

Organization of replication forks by the SeqA protein

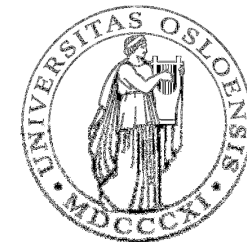
Kirsten Skarstad

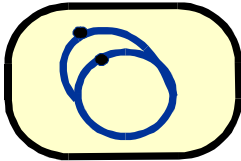
**Department of Cell Biology
Institute for Cancer Research**

The Norwegian Radium Hospital

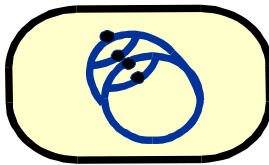


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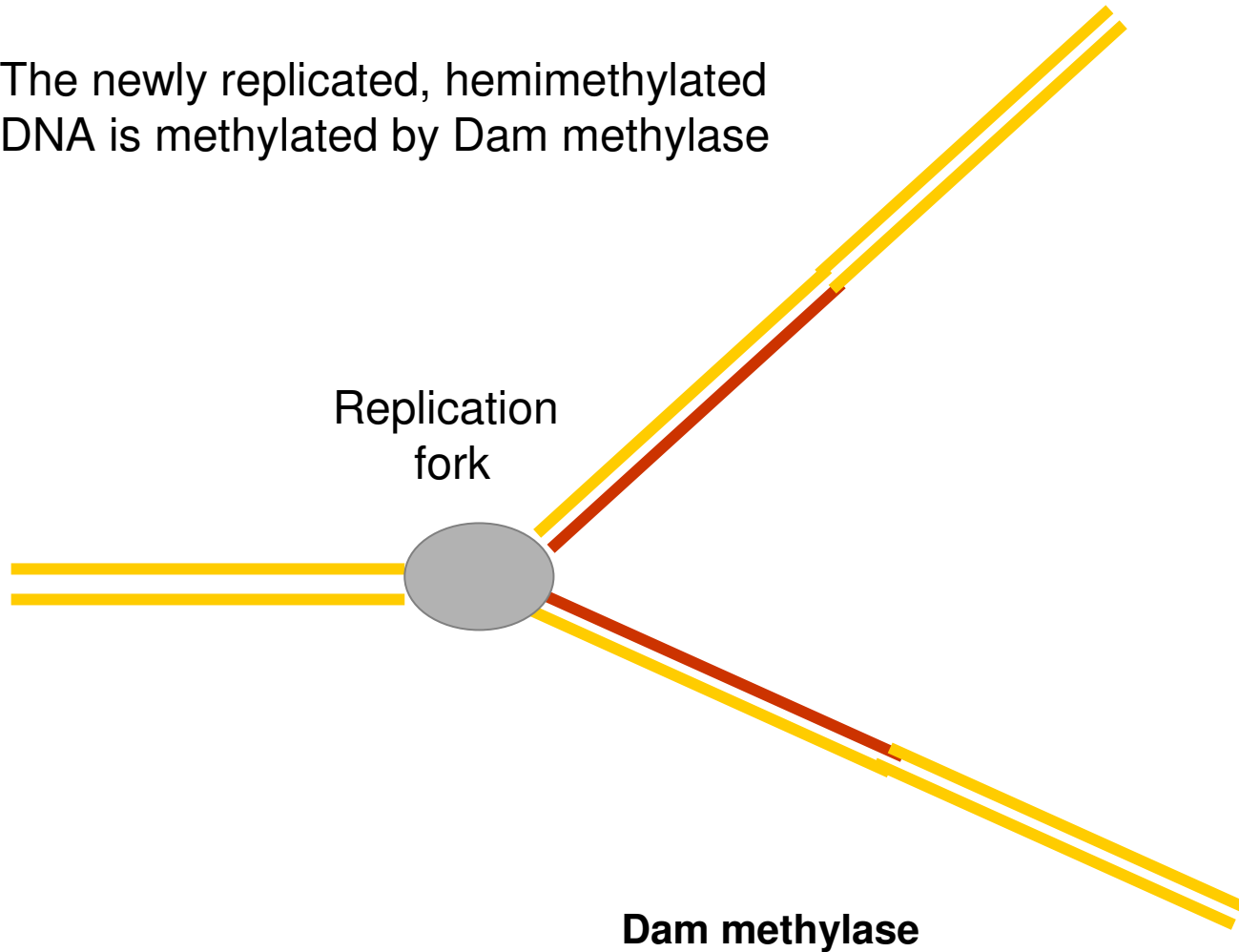




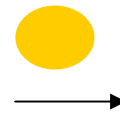
**SeqA protein specifically prevents
Initiation of replication from new origins**



The newly replicated, hemimethylated DNA is methylated by Dam methylase

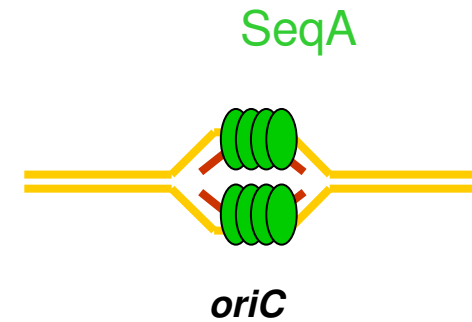
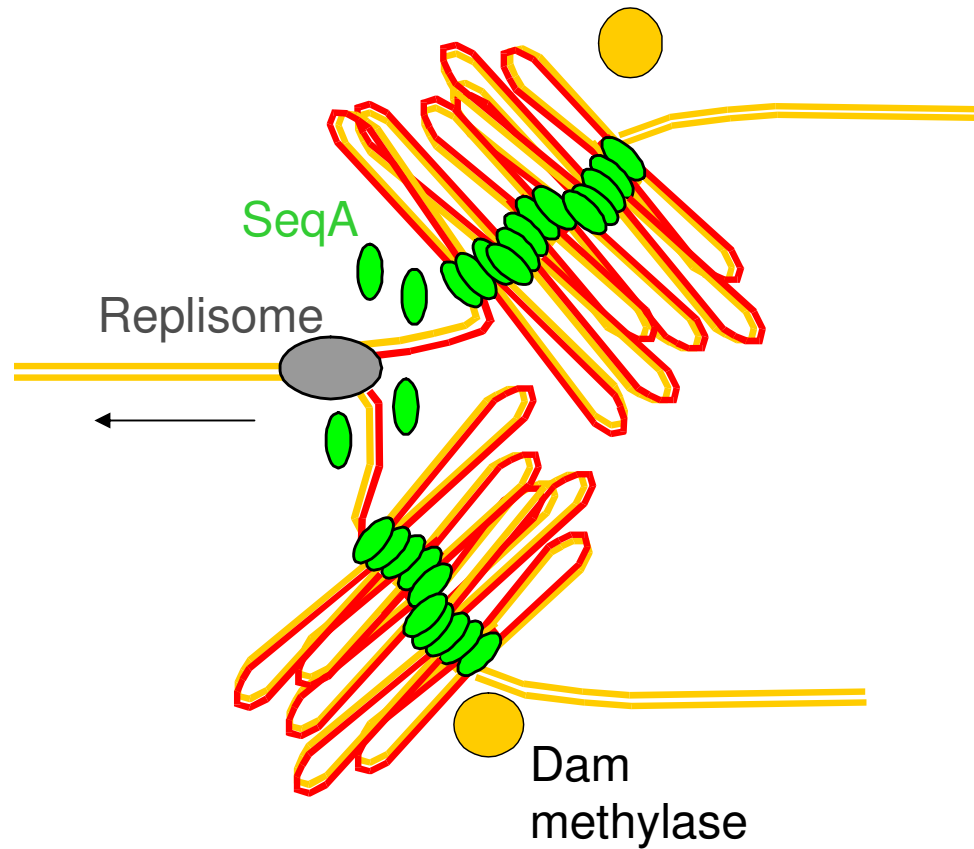


NNNNNGATCNNNNNGATCNNN
NNNNNCTAGNNNNNCTAGNNN

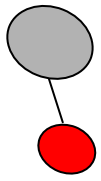


NNNNNGATCNNNNNGATCNNN
NNNNNCTAGNNNNNCTAGNNN

The SeqA protein binds to hemimethylated DNA at the replication forks



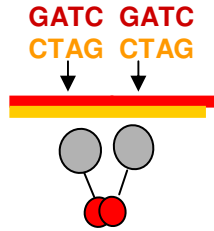
The binding of SeqA to new *oriC*s prevents re-replication



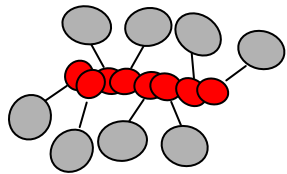
← 15 kDa CTD : binds GATC sites

← 6 kDa NTD : multimerization domain

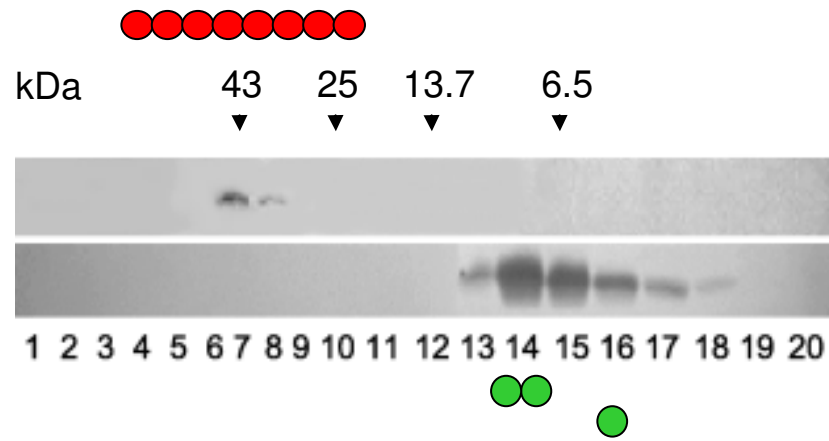
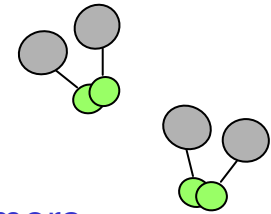
} SeqA



SeqA binds as a dimer to pairs of GATC sites



SeqA4 (A25T) has an N-terminal mutation and cannot form multimers, only dimers



SeqA-N

SeqA4-N

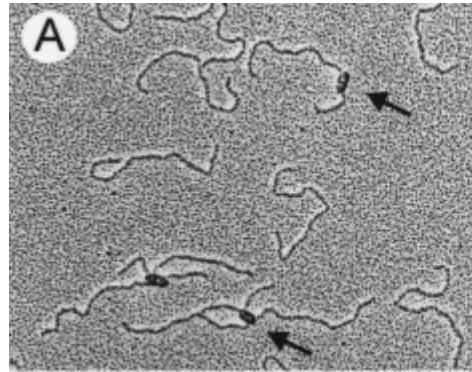
Fraction number from gelfiltration

1313bp *oriC* fragment

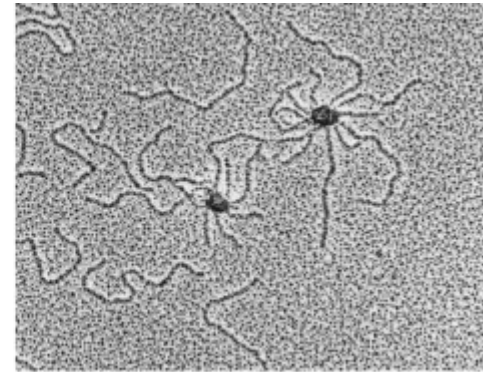


+ SeqA protein

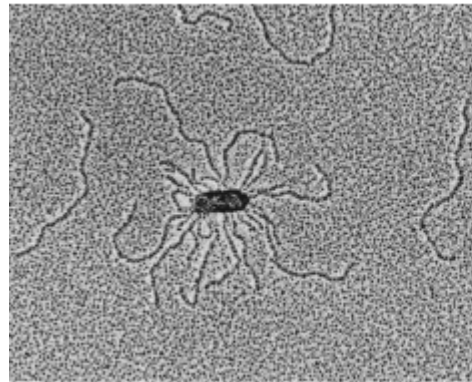
1:4



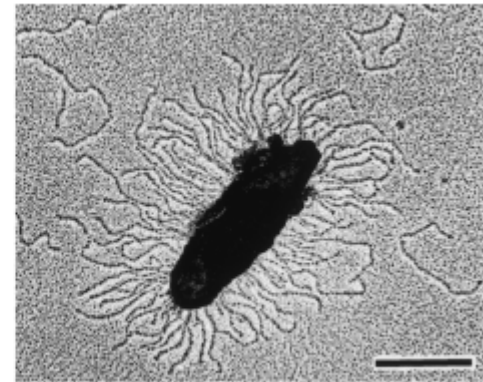
1:4



1:4

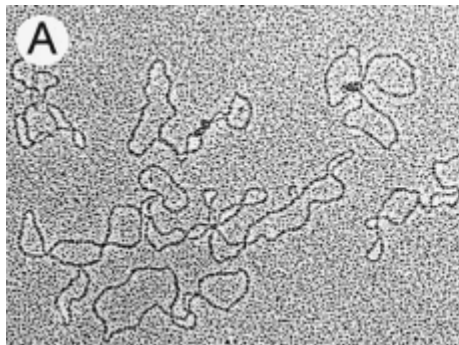


1:20



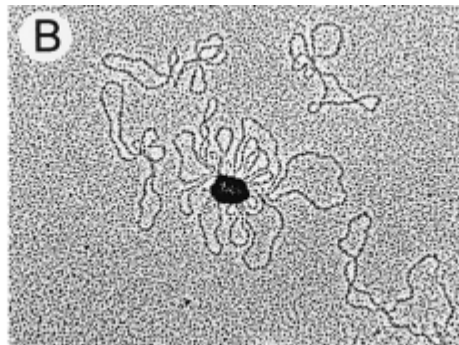
oriC plasmid

+ SeqA protein

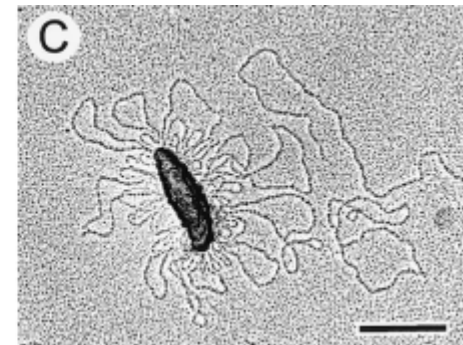


Molar ratios:

1:25



1:100



1:100

Fixed cells immunostained using purified SeqA antiserum

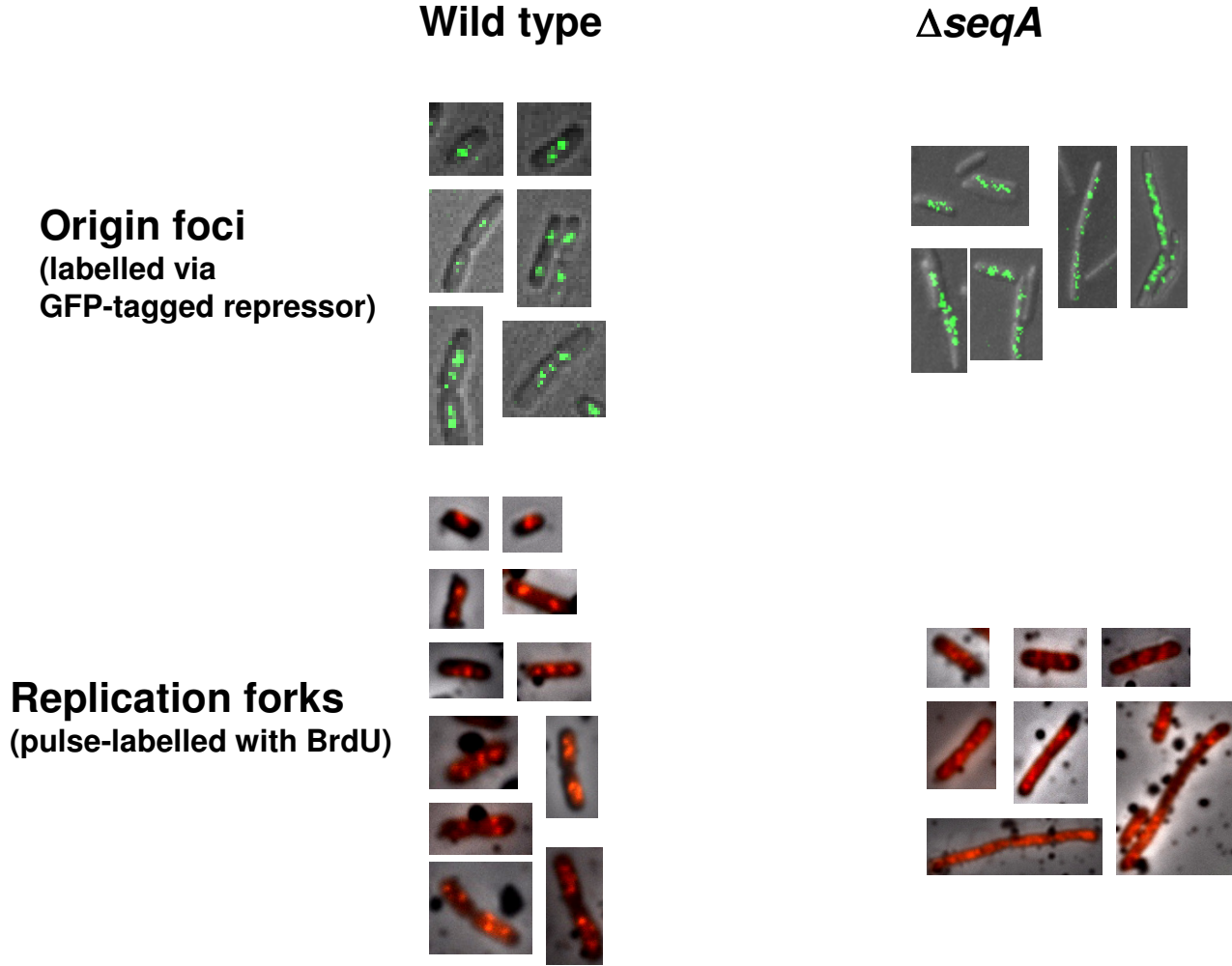


Cell grown in
minimal medium
with glucose-CAA

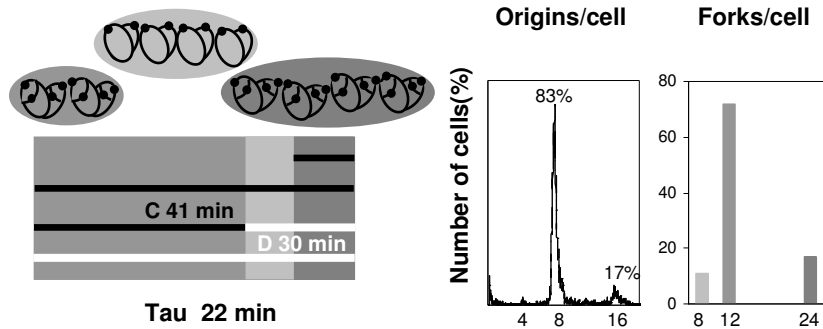


Cell grown in LB

SeqA is required for proper organization of forks and origins

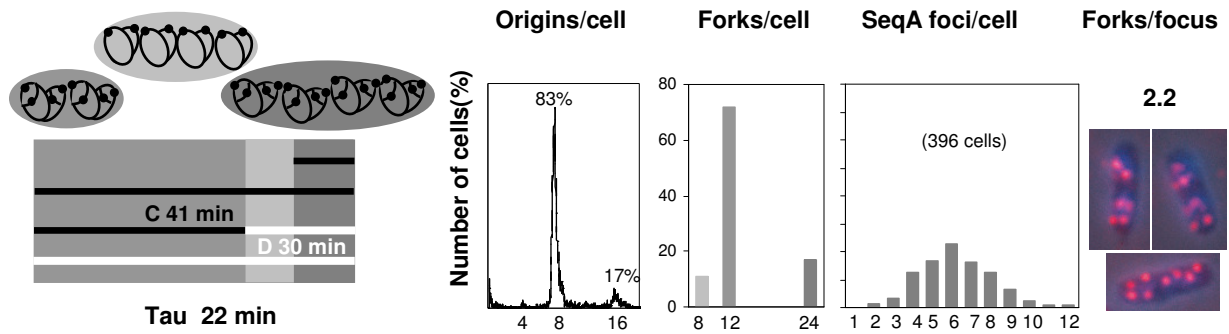


Cells grown in LB



The replication fork distribution is found by

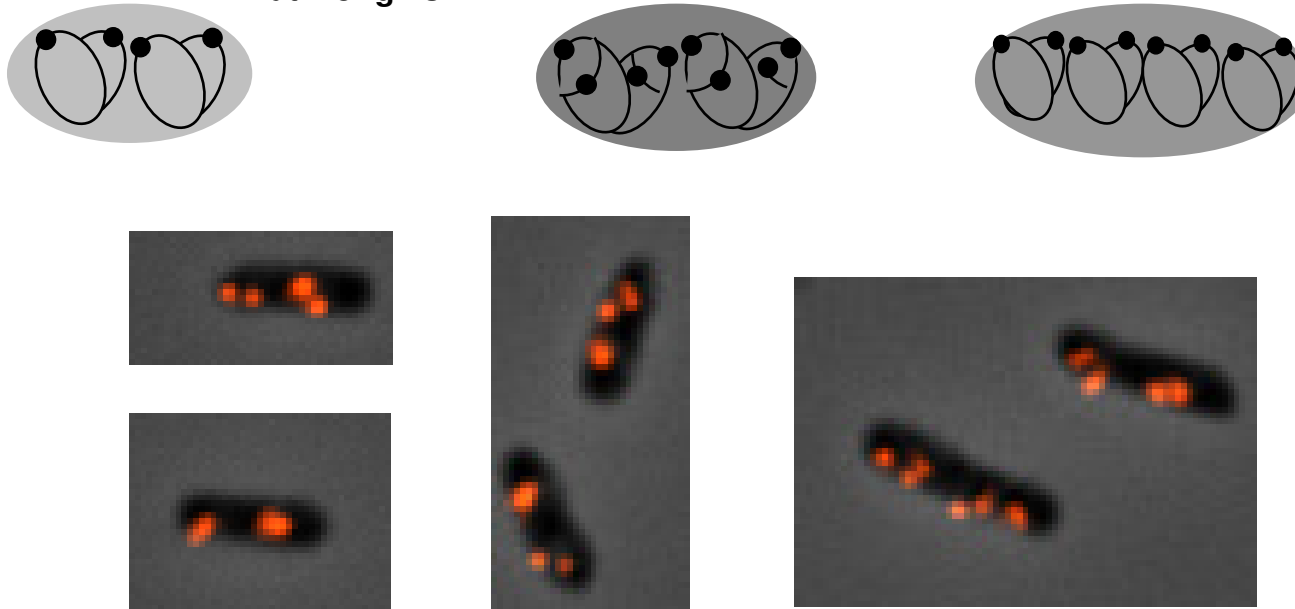
- 1) Southern measurement of ori/ter ratio
- 2) Flow cytometry measurement of DNA and origin distributions



Cells grown in LB had on average 2.2 forks per SeqA focus

A shorter replication period (and fewer forks) did not lead to fewer SeqA foci

Initiation of replication
at 4 origins



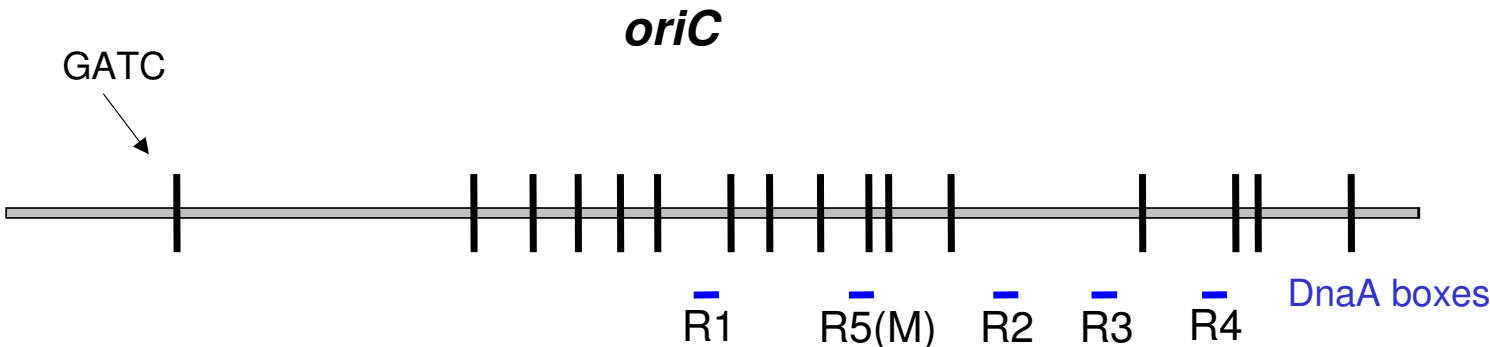
Shortly after initiation of replication there is no abrupt increase in the number of SeqA foci.

Cells then have 2, 3 or 4 SeqA foci which must accommodate 12 forks (4 old forks and 8 new forks).

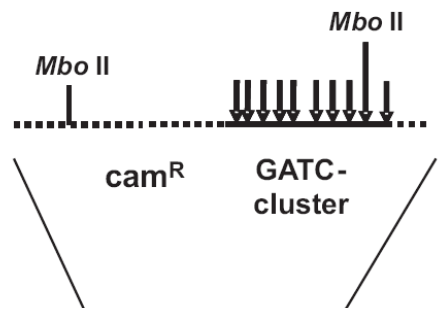
Two possibilities:

1. New origins and new forks are recruited to existing SeqA structures
2. New origins and new forks do not contain sufficient amounts of SeqA to be visible

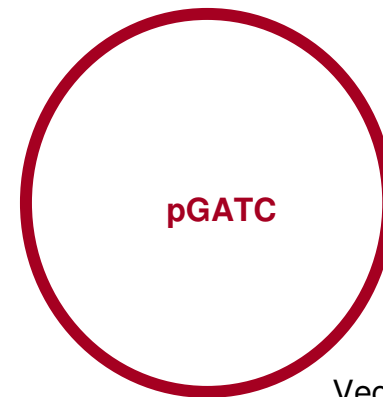
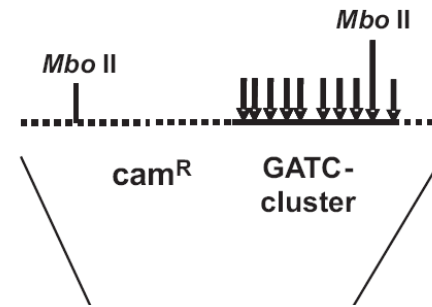
OriC stays sequestered (hemimethylated) 10-20 times as long as DNA in general and has 10 times as many GATCs as expected statistically



A sequence with the same frequency of GATC sites as *oriC* was found to be sequestered as long.



The presence of a plasmid with a GATC cluster
(DNA with 10 GATC sites spaced like in *oriC*)
did not affect replication timing,
the concentration of SeqA protein,
or the distribution of SeqA foci.



Vector:
pACYC184

Summary:

- There are on average 2.2-2.6 replication forks per SeqA focus**
- The numbers of SeqA structures are not governed directly by the numbers of forks**
- It is possible that new origins and forks are recruited to existing SeqA structures**

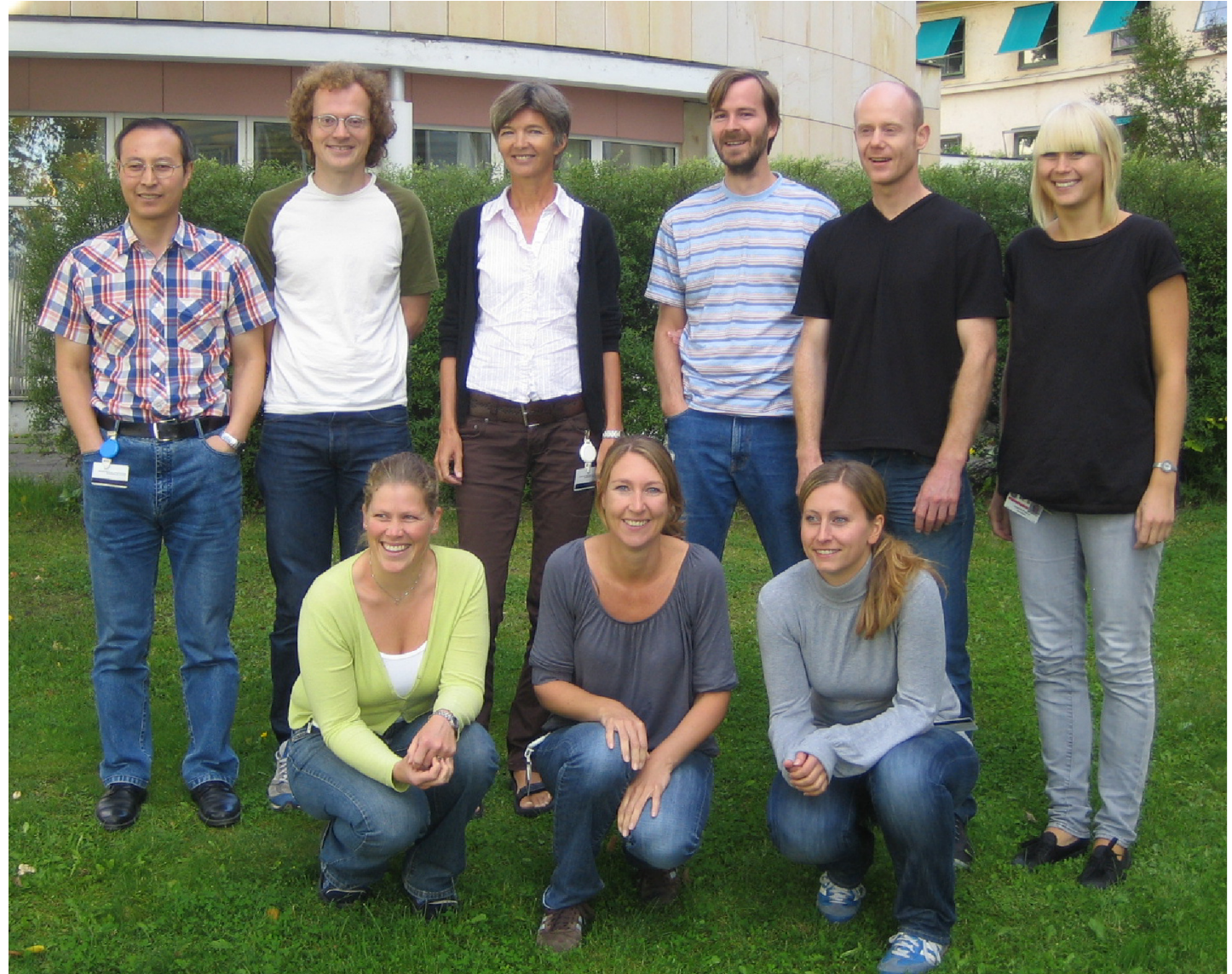
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