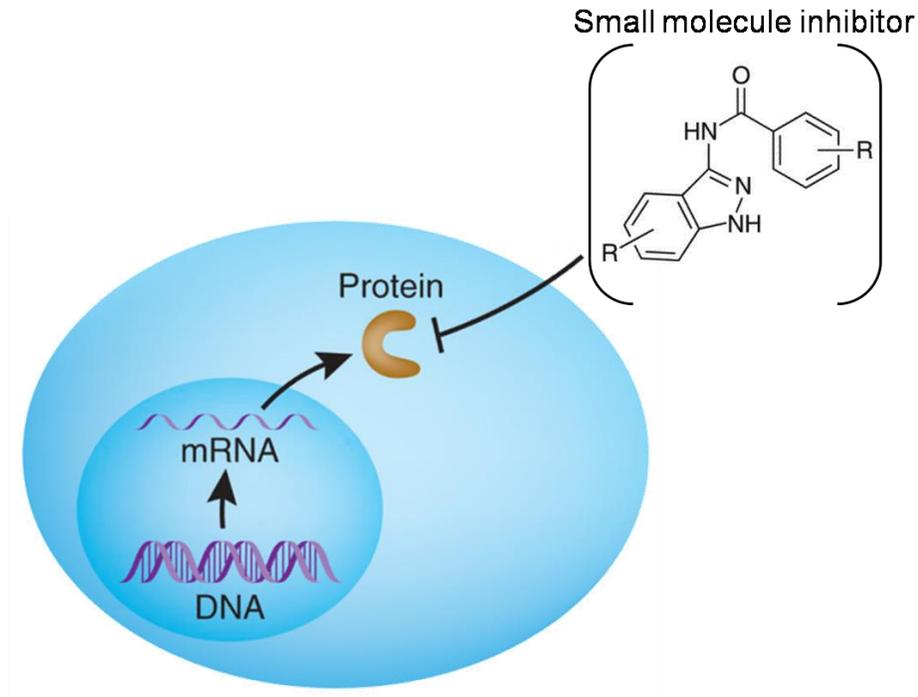


Genetica chimica: un approccio alternativo per lo studio di sistemi biologici complessi mediante l'utilizzo di *small-molecules*

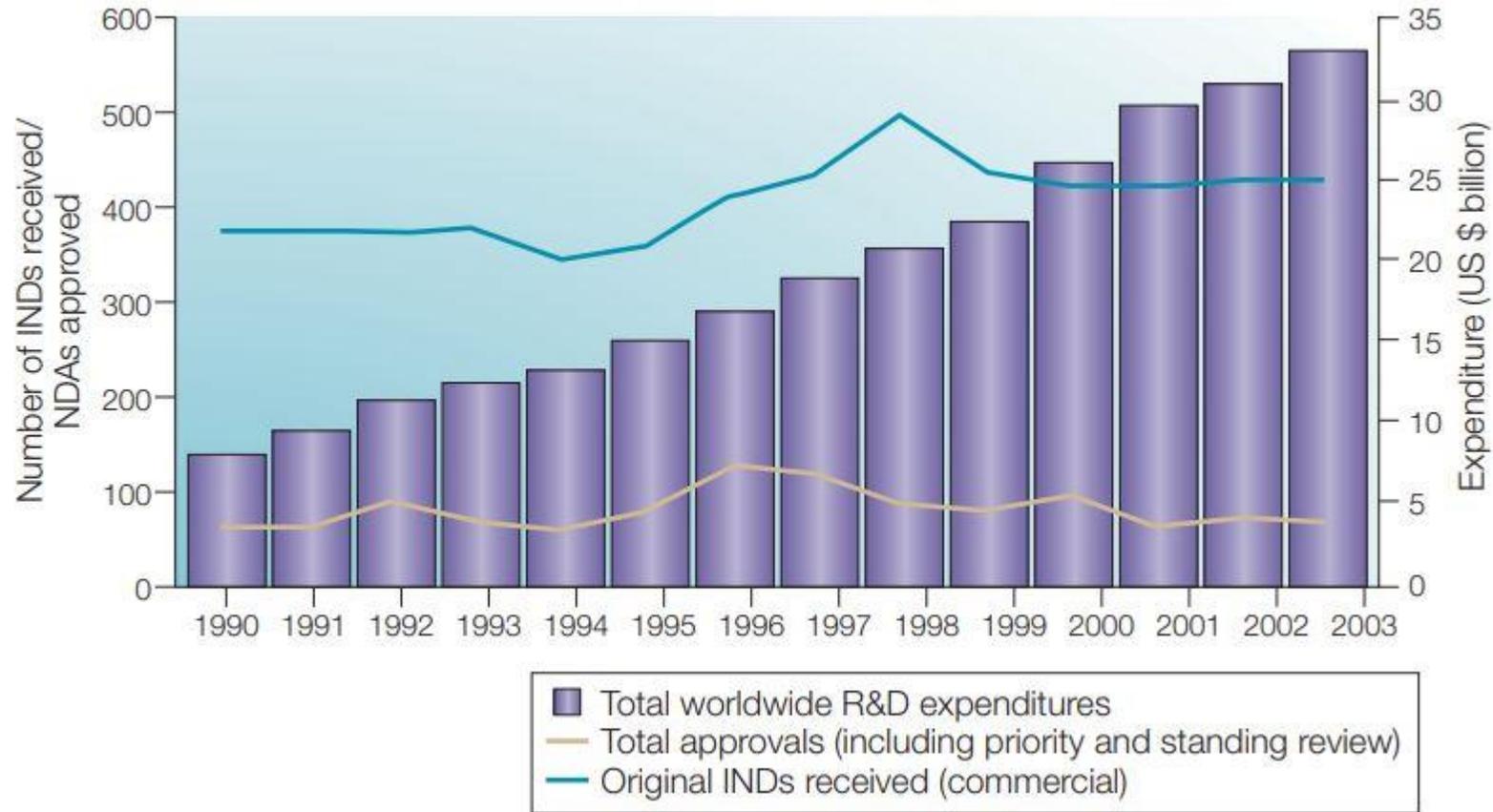


Basi teoriche e casi studio

In questa lezione:

- ▶ Genetica chimica: introduzione
- ▶ Caso studio I: *small-molecules* nella ricerca contro il cancro
- ▶ Caso studio II: molecole per lo studio dell'epigenetica
- ▶ Casi studio III: *repurposing* di *small-molecules* in pianta
- ▶ Conclusioni
- ▶ Letture consigliate

Gap produzione-approvazione



Introduzione

R&D di nuove small molecules

Drug repurposing

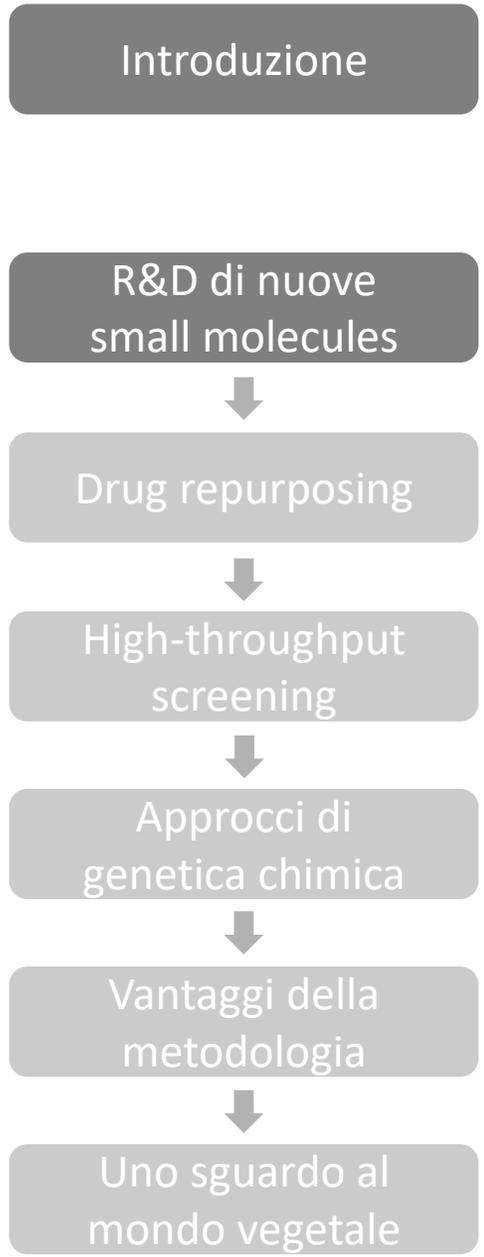
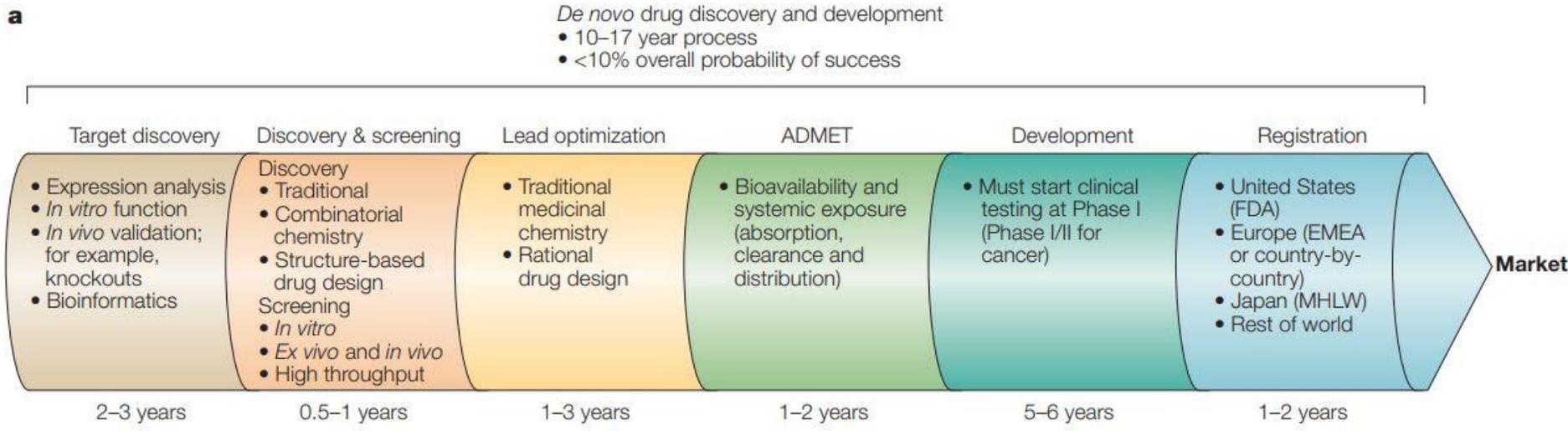
High-throughput screening

Approcci di genetica chimica

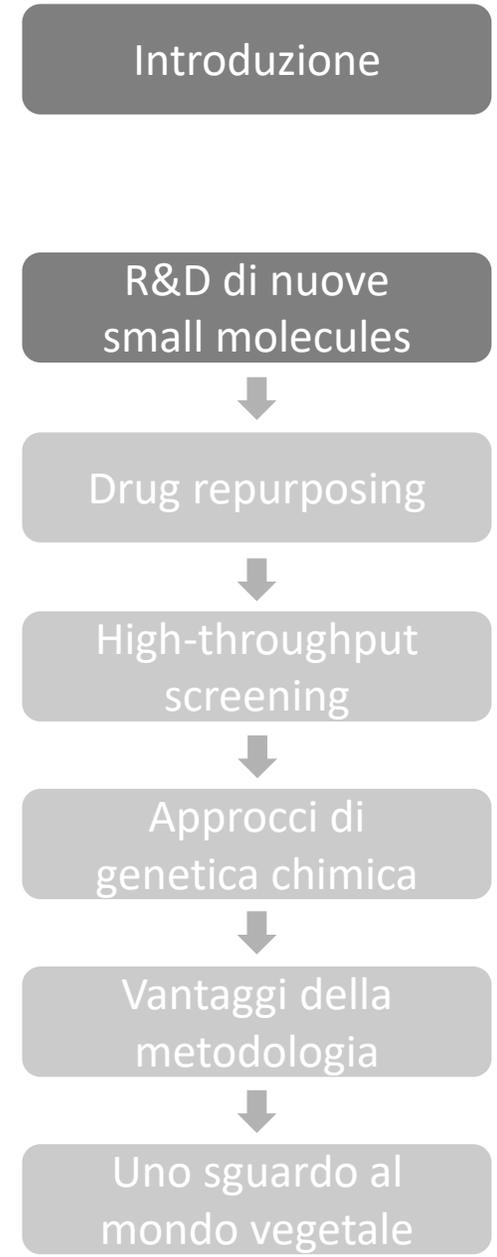
Vantaggi della metodologia

Uno sguardo al mondo vegetale

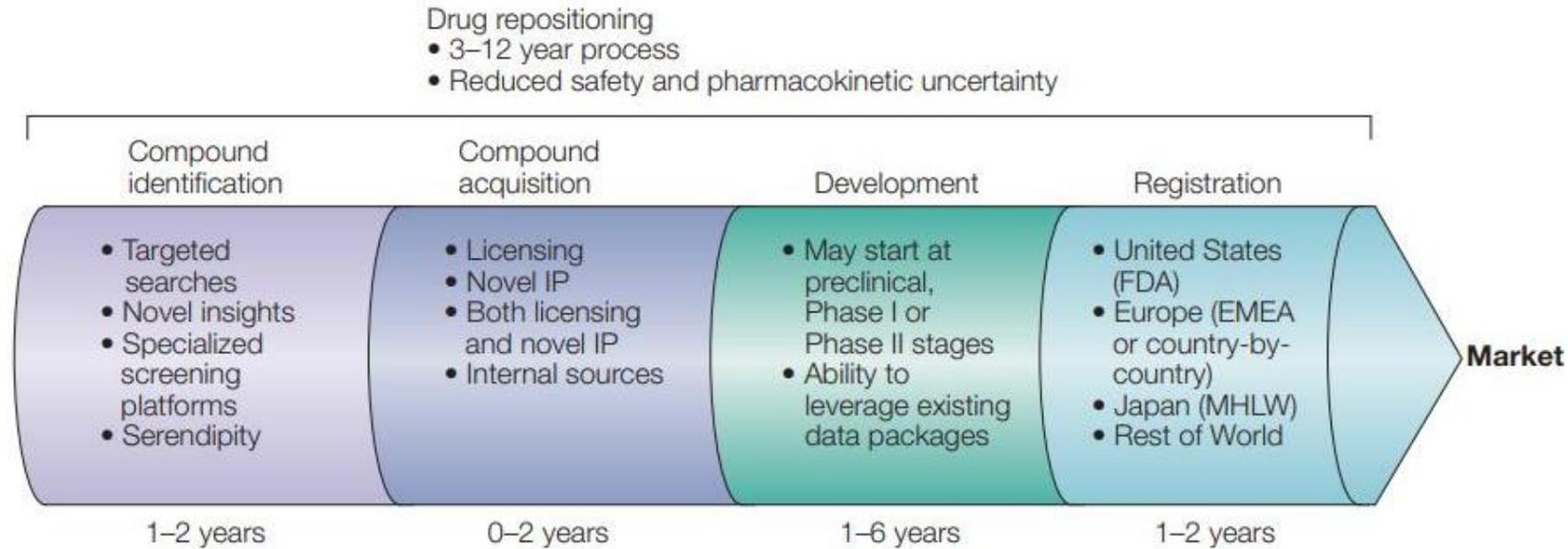
Processo di drug discovery *de novo*



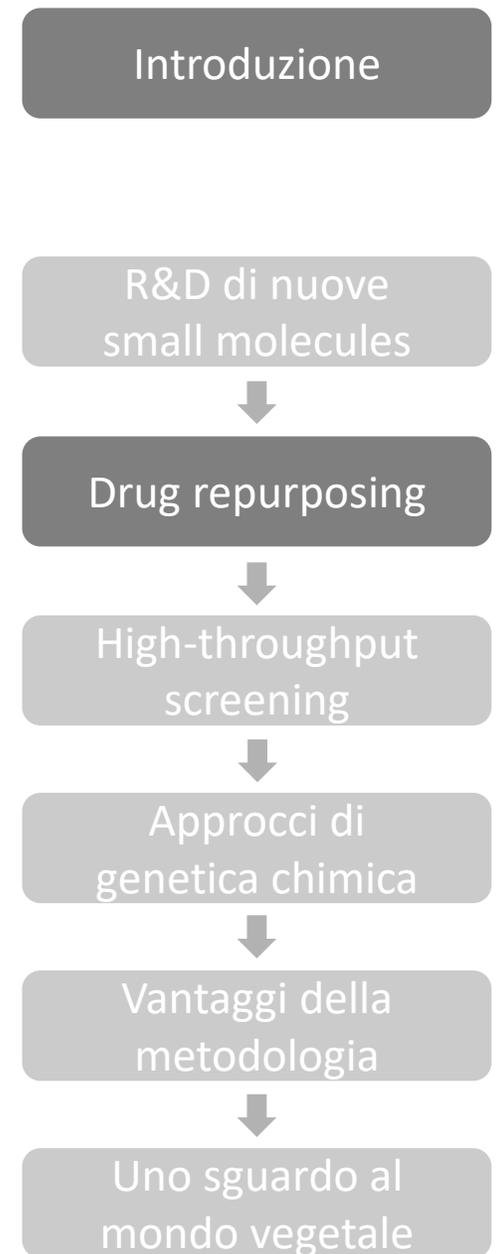
Risk-reward



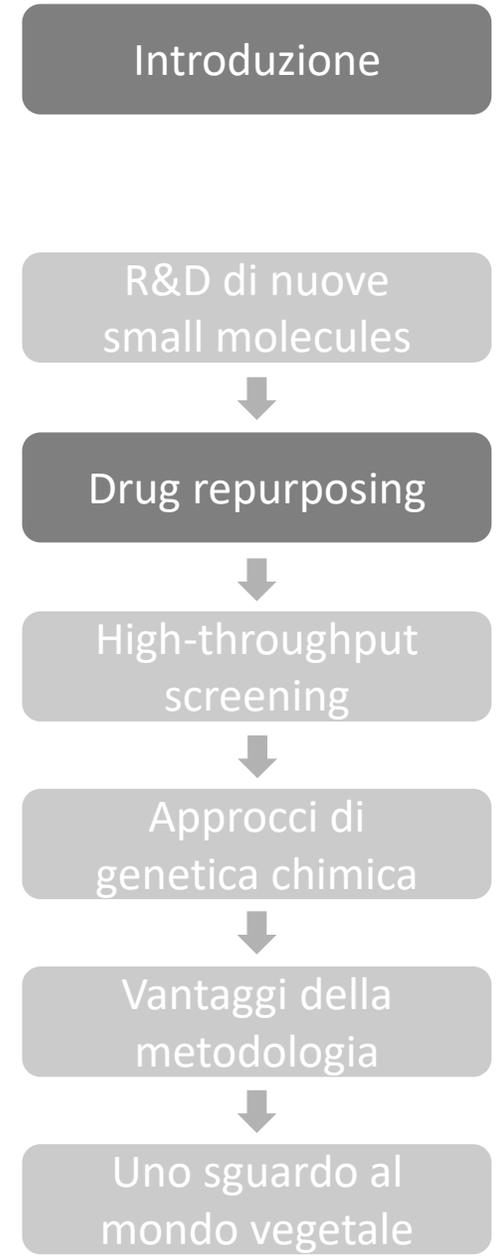
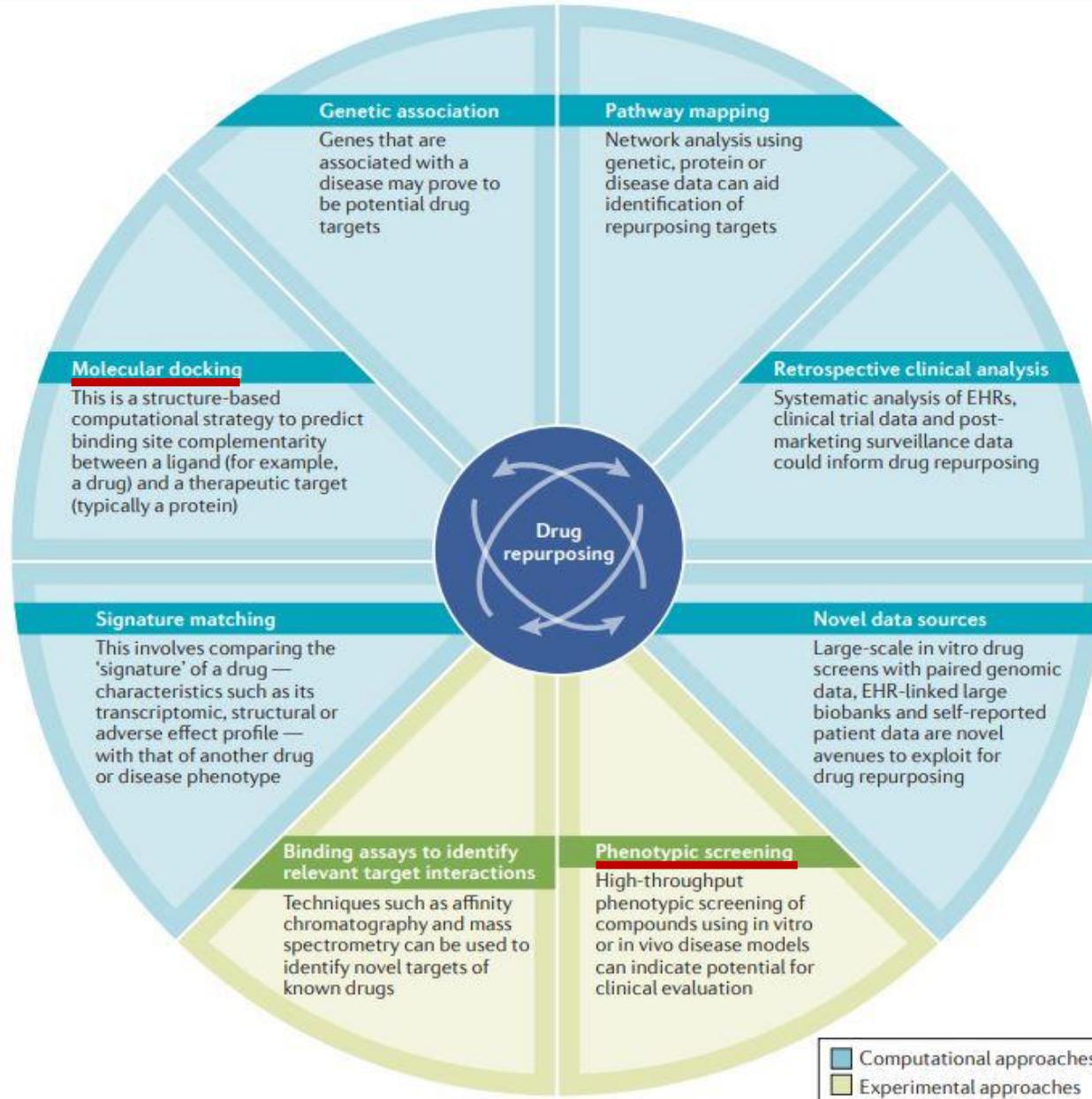
Processo di drug repurposing



Strategie di *repurposing* permettono di ridurre le tempistiche di ricerca e sviluppo assicurando un ridotto fattore di rischio, grazie alle precedenti fasi di sviluppo clinico che assicurano profili di sicurezza e farmacocinetiche ben noti

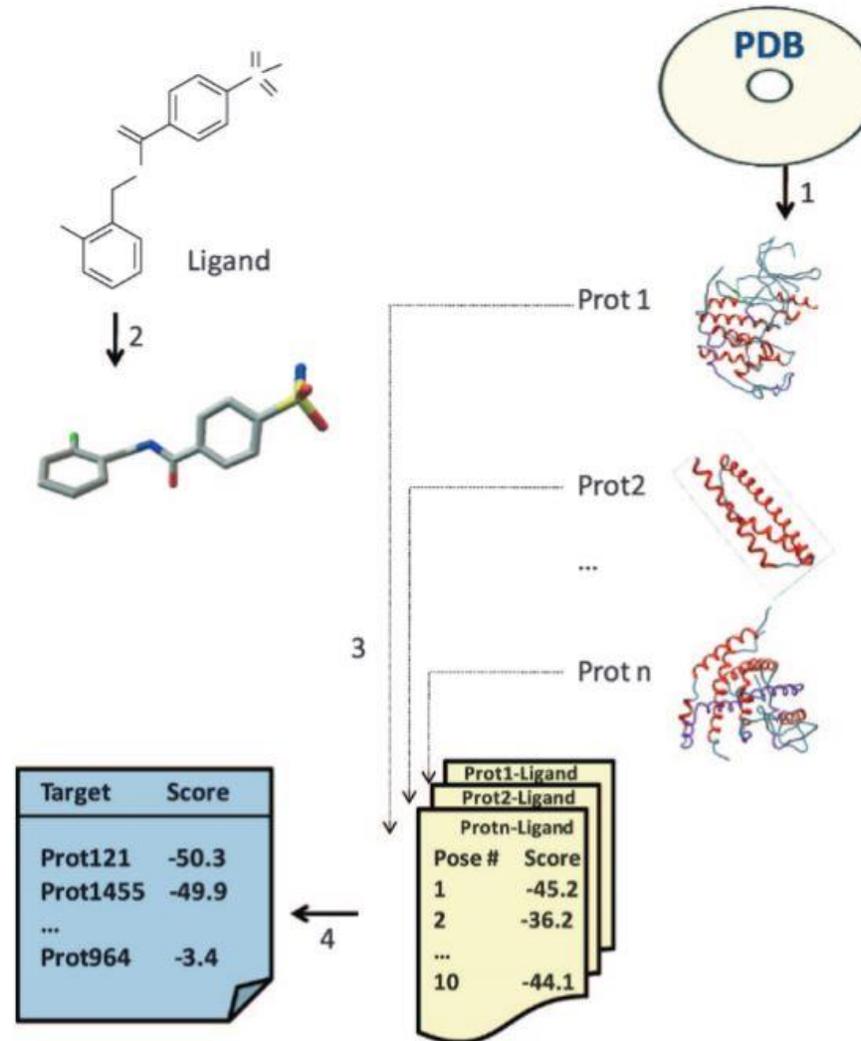


Approcci usati in drug repurposing



Docking molecolare

- ▶ Recupero delle informazioni sui siti di legame proteina-ligando da database
- ▶ Conversione di uno sketch 2-D del ligando in una struttura 3-D
- ▶ Docking seriale del ligando contro tutti i siti di legame
- ▶ Post-elaborazione dei *raw data* di docking per definire un elenco di *target*



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Database

Box 1 Selected resources for systems chemical biology

Genes

Entrez Gene: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>

Proteins

SwissProt: <http://expasy.org/sprot/>

Structures of biological macromolecules

PDB: <http://www.rcsb.org/pdb/home/home.do>

Structural Genomics Consortium: <http://www.sgc.utoronto.ca/>

Pathways

KEGG: <http://www.genome.jp/kegg/>

MetaCyc: <http://metacyc.org/>

BioCarta: <http://www.biocarta.com/genes/index.asp>

Reactome: <http://www.reactome.org/>

Receptors

GPCRdb: <http://www.gpcr.org/7tm/>

NHRs: <http://www.nursa.org/>

Ion channels: <http://www.iuphar-db.org/iuphar-ic/index.html>

Biochemical pathway reaction kinetics

SABIORK: <http://sabio.villa-bosch.de/SABIORK/>

BRENDA: <http://www.brenda.uni-koeln.de/>

Small molecules

PubChem: <http://pubchem.ncbi.nlm.nih.gov/>

Network simulators

Xyce: <http://www.cs.sandia.gov/Xyce/>

BioNetGen: <http://cellsignaling.lanl.gov/bionetgen/index.shtml>

Annotated biological models

<http://www.ebi.ac.uk/biomodels/>

Uncertainty analysis

DAKOTA: <http://www.cs.sandia.gov/DAKOTA/>

Cheminformatics tools

OpenEye software: <http://www.eyesopen.com/>

PubChem

PubChem

ChEMBL

ChEMBL

BindingDB



Introduzione

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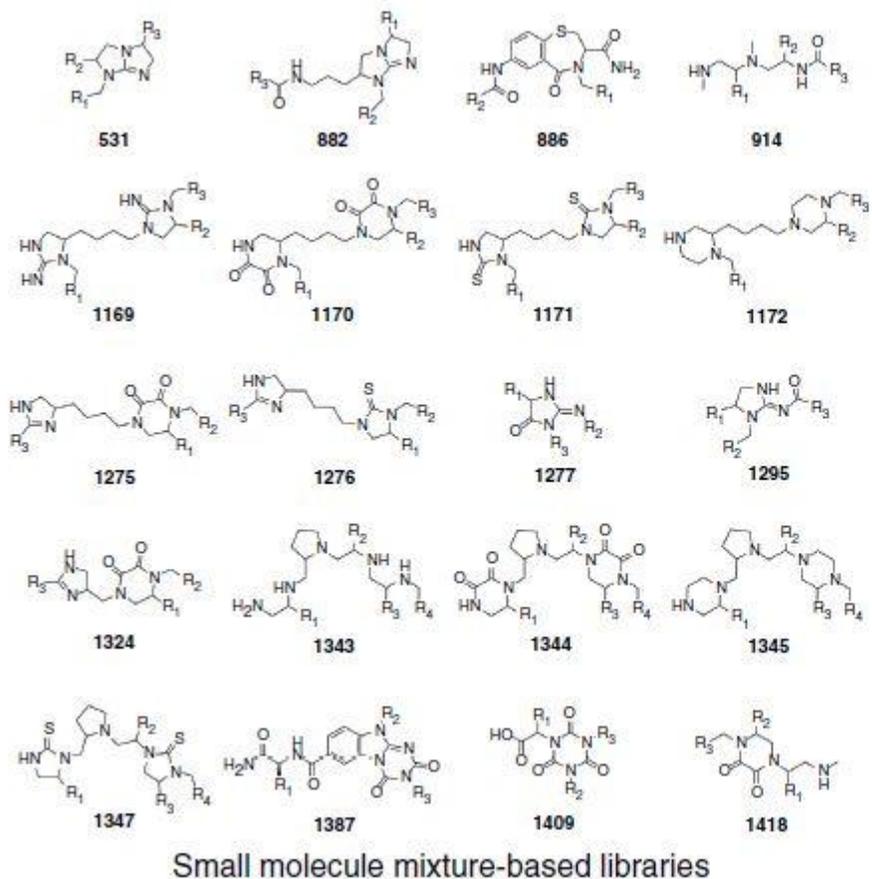
High-throughput
screening

Approcci di
genetica chimica

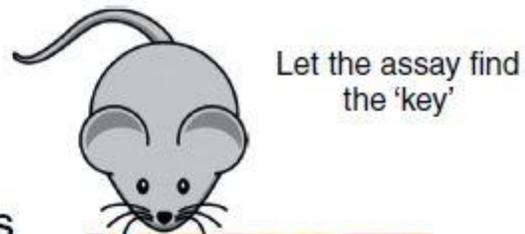
Vantaggi della
metodologia

Uno sguardo al
mondo vegetale

High-throughput screening



In vivo HTS



Deconvolution



Introduzione

R&D di nuove
small molecules



Drug repurposing



High-throughput
screening



Approcci di
genetica chimica

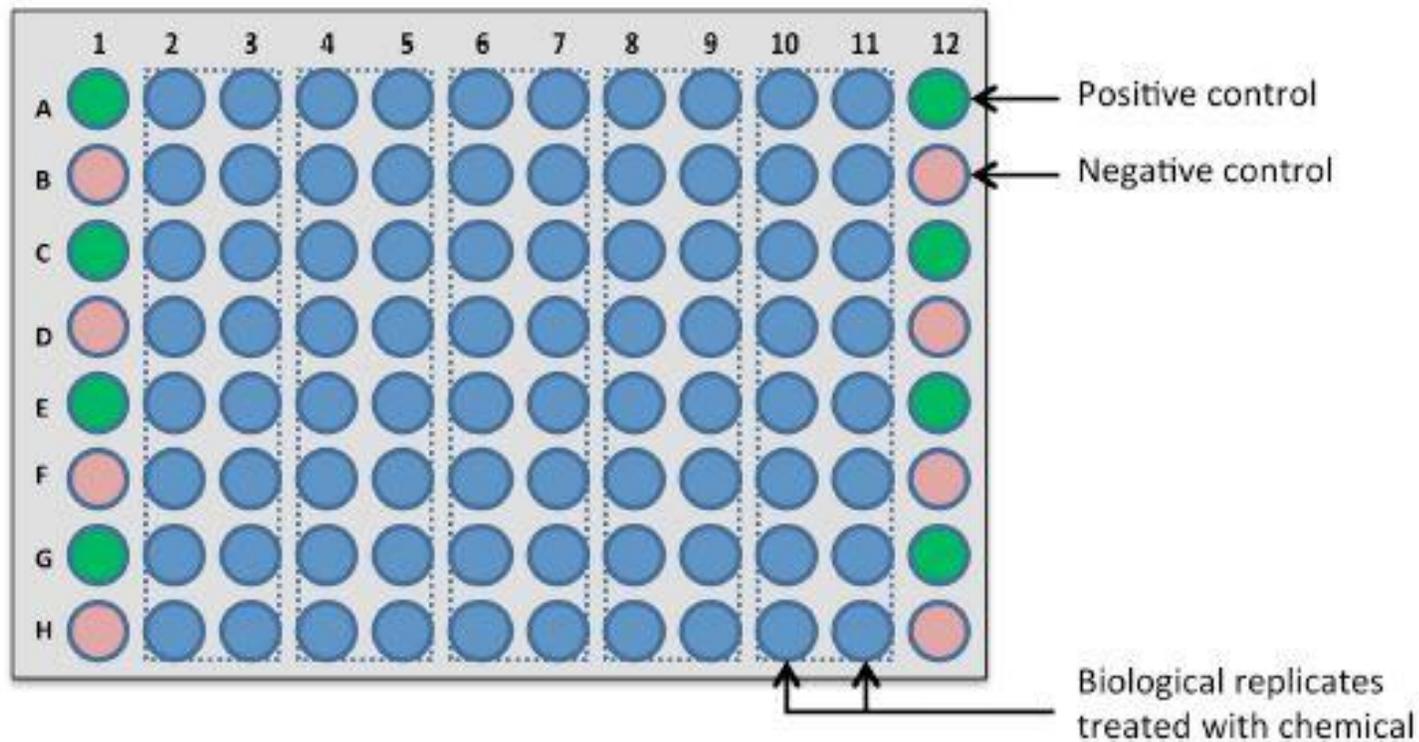


Vantaggi della
metodologia

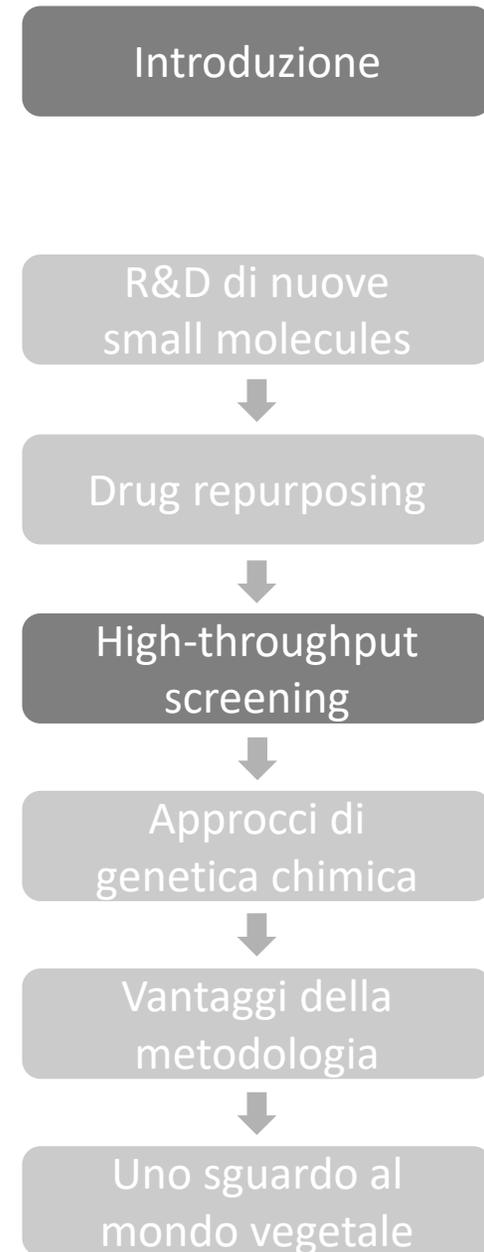


Uno sguardo al
mondo vegetale

Design della plate

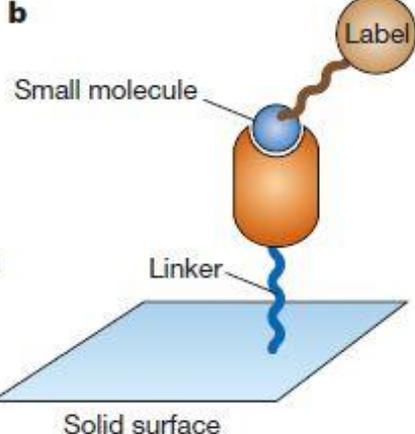
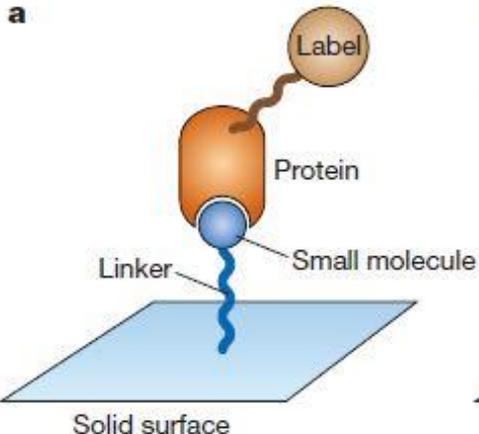


Ciascuna molecola viene testata in doppia replica biologica in una plate da 96 pozzetti, consentendo l'analisi di 40 molecole. Le colonne 1 e 12 sono dedicate ai controlli, 8 positivi e 8 negativi, distribuiti in ordine alternato.

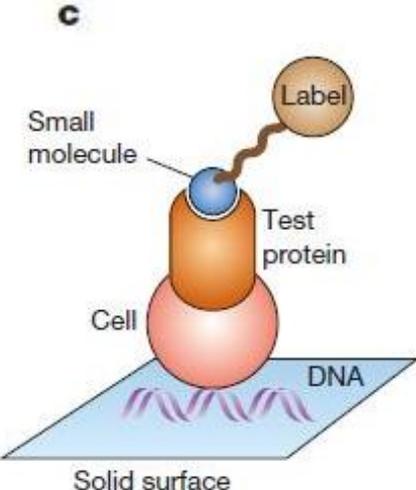


Metodi di screening

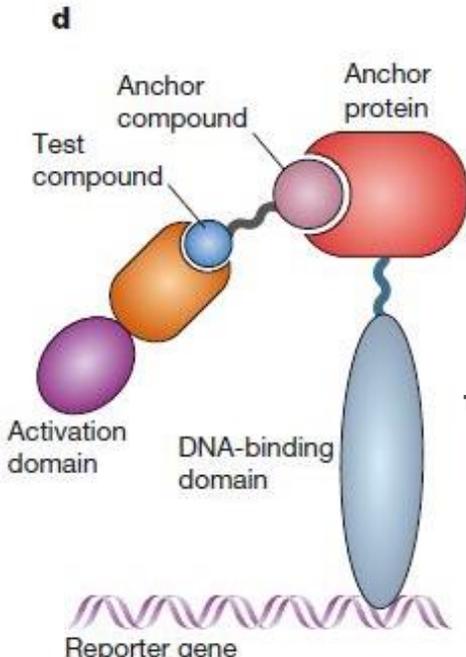
Small molecules covalently linked to a surface



Cells microarray that overexpress desired protein



Protein immobilized on a surface



Yeast threehybrid system

Introduzione

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Drug repurposing

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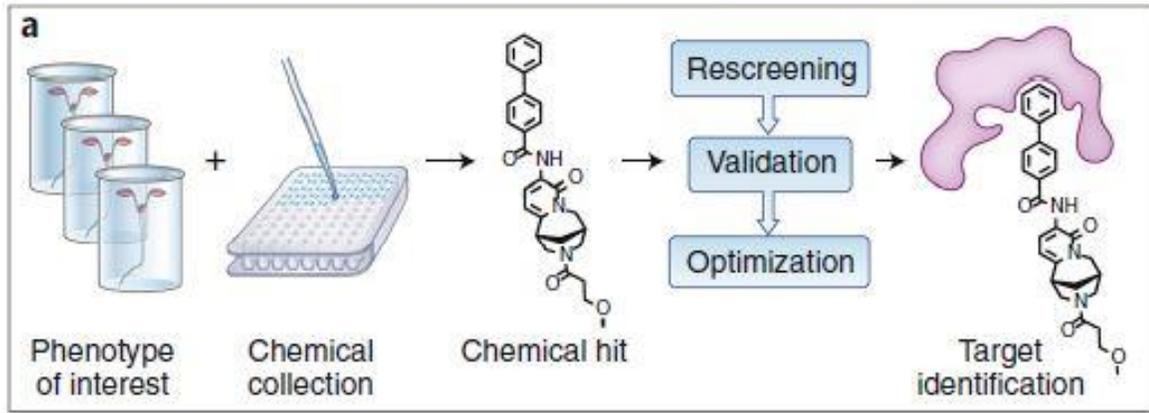
Vantaggi della metodologia

Uno sguardo al mondo vegetale

Step sperimentali nei due approcci

(Hypothesis-generating)

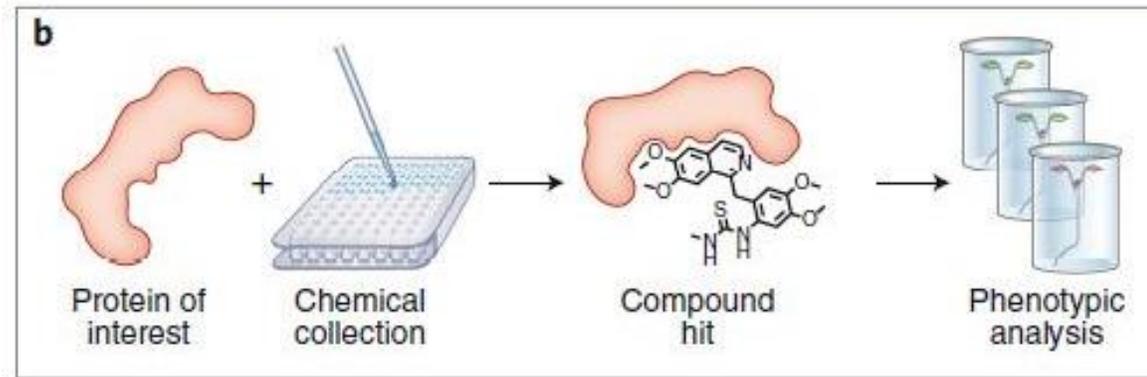
Forward chemical genetics



Partendo da un fenotipo noto, testiamo molecole che lo inducono. Quindi cerchiamo e validiamo il target della molecola. Alcune problematiche...

(Hypothesis-based)

Reverse chemical genetics



Partendo da un target di interesse, testiamo molecole bioattive. Quindi cerchiamo quali fenotipi vengono indotti per ricostruire la funzione.

Introduzione

R&D di nuove small molecules

Drug repurposing

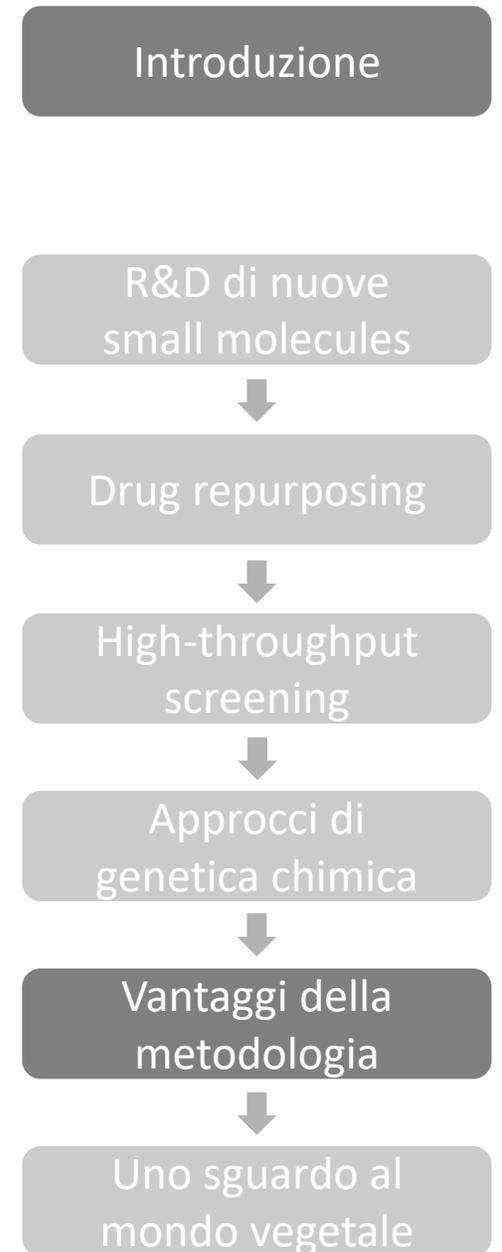
High-throughput screening

Approcci di genetica chimica

Vantaggi della metodologia

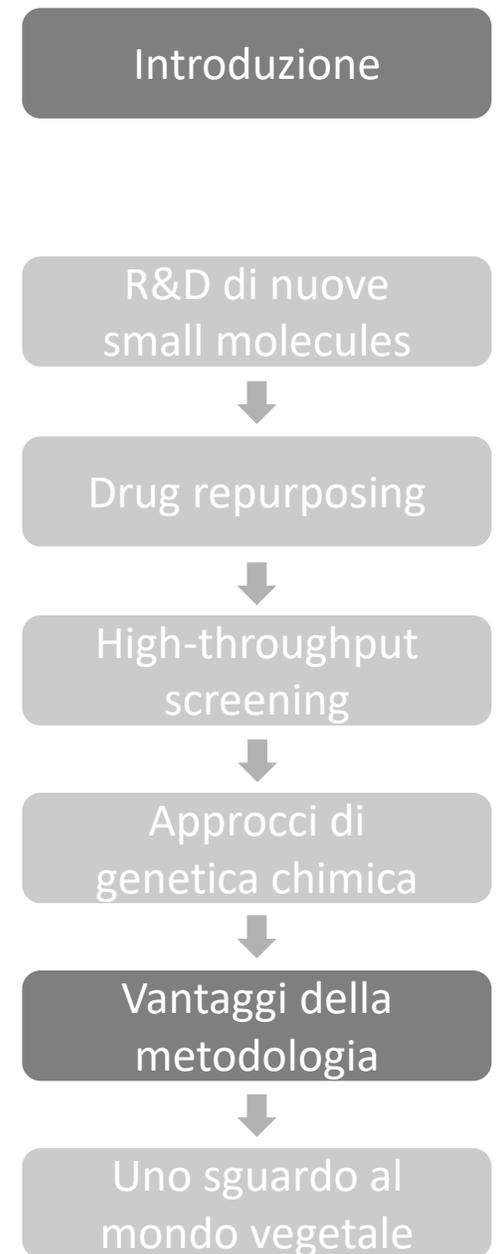
Uno sguardo al mondo vegetale

Quali sono i vantaggi di questa metodologia?

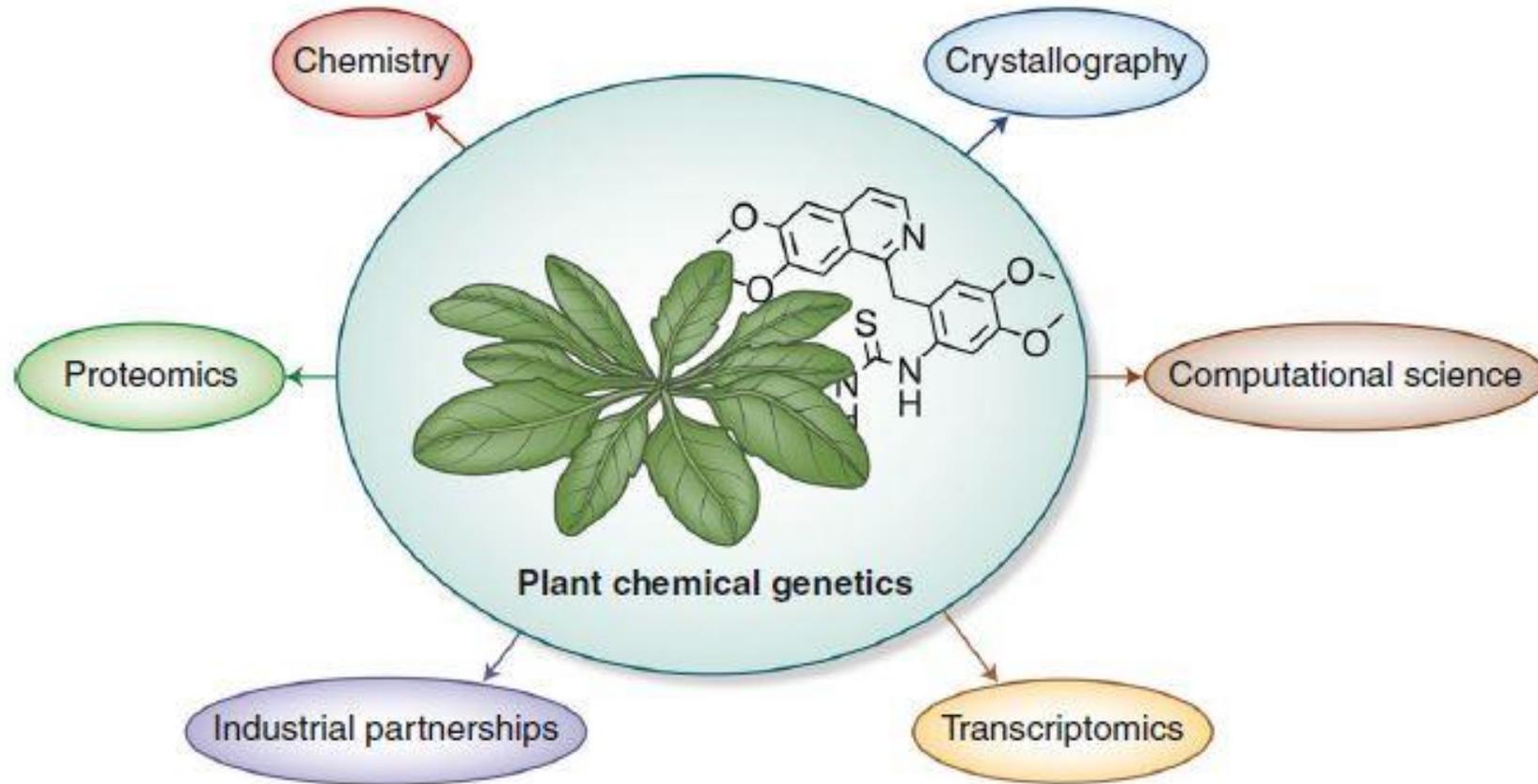


Analizziamone alcuni:

- ▶ Possibilità di utilizzo a differenti concentrazioni, permettendo l'analisi di un più ampio spettro di fenotipi
- ▶ Modulazione del tempo di esposizione alle molecole, con possibilità di rescue dinamico delle normali funzioni dell'organismo
- ▶ Possibilità di agire contemporaneamente su un ampio gruppo di proteine con funzioni ridondanti
- ▶ Applicazione topica delle molecole, al fine di agire solamente su uno specifico organo dell'organismo studiato
- ▶ Applicazione su specie non geneticamente alterabili, al fine di studiare geni omologhi
- ▶ Possibilità di studiare mutazioni embrioletali/che rendono l'organismo sterile



Genetica chimica in pianta



Introduzione

R&D di nuove
small molecules

Drug repurposing

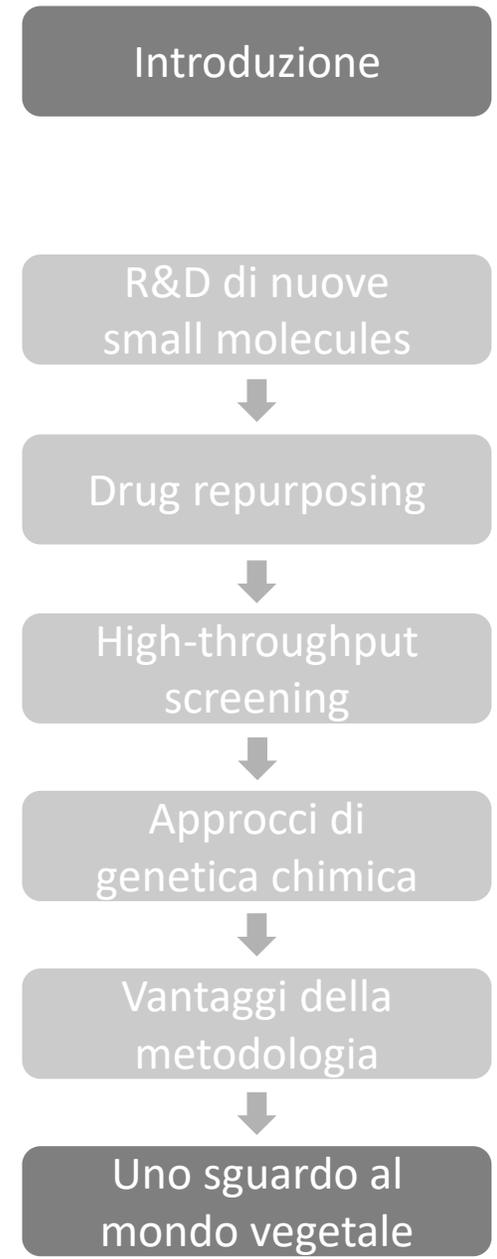
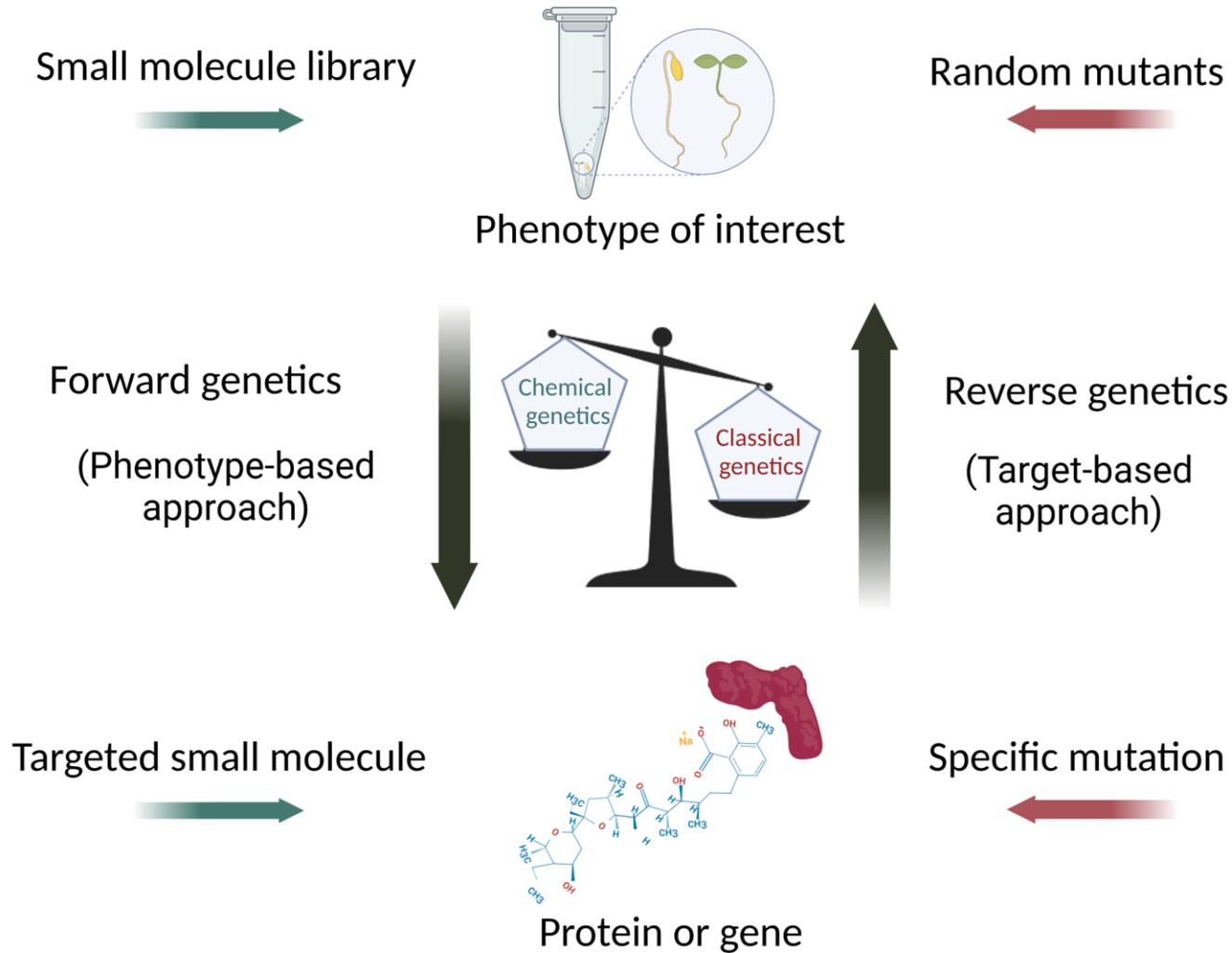
High-throughput
screening

Approcci di
genetica chimica

Vantaggi della
metodologia

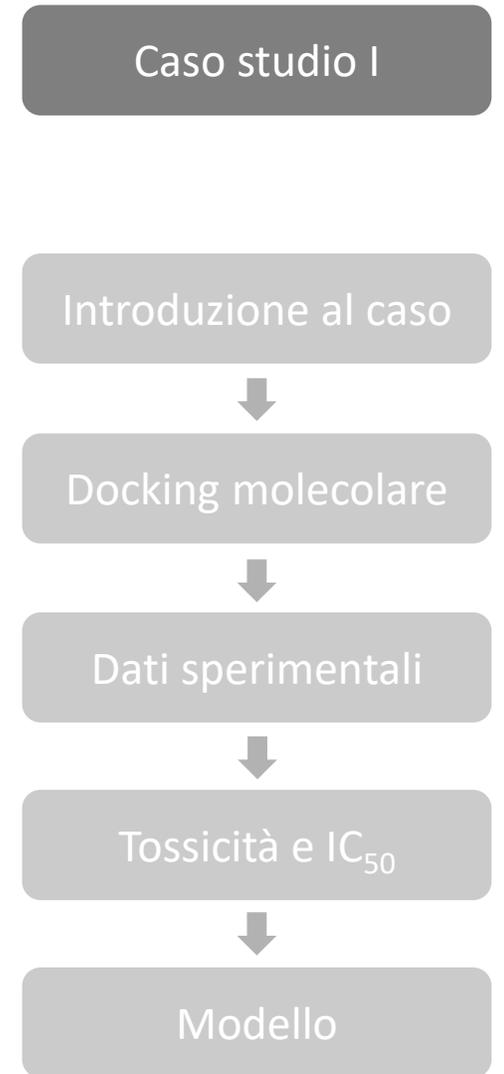
Uno sguardo al
mondo vegetale

Approcci in pianta



Caso studio I – cancer research

Ponatinib repurposing *In Vitro* and *In Vivo* (mouse) experiments

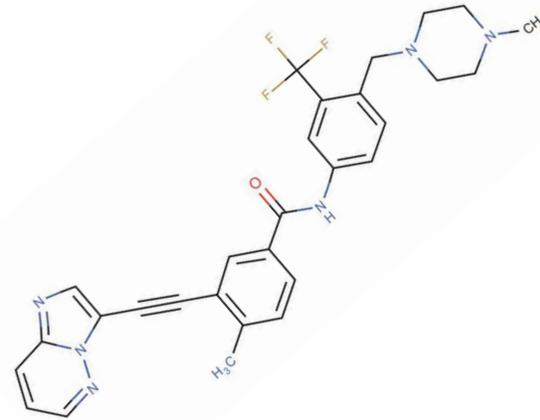
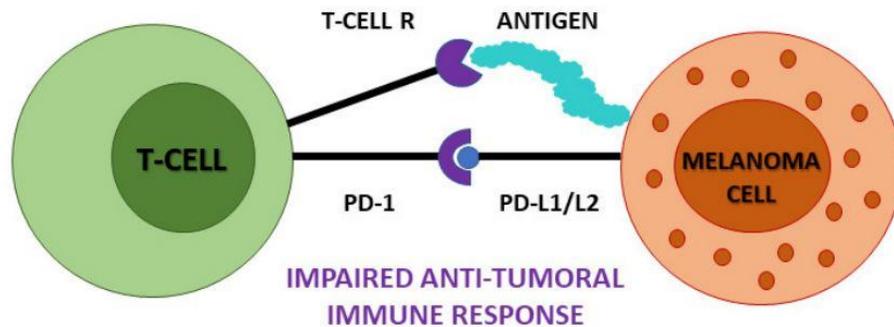


Overview

Repurposing Ponatinib as a PD-L1 Inhibitor Revealed by Drug Repurposing Screening and Validation by *In Vitro* and *In Vivo* Experiments

Anjali Barnwal, Sanjeev Das, and Jayanta Bhattacharyya*

T-CELL DEACTIVATION



Caso studio I

Introduzione al caso

Docking molecolare

Dati sperimentali

Tossicità e IC_{50}

Modello

Repurposing Ponatinib as a PD-L1 Inhibitor Revealed by Drug Repurposing Screening and Validation by *In Vitro* and *In Vivo* Experiments

Anjali Barnwal, Sanjeev Das, and Jayanta Bhattacharyya*

Research on the development of immune checkpoint inhibitors has been motivated by the clinical success of the use of antibodies to target immune inhibitory pathways, specifically the programmed cell death protein 1 (PD-1) and its ligand 1 (PD-L1).¹ In clinics, several studies showed that the inhibition of the PD-1/PD-L1 interaction enhances the T-cell response and tumor regression.^{2–4} Currently, more than 1000 clinical trials are evaluating the efficacy of antibodies targeting the PD-1/PD-L1 pathway and some have been approved for the treatment of many tumors such as non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), melanoma, Hodgkin's lymphoma, bladder cancer, etc.^{5–7} However, antibodies are associated with several disadvantages, which include a high production cost, lower stability, poor tissue penetration, and immunogenicity.⁸ Moreover, the PD-L1 antibody induces immune-related adverse effects (irAEs) due to long-lasting systemic immune activation, whereas small molecules could reduce this problem because of their shorter half-life in a systemic environment.⁹ To this end, the development of small-molecule inhibitors appears to be the

most effective alternative as it will overcome the problems associated with antibodies.

At present, the research progress on small-molecule inhibitors targeting the PD-1/PD-L1 pathway lags behind antibody development. Although recent studies reported a few series of small molecules, peptides, macrocyclic peptides, and peptidomimetics that target the PD-1/PD-L1 interaction, publicly disclosed validation in the animal model is rare.^{10–12} Conversely, the development of a new drug is associated with several bottlenecks, such as toxicity, cost, and the time required to bring it to the market. Hence, an existing drug used for a different target can be repurposed to minimize the time required for the development and to reduce the risk of failure

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Caso studio I

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Docking molecolare

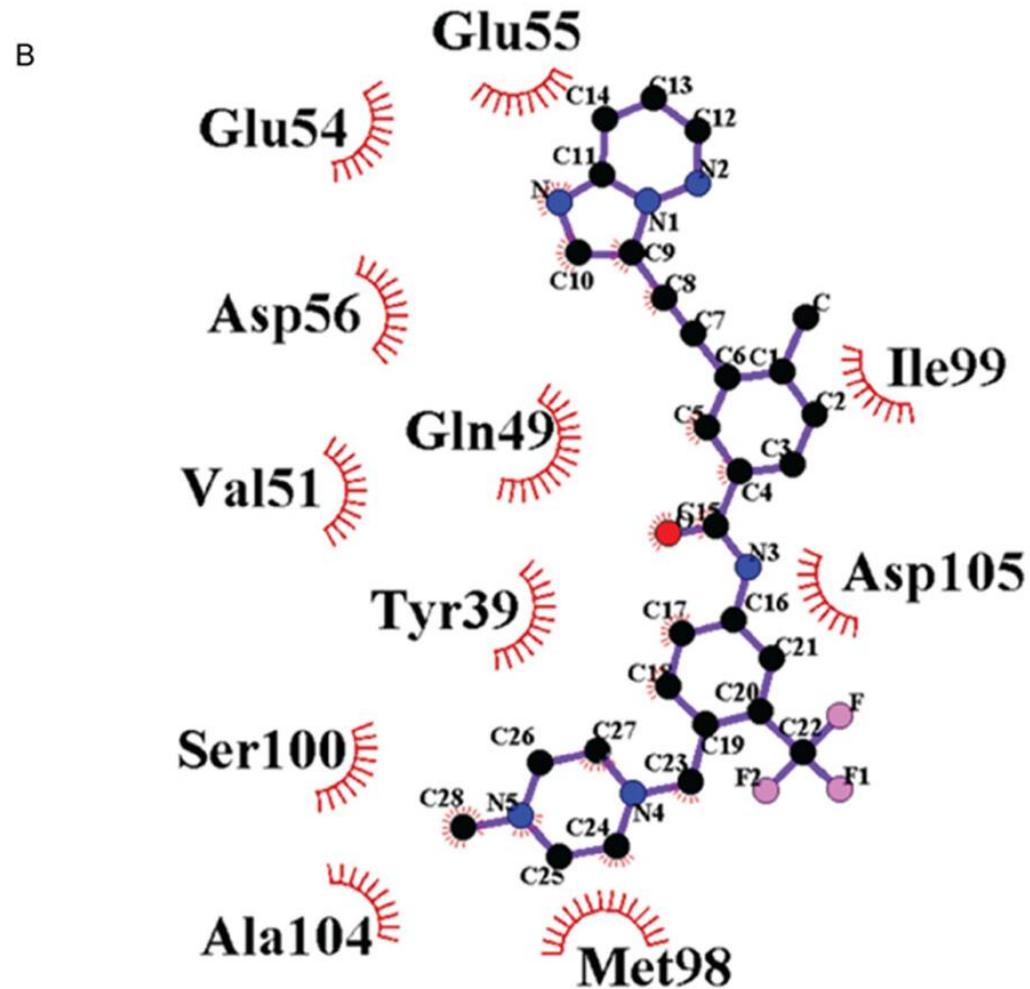
Dati sperimentali

Tossicità e IC₅₀

Modello

Interazione molecola-ligando

Studi di docking molecolare hanno permesso di determinare che il Ponatinib forma un legame idrofobico con i residui Glu-54, Glu-55, Asp-56, Gln-49, Val-51, Tyr-39, Ser-100, Ile-99, Met-98, Ala-104 e Asp-105 di PD-L1



Caso studio I

Introduzione al caso

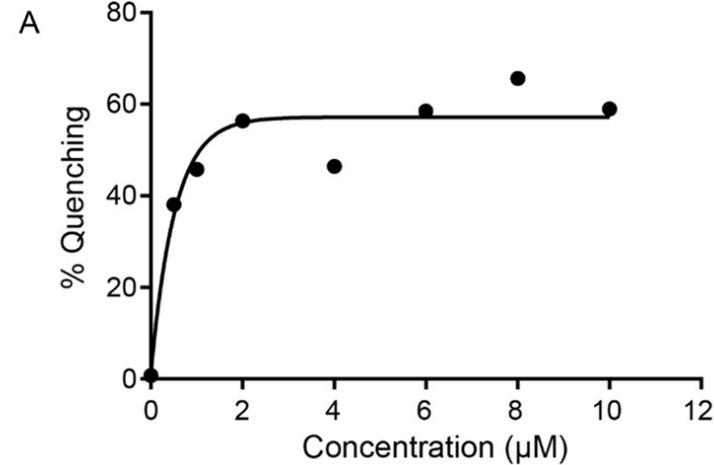
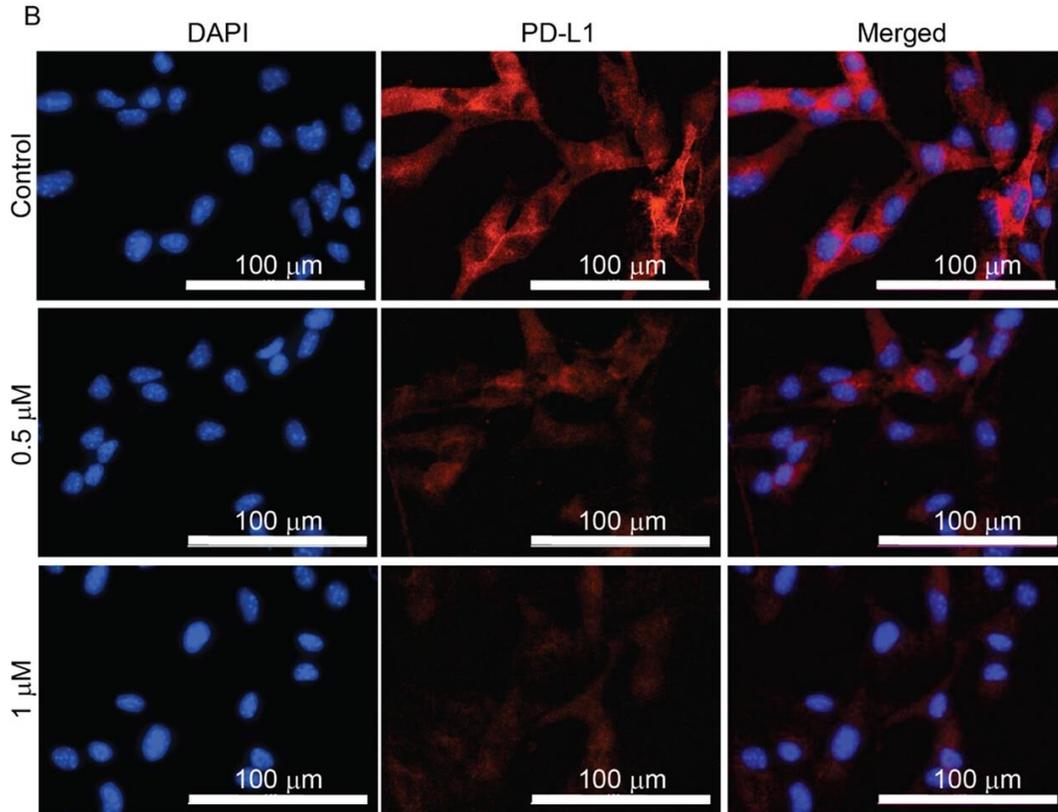
Docking molecolare

Dati sperimentali

Tossicità e IC_{50}

Modello

Ponatinib – PD-L1 in soluzione di cellule B16-F10



Il trattamento provoca un quenching della fluorescenza, ergo la quantità di PD-L1 libero è diminuita significativamente a seguito del trattamento con Ponatinib in modo dose-dipendente, indicando l'efficiente legame di proteina-ligando

Caso studio I

Introduzione al caso

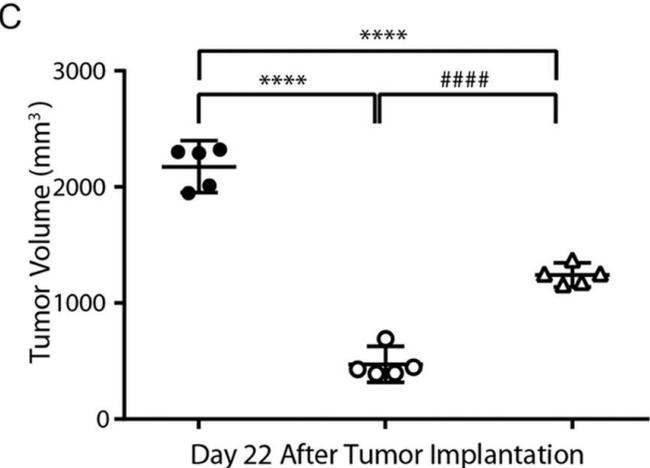
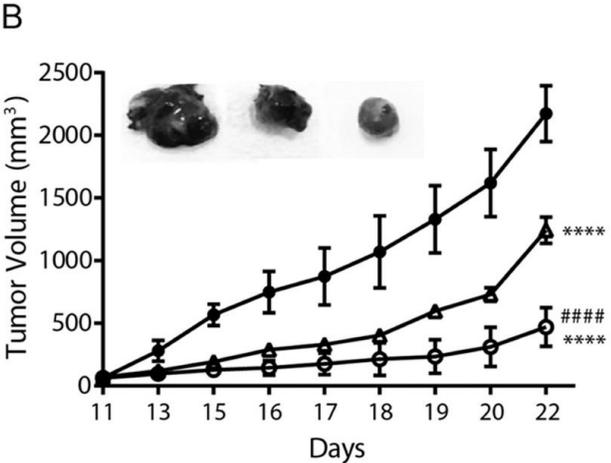
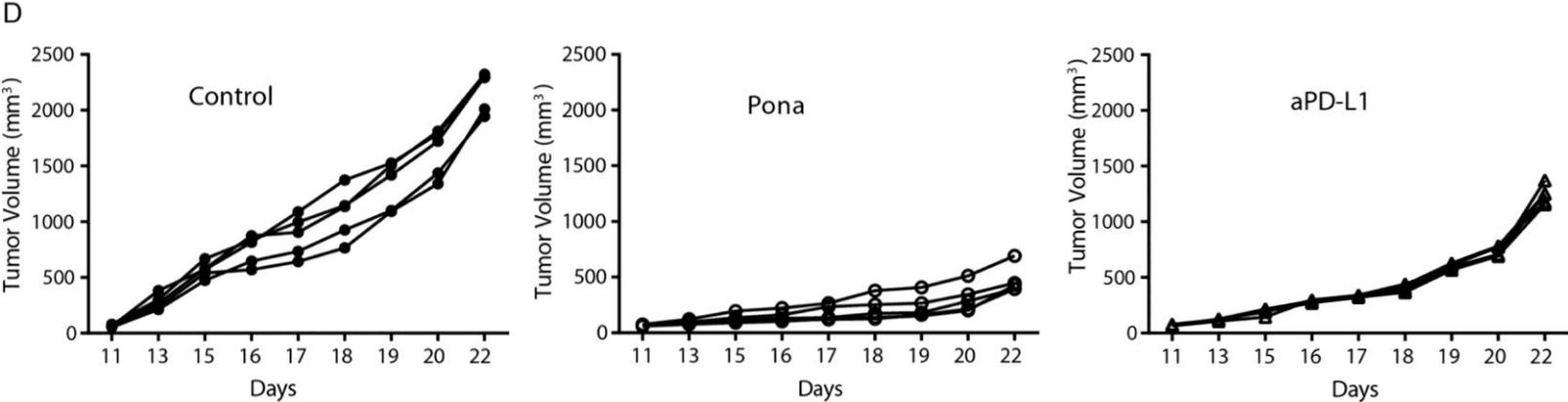
Docking molecolare

Dati sperimentali

Tossicità e IC_{50}

Modello

Effetti del trattamento



Caso studio I

Introduzione al caso

Docking molecolare

Dati sperimentali

Tossicità e IC₅₀

Modello

Due parametri fondamentali: tossicità and IC₅₀

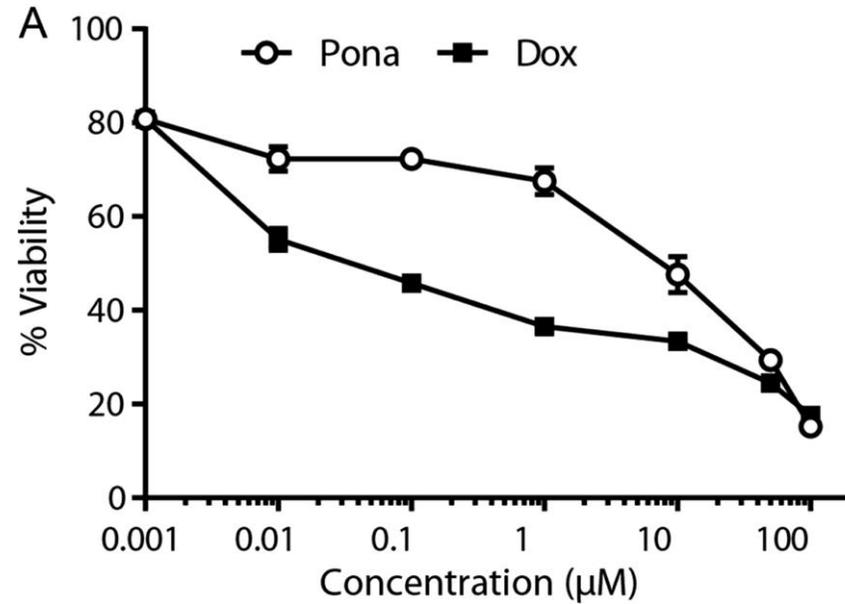


Table S2. IC₅₀ of Ponatinib and Doxorubicin in B16-F10 cells.

Treatment	IC ₅₀ (nM)
Ponatinib	16380
Doxorubicin	12.9

Caso studio I

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Dati sperimentali

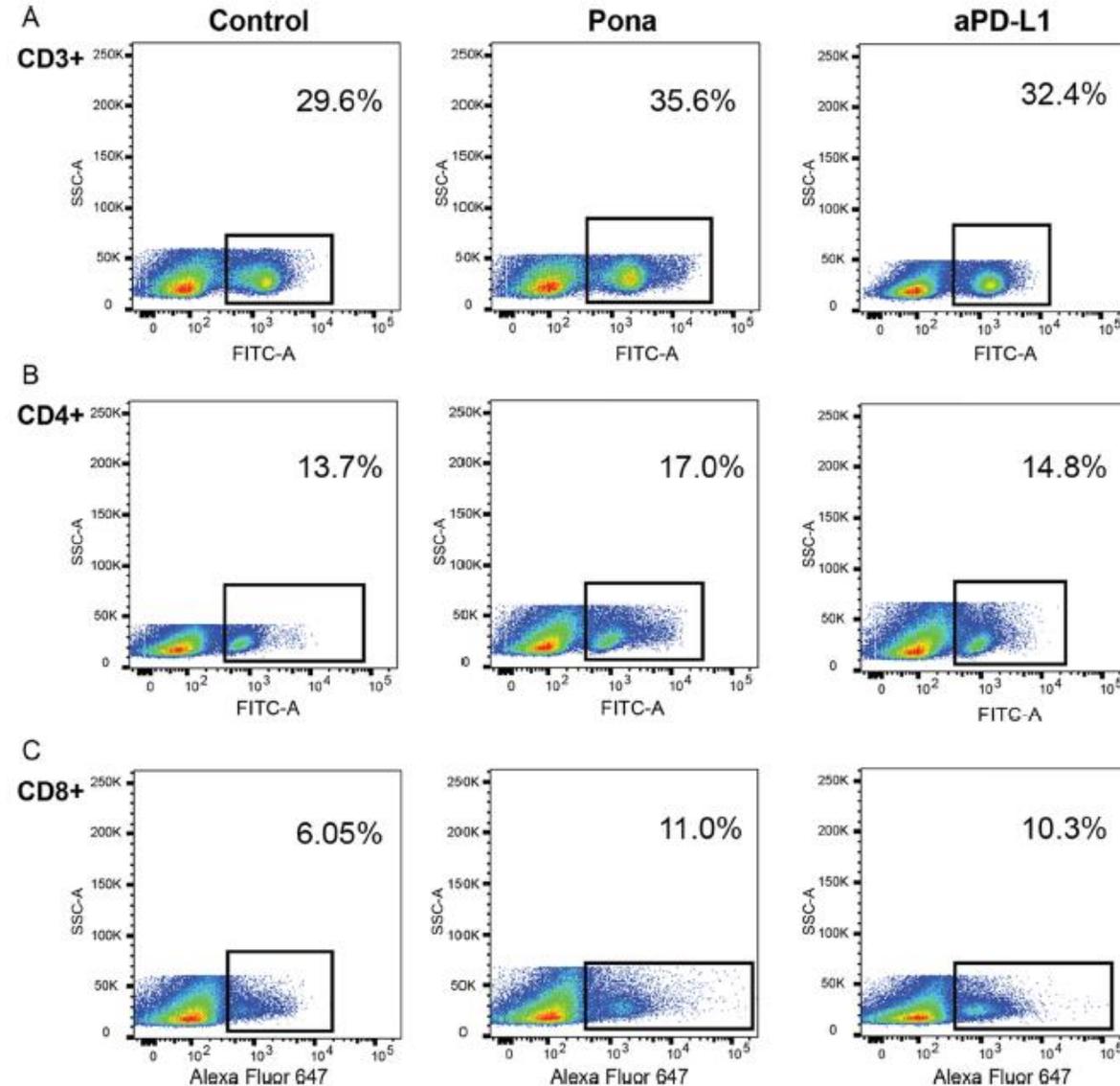
Tossicità e IC₅₀

Modello

Meccanismo di azione

Dato che il blocco della via di segnalazione PD-1/PD-L1 induce la proliferazione e l'attivazione delle cellule T, ne è stata valutata la quantità nella milza dei topi. È stato riscontrato un aumento nelle percentuali di cellule T trattate con Ponatinib rispetto al controllo.

Presi insieme, questi dati suggeriscono che il Ponatinib si leghi a PD-L1 e ritardi la crescita del tumore inducendo l'immunità antitumorale piuttosto che attaccandolo direttamente.



Caso studio I

Introduzione al caso



Docking molecolare



Dati sperimentali

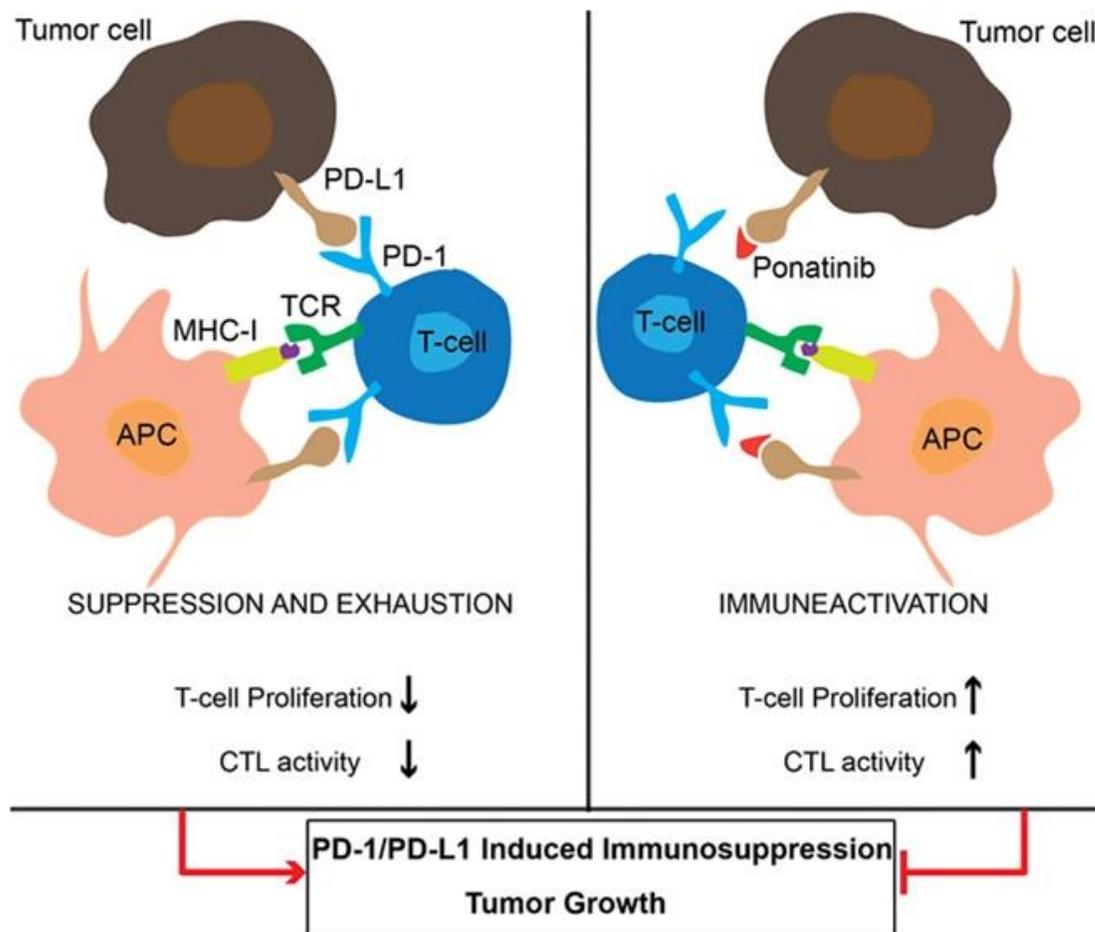


Tossicità e IC_{50}



Modello

“Ponatinib can be used as a potent small-molecule inhibitor of PD-L1 to overcome the disadvantages associated with antibodies”



Caso studio I

Introduzione al caso

Docking molecolare

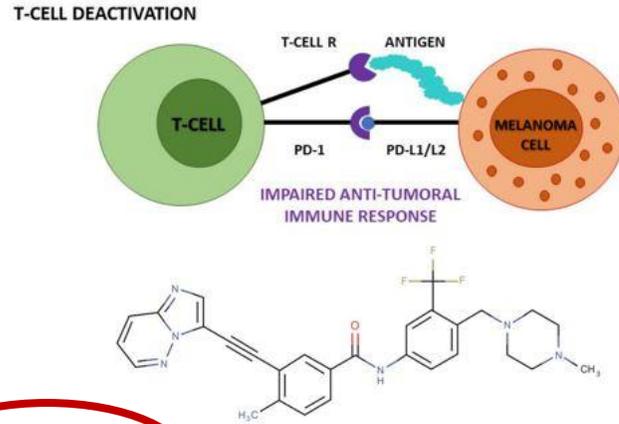
Dati sperimentali

Tossicità e IC_{50}

Modello

Consigli per l'esame:

Problema biologico e screening molecole



Massaro et al., 2017
Kamińska et al., 2021

Studiate bene i Supplementary forniti, sono un ottimo spunto per fare una analisi critica dell'articolo da presentare

Non avete background in merito al problema biologico proposto? Nel vostro paper sicuramente troverete qualche review citata che vi aiuterà e che potrete riportare come introduzione (poche slide però)

Due parametri fondamentali: tossicità and IC_{50}

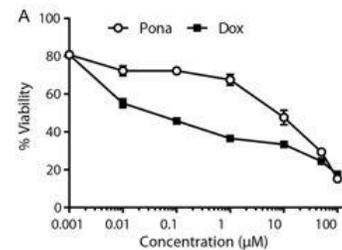


Table S2. IC_{50} of Ponatinib and Doxorubicin in B16-F10 cells.

Treatment	IC_{50} (nM)
Ponatinib	16380
Doxorubicin	12.9

Barnwal et al., 2023

Caso studio I

Introduzione al caso

Docking molecolare

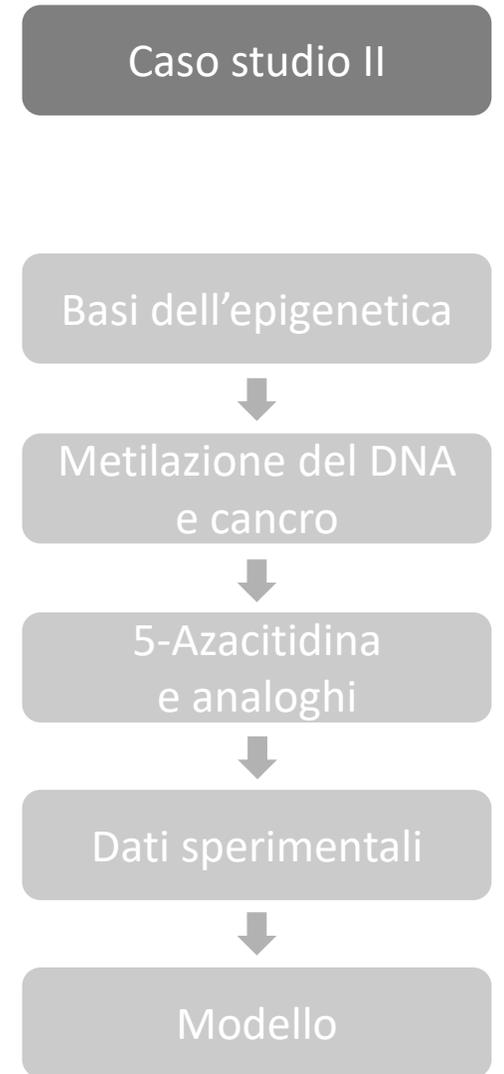
Dati sperimentali

Tossicità e IC_{50}

Modello

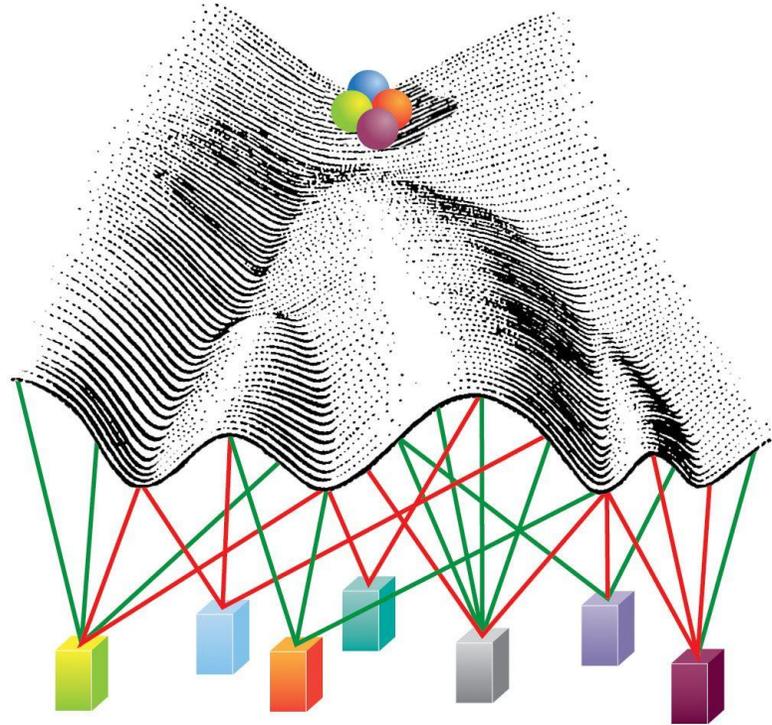
Caso studio II – epigenetic

5-Azacytidine and pancreatic cancer



Epigenetica: *on top of genetics*

“the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence”



The epigenetic landscape integrates the connected concepts of competence, induction, and regulative abilities of the genes into a single model designed to explain cellular differentiation, a long-standing problem in embryology. Waddington envisioned the epigenetic landscape as a series of ridges and valleys a cell can traverse on its way to a final tissue type.

Waddington, C.H., 1957

Caso studio II

Basi dell'epigenetica

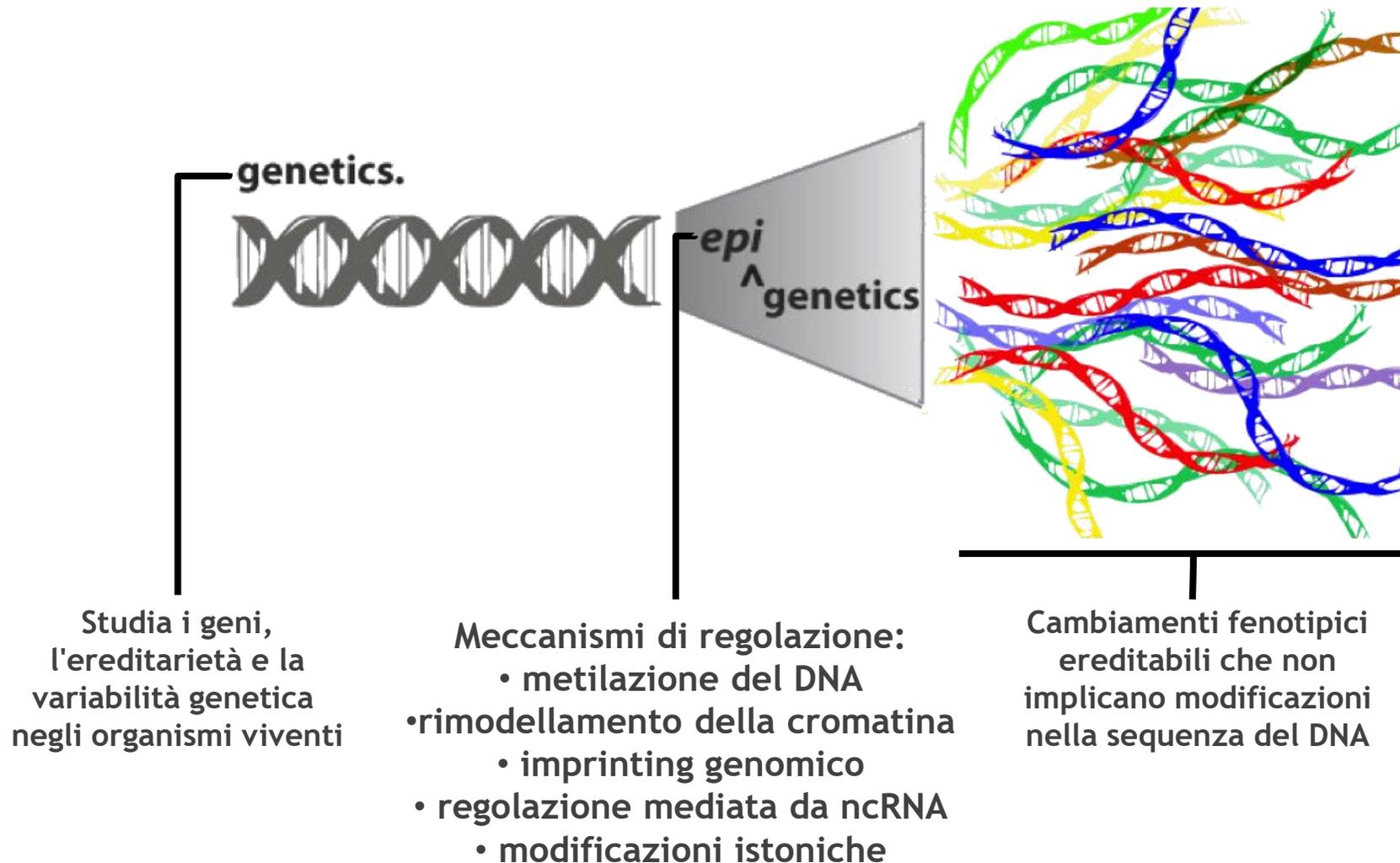
Metilazione del DNA
e cancro

5-Azacitidina
e analoghi

Dati sperimentali

Modello

Meccanismi di regolazione epigenetici



Caso studio II

Basi dell'epigenetica

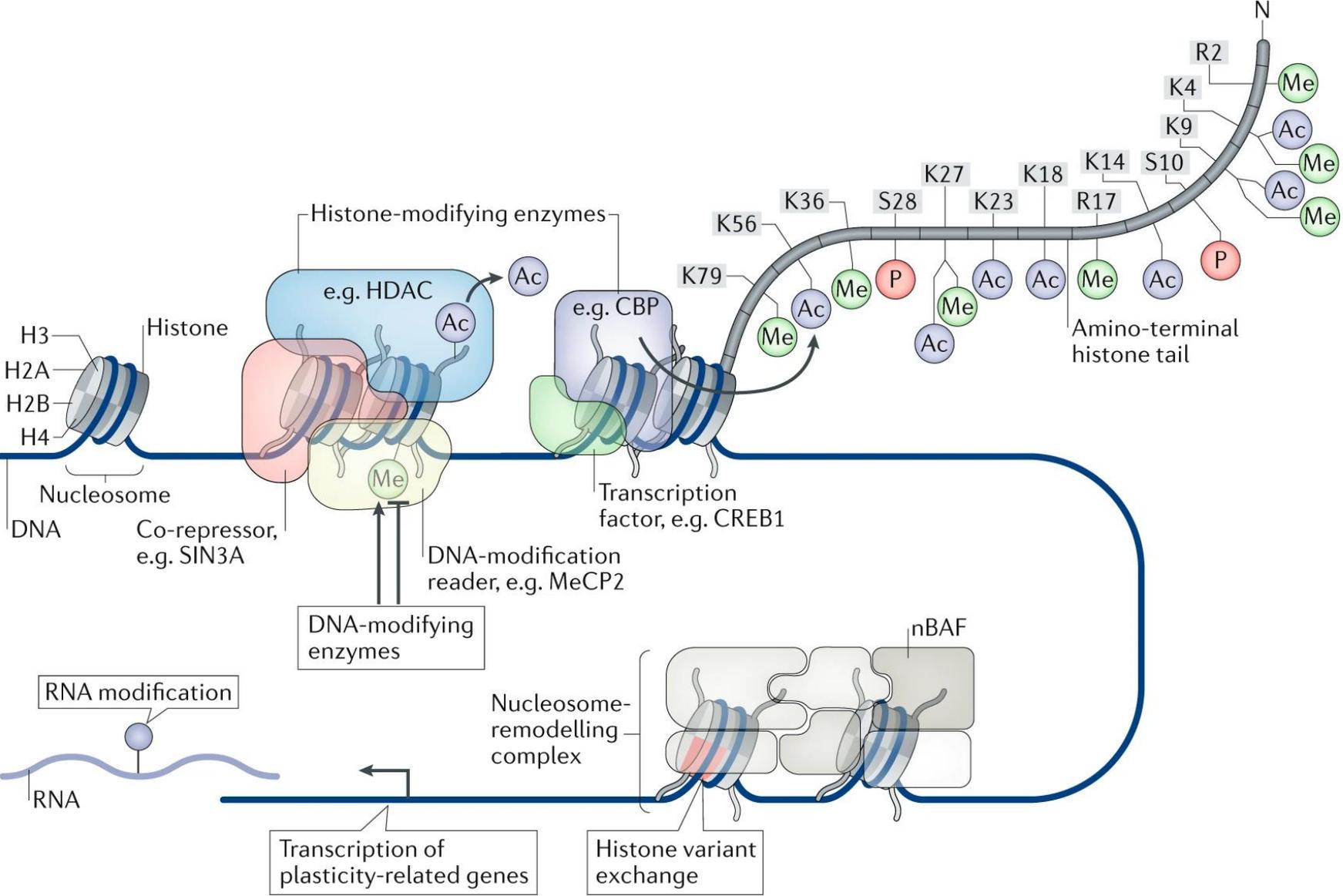
Metilazione del DNA e cancro

5-Azacidina e analoghi

Dati sperimentali

Modello

Panoramica: la complessità della regolazione



Caso studio II

Basi dell'epigenetica

Metilazione del DNA e cancro

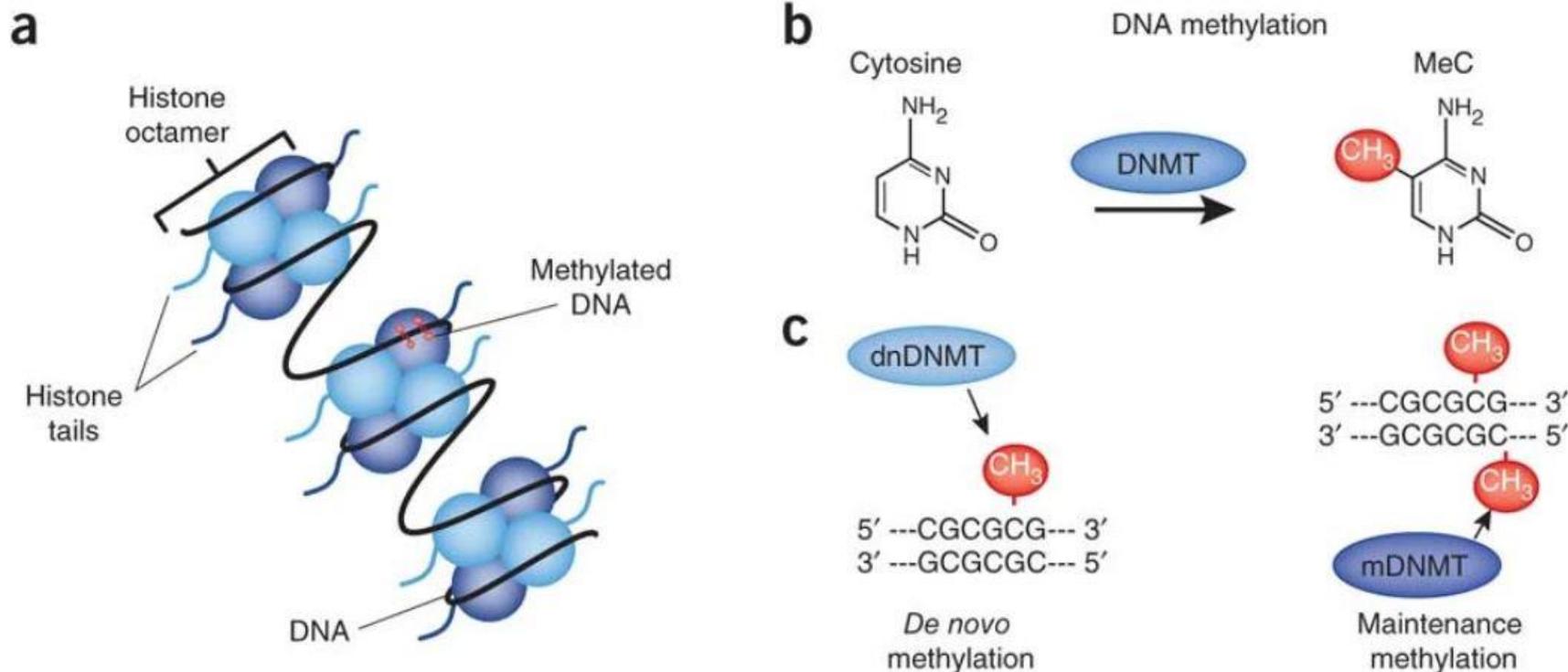
5-Azacidina e analoghi

Dati sperimentali

Modello

Metilazione del DNA – DNMT

La metilazione del DNA avviene sulla citosina, mediante l'aggiunta di un gruppo metilico nella posizione 5 dell'anello pirimidinico per mezzo di una DNMT.



DNMT3A e DNMT3B avviano la metilazione *de novo*, mentre DNMT1 è l'enzima di mantenimento che agisce sul DNA emimetilato a seguito della replicazione

Caso studio II

Basi dell'epigenetica

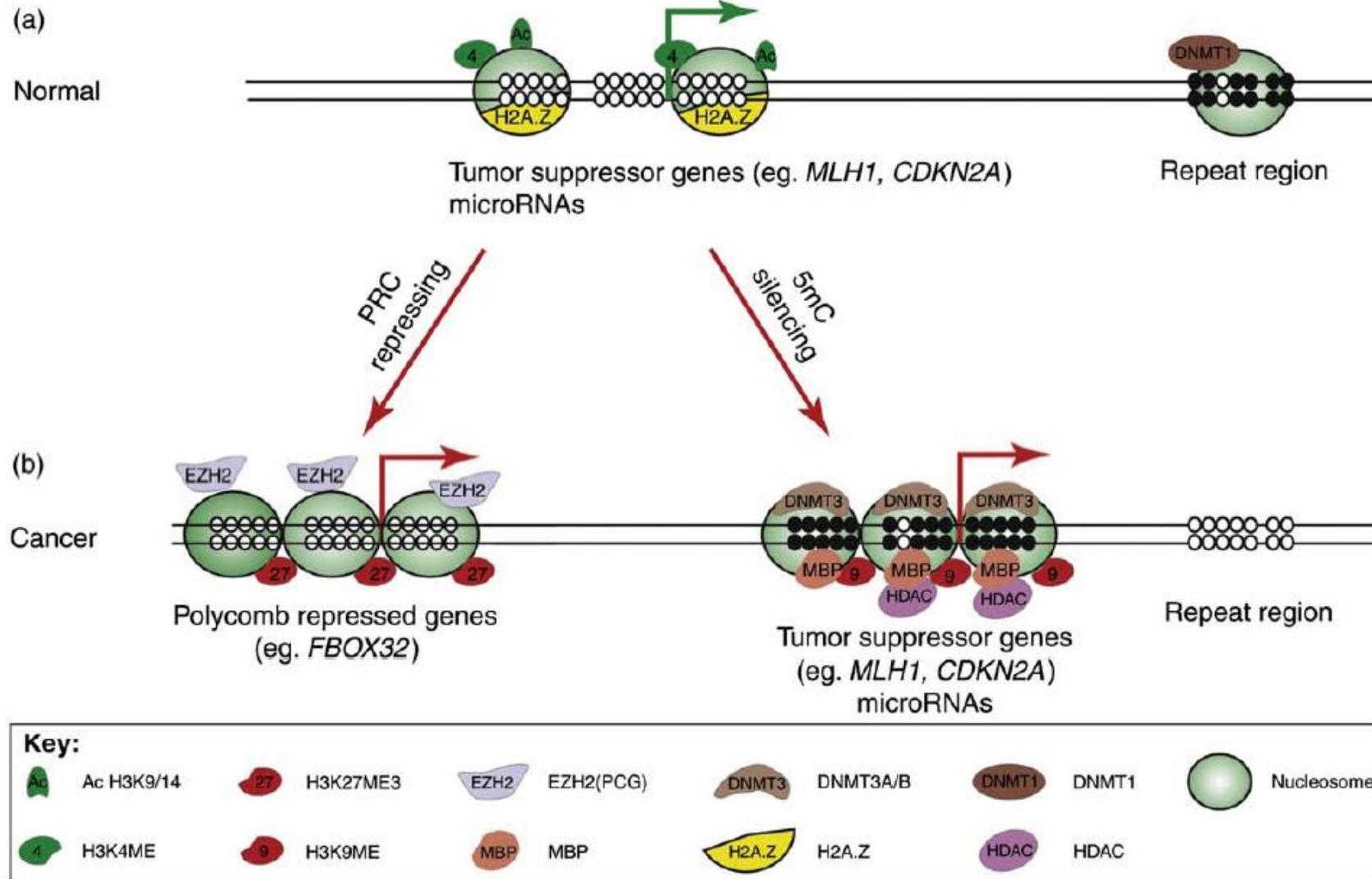
Metilazione del DNA e cancro

5-Azacidina e analoghi

Dati sperimentali

Modello

Alterazioni epigenetiche in cellule tumorali



Caso studio II

Basi dell'epigenetica

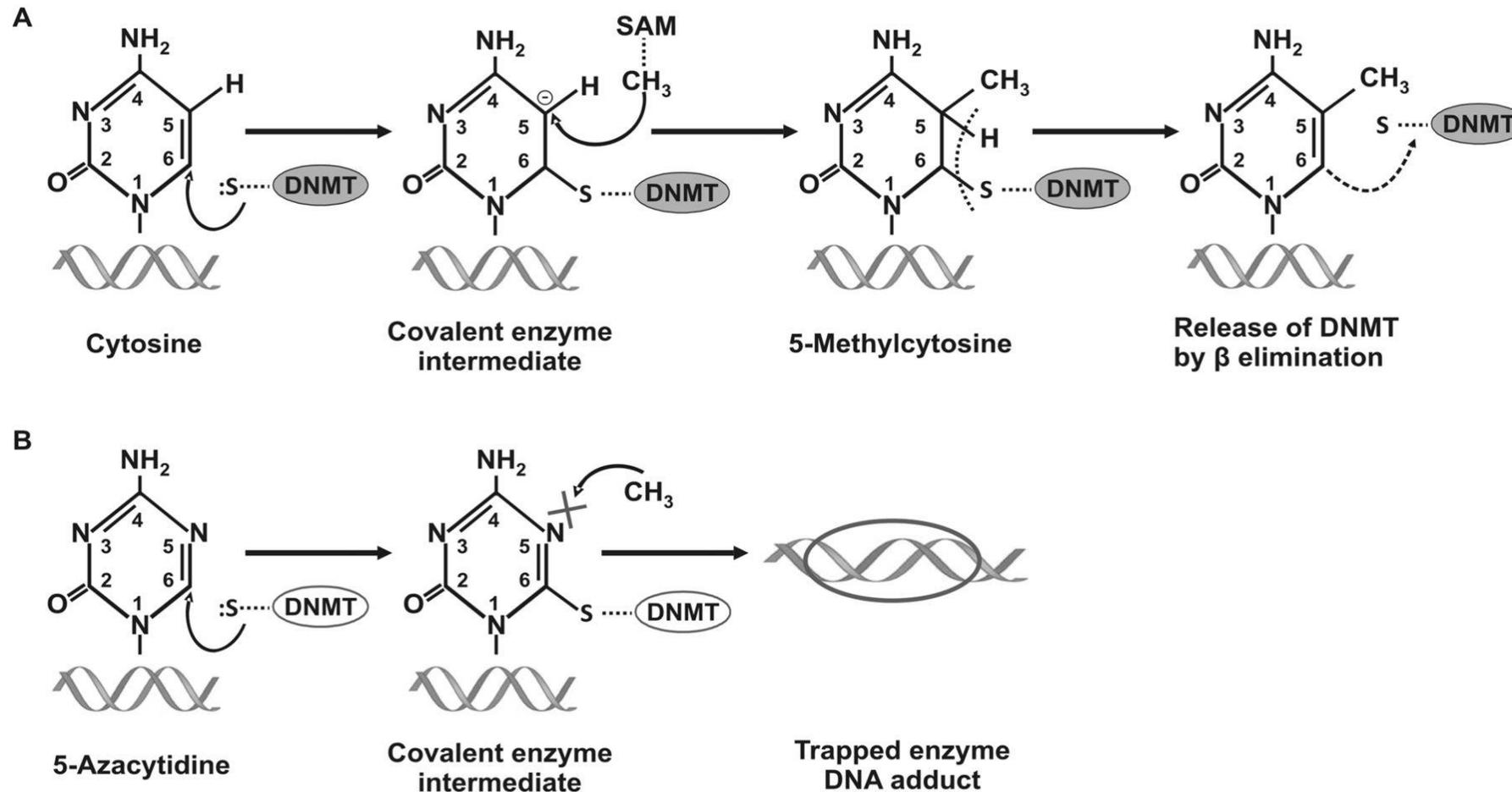
Metilazione del DNA
e cancro

5-Azacitidina
e analoghi

Dati sperimentali

Modello

5-azacytidine – meccanismo d'azione



Caso studio II

Basi dell'epigenetica

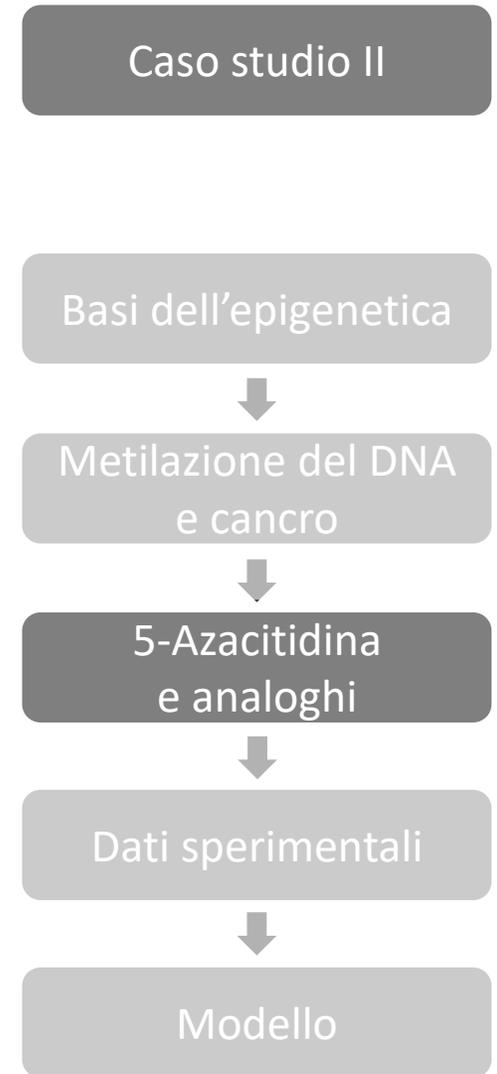
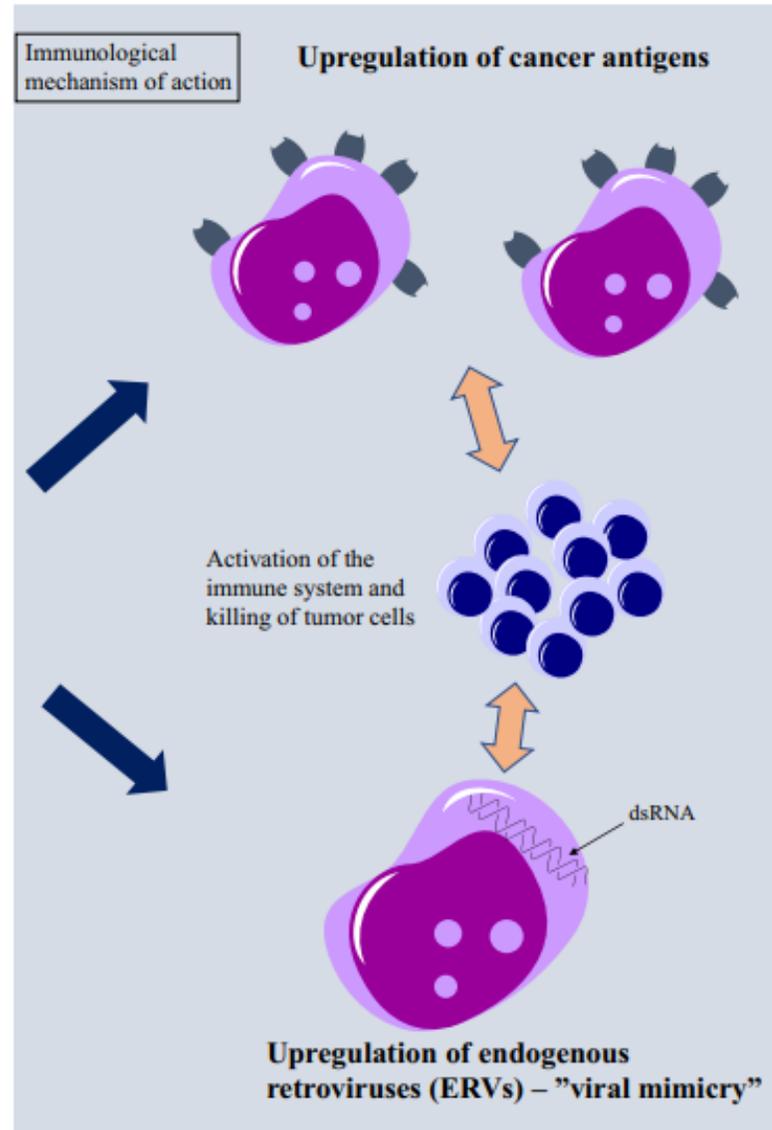
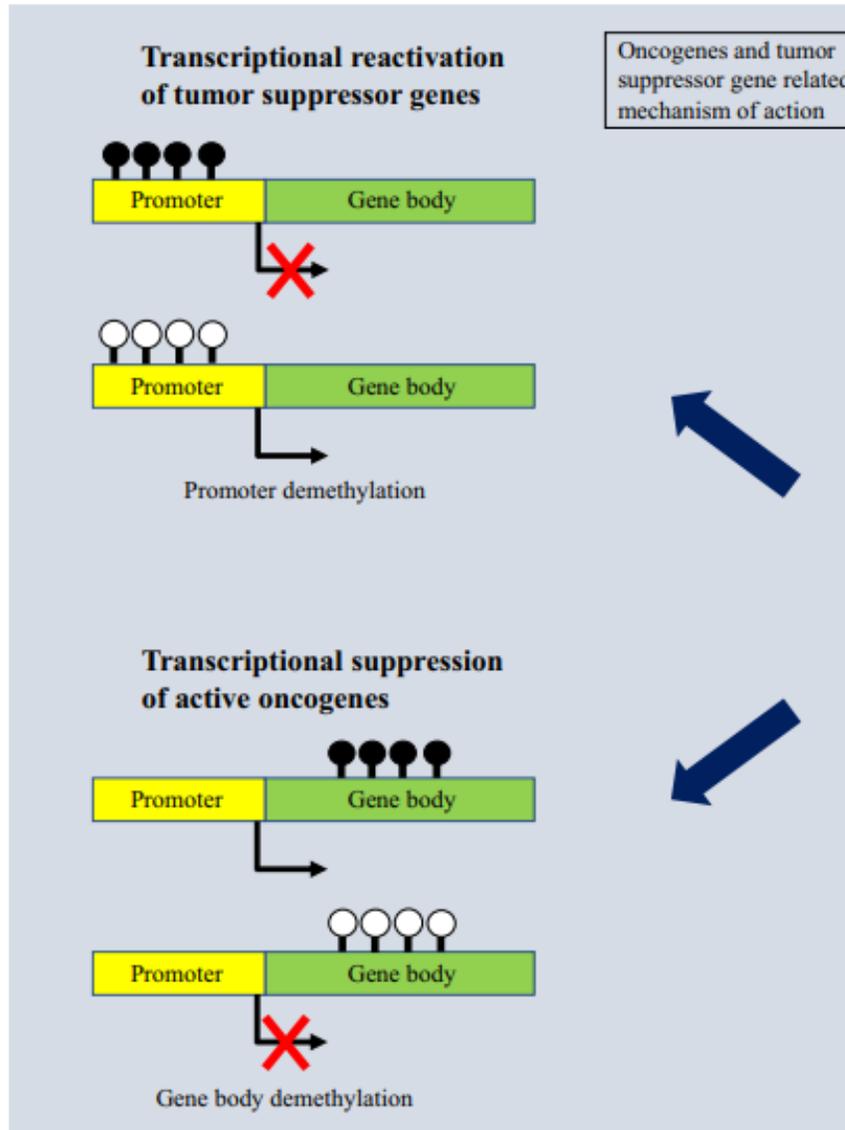
Metilazione del DNA
e cancro

5-Azacytidina
e analoghi

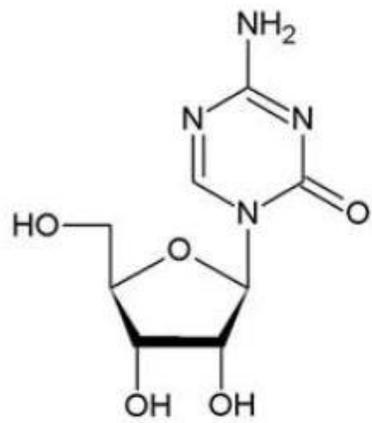
Dati sperimentali

Modello

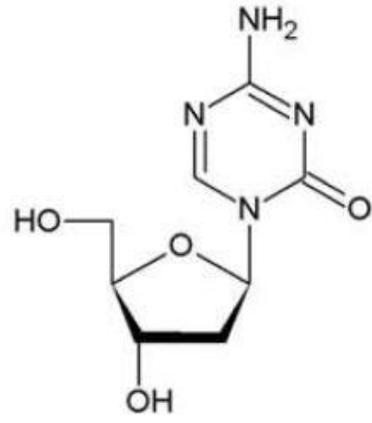
5-azacytidine – applicazioni



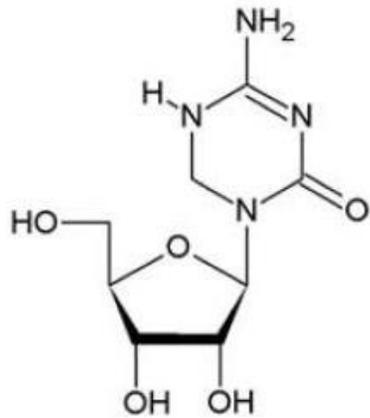
Nucleosidi analoghi



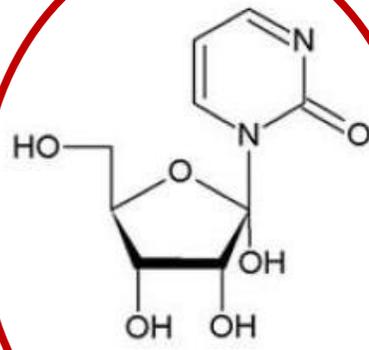
5-Azacytidine



5-Aza-2'-deoxycytidine



5,6-Dihydro-5-azacytidine



Zebularine

Caso studio II

Basi dell'epigenetica



Metilazione del DNA
e cancro



5-Azacitidina
e analoghi



Dati sperimentali



Modello

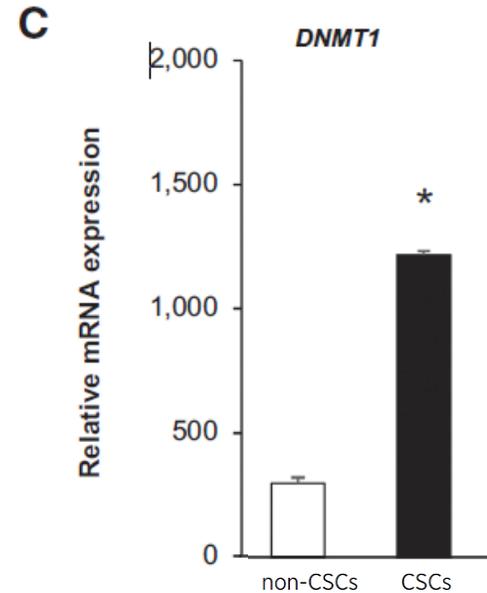
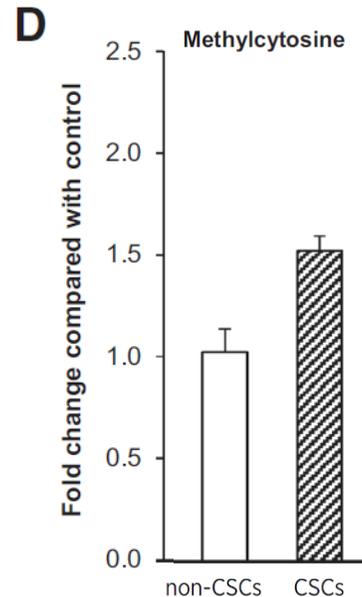
Overview

Tumor and Stem Cell Biology

Cancer Research

DNMT1 Inhibition Reprograms Pancreatic Cancer Stem Cells via Upregulation of the miR-17-92 Cluster

Sladjana Zagorac^{1,2}, Sonia Alcala^{2,3}, Gustavo Fernandez Bayon⁴, Tony Bou Kheir¹, Matthieu Schoenhals¹, Anna González-Neira⁵, Mario Fernandez Fraga⁴, Alexandra Aicher^{1,2}, Christopher Heeschen^{1,2}, and Bruno Sainz, Jr.^{2,3}



Caso studio II

Basi dell'epigenetica



Metilazione del DNA e cancro



5-Azacitidina e analoghi



Dati sperimentali



Modello

Effetti del trattamento con Zebularina

Caso studio II

Basi dell'epigenetica



Metilazione del DNA e cancro



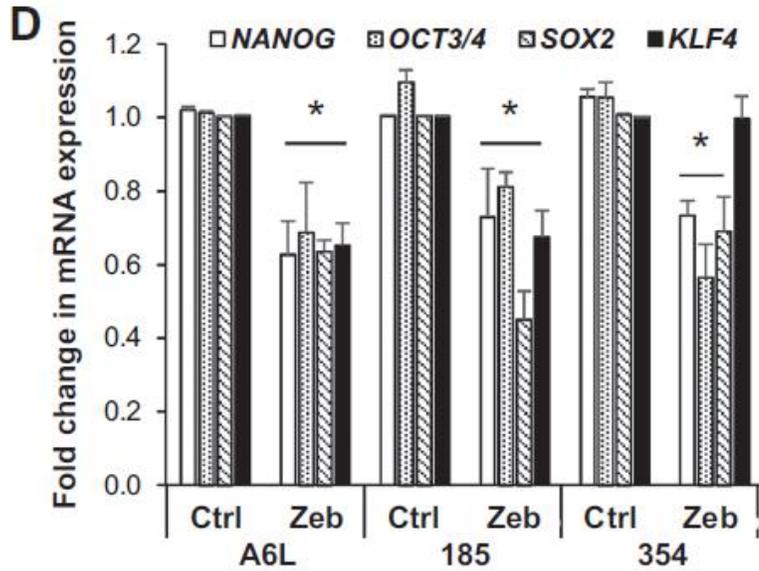
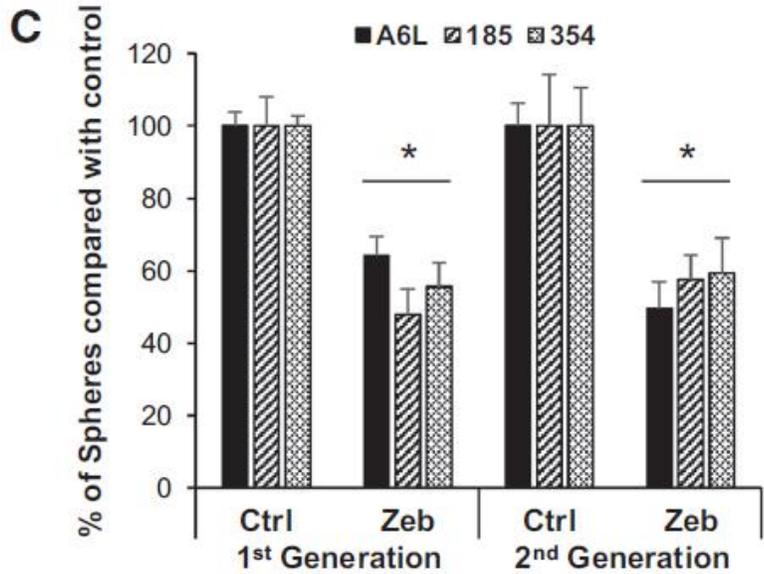
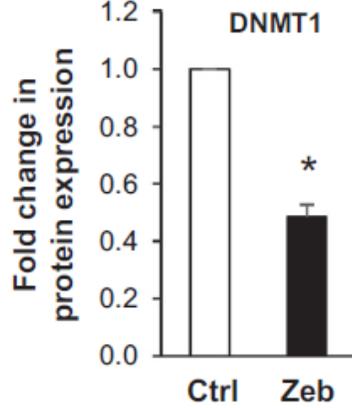
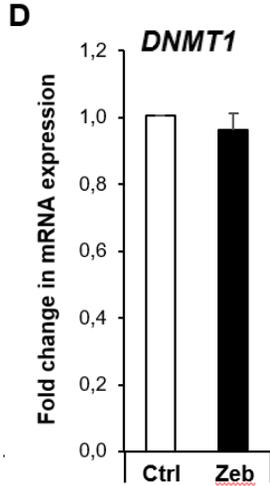
5-Azacitidina e analoghi



Dati sperimentali

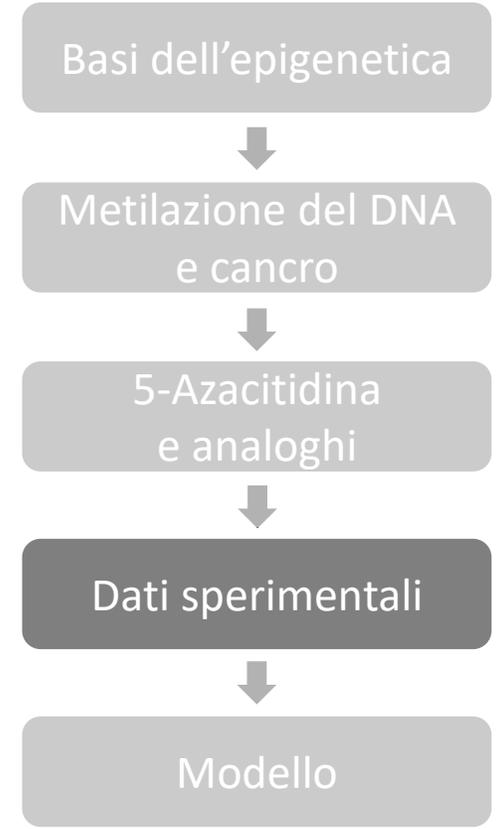
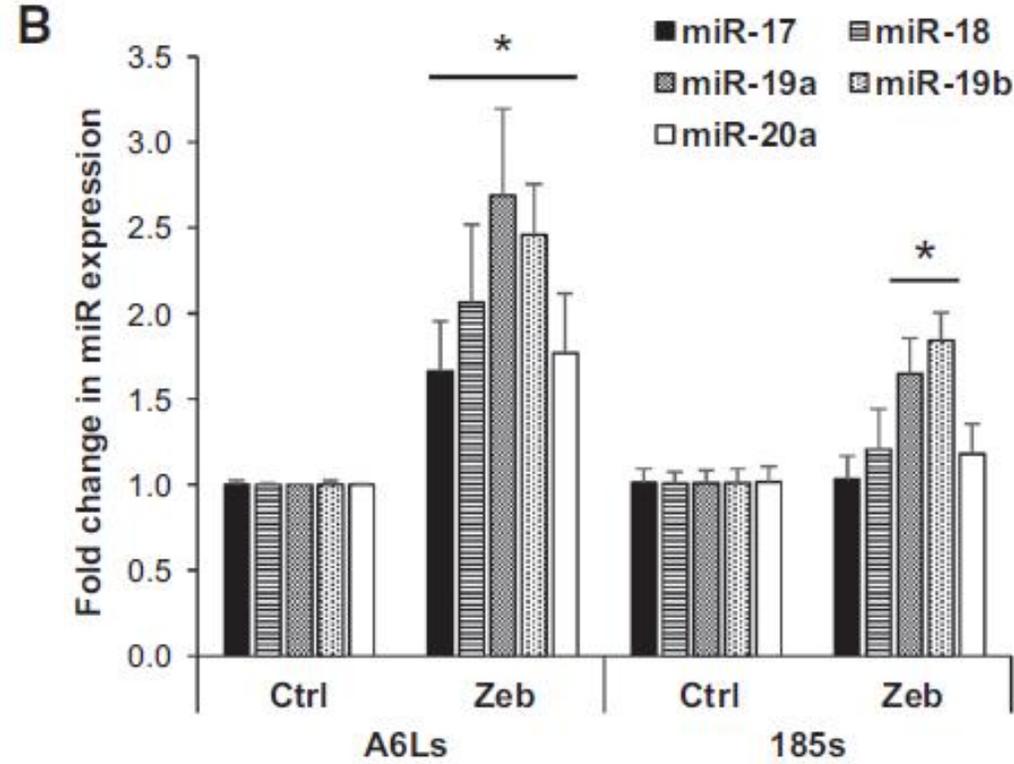
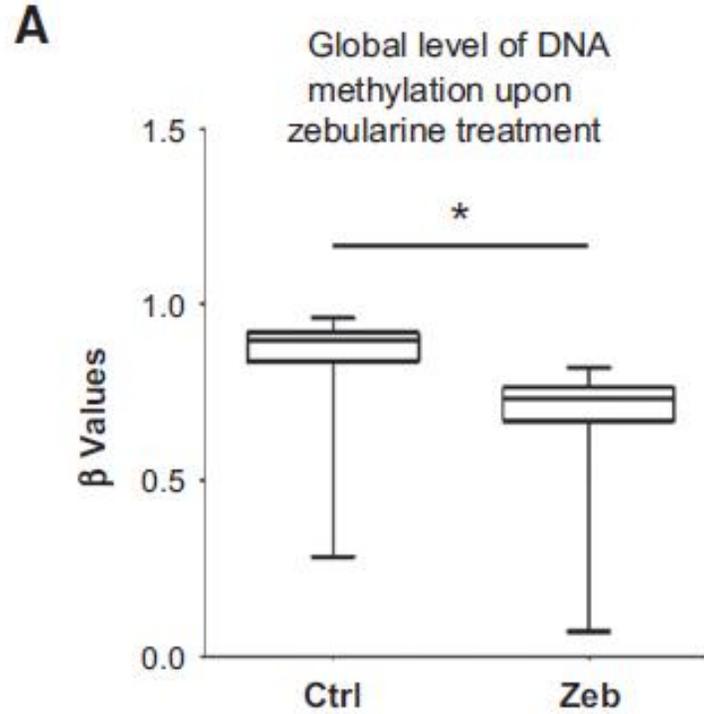


Modello



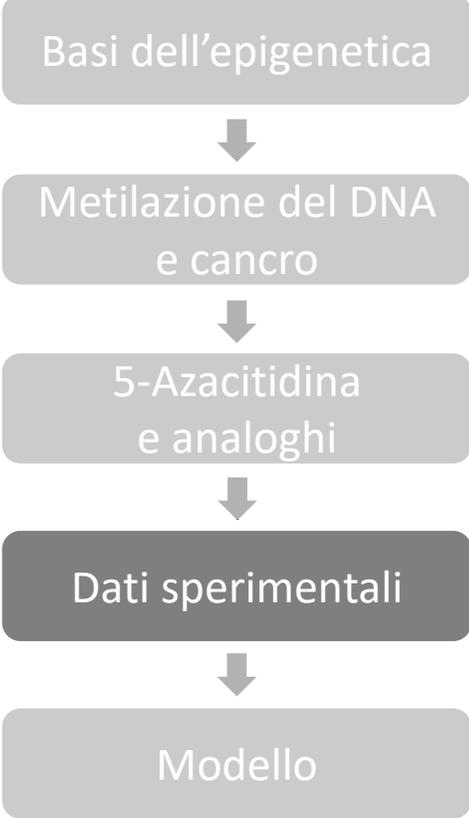
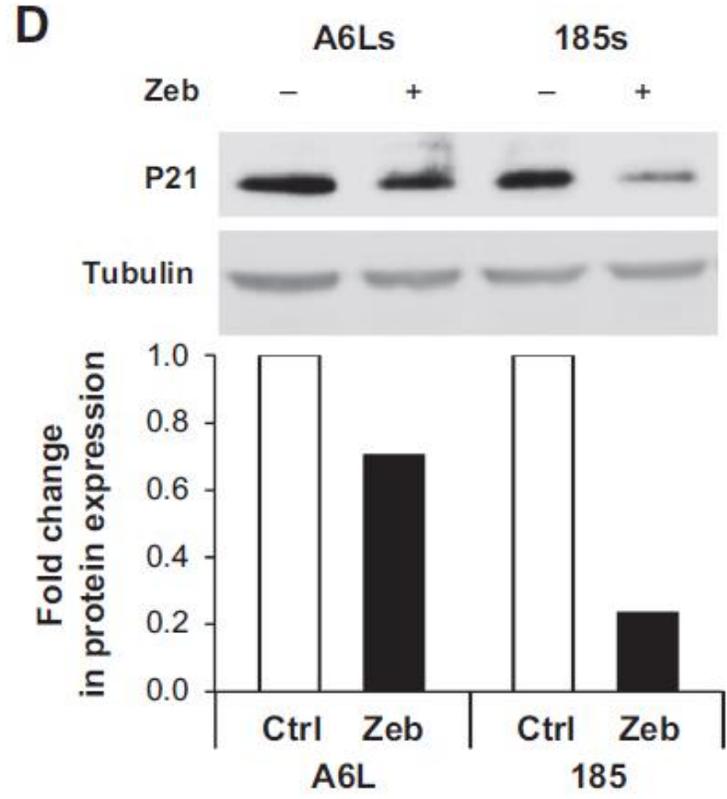
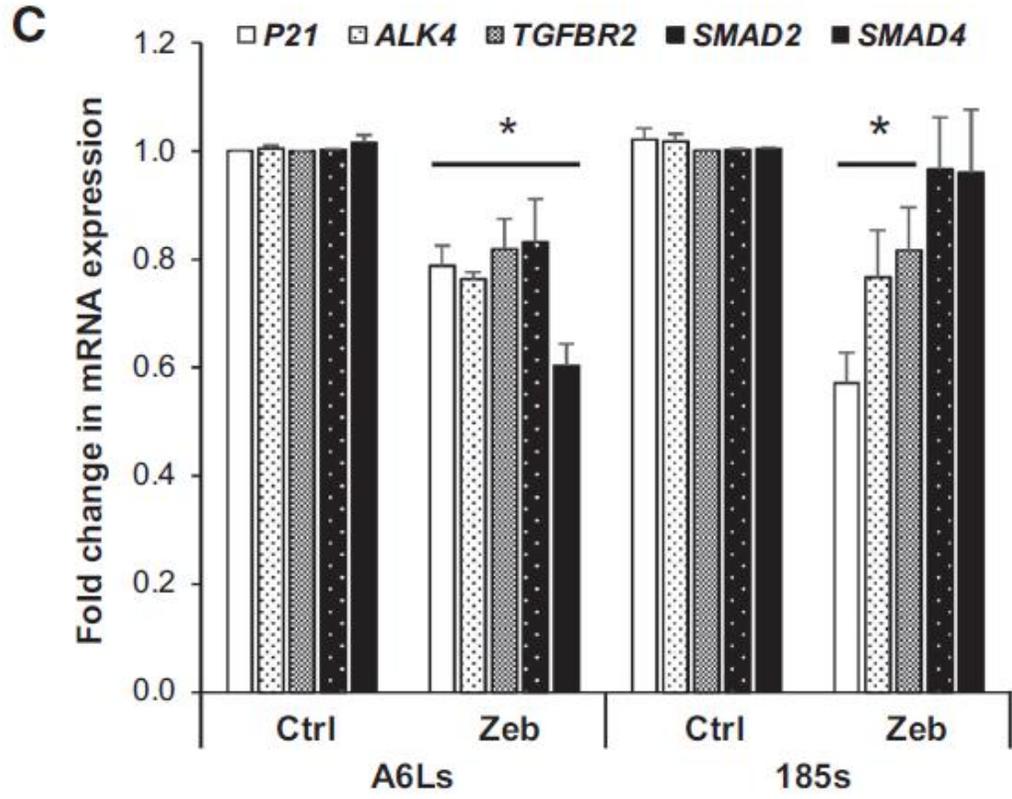
Attivazione del cluster miR-17-92

Caso studio II



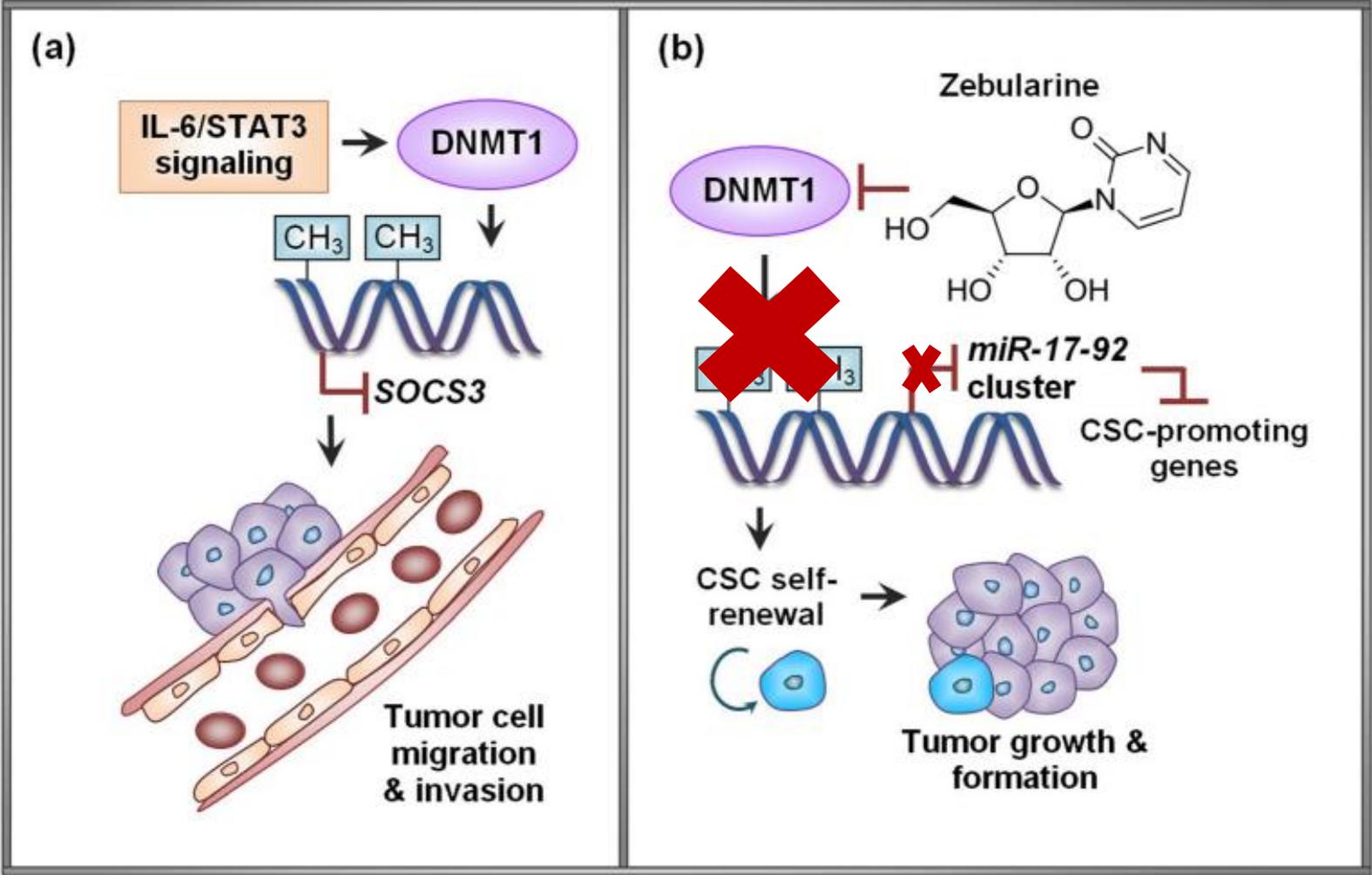
Target di miR-17-92

Caso studio II



Meccanismo di azione della Zebularina

Caso studio II



Basi dell'epigenetica

Metilazione del DNA e cancro

5-Azacitidina e analoghi

Dati sperimentali

Modello

Casi studio III – plant biology research

RDS 3434 and RDS 3061 repurposing for epigenetic studies in plant

Caso studio III

HMT: Polycomb
repressive complex II



RDS3434: Dati
sperimentali



HAT: p300/CBP
in plants

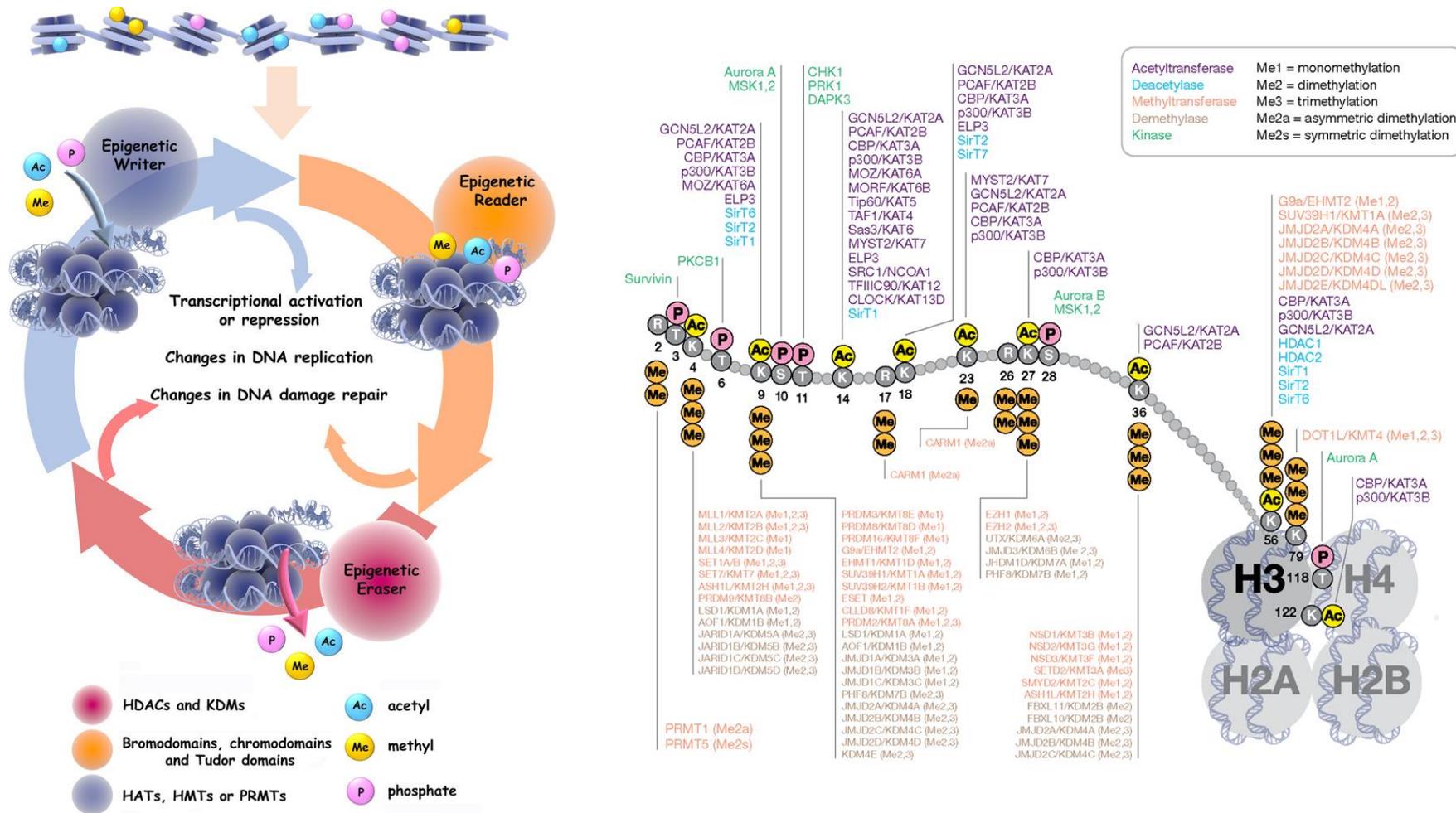


RDS3061: Dati
sperimentali

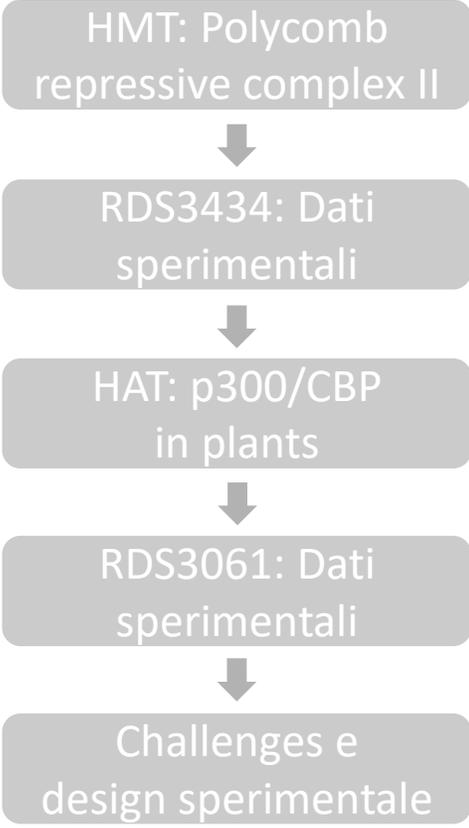


Challenges e
design sperimentale

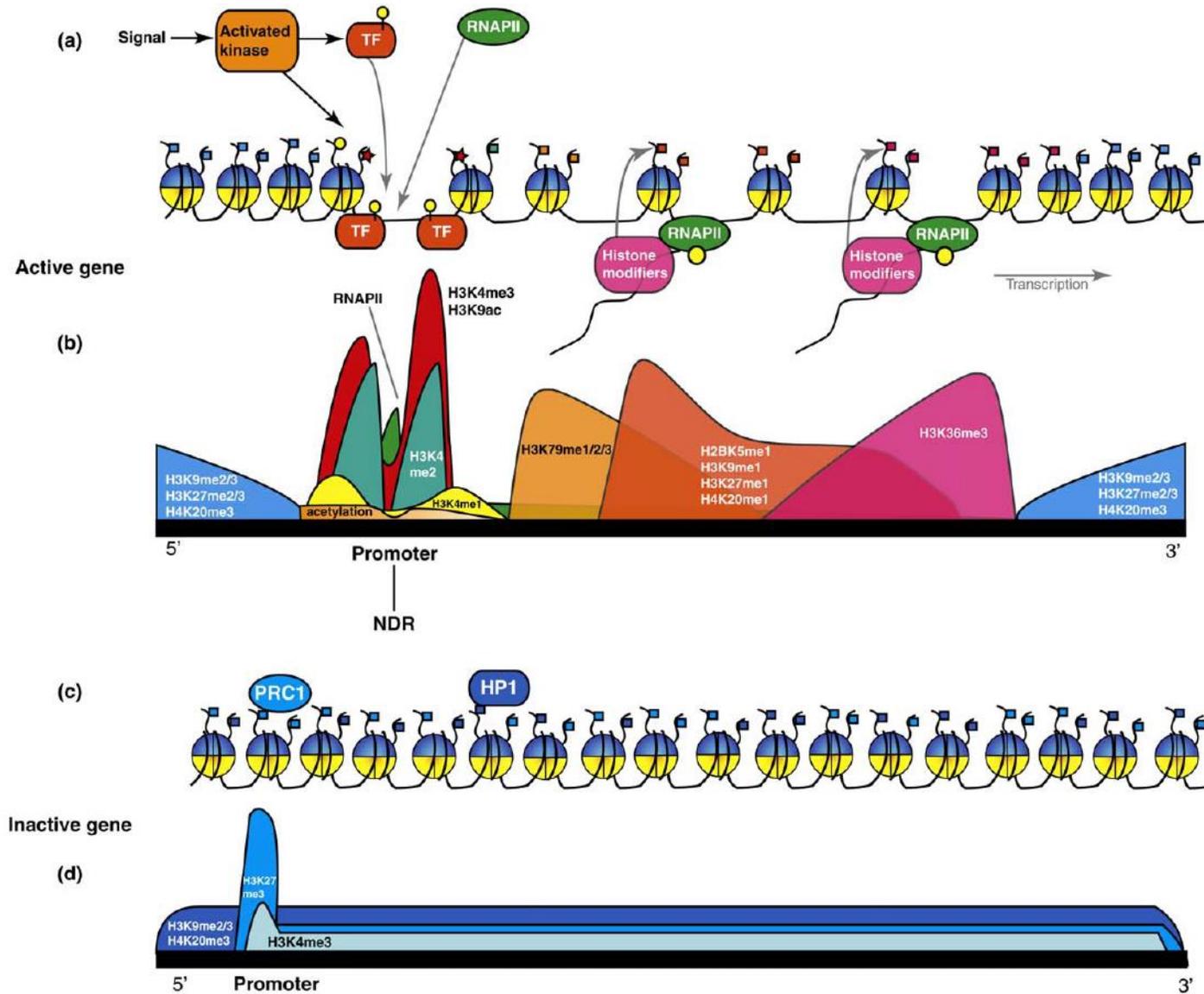
Un altro meccanismo epigenetico: PTM istoniche



Caso studio III



Il codice istonico



Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



HAT: p300/CBP in plants



RDS3061: Dati sperimentali



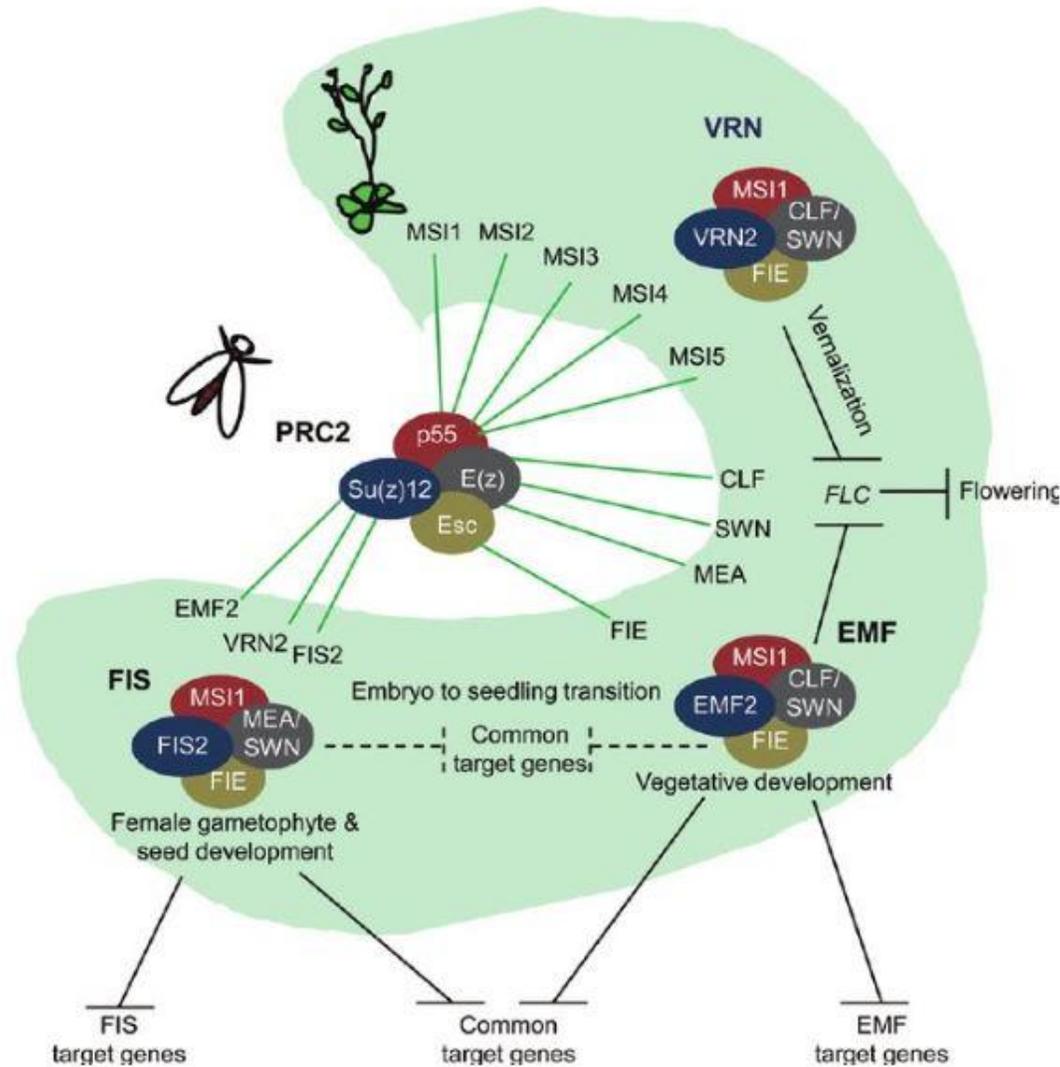
Challenges e design sperimentale

Polycomb Repressive Complex 2

Le proteine del gruppo Polycomb (PcG) costituiscono un sistema di repressione trascrizionale conservato in molti eucarioti, come *Drosophila*, mammiferi e piante.

PRC2 è composto da quattro subunità principali, che in *Drosophila* sono codificate da geni a copia singola.

In base alle combinazioni delle subunità, in *Arabidopsis* sono stati descritti tre complessi PRC2: EMF, VRN e FIS, che hanno funzioni distinte sebbene condividano alcuni geni bersaglio.



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

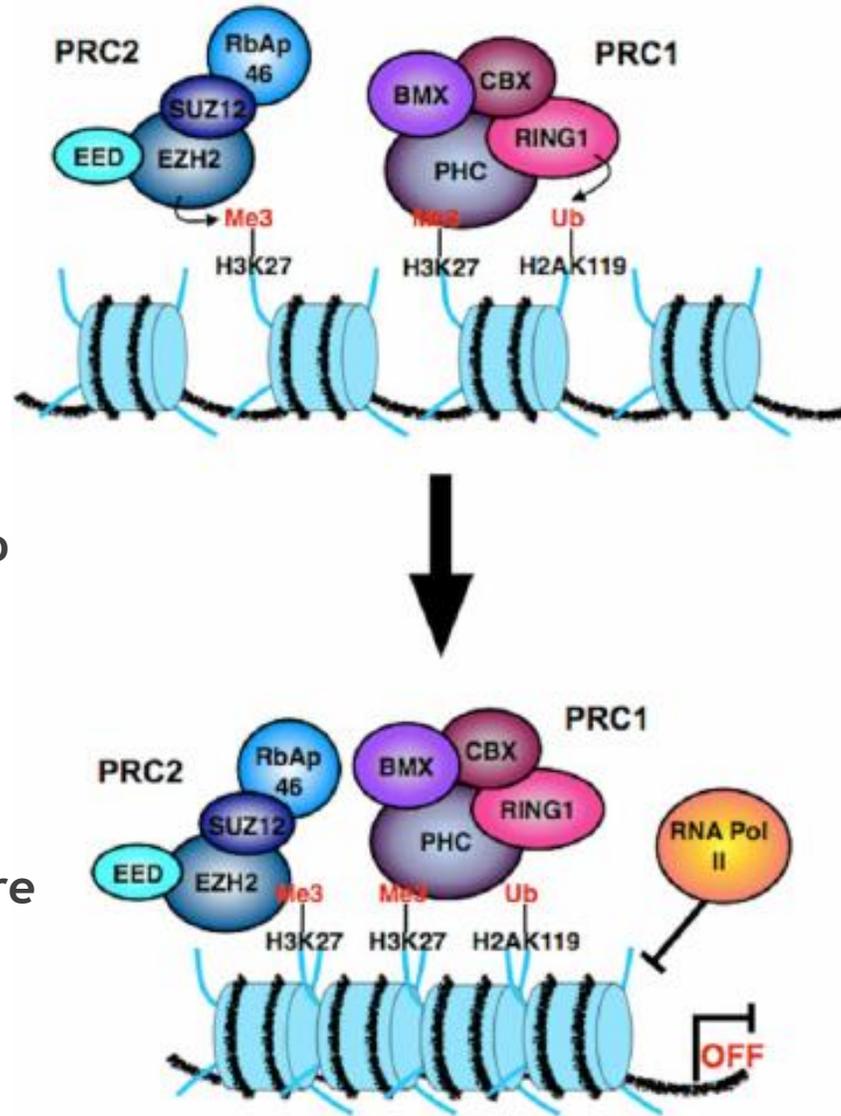
Challenges e design sperimentale

Metilazione istonica: marker di repressione di PRC1 e PRC2

PRC1 catalizza il trasferimento di un'ubiquitina sulla lisina 119 dell'istone H2A (H2Aub), mentre PRC2 trimetila la lisina 27 dell'istone H3 (H3K27me3).

Il marker H3K27me3 viene riconosciuto da PRC1 che stabilisce una repressione trascrizionale stabile mediante la monoubiquitinazione H2Aub.

I complessi possono comunque funzionare indipendentemente svolgendo funzioni diverse, a seconda delle proteine interagenti e dei geni bersaglio



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

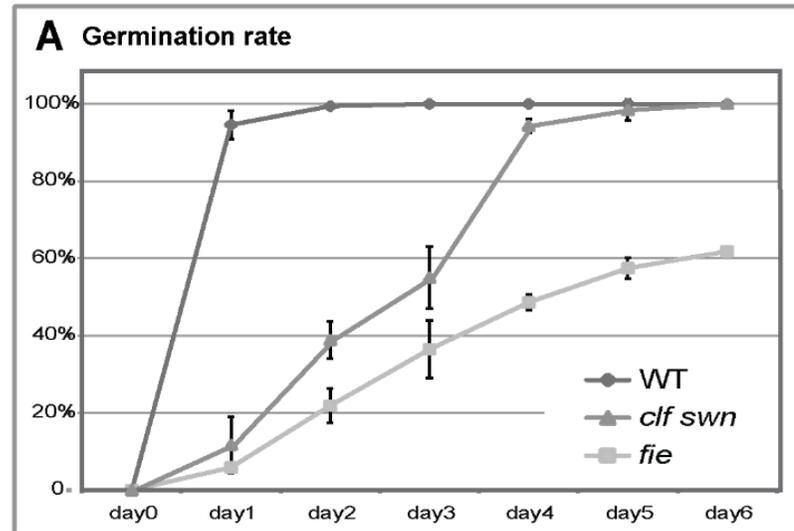
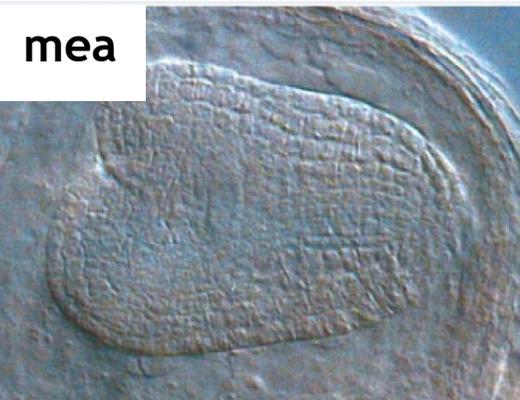
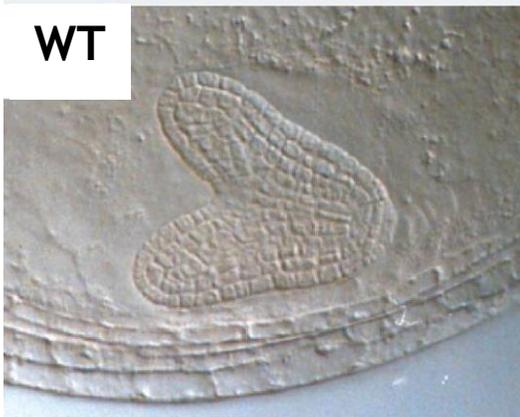
HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Mutanti PRC2

In pianta, mutazioni di PRC2 risultano embrioletali...



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

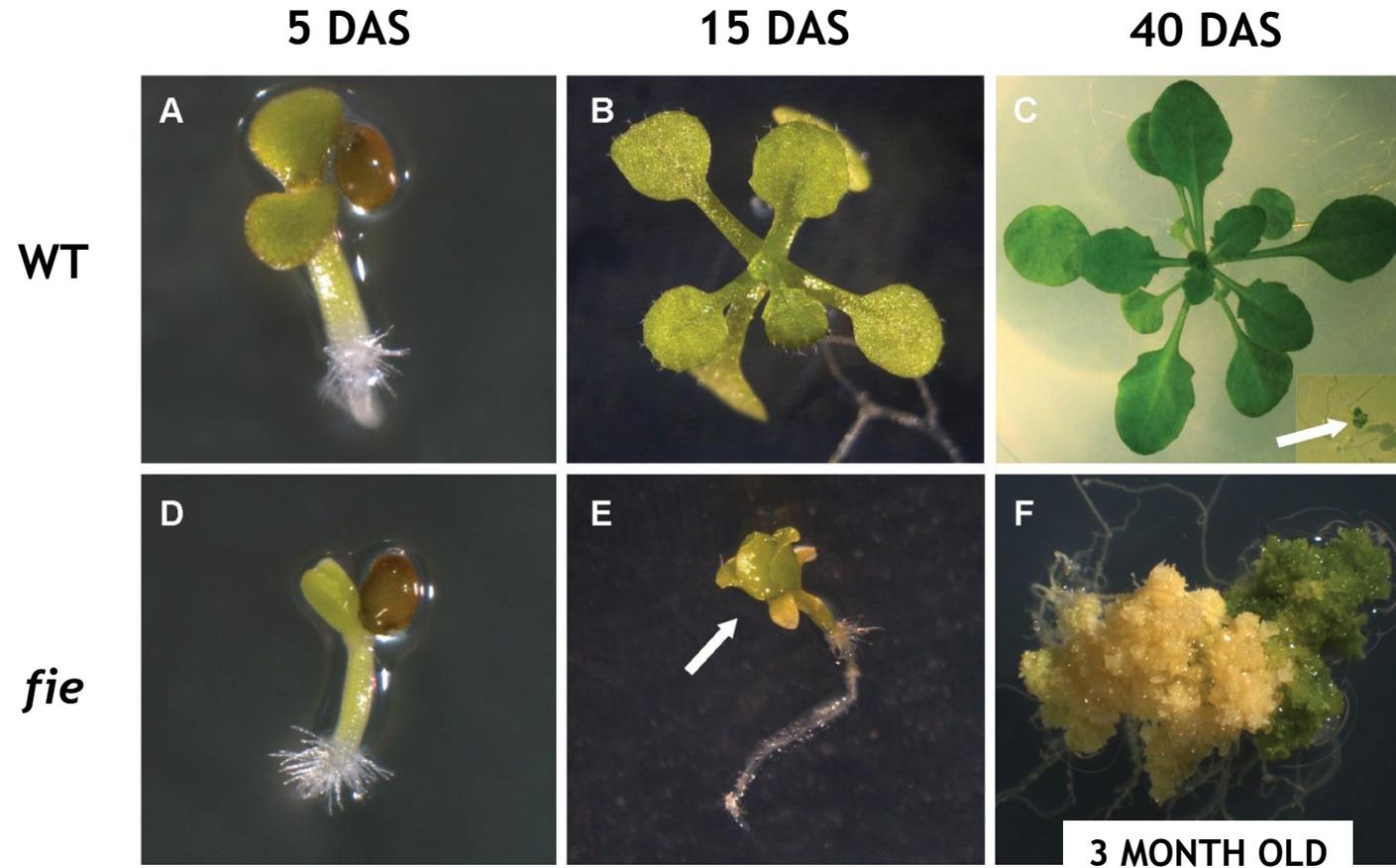
HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Mutanti PRC2

...oppure causa di sviluppo anomalo



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

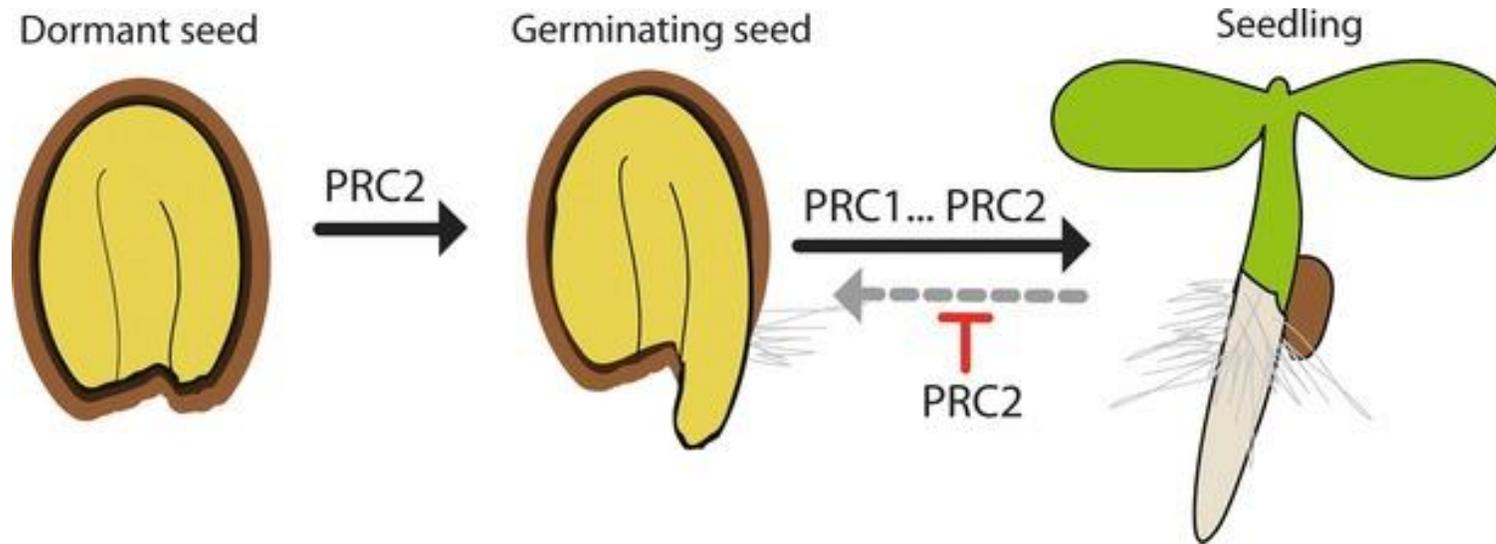
RDS3061: Dati sperimentali

Challenges e design sperimentale

Ciò ha ostacolato lo studio della funzione PRC2 negli anni

PRC2 nella transizione dormienza-germinazione

La fine della dormienza richiede l'attività di PRC2 per reprimerne i principali regolatori.



PRC2 è inoltre necessario per la repressione stabile dei geni embrionali. Disfunzioni di PRC2 consentono l'inizio dello sviluppo della plantula ma provocano una successiva reversione al programma di maturazione embrionale

Caso studio III

HMT: Polycomb repressive complex II

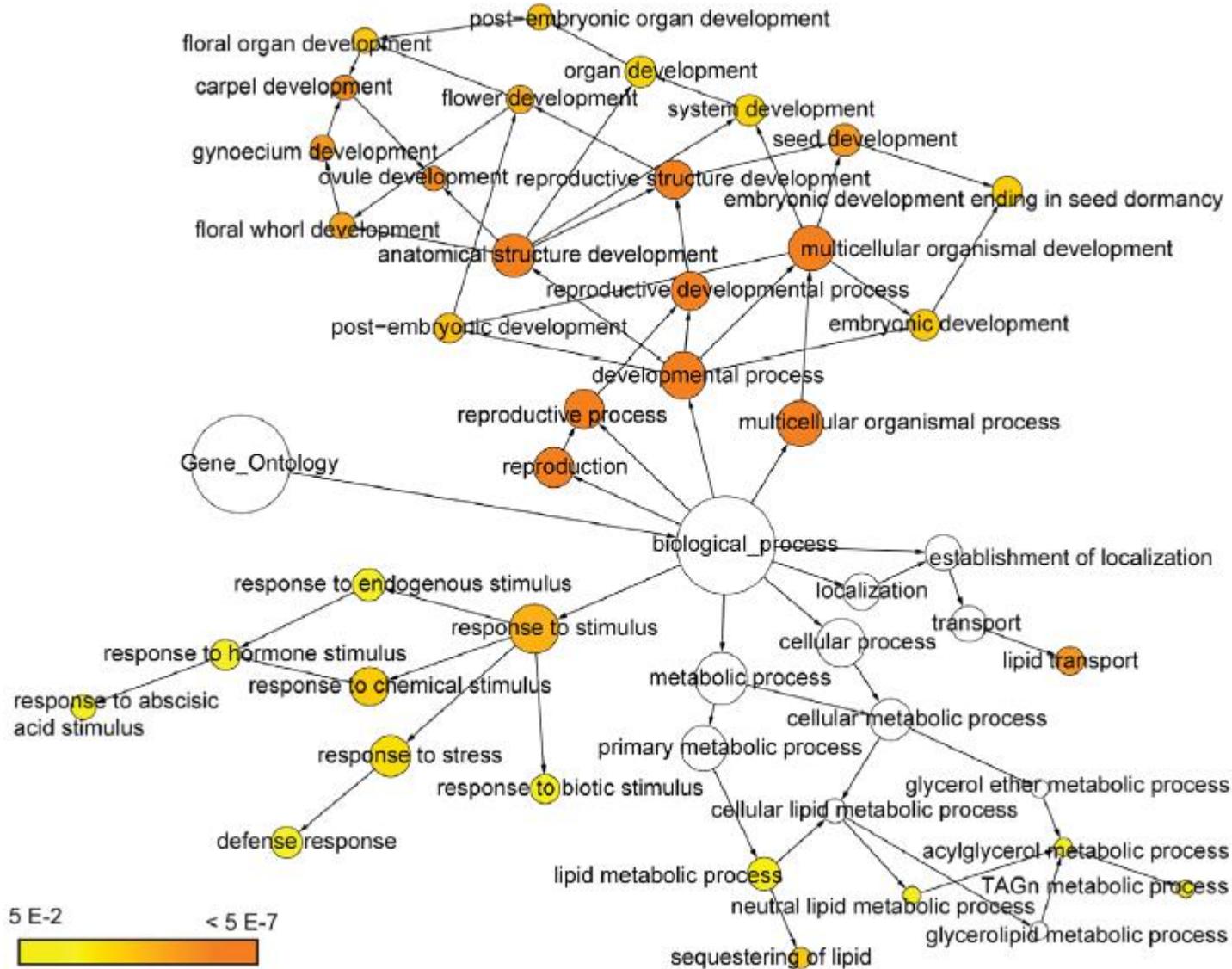
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Gene ontology



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Problematiche di studio

PLoS GENETICS

Polycomb Repressive Complex 2 Controls the Embryo-to-Seedling Phase Transition

Daniel Bouyer¹, Francois Roudier², Maren Heese¹, Ellen D. Andersen³, Nowack^{5,6}, Justin Goodrich⁷, Jean-Pierre Renou⁴, Paul E. Grini³, Vincen

¹Department of Molecular Mechanisms of Phenotypic Plasticity, Institut de Biologie Moléculaire des Plantes du
²Institut de Biologie de l'Ecole Normale Supérieure, CNRS UMR 8197-INSERM U 1024, Paris, France, ³Department
Norway, ⁴Department of Plant Genomics Research, CNRS/INRA, Evry, France, ⁵Department of Plant Systems
Biotechnology and Genetics, Ghent University, Ghent, Belgium, ⁷Institute of Molecular Plant Science, University of I

In the past few years, much progress has been made in the understanding of the *modus operandi* of PRC2. However, a major obstacle in studying the function of chromatin regulators is their essential role in early development as for instance mutants in ESC in *Drosophila* and its murine ortholog EED are embryonic lethal [13–15]. Similarly, PRC2 function is crucial already for endosperm formation in flowering plants by controlling the parent-of-origin dependent activity of a number of genes in the endosperm (imprinting). PRC2 function is maternal gametophytically required and loss of the maternal PRC2 function releases target genes from their repression leading to endosperm overproliferation and ultimately to seed abortion [16–19]. This requirement for endosperm formation has also precluded so far an analysis of PRC2 action during later stages of seed development and it also remained an open question whether PRC2 function is required for initial body plan formation in flowering plants during which an embryo with shoot, root, and one (Monocotyledons) or two (Dicotyledons) cotyledons is formed. In contrast to animals, the two stem cell populations established in embryogenesis, i.e. the root and shoot meristem, will produce the body of the adult plant and it has been shown previously that PRC2 is involved in postembryonic shoot meristem function [20].

Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



HAT: p300/CBP in plants



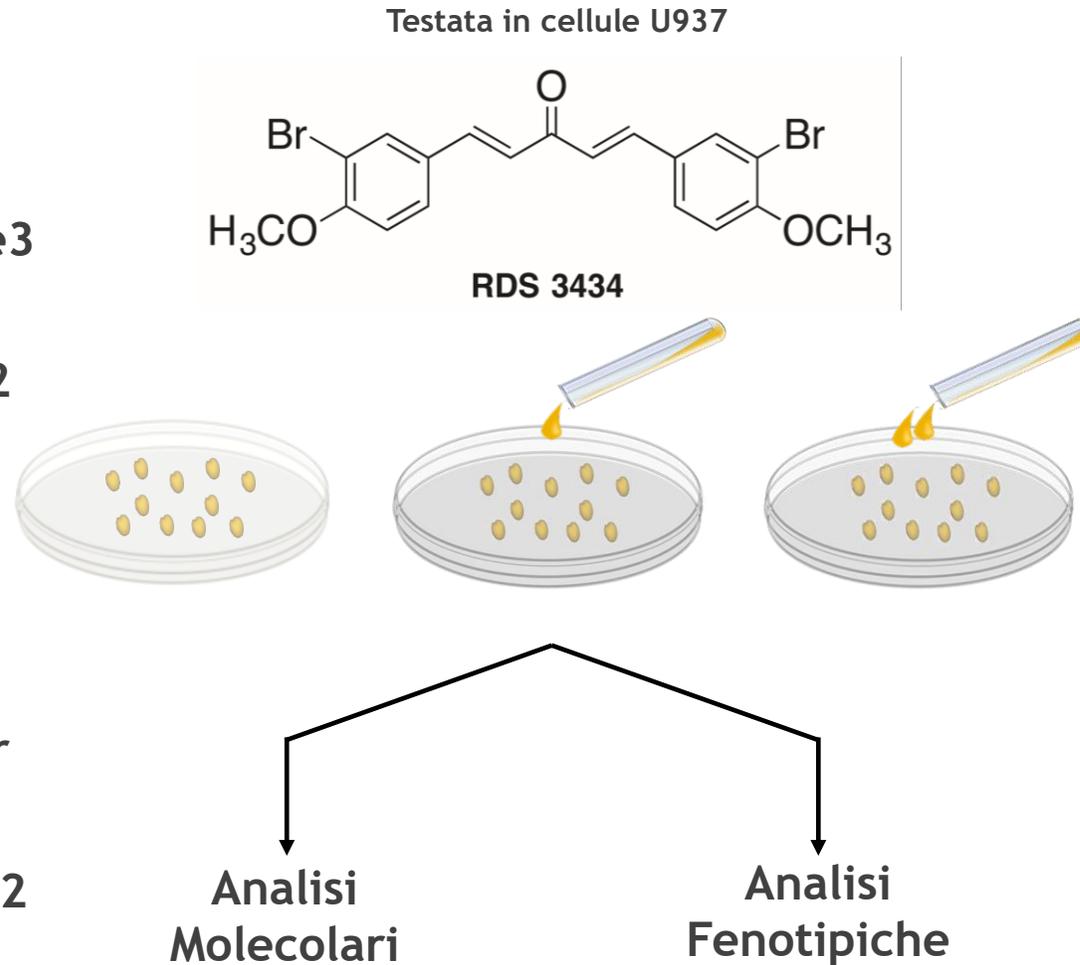
RDS3061: Dati sperimentali



Challenges e design sperimentale

RDS3434: *small-molecule* per studiare PRC2 in *Arabidopsis thaliana*

- ▶ Dimostrare che il trattamento con RDS3434 determina una diminuzione del marker H3K27me3
- ▶ Dimostrare che RDS3434 è selettivamente attivo contro PRC2
- ▶ Dimostrare che il trattamento con RDS3434 determina un aumento specifico e dose-dipendente dei livelli di espressione dei geni bersaglio di PRC2, con una conseguente riduzione del marker H3K27me3 sui loci corrispondenti
- ▶ Dimostrare che l'inibizione di PRC2 influisca sullo sviluppo di semi e plantule



Caso studio III

HMT: Polycomb repressive complex II

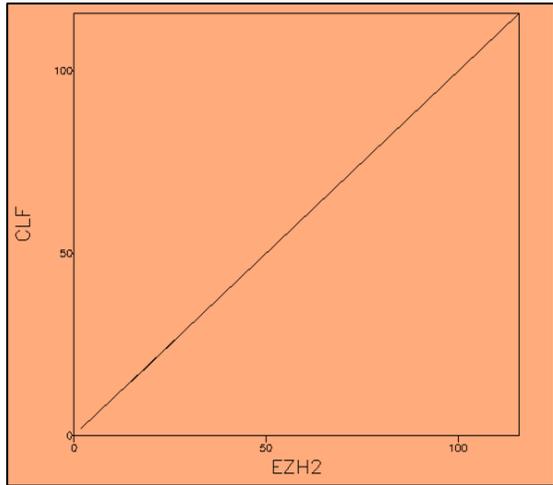
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

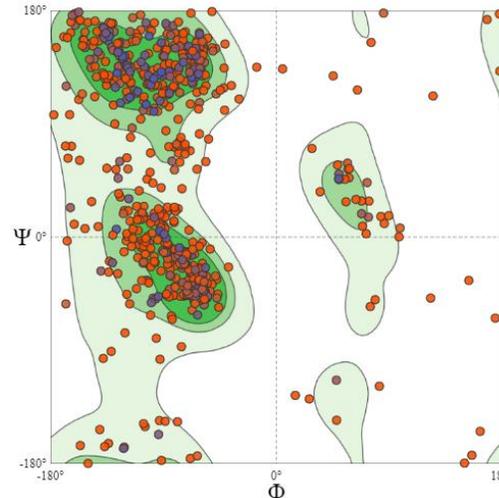
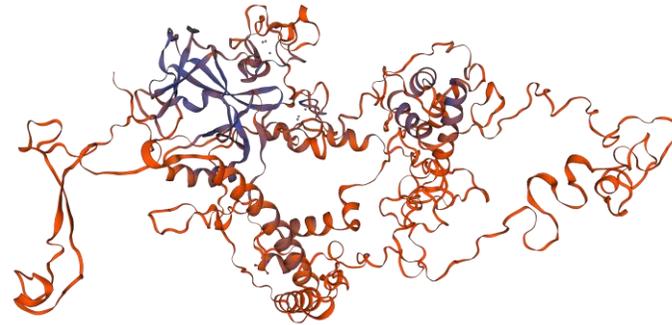
Challenges e design sperimentale

Elevata percentuale di identità del dominio catalitico EZH2 tra uomo e Arabidopsis



```
#-----  
#  
# Aligned_sequences: 2  
# 1: 752-867  
# 2: 612-727  
# Matrix: EBLOSUM62  
# Gap_penalty: 10.0  
# Extend_penalty: 0.5  
#  
# Length: 116  
# Identity: 75/116 (64.7%)  
# Similarity: 95/116 (81.9%)  
# Gaps: 0/116 ( 0.0%)  
# Score: 423.0  
#  
#-----
```

<https://www.ebi.ac.uk/Tools>



<https://swissmodel.expasy.org/>

Caso studio III

HMT: Polycomb repressive complex II

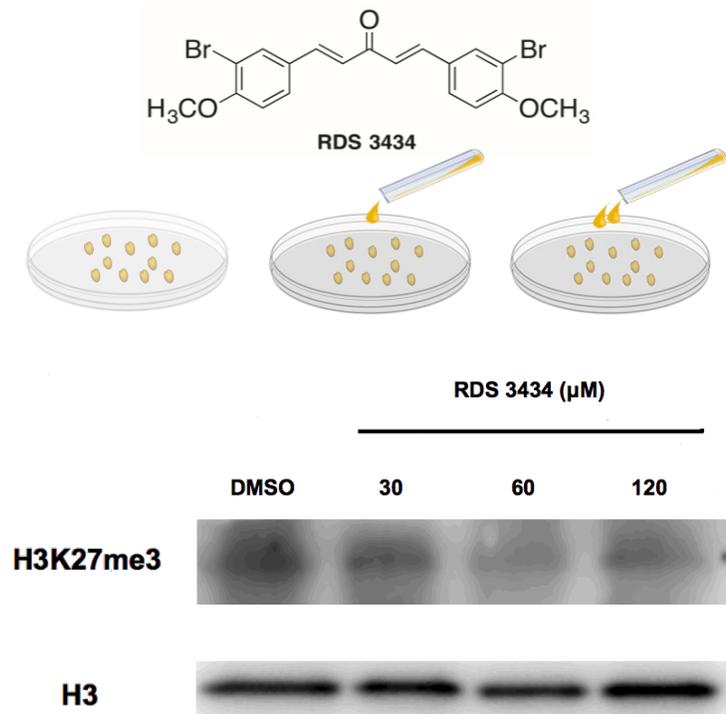
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

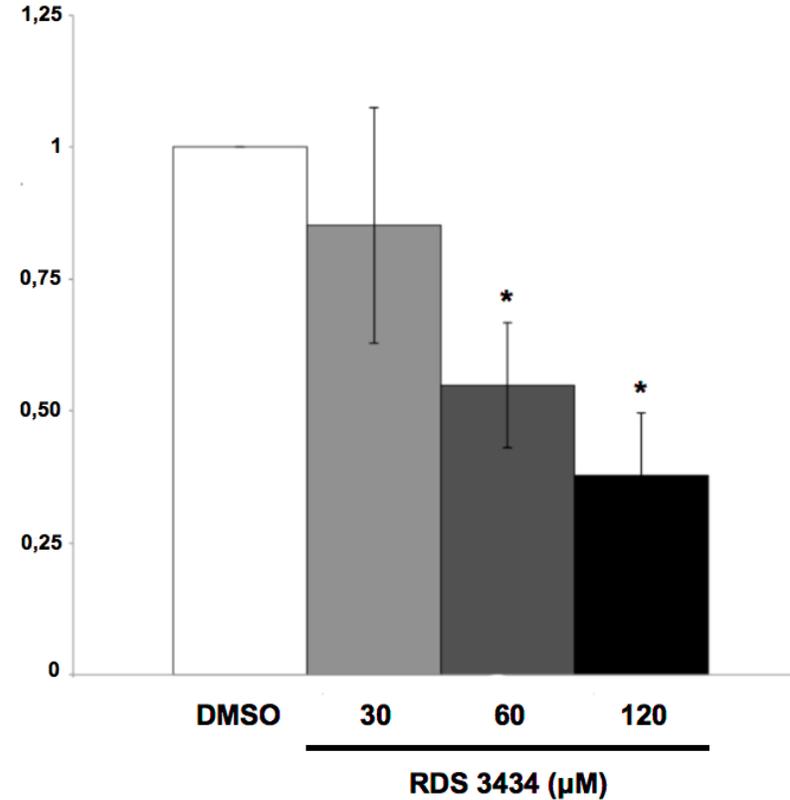
RDS3061: Dati sperimentali

Challenges e design sperimentale

Il trattamento con RDS 3434 determina una diminuzione dose-dipendente di H3K27me3



Relative H3K27me3 Protein Level



Caso studio III

HMT: Polycomb repressive complex II

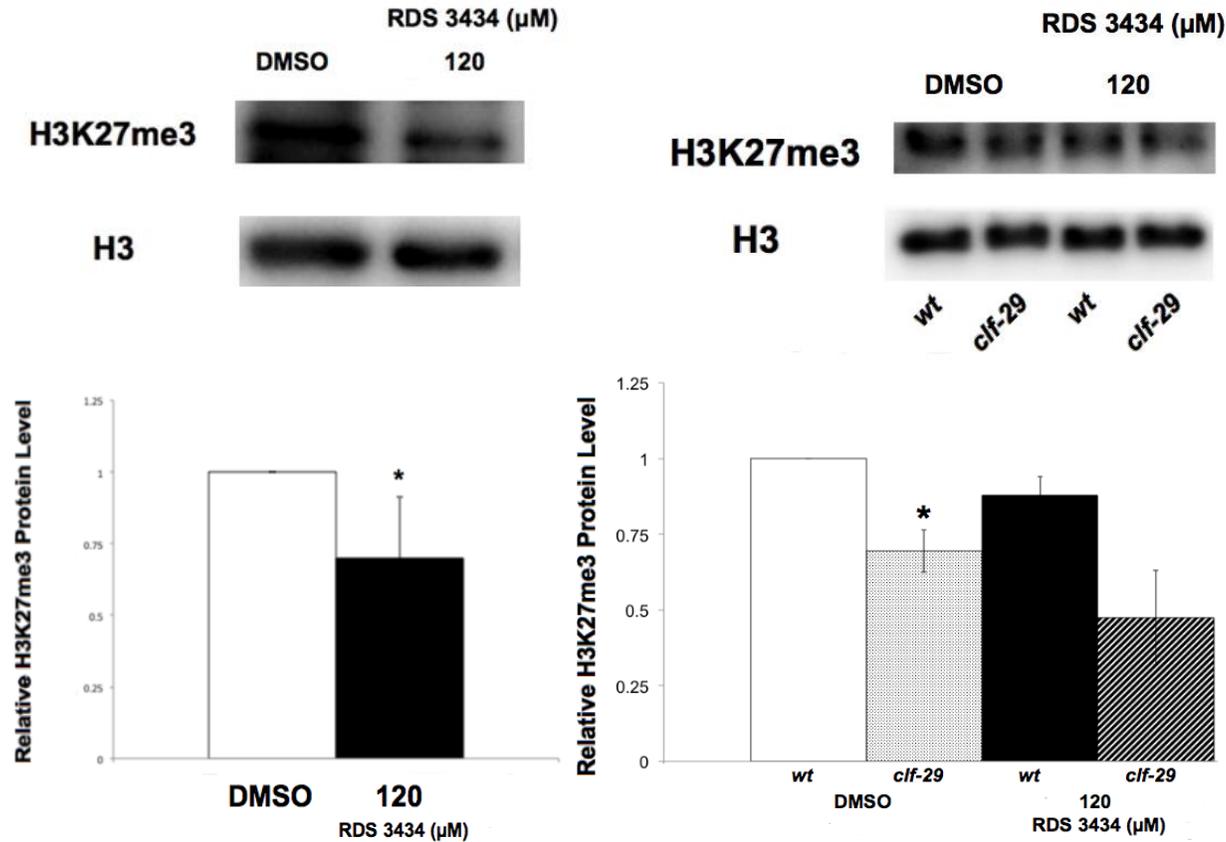
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Nel mutante *clf-29*, i livelli di H3K27me3 sono ulteriormente ridotti dal trattamento con RDS 3434



Caso studio III

HMT: Polycomb repressive complex II

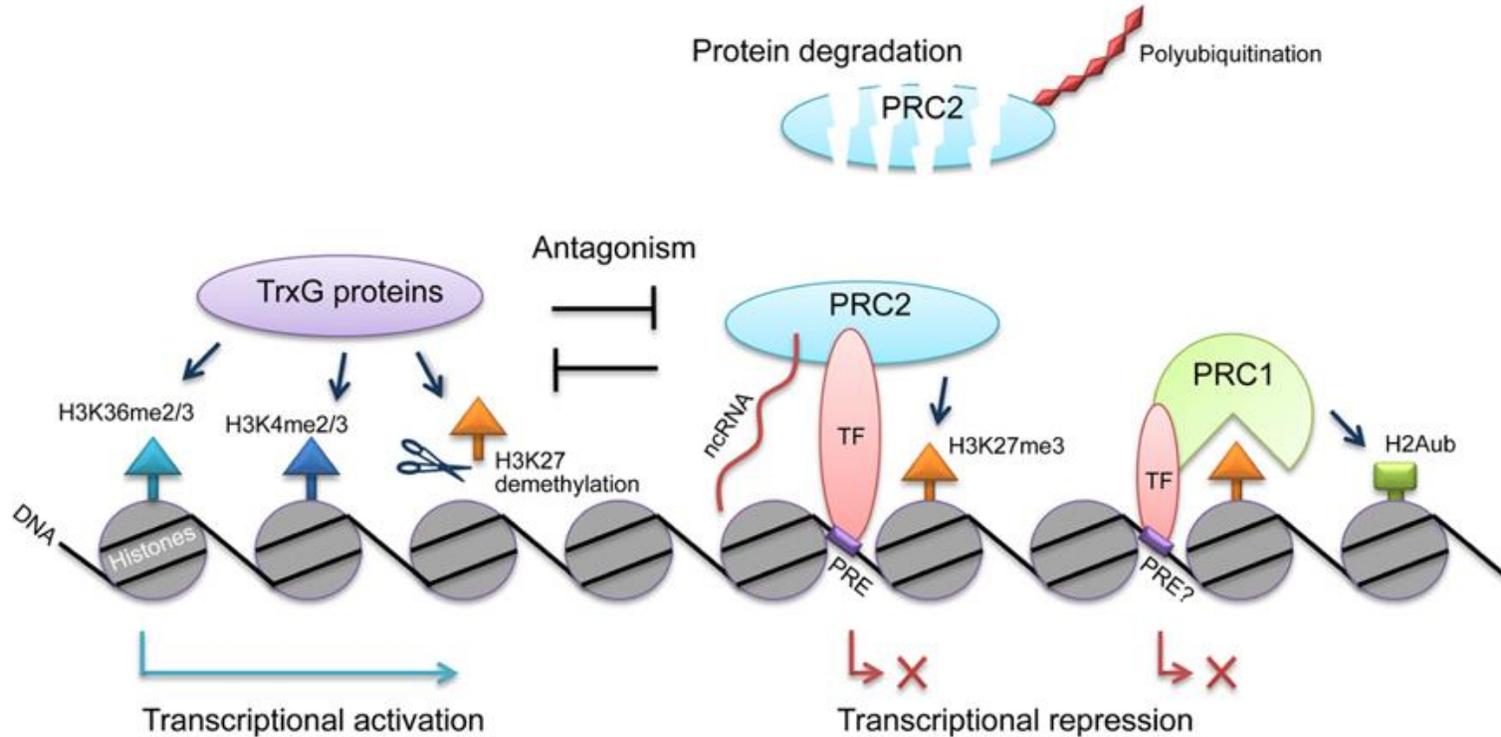
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

L'attività repressiva del marker epigenetico H3K27me3 è contrastata da H3K4me3



La trimetilazione della Lisina 4 dell'istone H3 è associata a geni trascritti attivamente ed è depositata dai complessi proteici Trihorax (trxG)

Caso studio III

HMT: Polycomb repressive complex II

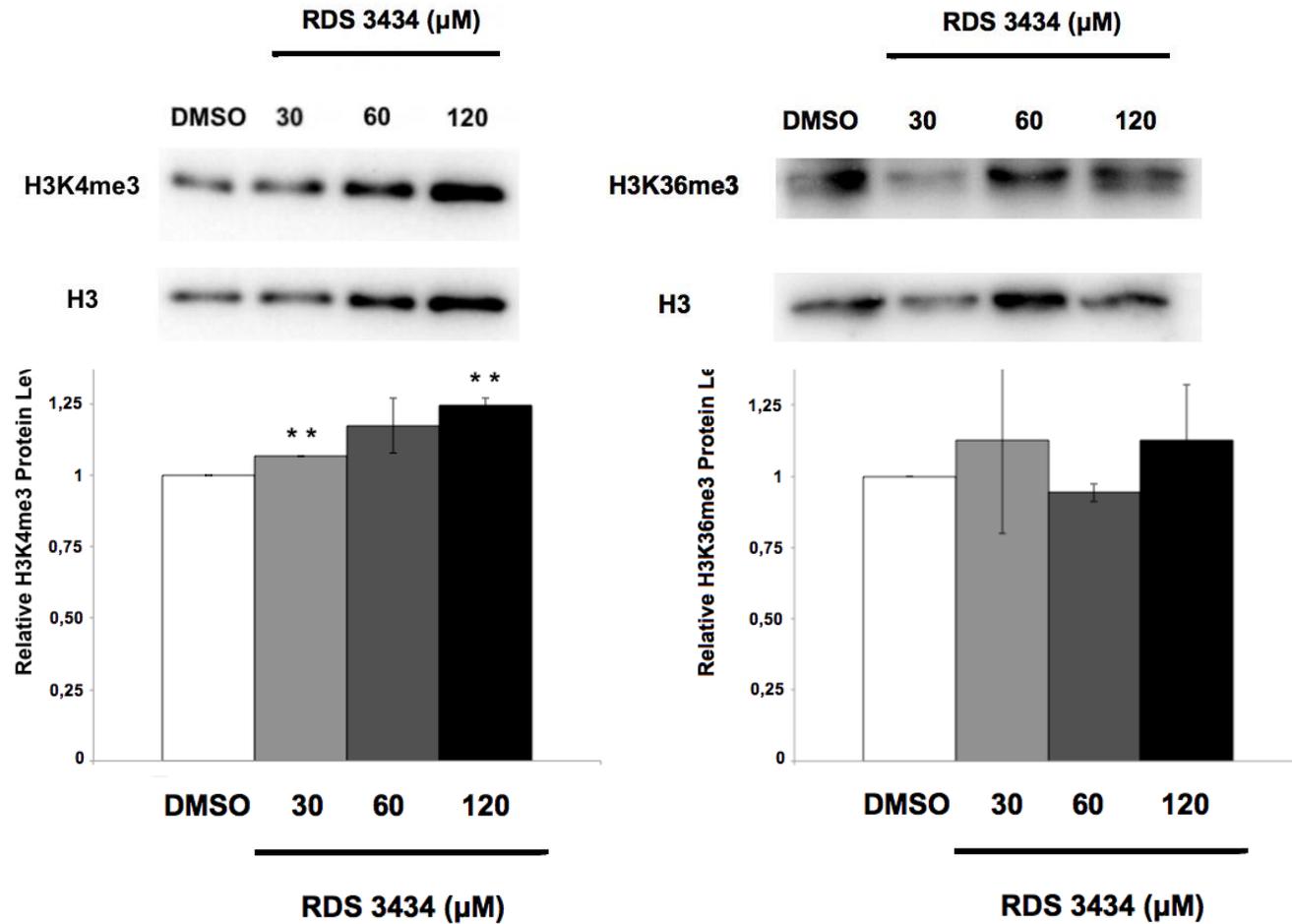
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

RDS 3434 è selettivamente attivo contro PRC2



Caso studio III

HMT: Polycomb repressive complex II

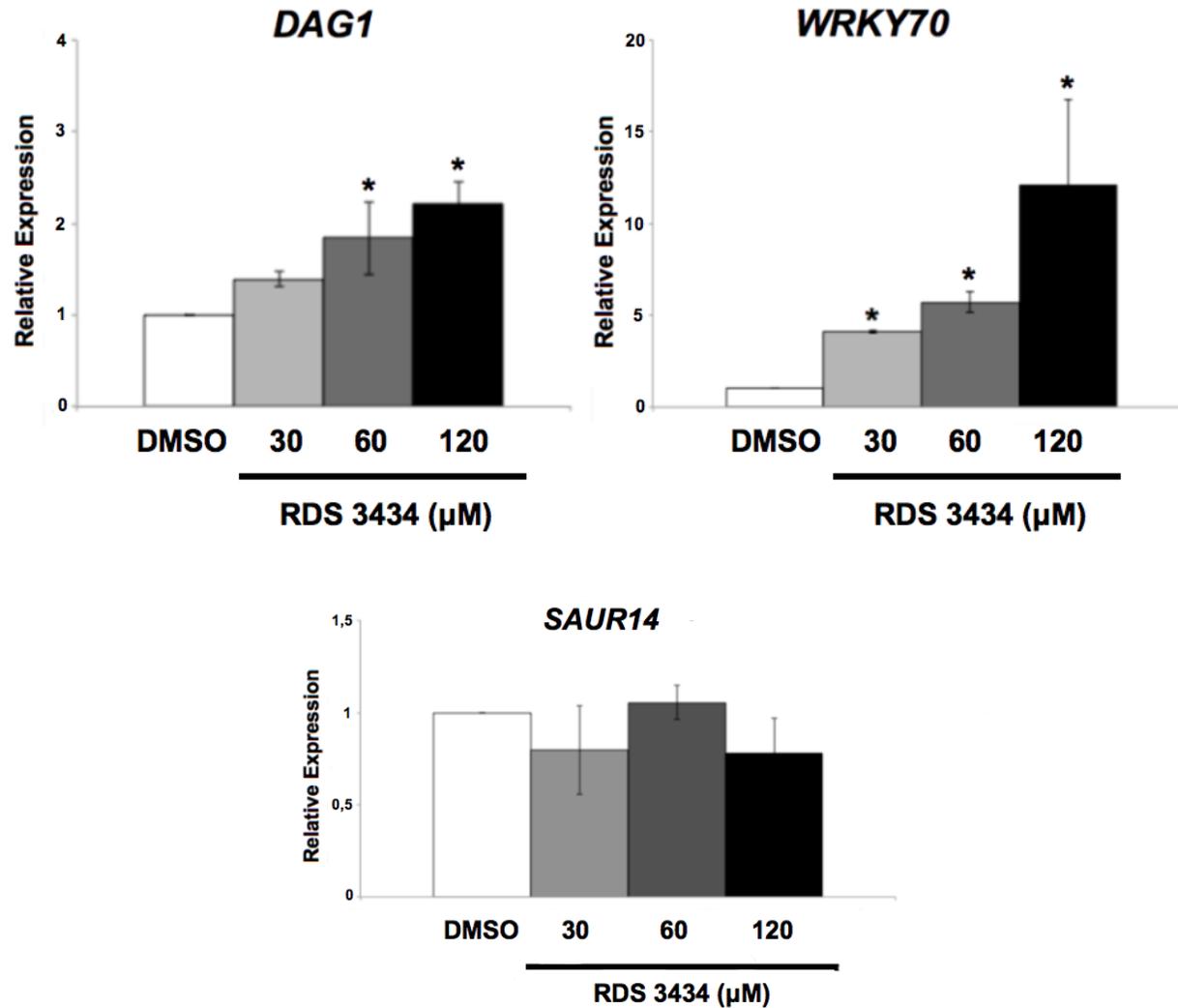
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Il trattamento con RDS 3434 aumenta selettivamente il livello di espressione dei geni bersaglio PRC2



Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



HAT: p300/CBP in plants

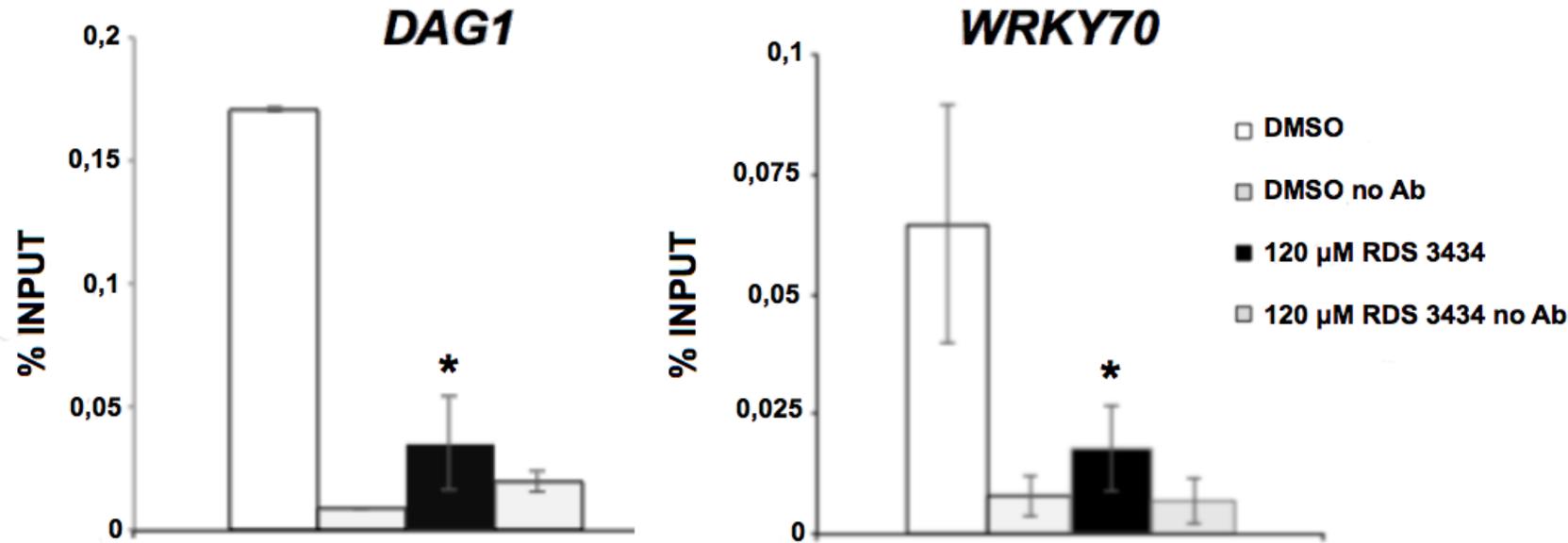


RDS3061: Dati sperimentali



Challenges e design sperimentale

Il trattamento con RDS 3434 riduce i livelli di H3K27me3 sui loci target di PRC2 (ChIP)



Caso studio III

HMT: Polycomb repressive complex II

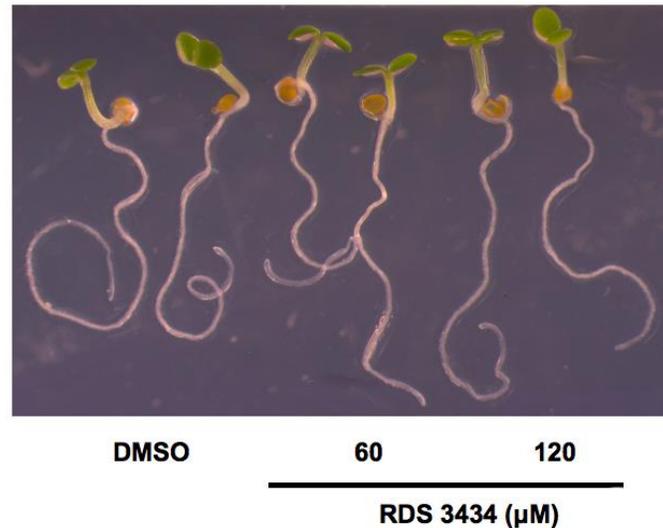
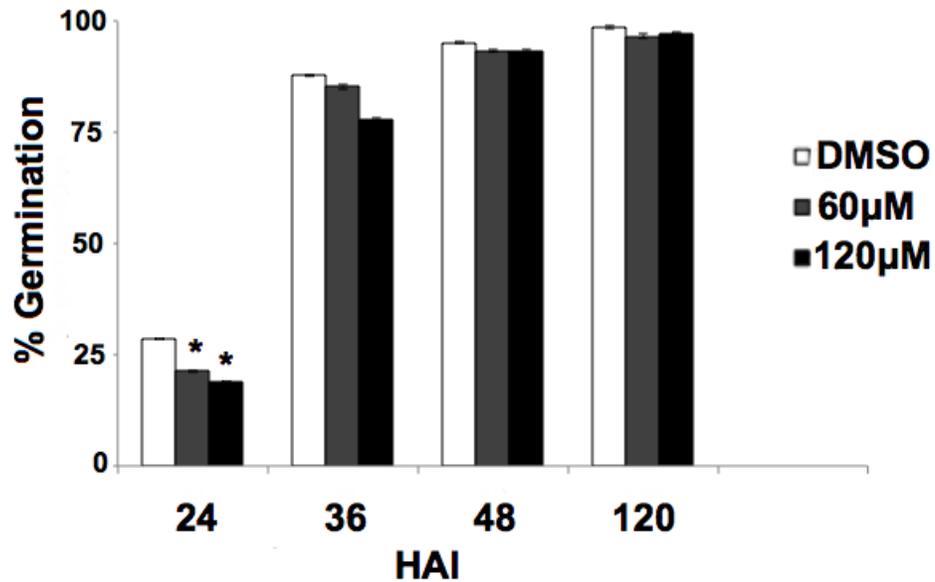
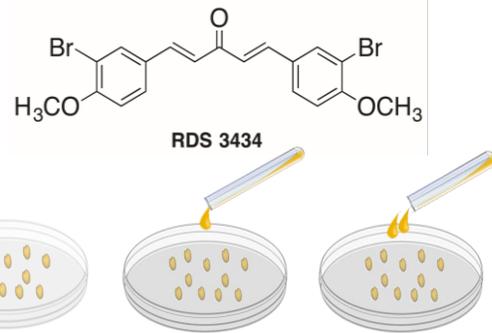
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

La riduzione dei livelli del marker H3K27me3 influisce sulla germinazione dei semi



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

La riduzione dei livelli del marker H3K27me3 influenza anche lo sviluppo delle radici (240μM)

Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



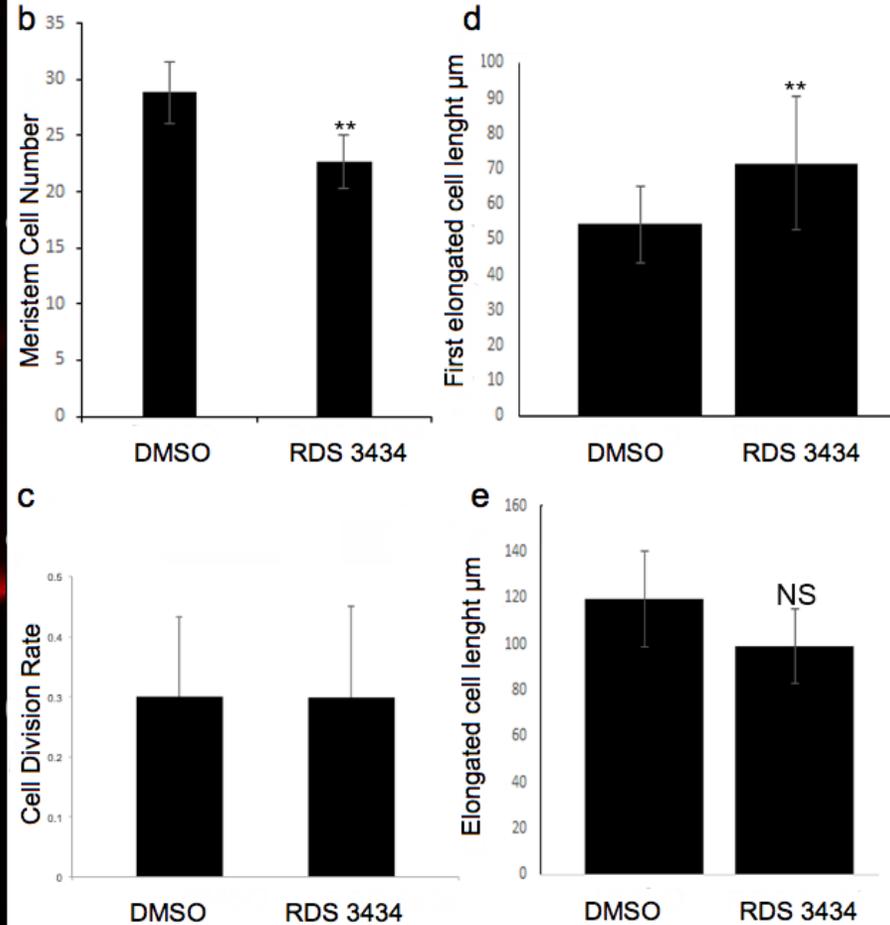
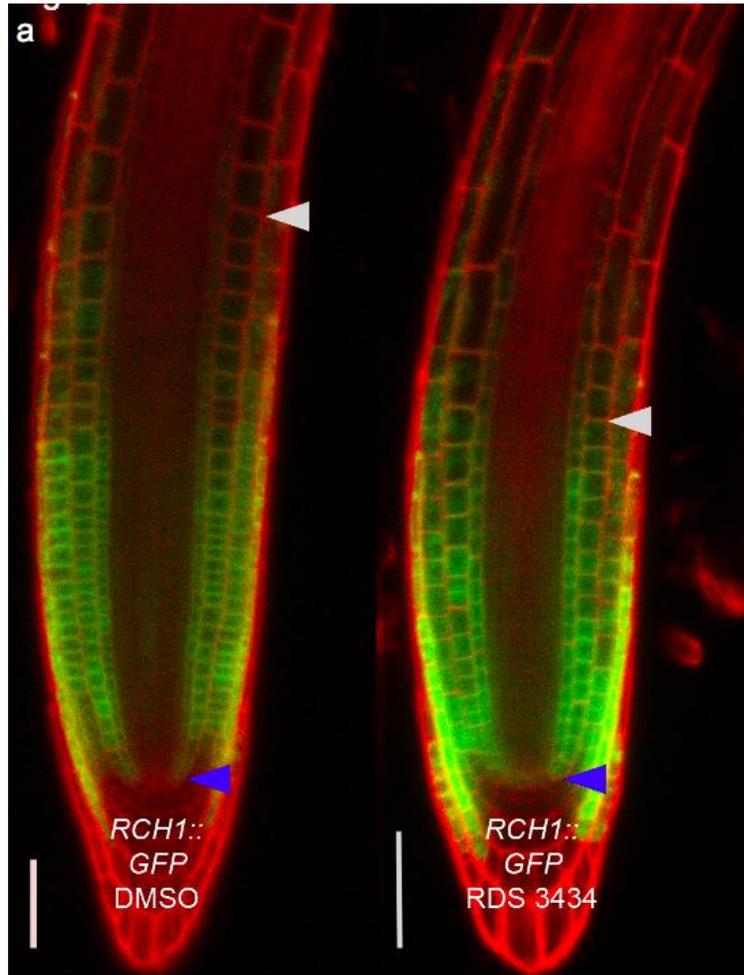
HAT: p300/CBP in plants



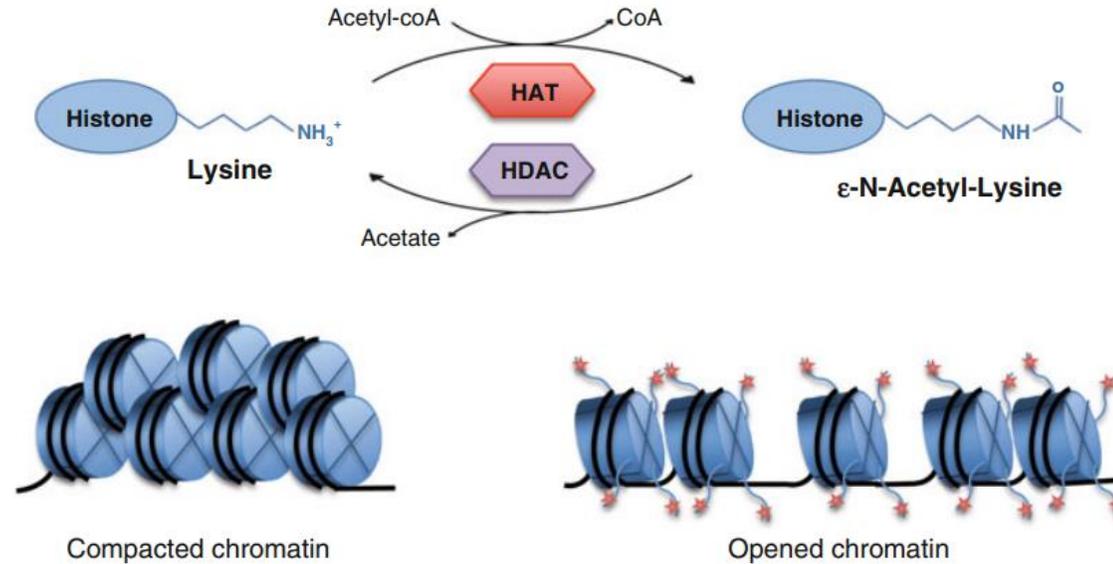
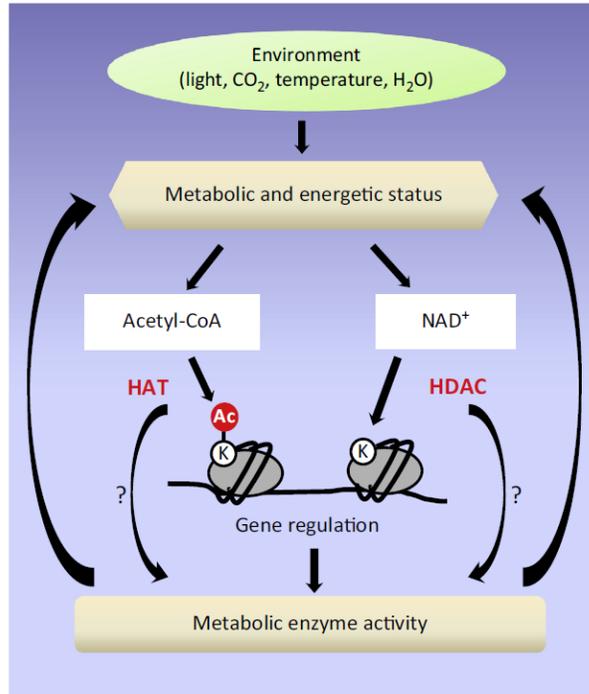
RDS3061: Dati sperimentali



Challenges e design sperimentale



Acetilazione istonica: marker di attivazione trascrizionale



Acetil-CoA e NAD, metaboliti fondamentali nella modulazione del flusso metabolico cellulare, sono utilizzati come substrati o cofattori nelle reazioni catalizzate da HATs e HDACs, rispettivamente. L'acetilazione è dunque un processo strettamente correlato allo stato energetico cellulare

Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Aumentare la disponibilità di molecole bioattive in pianta: studio di inibitori di acetiltransferasi istoniche

Caso studio III

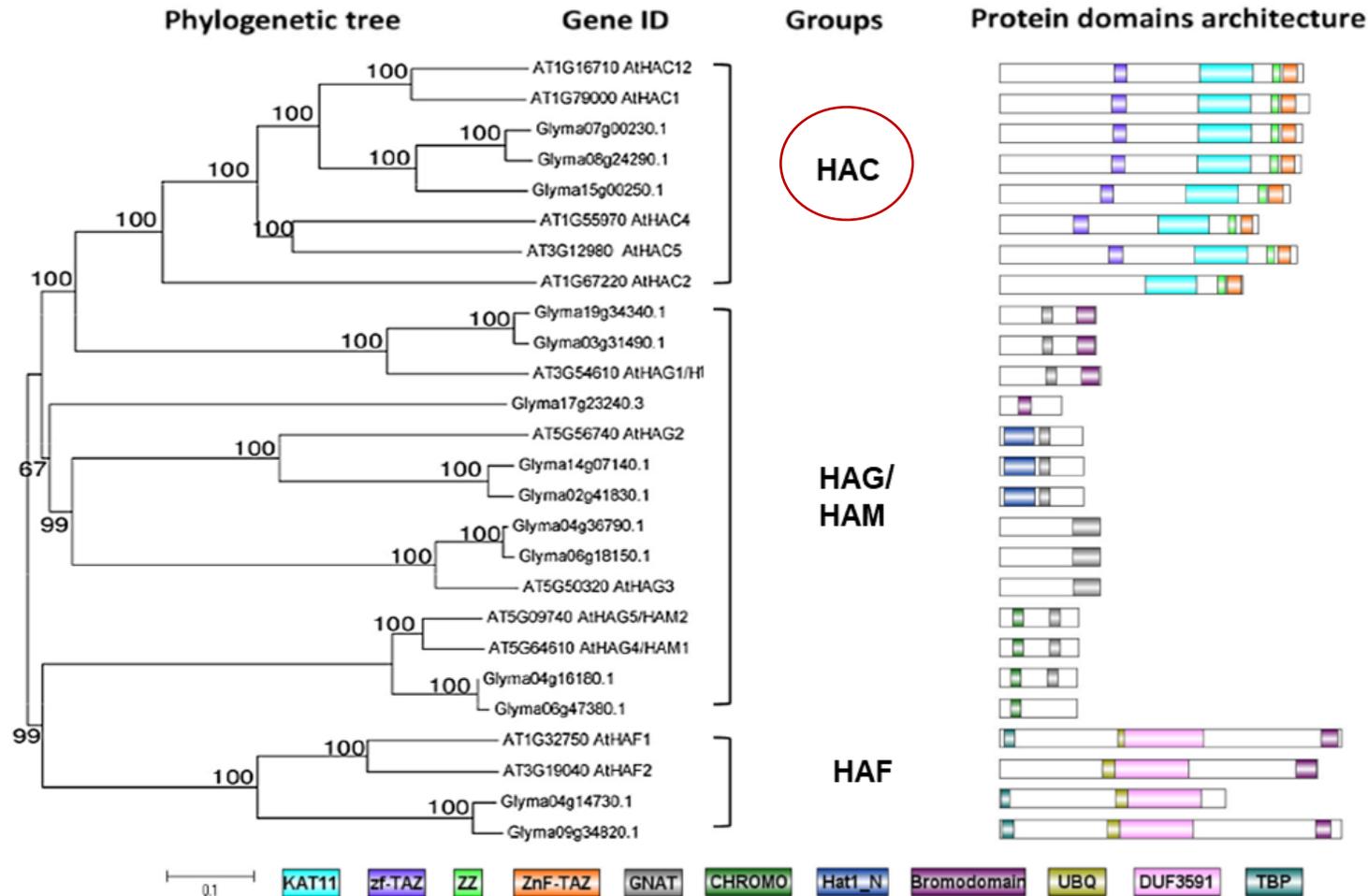
HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

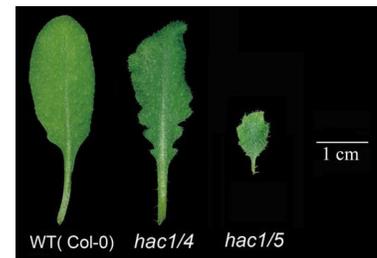
Challenges e design sperimentale



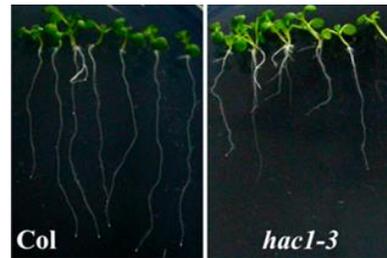
Target complessi: effetti pleiotropici nei processi di sviluppo

In *Arabidopsis* cinque geni appartenenti alla categoria HAC: HAC1, HAC2, HAC4, HAC5 e HAC12

con effetti pleiotropici nei processi di sviluppo:



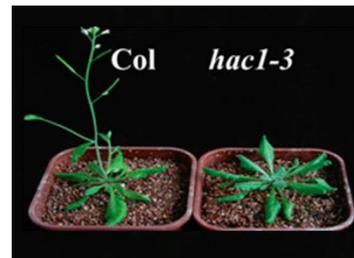
Foglie piccole, scure, increspate e dentellate



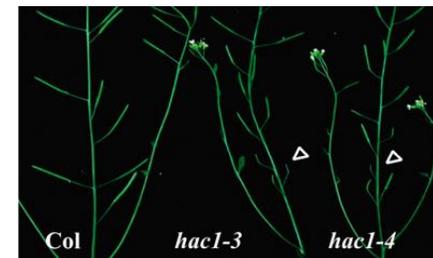
Accorciamento radice primaria



Accorciamento ipocotile e ipersensibilità all'etilene



Periodo fioritura ritardato



Accorciamento siliques

Caso studio III

HMT: Polycomb repressive complex II

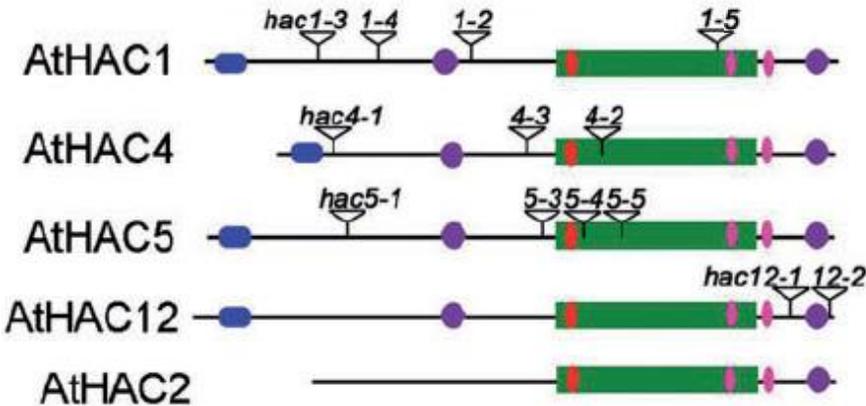
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

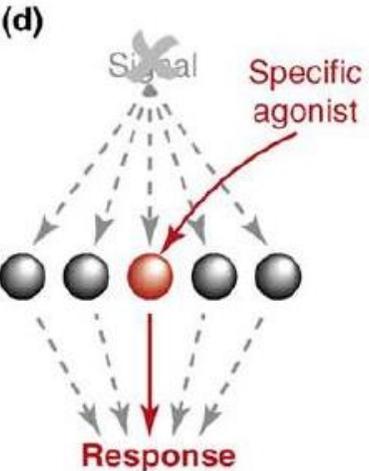
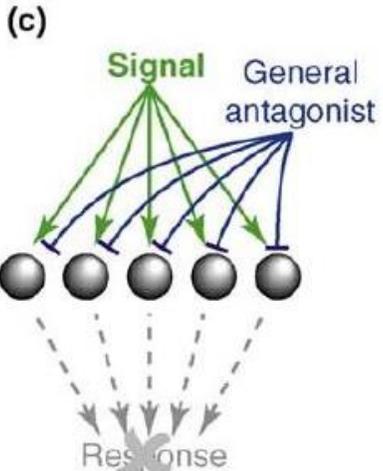
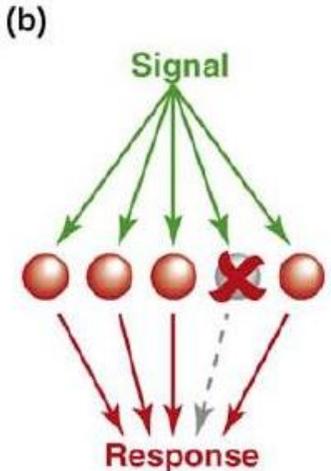
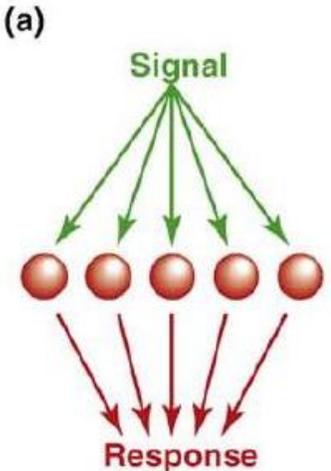
Challenges e design sperimentale

Problemi di ridondanza



Forward genetics

Chemical genetics



Caso studio III

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Challenges e design sperimentale

Toth and van der Hoorn, 2009
Li et al., 2013

One does not simply study epigenetic regulators



PCP
PLANT & CELL PHYSIOLOGY

Involvement of Arabidopsis Histone Acetyltransferase HAC Family Genes in the Ethylene Signaling Pathway

Chao Li^{2,3,4,*}, Jiang Xu^{1,2,4}, Jian Li², Qingyun Li² and Hongchun Yang²

¹Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, 100081 China

²National Institute of Biological Sciences, Beijing, 102206 China

³Department of Molecular Biology and Biochemistry, Lederle Graduate Research Tower, University of Massachusetts, Amherst, MA 01003, USA

Among all the mutants, the *hac1hac5* double mutant displayed the most severe phenotypes and the *hac1hac5hac4* mutant showed the second most severe phenotypes. Moreover, the *hac1hac5* double mutant and the *hac1hac5hac4* triple mutant are sterile. These results strongly indicate that HAC1, HAC12, HAC4 and HAC5 function synergistically, with HAC1 being dominant and HAC5 being the closest coordinator of HAC1.

Caso studio III

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RDS3434: Dati sperimentali



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RDS3061: Dati sperimentali

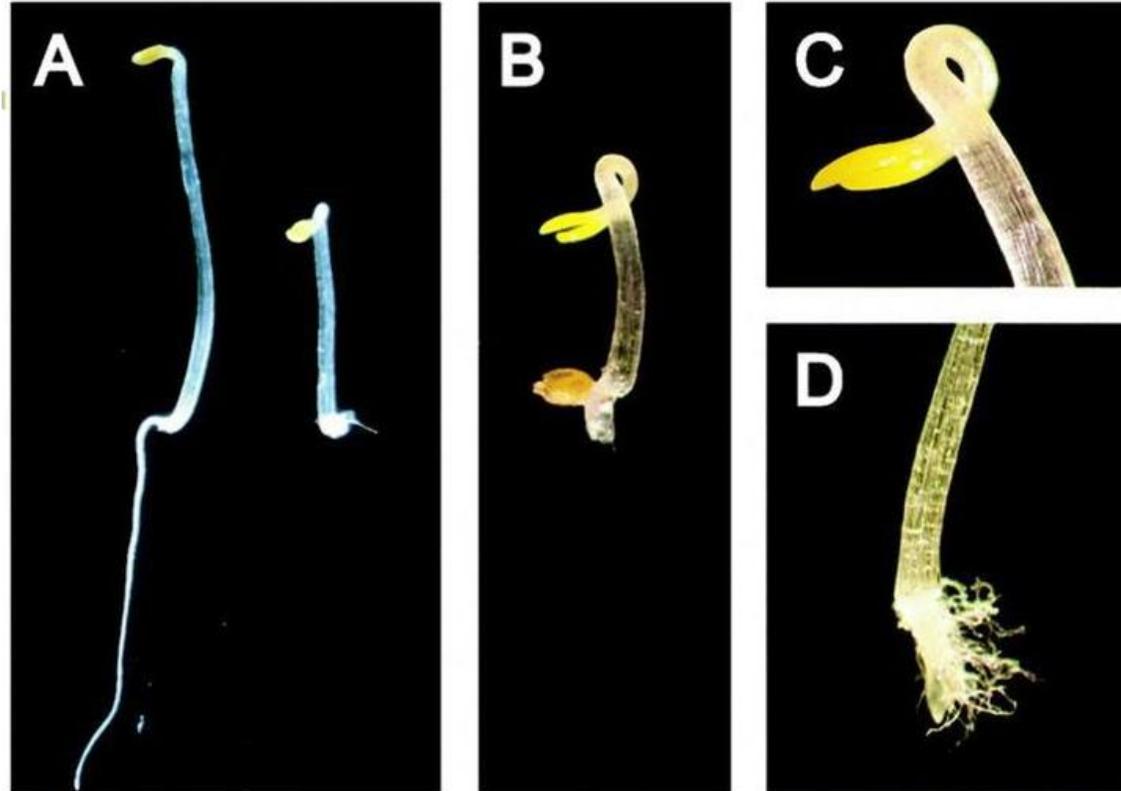


Challenges e design sperimentale

Risposta al fitormone etilene in plantula

Tripla risposta etilene:

- ▶ Ridotto allungamento cellulare
- ▶ Espansione radiale
- ▶ Formazione uncino apicale



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HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



HAT: p300/CBP in plants



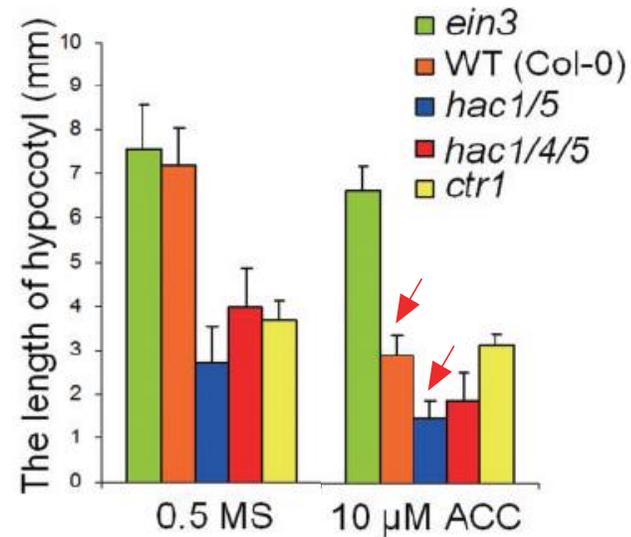
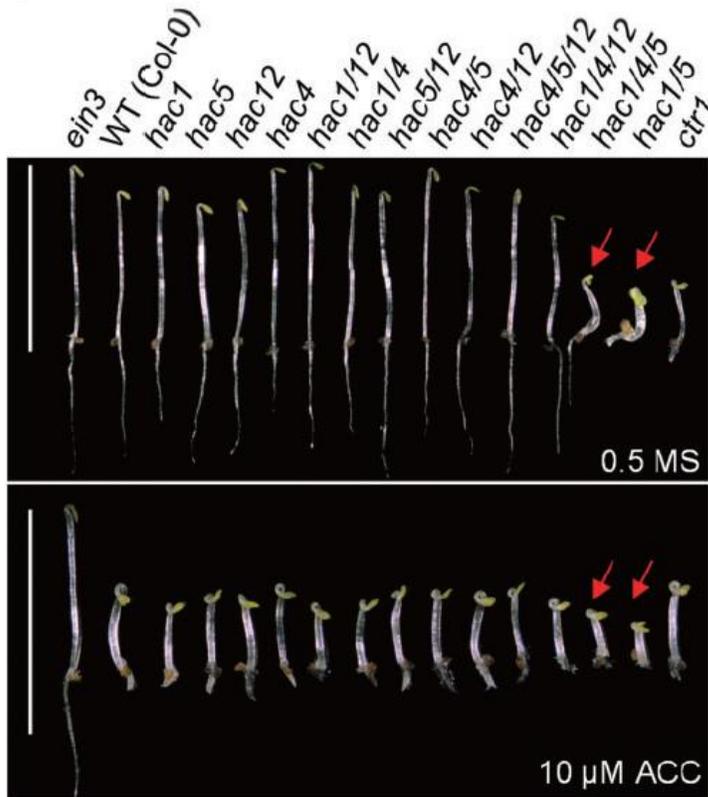
RDS3061: Dati sperimentali



Challenges e design sperimentale

I mutanti *hac* risultano ipersensibili all'etilene

Mutanti doppi e tripli delle *hac* sono stati studiati in relazione alla loro ipersensibilità all'etilene nel processo di allungamento dell'ipocotile



In presenza di ACC (precursore dell'etilene) i doppi mutanti *hac* ipocotile sono più corti rispetto al WT

Caso studio III

HMT: Polycomb repressive complex II

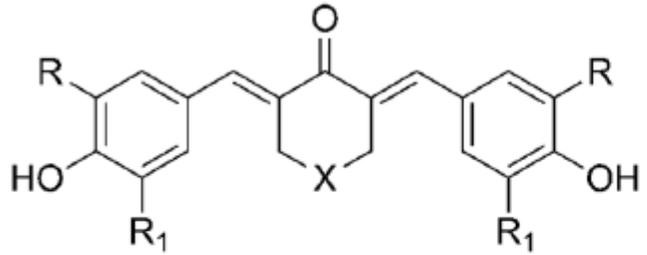
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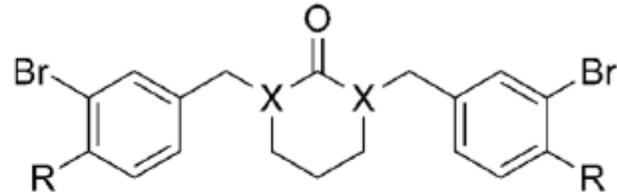
RDS3061: Dati sperimentali

Challenges e design sperimentale

Un approccio di genetica chimica per fenocopiare i mutanti *hac*



- 1a** X = CH₂; R = Br; R₁ = H
- 1b** X = S; R = Br; R₁ = H
- 1c** X = CH₂; R = F; R₁ = H
- 1d** X = CH₂; R = Cl; R₁ = H
- 1e** X = CH₂; R = Br; R₁ = Br
- 1f** X = CH₂; R = I; R₁ = H

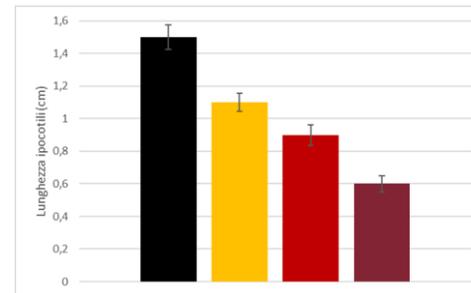


- 2a** X = N; R = OCH₃
- 2b** X = N; R = OH
- 2c** X = CH; R = OH



Control

Increasing inhibitor concentrations



Caso studio III

HMT: Polycomb repressive complex II

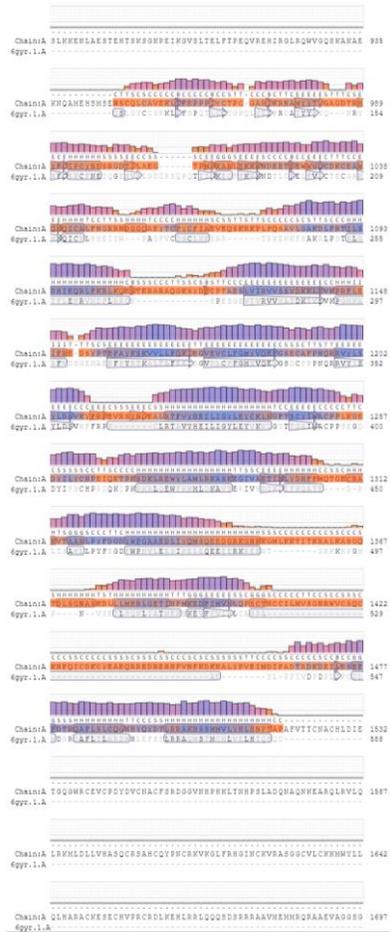
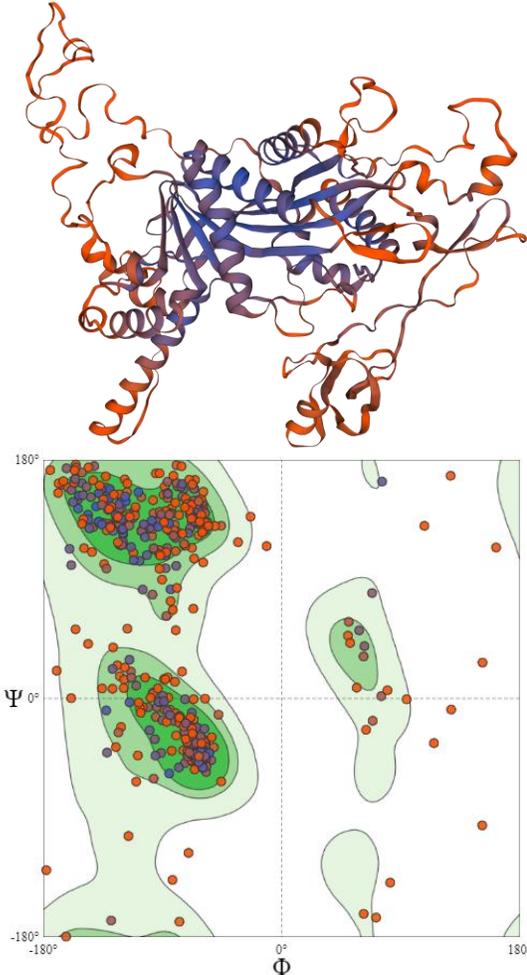
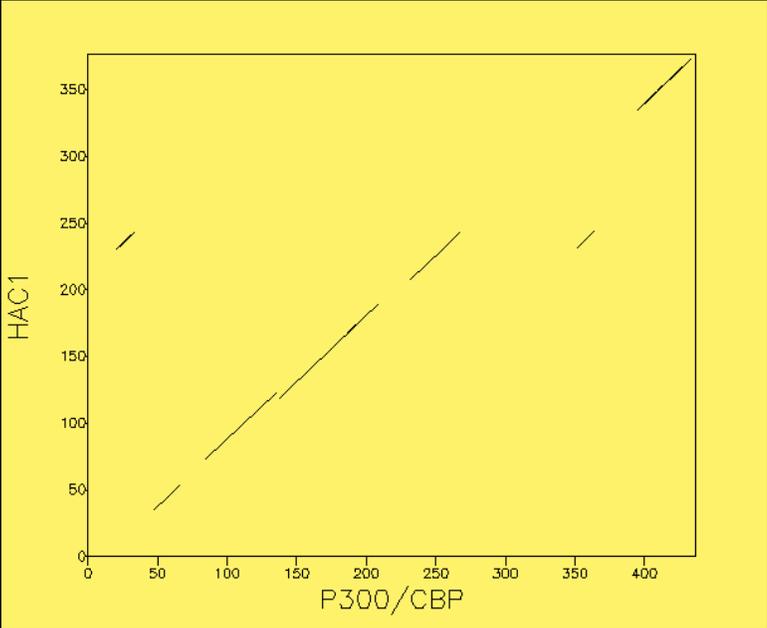
RDS3434: Dati sperimentali

HAT: p300/CBP in plants

RDS3061: Dati sperimentali

Challenges e design sperimentale

Buona percentuale di identità del del sito catalitico di P300/HAC tra uomo e pianta



Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



HAT: p300/CBP in plants



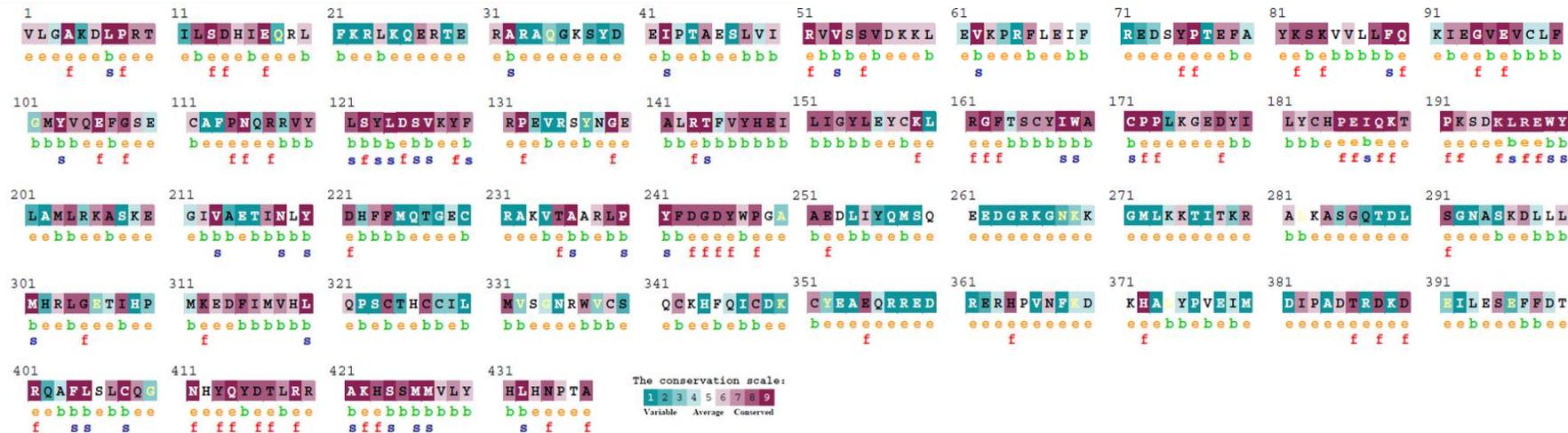
RDS3061: Dati sperimentali



Challenges e design sperimentale

Conservazione sito catalitico HAC1

La sequenza amminoacidica del dominio catalitico della proteina HAC1 è stata analizzata mediante l'uso del server ConSurf



Vengono evidenziati i residui più importanti da un punto di vista funzionale e strutturale. Da notare che sono gli stessi con cui l'inibitore entra maggiormente a contatto

Caso studio III

HMT: Polycomb repressive complex II

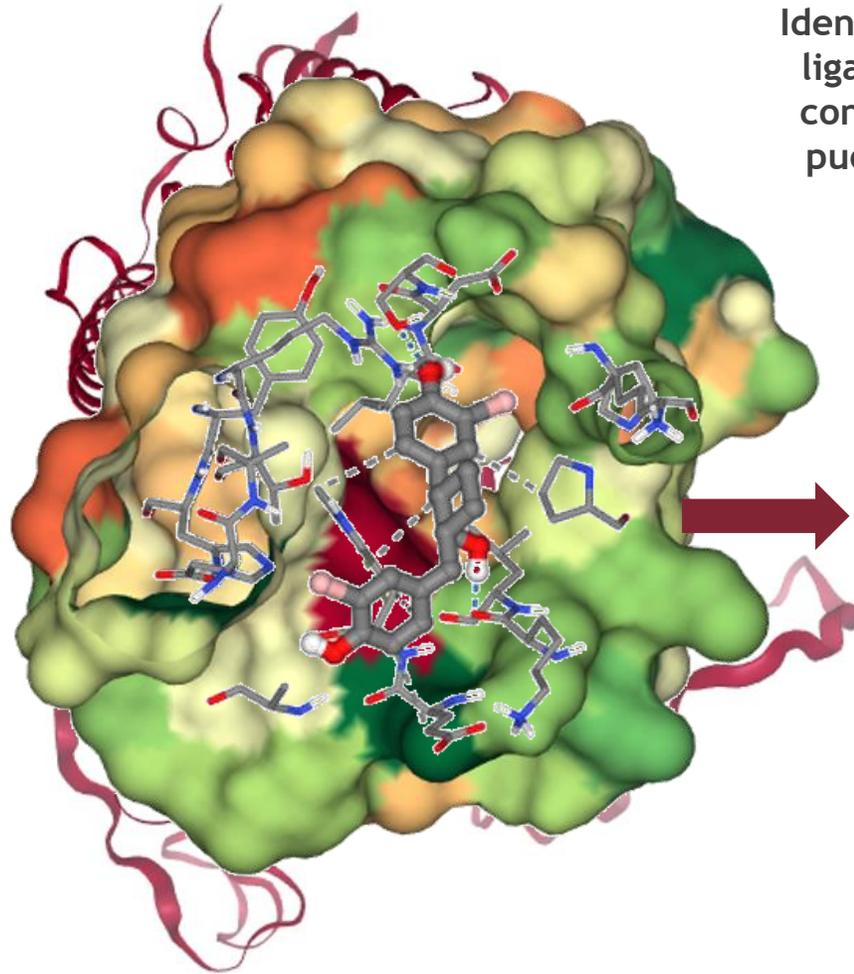
RDS3434: Dati sperimentali

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Challenges e design sperimentale

Interazioni proteina – *small-molecule*



Identificazione della conformazione bioattiva del ligando attraverso l'esplorazione in silico delle conformazioni energeticamente favorevoli che può assumere nell'interazione con il bersaglio

2.5 Å:

Prolina-191, Lisina-195, Leucina-196,
Glutammato-198, Triptofano-199

191
PKSDKLRWY
eeeeebbbb
ff fsffss

3.5 Å:

Leucina-124 e Serina-126

121
LSYLDVKYF
bbbbebbb
sfssfss fs

Caso studio III

HMT: Polycomb
repressive complex II



RDS3434: Dati
sperimentali



HAT: p300/CBP
in plants



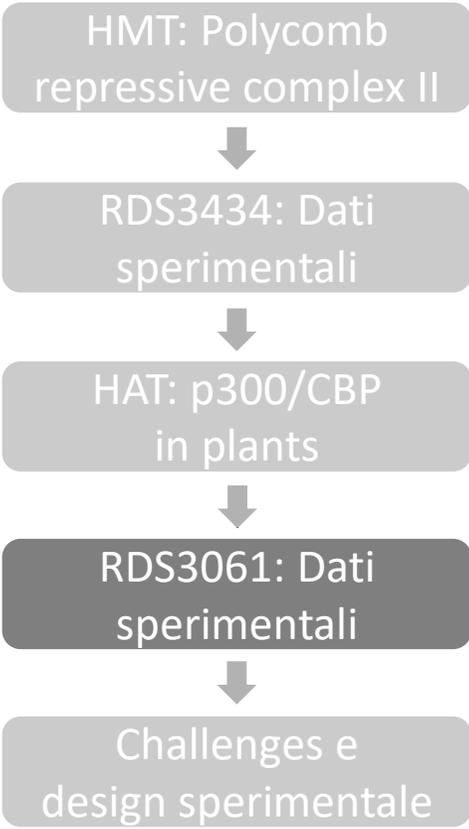
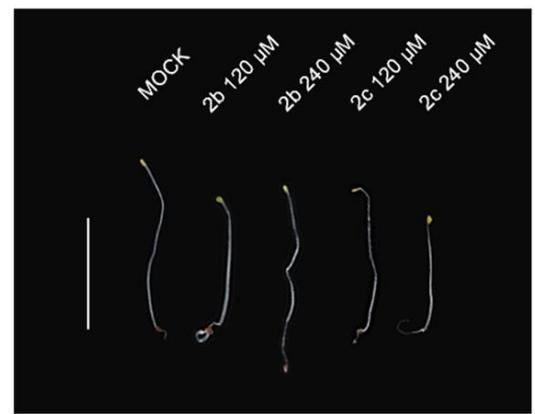
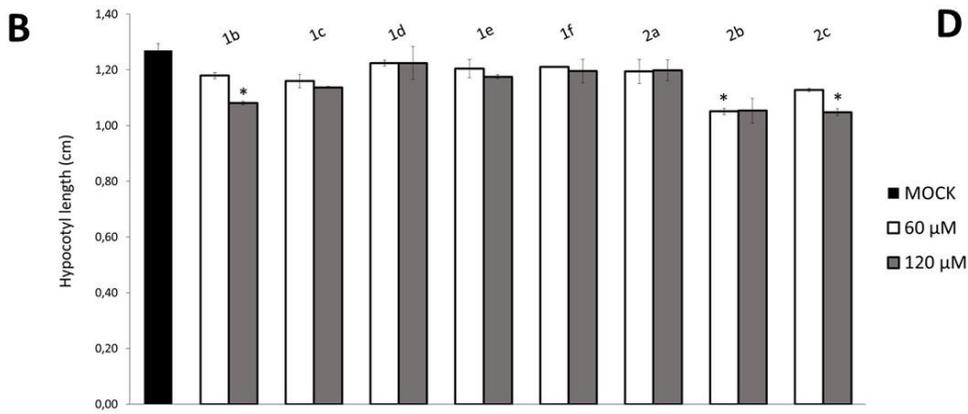
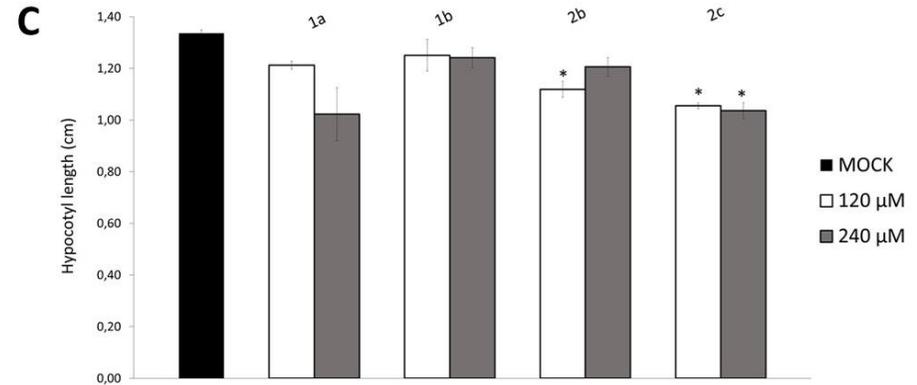
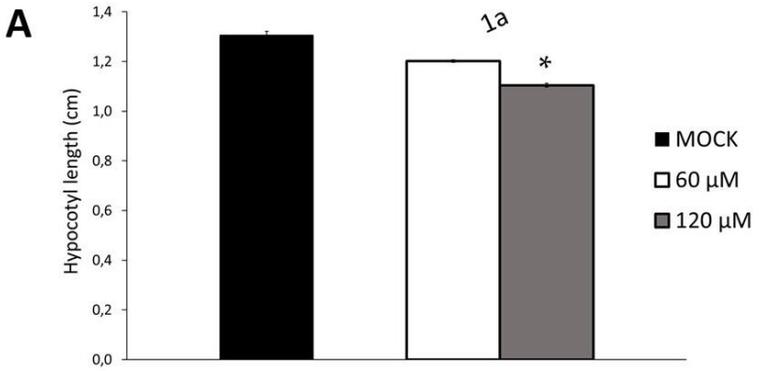
RDS3061: Dati
sperimentali



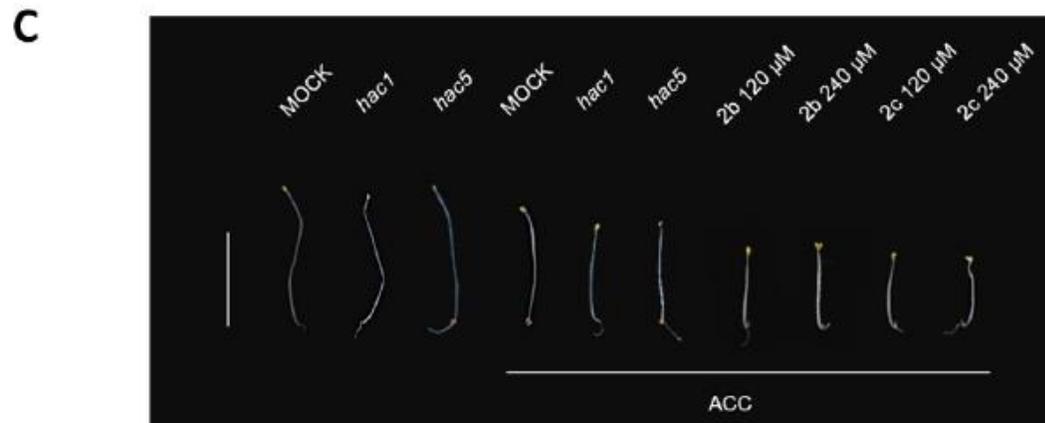
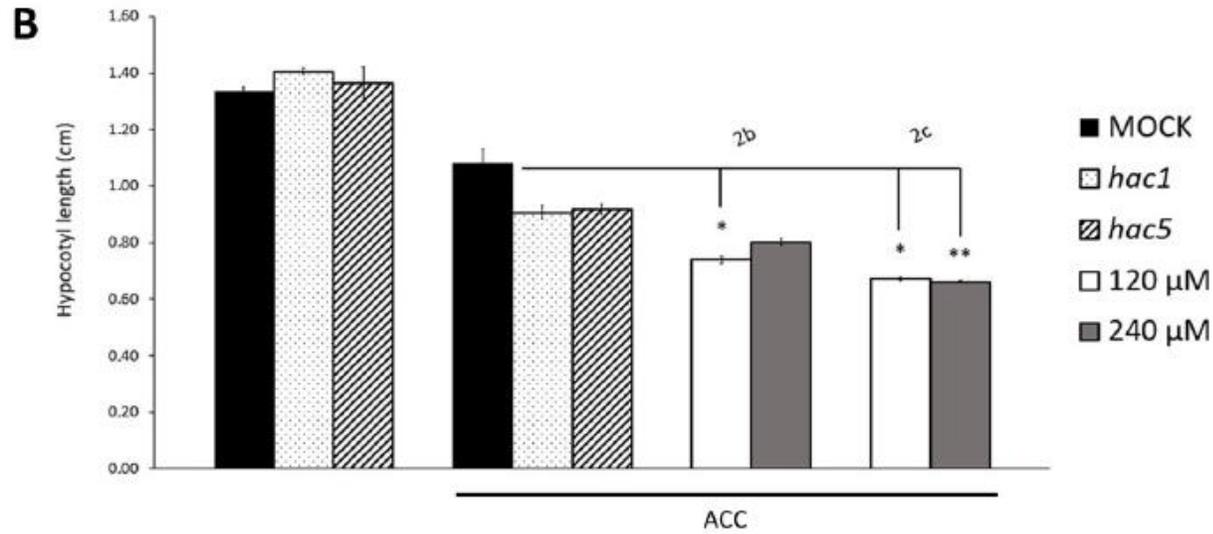
Challenges e
design sperimentale

Lead compound e primo screening

Caso studio III



Il trattamento chimico causa fenotipi più forti rispetto ai mutanti hac in presenza di ACC



Caso studio III

HMT: Polycomb repressive complex II

RDS3434: Dati sperimentali

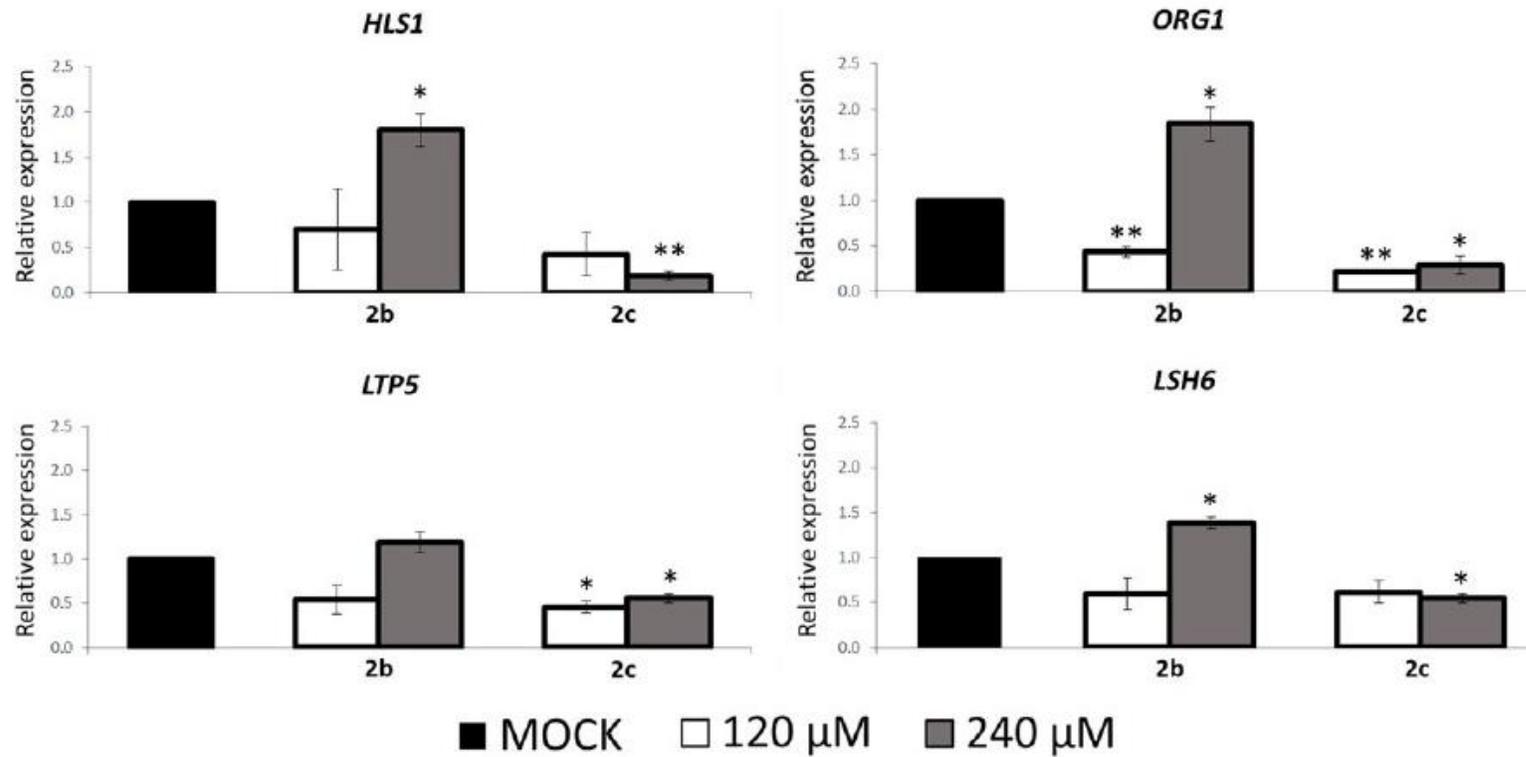
HAT: p300/CBP in plants

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Challenges e design sperimentale

Il trattamento con gli inibitori influisce sull'espressione dei geni bersaglio delle HAC

A



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HMT: Polycomb repressive complex II

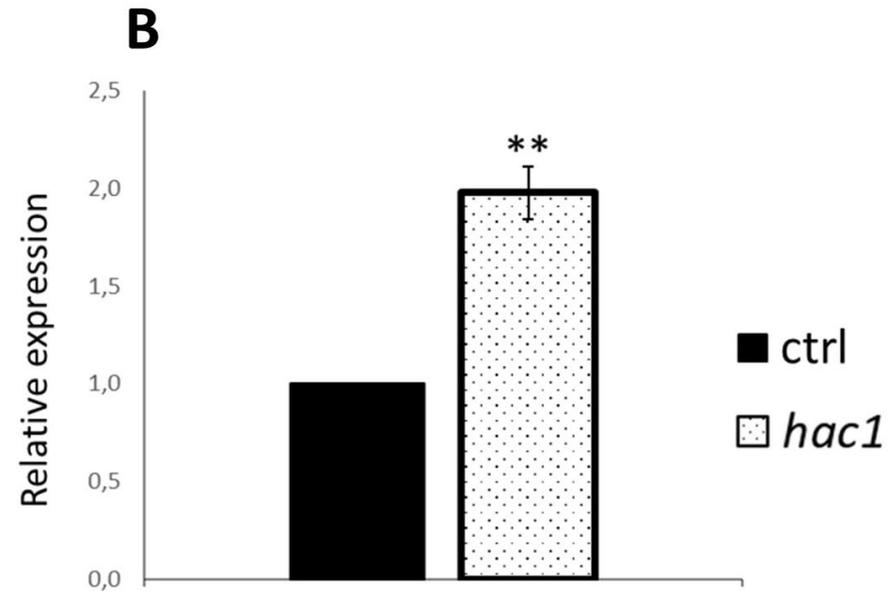
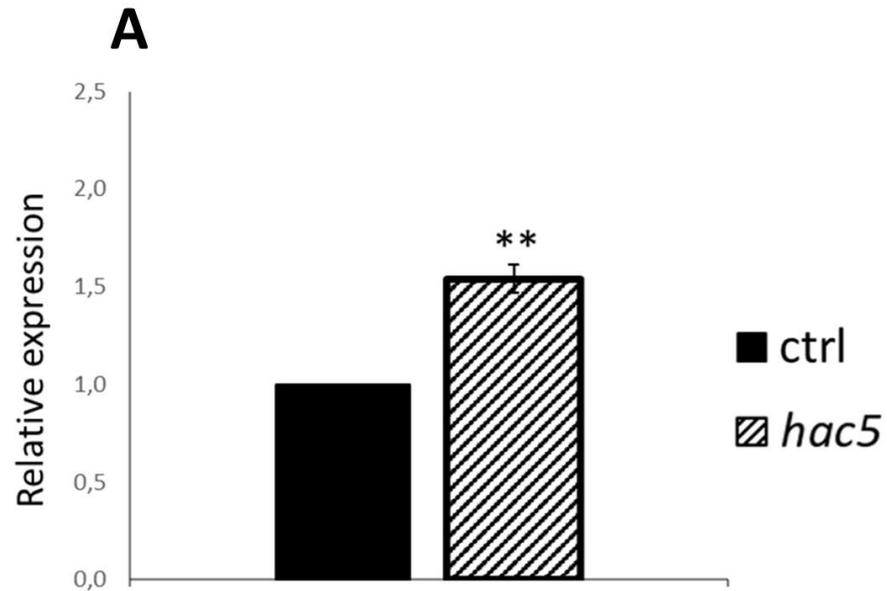
RDS3434: Dati sperimentali

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RDS3061: Dati sperimentali

Challenges e design sperimentale

I mutanti *hac* regolano mutualmente la loro espressione: effetto compensazione



Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



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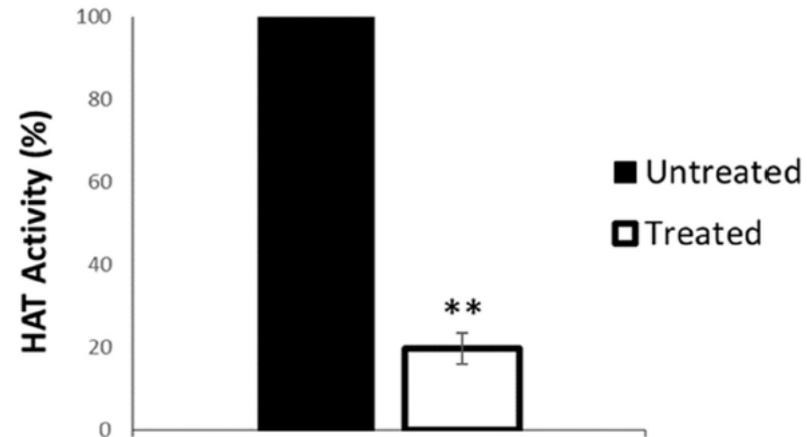
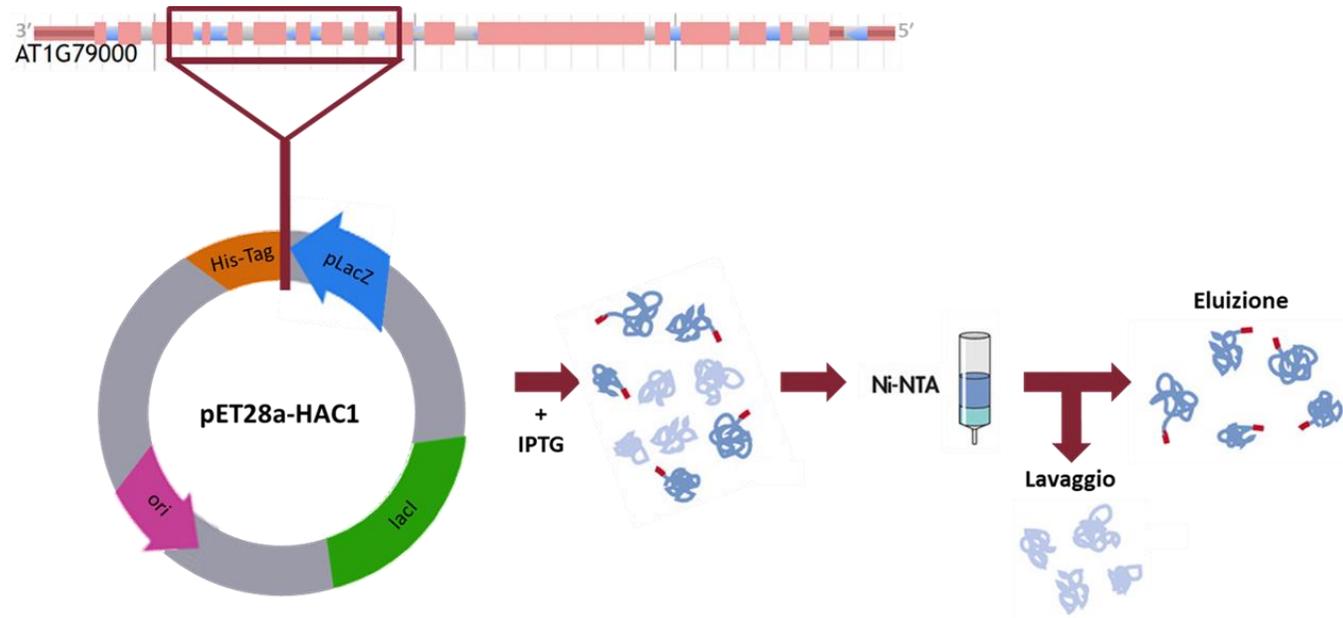


RDS3061: Dati sperimentali



Challenges e design sperimentale

Saggio di acetilazione *in vitro*



Caso studio III

HMT: Polycomb repressive complex II

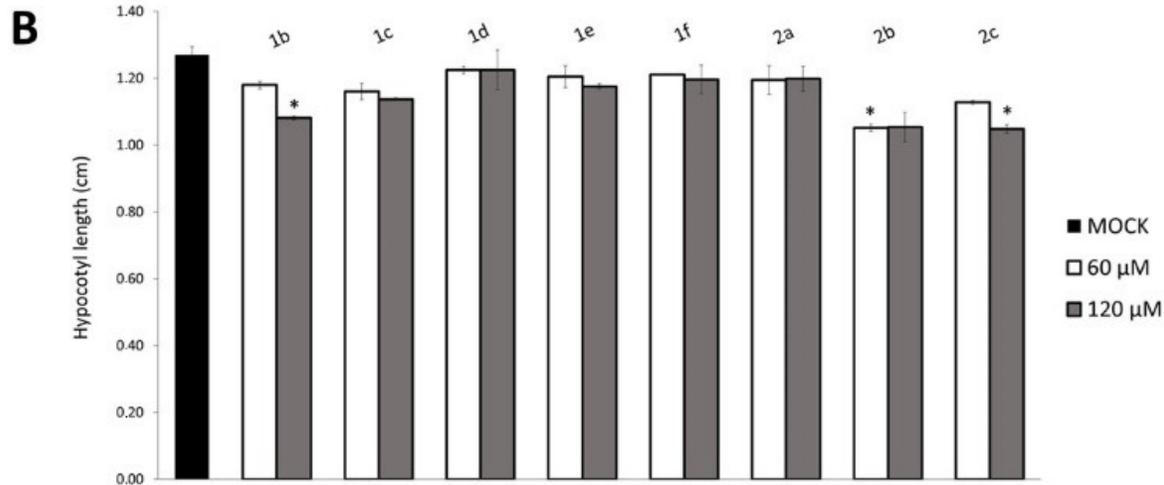
RDS3434: Dati sperimentali

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Challenges e design sperimentale

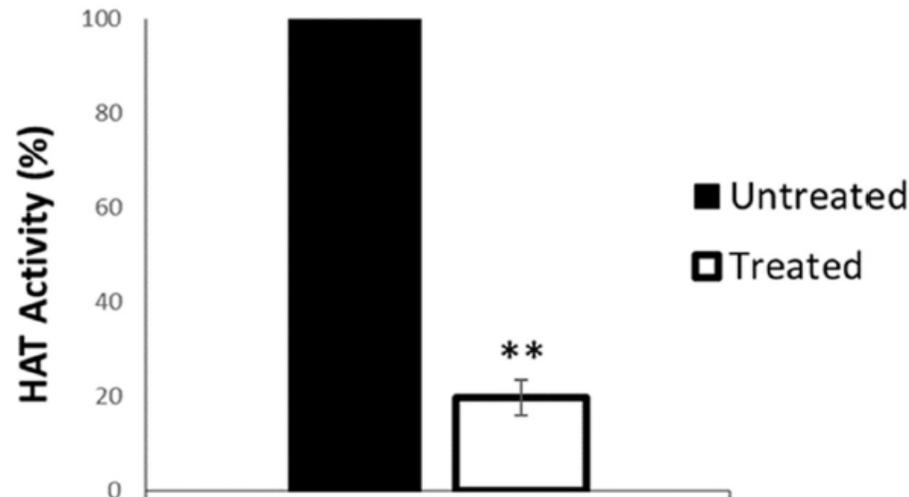
Challenges del drug repurposing



**Necessità di ampie librerie:
Non troverai un farmaco efficace
con un solo tentativo...**

**... ma allo stesso tempo gli
screening fenotipici
richiedono denaro e tempo**

**Poiché si utilizzano molecole
progettate per altri target, sono
necessarie saggi biochimici a
supporto dell'efficacia e della
specificità delle molecole**



Caso studio III

HMT: Polycomb
repressive complex II



RDS3434: Dati
sperimentali



HAT: p300/CBP
in plants

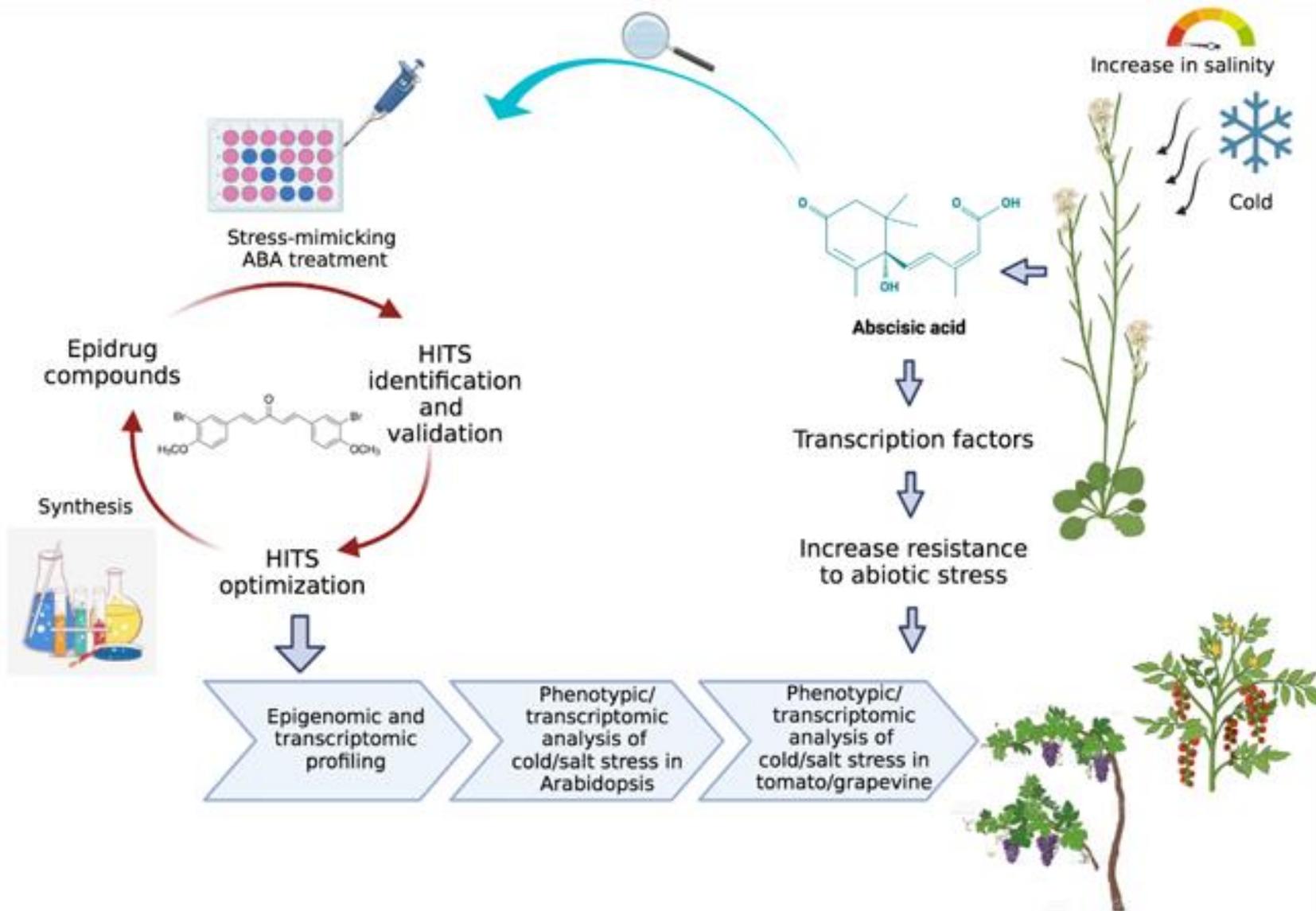


RDS3061: Dati
sperimentali



**Challenges e
design sperimentale**

Workflow e design sperimentale in pianta



Caso studio III

HMT: Polycomb repressive complex II

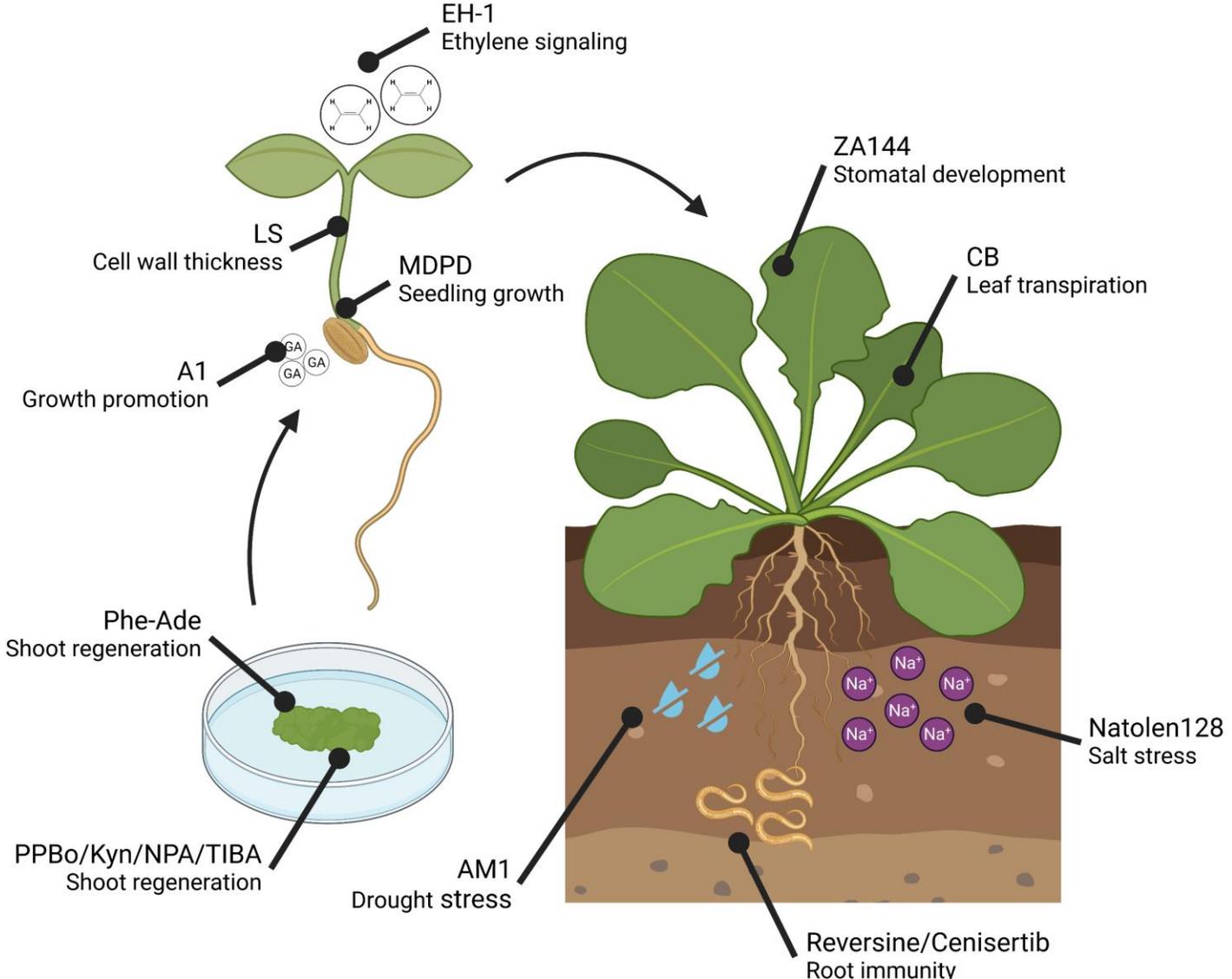
RDS3434: Dati sperimentali

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Challenges e design sperimentale

Molecole disponibili



Caso studio III

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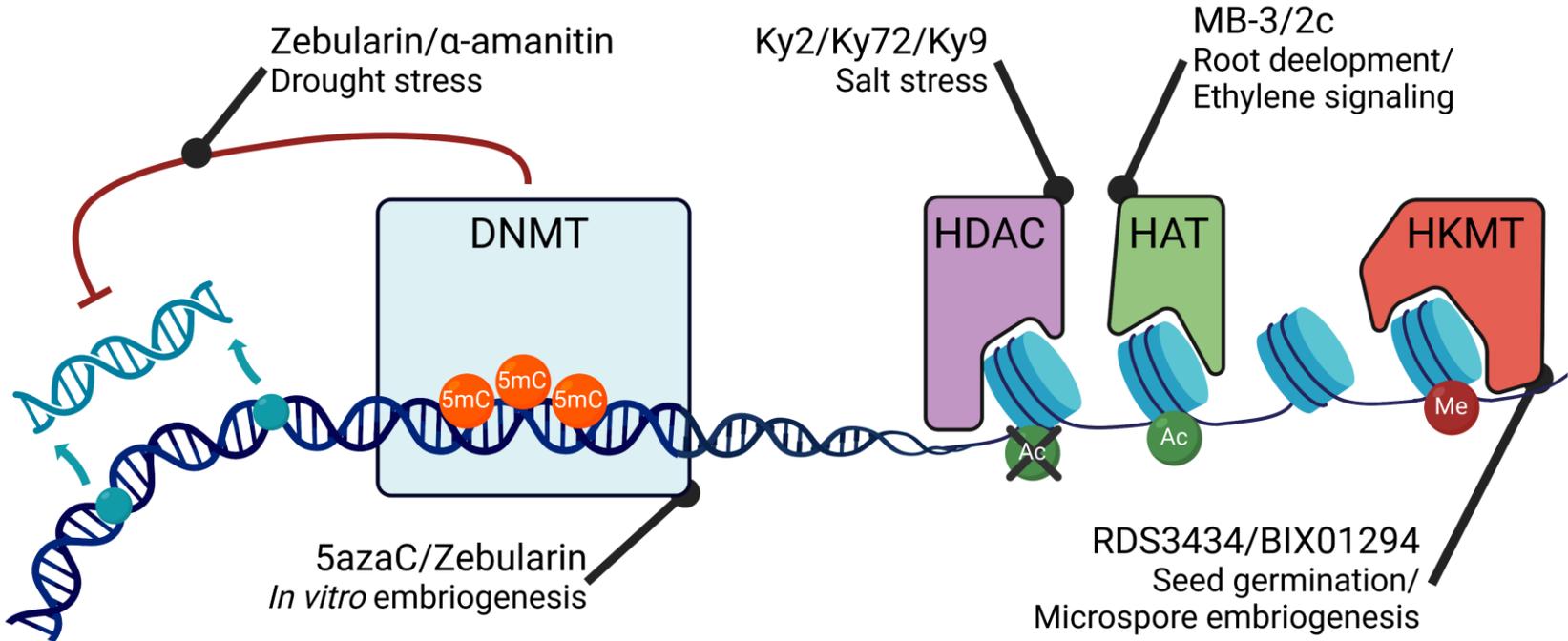
RDS3434: Dati sperimentali

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Challenges e design sperimentale

Molecole disponibili



Caso studio III

HMT: Polycomb repressive complex II



RDS3434: Dati sperimentali



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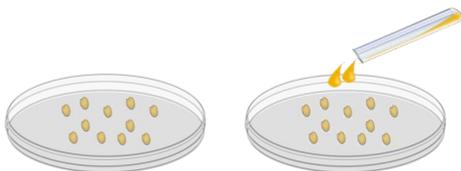


RDS3061: Dati sperimentali



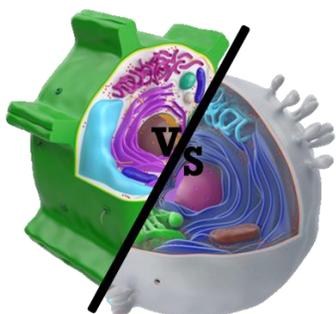
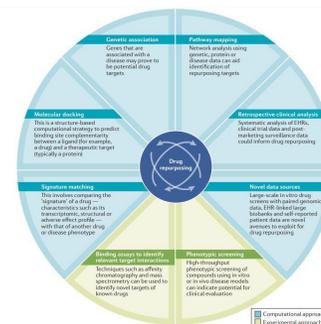
Challenges e design sperimentale

Ricapitoliamo i punti principali:



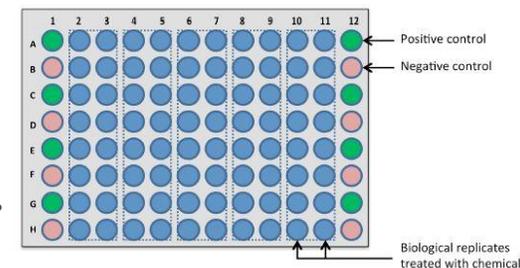
Strategie di genetica chimica, sia phenotype-based (forward) che target-based (reverse), risultano di estremo di interesse dalla ricerca applicativa a quella di base, fornendo un approccio alternativo e complementare alla genetica classica.

Sebbene il numero di small-molecules sia continuamente in aumento, le molecole testate e approvate sono una frazione esigua. Approcci di drug repurposing possono dare nuova luce a composti scartati, permettendo di risparmiare risorse.



Strategie di drug repurposing permettono inoltre di sfruttare la gigantesca disponibilità di molecole prodotte in campi molto finanziati, come la ricerca contro il cancro, in altri campi di ricerca mediante HTS e analisi preliminari *in silico*.

Research goes slowly: screening per identificare composti bioattivi in organismi diversi da quelli inizialmente target sono lunghi e dispendiosi. Normalmente le librerie testate contengono (decine di) migliaia di molecole, di cui solo una minima frazione viene ammessa a test successivi.



Lecture consigliate per approfondire l'argomento

Genetica chimica, drug repurposing, small-molecules vs. antibody, database

- ▶ Kawasumi, M., & Nghiem, P. (2007). Chemical genetics: elucidating biological systems with small-molecule compounds. *The Journal of investigative dermatology*, 127(7), 1577-1584. <https://doi.org/10.1038/sj.jid.5700853>
- ▶ Stockwell B. R. (2004). Exploring biology with small organic molecules. *Nature*, 432(7019), 846-854. <https://doi.org/10.1038/nature03196>
- ▶ Ashburn, T. T., & Thor, K. B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. *Nature reviews. Drug discovery*, 3(8), 673-683. <https://doi.org/10.1038/nrd1468>
- ▶ Imai, K., & Takaoka, A. (2006). Comparing antibody and small-molecule therapies for cancer. *Nature reviews. Cancer*, 6(9), 714-727. <https://doi.org/10.1038/nrc1913>
- ▶ Nicola, G., Liu, T., & Gilson, M. K. (2012). Public domain databases for medicinal chemistry. *Journal of medicinal chemistry*, 55(16), 6987-7002. <https://doi.org/10.1021/jm300501t>

Genetica chimica in biologia molecolare delle piante

- ▶ Tóth, R., & van der Hoorn, R. A. (2010). Emerging principles in plant chemical genetics. *Trends in plant science*, 15(2), 81-88. <https://doi.org/10.1016/j.tplants.2009.11.005>
- ▶ Robert, S., Raikhel, N. V., & Hicks, G. R. (2009). Powerful partners: Arabidopsis and chemical genomics. *The arabidopsis book*, 7, e0109. <https://doi.org/10.1199/tab.0109>
- ▶ Halder, V., & Russinova, E. (2019). Understanding the language of drugged plants. *Nature chemical biology*, 15(11), 1025-1028. <https://doi.org/10.1038/s41589-019-0389-0>
- ▶ Lepri, A., Longo, C., Messori, A., Kazmi, H., Madia, V. N., Di Santo, R., Costi, R., & Vittorioso, P. (2023) Plants and Small Molecules: An Up-and-Coming Synergy. *Plants (Basel, Switzerland)*, 12(8), 1729. <https://doi.org/10.3390/plants12081729>

Grazie per l'attenzione

Domande?