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The Perception of Light-Dark Transitions [and Discussion]

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The perception of light–dark transitions

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Transitions between light and darkness are particularly important where these serve as Zeitgebers to synchronize circadian rhythms. A special case is photoperiodism, which depends on the accurate detection of light–dark transitions and on the coupling of this information to a timing mechanism that appears to be based on the circadian clock.

Results from laboratory experiments are considered in relation to the natural changes experienced at dawn and dusk, and evidence is presented that the light–dark transitions that couple to the timing mechanism in short-day plants are perceived through changes in irradiance rather than through changes in light quality.

It has been generally accepted that the *light–dark* transition is sensed by a decrease of P_{fr} levels in darkness, whereas *dark–light* is sensed by the rapid formation of P_{fr} in the light. However, P_{fr} in light-grown plants appears to be rather stable and so changes in P_{fr} level after transfer to darkness may not be a sufficiently accurate method of detecting the light–dark transition in photoperiodism.

The paper reviews some of the evidence from photoperiodic experiments and concludes that the plant may discriminate between light and darkness through the continuous or intermittent formation of 'new' P_{fr}.

Introduction

The ability to discriminate between light and darkness is undoubtedly of great significance in the life of green plants. The initial emergence of the germinating seedlings into the light is accompanied by a profound change in the pattern of development from that characteristic of darkness to that characteristic of the light-grown plant, modulated with respect to the quantity and quality of radiation received. This strategy has presumably evolved to conserve stored reserves until photosynthesis is possible and this *dark–light* transition is primarily sensed by the phototransformation of dark-synthesized P_r into P_{fr}. Having once emerged into the light, plants are then normally subject to daily alternations of light and darkness, and a wide range of behavioural and developmental responses are keyed to this daily cycle.

There are, of course, many direct responses to light, and most of these are probably related to the needs of photosynthesis. A good example of such a response is stomatal opening, which appears to be controlled by light through two distinct systems. One of these operates through photosynthesis, with chlorophyll as its active chromophore. The second uses a photoreceptor located in the guard cells and is sensitive only to blue light (Zeiger *et al.* 1981). In both cases the continued formation of the excited state of the photoreceptor is essential for the response to light, and so discrimination between light and darkness is achieved.

The other main types of response to the daily light–dark cycle are those involving an interaction between a photoreceptor and an endogenous timing system. It is in such responses that transitions between light and darkness achieve their greatest significance because these are the major natural signals to which time measurement is coupled. The phases of overt circadian

rhythms such as leaf movements are, for example, entrained to the continuing light–dark cycle in such a way that the response changes with the time of day to the advantage of the plant. There have been relatively few studies of the photoreceptors that perceive the entraining light–dark transitions, but most evidence indicates that phytochrome is a major sensor in higher green plants, although a blue-light absorbing photoreceptor also functions in some cases Wilkins & Harris 1976; Satter & Galston 1981).

The timing of reproduction and other seasonal events such as dormancy is largely controlled by photoperiodism: a response to the durations of day or night (or both), which change seasonally and so give information about the time of year. The predictive value of photoperiodism is obvious and allows for life cycles to be coordinated to avoid unfavourable conditions such as low temperature in winter, or to be synchronized with favourable ones. Precise timing may be valuable in itself and not just with regard to season, allowing synchronism of events to the advantage of the plant. Synchronized flowering, for example, improves the chances of cross-pollination.

To locate the time of year accurately, time measurement must operate with great precision and be relatively insensitive to random variations in the environment. As might be expected, the greatest precision is often found in plants from the tropics, where the seasonal changes in daylength are much less than those at high latitudes. From observations made in West Africa, it was found that a difference of only 15 min of light per day was sufficient to determine whether or not flowering occurred in several species of tropical short-day plant (Njoku 1958). Such high precision in time measurement requires similarly high precision in the perception of the transitions between light and darkness. Because their flowering often occurs most rapidly in continuous light, the significance of light–dark transitions is less clear for long-day plants than for short-day plants, where flowering is primarily dependent on the duration of darkness (Vince-Prue 1979, 1981). Consequently the mechanism through which light–dark transitions are sensed is discussed in this paper in relation to the perception of the signals that synchronize photoperiodic time measurement in short-day plants. The discussion is largely based on results obtained with *Pharbitis nil*.

Characteristics of the short-day response

Perhaps the best known characteristic of photoperiodism in short-day plants is that their photoperiodic time-keeping is essentially a question of measuring the duration of uninterrupted darkness, which must exceed a certain critical value to allow flowering to occur (Vince-Prue 1975). Because the overall duration of darkness is critical for flowering, both the beginning (dusk) and end (dawn) of the night must be sensed precisely. A second important characteristic is that an inductive dark period can be rendered ineffective by an interruption with a short light treatment (night-break) given at a particular time. In experiments with *Pharbitis nil*, this light-sensitive period occurs 8–9 h after the transition to darkness irrespective of its duration, and similar results have been obtained with *Xanthium strumarium*. The night-break response is thus a transient period of light sensitivity related in time to the beginning rather than the end of the dark period. Although the time of night-break sensitivity may more or less coincide with the critical night length, it always occurs somewhat earlier (Takimoto & Hamner 1964) and, under some conditions, appreciably so (figure 1). After the transient light-sensitive phase is over, therefore, further reactions are necessary for the induction of flowering; these reactions are also

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inhibited by light. They are also temperature-sensitive in *Pharbitis* so that the critical night length, but not the time of night-break sensitivity, is influenced by temperature. However, when repeated cycles are used, as under natural conditions, the critical dark period is hardly influenced by temperature (Takimoto 1969).

The capacity to respond to an inductive dark period can be modified and in some cases eliminated by appropriate manipulation of the preceding photoperiod. In single-cycle short-day

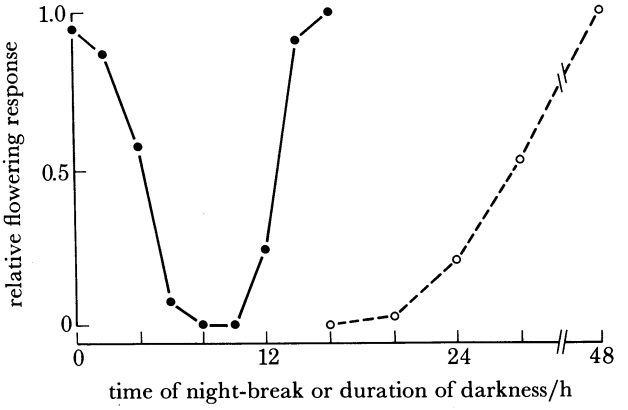

FIGURE 1. Time of sensitivity to a night-break and critical length of night in *Pharbitis nil*. Plants received a single dark period of various durations (○) or a 48 h dark period interrupted at different times with a 5 min red night-break (●). The temperature of the dark period was 18 °C in both cases. (From Takimoto & Hamner (1964).)

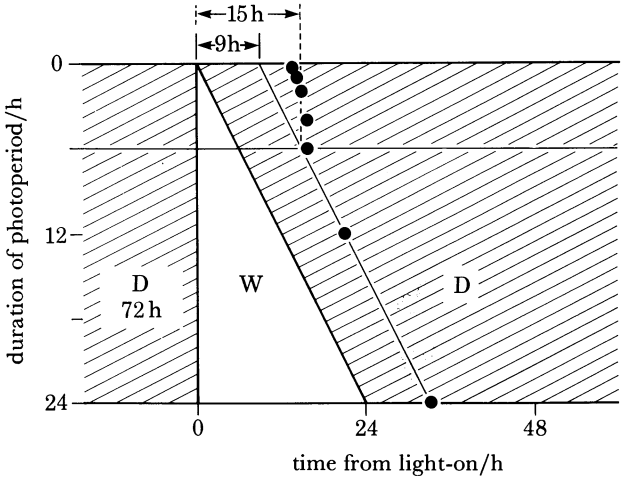

FIGURE 2. Time of maximum sensitivity to a red night-break as a function of the duration of the preceding photoperiod. The time of maximum sensitivity is indicated in real time (●) for the first circadian cycle only. (From Lumsden *et al.* (1982).)

plants, such as *Pharbitis*, where exposure to a single dark period is sufficient for flowering, there is apparently no maximum duration of photoperiod beyond which a subsequent dark period longer than the critical ceases to induce flowering. When the duration of light is decreased, however, there is a change in the timing characteristics and, when the photoperiod is 5 h or less, timing of night-break sensitivity is coupled to the beginning (15 h after light-on signal) rather than to the end (9 h after light-off signal) of the day (figure 2).

Time measurement can thus be coupled either to a light-on or to a light-off signal, the latter being perceived only after exposure to several hours of continuous light. Recent experiments have shown that the critical night length is timed in the same way, i.e. from the beginning or the end of the photoperiod depending on its duration (P. J. Lumsden, unpublished). A very similar pattern of response has been reported for another single-cycle short-day plant, *Xanthium strumarium* (Papenfuss & Salisbury 1967).

In *Pharbitis*, a skeleton photoperiod beginning and ending with a pulse of red light is sufficient to induce flowering in response to a subsequent dark period (Friend 1975). The time of sensitivity to a night-break remains characteristic of a single pulse rather than continuous light and occurs 15 h after light-on (from the second pulse) (Lumsden *et al.* 1982). It has been shown that P_{fr} is required for the flowering response under these conditions, and the reversibility of the first pulse by far-red light given 12 h later indicates the stability of the P_{fr} formed by the first pulse (Friend 1975). However, recent experiments (H. Saji, unpublished) have indicated that, with respect to night-break timing, a 24 h skeleton is perceived as continuous light (i.e. the night-break sensitivity occurs 9 h after light-off) when brief pulses of red light are given every hour. Thus, although a relatively stable P_{fr} is involved in the induction of flowering, exposure for several hours to continuous light or to frequent pulses of red light is necessary for timing to be coupled to a light-off signal.

What signals are responded to under natural conditions?

Under natural conditions the transitions between light and darkness occur through a gradually changing irradiance, especially at high latitudes: these are accompanied by a change in spectral quality (figure 3). Twilight spectra are relatively rich in blue and far-red wavelengths and relatively poor in orange–red light (Holmes & McCartney 1976; Smith & Morgan 1981). The pattern of spectral distribution becomes more exaggerated as the solar elevation declines and, during evening twilight on cloudless days, the ratio of red to far-red light decreases from a daylight value of about 1.1 to values in the region of 0.8 to 0.7 (Smith 1982). Recently reported higher values for daylight and twilight (Salisbury 1981) are due mainly to the chosen bandwidths and to the presentation of the data in terms of energy rather than quantum ratios; nevertheless the trend towards a reduced red:far-red ratio during twilight is clearly evident (figure 3). Either the decrease in irradiance or the lowering of the red:far-red ratio during evening twilight could therefore be the transition signal for the beginning of dark-time measurement at dusk. The opposite change could signal dawn. The ratio of either blue:green, or blue:red could also provide a reliable index of the progression through twilight (Smith 1982).

When does a plant begin to respond to darkness in the evening? Or to light at dawn? Relatively few experiments have considered these questions under natural conditions. In one such experiment in Japan, plants were transferred to darkness at different times during evening twilight and returned to daylight at different times in the morning, to determine when dark-time measurement began and ended (Takimoto & Ikeda 1961). Under these conditions, the inductive dark period for *Pharbitis nil* began when the natural irradiance had fallen to between 1–2 W m^{-2} in the evening and ended when it had increased to only about 0.004 W m^{-2} in the morning (Takimoto & Ikeda 1961). Thus, for *Pharbitis*, the biological night began near the time of astronomical sunset and ended at about the beginning of civil twilight. The responses of several other short-day species were also examined and showed considerable variation in the irradiance values at which the night began and ended. In another experiment in Hawaii, a

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twilight irradiance below about 0.15 W m^{-2} was found to be equivalent to photoperiodic darkness for sugar cane (Clements 1968). Although tropical twilights are relatively short, the twilight time during which the irradiance exceeded 0.15 W m^{-2} increased the effective daylength by some 26 min compared with the times of sunrise and sunset.

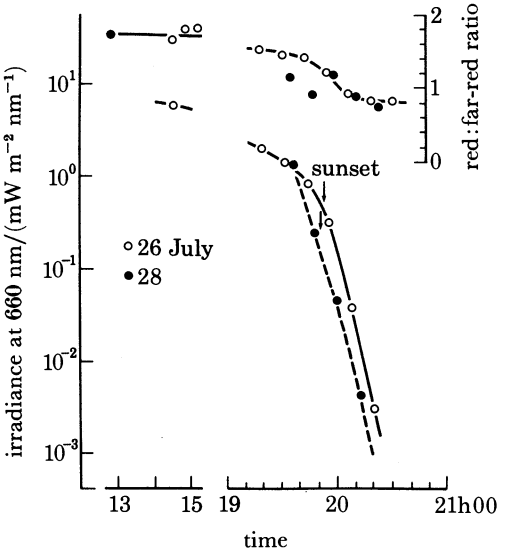

FIGURE 3. Changes with time in irradiance and in red:far-red ratio during evening twilight. Measurements were made on two separate days in Logan, Utah. (From Salisbury (1981).)

As changes in the red:far-red ratio were occurring together with irradiance during these natural twilight periods, this experimental approach does not rule out the possibility that irradiance was being sensed through the perception of an accompanying change in light quality, as has been proposed for the detection of canopy shade by phytochrome (Smith 1982). Smith has pointed out that the changes in red:far-red ratio during twilight are in fact very variable because of cloud conditions on the horizon, and so may not be precise signals for dusk perception. However, this is also true of twilight irradiance, which can vary considerably from day to day because of clouds. Such variations could lead to significant errors in timing, and indeed the effective photoperiod for *Pharbitis* has been shown to be 20–30 min longer on clear days than on cloudy ones (Takimoto & Ikeda 1960). The error would become very small, however, for plants such as *Xanthium* with low threshold irradiances (Salisbury 1963). It could also be reduced, by averaging, when repeated cycles are necessary for induction.

Changes in the quality and quantity of twilight occur together, and both can vary with cloud cover, latitude and season. Consequently experimental approaches with artificial light sources that vary each factor independently are necessary to determine whether either the quality or quantity of light, or both, are important natural cues for the perception of light–dark transitions in photoperiodism.

CHANGES IN END-OF-DAY LIGHT QUALITY

Most experimental approaches to end-of-day light quality have considered only its effects on the magnitude of the overall flowering response. It is abundantly clear from the results of many experiments that exposing plants to far-red light before entry to darkness can substantially

reduce or even eliminate flowering (Vince-Prue 1975, 1981) (figure 5). However, these conditions are far from natural, involving, as they usually do, either very short photoperiods or a single very long dark period. With longer photoperiods under natural 24 h cycles, an end-of-day light treatment with far red has been shown to promote flowering in some cases (Cumming 1963; Fredericq 1964; Esashi & Oda 1964) and so the possibility cannot be excluded that the low red:far-red ratio that occurs during evening twilight increases the flowering response when daylengths are close to the critical. However, as far as I am aware, this possibility has never been systematically tested under natural conditions.

These effects of end-of-day light quality on the flowering response do not, however, provide any information about the nature of the signal that initiates dark-time measurement, and it is necessary to design experiments specifically to determine how timing is affected. When the time of maximum sensitivity to a night-break was used as an indicator, an end-of-day treatment with 5 min of far red appeared to have almost no effect on timing in *Pharbitis* (Vince-Prue 1981; Takimoto & Hamner 1965*a*). Similarly, there was little or no effect on critical night length in *Chenopodium* (King & Cumming 1972) or *Xanthium* (Salisbury 1981) when the photoperiod was terminated with far-red or far-red-enriched light.

At most, therefore, there seems to be only a very small change in timing when the red: far-red ratio is reduced at the end of the day much more drastically than would ever occur under natural conditions. It must be emphasized, however, that these experiments have been carried out on only a limited range of species and usually with a single inductive cycle. It is necessary to investigate a wider range of plants and conditions, using both the time of night-break sensitivity and the critical night length as probes of time measurement to determine whether this conclusion is generally justified.

Changes in end-of-day irradiance

Varying the irradiance without changing the light quality has shown clearly that dark time measurement begins when the irradiance is below a critical value. In *Xanthium*, time measurement proceeded normally when plants were transferred to white light of low irradiance (*ca.* 100 mW m^{-2}) (Salisbury & Ross 1969), but in this experiment the change in light quantity was accompanied by some change in quality from daylight to incandescent. More convincing are the results of Takimoto (1967) for *Pharbitis*, where plants remained in fluorescent light throughout. Under these conditions time measurement began when plants were transferred to 40 mW m^{-2} but was completely prevented by light at *ca.* 1.0 W m^{-2} or more (figure 4). In both cases, night-breaks were used as a probe of time measurement. In an attempt to approximate more closely to natural conditions, we have recently looked at the effect of transferring plants through several different stepped gradients of irradiance without changing light quality (table 1). In all cases dark time measurement began when plants were transferred to *ca.* 2 W m^{-2} (continuous white fluorescent light) and a *change* in irradiance *per se* was unimportant. Although more experiments are needed with other plant species, there seems little doubt that dark time measurement can begin when the irradiance decreases below a threshold value in the absence of any change in light quality. Fewer studies have been made on the dawn signal, but the limited evidence indicates that here too a change in light quantity can terminate the dark period.

The fact that, under natural conditions, the irradiance fell from that perceived by *Xanthium*

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plants as light to that perceived as darkness within 5–12 min on two successive days (Salisbury 1981) demonstrates that the decrease in irradiance during evening twilight can give a precise environmental cue for photoperiodic timing.

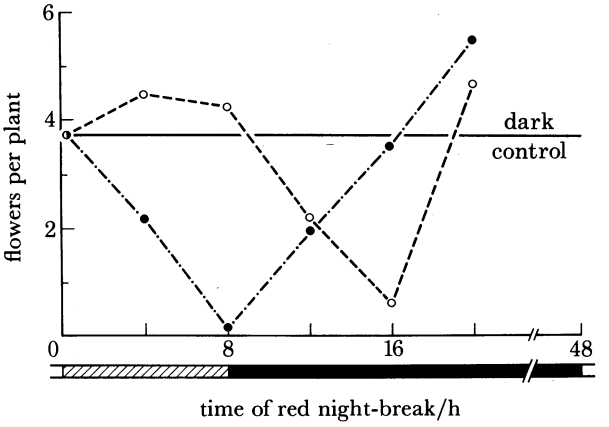

FIGURE 4. Effect of irradiance of an 8 h extension on the initiation of dark-time measurement in *Pharbitis nil*. After exposure to continuous white fluorescent light (16 W m^{-2}) plants received an 8 h extension with fluorescent light at 40 m W m^{-2} (●) or 1 W m^{-2} (○) before transfer to a 40 h inductive dark period. Time measurement was examined by exposing plants to a 15 min red night-break at various times. (From Takimoto (1967).)

TABLE 1. EFFECT OF STEPPED GRADIENTS OF IRRADIANCE AT THE END OF DAY ON DARK TIME MEASUREMENT IN *PHARBITIS NIL*

(The time of maximum sensitivity to a night-break normally occurs 9 h after transfer to darkness. All plants received a 32 h photoperiod followed by a 48 h inductive dark period. (Data of P. J. Lumsden.))

light treatment/(W m^{-2})					time of maximum night-break sensitivity from end of day/h
24 h	2 h	2 h	2 h	2 h	
80	60	40	20	2	7
40	30	20	10	2	7
20	15	10	5	2	7

PHOTORECEPTORS FOR PHOTOPERIODISM

Critical studies of the photoreceptor(s) for photoperiodism began with the discovery of the effect of a brief night-break that allowed the construction of action spectra for the control of flowering in short-day plants (Parker *et al.* 1946). These, together with demonstrations of red–far-red reversibility (Downs 1956) have shown that night-breaks are perceived through the formation of P_{fr}. Based on these results and on the observations of the disappearance of P_{fr} after transfer to darkness, it has generally been accepted that the dawn signal is sensed by the photochemical formation of P_{fr} and dusk by the lowering of P_{fr} levels through non-photochemical reactions (Vince-Prue 1982).

The assumption that phytochrome is the photoreceptor that discriminates between light and darkness in short-day plants largely rests on two kinds of evidence: (1) that the inhibition of flowering by a night-break operates through the formation of P_{fr} and (2) that P_{fr} is required during and sometimes after the photoperiod for the induction of flowering (Vince-Prue 1975).

However, neither of these is unequivocal evidence for a role for P_{fr} in the perception of the light–dark transitions that begin and end the critical night. The suppression of flowering by end-of-day far red (figure 5) and its reversal by red (Takimoto & Hamner 1965*b*) shows only that P_{fr} is required for flowering. Although the night-break is often assumed to be equivalent to dawn, sensitivity to a night-break often occurs before the end of the critical night (figure 1), so that the night-break and dawn may not necessarily be sensed in the same way.

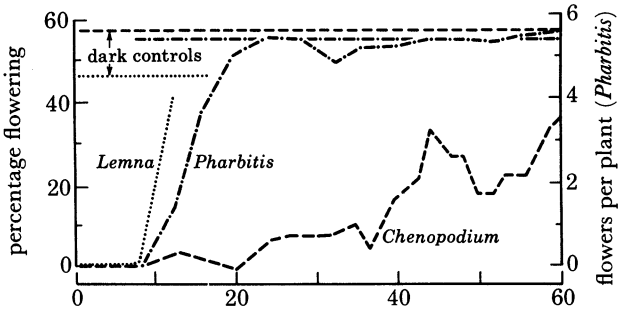

FIGURE 5. Effect on flowering of the time of giving a far-red light pulse during a single inductive dark period. Data for *Lemna paucicostata* 441 from Saji *et al.* (1982) and for *Chenopodium rubrum* from Cumming *et al.* (1965); plants were previously in continuous white light. *Pharbitis nil* was previously exposed to a single photoperiod of 23 h white light (data of P. J. Lumsden).

There is, however, no good evidence for the participation of photoreceptors other than phytochrome in the photoperiodic control of flowering. Photosynthesis, although undoubtedly necessary for the expression of flowering, does not appear to be directly involved in the perception of photoperiodic signals. In *Pharbitis*, for example, normal photoperiodic induction and time measurement have been shown to occur in photobleached seedlings in the absence of photosynthesis (King & Vince-Prue 1978). An essential contribution from a blue-absorbing photoreceptor has not been demonstrated and most of the reported effects of blue light can be explained on the assumption that phytochrome is the photoreceptor (Vince-Prue 1979). When given as a 24 h photoperiod, low-irradiance red light is just as effective as high-irradiance white light for photoperiodic induction in *Pharbitis*, whereas blue light is much less effective even when terminated by red (Vince-Prue 1981). Moreover, when given as an extension to a photoperiod in white light, a much lower irradiance of red light, compared with blue, is effective in preventing the initiation of dark time measurement (Takimoto 1967).

In *Pharbitis*, there is good evidence that the 'light-on' signal is perceived by phytochrome. A single 5 min pulse of red light is sufficient to initiate a circadian rhythm of sensitivity to a night-break and leads to the induction of flowering in response to a dark period longer than critical (King *et al.* 1982; Ogawa & King 1979). Although reversibility by far red has not been tested with respect to the initiation of timing (and may not be possible because of the requirement for P_{fr} for induction), the effectiveness of such a brief exposure to red light strongly suggests that phytochrome is the photoreceptor. The greater effectiveness of red light in preventing the beginning of dark timing when given as an extension of photoperiod (Takimoto 1967; Salisbury 1981) indicates that phytochrome is also the photoreceptor for the photoperiodic light-off signal.

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Perception of the light-off signal

If perception of a light–dark transition is to be accounted for solely in terms of P_{fr}, the precision of coupling to dark time measurement and the fact that 'darkness' does not begin appreciably sooner when P_{fr} is drastically lowered photochemically at the beginning of the night require that P_{fr} levels must fall rapidly through non-photochemical reactions. (An early proposal that non-photochemical loss of P_{fr} is itself the basic timer (Hendricks 1960) has been excluded by a variety of approaches (cf. Vince-Prue 1975).) Since most of the available evidence indicates that a decrease in irradiance below a threshold level constitutes the light-off signal, it is also necessary to propose that the loss of P_{fr} through non-photochemical reactions becomes significantly large at low irradiances and so allows dark time measurement to begin.

One problem with this hypothesis is that part of the total P_{fr} pool appears to be rather stable in darkness. Because most of the phytochrome has already decayed in fully de-etiolated plants, P_{fr} is present almost exclusively in this stable pool and the reduction in P_{fr} level after transfer to darkness would be small and very slow (Heim *et al.* 1981). Consequently non-photochemical changes in P_{fr} level may not be a sufficiently accurate method of detecting the light–dark transition that initiates dark time measurement in light-grown plants.

Much of the early evidence for stable P_{fr} came from physiological experiments in which far-red pulses were given at different times after transfer to darkness. In *Pharbitis*, the apparent stability of P_{fr} can easily be demonstrated and far-red pulses can be shown to depress flowering even after many hours in darkness (figure 5). These and similar results obtained in other experiments and with other plants (figure 5) are not consistent with the concept that the light–dark transition is sensed through a rapid non-photochemical lowering of P_{fr} unless there are kinetically separate pools of phytochrome with different physiological functions: an unstable pool of P_{fr} coupled to time measurement, and a stable pool required for the induction of flowering.

Spectrophotometric studies

The existence of two independent pools of P_{fr} has recently been suggested on the basis of spectrophotometric data from *Amaranthus caudatus* seedlings (Brockmann & Schäfer 1982). Moreover, measurements made on norfluorazon-treated seedlings of *Pharbitis nil* have shown that there was still some P_{fr} present in the unstable pool even after 3–4 days in continuous white light; this unstable P_{fr} had essentially disappeared 30–60 min after transfer to darkness (figure 5 in Heim *et al.* 1981). These results are very similar to earlier measurements made on *Pharbitis* cotyledons photobleached by exposing them to high-irradiance light for 24 h at low temperature (Vince-Prue *et al.* 1978). In these cotyledons, P_{fr} fell below the limit of detection within 30–40 min after the transfer to darkness, but sensitivity was too poor to detect the continued presence of P_{fr} in the stable pool.

More recently, detailed studies have been made on *Pharbitis* cotyledons that had either been photobleached at low temperature or after treatment with norfluorazon (Rombach *et al.* 1982). After exposure to continuous light for two 12 h periods, such as might be experienced under natural daylengths, evidence was found for both stable and unstable components of the total P_{fr}. There were also indications that P_{fr} could be lost both through reversion to P_r and by destruction to a non-photoreversible form. A rapidly reverting pool of P_{fr} was not observed in etiolated plants and the capacity for dark reversion appeared to develop only after exposure

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to light for several hours; it also disappeared in darkness. In this context it is interesting to note again (figure 2) that a light-off signal was not perceived in *Pharbitis* until the plants had been exposed to continuous light for more than 6 h.

The spectrophotometric evidence therefore does not exclude the possibility that there is a distinct pool of P_{fr} that undergoes rapid loss through non-photochemical reactions and so could be involved in the perception of the light–dark transition that couples to photoperiodic time measurement. Some of the P_{fr} is lost rapidly through non-photochemical reactions even in light-grown plants with $t_{\frac{1}{2}}$ values sufficiently short to give a precise transition signal (Hillman 1964; Heim *et al.* 1981; Vince-Prue *et al.* 1978; Rombach *et al.* 1982).

Physiological studies

A physiological approach to understanding how the light–dark transition might be perceived is to determine what experimental treatments act in the same way as continuous light and prevent the beginning of dark time measurement (Takimoto 1967; Salisbury 1981). In recent experiments with *Pharbitis*, we have routinely used a single 24 h photoperiod under continuous white fluorescent light at 80 W m^{-2} followed by a single 48 h dark period at 25 °C; plants are then transferred to continuous white fluorescent light. This treatment results in a high level of flowering, which is strongly inhibited when a red night-break is given at a particular time in the inductive dark period (Lumsden *et al.* 1982). The time of maximum sensitivity to a night-break is sharply defined at about 9 h after the light-off signal. When this 24 h photoperiod is extended for 6 h with continuous light, a delay of 6 h in the time of maximum night-break sensitivity would be expected, and the effectiveness of different kinds of extension treatments can therefore be evaluated. The 6 h extension period was always terminated with a 5 min exposure to red, to establish the same $P_{fr}:P_{total}$ ratio before transfer to darkness (figure 6).

As expected, an extension with 6 h of darkness terminated by 5 min red had little effect, and timing was essentially coupled to the initial transfer to darkness (figure 6). Continuous red light, on the other hand, effectively delayed the onset of dark timing, which was coupled to the end of the red extension. The extension light did not, however, have to be continuous, and complete delay occurred when red pulses lasting 1 or 5 min were given at hourly intervals; red pulses given at 2 or 3 h intervals gave a partial delay but were not equivalent to continuous light. These results are consistent with the concept that P_{fr} is lost rapidly in darkness and must be maintained by frequent pulses if the treatment is to be perceived as continuous light. A similar delay in dark time measurement after an extension with half-hourly pulses of red has also been observed in the opening of *Pharbitis* flowers (Kaihara & Takimoto 1980).

When the reversibility of the red pulses by far-red was examined, however, the results did not support the hypothesis that repeated exposures to light are necessary because P_{fr} is lost in the intervening dark periods. We have found no evidence for reversibility of the red pulses by far red (figure 6), as would be expected if this were so. Control treatments with far-red pulses given alone had much less effect than red, showing that it should have been possible to demonstrate reversibility under these conditions. Coupling of P_{fr} to the time-measuring system must therefore occur very rapidly, or alternatively the action of red pulses is not through P_{fr} formation.

One possibility consistent with these results is the concept of new P_{fr}, which was developed to explain somewhat similar observations in *Mesotaenium*. Repeated exposure to red every few minutes was needed to maintain chloroplast movement, even though P_{fr} was shown to be still

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present (Haupt & Reif 1979). The authors suggested that P_{fr} 'ages' in some way and is then no longer effective physiologically. In *Pharbitis*, a separate pool of phytochrome with a different physiological function would also have to be present because far-red pulses have demonstrated that P_{fr} continues to affect the flowering response for many hours (figure 5).

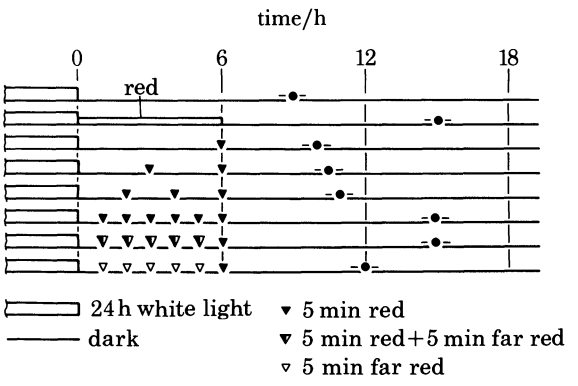

FIGURE 6. Effect of day-extension treatments on time of maximum sensitivity to a night-break for the control of flowering in *Pharbitis nil*. Plants were exposed to a single photoperiod of 24 h white light followed by a day extension for 6 h, as indicated on the figure, before transfer to a 48 h inductive dark period. Time measurement was examined by exposing plants to a 10 min red night-break at different times. The approximate time at which the night-break effect was greatest is indicated (–●–). Red (day-extension pulses and night-breaks) 55 μmol m^{-2} s^{-1}; far-red (700–780 nm) 9 μmol m^{-2}; continuous red, 12 μmol m^{-2}. (Data of P. J. Lumsden.)

Because the requirement for repeated exposure to light is not simply a function of the rapid loss of P_{fr} in darkness, the hypothesis that, with respect to the perception of light–dark transitions in photoperiodism, the plant senses 'continuous' light through the continuous or intermittent formation of new P_{fr} seems the most consistent with our experimental data at present.

CONCLUSIONS

Evidence has been presented that the light–dark transition that couples to the initiation of dark time measurement is perceived through a reduction in irradiance and that the photosensory pigment is phytochrome. The precise way in which this occurs is still uncertain but there is some evidence to suggest that this cannot be accounted for on the basis simply of the amount of P_{fr} that is present, as seems to be true of many other physiological responses under phytochrome control.

The general properties of phytochrome do not at first sight appear to make it a particularly good candidate for the precise sensing of light–dark transitions and for the function of discriminating between light and darkness. A better candidate would be a pigment that sensitizes reactions from the excited state. The characteristic photoreversibility of phytochrome, so appropriate for the detection of canopy shade through changes in light quality, appears to be of little consequence in the detection of the light–dark signals for photoperiodic time measurement. Consequently, light–dark sensing has usually been considered a function of the non-photochemical reactions that lead to a reduction in the amount of P_{fr} present in the tissue after transfer to darkness or to a sufficiently low irradiance. The photochemical formation of P_{fr} would then signal dawn.

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However, experimental evidence for the stability of P_{fr}, particularly in light-grown plants, suggests that these non-photochemical reactions may not be sufficiently rapid for the accurate sensing of the time of transfer to darkness. Another problem is the relative lack of sensitivity to changes in temperature (Vince-Prue 1975). A highly unstable pool of phytochrome with a specific function for the perception of photoperiodic Zeitgebers remains a possibility, and there is spectrophotometric evidence for an unstable pool of P_{fr} in both light-grown and dark-grown plants.

Physiological approaches using intermittent exposure to red light support the concept that P_{fr} may be lost rapidly in the intervening dark periods. However, the lack of reversibility by far-red argues against the conclusion that repeated exposures are required because of the rapid non-photochemical loss of P_{fr}. A concept consistent with these results is that P_{fr} ages in some way so that the perception of light as being continuous occurs only when new P_{fr} is regenerated sufficiently rapidly. If this is so, then phytochrome appears to operate somewhat differently in the detection of the continuous light signal for photoperiodic timing than in, for example, the perception of canopy shade.

Photoperiodism has been observed in an enormous range of organisms throughout the plant and animal world, and these organisms have adopted a variety of photoreceptors for the perception of photoperiodic Zeitgebers. Following the premise of Smith (1982), these photoreceptors must have been selected for their ability to acquire specific information about the light environment, namely to detect precisely the transitions between light and darkness that are coupled to photoperiodic time measurement. In higher plants, the photoperiodic photoreceptor appears to have more than one function: to detect light quality (responses to shade (Smith 1982)), to detect light direction (chloroplast orientation (Haupt 1982)) and to discriminate between light and darkness (photoperiodism). Thus phytochrome is able to detect different properties of light and it is not clear which , if any, of these can be identified as the fundamental perceptual function of the photoreceptor. Perhaps an original function has been modified and a different perceptual mechanism may have evolved to allow accurate sensing of the transitions between light and darkness in photoperiodism.

A final word must concern the blue-absorbing photoreceptor that can operate as a very sensitive detector of light quantity changes during twilight for the control of stomatal opening (Zeiger *et al.* 1981). It is interesting to speculate why this photoreceptor does not appear to have been adopted for plant photoperiodism.

I thank P. J. Lumsden for permission to quote from his unpublished results.

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Discussion

O. V. S. Heath, F.R.S. (10 *St Peter's Grove, London, U.K.*). Dr Vince-Prue's results with pulses of red light during the dark period much resemble those of Mansfield (1965) for effects of low-intensity red light in delaying the phase of a stomatal rhythm in darkness in *Xanthium pennsylvanicum*. He found that one or three interruptions of a 16 h dark period were ineffective but 64 interruptions were as effective as continuous red light. As in Dr Vince-Prue's experiment, following each period of red light with far red did not reverse the effect. For these reasons and also because maximum sensitivity was found at 703 nm, approximately midway between the main phytochrome absorption peaks, he concluded that if phytochrome was involved it was behaving very differently from its participation in the flowering behaviour of the same species (Borthwick *et al.* 1952).

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Daphne Vince-Prue. This very interesting observation raises the possibility that the photoperception characteristics that we observed for coupling with photoperiodic time measurement may be of general occurrence in time-based phenomena. It emphasizes the need to determine the wavelength sensitivity in our system.

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