A probabilistic model of plasmid replication regulation by a combination of handcuffing, iteron number and dimerization of RepA protein.

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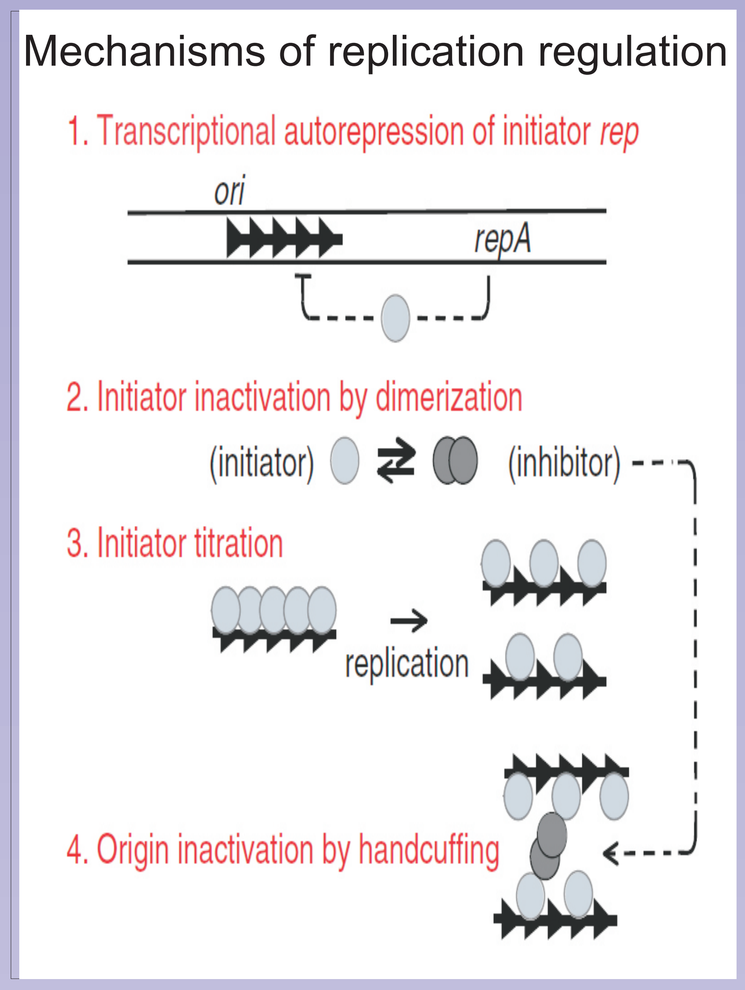
Plasmids are small circular DNA molecules in bacteria which replicate sharing the replication machinery with the bacterial chromosome. Since plasmids are much smaller than the chromosome, unless their replication is tightly regulated, their copy numbers can escalate quickly. A number of mechanisms of plasmid replication regulation are known in P1 plasmid of E.coli. Mechanisms such as autorepression of the replication initiator protein (RepA) and its dimerization are unable to explain copy number regulation by themselves. The number of RepA binding sites (iterons) are inversely related to copy numbers. Handcuffing of plasmids by RepA dimer is believed to be the main mechanism of arresting replication. We show here with a probabilistic model that for the handcuffing mechanism to work, a certain iteron number is critical without which handcuffing is unlikely to work. Further, RepA autorepression, dimerization, iteron number and handcuffing mechanism need to work in concert and no single mechanism in isolation is able to regulate plasmid replication effectively. The model also makes quantitative predictions that can be tested experimentally.

Why individual mechanisms don't work:

- For achieving a stable copy number, the process should involve a negative feedback by copy number. E.g. an increase in 'c' should decrease 'Rm'.
- RepA autorepression: 'c' cannot negatively regulate 'Rm'.
- Dimerization: Although the Rd/Rm ratio increases with 'c' the absolute value of Rm does not decrease.
- Titration of RepA by iterons: The ratio of RepA production to RepA binding is independent of 'c'. Therefore titration cannot work.
- Handcuffing: In the absence of iteron copies, the probability of handcuffing will always be very small compared to the probability of initiation. Therefore when n=1, handcuffing cannot arrest replication.



The model:

Let

Iteron number = n

Copy number = c

Rep A monomer concentration = Rm

Rep A dimer concentration = Rd

Keq= equilibrium constant for dimerization

Probability of replication initiation upon saturation of iterons by RepA binding will be a saturation function of Rm

$$p^n \quad \frac{Rm}{a \quad Rm}^n$$

Where a = constant

Similarly Probability of handcuffing

$$h \quad \frac{cpn}{K \quad cpn}$$

Therefore, with n saturated iterons,

$$h_n \quad 1 \quad 1 \quad \frac{cpn}{K \quad cpn}^n$$

At equilibrium, rate of replication equals the rate of dilution due to growth.

i.e. $p^n \; 1 \; h_n \quad D$

$$p^n \; 1 \quad 1 \quad 1 \quad \frac{cpn}{K \quad cpn}^n \quad D$$

This condition is satisfied when,

$$c \quad \frac{K}{p \; n} \quad \frac{p}{\sqrt[n]{D}} \quad 1$$

Therefore the equilibrium copy number will be a function of n as in fig 1.

fig 1:

fig.1.1

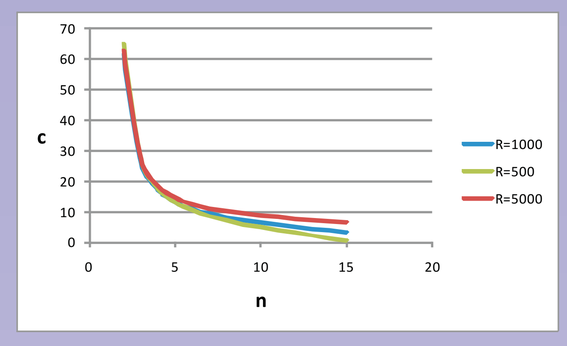


fig.1.2

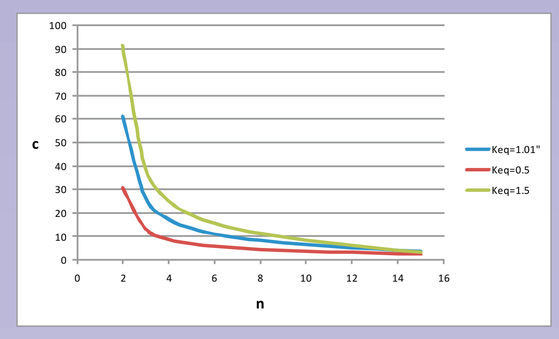


fig.1.3

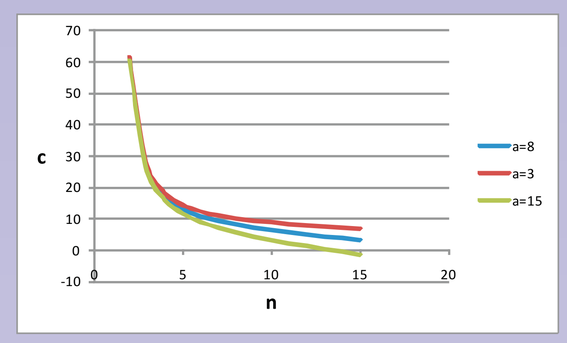


Fig 1: The effect of iteron number on copy number is affected by (1.1)R, (1.2)Keq and (1.3) a

Testing the hypothesis:

- No single mechanism can work in isolation, only a combination can work.
- Iteron number decreases the probability of initiation and increases probability of handcuffing. Iteron number may work through this mechanism rather than by titration. Also the predicted relation between 'n' and 'c' and effects of other parameters allow testing of the hypothesis.
- Copy-up by dimerization defect is a non-selfish copy-up but copy-up by iteron deletion is a selfish copy-up. Therefore polymorphism in iteron number will exist in wild population but polymorphism in RepA is unlikely to exist.

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