

# Microrganismi di interesse biotecnologico

**Un esempio di metaboliti secondari, prodotti da un iperparassita (*Cladosporium tenuissimum*) dei funghi della ruggine e dotati di attività antiproliferativa : i cladosporoli.**

# Microrganismi di interesse biotecnologico



## Protection

- Protect the plant directly from any infection that are likely to arrive.
  - 1) Biological control
  - 2) Chemical control

# Microrganismi di interesse biotecnologico

## 1) Biological control

- The process to reduce or control the pest level by using another micro-organism
- Use antagonistic microorganism – microorganism that antagonist to the pathogen
- The microorganism will destroy and inhibit the growth of pathogen.
- Environmentally friendly method.

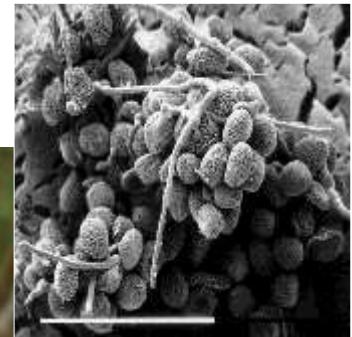
# Microrganismi di interesse biotecnologico

## BIOLOGICAL CONTROL METHODS

- The term biological control clearly implies control of a disease through some biological agency, and the term biological agency, means a living micro organism or macro organism other than the diseased or damaged plant acting as host and the pathogen or pest causing the disease or damage.
- According to Garrett (1965) “ biological control of plant disease may be defined as any condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism ( except man himself), with the result that there is a reduction in the incidence of the disease caused by the pathogen”.
- Eg: **Trichoderma viride**, a common saprophytic fungus, is able to parasitize the mycelia of other fungi.

# Microrganismi di interesse biotecnologico

The anamorphic ascomycete *Cladosporium tenuissimum* Cooke (teleomorph: *Davidiella*) found heavily parasitizing the aecial stage of *Cronartium flaccidum*



Can. J. Bot. 77: 339–347 (1999)

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**Molecular and conventional detection and identification of *Cladosporium tenuissimum* on two-needle pine rust aeciospores**

S. Moricca, A. Ragazzi, and K.R. Mitchellson

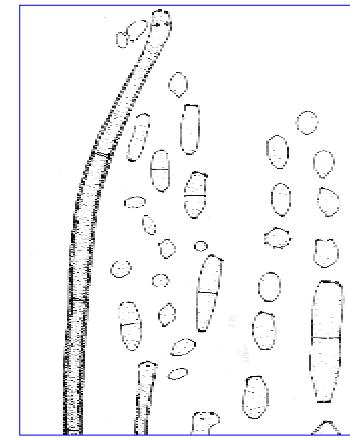
# Microrganismi di interesse biotecnologico

Kingdom:	Fungi
Division:	Ascomycota
Class:	Dothideomycetes
Order:	Capnodiales
Family:	Davidiellaceae
Genus:	<b><i>Cladosporium</i></b>

What does "*Cladosporium*" mean?

**κκλάδος** = arm = branch =  
projection (e.g. clade)  
= conidiophore/hyphal  
branch ...

**σπόρος** = sporo-, spor-,  
spori-, -sporium =  
seed = spore or  
propagule...



Every colony is a "sporulating colony"!!

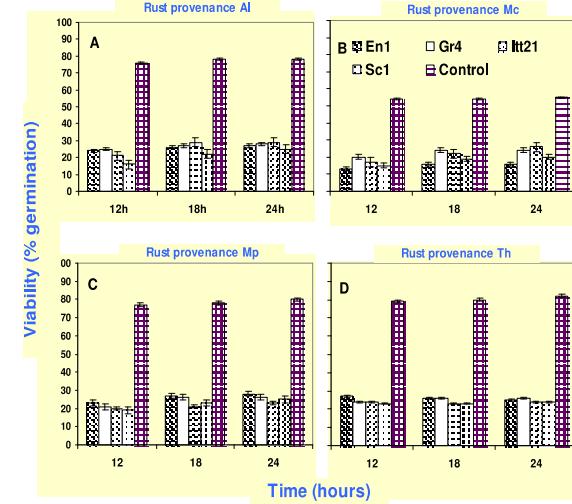
# Microrganismi di interesse biotecnologico

*In planta* antagonism assays: *Cronartium flaccidum/Pinus* spp. pathosystem



TABLE 3. Effect of treatment of pine seedlings with conidia of *Cladosporium tenuissimum* on suppression of pine stem rust<sup>a</sup>

Year	Pine species <sup>b</sup>	Disease severity				Disease incidence <sup>c</sup>							
		Stem infection <sup>d</sup>		Symptoms <sup>e</sup>		Start		End		% Disease reduction		% Seedling mortality	
		A	C	A	C	A	C	A	C	A	C	A	C
1999	Aleppo pine	+	+	+	+	S	S	M	S	24.9 c	0	3.3 b	13.3 b
	Austrian pine	+	+	-	+	M	M	M	S	58.3 b	0	0 c	6.9 d
	Italian stone pine	+	+	+	+	M	M	M	S	31.7 d	0	3.4 b	10.3 c
	Lanicio pine	+	+	-	+	S	S	M	S	54.6 c	0	0 c	6.6 d
	Maritime pine	+	+	+	+	S	S	M	S	23.8 e	0	7.1 a	17.2 a
	Scots pine	+	+	-	+	M	M	M	S	63.2 a	0	0 c	3.3 e
2000	Aleppo pine	+	+	+	+	S	S	M	S	31.9 d	0	6.6 b	13.7 b
	Austrian pine	+	+	-	+	M	M	M	S	57.9 b	0	3.3 c	10.3 c
	Italian stone pine	+	+	+	+	S	S	M	S	31.8 d	0	6.9 b	13.3 b
	Lanicio pine	+	+	-	+	S	S	M	S	45.6 c	0	3.4 c	10.3 c
	Maritime pine	+	+	+	+	S	S	M	S	19.1 c	0	10 a	21.4 a
	Scots pine	+	+	-	+	M	M	M	S	56.6 a	0	0 d	3.4 d



The culture filtrate strongly reduced spore viability *in vitro*

Biological Control

Antagonism of the Two-Needle Pine Stem Rust Fungi *Cronartium flaccidum* and *Peridermium pini* by *Cladosporium tenuissimum* In Vitro and In Planta

Salvatore Moriga, Alessandro Ragazzi, Keith Richard Mitchelson, and Gemma Assante

# Microrganismi di interesse biotecnologico

*In planta* antagonism assays: *Phaseolus vulgaris/Uromyces appendiculatus*



*Mycol. Res.* **108** (2): 170–182 (February 2004). © The British Mycological Society

DOI: 10.1017/S0953756203008852 Printed in the United Kingdom.

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## Histological studies on the mycoparasitism of *Cladosporium tenuissimum* on urediniospores of *Uromyces appendiculatus*

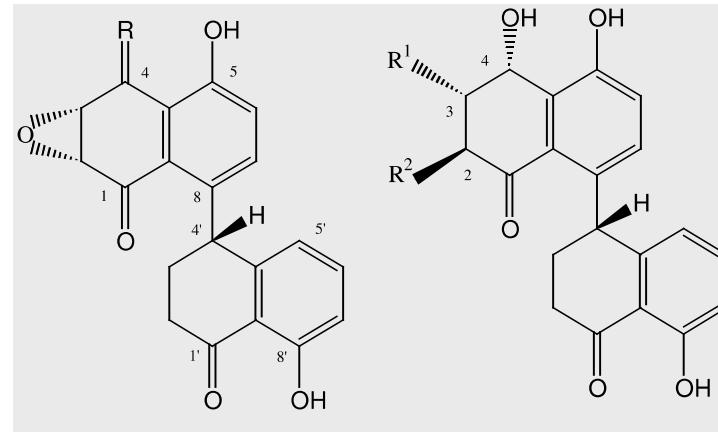
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Gemma ASSANTE<sup>1\*</sup>, Dario MAFFI<sup>1</sup>, Marco SARACCHI<sup>1</sup>, Gandolfinia FARINA<sup>1</sup>, Salvatore MORICCA<sup>2</sup>  
and Alessandro RAGAZZI<sup>3</sup>

# Microrganismi di interesse biotecnologico

The structures and stereochemistry of the metabolites produced by *C. tenuissimum* as elucidated by spectrometric and NMR analyses

## Cladosporols A - E



Cladosporol A:  $R = \beta\text{-H}, \alpha\text{-OH}$

Cladosporol B:  $R = O$

Cladosporol C:  $R^1 = R^2 = O$

Cladosporol D:  $R^1 = OH; R^2 = H$

Cladosporol E:  $R^1 = R^2 = OH$



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Phytochemistry 65 (2004) 2107–2111

PHYTOCHEMISTRY

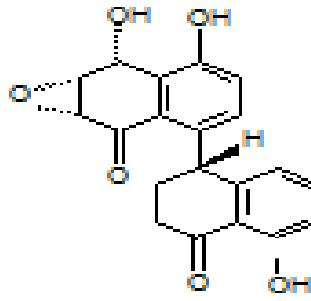
[www.elsevier.com/locate/phytochem](http://www.elsevier.com/locate/phytochem)

Secondary mould metabolites of *Cladosporium tenuissimum*,  
a hyperparasite of rust fungi <sup>☆</sup>

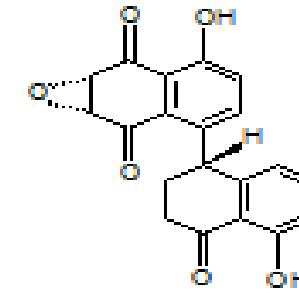
Gianluca Nasini <sup>a,\*</sup>, Alberto Arnone <sup>a</sup>, Gemma Assante <sup>b</sup>, Adriana Bava <sup>a</sup>,  
Salvatore Moricca <sup>c</sup>, Alessandro Ragazzi <sup>d</sup>

# Microrganismi di interesse biotecnologico

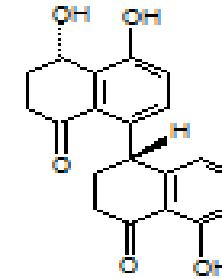
## The cladosporols



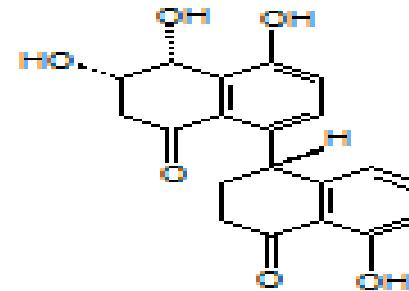
A



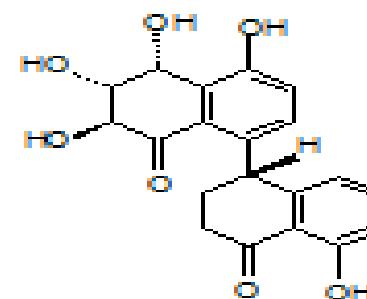
B



C



D



E

# Microrganismi di interesse biotecnologico

Demonstrated antifungal activity of *Cladosporium tenuissimum* and of its metabolites



- *C. tenuissimum* is a destructive mycoparasite (mechanical force/lytic action)
- *C. tenuissimum* produces fungicidal metabolites
- Cladosporols is an inhibitor of  $\beta$ -1,3-glucan synthetase

# Microrganismi di interesse biotecnologico

**I metaboliti secondari generati dal *Cladosporium tenuissimum*  
possono essere considerati dei veri e propri farmaci ?  
Possono essere usati, per esempio nel controllo delle malattie degenerative  
(cancro) nell'uomo ?**

# Microrganismi di interesse biotecnologico

## Le fasi della sperimentazione di un farmaco nuovo

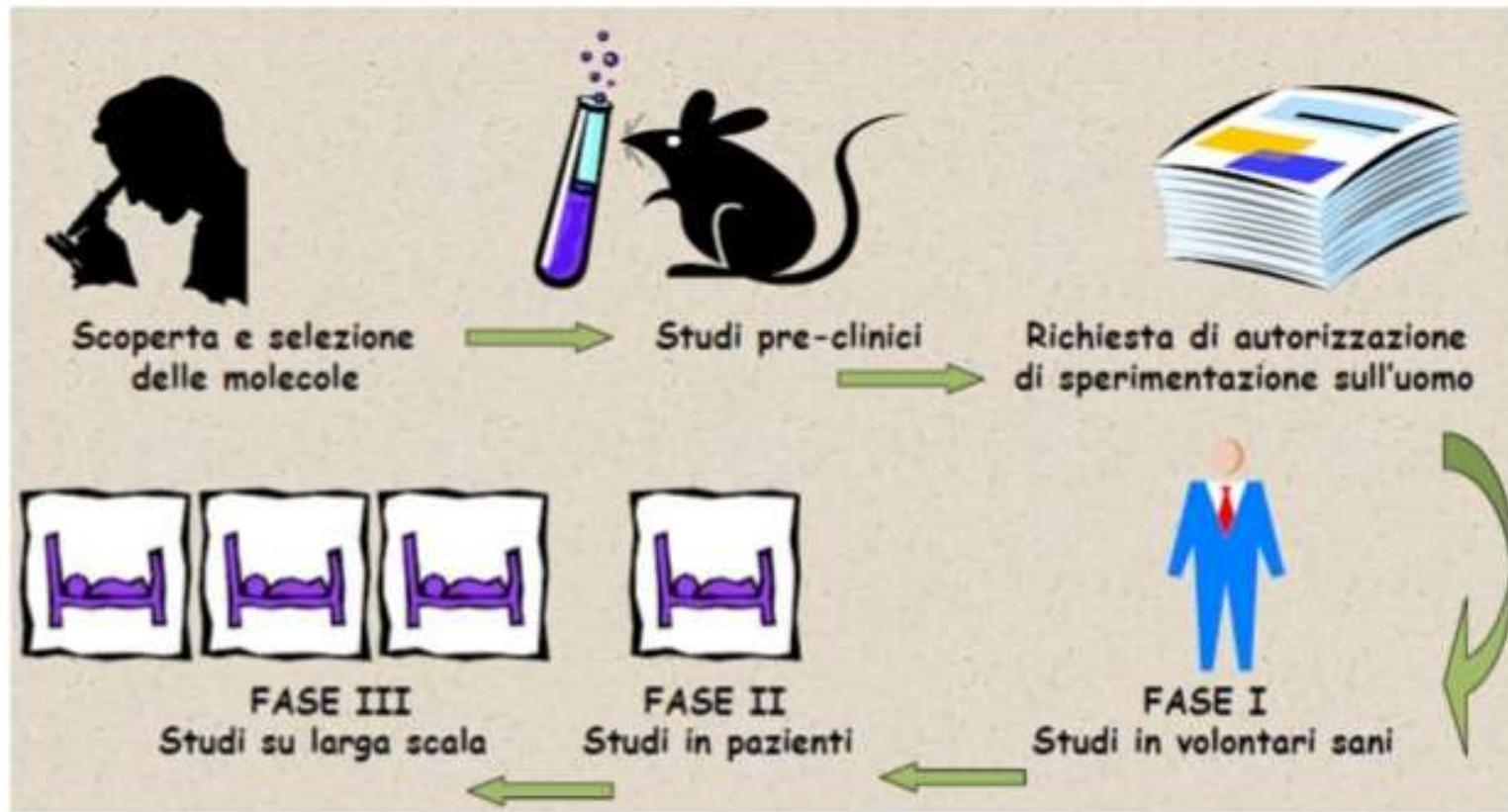


# Microrganismi di interesse biotecnologico

## Le fasi della sperimentazione di un farmaco nuovo



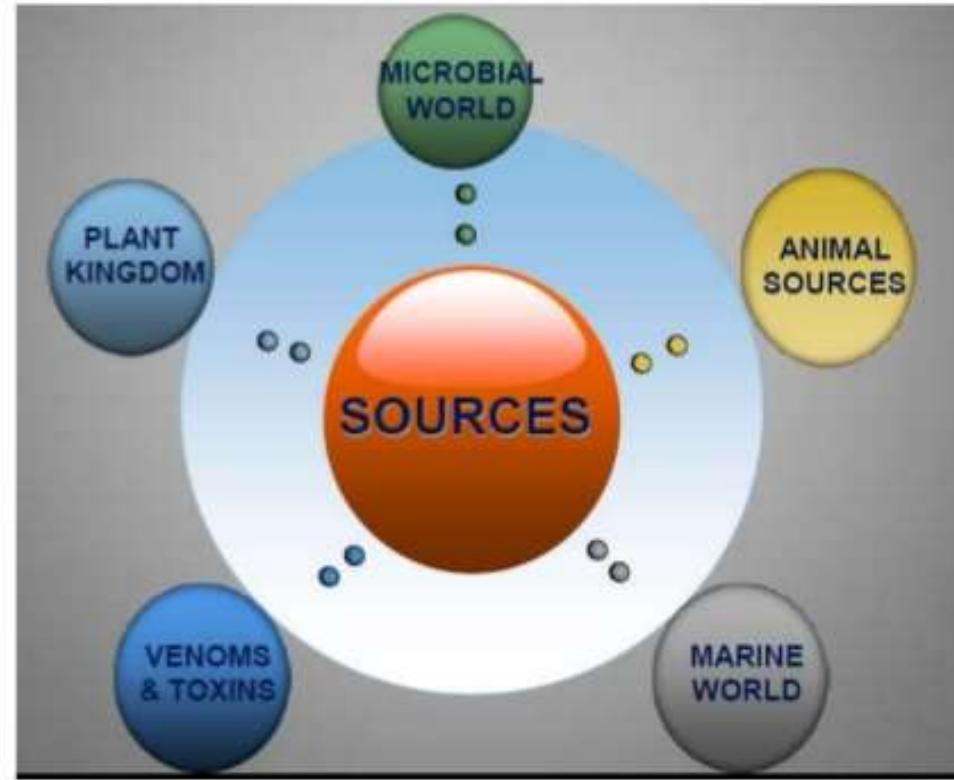
### Il percorso di un farmaco



# Microrganismi di interesse biotecnologico

## ROLE OF NATURAL PRODUCTS IN DRUG DEVELOPMENT

The natural products can be classify into



## HIGH THROUGHPUT SCREENING (HTS)

Identification of one or more positive candidates  
extracted from a pool of possible candidates  
based on specific criteria.



## INTRODUCTION

- High Throughput Screening (HTS) is a drug-discovery process widely used in the pharmaceutical industry. It leverages automation to quickly assay the biological or biochemical activity of a large number of drug-like compounds.



## ADVANTAGES OF HTS

- High sensitivity of assay (single molecule detection)
- High speed of assay (automation)
- Minimization of assay (microtiter plate assay)
- Low background signal
- Clear message (best: Yes/No answer)



## USES OF HTS:

- To screen for all kind of novel biological active compounds (libraries):
  - Natural products
  - Combinatorial Libraries (peptides, chemicals...)
  - Biological libraries
  
- To screen Micro arrays such as:
  - DNA chips
  - RNA chips
  - Protein chips





# Microrganismi di interesse biotecnologico

## HTS Assay Examples

- Receptor-binding
  - GPCR
- Enzyme
  - protease, kinase, phosphatase, lipase, others
- Bacterial growth
- Cell-based reporter gene
- Cell growth, cell viability
- Cytochrome P<sub>450</sub> inhibition
- Protein binding
- Protein production by cells
- ELISA

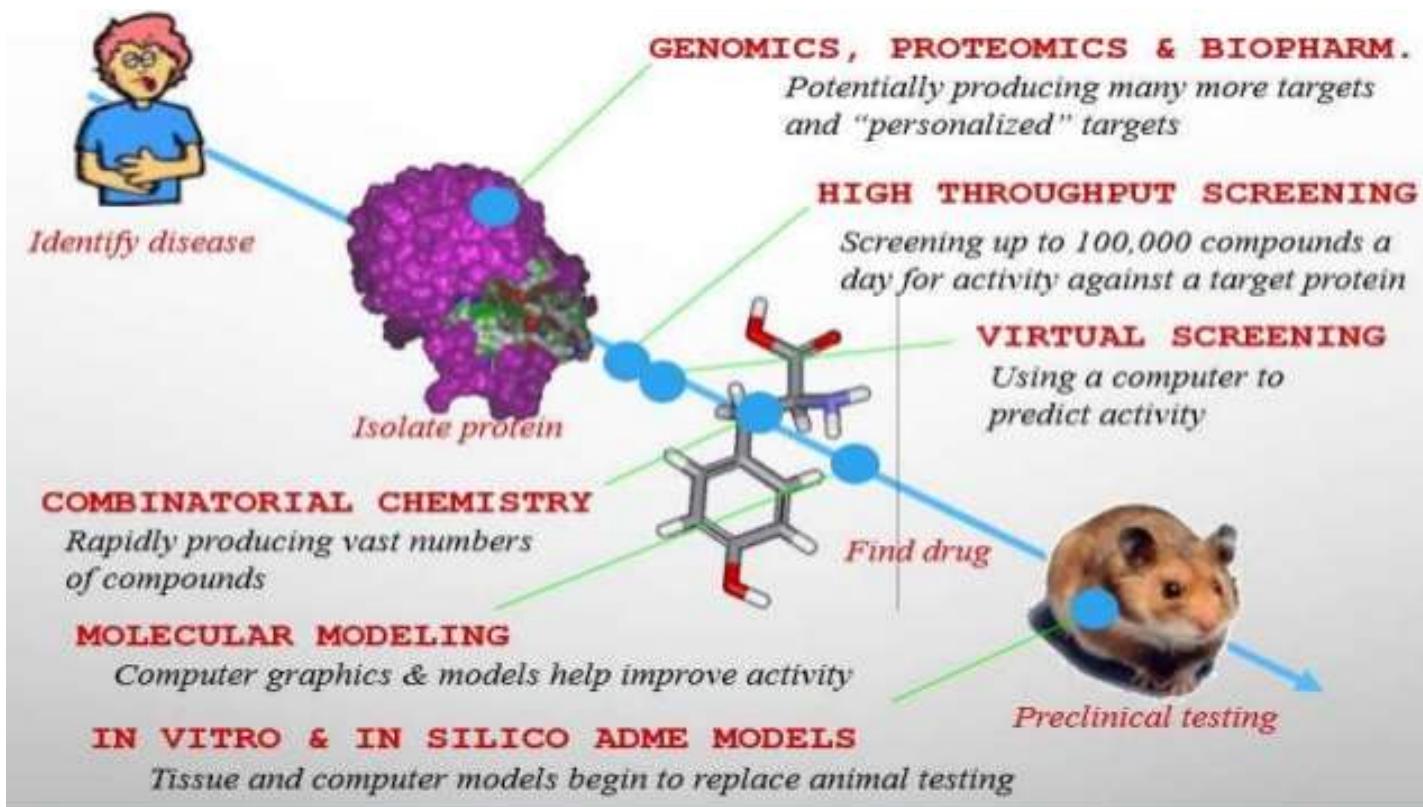
# Microrganismi di interesse biotecnologico

## Esempio di high throughput screening assay



# Microrganismi di interesse biotecnologico

## Esempio di high throughput screening assay

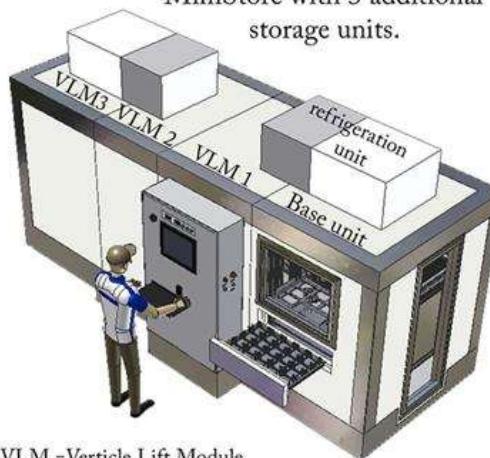


# Microrganismi di interesse biotecnologico

## High Throughput Screening Facility

### Compound Storage

MiniStore with 3 additional storage units.



VLM =Verticle Lift Module

### Liquid Handling



### Plate Readers



### Robotic Automation



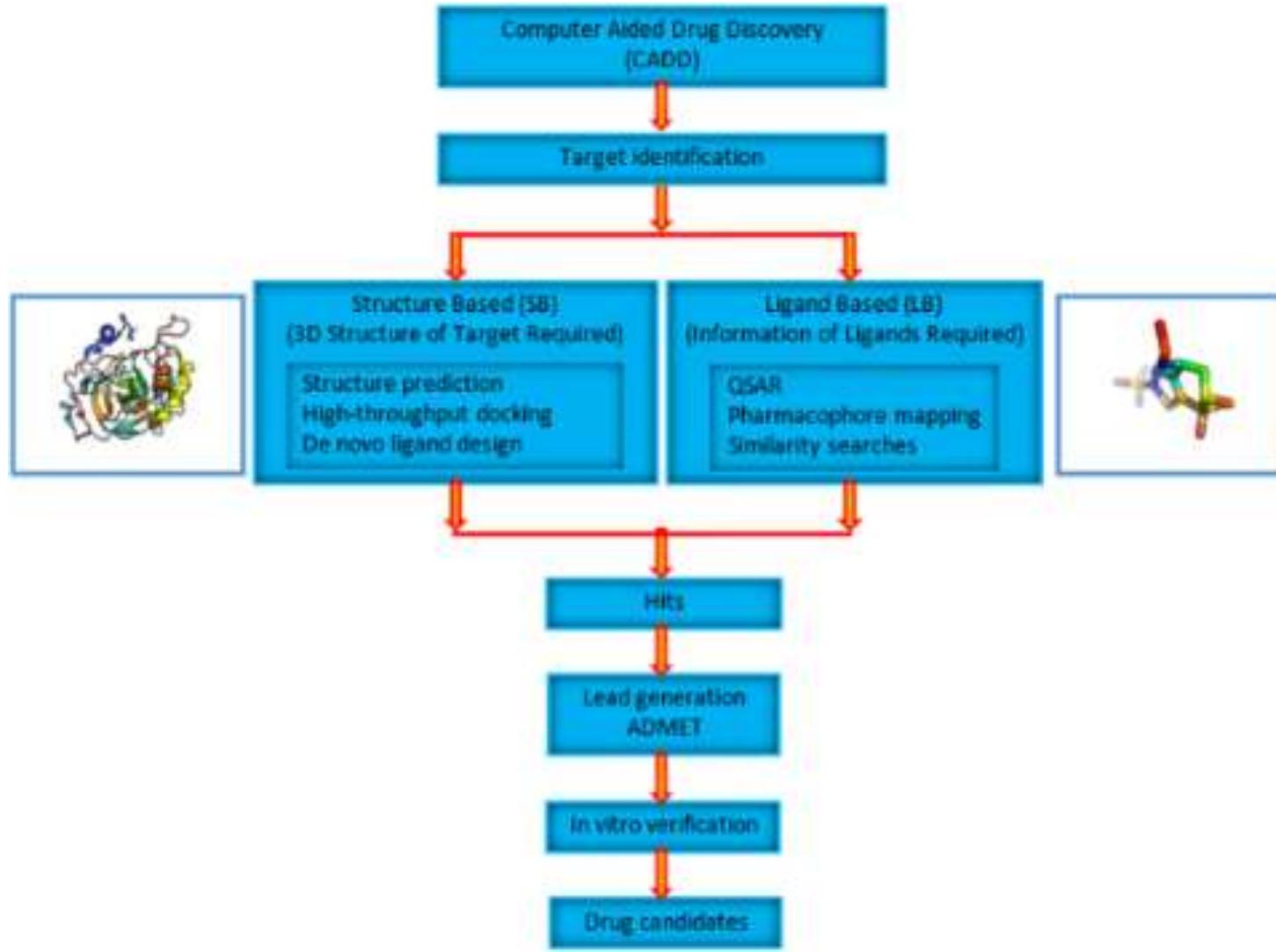
### Labware



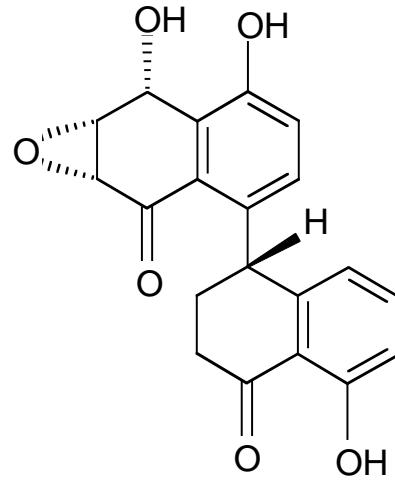


# Microrganismi di interesse biotecnologico

## Virtual high throughput screening assay



# Microrganismi di interesse biotecnologico

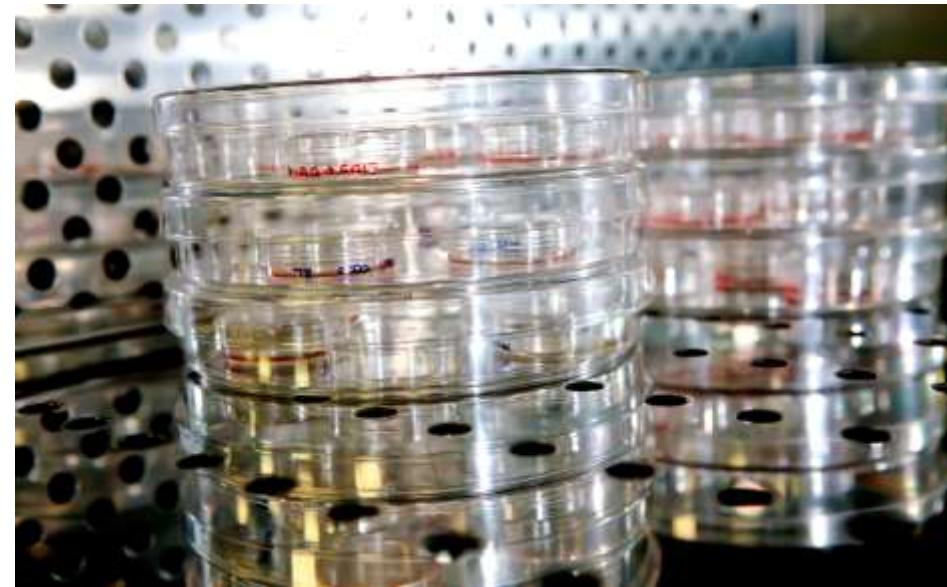


**Cladosporol A**



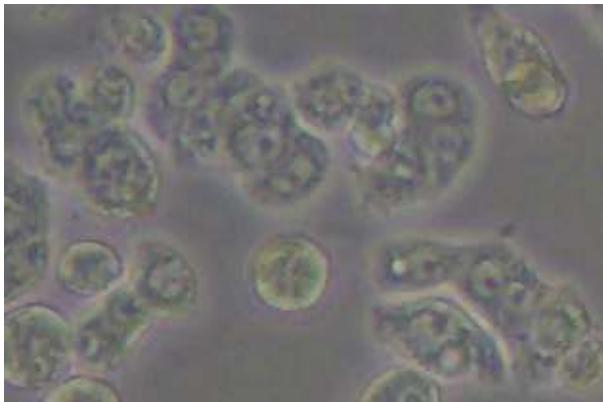
# Microrganismi di interesse biotecnologico

## Culture cellulari



# Microrganismi di interesse biotecnologico

## Morphological changes of untreated and cladosporol A-treated HT-29 cells



Untreated



5  $\mu$ M



10  $\mu$ M



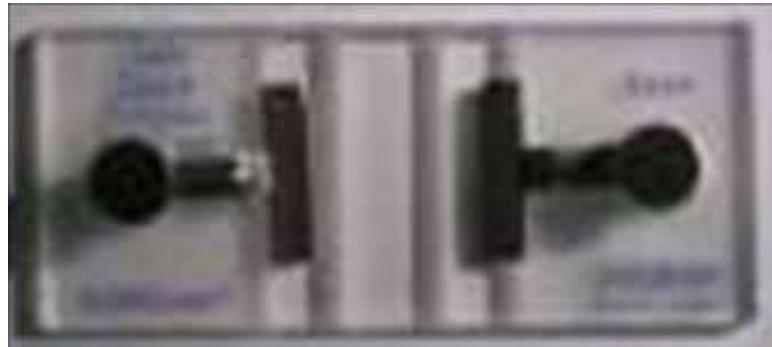
20  $\mu$ M

# Procedura per Conta Cellulare

- Preparare la sospensione cellulare
- Trasferire una piccola quantità di sospensione cellulare nella camera del vetrino, permettendo il riempimento per capillarità
- Contare le cellule nel quadrato centrale e nei quattro quadrati agli angoli
- Ciascun quadrato ha un **volume di 0.1mm<sup>3</sup>**

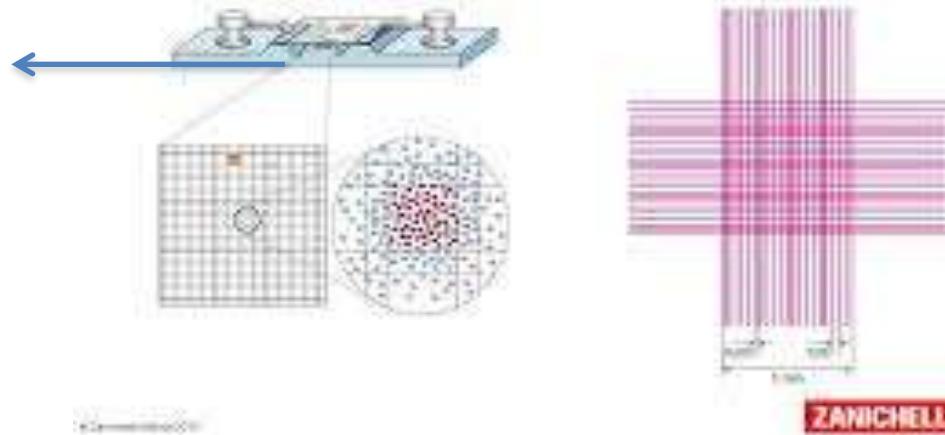
# Microrganismi di interesse biotecnologico

## Conta cellulare

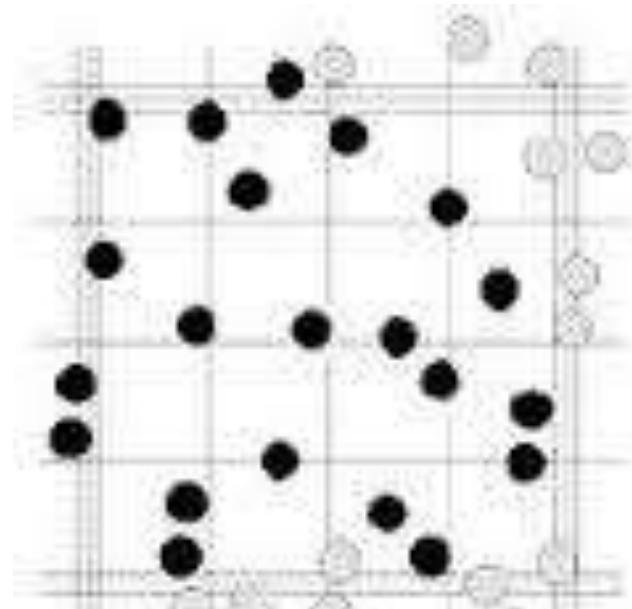


Il quadrato al cui interno si contano le cellule ha un lato di 1 mm, lo spessore tra vetrino portaoggetto e coprioggetto è di 0,1 mm, perciò il volume è di 0,1  $\mu\text{l}$ . Il fattore per riportare la conta a 1  $\mu\text{l}$  è 10, infatti  $0,1 \mu\text{l} \times 10 = 1 \mu\text{l}$ .

### Tecniche dirette: camere conta cellule



# Conta cellulare

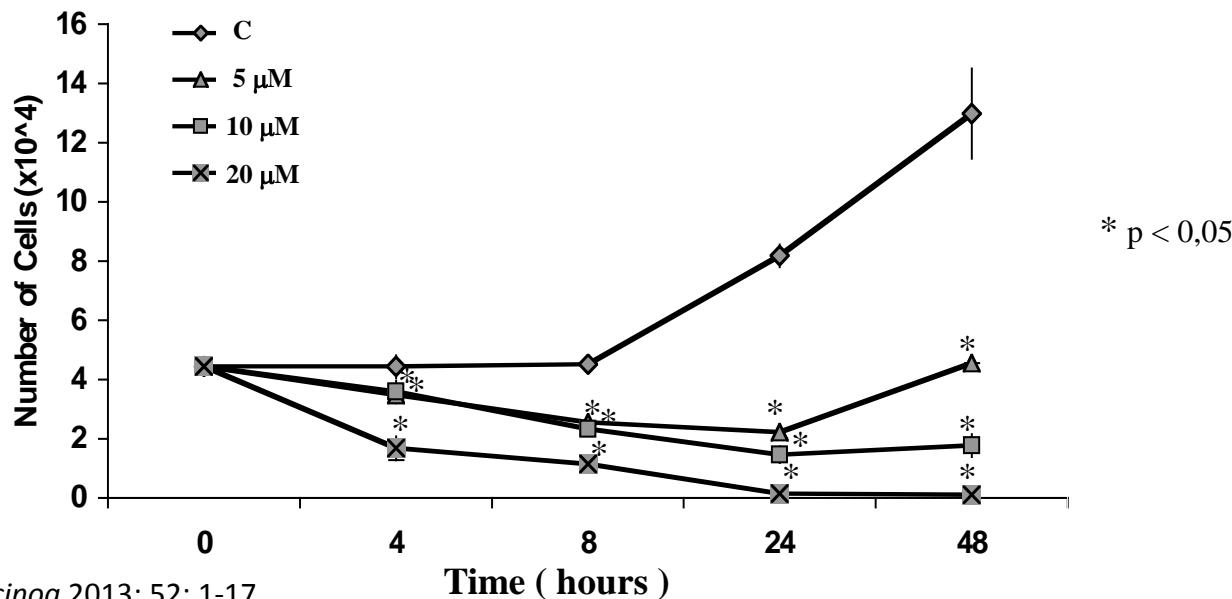
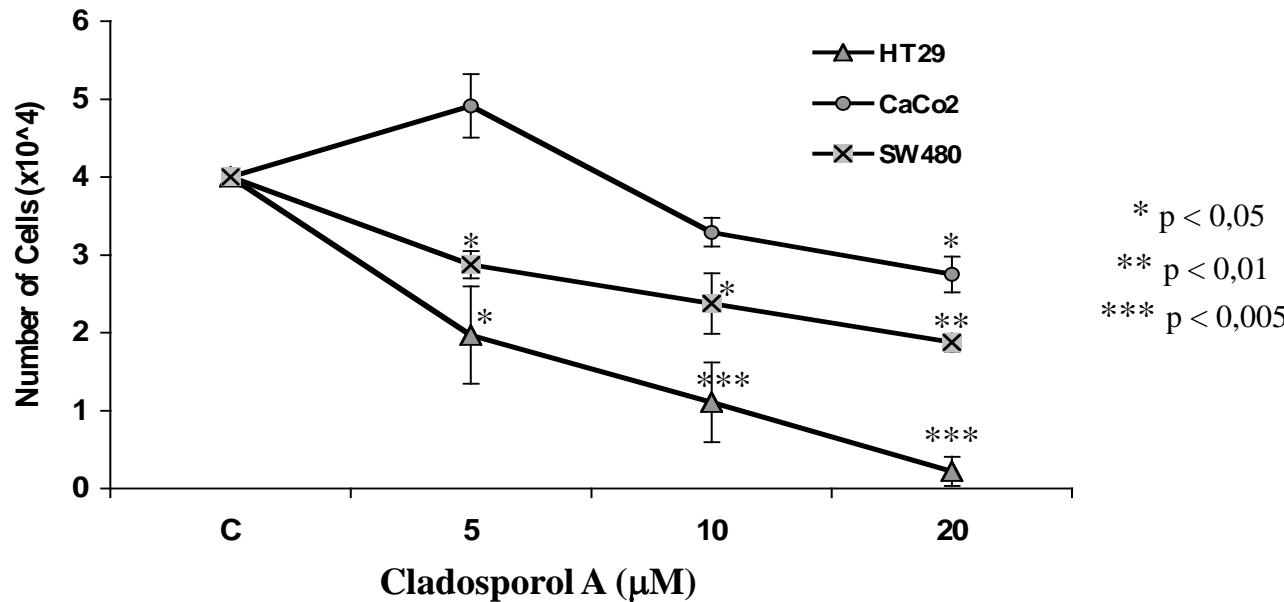


$$\text{media} = \frac{\text{n. cellule totali}}{\text{n. aree contate}} =$$

$$\frac{25 + 30 + 26 + 13 + 17 + 29 + 31 + 15 + 24 + 12}{10} = 22,2 \frac{\text{cell}}{\text{mm}^3}$$

$$x = \frac{\text{Media} \cdot 1 \text{ mm}^3}{\text{volume (mm}^3\text{)}} = \frac{22,2 \frac{\text{cell}}{\text{mm}^3} \cdot 1 \text{ mm}^3}{0,001 \text{ mm}^3} = 2,22 \cdot 10^4 \frac{\text{cell}}{\text{mm}^3} = \\ = 2,22 \cdot 10^7 \frac{\text{cell}}{\text{mL}}$$

# Cladosporol A inhibits HT-29 cell growth in a dose and time-response manner



# Microrganismi di interesse biotecnologico



## **RISULTATO N.1**

**Cladosporol A inhibits HT-29 cell growth in a dose and time-response manner**

## Alcune Questioni

**L'attività antiproliferativa del cladosporolo A da quali molecole è mediata ?**

**Quali meccanismi molecolari sono coinvolti in questi effetti antiproliferativi ?**

**Quali sono i controllori di questi meccanismi ?**

**Quali sono le vie di trasduzione che portano all'attivazione o all'inibizione della proliferazione cellulare ?**

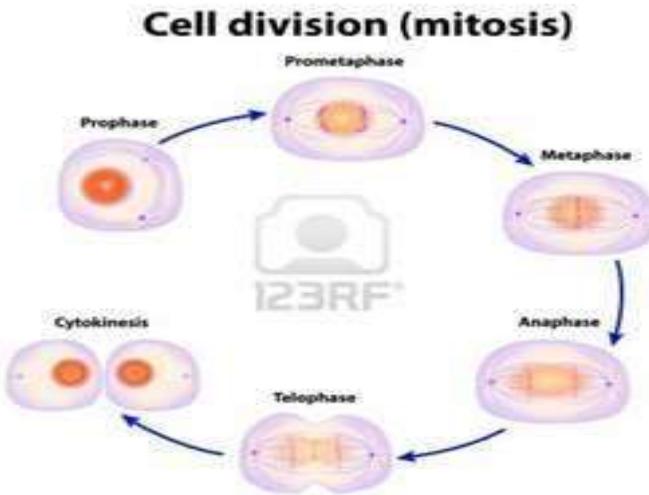
**E' possibile pensare a più vie di regolazione che sono stimolate dal cladosporolo A ?**

## Ciclo cellulare

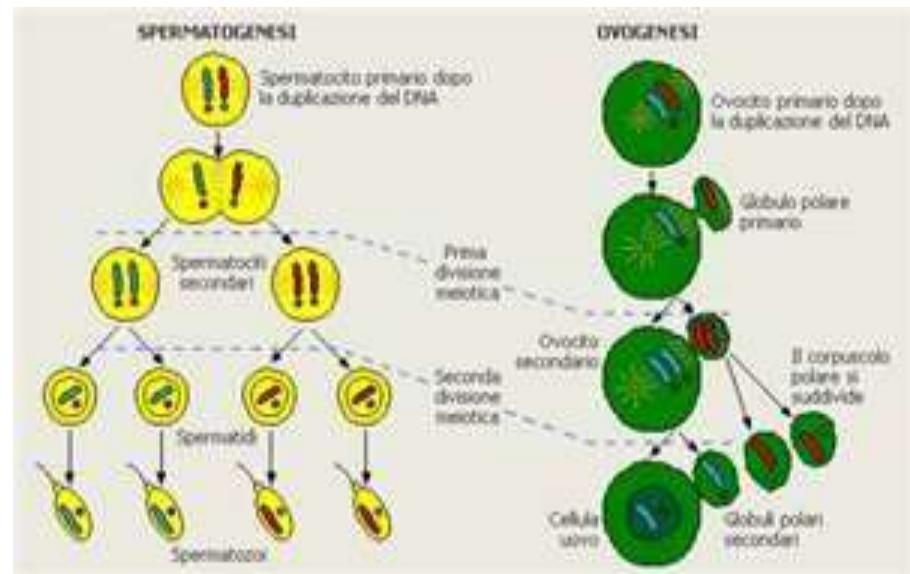
- La cellula si divide preparandosi inizialmente durante alcune fasi di maturazione e quindi entrando in divisione.
- Dividiamo questi due processi in:
  - Interfase
  - Divisione cellulare

## Divisione cellulare

- La divisione cellulare può essere di due tipi:
  - 1. Mitosi (diploidi)
  - 2. Meiosi (aploidi)



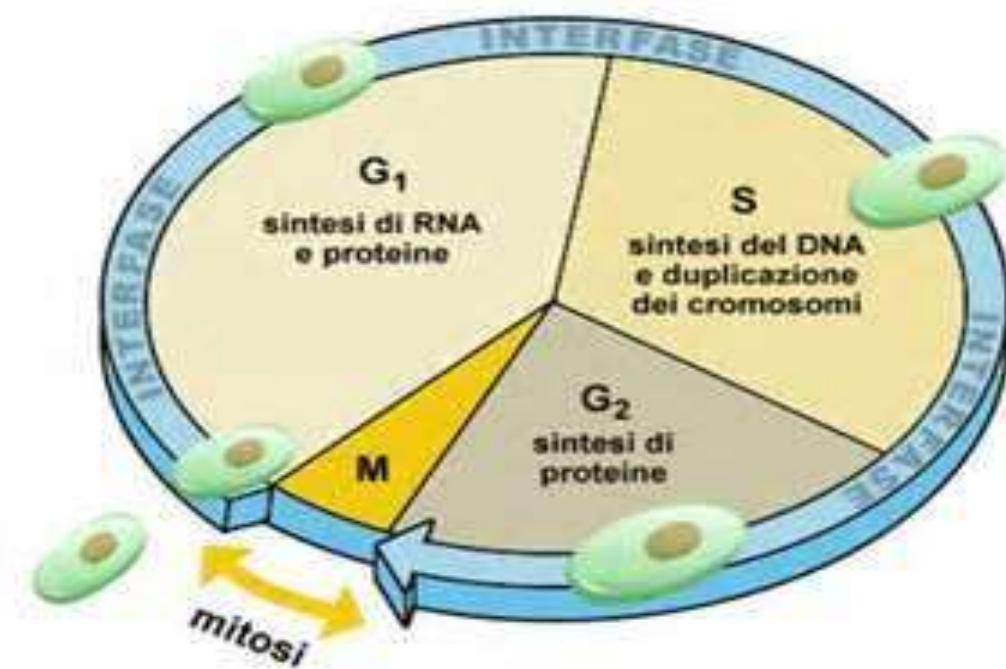
mitosi



meiosi

## Interfase

- L'interfase si divide in 4 processi:
- Fase G1 (gap1)
- Fase S (sintesi)
- Fase G2 (gap2)
- Fase M



# Microrganismi di interesse biotecnologico

## Cellule in coltura



In genere le cellule che sono utilizzate in esperimenti in vitro...

... sono popolazioni cellulari asincrone !!!



# Microrganismi di interesse biotecnologico

## Cellule in coltura

### Metodi di sincronizzazione

Normalmente in una coltura in accrescimento esponenziale le cellule si dividono in modo **NON SINCRONO** quindi in ogni istante sono presenti cellule in tutti gli stadi del ciclo cellulare.

Per poter studiare le variazioni biochimiche, morfologiche e fisiologiche che avvengono tra 2 divisioni cellulari si cerca di

**SINCRONIZZARE** la coltura in modo che **TUTTE LE CELLULE** in una coltura siano allo **STESO PUNTO** del **CICLO CELLULARE**.

1. Metodi che agiscono sul **metabolismo cellulare** portando le cellule tutte allo stesso punto del ciclo
2. **Metodi fisici** che permettono di separare nella popolazione le cellule allo stesso punto

# Microrganismi di interesse biotecnologico

## Cellule in coltura

1. Metodi che agiscono sul metabolismo cellulare portando le cellule tutte allo stesso punto del ciclo

### Sincronizzazione con la TEMPERATURA

un abbassamento della temperatura da 37°C a 25°C per 15 min induce divisione sincrona in Pneumococco. L'uso di sbalzi termici ad intervalli fissi è applicato anche ad altri microrganismi. Viene sfruttata la termodipendenza di qualche passaggio della divisione cellulare che avviene solo a T più elevata

### Sincronizzazione per CARENZA NUTRITIVA

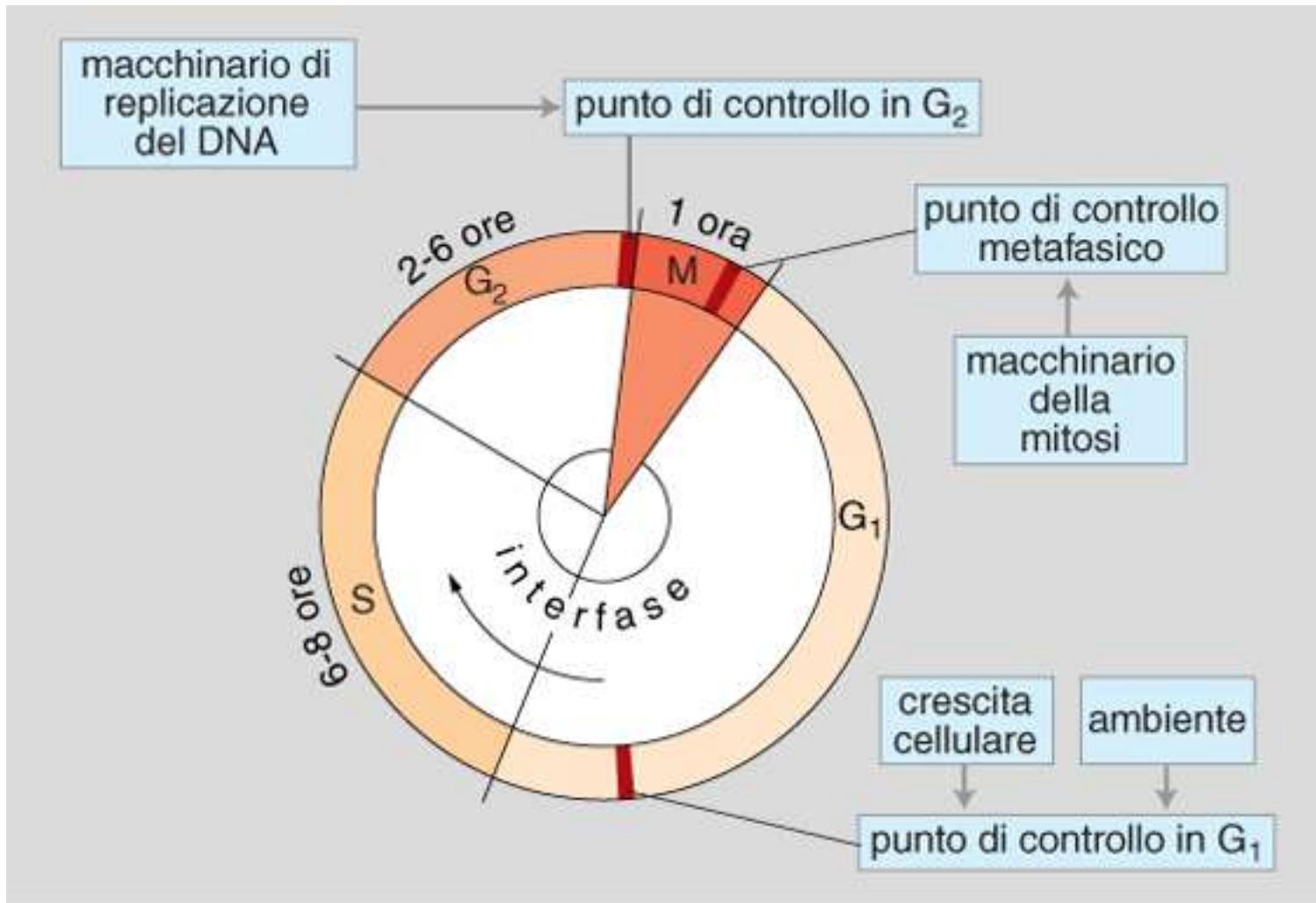
i microrganismi vengono posti in un terreno in cui mancano metaboliti essenziali (aminoacidi, vitamine basi azotate) : in questo modo le cellule raggiungono una condizione di blocco della crescita e non appena poste in terreno nutritivo completo presentano divisione sincrona

### Sincronizzazione per DILUIZIONE

le cellule vengono fatte crescere fino alla fase stazionaria ( $5 \times 10^9$  cellule/ml) poi diluite in terreno fresco

# Microrganismi di interesse biotecnologico

## Ciclo cellulare

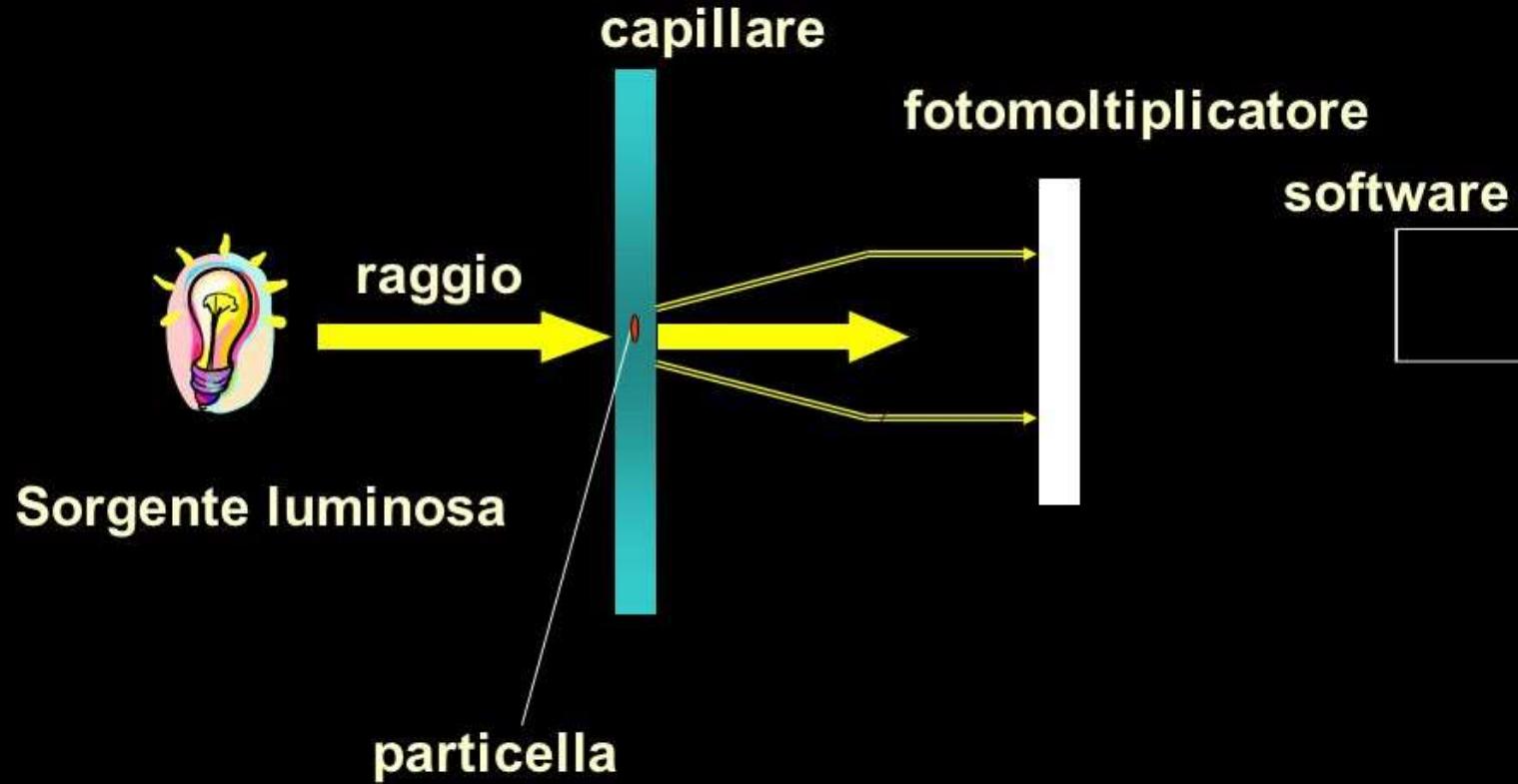


## Citofluorimetria a flusso

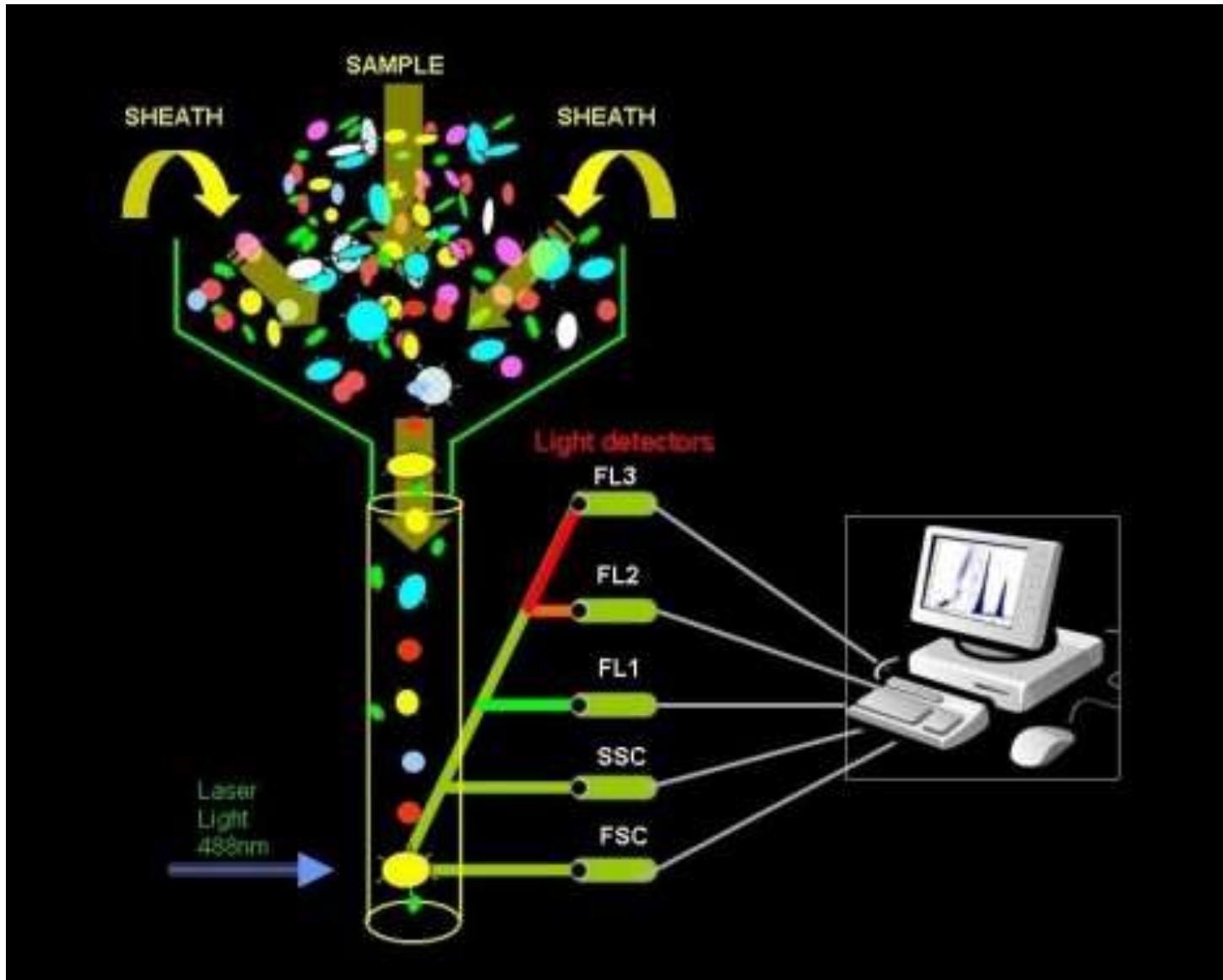


# Citofluorimetria a flusso

## Schema di un citometro a flusso



# Citofluorimetria a flusso



# Citofluorimetria a flusso

## Fluorocromi utilizzati più comunemente

Linee  
Laser  
Comuni

350      457      488      514      610      632  
300 nm    400 nm    500 nm    600 nm    700 nm



*Coniugato PE-TR*



*Texas Red*



*Ioduro di Propidio*



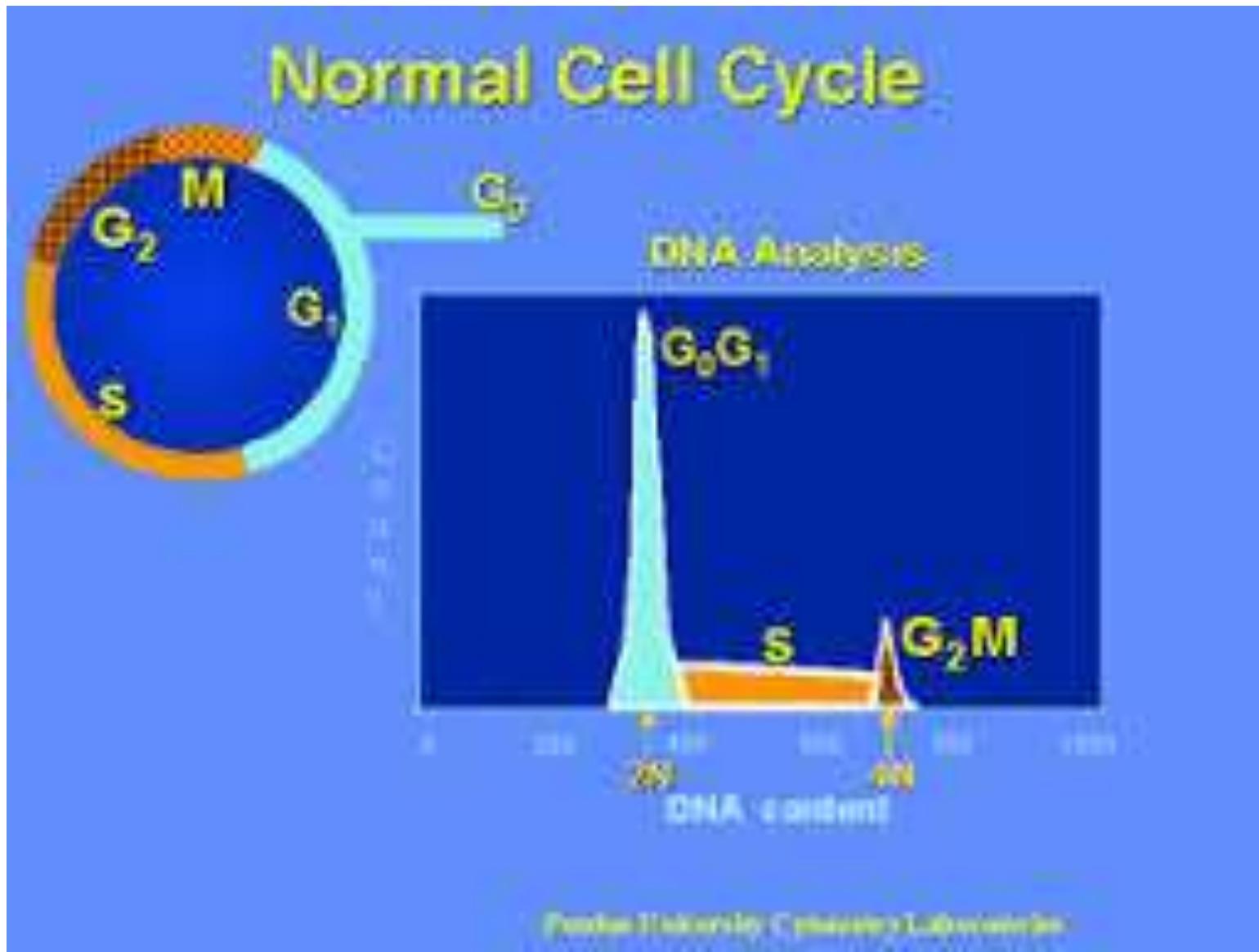
*Etidio Bromuro*

*PE (ficoeritrina)*

*FITC (fluoresceina)*

*Acido cis-  
Parinarico*

## Citofluorimetria a flusso



# Citofluorimetria a flusso

## Fluorocromi utilizzati più comunemente

Linee  
Laser  
Comuni

350      457      488      514      610      632  
300 nm    400 nm    500 nm    600 nm    700 nm



*Coniugato PE-TR*



*Texas Red*



*Ioduro di Propidio*



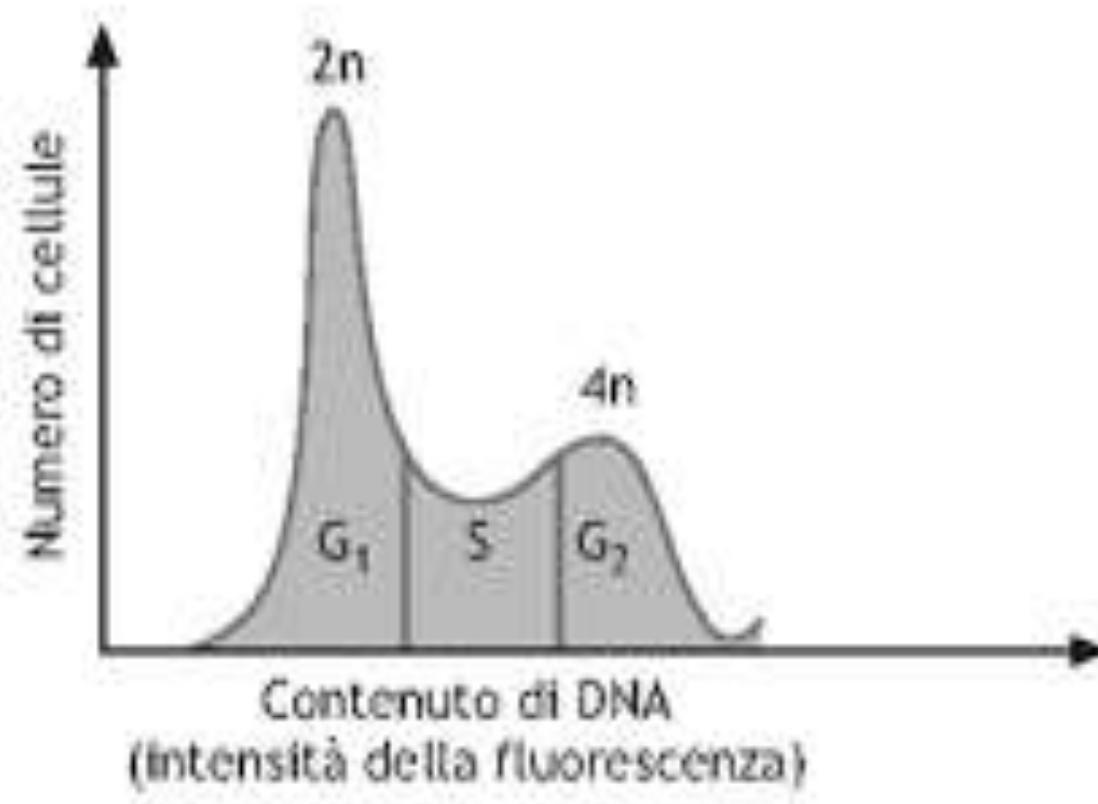
*Etidio Bromuro*

*PE (ficoeritrina)*

*FITC (fluoresceina)*

*Acido cis-  
Parinarico*

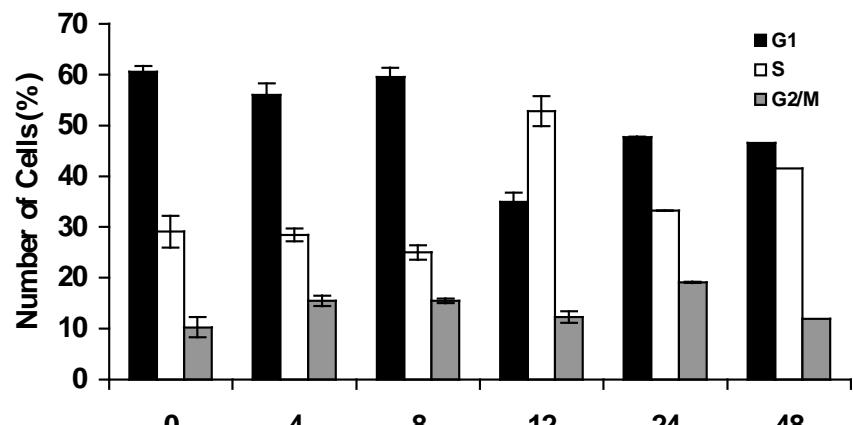
## Citofluorimetria a flusso



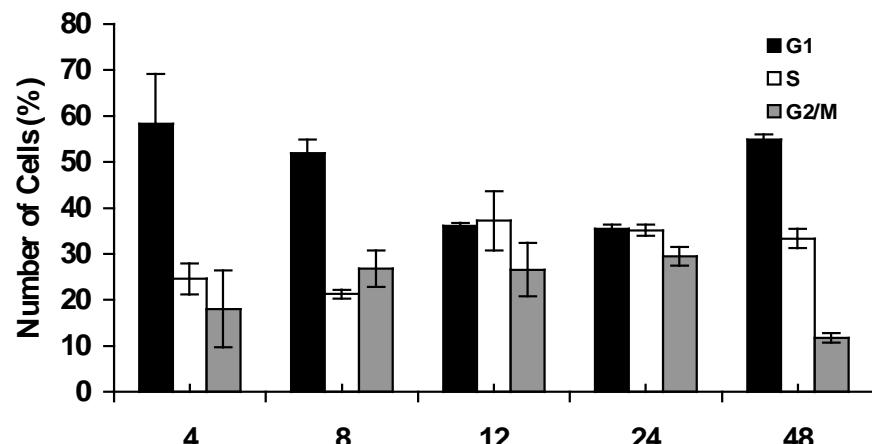
# Cladosporol A suppresses HT-29 cells growth via G1/S phase arrest

A

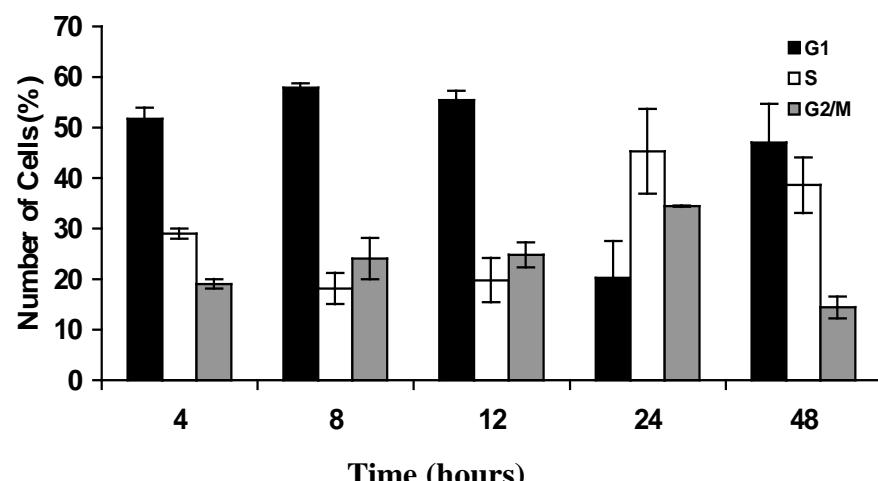
Untreated



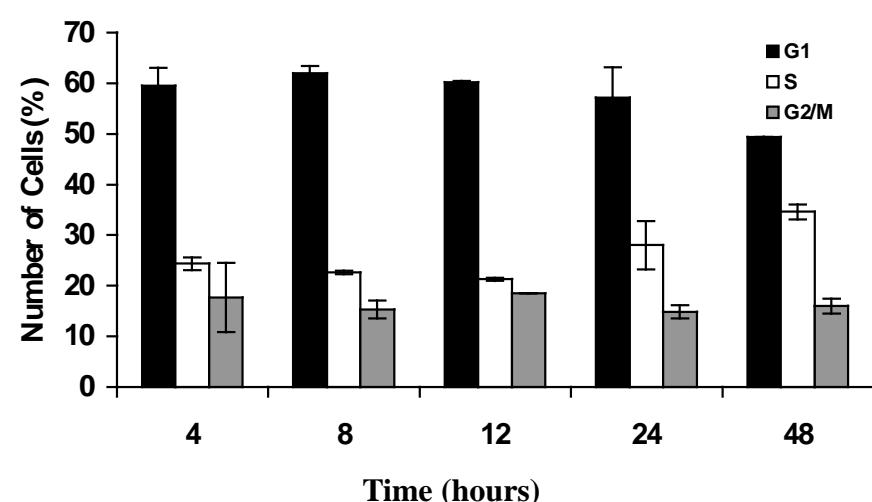
5  $\mu$ M



10  $\mu$ M



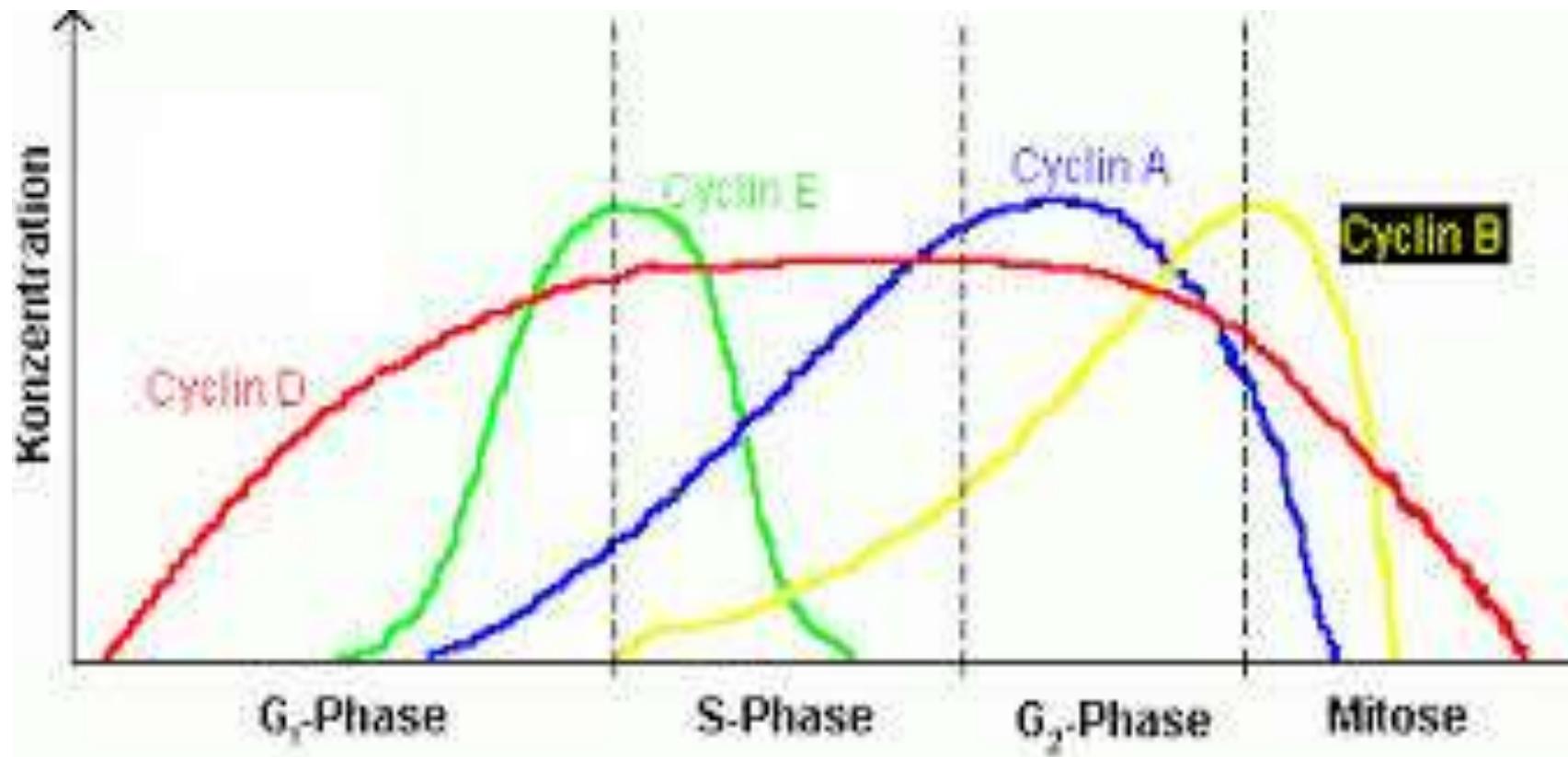
20  $\mu$ M



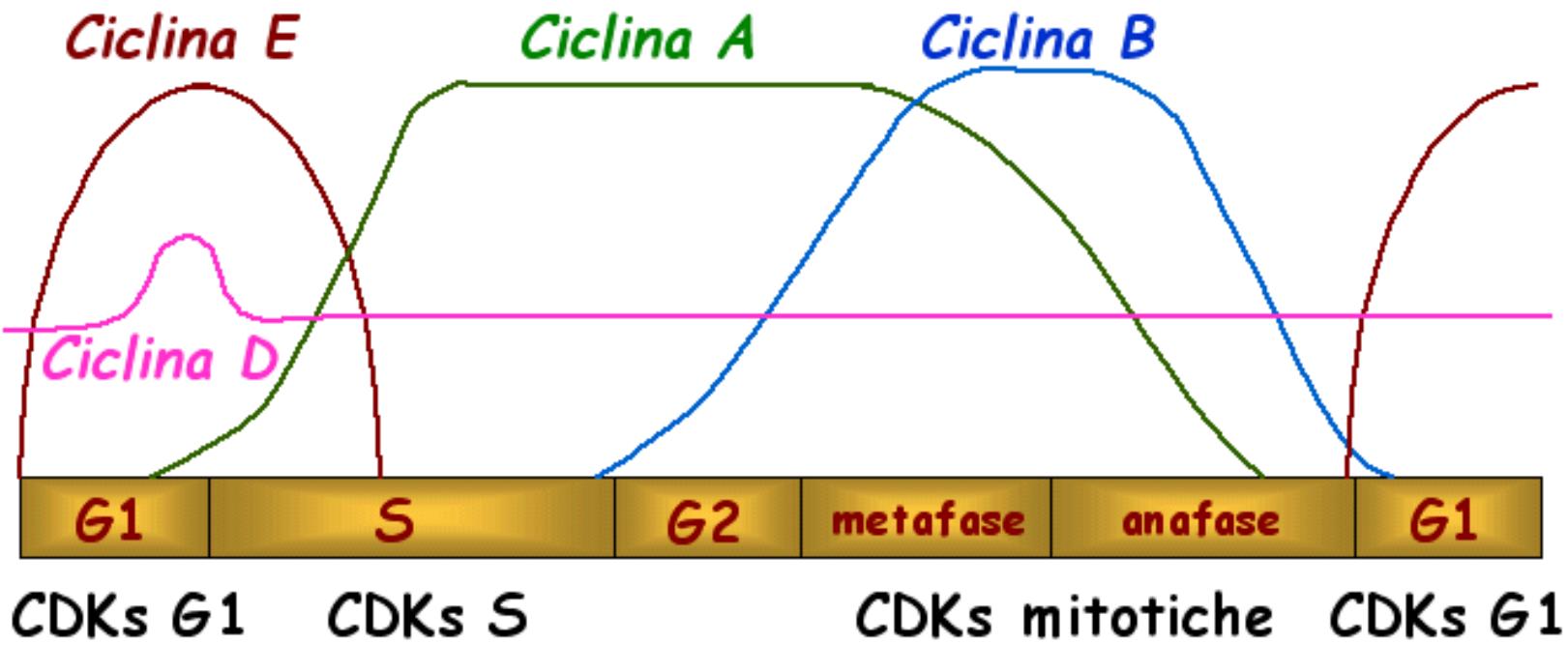
## **RISULTATO N.2**

**Cladosporol A suppresses HT-29 cells growth via G1/S phase arrest**

## Marcatori del ciclo cellulare

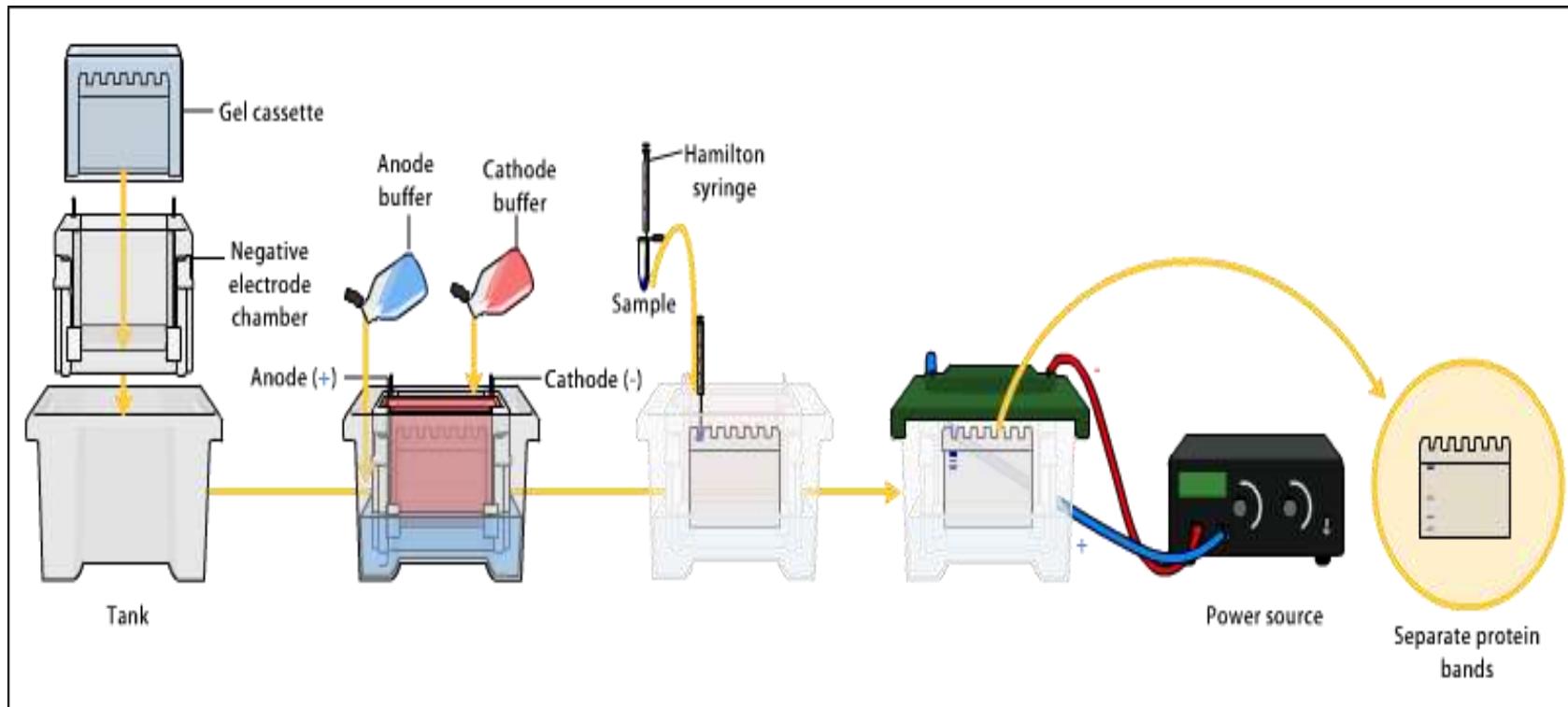


# Marcatori del ciclo cellulare



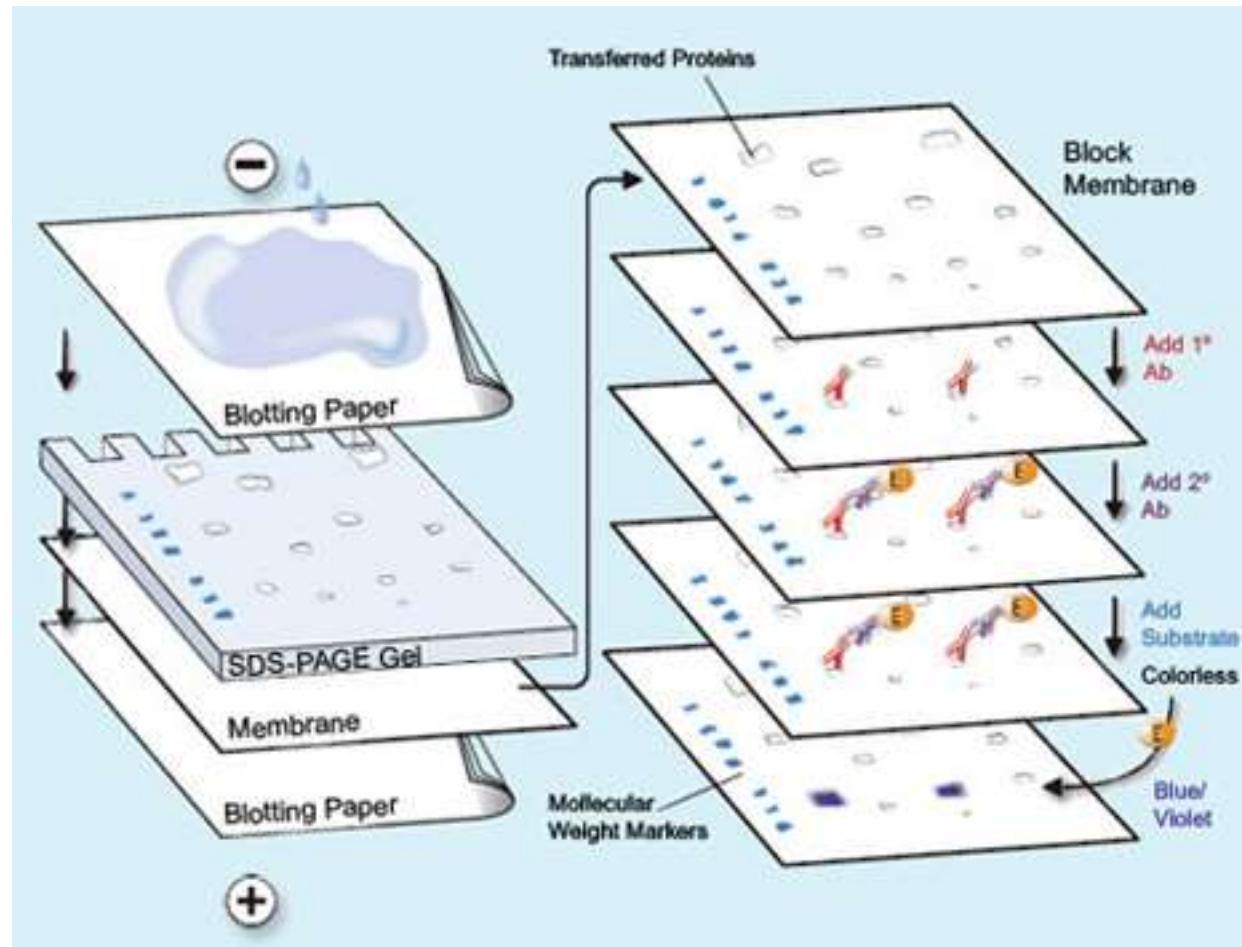
# Marcatori del ciclo cellulare

## Esperimento di Western Blotting

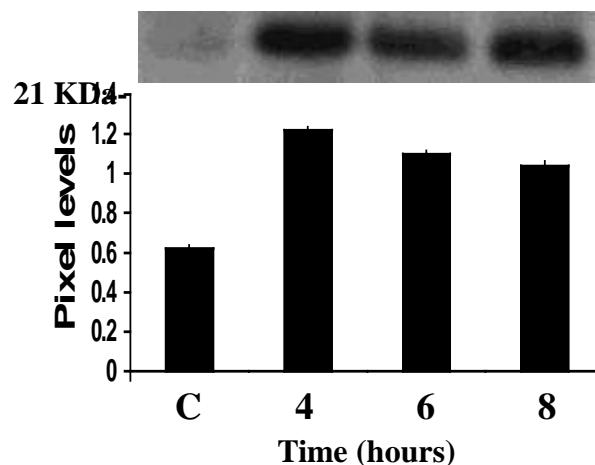
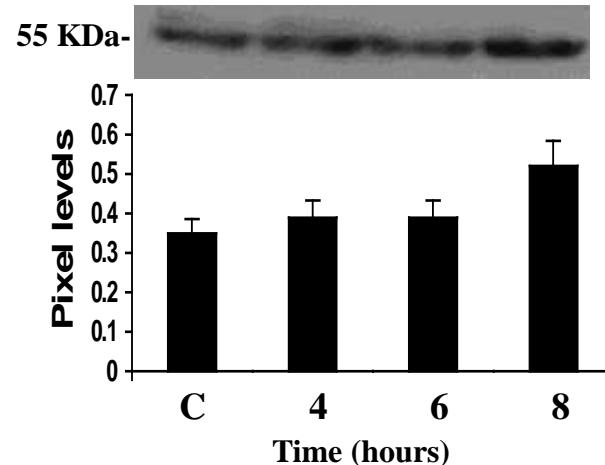
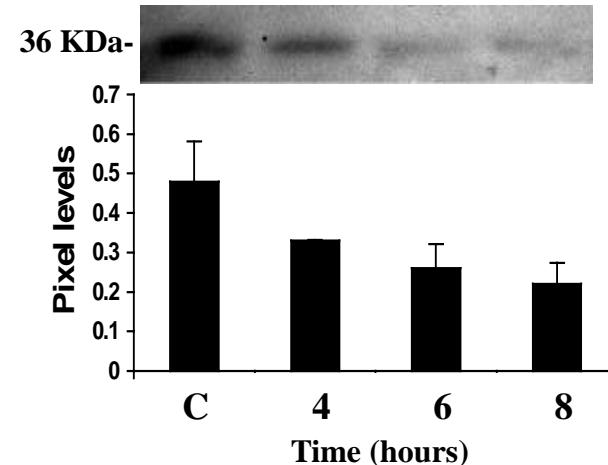
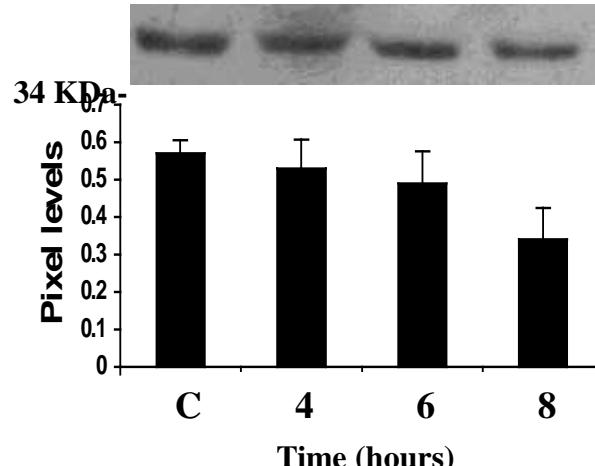
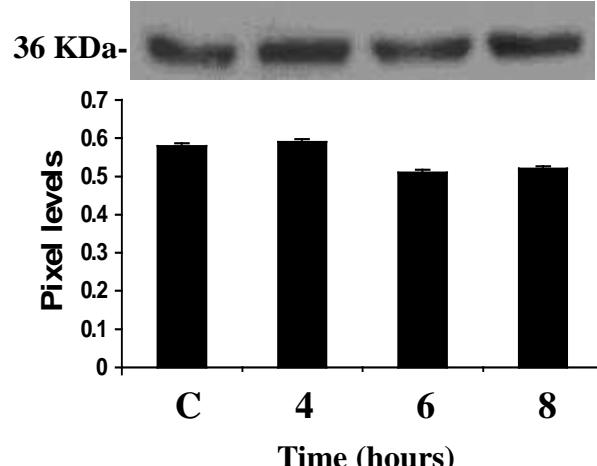
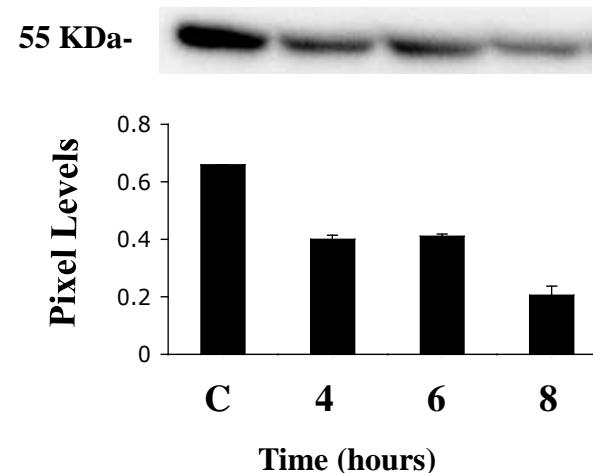


# Marcatori del ciclo cellulare

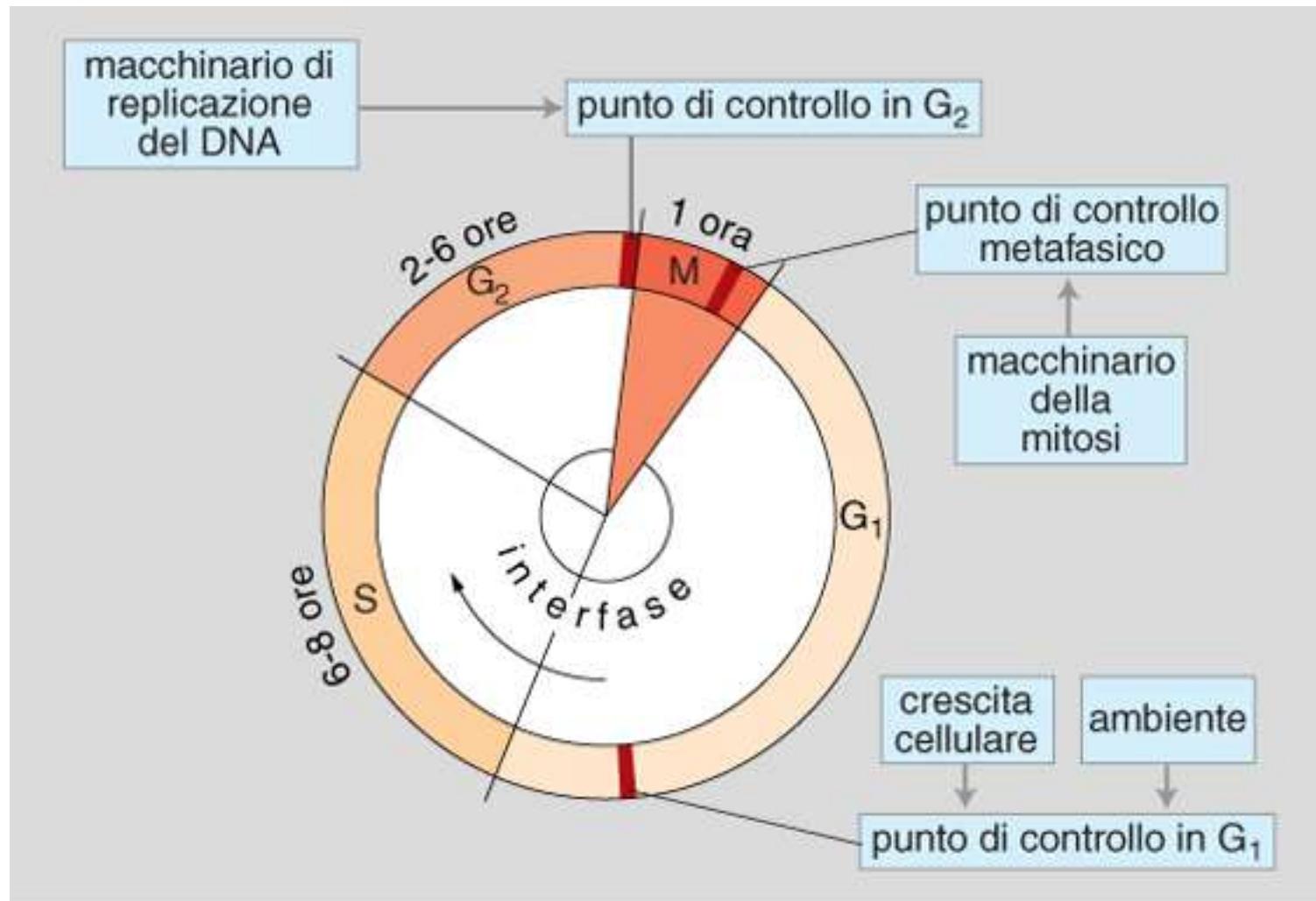
## Esperimento di Western Blotting



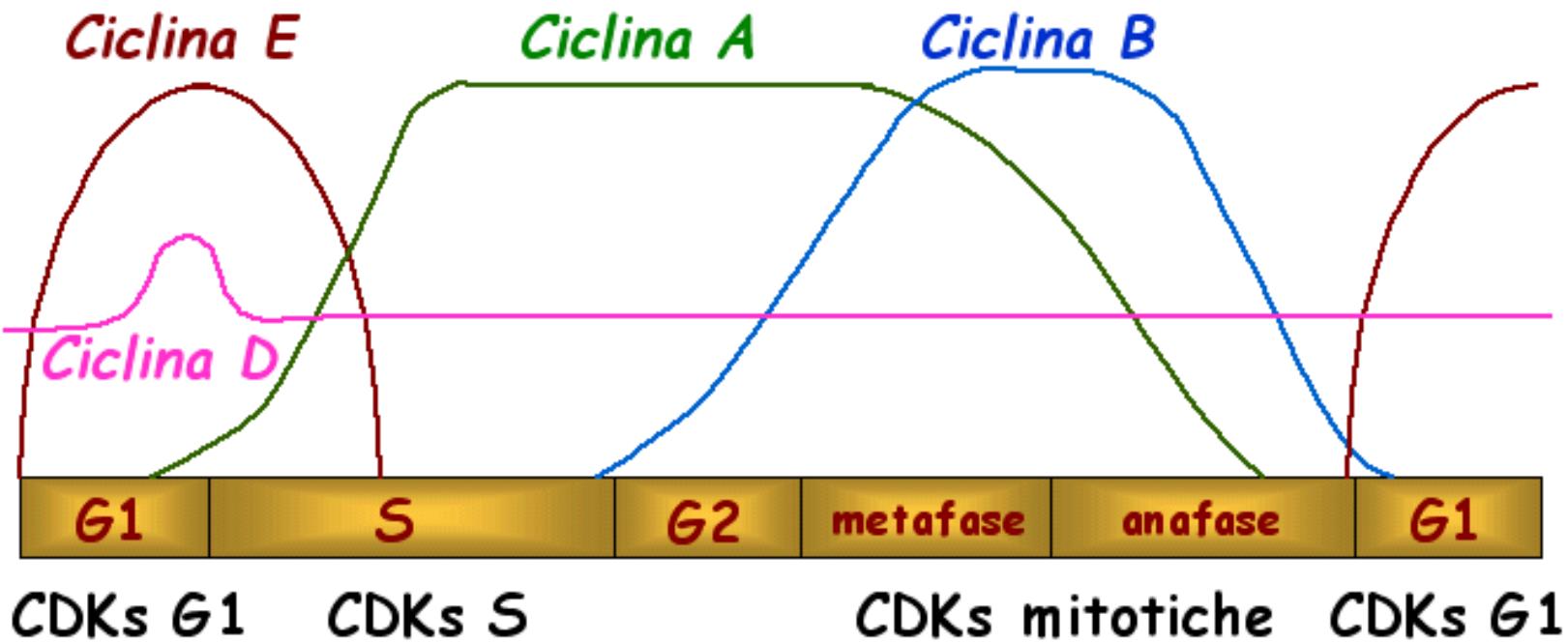
# Quantitative evaluation of cladosporol A-induced expression of cell cycle regulators in HT-29 cells

**A****p21****B****CyclinB1****C****CyclinD1****D****Cdc2-p34****E****PCNA****F****CyclinE**

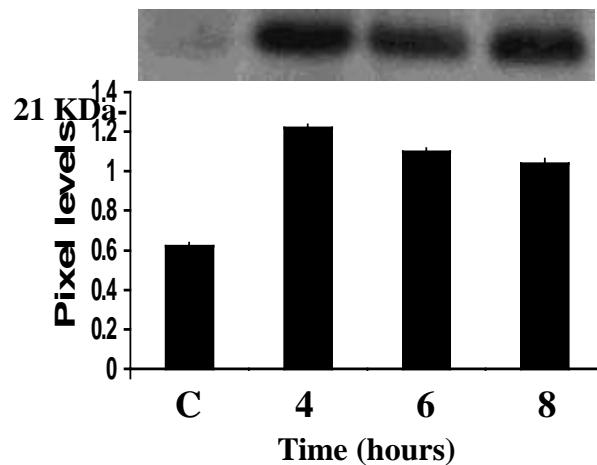
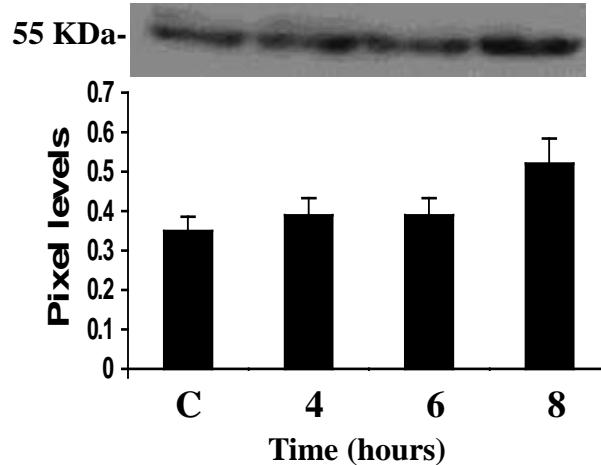
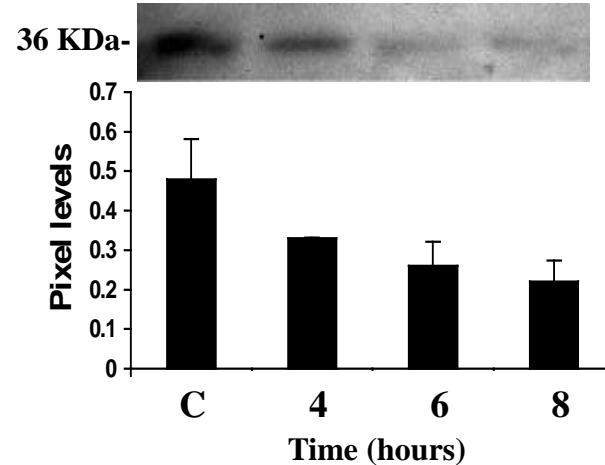
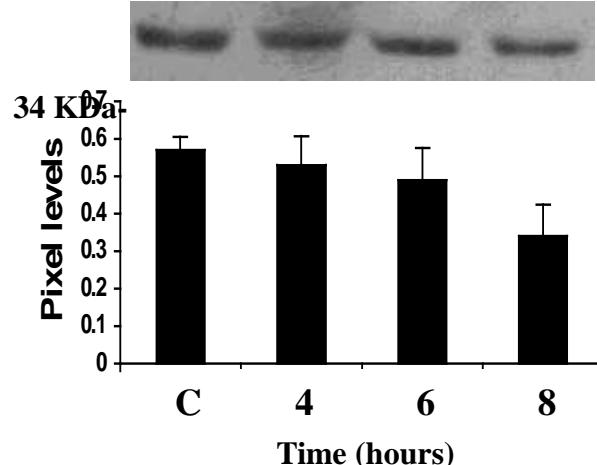
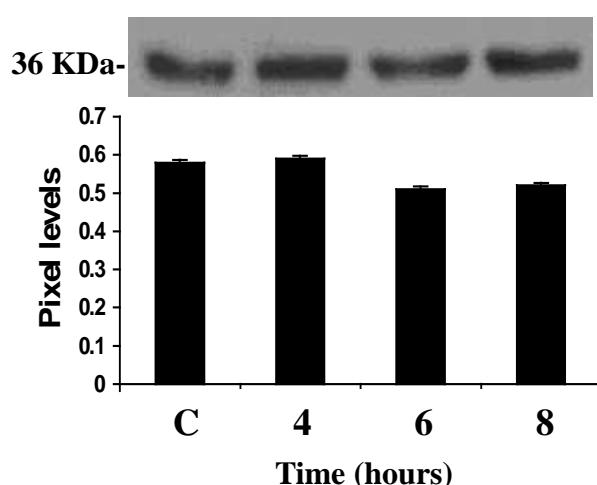
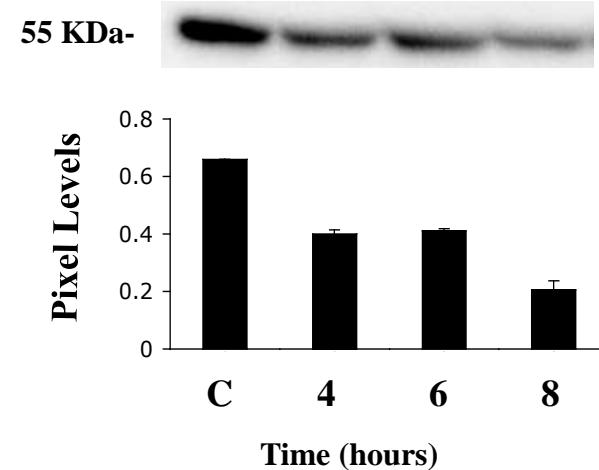
# Ciclo cellulare



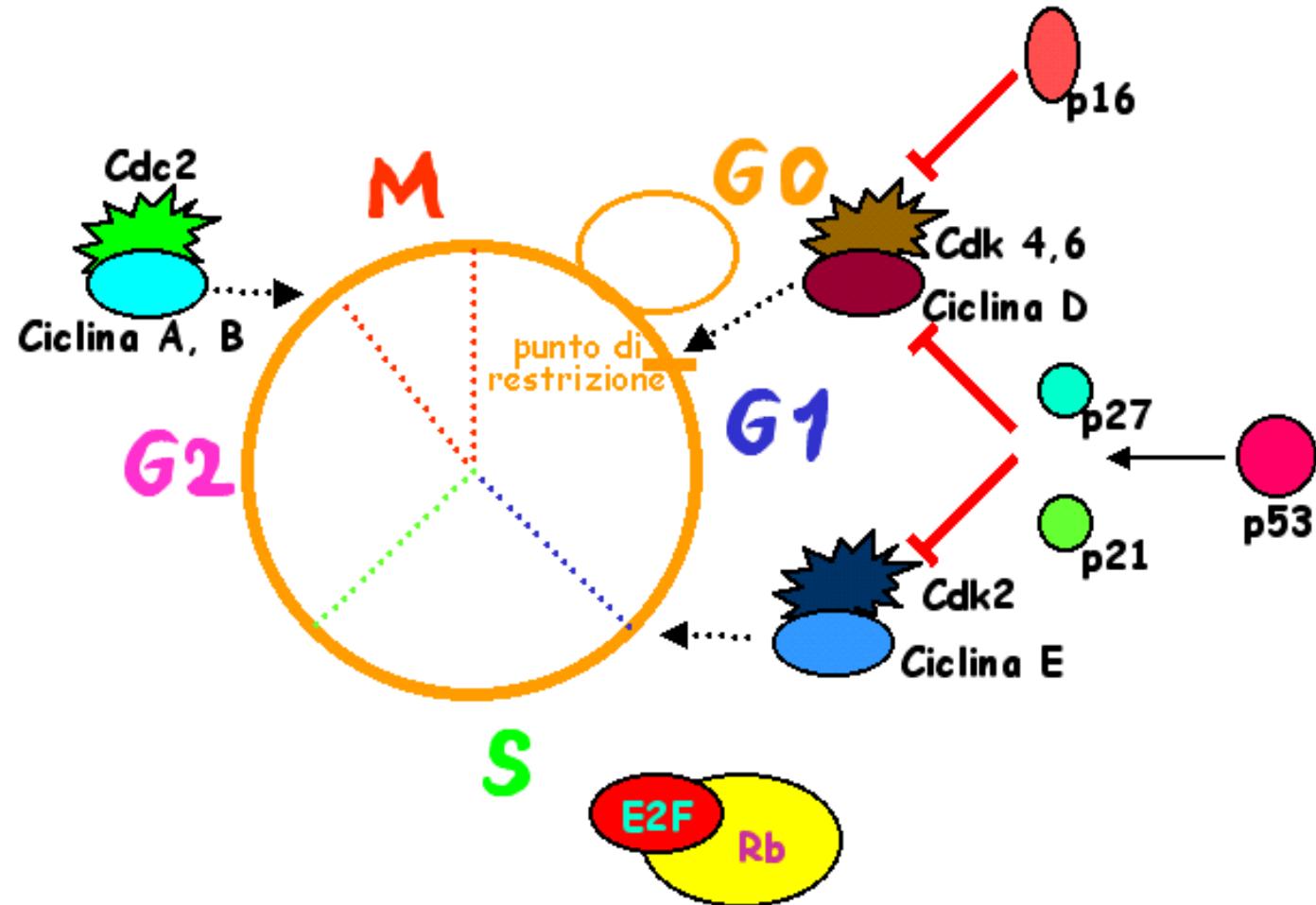
# Marcatori del ciclo cellulare



# Quantitative evaluation of cladosporol A-induced expression of cell cycle regulators in HT-29 cells

**A****p21****B****CyclinB1****C****CyclinD1****D****Cdc2-p34****E****PCNA****F****CyclinE**

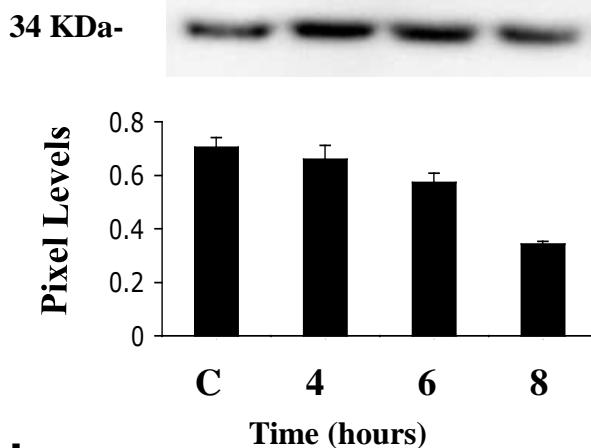
# Marcatori del ciclo cellulare



# Quantitative evaluation of cladosporol A-induced expression of cell cycle regulators in HT-29 cells

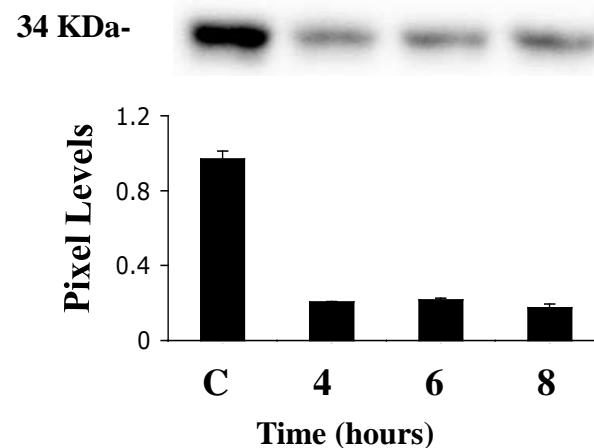
**G**

**CDK2**



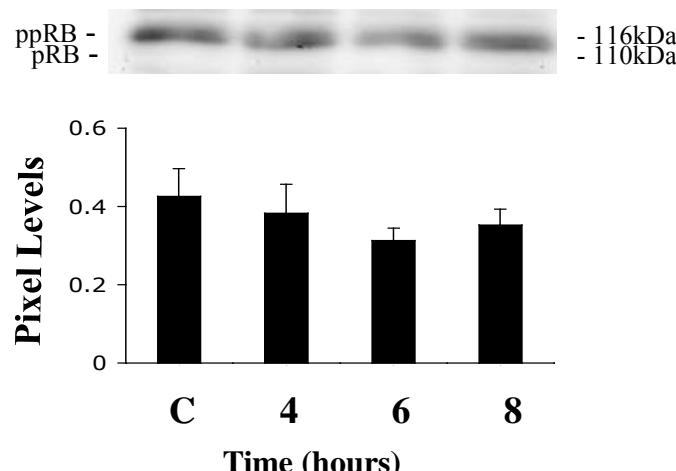
**H**

**CDK4**



**I**

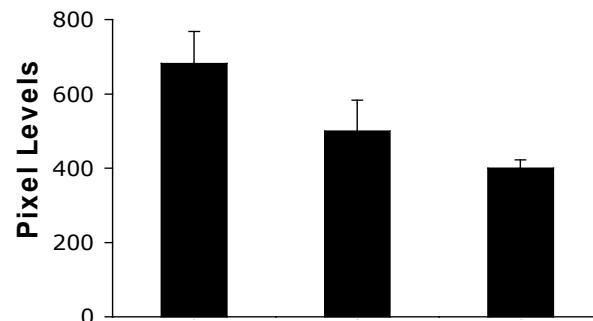
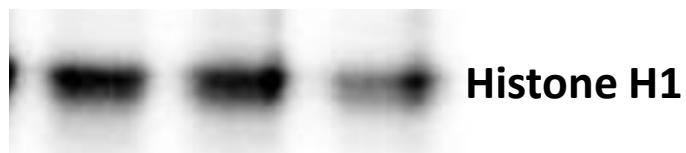
**pRB**



**IP: anti-CDK2**

**L**

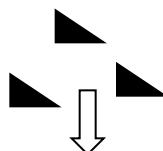
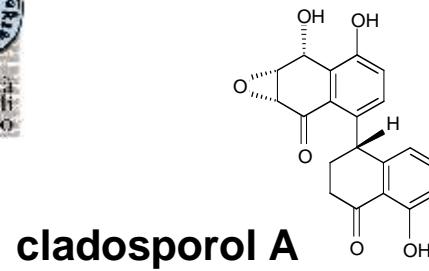
**20  $\mu$ M Cladosporol A  
(hours of treatment)**



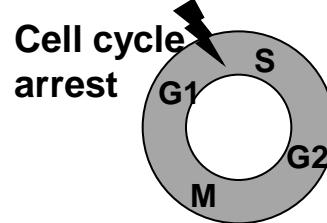
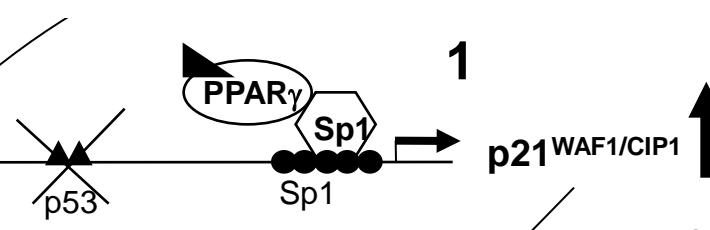
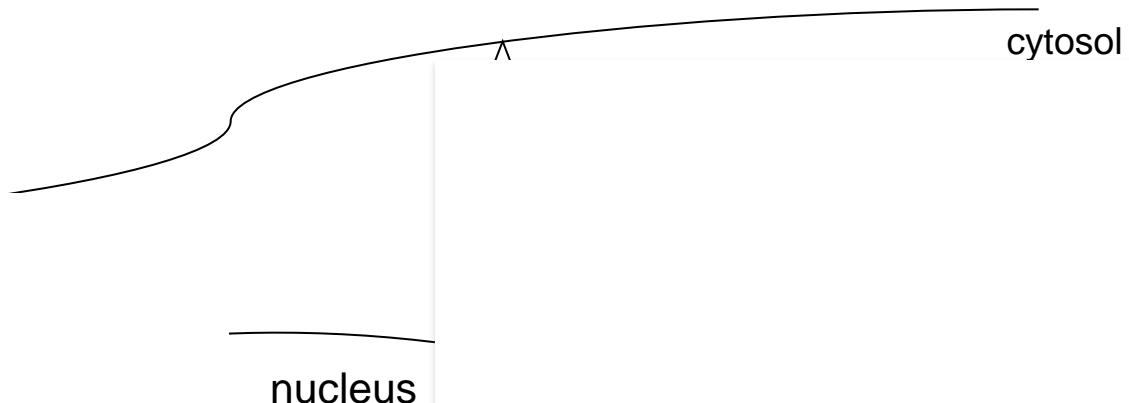
## **RISULTATO N.3**

**Reduction of cladosporol A-induced expression of cell cycle regulators in HT-29 cells (p21, cyclin D1, cyclin E, CDK2 and CDK4)**

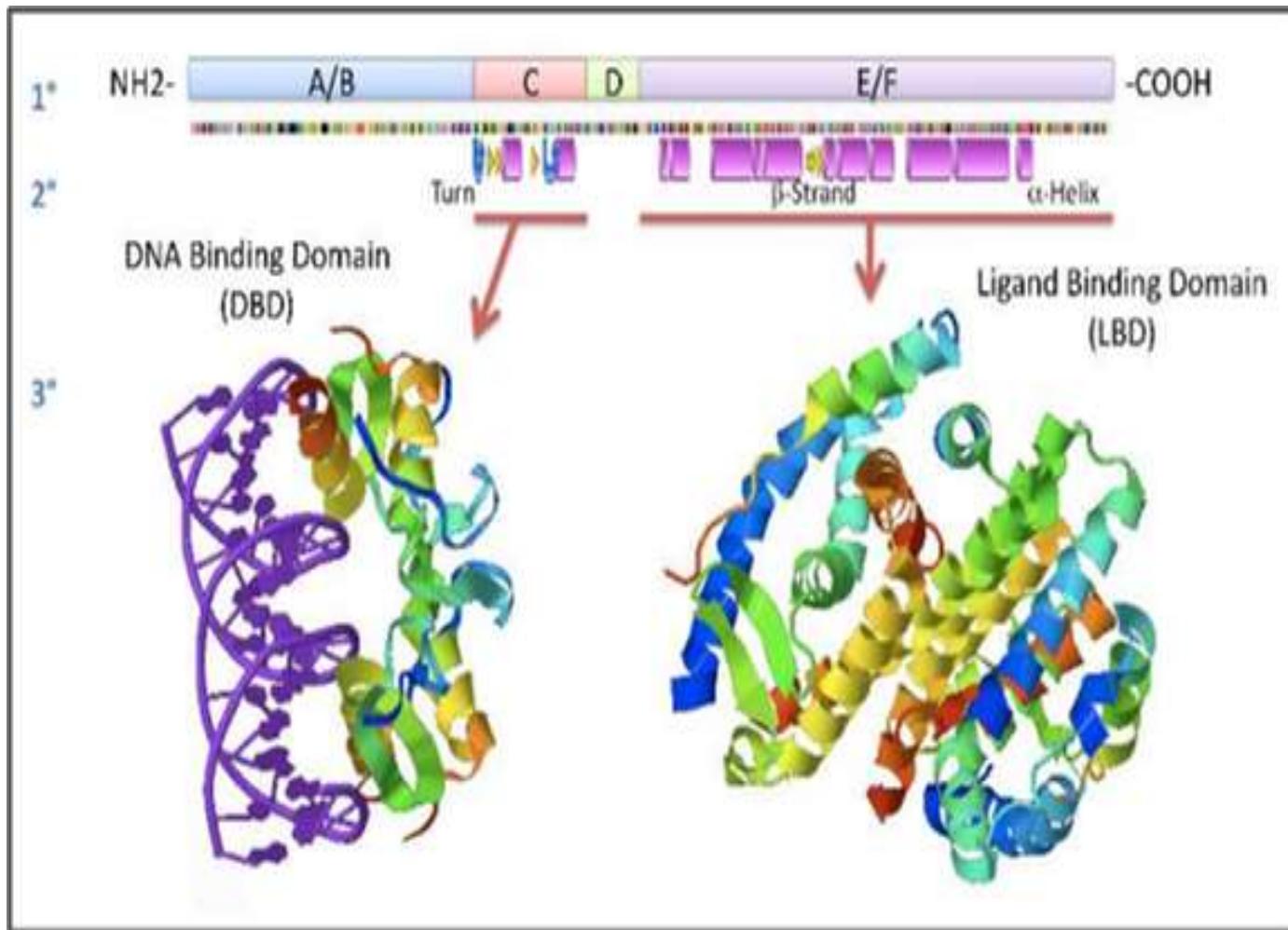
# Cosa abbiamo imparato fino ad oggi dalla lezione sui cladosporoli ?



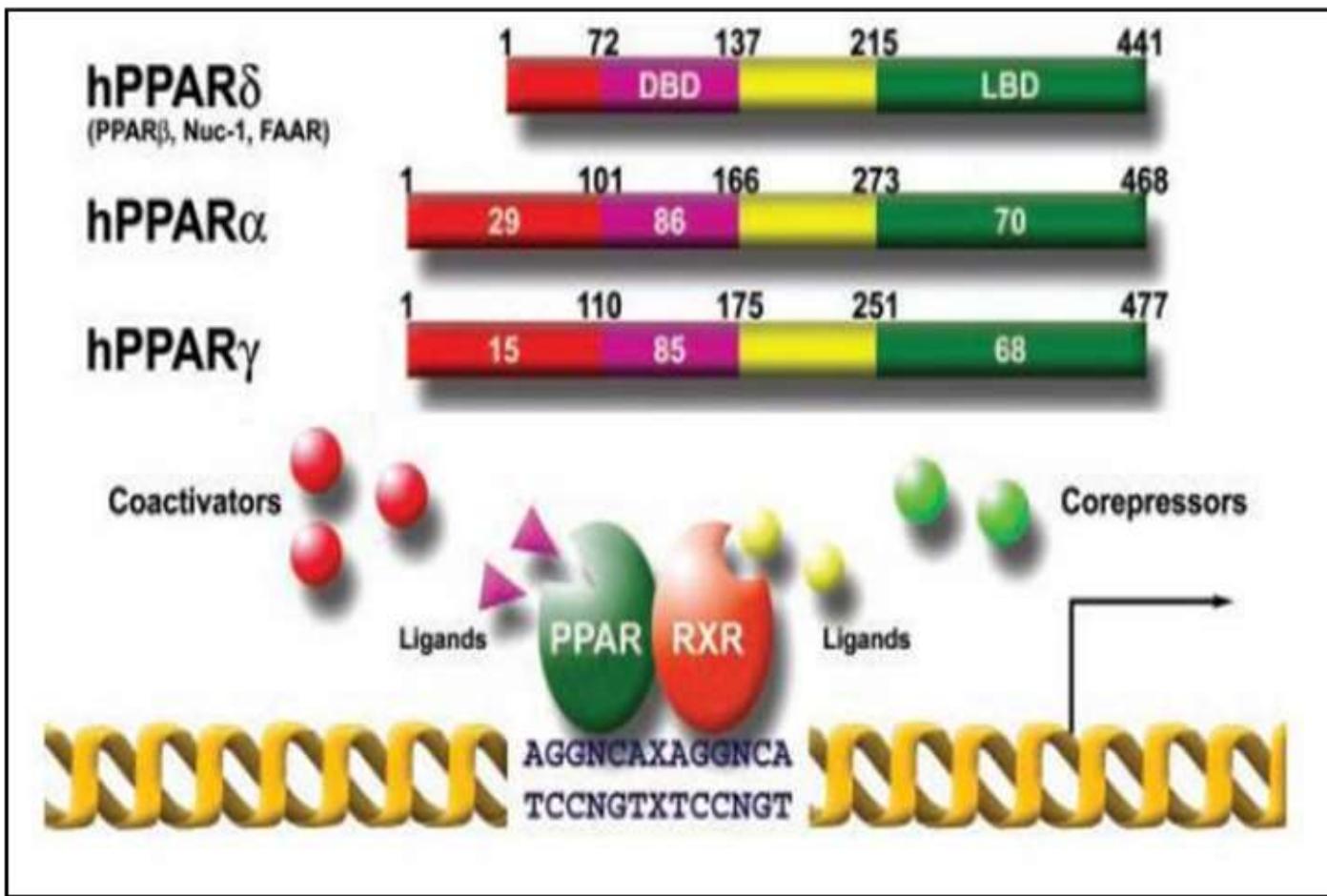
?



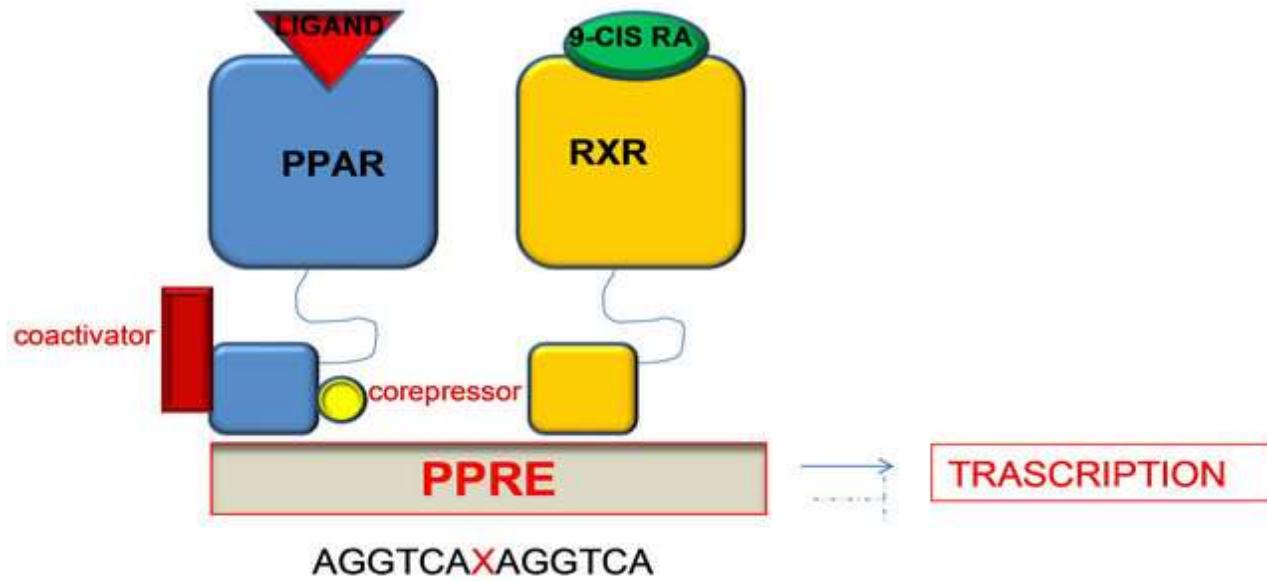
# I recettori nucleari



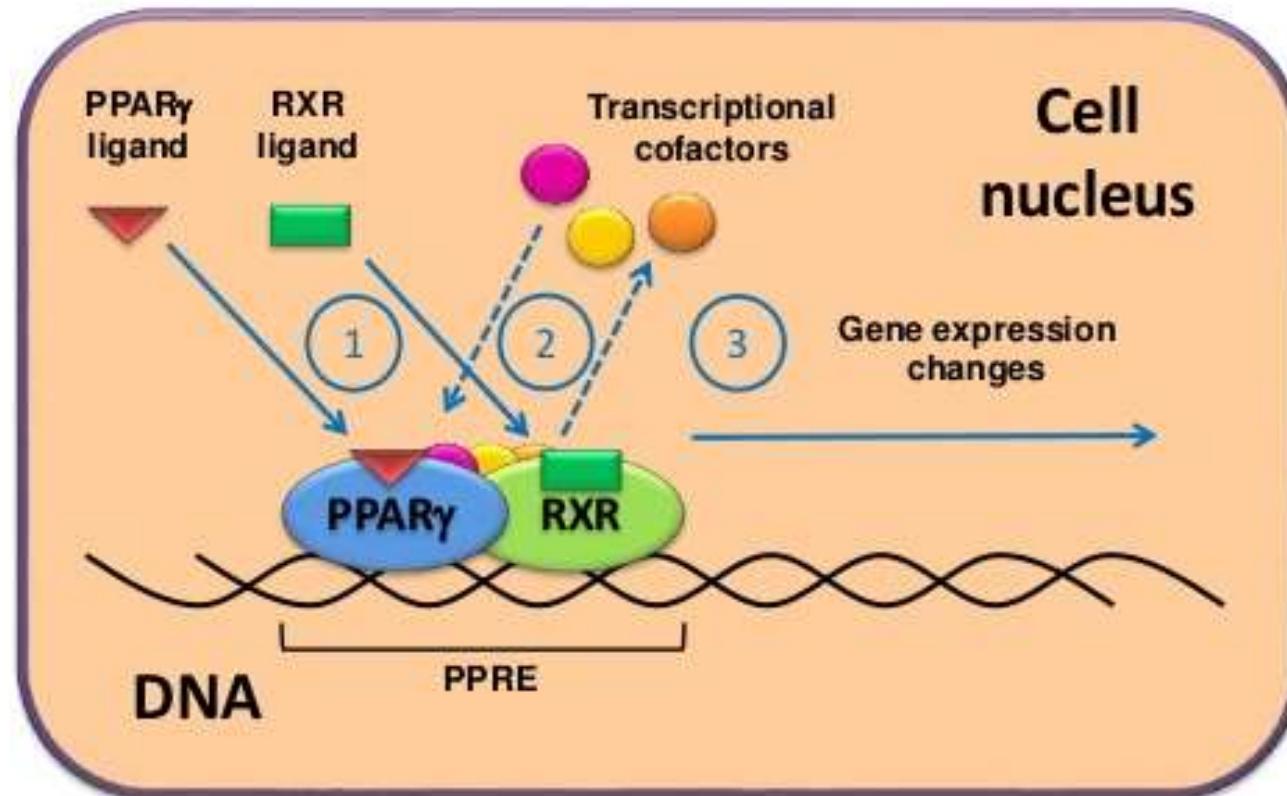
## I recettori PPARs (Peroxisome Proliferator-Activated Receptor)



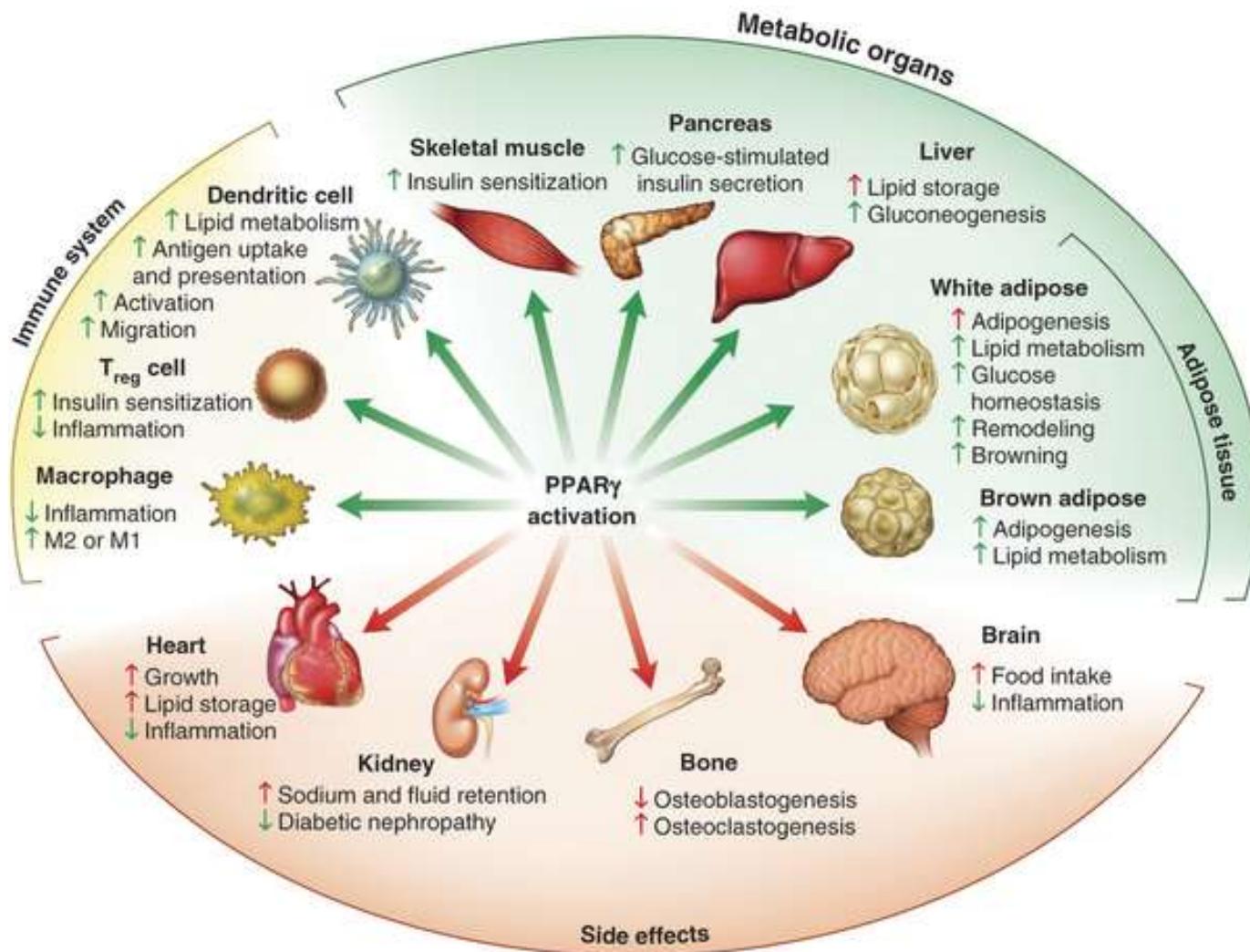
# Meccanismo di attivazione trascrizionale del recettore PPARgamma



# Meccanismo di attivazione trascrizionale del recettore PPARgamma

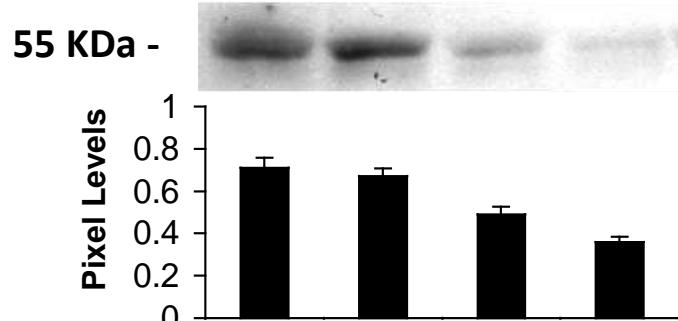


# Le funzioni del recettore PPARgamma

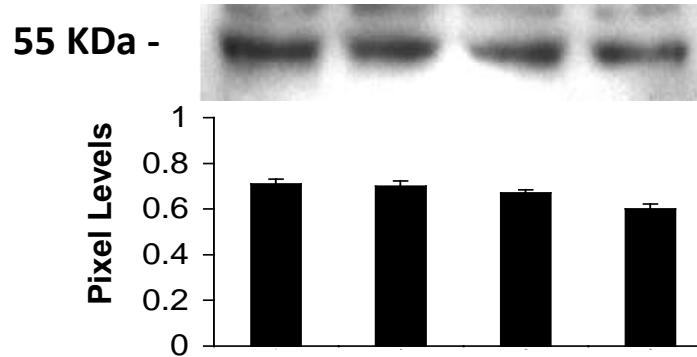


# Cladosporol A modulates PPARgamma expression in HT-29 cells

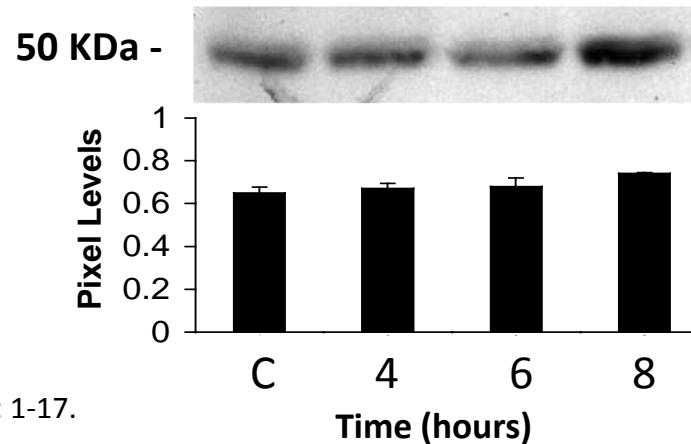
## PPAR $\gamma$



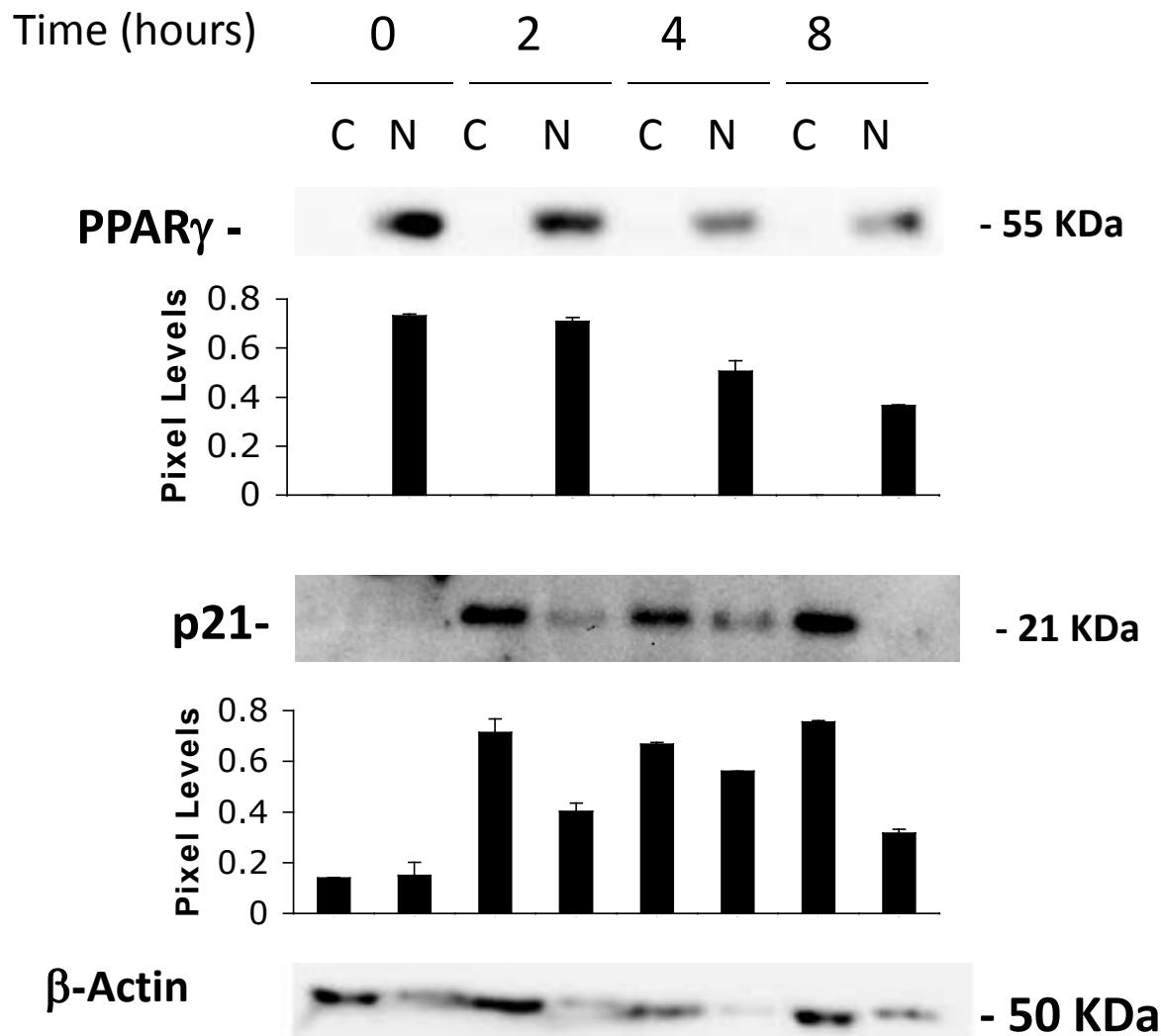
## PPAR $\alpha$



## RXR $\alpha$

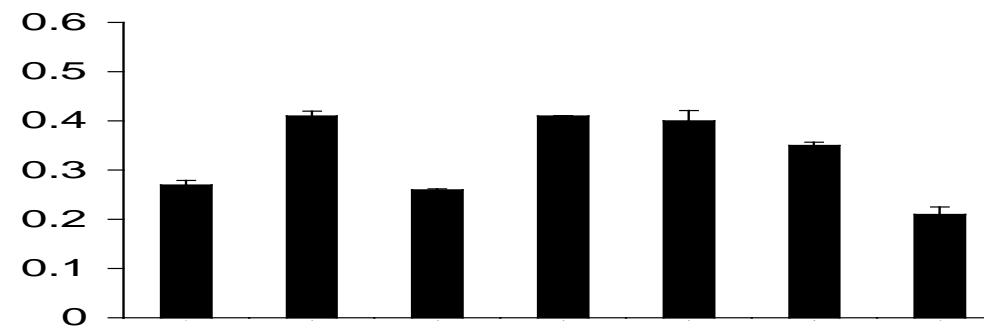
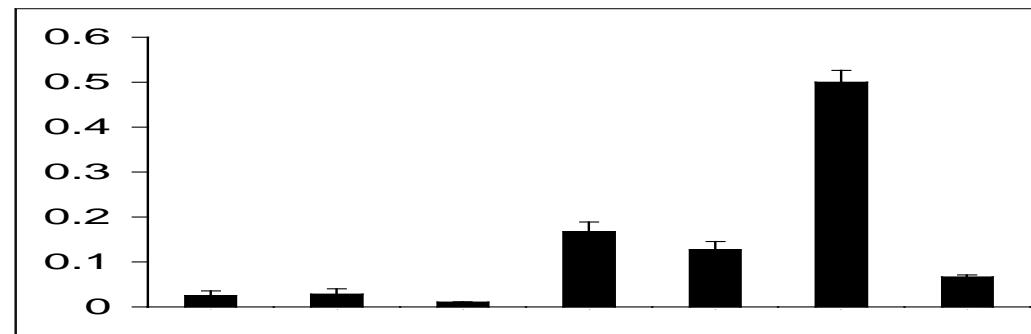


## Cladosporol A modulates PPARgamma expression in HT-29 cells



# Cladosporol A modulates PPARgamma expression in HT-29 cells

Time (hours)	-	1	1	2	2	3	3
Cladosporol A (20μM)	-	+	+	+	+	+	+
GW9662 (10μM)	-	-	+	-	+	-	+



## **RISULTATO N.4**

**PPAR $\gamma$  is the molecular target cladosporol A**

## **RISULTATO N.1**

**Cladosporol A inhibits HT-29 cell growth in a dose and time-response manner**

## **RISULTATO N.2**

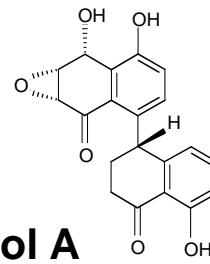
**Cladosporol A suppresses HT-29 cells growth via G1/S phase arrest**

## **RISULTATO N.3**

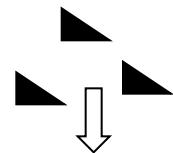
**Reduction of cladosporol A-induced expression of cell cycle regulators in HT-29 cells (p21, cyclin D1, cyclin E, CDK2 and CDK4)**

## **RISULTATO N.4**

**PPAR $\gamma$  is the molecular target cladosporol A**



cladosporol A



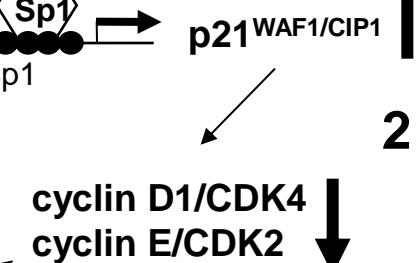
N AF1 DBD Hinge LBD AF2 C

PPAR $\gamma$

cytosol

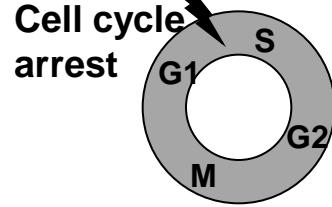
nucleus

1



2

cyclin D1/CDK4  
cyclin E/CDK2



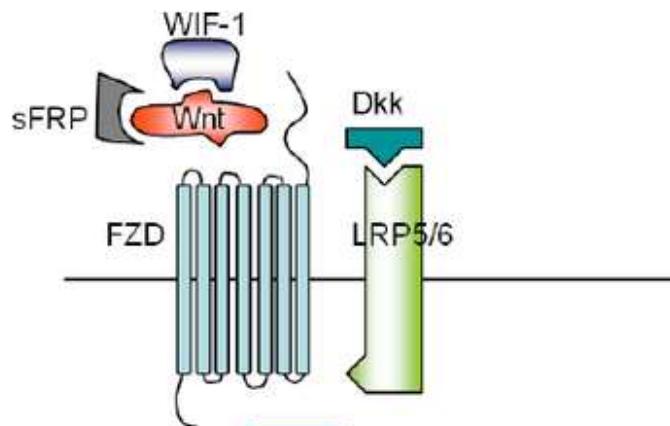
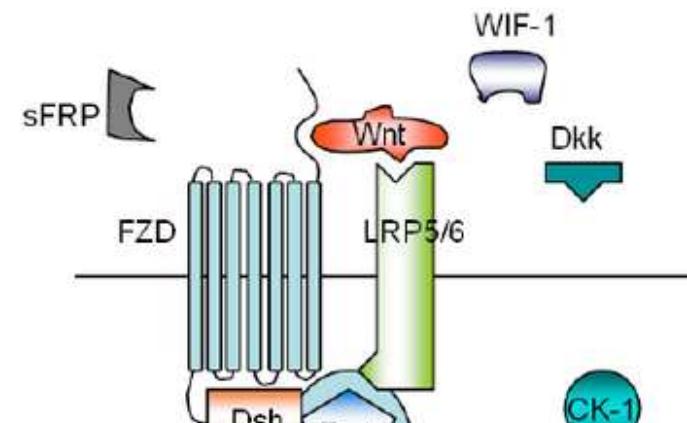
Cell cycle  
arrest

4

PPAR $\gamma$

# Via di attivazione del pathway di Wnt/beta-catenin

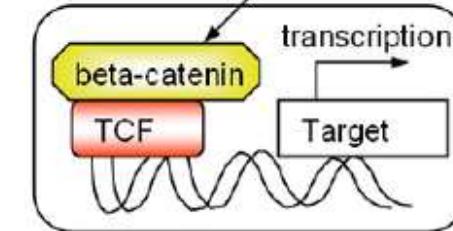
## WNT (Wingless-Type MMTV Integration Site Family)

**A****Wnt (-)****B****Wnt (+)**

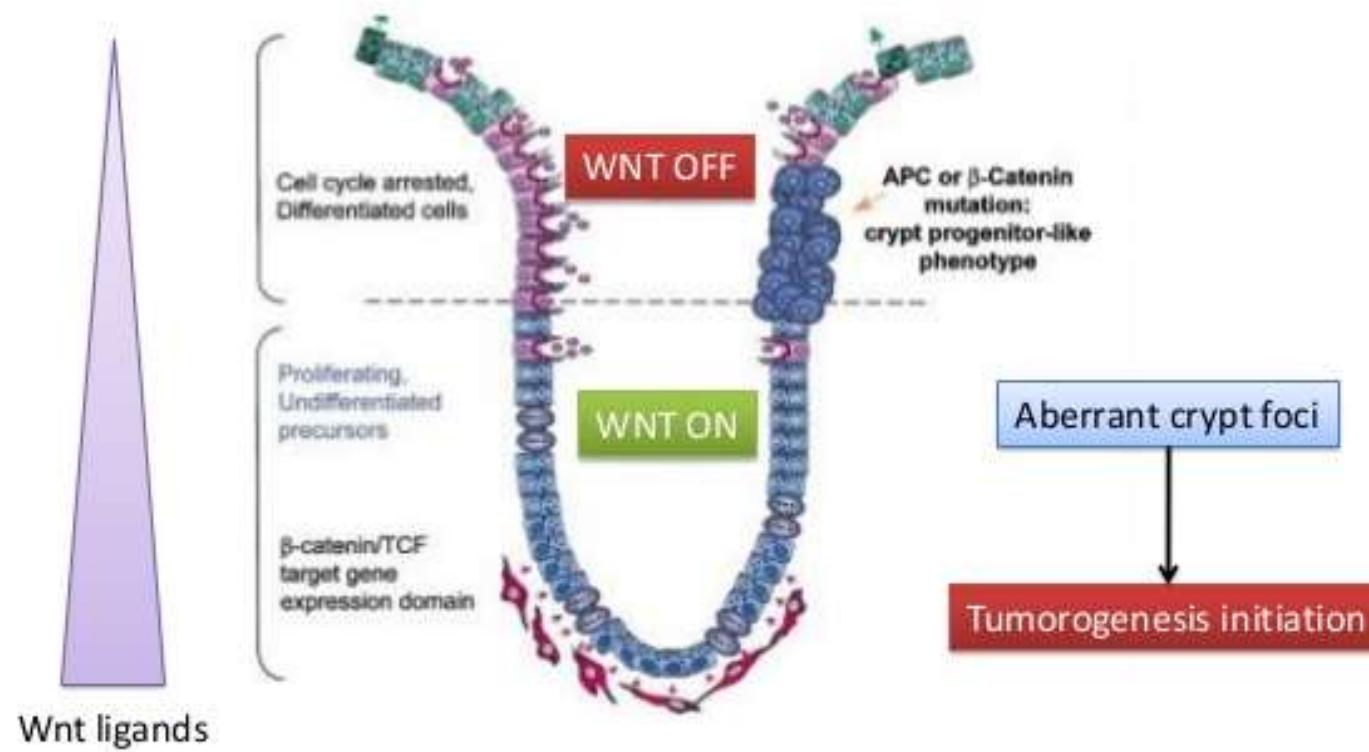
GSK3beta

APC

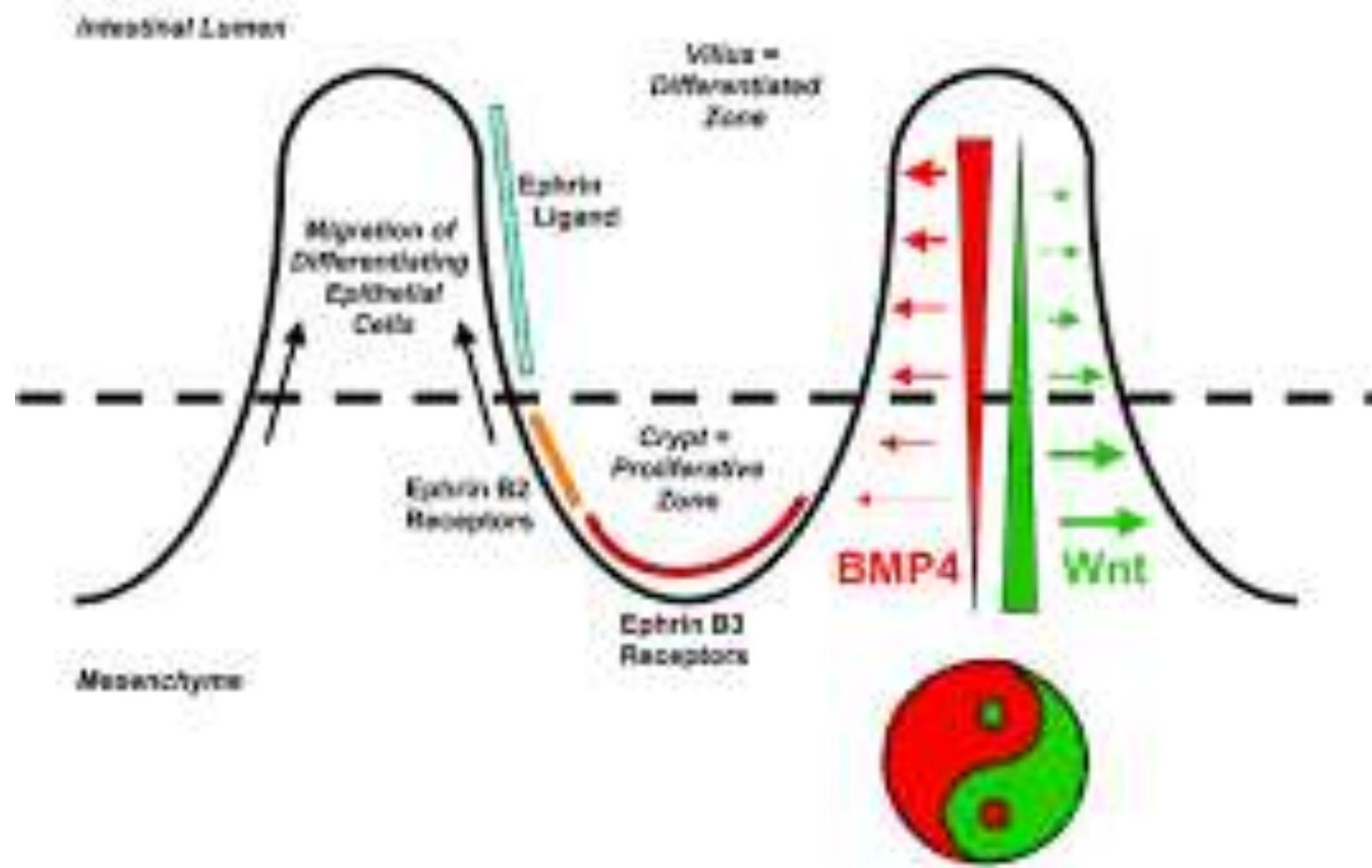
beta-catenin



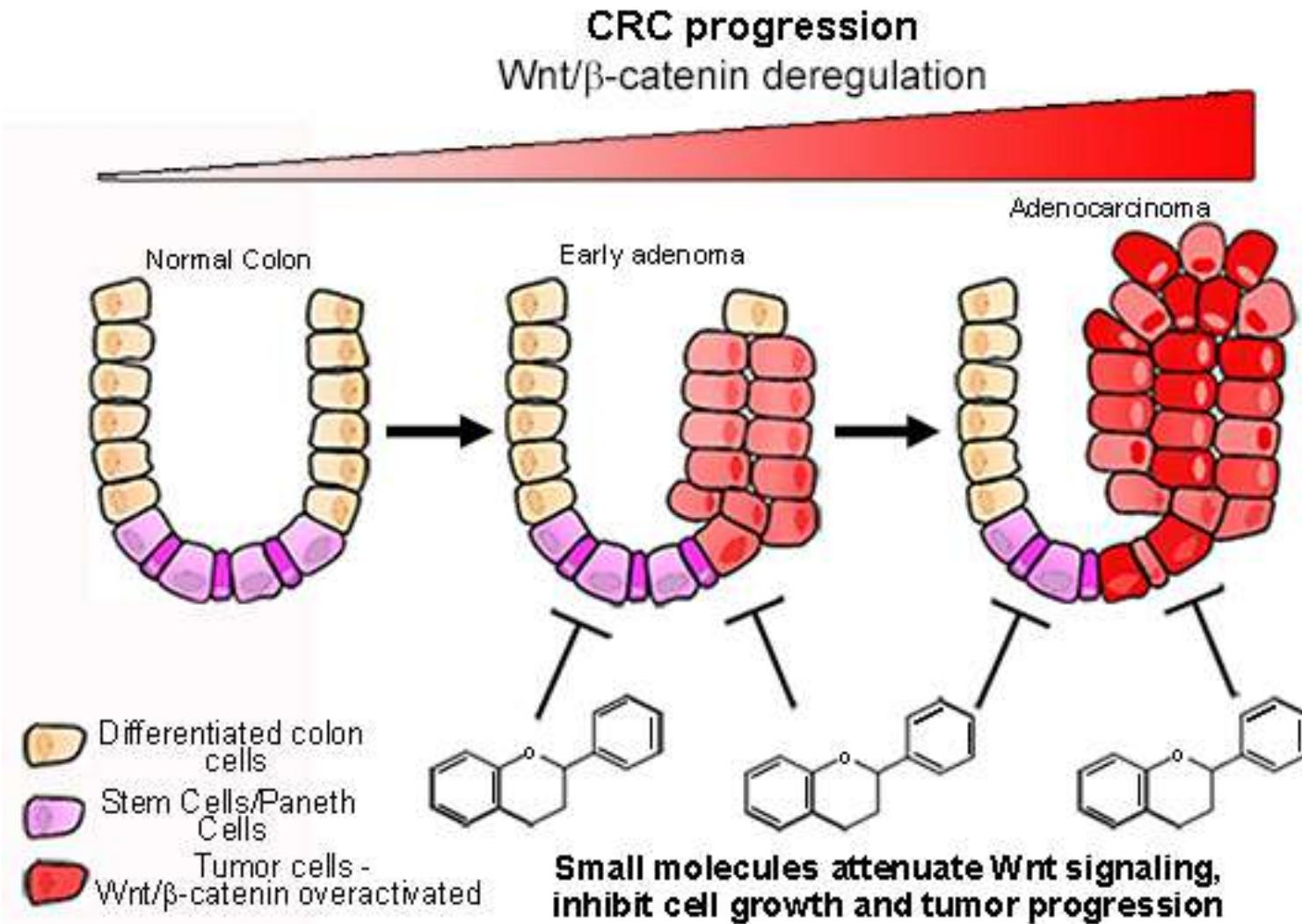
## Via di attivazione del pathway di Wnt/beta-catenin



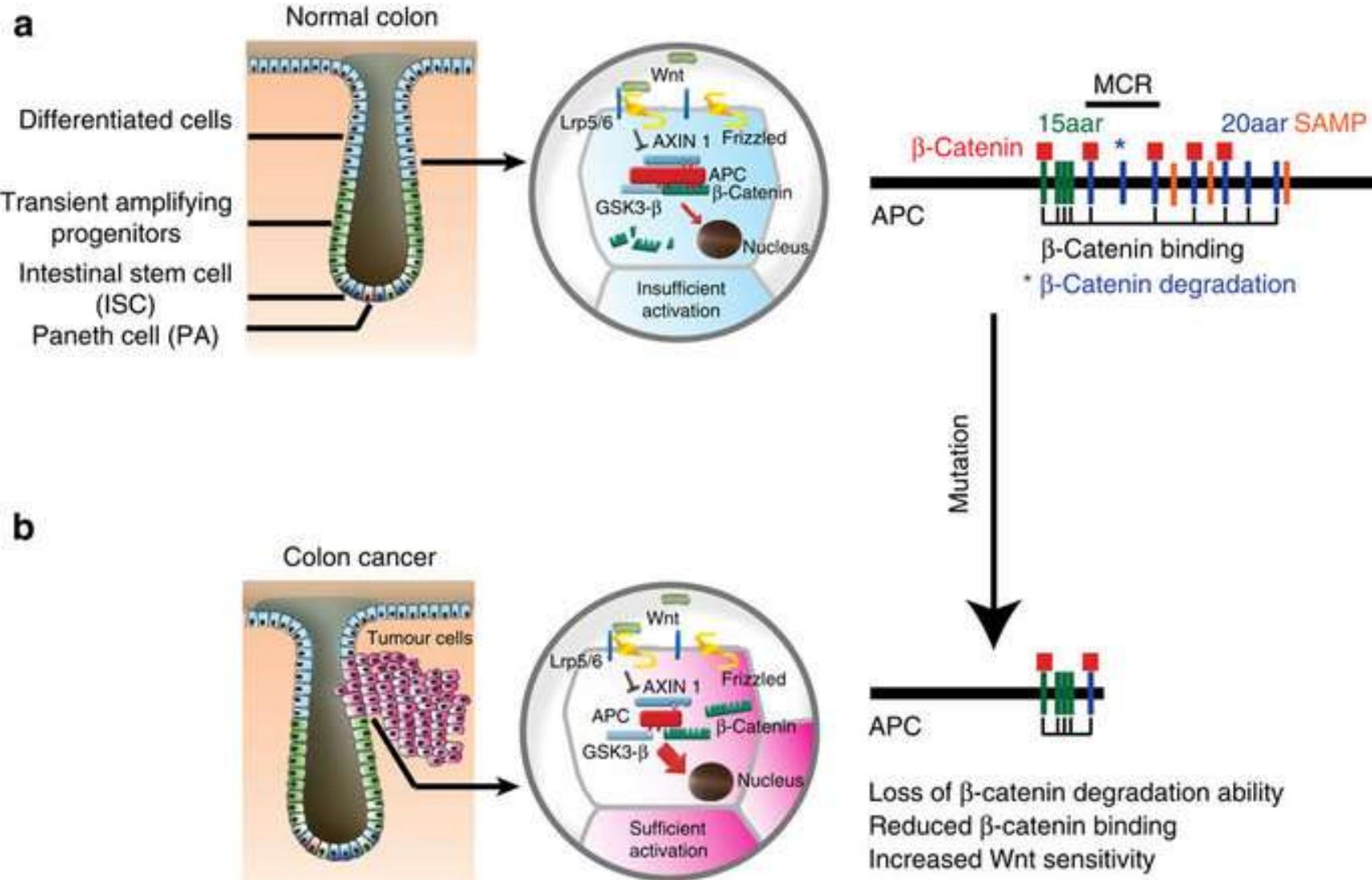
## Gradients of Wnt and BMP4 may define crypt / villus axis



## Il pathway di Wnt/beta-catenin nel tumore del colonretto



# Il pathway di Wnt/beta-catenin nel tumore del colonretto



# Il pathway di Wnt/beta-catenin nel tumore del colonretto

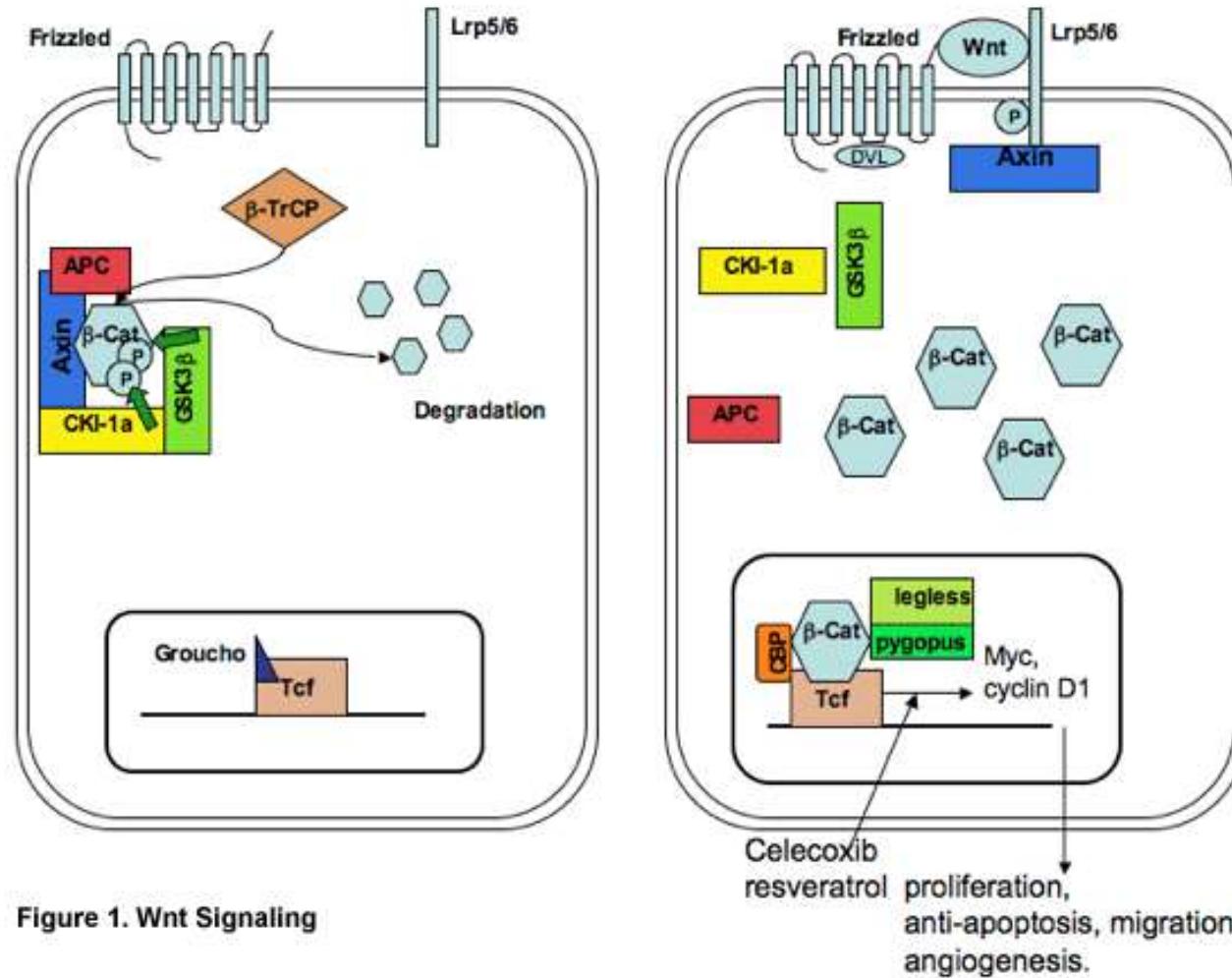


Figure 1. Wnt Signaling

APC = adenomatous polyposis coli;  $\beta$ -Cat =  $\beta$ -catenin;  $\beta$ -TrCP =  $\beta$ -transducin repeat-containing protein; CBP = CREB (cAMP response element-binding protein)-binding protein; CKI-1 $\alpha$  = Casein kinase I; DVL = disheveled; GSK3 $\beta$  = glycogen synthase kinase-3 $\beta$ ; Lrp5/6 = low-density lipid receptor 5/6; P = phosphate; Tcf = T-cell factor

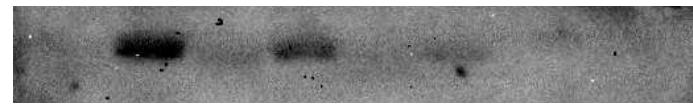
Note: GSK3 $\beta$  phosphorylates  $\beta$ -catenin and creates a motif for recognition of  $\beta$ -catenin binding with the E3-ubiquitin ligase complex SCF $^{\beta\text{-TrCP}}$ , which increases  $\beta$ -catenin degradation

# Cladosporol A interferes with the beta-catenin /TCF pathway and HT-29 cell proliferation

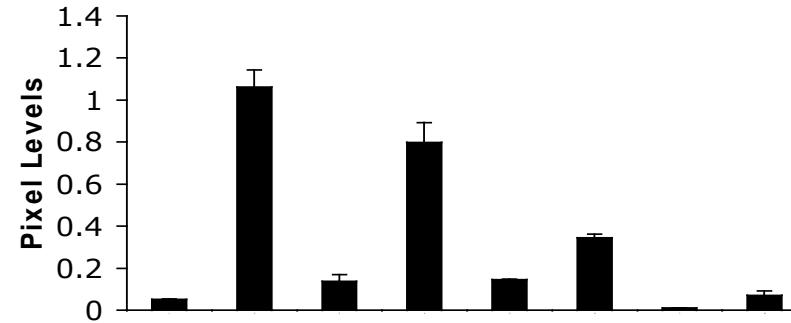
A

Time (hours)	0		2		4		8	
	C	N	C	N	C	N	C	N

$\beta$ -catenin -



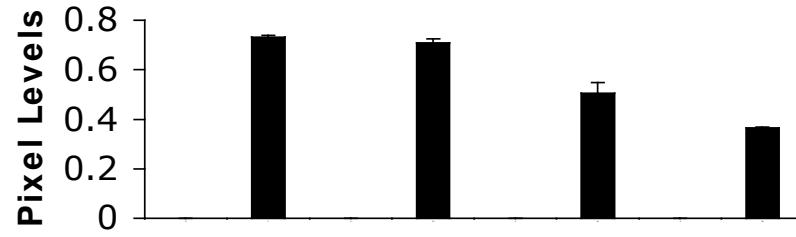
- 92 KDa



PPAR $\gamma$  -



- 55 KDa

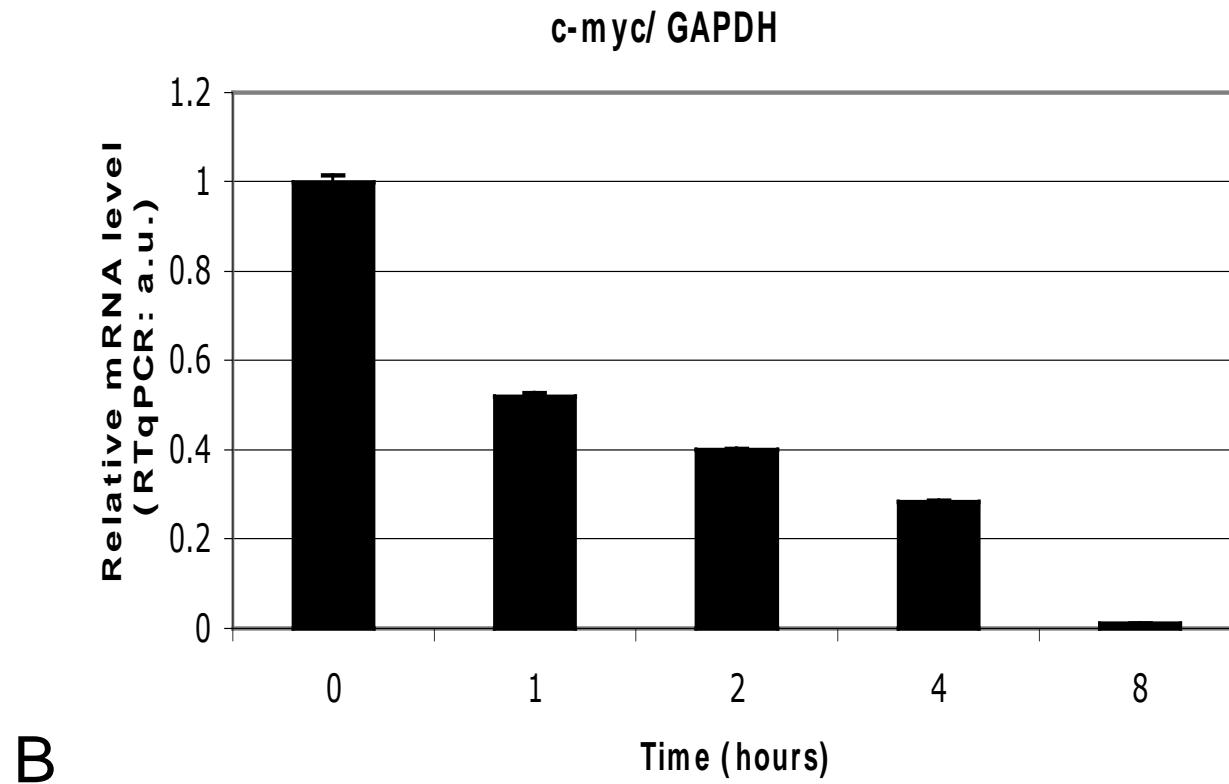


$\beta$ -Actin



- 50 KDa

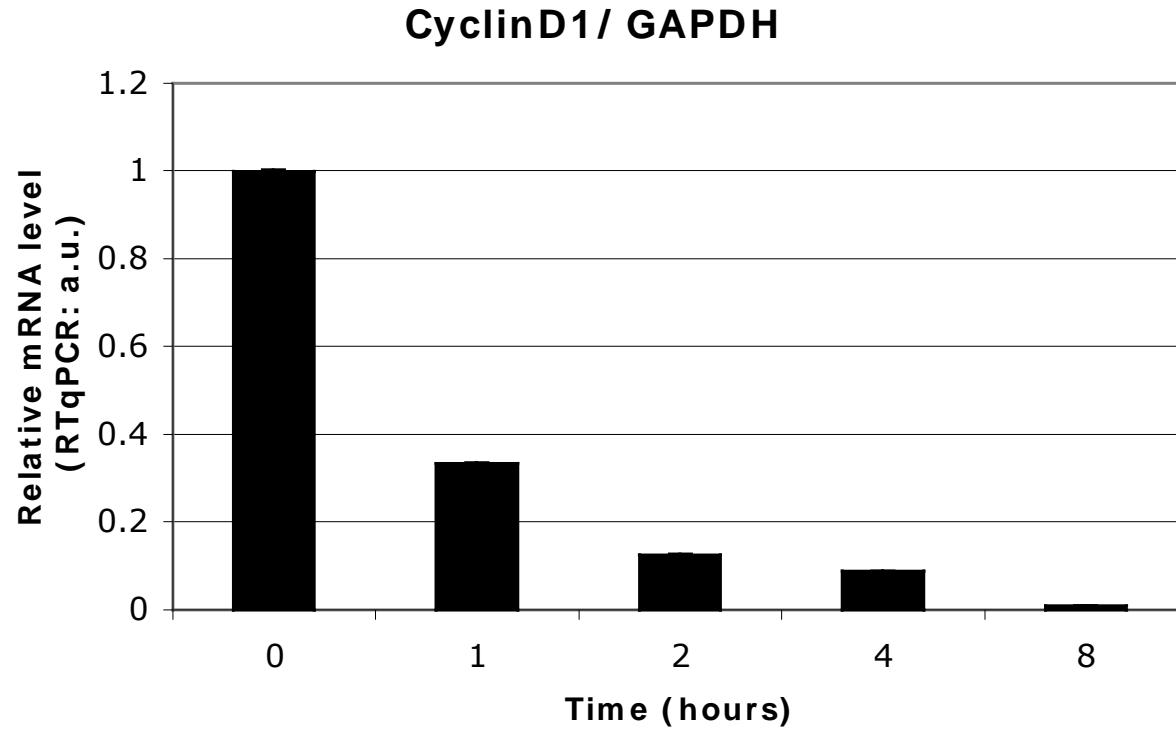
# Cladosporol A interferes with the beta-catenin /TCF pathway and HT-29 cell proliferation



# Cladosporol A interferes with the beta-catenin /TCF pathway and HT-29 cell proliferation



C



# Regulation of PPARgamma and beta-catenin expression in HT-29 cells

D

Time (hours)

	0		4		2		4		8	
	C	N	C	N	C	N	C	N	C	N
Cladosporol A 20µM	-	-	-	-	+	+	+	+	+	+
MG132 10µM	-	-	+	+	-	-	+	+	-	+



Cladosporol A 20µM

MG132 10µM

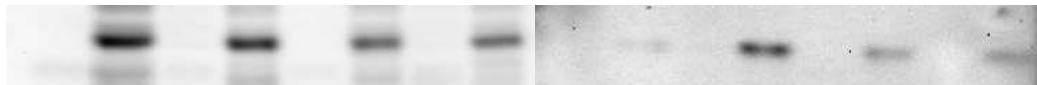
$\beta$ -catenin



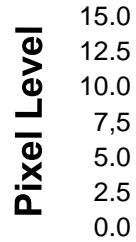
- 92 KDa



PPAR $\gamma$



- 55 KDa

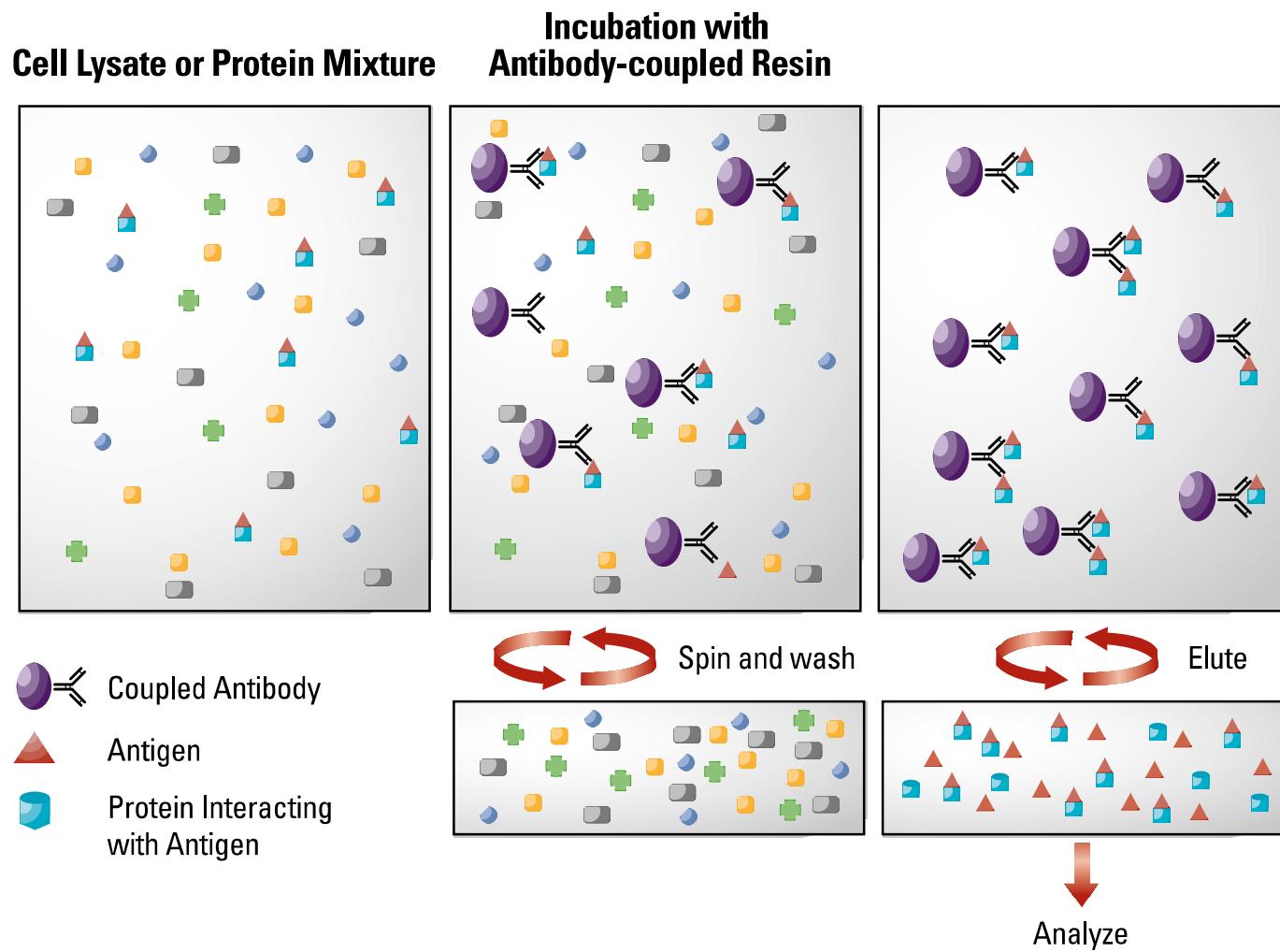


$\beta$ -actin

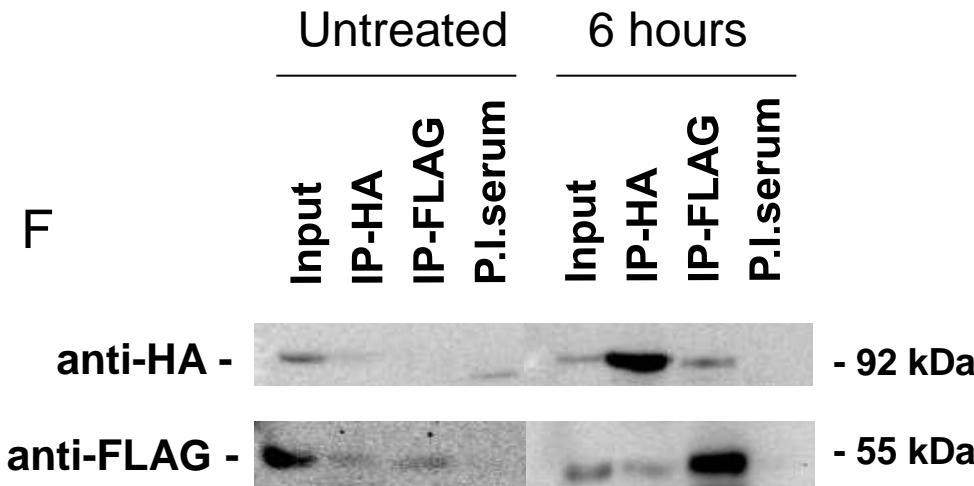
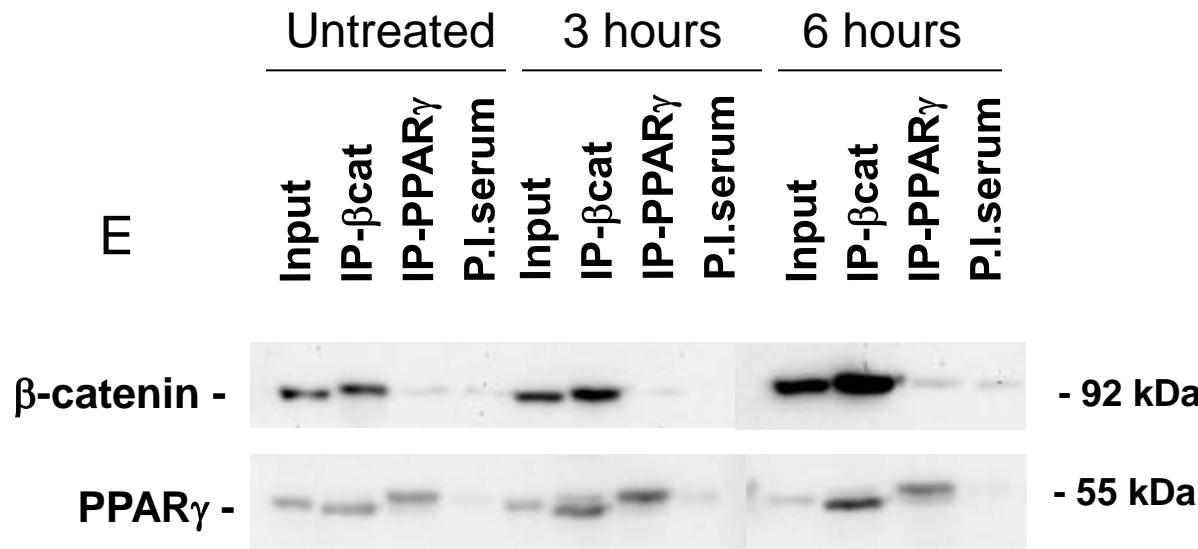


- 43 KDa

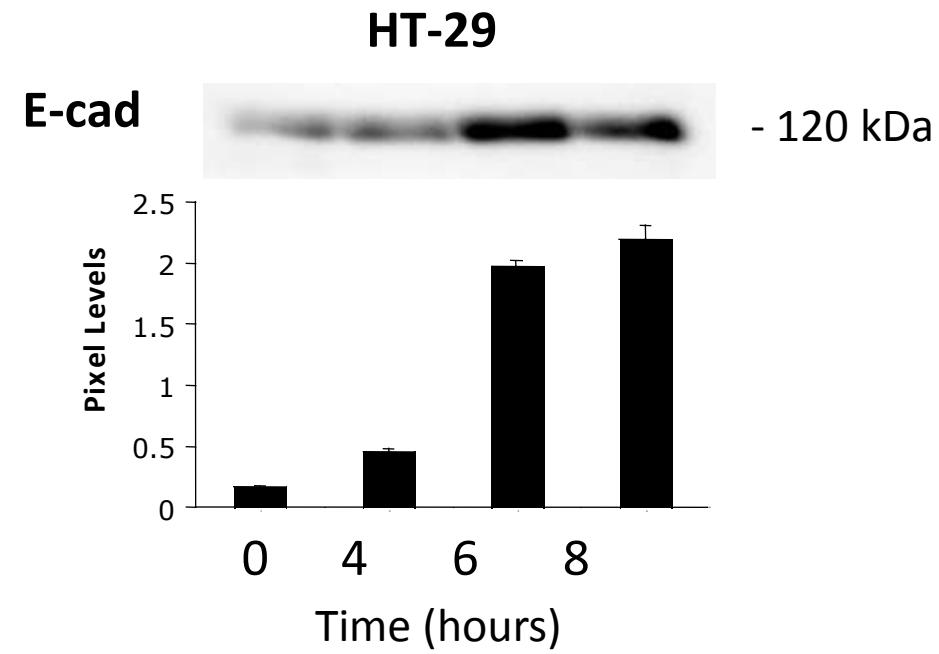
## Traditional procedure of co-immunoprecipitation of a protein



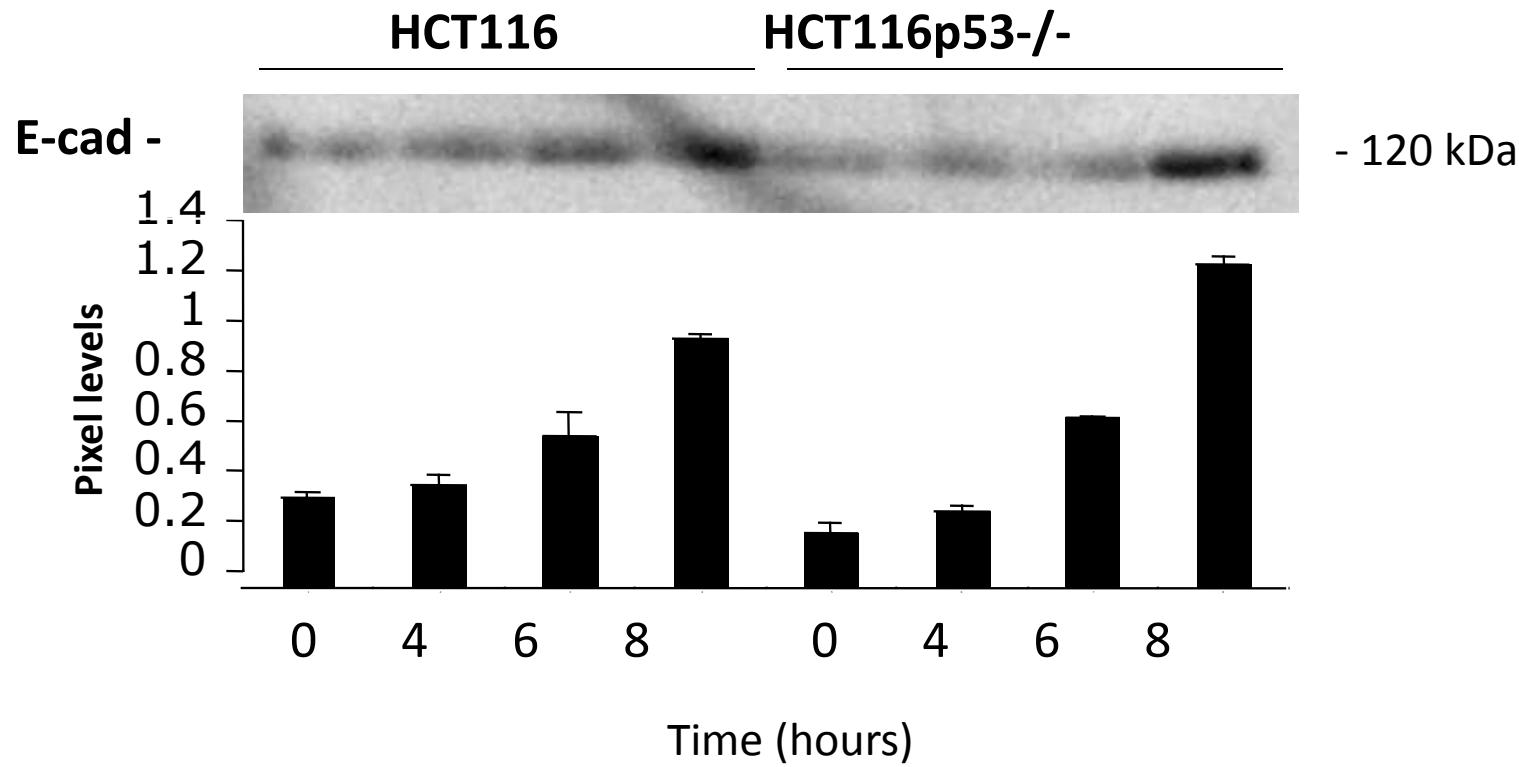
## PPARgamma and beta-catenin interaction in HT-29 cells



## E-cadherin gene expression in HT-29 cells after cladosporol A treatment

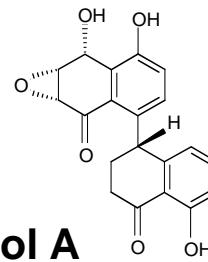


## E-cadherin gene expression in HT-116 cells after cladosporol A treatment



## **RISULTATO N.5**

**Cladosporol A interferes with the beta-catenin /TCF pathway and HT-29 cell proliferation**



**cladosporol A**

