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Booklets

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Title: Study of Hox protein-protein interactions in living cells using novel fluorescent techniques

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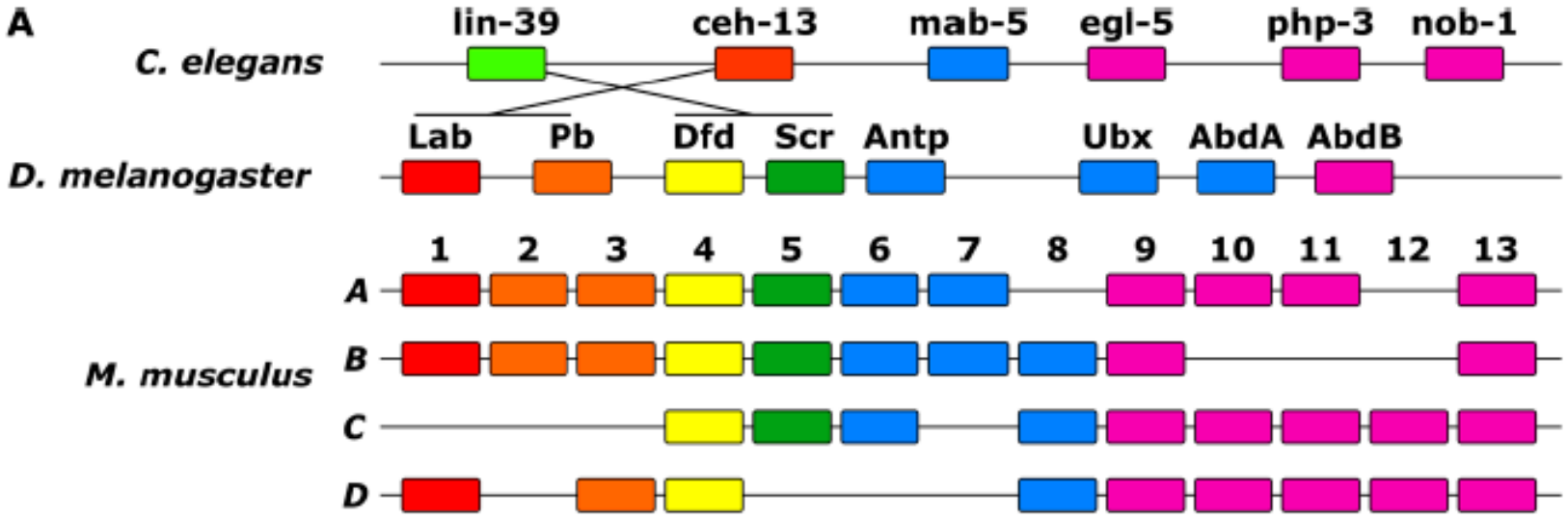
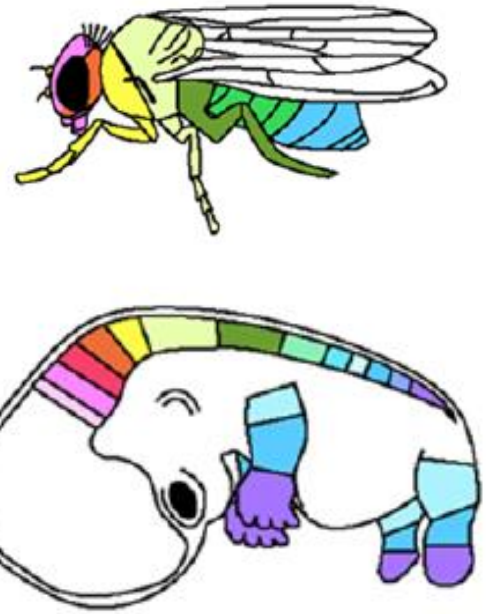
Hox proteins

- Transcription factors: development master regulators
- Confer identity to the anteroposterior (A-P) axis of metazoans

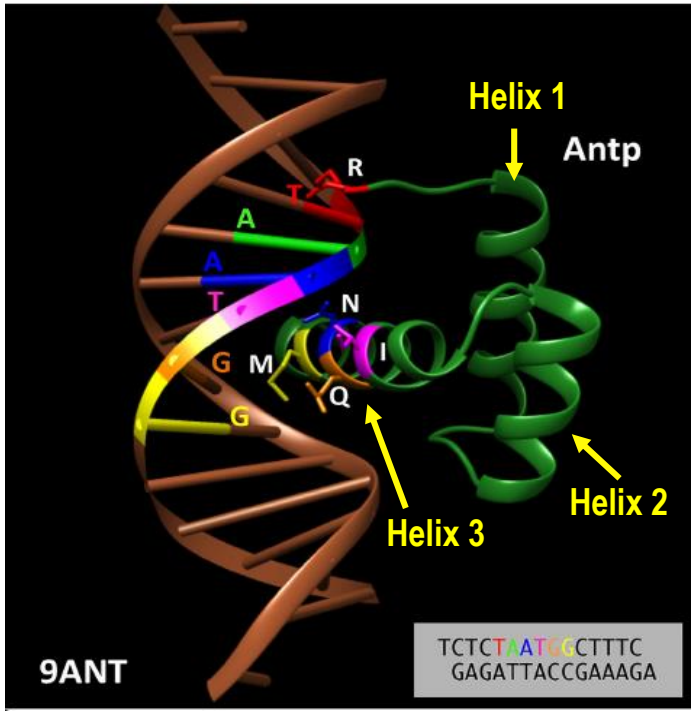




Lewis et al., 1978; Passner et al, 1999; Mann and Morata, 2000; Zandvakili y Gebelein, 2016; Ortiz-Lombardia et al., 2017

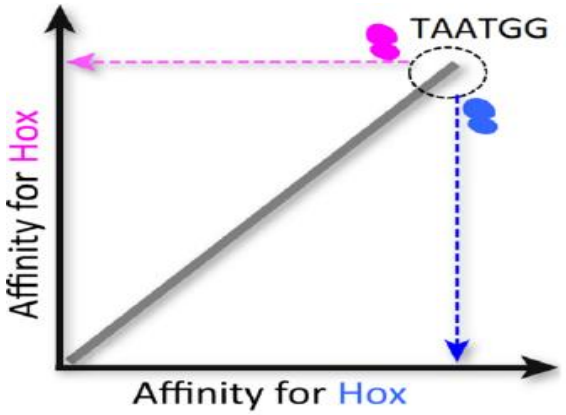
Functional specificity: the homeodomain

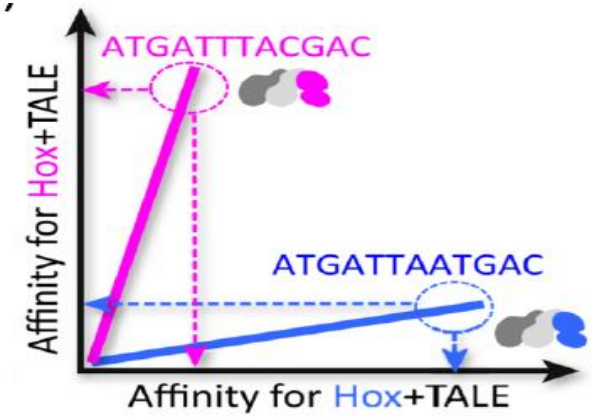
Highly conserved homeobox that codifies the homeodomain (HD)



If the HDs and their DNA binding sites are highly conserved, how homeoproteins specifically modulate target genes during embryo development?

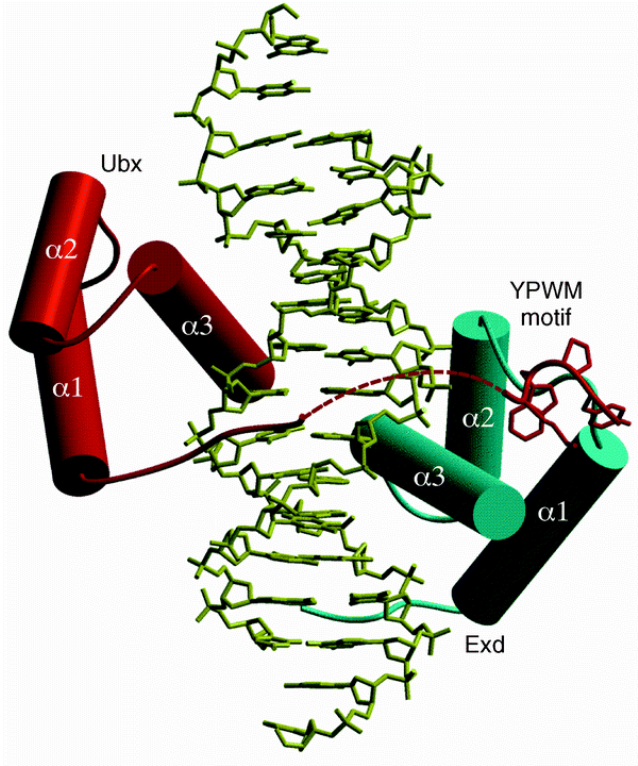
→ **Hox paradox**





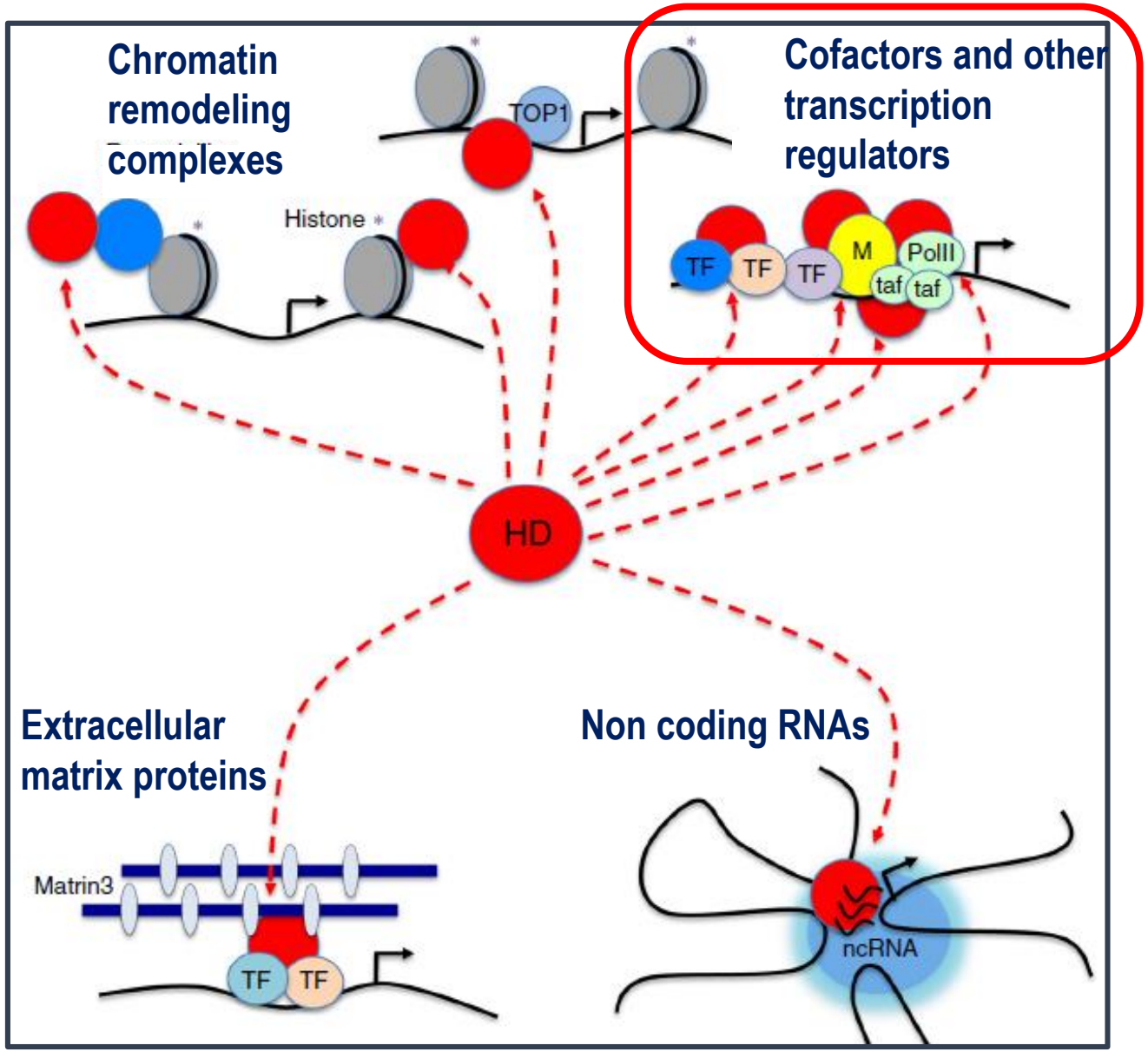
Gehring *et al*., 1990; Otting *et al.*, 1990; Qian *et al*., 1994; Hueber & Lohman, 2008; Ortíz-Lombardia, 2017

Hox protein-protein interactions



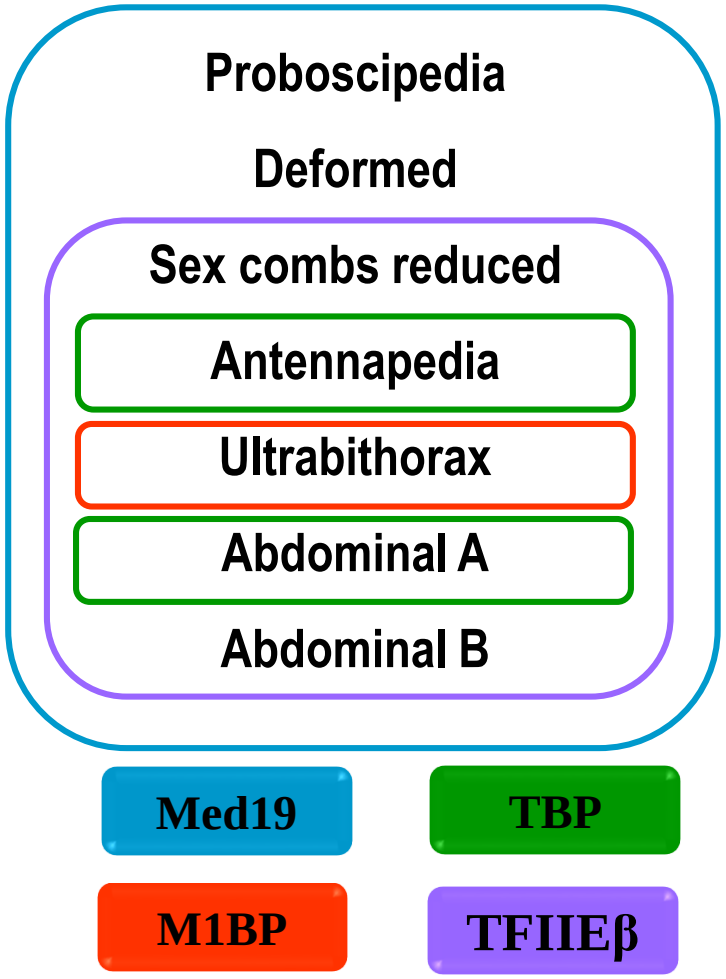
Homeoproteins interact with cofactors such as Exd for functional specificity.

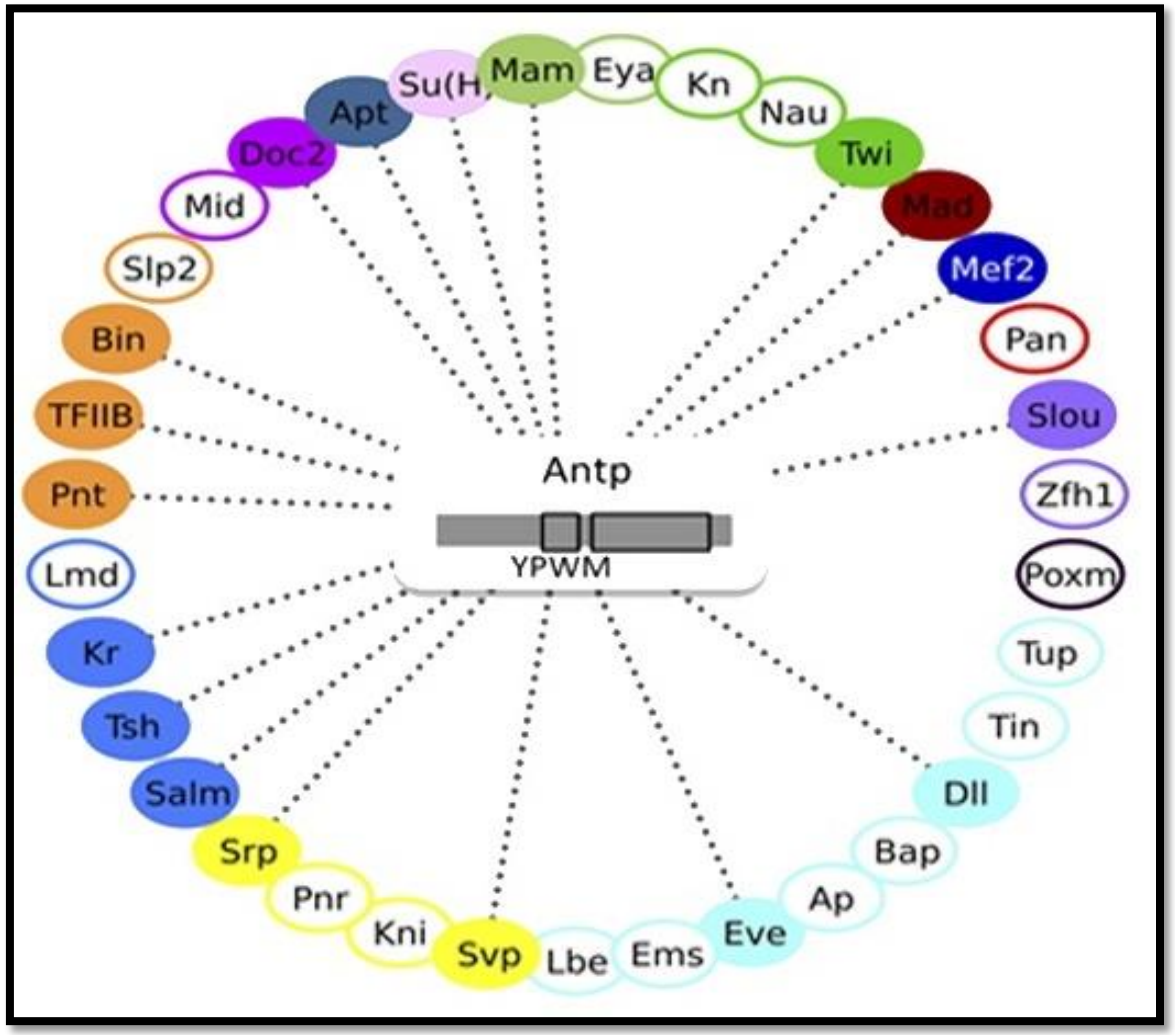
Many other interactors have been described “Hox interactome” (Baëza et al., 2015).



Passner et al., 1999; Bobola and Merabet, 2017

Hox transcription interactome towards development comprehension





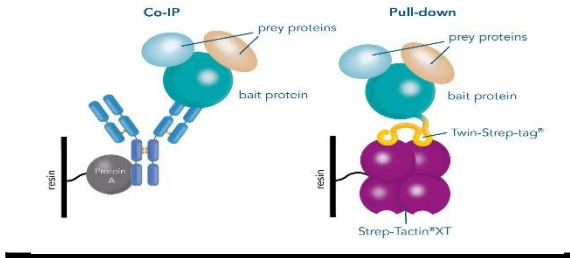
Boube *et al.*, 2014; Baëza *et al.*, 2015; Zouaz *et al.*, 2016 ; Altamirano-Torres et al., 2018

Analysis of protein-protein interactions (PPIs)

PPIs are key to cellular processes and function:

- ✓ Modify the kinetic properties of enzymes
- ✓ Construct a new binding site for small effector molecules
- ✓ Inactivate or suppress a protein
- ✓ Serve a regulatory role in either upstream or downstream level
- ✓ Change the specificity of a protein for its substrate through interaction with different binding partners

In vitro technics for PPIs analysis



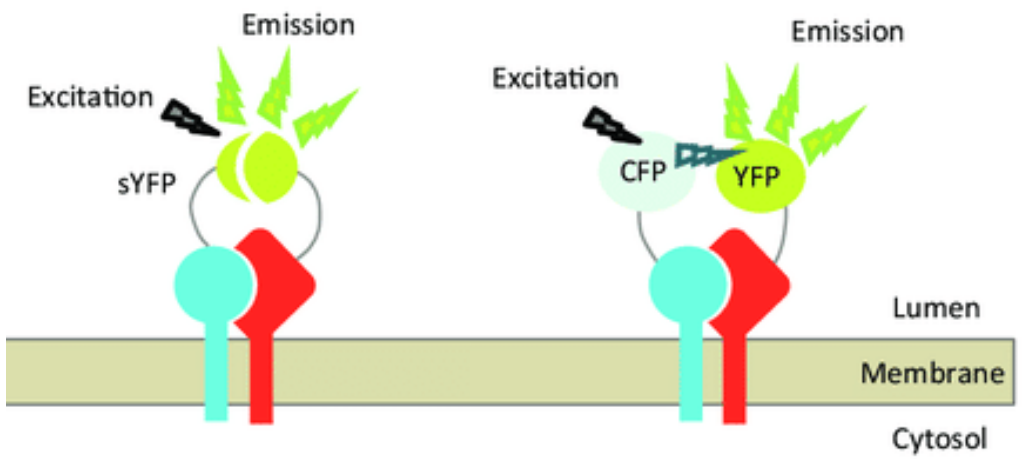
Rely on indirect detection of the PPI or require disruption of the cells, hence the compartment in which the PPI occurs cannot be determined.

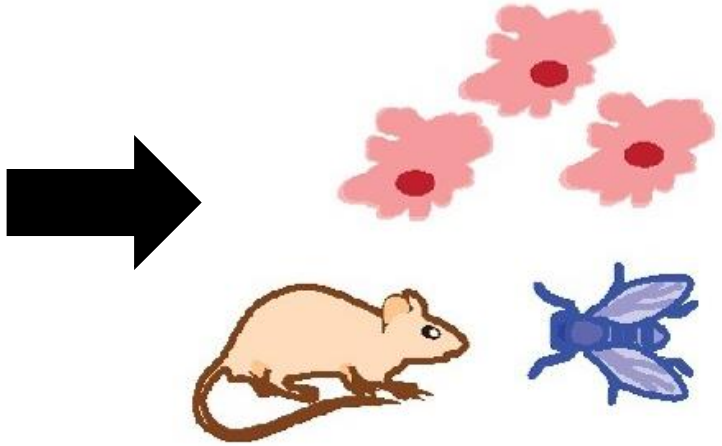
In vitro technique	Principle
Coimmunoprecipitation (Co-IP)	Target protein is recognized by a specific antibody immobilized to a support, which is then used to co-precipitate the antibody-protein complex from the cell lysate and identified by WB or MS
Pull-down assays	Glutathione S-transferase (GST)-tagged protein is captured by agarose beads. The immobilized protein captures its putative interacting partner from a cell lysate; the complexes are selectively eluted for WB analysis
Tandem affinity purification-mass spectroscopy (TAP-MS)	TAP-MS is based on the double tagging of the protein of interest on its chromosomal locus, followed by a two-step purification process and mass spectroscopic analysis.
Yeast two-hybrid (Y2H)	Monitor complex formation through transcriptional activation of reporter genes.

Fields and Song 1989; Rigaut et al., 1999; Nakatani and Ogryzko 2003; Brymora et al., 2004 ; Free et al., 2009

Fluorescent methods for PPIs detection

Detect protein-protein interactions in live conditions and visualize the location where they occur within the cell, tissue or organism, allowing us to better understand protein function *in vivo*.





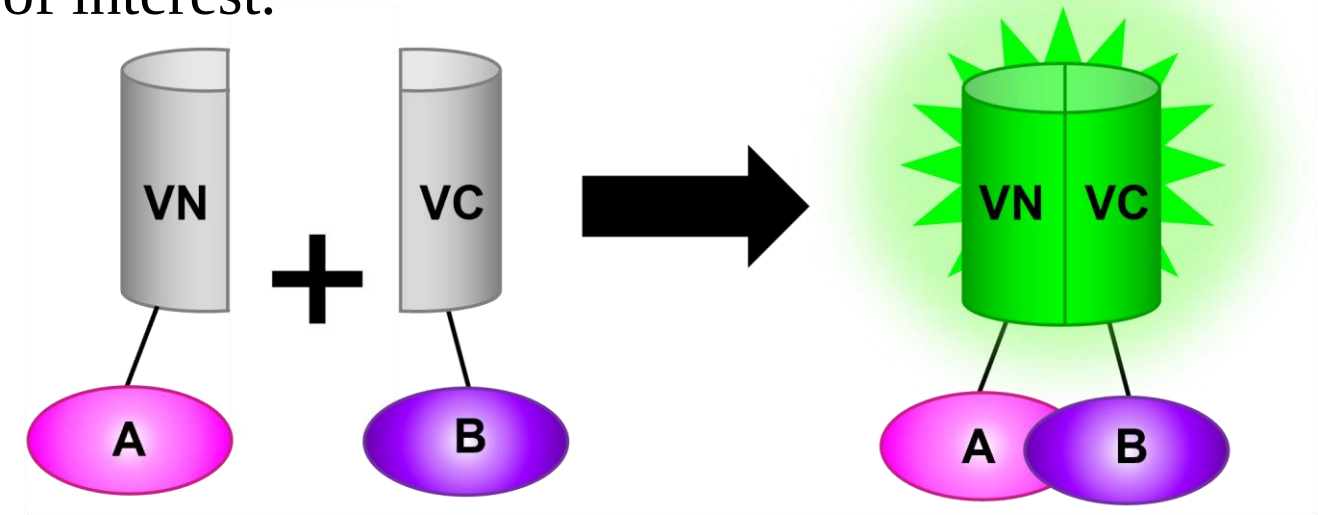
- Fluorescence anisotropy
- Fluorescence correlation spectroscopy (FCS)
- Fluorescence lifetime image microscopy (FLIM)
- Photonic crystal (PC) biosensors

- Bimolecular fluorescent complementation (BIFC)
- Forster resonance energy transfer (FRET)
- Competitive BiFC
- BiFC-FRET

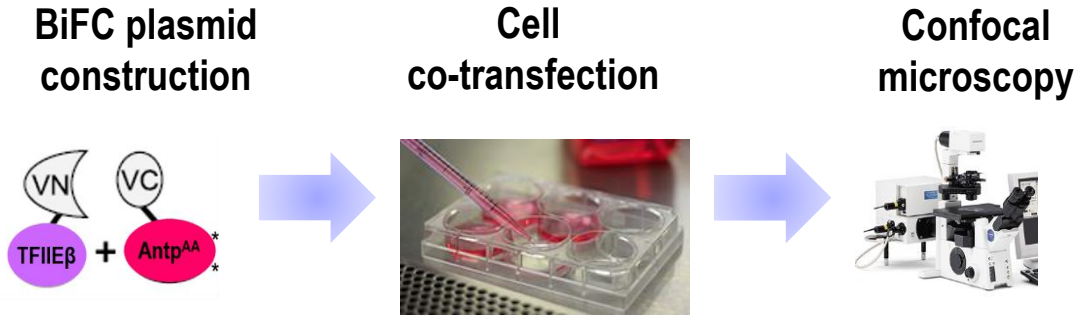
Delker *et al.*, 2022

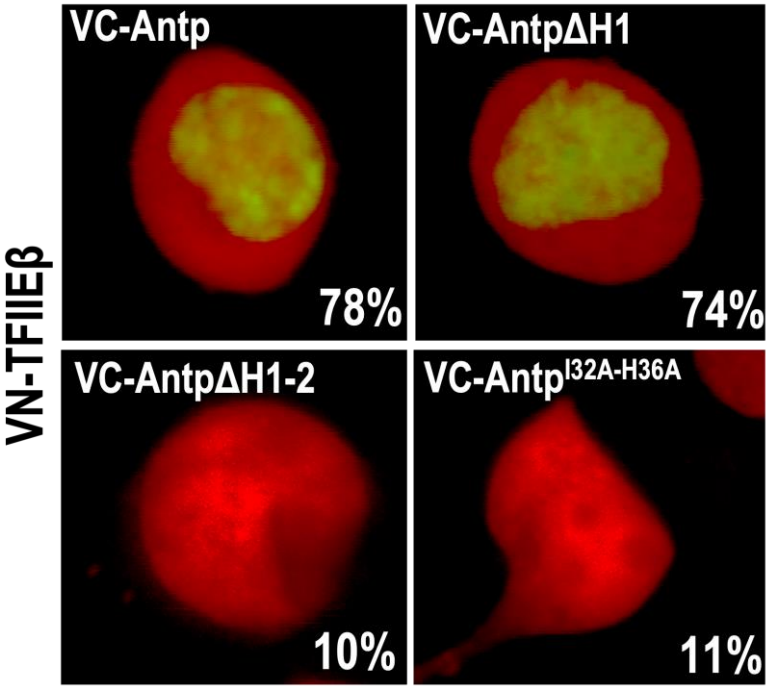
Bimolecular fluorescence complementation (BiFC)

Relies on a visible fluorescent signal given by the complementation between two fragments of a fluorescent protein that are each fused to a protein of interest.



The interaction between these proteins brings the fragments together and reconstitutes the fluorescence, indicating **protein-protein interaction**

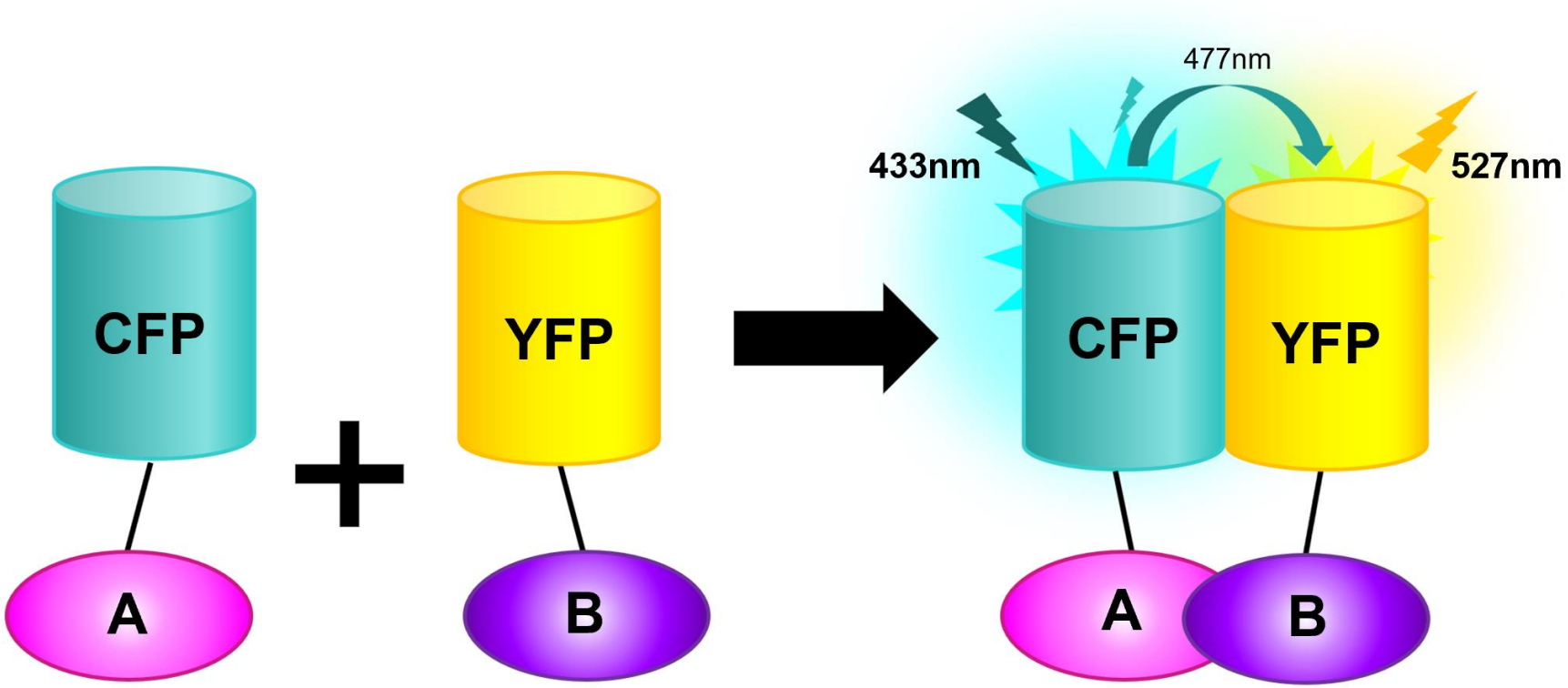




Hu *et al.*, 2002; Hu and Kerppola, 2003; Shyu *et al.*, 2006; Altamirano-Torres et al., 2018

Förster resonance energy transfer (FRET)

Detects the interactions *in vivo* by measuring the proximity between two fluorophores that are fused to proteins of interest, upon excitation.



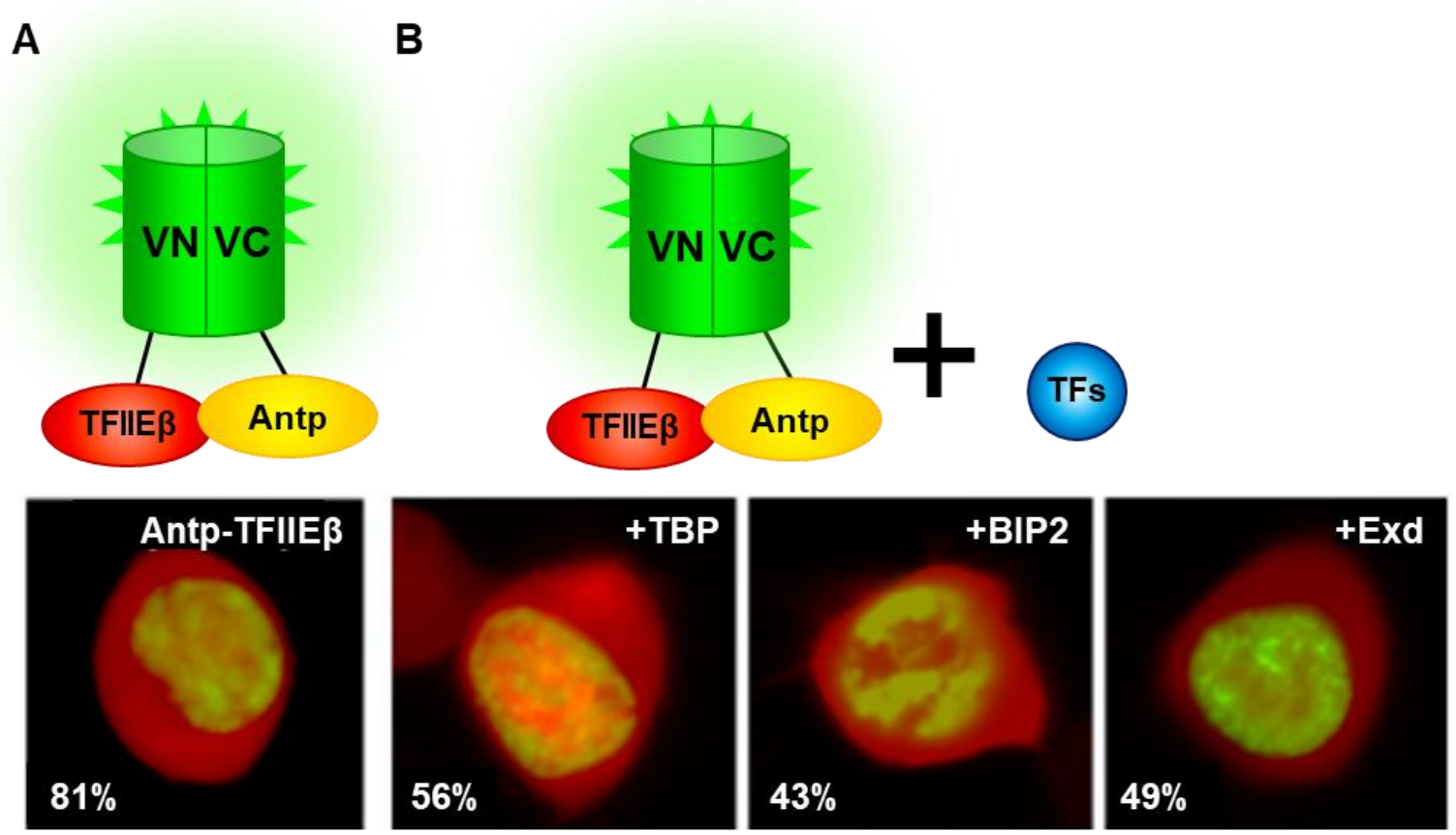
The energy transfer between a donor and an acceptor molecule should occur within a small distance (less than 10 nm) to validate the proximity between interest proteins and hence the interaction between them.

Shyu, *et al.*, 2006, Truong & Ikura, 2001, Parsons *et al.*, 2004, Kenworthy, 2001

Competitive BiFC

Can be used to detect possible candidates for trimeric interactions.

Here a “cold” competitive partner (not fused to a fluorescent protein fragment) is co-expressed with proteins fused to the VC and VN of the fluorophore, expecting a decrease in dimeric interaction.

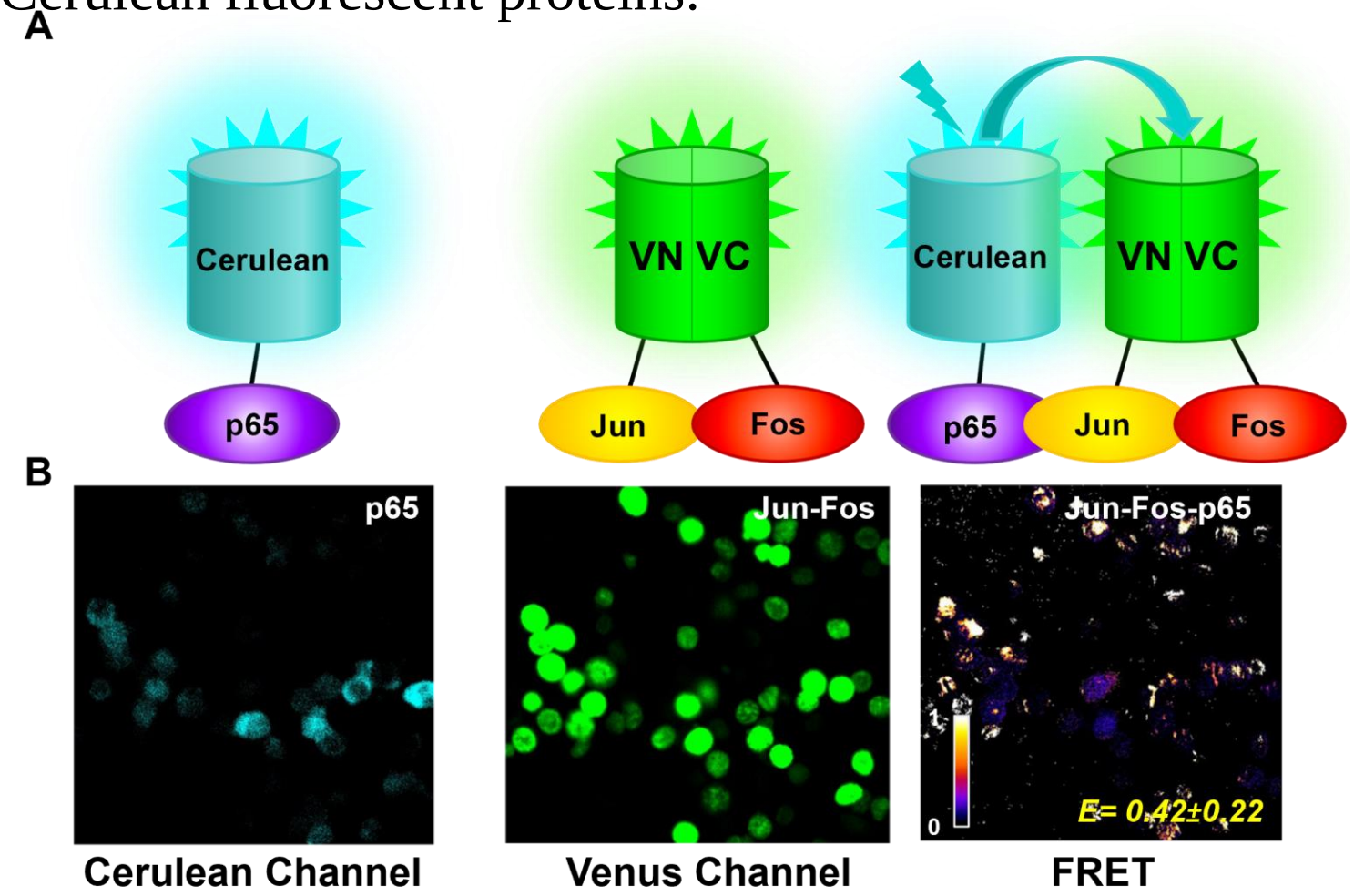


Baëza et al., 2015

BiFC-FRET

In living cells, trimeric complexes can be detect using a combination of BiFC and FRET (BiFC-FRET) using Venus and Cerulean fluorescent proteins.

BiFC allows reconstitution of a fluorescent protein (with proteins of interest fused to the fragments) and can be used as a donor, plus Cerulean fused to another protein used as an acceptor.



Shyu et al., 2008

Conclusions and perspectives

- Proteins rarely act alone, basically, all biological process at the cellular and organism levels require protein-protein to achieve their biological functions.
- Methods like co-immunoprecipitation, pull-down assays, BiFC, FRET and BIFC can be used to analyze the PPIs and understand the hox interactome.
- New fluorescent techniques have the advantage of verify the interactions in living cells and further analyze them in whole in model organisms to elucidated the functional implications of these interactions *in vivo.*
- PPIs complexes should be analyzed in an adequate biological context to determine their essential role to build and analyze interactome networks in cells and even whole organisms.

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