DNA replication fork repair by RecG: A propagating front model

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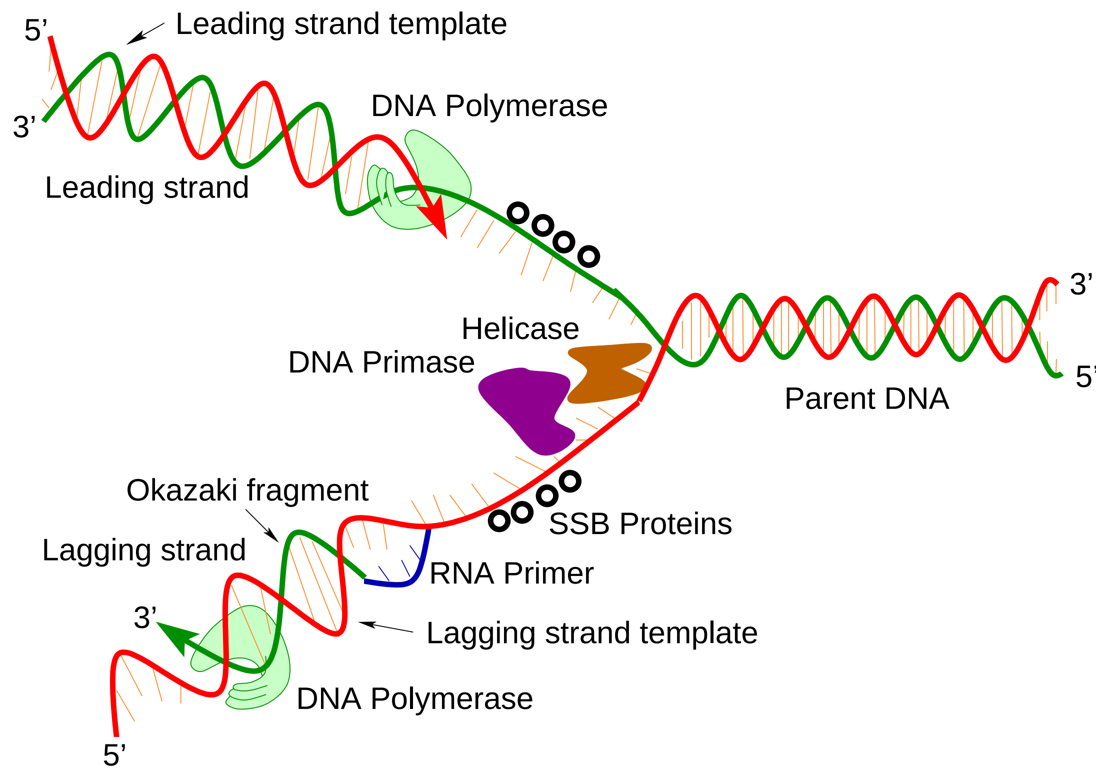
Replication

Replication



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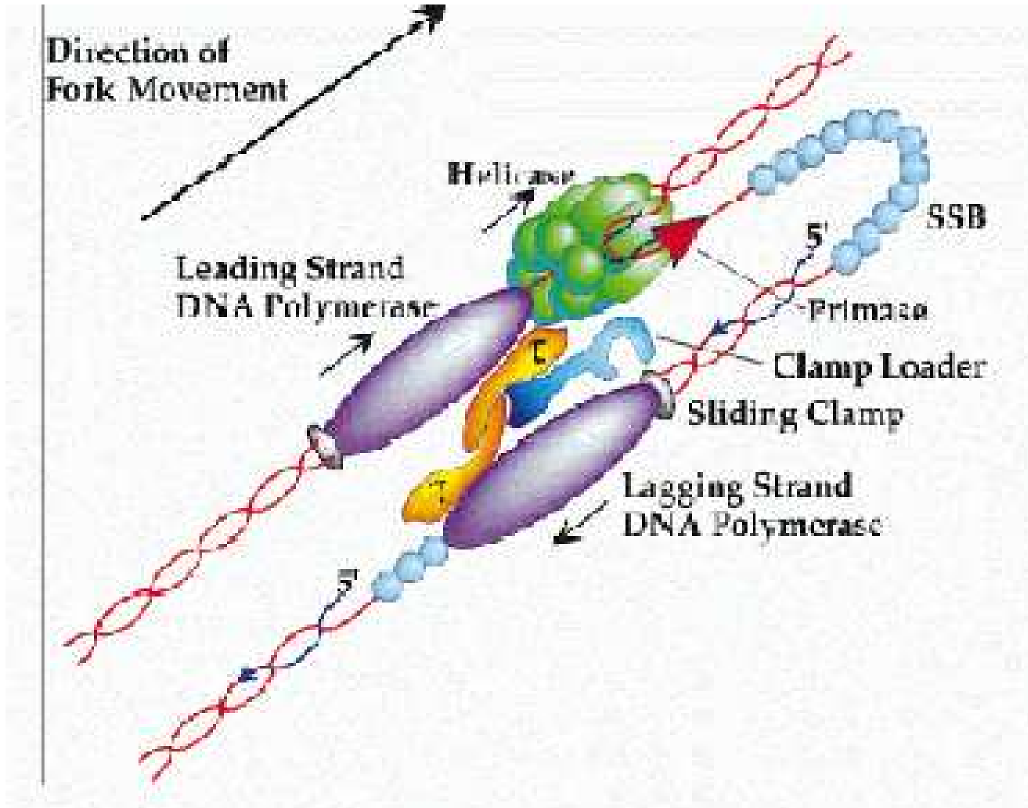

Figure 3. Model of the Replisome Based on Interactions Documented with *E. coli* Proteins

Where's the Problem?

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Y-fork?:

Helicase: A motor on DNA, moves towards the zipped phase of DNA and unzips it.

A helicase maintains the two strands at a separation larger than the zipped state.

Helicase: a fixed distance ensemble

- not the complete story

- Nick or Lesion on the leading strand

  Stalled fork

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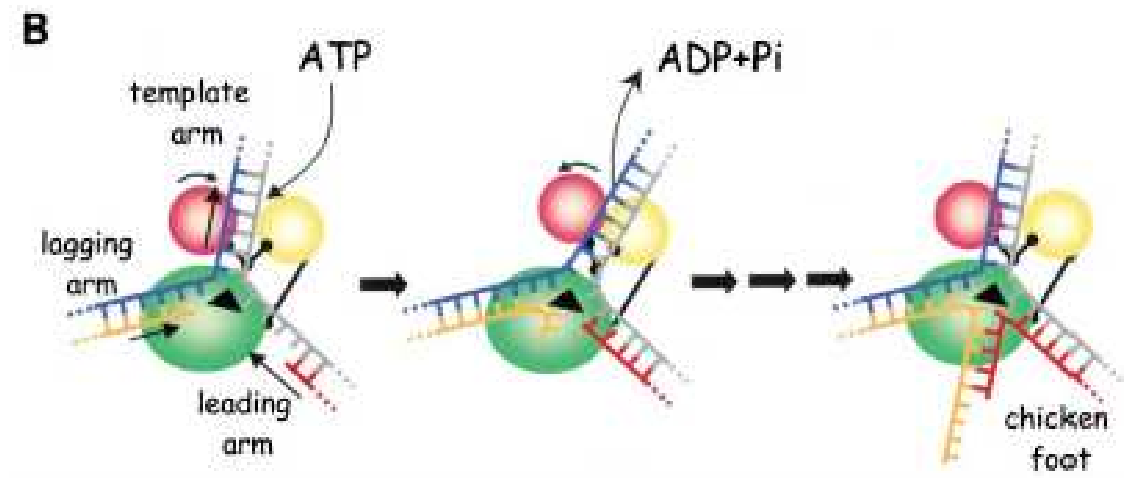
- RecG mediated fork reversal to a “chickenfoot” intermediate



[cell 107, 79 (2001)]

Questions..

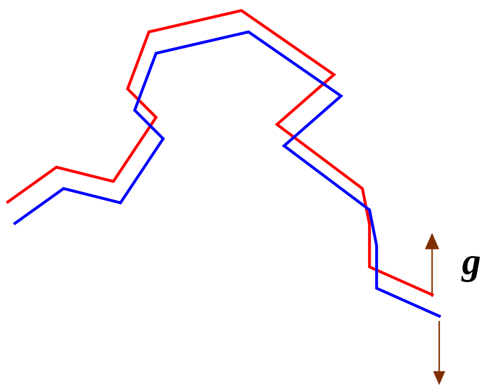
- Is there a common approach to address the difference between

  standard helicase going from unzipped to zippped phase - opening of Y-fork

  and
  RecG moving from zipped to unzipped phase - Y-fork reversal?

Ensembles and Unzipping transition

fixed force ensemble:



Free energy

$$\mathcal{F}_N(g,T)$$

$$\langle x \rangle = \frac{\partial \mathcal{F}_N(g,T)}{\partial g}.$$

fixed distance ensemble:

Free energy:

$$F_N(x,T)$$

$$\langle g \rangle = \frac{\partial F_N(x,T)}{\partial x}.$$

Existence of a force induced unzipping transition - FIRST order

competition between monomer attraction and bond orientation

Ensembles and Unzipping transition-III

fixed force ensemble:

Unzipping transition at
$g = g_c(T)$

Zipped state for $g < g_c(T)$

Unzipped state for $g > g_c(T)$

fixed distance ensemble:

For $x >$ bound state separation $\Longrightarrow$ phase coexistence.

Such a co-existing state is the Y-fork

interface $\Longrightarrow$ "Y-junction".

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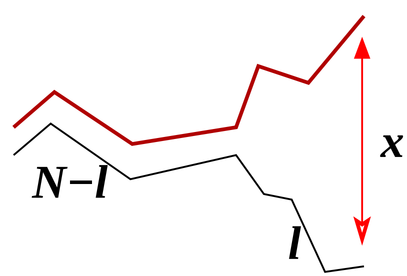

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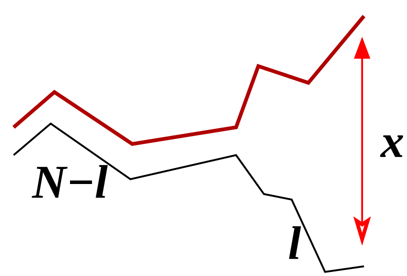
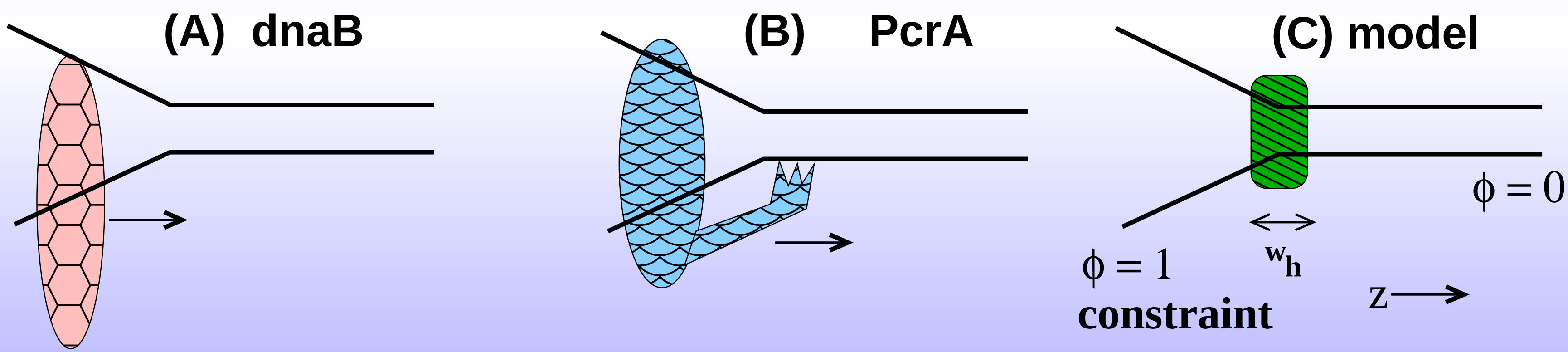
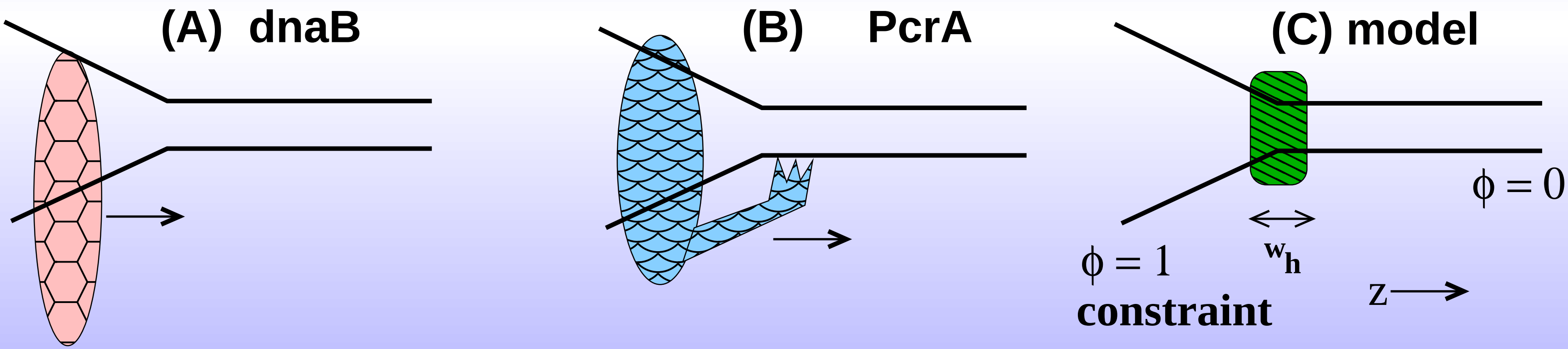
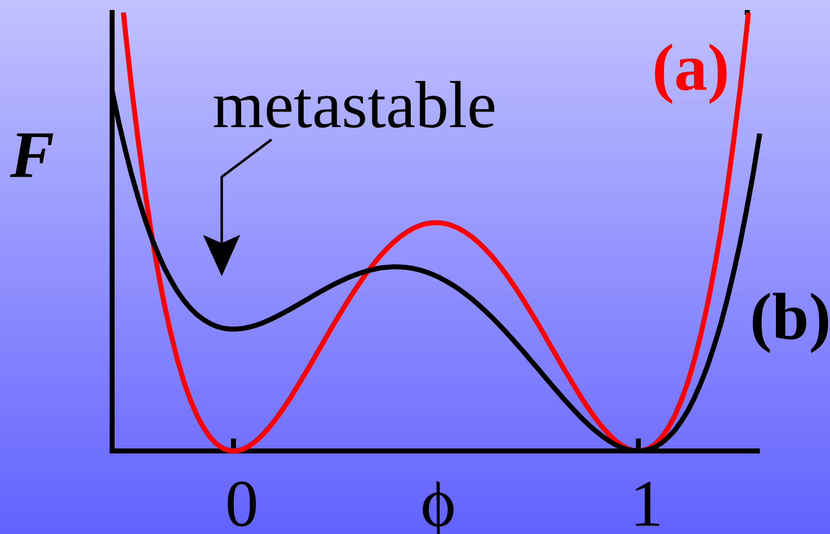


Hypothesis: Effect of helicase is to set the interface in motion



(A) dnaB
(B) PcrA
(C) model
ϕ = 0
ϕ = 1
w
h
constraint
z

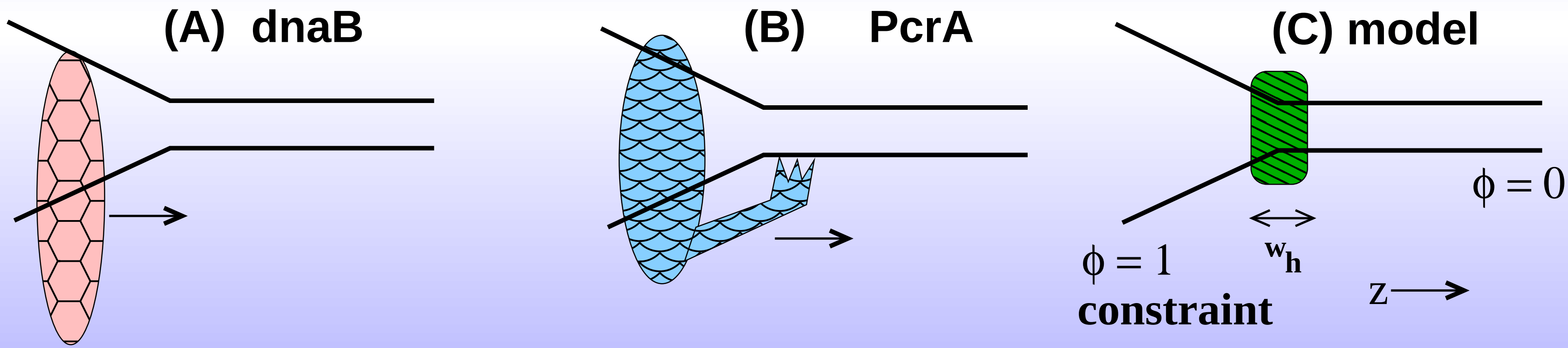


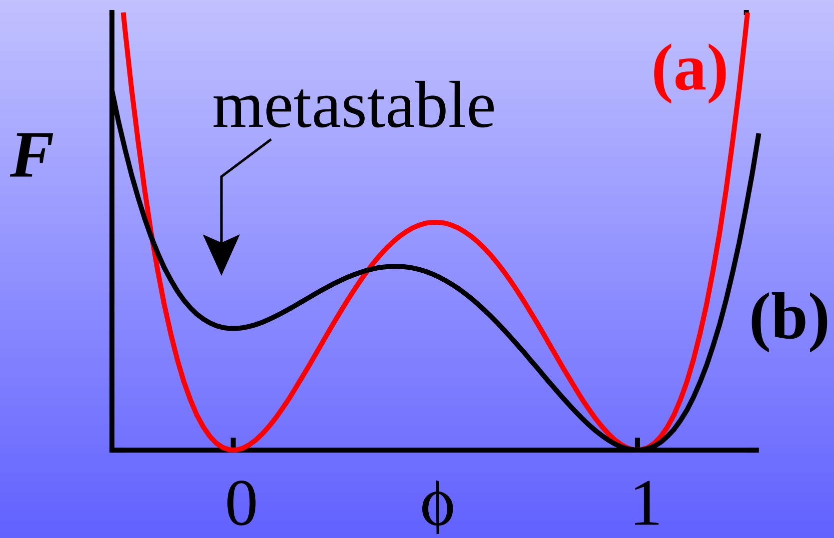


Landau Free energy:

$$-\frac{dF(\phi)}{d\phi} = \phi(\phi - \frac{1}{2} + h)(1-\phi)$$

ϕ: scaled separation





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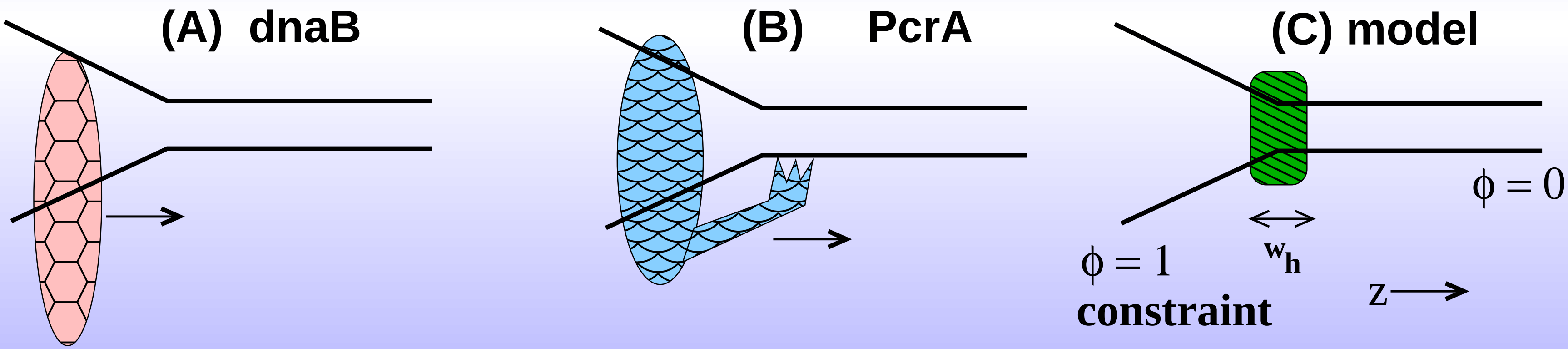

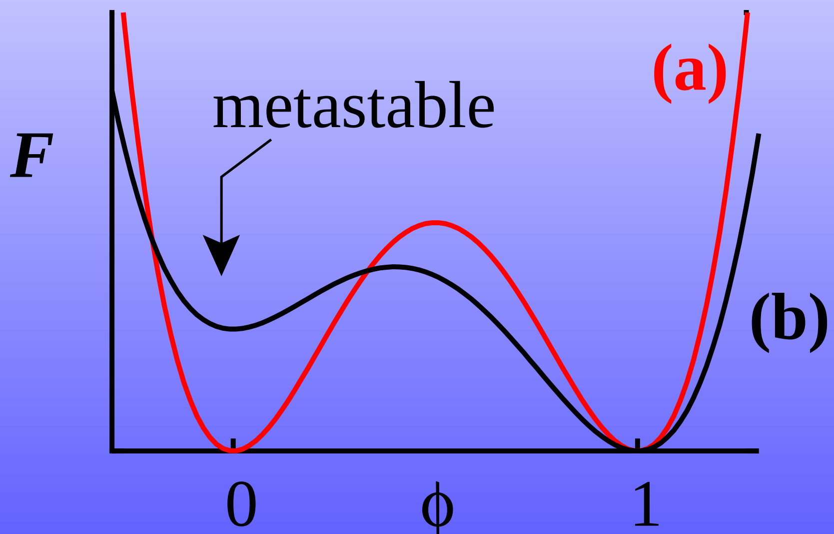

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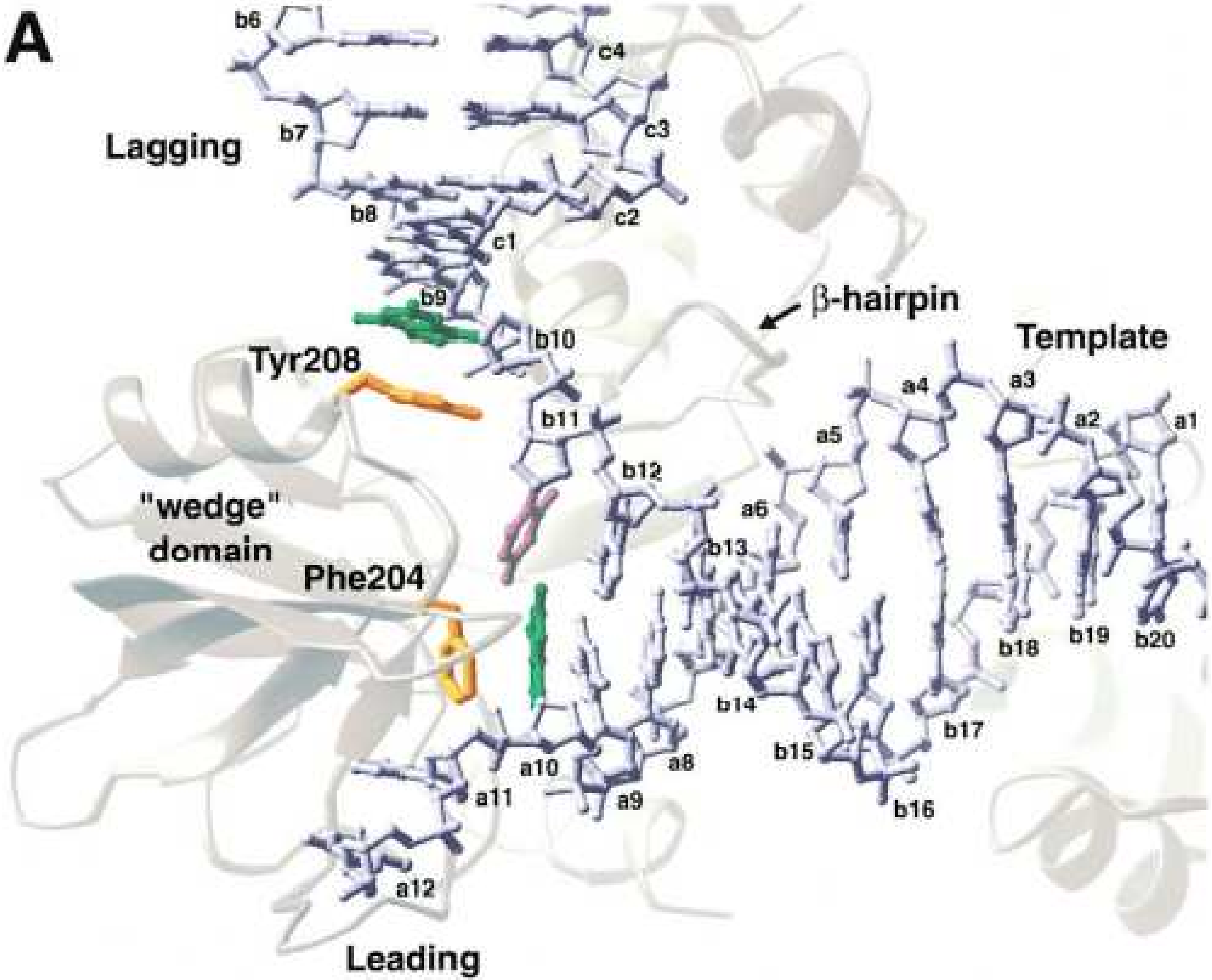
A
b6
c4
c3
b7
Lagging
b8
c2
c1
b9
β-hairpin
b10
Template
Tyr208
a4
a3
a2
a1
b11
a5
b12
"wedge"
domain
a6
b13
Phe204
b18
b19
b20
b14
b17
b15
a10
a8
a11
a9
b16
a12
Leading

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Similar to Fisher-Kolmogorov problem (1937) -
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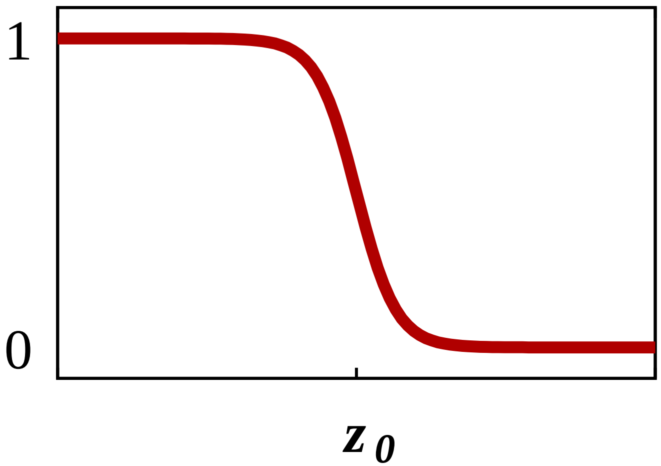
Goldstone Mode and Helicase



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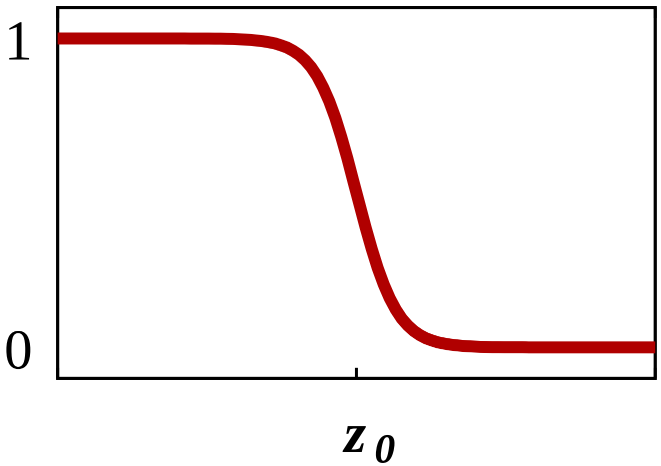

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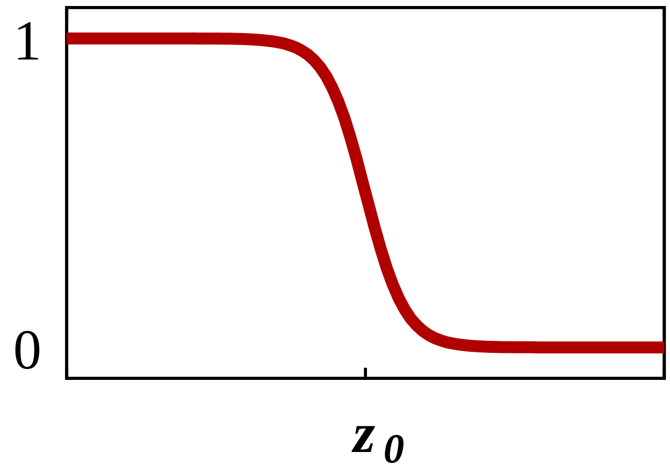

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$\phi(z,t) = \Phi(z-ct)$**?**, c:velocity

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Choose: $h(z,t) = H((z-ct)/W)$, $H(z) \neq 0$ for $|z| < 1$.

Better choice: implicit definition: $h \neq 0$ for $|\phi - \phi_h| \leq \delta\phi_w$

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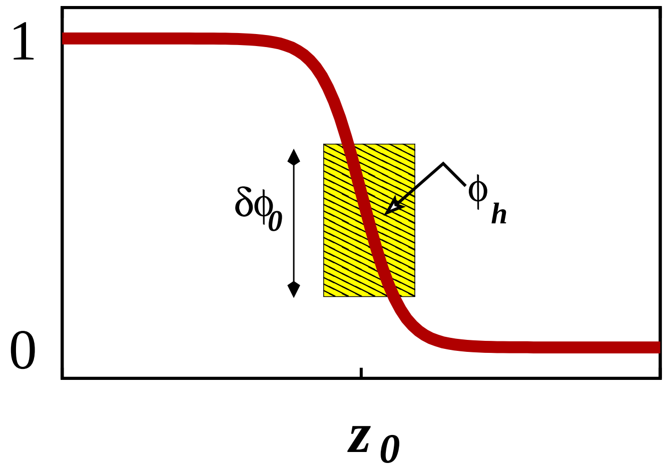

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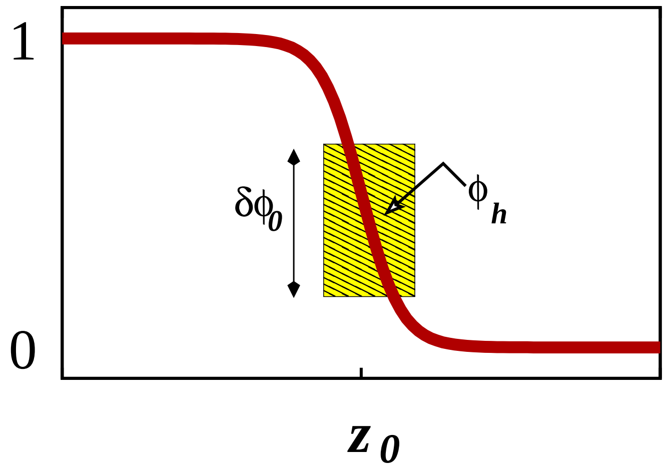

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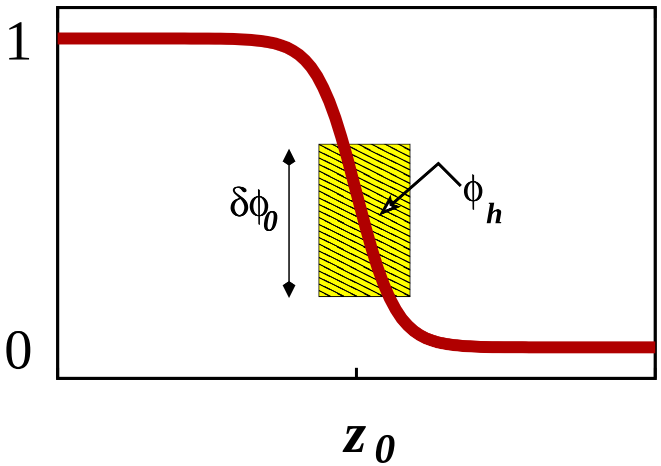

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$\phi = \phi_0(z) + \delta\phi(z - ct, t)$

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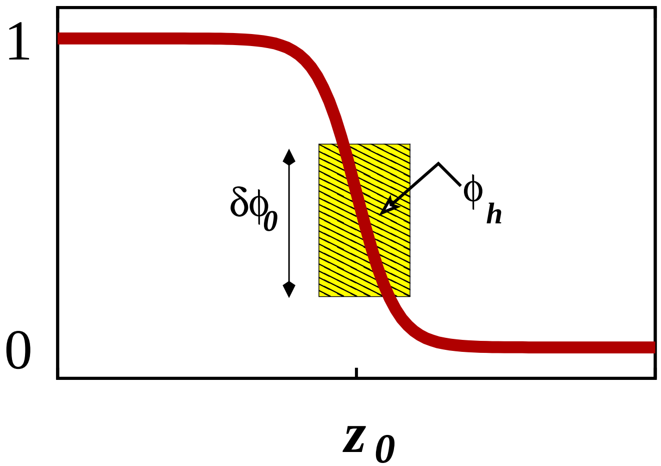

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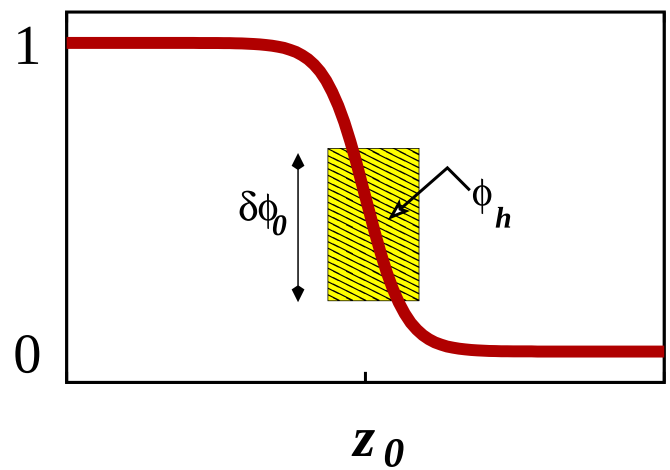

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To first order

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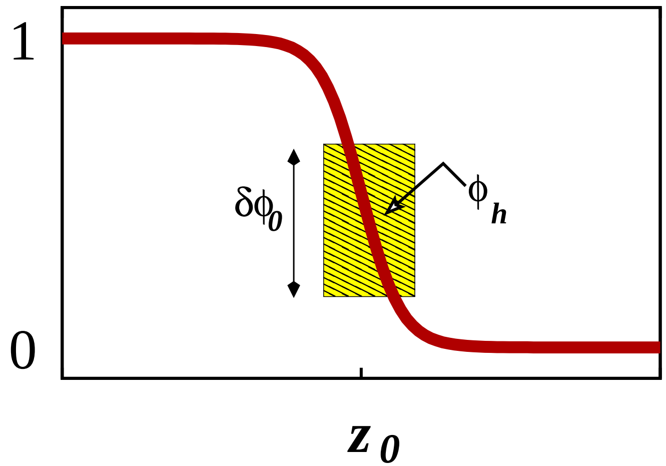

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For small c, $\boxed{\delta\phi = \frac{d\phi_0}{dz}\,(t-t_0)c}$

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Effective Model for RecG

Integrate out y, redefine the elastic constant:

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with $D' = D - \lambda^2 / K$.

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Perturbation theory, via Goldstone mode gives:

$$c = -\frac{1}{E_0}\left(\frac{\partial \phi_0}{\partial z}\right)^2 \Bigg|_{-}^{+}$$

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Perturbation theory, via Goldstone mode gives:

$$c = -\frac{1}{E_0}\left(\frac{\partial \phi_0}{\partial z}\right)^2 \Bigg|_{-}^{+}$$

What produced the minus sign?: monotonicity of ϕ_0.

Perturbation

Dynamics:

$$\frac{\partial \phi}{\partial t} = D\frac{\partial^2 \phi}{\partial z^2} - \frac{\partial^4 \phi}{\partial z^4} - h\frac{\partial^2 \phi}{\partial z^2} + \phi(\phi - \frac{1}{2})(1-\phi)$$

h is nonzero in a region near $\phi = 0$.

With negative D', preference for a modulated structure
The interface becomes unstable
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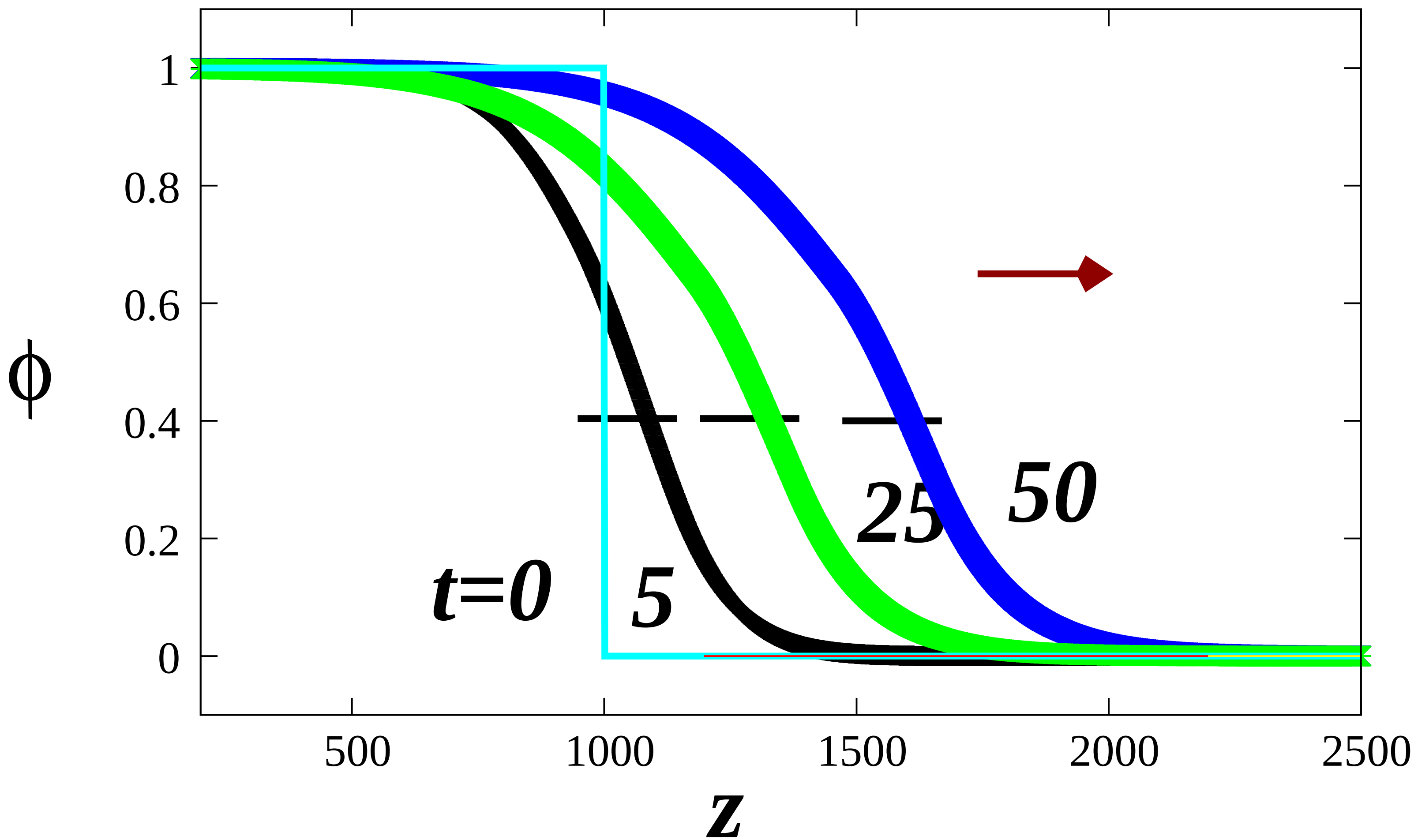
$$c = -\frac{1}{E_0}\left(\frac{\partial \phi_0}{\partial z}\right)^2\Bigg|_-^+$$

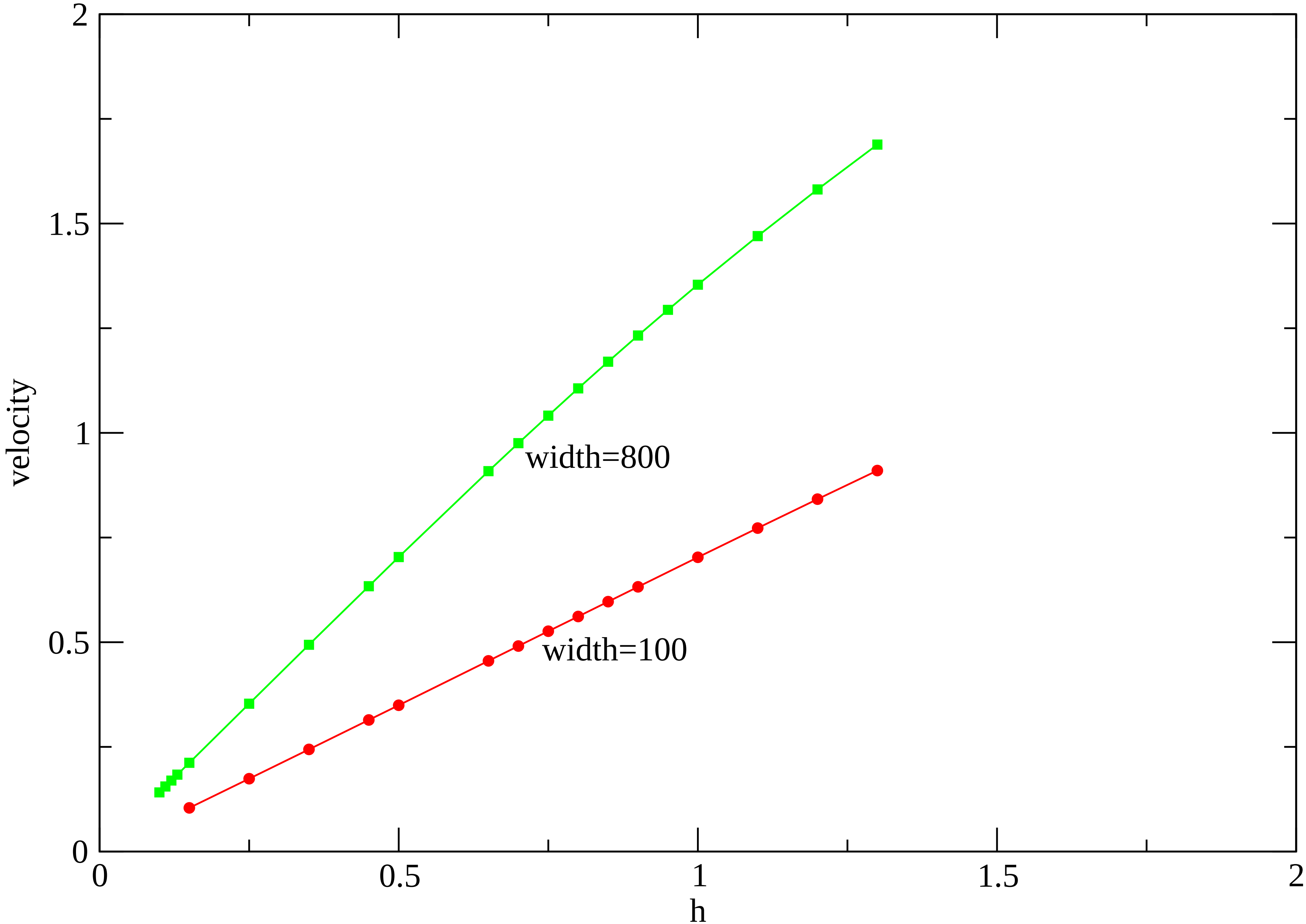
What produced the minus sign?: monotonicity of ϕ_0.

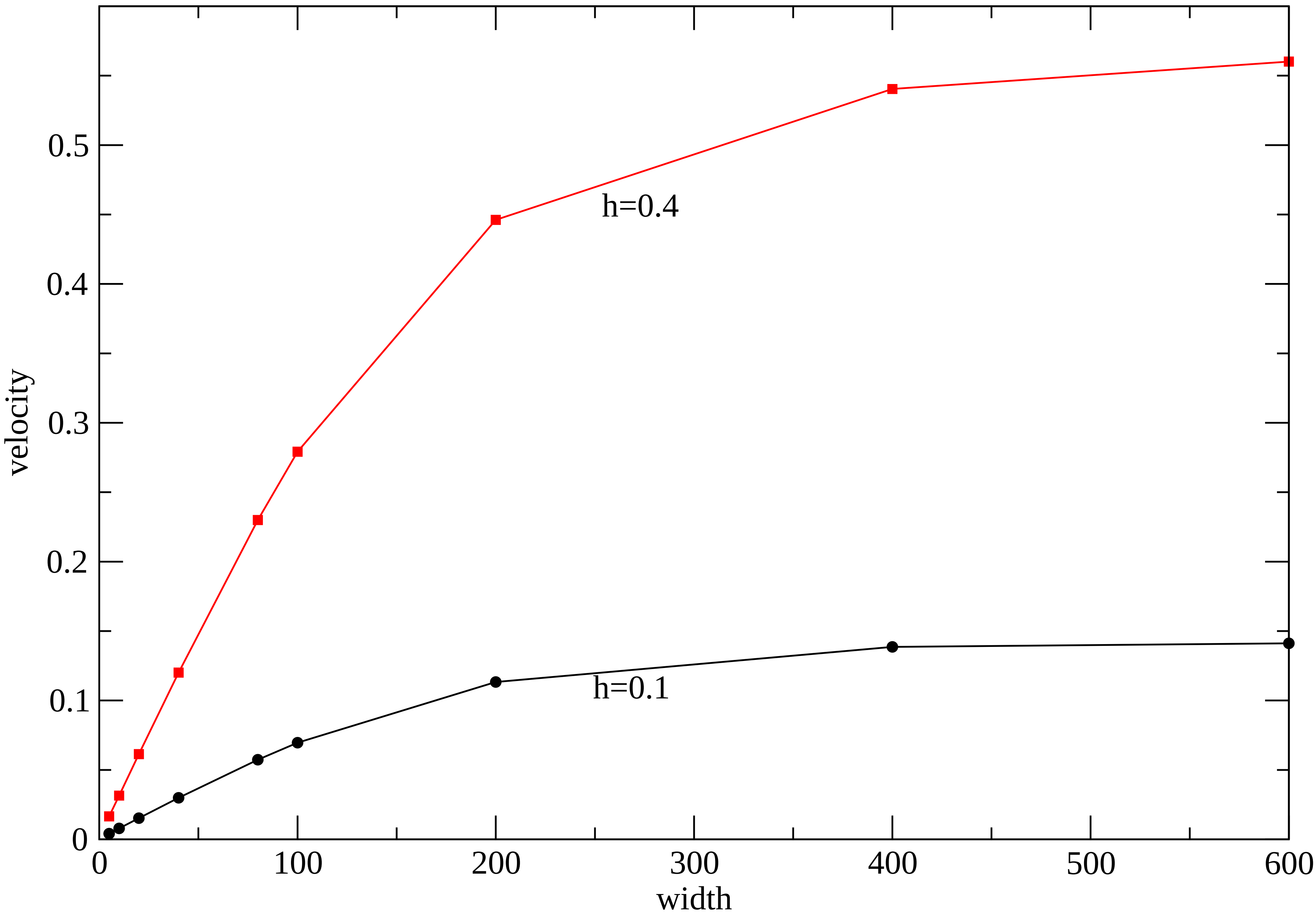
Helicase Profile

h=0.4d0, L=100



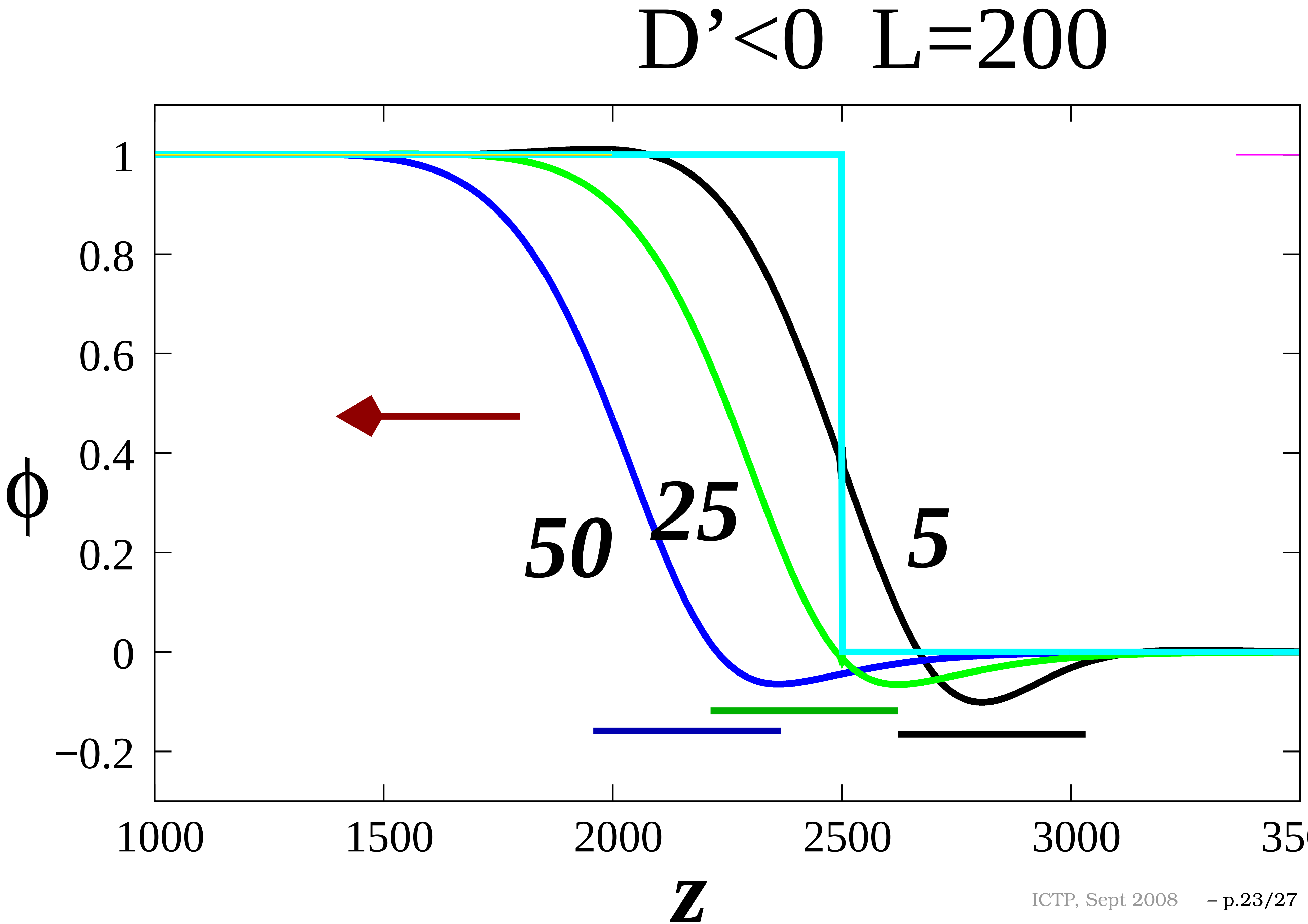
Helicase perturbation: $h\,\phi(1-\phi)$


2
1.5
1
0.5
0
velocity
width=800
width=100
0
0.5
1
1.5
2
h

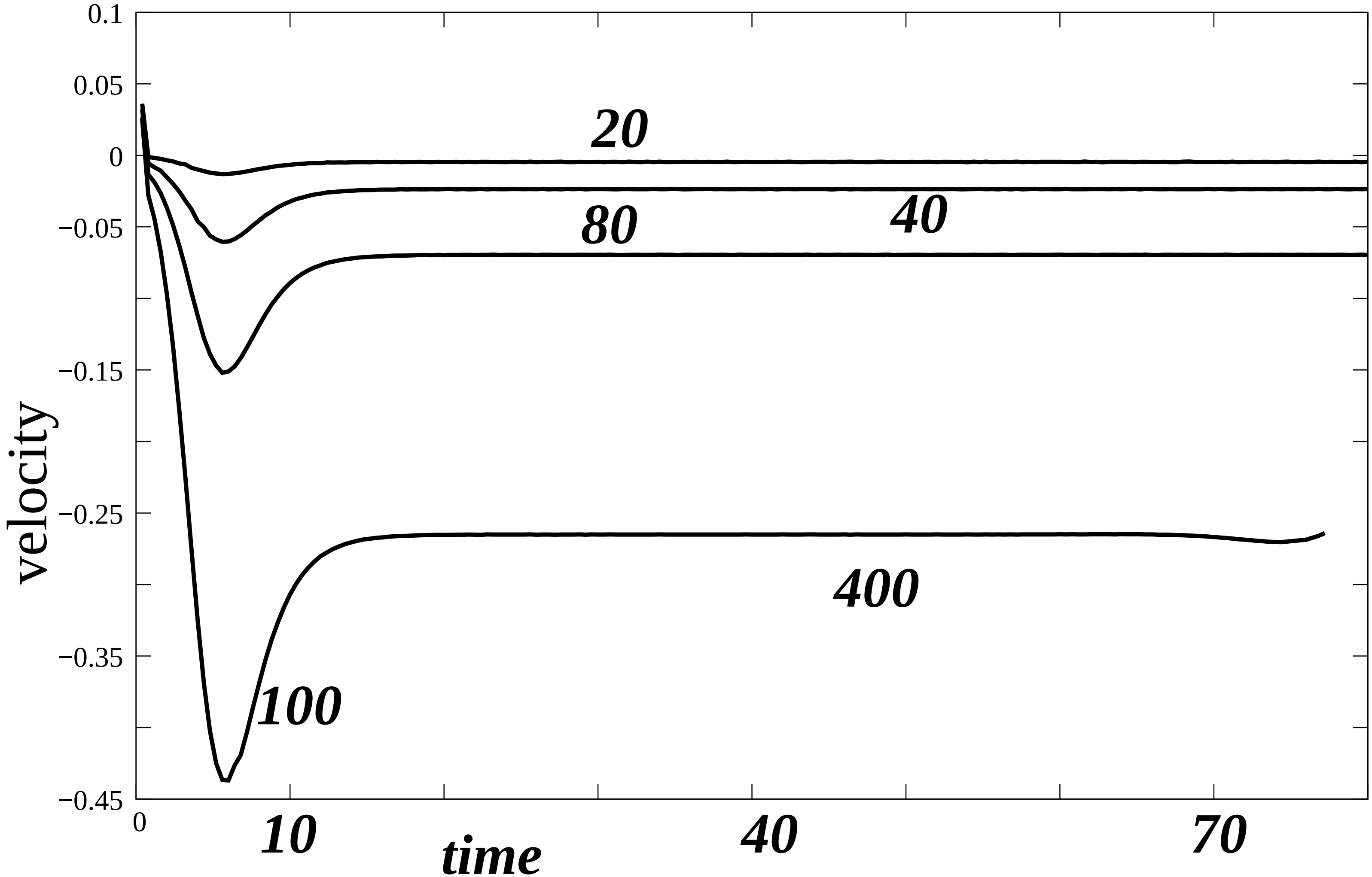


0.5
0.4
0.3
0.2
0.1
0
velocity
h=0.4
h=0.1
0
100
200
300
400
500
600
width

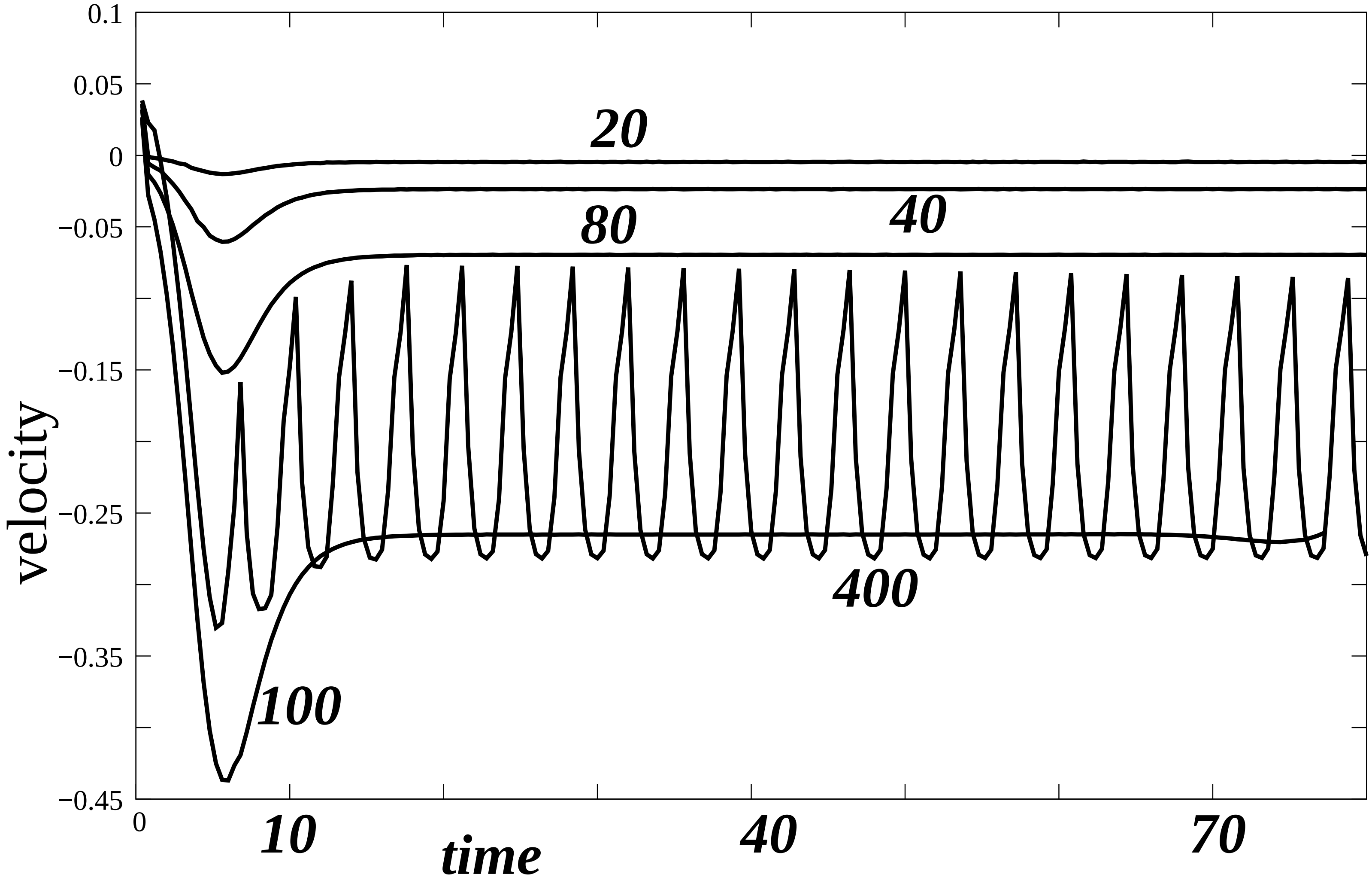
RecG profile



RecG velocity



RecG velocity



Summary

- Destabilization of the interface : a possible mode for reverse motion of RecG and Y-fork
- Replication to be analysed in a frame moving with the Y-fork.

ধন্যবাদ

"dhonnobad" - thank you in Bengali