

通过引入Cas9切割和同源重组修复的时空相关性 增加CRISPR基因驱动转化效率

Boosting Conversion of CRISPR Gene Drive
by Spatial and Temporal Correlation of Cas9 Cleavage to HDR and Inhibition of
NHEJ

小组编号: G03

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主要内容

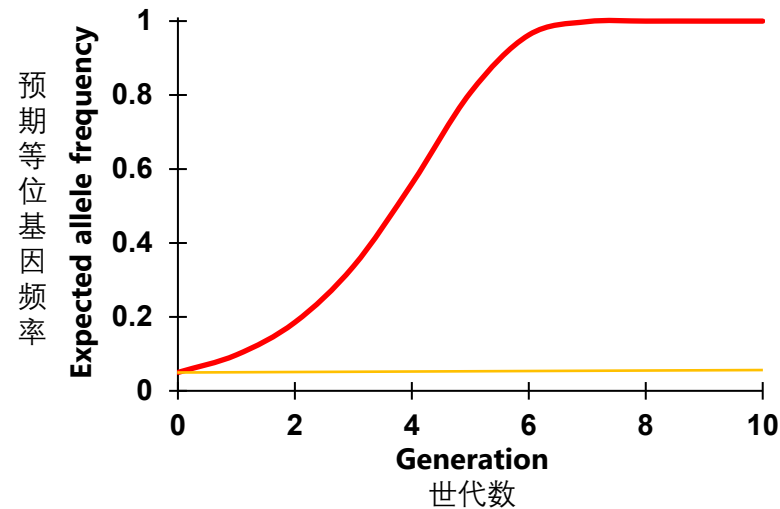
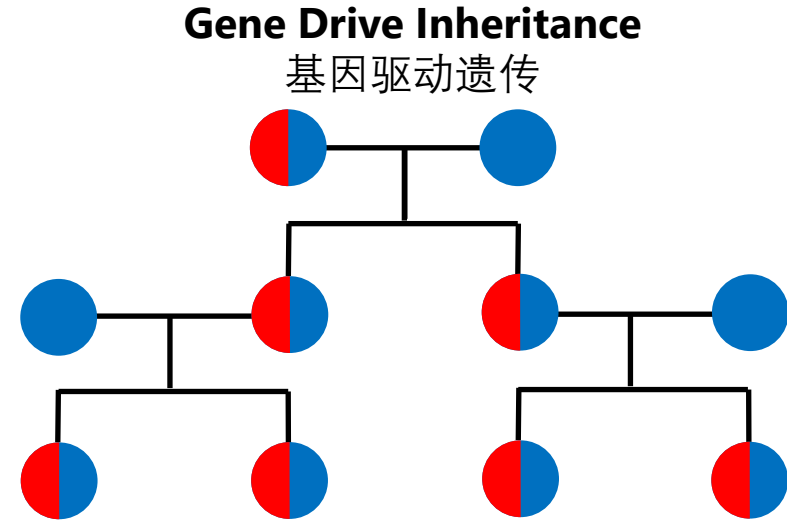
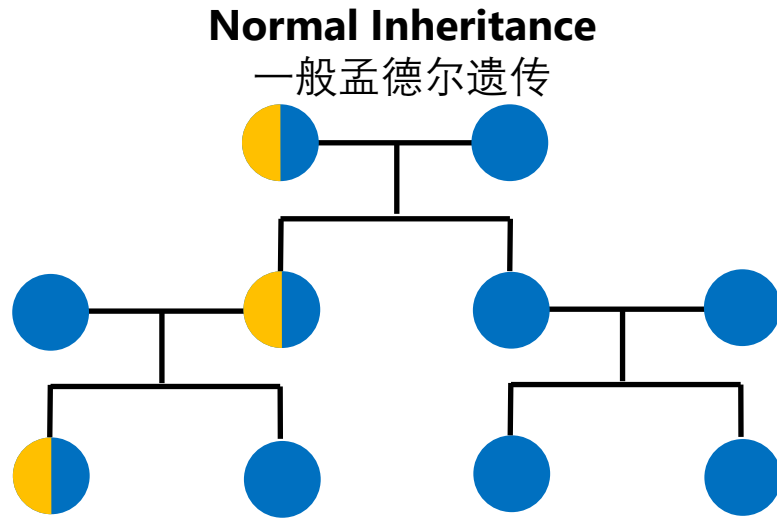
1. 研究背景
2. 研究目的
3. 生物信息预测结果



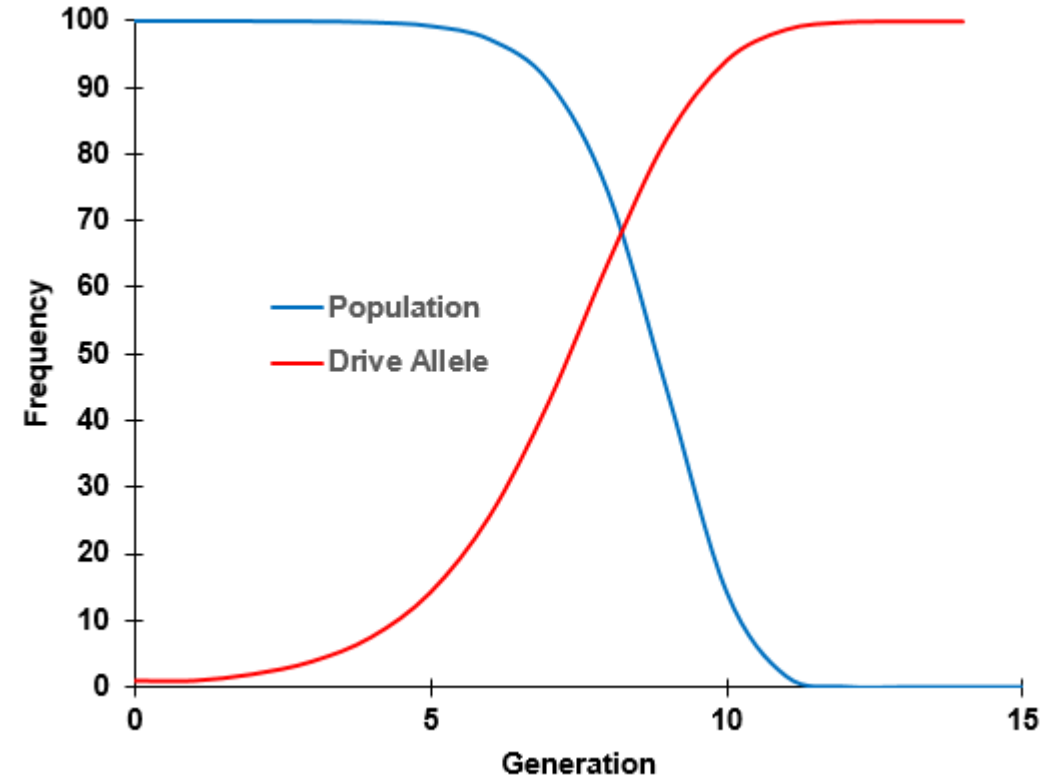
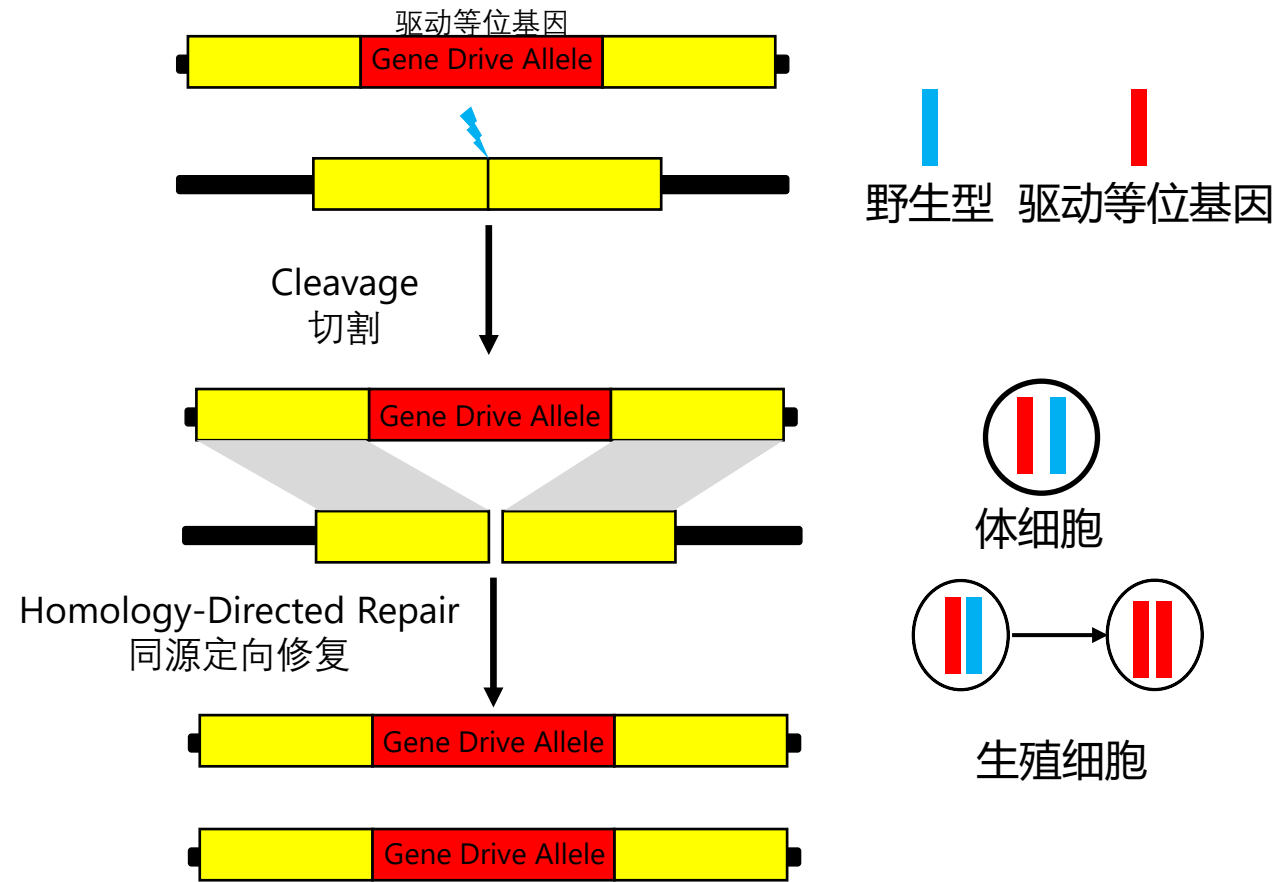
研究背景：遗传驱动

遗传驱动 (Gene Drive) 通过超孟德尔遗传使特定等位基因在特定种群中扩散

种群遗传控制和遗传驱动



CRISPR遗传驱动通过同源介导修复(Homology Directed Repair)进行复制

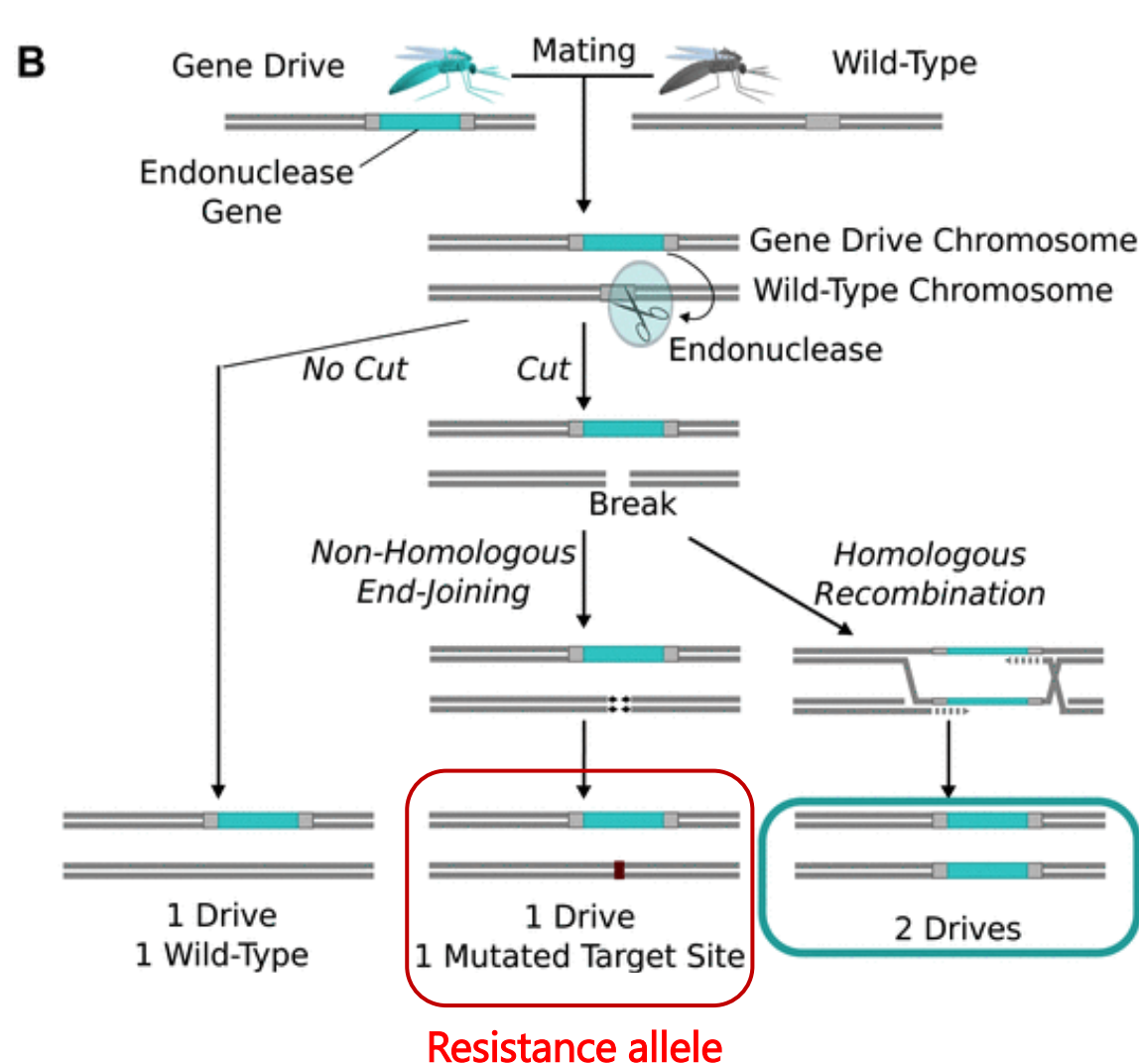


双链断裂的修复方式

非同源末端连接 (Non-Homologous end joining)
修复后的等位基因可能无法再被Cas9切割

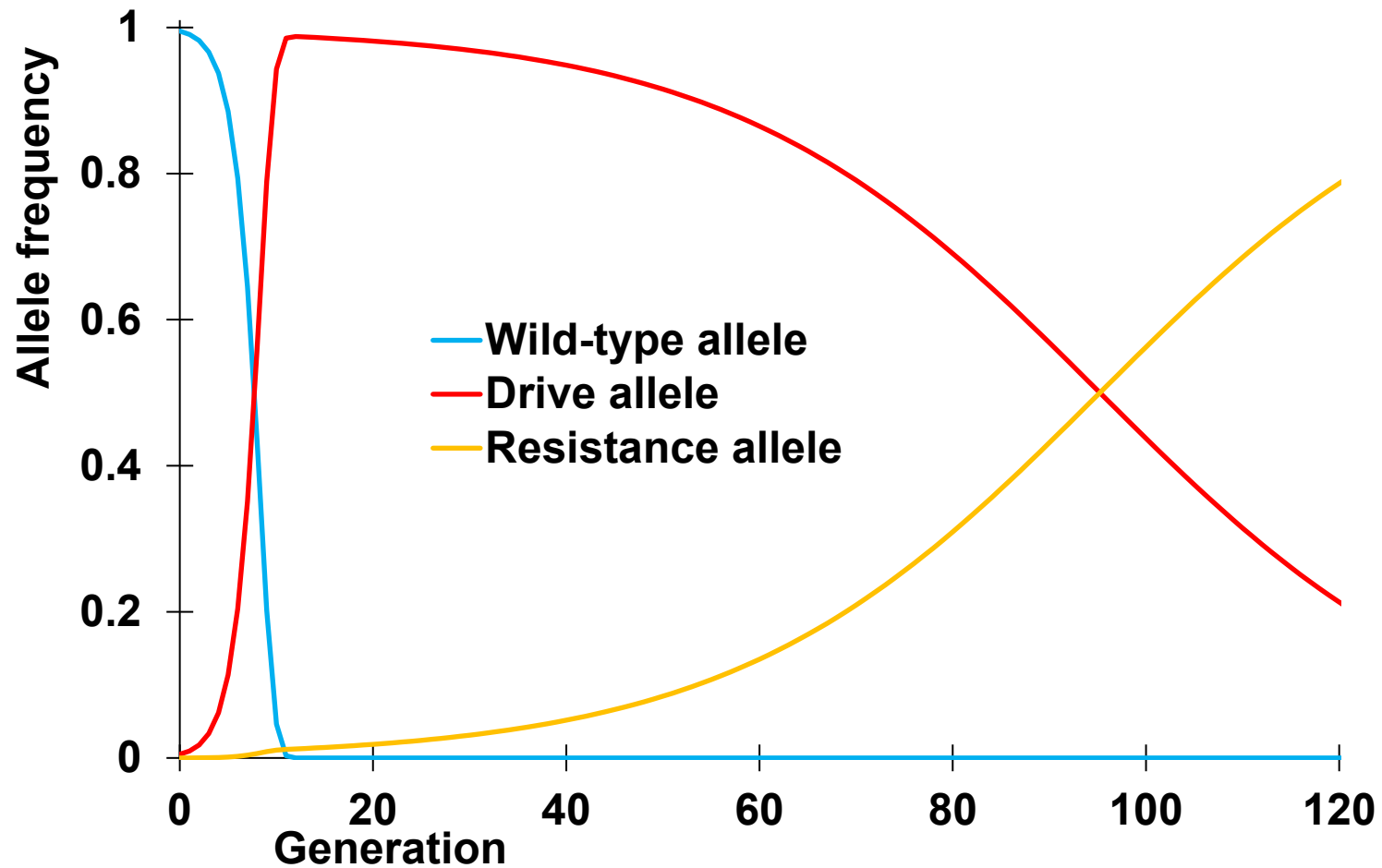
→形成抗性等位基因 (Resistance allele)

HDR可以复制驱动等位基因



抗性等位基因

抗性等位基因出现会导致驱动等位基因被取代，或降低驱动等位基因的转化效率，最终导致基因驱动的失败

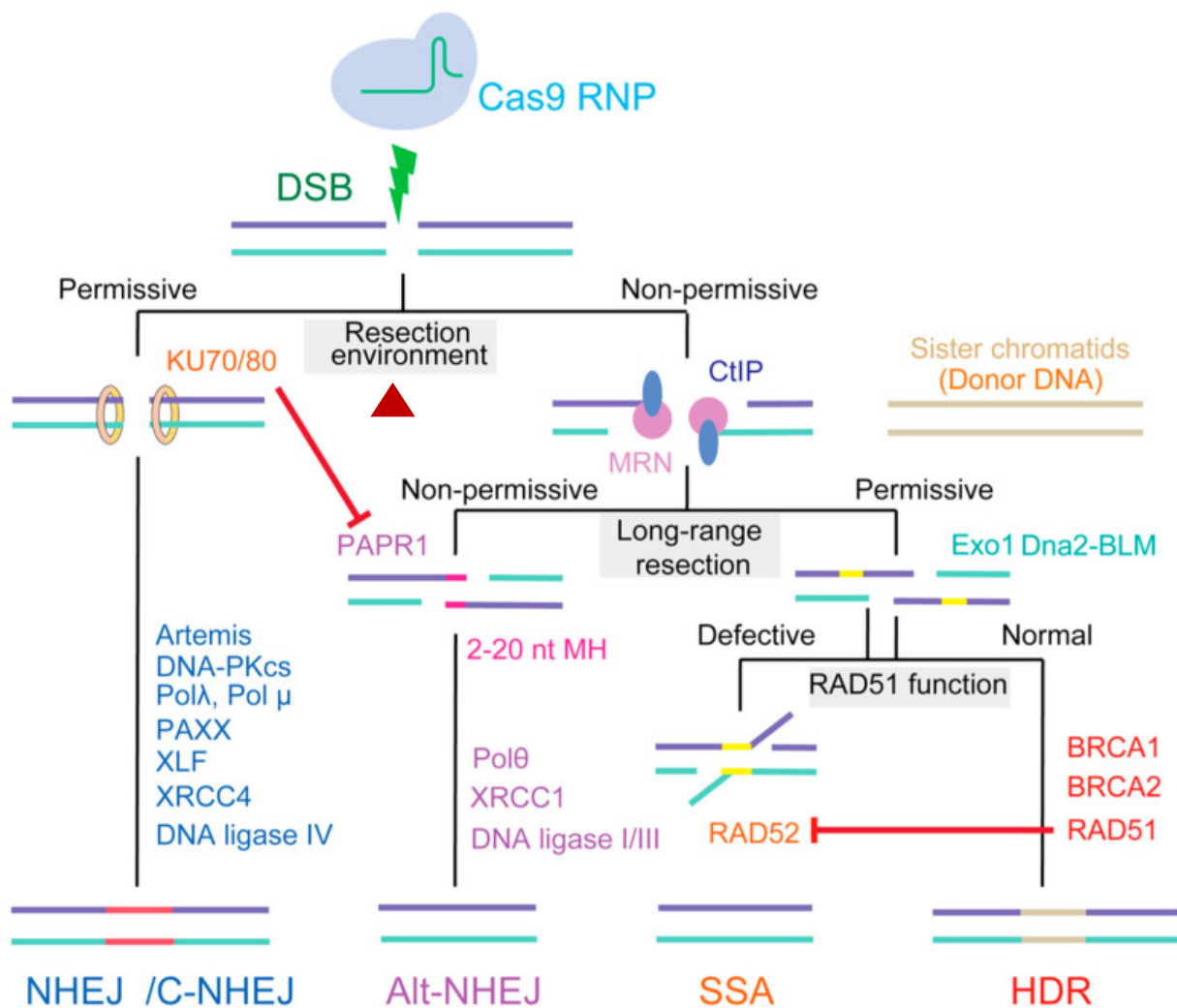




研究目的：Cas9切割与HDR的 时空关联性

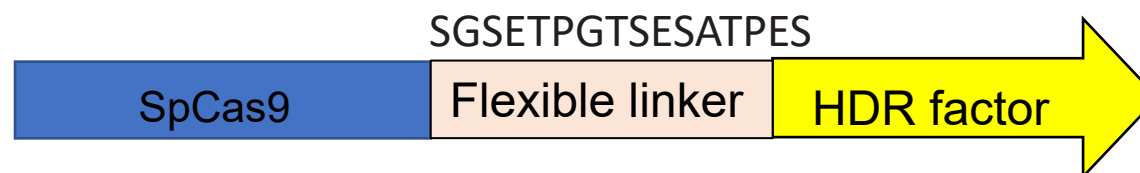
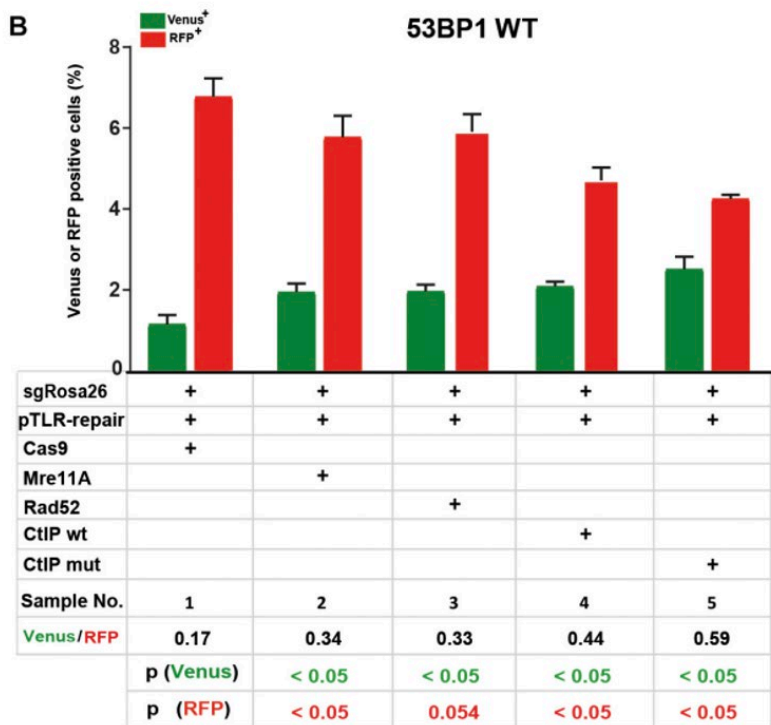
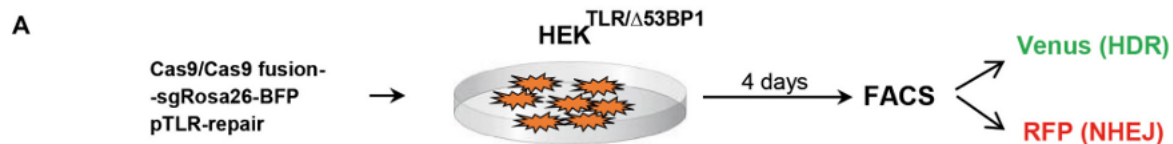
Cas9像一个乐高积木，可以和不同蛋白组成融合蛋白

HDR的起始需要特定蛋白的招募过程



CtIP作用于HDR的起始阶段，且招募下级蛋白

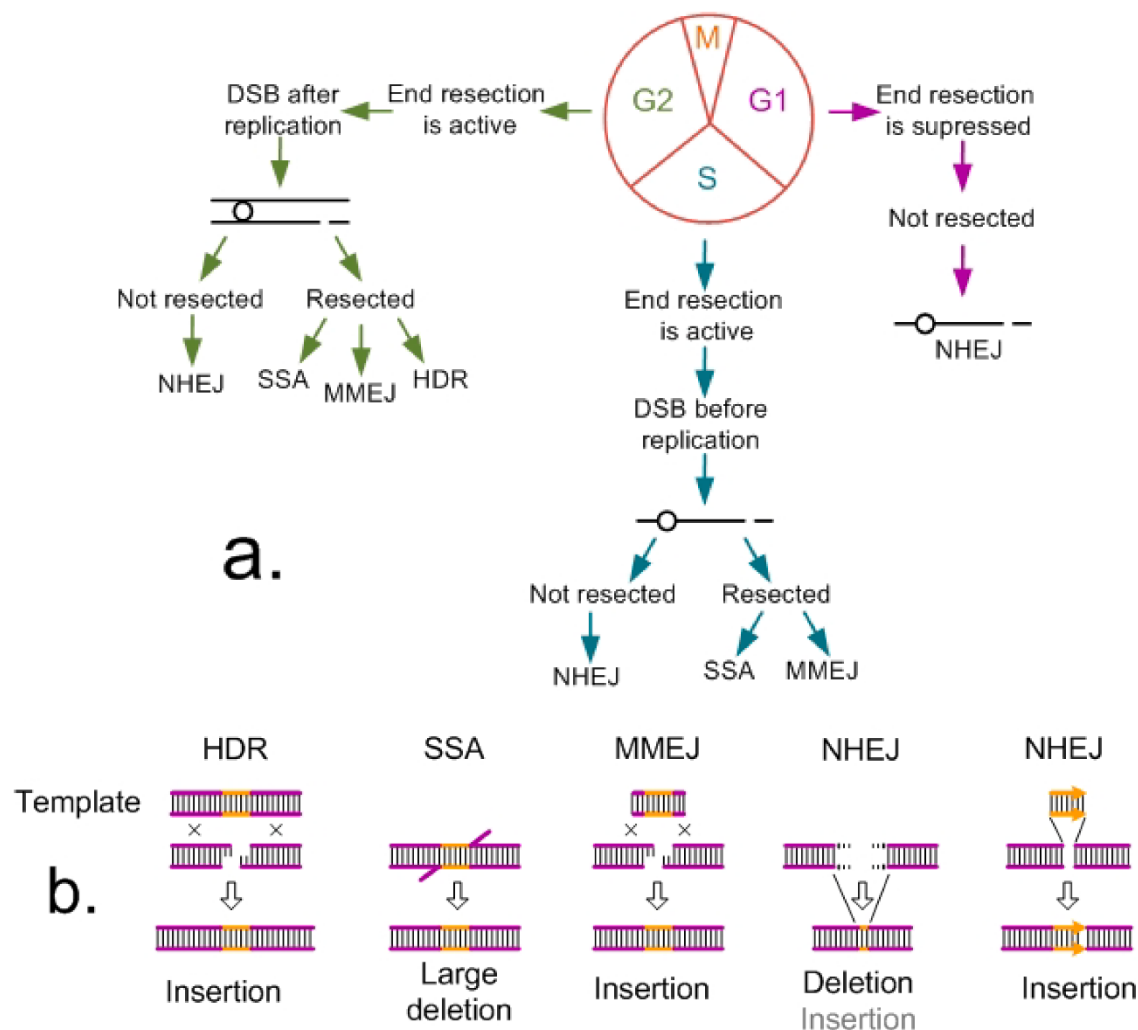
Cas9切割与HDR的空间关联性：将Cas9与CtIP连接



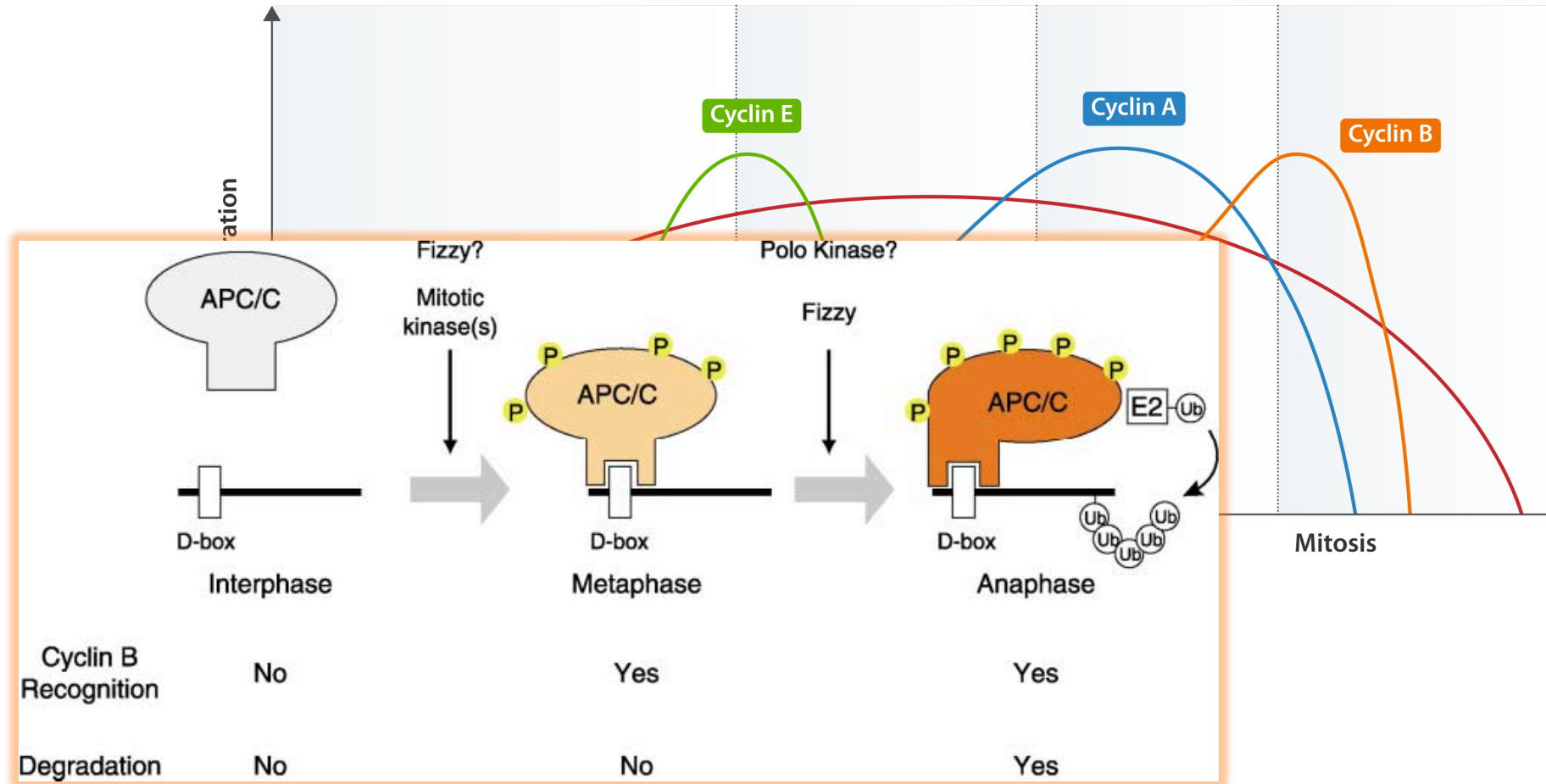
连接Cas9和CtIP后, (HDR/NHEJ) 比例上升

HDR倾向于在细胞周期的S和G2期进行

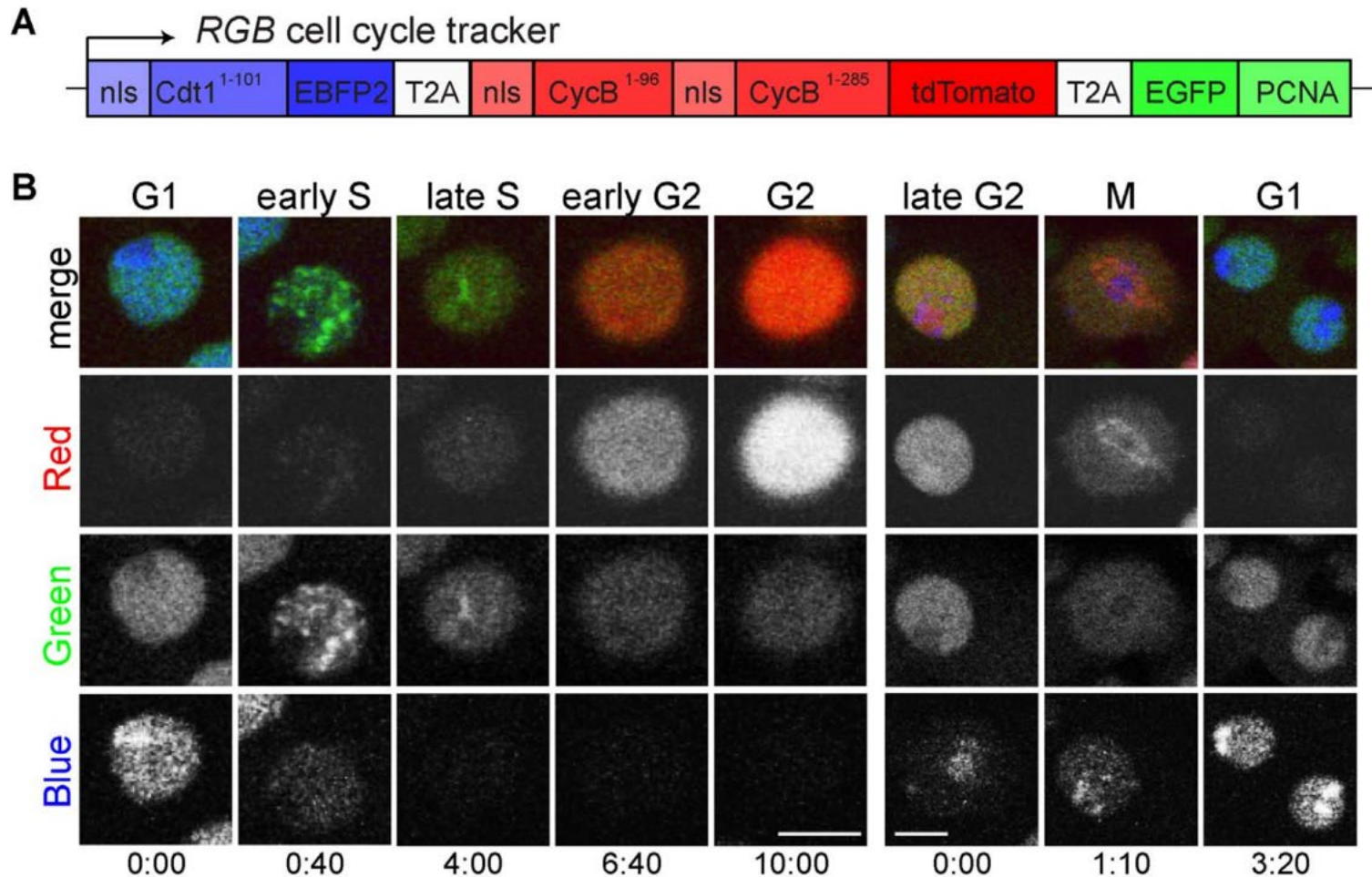
在细胞周期的S和G2期，DNA末端切割（End Resection）机制活跃，为HDR发生提供前提



Cyclin B的细胞周期依赖性降解



CycB的降解子融合蛋白



CycB降解子-tdTomato
在S,G2和M期存在



CycB降解子-Cas9只在
S,G2和M期切割?



生物信息预测结果

利用人CtIP进行BLAST搜索，并构建系统发生树

果蝇CtIP预测结构的结构域分析

融合蛋白的预测结构分析

人CtIP BLAST结果

Drosophila melanogaster:

NCBI BLAST 在nr database未能找到显著相似性;
UniProt BLAST 找到2个相似蛋白(Q9VPF5, X2JBC3)

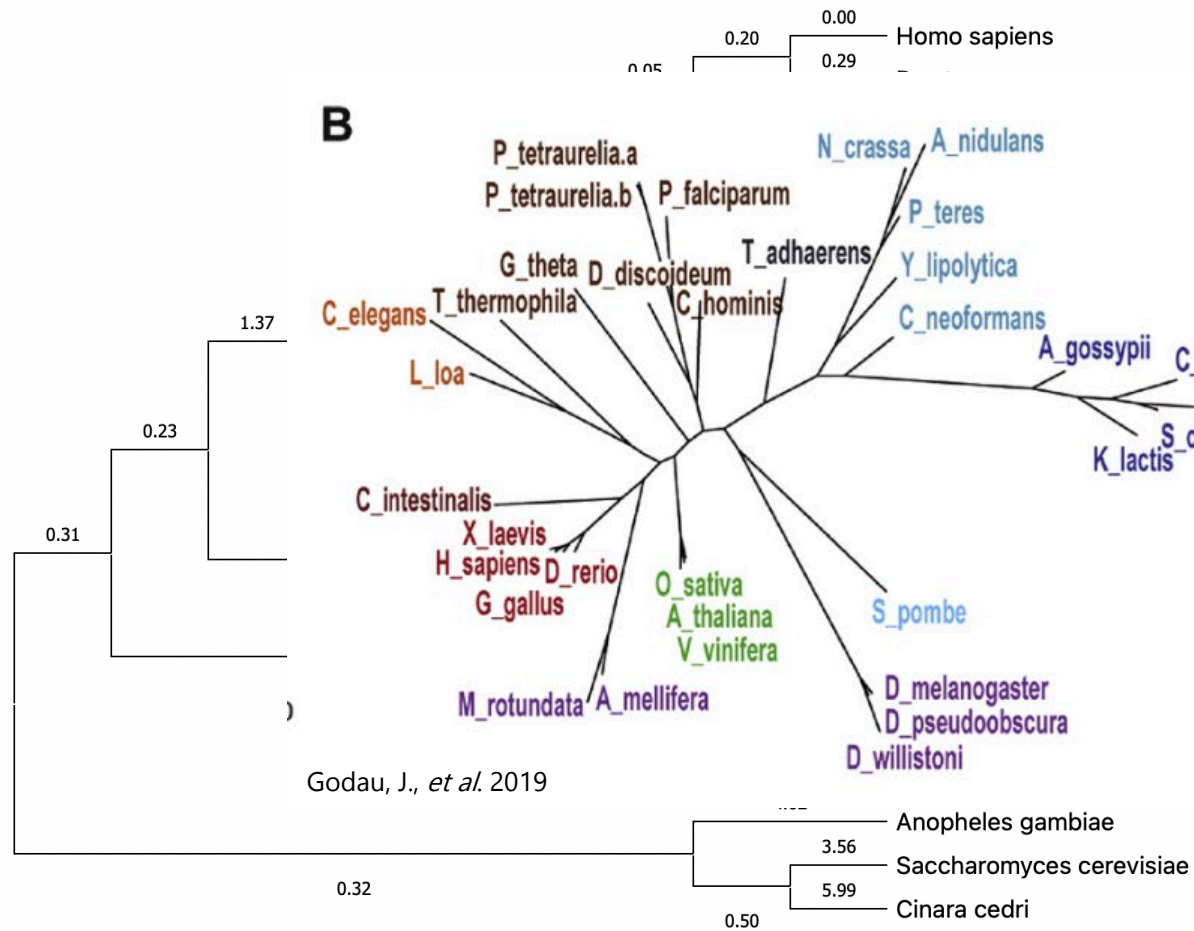
Mosquitoes :

NCBI BLAST 在nr database未能找到显著相似性;
UniProt BLAST 找到两个相似蛋白 (A0A6I8u298, A0A1S4GIH4)

Percent Identity Matrix

<input type="checkbox"/> sp Q99708 CTIP_HUMAN	100.00%	16.05%	21.45%	14.71%	19.23%
<input type="checkbox"/> tr A0A6I8U298 A0A6I8U298_AEDAE	16.05%	100.00%	23.51%	15.81%	22.41%
<input type="checkbox"/> tr A0A1S4GIH4 A0A1S4GIH4_ANOGA	21.45%	23.51%	100.00%	25.16%	21.10%
<input type="checkbox"/> tr Q9VPF5 Q9VPF5_DROME	14.71%	15.81%	25.16%	100.00%	92.24%
<input type="checkbox"/> tr X2JBC3 X2JBC3_DROME	19.23%	22.41%	21.10%	92.24%	100.00%

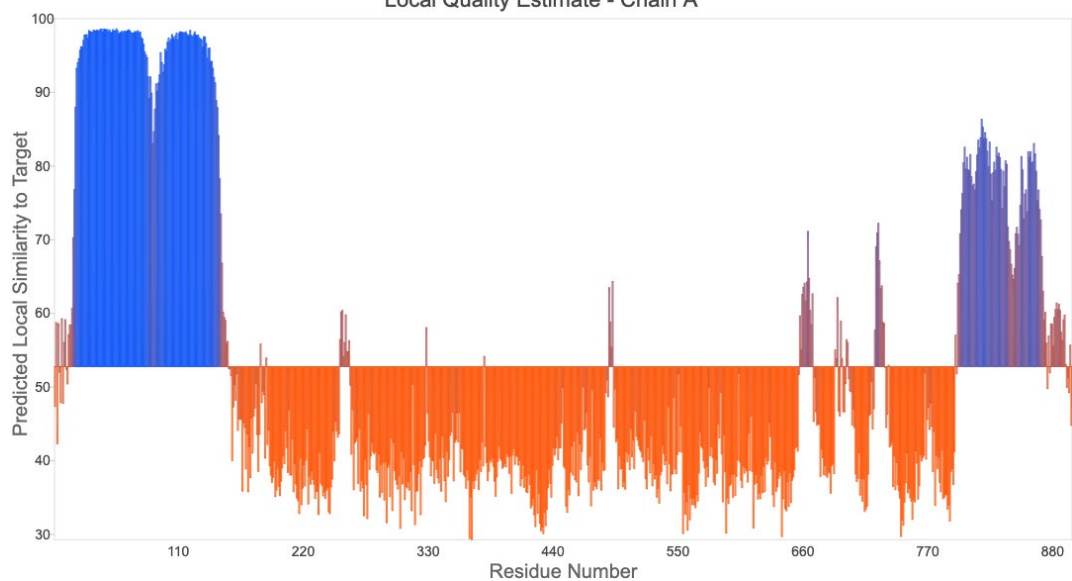
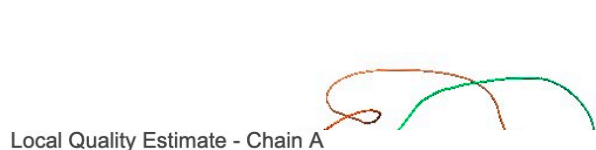
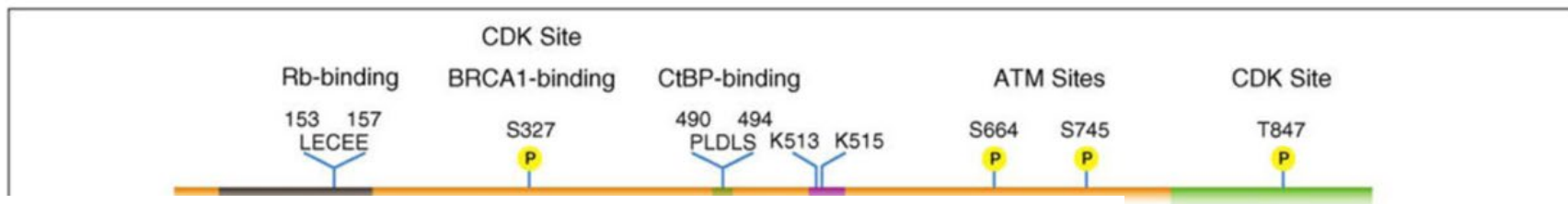
利用MEGA11进行CtIP系统发生树构建



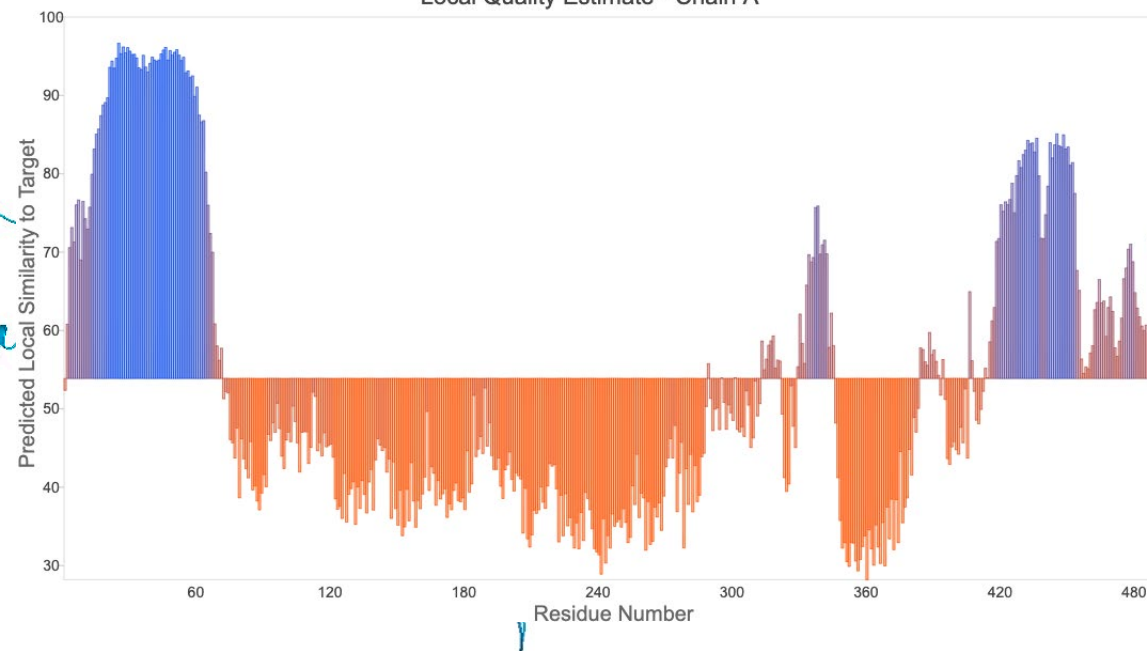
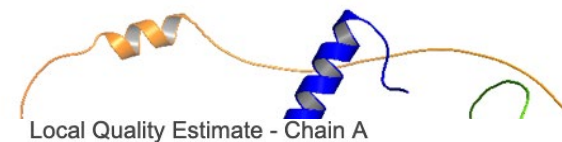
利用UniProt中经人工审阅的序列、部分昆虫CtIP序列和BLAST找到的序列建树并不一致

只采取保守的C端序列进行建树，与演化关系并不一致

果蝇CtIP蛋白的预测结构

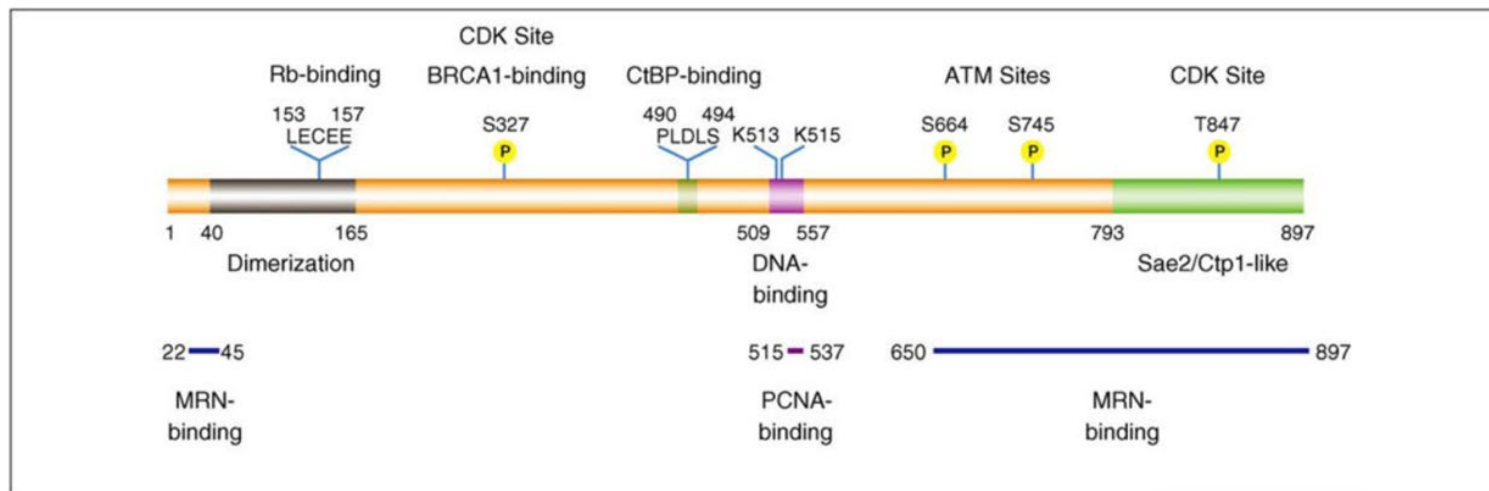


Q99708 CTIP_HUMAN(Alphafold2 prediction)



Q9VPF5 Q9VPF5_DROME(Alphafold2 prediction)

果蝇CtIP蛋白的预测结构

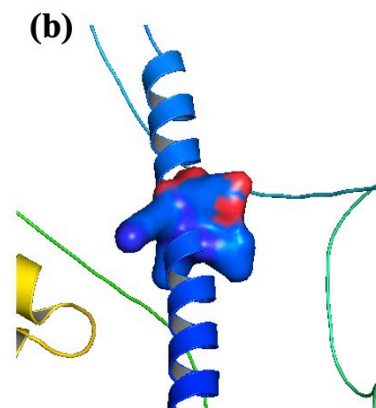
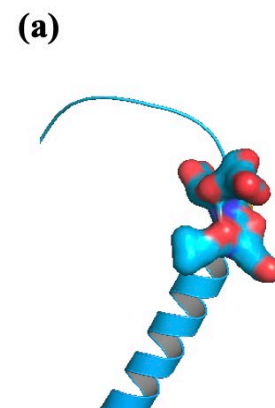
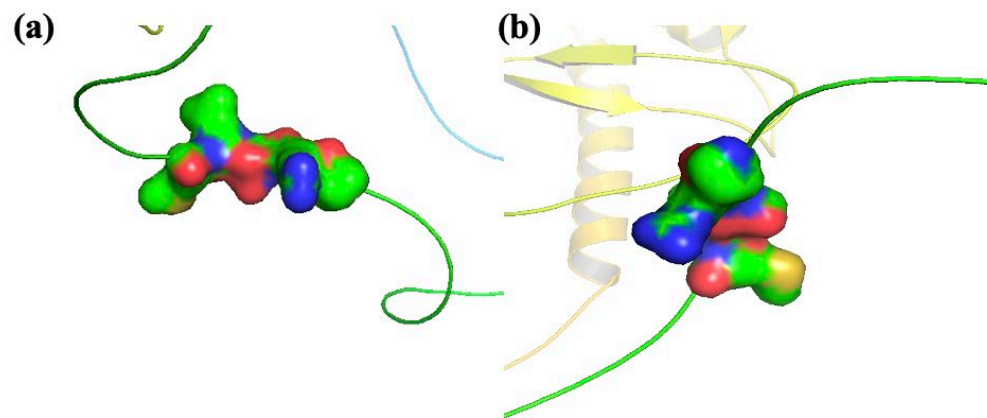


490-494:PLDLS

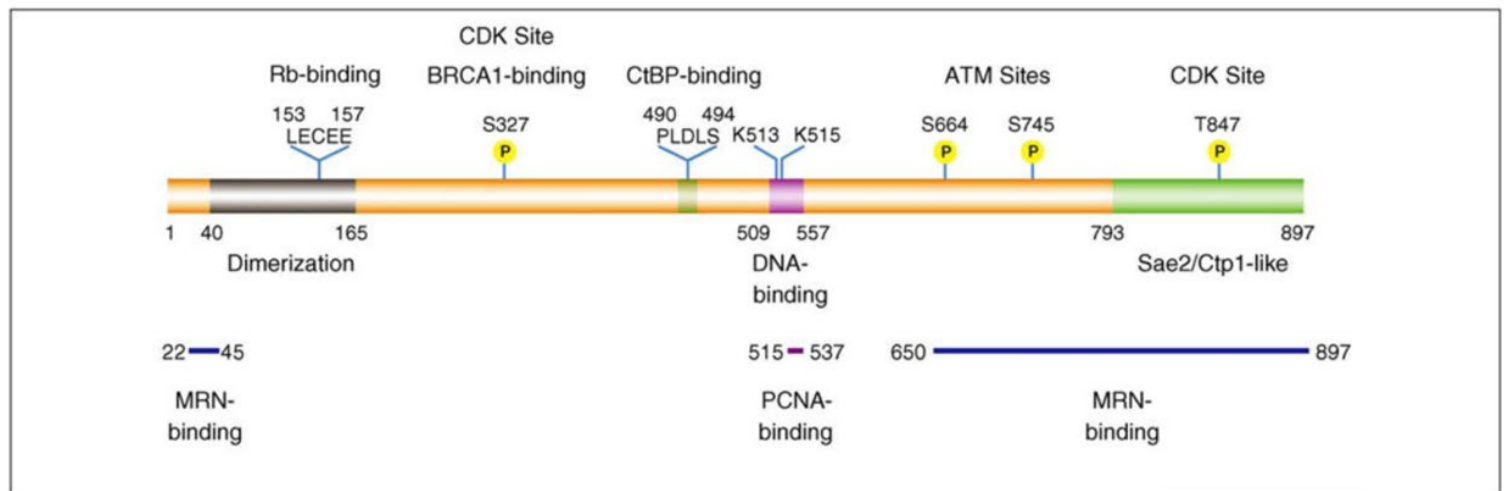
219-223:PRSLM

153-157:LECEE

58-62:LLKER

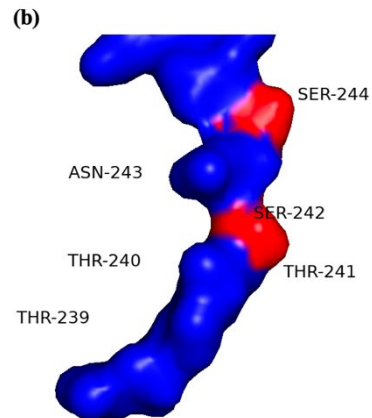
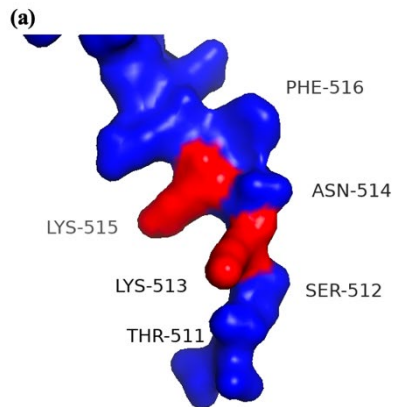


果蝇CtIP蛋白的预测结构

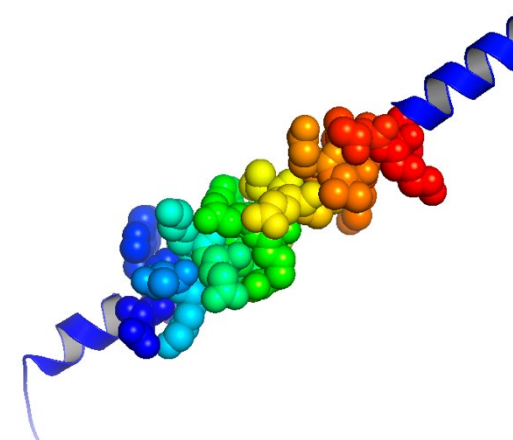


509-557: 无序结构
K513 & K515

238-266: 无序结构
S242 & S244

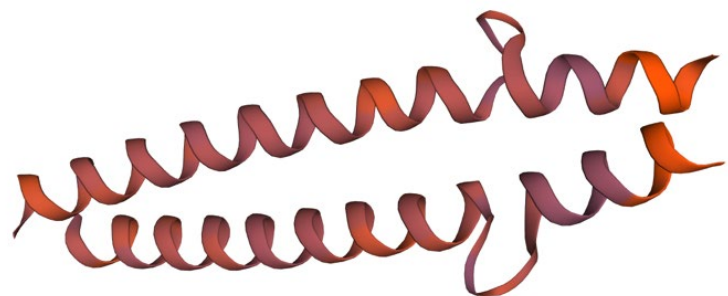
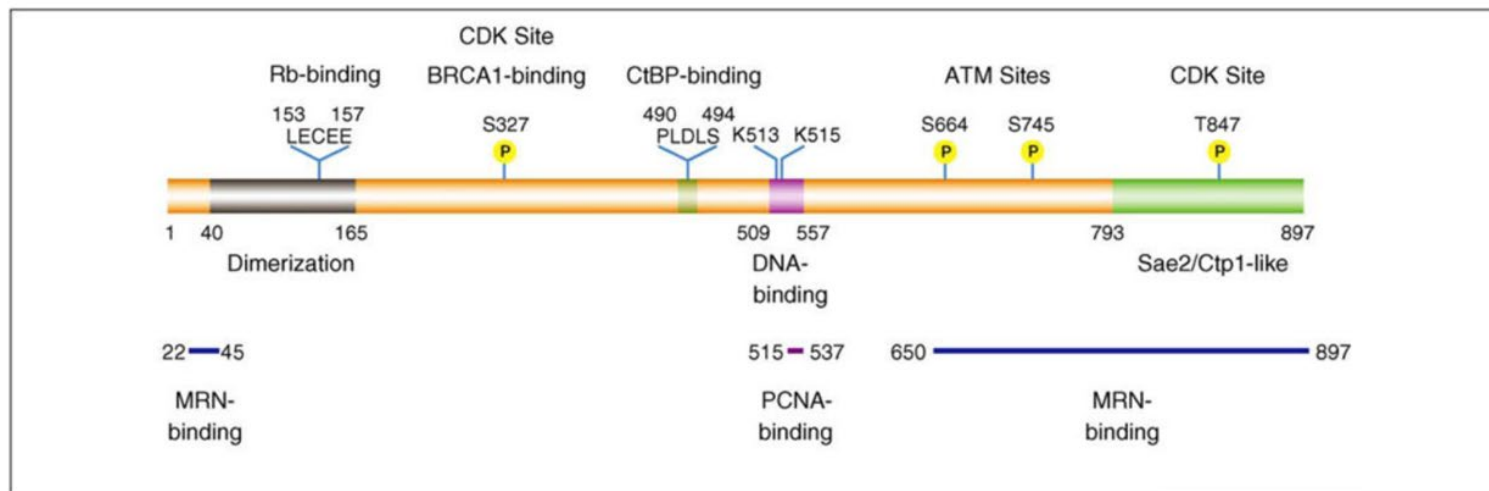


22-45



没有对应结构域?

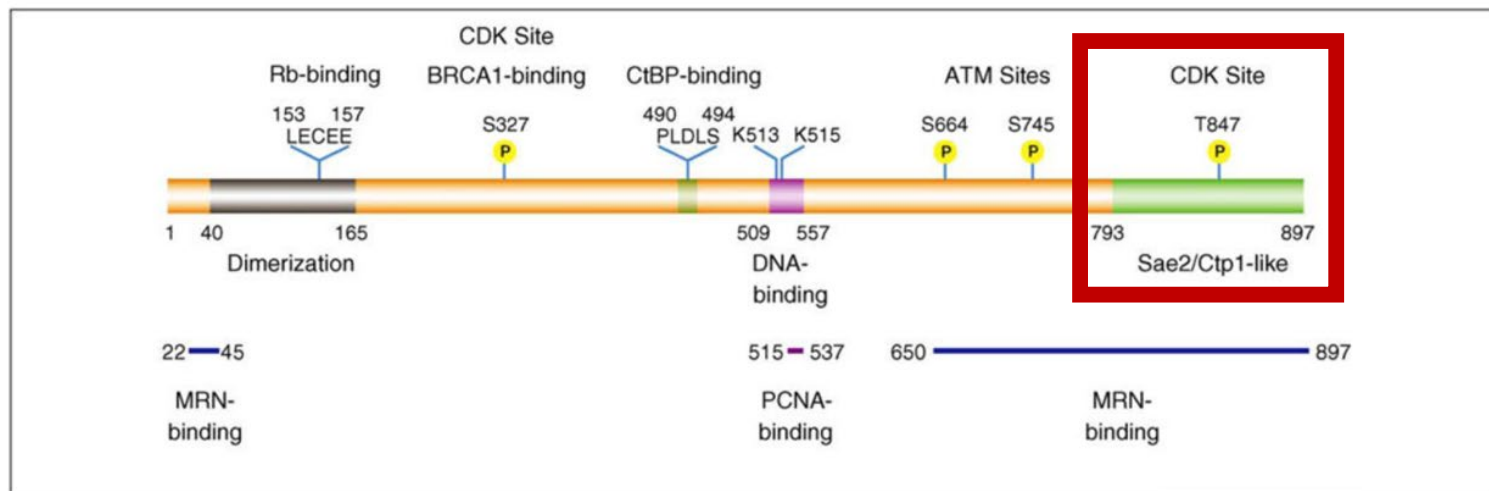
果蝇CtIP蛋白的预测结构



Q9VPF5 Q9VPF5_DROME SMR 预测:
N端存在与二聚化相关的螺旋

模版: Myc proto-oncogene protein (Human)

果蝇CtIP蛋白的预测结构



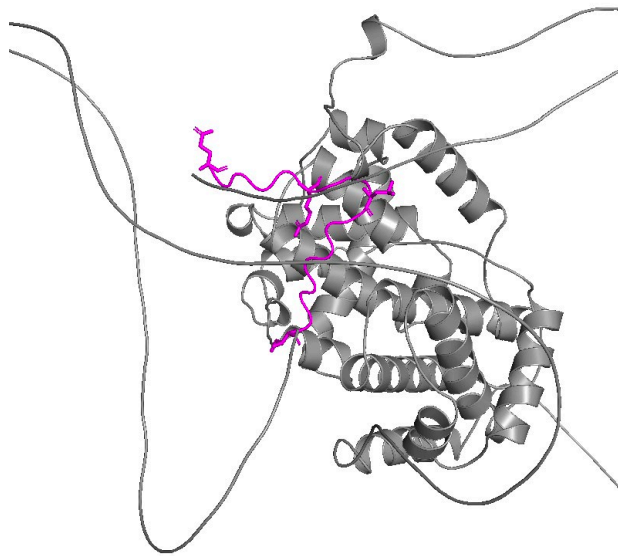
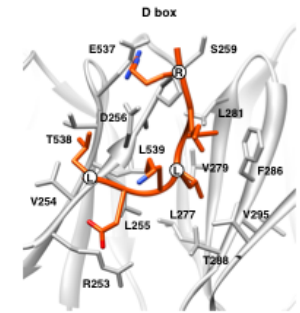
Residue conservation



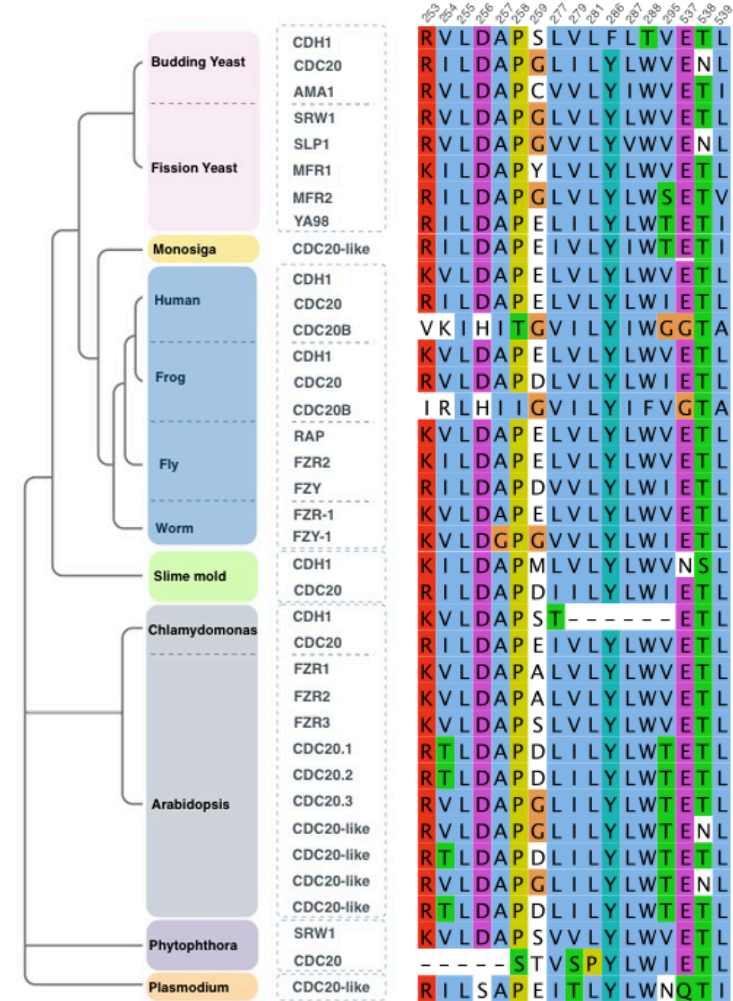
Legend: 0- Highly variable, 1, 2, 3, 4, 5- Neutral, 6, 7, 8- Highly conserved

Lambda Predict Protein(λ PP): C端高度保守;
T847 -> T462

CycB的降解子(degron) – D Box

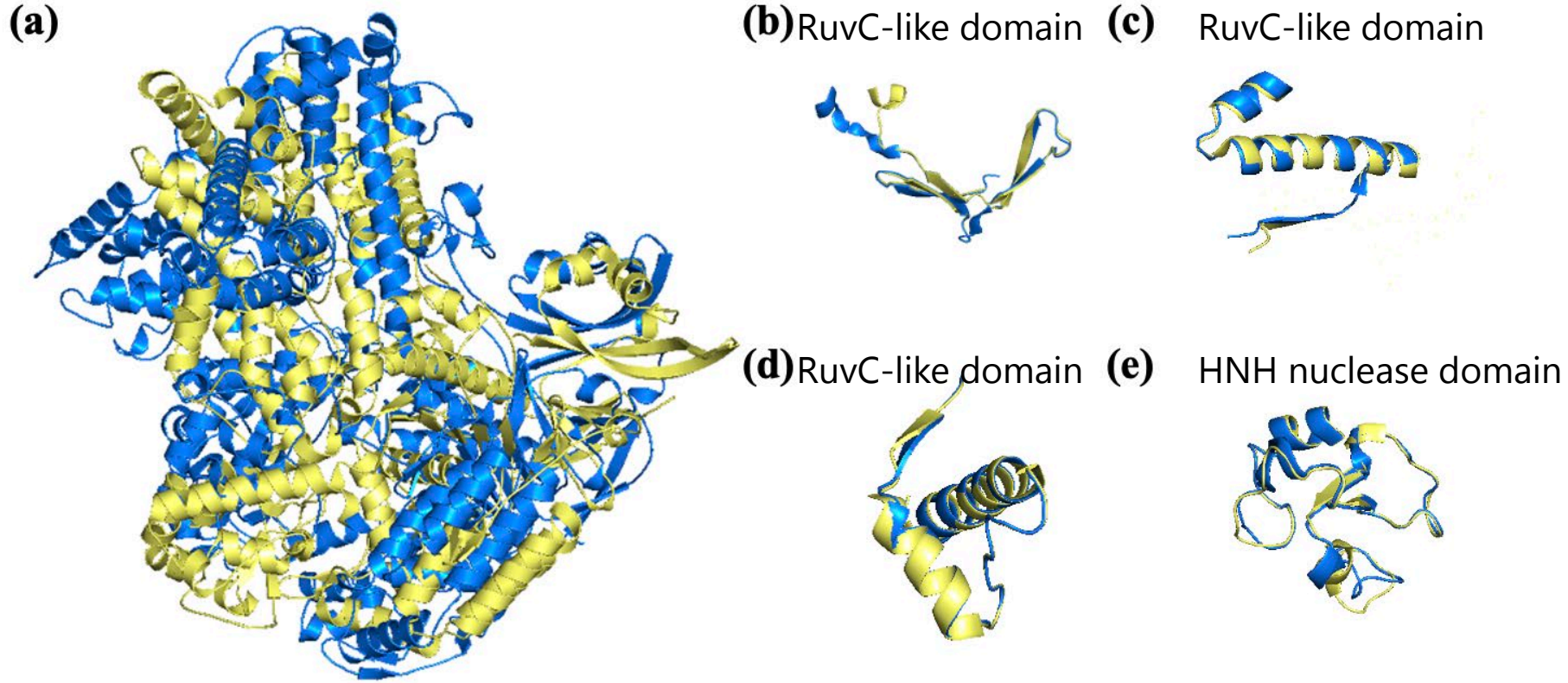


D Box of *Drosophila melanogaster* CycB(Structure Predicted by Alphafold2)

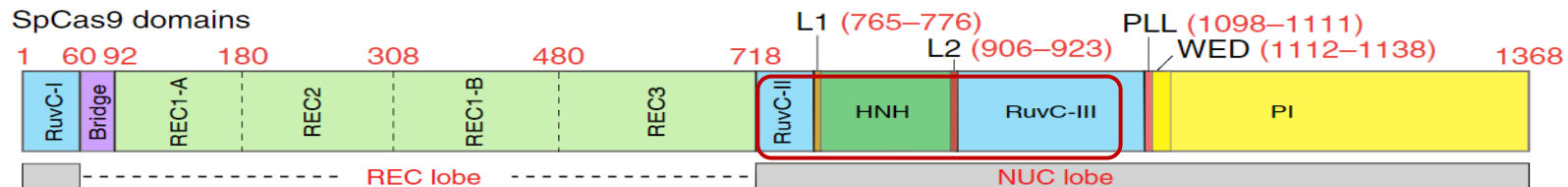


融合蛋白的结构预测

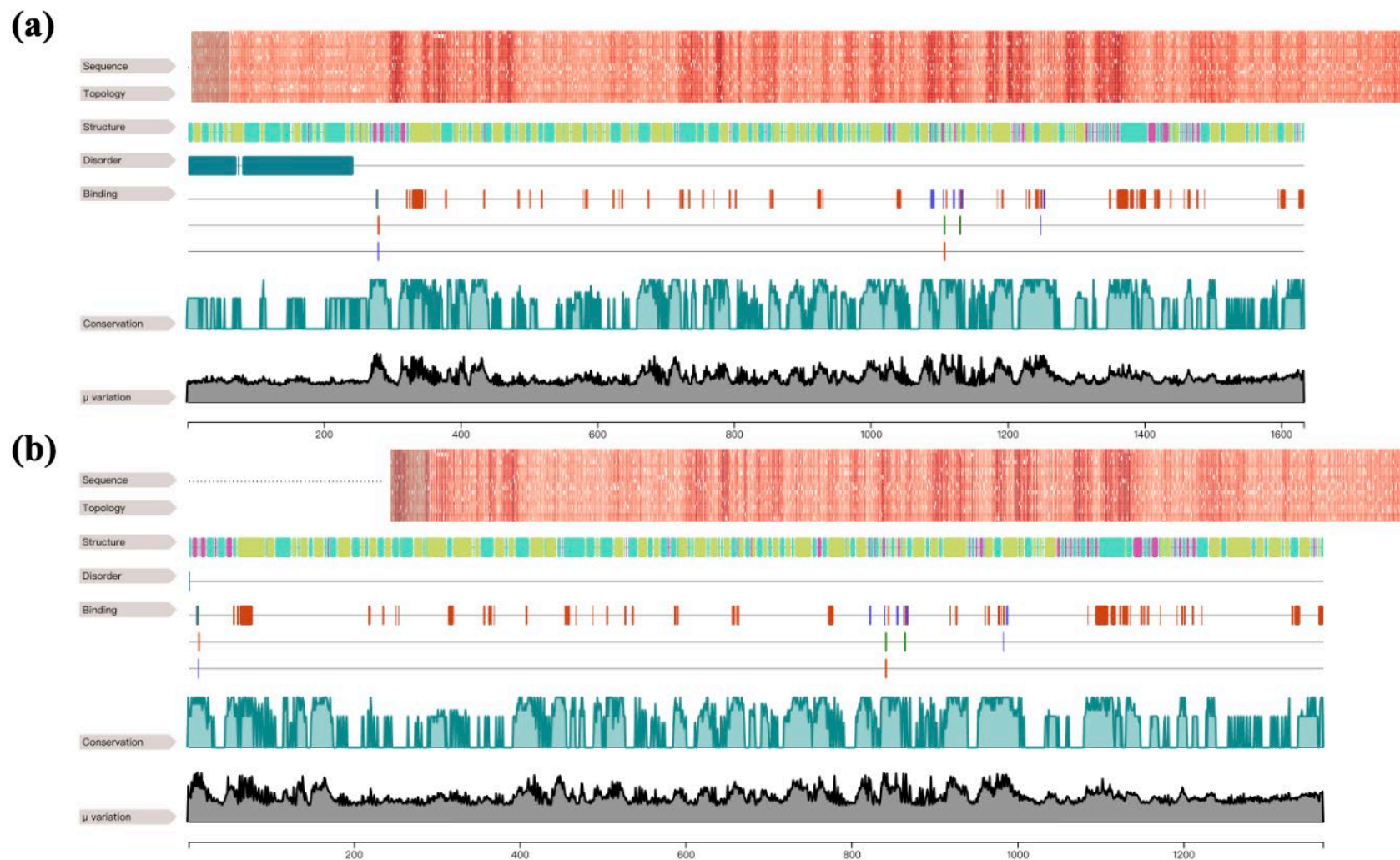
Alphafold2 预测Cas9的结构与测定Cas9结构在关键结构域结构吻合



Alphafold2 prediction(Blue) of SpyCas9 matches measured(Yellow) structure of SpyCas9



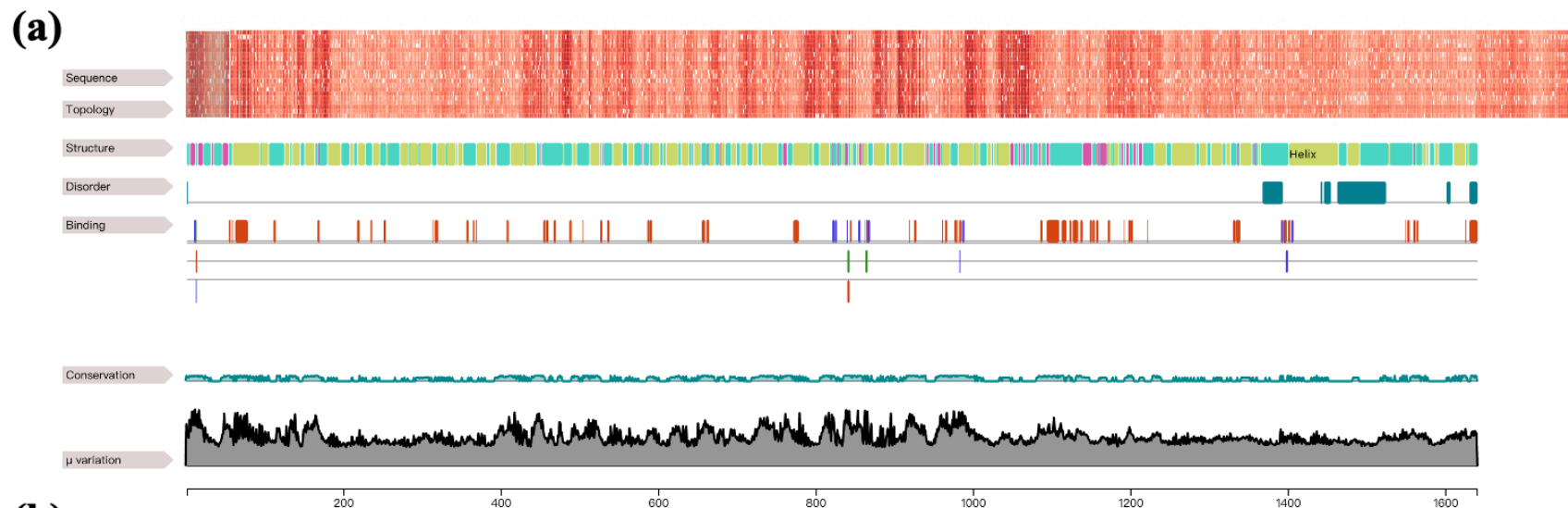
融合蛋白的结构预测



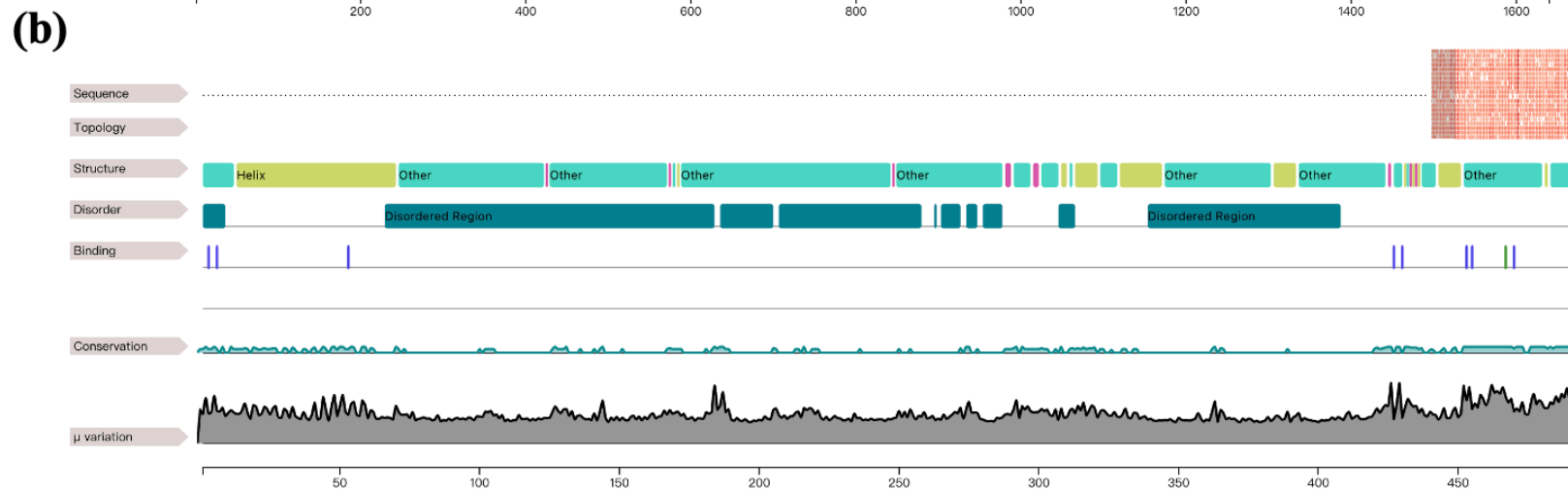
定位在细胞核

λ PP: CycB1-266-Spycas9 (a) and SpyCas9 (b)

Structural Prediction of fusion protein



定位在细胞核

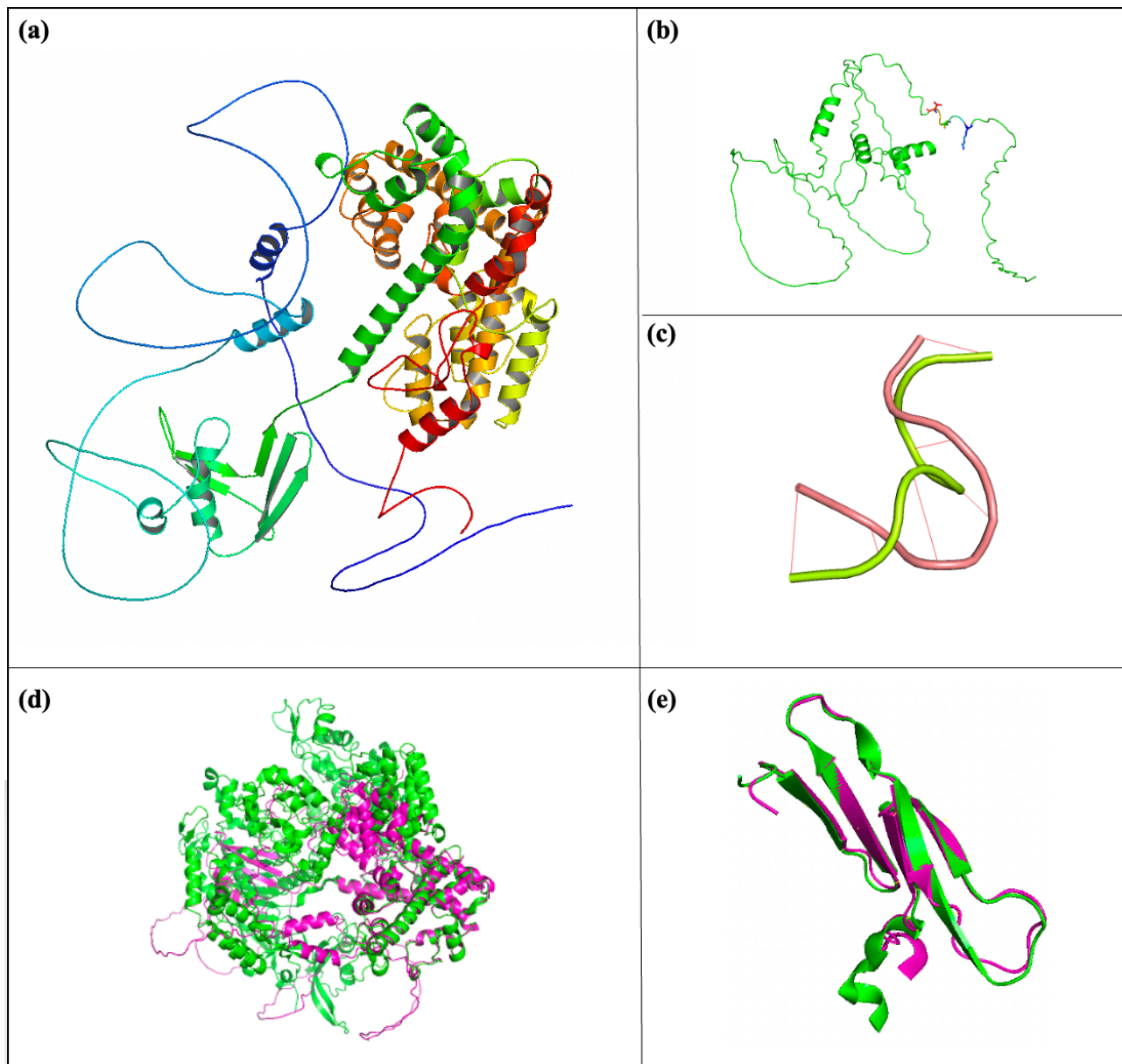


序列保守性出现变化

λ PP: *Spycas9-D.melanogaster CtIP*(a) and *D.melanogaster CtIP*(b)

融合蛋白结构预测

AlphaFold2 预测融合蛋白
1-780AA的结构



融合蛋白(粉色) 与
SpyCas9蛋白(绿色)结构
比对

融合蛋白中与D Box区段
对应的结构

预测融合蛋白D Box序列
(绿色) 和 CycB D Box序列
(粉色)比对

Cas9 蛋白 RuvC-like 结构
域(1-62)比对



总结与启示

1. 在融合蛋白中CtIP和CycB D Box 的功能可能受到影响 (保守性变化、预测结构变化)
2. 在融合蛋白中Cas9的部分内切酶活性可能保留 (预测结构相似)
3. 由于未预测完整融合蛋白的结构，以上结论的也需要进一步利用生物信息工具预测，或通过实验验证
4. 运用多种生物信息学工具相互验证，同时重点参考已有文献。

感谢罗静初老师和饶希晨学长的指导与帮助
感谢Dr. Jackson Champer的指导

感谢大家的倾听与交流～

