

# Diferenciace heterocyst

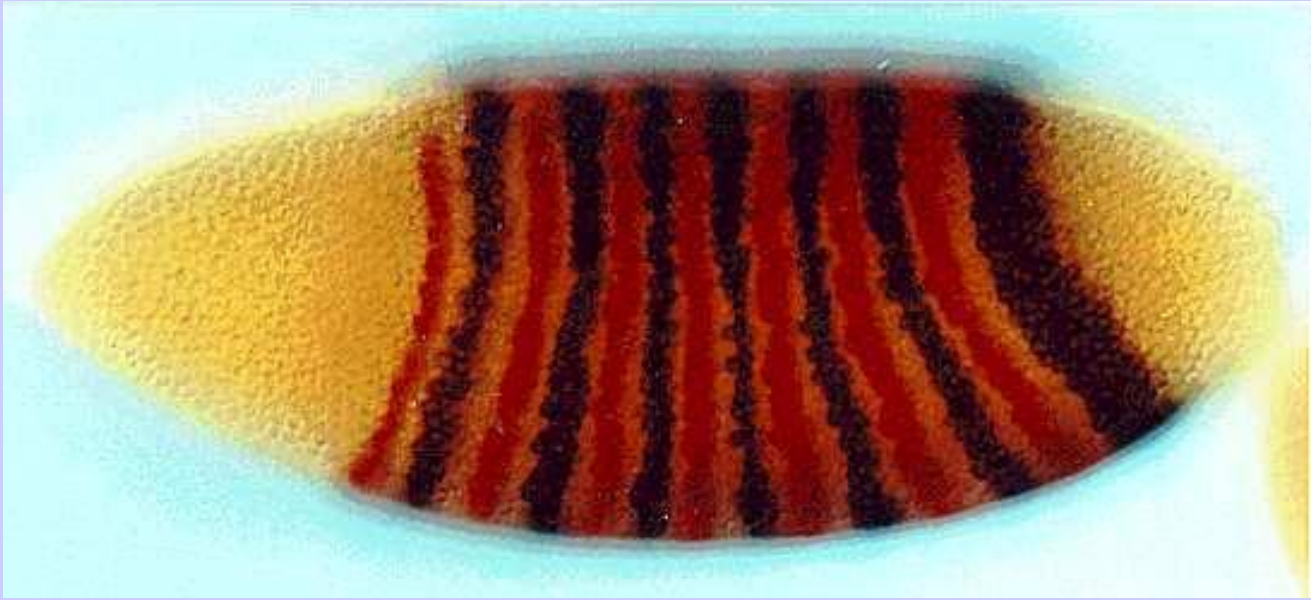
Martin Tichý

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*Institute of Physical Biology, University of South Bohemia, Nové Hradý*  
*Czech Republic*



*Anabaena* sp. PCC 7120 je Nostoc

heterocysty nejsou cysty



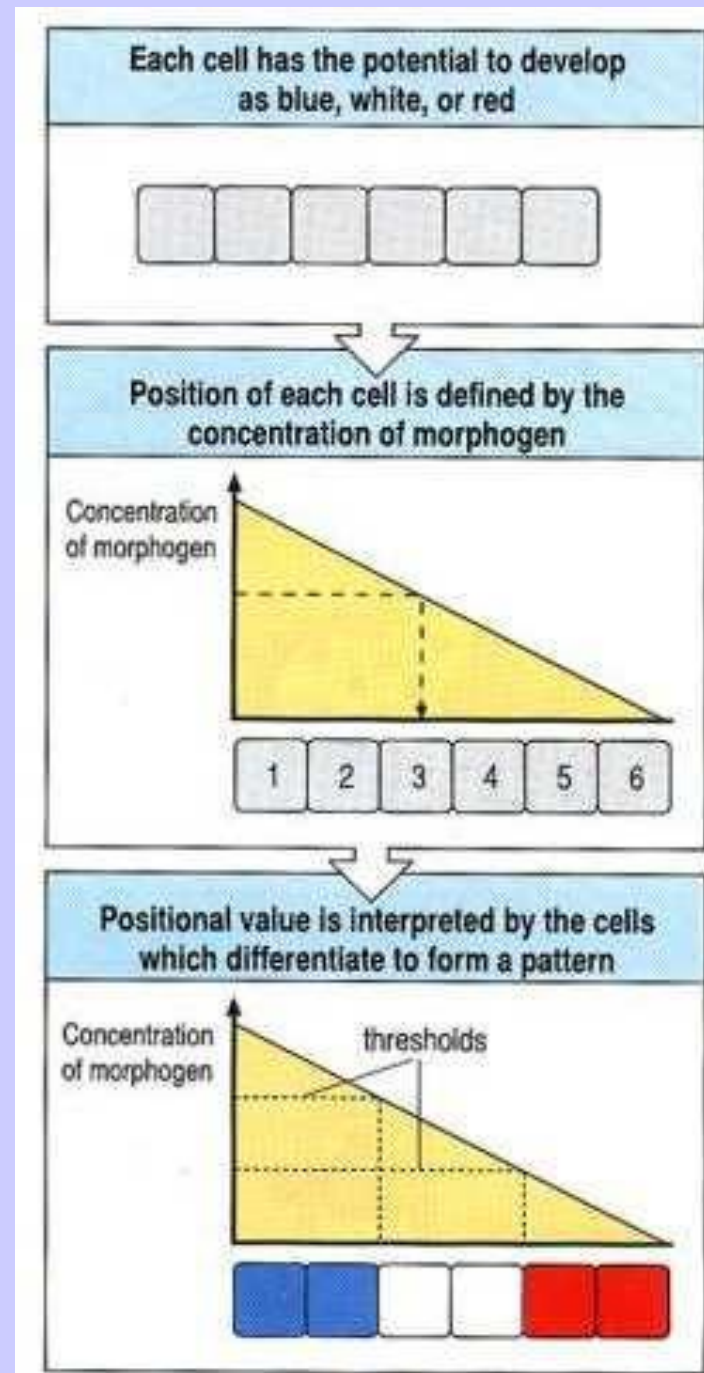
# **Paradox of developmental biology**

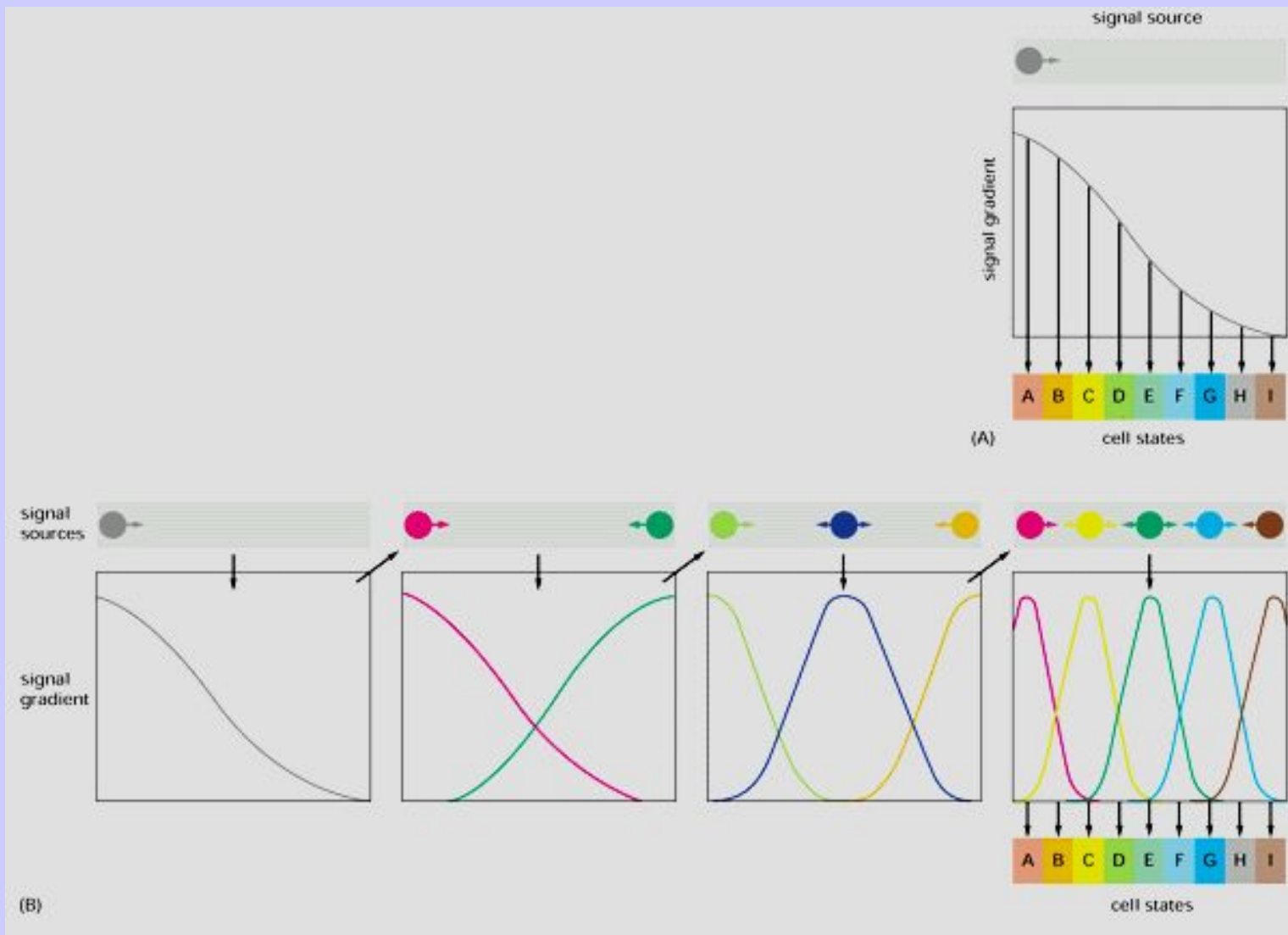
How is it that a single cell gives rise to a multicellular organism composed of 100s of different cell types – yet all the cell types have the same genes?

**Patterning can involve the interpretation of positional information.**



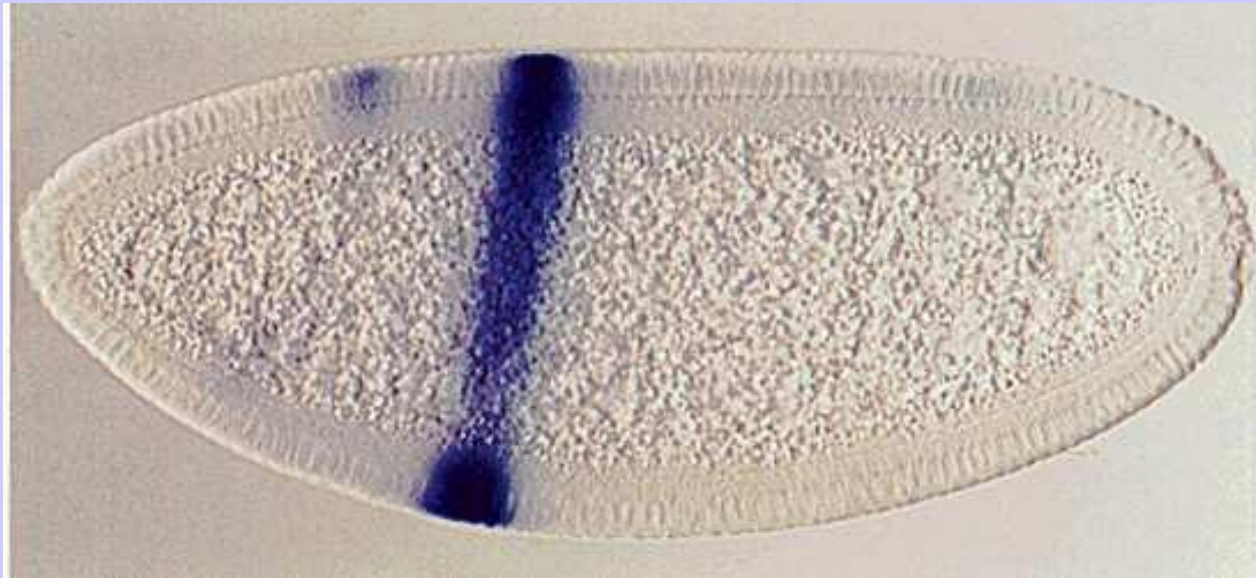
French flag analogy





**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

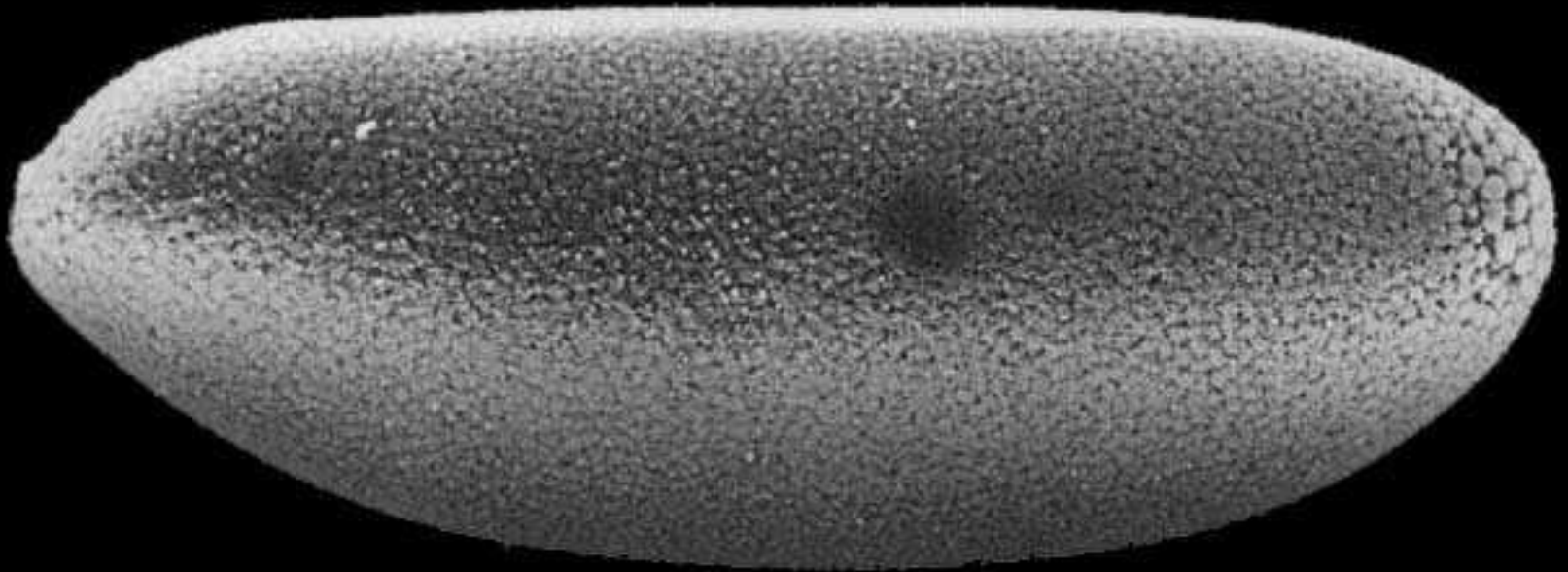




**Figure 21-65.** The formation of *ftz* and *eve* stripes in the *Drosophila* blastoderm. Genes *ftz* and *eve* are both pair-rule genes. Their expression patterns (shown in *brown* for *ftz* and in *gray* for *eve*) are at first blurred but rapidly resolve into sharply defined stripes. (From P.A. Lawrence,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



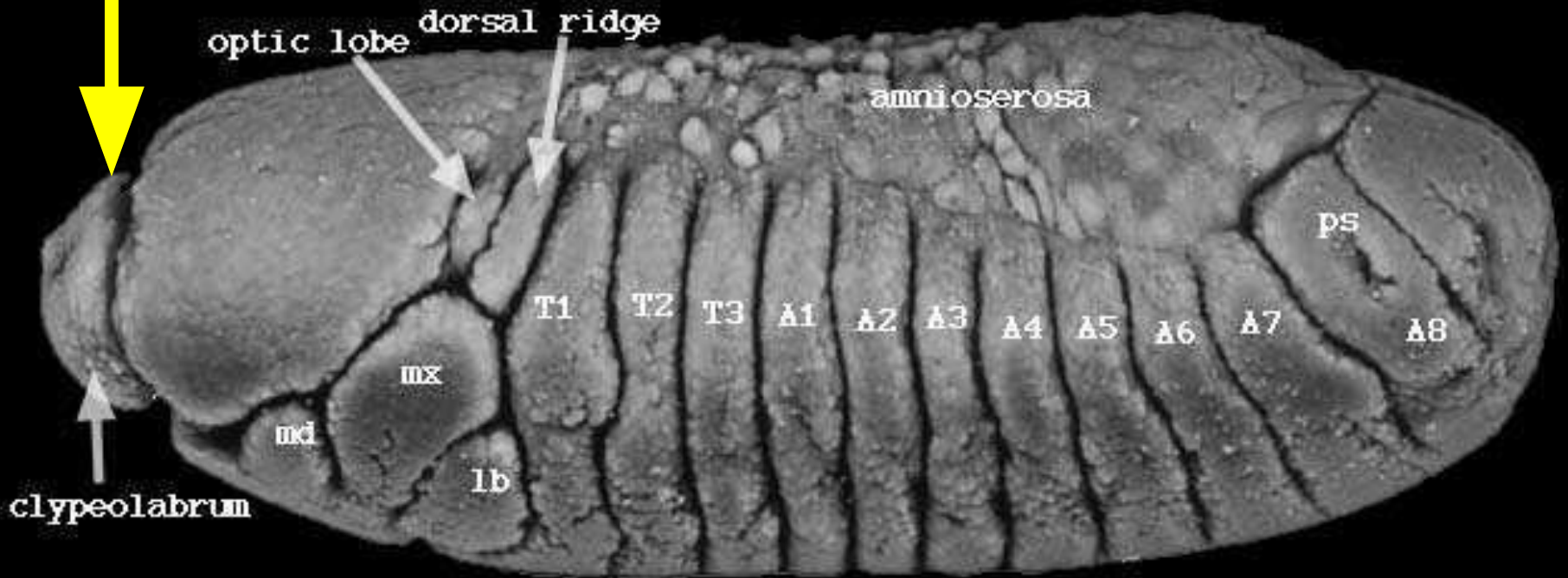
# The Course of Development



# The Course of Development

Time

*Complicated*

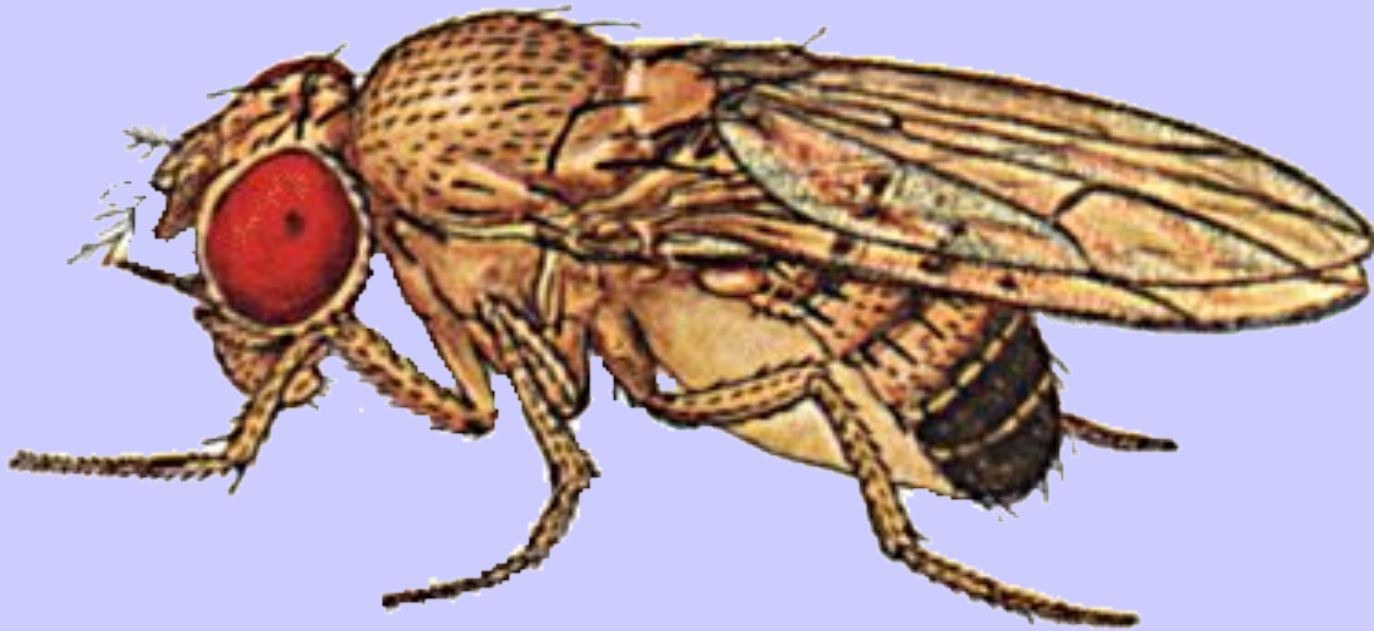


Events in time and space . . .

# The Course of Development

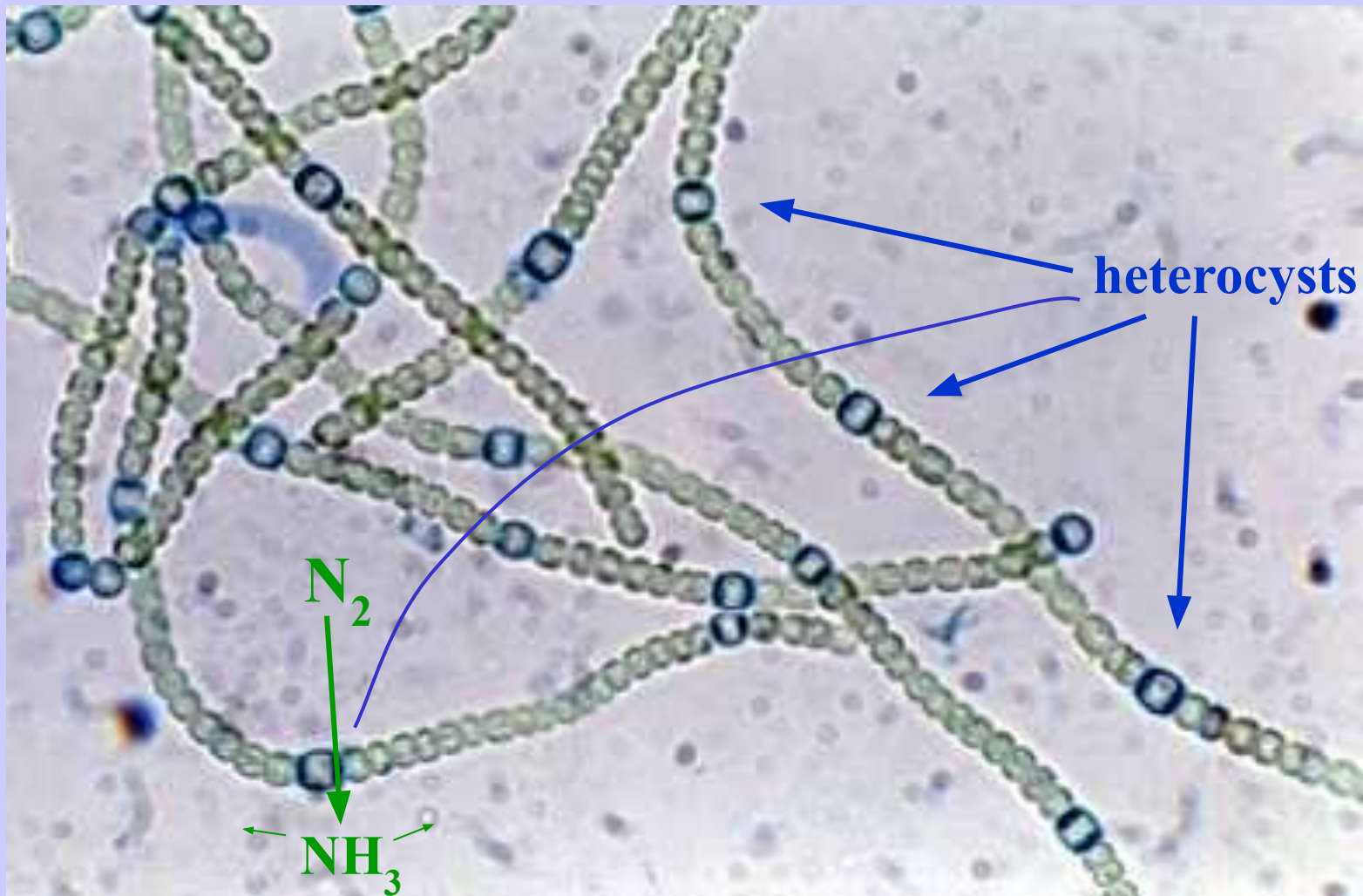
Time

*Really Complicated*



# Cyanobacteria

Anabaena grown without fixed nitrogen



Matveyev and Elhai (unpublished)

# Paradox of developmental biology

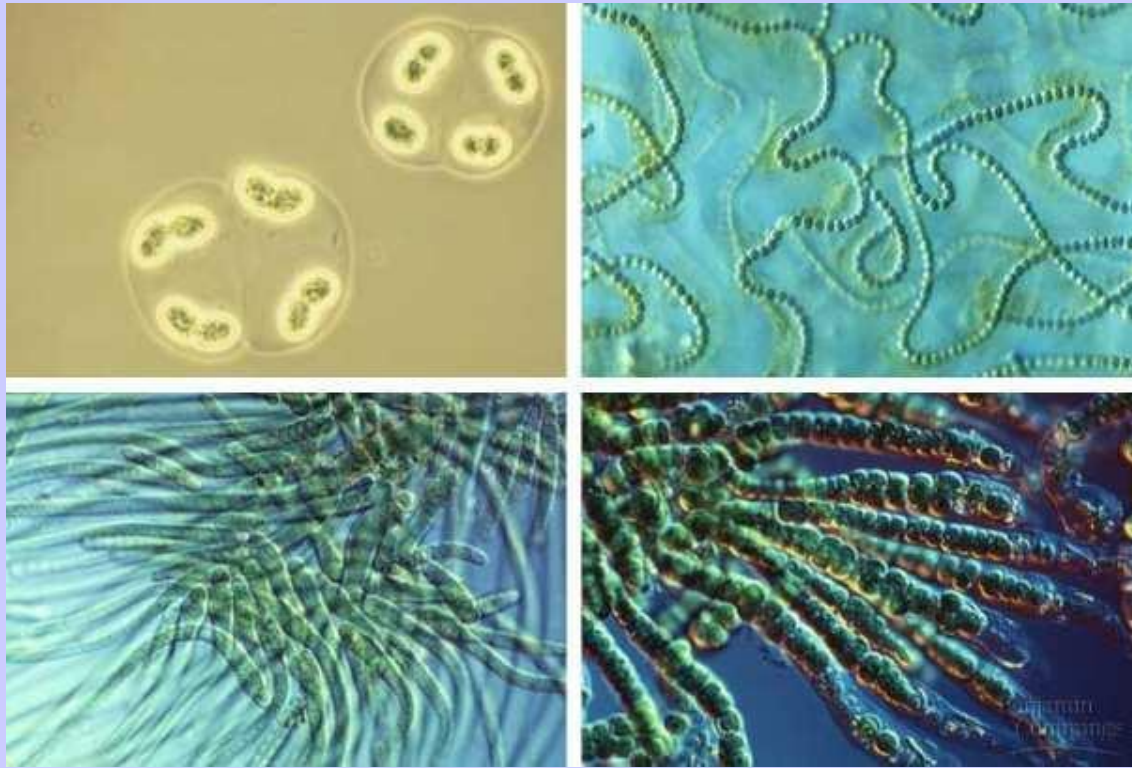
How is it that a single cell gives rise to a multicellular organism composed of 100s of different cell types – yet all the cell types have the same genes?

## How Cyanobacteria Count to 10

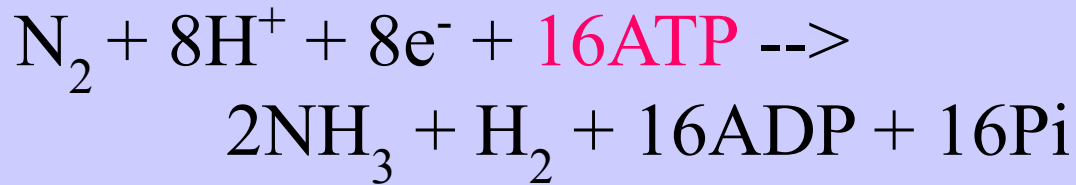
Robert Haselkorn

Jak každá desátá buňka ví že má být heterocystou

# Fixace dusíku



# Biochemistry of N<sub>2</sub> fixation



Note: *Very expensive*

Reason why N<sub>2</sub> 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  $O_2$**

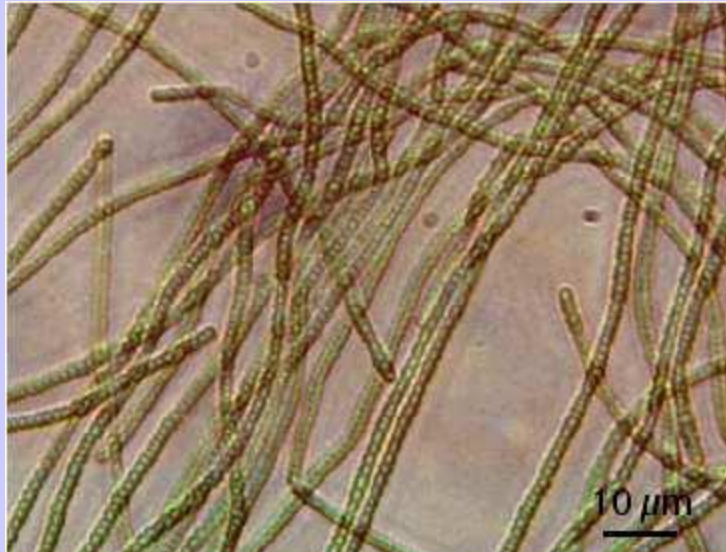
Protects nitrogenase ( $N_2$  fixing enzyme) from  $O_2$

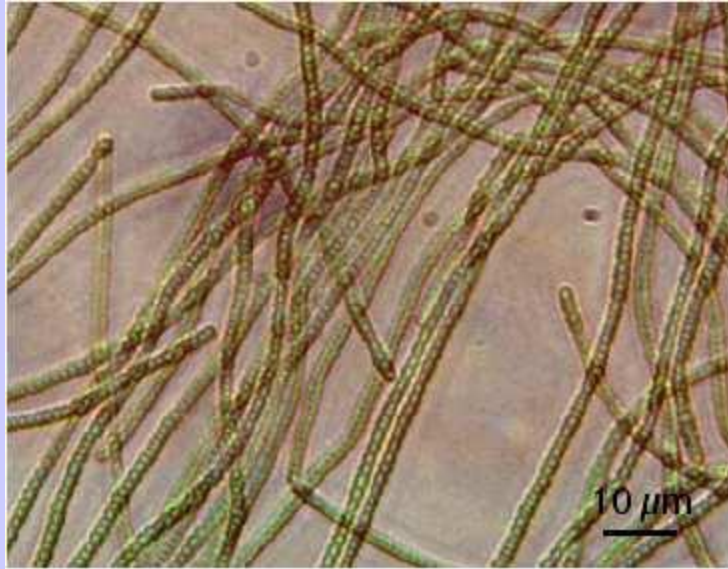
Outside sources of  $O_2$

$O_2$  produced by cyanobacteria

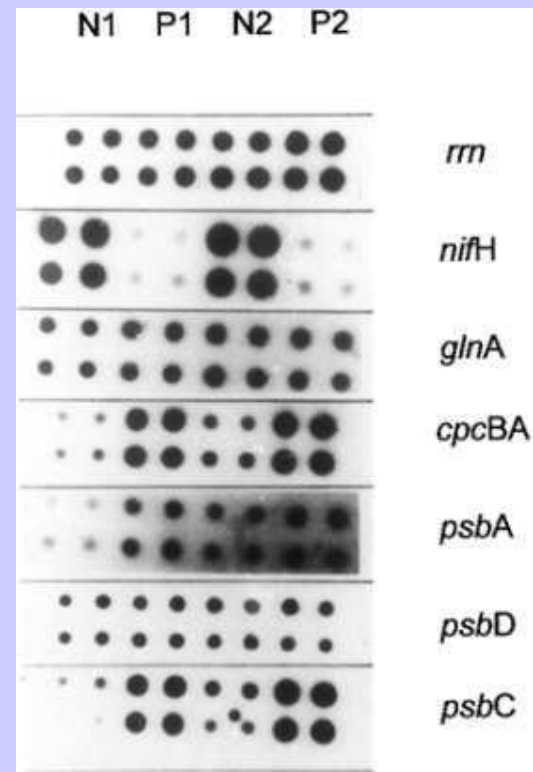
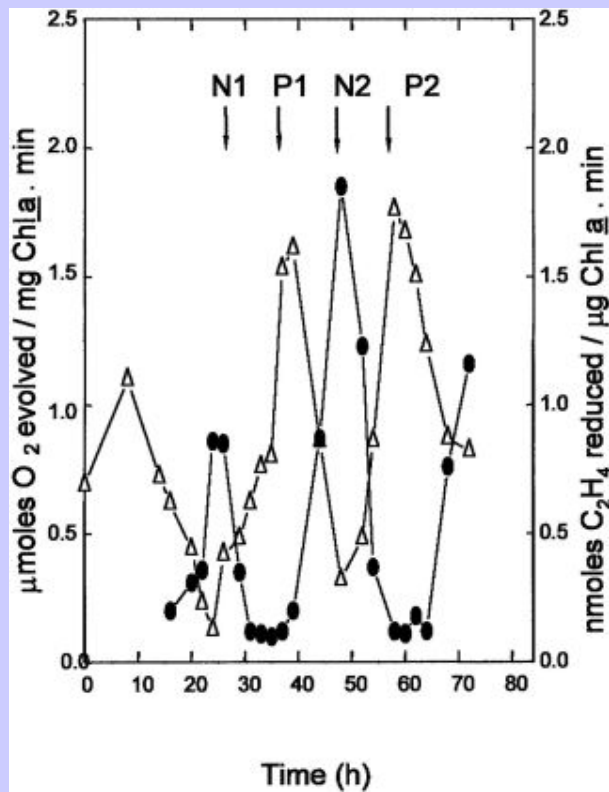


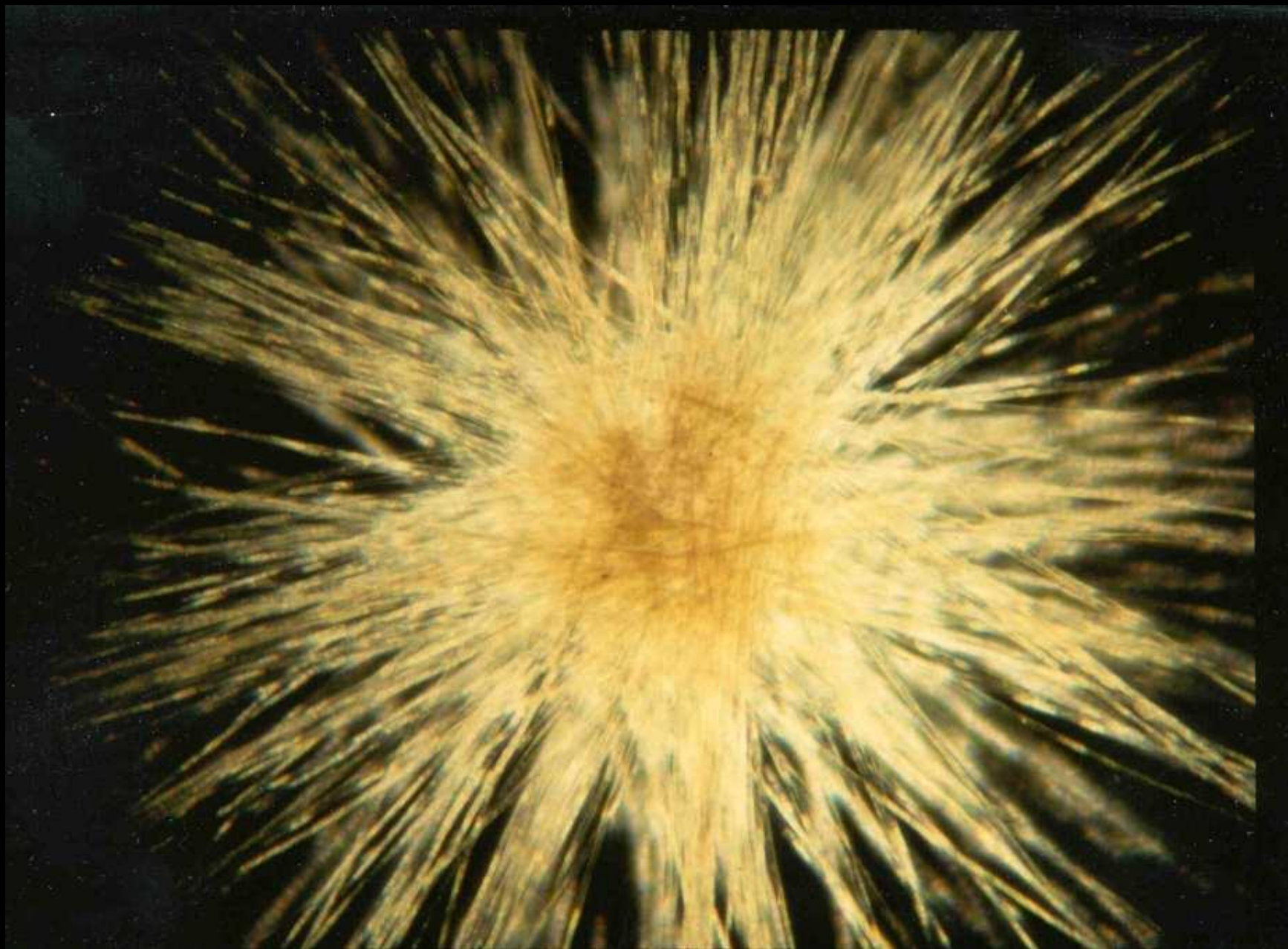
Ne všechny sinice schopné fixovat dusík tvoří heterocysty





*Plectonema boryanum* IAM-M101





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.

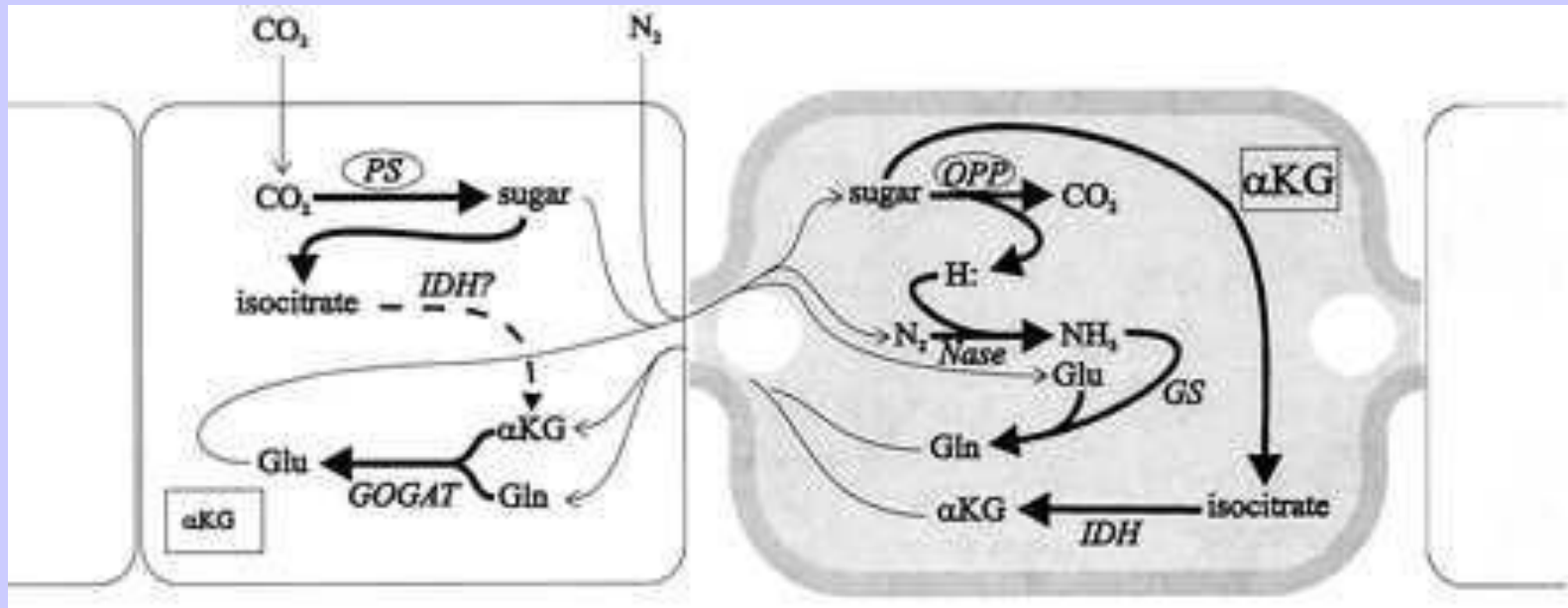
# What are heterocysts?

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<sub>2</sub>-producing end of the photosynthetic electron transport chain, is dismantled during heterocyst differentiation, so that the heterocyst need contend only against O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> that overcomes these barriers is consumed by the high oxidase activity associated with heterocysts.



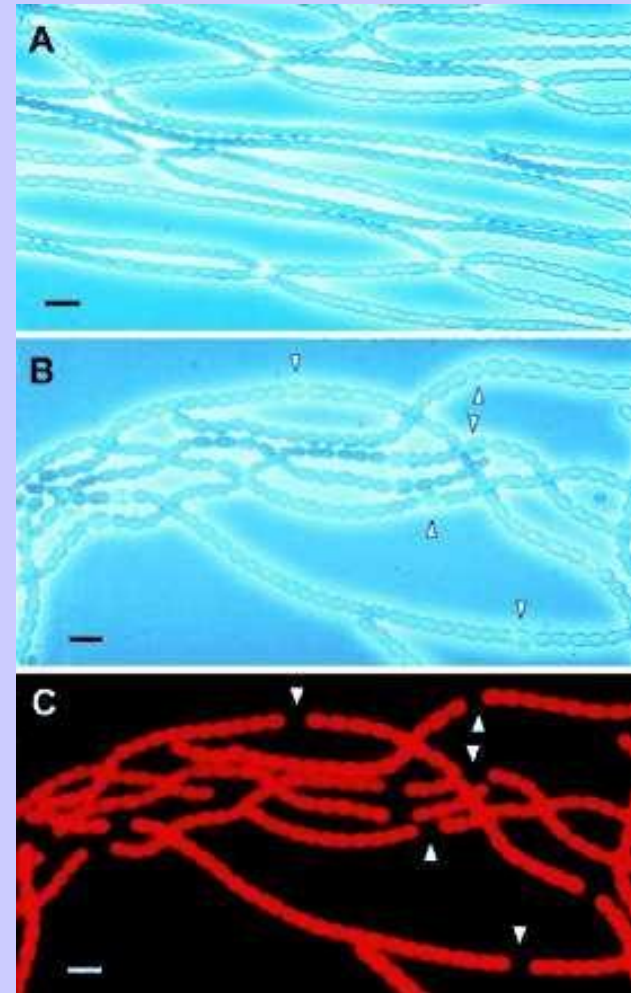
Dělba práce



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  $\mu\text{m}$ .



# Heterocysts only when needed

*Anabaena* filament growing on nitrate



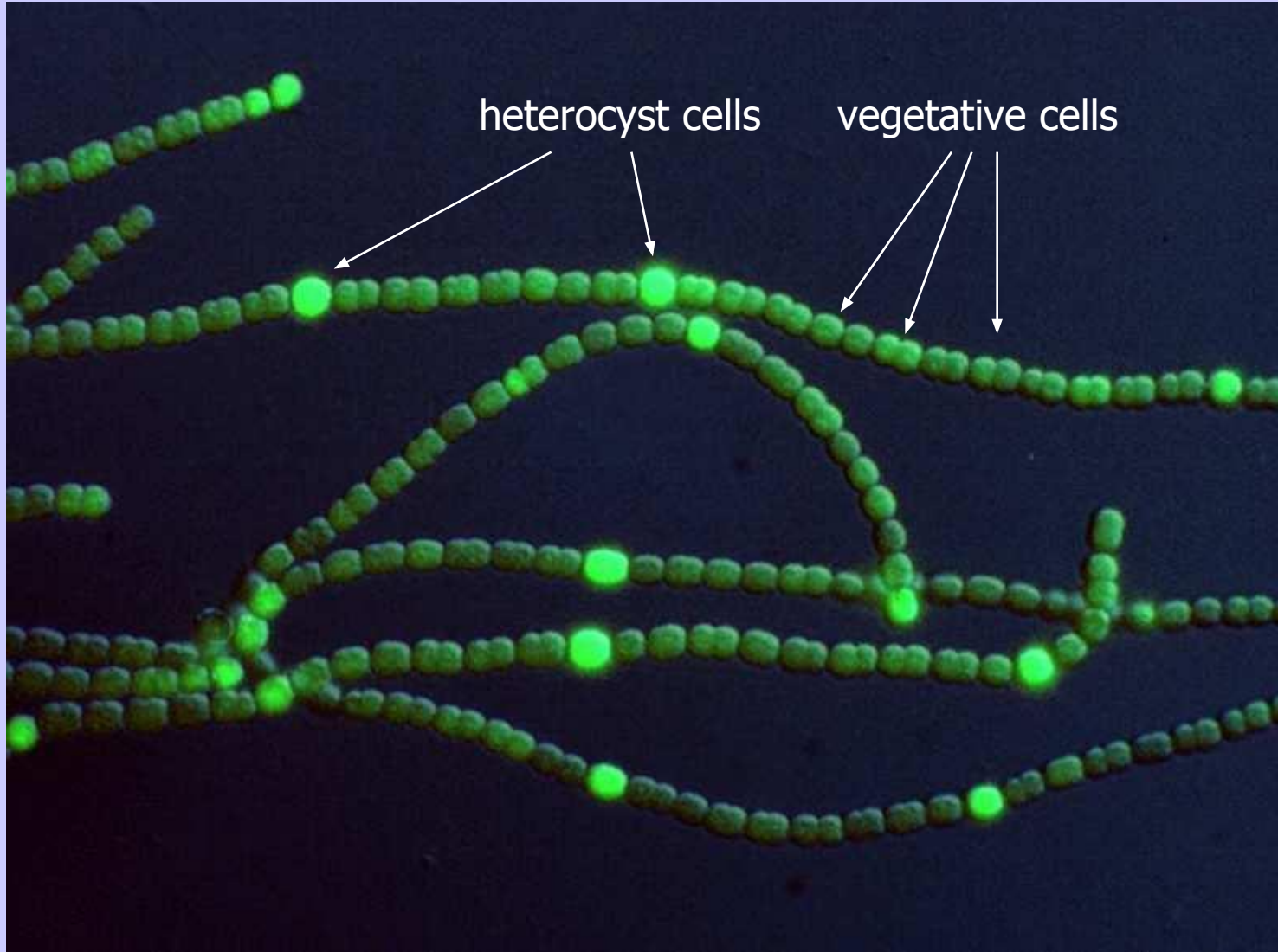
removal  
of nitrate

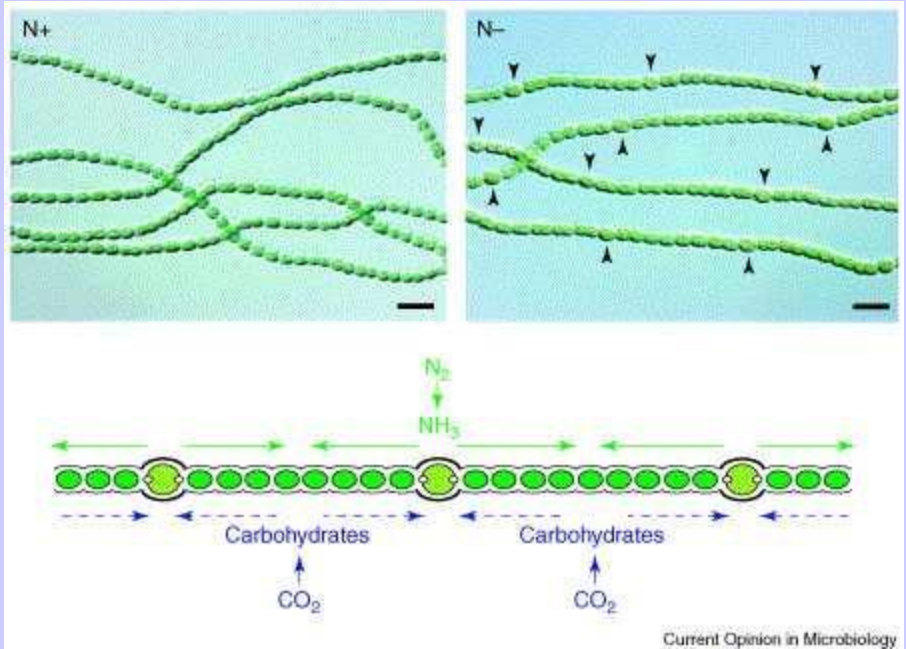
18  
hours



*Anabaena* filament growing on  $N_2$

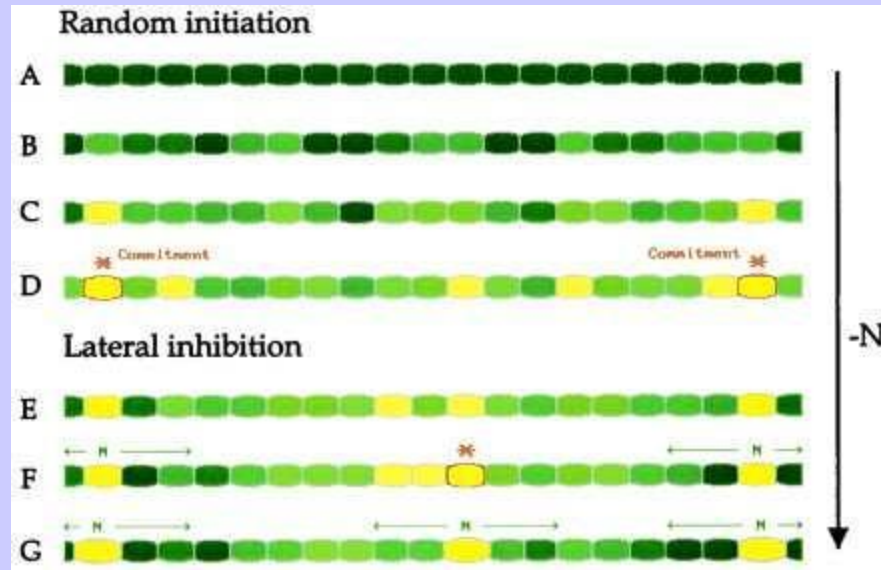
# Anabaena





# 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  $c = c_{\min}$

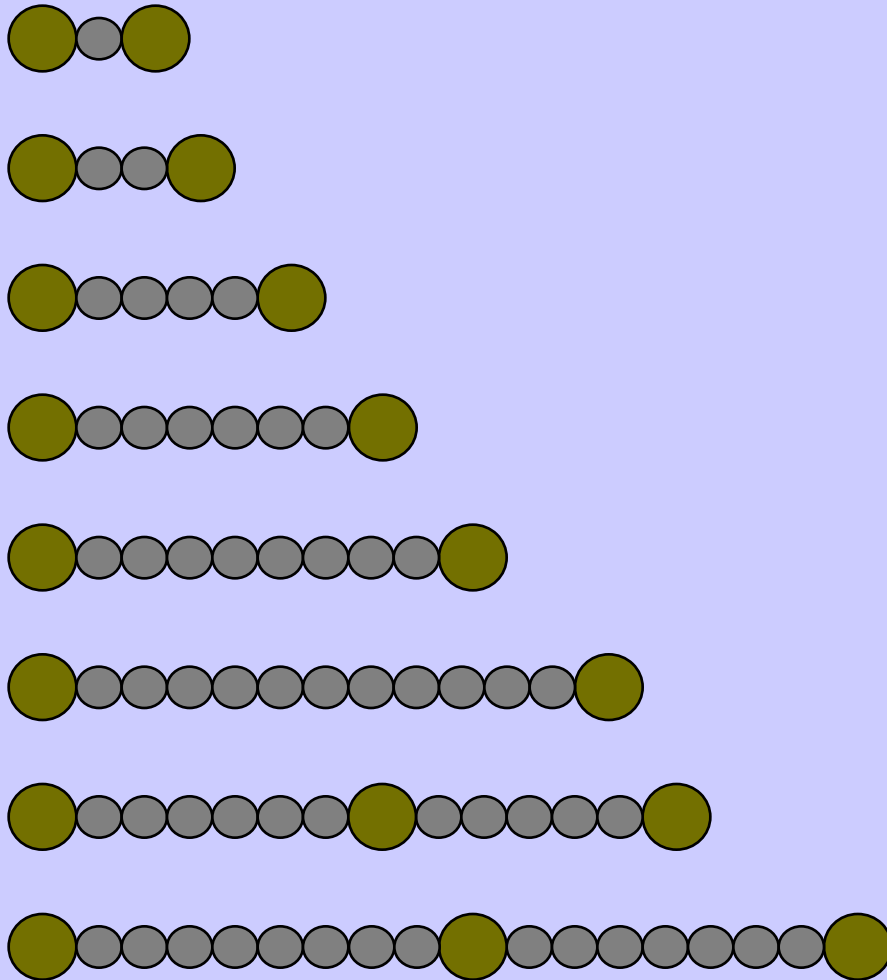
produce  $F_h(s, c)$

$F_h(s, c)$ :

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

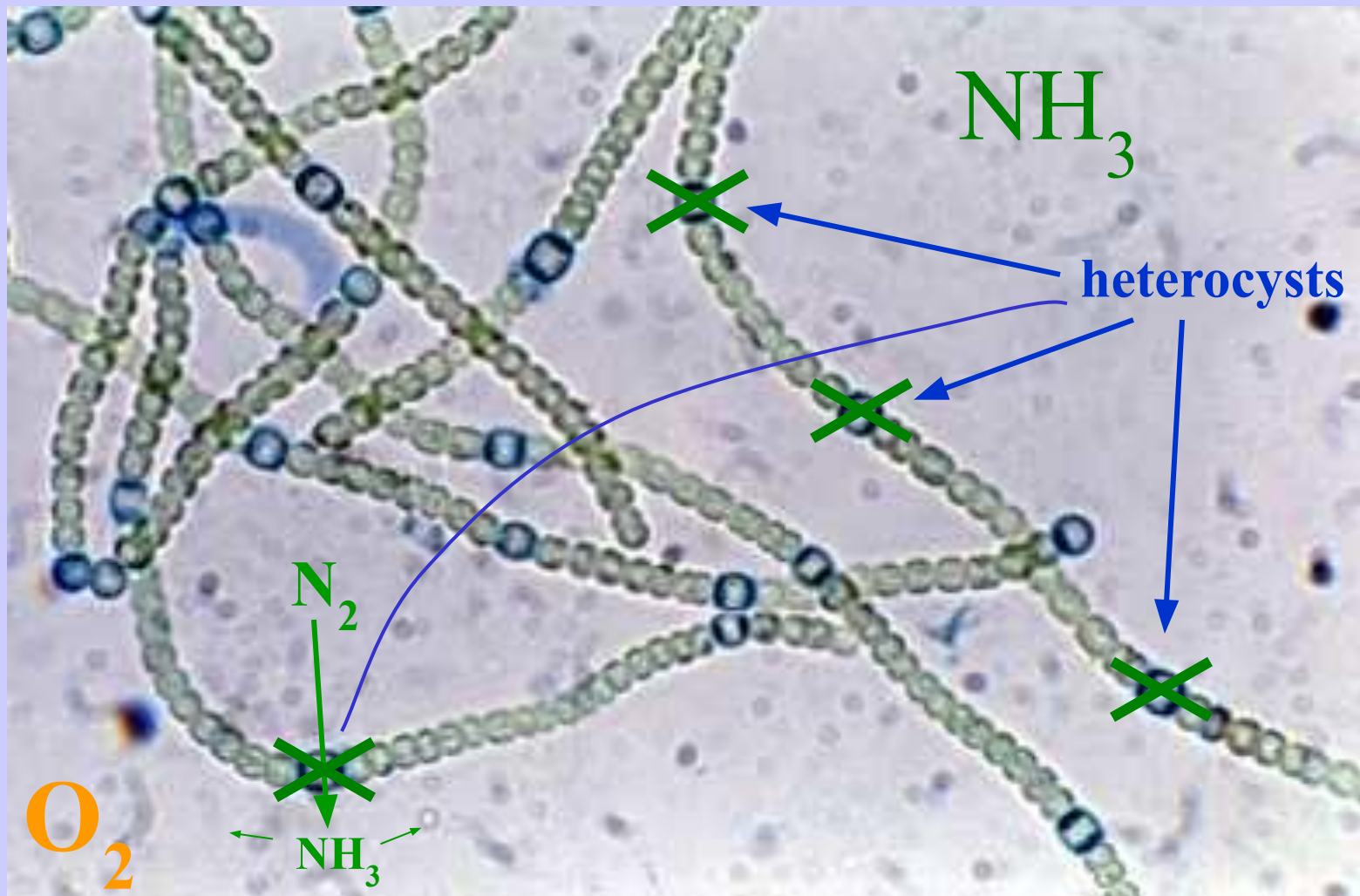
$dc/dt = r_c \cdot (c_{\max} - c)$

# Anabaena – continuous model





# *Case of the Hidden Heterocyst*

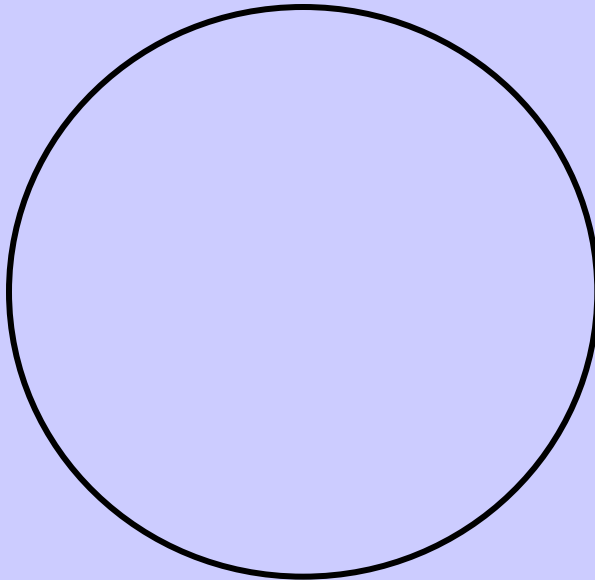


Matveyev and Elhai (unpublished)

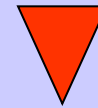
# *Case of the Hidden Heterocyst*

Strategy to find heterocyst differentiation genes

*Nostoc* genome



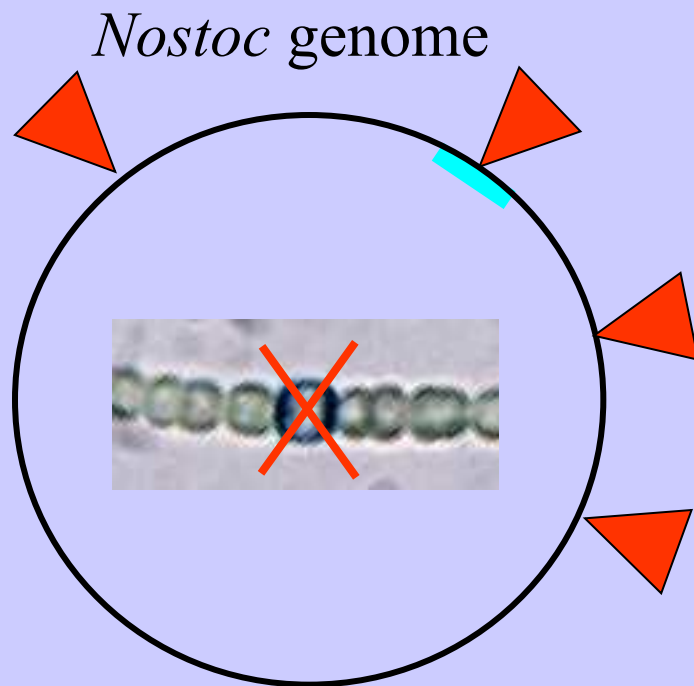
Transposon



1. Use transposon mutagenesis

# *Case of the Hidden Heterocyst*

Strategy to find heterocyst differentiation genes



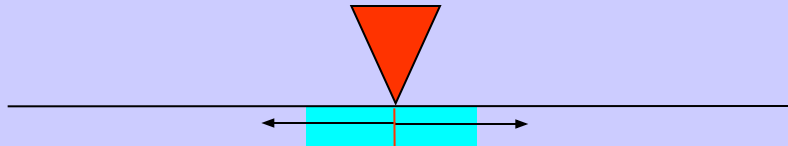
Transposon

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



```
AAGCTTGACCAAAAAGTTAAAACACTGACGGCAAATAAT
CAATGACTATCAGACAGAGAATCATCGTGCTGTCAGTAA
AACCTCTGATTTTCGATCTTTACCATAATTGTTATGTTGT
AATGACTAACCAGACTATCTTTTACAGAGCTTCTGGTTA
ACACTTGTCTAATTAGACATTGATAATGTTTGTGGGGGT
TGGTCATCAGGAATGGTAAATAGCAATTACCCTTCAGAC
TTTCCTATGAGACGCTCCGCCAACGAGCAGTGTCTCTTA
AAGAACGTTATGAGCGCTCAGTTAACTTCAGAAATTCAC
GGCGGAAATCCATAGTTATTATTACTTATGACTAAAACA
AAATTACTATGGCGGCTTGTTTAAATATAGATTCTGTGTT
CTGAGAAATGACTTTTAAAGTCCCACCTAACTTTTCTC
ATCTATTGCTATATTTTCGACTTTAAAACCTTATAGTAGAT
GGCTTAATTCTCAAATAACAAACTCATTTTTAGTAGATA
TTTCATGCAAACCTGAGGTTTTTAGTGATATTTCCCTT
ATTGAGTACAGCCACTCCACAAACCTTAGAATGGCTACT
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CGTTGTATCTGGATGGGTAAAAGTTTCGGCGCACCTGTGG
A
```

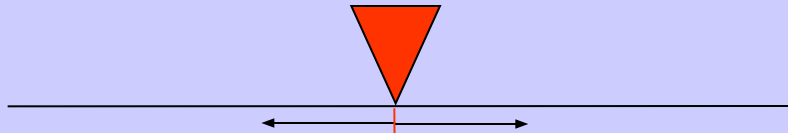
1. Use transposon mutagenesis to find a mutant defective in heterocyst differentiation
2. Sequence out from transposon

# *Case of the Hidden Heterocyst*

## Strategy to find heterocyst differentiation genes

*Nostoc* genome

*Do it*



```
AAGCTTGACCAAAAAGTTAAAACACTGACGGCAAATAAT
CAATGACTATCAGACAGAGAATCATCGTGCTGTCAGTAA
AACCTCTGATTTTCGATCTTTACCATAATTGTTATGTTGT
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GGCTTAATTCTCAAATAACAAACTCATTTTTAGTAGATA
TTTCATGCAAACCTGAGGTTTTTAGTGATATTTTCCCCTT
ATTGAGTACAGCCACTCCACAAACCTTAGAATGGCTACT
CAATATTGCAATTGATCATGAATATCCCAGTGGTACAGC
AGTTTTTAAATGGAAGATGCCTGGGGTAATGCAGTTTATTT
CGTTGTATCTGGATGGGTAAAAGTTTCGGCGCACCTGTGG
```

A

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

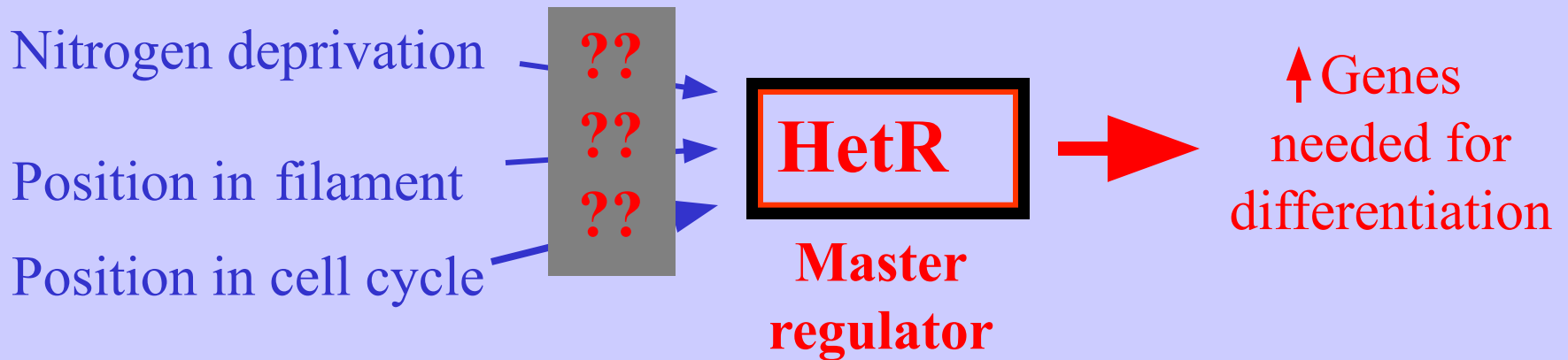
by 6 h, 20-fold increase within spatially separated cells

positive autoregulation



# Differentiation in cyanobacteria

## Integration of signals through HetR





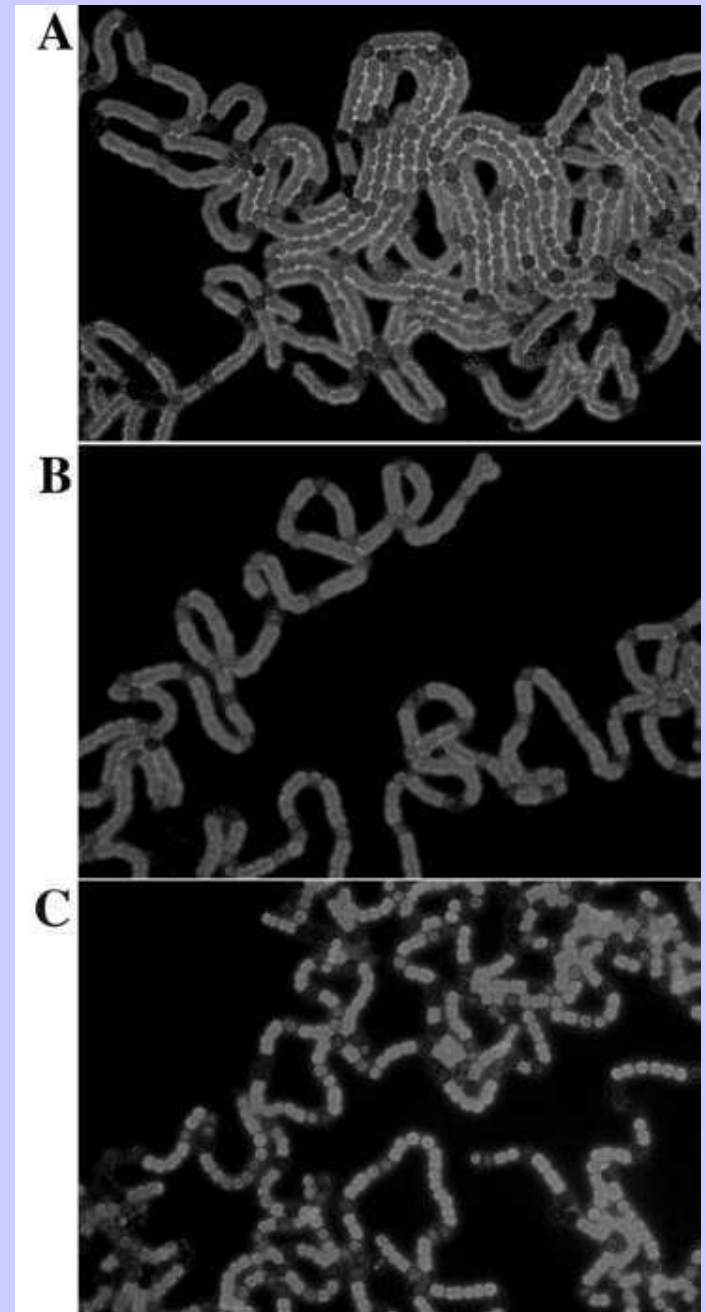


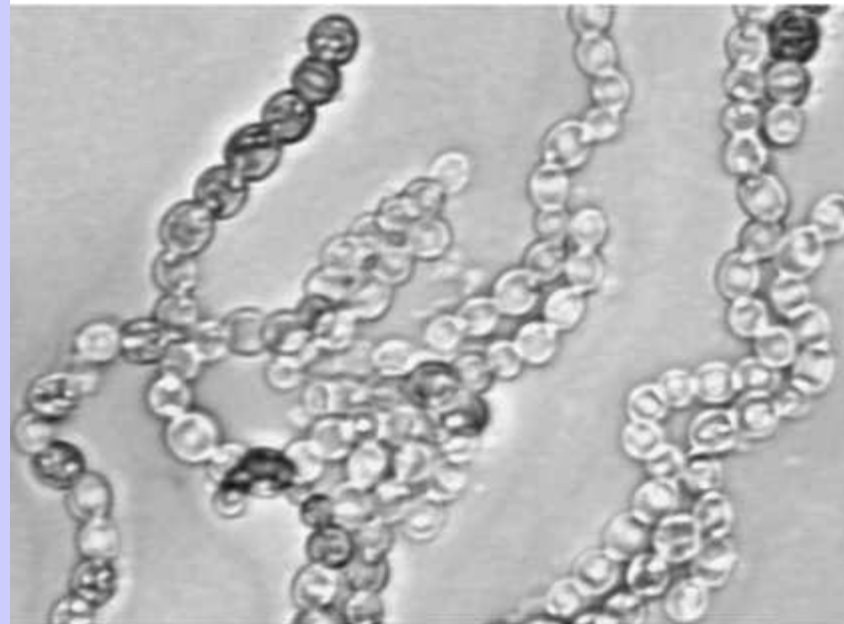
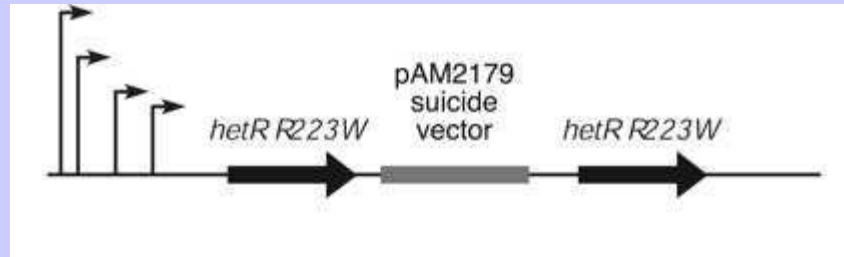




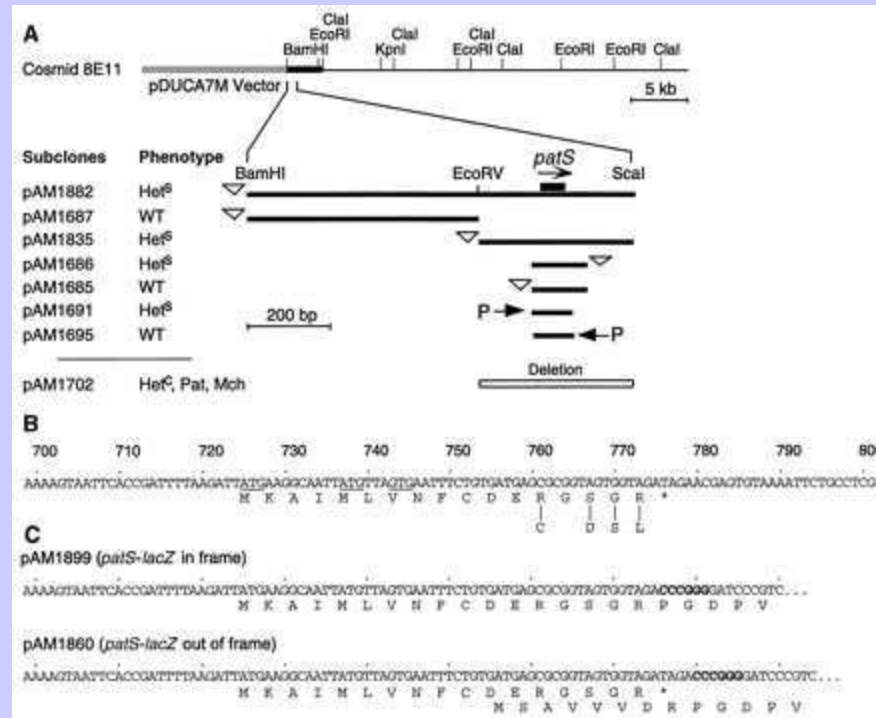
# HetR overexpression

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.





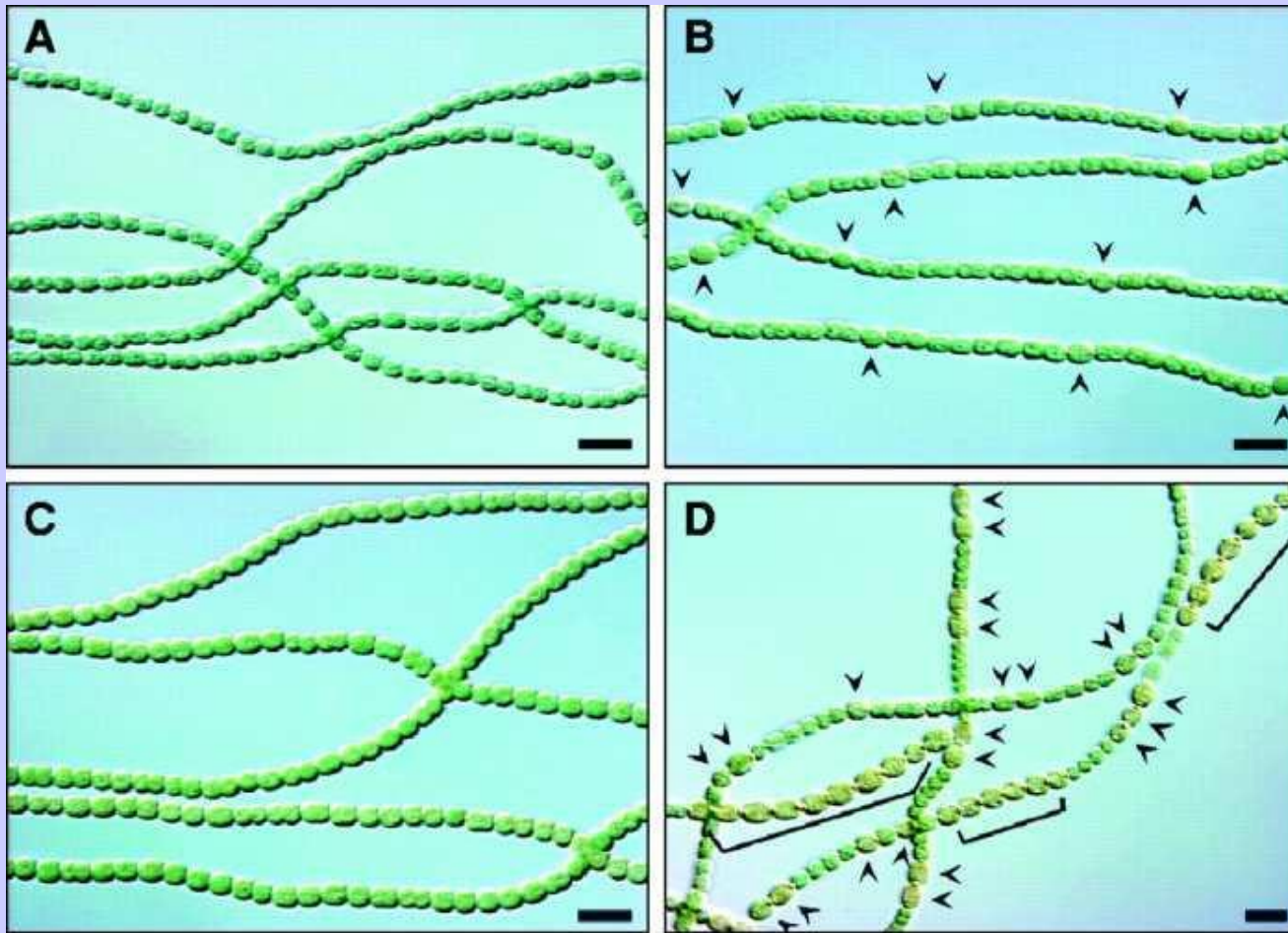
# PatS



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

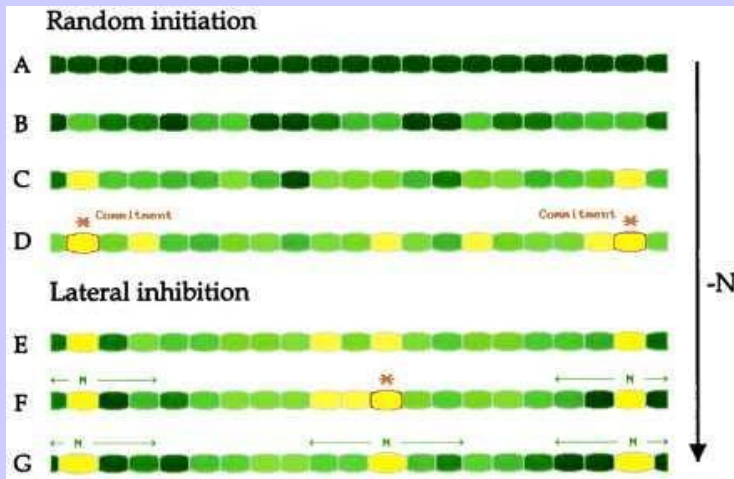
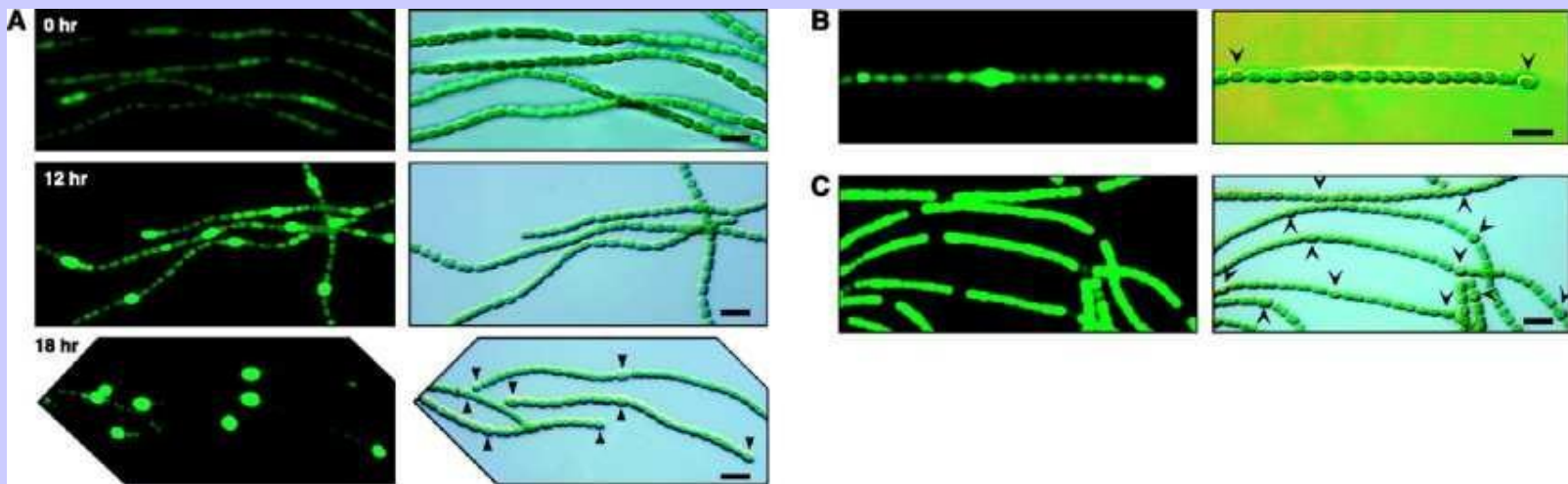


Wild-type filaments (A) grown in BG-11 medium and (B) after the nitrogen step-down in BG-11<sub>0</sub> to induce heterocysts (arrowheads) are shown. (C) Overexpression of *patS* prevented heterocyst formation in BG-11<sub>0</sub>, and (D) deletion of *patS* resulted in supernumerary heterocysts with an abnormal pattern in BG-11<sub>0</sub>. Differential interference contrast micrographs were taken before (A) and 24 hours after (B through D) heterocyst induction.

RGSGR

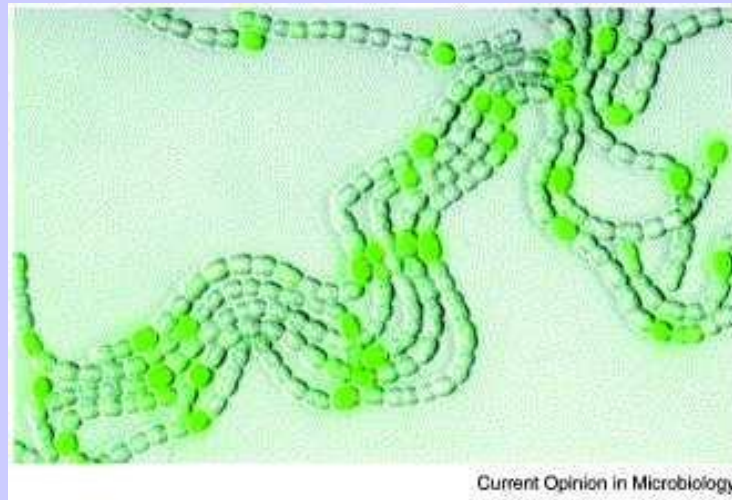
The exogenous addition of a pentapeptide corresponding to the last five COOH-terminal residues of PatS also inhibited heterocyst differentiation, indicating that a processed form of PatS may be a diffusible inhibitory signal regulating development.





Řesně 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.

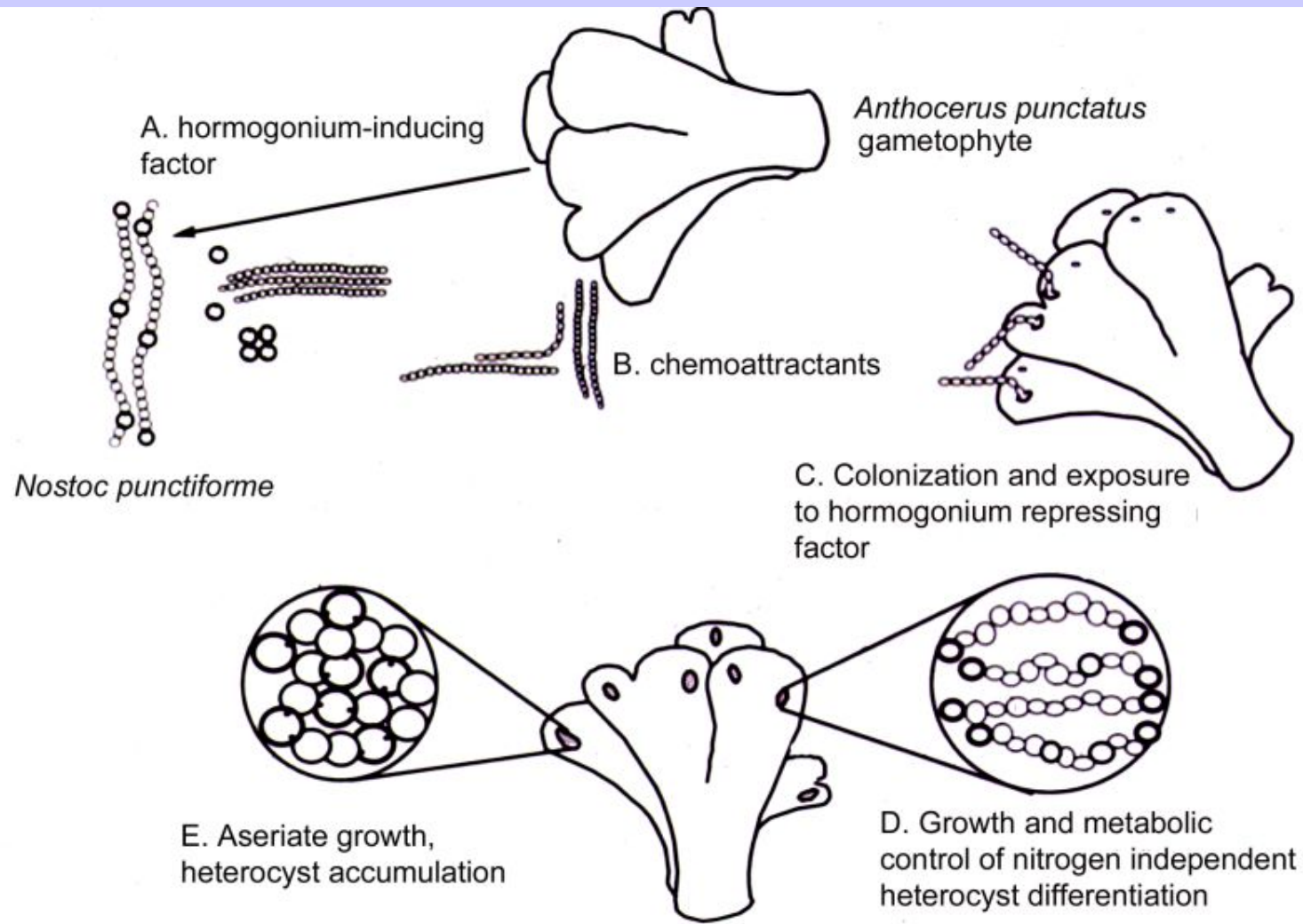


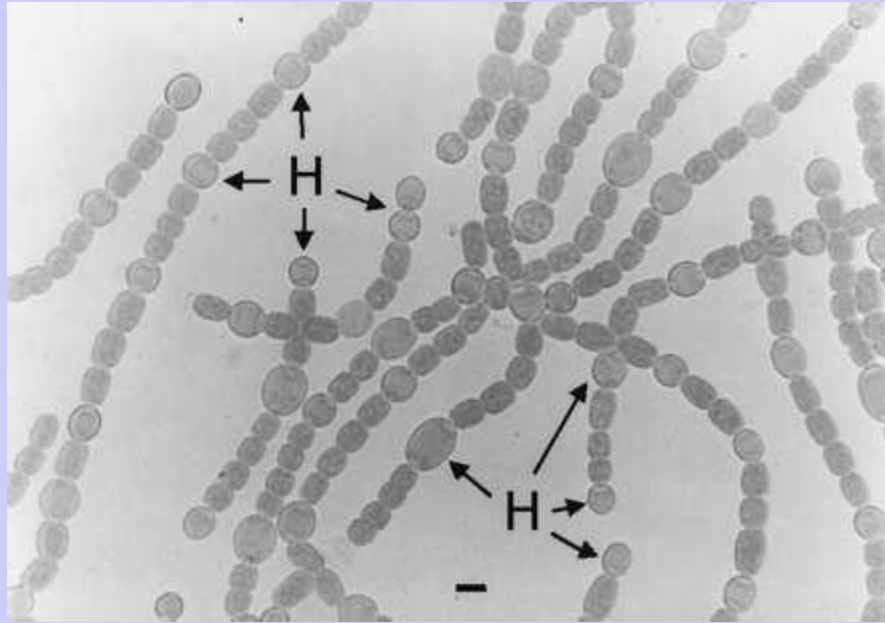
Current Opinion in Microbiology

- 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.

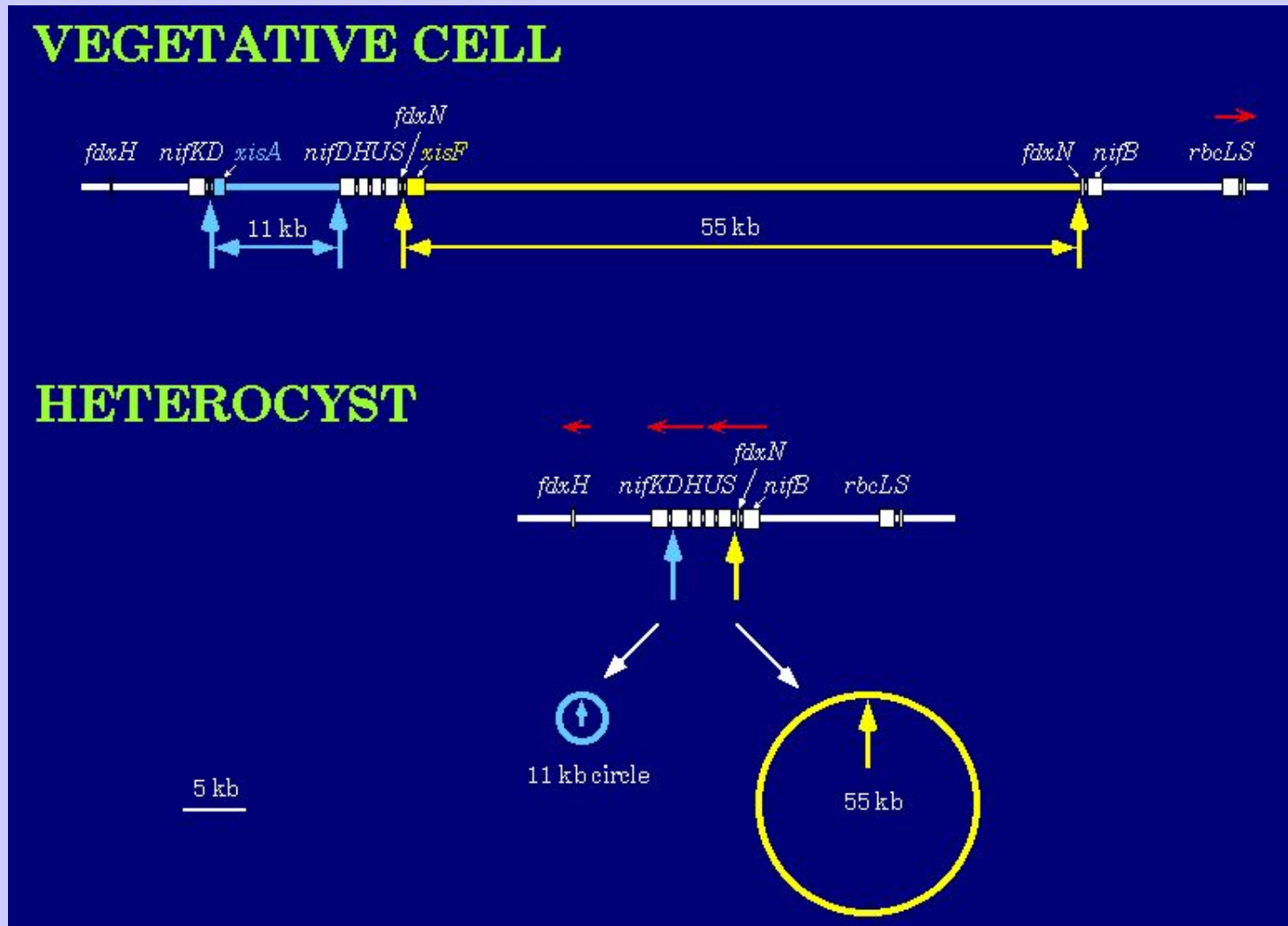


***Anthoceros punctatus.* + *Nostoc punctiforme***

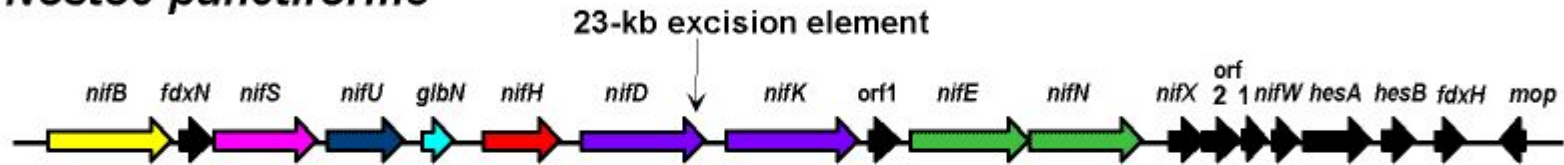




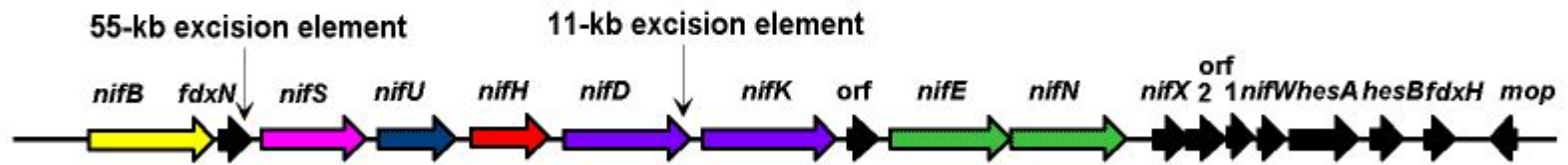
# Další události nezbytné k aktivaci *nif* genů



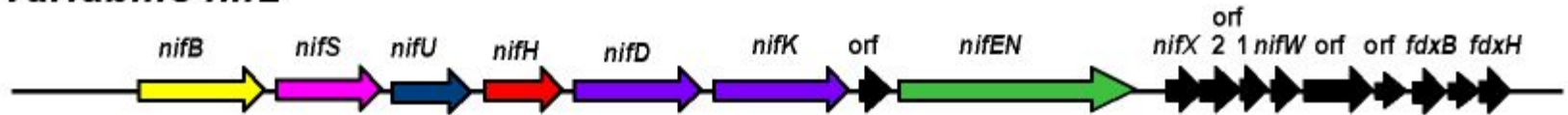
## *Nostoc punctiforme*



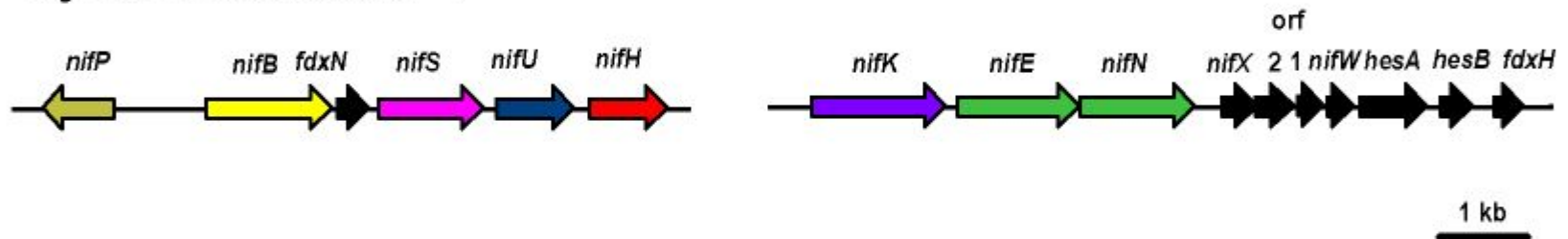
## *Anabaena* sp. PCC 7120



## *A. variabilis* *nif2*



## *Synechococcus* RF-1



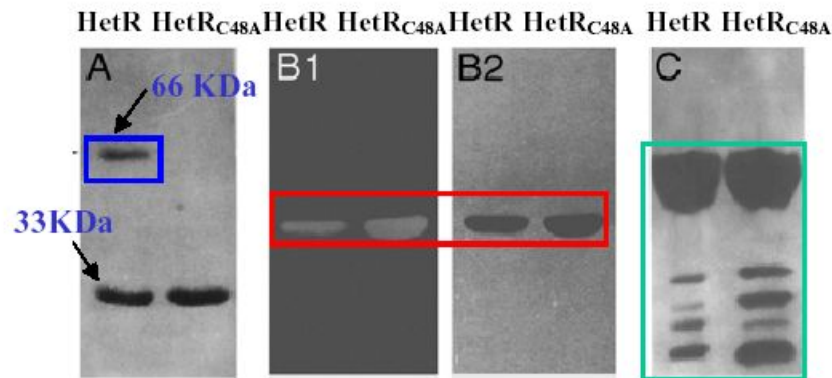
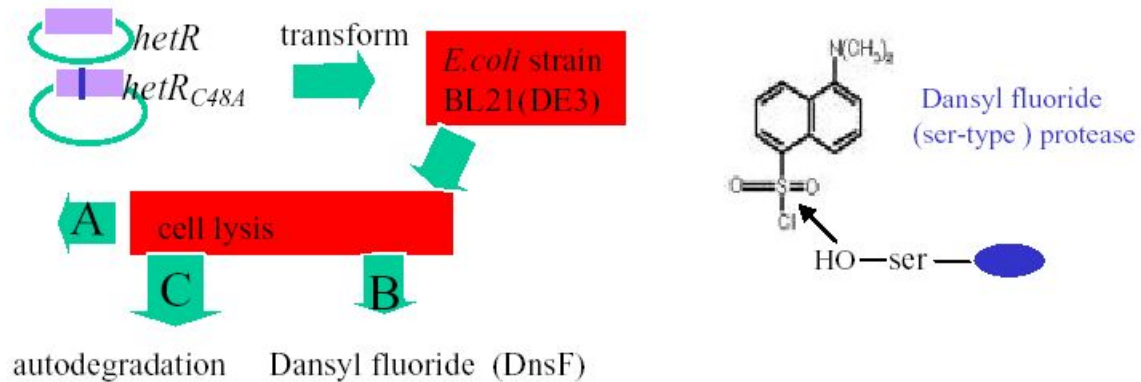
HetR homodimer is a DNA-binding protein required for heterocyst differentiation, and the DNA-binding activity is inhibited by PatS

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

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



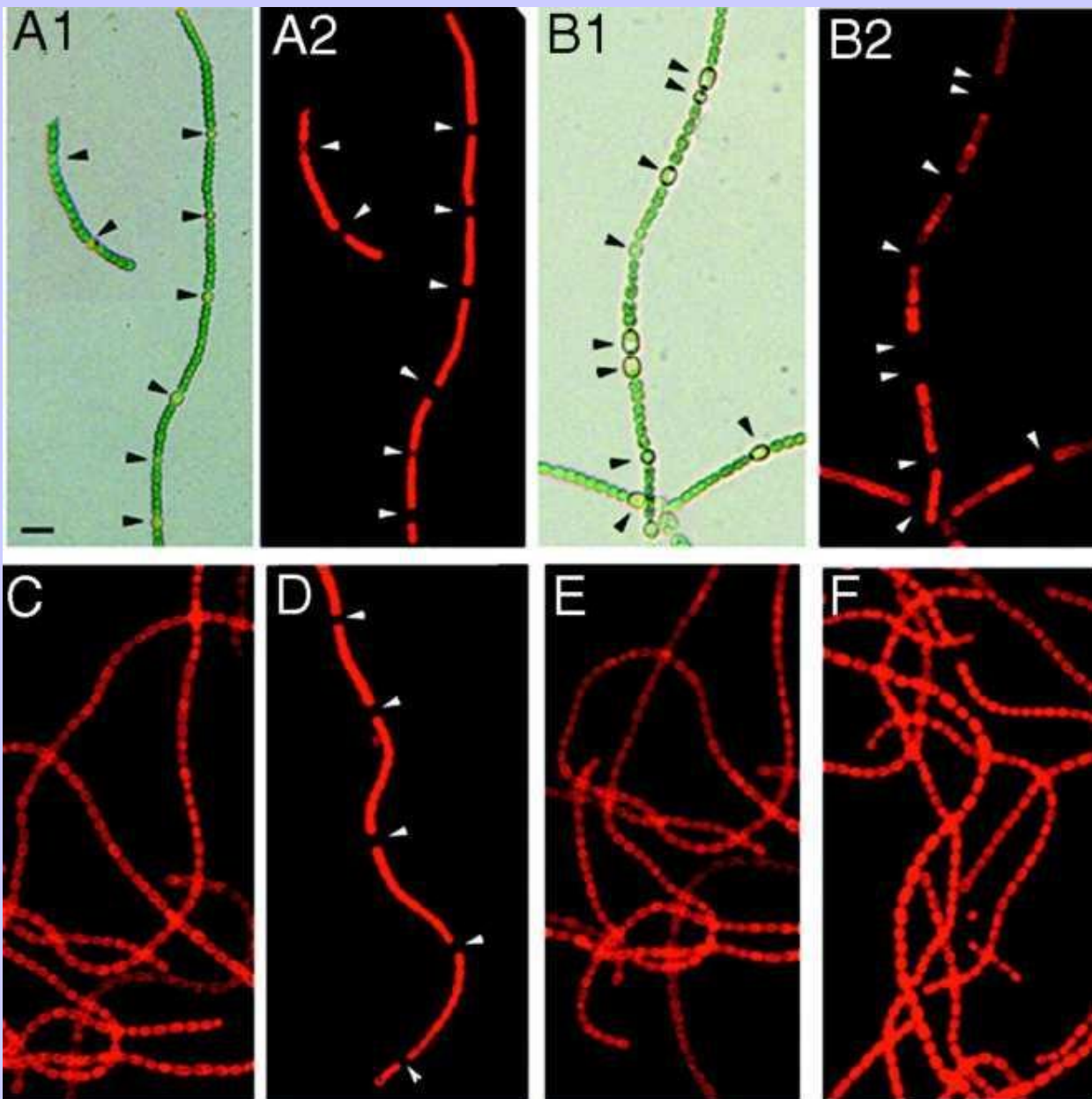
# In vitro In vivo HetR homodimer

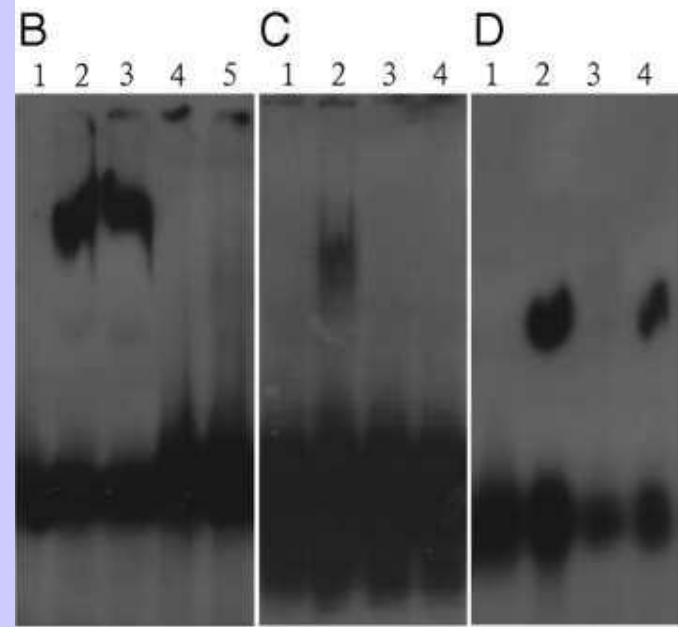
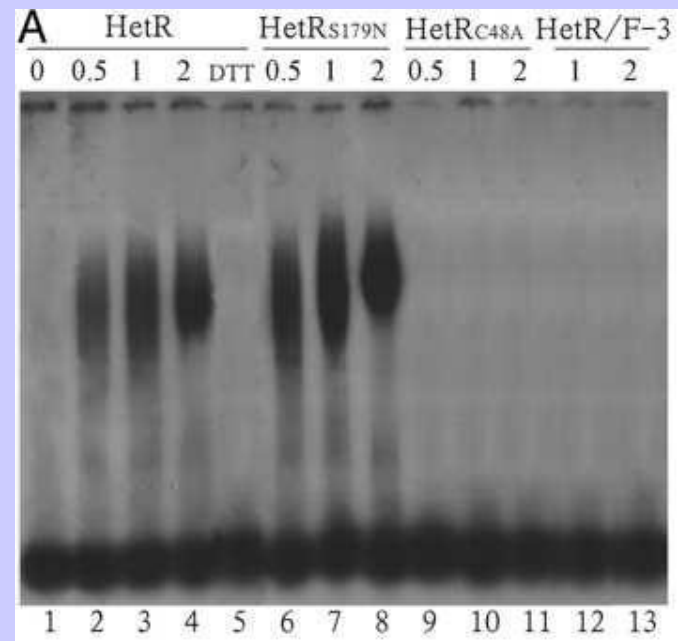


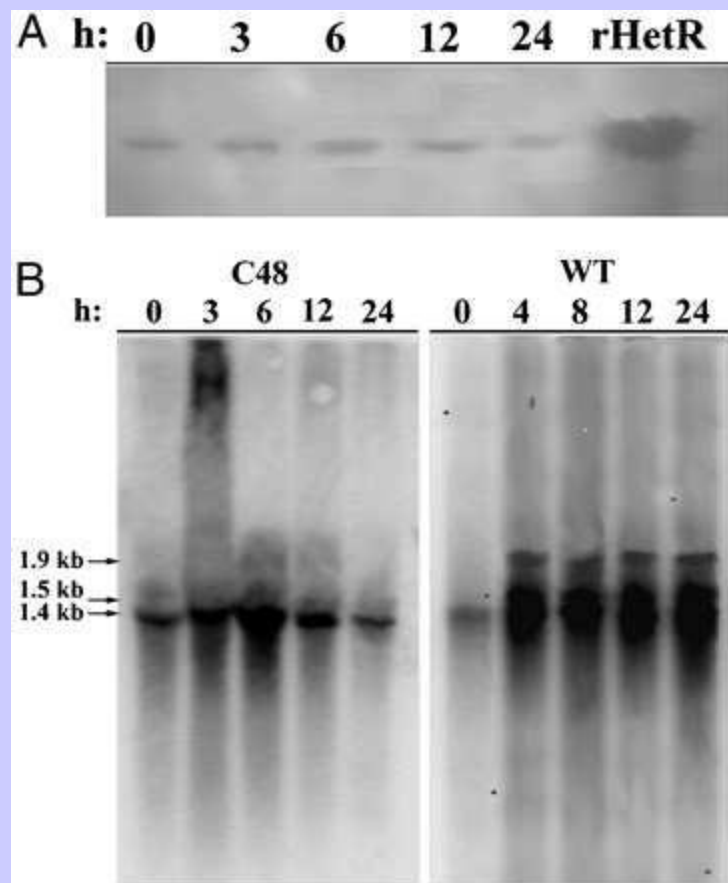
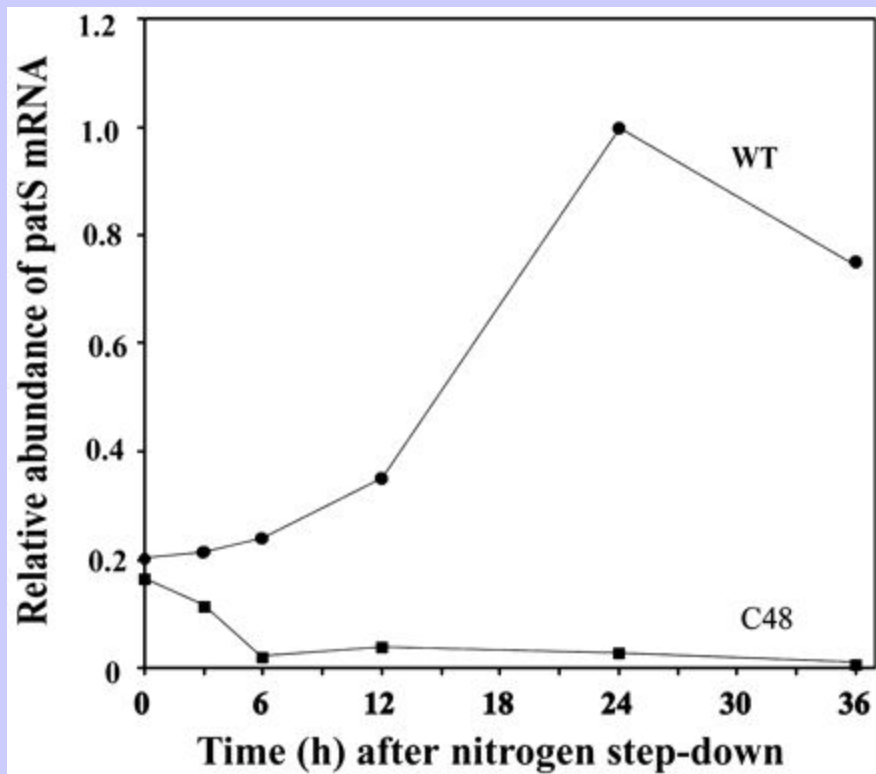
A HetR<sub>C48A</sub> dimer

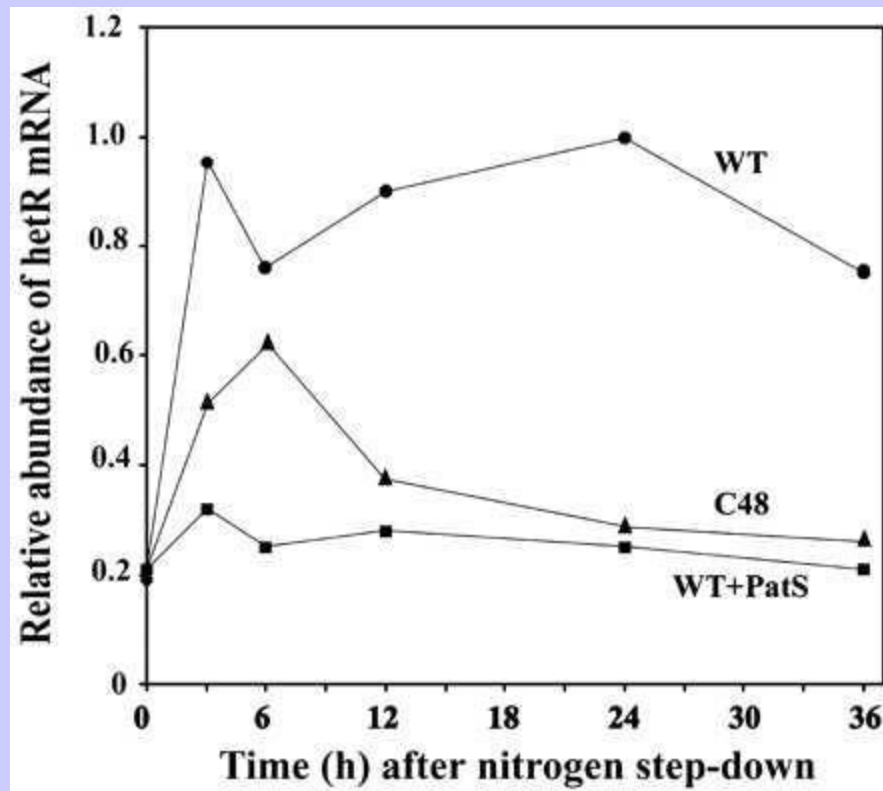
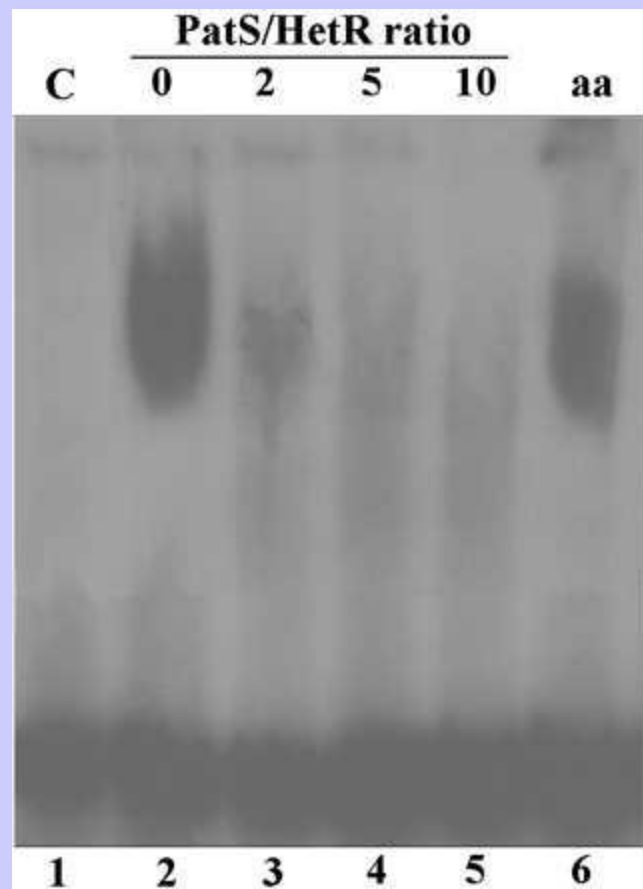
B<sub>1</sub>, B<sub>2</sub> ser-type protease-DnsF  
HetR HetR<sub>C48A</sub>

C HetR HetR<sub>C48A</sub> protease (Autodegradation)



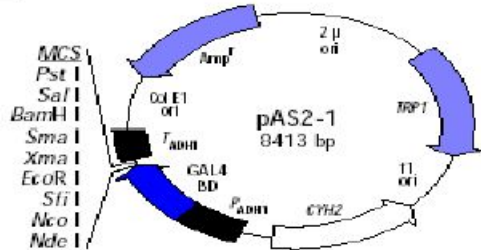




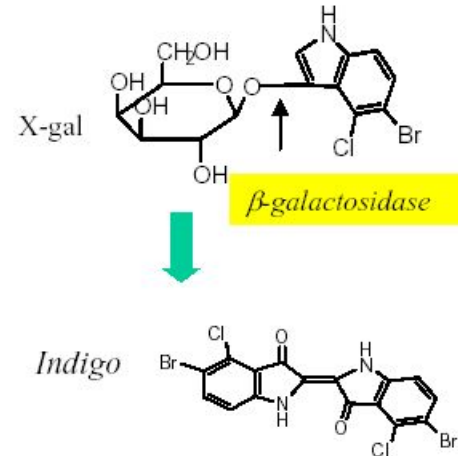
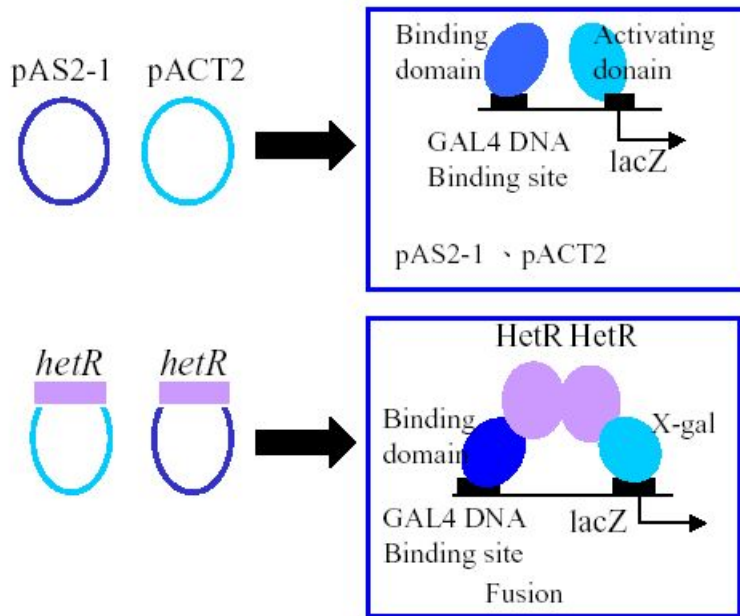
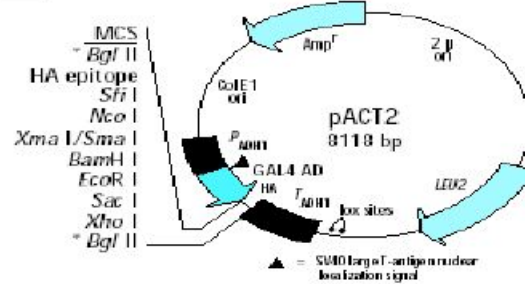


# Yeast-Two Hybrid assay

**A**

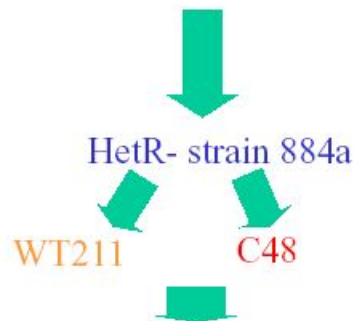


**B**



## In vitro in vivo HetR homodimer (2)

In vivo HetR Protein homodimer ◦

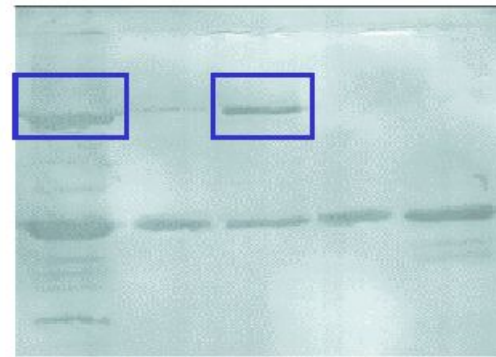


cellular extract  
(1mM PMSF)

immunoblott



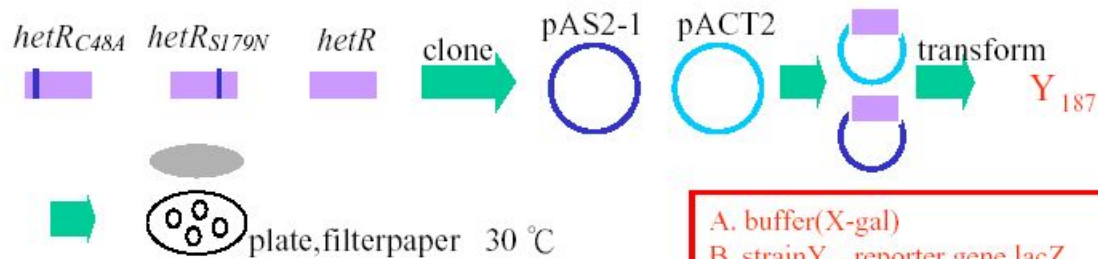
DTT - + - + -



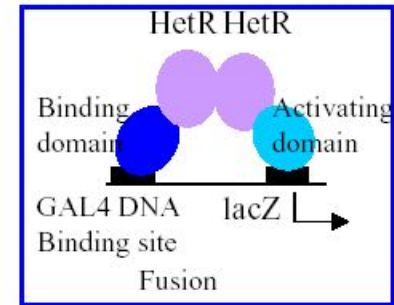
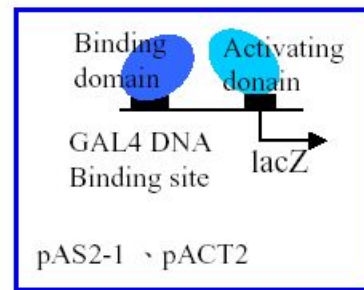
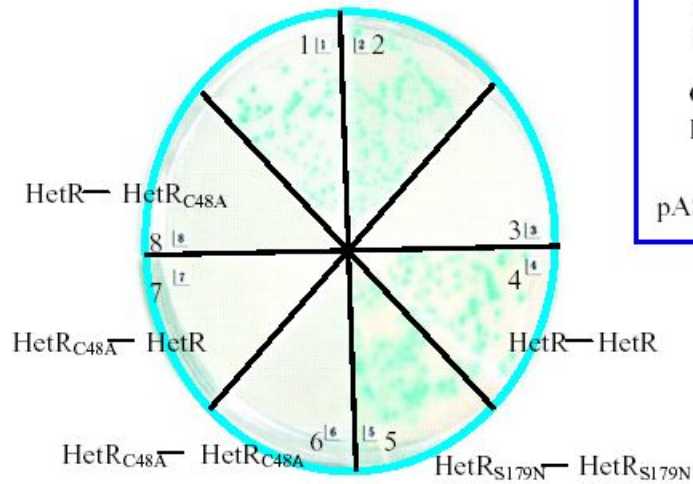
Lane1 rHetR  
Lane2 WT211  
Lane3 WT211  
Lane4 C48  
Lane5 C48

# HetRC48A does not form dimer

Yeast-Two Hybrid assay:

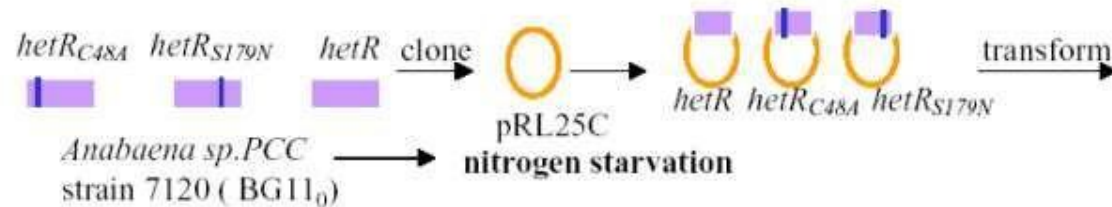


A. buffer(X-gal)  
 B. strain  $Y_{187}$  reporter gene  $lacZ$

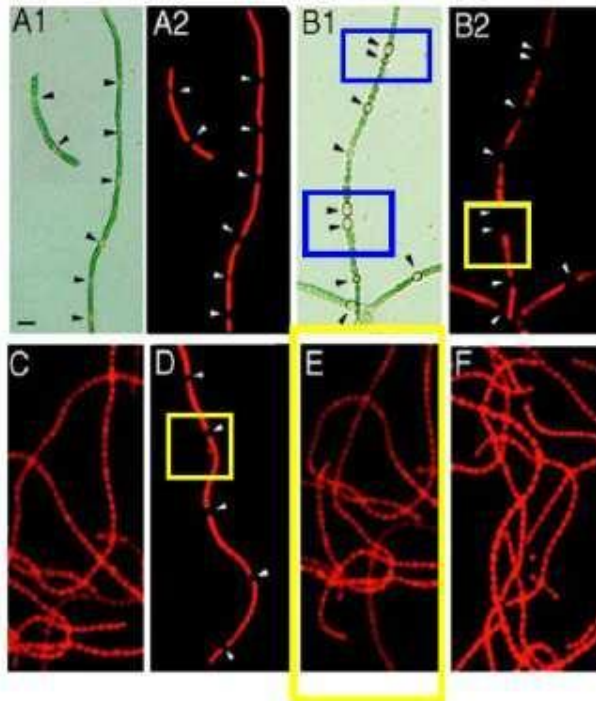




## Cys48 mutation does not interfere with WT HetR function



實驗結果:



A1 and A2 : WT

*Anabaena sp. PCC strain 7120*

C :  $hetRC48A$  ◦

B1 and B2 : pRL25C ( $hetR$ ) *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

