

Bimolecular Fluorescence Complementation (BiFC): Principle and Applications

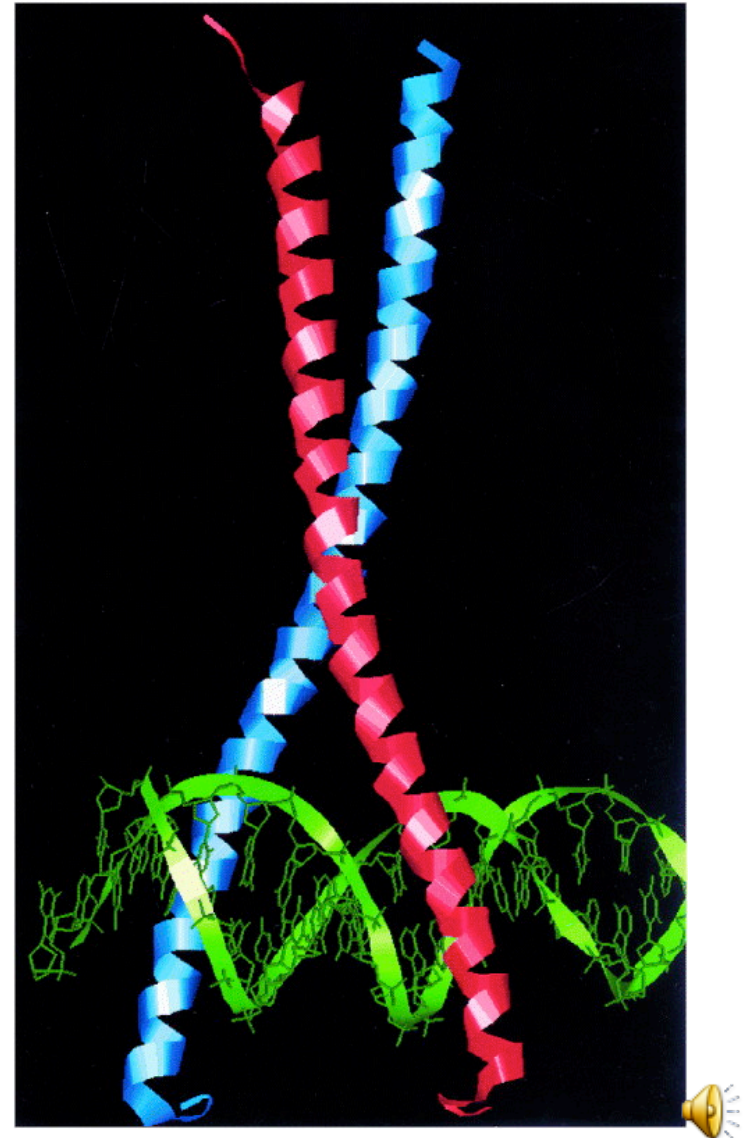
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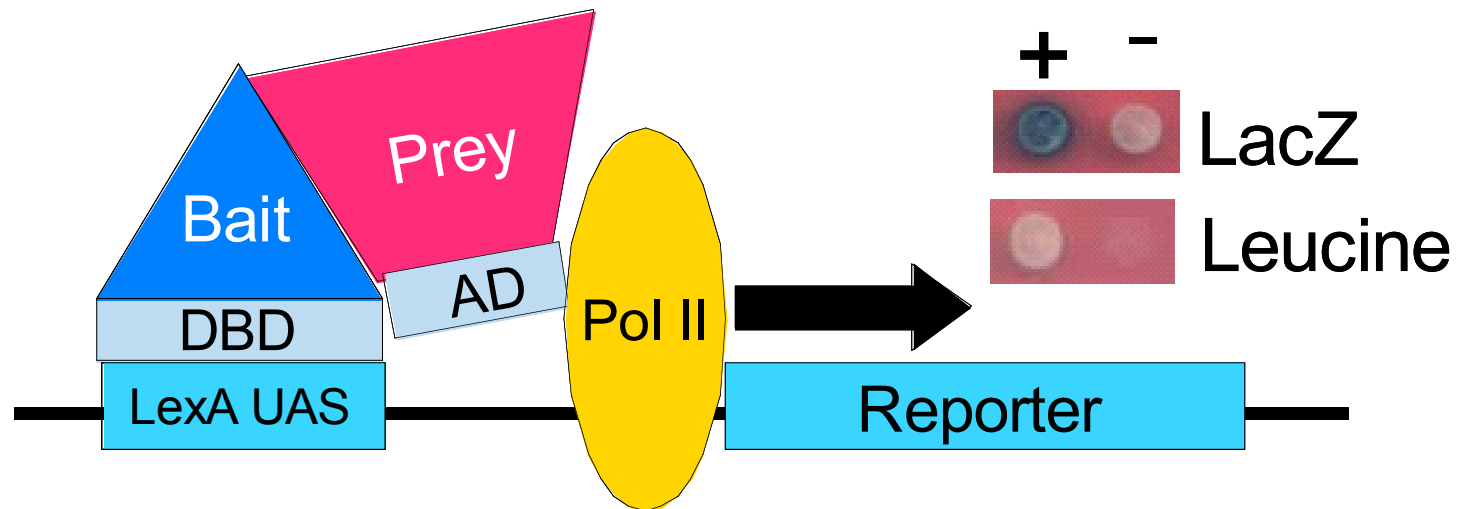


Protein-Protein Interactions

- Protein-protein interactions and interactions between proteins and other macromolecules are necessary for cell survival
- Identifying interactions between proteins involved in common cellular functions provides us with a broad view of how they work cooperatively in a cell



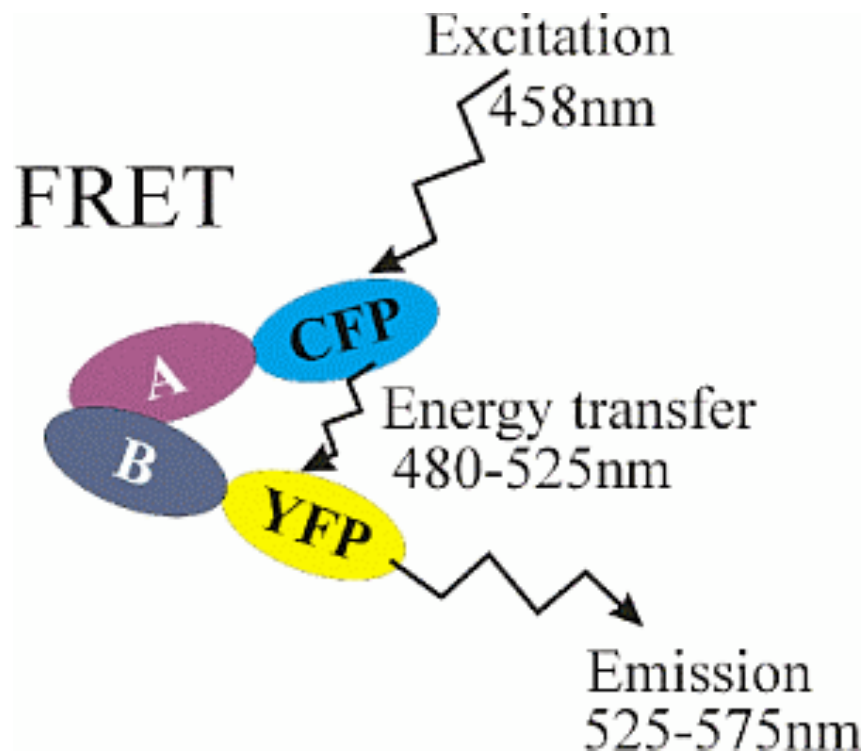
Identifying Protein-Protein Interactions: Yeast-Two Hybrid



- Detects pairwise and transient interactions between proteins
- Inexpensive and eukaryotic *in vivo*
- Requires overexpression of proteins
- Must verify interactions by other biochemical approaches
- Fusion proteins must localize to nucleus



Identifying Protein-Protein Interactions: FRET



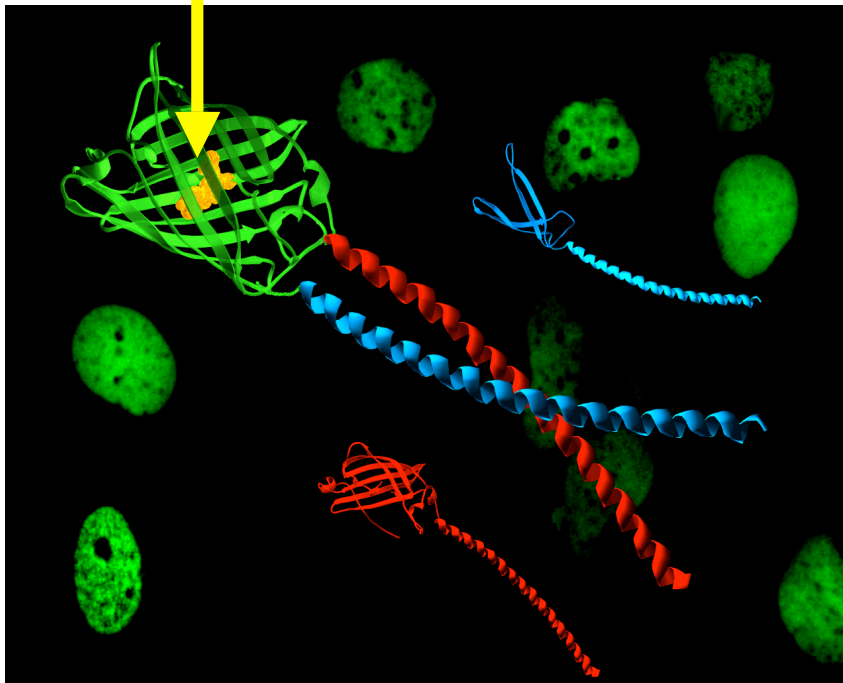
Fluorescence Resonance Energy Transfer

- Tool to quantify molecular dynamics
- Fluorophores must be in close proximity
- Necessitates overexpression of fusion proteins
- Requires irreversible photo-bleaching
- Compromised by photochemical transformations of GFP



Bimolecular Fluorescence Complementation (BiFC)

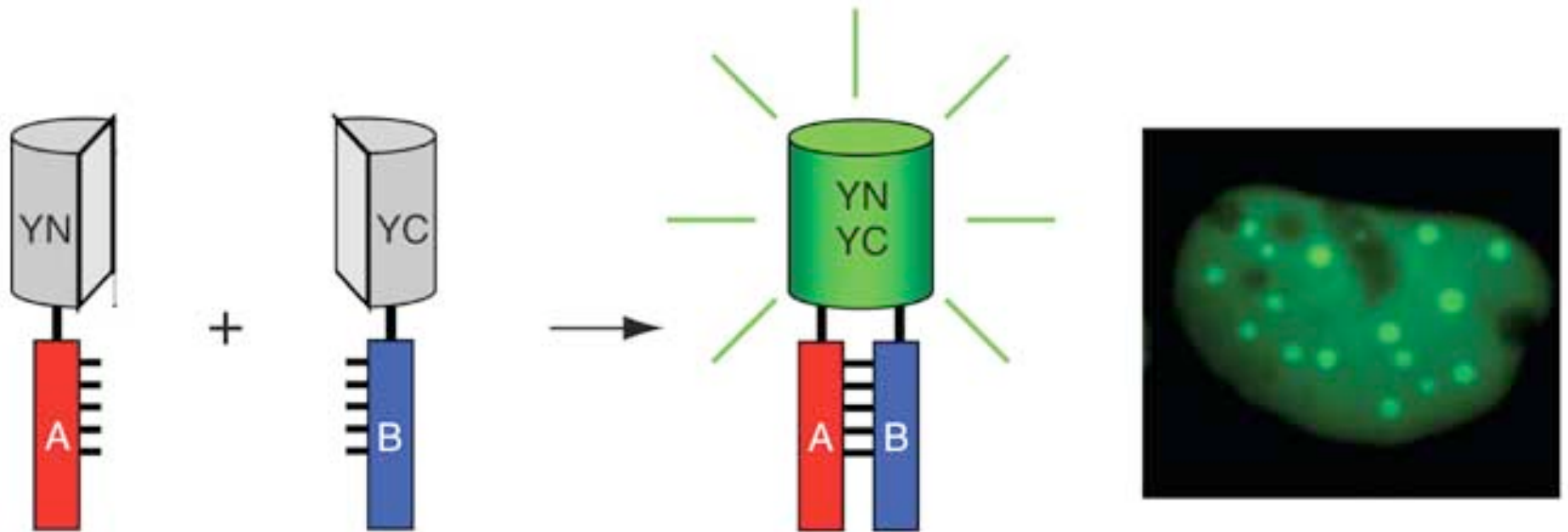
Fluorescence!



- New method for investigating and directly visualizing protein-protein interactions in living, mammalian cells
- Based on formation of a fluorescent complex through the association of two fragments of a fluorescent protein when they are brought together by an interaction between proteins fused to the fragments



Bimolecular Fluorescence Complementation (BiFC)



- Two fragments (YN and YC) of the yellow fluorescent protein (YFP) are fused to two putative interaction partners (A and B)
- An interaction between the proteins facilitates association between the fragments to produce a bimolecular fluorescent complex
- YN and YC do not interact on their own



Bimolecular Fluorescence Complementation (BiFC): Pros

- Enables direct visualization of protein interactions and does not rely on their secondary effects
- Interactions can be visualized in living cells, eliminating potential artifacts associated with cell lysis or fixation
- Proteins are expressed in a relevant biological context, ideally at levels comparable to their endogenous counterparts
- Enables determination of the subcellular sites of protein interactions
- BiFC assay does not require stoichiometric complex formation but can detect interactions between subpopulations of each protein
- BiFC does not require specialized equipment, apart from an inverted fluorescence microscope equipped with objectives that allow imaging of fluorescence in cells

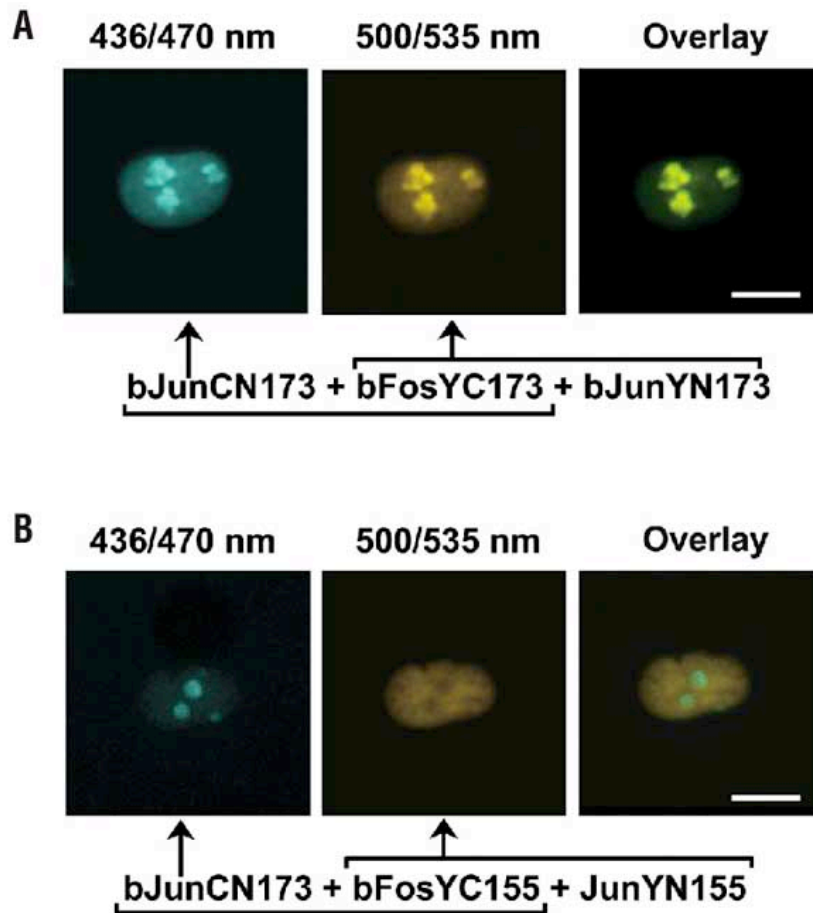


Bimolecular Fluorescence Complementation (BiFC): Cons

- Slow, irreversible formation of fluorescent complex limits use of assay for studying rapid changes in protein interactions
- Association of fragments of fluorescent protein does not require specific distance of interacting partners but does require that the linkers that tether them to the interaction partners have sufficient flexibility to enable them to associate
- Fluorescence complementation does not establish that fusion proteins interact directly, but that they are in the same complex in the cell



Multicolor BiFC



- Many proteins have alternative interaction partners in each cell, many are mutually exclusive and result in competition for shared interaction partners in the cell
- Based on complementation between fragments of fluorescent proteins with different spectral characteristics
- Allows simultaneous visualization of multiple protein complexes in the same cell
- Enables analysis of the competition between alternative interaction partners for complex formation

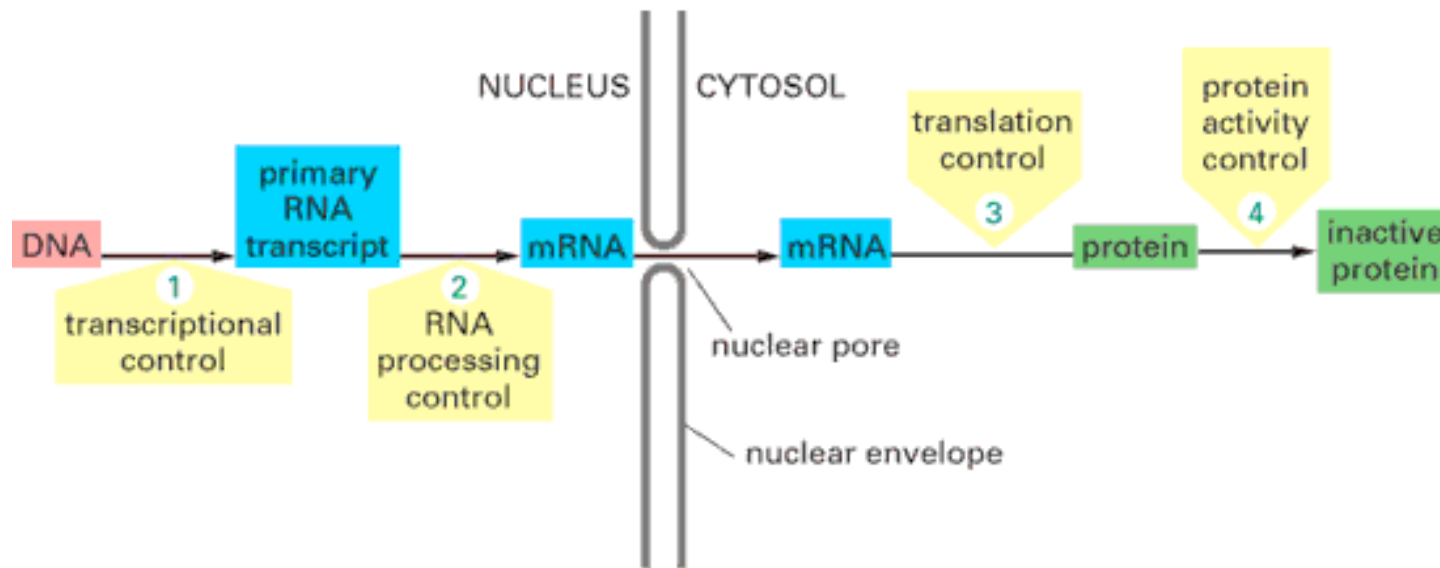


Making BiFC Better

- YPF chromophore maturation sensitivity to higher temperatures requires preincubation at lower temperatures prior to visualization
- Researchers identified new fluorescent protein fragments derived from **Venus** and **Cerulean**
- New combinations exhibit a 13-fold higher BiFC efficiency
- Reduces amount of plasmid required for transfection
- Shortens incubation time → 2-fold increase in specific BiFC signals



Regulation of Gene Expression

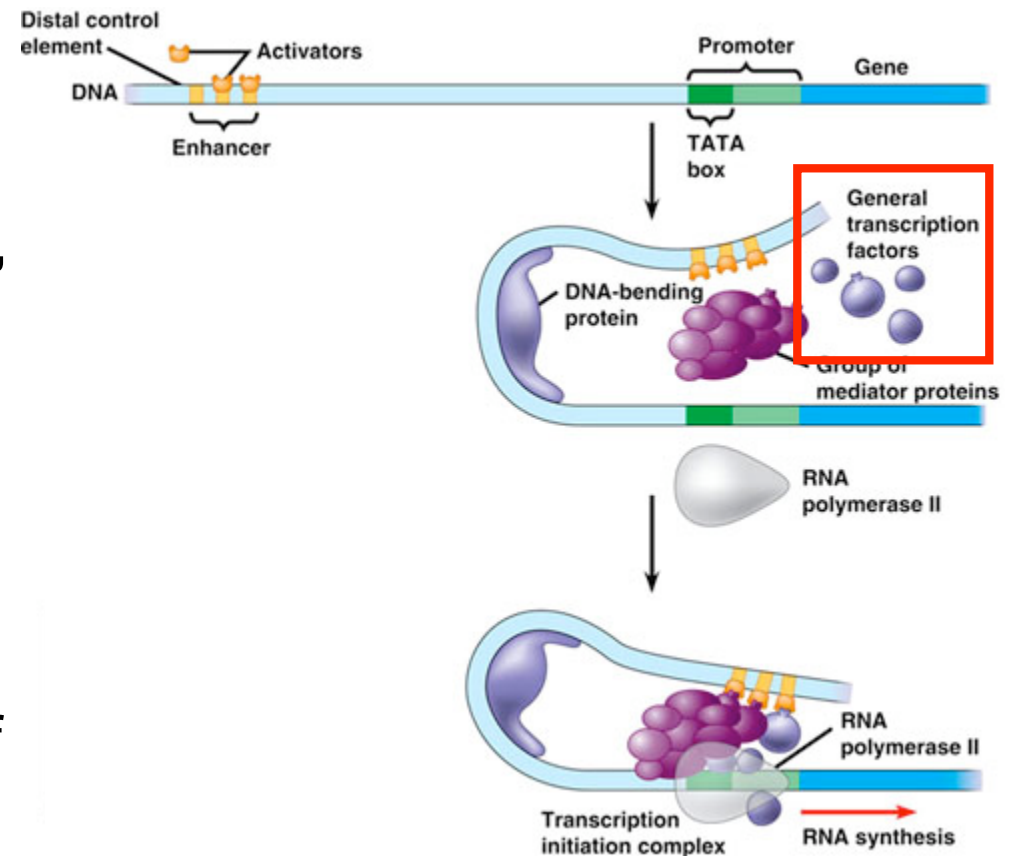


- Gene expression is the process by which RNA is transcribed from DNA and then translated into the structures and functions of a cell (i.e. proteins)
- Regulation of gene expression is the cellular control of the amount and timing of the appearance of the functional gene product
- Gene regulation is tightly monitored by several specificity factors, activators, and repressors and gives the cell control over structure and function



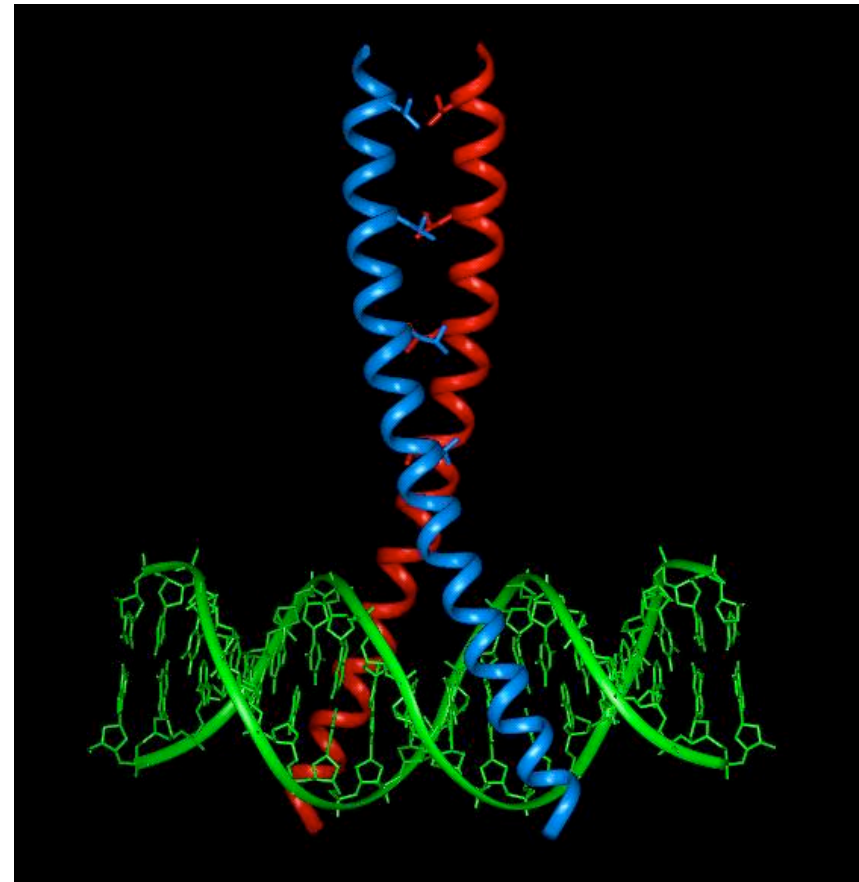
Using BiFC to Study Transcription Regulatory Proteins

- Transcriptional regulation involves combinatorial interactions between several transcription factors, which allow for a sophisticated response to multiple conditions in the environment
- BiFC analysis has been crucial to understanding the hierarchy of determinants of subcellular localization in different transcription factor families



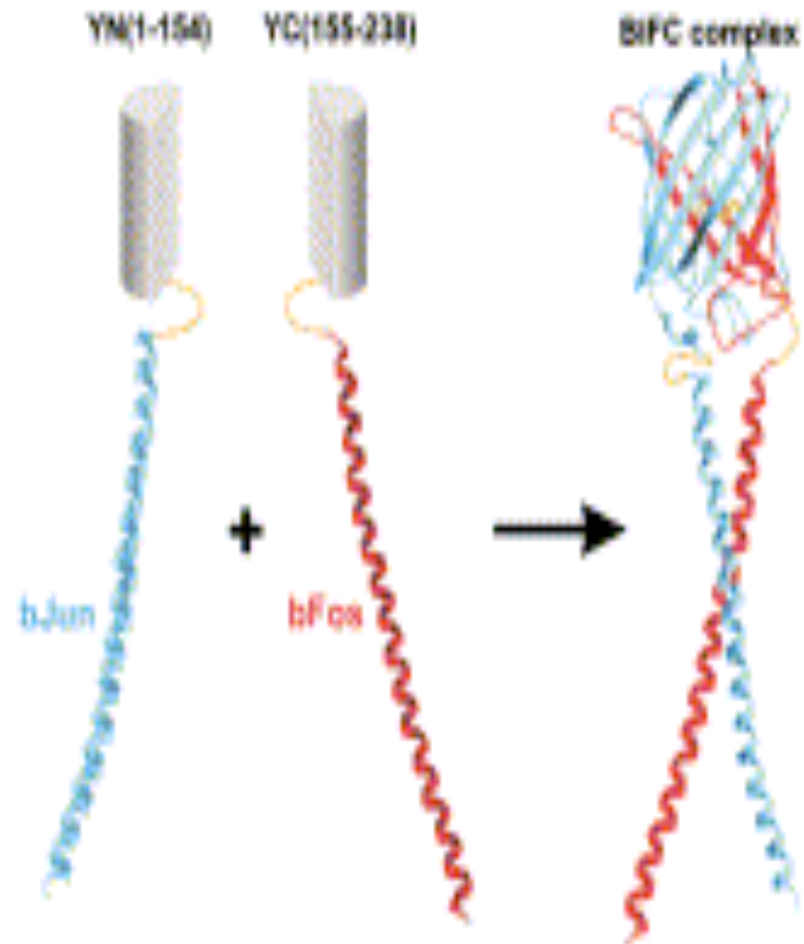
Leucine Zipper Motifs

- The leucine zipper motif is a structure made up of two α -helical segments of protein that have leucines facing each other along the length of the helices, allowing them to dimerize and form a symmetric interface that can bind to the DNA on both sides of the double helix
- The leucine zipper motif is present in three oncogene products: Fos, Jun and Myc, which all act as transcription factors as part of the AP-1 complex in yeast

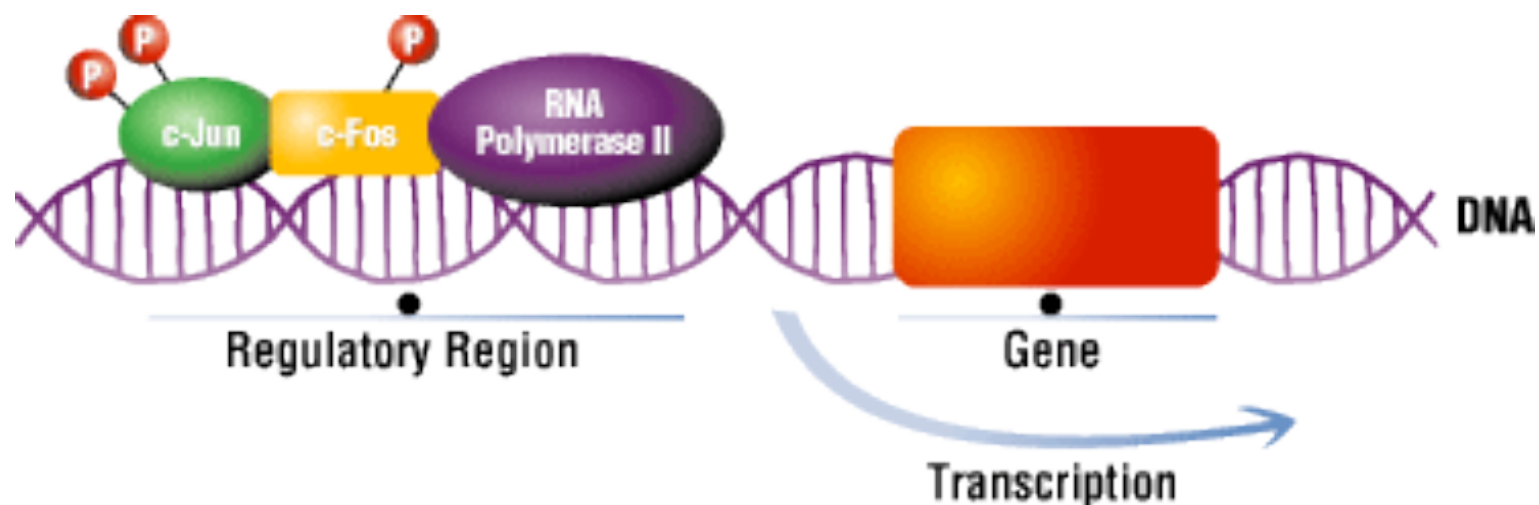


Leucine Zipper Motifs

- Leucine zipper regulatory proteins are important regulators of normal development
- If they are overproduced or mutated in a vital area, they may generate cancer
- These proteins interact with the DNA as dimers (homo- or hetero-) and are also called basic zipper proteins (bZips)



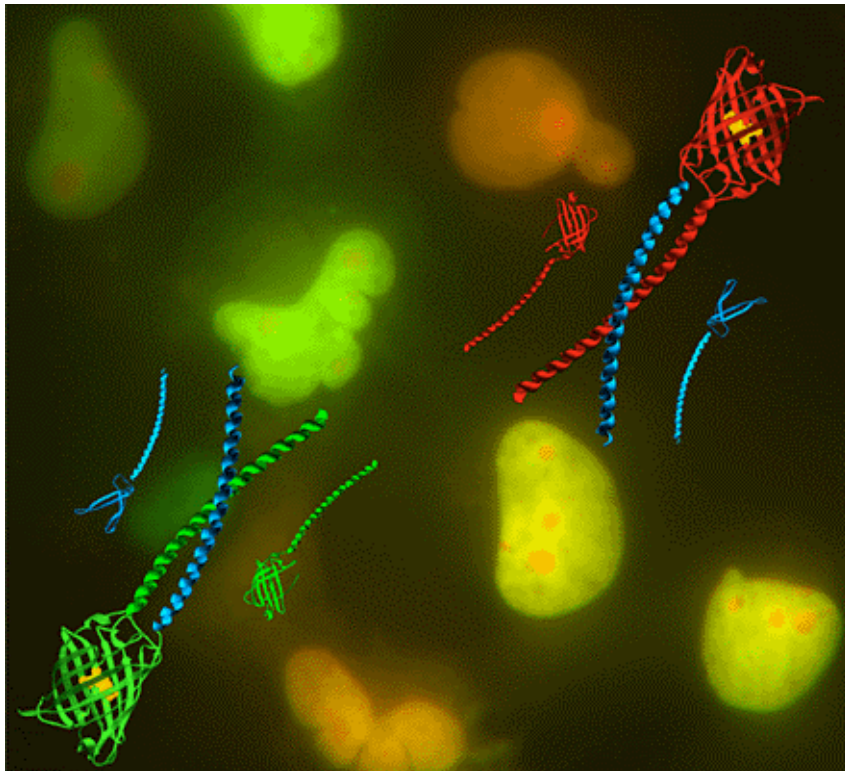
Activator Protein-1 (AP-1)



- AP-1 belongs to the basic region leucine zipper (bZIP) family of transcription factors and functions as homodimers or heterodimers formed among the members of Fos, Jun, ATF2 and Maf family of proteins to regulate gene expression
- AP-1 binds to DNA sequences (TRE: transcription response element) in the promoter region of many genes
- AP-1 activity can be induced by both physiological stimuli and environmental stresses, thereby regulating a wide range of cellular processes including cell proliferation, differentiation, death, and stress responses
- Deregulated AP-1 activity is implicated in many human diseases including cancer



Multicolor BiFC and bZIP Family



- The fluorescence of complexes formed by full-length Jun with the bZIP domain of Fos is shown in green
- The fluorescence of complexes formed by the bZIP domain of Jun with the bZIP domain of Fos is shown in red
- The complexes exhibit distinct subnuclear distributions in the same cells



Application of BiFC: Determining Subcellular Localization

- ATF2 (Activating Transcription Factor 2) belongs to the bZIP family and is a member of AP-1
- c-Jun, a proto-oncoprotein, is a major dimerization partner of ATF2
- c-Jun-ATF2 heterodimers are responsible for activating target genes involved in stress response, including *c-jun*



Application of BiFC: Determining Subcellular Localization

- Researchers used BiFC to present evidence that ATF2 monomers and ATF2 homodimers are localized predominantly in the cytoplasm
- ATF2 possesses a nuclear export signal (NES) in its leucine zipper region and two nuclear localization signals (NLS) in its basic DNA-binding region
- These signals contribute to the shuttling of the protein between the cytoplasm and the nucleus
- Dimerization with c-Jun in the nucleus prevents export of ATF2 and is essential for transcriptional activation of the *c-jun* promoter
- ATF2 and c-Jun mutually regulate each other by altering the dynamics of subcellular localization and by positively impacting transcriptional activity



Bibliography

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