Bioinformatic analysis of Ran

Ran蛋白的生物信息学分析

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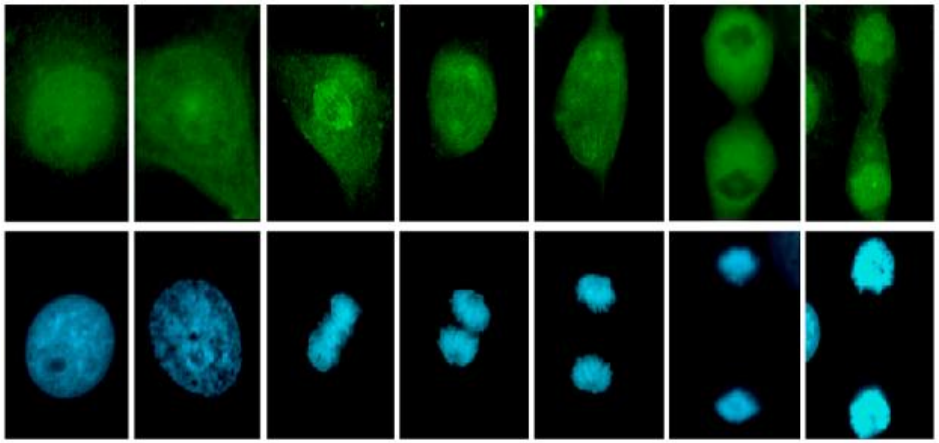
Part I: Background

The Ran (Ras-like Nuclear) GTPase

1. a member of Ras superfamily/small GTPase superfamily

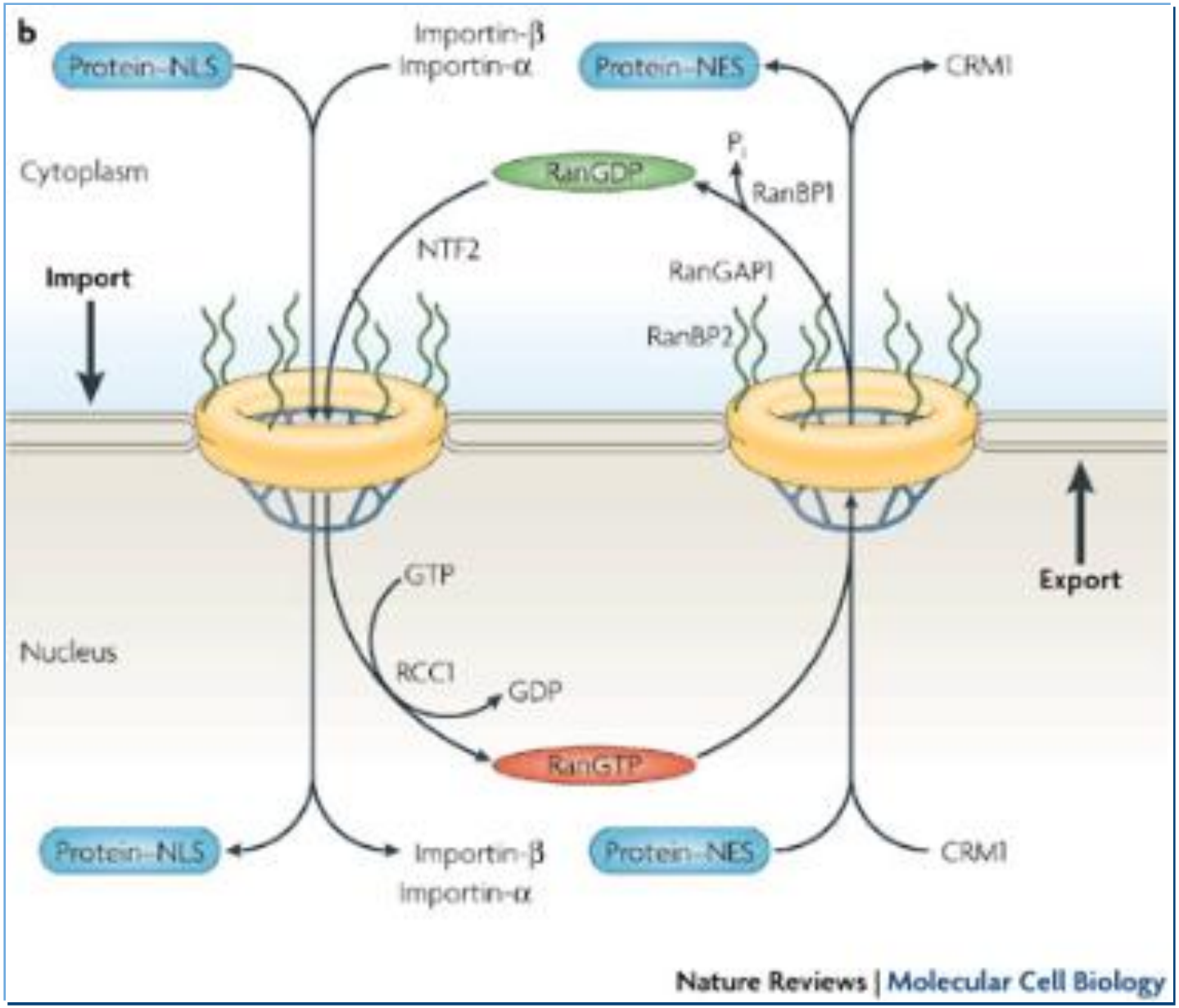
2. nuclear localization



3. very conservative (from yeast to human)

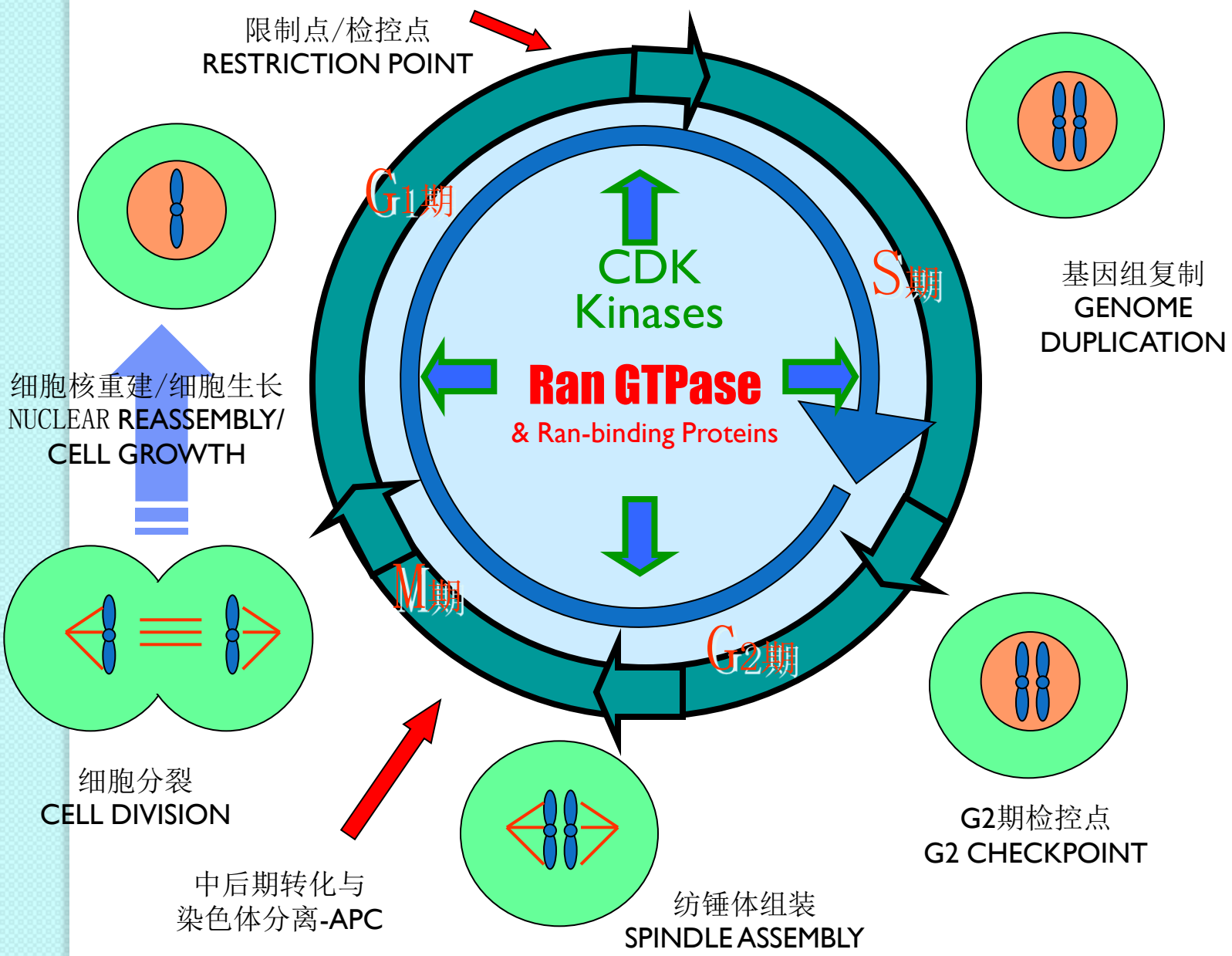
4. molecular weight, ~25Kd

5. GTPase activity



Nature Reviews Molecular Cell Biology **9**, 464-477 (June 2008)

A typical eukaryotic cell division cycle



Part II: Bioinformatic analysis of Ran

Ran (Human)

- Ref seq No.(mRNA) : NM_006325.3
- Gene Location:12q24.3
- Ref seq No.(Protein) : NP_006316.1
- Uniprot ID: P62826
- Protein length: 216AA
- No other isoforms in human
- Highly conservation

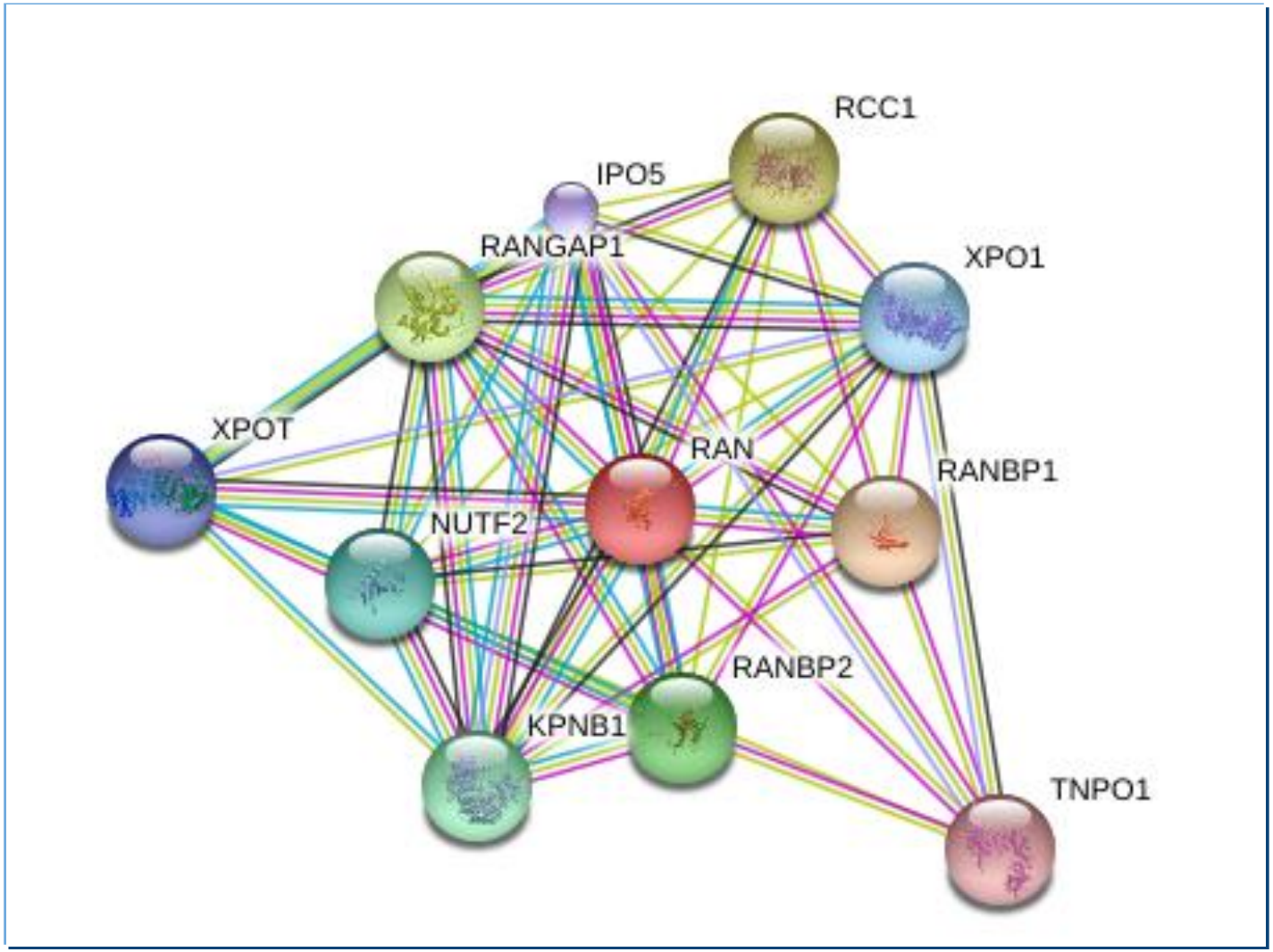
Results of Uniref100

Members Customize

	Cluster member(s)	Entry name	Status	Protein names	Organisms
☑	P62826	RAN_HUMAN	★	GTP-binding nuclear protein Ran	Homo sapiens (Human)
☑	Q3T054	RAN_BOVIN	★	GTP-binding nuclear protein Ran	Bos taurus (Bovine)
☑	P62825	RAN_CANFA	★	GTP-binding nuclear protein Ran	Canis familiaris (Dog) (Canis lupus familiaris
☑	Q4R4M9	RAN_MACFA	★	GTP-binding nuclear protein Ran	Macaca fascicularis (Crab-eating macaque) (Cyn
☑	P62827	RAN_MOUSE	★	GTP-binding nuclear protein Ran	Mus musculus (Mouse)
☑	Q5R556	RAN_PONAB	★	GTP-binding nuclear protein Ran	Pongo abelii (Sumatran orangutan)
☑	P62828	RAN_RAT	★	GTP-binding nuclear protein Ran	Rattus norvegicus (Rat)

Date of job execution	Jun 2, 2012
Running time	23.5 seconds
Identical positions	216
Identity	100%
Similar positions	0
Program	clustalo

Protein interaction （String）：



Ran-binding Proteins (1)

-------Regulators and effectors

1. the GTPase activating protein, RanGAP
2. the GDP-GTP exchange factor, RanGEF, RCC1
3. Ran-binding protein 1, RanBP1

Ran-binding Proteins (2)

-------Regulators and effectors

1. importins α, β,
   (NLS) （SV40 T-ag：PKKKRKV132；Nucleoplasmin： KR-10 a a -KKKL171）

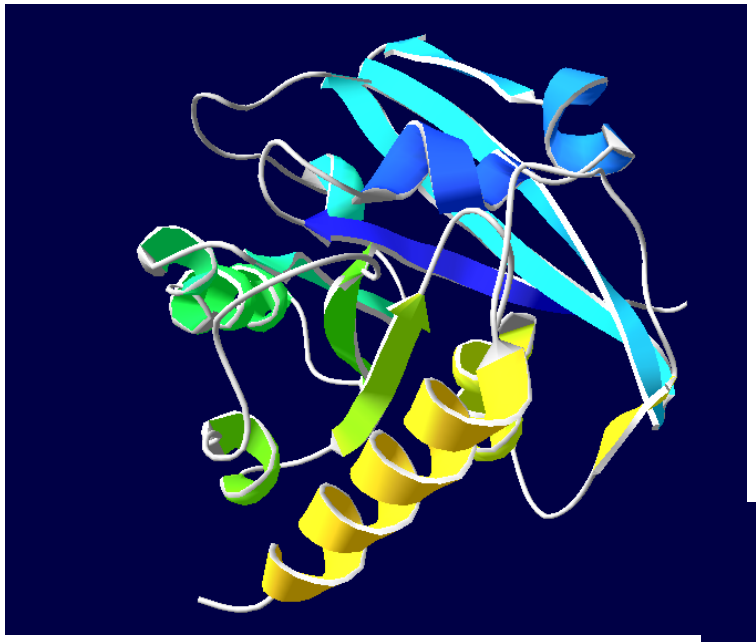
2. NTF2 (Nuclear Transport Factor 2, p10）

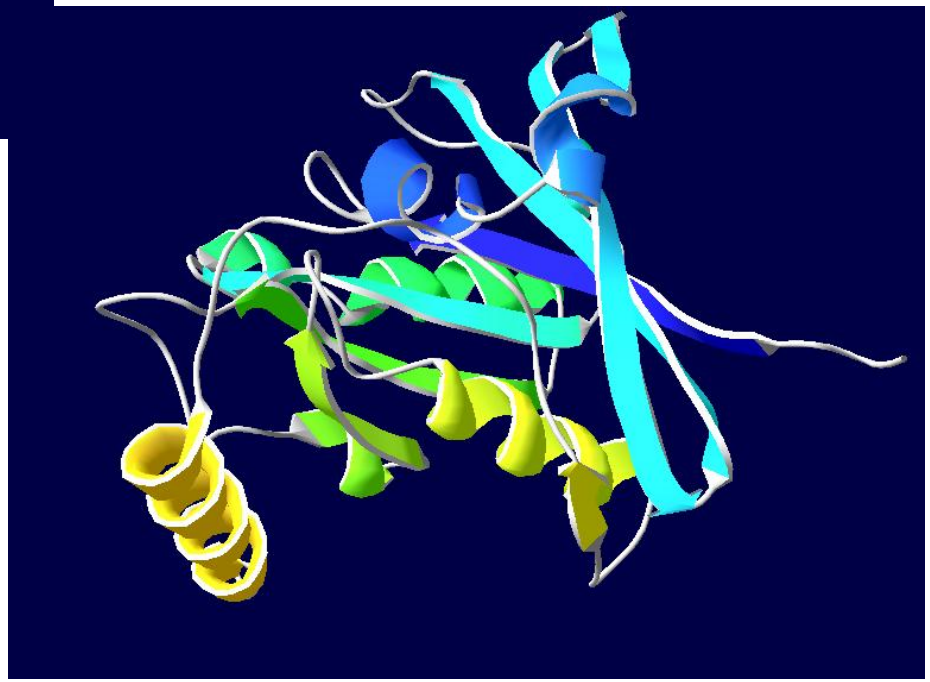
3. Exportin (CRM1, Chromosome Maintenance Region 1)
   (NES)（MAPKK：D LQ K K L E E L E LD）

4. nucleoporins (FxFG)

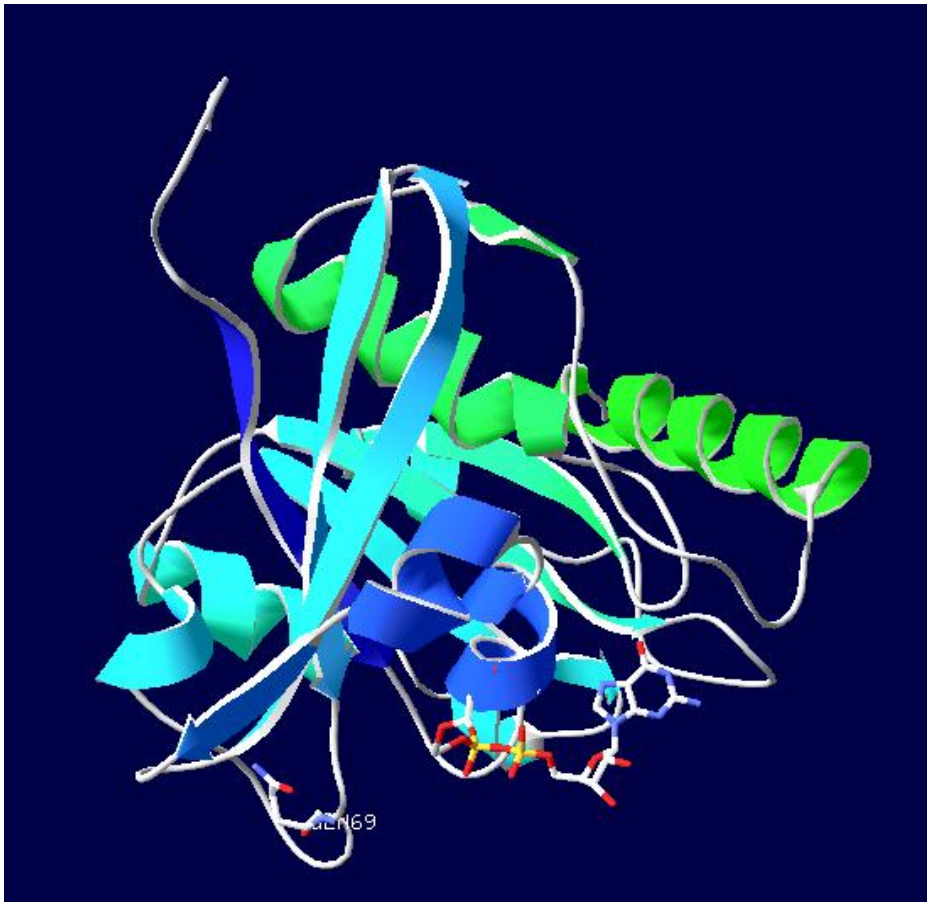
5。 Ran mutants: RanT24N (苏24天冬)(GDP),
RanQ69L (谷69亮)(GTP)

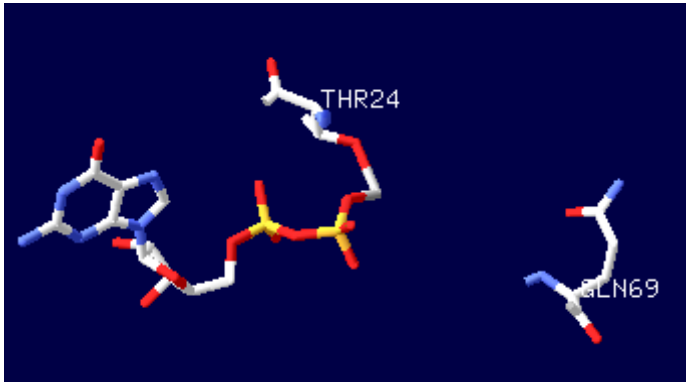
3D structure of Ran



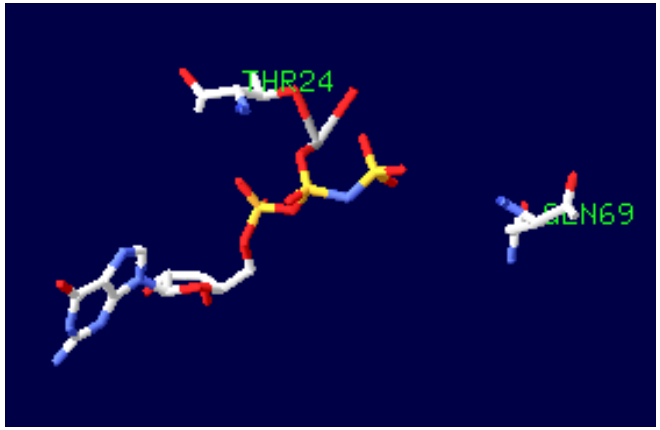


RanGDP 3GJ0

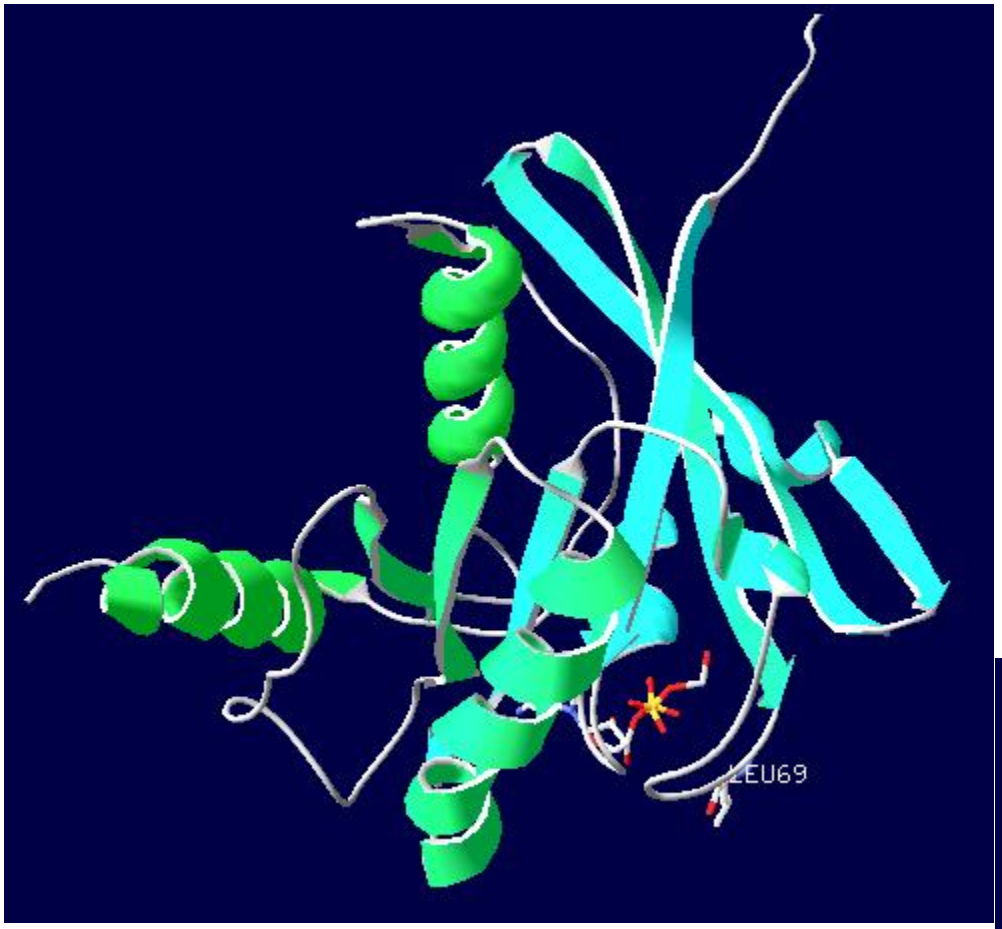


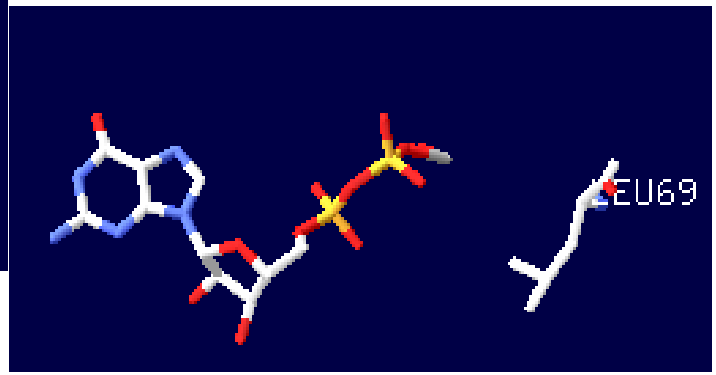


RanGTP 1RRP



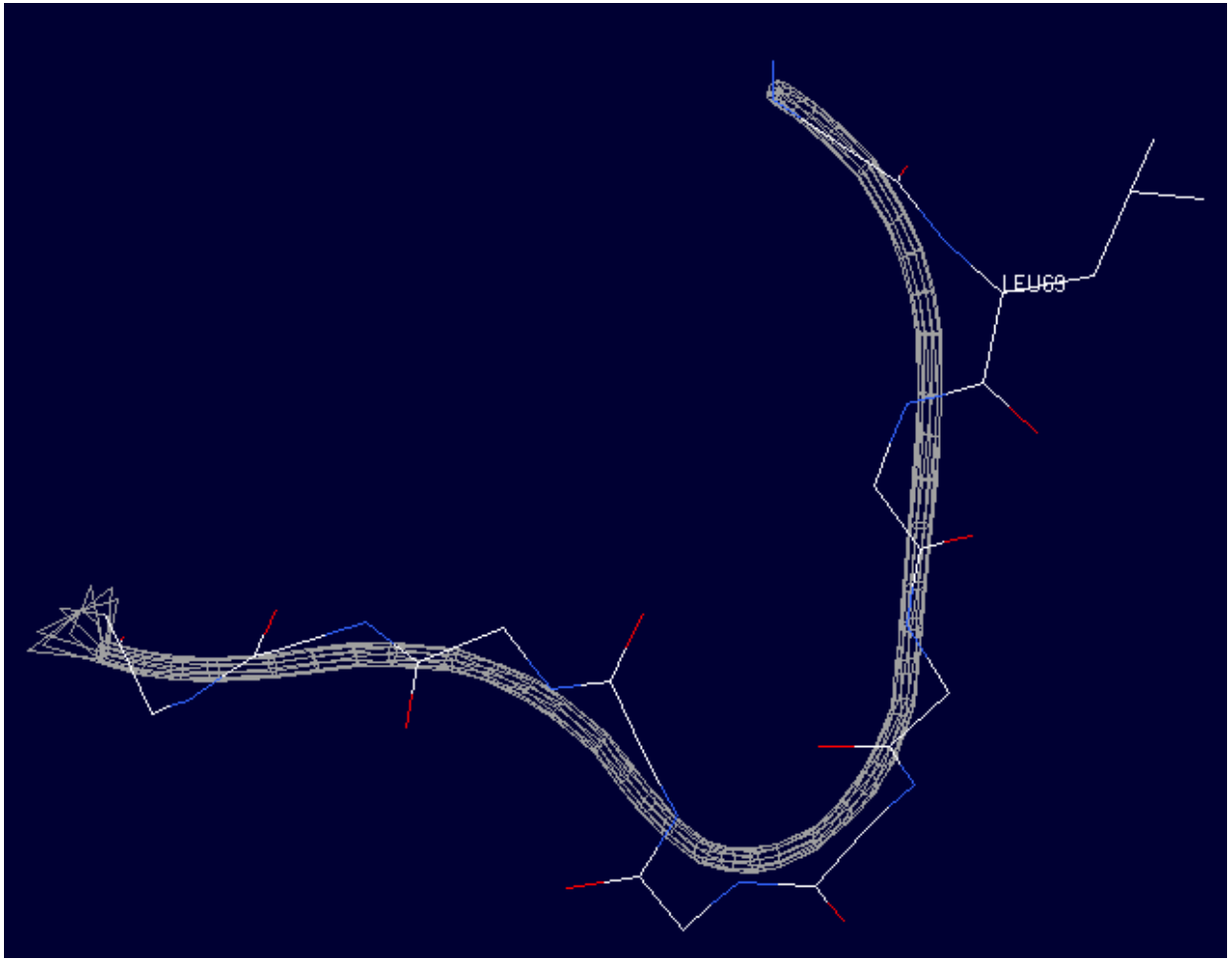
Ran Q69L Mutant 3RAN

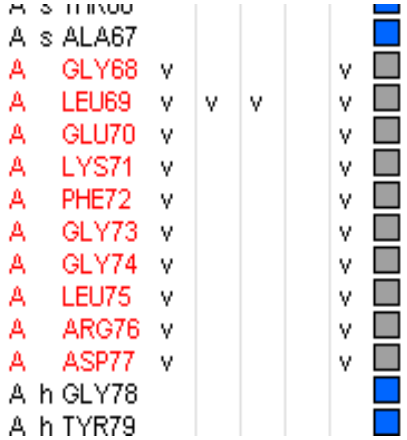




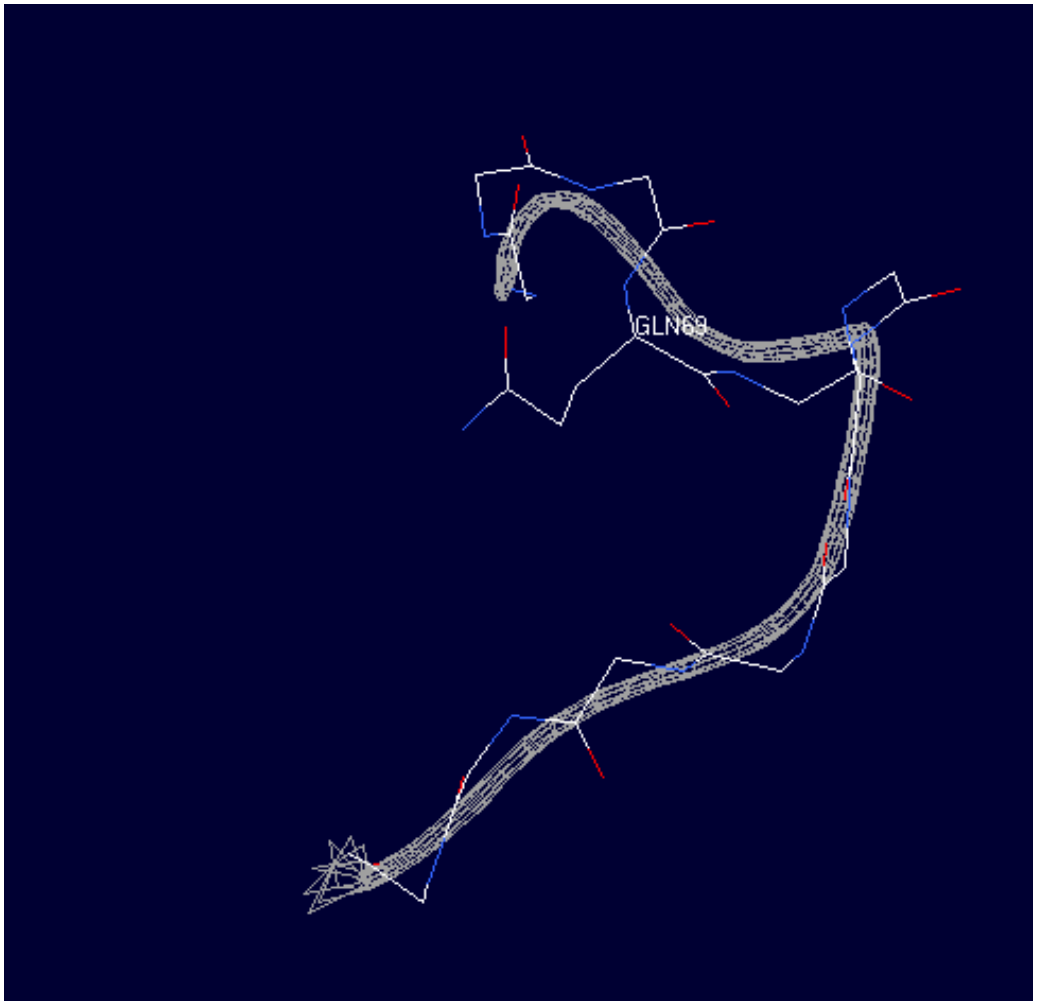
Structural differences of Ran mutants:

- Q69L





- WT



A s TRP64
A s ASP65
A THR66 v v
A ALA67 v v
A GLY68 v v
A GLN69 v v v v
A GLU70 v v
A LYS71 v v
A PHE72 v v
A GLY73 v v
A GLY74 v v
A LEU75 v v
A ARG76 v v
A ASP77 v v
A h GLY78

Phosphorylation prediction 1:

NetPhos 2.0 Server

The NetPhos 2.0 server produces neural network predictions for serine, threonine and tyrosine phosphorylation

Kinase specific phosphorylation predictions are available at: http://www.cbs.dtu.dk/services/NetPhosK/

Instructions | Output format

SUBMISSION

Paste a single sequence or several sequences in FASTA format into the field below:

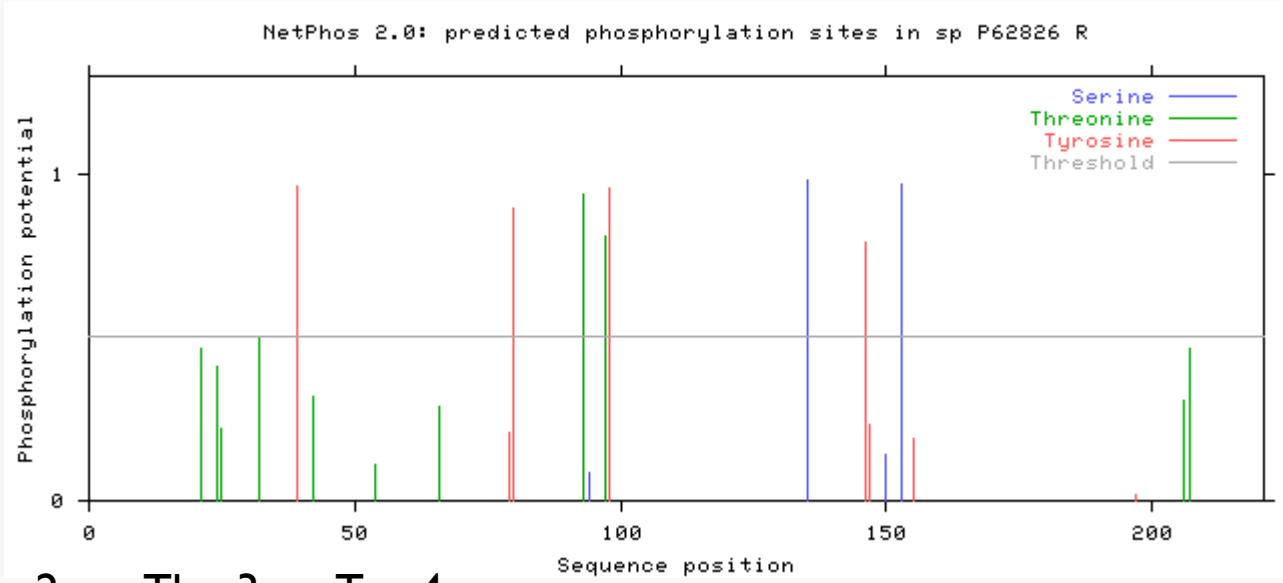
Submit a file in FASTA format directly from your local disk:

选择文件 没有选择文件

Predict on: ☑ tyrosine ☑ serine ☑ threonine

☑ **Generate graphics** Submit Clear fields

Result:



Ser: 2 Thr: 3 Tyr: 4

Serine predictions

Name	Pos	Context	Score	Pred
sp_P62826_R	94	FDVTSRVTY	0.085	.
sp_P62826_R	135	VKAKSIVFH	0.984	*S*
sp_P62826_R	150	YYDISAKSN	0.144	.
sp_P62826_R	153	ISAKSNYNF	0.970	*S*

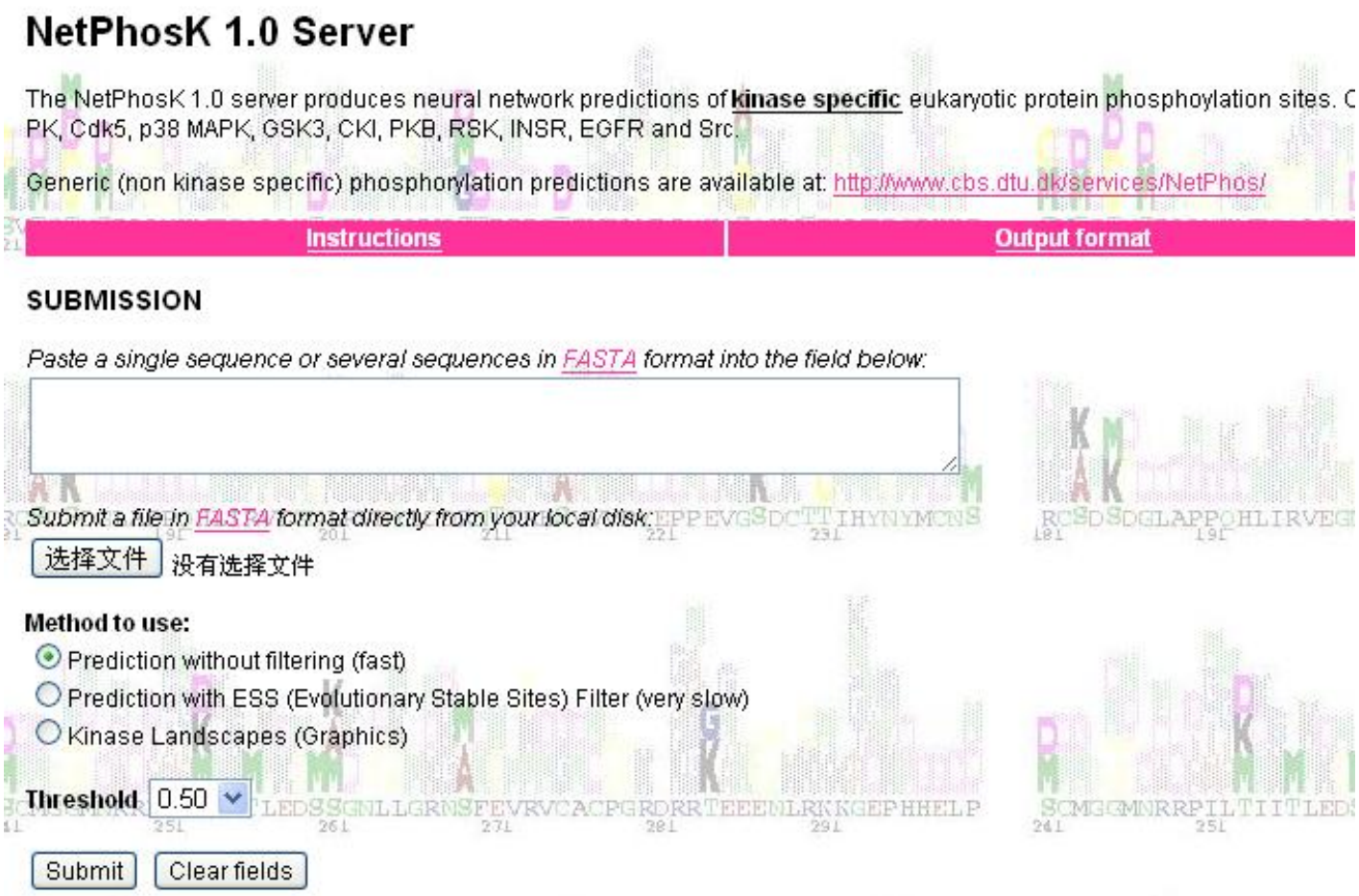
Threonine predictions

Name	Pos	Context	Score	Pred
sp_P62826_R	21	GDGGTGKTT	0.466	.
sp_P62826_R	24	GTGKTTFVK	0.408	.
sp_P62826_R	25	TGKTTFVKR	0.219	.
sp_P62826_R	32	KRHLTGEFE	0.501	*T*
sp_P62826_R	42	KYVATLGVE	0.317	.
sp_P62826_R	54	LVFHTNRGP	0.109	.
sp_P62826_R	66	NVWDTAGQE	0.290	.
sp_P62826_R	93	MFDVTSRVT	0.938	*T*
sp_P62826_R	97	TSRVTYKNV	0.807	*T*
sp_P62826_R	206	EVAQTTALP	0.307	.
sp_P62826_R	207	VAQTTALPD	0.464	.

Tyrosine predictions

Name	Pos	Context	Score	Pred
sp_P62826_R	39	FEKKYVATL	0.965	*Y*
sp_P62826_R	79	LRDGYYIQA	0.209	.
sp_P62826_R	80	RDGYYIQAQ	0.898	*Y*
sp_P62826_R	98	SRVTYKNVP	0.959	*Y*
sp_P62826_R	146	KNLQYYDIS	0.793	*Y*
sp_P62826_R	147	NLQYYDISA	0.236	.
sp_P62826_R	155	AKSNYNFEK	0.193	.
sp_P62826_R	197	LAAQYEHDL	0.021	.

Phosphorylation prediction II:



Results :

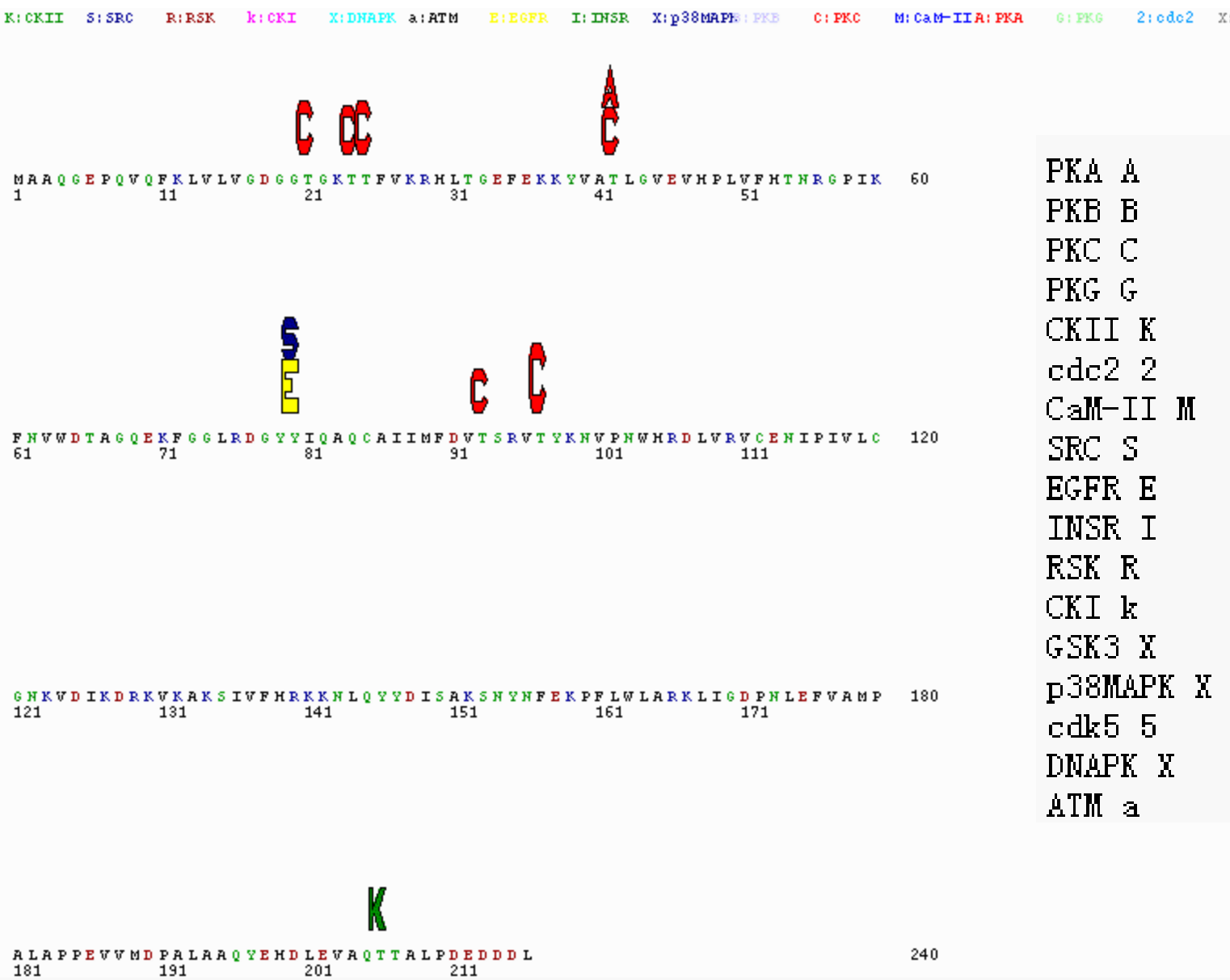
```
Method: NetPhosK without ESS filtering:
Query:  sp_P62826_RAN_HUMAN

Site   Kinase   Score
----------------------
T-21      PKC    0.66
T-24      PKC    0.59
T-25      PKC    0.66
T-42      PKC    0.56
T-42      PKA    0.52
Y-80      SRC    0.52
Y-80     EGFR    0.64
T-93      PKC    0.53
T-97      PKC    0.85
T-206    CKII    0.50
----------------------
Highest Score:   0.85 PKC at position 97
```

期望阈值改为0.70

```
Method: NetPhosK without ESS filtering:
Query:  sp_P62826_RAN_HUMAN

Site   Kinase   Score
----------------------
T-97      PKC    0.85
----------------------
Highest Score:   0.85 PKC at position 97
```


K:CKII S:SRC R:RSK k:CKI X:DNAPK a:ATM E:EGFR I:INSR X:p38MAPK B:PKB C:PKC M:CaM-II A:PKA G:PKG 2:cdc2 X:GSK3 5:cdk5
MAAQGEPQVQFKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPLVFHTNRGPIK 60
1 11 21 31 41 51
FNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKNVPNWHRDLVRVCENIPIVLC 120
61 71 81 91 101 111
GNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFLWLARKLIGDPNLEFVAMP 180
121 131 141 151 161 171
ALAPPEVVMDPALAAQYEHDLEVAQTTALPDEDDDL 240
181 191 201 211
PKA A
PKB B
PKC C
PKG G
CKII K
cdc2 2
CaM-II M
SRC S
EGFR E
INSR I
RSK R
CKI k
GSK3 X
p38MAPK X
cdk5 5
DNAPK X
ATM a

- Ser135被PAK4磷酸化 促进分裂期Ran与纺锤体组分结合（J Cell Biol. 2010 Sep 6;190(5):807-22.）
- Ser135被Plk1磷酸化 促进双极纺锤体的形成 （Biochem Biophys Res Commun. 2006 Oct 13;349(1):144-52.）

Ps：其他蛋白修饰预测工具

http://www.cbs.dtu.dk/services/

- O-糖基化：NetOGlyc
- N-糖基化：NetNGlyc
- 蛋白酶体降解位点：Prop


Thank
You!

Nat Cell Biol. 2010 Jul;12(7):703-10. Epub 2010 Jun 6.

Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP.

Dishinger JF, Kee HL, Jenkins PM, Fan S, Hurd TW, Hammond JW, Truong YN, Margolis B, Martens JR, Verhey KJ.

Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.

Abstract

The biogenesis, maintenance and function of primary cilia are controlled through intraflagellar transport (IFT) driven by two kinesin-2 family members, the heterotrimeric KIF3A/KIF3B/KAP complex and the homodimeric KIF17 motor. How these motors and their cargoes gain access to the ciliary compartment is poorly understood. Here, we identify a ciliary localization signal (CLS) in the KIF17 tail domain that is necessary and sufficient for ciliary targeting. Similarities between the CLS and classic nuclear localization signals (NLSs) suggest that similar mechanisms regulate nuclear and ciliary import. We hypothesize that ciliary targeting of KIF17 is regulated by a ciliary-cytoplasmic gradient of the small GTPase Ran, with high levels of GTP-bound Ran (RanGTP) in the cilium. Consistent with this, cytoplasmic expression of GTP-locked Ran(G19V) disrupts the gradient and abolishes ciliary entry of KIF17. Furthermore, KIF17 interacts with the nuclear import protein importin-beta2 in a manner dependent on the CLS and inhibited by RanGTP. We propose that Ran has a global role in regulating cellular compartmentalization by controlling the shuttling of cytoplasmic proteins into nuclear and ciliary compartments.

Nat Cell Biol. 2012 Mar 4;14(4):431-7. doi: 10.1038/ncb2450.

A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia.

Kee HL, Dishinger JF, Blasius TL, Liu CJ, Margolis B, Verhey KJ.

Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.

Abstract

The cilium is a microtubule-based organelle that contains a unique complement of proteins for cell motility and signalling functions. Entry into the ciliary compartment is proposed to be regulated at the base of the cilium. Recent work demonstrated that components of the nuclear import machinery, including the Ran GTPase and importins, regulate ciliary entry. We hypothesized that the ciliary base contains a ciliary pore complex whose molecular nature and selective mechanism are similar to those of the nuclear pore complex. By microinjecting fluorescently labelled dextrans and recombinant proteins of various sizes, we characterize a size-dependent diffusion barrier for the entry of cytoplasmic molecules into primary cilia in mammalian cells. We demonstrate that nucleoporins localize to the base of primary and motile cilia and that microinjection of nucleoporin-function-blocking reagents blocks the ciliary entry of kinesin-2 KIF17 motors. Together, this work demonstrates that the physical and molecular nature of the ciliary pore complex is similar to that of the nuclear pore complex, and further extends functional parallels between nuclear and ciliary import.