ECOLOGY

Synchronous Spawning: When Timing Is Everything

Mark Hay

Calcified green seaweeds are among the world's most successful plants, having persisted for 150 million years. They build structures that can cover an area of 50 km^2 and be 20 m thick, and they produce much of the carbonate material in the tropics (*1*). Many organisms depend on these plants as hosts or as critical nursery habitats (*2*). The success of these seaweeds is linked to some of their most fascinating traits: They have efficient chemical and structural defenses against herbivores (*3*); siphonous construction (a system of mulitnucleate tubular filaments lacking crosswalls), which allows mobilization and reallocation of internal resources within hours; and a surprising ability to conduct important life processes at specific times of day when these activities are most advantageous (*4*, *5*). This ability to time reproduction and growth is similar to behaviors commonly associated with animals, not plants. An impressive field investigation by Clifton, reported in this issue on page 1116 (*4*), provides an intriguing example of this timing ability.

The author documents 17 species in five genera (*Halimeda*, *Penicillus*, *Udotea*, *Rhipocephalus*, and *Caulerpa*) that, within a single night, produce gametangia on their exterior surfaces and appear to move their entire protoplasm into these structures (see figure). During the subsequent 24 hours, the protoplasm is converted into gametes, and at dawn the next morning, gametes are synchronously released at times that are species- and gender-specific. Distantly related species overlap in their diel timing of gamete release, but closely related species partition spawning into nonoverlapping 5- to 15-min periods that are separated by only minutes, presumably limiting hybridization. Seaweeds die after spawning. Converting vegetative protoplasm into gametes, synchronously releasing these, and dying all occur in 36 hours or less, with two-thirds of this period being at night.

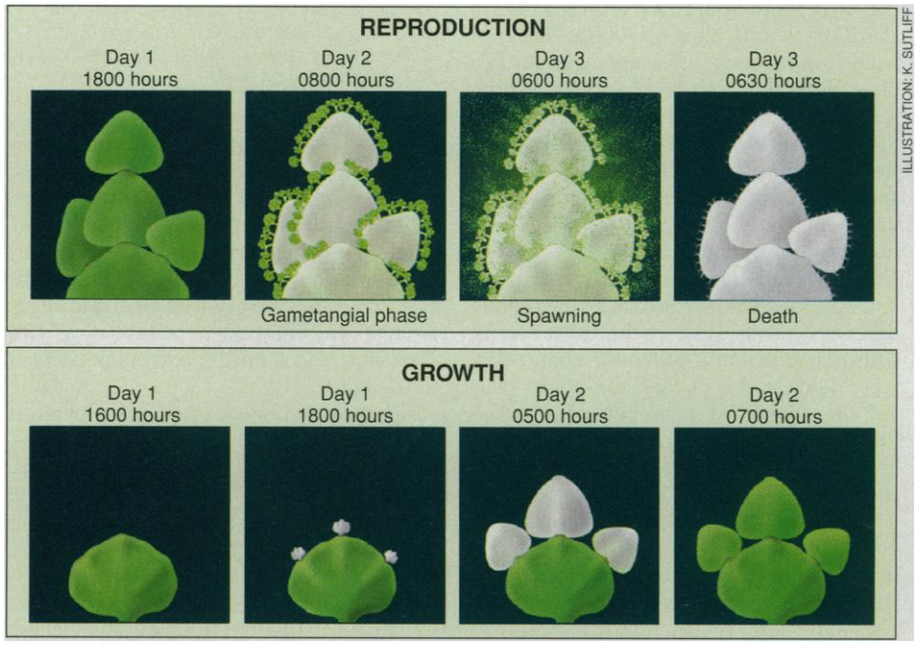
Because fertilization occurs in the water column, processes that concentrate gametes enhance fertilization. Synchronous spawning concentrates gametes in time, but more subtle interactions also occur. Clifton found that male gametes were always released a few minutes before female gametes and that unfertilized gametes remained mobile for 45 to 60 min. This suggests that the larger, more mobile, and positively phototactic females may be fertilized as they swim up through a cloud of previously released male gametes. Concentration of gametes on a microspatial scale could be enhanced if gametes released pheromones, as occurs in brown algae (*6*), but this has not been investigated.

In the seaweeds that Clifton studied, only 3 to 5% of the population commonly spawn on the same day. In contrast, most corals, which can also use synchronized spawning, synchronize all reproductive individuals in the population on the same days of the year, as well as the same diel time period (*7*). Additionally, synchronously spawning invertebrates release their gametes in the evening rather than at dawn, apparently to avoid day-active consumers (*8*). The fact that Clifton documented dawn spawning for each of the 17 seaweeds he studied suggests that this is a conservative trait, and possibly one under strong selection.

What might be the advantages of dawn spawning? Gametes could use photic cues, so that females would swim up through the previously released males during periods of morning calm, thus increasing fertilization success. The zygote would have a full day for photosynthesis after settlement. Gametes could avoid predation by nocturnal zooplankton. Adult plants might be unable to retain chemical defenses against day-active herbivorous fishes once the plants begin converting their protoplasm into gametes, so a dawn release might avoid consumption of gametes while they are still on the adult.

The seaweeds studied by Clifton also use time in other ways. Most seaweeds elongate during the day when they are photosynthetically active. In contrast, the siphonous seaweeds rapidly produce new growth at night while herbivorous fishes are inactive (*5*). The nocturnal growth is uncalcified, unpigmented, and more nutritious than older tissues (see figure); however, it is also better provisioned with chemical defenses. The valuable chlorophyll molecules are retained in the well-defended adult tissues until minutes before sunrise when they are moved into the new tissues. As photosynthesis occurs, the new growth becomes calcified and less valuable nutritionally. Coincident with these changes, chemical defenses decline (*5*). As with Clifton's data (*4*), these patterns suggest that siphonous tropical seaweeds are exhibiting complex behaviors where protoplasmic resources are carefully manipulated on a diel time scale so as to minimize costs and increase gains.

Despite the ecological and geological importance of siphonous seaweeds, their basic ecology is poorly understood. Additionally,



Synchronized seaweeds. Gamete production (upper) and vegetative growth (lower) in the genus *Halimeda*.

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the physiology, biochemistry, and cellular processes that allow the rapid conversion and movement of protoplasm necessary for both gametagenesis and nocturnal growth are unknown. Clifton's (*4*) investigation begins to fill this void and also documents a phenomenon of plant behavior that was previously unknown for seaweeds. This system offers interesting parallels and contrasts with other synchronized events such as mass spawning in corals, mass seed set in bamboo, and temporal release of pheromones in insects.

As a final note, although society has become increasingly aware of how ecological and environmental knowledge influences our lives, the facilities and opportunities for conducting long-term field investigations are very limited. The Smithsonian Tropical Research Institute in Panama supported Clifton's work, and it is one of the few places where in-depth field investigations can still be conducted. Such facilities and institutions are valuable resources that should be recognized and supported.

An enhanced version of this Perspective, with live links, can be seen in *Science* Online at http://www.sciencemag.org/

References

1. L. Hillis-Colinvaux, *Adv. Mar. Biol.* **17**, 1 (1980); P. J. Davies and J. F. Marshall, *Proc. 5th Int. Coral Reef Congr.* **5**, 1 (1985).
2. M. E. Hay, *Proc. 8th Int. Coral Reef Symp.*, in press, 1997.
3. V. J. Paul and W. Fenical, *Mar. Ecol. Prog. Ser.* **34**, 157 (1986); M. E. Hay, *J. Exp. Mar. Biol. Ecol.* **200**,103 (1996).
4. K. E. Clifton, *Science* **275**, 1116 (1997).
5. M. E. Hay *et al.*, *Oecologia* **75**, 233 (1988).
6. W. Boland, in *Chemical Ecology*, T. Eisner and J. Meinwald, Eds. (National Academy of Sciences, Washington, DC, 1995), pp. 87–102.
7. R. H. Richmond and C. L. Hunter, *Mar. Ecol. Prog. Ser.* **60**,185 (1990).
8. N. Lindquist and M. E. Hay, *Ecol. Monogr.* **66**, 431 (1996).

CELL BIOLOGY

Controlling Cell Death

Pierre Golstein

In many situations—both normal and pathological—cells die as a result of an orderly, stereotyped cascade of cellular events. On pages 1122, 1126, 1129, and 1132 of this issue (*8*, *9*, *12*, *13*), four reports describe the molecular basis of crucial steps in this cascade. The importance of understanding the basis of this programmed cell death was spectacularly demonstrated recently through the rescue with cell death inhibitors of mice undergoing acute liver destruction (*1*, *2*).

Programmed cell death is genetically determined, as demonstrated in the nematode *Caenorhabditis elegans*. Cell death occurring during development of this worm involves the molecules CED-3 and CED-4, which are necessary for cell death to occur, and CED-9, which protects cells from death. Genetic evidence shows that CED-9 acts as an inhibitor of CED-4 to prevent the death-inducing activity of CED-3 (*3*). In mammals, CED-3 homologs are a family of at least 10 cysteine proteases with aspartate specificity, formerly called the ICE (interleukin-1β–converting enzyme) family, now called caspases (*4*). CED-9 corresponds in mammals to a family with many members (*5*); the prototype is the Bcl-2 molecule; family members will be referred to here as Bcl-2s. The mammalian equivalents of the CED-4 molecule are not yet known.

The programmed cell death cascade can be conveniently divided into several stages (Fig. 1A). Multiple signaling pathways lead from death-triggering extracellular agents to a central control and execution stage. In this stage, the activation of CED-3/caspases occurs, which is controlled by CED-9 /Bcl-2s and presumably also involves CED-4. Bcl-2 is found mainly at the mitochondrial membrane (*6*), which, together with other arguments, suggests that mitochondria may also play a part at this stage (*7*). Downstream, caspase activation leads directly or indirectly to the characteristic "apoptotic" structural lesions accompanying cell death—cytoplasmic and chromatin condensation and DNA fragmentation. The control and execution stage thus requires at least four components, CED-3/caspases, CED-9/Bcl-2s, CED-4, and mitochondria. How do these components interact at the molecular level?

In two papers in this issue (*8*, *9*), the molecular link between Bcl-2s and caspases is shown to involve CED-4 (or its mammalian equivalent). In an impressive paper, Chinnaiyan *et al.* (*8*) initially show that overexpression of *C. elegans* CED-4 in mammalian cells leads to cell death. These transfected cells allowed further investigation on CED-4, getting around the absence of a known mammalian equivalent of CED-4 and of *C. elegans* cell lines. CED-4–induced mammalian cell death can be blocked by the overexpression of Bcl-x_L (one of the death-protective Bcl-2s) and by caspase inhibitors. These results indicate that in mammalian cells CED-4, under the control of Bcl-2s, causes death through caspase activation. Chinnaiyan *et al.* then show by coimmunoprecipitation a direct physical interaction between the proteins encoded by the genes *ced-4* and *ced-9* (or *bcl-x_L*) and the proteins encoded by *ced-4* and *ced-3* (or the mammalian caspases ICE and FLICE, which have a large prodomain, but not CPP32 or Mch-2, which have smaller prodomains). These transfection experiments made clever use of mutant molecules with the binding but not the killing properties of the wild-type molecules, to prevent induction of cell death with its resulting decrease in protein yield.

CED-4 can independently and simultaneously bind both CED-9 and CED-3, as shown by coprecipitation of CED-3 and CED-9 only when CED-4 was also cotransfected. Similar results were obtained for Bcl-x_L and ICE; however, in this case there was no requirement for CED-4, consistent with an endogenous mammalian equivalent of CED-4 that is able to link Bcl-2s and caspases. A reservation is that the techniques used do not allow detection of other molecules that might participate in, and are perhaps required for, the CED-3–CED-4–CED-9 complexes.

Where in the cells do these molecular interactions take place? Wu *et al.* (*9*) show by immunostaining plus confocal microscopy that the expression of CED-4 was diffuse in the cytoplasm when CED-4 was expressed in isolation, whereas it formed a compact granular pattern when CED-9 was coexpressed with CED-4. Similarly, in fractionation experiments CED-4 shifted upon expression of CED-9 from a cytosolic to a membrane-organelle fraction rich in mitochondria. Because Chinnaiyan *et al.* (*8*) showed that the binding of CED-9 to CED-4 did not prevent the binding of CED-4 to CED-3, when CED-9 drags CED-4 to (presumably) mitochondria, it must also drag CED-3 there. While doing this, CED-9 also modifies CED-4 to ultimately prevent CED-3/caspase activation.

These reports, added to the previous genetic, biochemical, and functional data, establish a nicely simple paradigm: CED-9/Bcl-2, at the mitochondrial membrane, binds and modulates CED-4, which in turn binds CED-3/caspases (Fig. 1B). Binding of CED-4 to CED-3/caspases may lead to caspase activation if CED-4 is not bound by CED-9/Bcl-2.

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