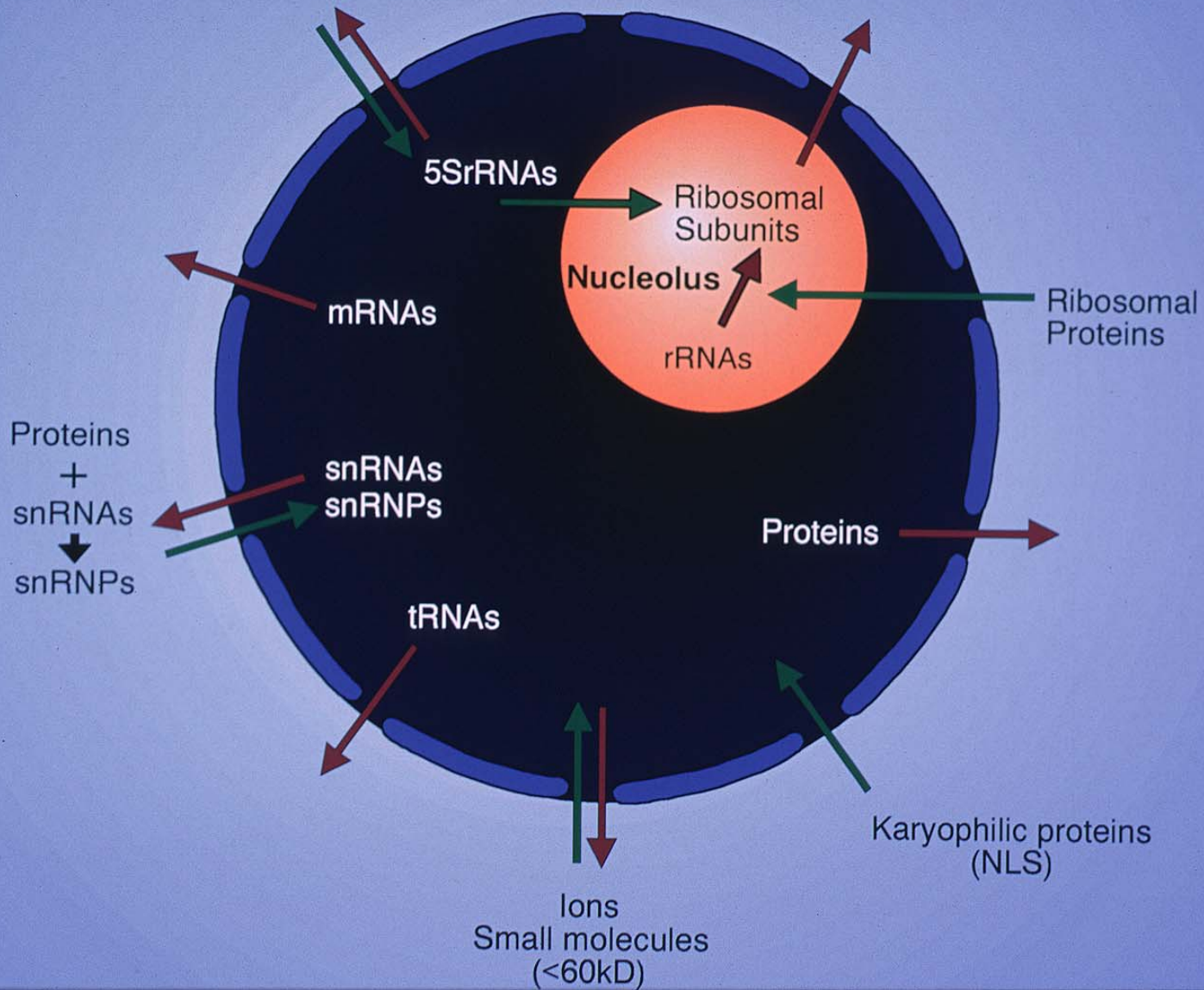
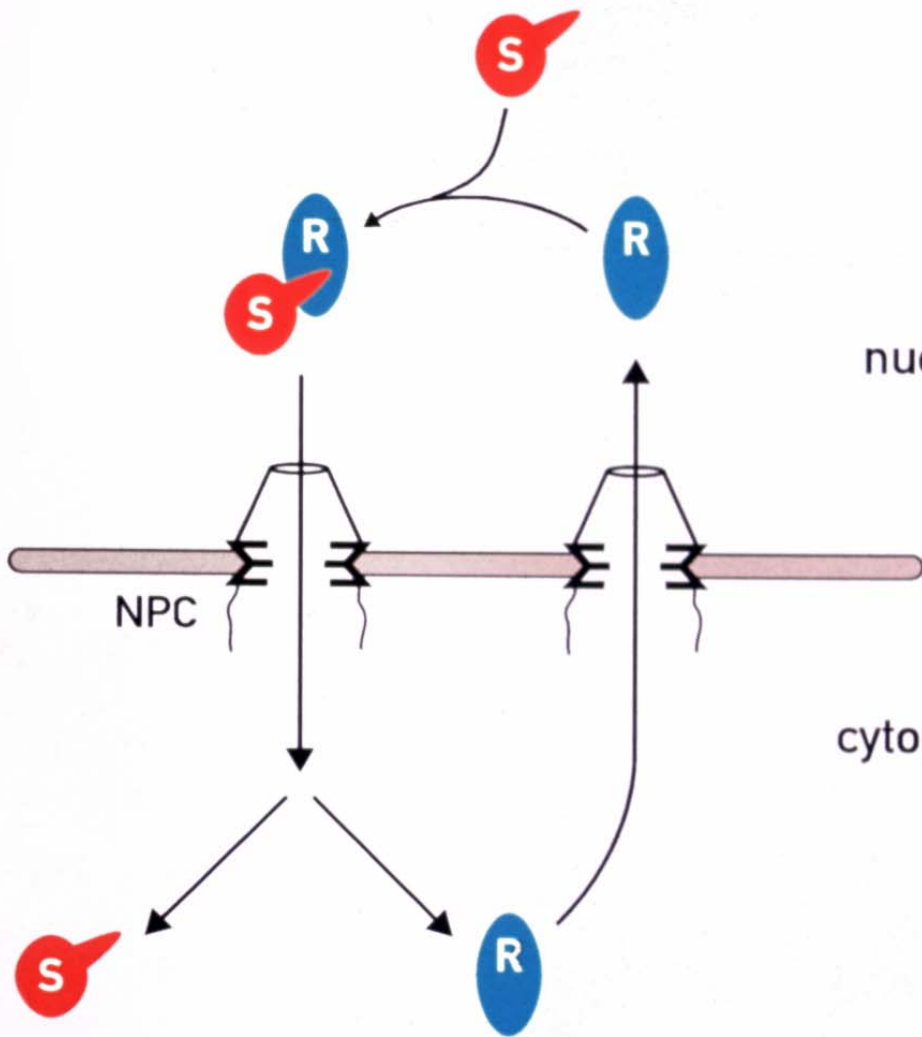


# NUCLEAR IMPORT/EXPORT

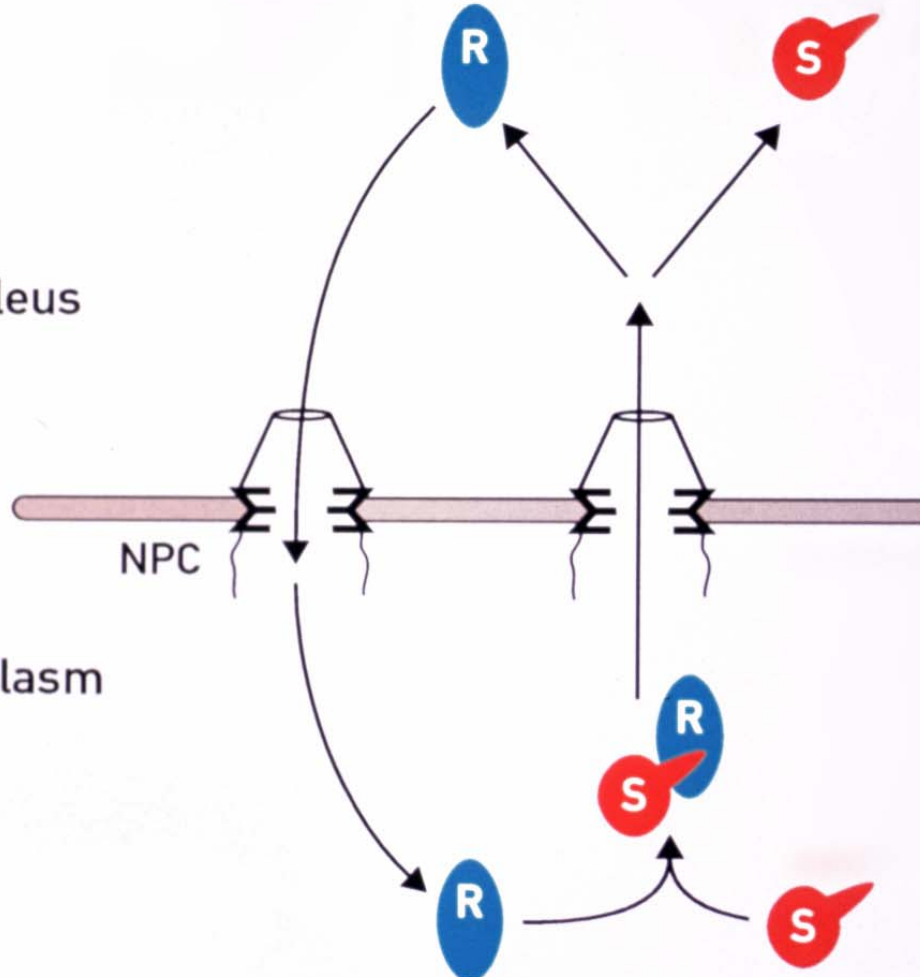




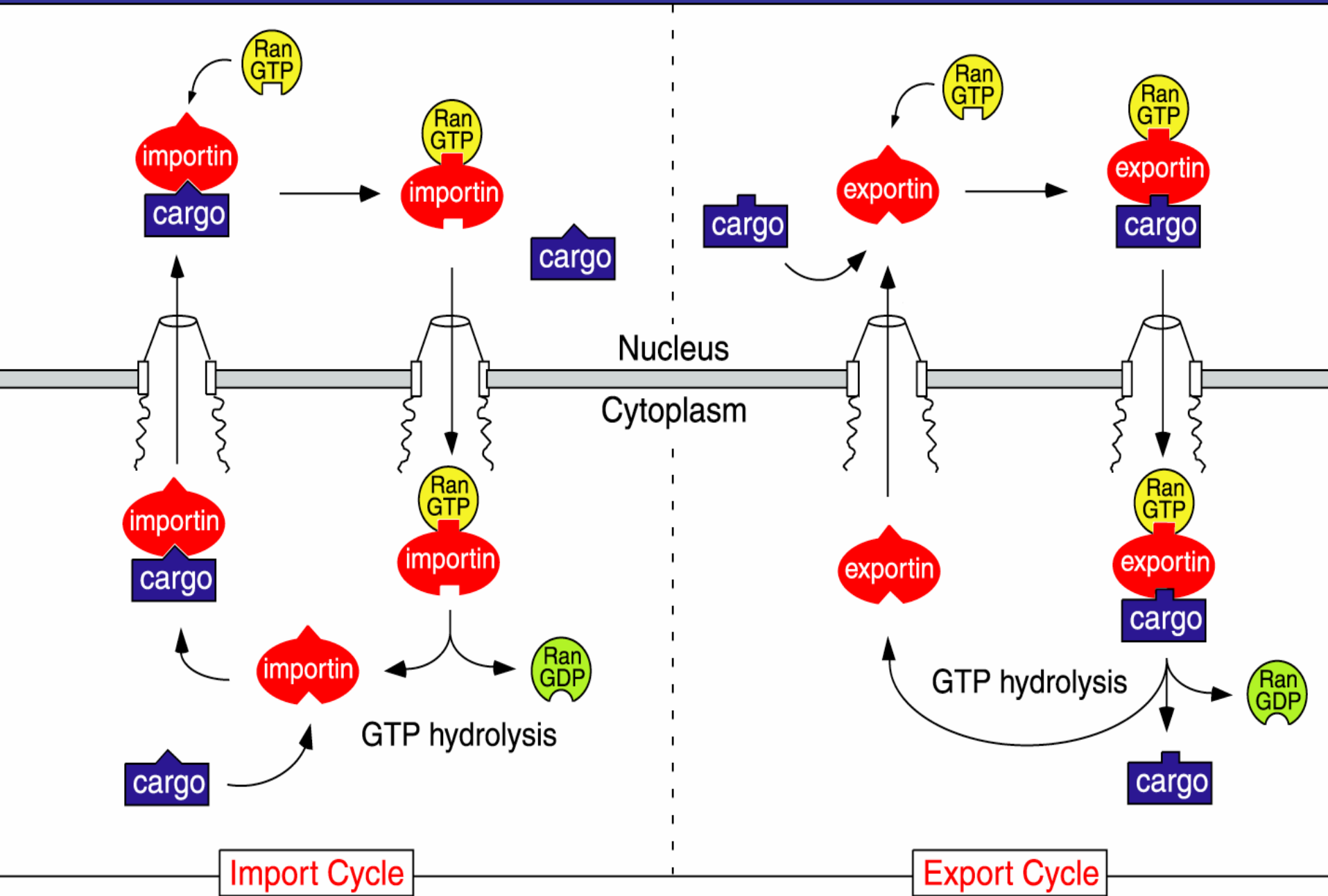
**EXPORT**

nucleus

cytoplasm

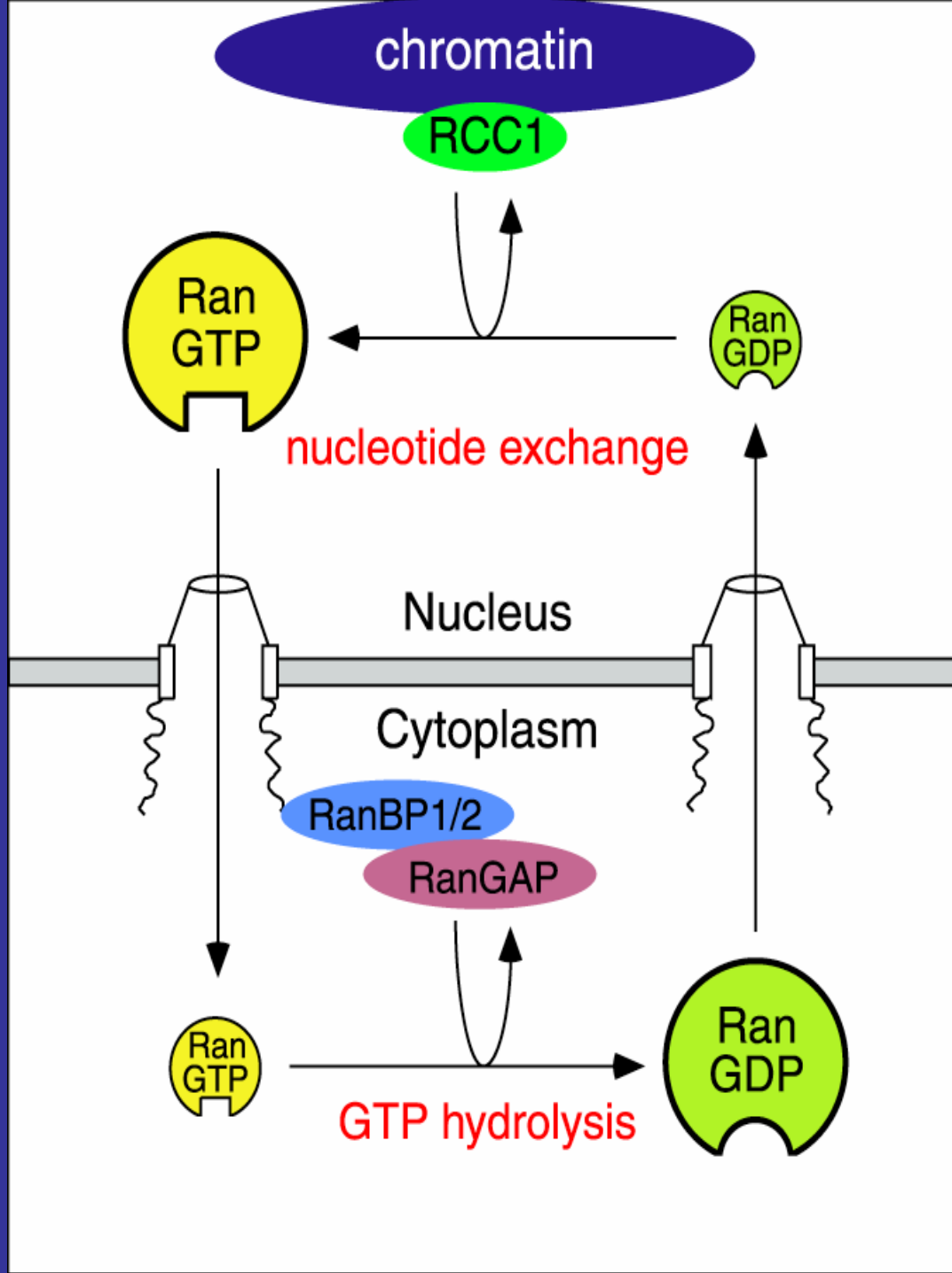


**IMPORT**



Import Cycle

Export Cycle





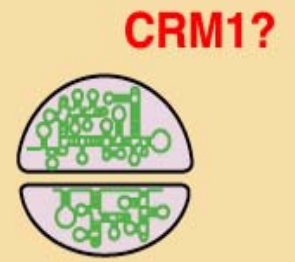
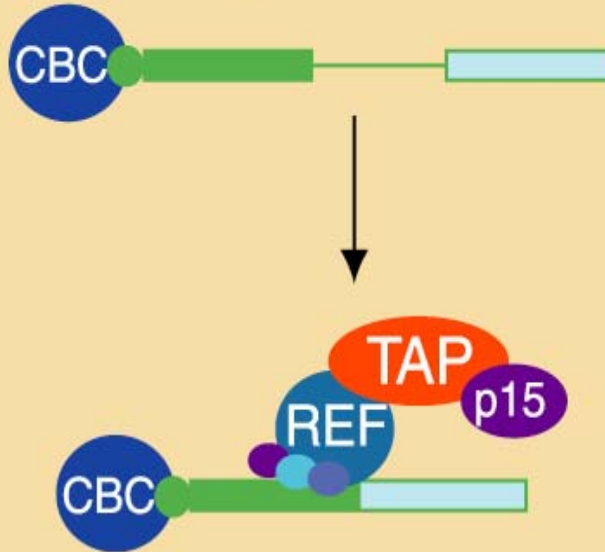
# RNA export pathways

tRNAs

UsnRNAs

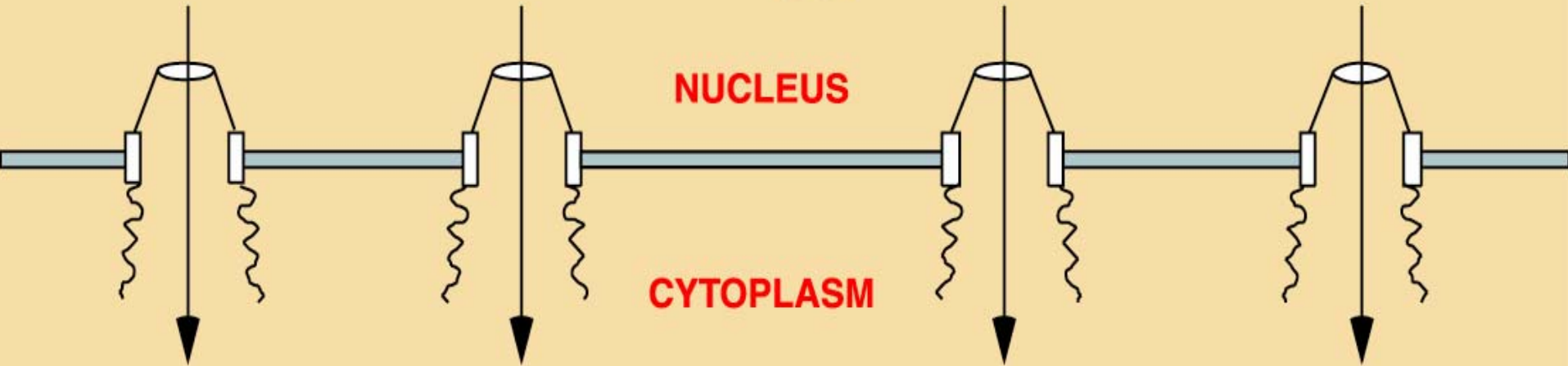
mRNAs

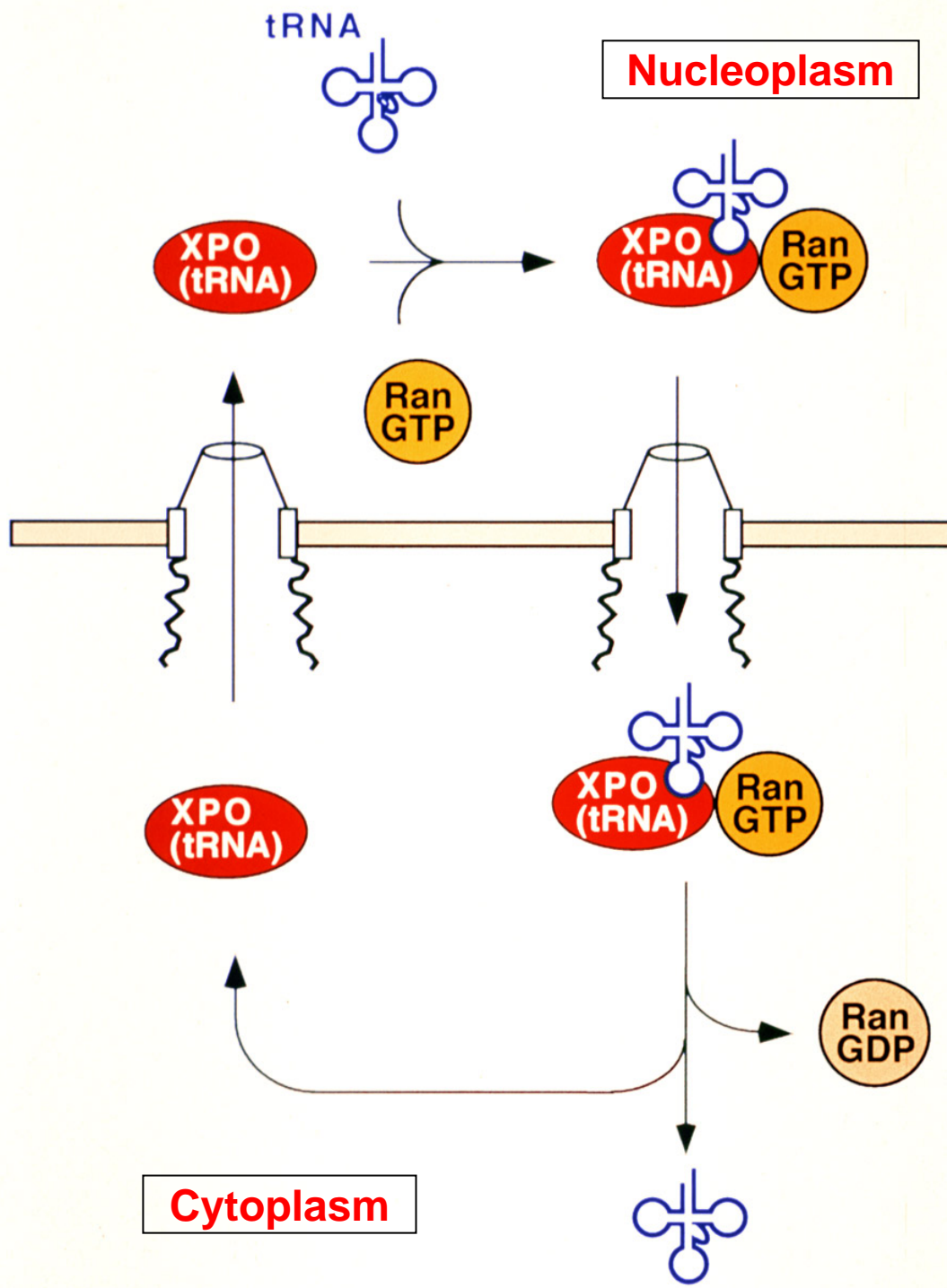
rRNAs



NUCLEUS

CYTOPLASM









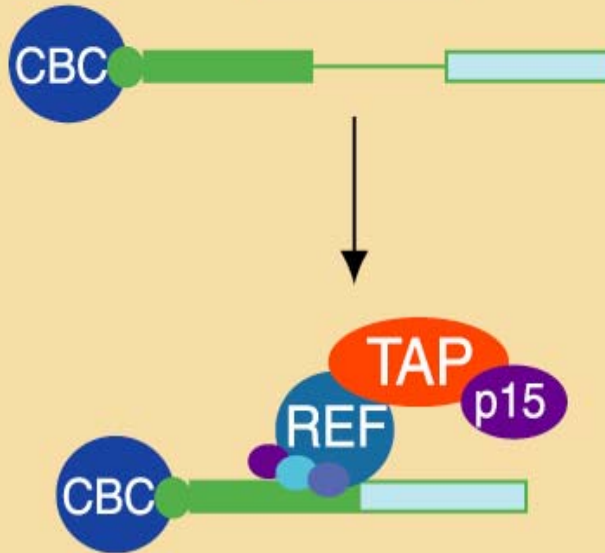
# RNA export pathways

tRNAs

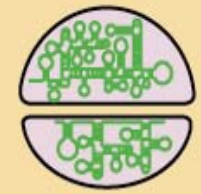
UsnRNAs

mRNAs

rRNAs

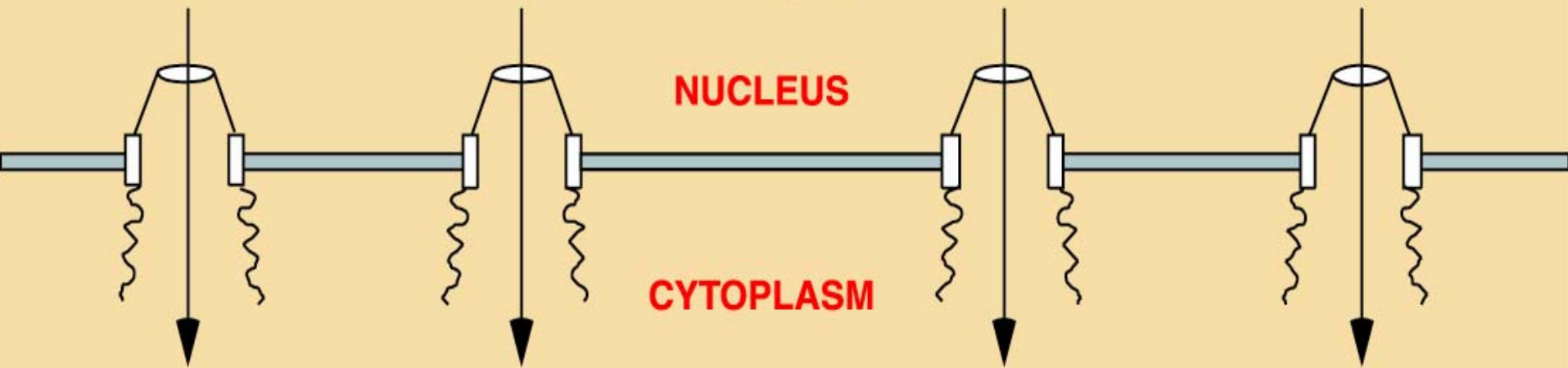


CRM1?



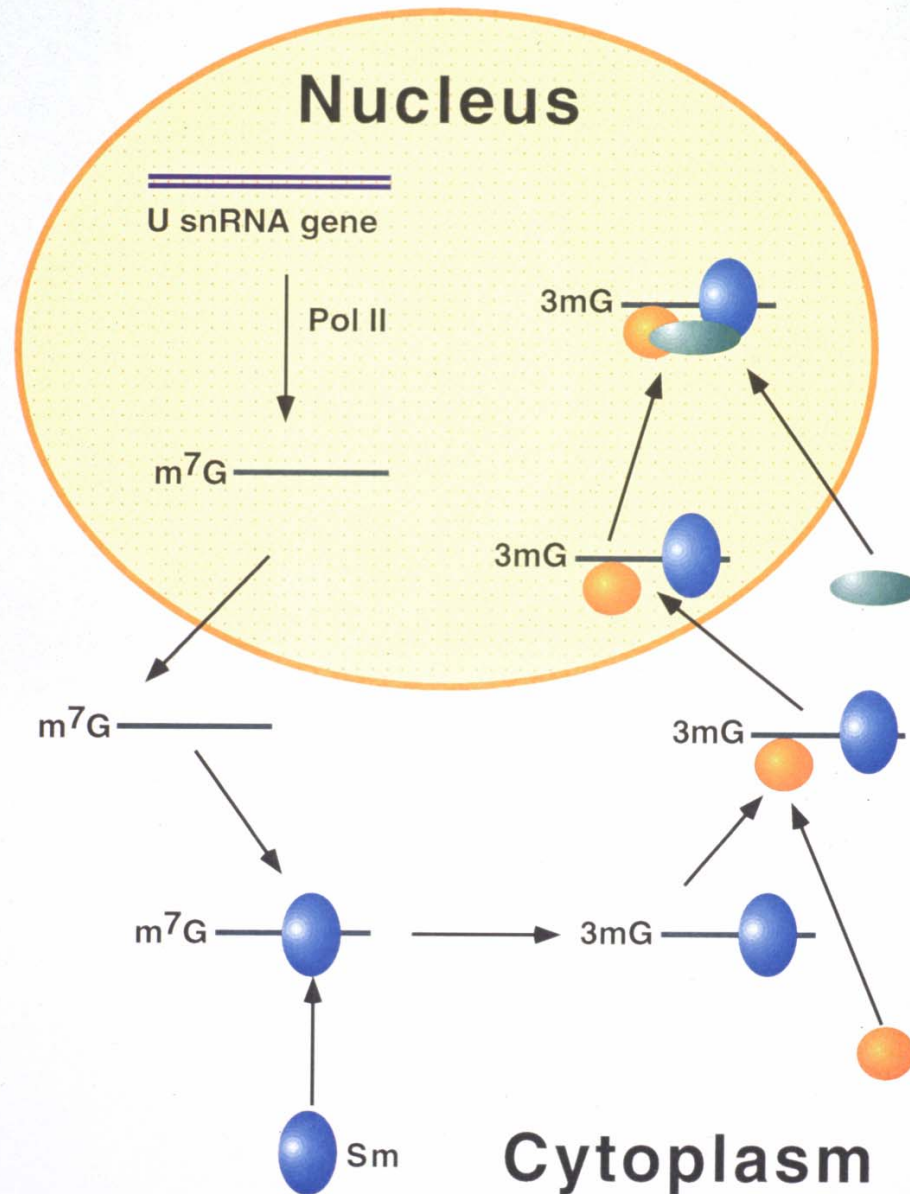
NUCLEUS

CYTOPLASM





# U snRNA BIOGENESIS

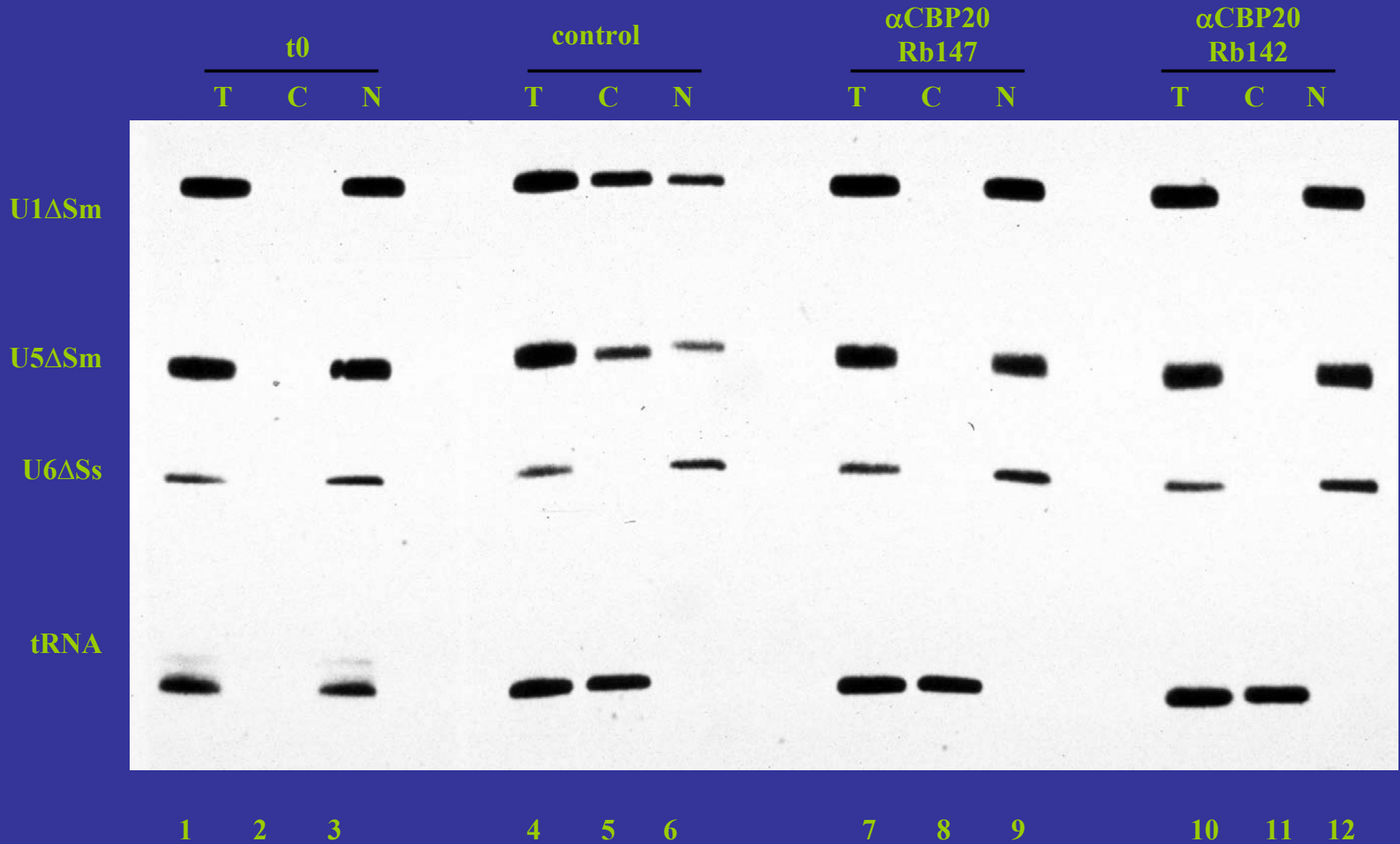


# A NUCLEAR CAP BINDING COMPLEX

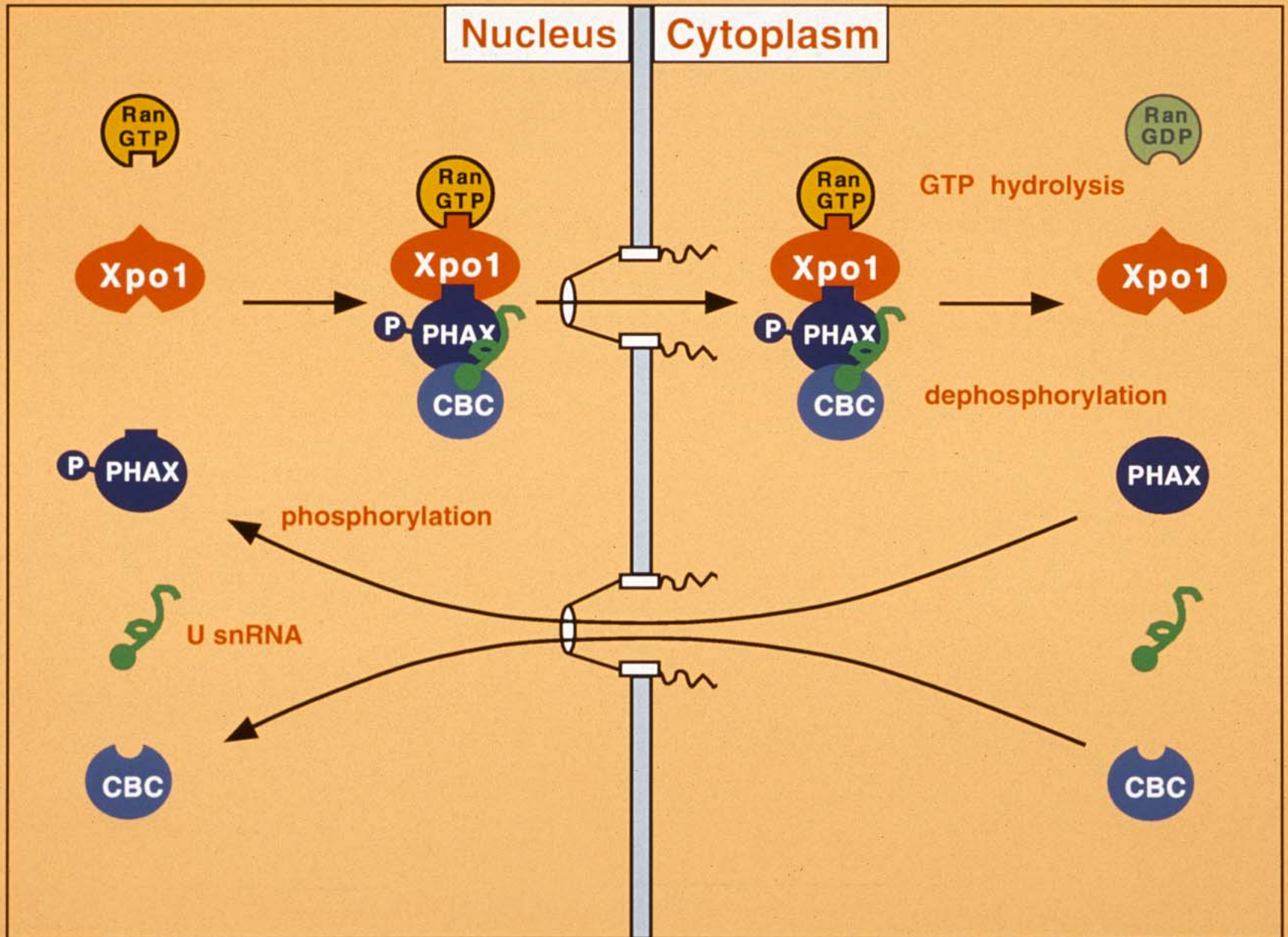


**involved in pre-mRNA processing  
and U snRNA nuclear export**

# Anti-CBP 20 antibodies inhibit U snRNA export



# Model of U snRNA export





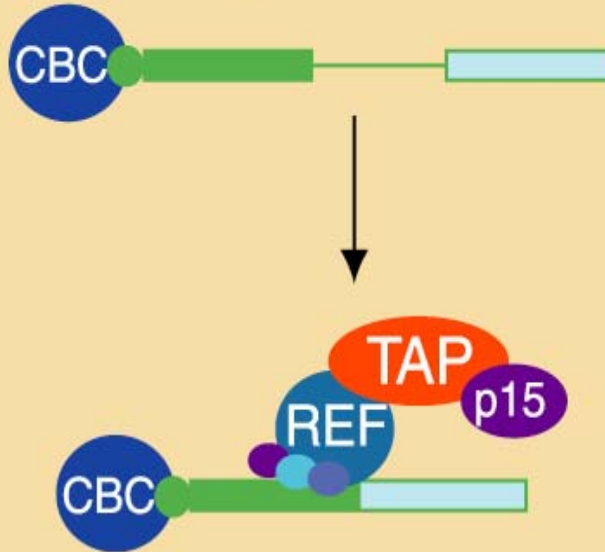
# RNA export pathways

tRNAs

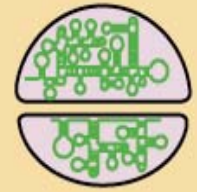
UsnRNAs

mRNAs

rRNAs

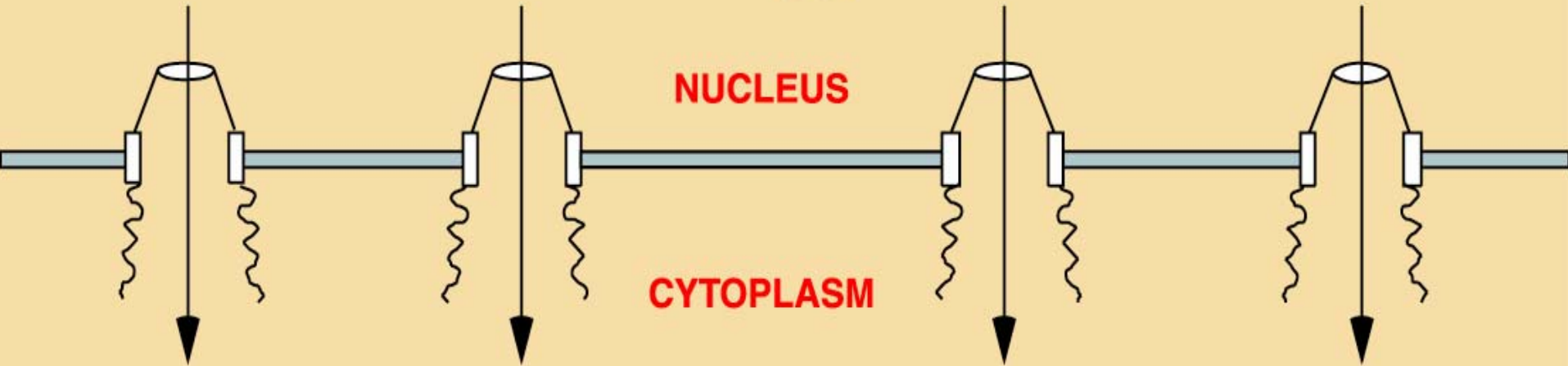


CRM1?

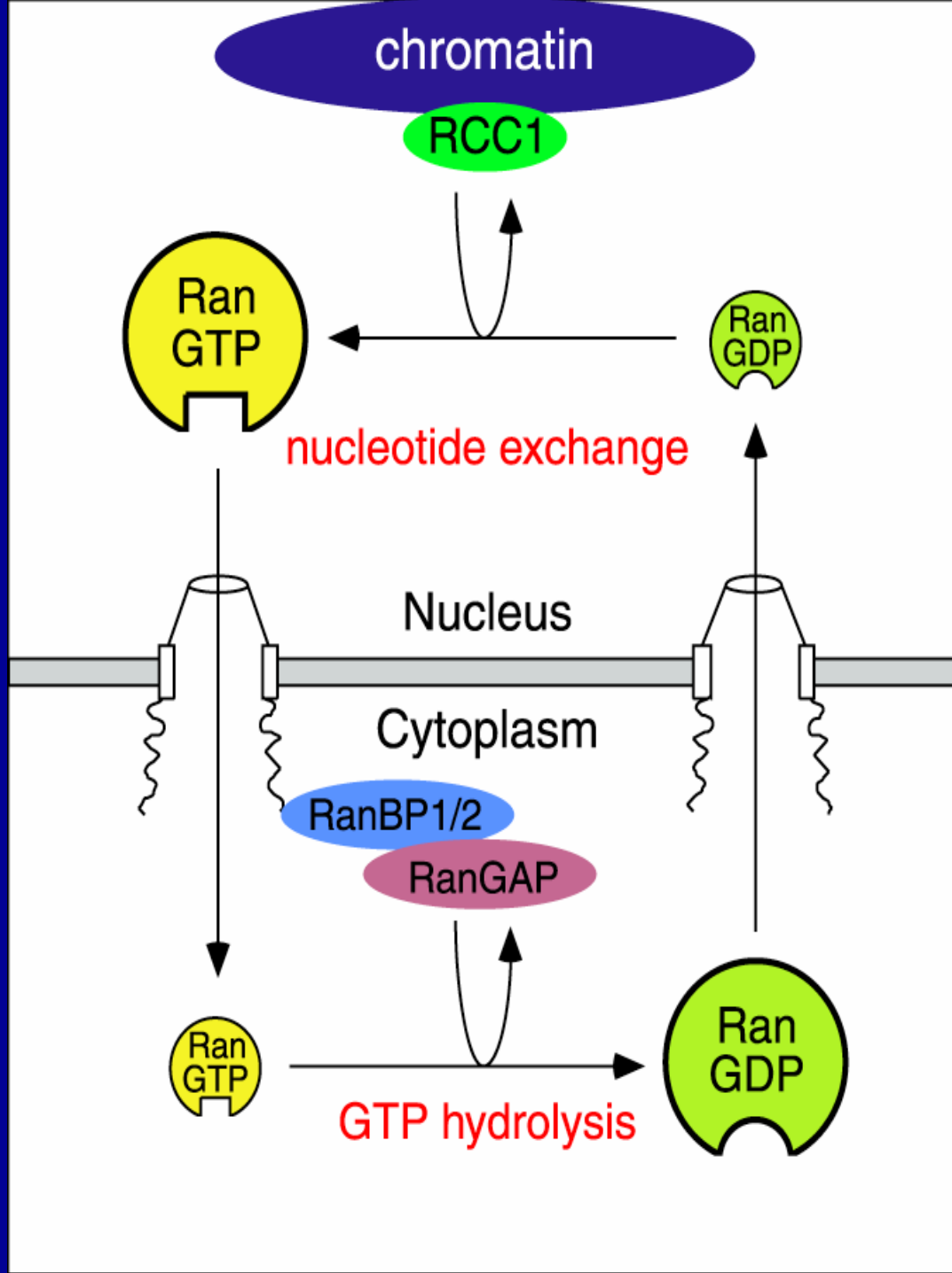


NUCLEUS

CYTOPLASM



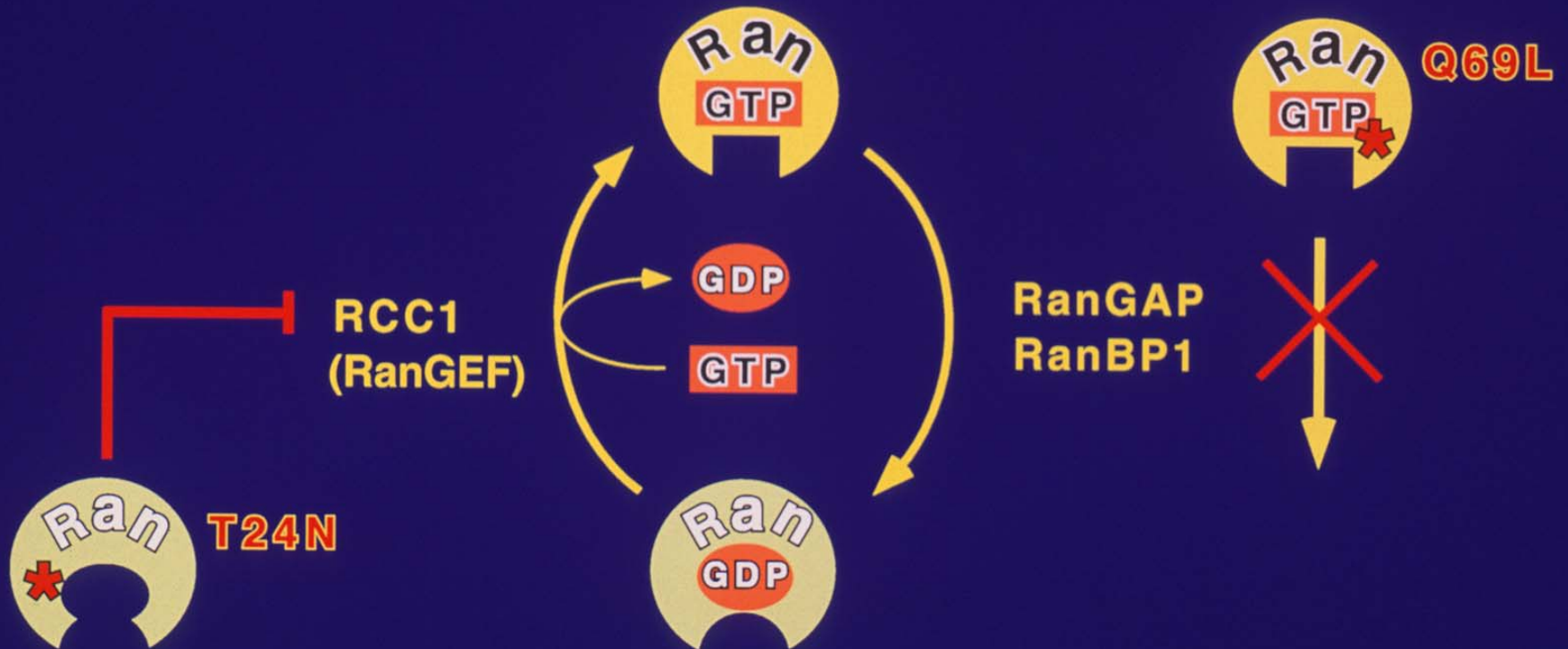




# MEIOTIC SPINDLE FORMATION

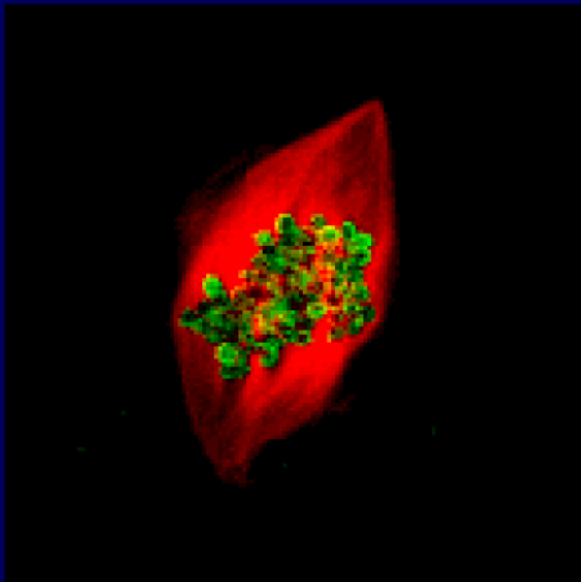


# The Ran GTPase cycle

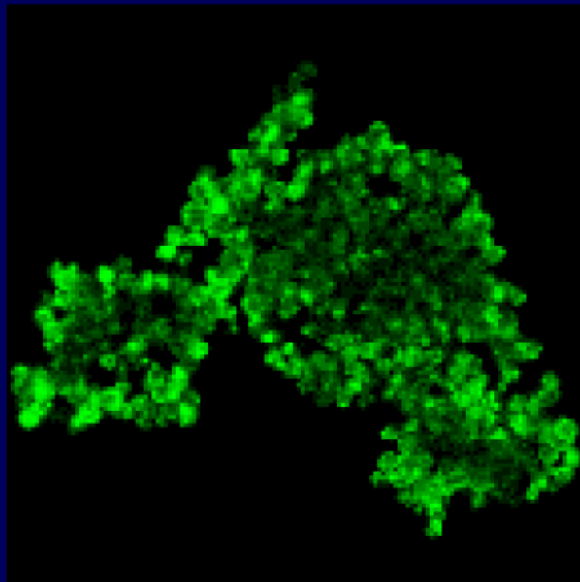


# RCC1 activity is required for chromatin-induced spindle formation

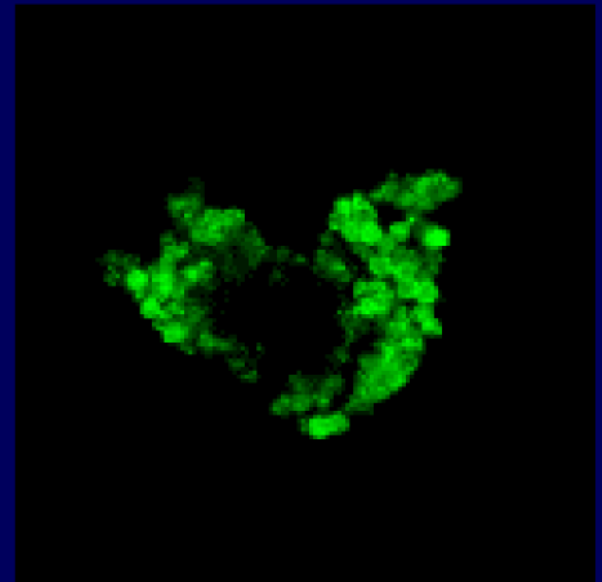
control



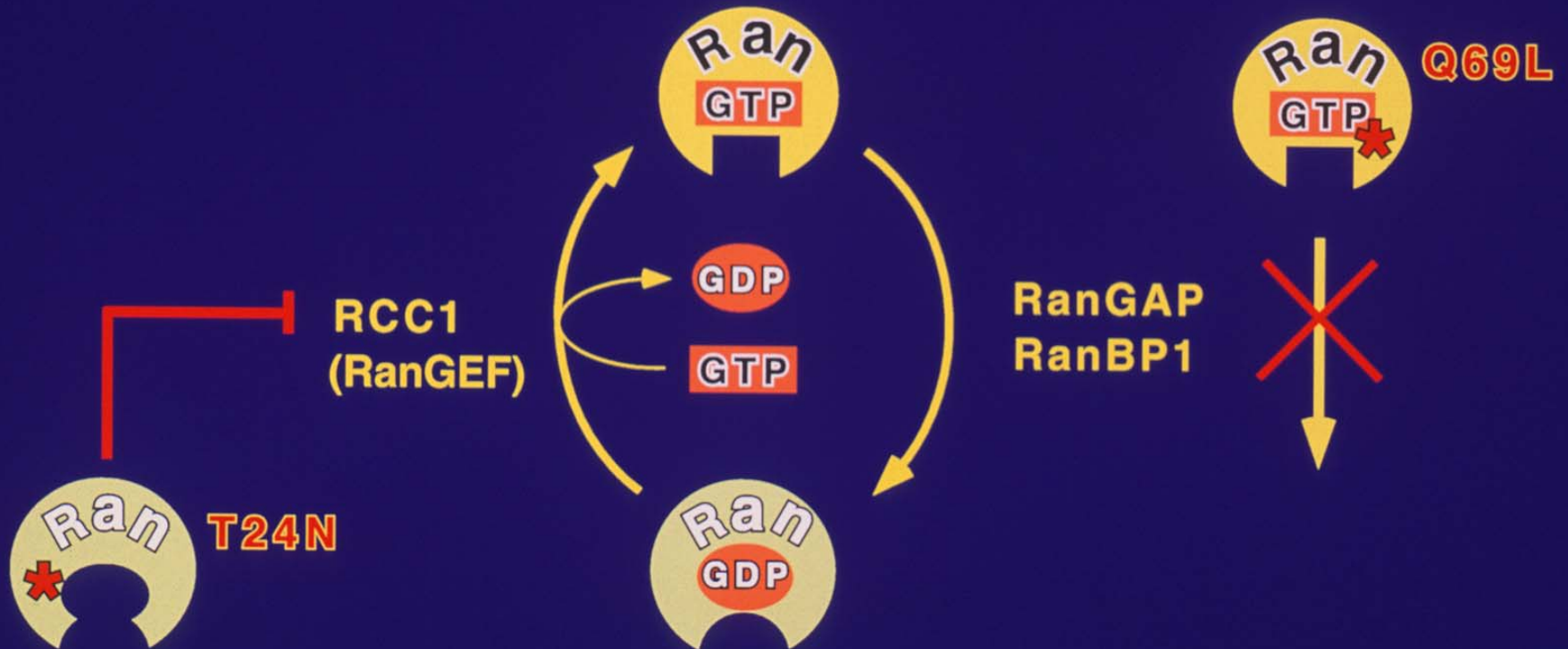
+ Ran T24N



+ RanGAP  
+ Ran BP1

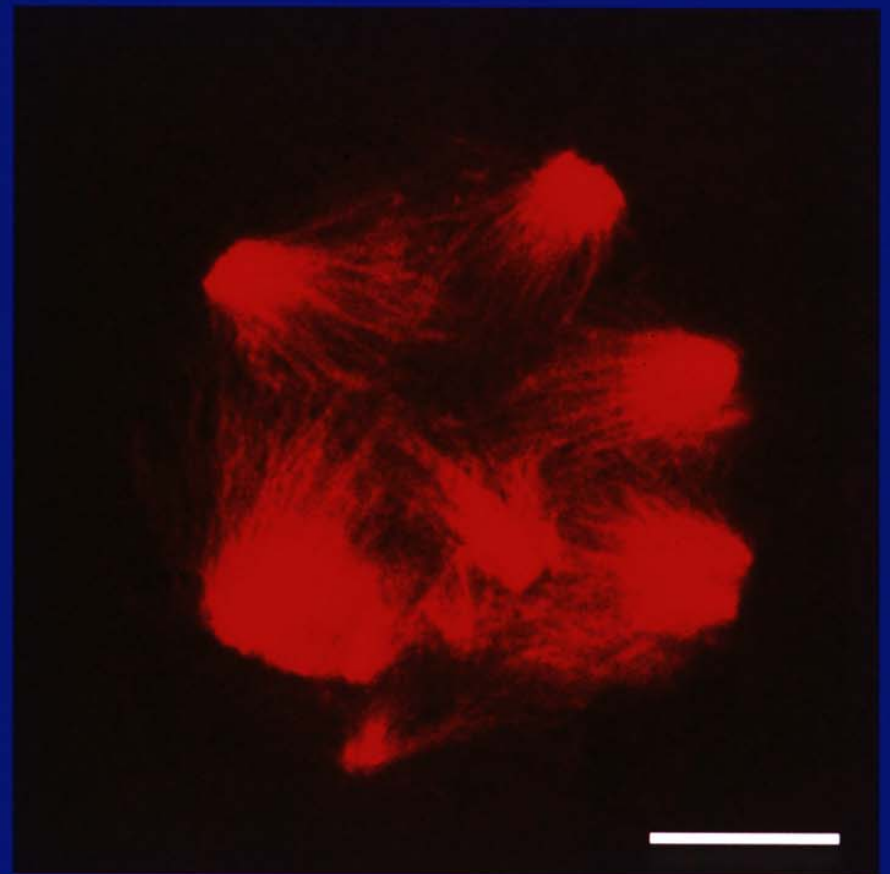
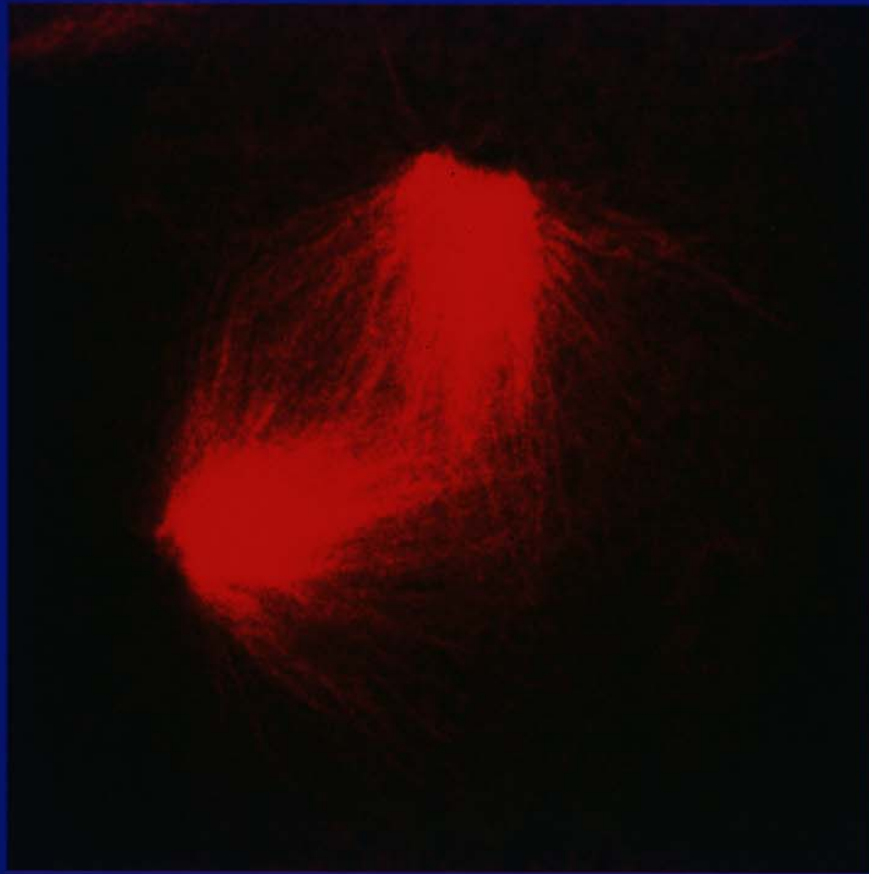


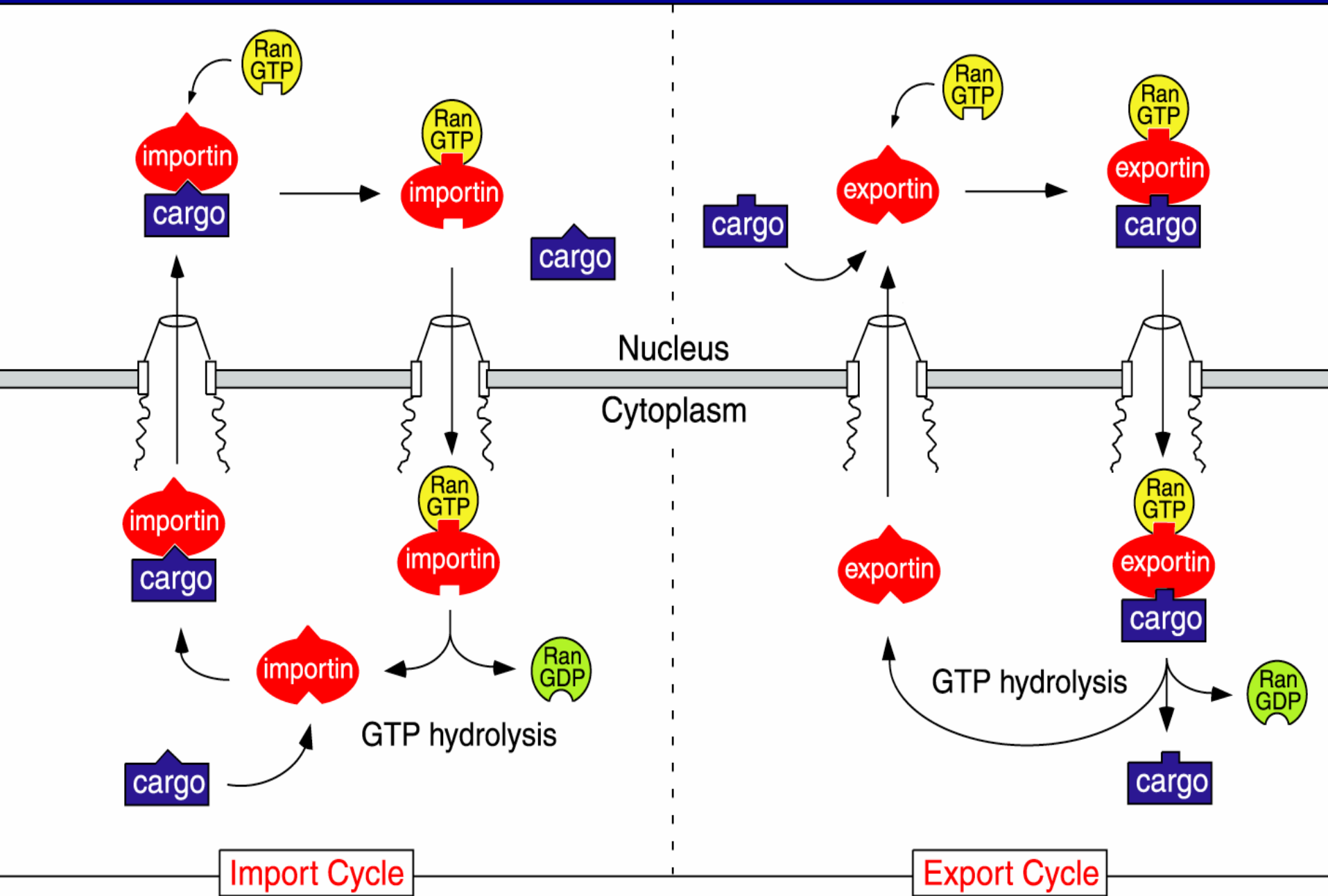
# The Ran GTPase cycle





## Excess RanGTP induces formation of spindle-like structures

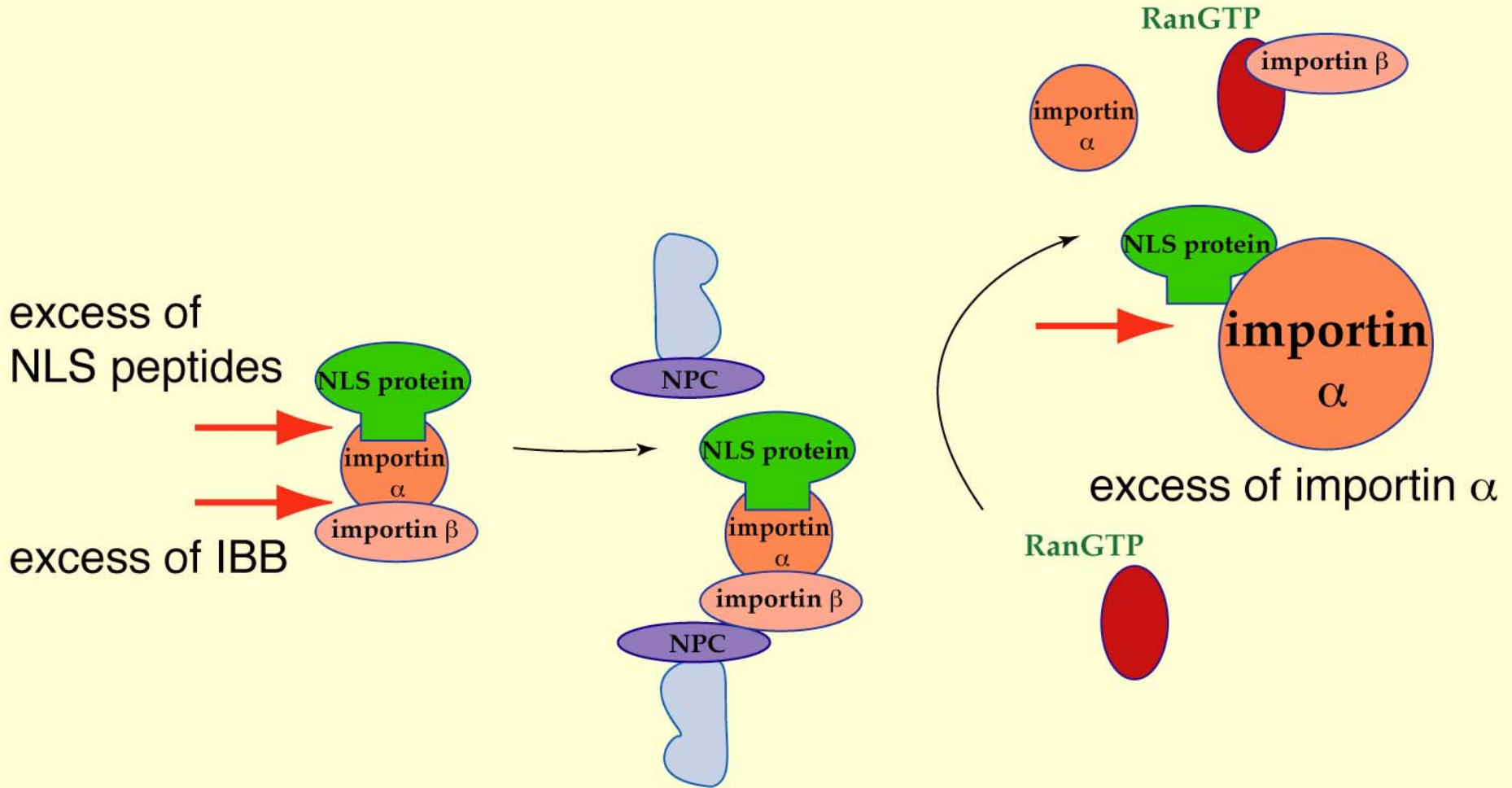




Import Cycle

Export Cycle

# Import of NLS containing proteins

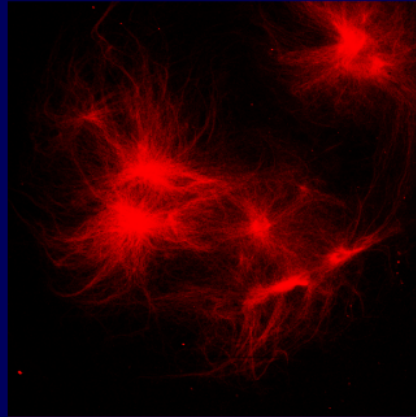


# NLS and IBB promote microtubule assembly

buffer



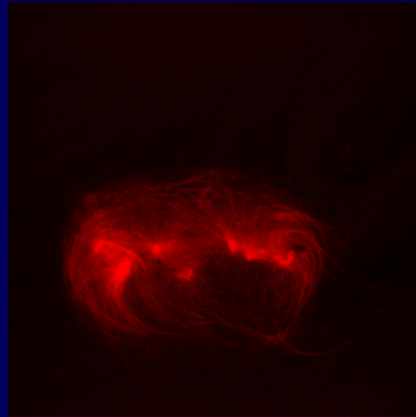
BSA-NLS



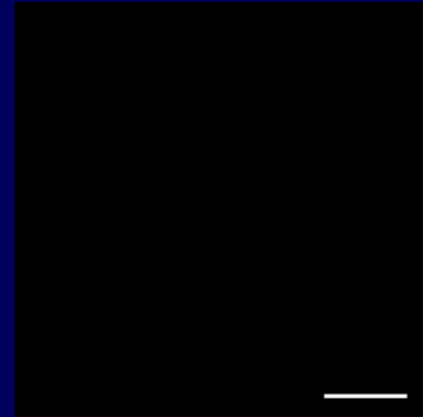
BSA-rev.NLS



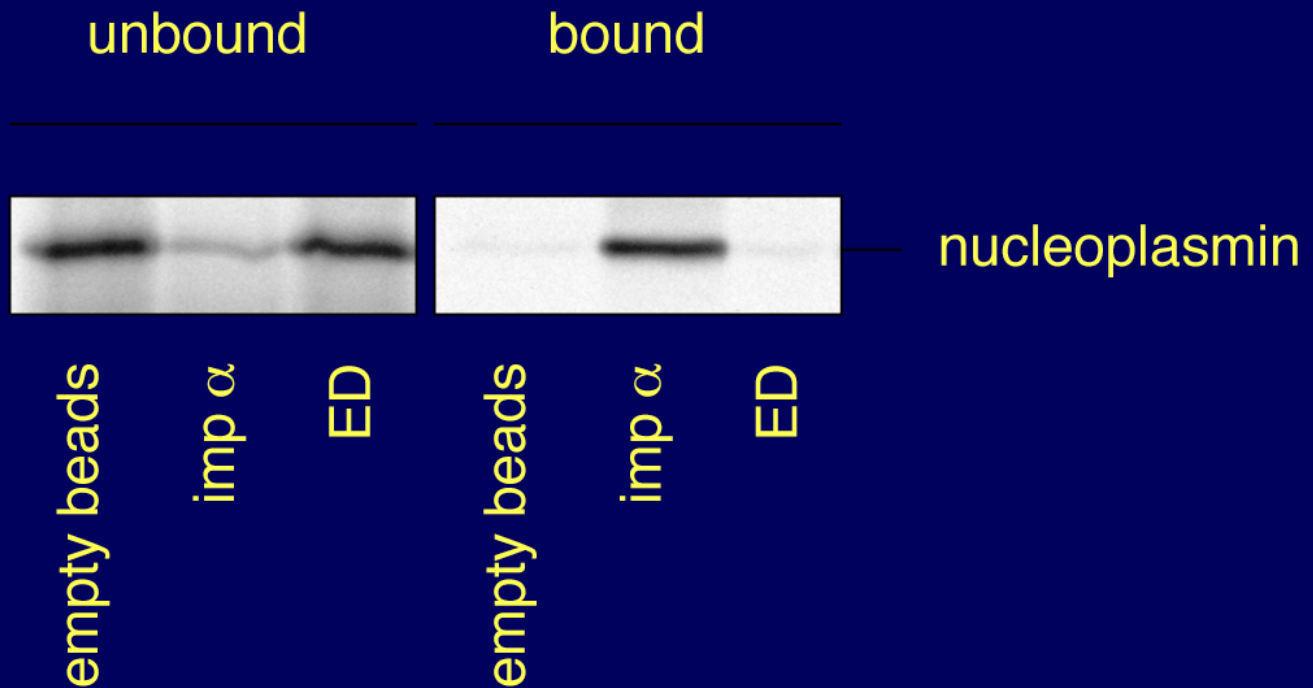
IBB



tr.IBB



## A mutant of importin $\alpha$ deficient in NLS binding



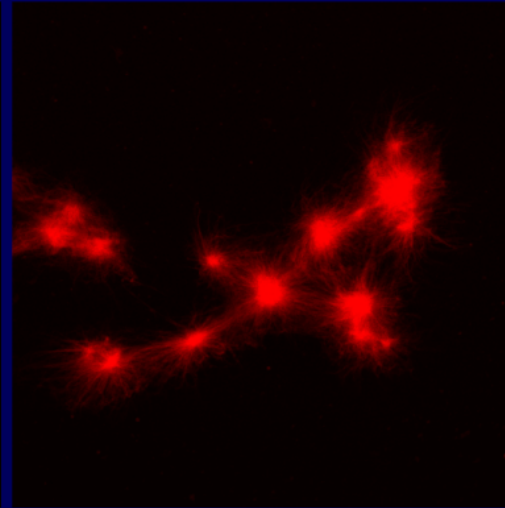


# Addition of importin $\alpha$ inhibits microtubule assembly

buffer



RanQ69L.GTP



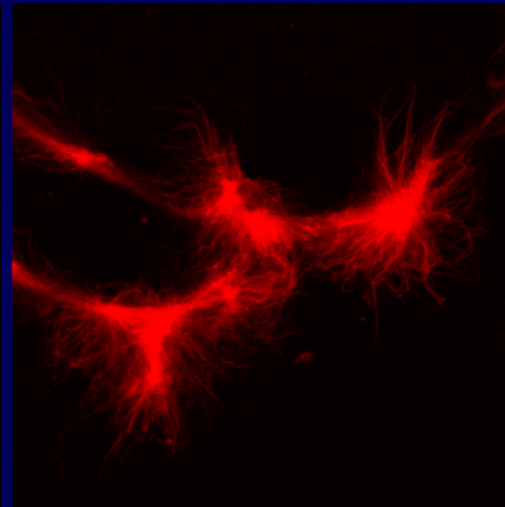
RanQ69L.GTP +

imp  $\alpha$

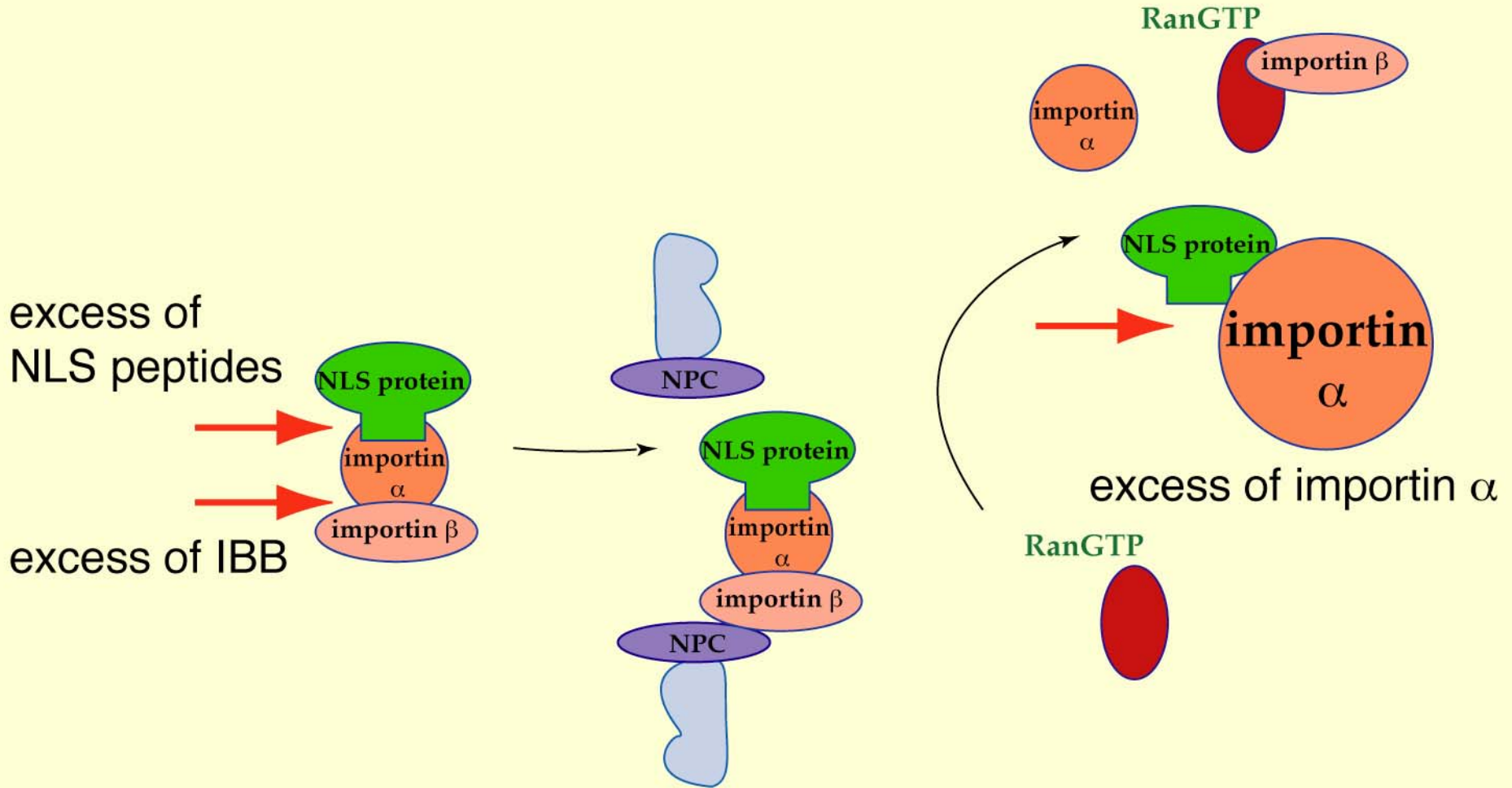


RanQ69L.GTP +

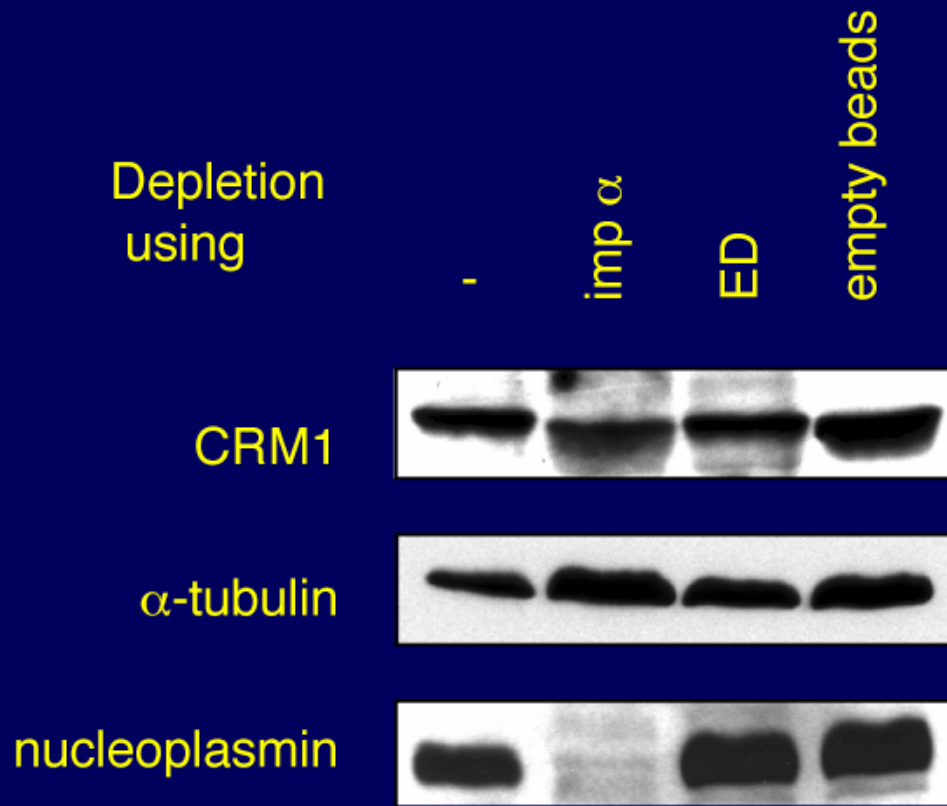
ED



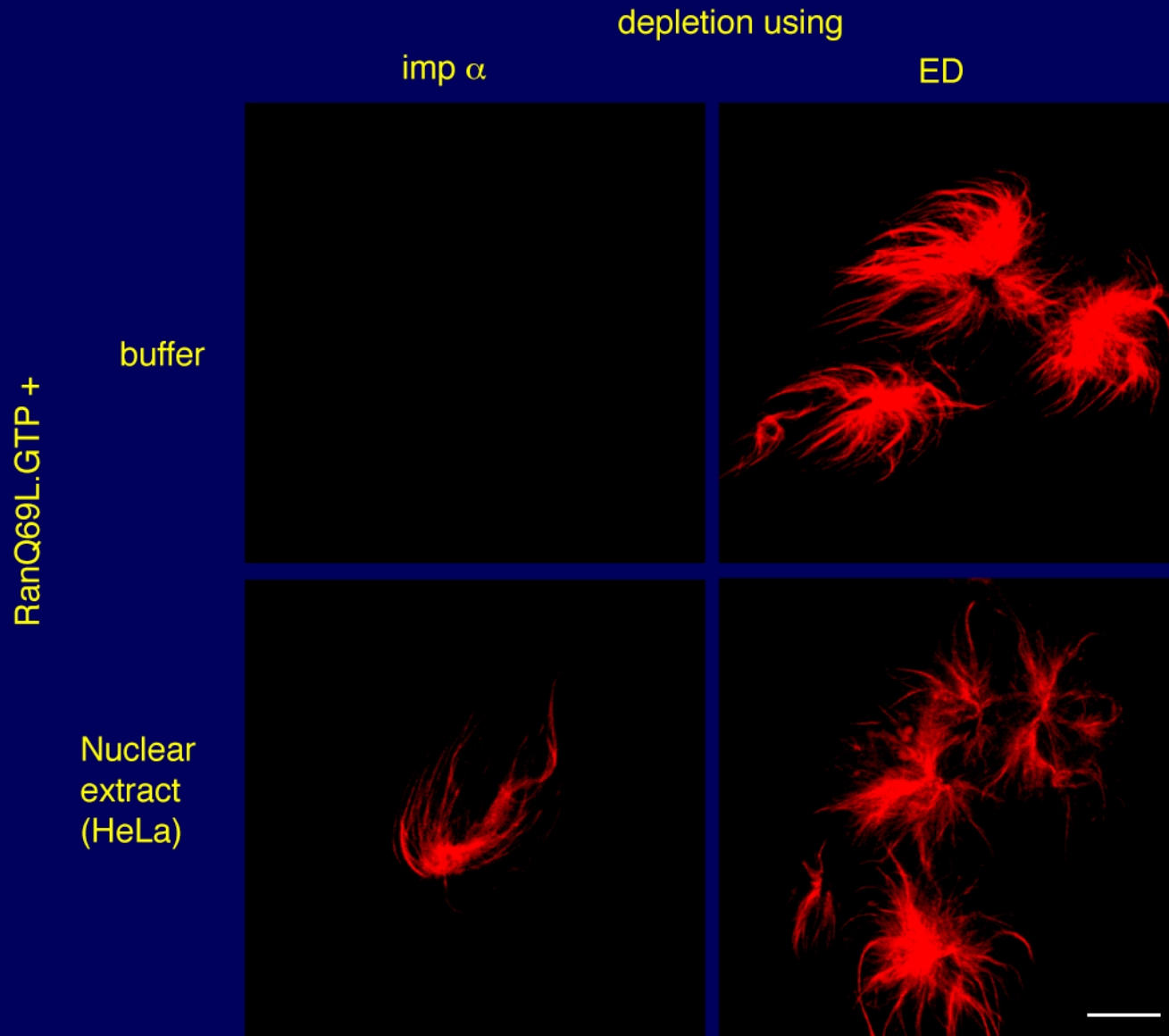
# Import of NLS containing proteins



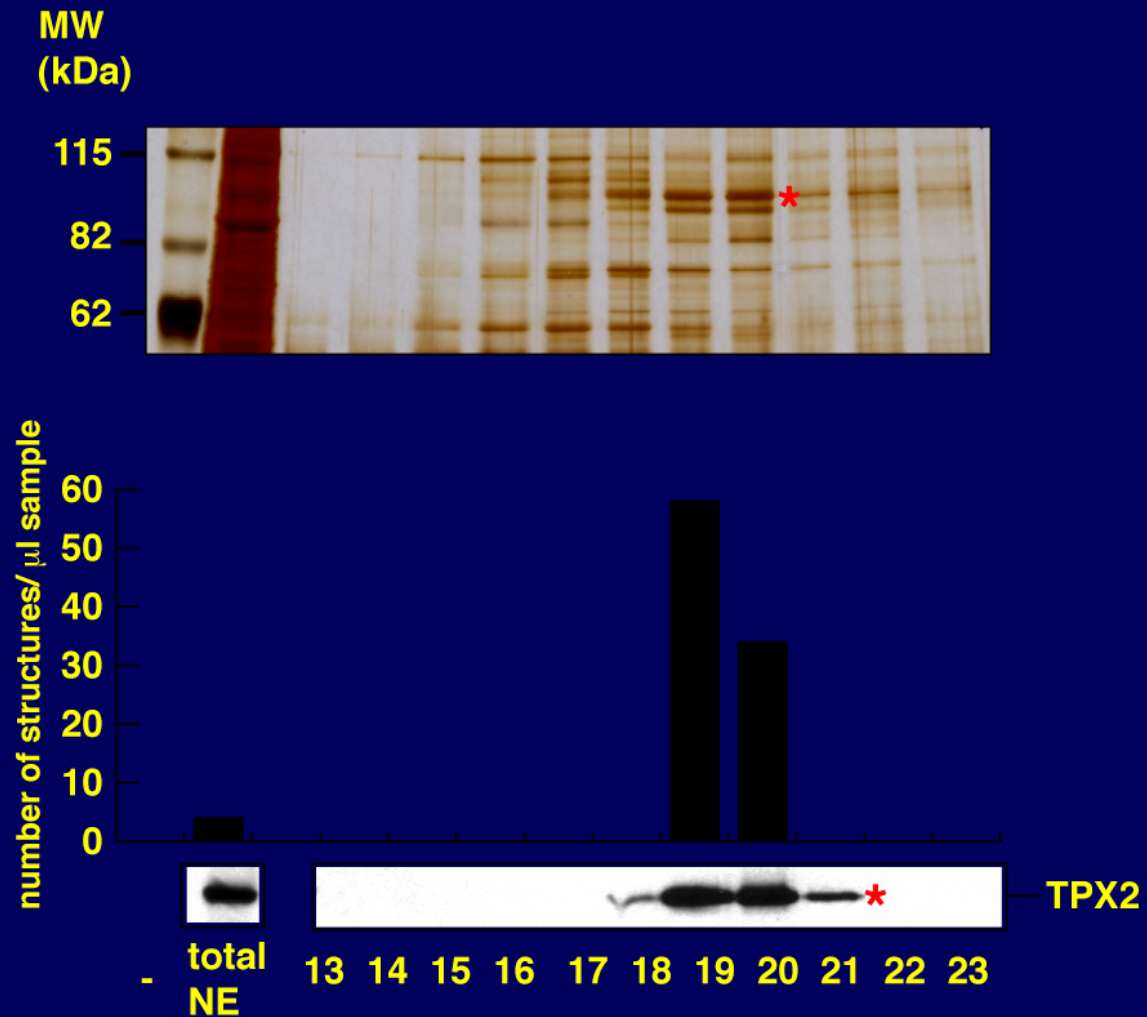
# Depletion of NLS proteins from *Xenopus* M-phase extracts



# Depletion of NLS proteins inhibits microtubule assembly

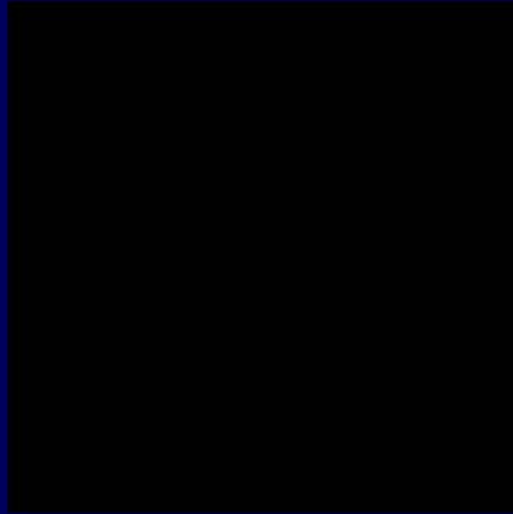


# TPX2 cofractionates with the microtubule assembly activity

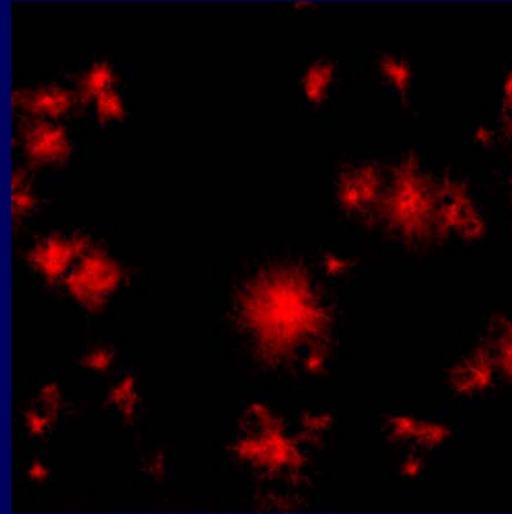


# Recombinant TPX2 induces aster formation in M-phase extracts

buffer



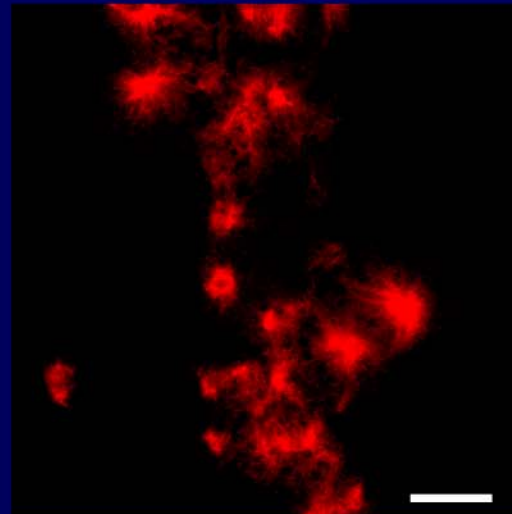
rec. TPX2



rec. TPX2  
+ importin  $\alpha$



rec. TPX2  
+ RanGAP  
+ RanBP1





## TPX2 complements depletion of NLS proteins

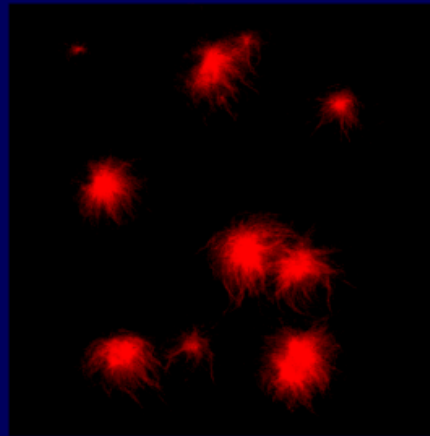
NLS protein  
depleted extracts  
+ RanQ69L.GTP

---

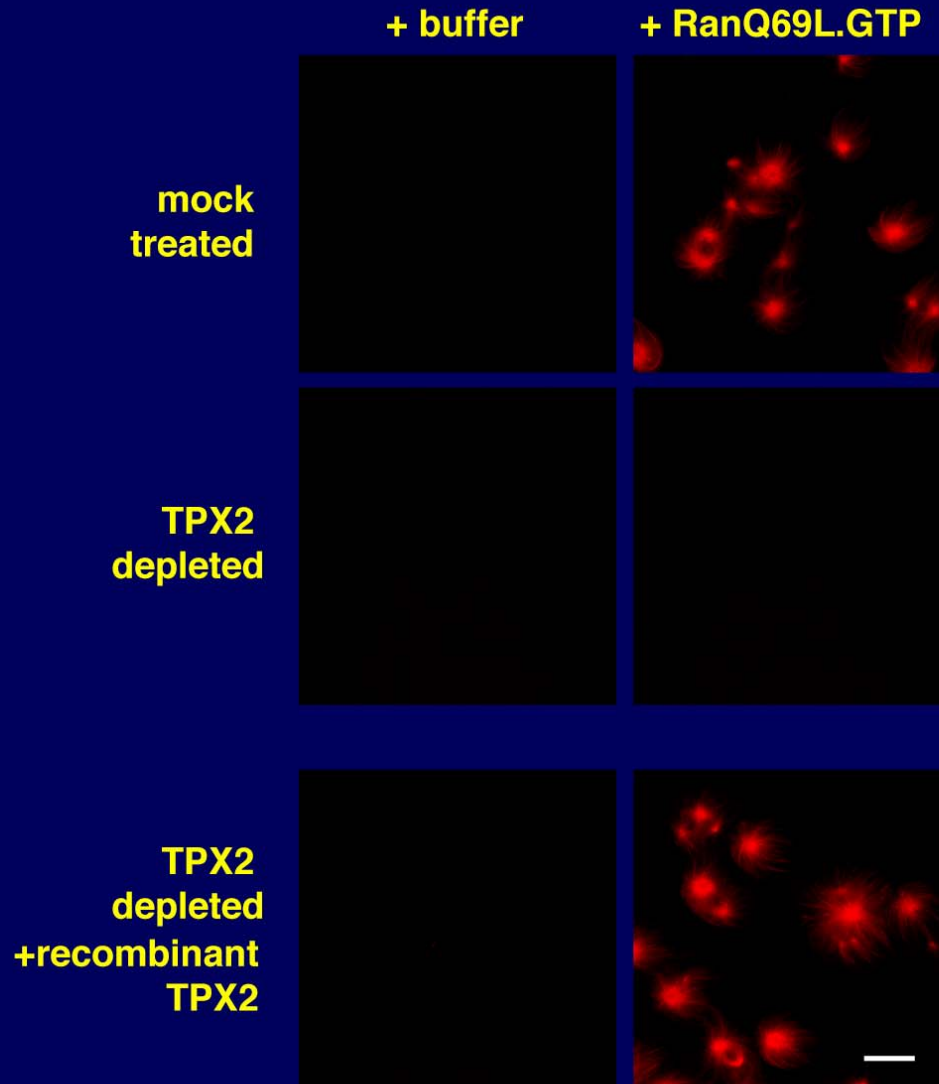
-

+ TPX 2

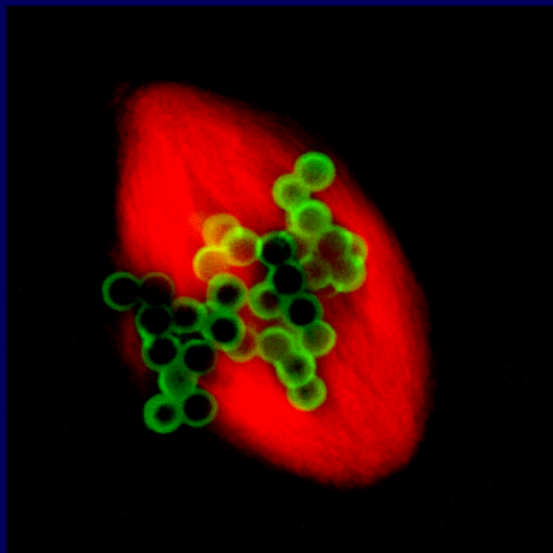
non-treated  
extracts  
+ TPX 2



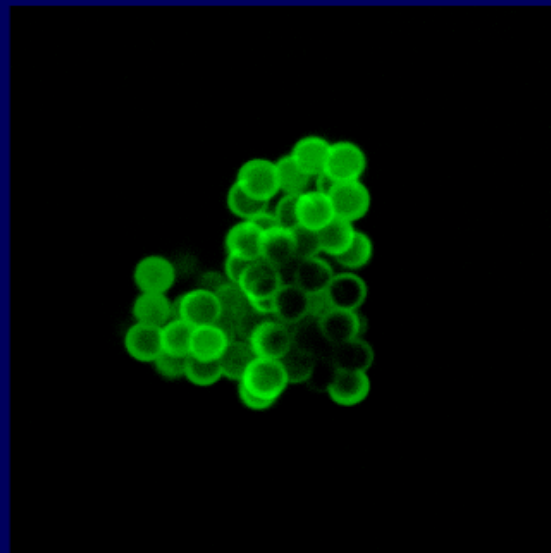
# TPX2 depletion prevents Ran.GTP-induced spindle assembly



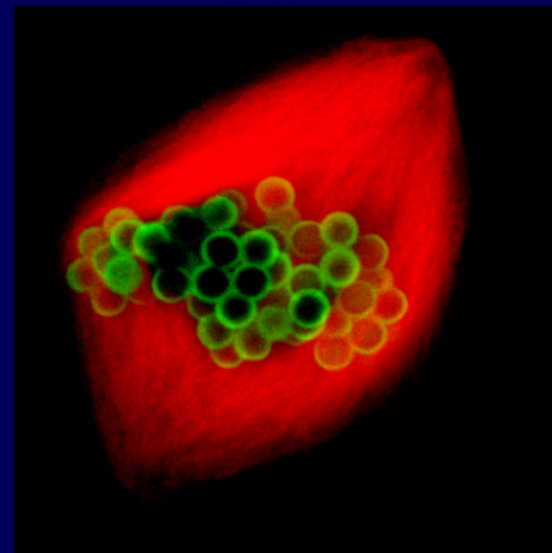
# TPX2 depletion prevents chromatin-induced spindle assembly



mock  
treated

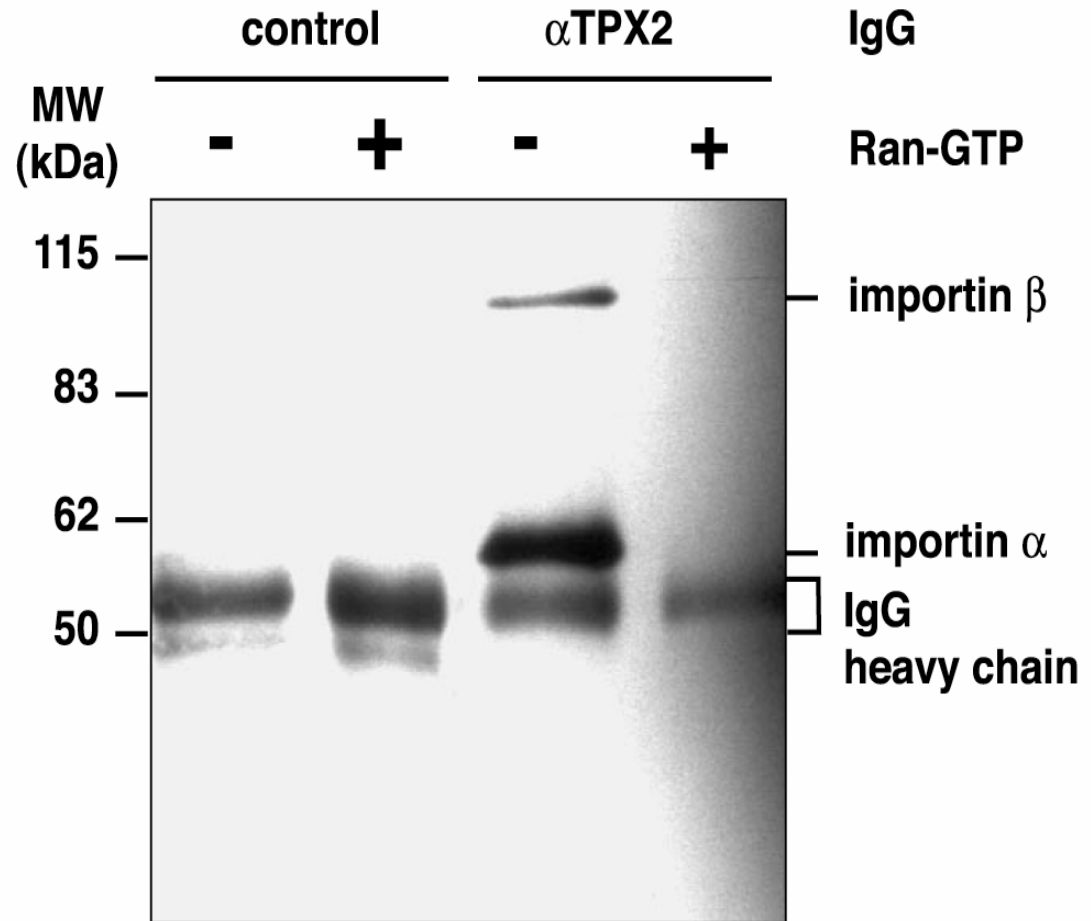
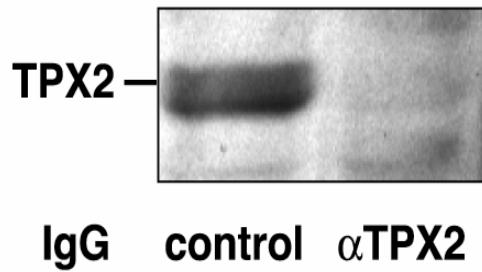


TPX2  
depleted

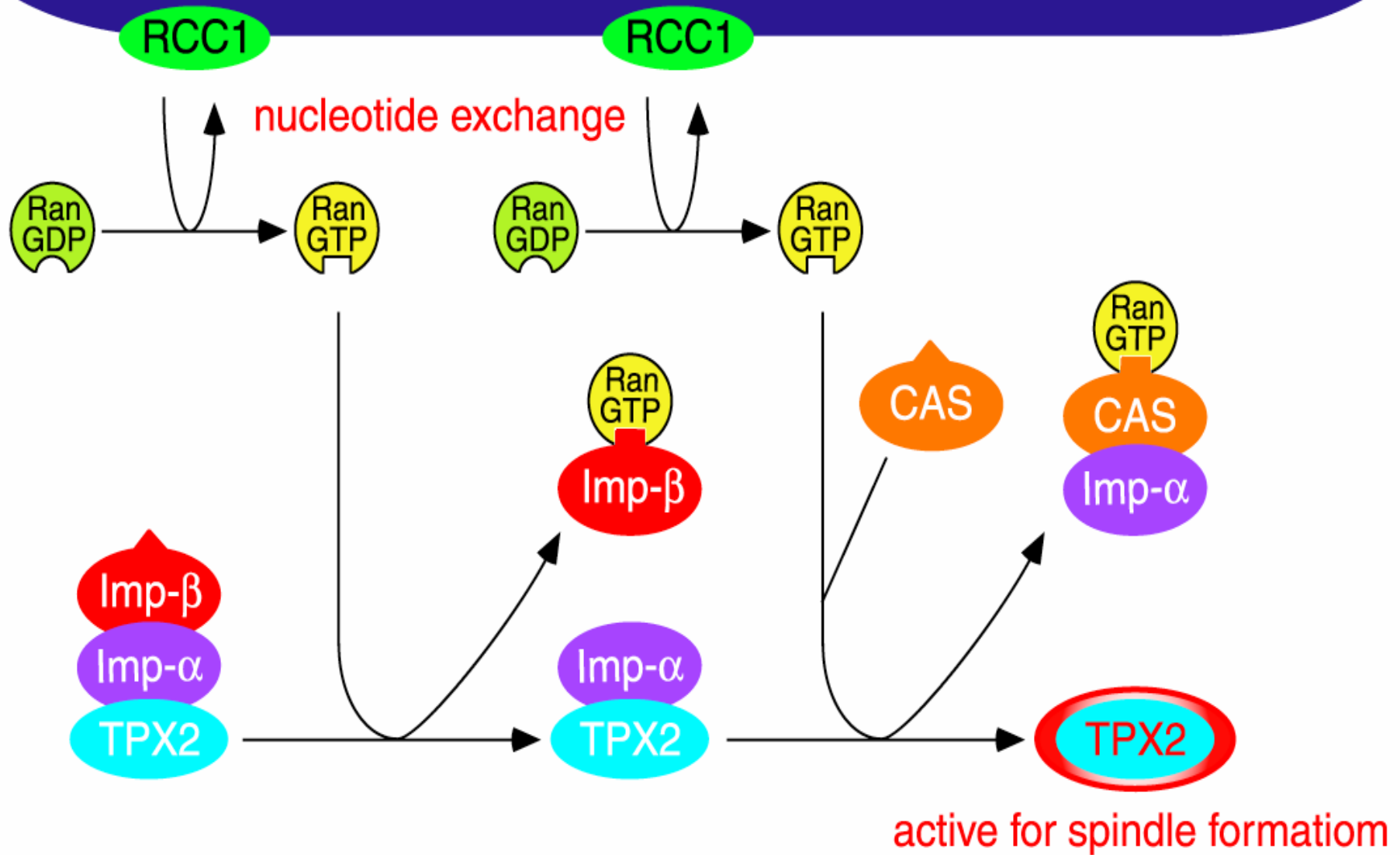


TPX2  
depleted  
+ recombinant  
TPX2

# TPX2 associates with importin $\alpha$ and $\beta$ in *Xenopus* M-phase extracts



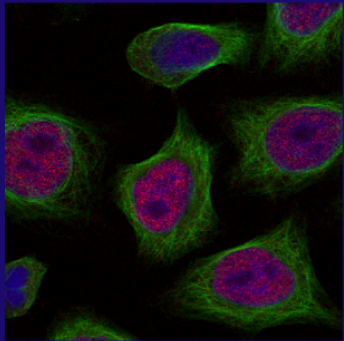
# chromatin



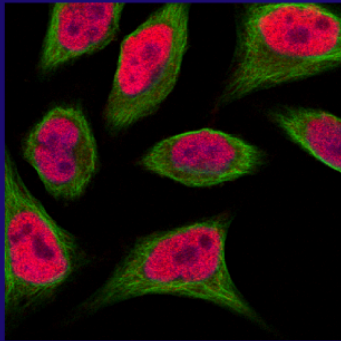
## Expression of TPX2 during the cell cycle in human cells

time after release from  
S-phase (hours)

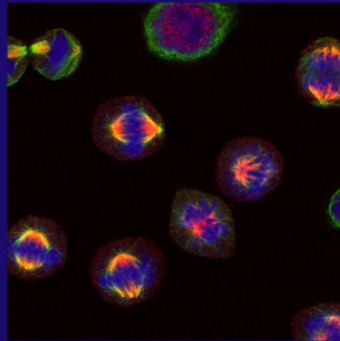
0



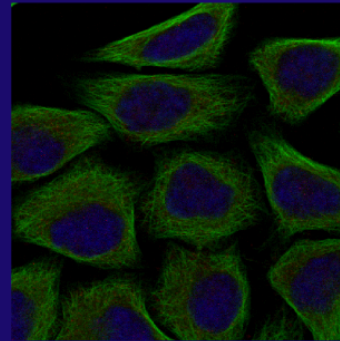
8



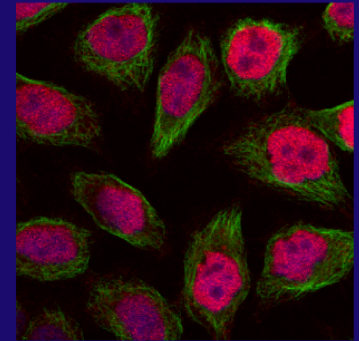
10



16



24



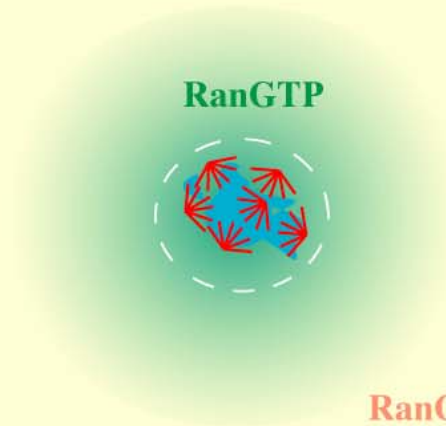
blue: DNA  
green: tubulin  
red: TPX2



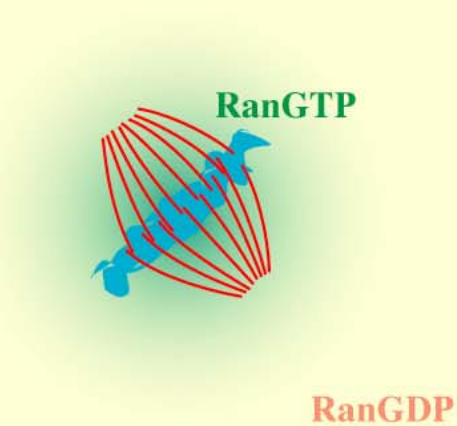
# Meiotic and mitotic spindle assembly



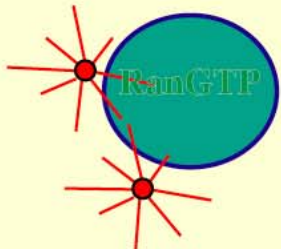
RanGDP



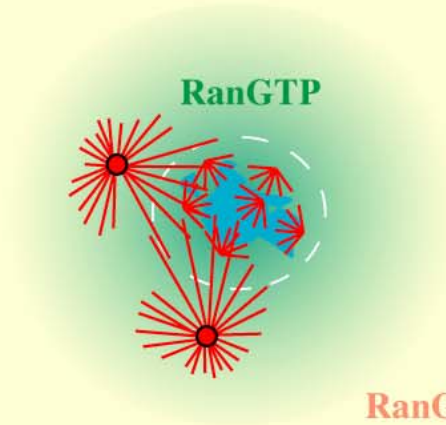
RanGDP



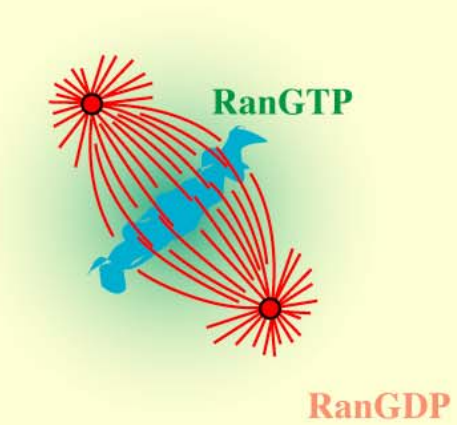
RanGDP



RanGDP



RanGDP



RanGDP

# Inhibition of RCC1 function leads to defects in mitotic spindle formation

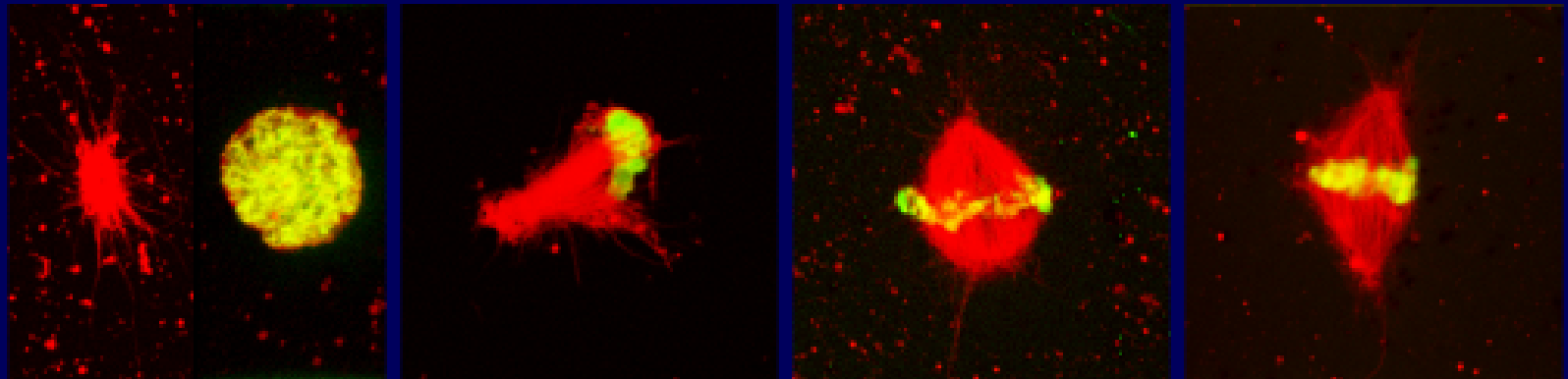
15 min

30 min

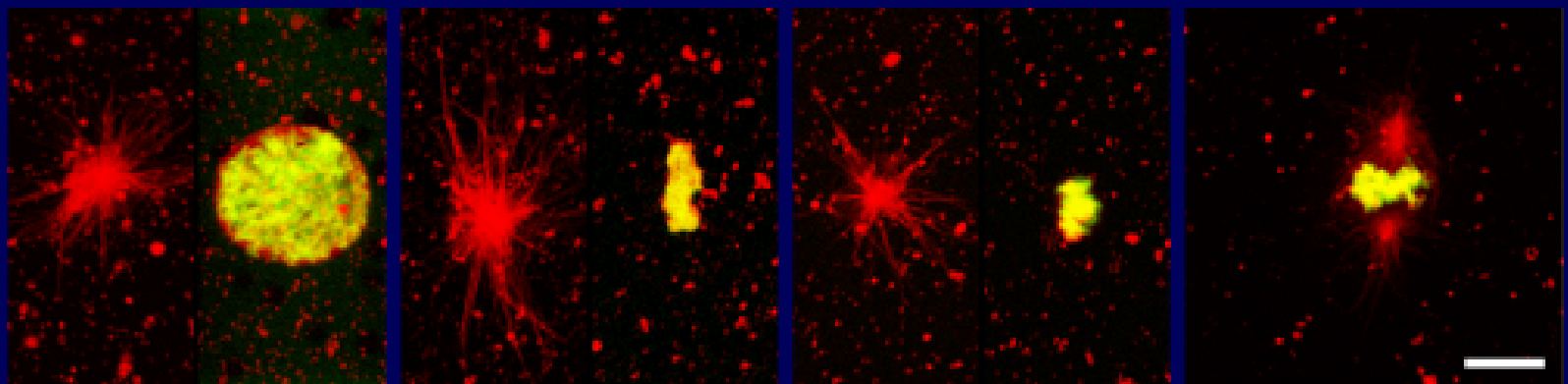
45 min

60 min

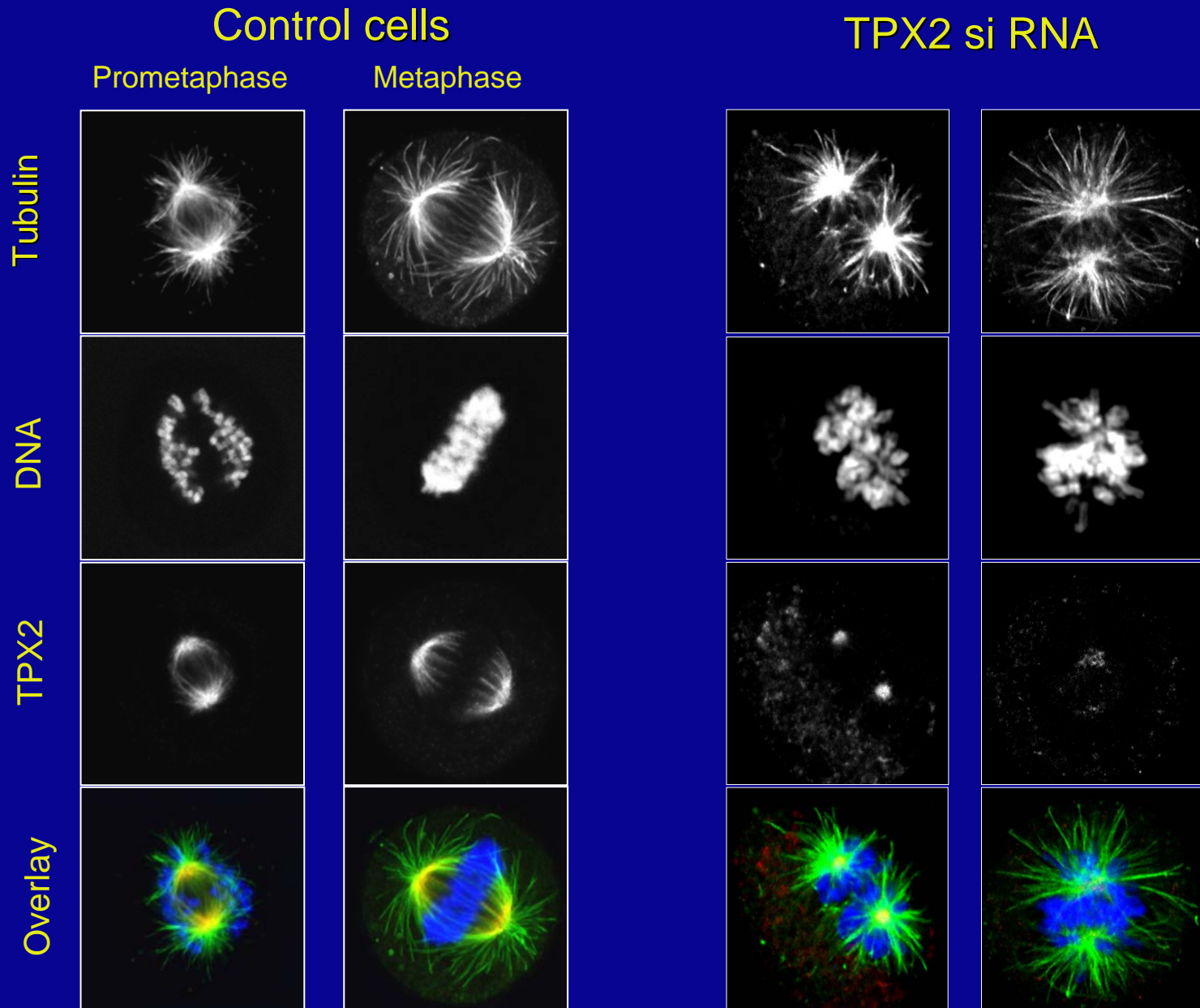
Control



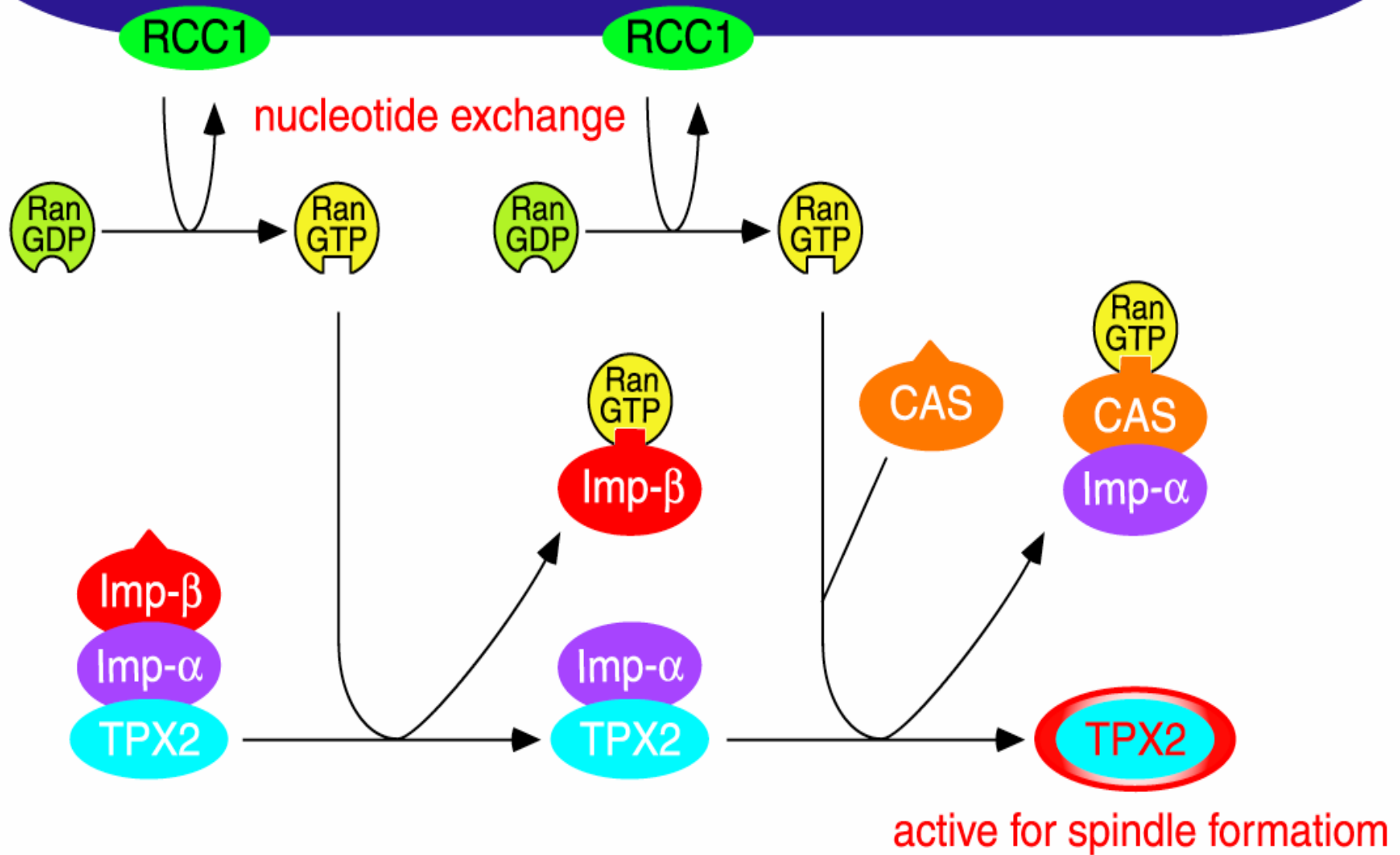
+Ran  
T24N



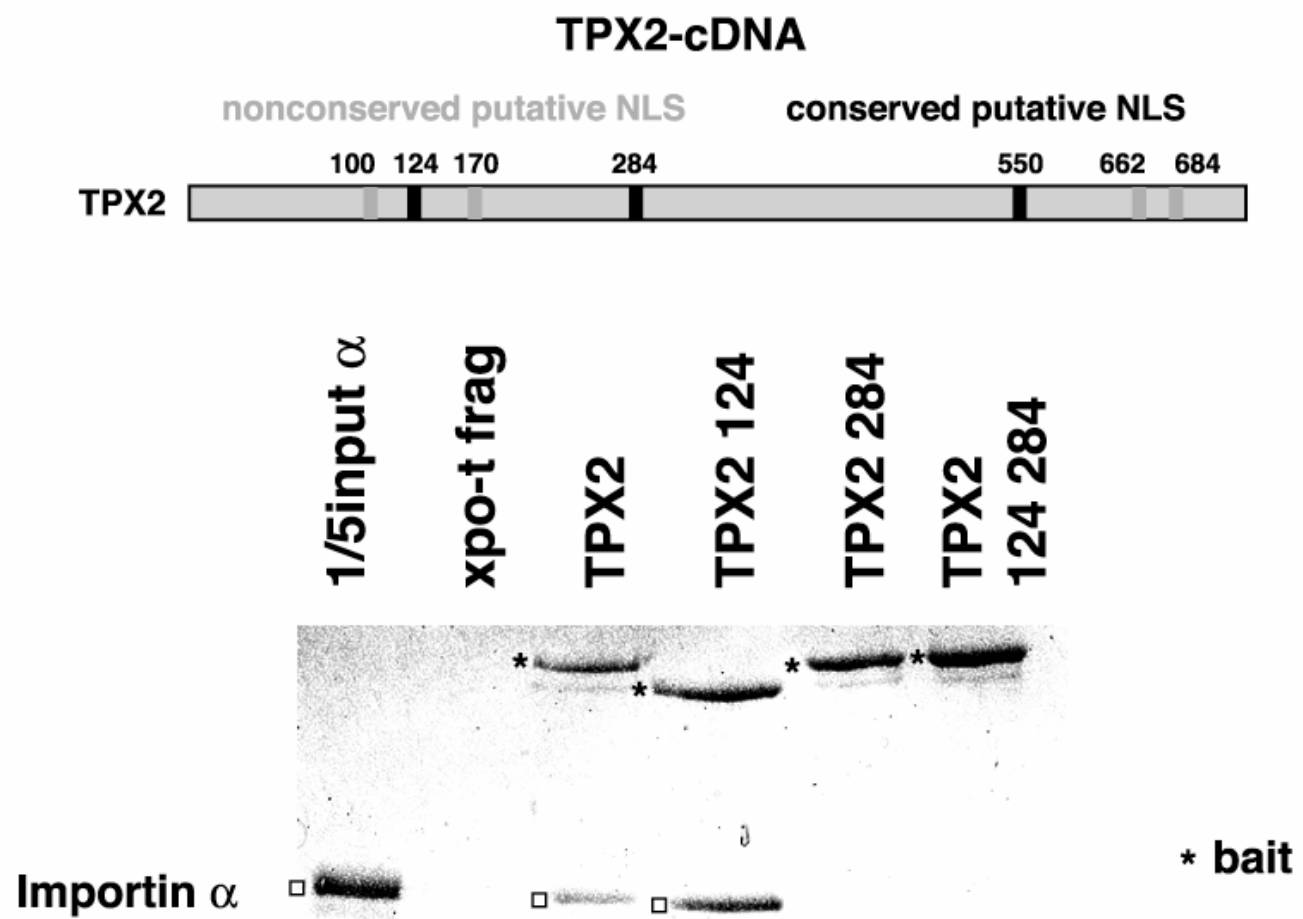
# Suppression of hTPX2 expression in HeLa cells by siRNA



# chromatin



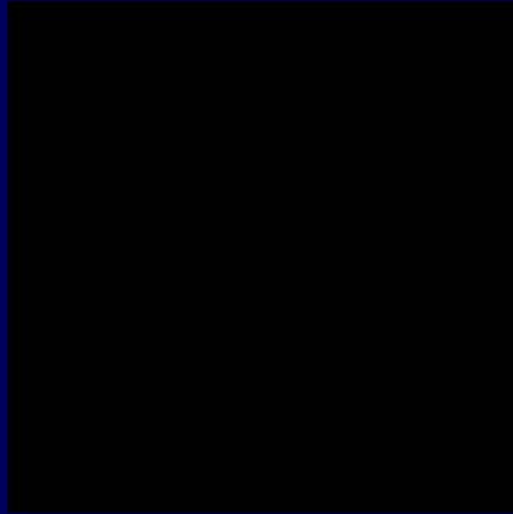
# Mapping of the Importin $\alpha$ binding site of TPX2



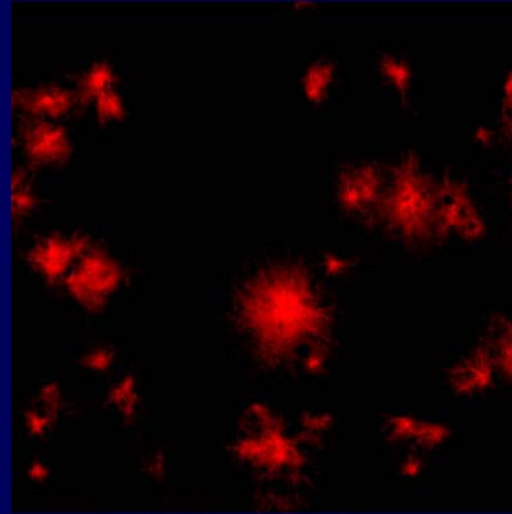


# Recombinant TPX2 induces aster formation in M-phase extracts

buffer



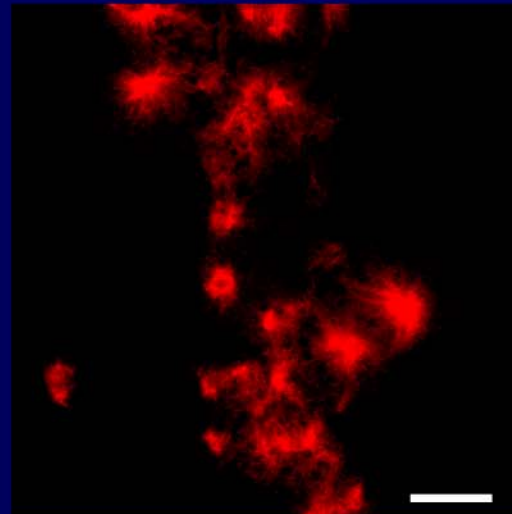
rec. TPX2



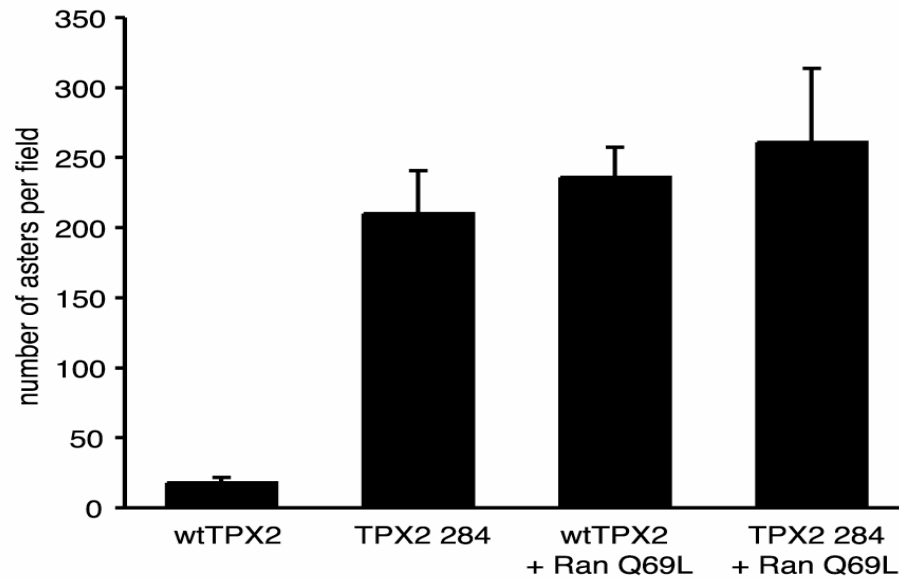
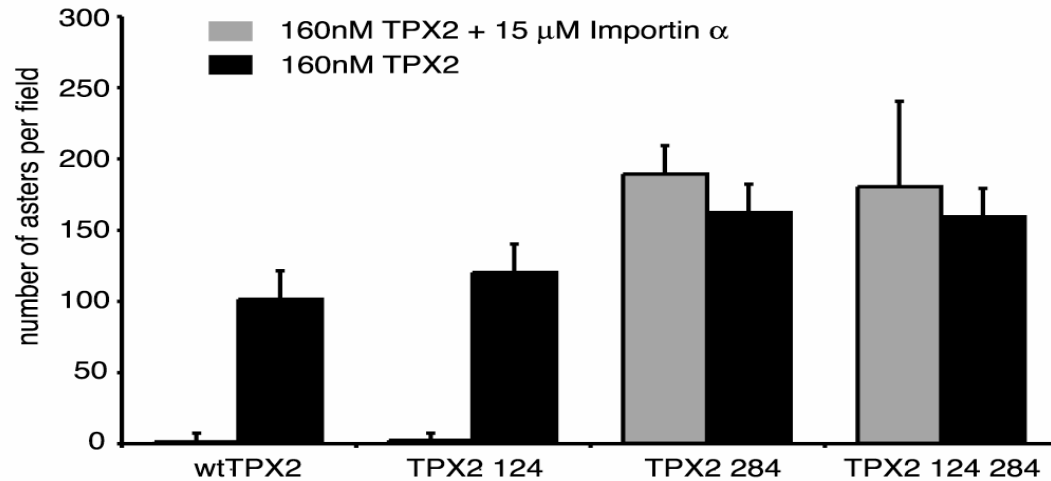
rec. TPX2  
+ importin  $\alpha$



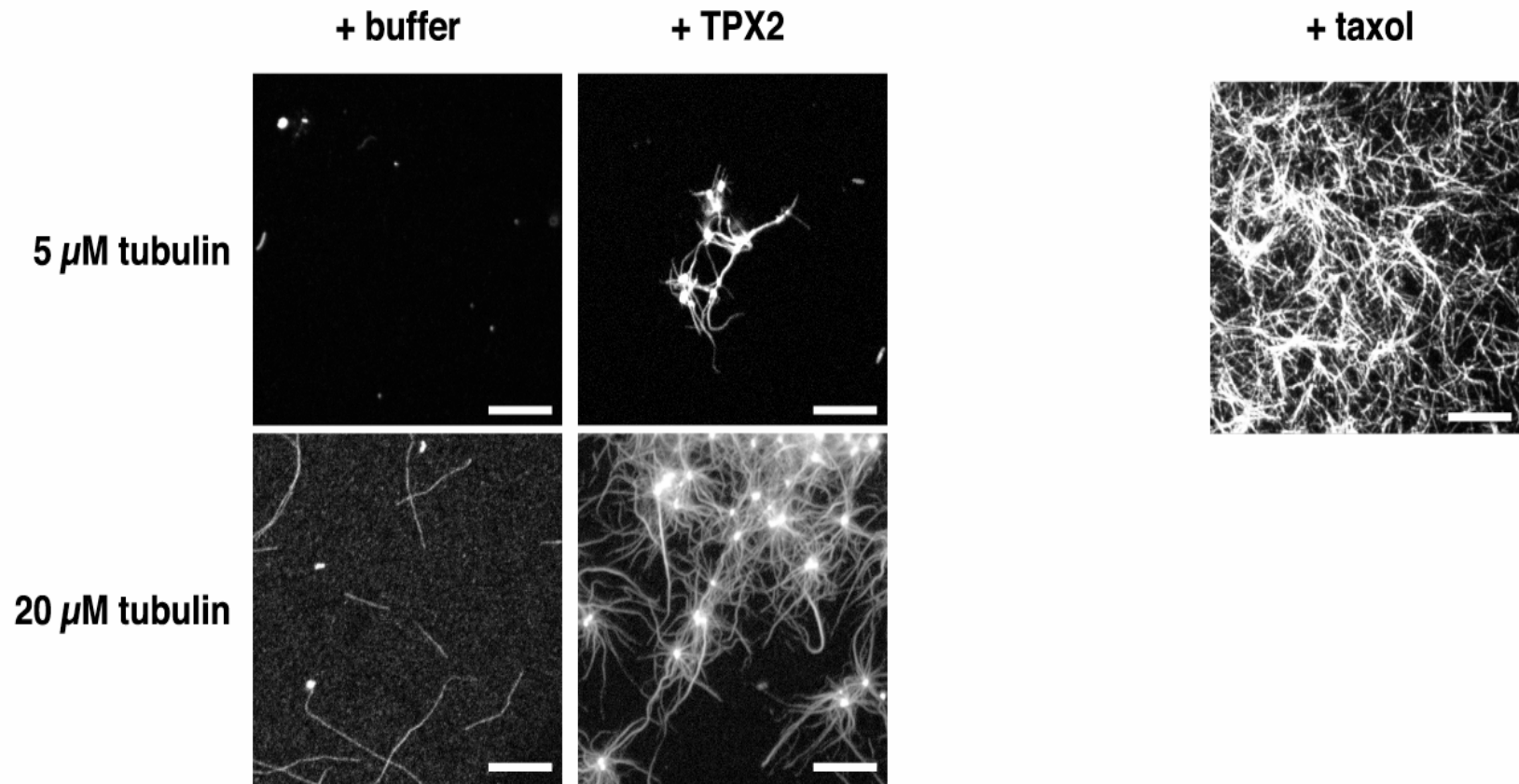
rec. TPX2  
+ RanGAP  
+ RanBP1

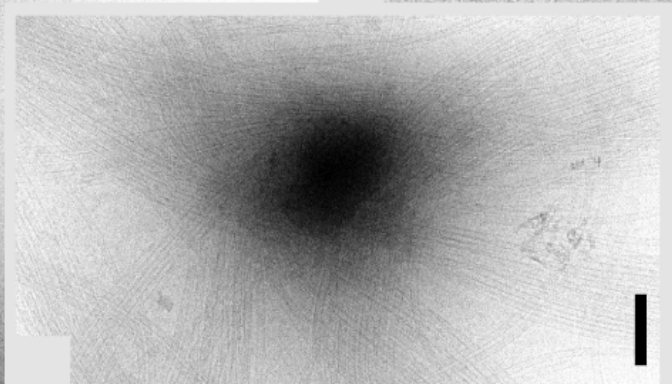
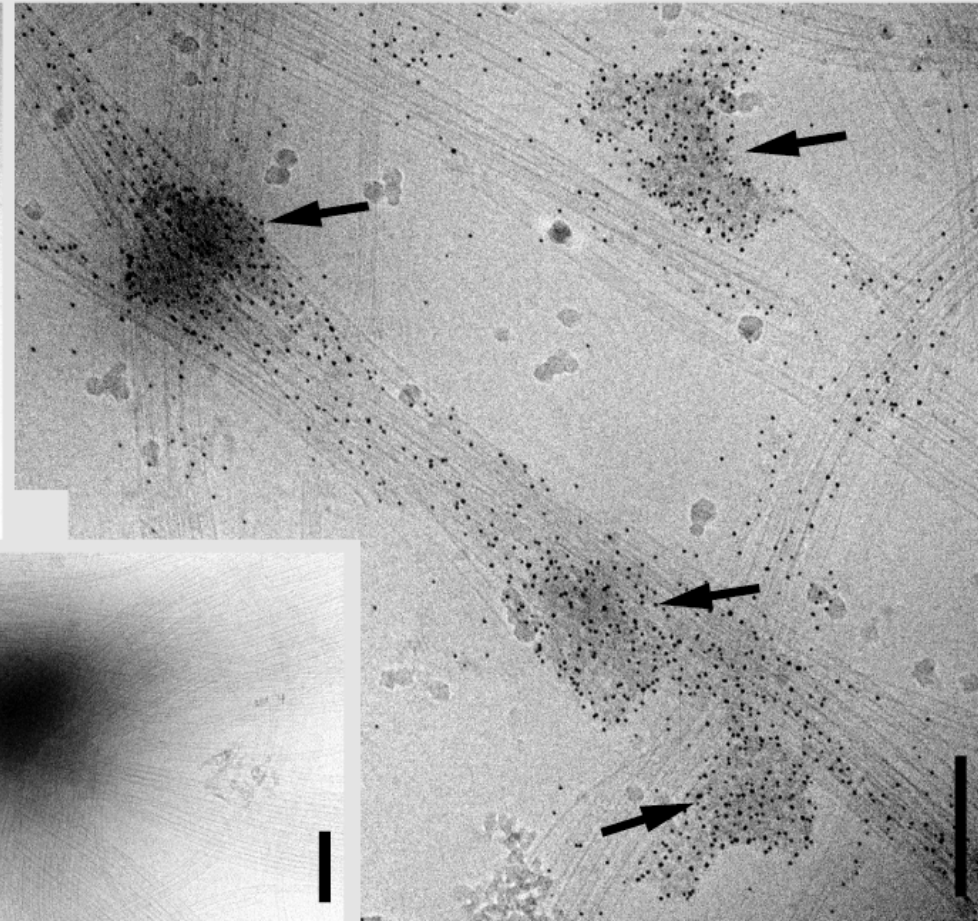
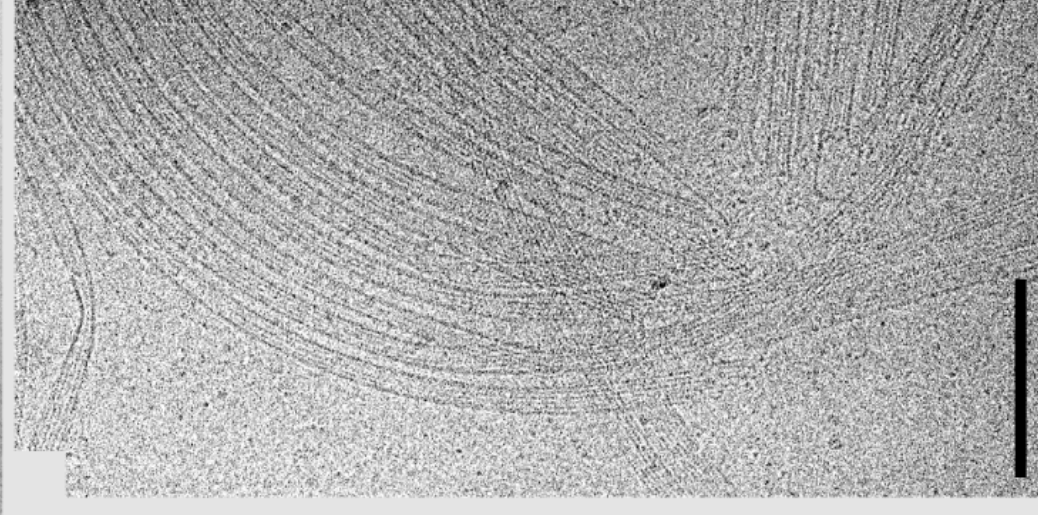
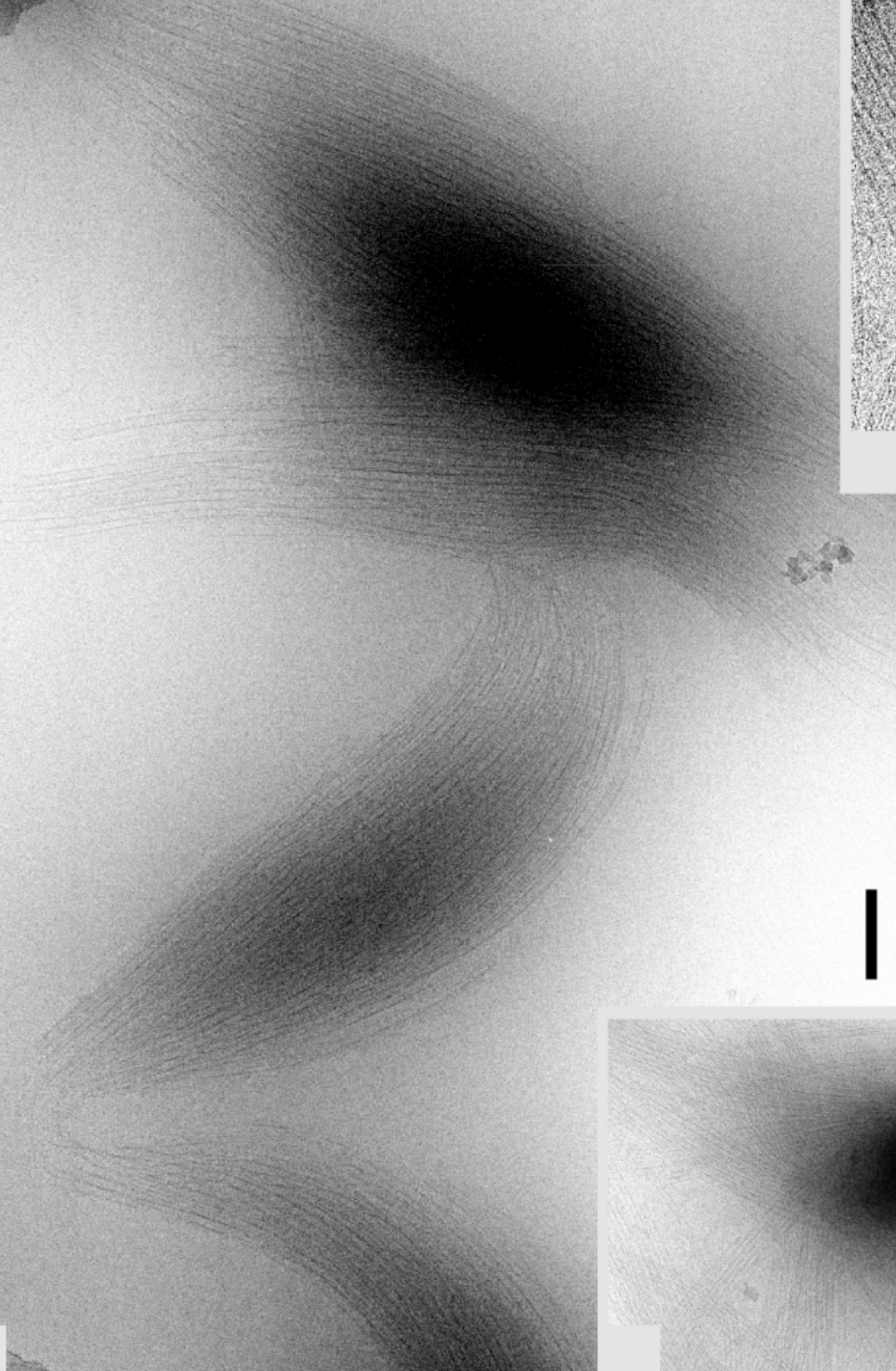


# TPX2 $\Delta$ NLS is not inhibited by endogenous Importin $\alpha$ in *Xenopus* M-phase extracts

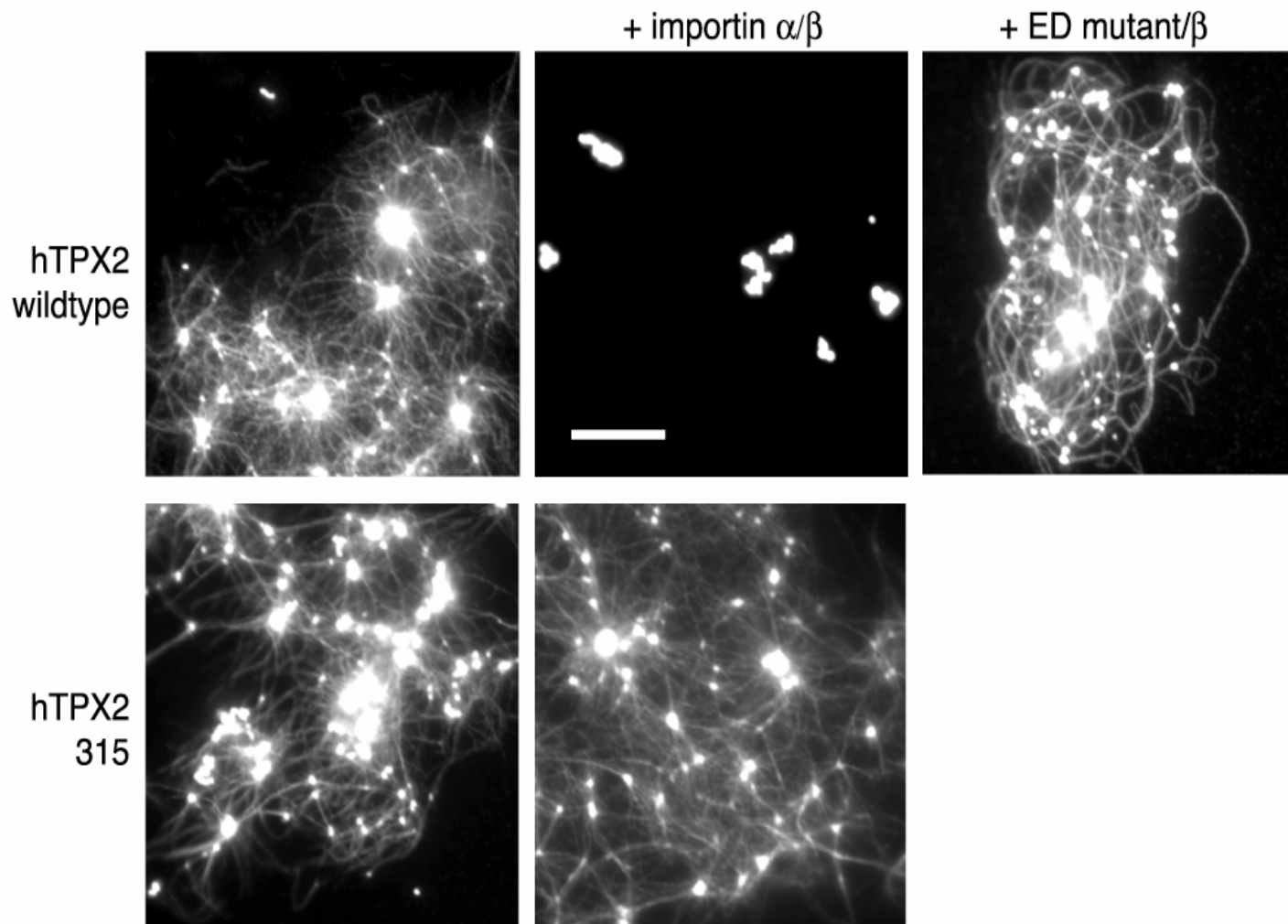


# TPX2 nucleates and organizes microtubules in a solution of purified tubulin



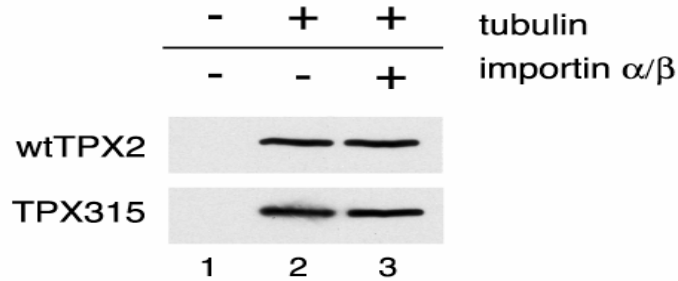


# TPX2 nucleating activity is inhibited by importin $\alpha$ in a purified system





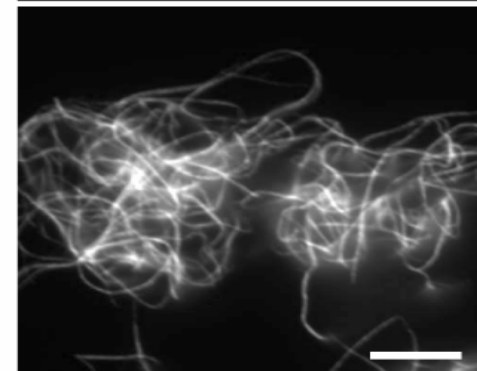
# TPX2 interaction with microtubules is not inhibited by importin $\alpha$



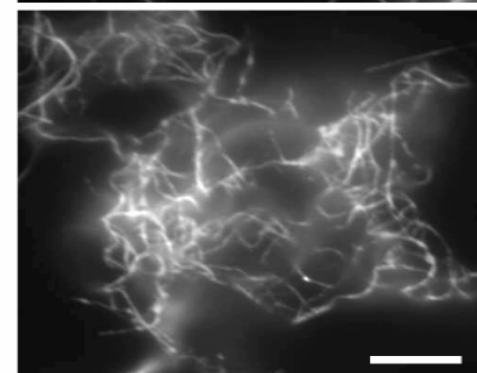
+ buffer



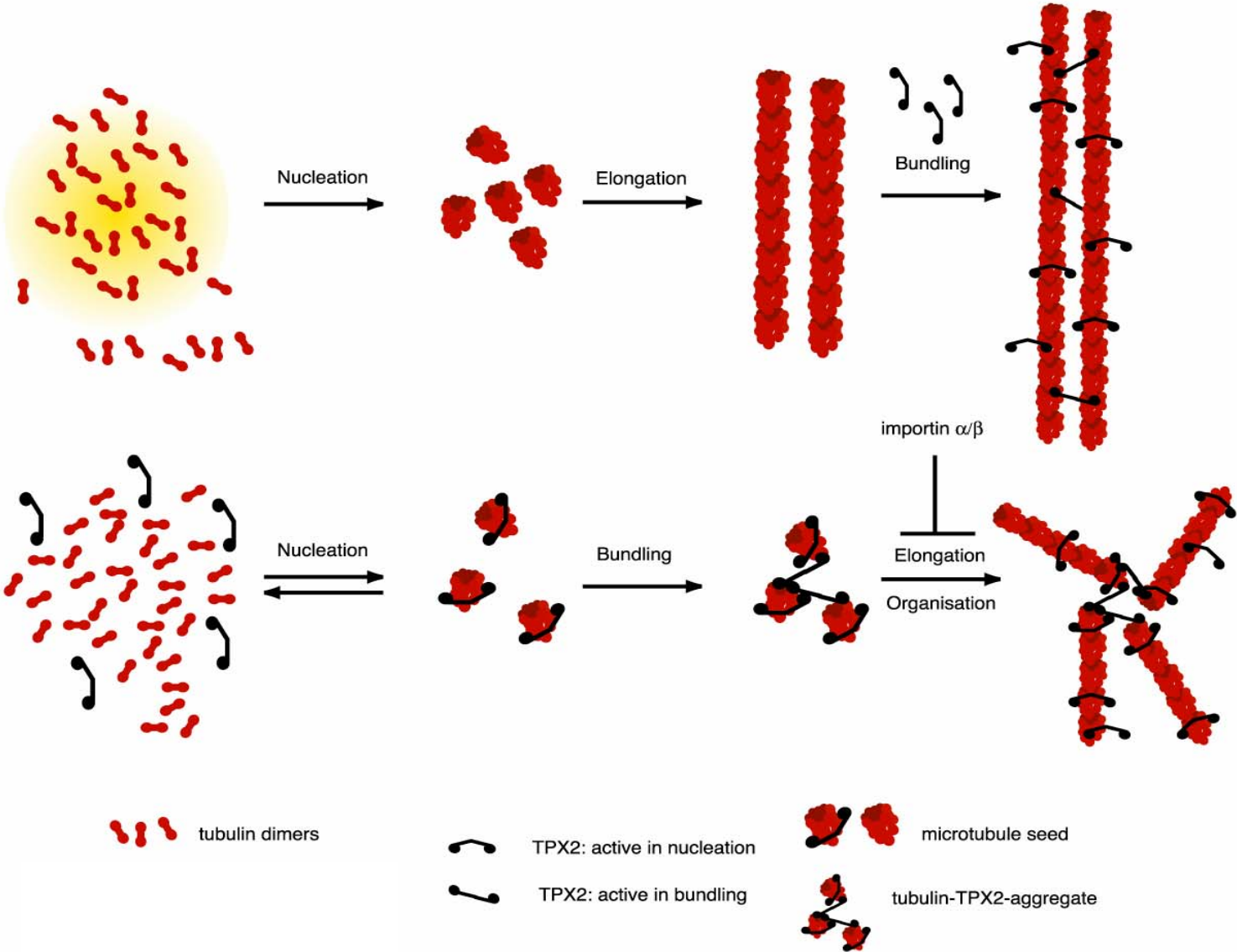
+ TPX2



+ TPX2  
+ importin  $\alpha/\beta$



# Model: TPX2 nucleates and organizes microtubules



WT

1:18

2:36

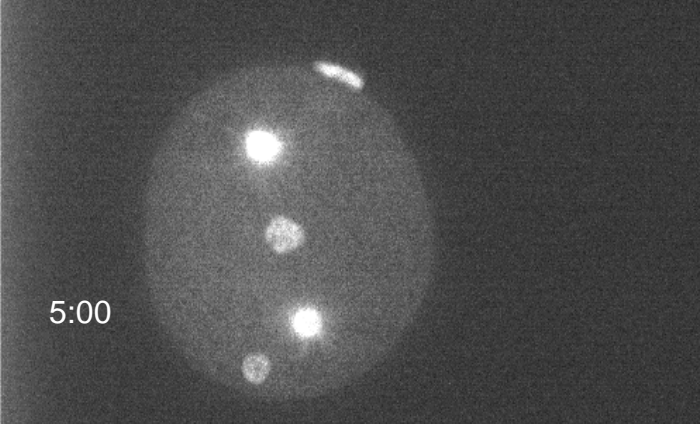
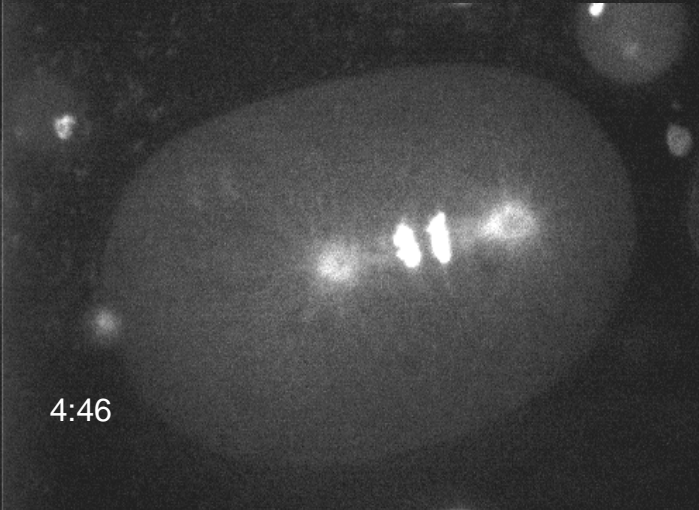
4:46

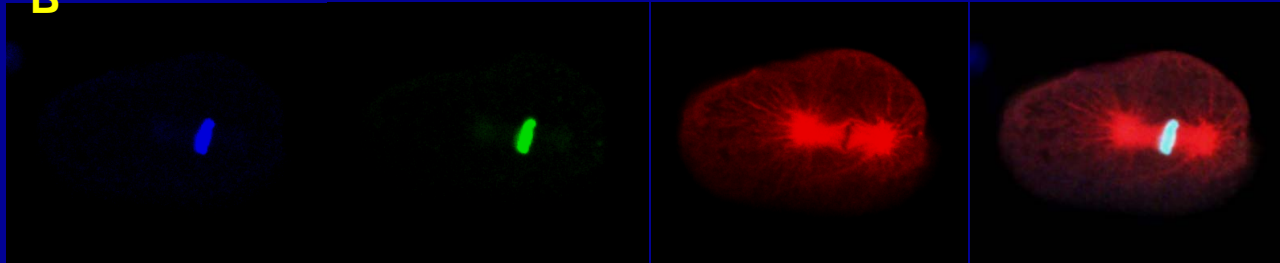
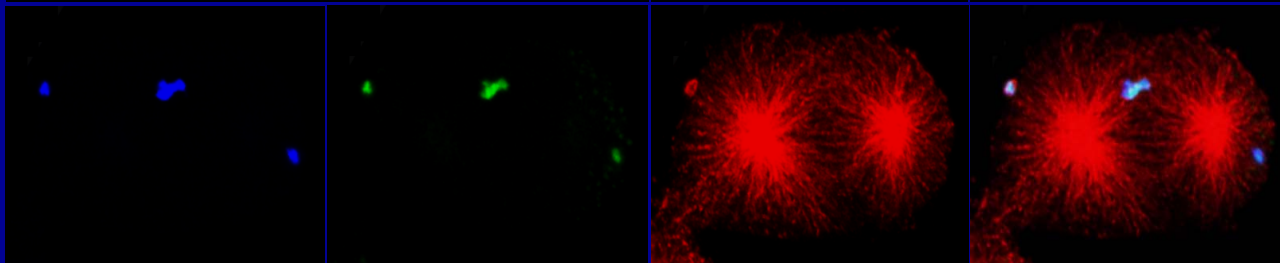
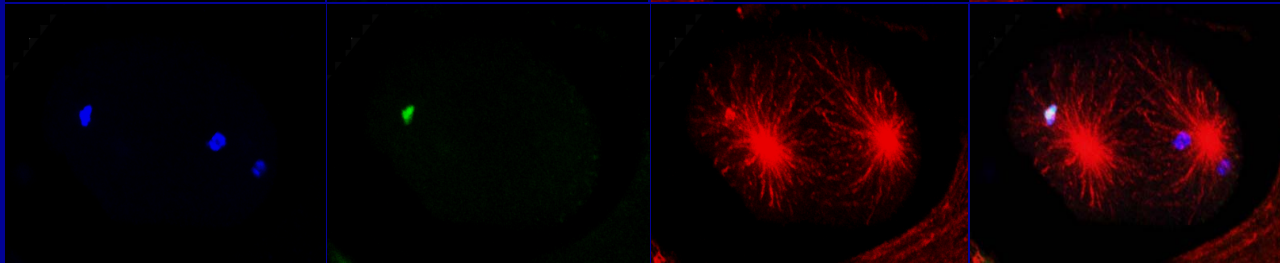
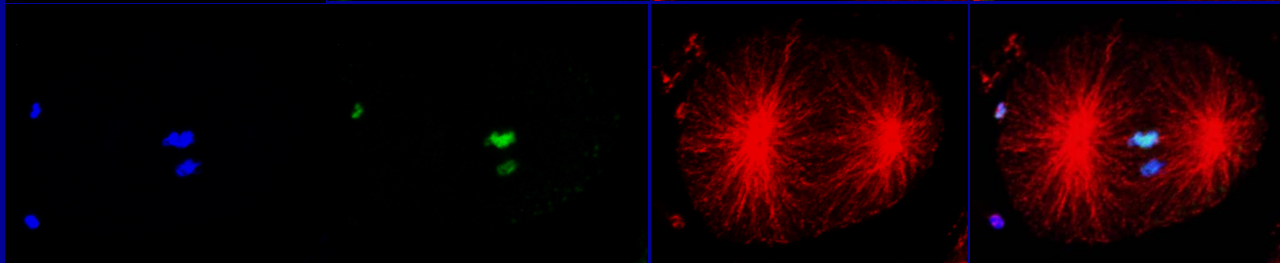
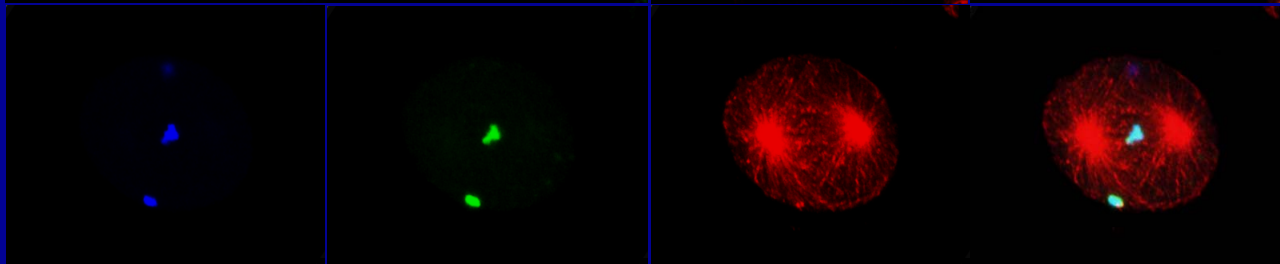
5:00

7:23

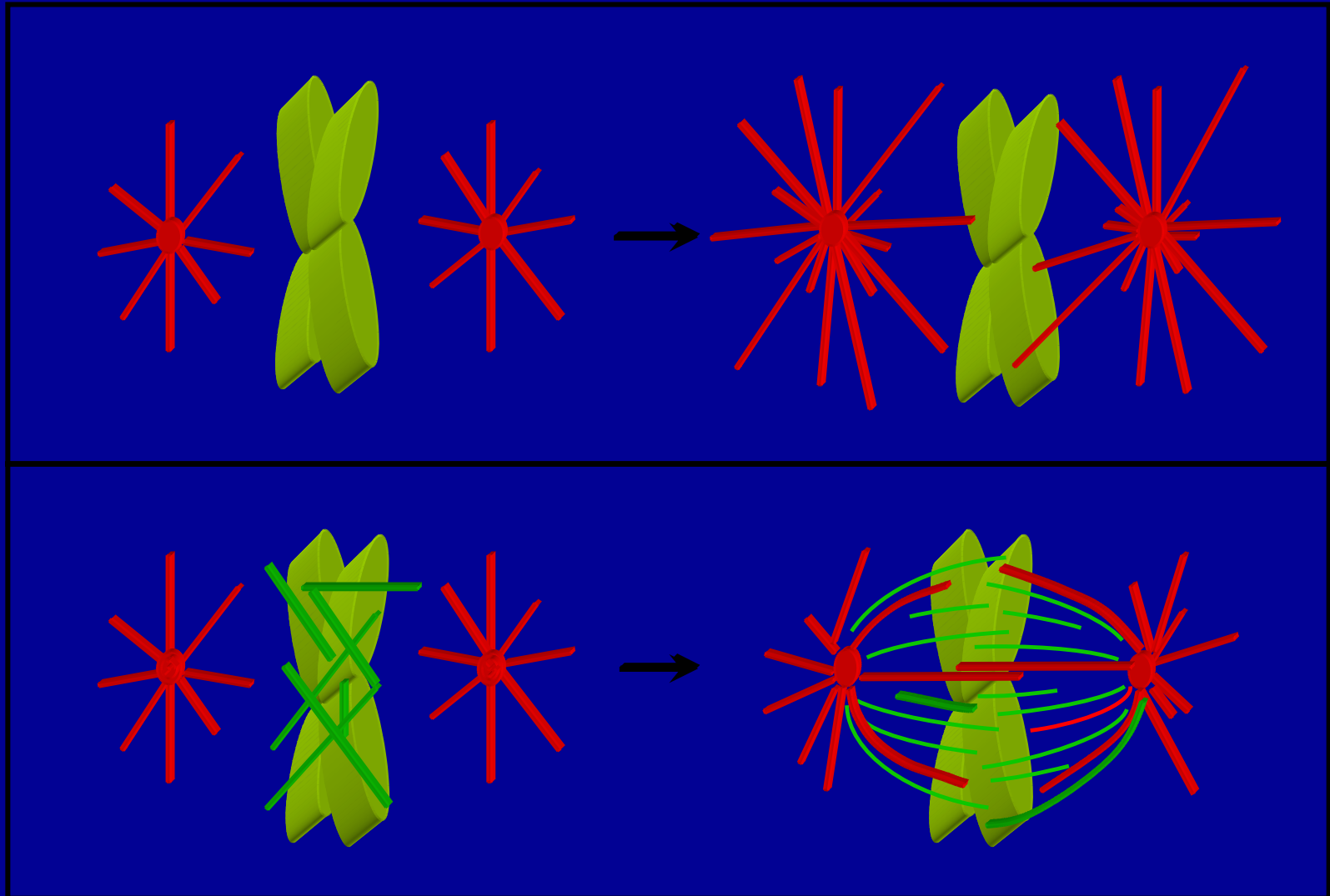
8:28

Ran  
dsRNAi



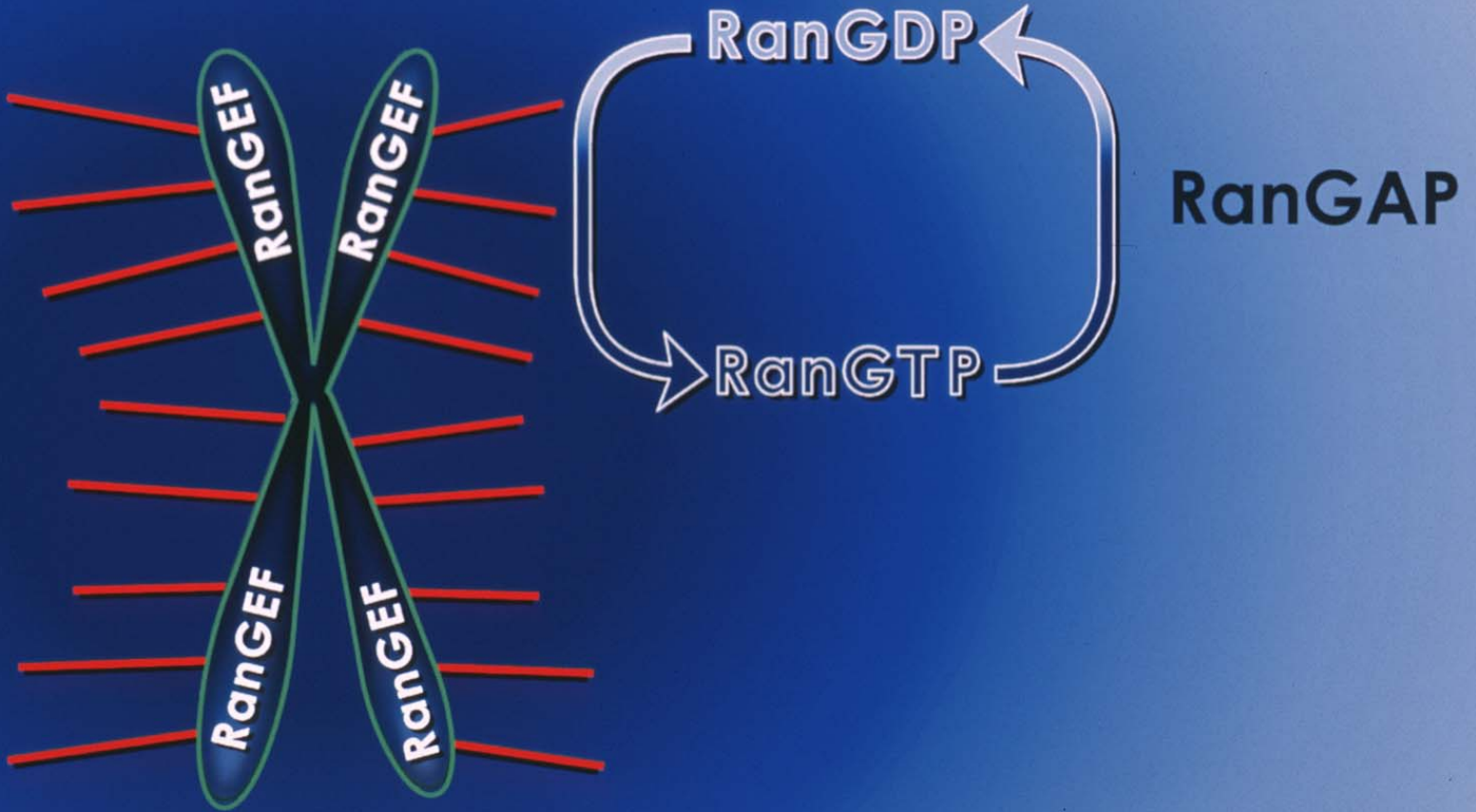
**GFP::hisH2****Phospho-hisH3****Tubulin****Overlay****B****Wild-Type****RanGAP****RanBP2****Importin  
alpha-2****Importin  
beta**

# The Induction of Microtubule Polymerisation by Chromatin is essential for Spindle Formation in Somatic Cells



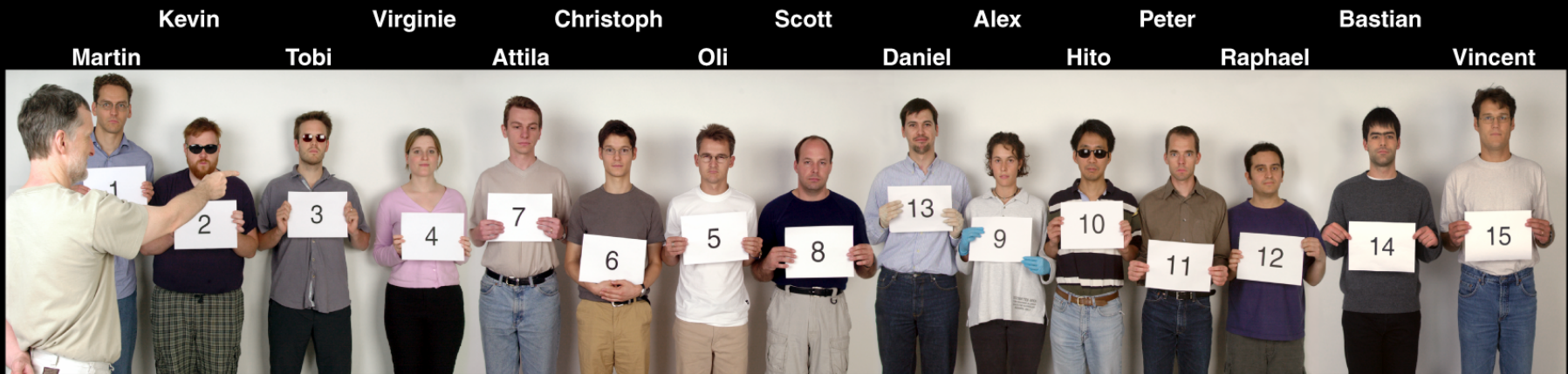


# Chromosome-induced local RanGTP





# Mattaj lab 2001



Iain



**EMBL**  
Gene Expression Programme