Phytochrome in Cultured Wild Carrot Tissue

II. DARK TRANSFORMATIONS

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ABSTRACT

Disappearance and transformation of phytochrome in light-treated, dark-grown cultured tissue of wild carrot (Daucus carota L.) have been analyzed. Evidence is presented for the existence of two populations of far red-absorbing phytochromes: one which is subject to reversion, and a second which undergoes loss of photoreversibility. Phytochrome levels and transformation characteristics have been compared for several strains and developmental stages of cultured wild carrot tissue.

RESULTS AND DISCUSSION

The kinetics of dark-transformations of phytochrome in cultured wild carrot tissue after a saturating red irradiation (Fig. 1) is similar to the kinetics seen in similarly treated pea and bean stem tissue (2, 5). The graph resulting from plotting the log of Pfr disappearance against time clearly shows two phases (Fig. 2, line A). The first, more rapid phase corresponds with the period of Pr appearance. The second phase, which is usually linear for up to 10 to 12 hr, corresponds with a period of little or no Pr appearance. The linear phase most likely represents a period of involvement of Pfr in a single reaction in which the rate is limited mainly by the concentration of unreacted Pfr. By extrapolating the linear phase to zero time and by plotting the difference between the extrapolated and original lines, a new line is obtained which corresponds with the period of divergence of the graphs of Pfr and total phytochrome (e.g., Fig. 1). Because the rate of synthesis of new phytochrome is so low in this tissue (6), the divergence of these lines must be due to reversion of Pfr to Pr. The apparent half-time of this reversion is 50 min.

Given the validity of the extrapolation and subsequent interpretations, it can be further reasoned from kinetic analysis that two major populations of Pfr phytochrome exist in this material. One population (approximately one-third of the total) reverts rapidly to Pr, whereas the other undergoes slow loss of photoreversibility. If these two reactions competed for the same population of Pfr and if both were first order reactions, its disappearance would also exhibit simple first order kinetics, rather than the curvilinear graph observed.

This interpretation is incomplete because semilog plots of total phytochrome levels are not linear throughout. The major portion (80–90%) of the loss appears to occur rapidly and exponentially in the first 6 hr while the rest is lost at a much slower rate.

Our interpretation is supported by the work of Correll et al. (1), who, by using purified phytochrome extracted from etiolated rye seedlings, presented evidence of two populations of Pfr that decayed by first order kinetics at roughly 100-fold different rates. In a note at the end of this article, the authors interpreted earlier in vivo analyses of Pfr disappearance in a similar way: "The bleaching appeared to be first order for the first 30 minutes at which time over 50 per cent of the reaction had occurred." The time courses of \( P_{660} \) and \( P_{730} \), and \( P_{660} + P_{730} \) changes in the in vivo assay (5) are very similar to our Figure 1.

The loss of the photoreversibility of Pfr, after red light treatment, has been shown to be a simple first order reaction in etiolated Amaranthus seedlings by Kendrick and Frankland (4). The analysis of their material is made easier because of the absence of reversion of Pfr to Pr.

In Table I different clones and different stages of development of cultured wild carrot tissue are compared. The average Pfr level of our dark-grown tissue (clone 1) was 0.33 \( \Delta(\text{OD})/g \) fresh

1 Abbreviations: Pr and Pfr: red- and far red-absorbing phytochrome.
weight of packed tissue. Although not precisely comparable because of differences in absorbance properties of the tissues, this compared favorably with the highest values of $1.2 \Delta(\Delta OD)/g$ fresh weight of pea epicotyl hook tissue measured in a similar way by Furuya and Hillman (2). Clone S and clone 1 were derived from the same parent culture. Both are diploid and differ somewhat in growth rate and gross appearance, but not in capacity for embryogenesis.

![Graph](https://example.com/graph.png)

Fig. 1. Dark transformations of phytochrome in dark-grown wild carrot tissue after a short, saturating red light treatment. Pr equals total minus Pfr.

![Graph](https://example.com/graph2.png)

Fig. 2. Semilogarithmic plot of Pfr disappearance (A) showing extrapolation of the slower phase and calculation of the time course of the rapid phase (B) as the difference between the original and extrapolated graphs. Data represent the averages of eight experiments.

The large difference in the phytochrome content of the two clones suggests that this characteristic may be genetically controlled. The comparatively low level of phytochrome in plantlets derived from clone 1 tissue probably reflects the correspondingly lower proportion of meristematic tissue in the rapidly expanding plantlets.

The time required for disappearance of one-half of the initial Pfr is approximately the same in all materials except rapidly developing embryos. The shorter half-life in embryos is consistent with their generally higher rates of metabolism. The following half-times for Pfr disappearance at 25 C and after a saturating red irradiation have been reported: bean hypocotyl hook tissue, 20 min (5); pea epicotyl hook tissue, 60 min (2); etiolated Amaranthus seedlings, 20 min (4).

Kinetic analyses were not made for dark transformation in embryo or plantlet materials because of the small number of replications; however, both the parameters given in Table I and the shape of the curves in the plotted data (not presented) indicate that reversion and destruction occur. When Pfr is reduced below the level of detectability, decrease in total pigment ceases and a period of slow restoration begins (6).

In summary, this work adds to the growing evidence for the existence of multiple species of phytochrome in vivo. At least two forms of Pfr appear to exist simultaneously in this tissue. There appears to be little difference in the quantity of measurable phytochrome in young adventive embryos and the undifferentiated tissue from which they were derived. A significantly larger portion of the Pfr formed in embryos appears subject to loss of photo-reversibility. The kinetics of dark transformations is similar in undifferentiated tissues and in adventive embryos and plantlets derived from it. Different diploid clones originally of common origin have developed inherently different levels of measurable phytochrome.

**LITERATURE CITED**


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**Table I. Initial Phytochrome Levels, Half-life of Pfr Disappearance, and Extent of Phytochrome Destruction following a Single Saturating Red Irradiation of Dark-grown Wild Carrot Tissue**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Level</th>
<th>Half-life</th>
<th>Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undifferentiated1</td>
<td>33</td>
<td>2.0</td>
<td>63</td>
</tr>
<tr>
<td>Embryos1</td>
<td>34</td>
<td>1.3</td>
<td>75</td>
</tr>
<tr>
<td>Plantlets1</td>
<td>17</td>
<td>1.8</td>
<td>65</td>
</tr>
<tr>
<td>Clone 2</td>
<td>25</td>
<td>2.1</td>
<td>59</td>
</tr>
<tr>
<td>Clone S</td>
<td>60</td>
<td>2.2</td>
<td>52</td>
</tr>
</tbody>
</table>

1 Clone 1.