CHAPTER 0

ENDOGENOUS AND EXOGENOUS REGULATIONS OF CHLOROPLAST PHOTOCHEMICAL ACTIVITY - AN OVERVIEW

0.1. ENVIRONMENTAL PERIODICITIES AND BIOLOGICAL OSCILLATIONS

0.1.1. Periodicity in the Environment
0.1.2. Biological Time Keeping
0.1.3. Origin of the Biological Rhythms
0.1.4. Types of Biological Oscillations
0.1.5. Prevalence of Rhythmic Phenomena in Plants
0.1.6. Photoperiodic Control

0.2. GENERAL PROPERTIES OF CIRCADIAN RHYTHMS

0.2.1. Terms and definitions Commonly used in the Study of Biological Rhythms
0.2.2. Circadian Period
0.2.3. Ubiquity of Circadian Rhythms in Eukaryotic Organisms
0.2.4. Entrainability of Circadian Rhythms
0.2.5. Persistence under Constant Conditions
0.2.6. Temperature Compensation
0.2.7. Effects of Continuous Light
0.2.8. Genetic Basis of Circadian Rhythms
0.2.9. Effects of Aging on Rhythmic Phenomena

0.3. PERTURBATION OF RHYTHMIC PHENOMENA BY EXTERNAL FACTORS

0.3.1. Phase Shifts Induced by Light and Temperature
0.3.2. Effect of Chemical on Circadian Rhythms

0.4. MODELS FOR CIRCADIAN CLOCKS

0.4.1. Why Membrane Models?
0.4.2. Evidences for the Involvement of Membranes in Circadian Rhythms
0.4.3. Membrane Models for Circadian Rhythms
  0.4.3.1. Membrane Feedback Model of Sweeney
  0.4.3.2. Membrane Feedback Model of Njus
  0.4.3.3. Coupled Translation-Membrane Model
  0.4.4.4. Model of Ion-Mediated Translational Control

0.5. RHYTHMIC PHENOMENA IN PHOTOSYNTHESIS
  0.5.1. Rhythms in Stomatal Movements
  0.5.2. Rhythms in Chloroplast Movement and Chlorophyll Contents
  0.5.3. Rhythms in CO₂ Fixation
  0.5.4. Oscillations in Photosynthetic Electron Transport

0.6. PHOTOSYNTHETIC ELECTRON TRANSPORT
  0.6.1. Hill and Bendall's Z Scheme of Photosynthesis
  0.6.2. Photosystem II Mediated Electron Transport
    0.6.2.1. The Oxygen Evolving Complex
    0.6.2.2. Photosystem II Reaction Center
    0.6.2.3. Reducing side of PS II
  0.6.3. Photosystem I Related Electron Transport
    0.6.3.1. Intersystem Electron Carriers
    0.6.3.2. Photosystem I
  0.6.4. Photophosphorylation
  0.6.5. Thylakoid Membrane Composition
    0.6.5.1. Morphology of the Chloroplast Membranes
    0.6.5.2. Thylakoid Lipids
    0.6.5.3. Thylakoid Proteins
      0.6.5.3.1. Intrinsic Proteins of the Thylakoid Membrane
      0.6.5.3.2. Extrinsic Proteins of the Thylakoid Membranes
  0.6.6. Organization of Thylakoid Membranes
  0.6.7. Consequences of Lateral Heterogeneity in Thylakoids

0.7. LEAF SENESCEENCE AND PHOTOSYNTHESIS
  0.7.1. Senescence Induced Changes in Chloroplast Structure
  0.7.2. Changes in Electron Transport Capacity
0.8. PHYTOCHROME CONTROL OF LEAF SENESCENCE 50
0.9. HORMONAL CONTROL OF LEAF SENESCENCE 50
0.10. INTERACTION BETWEEN GROWTH REGULATORS AND LIGHT DURING SENESCENCE 51
0.11. SCOPE OF THE PRESENT STUDY 51
ENVIRONMENTAL PERIODICITIES & BIOLOGICAL OSCILLATIONS

Life on the planet earth is profoundly influenced by the environmental factors. Changes in the environment have been known to occur with an astounding precision and rhythmicity. Physical environment comprising of light, temperature, humidity etc., exhibits strong periodicity owing to the planetary movements. The complex structural organization among the living systems has necessarily evolved through the twin processes of natural selection and adaptation in response to these environmental periodicities (Edmunds, 1984). In both plants and animals rhythmic events are widespread and are very common among a number of physiological and behavioural processes (Bünning, 1973).

The spatial organization and its functional relationship in all the living systems reflects a complex pattern of interactions. Of great adaptive significance to the organism is its capacity to respond to the time pattern of the environment. The capacity of the living systems to adapt to the temporal pattern of the environmental factors has been called endogenous rhythm, physiological clock, circadian rhythm etc. These rhythmic responses have enabled the biochemical machinery in the cells to tune to the daily changes in their environment in order to position themselves in time with respect to favourable biotic and physical conditions (Edmunds, 1984; Engelmann and Schrempf, 1980).

0.1.1. Periodicity in the Environment

Since the basis of endogenous biological rhythms is the periodicity in the environmental factors, it is of
importance to examine the nature of these oscillations resulting out of planetary movements. The time taken for the earth's rotation, measured with reference to the stars is called a sidereal day, and is 23 h 56 min 4 s. However, the solar day, for an observer on this planet is about 24 h 4 min. The solar day length differs by about 16 min depending upon whether the earth is nearer to the sun or away from it. Though it is not exactly 24 h, the solar day is taken as 24 h to denote the natural day-night cycle to which the living systems are exposed. The cyclic change of day and night is correlated with rhythms of temperature and humidity with a strong 24 h periodicity (Saunders, 1977).

While the rotation of the earth on its polar axis gives rise to the dominant cycle of day and night, the revolution of the earth around the sun (365 d 6 h 9 min 10 s) gives rise to the unfailing procession of the seasons. Similarly the complex movements of the moon in relation to the earth and the sun gives rise to the lunar month (measured as the interval between successive new moons from a point on the earth's surface is 29.53 d) and to the tidal cycles (12.4 h and the lunar day, 24.8 h). Organisms have had ample time in their evolutionary history to respond to these environmental periodicities in the form of endogenous biological rhythms which have now become an integral part of the organization of plants and animals (Saunders, 1977).

0.1.2. Biological Time Keeping

All eukaryotic organisms have evolved various forms of time keeping to suit their life functions to the highly predictable cyclic variations in the environment. This functional biochronometry in living systems is of great adaptive significance to them, since it confers upon them the ability to
anticipate and avoid adverse events with the help of temporal organization (Brady, 1982; Edmunds, 1984; Saunders, 1977). The circadian system of an animal or plant becomes entrained to the natural 24 h periodicity of light and temperature and adopts a particular phase relationship to it. Entrainment offers a selective advantage to the organism through phase-control and period-control (Brady, 1982).

By achieving steady state entrainment to the environmental synchronizers or zeitgeber the organism can partition its activities into some kind of temporal order, and thereby perform behavioural and physiological activities at the right time of the day (Saunders, 1977). At the physiological level, for example, Halberg (1960) pointed out that the selective advantage of the 'right' amount of the 'right' substance available at the 'right' place is determined by its occurrence at the 'right' time. The significance of such temporal adaptation can be illustrated with examples from the plants. For instance, the periodic opening and closing of the flowers at certain times of the day is correlated with the 'time memory' of the honey bees to synchronise their visits to these flowers, thus resulting in increased efficiency of pollination and foraging of the bees (Saunders, 1977).

Functional biochronometry in plants and animals has been explained in terms of a clock mechanism capable of accurately measuring the time even in the absence of synchronizing external correlates generated by the planetary movements (Brady, 1982; Edmunds, 1984). This clock has been named the physiological clock or biological clock (Brady, 1982; Bünning, 1973; Saunders, 1977) and is capable of three kinds of time keeping:
(i) Time-set; time set for an event to occur relative to the environmental time.

(ii) Measure duration; time the length of something such as the number of hours of light or darkness in a day (photoperiodism).

(iii) Find local time; for example in order to be able to navigate by celestial cues.

Thus these biological clocks use oscillations of various periodicity ranging from seconds to a year. Details of the classification of various types of biological rhythms are given elsewhere (0.1.4.).

0.1.3. Origin of the Biological Rhythms

Biological oscillations of varying periodicity, particularly the circadian rhythms seem to have evolved quite early in the evolutionary history of the plants and animals. Oatley and Goodwin (1971) have suggested that most aspects of cell dynamics are oscillatory wherever temporal constraints are required to separate incompatible events in a process. For example, Pittendrigh (1966) suggested that the circadian rhythms may have evolved to temporally separate replication of the genetic message in eukaryotic cells from the other metabolic processes and restrict it to the dark periods in order to avoid the deleterious effects of the UV radiation on the nucleic acids during the day time.

Paietta (1982) recently has proposed that one of the original selective forces involved in evolution of circadian rhythms was the joint effect of light-dark (LD) cycles and the increasing concentrations of free oxygen early in eukaryotic evolution. According to him diurnal rhythmicity
would have provided a protective mechanism for minimizing the deleterious effect of diurnal photo-oxidation, by preventing the photodestruction of sensitive components through excited state quenching by pigments and through restricting the highly photosensitive processes to darkness.

0.1.4. Types of Biological Oscillations

All the diverse biological oscillations have been broadly grouped under four major categories (Brady, 1982; Büning, 1973; Edmunds, 1984; Palmer et al., 1976).

(i) Persistent rhythms: rhythmic oscillations which exhibit daily (circadian), tidal (circatidal), lunar and yearly periods. These rhythms are commonly displayed in most if not all eukaryotic organisms but not in prokaryotes.

(ii) Zeitgedachtnis or time sense or time memory of bees and humans.

(iii) Seasonal photoperiodism, where essentially a daily measurement of the length of the day (or night) is made.

(iv) Celestial orientation and navigation with the help of sun, moon or stars as direction givers.

The various types of biological oscillations outlined above have a range of periods which are considerably longer than those that denote ultrafast and rapid chemical reactions and biochemical rhythmicities pertaining to the intermediary metabolism (e.g. glycolytic oscillations) and to those rhythms commonly observed in the epigenetic and genetic time domains (Lloyd et al., 1982).

The circadian[circa (about) + dies (day)] rhythms in a restricted sense have a period approximately equal to 24 h and are entrainable by temperature and light cycles
(Bünning, 1973). Endogeneity of these rhythms is demonstrated by their ability to persist under free-running conditions held constant with respect to major environmental zeitgebers. Circadian rhythms are by far the most commonly noted and extensively studied type of biological oscillations (Brady, 1982; Bünning, 1973; Edmunds, 1984; Mansfield and Snaith, 1984; Saunders, 1977). Other oscillations with period lengths shorter than 20 h called ultradian rhythms (Lloyd et al., 1982; Wagner, 1977) and those with periods longer than 30 h namely infradian rhythms (Edmunds, 1984) have also been investigated in a number of physiological processes in plants.

0.1.5. Prevalence of Rhythmic Phenomena in Plants

Functional biochronometry in plants is reflected through the rhythmic oscillations in a number of physiological and developmental processes encompassing functions as diverse as photosynthesis, seed germination, leaf movement and bioelectricity. Table 0.1 presents a list of diverse rhythmic phenomena in plants with period lengths varying from seconds to a year.

Most commonly noted and one of the first to be studied in plants is the rhythmic movement in leaves (Bünning, 1973; DeMairan, 1729; Pfeffer, 1875). The capacity of plants to measure the seasonal changes in the day-length was reflected in the seasonal cycles of flowering and fruiting. This phenomenon of photoperiodism was demonstrated first in plants by Garner and Allard (1920; 1923). Eversince these pioneering studies, a large number of rhythmic processes in plants have been identified and studied in great detail.
Table 0.1:
Rhythmic phenomena in plants (selected examples taken from Edmunds, 1984; Wagner, 1977).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Rhythmic phenomenon</th>
<th>Period length</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipomoea, Pisum, Xanthium</td>
<td>Action potentials</td>
<td>0.1 - 10s</td>
<td>Pickard, 1972, 1973</td>
</tr>
<tr>
<td>Lupinus angustifolius</td>
<td>Refractory period</td>
<td>12 min</td>
<td>Paszewski and Zawadski, 1976</td>
</tr>
<tr>
<td>Mimosa pudica</td>
<td>Changes in propagation direction</td>
<td></td>
<td>Stoeckel and Pfrisch, 1975</td>
</tr>
<tr>
<td>Acetabularia</td>
<td>Transcellular current</td>
<td></td>
<td>Novak and Sironval, 1976</td>
</tr>
<tr>
<td>Phaseolus</td>
<td>Electric potentials</td>
<td>5 min, 12 min</td>
<td>Aimi and Shibasaki, 1975</td>
</tr>
<tr>
<td>Phaseolus</td>
<td>Rotational leaf movement</td>
<td>53 min</td>
<td>Alford and Tibbits, 1971</td>
</tr>
<tr>
<td>Avena</td>
<td>Water uptake</td>
<td>30 min</td>
<td>Johnsson, 1973</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>Respiration</td>
<td></td>
<td>Pallas et al., 1974</td>
</tr>
<tr>
<td></td>
<td>Photosynthesis</td>
<td></td>
<td>-do-</td>
</tr>
<tr>
<td>Chenopodium rubrum</td>
<td>Respiration, Photosynthesis, flowering, chlorophyll accumulation</td>
<td>All Circadian</td>
<td>Chia-Looi and Cumming, 1972</td>
</tr>
<tr>
<td></td>
<td>Enzyme activities</td>
<td>4-6 h</td>
<td>Deitzer et al., 1974</td>
</tr>
<tr>
<td></td>
<td>-do-</td>
<td></td>
<td>Semi-circadian Frosch et al., 1973</td>
</tr>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
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<tr>
<td><em>Gonyaulax polyedra</em></td>
<td>Bioluminescence</td>
<td>All circadian, Edmunds, 1984</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cell division, photosynthesis, chloroplast ultrastructure</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Euglena gracilis</em></td>
<td>Cell division</td>
<td>All circadian, Edmunds, 1984</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phototaxis, photosynthesis, cell shape</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acetabularia</em></td>
<td>Photosynthetic O₂ evolution, chloroplast number, shape, ultrastructure</td>
<td>Edmunds, 1984</td>
<td></td>
</tr>
<tr>
<td><em>Bryophyllum fedschenkoii</em></td>
<td>CO₂ compensation</td>
<td>Circadian</td>
<td>Jones, 1973</td>
</tr>
<tr>
<td></td>
<td>CO₂ emission</td>
<td>Circadian</td>
<td>Wilkins, 1960</td>
</tr>
<tr>
<td><em>Chenopodium rubrum</em></td>
<td>Energy charge</td>
<td>Circadian</td>
<td>Wagner et al., 1974</td>
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<tr>
<td></td>
<td>Mitosis</td>
<td>20 h</td>
<td>King, 1975</td>
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<tr>
<td></td>
<td>Flower induction</td>
<td>Circadian</td>
<td>Cumming, 1972; King, 1975</td>
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<td></td>
<td>and flower stimulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Allium cepa</em></td>
<td>Mitosis</td>
<td>semi-circadian</td>
<td>Bishop and Klein, 1971,1973</td>
</tr>
<tr>
<td><em>Eucalyptus occidentalis</em></td>
<td>Seed germination</td>
<td>Annual</td>
<td>Zohar et al., 1975</td>
</tr>
</tbody>
</table>


Some of the important rhythms observed in the physiological and metabolic activities in higher plants include leaf and petal movements, photosynthesis, respiration, growth rate, stomatal movements, dark CO₂ fixation and flowering (Mansfield and Snaith, 1984; Wagner, 1977). In the unicellular algae and dinoflagellates, rhythmic changes at the physiological level have been observed in cell division, motility of the cells, photosynthesis, phototaxis, cell shape, bioluminescence, chloroplast number, shape and migration etc. Oscillations at the biochemical level, in activity of a number of enzymes, photosynthetic pigments, RNA and DNA contents, electric potential etc., have also been documented (Edmunds, 1984).

Rhythmic phenomena have been investigated in almost all the major groups of plants and thus seem to be ubiquitous in nature. Unicellular dinoflagellates such as *Gonyaulax Euglena*, green algae such as *Acetabularia*, higher plants such as *Phaseolus*, *Albizzia*, *Glycine max*, *Chenopodium* etc., have been extensively used in the studies on circadian rhythms.

### 0.1.6. Photoperiodic Control

Many organisms use day-length measurements to regulate the physiological reactions with the help of a time-keeping mechanism called photoperiodic control. Various processes of growth and development in plants are known to depend on the day length and therefore perception of changes in length of the daily photoperiod as a function of the changing seasons is of critical importance in these processes (for example flowering). The photoperiodic control in flowering was first demonstrated by Garner and Allard (1920). The surprising degree of accuracy in the time measurement in plants with respect to the length of the photoperiod has been shown in many cases (Bünning, 1973). Often, the deviation in such
time perceptions is as short as a few minutes over several weeks (Bünning, 1973).

Time measurements in photoperiodic reactions are largely temperature independent as in the case of circadian rhythms. Also the photoperiodic information about seasonal changes is not light intensity dependent, and requires only low light intensity (Bünning, 1973). Hendricks (1960) and Evans (1969) suggested that the red-far-red pigment system, namely phytochrome plays an important role in photoperiodic reactions of plants. Now, phytochrome has clearly been shown as the decisive photoreceptor in the photoperiodic response of plants (Bünning, 1973; Thomas and Vince-Prue, 1984).

It is believed that the various species investigated for their photoperiodic response may have different time-measuring systems. Two different mechanisms have been postulated as the basis for the photoperiodic control. The first hypothesis - 'hour glass process' suggests that photoperiodic timing is a consequence of a series of unidirectional biochemical reactions proceeding sequentially to completion. The alternative hypothesis, supported by most of the experimental results, relates timing to the progress of a circadian rhythm in responsivity to light. The accuracy and reliability of day-length measurements are said to be mainly caused by the involvement of the circadian clock (Bünning, 1973; Evans, 1969; King and Cumming, 1972; Thomas and Vince-Prue, 1984).

0.2. GENERAL PROPERTIES OF CIRCADIAN RHYTHMS

Circadian rhythms with a period approximately equal to 24 h are known in physiological and behavioural aspects of both plants and animals (Brady, 1982; Bünning, 1973; Saunders, 1977). There is evidence to suggest that these 24 h rhythms may also be the basis for other oscillatory phenomena such
as the tidal rhythm and photoperiodism (Brady, 1982). The general characteristics of these rhythms are summarized in Table 0.2.

0.2.1. Terms and Definitions Commonly used in the Study of Biological Rhythms

(i)  **Amplitude**: the peak to trough difference in a biological oscillation (Fig. 0.1).

(ii) **Biological rhythm**: a cyclical variation exhibited in a biological function.

(iii) **Endogenous**: rhythms or other biological time keeping controlled from within the organism by some kind of physiological clock.

(iv) **Entrainment**: the synchronization of a biological rhythm to a *Zeitgeber* so that both have the same period.

(v) **Free-running**: rhythms running at their own natural frequency in constant conditions and which are therefore not entrained to a *Zeitgeber* such as a day-night cycle.

(vi) **Frequency**: the reciprocal of the period of a rhythm e.g. once per 24 h.

(vii) **Period**: The length of one complete cycle of a rhythm (Fig. 0.1); reciprocal of frequency.

(viii) **Phase**: a particular reference point in the cycle of a rhythm, e.g. the peak of O₂ evolution rhythm in *Gonyaulax*.

(ix) **Phase-response curve**: the 24 h profile of an organism phase shifts in response to environmental signals.

(x) **Phase shift**: a single displacement of an oscillation along the time-axis following a perturbation. It may involve either an advance (+ΔØ) or a delay (- ΔØ) Fig. 0.1.
Table 0.2:

Properties of circadian rhythms

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1.</td>
<td><strong>Period</strong></td>
<td>about 24 h</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Ubiquity</strong></td>
<td>among eukaryotes, at every level of organization. Not in prokaryotes</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Entrainability</strong></td>
<td>by light and temperature cycles, and by social cues.</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Persistence</strong></td>
<td>under free-running conditions held constant with respect to major environmental Zeitgeber</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Phase-shiftability</strong></td>
<td>by single perturbation of light, temperature or chemicals.</td>
</tr>
<tr>
<td>6.</td>
<td><strong>Temperature compensation</strong></td>
<td>temperature independent free-running period within the physiological range</td>
</tr>
<tr>
<td>7.</td>
<td><strong>Genetic basis</strong></td>
<td>endogeneity</td>
</tr>
</tbody>
</table>

Details are given in the text (From Edmunds, 1984).
Fig. 0.1: Properties of biological oscillations.

0.1 a. A biological oscillation (sine wave) firstly entrained by an environmental zeitgeber (square wave), and then free-running in constant darkness.

0.1 b. A biological oscillation in free-run subjected to a perturbation $P$ causing a delay phase-shift ($-\Delta \varphi$).

0.1 c. A biological oscillation in free-run subjected to a perturbation $P_1$ causing an advance phase-shift ($+\Delta \varphi$). D, dark; L, light, DD, Constant darkness; T, period of zeitgeber; $\bullet$ and O, phase points; $\varnothing$, phase; $\tau$, period; $\psi$, phase angle (from Saunders, 1977).
Zeitgeber: from the German for 'time-giver'. A periodic environmental signal that entrains some biological rhythms, for example a natural or artificial day-light cycle or a temperature cycle.

0.2.2. Circadian Period

The most striking feature of circadian rhythms, as suggested by the name, is the period approximating the natural day-light cycle of 24 h. Under entraining conditions, the period is always 24 h but it seldom equals 24 h under free-running conditions, where the synchronizing environmental factors such as light and temperature are held constant. In many cases the circadian rhythms continue to free-run several days under constant conditions (e.g. leaf movement rhythms, Lee and Satter, 1983). In some cases the oscillations damped off after a few cycles such as the petal movement of the Kalanchoe flower (Brady, 1982; Büning, 1973).

The circadian period has a range of 22 to 28 h in most cases under free-running conditions. For example, the diurnal leaf movement in Phaseolus has a period of about 27 h and the rhythm of spore release in the green alga Oedogonium has been observed to exhibit a periodicity of about 22 h (Bünning, 1973). Likewise, in Avena the coleoptile growth rate was rhythmic with periods of 23.3 h (Ball et al., 1957). The accuracy and constancy of this free-running period length is remarkable. A deviation of usually less than one hour or even less than 15 to 20 minutes has been recorded in many plants (Brady, 1982; Bünning, 1973).

0.2.3. Ubiquity of Circadian Rhythms in Eukaryotic Organisms

Circadian rhythms have so far been reported only in eukaryotic organisms (Hastings et al., 1976). These rhythms are known
to occur at all levels of eukaryotic organization beginning with unicellular algae such as *Gonyaulax* and protozoans like *Tetrahymena* and *Paramecium* to the most complex organisms such as higher plants and animals (Bünning, 1973; Edmunds, 1984). There is no concrete report on the occurrence of circadian oscillations in any prokaryotic systems. But two reports on the circadian rhythms in bacteria need a mention here (Halberg and Connor, 1961; Sturtevant, 1973a,b). These findings, however, have not been subsequently confirmed by other workers (Engelmann and Schrempf, 1980).

0.2.4. **Entrainability of Circadian Rhythms**

Synchronization is another feature of circadian rhythms. Environmental factors such as light-dark cycles and temperature cycles act as natural synchronizers of the circadian oscillators. These synchronizers are referred to as *Zeitgeber*, a German term commonly in vogue. However, in the case of human beings, more than the light-dark cycles, certain common social time cues are prominent in synchronizing the circadian system (Aschoff et al., 1975). There are some exceptions to the synchronization of circadian oscillations by the light signal in the case of plants also. For example in *Penicillium diversum*, light failed to entrain the rhythm (Bourret et al., 1969), while hormones have been shown to mediate synchronization by light (Koukkari, 1979).

0.2.5. **Persistence under Constant Conditions**

The most important criterion that must be satisfied in any attempt to show a rhythmic pattern to be endogenous is its ability to persist in the absence of *Zeitgeber*. Pittendrigh (1954) formulated a set of five formal rules to test the endogenous nature of a rhythm. Of these the requirement
for persistence in constant conditions has been given primary importance (Mansfield and Snaith, 1984; Wilkins, 1969).

0.2.6. Temperature Compensation

The physiological and other processes in which rhythms are observed are usually influenced by temperature. Therefore a clock mechanism must be temperature compensated to work properly under different ambient temperatures. Temperature compensation property of the circadian rhythms has been widely studied in many organisms (Bünning, 1973; Engelmann and Schrempf, 1980; Saunders, 1977). The period of free-running rhythms remains remarkably unaffected by the ambient temperature with temperature coefficient ($Q_{10}$) values slightly above or below 1.0 depending upon the organism (Engelmann and Schrempf, 1980). For example, the rhythm of leaf movement in darkness in *Phaseolus* (Leinweber, 1956) has periods of 28.3 h at 15°C and 28.0 h at 25°C ($Q_{10} = 1.01$), and that of CO$_2$ output in *Bryophyllum* (Wilkins, 1962) has periods of 23.9 h at 16°C and 22.4 h at 26°C ($Q_{10} = 1.06$). Table 0.3 shows some selected examples of temperature compensation of the free-running circadian periods.

0.2.7. Effects of Continuous Light

Continuous light has been shown to influence the plant rhythms differentially. In *Phaseolus coccineus* period for the leaf movement rhythm varied with quality of the light in continuous light (LL) treatment. Longest period length (28.1 h) was observed in red light (610-690 nm) and shortest period (24.7 h) in the far-red (690-850 nm) light (Bünning, 1973). In the green alga *Oedogonium cardiacum* and the marine dinoflagellate *Gonyaulax polyedra* the endogenous rhythmicity showed strong damping in continuous darkness (DD) but persisted in dim
Table 0.3:
Examples showing the temperature compensation of the free-running circadian period in plant rhythms (Adopted from Saunders, 1977)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Rhythmic phenomenon</th>
<th>Temperature range, °C</th>
<th>Period range, in hours</th>
<th>Temperature coefficient Ω₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euglena gracilis</em></td>
<td>Phototaxis in DD to test light</td>
<td>16.7 - 33</td>
<td>26.2 - 23.2</td>
<td>1.01 - 1.1</td>
</tr>
<tr>
<td><em>Oedogonium cardiacum</em></td>
<td>Sporulation</td>
<td>17.5 - 27.5</td>
<td>20 - 25</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Gonyaulax polyedra</em></td>
<td>Bioluminescence</td>
<td>18 - 25</td>
<td>22.9 - 24.7</td>
<td>0.9</td>
</tr>
<tr>
<td><em>Neurospora crassa</em></td>
<td>Cell division</td>
<td>18.5 - 25</td>
<td>23.9 - 25.4</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Phaseolus multiflorus</em></td>
<td>Zonation of growth in dim red light</td>
<td>24 - 31</td>
<td>22 - 21.7</td>
<td>1.03</td>
</tr>
<tr>
<td><em>Bryophyllum</em></td>
<td>Leaf movement in DD</td>
<td>15 - 25</td>
<td>28.3 - 28.0</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>CO₂ output</td>
<td>16 - 26</td>
<td>22.4 - 23.9</td>
<td>1.06</td>
</tr>
</tbody>
</table>


LL (Edmunds, 1984; Saunders, 1977). However, under bright LL the rhythm in photosynthesis in *Gonyaulax* and *Euglena* disappeared totally, contrary to the dim LL condition (Lonergan and Sargent, 1978; Hastings et al., 1961). In *Acetabularia*, photosynthetic $O_2$ evolution rhythm was shown to persist under high light intensity in LL (Terborgh and McLeod, 1967). Also there is a report on the persistence of $CO_2$ fixation rhythm in bright LL in *Arachis hypogaea* (Pallas et al., 1974).

### 0.2.8. Genetic Basis of Circadian Rhythms

It has been clearly shown that the characteristic features of biological rhythms such as the period length and temperature compensation are genetically controlled by a single gene (Saunders, 1977). This finding has lent additional support to the proposition that the biological clock is a product of natural selection and is an intrinsic part of the organism's physiology. Experimental evidences leading to the conformation of a genetic basis for circadian rhythms were obtained from various plant and animal systems.

From the crossing studies with *Phaseolus* seedlings showing different periods in their leaf movements (Büning 1932; cited in Büning, 1973) believed that the rhythmicity was inherited, probably by polygenic system. It has subsequently been found out from studies with natural and induced mutants that a single gene controlled the oscillations (Saunders, 1977). Clock mutants of *Drosophila melanogaster* and *Neurospora crassa* obtained by chemical mutagenesis have also shown that a single gene is involved in the rhythm control (Feldman, 1982; Feldman et al., 1979; Konopka and Benzer, 1971). Bruce (1972), crossing wild type strains of *Chlamydomonas reinhardtii* with 24 h and 21 h periods of phototactic responsiveness, also arrived at a similar conclusion that the circadian rhythm was controlled by a single gene.
0.2.9. Effects of Aging on Rhythmic Phenomena

In many animal systems, at the very early stages of development such as at the embryonic stage or soon after birth (e.g. in humans) none of the known rhythms could be noted. The general arrhythmicity slowly develops into normal patterns of rhythmic oscillations at a latter stage (Saunders, 1977). Similar to the discrepancies in rhythmic phenomena at the early developmental stages, aging has been known to affect the characteristics of rhythms such as amplitude and period (Samis and Capobianco, 1978). Prominent change in the frequency of the oscillations at old age in rodents was reported by Pittendrigh and Daan (1974). In leaf movement rhythms of Phaseolus, Pfeffer (1875) observed that the rhythmic waveform was affected by the leaf age. A similar result was obtained by Chen et al. (1984) with Albizzia, where the period was not affected but the waveform changed due to aging.

0.3. PERTURBATION OF RHYTHMIC PHENOMENA BY EXTERNAL FACTORS

0.3.1. Phase Shifts Induced by Light and Temperature

One of the criteria used in deciding whether a given oscillation is endogenous or not is the ability to phase shift the rhythm. It should be possible to alter the phase of the free-running rhythm by abrupt changes in one or more environmental parameters normally held constant (Mansfield and Snaith 1984; Pittendrigh, 1954). Both light and temperature treatments have been shown to be effective in causing a phase shift in plant rhythms. For example the CO₂ output rhythm in Bryophyllum could be phase shifted by sudden changes in light intensity (Wilkins, 1960). Similarly Wilkins (1962) found that a 10°C temperature rise for a few hours from a constant 26°C resulted in a phase-shift in the free-running rhythm which was identical to that produced by light.
Free-running oscillations in *Drosophila pseudobscura* perturbed by short light signals at different circadian times exhibited substantial phase shifts in the steady state of the rhythm (Pittendrigh, 1965). Bruce (1960) and Sweeney and Hastings (1960) have shown that a variety of plants and cold-blooded animals exhibit phase shifts in their free-running circadian rhythms when a 'sinusoidal' or 'square-wave' temperature cycle is introduced.

0.3.2. Effect of Chemicals on Circadian Rhythms

Involvement of membranes in the rhythmic processes has been strongly favoured by many investigators (Engelmann and Schrempf, 1980). If membranes are essentially involved, use of a chemical substance that alters the membrane structure or interferes with its functions would produce significant alterations in the characteristics of the rhythms. This assumption has been proved valid through a number of experiments. Chemicals with proven effects on the membranes such as fusaric acid, steroids, vanillic acid, etc., produced large phase shifts in the circadian rhythms (Kiessig et al., 1979; Sundararajan et al., 1978; Zucker, 1978).

Ions such as $K^+$, when given as a pulse of few hours duration, shifted the phase of circadian rhythms in *Musculus* (Bünning and Moser, 1973), *Albizzia* (Satter et al., 1973) and *Gonyaulax* (Sweeney and Herz, 1977). $Li^+$ was found to increase the period length of circadian rhythms in plants such as *Kalanchoe* (Engelmann, 1973); *Phaseolus vulgaris* (Engelmann and Schrempf, 1980) and *Lemna* (Kondo and Tsuzuki, 1978). A number of ionophores such as gramicidin, nonactin, nigericin, valinomycin, etc., have been tested for their effect on the phase properties of the circadian rhythms in plants (Engelmann
Abscisic acid, heavy water and alcohols are some of the other chemical perturbants tested in the case of plant and animal rhythms for their ability to affect the phase and period characteristics of these oscillations (Bruce and Pittendrigh, 1960; Engelmann and Schrempf, 1980; Kastenmeier et al., 1977; Maurer and Engelmann, 1974; Taylor et al., 1979).

0.4. MODELS FOR CIRCADIAN CLOCKS

A large number of models have been proposed to explain the molecular basis of the endogenous circadian rhythms in organisms. However, none of these models either collectively or singly explain the mechanisms underlying the functioning of the biological clock satisfactorily. Most of these models often represent synthesized versions of various models proposed earlier, incorporating the strong points from them.

Some of the prominent models attempting to account for circadian variations in different physiological processes of a cell have been listed in Table 0.4. The existing molecular models on biological rhythms have been grouped into three or four broad categories by Edmunds (1976). (A) Molecular models based on the properties of the molecules themselves for generating 24 h rhythms (Queiroz-Claret and Queiroz 1981). (B) Network models explained in terms of biochemical feedback-loops in energy metabolism such as the glycolytic oscillations. (C) Transcriptional "tape-reading" models such as the chronon model by Ehret and Trucco (1967). In this model sequential transcription of long DNA polycistron complexes in eukaryotes coupled with time-consuming, rate-limiting, temperature-independent-diffusion steps by mRNA to the ribosomes for translation have been used to explain the

TH 2052
Table 0.4:

A list of models proposed to explain the molecular basis of circadian rhythms. The various models have been grouped into four major categories as explained in the text (section 0.4). Taken from Edmunds, 1984.

<table>
<thead>
<tr>
<th>Major category</th>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Molecular <strong>(in vitro)</strong></td>
<td>Periodicity in the structure and properties of molecules</td>
<td>Queiroz-Claret and Queiroz (1981)</td>
</tr>
<tr>
<td>B. &quot;Network&quot; (Biochemical feedback loops)</td>
<td>Glycolytic oscillator</td>
<td>Hess and Boiteux (1971)</td>
</tr>
<tr>
<td></td>
<td>Cell energy metabolism = the clock</td>
<td>Sel'kov (1975); Reich and Sel'kov (1981)</td>
</tr>
<tr>
<td></td>
<td>Coupled oscillator</td>
<td>Winfree (1967); Goodwin and Cohen (1969); Pavlidis and Kauzmann (1969); Wagner and Cumming, (1970); Pavlidis (1971); Wagner, 1977</td>
</tr>
<tr>
<td></td>
<td>Cyclic AMP model</td>
<td>Cummings (1975)</td>
</tr>
<tr>
<td></td>
<td>Heterodyne Endosymbiont hypothesis</td>
<td>Levandowsky (1981)</td>
</tr>
<tr>
<td>C. Transcriptional (Tape-reading)</td>
<td>Chronon model</td>
<td>Ehret and Trucco (1967); Wille et al. (1972)</td>
</tr>
<tr>
<td>Major category</td>
<td>Model</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>D. Membrane models</td>
<td>Chronogene-cytochron model for cell cycle clocks</td>
<td>Edmunds and Adams (1981)</td>
</tr>
<tr>
<td></td>
<td>Membrane feedback model: active transport of molecule</td>
<td>Sweeney, 1974</td>
</tr>
<tr>
<td></td>
<td>Membrane feedback model: limit cycle behaviour</td>
<td>Njus et al., (1974)</td>
</tr>
<tr>
<td></td>
<td>Coupled translation-membrane model</td>
<td>Schweiger and Schweiger(1977)</td>
</tr>
<tr>
<td></td>
<td>Ion-mediated translational control model</td>
<td>Burgoyne (1978)</td>
</tr>
</tbody>
</table>
observed circadian periods. (D) Membrane models based on the transport activity and other properties of the membranes (Engelmann and Schrempf, 1980).

0.4.1. Why Membrane Models?

The molecular models proposed to explain the circadian periodicities generally suffer a major setback, namely, the high frequency oscillations in the structure and properties of the molecules involved. Although these high frequency oscillations can be transformed to slower oscillations by different mechanisms, such as coupling with other oscillators (Selkov, 1972) or repeated induction/repression of transcriptional events as in the chronon model (Ehret and Trucco, 1967), membranes as diffusion barriers provide easily for longer time delays. Moreover, membranes have been used to explain temperature compensation of circadian oscillators (Njus et al., 1974).

0.4.2. Evidences for the Involvement of Membranes in Circadian Rhythms

Membranes are an essential part of the cell machinery. It is therefore logical to expect that they must be involved either directly or indirectly in the mechanism of circadian rhythms. If this argument is true, then any treatment perturbing the membrane structure or interfering with its functions should influence the characteristics of the rhythmic phenomena. There is a large body of experimental evidence in support of this assumption. In both animals and plants it has been found that membrane perturbations lead to phase shifts (both advance and delay phase shifts) and change in the period length (Engelmann and Schrempf, 1980).
Following is a brief account of the various treatments which perturbed the structure and functions of the membranes leading to significant changes in the rhythmic processes.

Imposed changes in the fatty acid composition of the membrane lipids in *Neurospora* (Roeder et al., 1977) affected the period of the rhythm. Fusaric acid, which interferes with the lipid moiety of membranes phase shifted the leaf movement rhythm in *Gossypium* (Sundararajan et al., 1978). The membrane depolarizer, vanillic acid induced large delay phase shifts in the bioluminescence rhythm in *Gonyaulax* (Kiessig et al., 1979).

Ions are especially interesting, since the concentration of some of them oscillates in a circadian fashion. K\(^+\), Li\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) have been shown to influence the rhythms in various systems (Engelmann and Schrempf, 1980; also see 0.3.2.). Detergents, such as dogitornin (Salman, 1971), alcohols (Engelmann and Schrempf, 1980), inhibitors of mitochondrial functions such as DNP, CN\(^-\) (Njus, 1976), CCCP, a proton carrier (Felle and Bentrup, 1977) are some of the other chemical treatments that lend support to the argument that membranes are involved in the circadian oscillations.

Rhythmicity in the light reactions of photosynthesis in various organisms (Lonergan, 1984b) and ultrastructural changes in the thylakoid membranes and their stacking (Vanden Driessche and Mars, 1972) also indicate that membranes have an important role to play in the rhythmic phenomena.

**0.4.3. Membrane Models for Circadian Rhythms**

**0.4.3.1. Membrane Feedback Model of Sweeney**

Based on the experimental results obtained from the various rhythmic phenomena in the unicellular green alga *Acetabularia*,
Sweeney (1974) proposed a membrane feedback model. This model proposes that the cells possess a master clock, which derives different rhythms. She has considered the thylakoid membranes of the chloroplasts as the base of her model. The mechanism of the model consists of active transport by an hypothetical substance X (possibly K\(^+\) in cells) into the organelles, and its passive leakage after attaining a threshold concentration inside. This leakage establishes an equilibrium and initiates the cycle again. The long diffusion paths involved in the transport processes are thought to account for the long period lengths.

Although light mediated changes in the transport rate are said to result in a phase shift, no provision for a photoreceptor is made in the model. A number of experimental findings supporting this model are now available (Adamich et al., 1976; Sweeney, 1976; Sweeney and Herz, 1977). However, the major objection to this model is that it is entirely based on the chloroplast membranes and that it does not explain the rhythms in those organisms which do not have chloroplasts (animals, fungi etc.) or in those which posses tissues without these organelles (for e.g. roots).

0.4.3.2. Membrane Feedback Model of Njus

A model developed in lines similar to those of Sweeney (1974) was published by Njus et al (1974). This model takes plasma membrane as the site of rhythm control and tends to explain the transport by K\(^+\) (analogous to substance X of Sweeney, 1974). According to this model translational diffusion of membrane particles, probably proteins, in connection with time-dependent cooperative phenomena would lead to the long periods of the circadian rhythms.
A provision for temperature compensation has been included by Njus et al (1974) and is explained by homeostasis of membrane fluidity (Baranska and Wlodauer, 1969; Patterson, 1970). Phase shifts by light and low temperature are said to be brought about by opening of the ion gates. There are however several drawbacks in this model particularly concerning the phase shifting effects of $K^+$ ion pulses (Engelmann and Schrempf, 1980).

**6.4.3.3. Coupled Translation-Membrane Model**

This model was proposed by Schweiger and Schweiger (1977). Organelles particularly chloroplast membranes have been used as the central point of the model. A specific process of loading and unloading of certain "essential proteins" synthesized on the cytoplasmic ribosomes into and out of the chloroplasts are thought to account for the rhythmic time delays. Loading of the chloroplasts with these proteins changes the functional state of the thylakoids which in turn lead to variations in the ion concentration. This is said to inhibit either the synthesis or the transport of the essential proteins. When the loading is stopped, unloading of the thylakoid membranes with final degradation of the essential proteins is said to occur. Light and darkness change the thylakoid membranes by opening the ion gates as in the feedback model of Njus et al (1974). This model also suffers from drawbacks similar to those encountered in Sweeney's (1974) membrane model.

**6.4.3.4. Model of Ion-Mediated Translational Control**

Burgoyne (1978) by combining the essential features of the membrane feedback model of Njus et al.(1974) and the coupled translational-membrane model of Schweiger and Schweiger
(1977) and incorporating the findings of influences of monovalent ion concentrations on translation of mRNA, has proposed a model of ion-mediated translational control.

According to this model, the rate of synthesis of a membrane protein involved in ion transport is feed-back controlled by the intracellular concentration of monovalent ions. It may be Na⁺/K⁺-ATPase or a modulator of its activity. Synthesis and insertion of this protein into the membrane would result in a change in the ion concentration, which in turn would inhibit the protein synthesis. The model accounts for the long time constant of the circadian rhythm with the help of long half-life of the membrane protein and the time lag between its synthesis and subsequent transport and insertion into the plasma membrane. The model thus leads to a number of predictions that could be tested experimentally (Engelmann and Schrempf, 1980). The amount of protein, its synthesis and the ion concentration should vary in a circadian manner. Most importantly, the translation of the mRNA coding for the protein should be sensitive to the ion concentration changes during the circadian cycle.

0.5. RHYTHMIC PHENOMENA IN PHOTOSYNTHESIS

Among the various physiological and biochemical processes exhibiting time-keeping rhythmic phenomena photosynthesis figures prominently (Edmunds, 1984; Lonergan, 1984b; Sweeney and Prezelin, 1978). Various reactions involved in the photosynthetic electron transport and carbon fixation have been identified to be rhythmic over the day (Hillman, 1976; Lonergan 1984b; Queiroz, 1974).
0.5.1. Rhythms in Stomatal Movements

The opening and closing movements of stomata have been studied in a number of higher plants (Chia-Looi and Cumming, 1972). The fluctuations in the stomatal aperture size were affected in a circadian fashion and were responsive to phase shifting by light and darkness (Brun, 1962; Hopmans, 1971; Mansfield and Heath, 1963; Martin and Meidner, 1972; Meidner and Mansfield, 1965; Stalfelt, 1963). Attempts have been made to correlate the circadian stomatal movements with the photosynthesis rhythms (Scarth and Shaw, 1951), where positive relationships have been observed.

0.5.2. Rhythms in Chloroplast Movement and Chlorophyll Contents

Mostly in algal systems, a rhythmic movement of the chloroplasts has been noted (Sweeney, 1979). Aggregation and dispersal movements (Hopkins, 1965) and periodic withdrawal of the chloroplasts from the margins of the coenocytic algal blades as in Caulerpa (Dawes and Barilotti, 1969) have been commonly observed. Also ultrastructural changes in the thylakoid membranes have been reported (See Chapter 1, section 1.1.2. for details).

There are varied reports on rhythmicity in the cellular pigments content. In dinoflagellate cells cultured under laboratory conditions and in the natural field populations diurnal variations in the chlorophyll content have been observed (Eppley et al., 1967; Jørgensen, 1966; Owens et al., 1980). However, many reports suggest that the chlorophyll content does not undergo any change during the day (Lonergan, 1984b). A detailed review on changes in the chlorophyll content is presented in Chapter 1, section 1.1.4.
0.5.3. Rhythm in CO₂ Fixation

Rhythmic oscillations in the acid metabolism of plants belonging to the family Crassulaceae have been extensively investigated (Mansfield and Snaith, 1984; Queiroz, 1974). Measurements of CO₂ output into the free air by *Bryophyllum fedtschenkoi* (Wilkins, 1959) and CO₂ compensation point in the same plant (Jones, 1973) have clearly demonstrated circadian oscillations in this CAM plant. Recently a rapid oscillation in the levels of fructose bisphosphate in different plant tissues under stress conditions has been reported (Paz et al., 1985).

A rhythm in the activity of phosphoenolpyruvate carboxylase enzyme has been well documented (Mukerji, 1968; Queiroz, 1968; 1969). This rhythm was found to be synchronous to the rhythm in CO₂ fixation. Similarly the rhythmic CO₂ output in CAM plants was related directly to the rhythm in the activity of malic enzyme (Queiroz, 1974).

Rhythms in the photosynthetic CO₂ fixation by C₃ plants such as algae and higher plants have been discussed at length in Chapter 1, section 1.1. No rhythmic changes in the activities of any of the Calvin cycle enzymes have been reported (See section 1.1 for a detailed review of rhythms in electron transport and enzymic reactions).

0.5.4. Oscillations in Photosynthetic Electron Transport

Apart from the whole cell O₂ evolution and ^14^CO₂ fixation rhythms studied in dinoflagellates and other marine algae (Lonergan, 1984b), a number of studies have been carried out on the electron transport reactions such as whole chain electron flow, partial reactions mediated by photosystem II and photosystem I by many investigators (Lonergan, 1984b).
While in all the reports a rhythm in the whole chain electron transport has been described, attempts to identify rhythmicity in the partial photosystem reactions have not yielded positive results (Lonergan, 1981; Lonergan and Sargent, 1979). However, two reports on the rhythms in photosystem II activity are also available (Okada and Horie, 1979; Samuelsson et al., 1983).

0.6. PHOTOSYNTHETIC ELECTRON TRANSPORT

It was hypothesised that the first ever living cell was a photoautotroph (Broda, 1975). Ever since this first step in evolution, a great deal of biological diversity in the forms of autotrophic energy production has taken place (Broda, 1975). Of these, photosynthesis is a process by which green plants, green, red and brown algae, cyanobacteria and photosynthetic bacteria trap the free energy of light and transform it into chemically stable form to suit their metabolic needs. The various physical and chemical mechanisms involved in the complex pathways of energy transduction in photosynthesis have been reviewed in recent years elaborately (Barber, 1977; Clayton, 1980; Govindjee, 1975; Govindjee, 1982).

The initial event of light quanta absorption by the chlorophyll molecules bound to proteins within the thylakoid membranes leads to the formation of radical pair of the primary electron donor and acceptor against a thermodynamic potential gradient. Through cooperation of two such photoacts plants and cyanobacteria oxidize water and release molecular oxygen and produce NADPH and ATP for CO₂ fixation.

0.6.1. Hill and Bendall's Z Scheme of Photosynthesis

The model for interaction of the two light reactions in the green plant photosynthesis proposed by Hill and Bendall (1960)
is based on the series interaction of two photoreactions via a connecting electron transport chain. This extremely accommodative model has stood the test of time and remains to be one of the widely accepted schemes, popularly known as Z scheme (Bishop, 1971; Govindjee, 1982; Vernon and Avron, 1965; Williams, 1977). A recent version of the Z scheme incorporating the current advances in the understanding of photosynthetic electron transport is given in Fig. 0.2. Following is a brief account of the salient features of the Z scheme.

Photosystem II (PSII), associated with the oxidation of water and Photosystem I (PSI), associated with the reduction of NADP$^+$ are serially connected by an intersystem electron transport chain. Absorption of light by the light harvesting antenna system of PSII and the charge separation at the reaction centre (RC) lead to the formation of a strong oxidant which retrieves electrons from water and a weak reductant, PQH$_2$. The second photoreact occurring at the RC of PSI results in the production of a strong reductant NADPH which enters the CO$_2$ assimilation pathway and a weak oxidant P700$^+$. These two photoreactions are kept operating linearly by the repeated oxidation-reduction reactions of the intermediate electron carriers such as the plastoquinones, cytochrome b$_6$-f complex and plastocyanin. The non-cyclic electron transport is energetically coupled to the synthesis of ATP through photophosphorylation, required for photosynthetic carbon fixation.

0.6.2. Photosystem II Mediated Electron Transport

0.6.2.1. The Oxygen Evolving Complex

The oxygen evolving complex (OEC) of photosynthesis is known to be located near the inner side of the thylakoid membranes
Fig. 0.2: Modified and updated version of the Z scheme of electron transport in the thylakoid membranes. (Data collected from Govindjee et al., 1985; Haehnel, 1984; van Gorkom, 1985 have been incorporated into this scheme).
(Fowler and Kok, 1974; Renger, 1976; Wydrzynski et al., 1978; Zilinskas and Govindjee, 1974). The present understanding of OEC structure indicates that it is composed of at least four polypeptides; an intrinsic 27-34 kilodalton (KD) polypeptide, possibly associated with manganese (Mn) and three peripheral polypeptides of 33, 24 and 18 KD (Govindjee et al., 1985). The binding and oxidation of water probably takes place at a cluster of four Mn ions (Cheniae, 1980; Critchley, 1985; Hansson et al., 1984; Kusonoki, 1984; Sauer, 1980; Wydrzynski and Sauer, 1980). O₂ is evolved upon the accumulation of four oxidizing equivalents which occur in four successive redox states called $S_0$, $S_1$, $S_2$, and $S_3$ originally proposed by Kok et al. (1970). The manganese cluster kept in place by an extrinsic 33 KD polypeptide has been assumed to accumulate the oxidizing equivalents (Govindjee et al., 1985; Inoue et al., 1983; van Gorkum, 1985).

Calcium and chloride ions are essential for the functioning of OEC (Dekker et al., 1984; Govindjee et al., 1985; Hind et al., 1969; Inoue et al., 1983; Izawa et al., 1983; Kelley and Izawa, 1978; Ono and Inoue, 1983; Sandusky and Yocum, 1983). Several working hypotheses have been proposed to account for the Cl⁻ ion requirement but no direct experimental evidence is yet available (Govindjee et al., 1985). An indirect role for Cl⁻ in O₂ evolution might be as an allosteric effector, stimulating the water-splitting reactions by binding to a site or sites other than the active site (Coleman and Govindjee, 1984; Critchley, 1985; Govindjee et al., 1985).

0.6.2.2. Photosystem II Reaction Center

Photosystem II is a chlorophyll-containing supramolecular protein complex capable of light induced O₂ evolution and
reduction of plastoquinone (PQ). Light absorbed by PSII antenna pigment, results in the oxidation of a specific chlorophyll (Chl) molecule P680 (Döring et al., 1968) and reduction of an intermediary transient acceptor pheophytin a (Klimov, 1984; Klimov and Kransnovski, 1981; Klimov et al., 1977; Klimov et al., 1985). The following electron transport from pheophytin a to QA is likely to be the electrogenic step in the charge separation of PSII (van Gorkom et al., 1983). On the donor side, the oxidized P680+ is rereduced by the secondary donor(s) Z whose chemical nature is believed to be a strongly bound plastoquinol molecule that is oxidized to semiquinone cation (Dekker, 1985; Dekker et al., 1984).

The PSII reaction centre complex isolated from diverse classes of oxygenic photosynthetic organisms such as green algae and higher plants (Satoh, 1985) was identical and consisted of five different polypeptides of 51-44, 44-40, 34-30, and 10-7 KD molecular weight (Satoh et al., 1983). It has been shown that one of the polypeptides, about 32 KD is the species responsible for the binding of atrazine/DCMU type herbicides and hence the Q_b binding protein (Kyle, 1985; Satoh et al., 1983).

Melis and Homann (1975) found that the photoreduction of Q was heterogenous and attributed this to the existence of two types of PSII centres called PSII_α and PSII_β. It has been later found that the PSII_α and β centres are independent systems with different antennae (Thielen and van Gorkom, 1981a; Thielen et al., 1981). PSII_α centres have been shown to be restricted to the appressed membranes whereas the PSII_β centres (generally about 30% of PSII centres) are bound in the non-appressed membranes (Anderson and Melis, 1983; Melis and Thielen, 1980). Though different roles are assigned for the PSII_β centres, electron transport involving these centres is said to be of quantitative importance only at high light intensities (van Gorkom, 1985).
0.6.2.3. Reducing Side of PS II

The electron transfer from \( Q_{A}^- \), which has been identified as a plastosemiquinone -Fe\(^{2+}\) complex (Nugent et al., 1981; Okamura et al., 1982), involves a two-electron gate (\( Q_B \)) mechanism (Bouges-Bocquet, 1973; Velthuys & Amez, 1974). The secondary electron accepter \( Q_B^- \), also a plastoquinone molecule is bound to the 32 KD polypeptide which is known for its binding sites for herbicides. \( Q_B^- \) is also known for its role in the bicarbonate effect on electron transport (Cramer and Crofts, 1982; Vermas and Govindjee, 1981).

A large pool of PQ molecules which is reduced by the secondary acceptor \( Q_B^- \) is located in the hydrophobic zone of the thylakoid lipid bilayer (Whitmarsh, 1986). PQ has been assigned the role of a mobile electron carrier in order to account for the electron transfer between PSII and PSI in view of the lateral heterogeneity of the thylakoid membranes (Millner and Barber, 1984; Whitmarsh, 1986). PQ is reduced by \( Q_B^- \) to \( PQH_2 \), where two \( H^+ \) are taken from the outside of the thylakoid into the lumen, thus generating a proton gradient necessary for ATP synthesis (Govindjee and Govindjee, 1975).

0.6.3. Photosystem I related Electron Transport

0.6.3.1. Intersystem Electron Carriers

Cytochrome b\(_6\)f complex, a supramolecular intrinsic protein complex located in both the appressed and non-appressed regions of the thylakoid membranes (Barber, 1983a; Haehnel, 1984; Whitmarsh, 1986) oxidizes \( PQH_2 \) and transfers the electrons to plastocyanin. Cyf b\(_6\)f complex also probably supports a Q cycle mechanism (Cox and Olsen, 1982; Hurt and Hauska, 1982). Plastocyanin (PC), the other mobile electron carrier
involved in the intersystem electron transport (Whitmarsh, 1986) is an extrinsic protein located in the thylakoid lumen (Barber, 1983a). PC reduces the oxidized PSI reaction centre, P700⁺.

0.6.3.2. Photosystem I

PSI is a hydrophobic membrane spanning intrinsic protein complex comprising of the reaction centre P700 (Barber, 1983a; Haehnel, 1984; Kaplan and Arntzen, 1982; Kok, 1957). P700 is the primary electron donor of light reaction I consisting of a chemically modified monomeric Chl a (Wasielewski et al., 1981). The charges separated in the reaction centre are stabilized by fast electron transfers through the chain of electron acceptors as shown below:

\[
\text{P700} \rightarrow A \rightarrow A_1 \rightarrow X \rightarrow \text{(Centre B - Centre A)}
\]

The final electron acceptors in the electron transport chain of PSI complex are the sulfur centres A and B which consist of bound ferredoxins with Fe₄S₄ centres (Cammack and Evans, 1975; Malkin, 1982). Ultimately NADP⁺ is reduced to provide the required reducing power to drive the Calvin cycle enzymic reactions leading to the assimilation of CO₂.

0.6.4. Photophosphorylation

The processes of photophosphorylation and ATP synthesis were first demonstrated in whole cell photosynthesis and isolated chloroplasts, respectively (Arnon et al., 1954). Electron transport and phosphorylation are coupled, in the sense that no phosphorylation will occur unless electron transport is proceeding (Jagendorf, 1977). However, a small amount of electron transport takes place in the absence of phosphorylating conditions (i.e. in the absence of ADP and P₇), known
as basal electron transport. This rate can be enhanced in the presence of ADP and Pi and the enhancement is often given as a ratio, referred to as the 'photosynthetic control' (Jagendorf, 1977).

A number of synthetic and natural chemicals are known to enhance the rate of basal electron transport even in the absence of ADP and Pi. These chemicals are said to be uncouplers (Good, 1977) of the electron transport from the restraints of inactive but coupled energy conservation machinery. A few other compounds have been found to block ATP synthesis by inhibiting the terminal reactions and are called 'energy transfer inhibitors' (McCarty, 1977). These energy transfer inhibitors prevent both ATP synthesis and the additional electron flow induced by and dependent on phosphorylation, but do not affect the basal or uncoupled electron flow (Jagendorf, 1977).

In the entire chain of electron transport two phosphorylation sites have been identified by analyzing the P/A ratios (Izawa and Good, 1968; Reeves and Hall, 1973; Trebst, 1974). The first site occurs between water and PSII, and the second is between Q and Cyt f of the intersystem electron transport chain.

Three major hypotheses have been proposed to account for the mechanism of coupled electron flow and ATP synthesis. The "chemical hypothesis" (Chance and Williams, 1956; Chance, 1977) envisages the formation of direct chemical bonds with electron carriers leading to synthesis of ATP from ADP and Pi. The 'conformational hypothesis' proposed by (Boyer, 1974; 1977) suggests that the transfer of energy from redox reactions to ATP synthesis is mediated by conformational changes in the enzymes of phosphorylation or the thylakoid
membrane or both. Mitchell's (1961; 1977; Ferguson, 1985) "Chemiosmotic hypothesis", which is most widely accepted, depends on vectorial translocation of protons across the proton-impermeable membrane as a necessary consequence of electron transport, and the membrane localized or delocalized proton electrochemical gradient drives ATP synthesis (Ferguson, 1985).

The enzyme responsible for ATP formation, ATP synthetase is localized on the thylakoid membranes as two discrete units namely, coupling factor zero (CF₀), an intrinsic protein complex and coupling factor 1 (CF₁), an extrinsic protein complex on the outerside of the thylakoid vesicle (Barber, 1983a; Haehnel, 1984; Jagendorf, 1977). Both CF₀ and CF₁ are comprised of a number of subunits. Three sub units of CF₀ are known to constitute the proton translocating channel (Nelson, 1982). CF₁ complex probably has a stoichiometry of subunits of \( \frac{3}{2}, \frac{3}{2}, \frac{1}{2}, \frac{1}{2}, \frac{1}{2}, \frac{1}{2} \) and \( 2 \xi \) (See Fig. 3; Merchant and Selman, 1985).

0.6.5. Thylakoid Membrane Composition

0.6.5.1. Morphology of the Chloroplast Membranes

Thylakoids represent one of the most complex folded membrane systems that are organized into appressed regions and non-appressed regions (Arntzen and Briantais, 1975; Coombs and Greenwood, 1976; Kaplan and Arntzen, 1982). The membrane in the appressed (stacked) regions exhibit close membrane-membrane interactions. The thylakoids being closed vesicles have inner and outer surfaces with an internal lumenal compartment. The degree of membrane appression is not a rigid phenomenon, instead it responds reversibly to the cation concentration (Barber and Chow, 1979) and this stacking and
destacking response is dependent on the valency of the non-binding cation species (Barber, 1980; Barber et al., 1977). Whereas electrostatic neutralization of the exposed membrane charges by the binding of certain cations also induces stacking of the thylakoids (Barber, 1980a; Barber, 1983b). Apart from the above mentioned stacking processes studied in vitro, variations in the degree of appression of the thylakoids depending upon the growth conditions have also been extensively studied in vivo (Anderson 1982a). Moreover, the intramembrane movements of the proteins have also been commonly observed in response to ionic conditions (Staehelin, 1976; Wang and Packer, 1973) and to protein phosphorylation (Kyle et al., 1983).

### 0.6.5.2. Thylakoid Lipids

The acyl lipids of the thylakoid membranes constitute about 50% of the thylakoid mass and are highly enriched with electroneutral galactolipids as compared to phospholipids. About 75% of the acyl lipids are mono and digalactosyldiacyl glycerols and phospholipids and phosphatidylglycerol constitute 15% and 10%, respectively (Barber, 1983a). The thylakoids have little or no sterols and have highly unsaturated fatty acids (Chapman et al., 1983) with a predominance (~90%) of linolenic acid (Quinn and Williams, 1978). Time dependent fluorescence anisotropy studies have shown that higher plant thylakoids are extremely fluid (Ford and Barber, 1983).

### 0.6.5.3. Thylakoid Proteins

The thylakoid membranes have intrinsic as well as extrinsic proteins. There are five supramolecular intrinsic membrane spanning protein complexes in higher plant thylakoids (Barber, 1983a). These are PSI and PSII complexes, light harvesting
chlorophyll a/b pigment complex (LHCP), Cyt b₆-f complex and the CFₒ complex of the ATP synthetase (Barber, 1983a; Haehnel, 1984; Whitmarsh, 1986). Figure 0.3 shows the various intrinsic and extrinsic membrane proteins involved in electron transport as well as chloroplast ATP synthetase.

0.6.5.3.1. Intrinsic Proteins of the Thylakoid Membranes

The PSI complex contains about 30% of the total chlorophylls in normal sun plants (Thornber et al., 1979) with its molecular weight at about 800 KD (Hiller and Goodchild, 1981) and a diameter of 106 Å (Mullet et al., 1980). Each complex has been estimated to contain one P700 with 150-200 Chl a molecules and the associated primary electron acceptors A₁, A₂ and two iron sulfur centers (Anderson, 1980a; Bengis and Nelson, 1977).

The PSII complex contains 10-15% of the total chlorophylls with about 60 Chl a per P680 (Anderson, 1980a). The Chl binding polypeptides have no Chl b and weigh about 50 and 43 KD (Anderson, 1980b; Braumann et al., 1982; Satoh, 1981). Other polypeptides are associated with the OEC and RC components. The molecular weight is in the region of 600 KD and the diameter about 80 Å (Armond et al., 1977; Hiller and Goodchild, 1981).

The LHCP contains two polypeptides of 26 and 28KD and has a Chl a/b ratio close to 1.5 (Braumann et al., 1982; Thornber, 1975). LHCP only functions in a light harvesting capacity with about 240 Chl per P680 (Anderson, 1980a; Thornber and Barber, 1979).

Cyt b₆-f complex, which acts as a plastoquinol-plasto-cyanin oxidoreductase has been found to contain five poly-
Diagramatic representation of the various intrinsic and extrinsic protein complexes of the thylakoid membranes involved in electron transport and proton translocation (from Anderson, 1984).
peptides; 34, 33, 23.5, 20, 17.5 KD. There are two Cyt b-563, one Cyt f, and Rieske Fe-S center with two non-heme irons and bound plastoquinone-9 per each complex (Hurt and Hauska, 1981).

Finally, the ATP synthetase (CF\textsubscript{o}) complex consists of three main polypeptides with molecular weights 15, 12 and 8 KD, called subunits I, II and III, respectively (Nelson, 1982). While subunit I acts as a binding protein between CF\textsubscript{o} and the CF\textsubscript{l}, the subunits II and III are related to the formation of proton conducting channel.

0.6.5.3.2. Extrinsic Proteins of the Thylakoid Membranes

Extrinsic proteins on the outer surface of the membranes consist of (i) Ferredoxin - a low molecular weight (11 KD) polypeptide with 2 Fe-2S active centres and 95-100 amino acid residues (Hall and Rao, 1977); (ii) Ferredoxin-NADP oxido reductase, a flavoprotein (40 KD) relatively tightly bound to the thylakoid surface (Forti, 1977); (iii) ATP synthetase (CF\textsubscript{l}) complex a spherical complex of about 325 KD which consists of 5 subunits (Nelson, 1982; Schlodder et al., 1982).

Extrinsic proteins on the inner side of the membranes consist of plastocyanin, a one copper atom-containing protein of about 10.5 KD loosely bound to the inner surface (Haehnel et al., 1981). This protein is a mobile electron carrier and resembles a flattened barrel with the Cu atom located near one end (Colman et al., 1978). There are at least three other extrinsic polypeptides related to the OEC of PSII present on the inner surface of the thylakoids (Govindjee et al., 1985).
0.6.6. Organization of Thylakoid Membranes

The various intrinsic protein complexes present in the thylakoids are distributed among the appressed and non-appressed zones of the membranes in a heterogeneous manner (Barber, 1983a; Haehnel, 1984; Whitmarsh, 1986). Figure 0.4 illustrates the current ideas on the organization and distribution of various protein complexes in the thylakoid membranes. The PSII complexes have been found to be almost entirely restricted to the appressed membranes, whereas the stromal non-appressed membranes are enriched with PSI complexes (Arntzen et al., 1972; Sane et al., 1970). Some studies (e.g. Anderson, 1982a; Anderson and Melis, 1983; Andersson and Haehnel, 1982) have reported that while about 85% of the PSII are restricted to the granal stacks, 15% are also found in the stromal membranes. However, all the PSI were found only in the non-appressed membranes.

The LHCP, as it is functionally related to PSII is also entirely limited to the grana (Albertsson et al., 1982; Andersson, 1978; Andersson and Anderson, 1980; Barber, 1980a,b). These complexes are reported to be spanning the membrane with exposed ends on both sides (Andersson et al., 1982; Mullet and Arntzen, 1980). Both CF₀ and CF₁ units of the ATP synthetase have been located exclusively in the stromal membranes (Andersson and Haehnel, 1982).

Several studies have indicated that the Cyt b₆-f complex is randomly distributed between appressed and non-appressed regions (Anderson, 1982b; Cox and Andersson, 1981; Mansfield and Bendall, 1984; Takano et al., 1982; Whitmarsh, 1986). However, Barber (1983a) and Ghirardi and Melis (1983) have argued that the lateral distribution of Cyt b₆-f complex is restricted to the boundaries between the appressed and non-appressed regions alone.
Fig. 0.4 Distribution of protein complexes of thylakoids between the appressed membranes of grana and non-appressed membranes of the stroma (from Anderson, 1984).
Plastoquinone, the hydrophobic mobile electron carrier appears to be located in the hydrophobic core of the lipid bilayer and distributed in both the appressed and non-appressed regions of the thylakoids (Jennings et al., 1983). However, Peters et al., (1983) have shown that the mobile carrier plastocyanin is preferentially located in the stroma-exposed regions of the thylakoid membranes.

0.6.7. Consequences of Lateral Heterogeneity in Thylakoids

The above mentioned lateral distribution of the various intrinsic and extrinsic protein complexes in the thylakoids necessitate an optimal spatial relationship between these complexes in order to achieve non-cyclic electron flow (Fig. 0.4). Electron transport from PSII to PSI, which are spatially separated between the appressed and non-appressed regions, respectively, requires the long-distance diffusion of the intersystem electron carriers. In view of the intrinsic nature of Cyt b₆-f complex, PQ and PC are the likely candidates for shuttling electrons between the two photosystem (Millner and Barber, 1984; Whitmarsh, 1986). Detailed studies have shown that indeed, the large diffusion coefficients of these two mobile carriers facilitate such electron shuttling (Whitmarsh, 1986).

The other important consequence of the lateral heterogeneity of thylakoid membranes is the possibility of lateral motion of the intrinsic proteins within the non-appressed and the appressed regions and also between these two zones (Barber, 1983a). A physiological mechanism for the balanced relative excitation of both PSII and PSI, so as to achieve optimal electron flow, involves the interchange of intrinsic proteins between the granal and stromal membranes through
a regulation of the electrical charge in the exposed regions of the proteins. This regulatory mechanism is called as State I - State II transitions (Myers, 1971; Barber, 1976).

Recent studies have clearly shown that a redox controlled phosphorylation/dephosphorylation on the exposed surfaces of LHCP (Allen et al., 1981; Horton and Black, 1980; Haworth et al., 1982) controls the relative distribution of excitation energy between the two photosystems. Phosphorylation induced mobility of the LHCP and its subsequent association with the PSI has been demonstrated by many workers (Islam and Jennings, 1985; Kyle et al., 1983; Kyle et al., 1984; Torti et al., 1984). Such lateral mobility results in an increase in the absorptive cross section of PSI thus leading to increased energy transfer (Black et al., 1986). These State I-State II transitions are known to provide effective regulatory mechanism in vivo under various light conditions and energy distribution (Melis, 1984; Melis et al., 1985).

0.7. LEAF SENESCENCE AND PHOTOSYNTHESIS

One of the most prominent phases of leaf development is the phase of senescence, which is usually characterized by the appearance of signs of cell and tissue degradation. The process of accumulation of deleterious changes in the structural and functional machinery of the cells that ultimately lead to its collapse is referred to as senescence (Leopold, 1980). These changes provide for an endogenous regulation of death. However, the influence of the environmental factors such as light, temperature and water stresses also condition the sequence of events occurring in a cell during senescence (Biswal and Biswal, 1984; Sestak, 1985).
Photosynthesis, being the primary metabolic process in plants is adversely affected by senescence-induced changes and is reflected in terms of a reduction in the biomass production. While the structure and function of the chloroplasts, have been understood fairly well in normal mature plants (see section 0.6), many attempts have been made to characterize the nature of changes in this organelle, during the declining phase of leaf development (Sestak, 1985; Thimann, 1980).

0.7.1. Senescence Induced Changes in Chloroplast Structure

Among the first microscopically visible changes in leaf senescence are those in the chloroplasts (Butler and Simon, 1971). Loss of starch deposits and swelling of the chloroplasts are characteristic events that have been commonly noted in many plant systems (Das and Leopold, 1964; Dennis et al., 1967; Shaw and Manocha, 1965). It has been observed that the thylakoids become less dense and osmophilic granules appear close to them (Barton, 1966; Butler, 1967; Mlodzianowski and Ponitka, 1973). In most cases disappearance of plastid and other organellar membranes was observed routinely during the later stages of senescence (Thimann, 1980). Such weakening and dissolution of membranes always lead to large changes in the permeability of cells in senescing tissues (Das and Leopold, 1964; Draper and Simon, 1971; Sacher, 1957; 1959). Movement of free fatty acids from the chloroplasts into other parts of the cell have been ascribed to the increased leakage in chloroplast membranes (Draper and Simon, 1971).

Breakdown of a number of proteins in the thylakoid membranes, especially the light harvesting chlorophyll proteins associated with PSII and PSI antenna systems is one of the major events in the pattern of structural disintegration (Bricker and Newman, 1982). Disappearance of other
membrane proteins such as the CF\textsubscript{0} and CF\textsubscript{1} of the ATP synthetase has also been observed (Camp et al., 1982). Results from various laboratories suggest that during leaf senescence whole chloroplasts are lost rather than general degradation of chloroplasts (Camp et al., 1982; Peoples et al., 1980; Wittenbach et al., 1982). It has been suggested by Thimann (1980) that the changes in the fine structure and membrane integrity may be 'results' rather than the 'causes' of senescence.

0.7.2. Changes in Electron Transport Capacity

One of the primary symptoms of senescence that can be correlated to the loss of photochemical activity is the decline in Chl content (Biswal and Biswal, 1984; Sestak, 1977a; Sestak, 1985; Thimann, 1980). Concomitant with the changes in membrane properties, extensive impairment of the electron transport activity in chloroplasts was noted (Biswal and Biswal, 1984; Sestak, 1977b; Stoddart and Thomas, 1982; Thomas and Stoddart, 1980). Rapid decline in the whole chain electron transport activity (Holloway et al., 1983; Jenkins and Woolhouse, 1981a), and partial electron transport activity estimated as PSII and PSI assays in a variety of plants (Biswal and Mohanty, 1976b; Bricker and Newman, 1982; Grover et al., 1986a; Jenkins and Woodhouse, 1981a,b) are commonly observed in chloroplasts isolated from detached or intact senescing leaves. Profound variations in the intersystem electron transport carriers such as plastoquinone, plastocyanin and cytochrome b\textsubscript{6}-f complex have been attributed to be responsible for reduced flow of electrons through the whole chain (Dupont and Siegenthaler, 1986; Holloway et al., 1983; Jenkins and Woolhouse, 1981a,b; Sabat et al., 1985).
0.8. PHYTOCHROME CONTROL OF LEAF SENESCENCE

Light is known to act as senescence retardant (Biswal and Biswal, 1984). Apart from its role via photosynthesis, light has been shown to effectively counteract the deteriorative changes in the chloroplast structure and function by its action through photomorphogenesis (Biswal and Biswal, 1984). The photoreceptor responsible for this retarding effect of light is phytochrome. De Greef et al. (1971), Biswal and Sharma (1976) and Pfieffer and Kluedgen (1980) have all shown that phytochrome maintains the chloroplast membrane integrity and regulates the loss of Chl content under detached senescing conditions. Many recent reports have further strengthened the belief that phytochrome is active in senescence retarding role of light (Biswal et al., 1982; 1983a; Cuello et al., 1984). It is possible that phytochrome may be involved in the regulation of synthesis of the 'senescence retarding proteins' (Cuello et al., 1984).

0.9. HORMONAL CONTROL OF LEAF SENESCENCE

Plant growth substances exert both senescence stimulatory and inhibitory effects (Noodén, 1980). While the natural growth regulators such as abscisic acid and ethylene induce senescence whether applied exogenously or by an alteration of endogenous level (Even-Chen & Itai, 1975; Karanov and Pogoncheva 1976; Osborne, 1973), others such as auxin, gibberellic acid and cytokinin have been unequivocally shown to retard senescence (Noodén, 1980; 1986).

It is important to note that the protective action of growth regulators on the senescence mediated changes is only prominent when used on a detached system. The intact
system does not always respond positively (Noodén, 1980). This is probably because of the endogenous level of these substances in the attached leaves. Cytokinin and its synthetic analogues have been widely used in senescence studies (Noodén and Leopold, 1978).

0.10. INTERACTION BETWEEN GROWTH REGULATORS AND LIGHT DURING SENESCENCE

Both light and the growth substances have positive influence on the senescence process in leaves, in the sense that they counter or reduce the extent of deteriorative changes in the system (Biswal and Biswal, 1984; Thimman, 1980). These two senescence retardants are also known to exhibit an interrelationship (Gepstein and Thimann, 1981). The endogenous level of ethylene was reduced when the dark senescing leaves were exposed to light (Gepstein and Thimann, 1981). Similarly, Wareing and Thompson (1976) showed that the endogenous levels of cytokinins and ethylene were reciprocally affected by short pulses of red light. This type of interaction between light and growth regulators was also shown by Mishra and Kar (1973) and Singh and Misra (1975). However, the nature of this interaction is not clearly understood yet. There have been no studies to investigate the possible interaction between light and the growth regulators applied exogenously as compared to the endogenous pool of these substances.

0.11. SCOPE OF THE PRESENT STUDY

Rhythmic oscillations in the photosynthetic reactions in a number of algal species have been investigated by many research groups (Edmunds, 1984; Lonergan, 1984b). However, studies of similar nature in higher plants are restricted to a limited number of systems and the phenomenology is less
thoroughly investigated as compared to their microbial counterparts (Lonergan, 1984b; Hillman, 1976; Wagner, 1977). The phenomenological reports in various systems include studies on the whole cell of CO₂ fixation, chlorophyll contents, whole cell O₂ evolution, photochemical activities of isolated chloroplasts, various parameters of Chl fluorescence, enzymic reactions of Calvin cycle, etc. (Edmunds, 1984; Lonergan, 1984b). Most of these studies reflected circadian-type of oscillations with a few exceptions (Lonergan, 1984a; Cumming, 1972; Wagner and Cumming, 1970).

None of the enzymic reactions involved in the carbon fixation pathway were found to oscillate in any of the systems, although the whole cell ¹⁴CO₂ fixation itself was rhythmic (Edmunds, 1984; Pallas et al., 1974). Therefore, the possible location of the rhythm control was attributed to the light reactions of photosynthesis. Efforts made to identify the possible reactions involved in such a rhythm control in the electron transport chain did not yield any positive conclusions since the partial electron flow via PSII or PSI, which together constitute the whole chain electron transport was not rhythmic, while the latter showed distinct oscillatory patterns (Lonergan, 1984b). However, two reports on rhythmicity in PSII activity are available in the case of algal systems (Okada and Horie, 1979; Samuelsson et al., 1983), but no such observation has yet been made in higher plants (Lonergan, 1981).

Various groups engaged in the study of rhythms have attempted to explain the molecular basis of these oscillations with the help of a large number of molecular models. However, no definite conclusions have been arrived at, so far. The possibility of thylakoid membranes being responsible for the generation of rhythmic variations in the photosynthetic
light reactions is currently being favoured (Lonergan, 1981; Lonergan, 1983; Prezelin and Sweeney, 1977).

In the first part of the present study, we have attempted to investigate and characterize endogenous rhythmic phenomena in the electron transport reactions of wheat chloroplasts. Time-dependent rhythmic variations in photosynthesis are known to influence the primary productivity (Harding et al., 1982b) and therefore a study of the phenomenology of rhythms in a crop plant such as wheat assumes an agronomic significance. We have attempted here, to investigate the whole chain as well as partial electron transport reactions in considerable detail under a variety of growth and illumination conditions. Rhythm patterns observed in the PSII and PSI reactions have been further probed, in order to identify possible location(s) of the rhythm control in the electron transport chain. Finally, a study has also been made under constant darkness and under senescent conditions to consolidate the assertion that the observed rhythms in wheat are endogenous in nature. We have attempted to explain the oscillations observed in wheat chloroplast photochemical activity in terms of time-dependent reversible changes in the membrane structure.

Second part of the present study describes the nature of structural and functional changes occurring in wheat chloroplasts during leaf senescence.

The nature of deteriorative changes taking place in the chloroplasts during leaf senescence has been extensively studied in a wide variety of plant systems (Sestak, 1985; Thimann, 1980). Since during the senescent phase of development photosynthetic capacity of the leaves undergoes a drastic change, crop productivity is affected to an appreciable
extent. Therefore, a study of changes in the molecular events and their sequence of occurrence in the chloroplasts during leaf senescence is essential from basic understanding point of view as well as from its agronomic value.

Light, an important environmental factor during leaf senescence is just as essential as in the case of endogenous rhythmic regulation of the physiological functions. Of the various environmental factors, light has been singled out as an effective retardant of the senescence induced changes in chloroplasts (Biswal and Biswal, 1984). It has been found that light acts both via photosynthesis and photomorphogenesis. Similarly, many plant growth regulating substances are known to retard senescence (Woolhouse and Jenkins, 1983; Noonan and Leopold, 1978). While the role of these two senescence retardants has been studied separately, detailed investigations of their possible interactions are extremely limited (Gepstein and Thimann, 1981; Mishra and Kar, 1973).

In this part of the present study, we have attempted to investigate the influence of light as a function of its spectral quality on the chlorophyll contents and on the photochemical activities in detached senescing wheat leaves. Possibility of an interaction between light and a synthetic analogue of cytokinin, benzimidazole has also been explored. The spectral quality of light used in this study included white, red and far-red light. The detached leaf system was chosen as a convenient laboratory model. The observations made with the detached leaves were also extended to intact leaf system in order to ascertain the validity of the conclusions drawn with the former. Antagonistic interaction between the growth regulator and light and its differential expression in detached and intact leaf systems have been discussed. Since light retardation of senescence is also ascribed to
its action via phytochrome, we have checked the possibility of phytochrome involvement in regulation of senescence under our experimental conditions.

In toto, we have attempted to characterize the photochemical activities of wheat chloroplasts under endogenous (rhythmic) regulations and exogenous (environmental) control during the mature and senescent phases of primary leaf development.