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Apparent Endogenous Circadian Rhythm in *Ent*-Kaurene Biosynthesis

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Abstract Net synthesis of [^{14}C]*ent*-kaurene from [^{14}C]2-mevalonic acid was assayed in cell-free enzyme extracts prepared from Alaska pea (*Pisum sativum* L.) seedlings throughout 44 h of a regimen consisting of a 16-h day and an 8-h night. Activities generally followed an upward trend during the dark period and a downward trend during the photoperiod. Activity was also assayed in enzyme extracts prepared at intervals during a 12-h photoperiod and a following, continuous 36-h dark period after entrainment of plants to a regimen of 12-h days and 12-h nights. *Ent*-kaurene synthesis activity again followed an upward trend in enzyme extracts prepared during what would have been the entrainment dark period, and a downward trend during the entrainment photoperiod. The apparent endogenous rhythm of *ent*-kaurene biosynthesis may have implications for the regulation of gibberellin biosynthesis.

Previously, it was reported from my laboratory that there was no indication of endogenous circadian rhythm in the capacity for *ent*-kaurene biosynthesis from mevalonic acid in cell-free enzyme extracts prepared from shoot tips of 10-day-old Alaska pea (*Pisum sativum* L.) seedlings (Ecklund and Moore 1974). Reexamination of the previous data and additional investigations have indicated, on the contrary, that there may be an endogenous circadian rhythm of *ent*-kaurene biosynthesis. Since *ent*-kaurene is a key intermediate in the biosynthetic pathway of the gibberellins (GAs), the new finding may have significant implications for regulation of GA biosynthesis.

Materials and Methods

All the material and methods utilized in these investigations, except for the particular photoperiodic regimens used, including routine culture of the plants, preparation of enzyme extracts, and in vitro assays of [^{14}C]*ent*-kaurene and [^{14}C]squalene, were as described previously (Ecklund and Moore 1974). In the first experiment, enzyme assays were performed using shoot tips of 10-day-old Alaska pea seedlings during 44 continuous hours of a regimen consisting of a 16-h day and an 8-h night. For the second experiment, seedlings were first entrained to a regimen of a 12-h day and a 12-h night for 10 days, then enzyme assays were performed during a 12-h photoperiod and a following, continuous 36-h dark period. The only other notable difference is that the reaction mixtures which yielded the data for Fig. 1 were incubated for 150 min, rather than the usual 60 min as used for the experiment depicted in Fig. 2.

Results and Discussion

In a first experiment to test the possibility of an endogenous circadian rhythm of *ent*-kaurene biosynthesis, plants were grown in a greenhouse under a 16-h day and an 8-h night. Beginning when the plants were 10 days old, shoot tips were harvested at certain time intervals throughout the light and dark periods over 44 h of the 16:8 day:night regimen, and [^{14}C]*ent*-kaurene synthesis from [^{14}C]2-mevalonate was assayed in cell-free reaction mixtures. Generally, there were upward trends and higher activities during each dark period and downward trends and lower activities during each photoperiod (Fig. 1). Interestingly, [^{14}C]squalene, the biosynthesis of which also was assayed, showed an upward trend during each photoperiod.

The second experiment entrained Alaska pea seedlings to a regimen consisting of a 12-h day and a 12-h night for 10 days in a growth chamber, and then exposed them to a continuous 36-h dark period following a 12-h light period. Shoot tips were harvested at intervals, and [^{14}C]*ent*-kaurene synthesis was assayed in enzyme extracts. Activity clearly followed an upward trend during the periods coin-

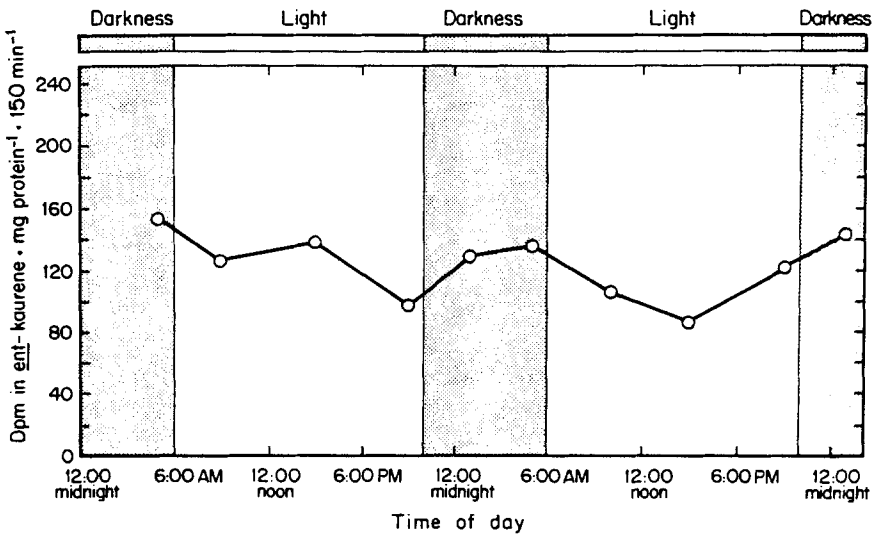

Fig. 1. Net synthesis of [^{14}C]*ent*-kaurene from [^{14}C]2-mevalonate in cell-free enzyme extracts of shoot tips of pea seedlings throughout 44 h of a regimen consisting of a 16-h day and an 8-h night. The bar at the top and the alternate light and shaded areas indicate the day:night regimen. Reaction mixtures were incubated for 150 min. Data points are means of triplicate assays.

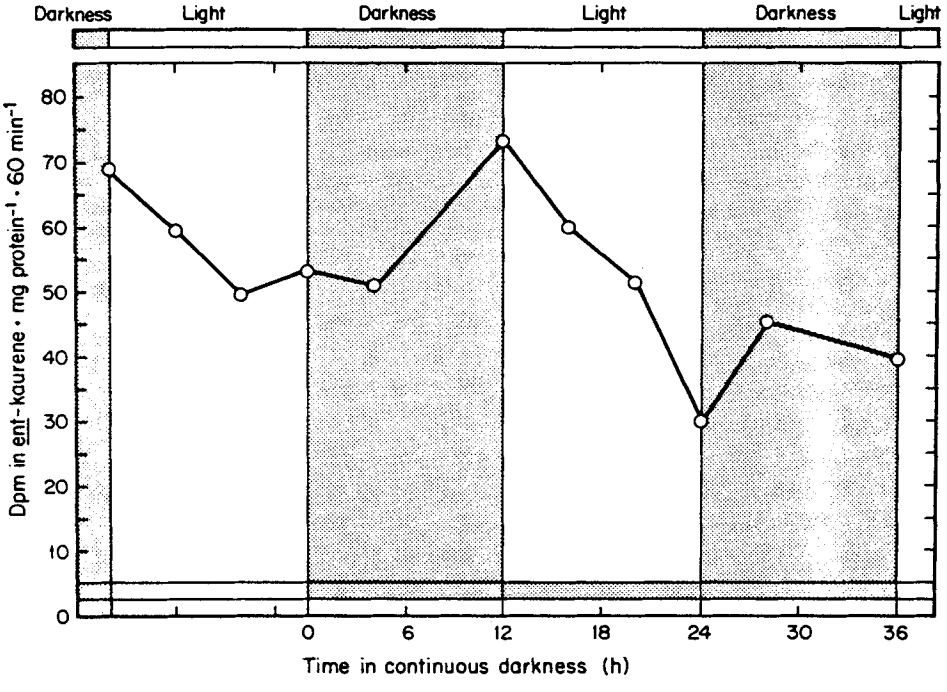

Fig. 2. [^{14}C]*Ent*-kaurene synthesis activity in cell-free enzyme extracts of shoot tips of pea seedlings exposed to a 12-h photoperiod followed by a continuous 36-h dark period after entrainment for 10 days to a regimen consisting of a 12-h day and a 12-h night. The bar at the top and the alternate light and shaded areas show the day:night regimen to which the plants were entrained prior to harvesting the plants and performing the assays. The bar at the bottom shows the 12-h photoperiod followed by a continuous 36-h dark period during which the assays were performed. Incubation time was 60 min. Data points are means of duplicate assays.

ciding with the entrainment dark period and followed a downward trend during the period coinciding with the entrainment light period (Fig. 2). Overall there was a downward trend throughout the long dark period, which was not unexpected.

Thus, evidently, there is an endogenous circadian rhythm of *ent*-kaurene biosynthesis in Alaska pea seedlings, that is, to the extent that the activities observed in cell-free enzyme extracts reflect activities which occur in vivo. Since *ent*-kaurene is considered to be a key intermediate in the pathway of biosynthesis of the GAs, this finding may have significant implications regarding the regulation of GA biosynthesis.

It was somewhat surprising to find higher *ent*-kaurene synthesis activities during the dark period than during the photoperiod, since, for one reason, *ent*-kaurene synthesis has a relatively large requirement for ATP, the synthesis of which is assumed to occur at a maximum rate during photosynthesis. It is noteworthy that some other metabolic processes which compete for mevalonic acid as substrate—squalene synthesis, for example—evidently do occur at generally higher rates during the photoperiod.

The results reported here do not conflict with previous observations that *ent*-kaurene synthesis activities are much higher in enzyme extracts of shoot tips of green seedlings than those of etiolated seedlings (Ecklund and Moore 1974). It seems reasonable to speculate that the apparent endogenous circadian rhythm is not functional in etiolated seedlings but is turned on upon photomorphogenesis to a green plant.

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References

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