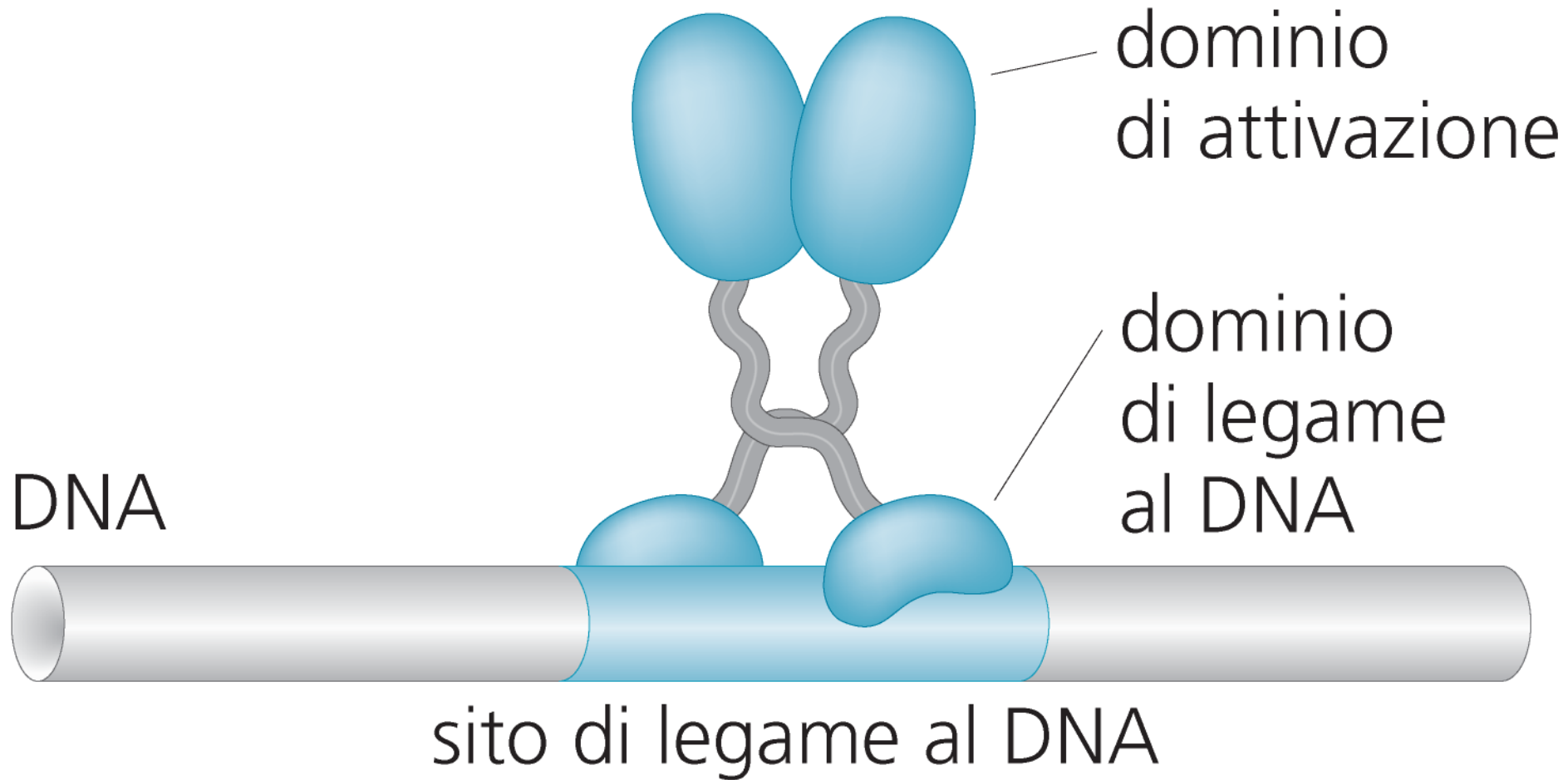
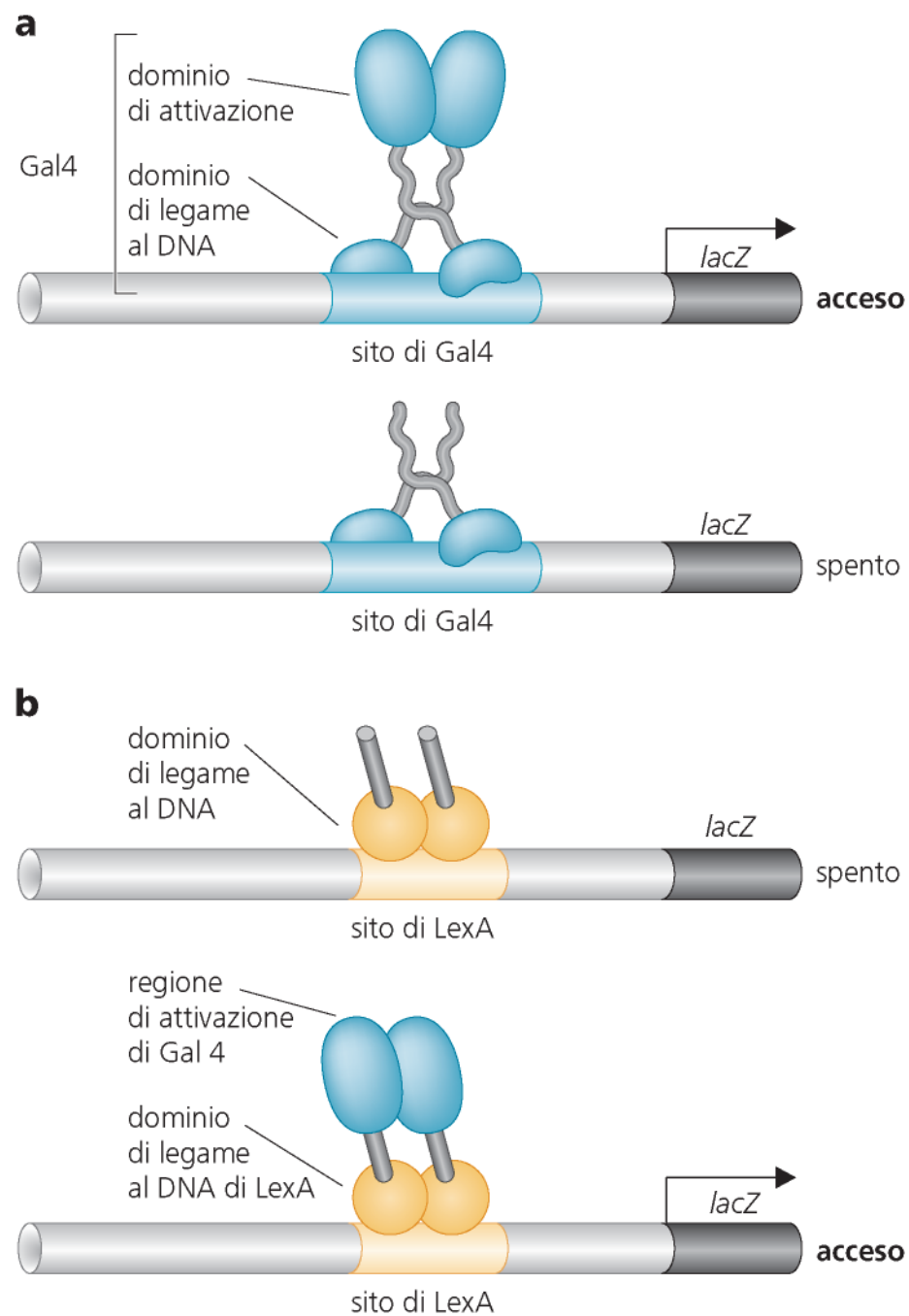


Struttura Attivatori in Eukaryotes

GLI ATTIVATORI TRASCRIZIONALI



DBD e AD possono essere separati



*Studying protein interactions:
Two Hybrid in yeast*

Two-hybrid system

Il sistema del doppio ibrido (Two-hybrid system) è una metodologia basata sulla modularità dei fattori di trascrizione che permette di identificare e clonare proteine che interagiscono con altre proteine.

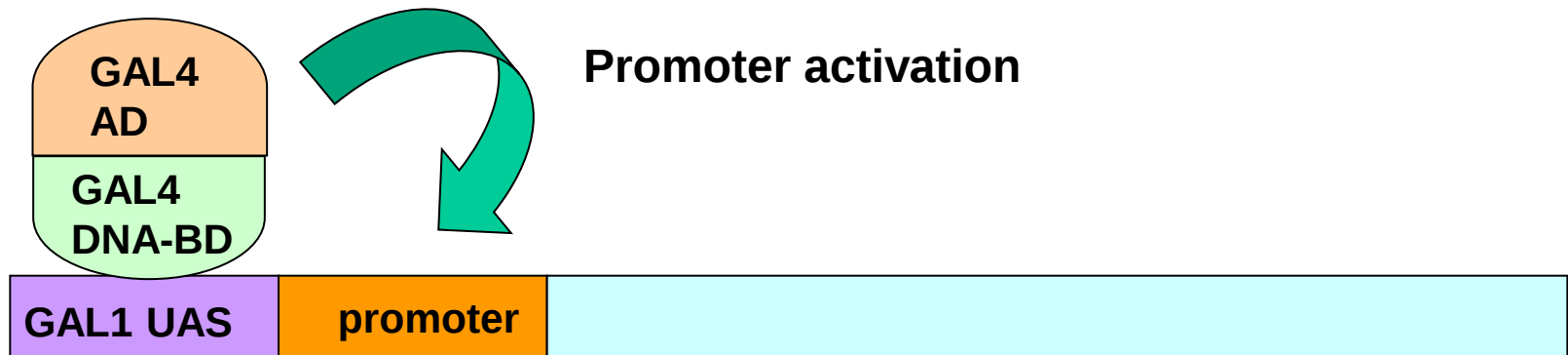
Il problema generale di caratterizzare interazioni proteina-proteina, identificando eventuali partner proteici di una proteina data, può venire risolto invari modi, tra cui:

- co-immunoprecipitazioni (utilizzo di anticorpi e risoluzione su gel nativi)
- cross-linking (con reagenti, come la glutataraldeide, che formano legami covalenti tra le proteine che interagiscono)
- colonne di affinità con partner di interazione
- co-purificazioni in colonne cromatografiche
- screening di librerie di espressione
- utilizzo di phage display libraries

Il two-hybrid system si distingue perché arriva a **identificare e clonare contemporaneamente** il gene che codifica per il partner proteico senza bisogno di informazioni preliminari sui partner proteici o di strumenti precostituiti (per es. anticorpi)

The GAL4 Transcription Factor

The yeast GAL4 transcription factor is a protein consisting of two major domains, a DNA binding domain (DNA-BD) and a transactivation domain (AD). Its normal role is to bind the GAL1 UAS element and activate transcription from the adjacent promoter.



For the purposes of the Y2H system, the coding sequences for these two domains are separated and expressed from different plasmids.

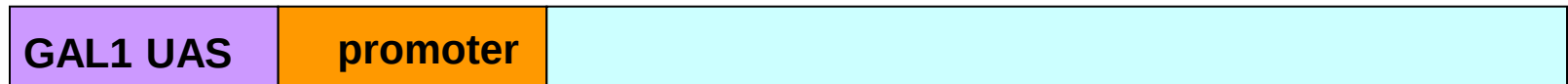
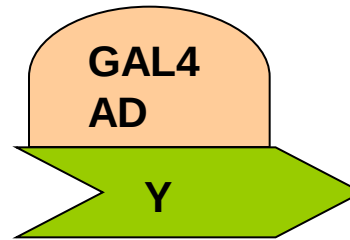
Yeast 2-hybrid

1. The hybrid of the GAL4 DNA-binding domain (BD) and protein X binds to the GAL1 UAS but cannot activate transcription without the activation domain.

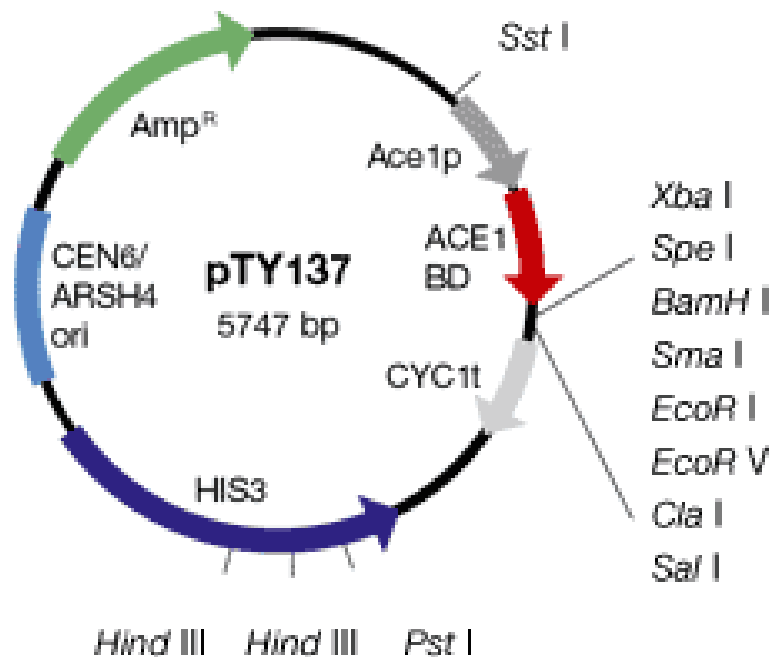


Yeast 2-hybrid

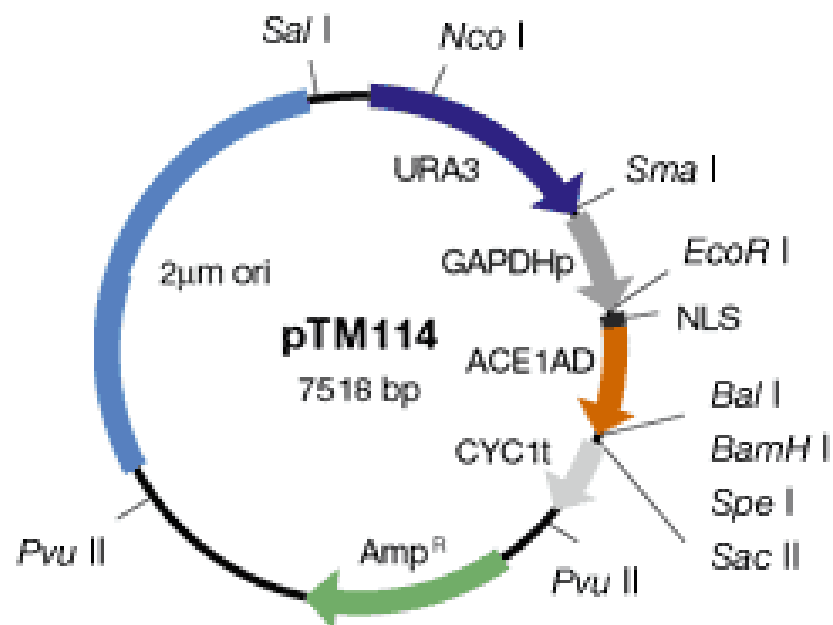
2. The hybrid of the GAL4 activation domain (AD) and protein Y cannot localise to the UAS by itself and thus does not activate transcription.



Bait-Plasmid



Prey-Plasmid



Yeast 2-Hybrid System: Summary

Interaction between the X and Y portions of two hybrid proteins *in vivo* reconstitutes GAL4 transcription factor function and results in expression of a gene.



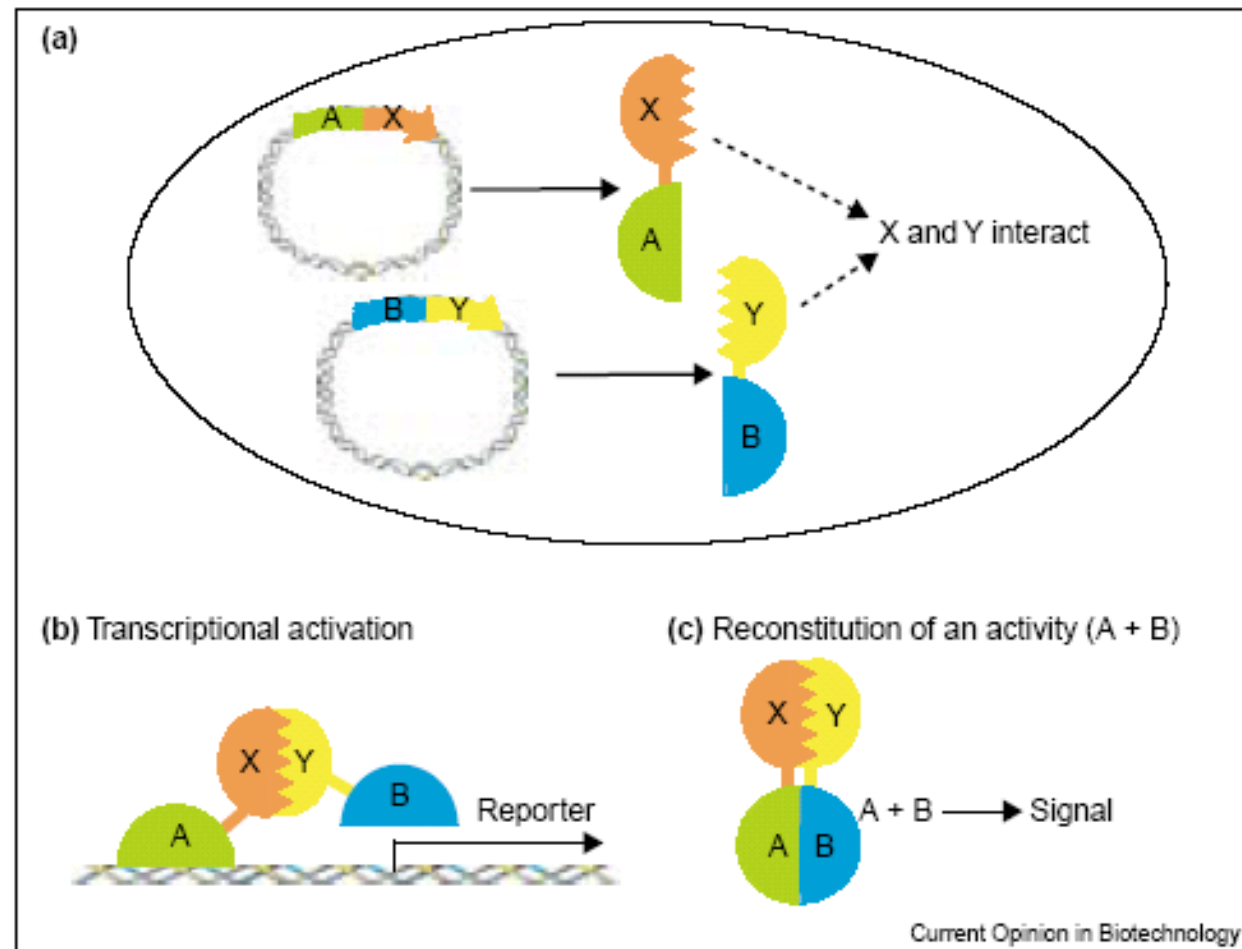
Yeast 2-Hybrid System: Summary

Interaction between the X and Y portions of two hybrid proteins *in vivo* reconstitutes GAL4 transcription factor function and results in expression of a reporter gene.

Expression of β -galactosidase can be assayed using X-GAL



In vivo selection systems. (a) DNA encoding the target (X, red) and bait (Y, yellow) proteins is fused to either part (A or B) of the reporter system. Co-expression in a cell yields the fusion proteins A–X and B–Y. (b) The yeast two-hybrid system. Interaction between X and Y results in functional pairing of the DNA-binding domain (A) and the transcriptional activator (B) allowing transcription of a reporter gene and leading to a detectable phenotype. (c) The protein fragment complementation assay. Interaction between X and Y results in reconstitution of an active enzyme (A–B), which gives rise to a detectable signal such as fluorescence or biosynthesis of an essential growth factor.



(A)

