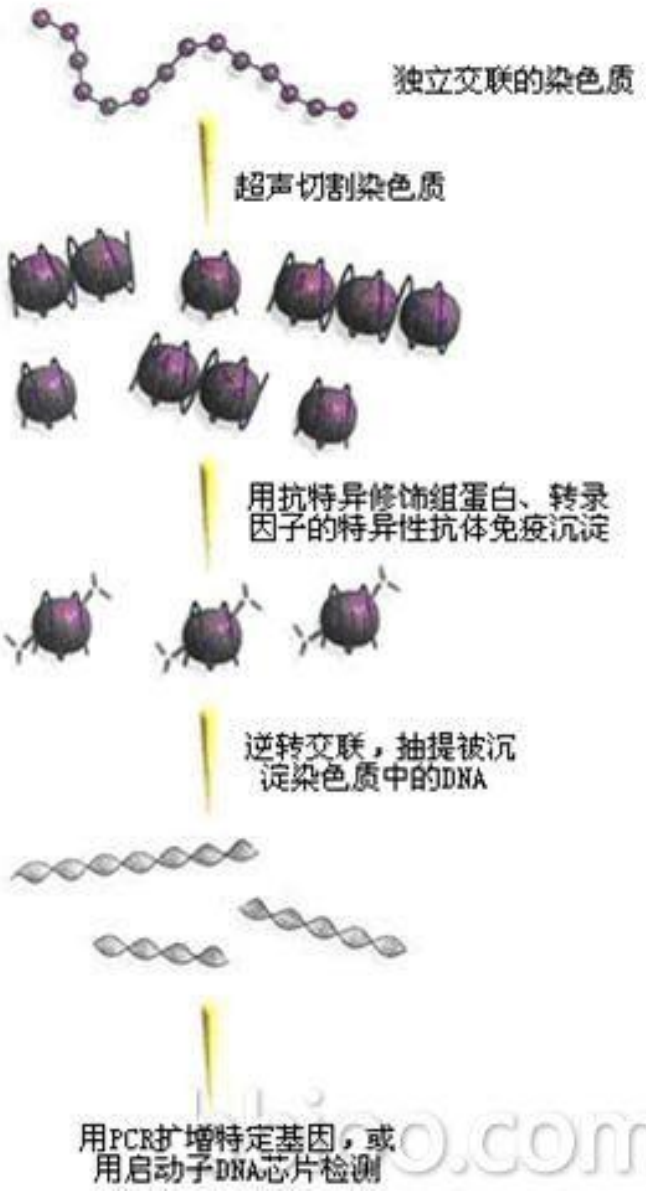
Measuring Chromatin Interaction Dynamics on the Second Time Scale at Single-Copy Genes

（单拷贝基因染色质互作动力学的秒尺度测量）

熊乐

Chromatin Immunoprecipitation assay (ChIP)



1.细胞固定

↓

2.染色质断裂

↓

3.染色质免疫沉淀

↓

4.交联反应的逆转和DNA的纯化

↓

5.DNA的鉴定

现有方法的不足

1.ChIP

无时间尺度

2.Live-cell imaging approachs

空间分辨率低

3.Competition ChIP

时间分辨率低

- 现有的测量方法无法测量单拷贝基因秒尺度范围内染色质互作动力学过程

Cross-linking kinetic (CLK) model

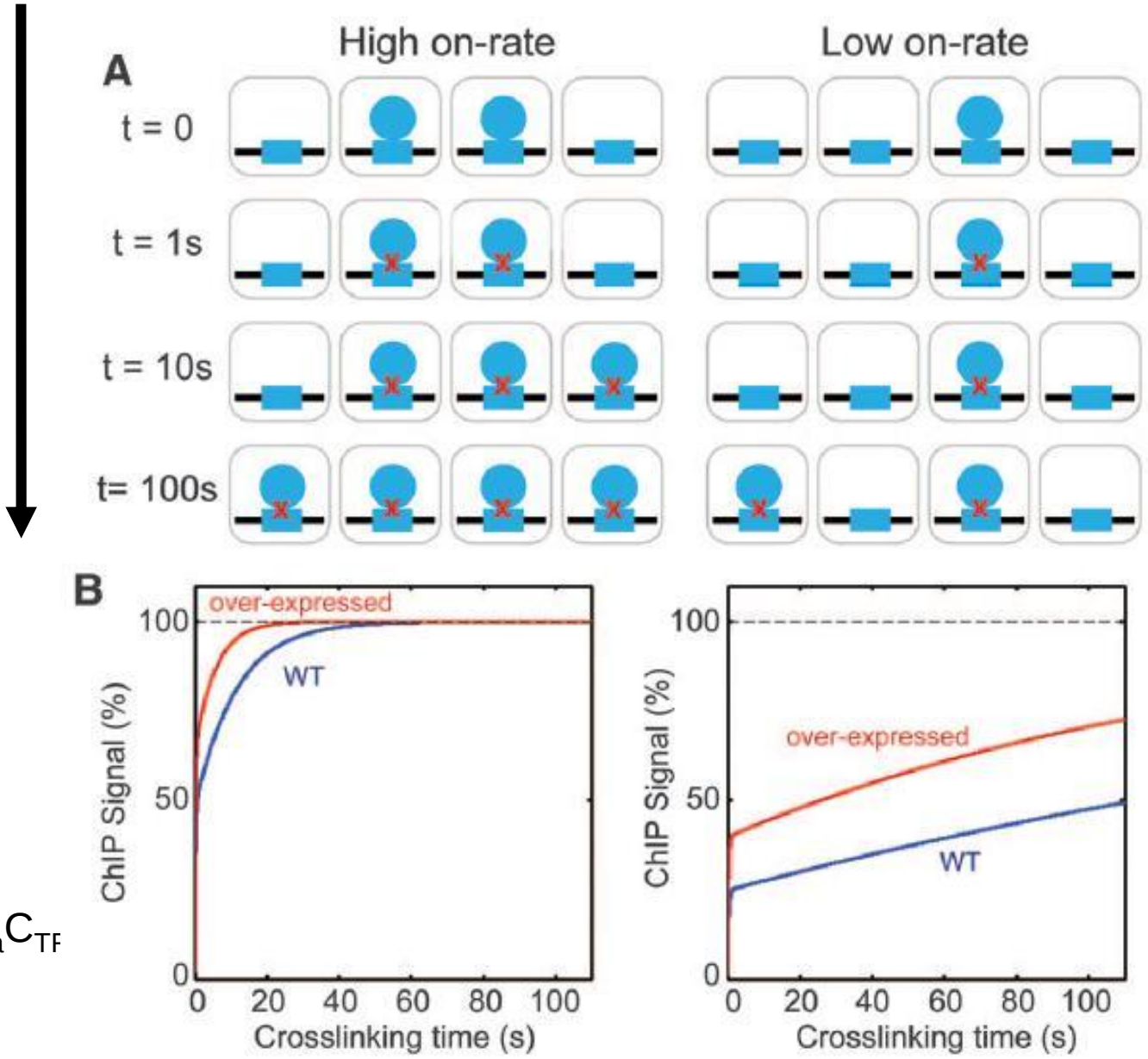
- A mathematical model based on standard principles of chemical kinetics that describes the dependence of ChIP signal on formaldehyde(甲醛) cross-linking time.

On rate: $C_{TF,}$ chromatin binding factor concentration;
Ka, the product of the second order rate constant.

Off rate: K_d;and the half-life, $t_{1/2}$ =ln2/Kd.

$\theta_b^{0:}$:the fraction of bound chromatin sites at steady state.

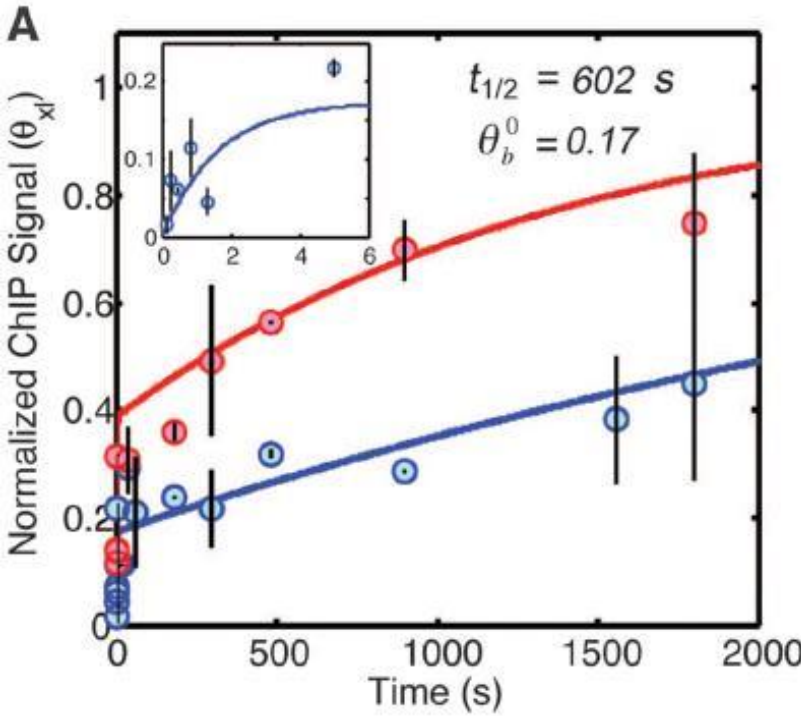
Formaldehyde incubation times

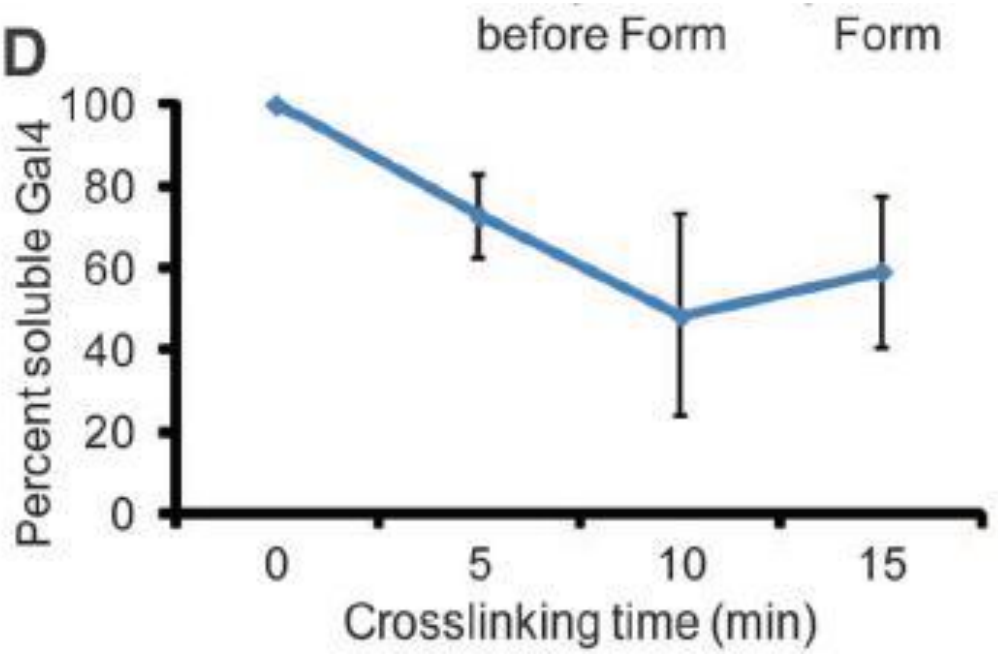


Biphasic behavior：
1.cross-linking kinetics
2.at a rate driven by K_aC_{TF}

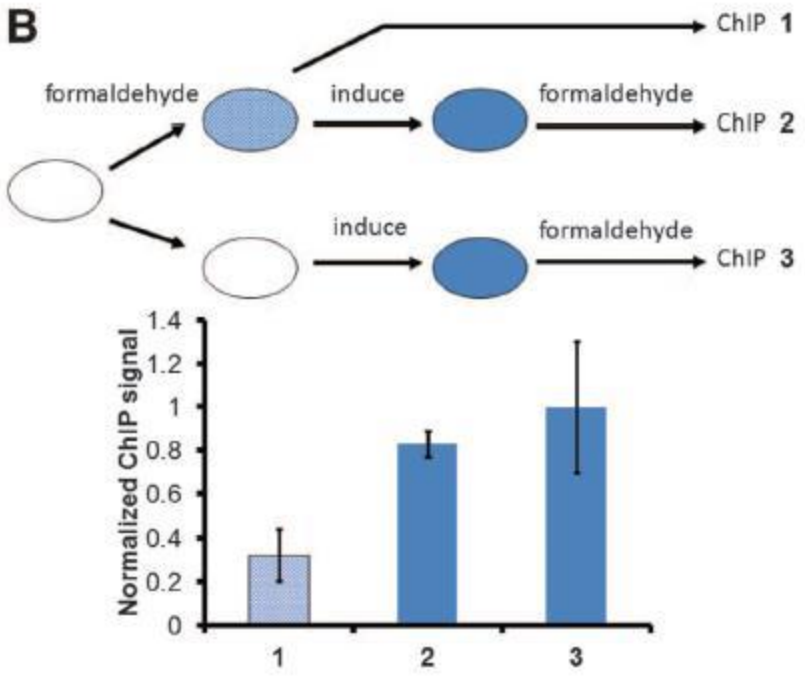
Test CLK model(1)

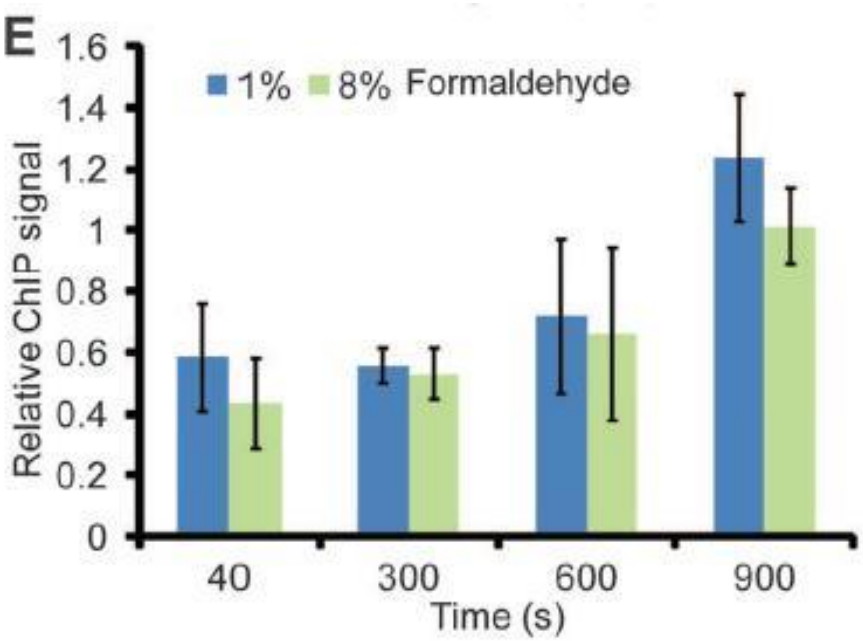
Gal4-GAL3 promoter





Test CLK model(1)





Conclusion

Problem:

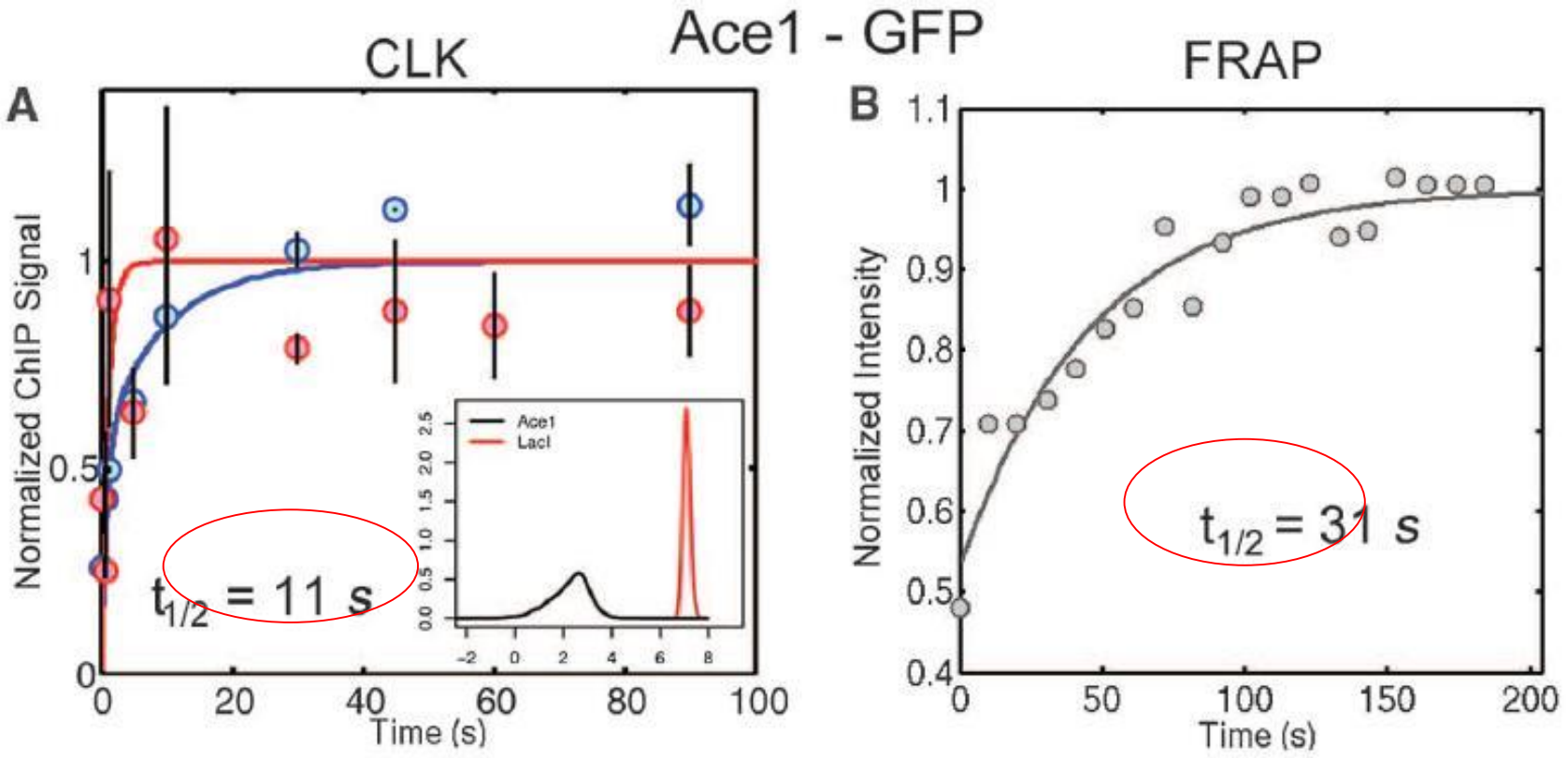
The in vivo stability of the Gal4-promoter interaction has been the subject of debate.

Answer:

CLK analysis revealed that the Gal4-GAL3 interaction had a t1/2 of about 10 min , suggesting that a single Gal4 complex facilitates multiple rounds of transcription initiation.Combined with the low fractional promoter occupancy(~0.17), we conclude that the GAL3 gene is likely transcribed in infrequent bursts.

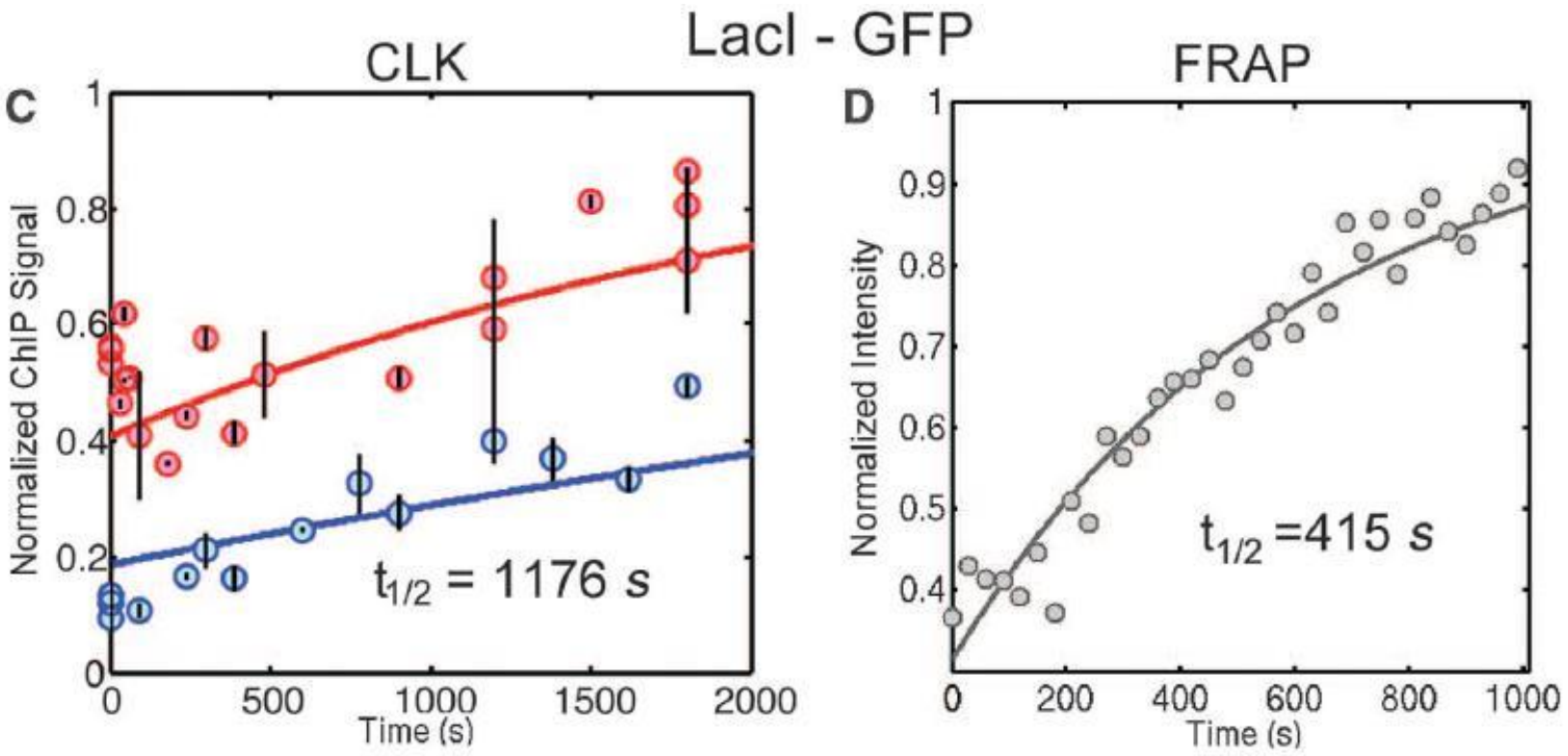
Test CLK model(2)

Comparison of TF-chromatin dynamics by CLK and FRAP



Test CLK model(2)

Comparison of TF-chromatin dynamics by CLK and FRAP

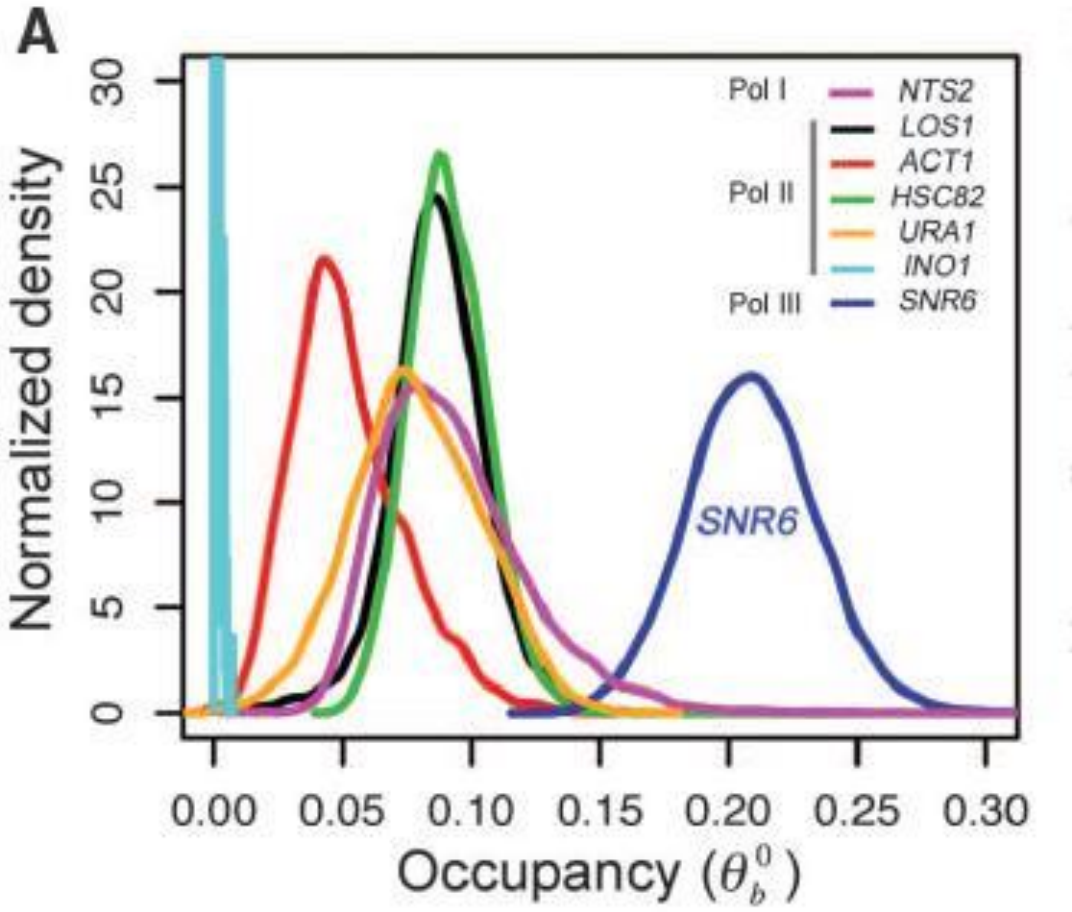


Conclusion

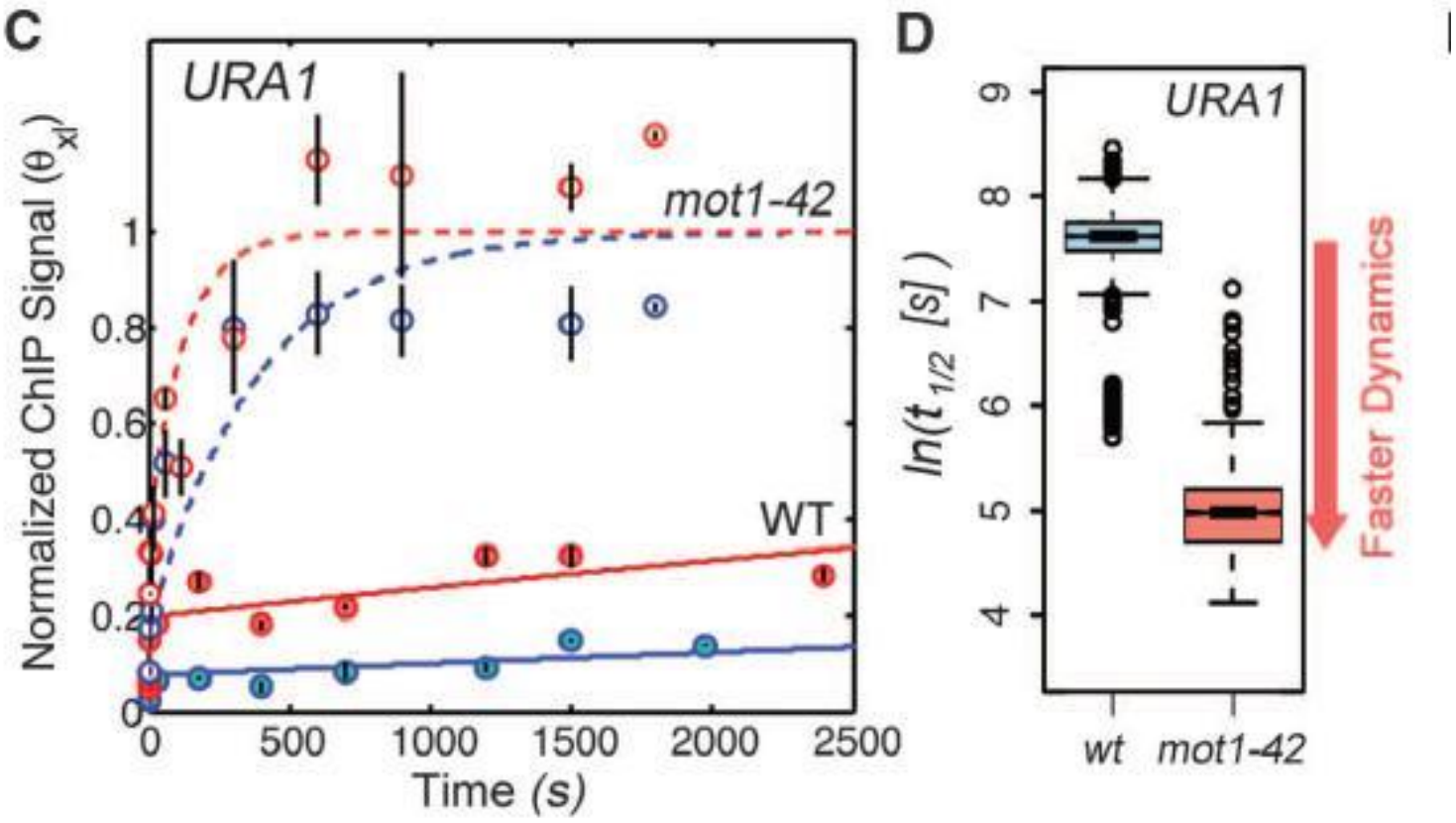
Compared with other methods, the CLK method increases the time resolution of chromatin dynamics at single-copy loci by two to three orders of magnitude.

Test CLK model(3)

TATA-binding protein (TBP) with different promoters

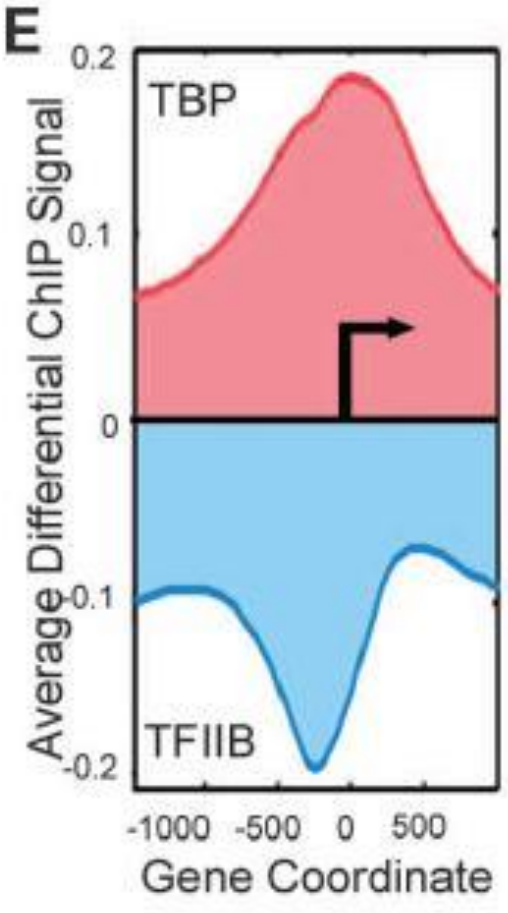


Mot1 调控TBP影响URA1表达水平。



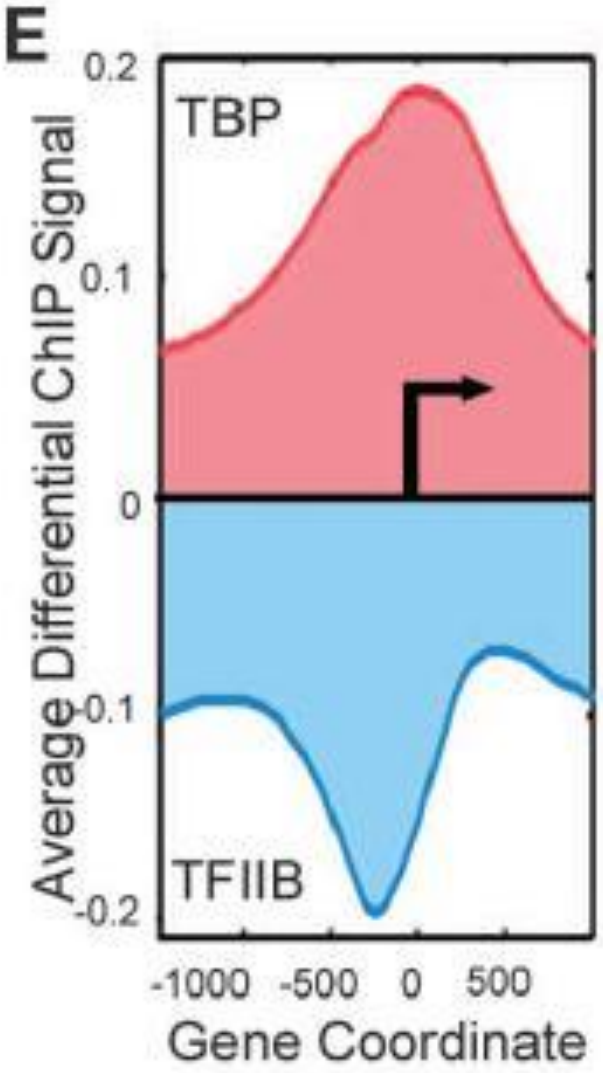
Test CLK model(3)

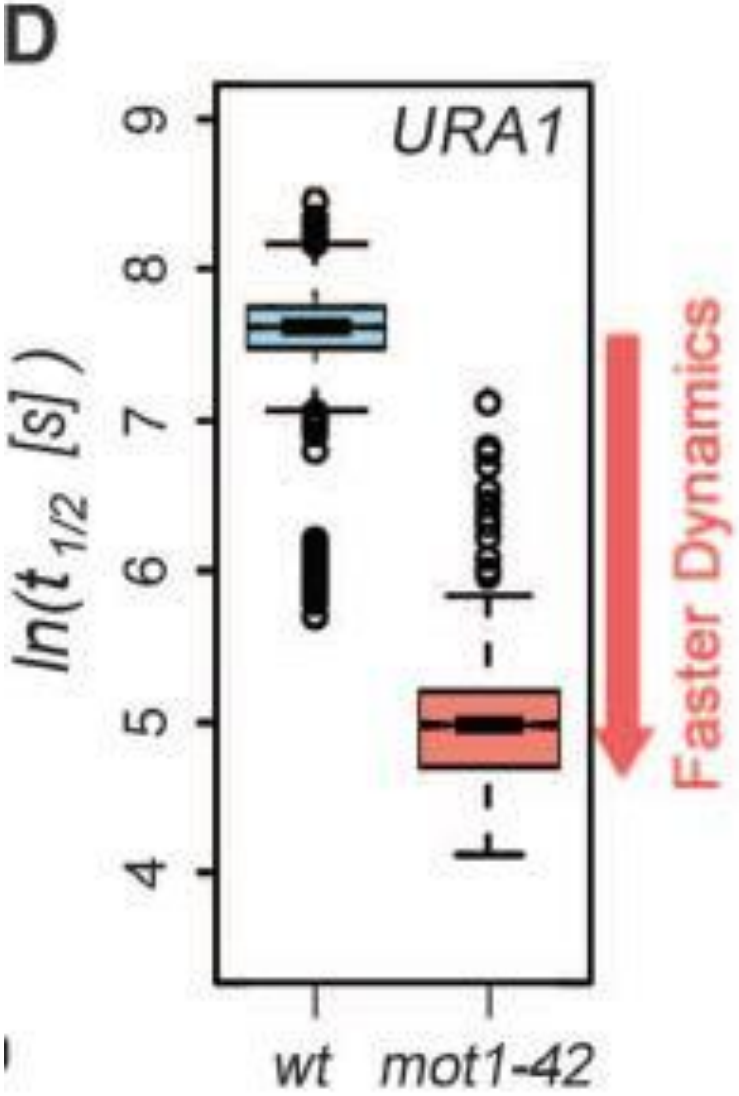
The genome-wide TBP ChIP signal at a single cross linking time in WT and mot1-42 cells.



Mutation of Mot1 increased the TBP Chip signal at pol II promoter.

Mutation of Mot1 decreased the TF IIB Chip signal at pol II promoter.


E
TBP
TFIIB
Average Differential ChIP Signal
0.2
0.1
0
-0.1
-0.2
-1000
-500
0
500
Gene Coordinate


D
URA1
ln(t1/2 [s])
4
5
6
7
8
9
wt
mot1-42
Faster Dynamics

- There is no detectable stable chromatin-bound TBP as judged by live-cell imaging but there are stable TBP complexes as judged by competition ChIP.

- The CLK results show that TBP fractional occupancies are low. Thus, although there are stable TBP-promoter complexes in vivo, most promoters are not occupied at steady state. The unexpectedly low occupancies are consistent with results showing that transcription in vivo occurs via uncoordinated stochastic cycles separated in time.

总结：基于ChIP技术的Cross-linking kinetic (CLK) model 能够在秒尺度范围内刻画染色质单拷贝基因相互作用关系。