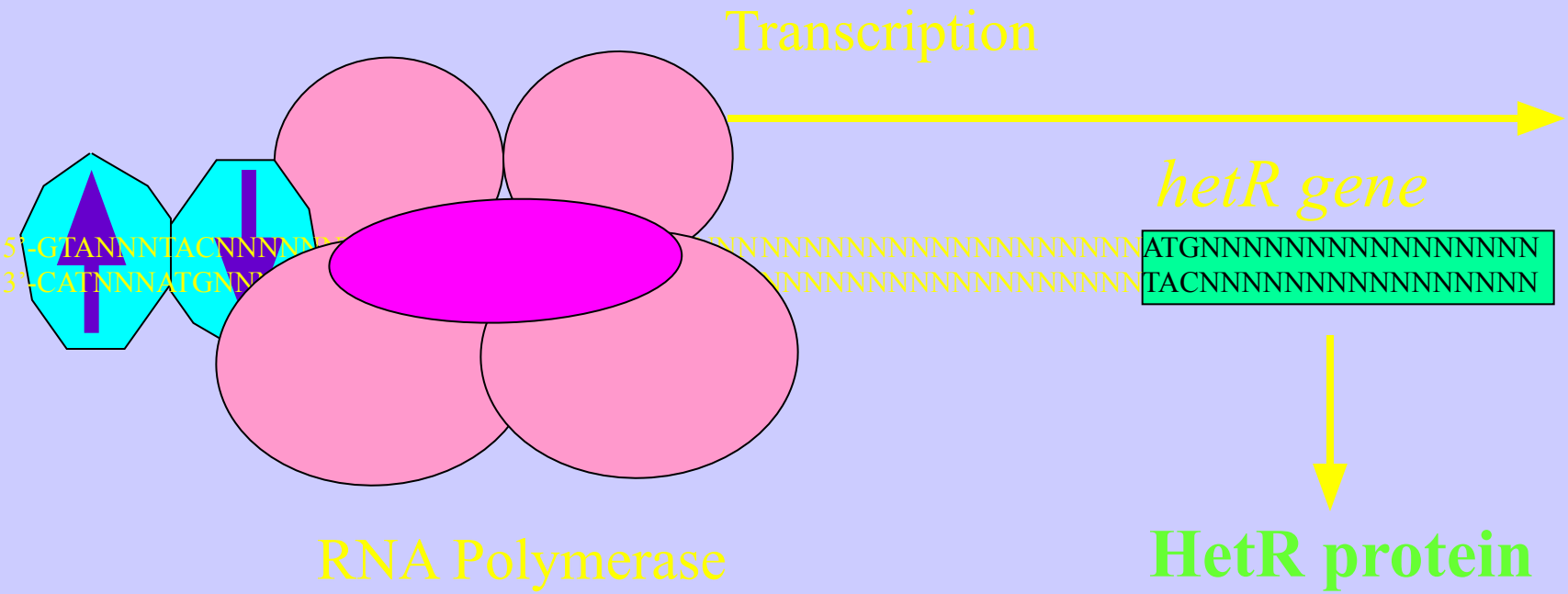
How might *hetR* be controlled?

Absence of fixed nitrogen

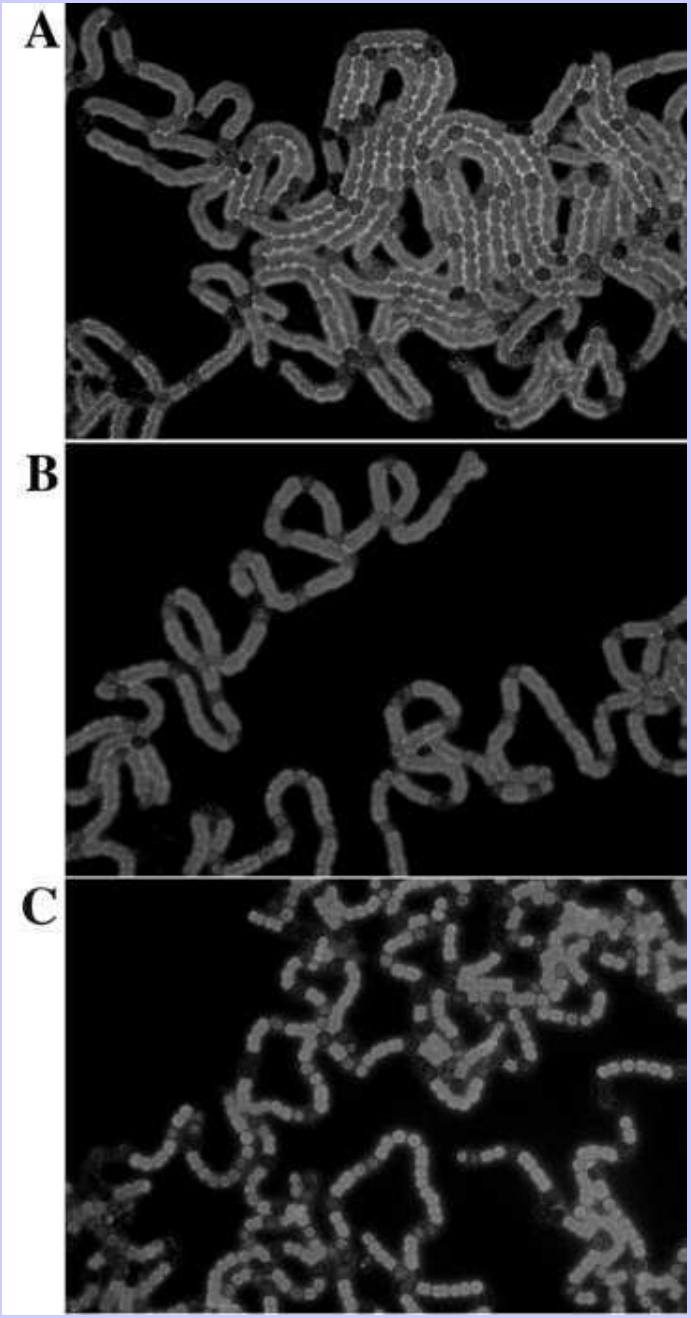


How might *hetR* be controlled?

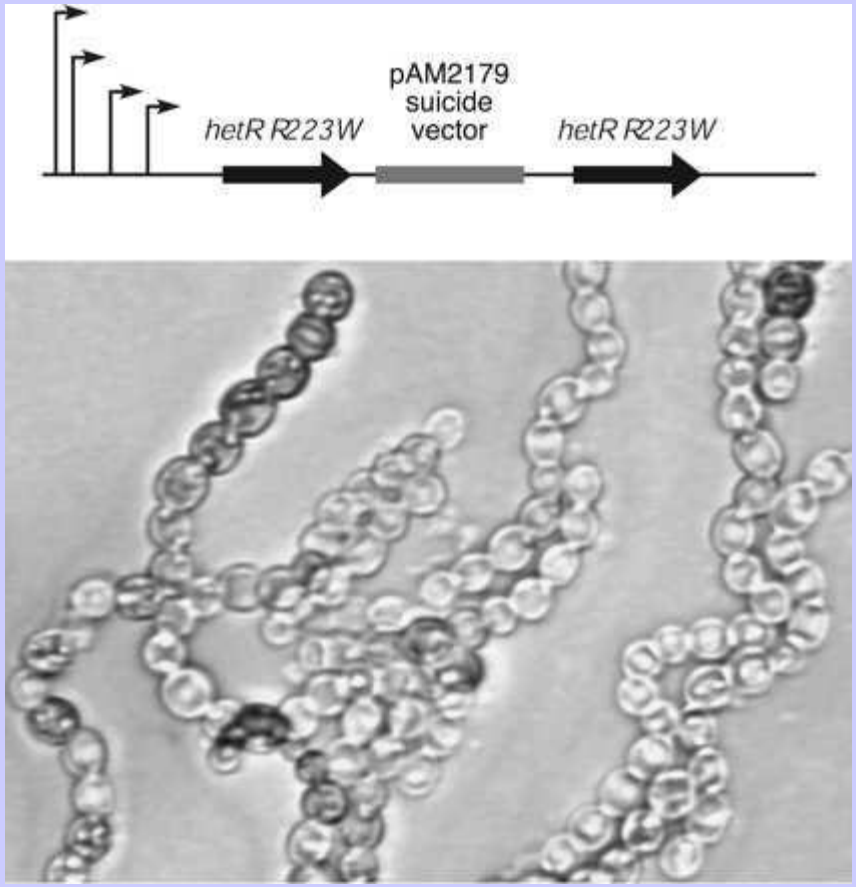
Absence of fixed nitrogen



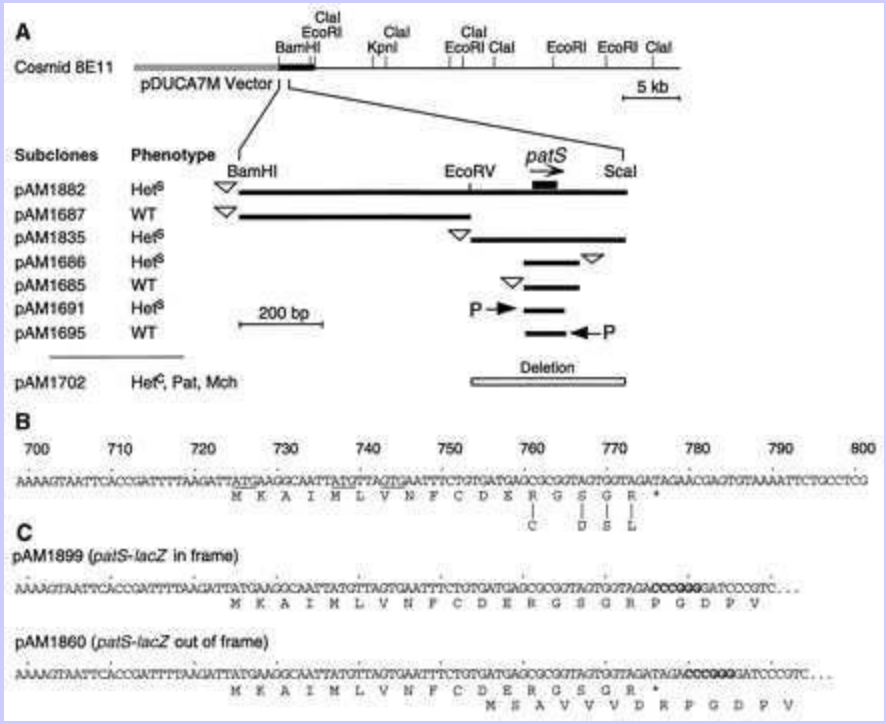
HetR overexpression



The key result of this experiment is that all of the upstream controls of HetR expression can be bypassed; expression of HetR alone suffices to turn on the differentiation pathway.


pAM2179
suicide
vector
hetR R223W
hetR R223W

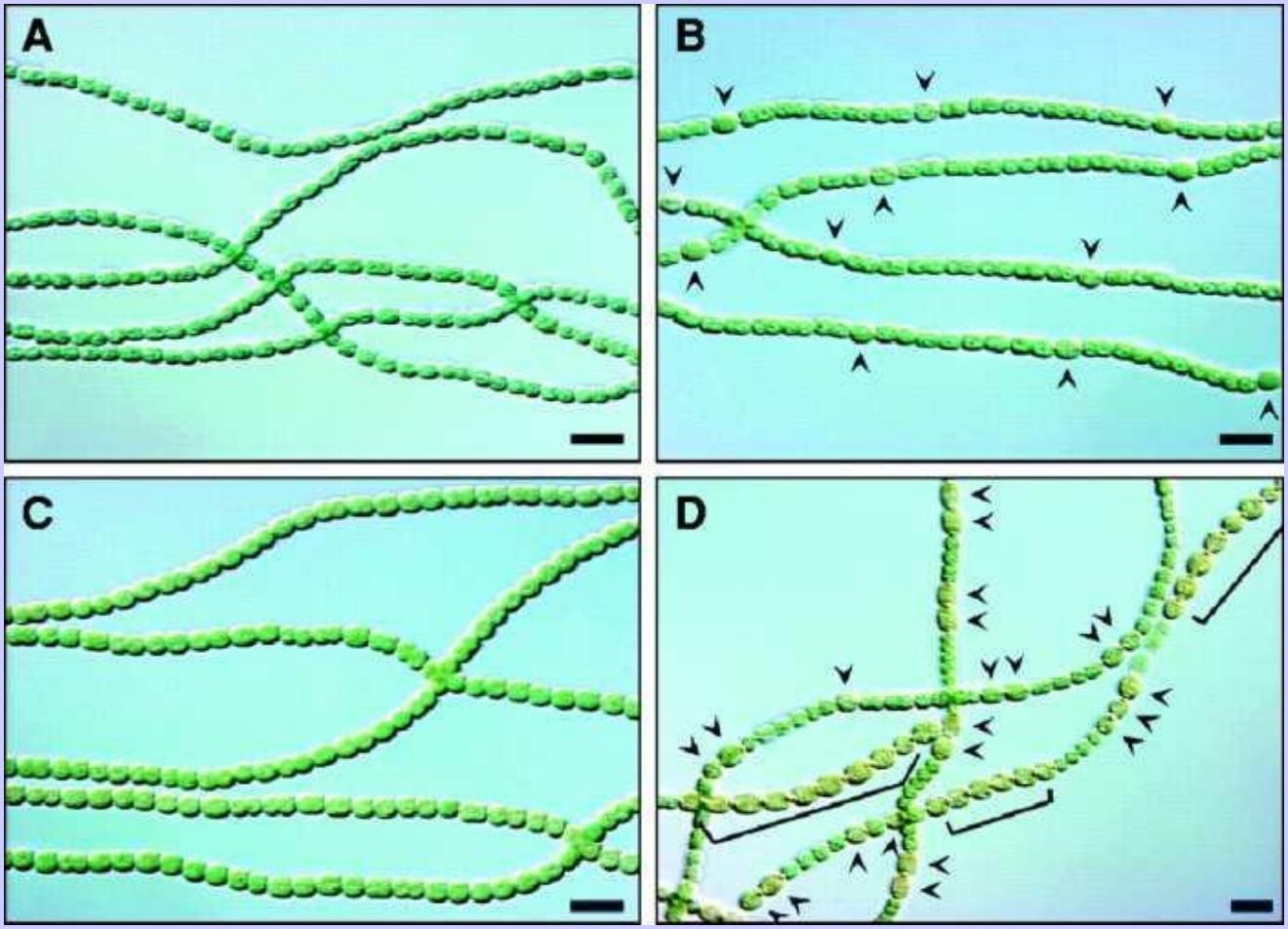
PatS



overexpression of *patS* completely blocks heterocyst development

patS encode a 17- or 13-amino-acid peptide, is crucial for the formation and maintenance of the normal heterocyst pattern

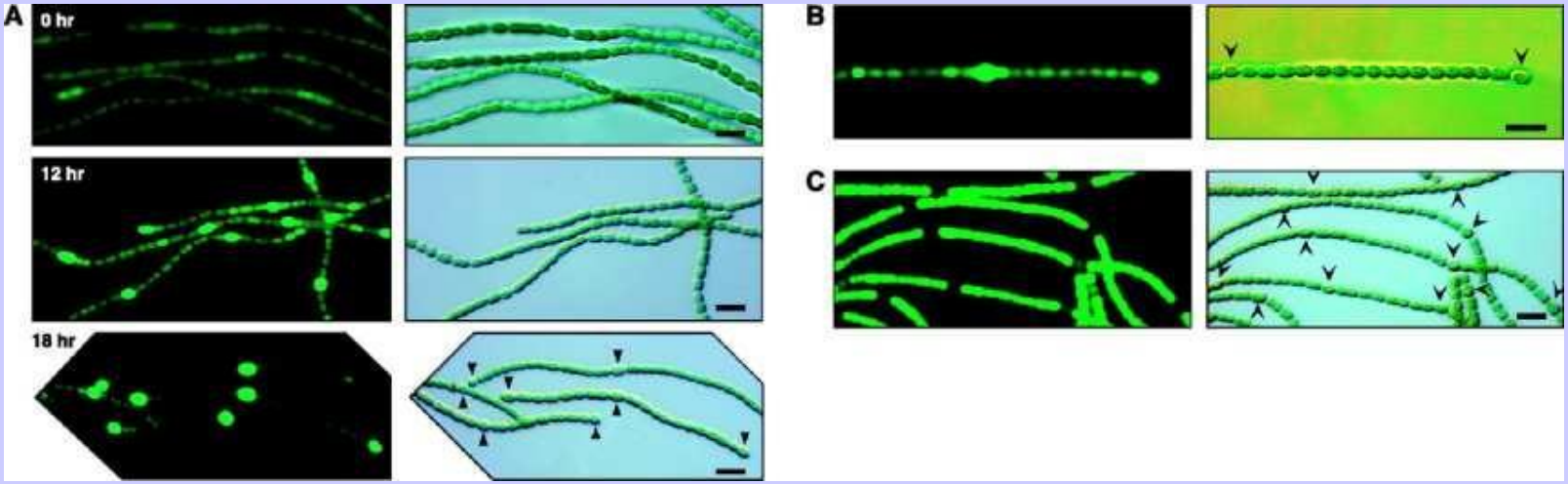
patS controls heterocyst development in *Anabaena* PCC 7120

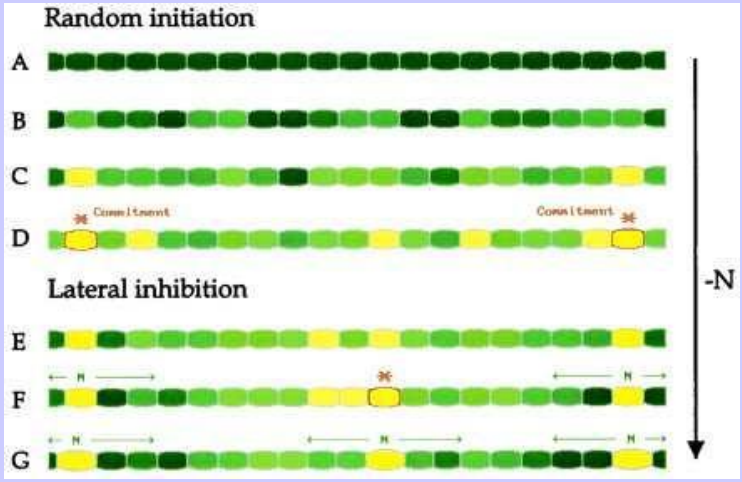


Wild-type filaments (**A**) grown in BG-11 medium and (**B**) after the nitrogen step-down in BG-11_0 to induce heterocysts (arrowheads) are shown. (**C**) Overexpression of *patS* prevented heterocyst formation in BG-11_0, and (**D**) deletion of *patS* resulted in supernumerary heterocysts with an abnormal pattern in BG-11_0. Differential interference contrast micrographs were taken before (A) and 24 hours after (B through D) heterocyst induction.

RGSGR

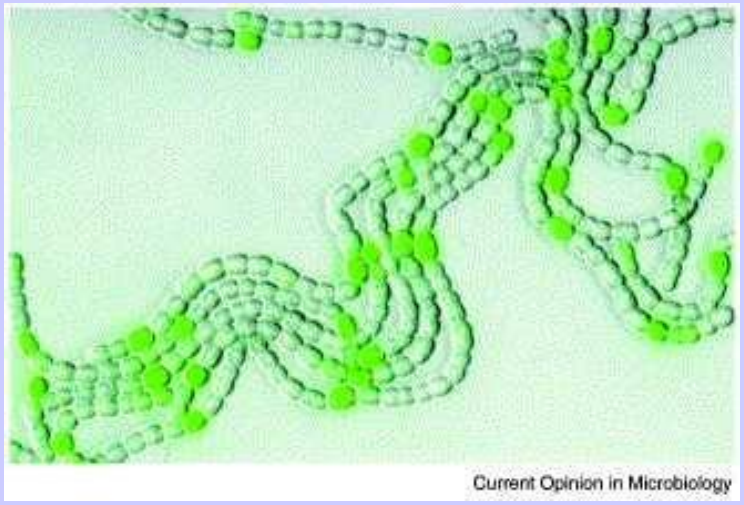
The exogenous addition of a pentapeptide corresponding to the last five COOH-terminal residues of PatS also inhibited heterocyst differentiation, indicating that a processed form of PatS may be a diffusible inhibitory signal regulating development.





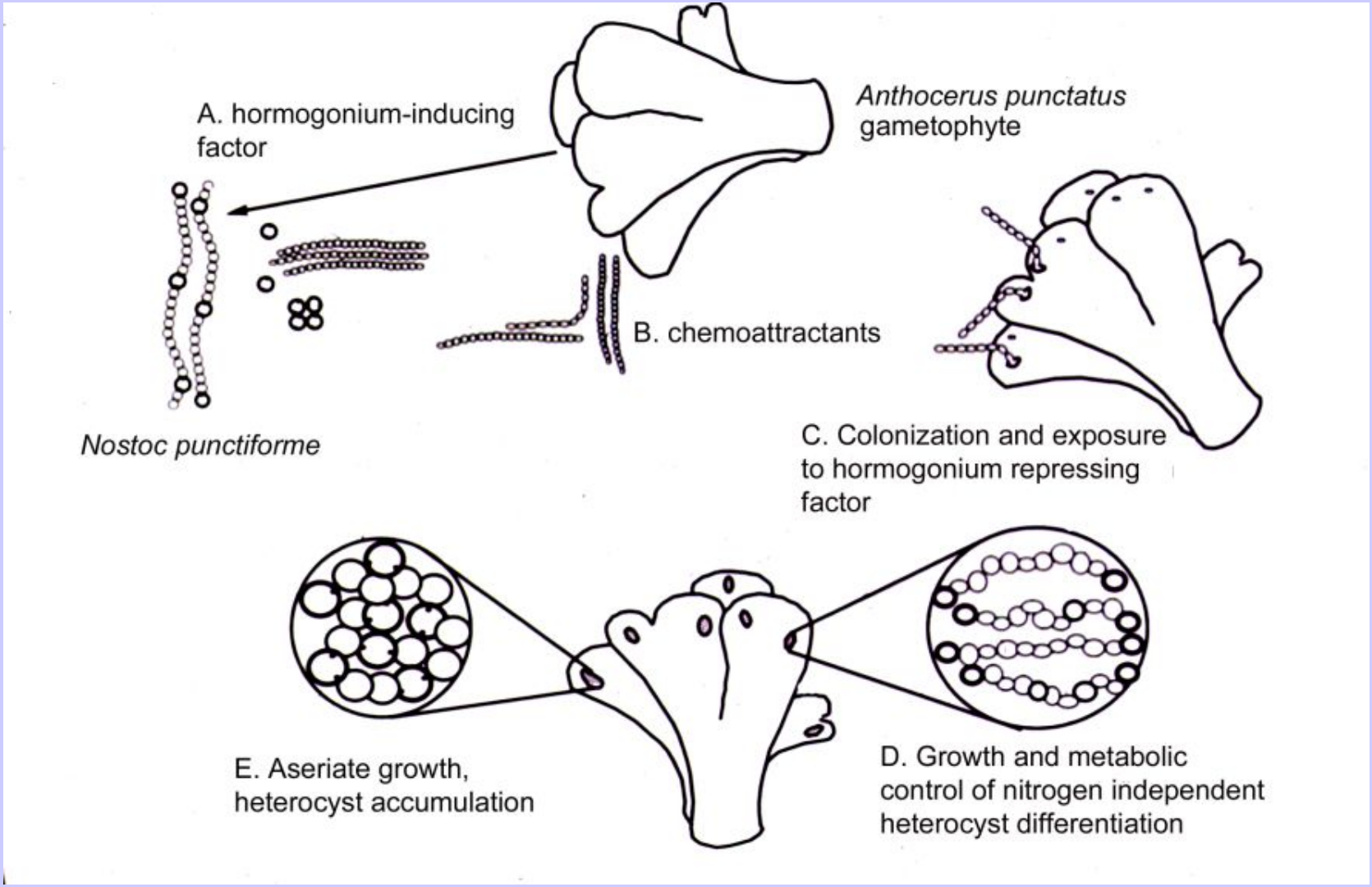
Přesně jako v modelu
z roku 1975

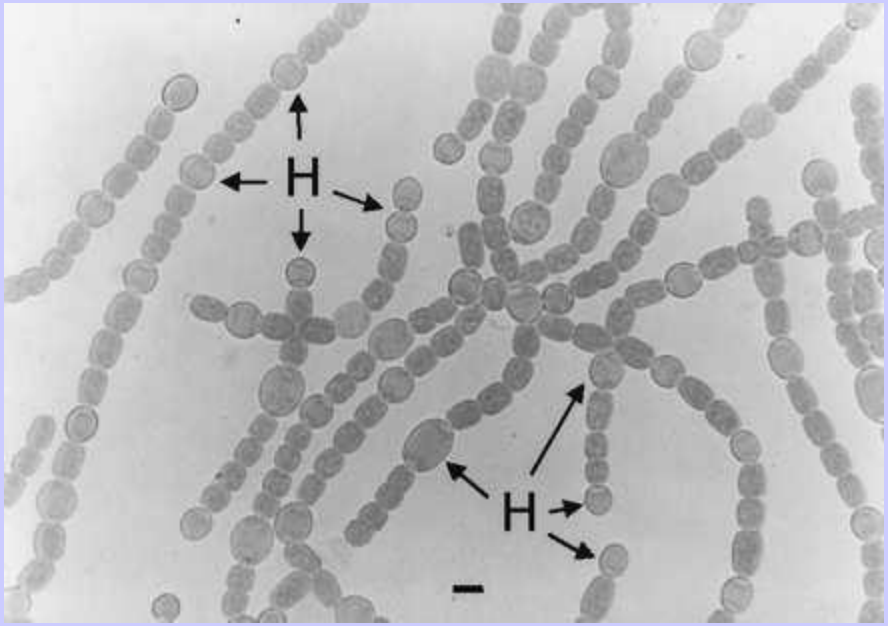
The inhibition of neighboring cells by select differentiating cells (lateral inhibition) is an important mechanism of pattern formation in eukaryotic organisms.



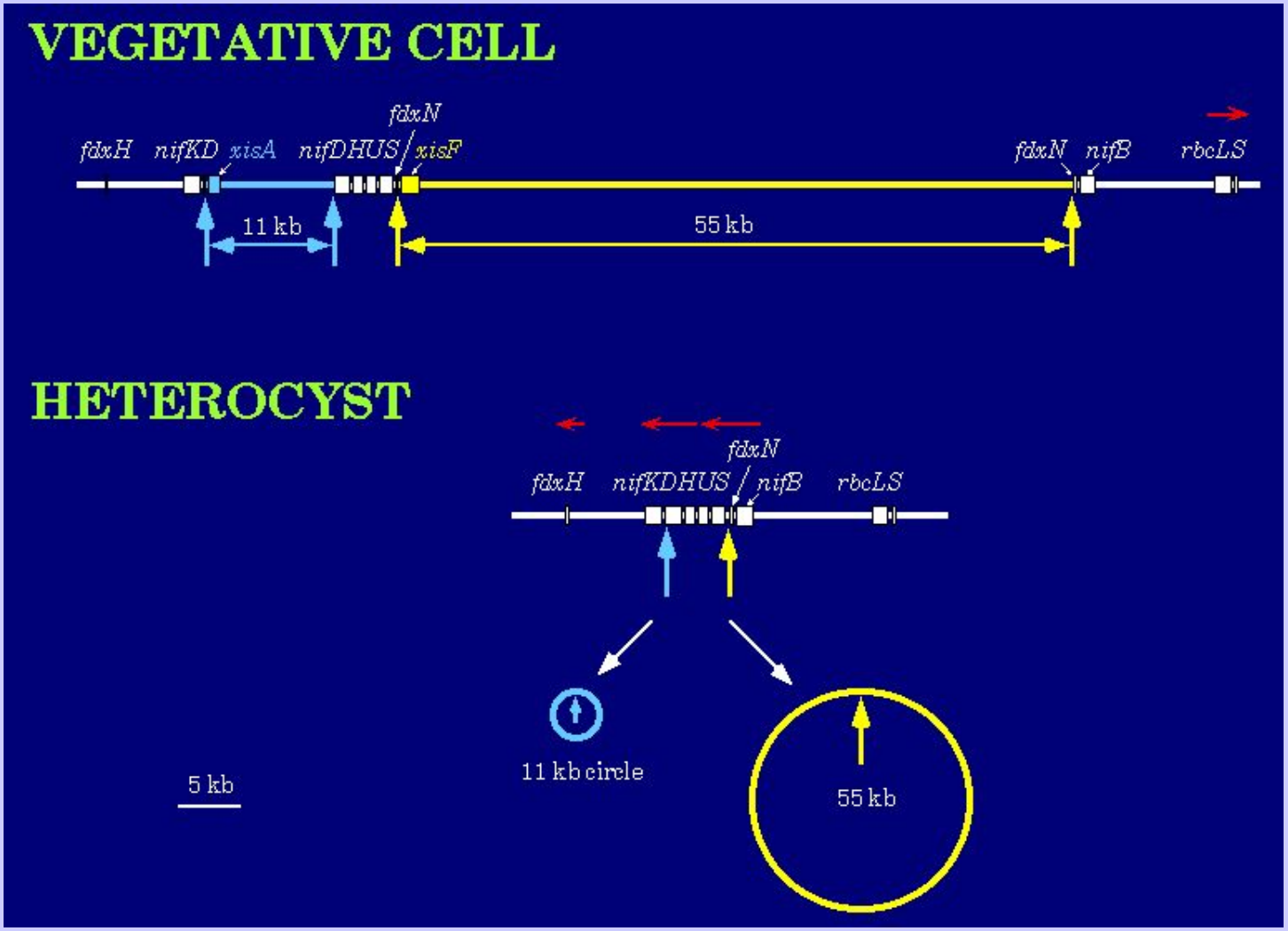
- Because it takes ~20 hours for heterocysts to mature and begin supplying fixed nitrogen to the filament, a specialized early inhibitory signal is required to allow only a fraction of starving cells to terminally differentiate.
- The first cells to differentiate increase the production of PatS to inhibit neighboring cells from forming heterocysts. PatS-producing cells must themselves be refractory to the PatS signal.

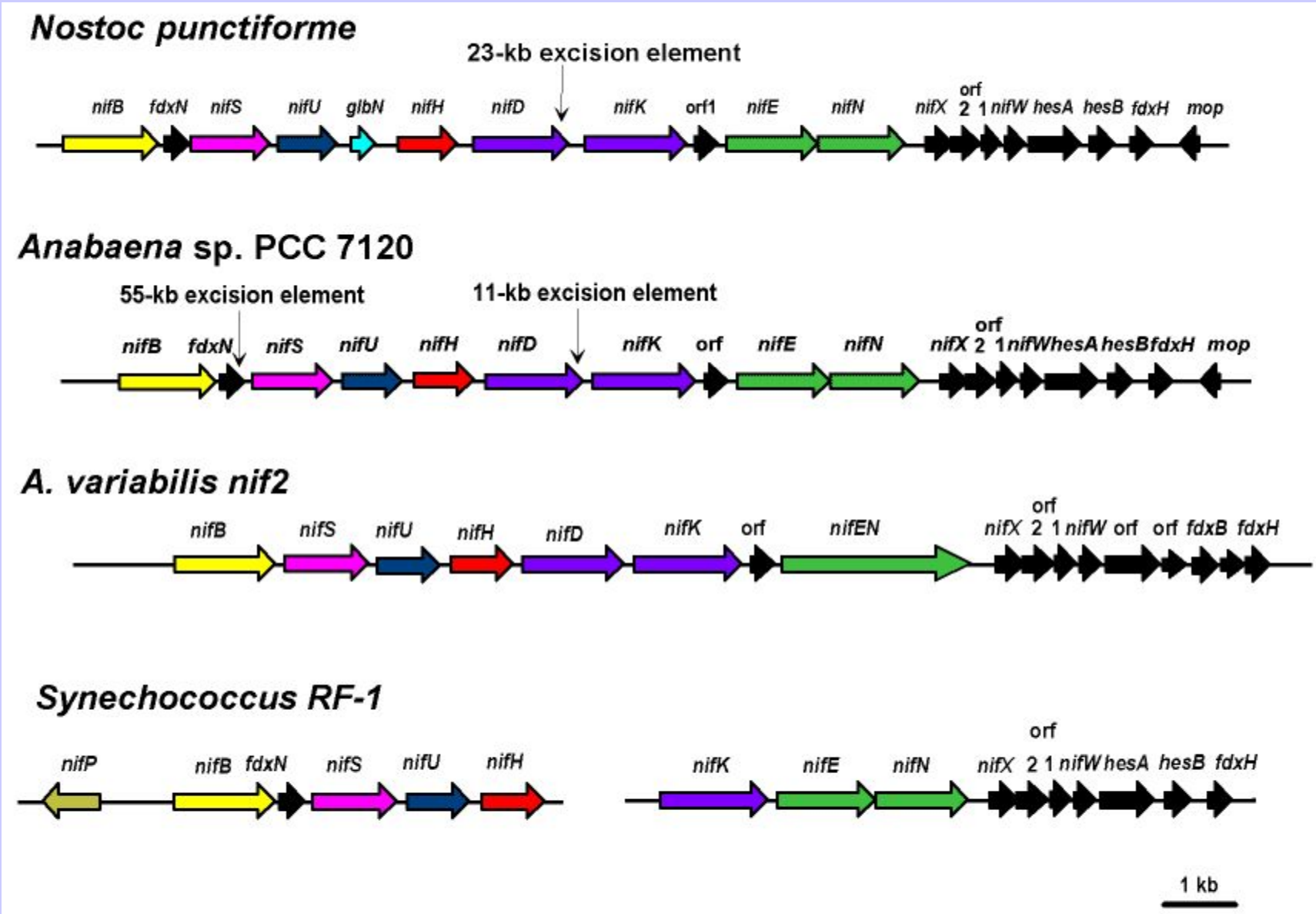
Anthoceros punctatus*. + *Nostoc punctiforme


A. hormogonium-inducing factor
Anthocerus punctatus gametophyte
B. chemoattractants
Nostoc punctiforme
C. Colonization and exposure to hormogonium repressing factor
E. Aseriate growth, heterocyst accumulation
D. Growth and metabolic control of nitrogen independent heterocyst differentiation


H
H

Další události nezbytné k aktivaci *nif* genů



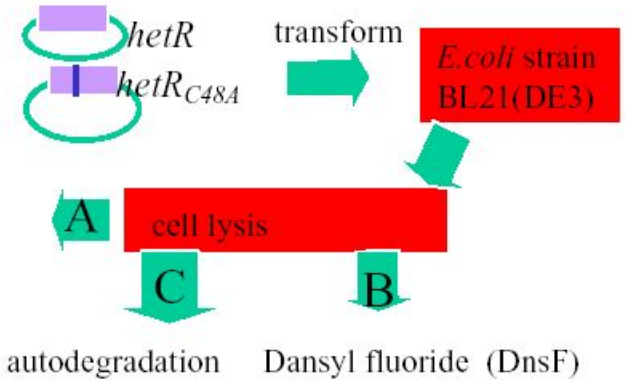

Nostoc punctiforme
23-kb excision element
nifB fdxN nifS nifU glbN nifH nifD nifK orf1 nifE nifN nifX orf 2 1 nifW hesA hesB fdxH mop
Anabaena sp. PCC 7120
55-kb excision element
11-kb excision element
nifB fdxN nifS nifU nifH nifD nifK orf nifE nifN nifX orf 2 1 nifW hesA hesB fdxH mop
A. variabilis nif2
nifB nifS nifU nifH nifD nifK orf nifEN nifX orf 2 1 nifW orf orf fdxB fdxH
Synechococcus RF-1
nifP nifB fdxN nifS nifU nifH
nifK nifE nifN nifX orf 2 1 nifW hesA hesB fdxH
1 kb

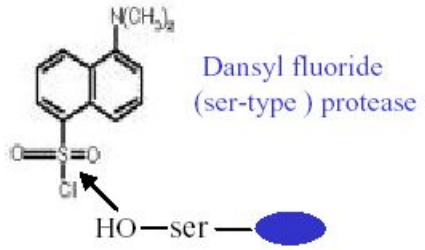
HetR homodimer is a DNA-binding protein required for heterocyst differentiation, and the DNA-binding activity is inhibited by PatS

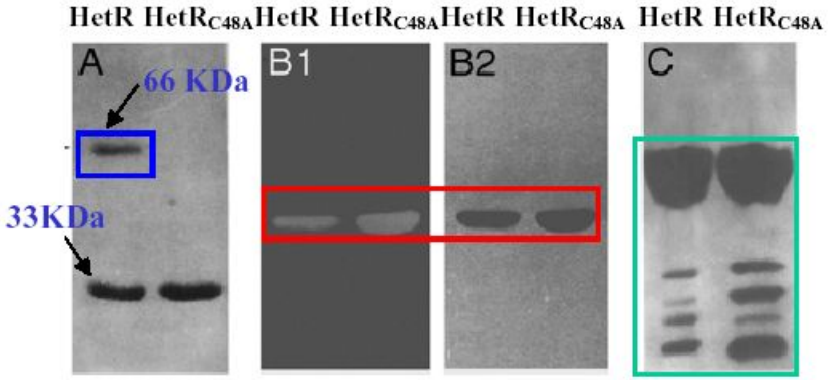
Huang, X., Dong, Y. Q., and Zhao, J. D. (2004)

Proc. Natl. Acad. Sci. USA,**101**, 4848-4853.

In vitro In vivo HetR homodimer



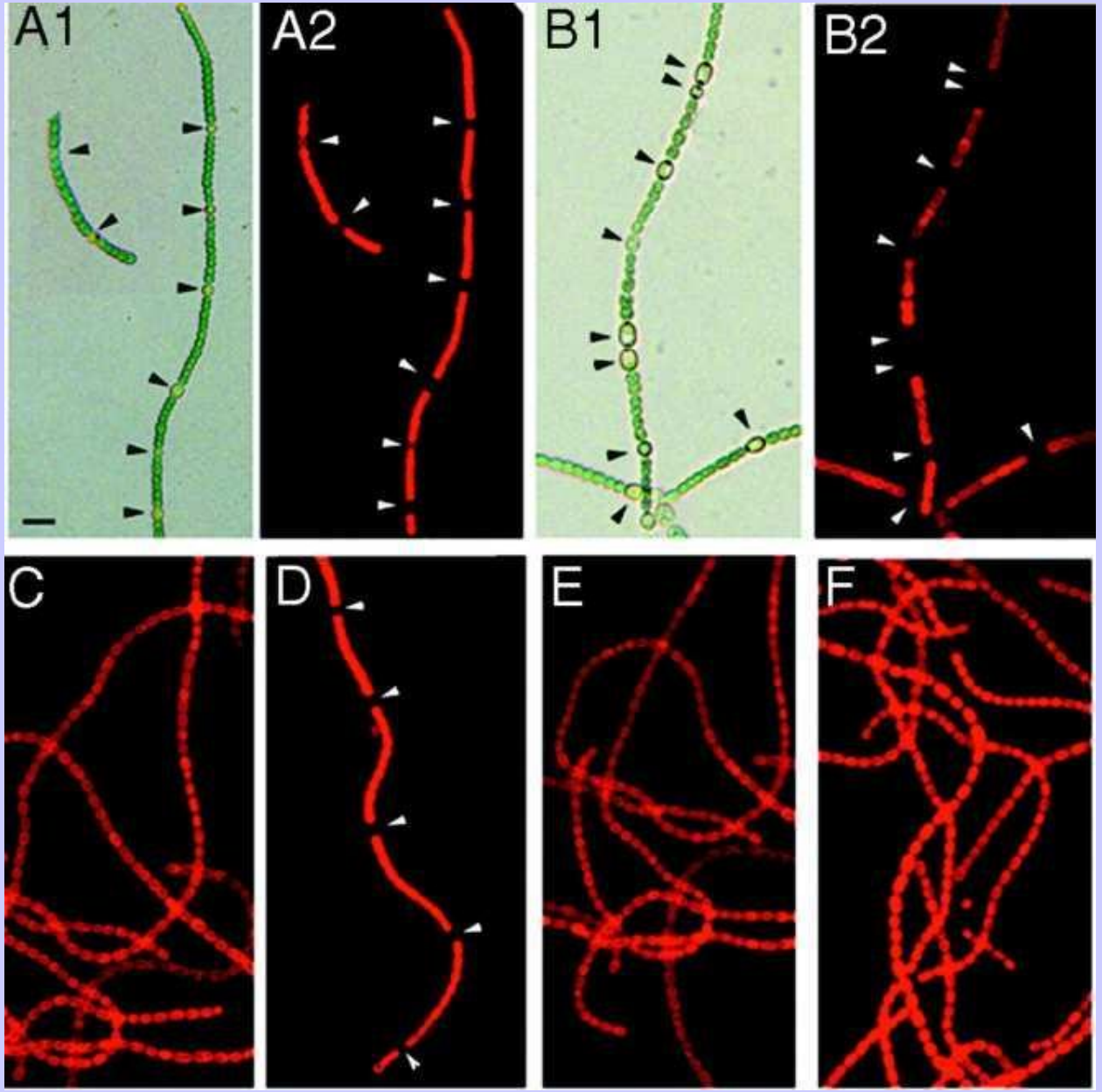


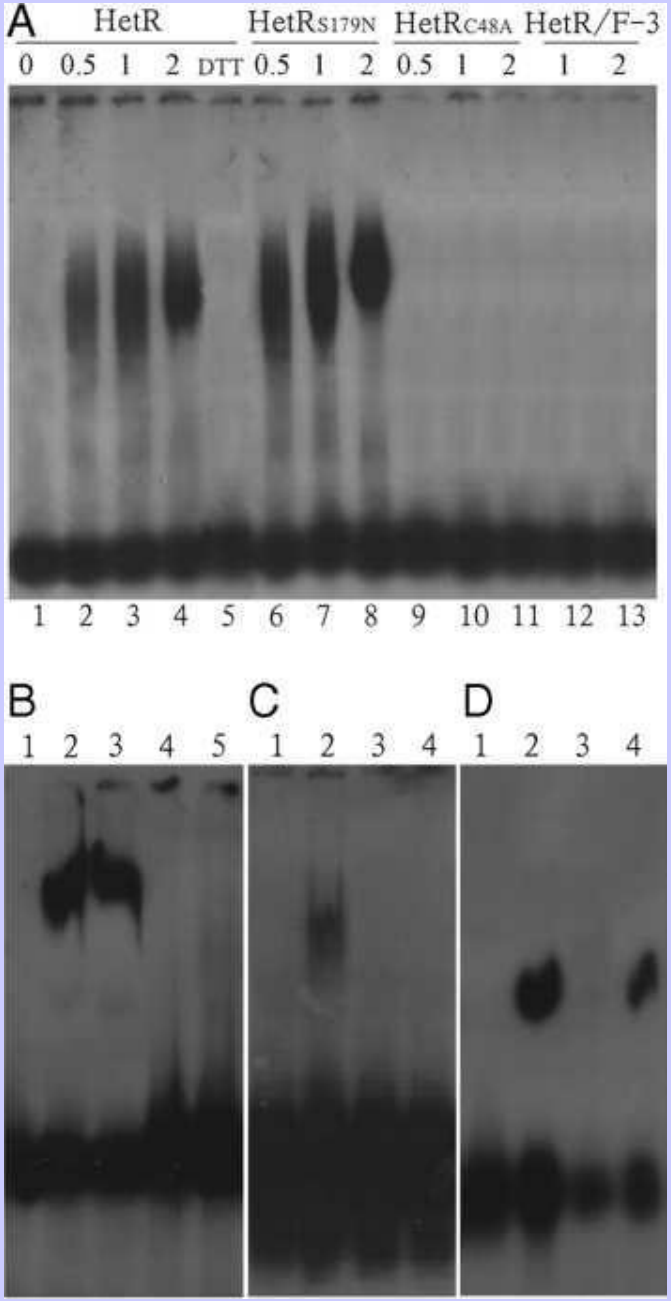


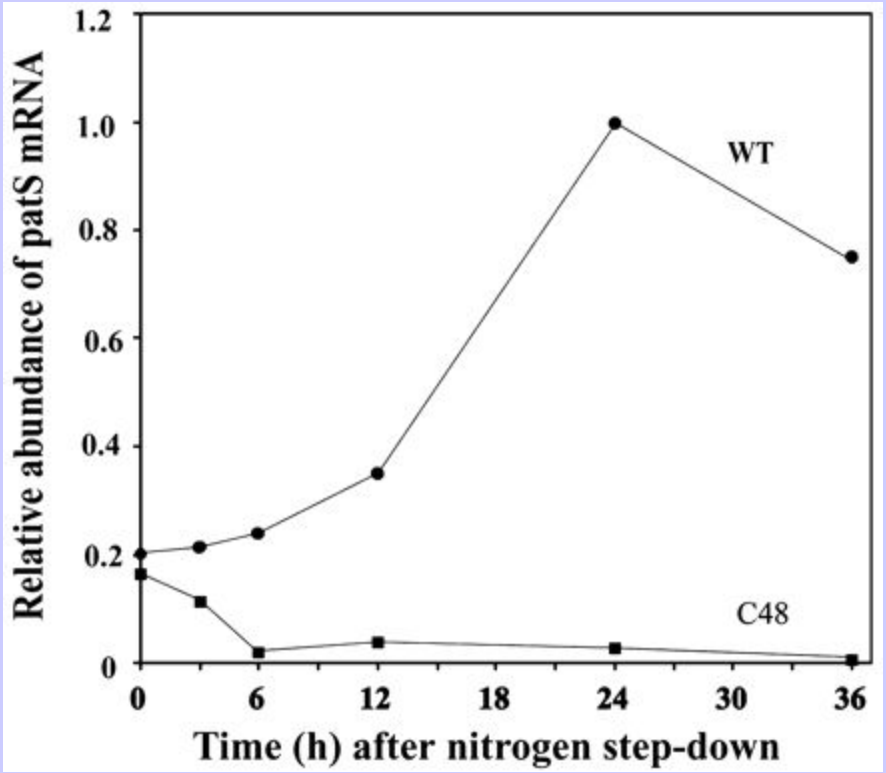
A HetR$_{C48A}$
dimer

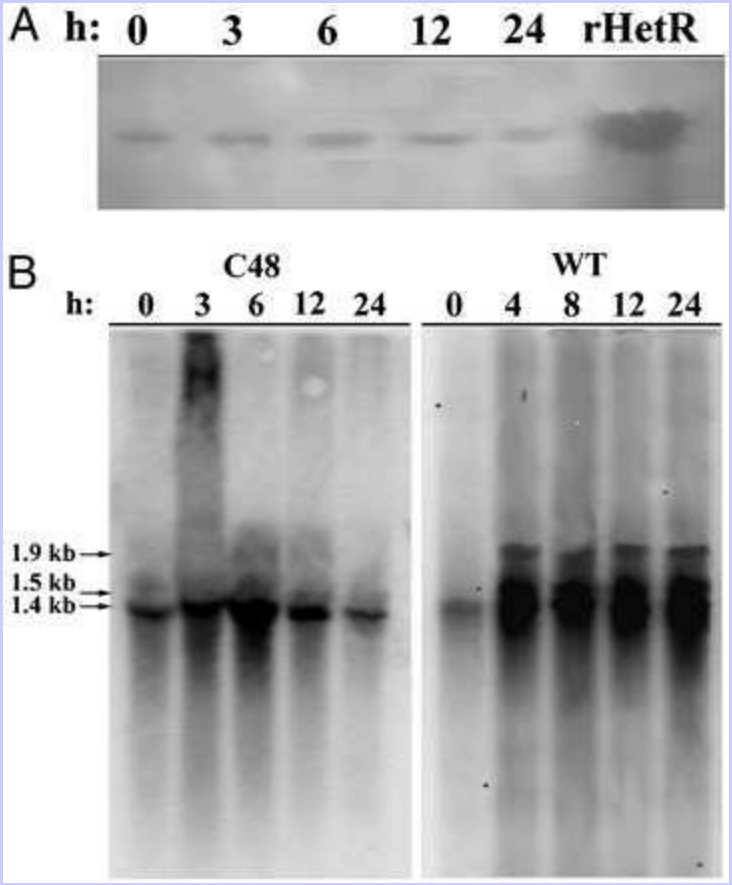
B_1, B_2
ser-type protease-DnsF
HetR HetR$_{C48A}$

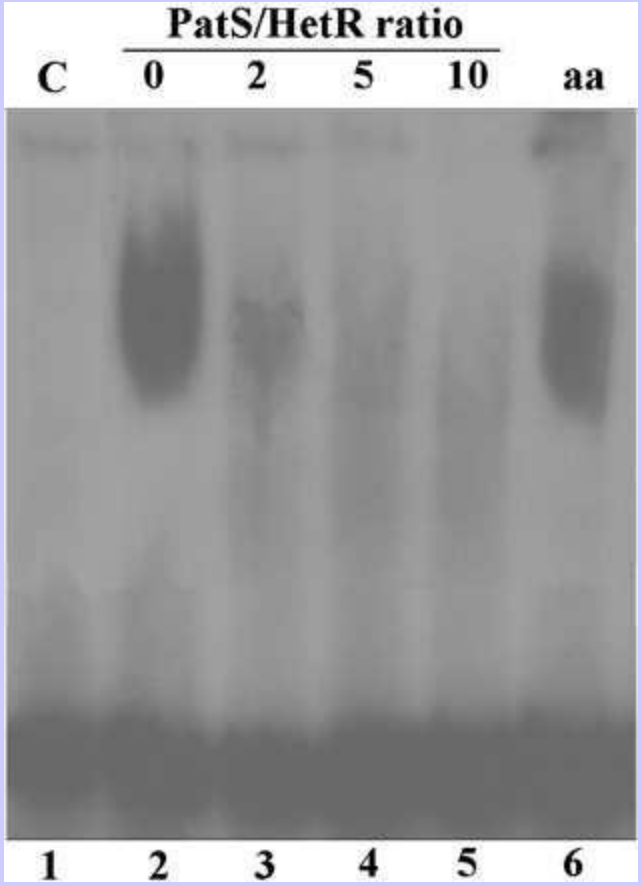
C HetR HetR$_{C48A}$
protease
(Autodegradation)

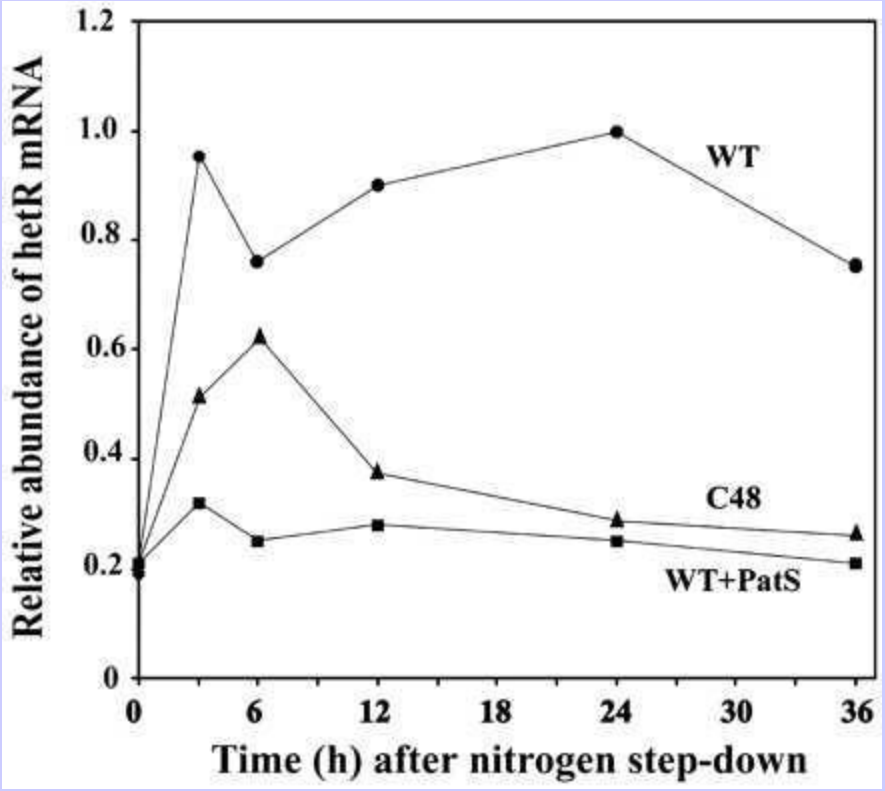

A1
A2
B1
B2
C
D
E
F


A
HetR
HetRS179N
HetRC48A
HetR/F-3
0 0.5 1 2 DTT 0.5 1 2 0.5 1 2 1 2
1 2 3 4 5 6 7 8 9 10 11 12 13
B
1 2 3 4 5
C
1 2 3 4
D
1 2 3 4

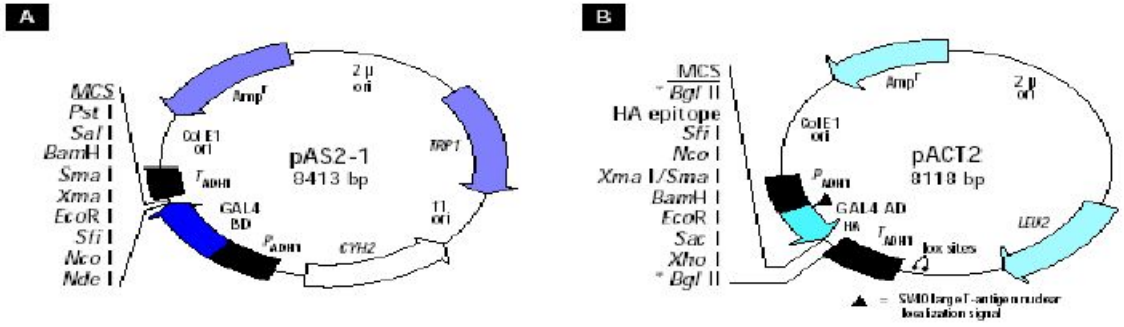

Relative abundance of patS mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
WT
C48
0
6
12
18
24
30
36
Time (h) after nitrogen step-down

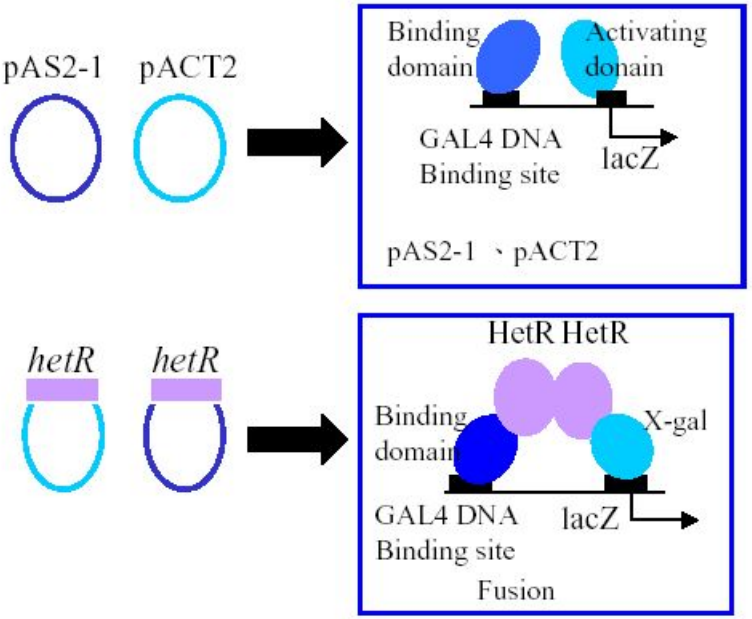

A
h: 0 3 6 12 24 rHetR
B
C48
WT
h: 0 3 6 12 24
0 4 8 12 24
1.9 kb
1.5 kb
1.4 kb

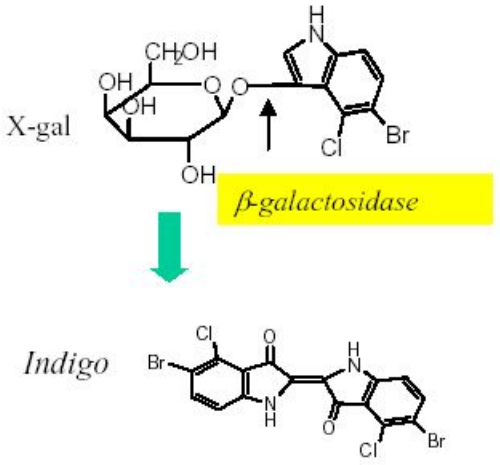

PatS/HetR ratio
C
0
2
5
10
aa
1
2
3
4
5
6


Relative abundance of hetR mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
WT
C48
WT+PatS
0
6
12
18
24
30
36
Time (h) after nitrogen step-down

Yeast-Two Hybrid assay

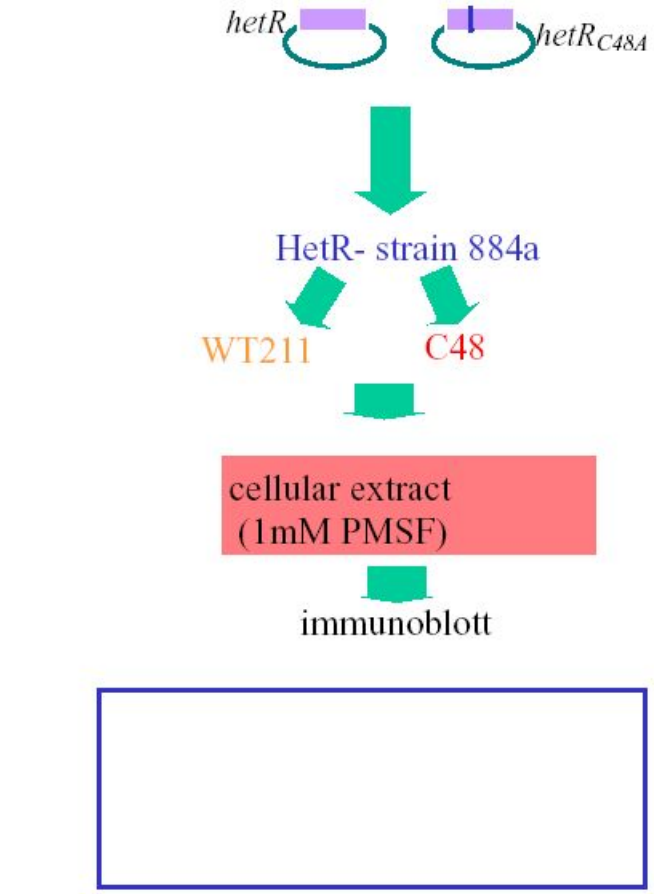


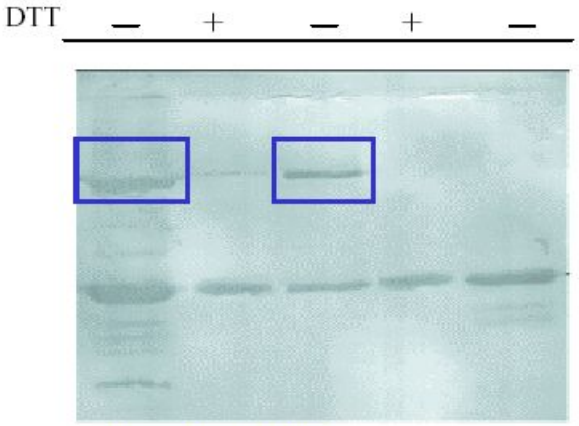




In vitro in vivo HetR homodimer (2)

In vivo HetR Protein homodimer ◦

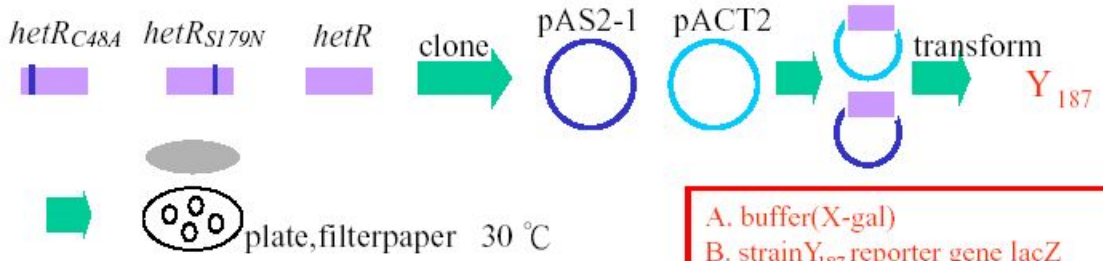




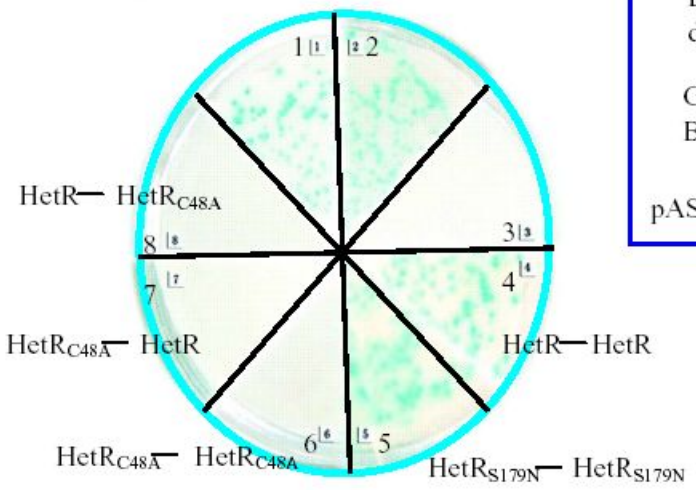
Lane1 rHetR
Lane2 WT211
Lane3 WT211
Lane4 C48
Lane5 C48

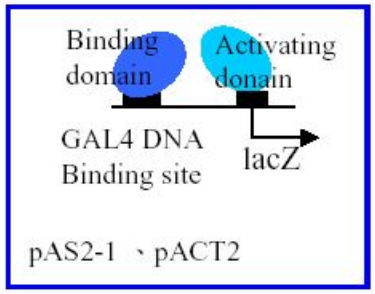
HetRC48A does not form dimer

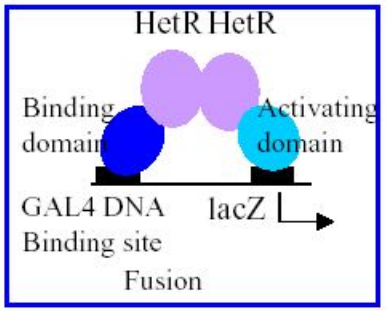
Yeast-Two Hybrid assay:



A. buffer(X-gal)
B. strain Y_{187} reporter gene lacZ



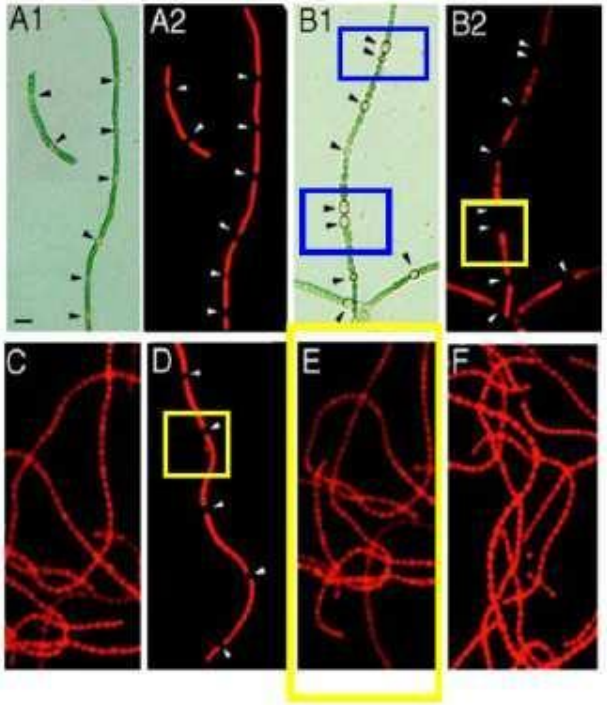




Cys48 mutation does not interfere with WT HetR function

Anabaena sp.PCC strain 7120 ($BG11_0$) → pRL25C **nitrogen starvation**

實驗結果:



A1 and A2 : WT *Anabaena sp.*PCC strain 7120

C : ***hetRC48A*** ◦

B1 and B2 **: p**RL25C *(hetR)Anabaena* ◦

D : pRL25C($hetR_{C48A}$) *Anabaena* ◦

E : pRL25C($hetR_{S179N}$) *Anabaena* ◦

F : 1μM PatS *Anabaena*

HetRS179N makes dimers, but no protease

HetR binds DNA - by EMSA