

REGOLAZIONE DEL CICLO CELLULARE

www.fisiokinesiterapia.biz

Siti utili

Animazione sul ciclo cellulare e su come è stata scoperta la sua regolazione:

<http://www.usask.ca/biology/biol316/visgen/CELL.mov>

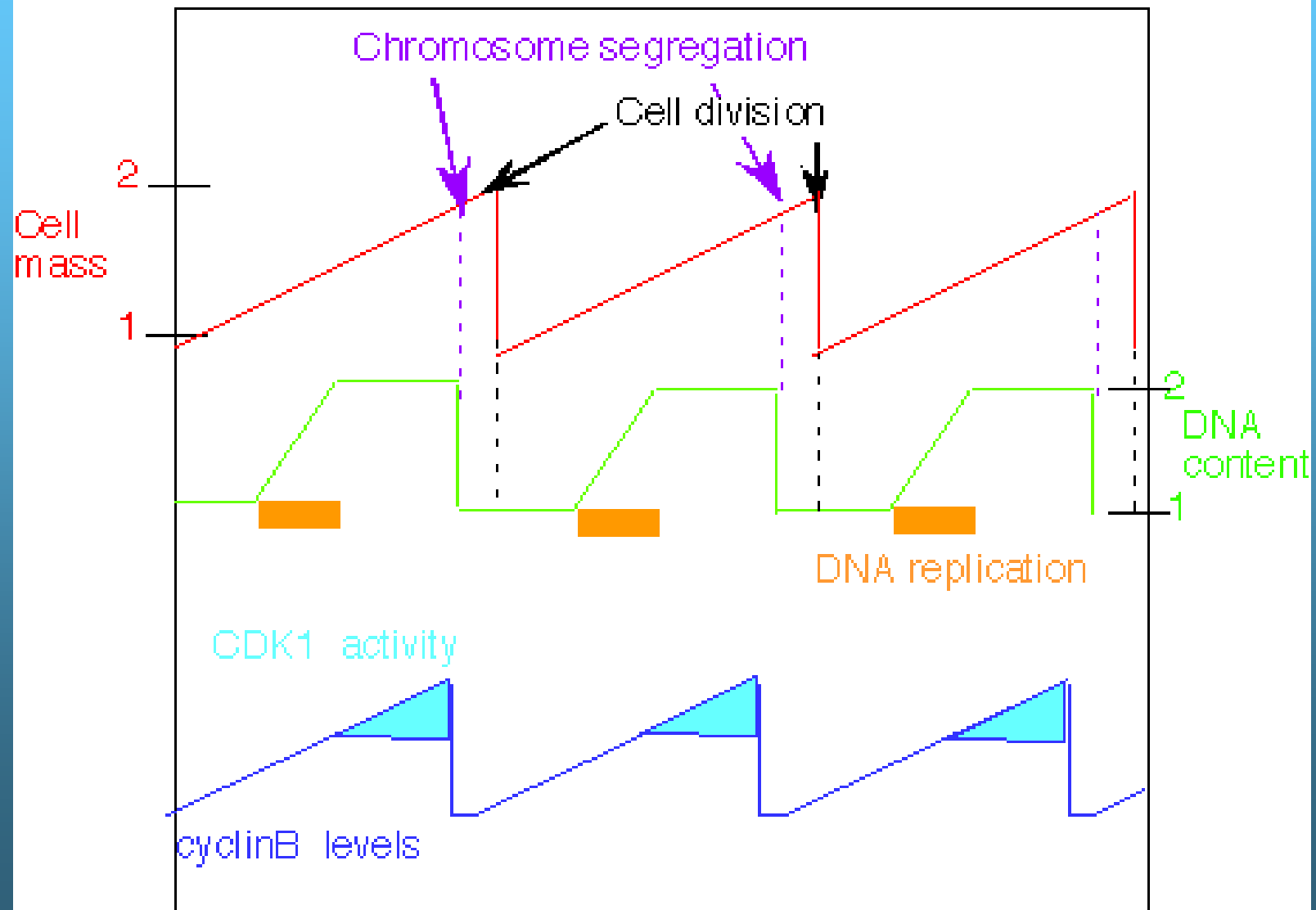
Sito sul lievito *Saccharomyces cerevisiae*:

http://biochemie.web.med.uni-muenchen.de/Yeast_Biology/

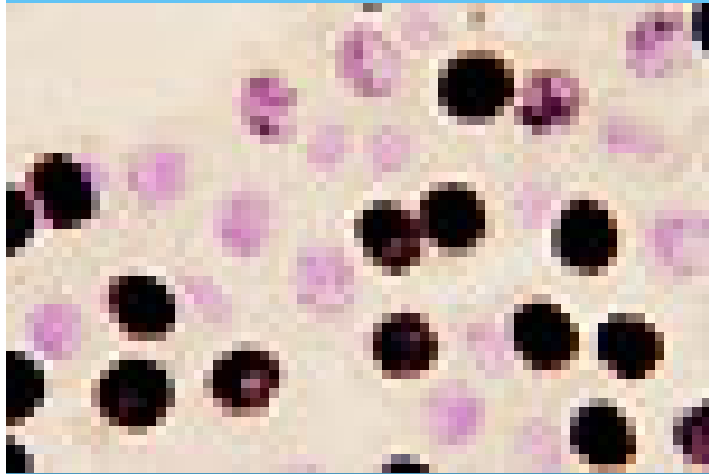
Ottimo sito sulla regolazione del ciclo cellulare, con spiegazioni dettagliate:

<http://www-rcf.usc.edu/~forsburg/cclecture.html>

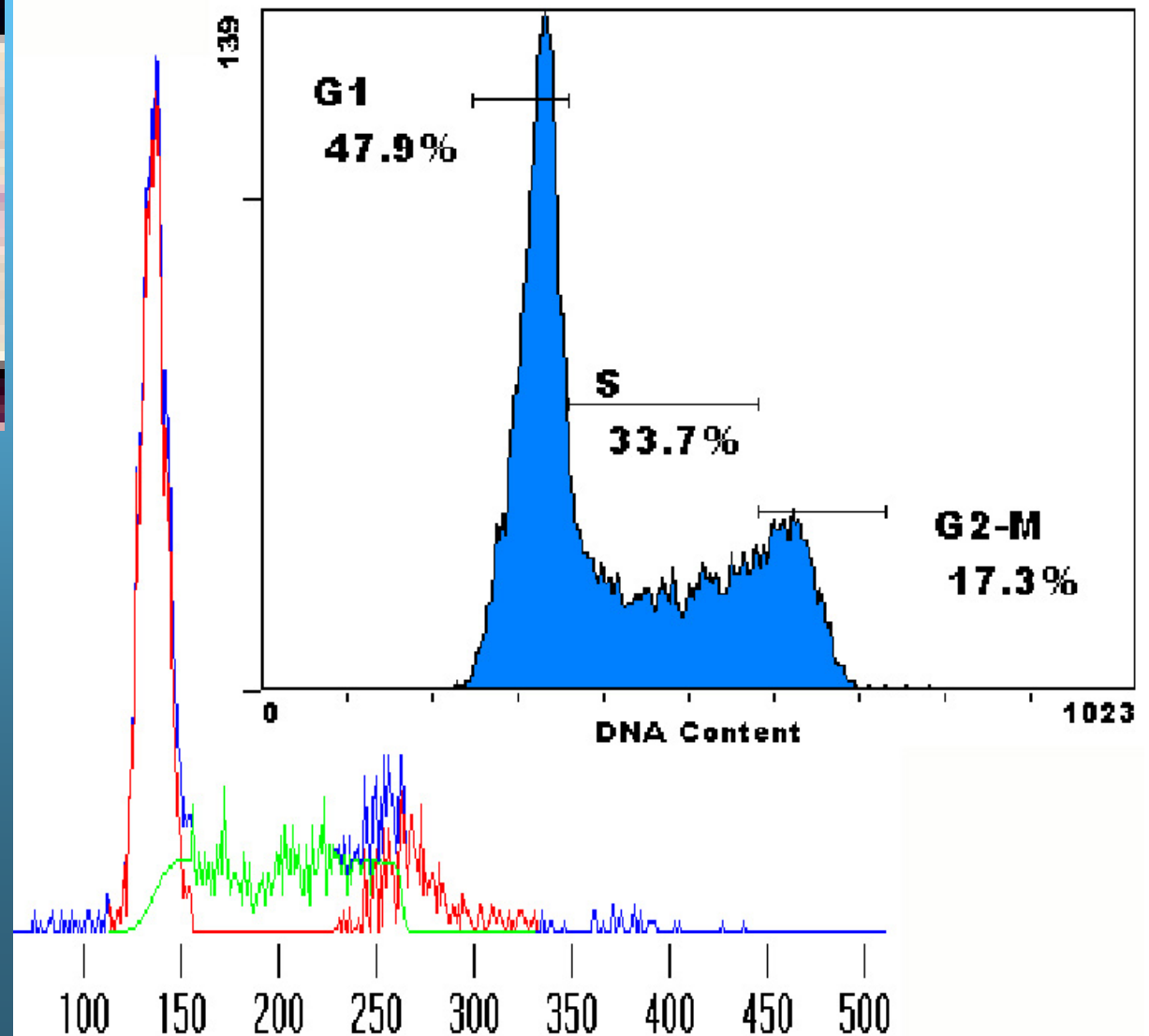
Il ciclo cellulare e il primo sistema di regolazione identificato



Alcune metodiche



E, naturalmente,
l'analisi genetica, a
partire da semplici
cellule aploidi, i
lieviti....

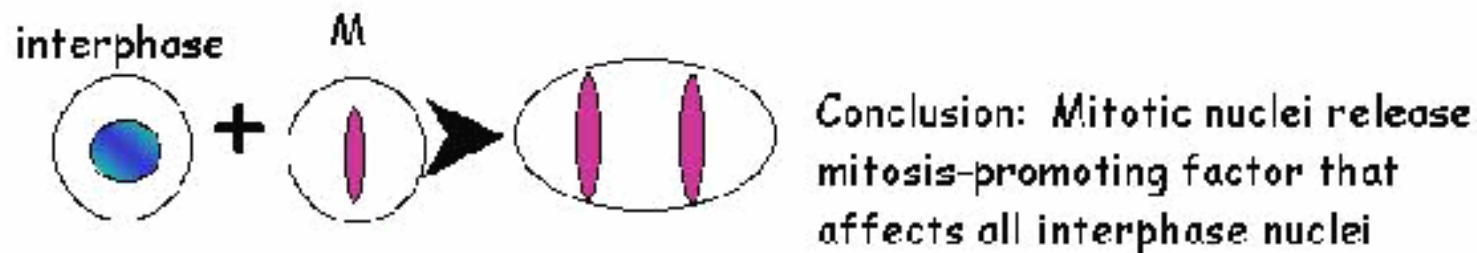
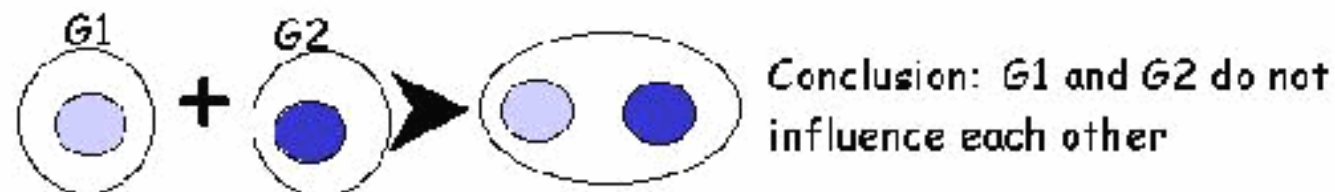
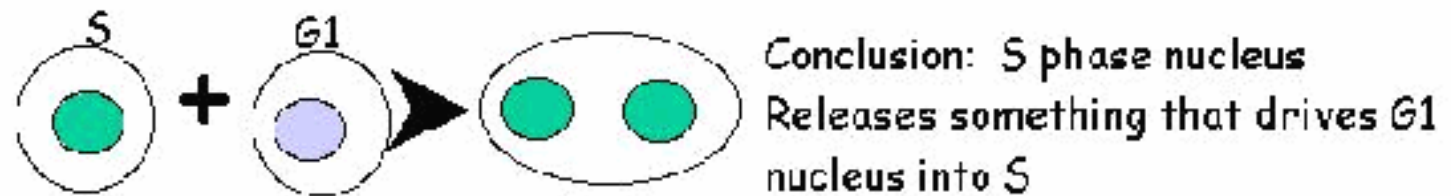


rassegna delle principali scoperte

- Esperimenti di fusione cellulare
 - Esperimenti con i lieviti
- Esperimenti con uova di *Xenopus*
- Esperimenti con embrioni di riccio di mare

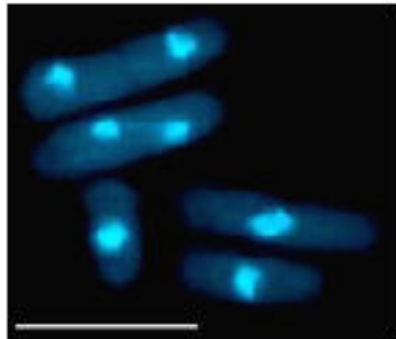
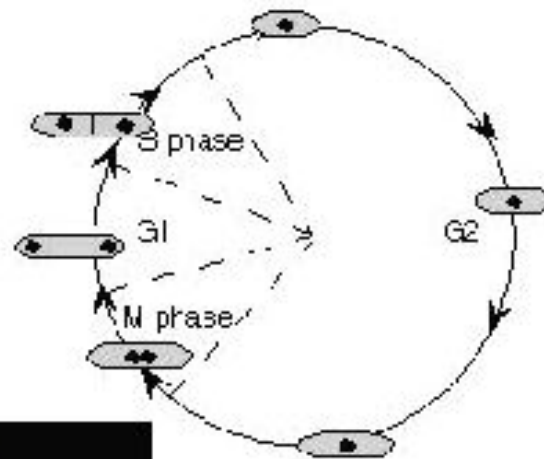
Esperimenti di fusione cellulare

Classic Experiment: Rao and Johnson Nuclear fusion



I lieviti

Yeast: genetic key to the cell cycle



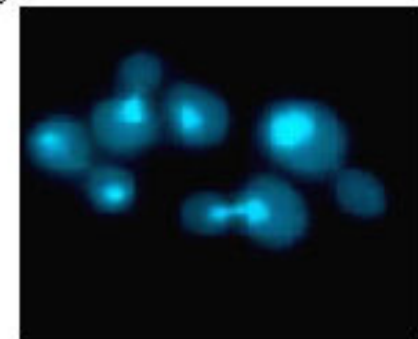
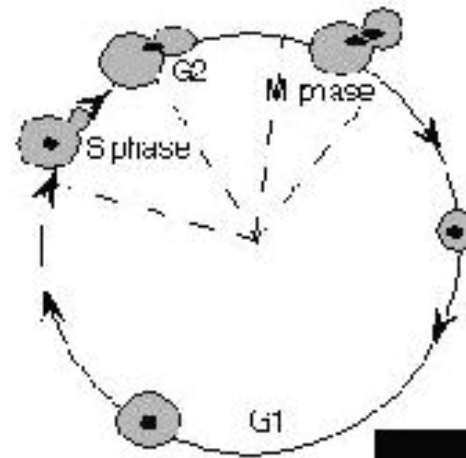
pombe
significa
birra in
Swahili

Schizosaccharomyces pombe

"Fission yeast"

Long G2, short G1

Nurse2: isolate mutants blocked at specific cell cycle points: growth without division (cdc phenotype). Assess DNA content, nuclear morphology



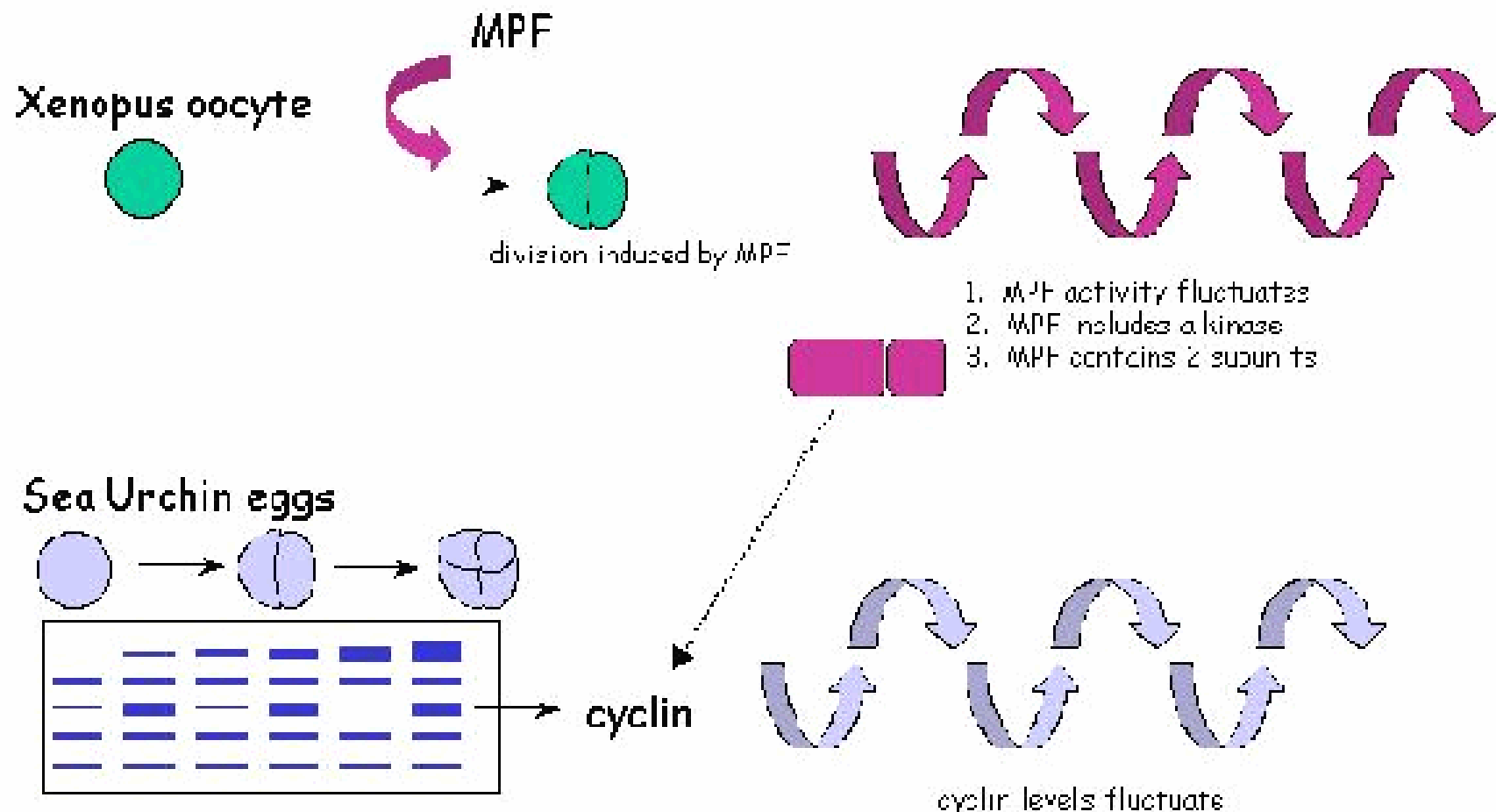
Saccharomyces cerevisiae

"Budding yeast" (brewer's, baker's)

Long G1, short G2

Hartwell: isolate mutants blocked at specific cell cycle points: growth without division. Assess DNA content, nuclear morphology, and bud morphology to determine where arrested.

Le uova di *Xenopus* e quelle di riccio di mare

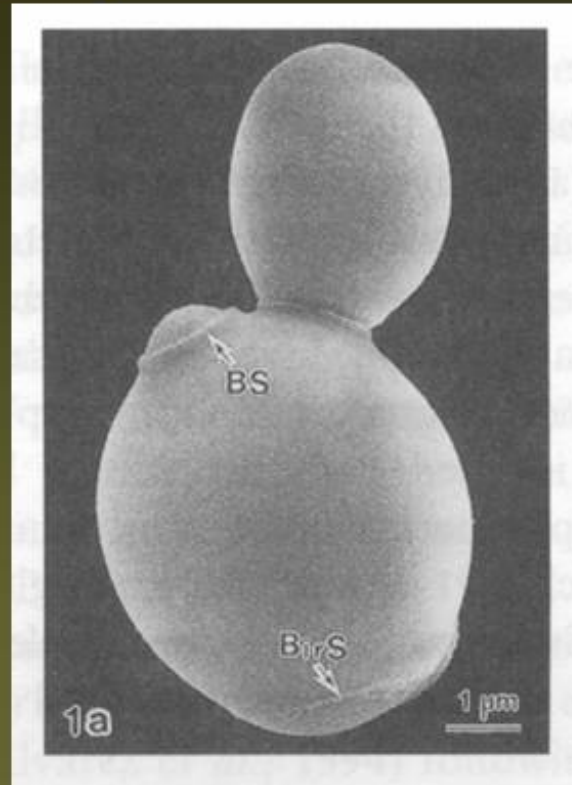
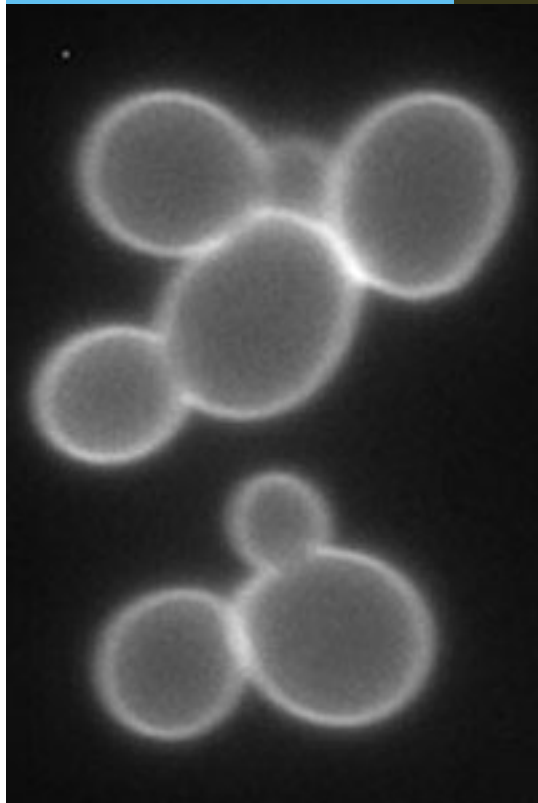


$\text{MPF} = \text{cyclin B} + \text{Cdc2 kinase}$

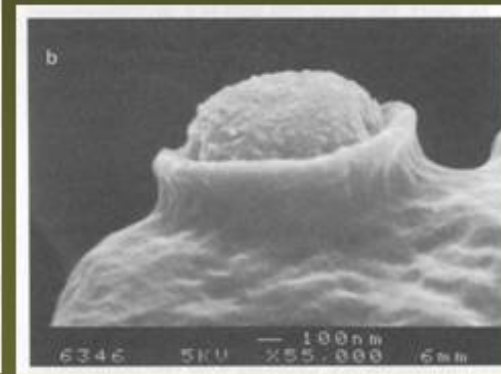
il lievito gemmante *S. cerevisiae*



YEAST BUD AND BUD SCAR



Buds normally develop close to the last bud (bud polarity)



<http://www-micro.msb.le.ac.uk/video/Scerevisiae.mov>

Cosa è l'analisi genetica nel lievito

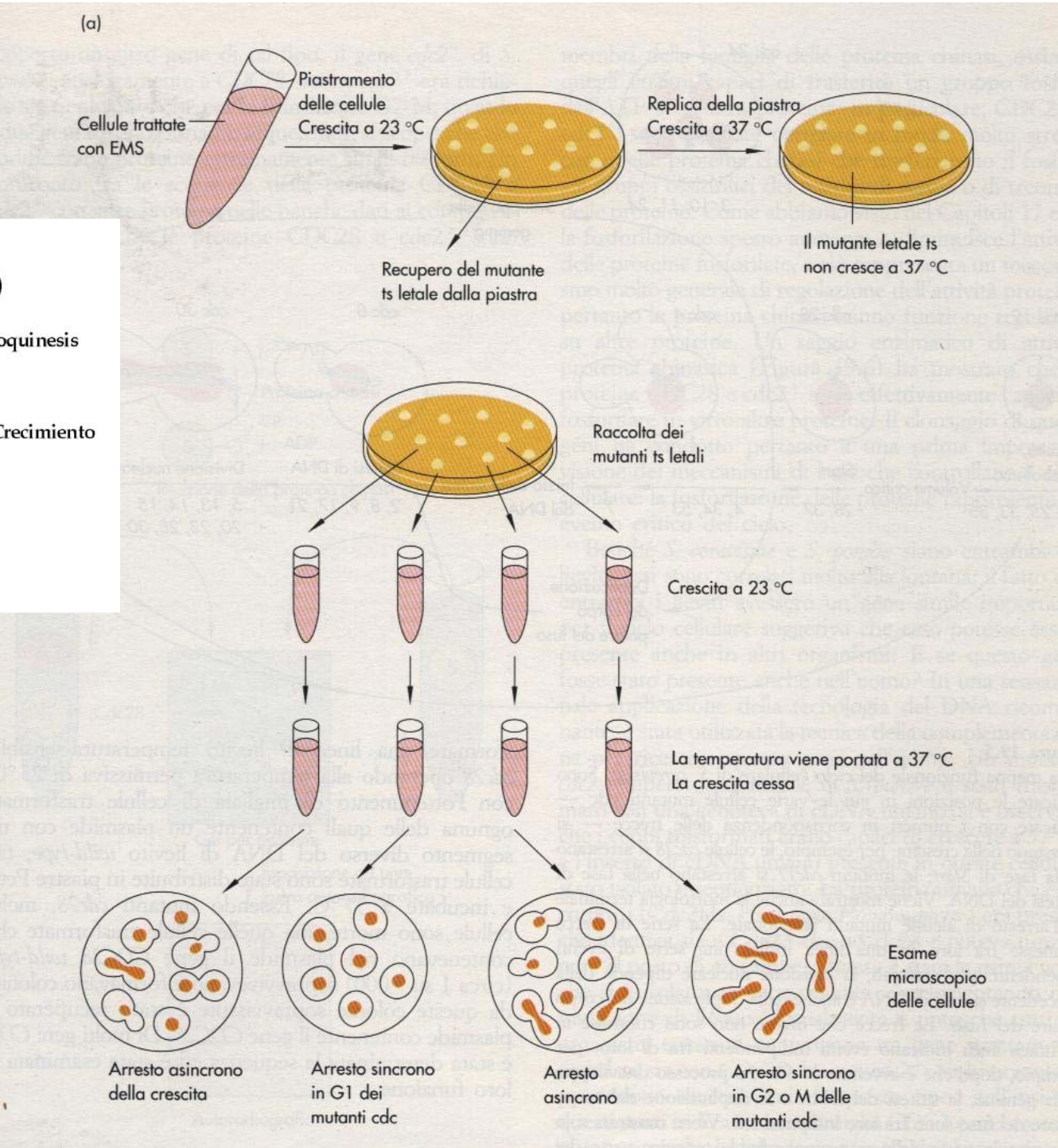
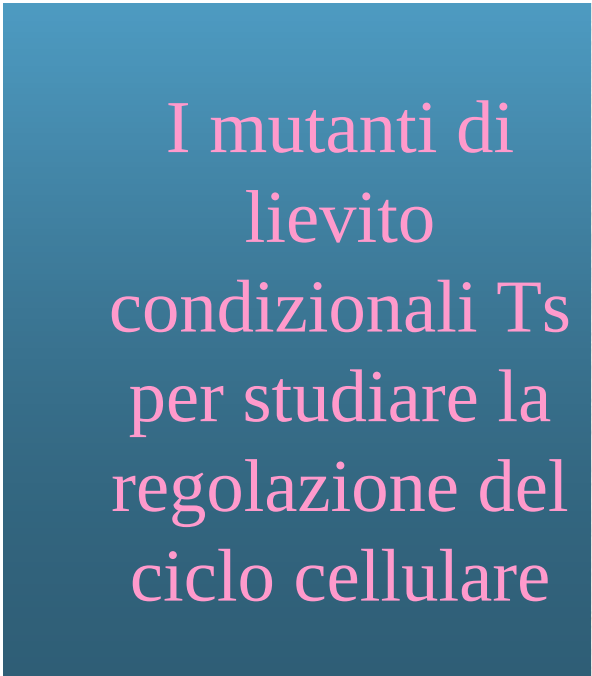
- L'idea base è stata quella di identificare i geni necessari per la regolazione del ciclo cellulare studiando i **mutanti**.

Punti forti a) i lieviti sono aploidi; b) le mutazioni da cercare sono quelle che bloccano la divisione; c) usare mutanti condizionali per la temperatura: le proteine mutate non funzionano a 37°C, a 23°C sì, quindi si cercheranno le mutazioni che bloccano il ciclo a 37°C; d) utilizzare sistemi semplici (morfologia) per identificare lo stadio in cui è avvenuto il blocco del ciclo e poi identificare i processi chiave: c'è stata duplicazione del DNA? E la condensazione dei nuclei? Il fuso mitotico è presente?; mutanti **cdc** ("cell division cycle").

- Usando le metodiche di complementazione, è possibile vedere quanti sono i geni implicati in un determinato blocco. Ad es., tutti i mutanti che si bloccano in G₂ corrispondono ad altrettanti geni?

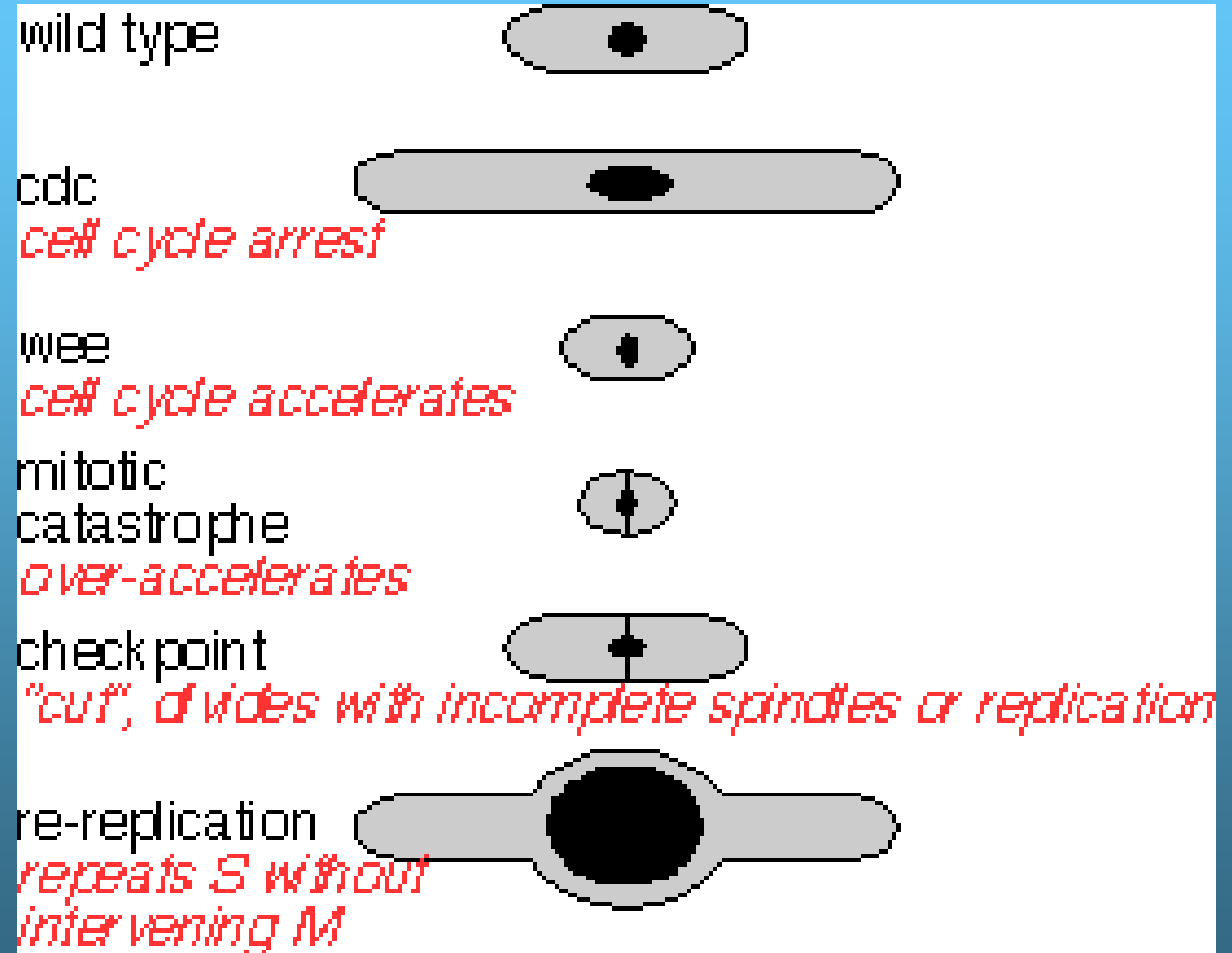
- Usando le metodiche di epistasi si combinano tra loro gli effetti di

the 1990s, the number of people in the United States who are 65 years of age or older has increased by 50 percent, and the number of people 75 years of age or older has increased by 100 percent. The number of people 85 years of age or older has increased by 200 percent. The number of people 90 years of age or older has increased by 400 percent. The number of people 95 years of age or older has increased by 800 percent. The number of people 100 years of age or older has increased by 1,600 percent. The number of people 105 years of age or older has increased by 3,200 percent. The number of people 110 years of age or older has increased by 6,400 percent. The number of people 115 years of age or older has increased by 12,800 percent. The number of people 120 years of age or older has increased by 25,600 percent. The number of people 125 years of age or older has increased by 51,200 percent. The number of people 130 years of age or older has increased by 102,400 percent. The number of people 135 years of age or older has increased by 204,800 percent. The number of people 140 years of age or older has increased by 409,600 percent. The number of people 145 years of age or older has increased by 819,200 percent. The number of people 150 years of age or older has increased by 1,638,400 percent. The number of people 155 years of age or older has increased by 3,276,800 percent. The number of people 160 years of age or older has increased by 6,553,600 percent. The number of people 165 years of age or older has increased by 13,107,200 percent. The number of people 170 years of age or older has increased by 26,214,400 percent. The number of people 175 years of age or older has increased by 52,428,800 percent. The number of people 180 years of age or older has increased by 104,857,600 percent. The number of people 185 years of age or older has increased by 209,715,200 percent. The number of people 190 years of age or older has increased by 419,430,400 percent. The number of people 195 years of age or older has increased by 838,860,800 percent. The number of people 200 years of age or older has increased by 1,677,721,600 percent. The number of people 205 years of age or older has increased by 3,355,443,200 percent. The number of people 210 years of age or older has increased by 6,710,886,400 percent. The number of people 215 years of age or older has increased by 13,421,772,800 percent. The number of people 220 years of age or older has increased by 26,843,545,600 percent. The number of people 225 years of age or older has increased by 53,687,091,200 percent. The number of people 230 years of age or older has increased by 107,374,182,400 percent. The number of people 235 years of age or older has increased by 214,748,364,800 percent. The number of people 240 years of age or older has increased by 429,496,729,600 percent. The number of people 245 years of age or older has increased by 858,993,459,200 percent. The number of people 250 years of age or older has increased by 1,717,986,918,400 percent. The number of people 255 years of age or older has increased by 3,435,973,836,800 percent. The number of people 260 years of age or older has increased by 6,871,947,673,600 percent. The number of people 265 years of age or older has increased by 13,743,895,347,200 percent. The number of people 270 years of age or older has increased by 27,487,790,694,400 percent. The number of people 275 years of age or older has increased by 54,975,581,388,800 percent. The number of people 280 years of age or older has increased by 109,951,162,777,600 percent. The number of people 285 years of age or older has increased by 219,902,325,555,200 percent. The number of people 290 years of age or older has increased by 439,804,651,110,400 percent. The number of people 295 years of age or older has increased by 879,609,302,220,800 percent. The number of people 300 years of age or older has increased by 1,759,218,604,441,600 percent. The number of people 305 years of age or older has increased by 3,518,437,208,883,200 percent. The number of people 310 years of age or older has increased by 7,036,874,417,766,400 percent. The number of people 315 years of age or older has increased by 14,073,748,835,532,800 percent. The number of people 320 years of age or older has increased by 28,147,497,671,065,600 percent. The number of people 325 years of age or older has increased by 56,294,995,342,131,200 percent. The number of people 330 years of age or older has increased by 112,589,990,684,262,400 percent. The number of people 335 years of age or older has increased by 225,179,981,368,524,800 percent. The number of people 340 years of age or older has increased by 450,359,962,737,049,600 percent. The number of people 345 years of age or older has increased by 900,719,925,474,099,200 percent. The number of people 350 years of age or older has increased by 1,801,439,850,948,198,400 percent. The number of people 355 years of age or older has increased by 3,602,879,701,896,396,800 percent. The number of people 360 years of age or older has increased by 7,205,759,403,792,793,600 percent. The number of people 365 years of age or older has increased by 14,411,518,807,585,587,200 percent. The number of people 370 years of age or older has increased by 28,823,037,615,171,174,400 percent. The number of people 375 years of age or older has increased by 57,646,075,230,342,348,800 percent. The number of people 380 years of age or older has increased by 115,292,150,460,684,697,600 percent. The number of people 385 years of age or older has increased by 230,584,300,921,369,395,200 percent. The number of people 390 years of age or older has increased by 461,168,601,842,738,790,400 percent. The number of people 395 years of age or older has increased by 922,337,203,685,477,580,800 percent. The number of people 400 years of age or older has increased by 1,844,674,407,370,955,161,600 percent. The number of people 405 years of age or older has increased by 3,689,348,814,741,910,323,200 percent. The number of people 410 years of age or older has increased by 7,378,697,629,483,820,646,400 percent. The number of people 415 years of age or older has increased by 14,757,395,258,967,641,292,800 percent. The number of people 420 years of age or older has increased by 29,514,790,517,935,282,585,600 percent. The number of people 425 years of age or older has increased by 59,029,581,035,870,565,171,200 percent. The number of people 430 years of age or older has increased by 118,059,162,071,741,130,342,400 percent. The number of people 435 years of age or older has increased by 236,118,324,143,482,260,684,800 percent. The number of people 440 years of age or older has increased by 472,236,648,286,964,521,369,600 percent. The number of people 445 years of age or older has increased by 944,473,296,573,929,042,739,200 percent. The number of people 450 years of age or older has increased by 1,888,946,593,147,858,085,478,400 percent. The number of people 455 years of age or older has increased by 3,777,893,186,295,716,170,956,800 percent. The number of people 460 years of age or older has increased by 7,555,786,372,591,432,341,913,600 percent. The number of people 465 years of age or older has increased by 15,111,572,745,182,864,683,827,200 percent. The number of people 470 years of age or older has increased by 30,223,145,490,365,729,367,654,400 percent. The number of people 475 years of age or older has increased by 60,446,290,980,731,458,735,308,800 percent. The number of people 480 years of age or older has increased by 120,892,581,961,462,917,470,617,600 percent. The number of people 485 years of age or older has increased by 241,785,163,922,925,834,941,235,200 percent. The number of people 490 years of age or older has increased by 483,570,327,845,851,669,882,470,400 percent. The number of people 495 years of age or older has increased by 967,140,655,691,703,339,764,940,800 percent. The number of people 500 years of age or older has increased by 1,934,281,311,383,406,679,529,881,600 percent. The number of people 505 years of age or older has increased by 3,868,562,622,766,813,359,059,763,200 percent. The number of people 510 years of age or older has increased by 7,737,125,245,533,626,718,119,526,400 percent. The number of people 515 years of age or older has increased by 15,474,250,491,067,253,436,239,052,800 percent. The number of people 520 years of age or older has increased by 30,948,500,982,134,506,872,478,105,600 percent. The number of people 525 years of age or older has increased by 61,897,001,964,269,013,744,956,211,200 percent. The number of people 530 years of age or older has increased by 123,794,003,928,538,027,489,912,422,400 percent. The number of people 535 years of age or older has increased by 247,588,007,857,076,054,979,824,844,800 percent. The number of people 540 years of age or older has increased by 495,176,015,714,152,109,959,649,689,600 percent. The number of people 545 years of age or older has increased by 990,352,031,428,304,219,919,299,379,200 percent. The number of people 550 years of age or older has increased by 1,980,704,062,856,608,439,838,598,758,400 percent. The number of people 555 years of age or older has increased by 3,961,408,125,713,216,879,677,197,516,800 percent. The number of people 560 years of age or older has increased by 7,922,816,251,426,433,759,354,395,033,600 percent. The number of people 565 years of age or older has increased by 15,845,632,502,852,867,518,708,790,067,200 percent. The number of people 570



Esempio di analisi genetica nel lievito *S. pombe*

cdc25	long
wee1	short
cdc2-L	
	long
cdc2-w	
	short OP
cdc25 short	
OPwee1	long
cdc13	long



Applicazione ad esperimenti di epistasi

Gli esperimenti furono poi suffragati da prove biochimiche. E, soprattutto, si vide che cdc2 corrispondeva alla kinasi e cdc13 alla ciclina che, combinate insieme, davano l'attività MPF degli ovociti di *Xenopus* e degli embrioni di riccio di mare

cdc2-L wee1	long
cdc2-L OP cdc25	long
cdc2-w cdc25	short
cdc2-w OP wee1	short
cdc25 wee1	normal

Data (epistasis expt)

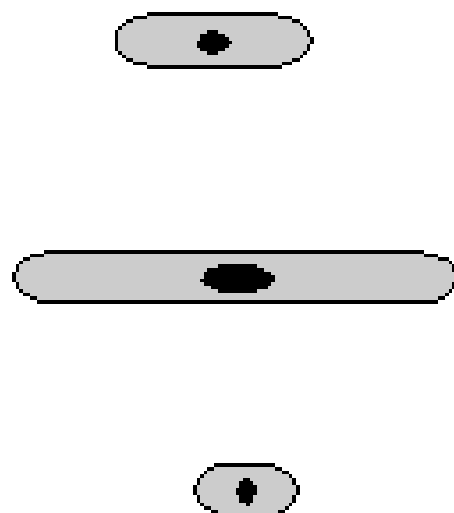
ts = LOF, OP = GOF

model

Phenotype

single mutant

double mutant



wild type

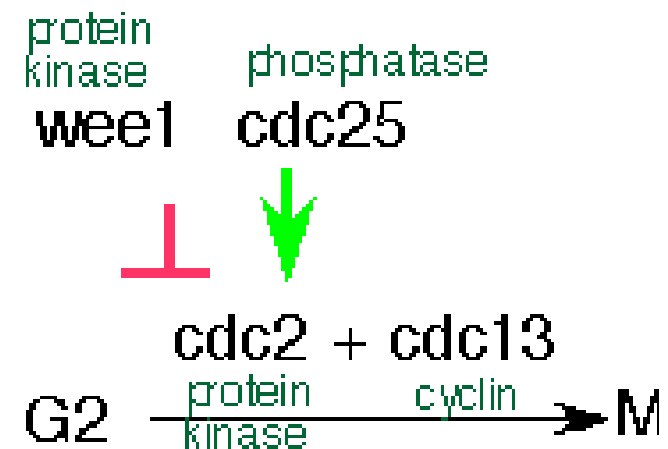
cdc25^{ts} wee1^{ts}

*cdc2^{ts}
cdc13^{ts}
cdc25^{ts}
OP wee1⁺*

*cdc2^{ts} wee1^{ts}
cdc2^{ts} OP cdc25⁺*

*wee1^{ts}
OP-cdc25⁺*

*cdc2-w cdc25⁺
cdc2-w OP wee1⁺*

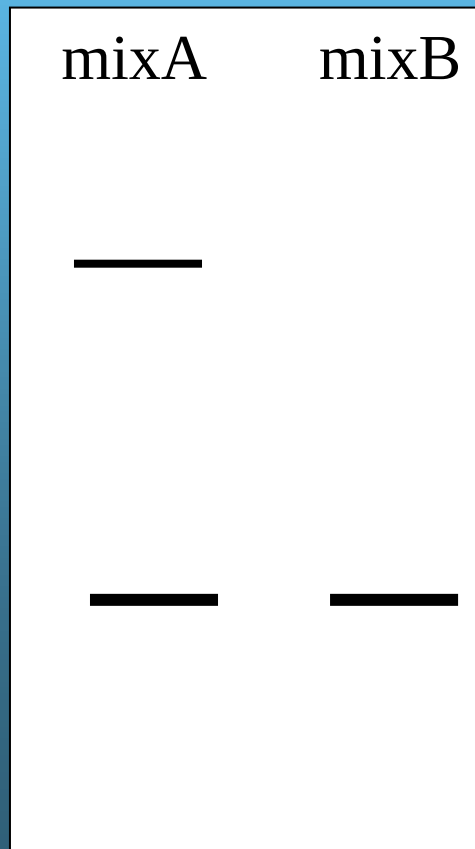


Dimostrazione che Cdc28/Cdc2 è una kinasi

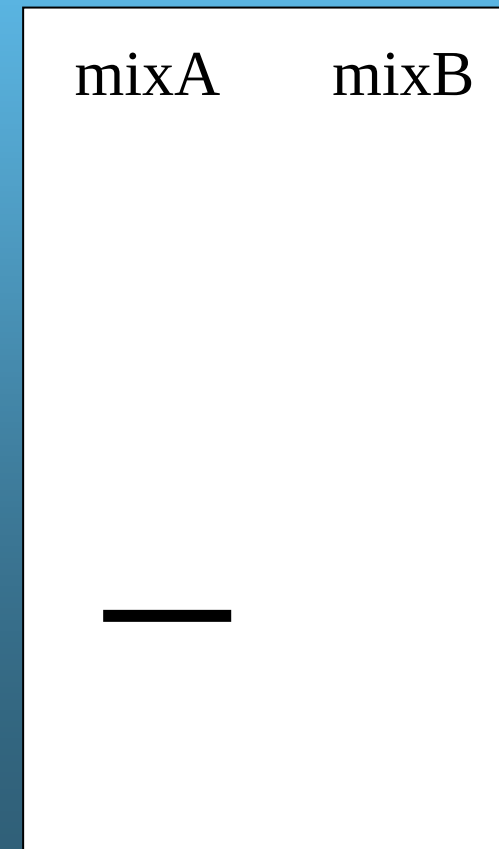
Mix A = Cdc28 +
proteina substrato + γ -
 ^{32}P -ATP

Mix B = proteina
substrato + γ - ^{32}P -ATP

elettroforesi



autoradiografia



Dimostrazione dell'attività MPF in cellule uovo di *Xenopus*

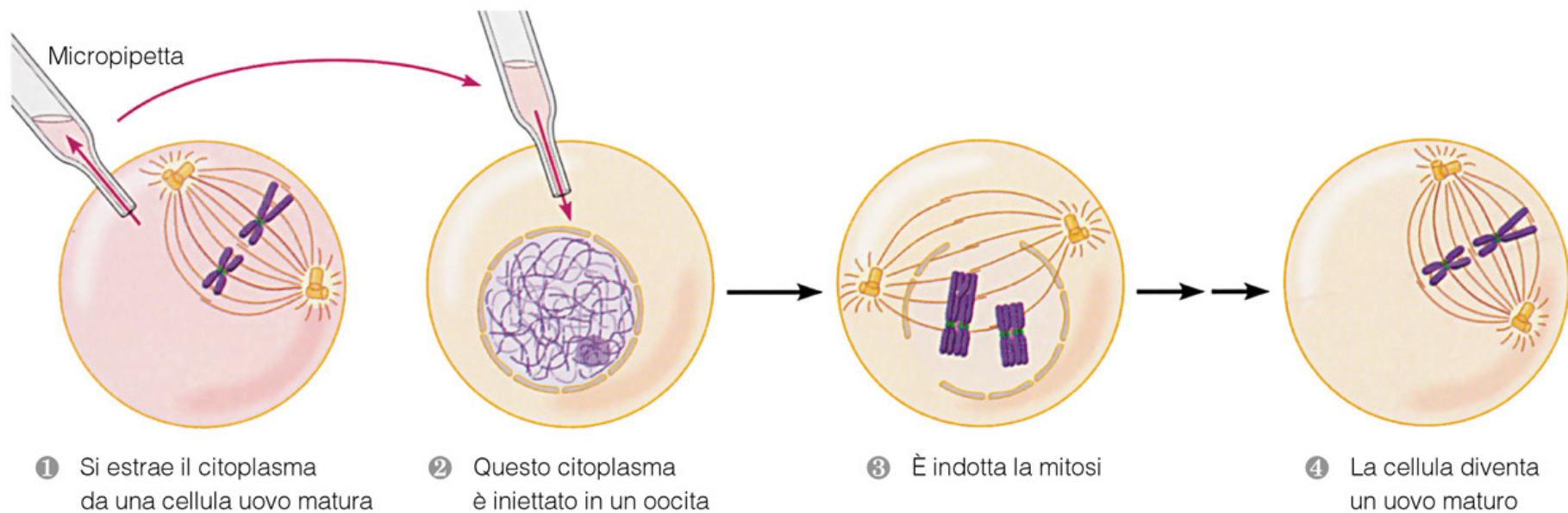


Figura 17-32

Ciclicità dell'attività MPF

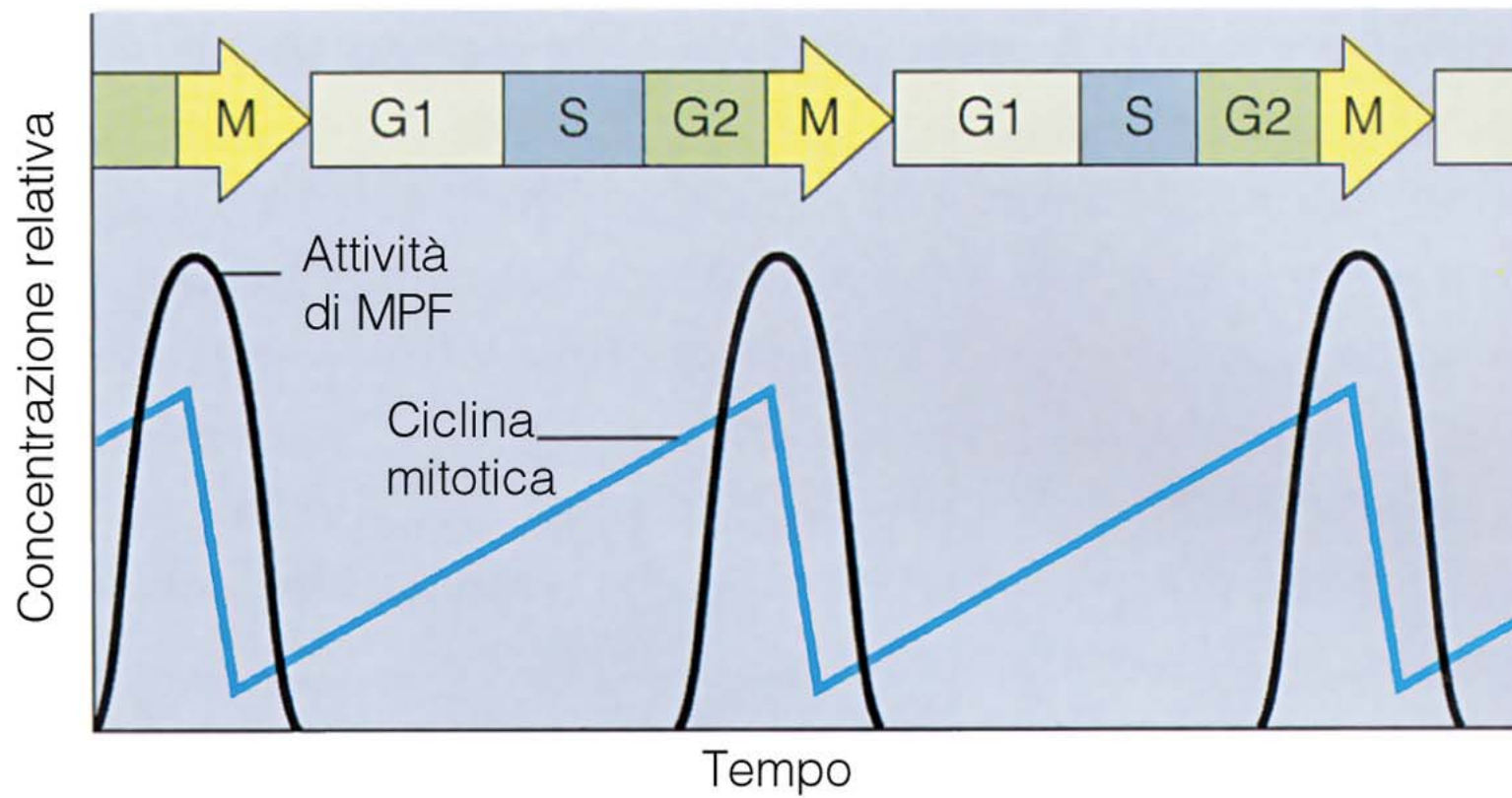
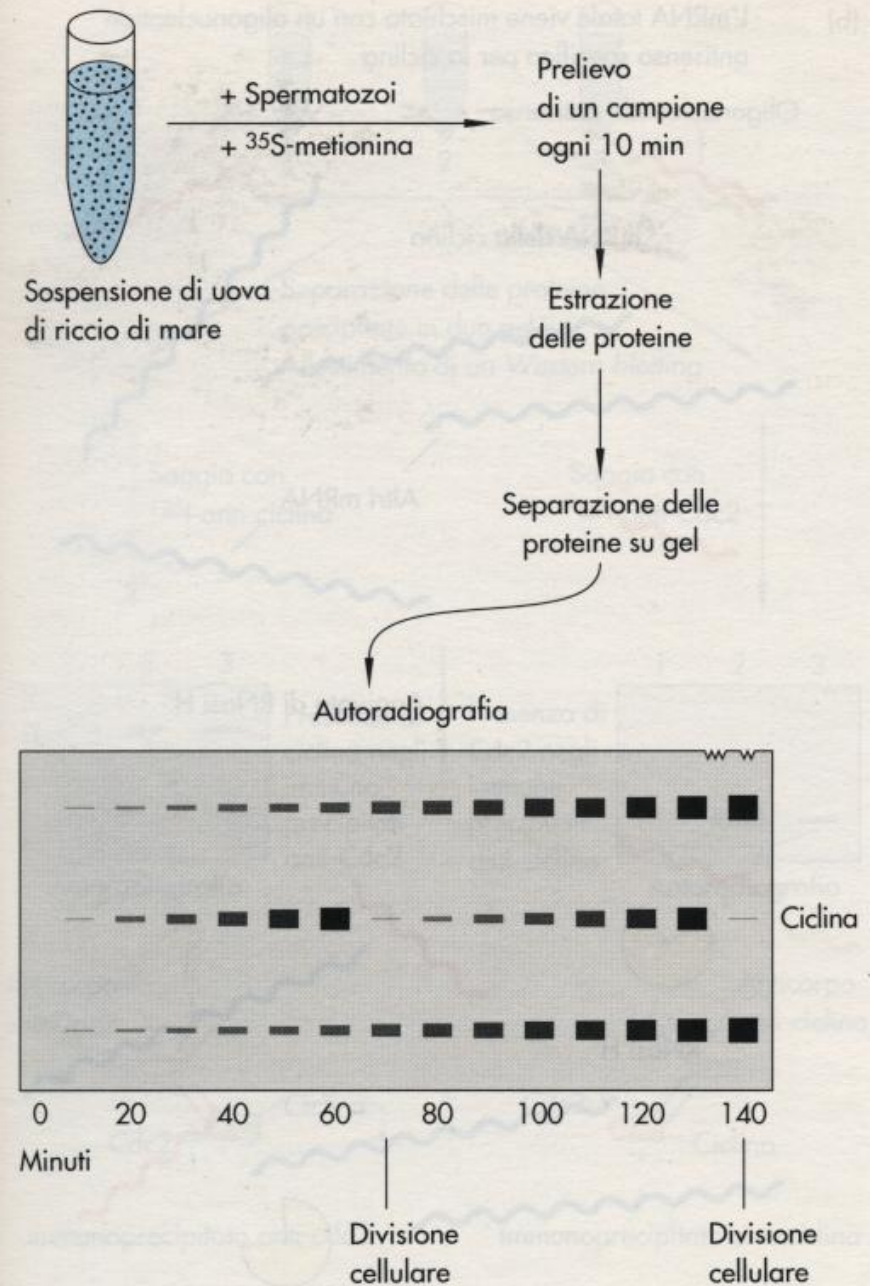
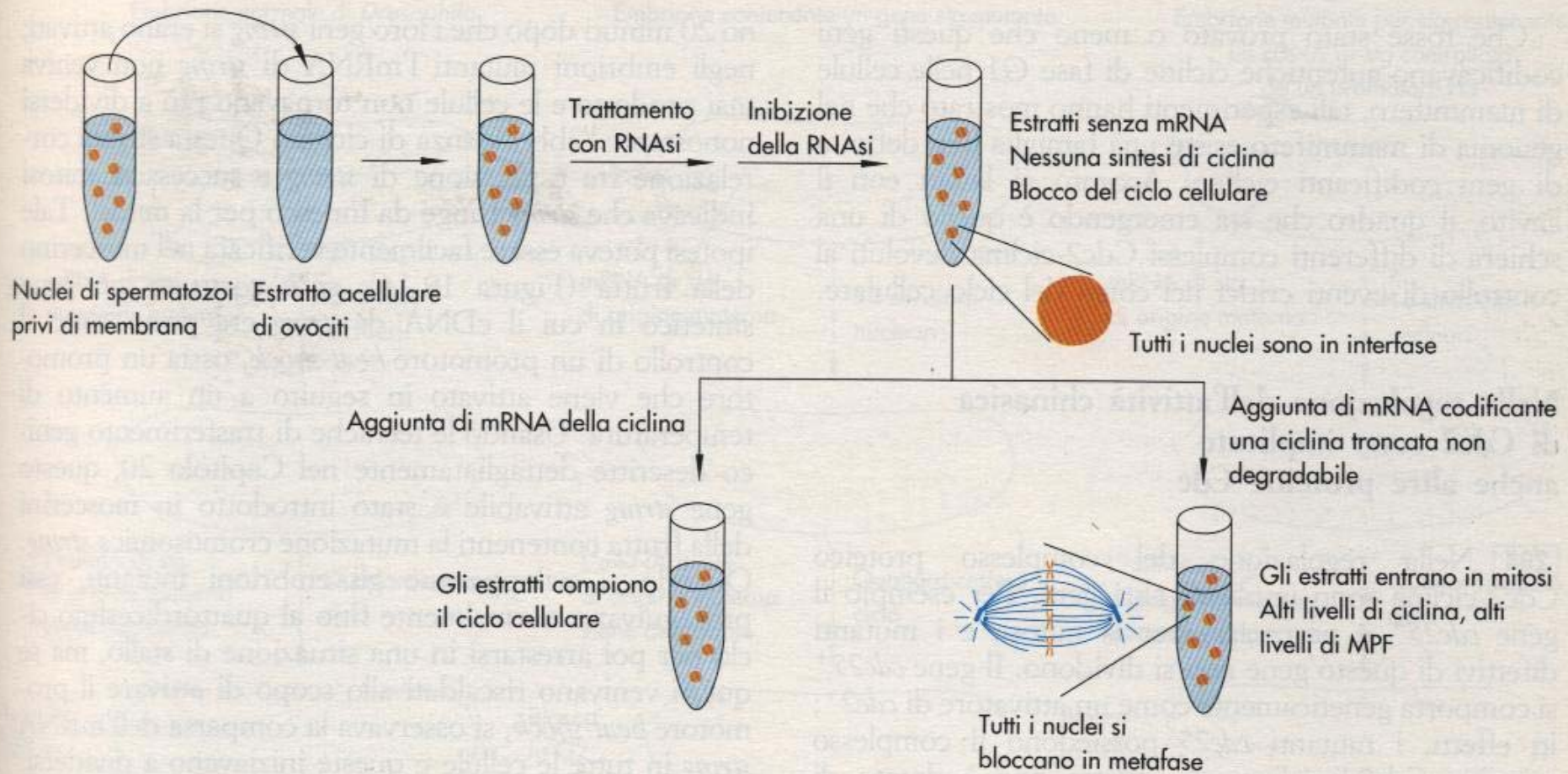


Figura 17-33

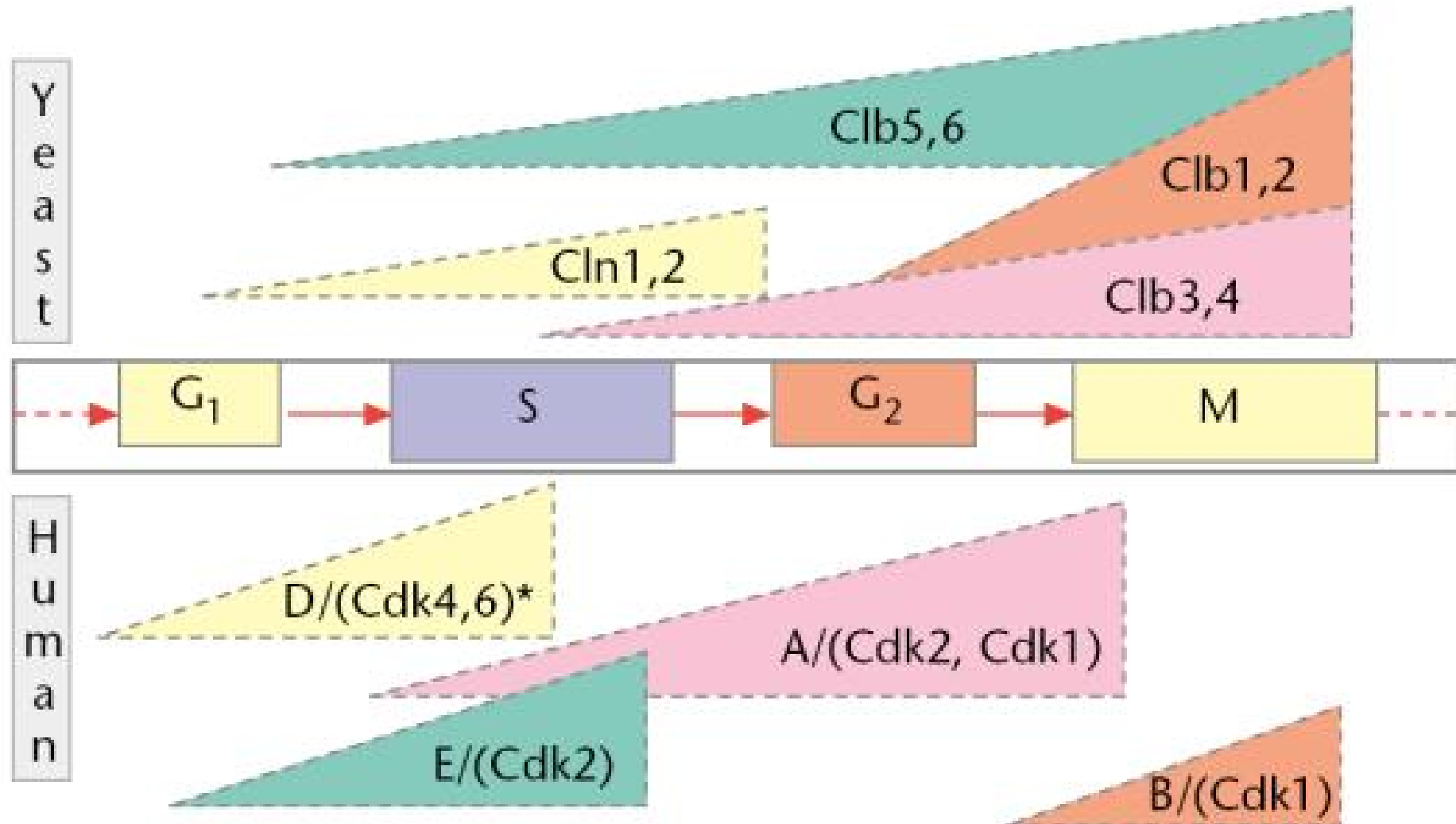
Identificazione della ciclina nei primi stadi embrionali di riccio di mare



Dimostrazione che la ciclina deve essere neosintetizzata e demolita nel corso del ciclo cellulare



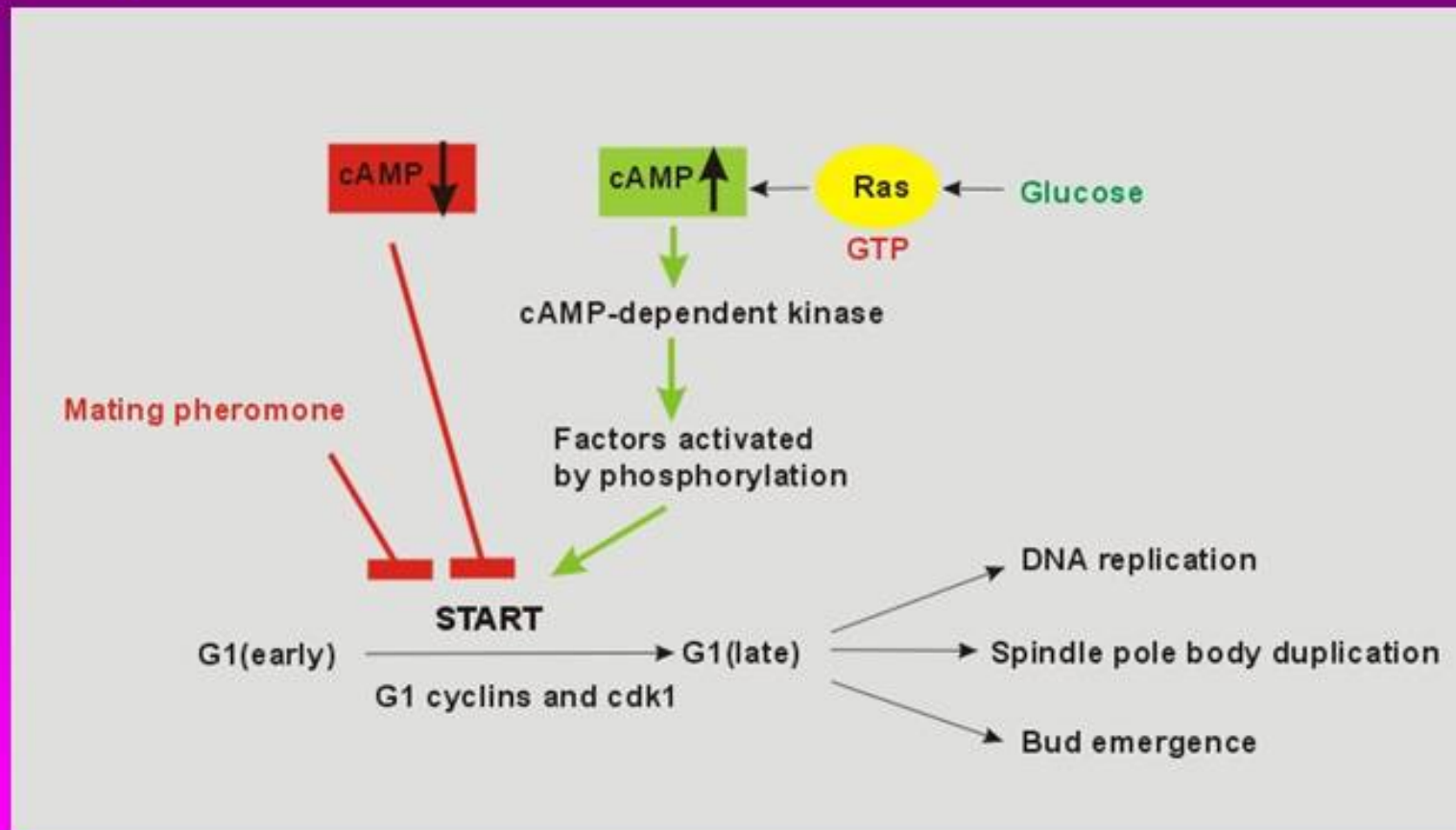
Confronto tra cicline del lievito e dell'uomo



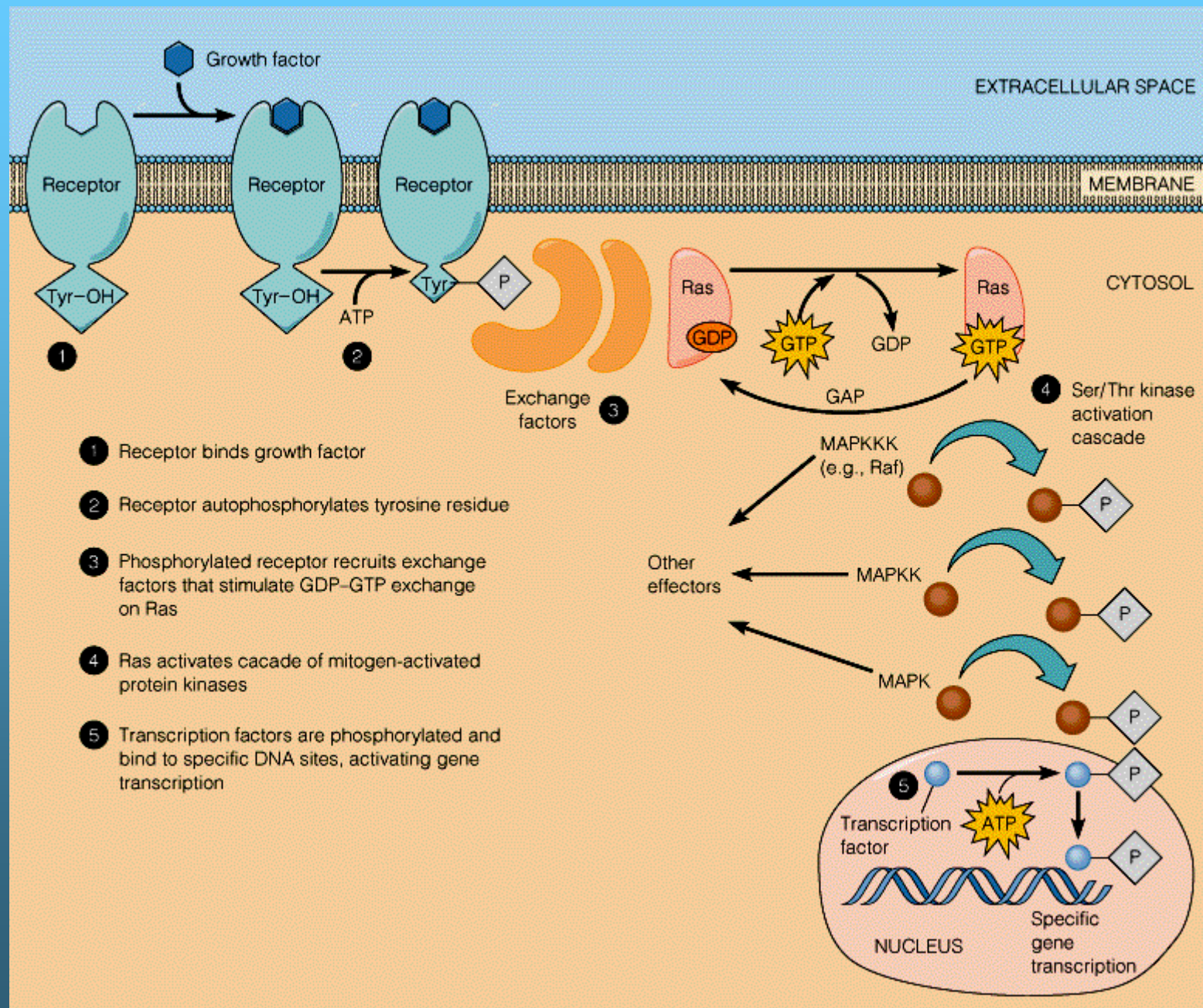
La funzione di Ras nella progressione di G₁ nel lievito



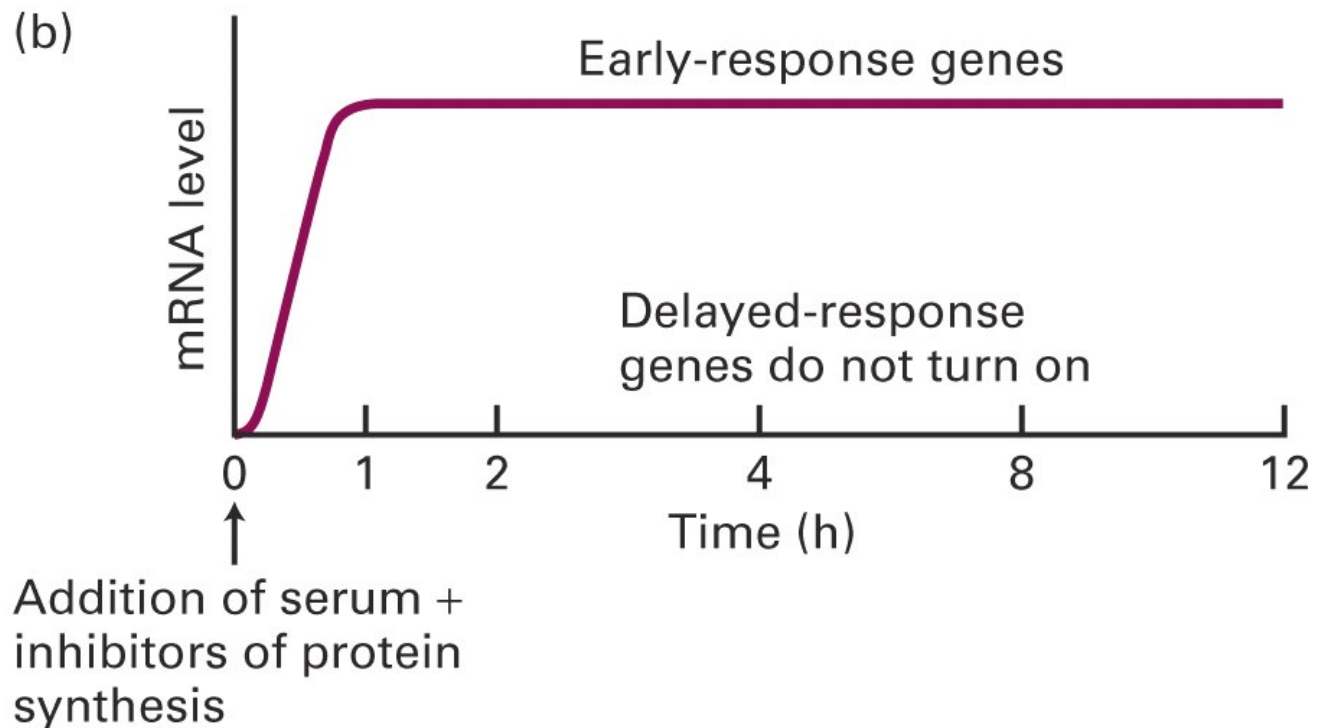
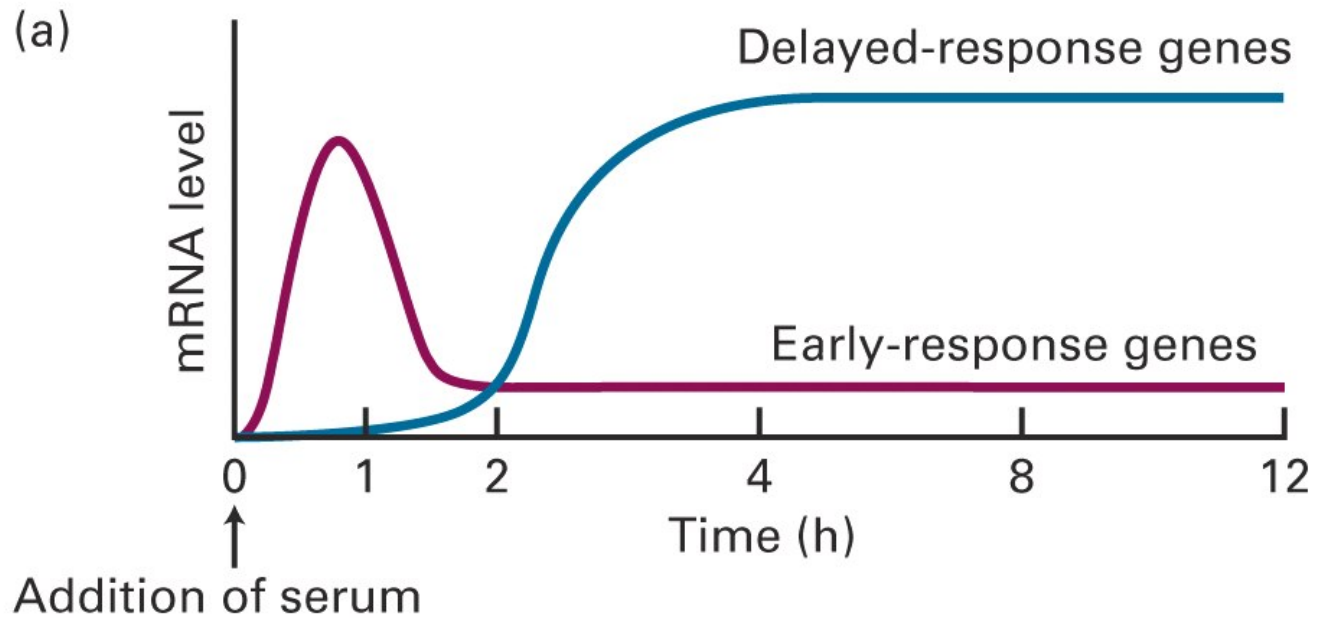
G1 REGULATION IN YEAST



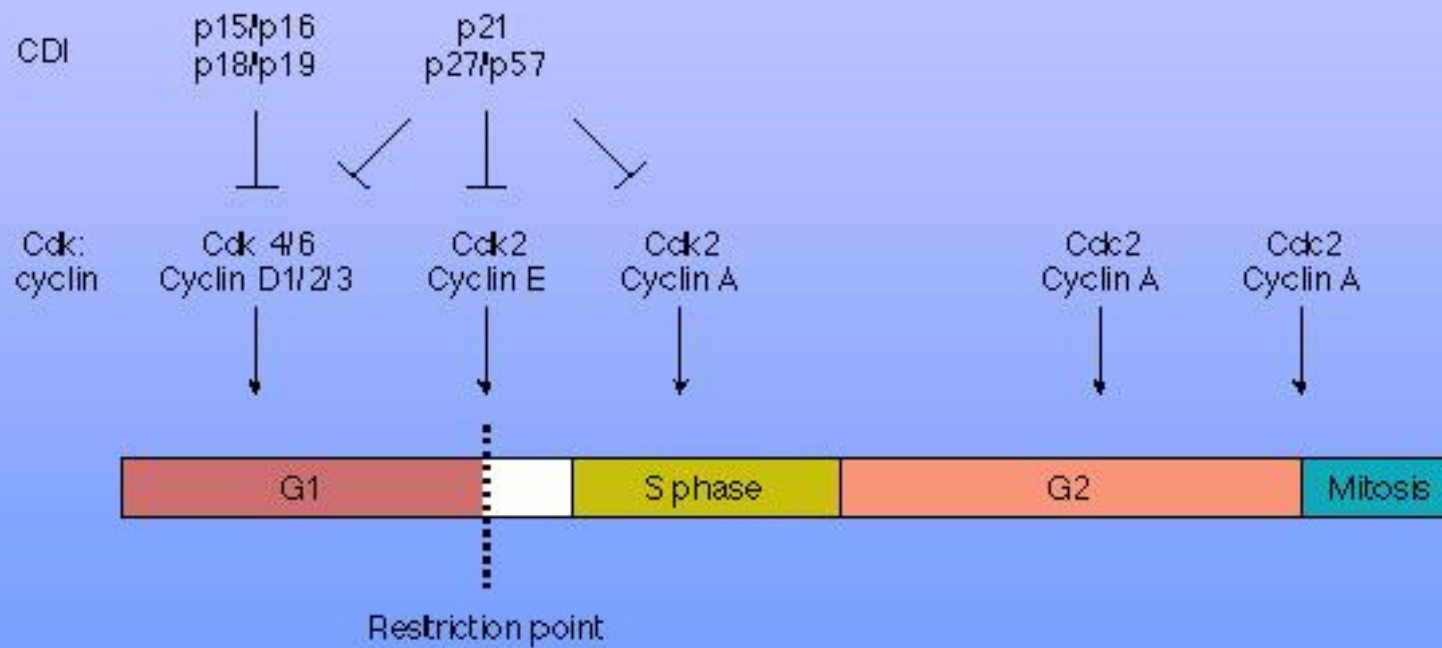
I segnale di Ras negli eucarioti superiori



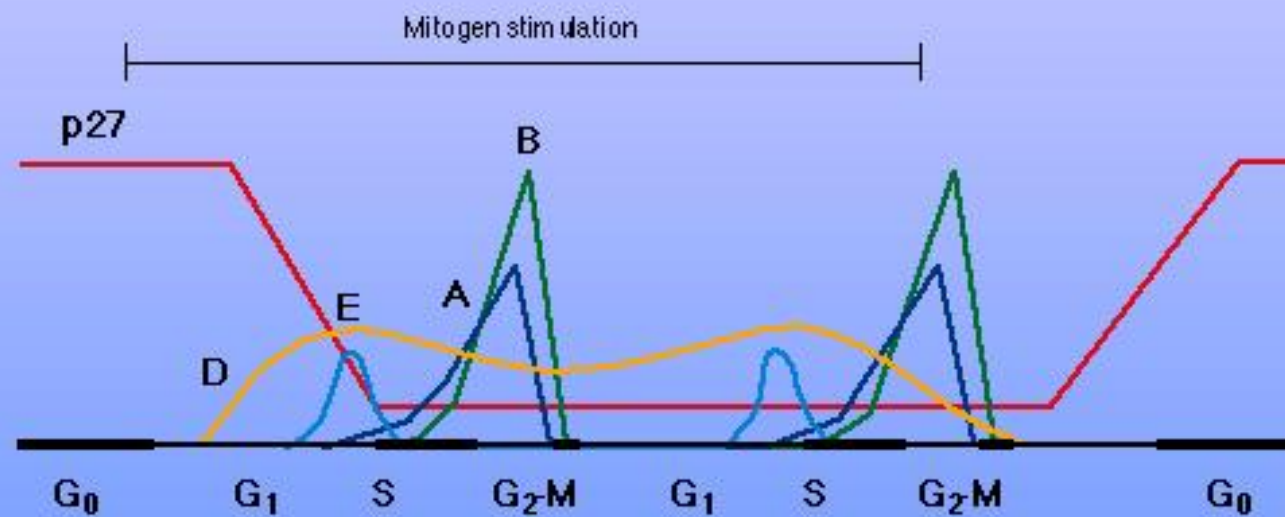
Controllo della proliferazione nelle cellule degli organismi pluricellulari



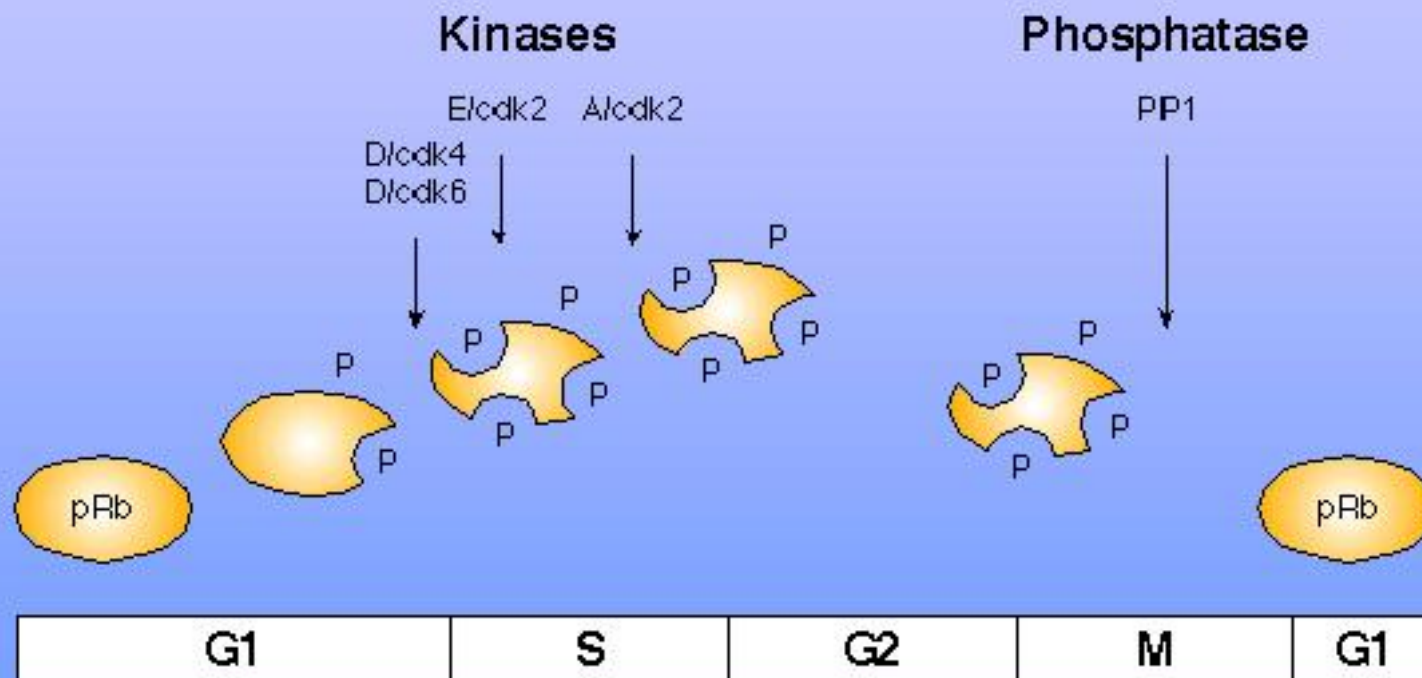
The mammalian cell cycle



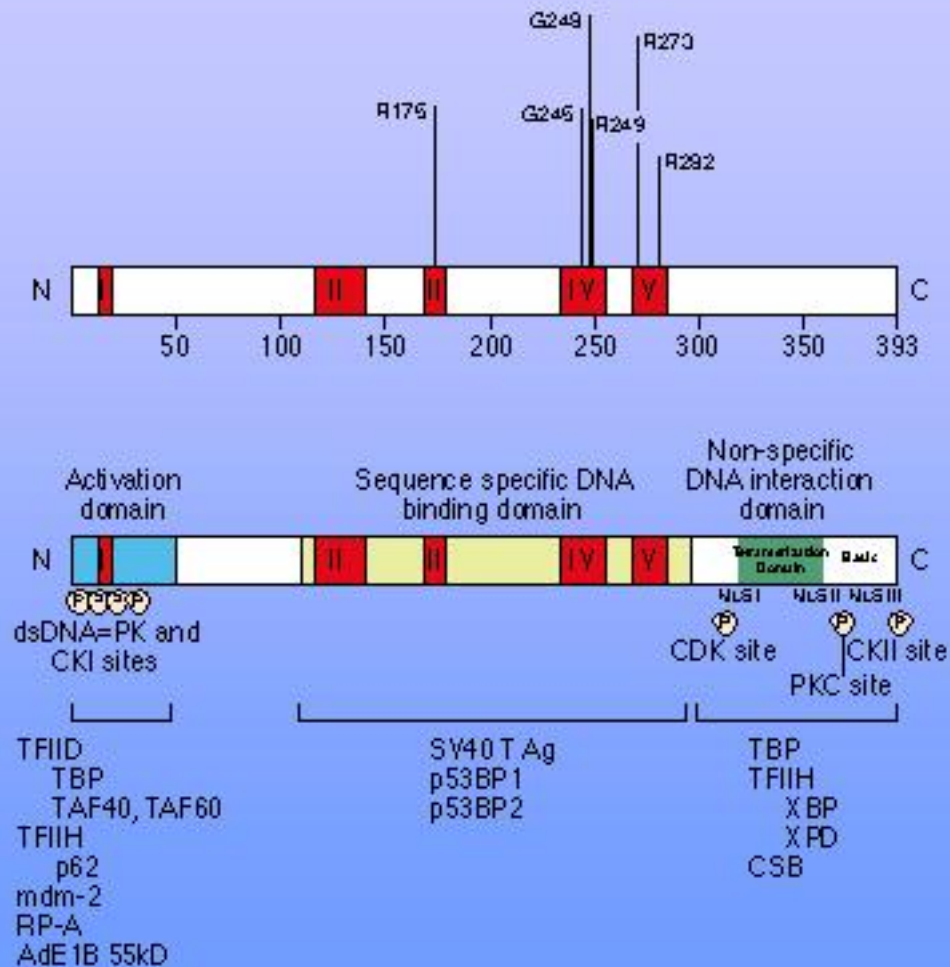
Cyclin regulation during cell cycle progression



Control of pRb

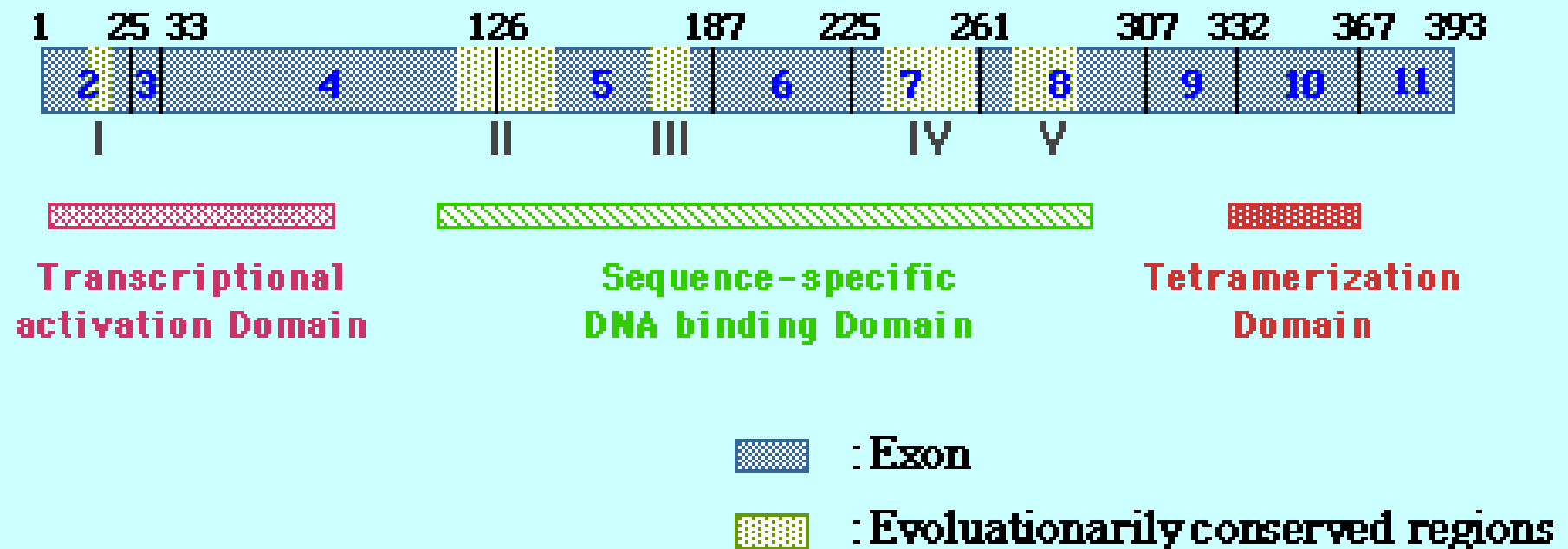


The p53 tumour suppressor protein



La proteina p53

Structural organization of p53 protein



p53, il *guardiano del genoma*

p53 contiene all'N-terminale un forte dominio di attivazione genica; in forma di tetramero si lega al DNA in maniera specifica. Oltre ad attivare la trascrizione dei geni in cui è presente il suo elemento-risposta, è in grado di inibire la trascrizione di altri geni.

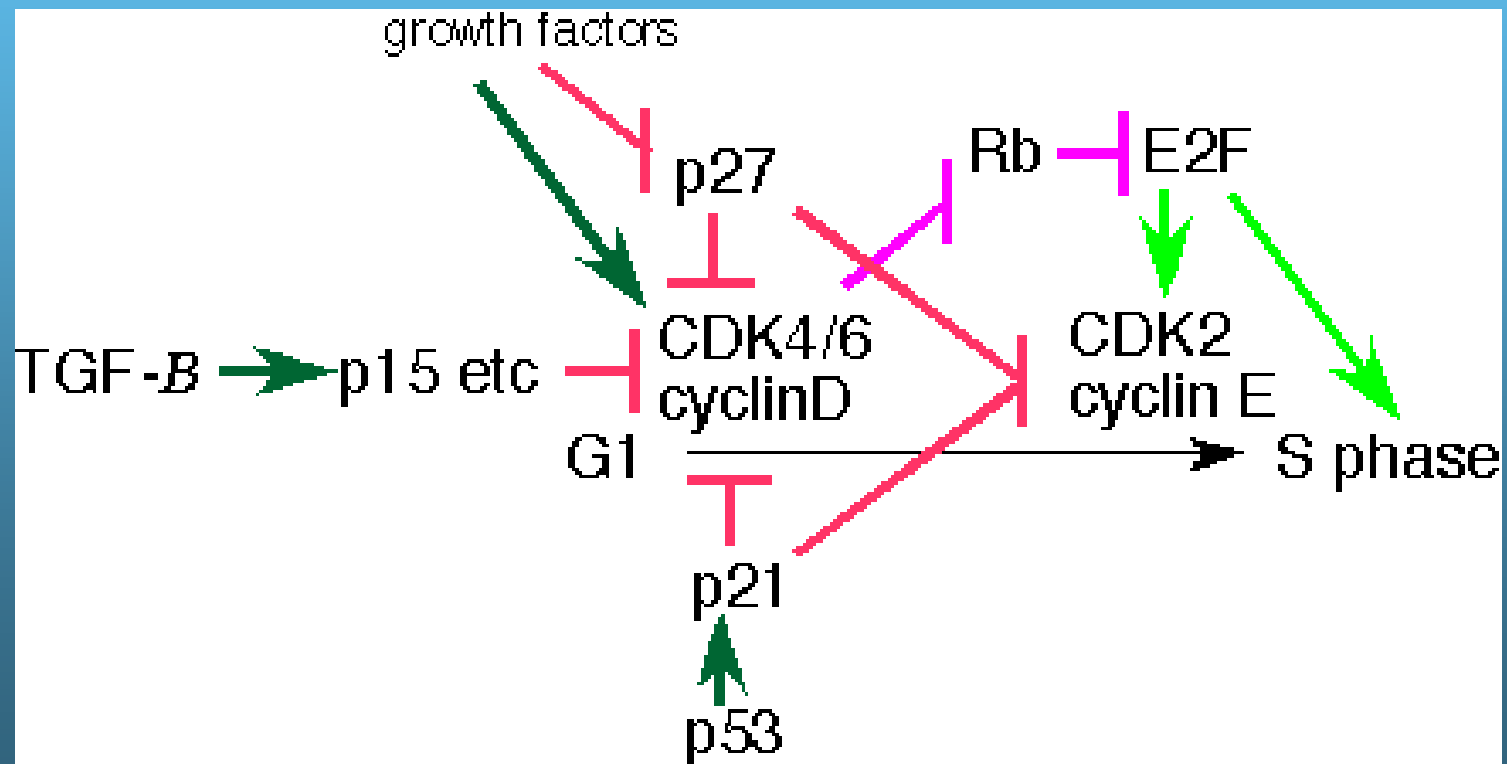
Diversi virus tumorali esprimono prodotti che si legano a p53 e ne inattivano le funzioni (es. SV40 large T antigen, le proteina E1B 55-kD dell'adenovirus e E6 delle forme oncogene del papillomavirus umano HPV E6).

p53 si trova spesso mutata nei tumori.

Nelle cellule, p53 può associarsi ad una proteina di 90kD, il prodotto dell'oncogene *mdm-2*, che è amplificato in molti tumori. Il legame con mdm2 inattiva le funzioni di p53 (inibizione, avvio alla distruzione, sequestro nel nucleolo...)

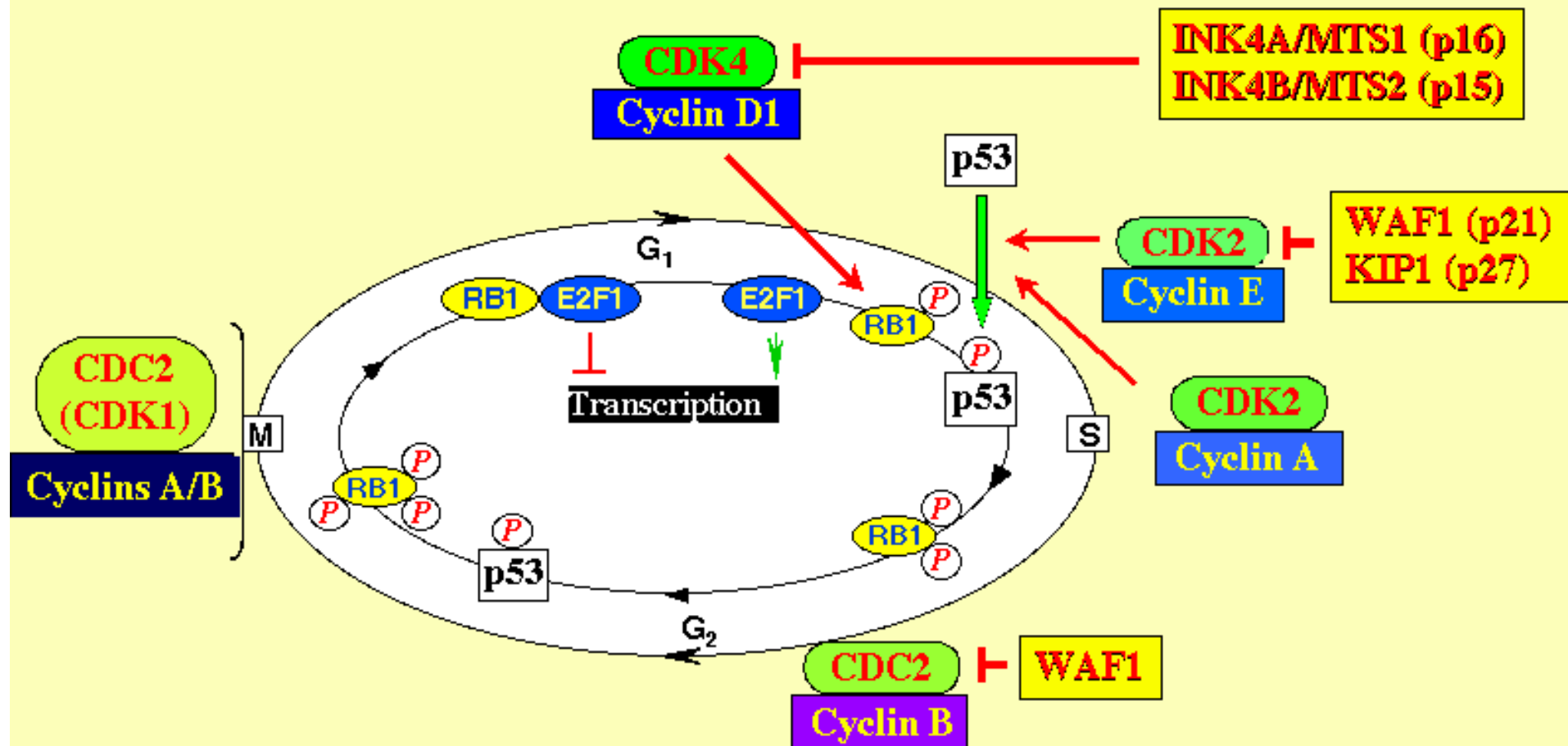
Alti livelli di p53 (*normale, non mutante*) portano all'arresto del ciclo cellulare e/o all'apoptosi. Come fattore del checkpoint, la sua funzione equivale a quella del gene *fad9* del lievito.

Controllo della progressione in G₁

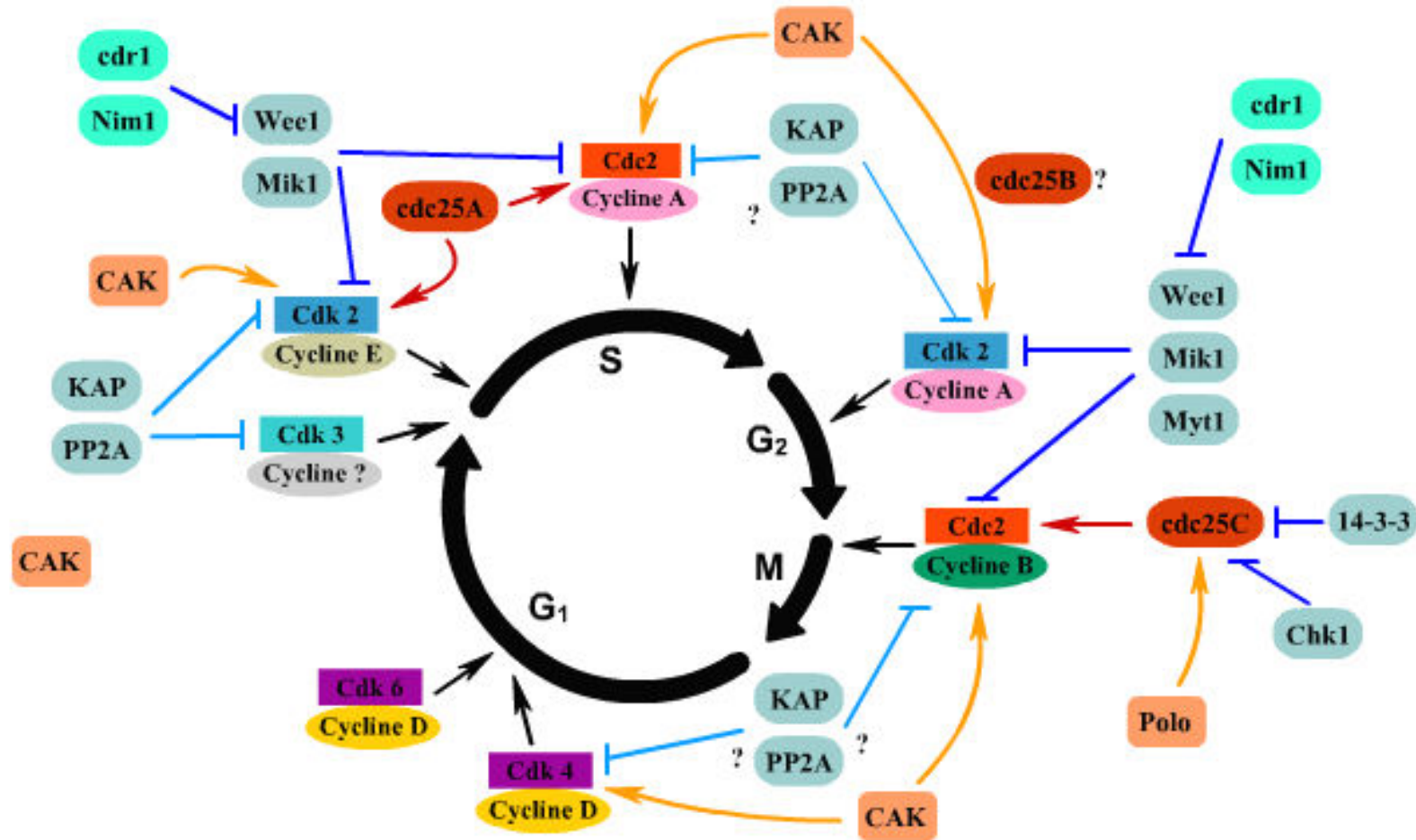


Gli inibitori dei complessi ciclina-kinasi

CYCLIN-DEPENDENT KINASE INHIBITORS



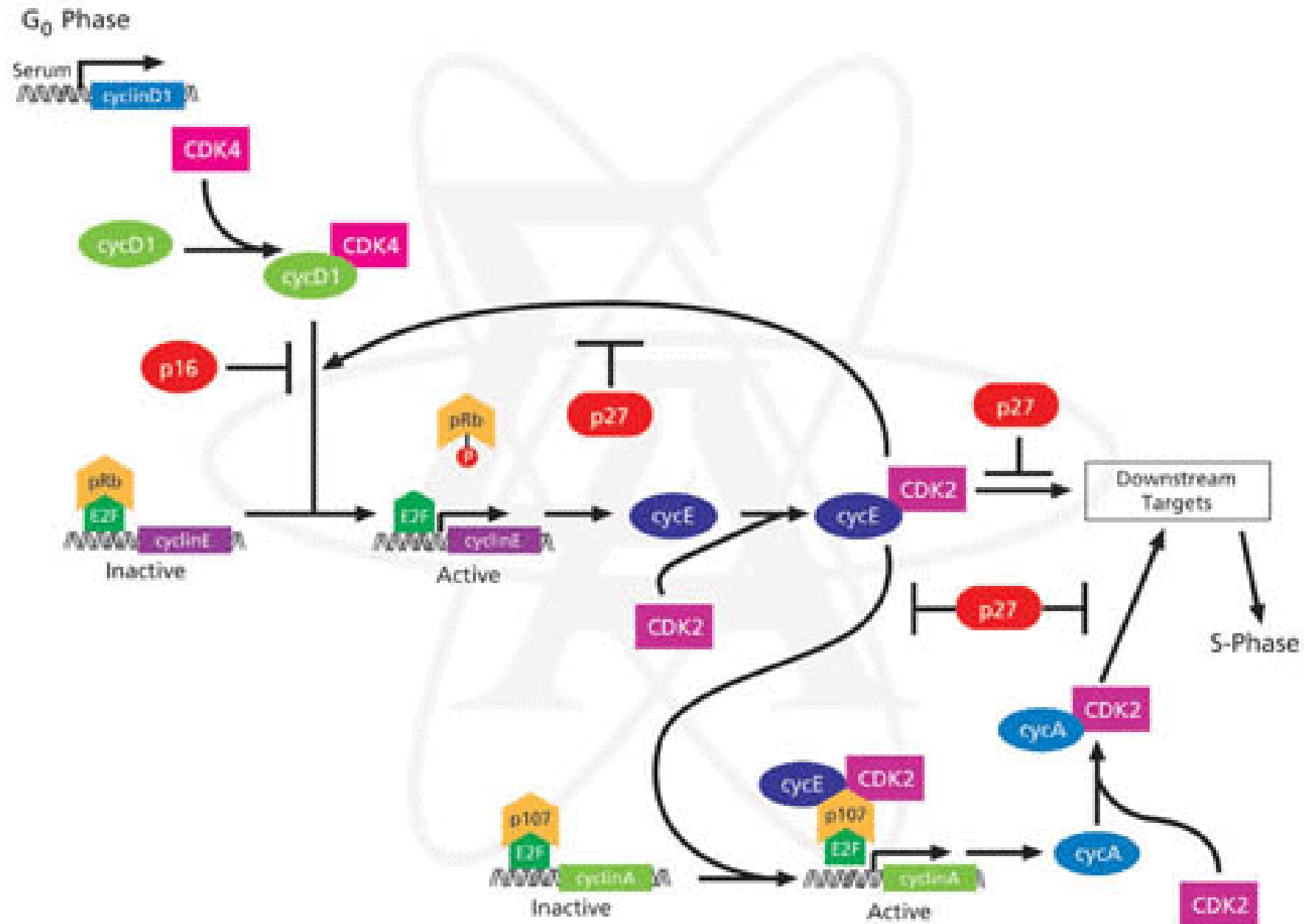
La regolazione per fosforilazione dei complessi ciclina-CDK



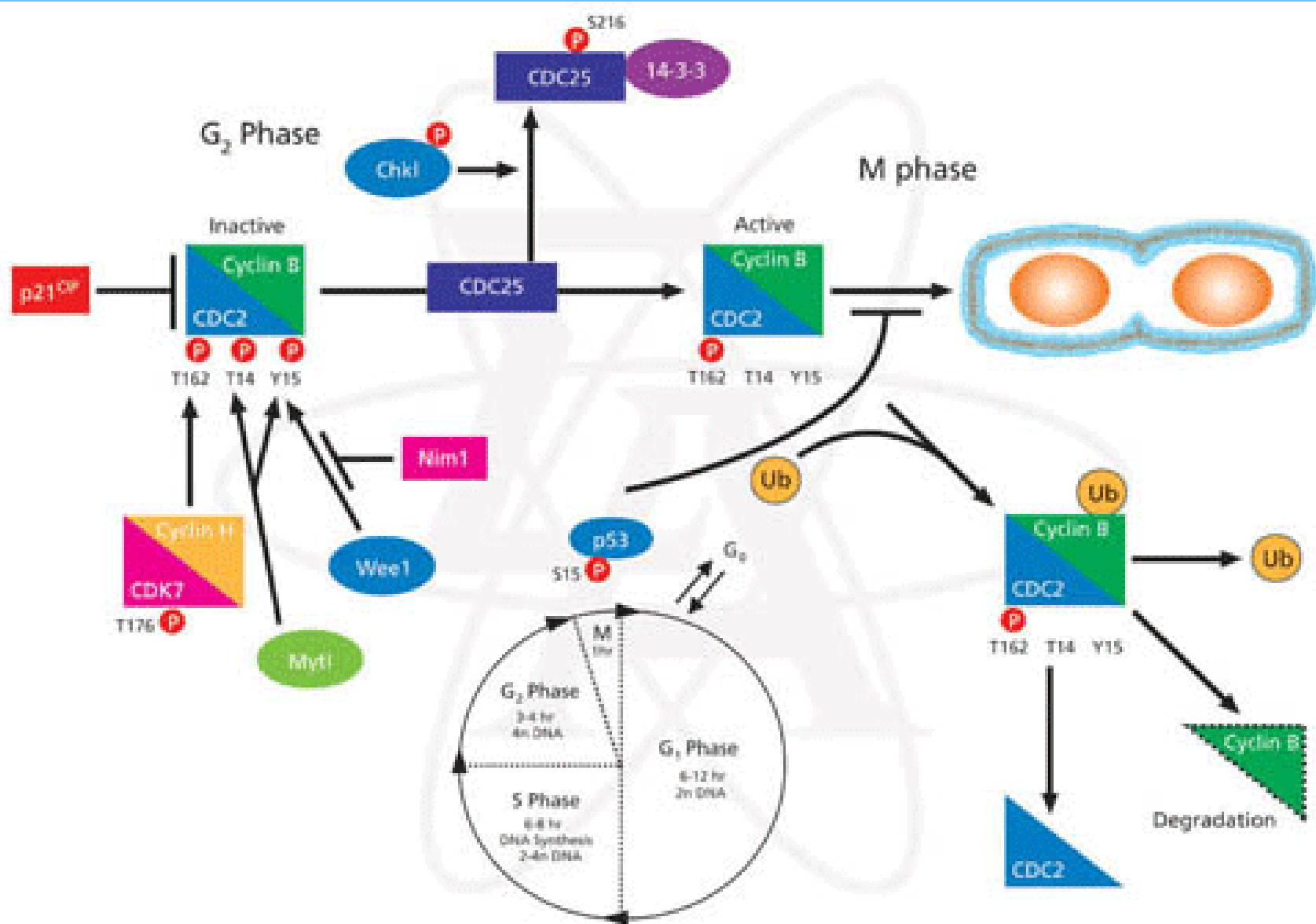
- ⊕ → Activation par déphosphorylation des phosphates inhibiteurs
- ⊕ → Activation par phosphorylation activatrice
- ⊖ —| Inactivation par phosphorylation inhibitrice
- ⊖ —| Inactivation par déphosphorylation du phosphate activateur

Régulation des complexes cdk/cyclines par phosphorylation

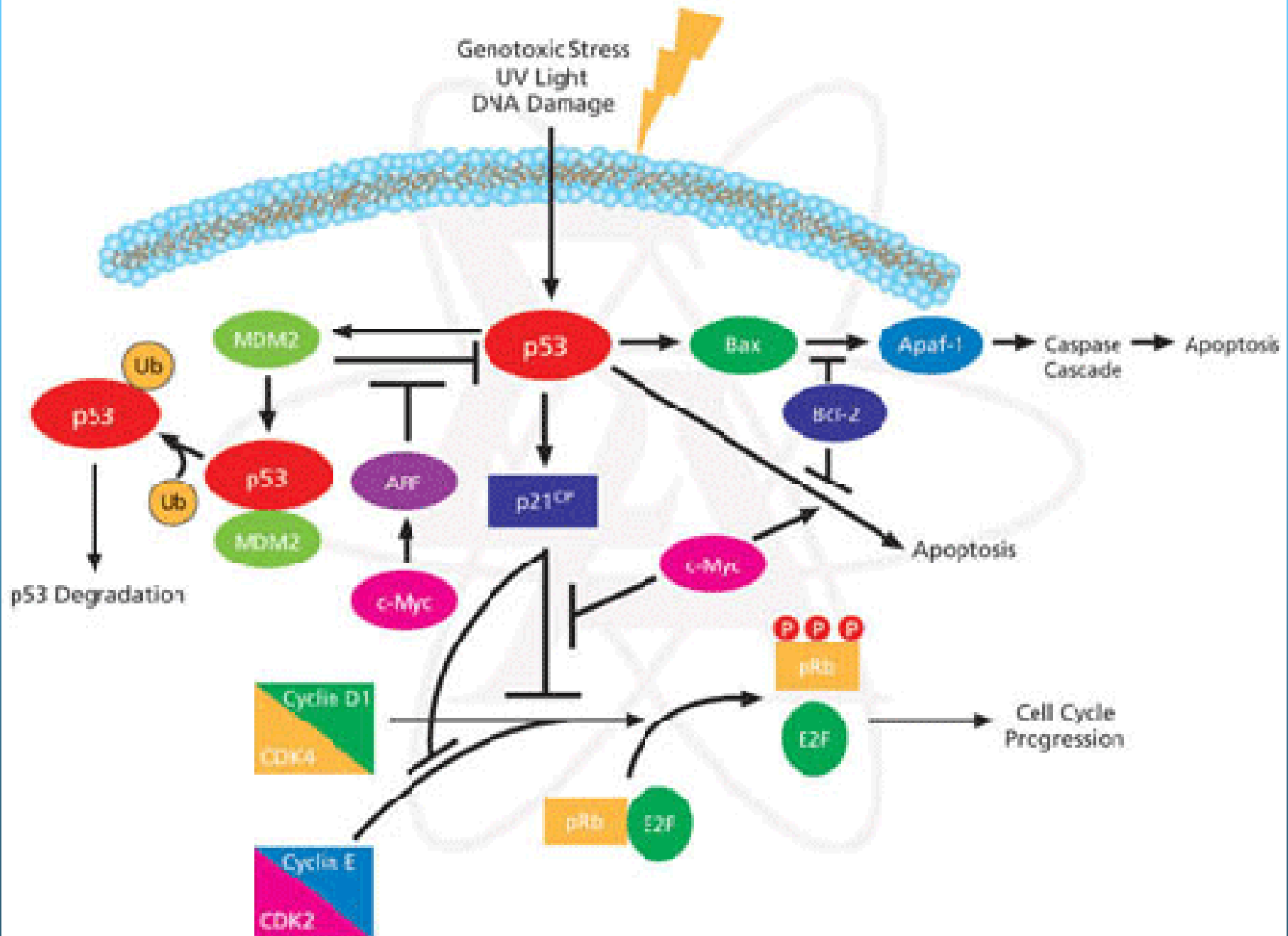
La progressione in G_1 e la transizione $G_1 \rightarrow S$



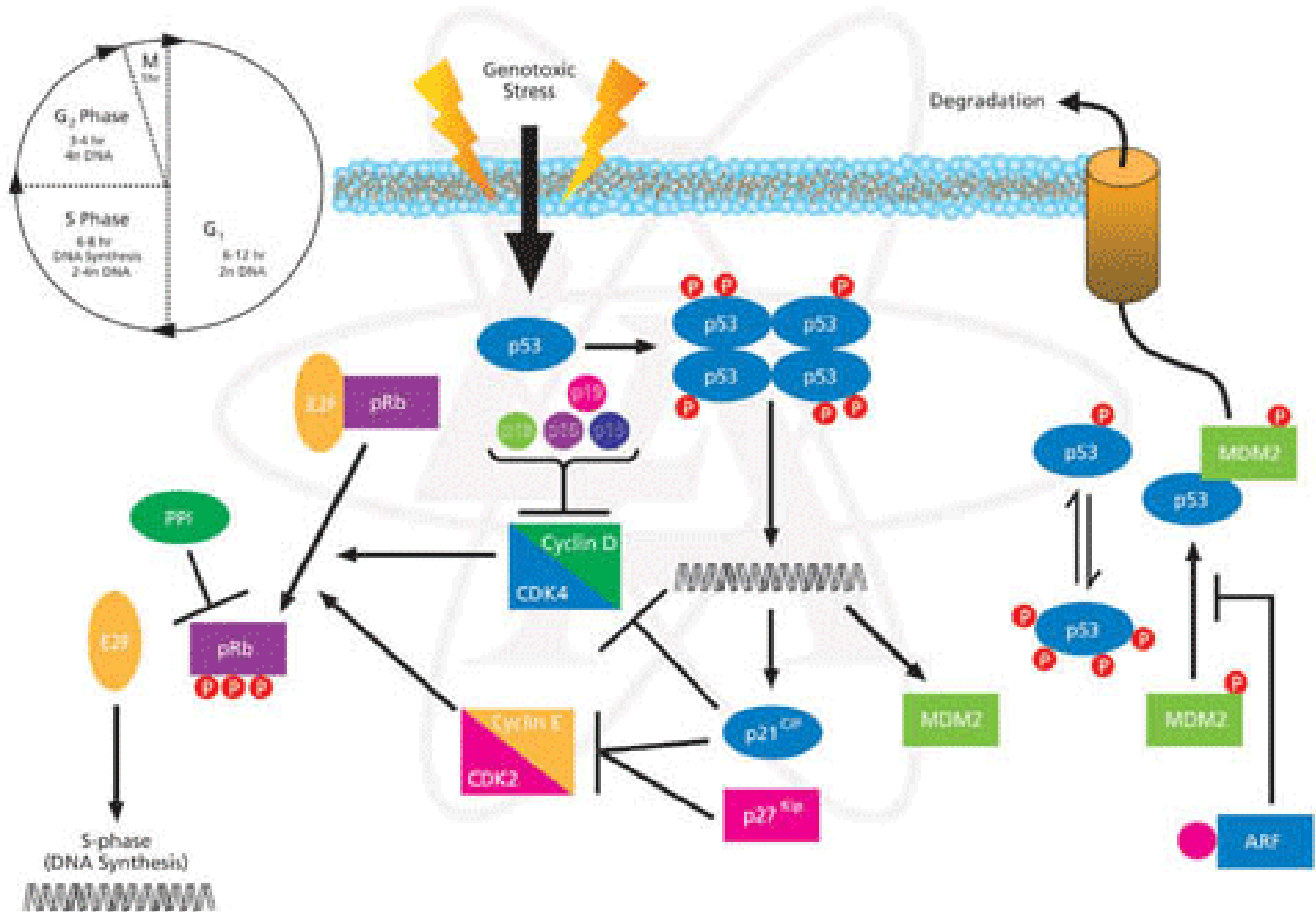
Attività MPF: il complesso ciclina B-CDK1 (detto anche CDC2) e la sua regolazione



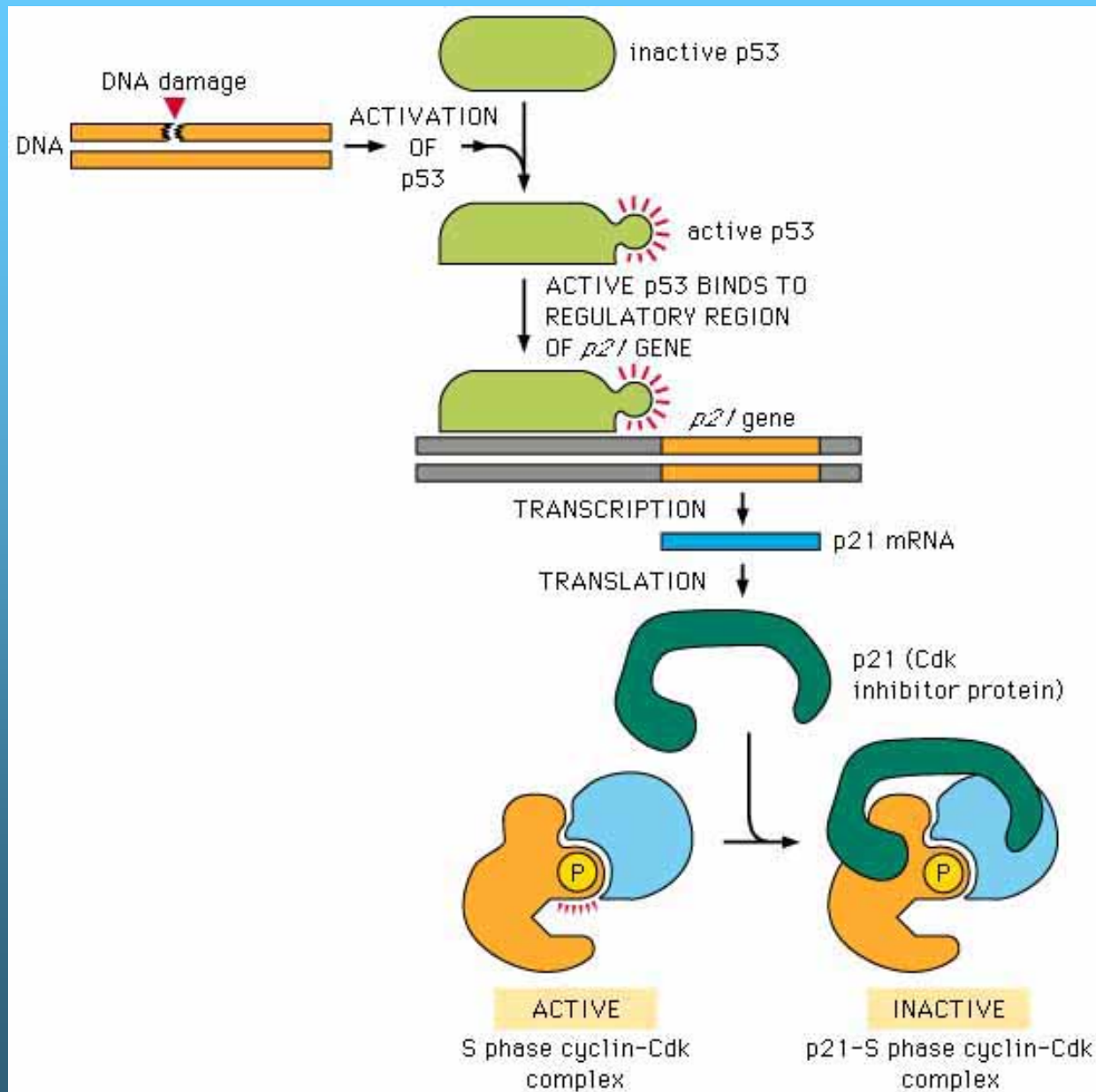
Come p53 regola la fase G₁ in caso di danno al DNA



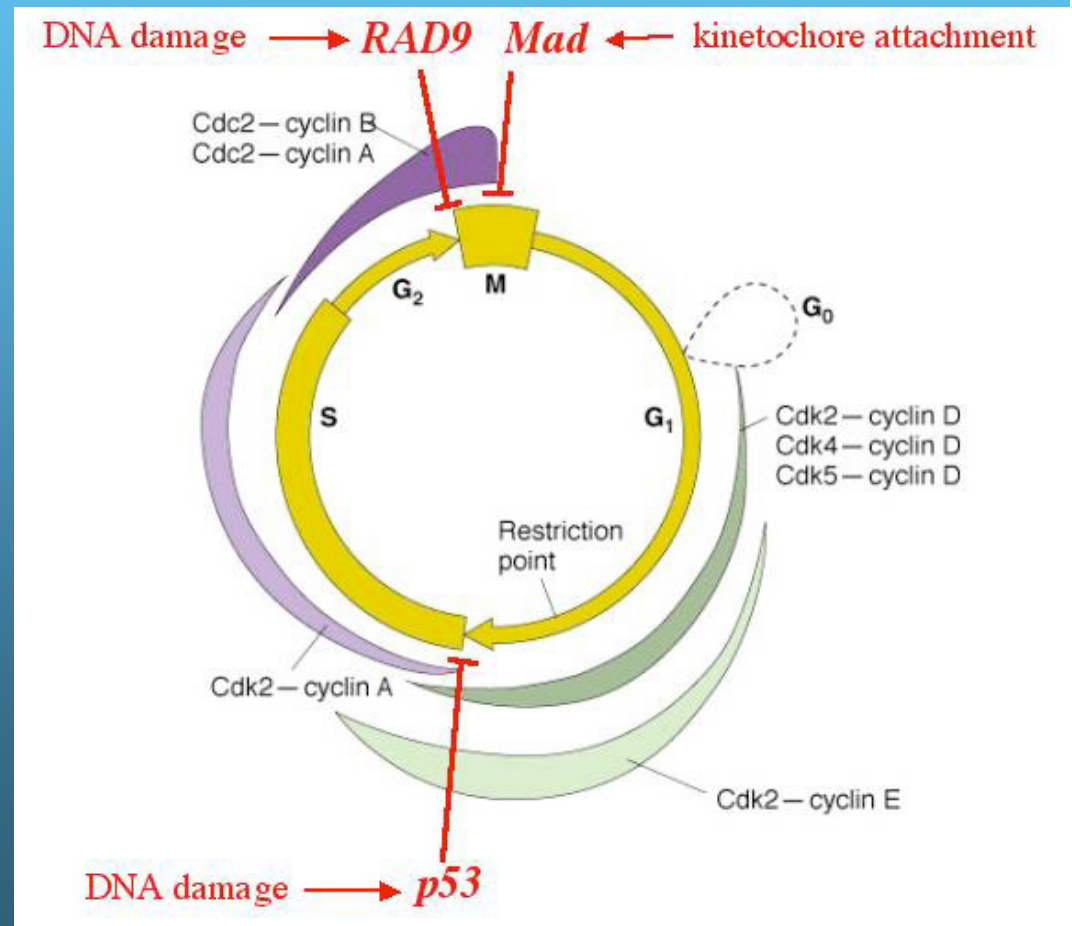
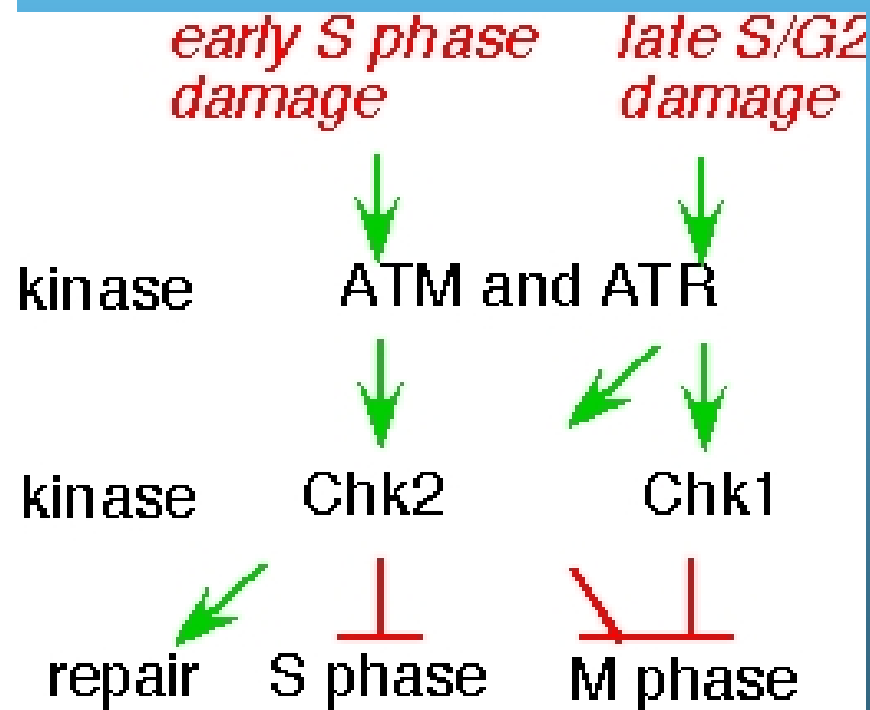
Dettagli della regolazione di p53



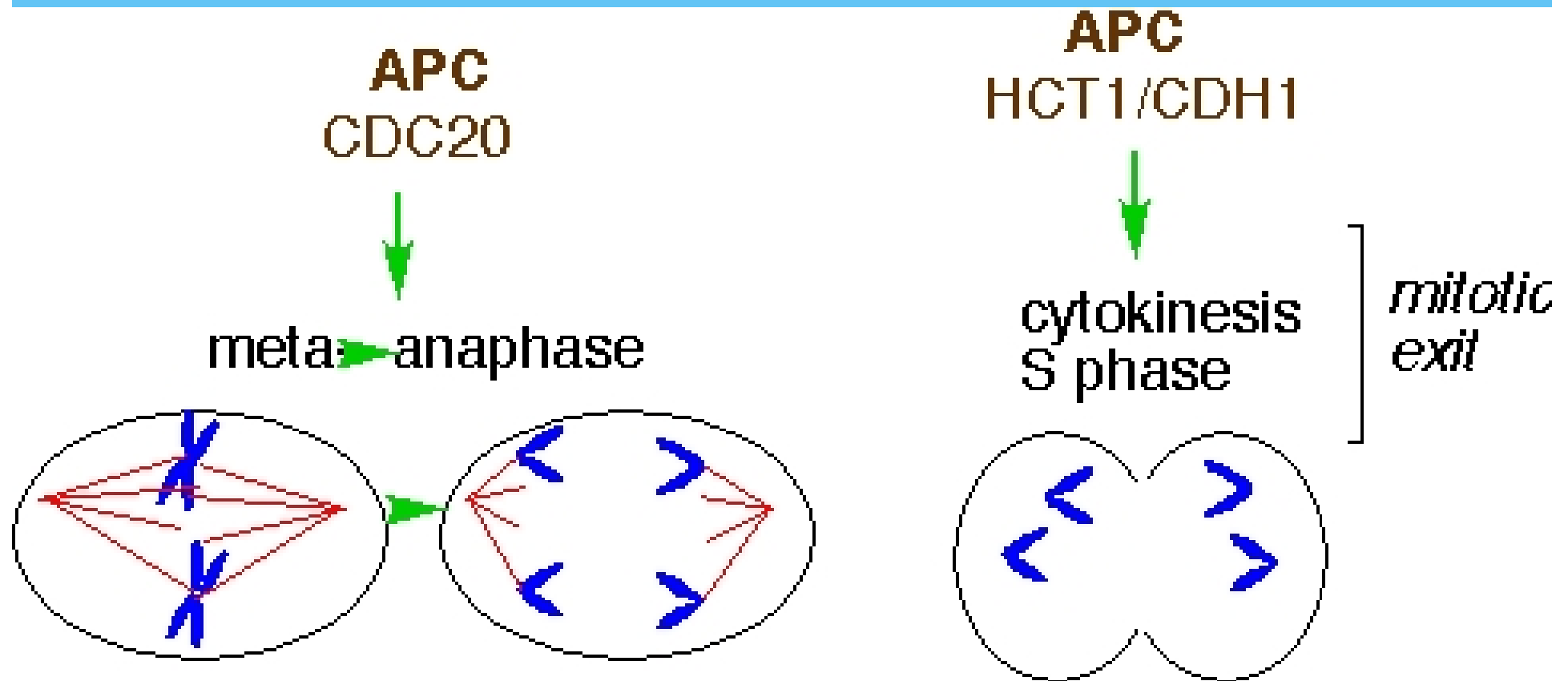
Come p53 regola la fase S in caso di danno al DNA



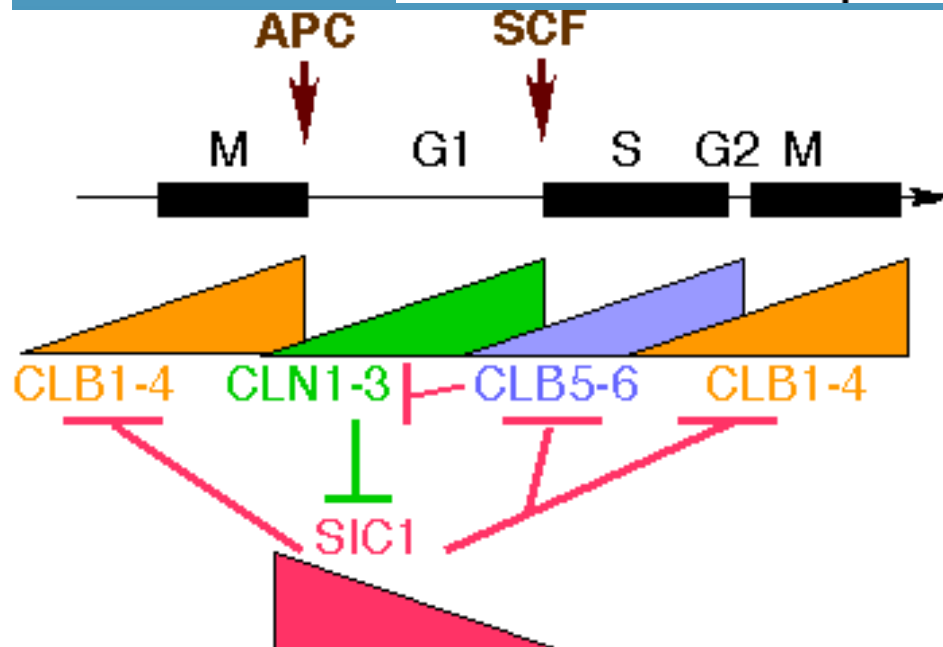
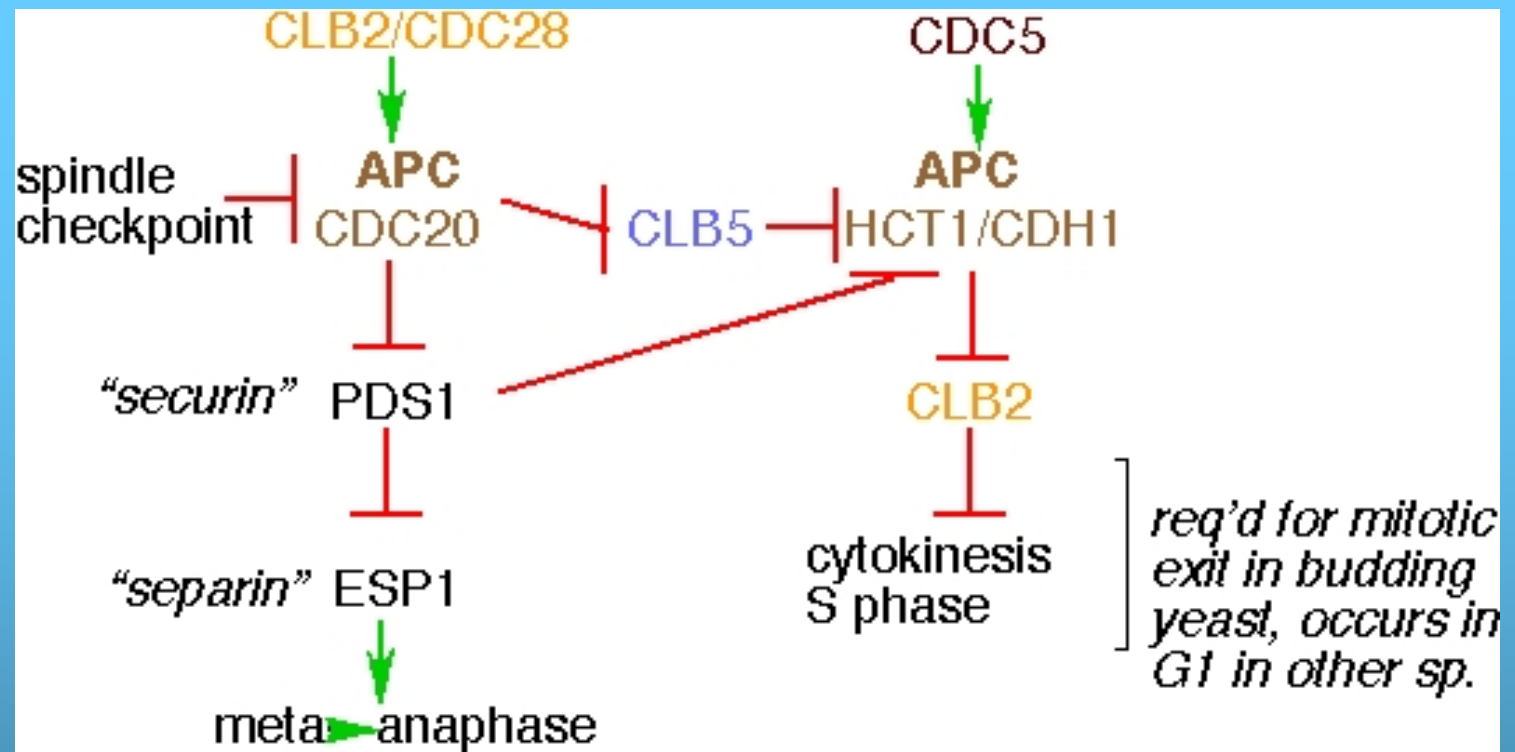
Checkpoints del danno



La proteolisi come metodo di controllo in metafase

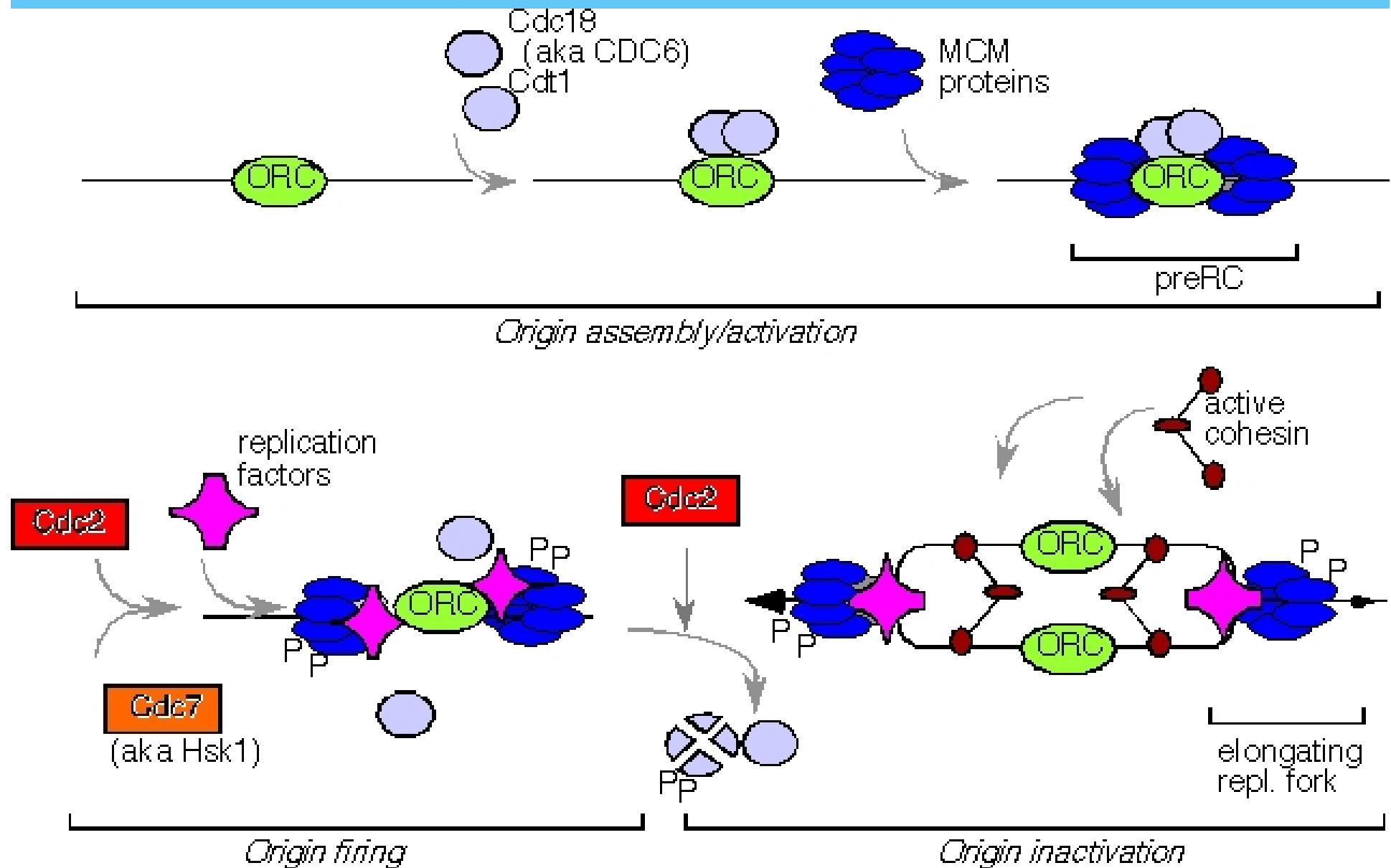


APC e SCF sono complessi molto simili tra loro

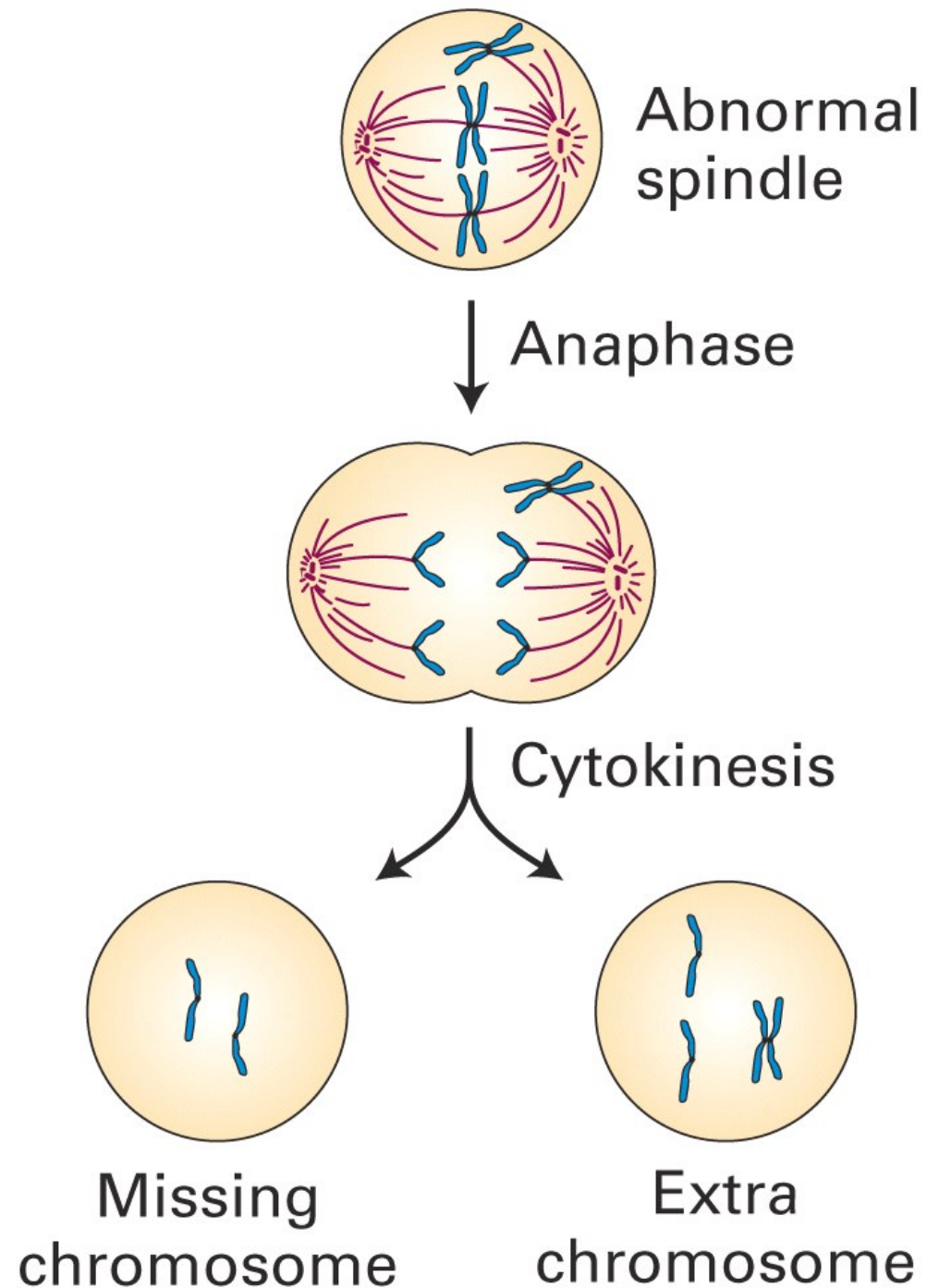


SCF degrada le proteine che inibiscono l'ingresso in fase S, come, ad es. SIC1, l'inibitore del complesso ciclina A/cdk2

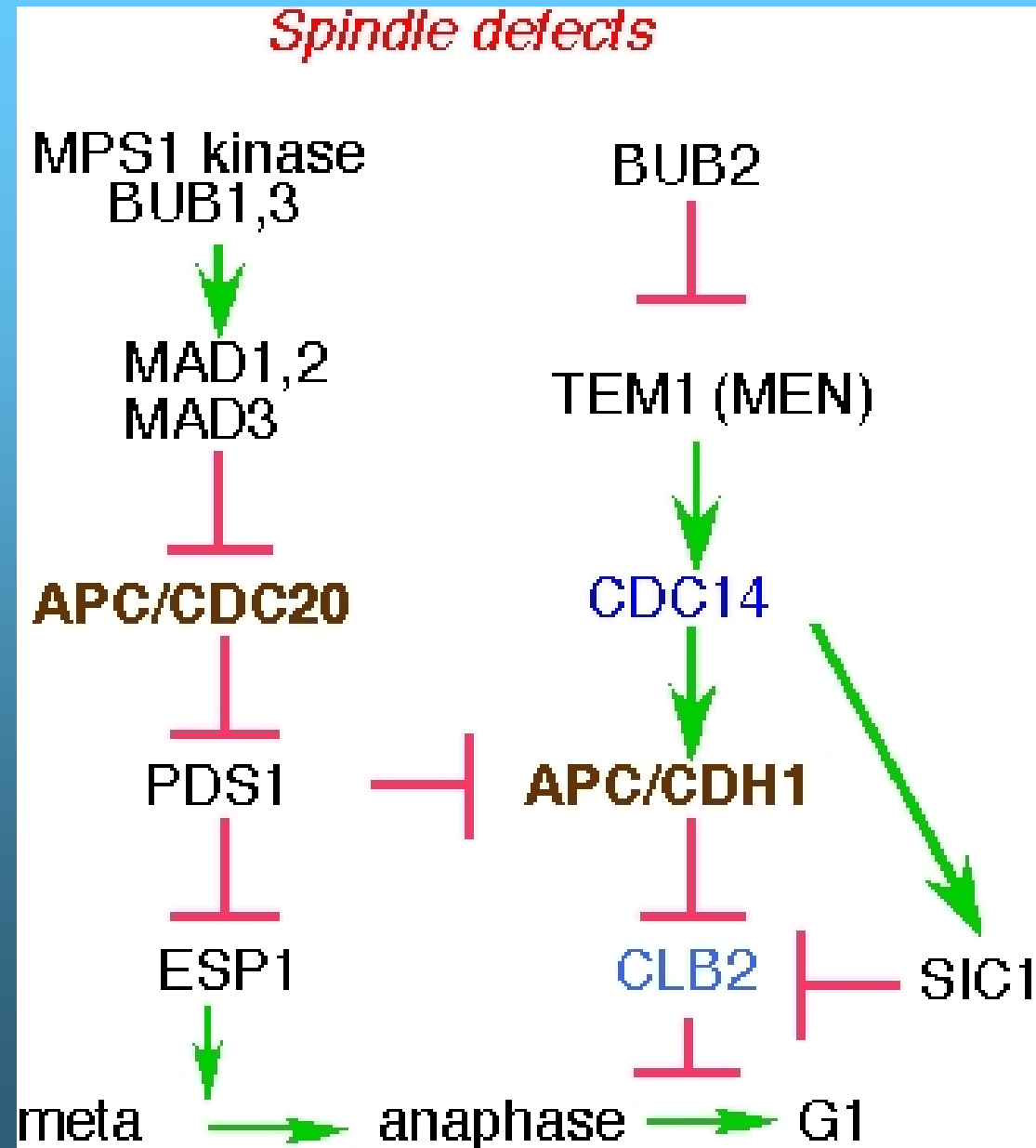
Il controllo della replicazione del DNA



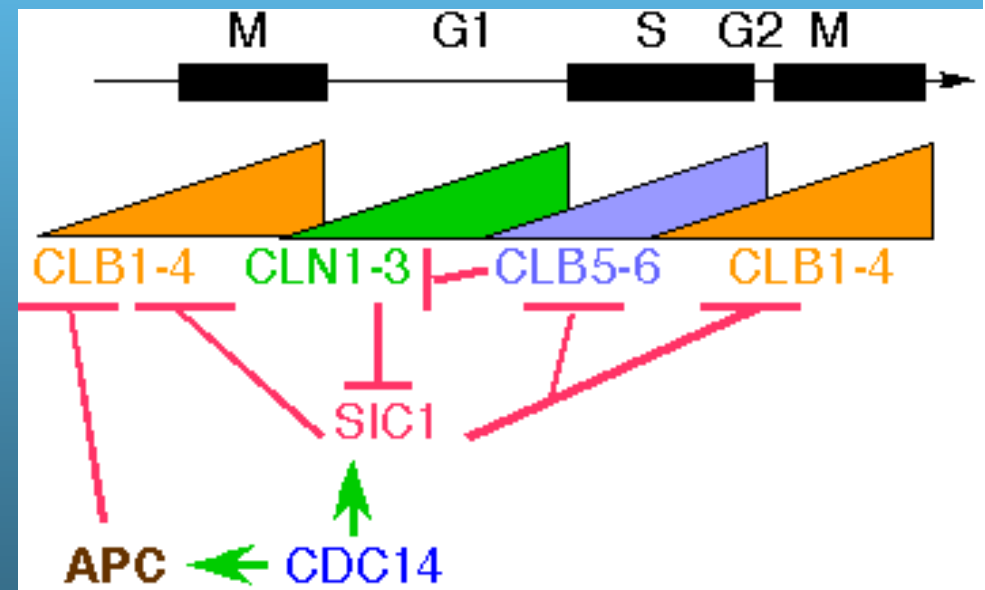
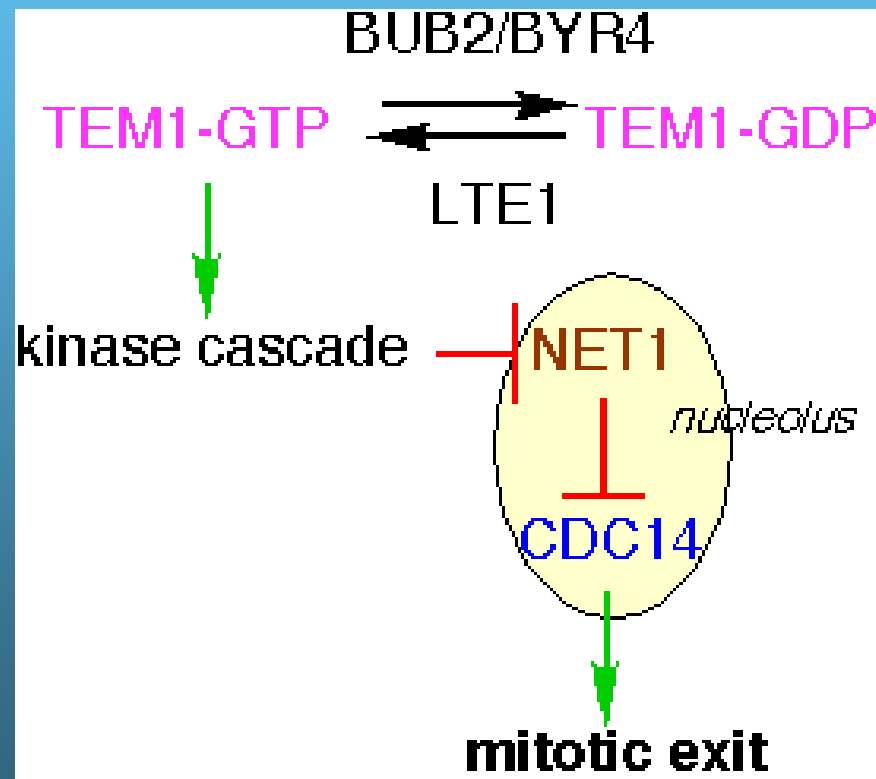
**Il controllo in anafase
impedisce la
segregazione errata dei
cromatidi fratelli**



Il controllo del fuso mitotico



L'uscita dalla mitosi



La *rete* che controlla l'uscita dalla mitosi

- La rete di controllo dell'uscita dalla mitosi (MEN) coordina APC con l'inattivazione delle kinasi. Consiste essenzialmente in una cascata di kinasi attivata da GTPasi, che porta all'attivazione della fosfatasi CDC14.
- Nel lievito gemmante, la cascata ha inizio con lo spostamento di uno dei poli del fuso nella gemma, il che porta al rilascio di CDC14 sequestrata nel nucleolo.
- L'attività MEN è inibita se il checkpoint del fuso non dà il “permesso”
- CDC14 defosforila CDH1, incrementando la velocità di distruzione delle cicline mitotiche
- CDC14 induce la sintesi di SIC1 e lo defosforila
- Negli eucarioti superiori, CDC14 defosforila RB
- Tutte le proteine che sono state fosforilate per effetto delle kinasi si trovano alla fine del ciclo/inizio di G_1 defosforilate.