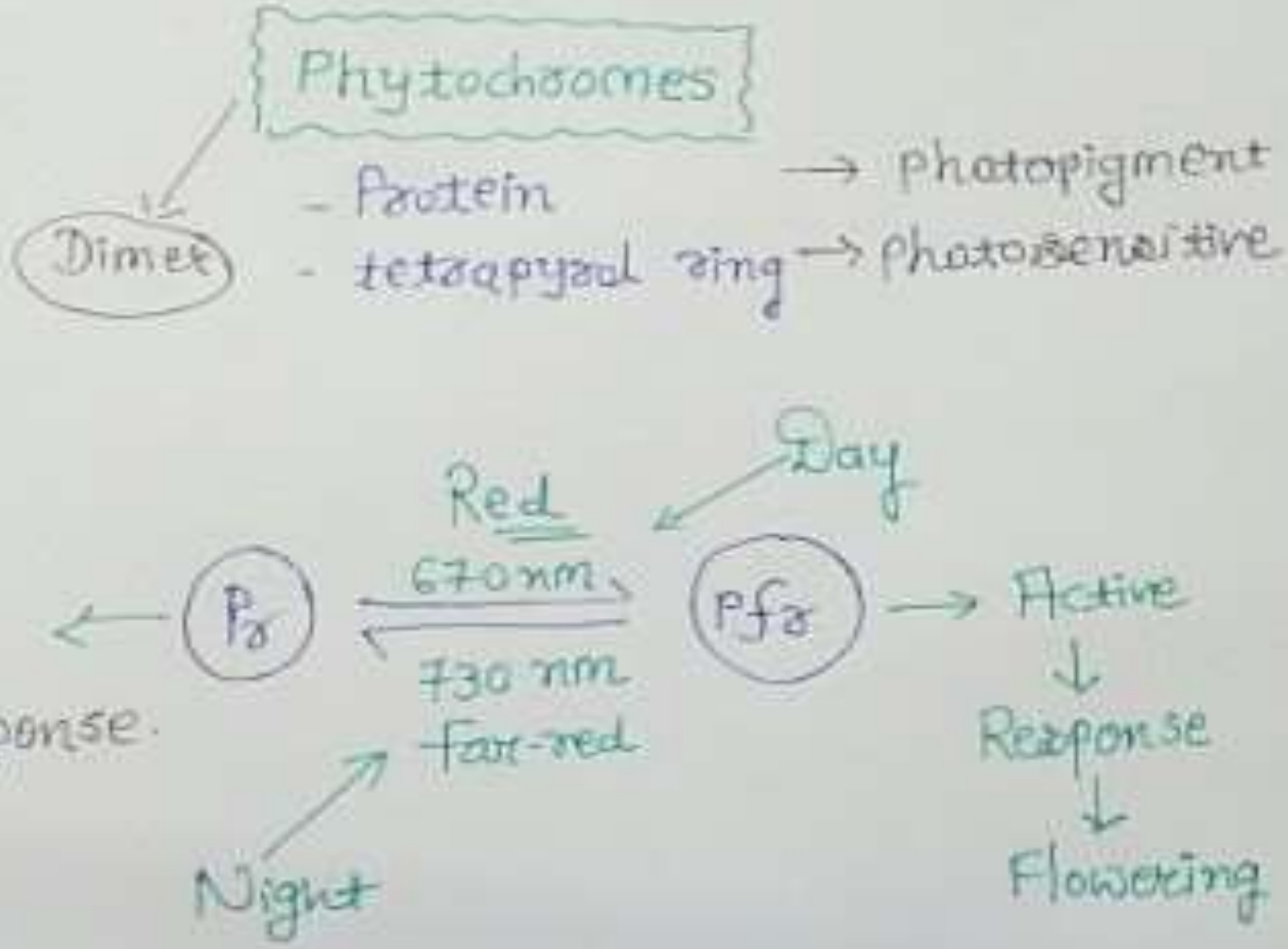
PHYTOCHROME AND CRYPTOCHROME

CONTENTS

PHYTOCHROME

INTRODUCTION



- Phytochrome is a pigment found in some plant cells that has been proven to control plant development.

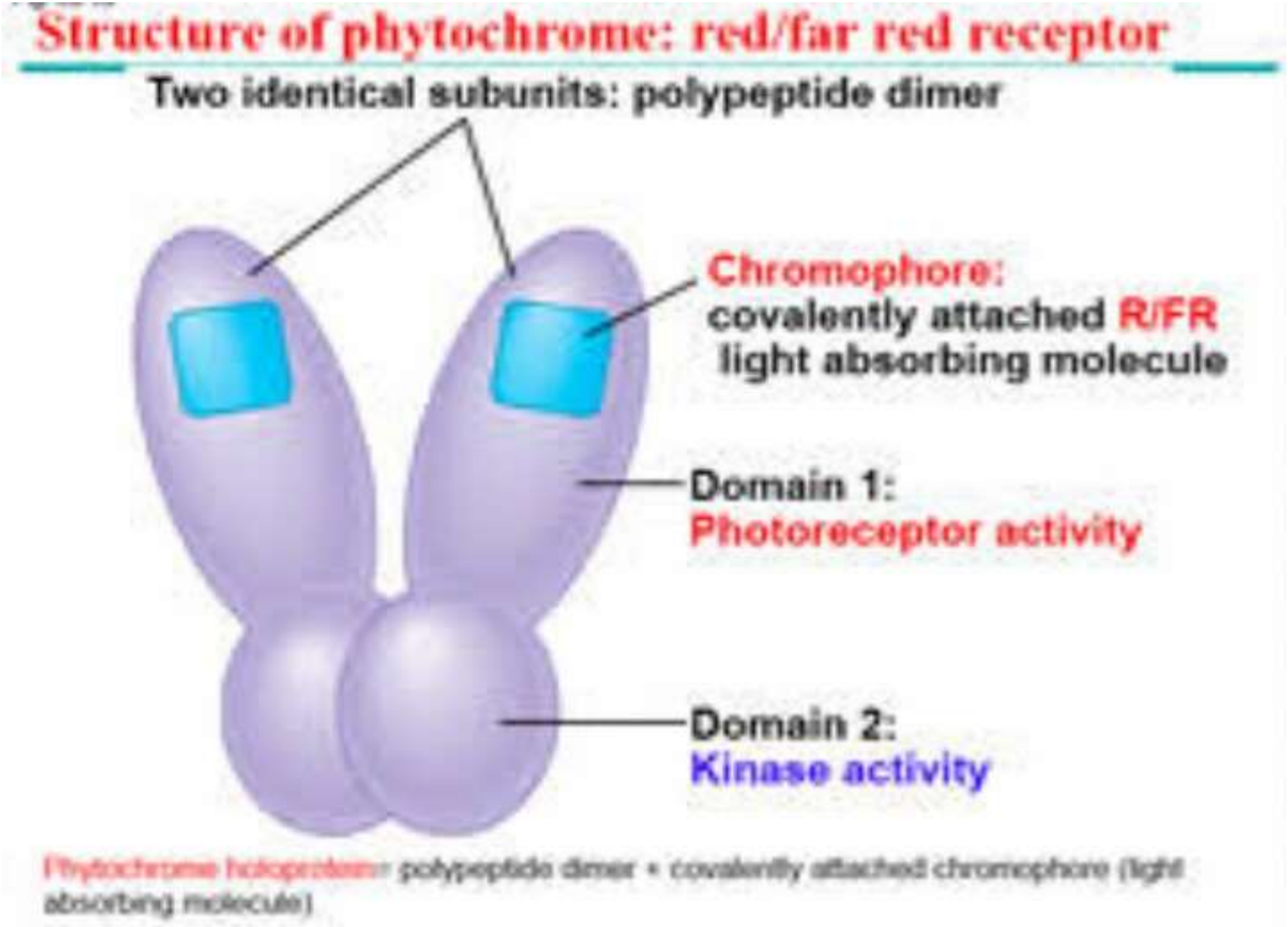
- This pigment has two forms or “phases” in can exist in p-red light sensitive (pr) and p-far red light sensitive (pfr) forms.

- The actual plant response is very specific to each species and some plants do not respond at all.

- Many flowering plants use it to regulate the time of flowering based on the length of day and night and set circadian rhythms.

- Biochemically, phytochrome is a protein with a bilin chromophore.

STRUCTURE


Structure of phytochrome: red/far red receptor
Two identical subunits: polypeptide dimer
Chromophore:
covalently attached R/FR
light absorbing molecule
Domain 1:
Photoreceptor activity
Domain 2:
Kinase activity
Phytochrome holoprotein = polypeptide dimer + covalently attached chromophore (light absorbing molecule)

- Phytochrome consists of two identical chains. Each chain has a PAS domain and GAF domain.

- The PAS domain serves as a signal sensor and the the GAF domain is responsible for binding to cGMP and also senses light signals.

- Together, these subunits form the phytochrome region.

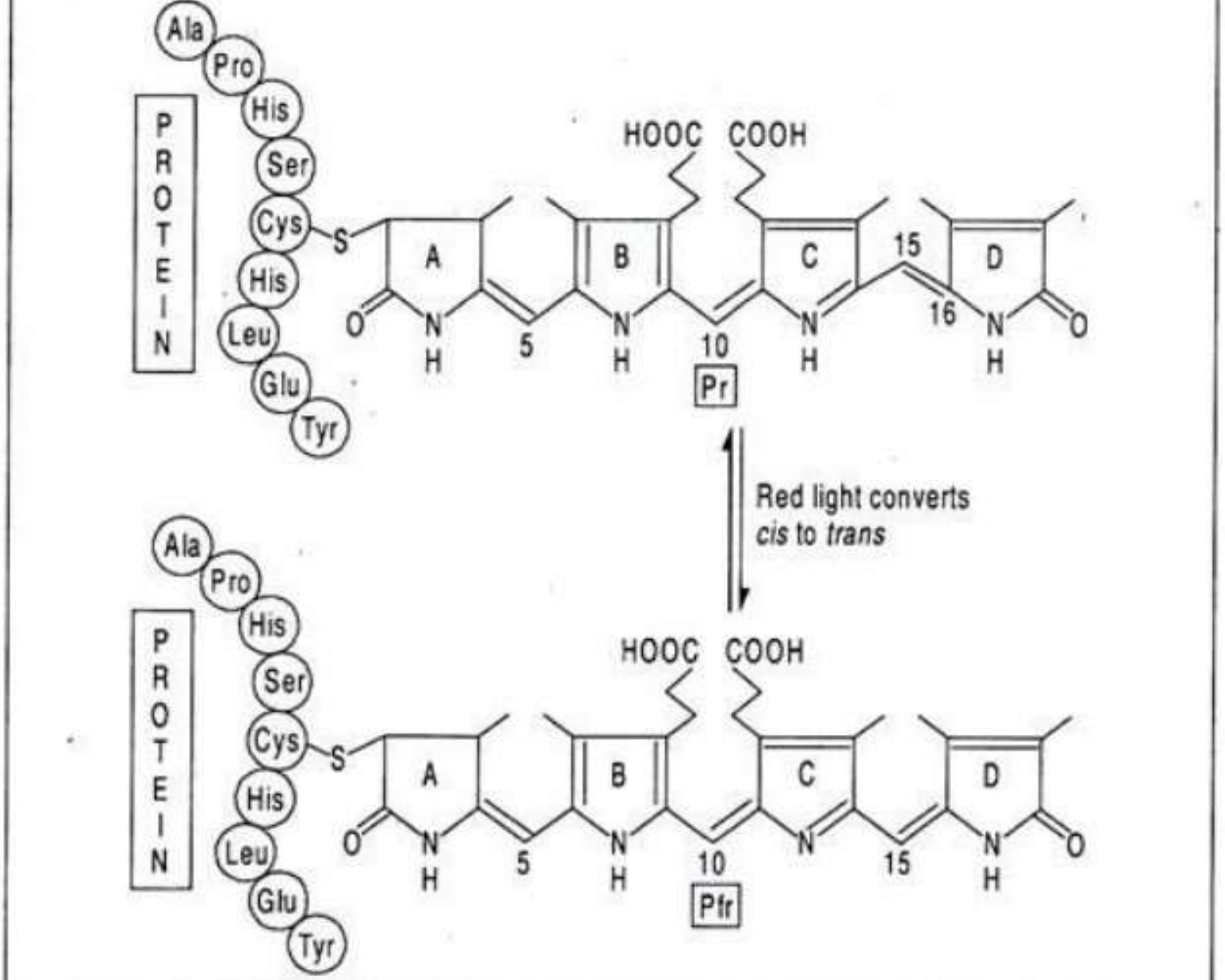


Fig. 14.2 : Structure of the phytochrome chromophore and its binding to the apoprotein. The chromophore is covalently linked to the protein at cysteine residue via a thioether bond. The chromophore undergoes *cis-trans* isomerization at carbon 15 in response to red and far-red light

HISTORY AND DISCOVERY

- The phytochrome pigment was discovered by Sterling Hendricks and Harry Borthwick at the USDA-ARS Beltsville Agricultural Research center in Maryland during a period from the late 1940s to the Early 1960s.

- they discovered that red light was very effective for promoting germination or triggering flowering responses.

- The red light responses were reversible by far-red light indicating the presence of a photoreversible pigment.

- The phytochrome pigment was identified using a spectro-photometer in 1959 by biophysicist warren Butler and biochemist Harold Siegelman.

- Butler was also responsible for the name, phytochrome.

- In 1983 the laboratories of Peter Quail and clark Lagarias reported the chemical purification of the intact phytochrome molecule, and in 1985 the first phytochrome gene sequence was published by Howard Hershey and peter Quail.

CHARACTERISTICS

- The action spectrum of the light needed for these responses shows a peak in the red at about 660 nm.

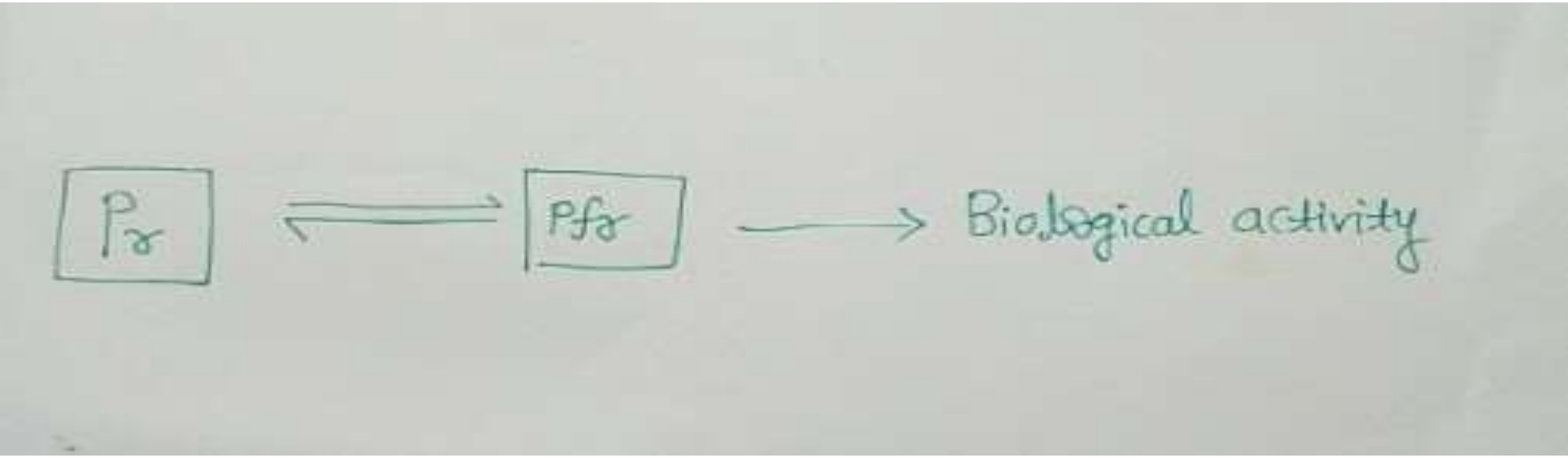
- These responses can be reversed by an application of far-red light soon after the red treatment.

- Sensitive spectrophotometers can measure a decrease in absorbance at 730 nm when sensitive plant tissues are exposed to red light.

- The change in absorbance is caused by the conversion of the photoreceptor from one structural form to another.

- The red-absorbing form changes to the far-red absorbing from when it absorbs red light and back again when it absorb far-red light.

- The phytochrome molecule is the photoreceptor for red light responses. It exists in two forms, Pr and Pfr.



❖The Pr form:

- Absorbs at a peak of 666 nm
- Is the form synthesized in dark-grown seedlings.
- When Pr absorbs red light, it is converted to the Pfr form.

❖The Pfr form:

- Absorbs at a peak of 730 nm.
- The Pfr form is the active form that initiates biological responses.
- When Pfr absorbs far red light, it converted to the Pr form in the dark overtime=dark reversion; Pfr is also susceptible to proteinases.

- Pfr absorbs some red light, so in red light, there is a balance of 85% Pfr and 15% Pr.

- Pr absorbs very little far red light, so in far red light, there is a balance of 97% Pr to 3% Pfr.

FUNCTION

- Many flowering plants use it to regulate the time of flowering based on the length of day and night and to set circadian rhythms.

- It also regulates other responses including the germination of seeds, elongation of seedlings, the size, shape and number of leaves, the synthesis of chlorophyll, and the straightening of the epicotyl or hypocotyl hook of dicot seedlings.

- It is not found in the leaves of most plant.

CRYPTOCHROMES

INTRODUCTION

- Cryptochrome is a class of flavoprotein that encompass a blue light driven reaction cycle.

- Photolyase and cryptochrome are functionally different, but possess similar photoactive domains.

- Cryptochrome is involved in the circadian clocks of plants and animals, and the sensing of magnetic fields in a number of species.

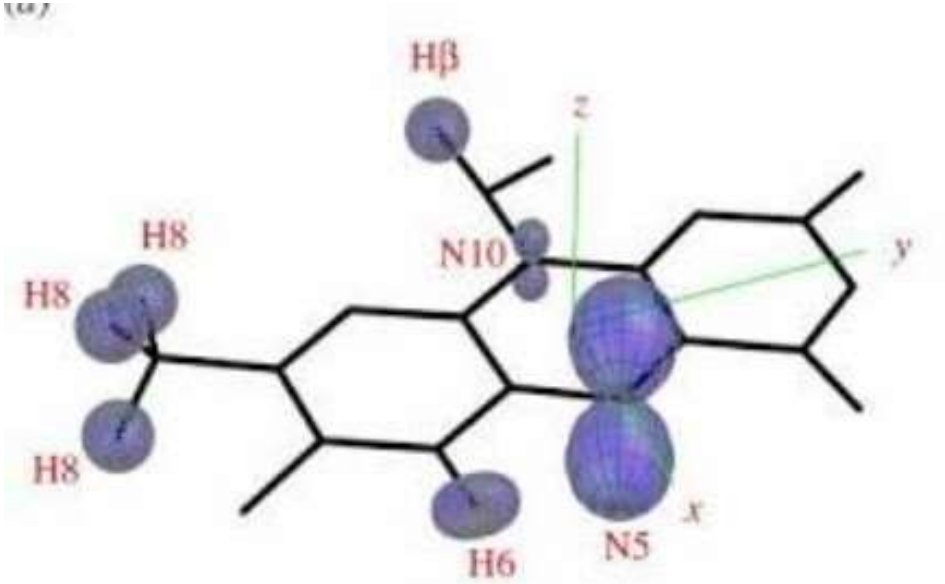
HISTORY AND DISCOVERY

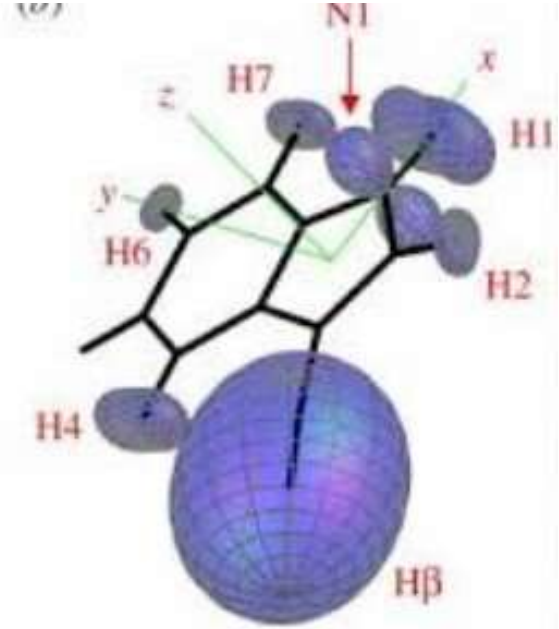
- Although Charles Darwin first documented plant responses to blue light in the 1800s, it was not until the 1980s that research began to identify the pigment responsible.

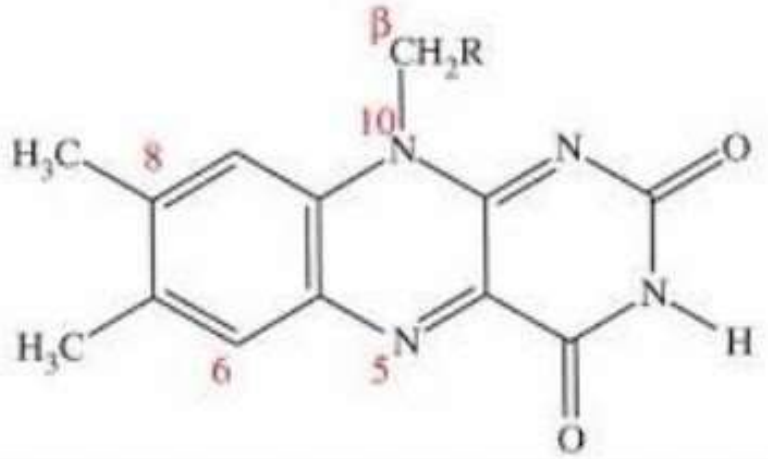
- By 1995, it became clear that the products of the HY4 gene and its two human homologous did not exhibit photolyase activity and were instead a new class of blue light photoreceptor hypothesized to be circadian photo pigments.

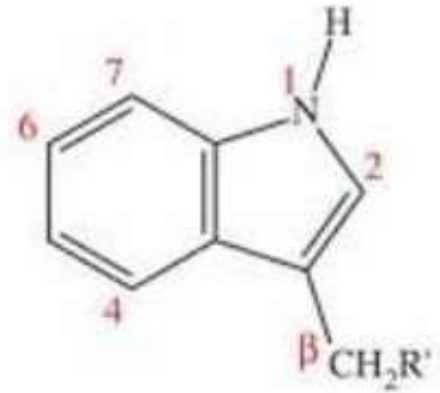
- In 1996 and 1998, cry homologous were identified in drosophila and mice, respectively.

STRUCTURE


Hβ
z
H8
H8
H8
N10
y
x
H6
N5


N1
H7
x
z
H1
y
H6
H2
H4
Hβ


β
CH2R
10
H3C
8
N
N
O
N
H3C
6
5
N
H
O


H
7
1
N
6
2
4
β
CH2R'

- The structure of cryptochrome involves a fold very similar to that of photolyase, with a singal molecule of FAD.

- These proteins have variable lengths and surfaces on the c-terminal end,.

- The Ramachandran plot shows that the secondary structure of the CRY1 protein is primarily a right-handed alpha helix with little to no steric overlap.

PROPERTIES

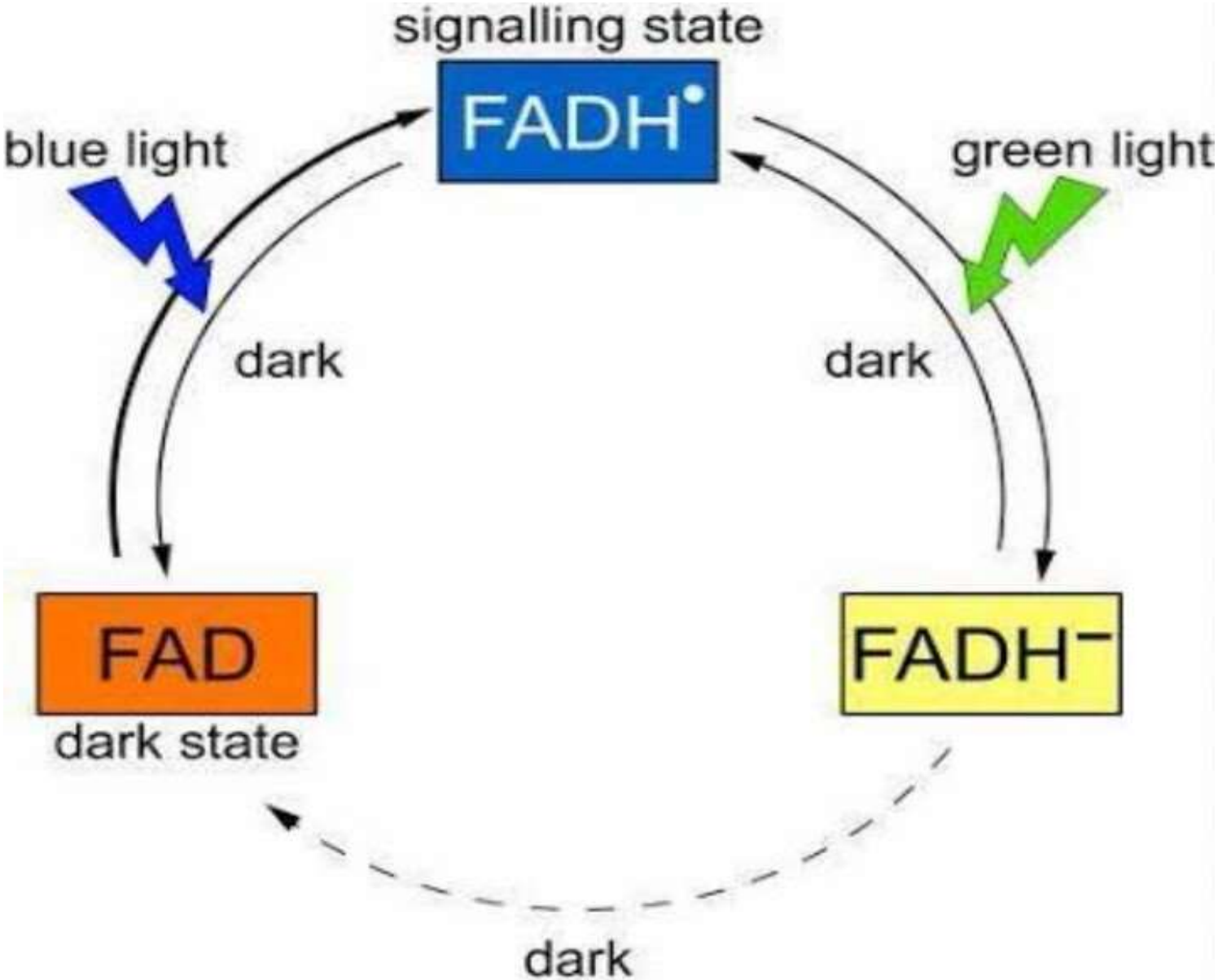
- Purification of recombinant flavoproteins such as those in the photolyse / cryptochrome family of blue photoreceptors often leads to loss of chromophores during purification and chromophore oxidation.

- For this reason, flavin composition and redox state in recombinant flavoproteins must be supplemented with supporting data to conclude the chromophore composition and redox state of the flavin in vivo .

- Arabidopsis cryptochrome which are known to function as photoreceptors, have been purified with near-stoichiometric catalytic flavin .

- The recombinant dCry photoreceptor, however, contains less than 5% flavin , an apparent contradiction to its known photoreceptive function.

MECHANISM


signalling state
FADH•
blue light
green light
dark
dark
FAD
dark state
FADH⁻
dark

Autophosphorylating kinase activity of cryptochrome:

- Phosphorylation has been found to contribute to regulatory processes of many photoactive enzymes.

- Phosphorylation activity of cryptochromes, however has not been extensively studied and the current data are contradictory.

- The most heavily studied cryptochrome kinase activity is that of *Arabidopsis Cry1 and Cry2; both of which play* important roles in light-stimulated photo morphogenic responses in plants and have been purified with near-stoichiometric levels of flavin.

- AtCry1 has shown stoichiometric ATP binding within theactive site of the enzyme.

- Both autophosphorylation and ATP binding were shown for Cry1 as well, however flavin dependence was not tested since human cryptochromes have yet to be purified with greater than trace amounts of flavin.

● *Photophysical Cryptochrome Properties:*

●As mentioned previously, absorption transients of excited flavin have been isolated in photolyase .

● In photolyase, this technology has proven the long predicted radical mechanism of photoreactivation .

- Excited flavin absorption, in the presence of T<>T substrate, was measured at 690 nm between 0.05 ns and 2.8 ns after excitation to observe the decay rate of the excited flavin.

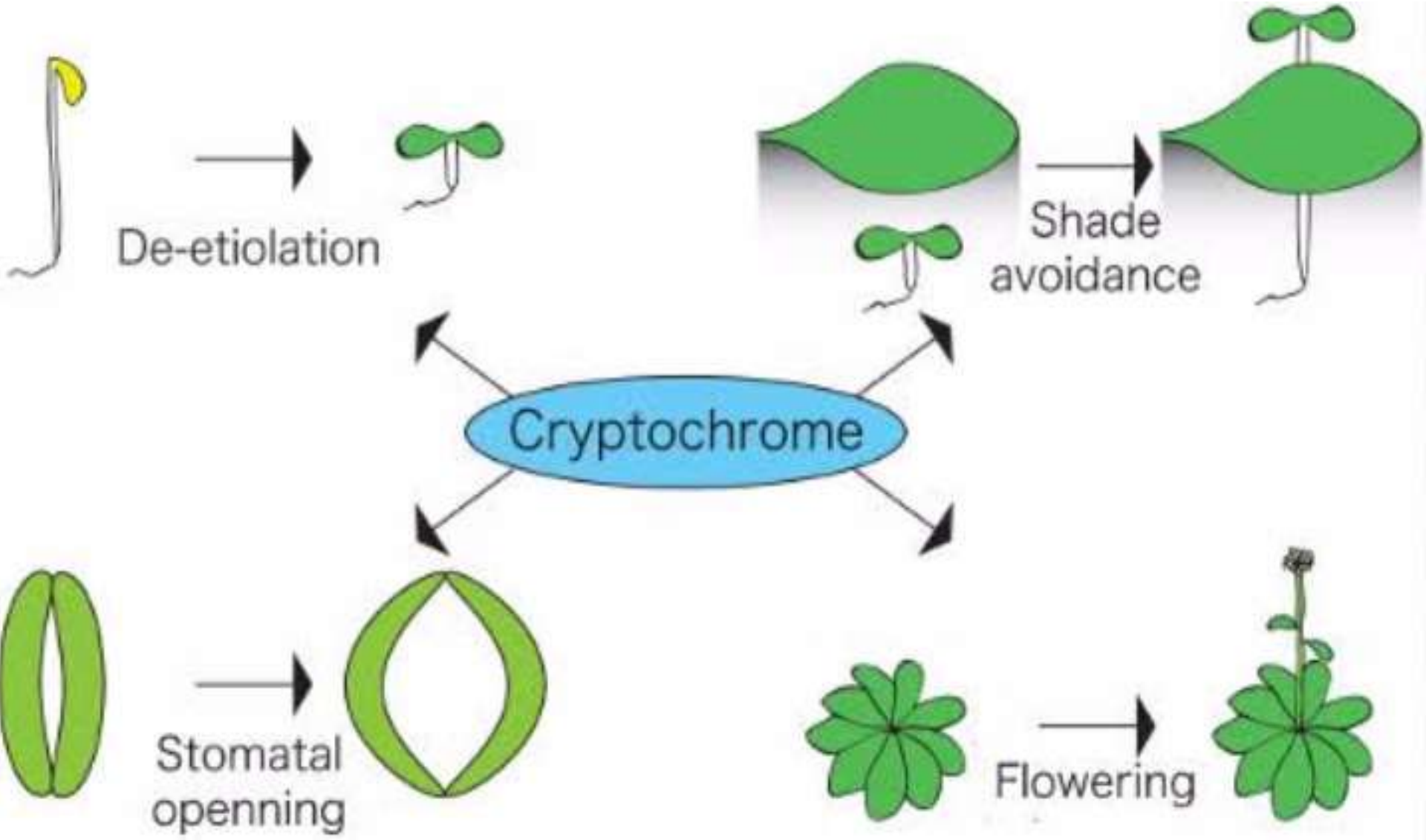
- At this high wavelength, the only absorbing species is FADH-.

- However, at shorter wavelengths above 500 nm both $FADH^-$ and the neutral radical absorb.

- Therefore the difference between the $FADH^-$ decay curve at 690 nm and those recorded at either 625 nm or 510 nm can be attributed to formation of FADH°.

- Since there is no known substrate for cryptochromes, identification of reaction intermediates is not possible at this time.

FUNCTION


De-etiolation
Shade avoidance
Cryptochrome
Stomatal openning
Flowering

REFERENCES

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- WWW.WIKIPEDIA .COM
- WWW.GOOGLE.COM
- WWW.YOUTUBE.COM/PHYTOCHROME


THANK
YOU