CELL CYCLE REGULATION IN THE DINOFLAGELLATE, GAMBIERDISCUS TOXICUS: MITOSIS IS COUPLED TO THE DIURNAL CYCLE BY A BLUE LIGHT DEPENDENT SIGNAL

F.M. Van Dolah[1,2,3], T.A. Leighfield[1,3], and L.M. Sugg[3]
[1]Marine Biotoxins Program, U.S. National Marine Fisheries Service,
Charleston Laboratory, Charleston, SC
[2]Medical University of South Carolina, Charleston, SC
[3]University of Charleston, Charleston, SC

Naturally occurring toxins produced by marine microalgae are the major source of food poisonings associated with consumption of seafoods. The frequency, severity and global distribution of blooms of toxic microalgae have increased during the past twenty years. In order to gain understanding of how environmental cues may trigger the rapid growth and reproduction of dinoflagellates that constitutes a "bloom", we have undertaken the current project to identify molecular mechanisms which regulate the cell division cycle in dinoflagellates. Our current work focuses on Gambierdiscus toxicus, a dinoflagellate implicated to be the primary source of toxins responsible for ciguatera fish poisoning. Ciguatera is a potentially fatal neurological syndrome associated with tropical reefs, which afflicts more than 50,000 people annually on a world wide basis.

We have previously reported that cell division in Gambierdiscus toxicus is phased to the diurnal cycle, such that cells divide only during a three hour window late in the dark phase when grown in a 16:8 hour light:dark (L:D) cycle (Van Dolah et al., J. Phycol. 31:395-400, 1995). Formally, cell division phased to the diurnal cycle may be regulated by either the D:L transition (e.g., mitosis occurs 22 h after the onset of light) or the L:D transition (e.g., mitosis occurs 6 h after the onset of dark). To distinguish which transition is critical to cell cycle control in G. toxicus, cells were transferred from a 16:8 to a 12:12 L:D cycle, and the timing of mitosis determined by mitotic index (% of cells possessing replicating nuclei, determined in propidium iodide stained cells). Under these conditions, mitosis continued to occur 6 h after the onset of the dark phase (Figure 1). Furthermore, if cells are placed in 24 h light, such that the L:D transition does not occur, no cells proceed through the cell cycle to mitosis. This indicates that the L:D transition is involved in phasing cell division to the diurnal cycle.

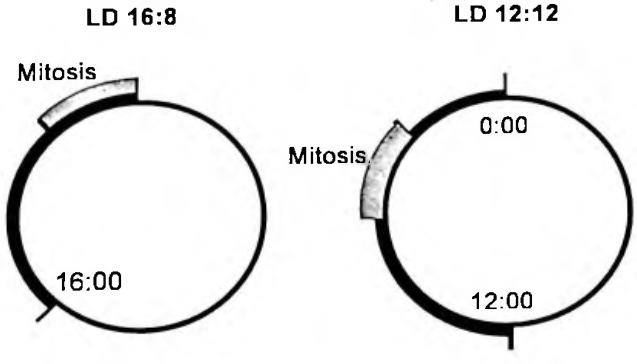

Figure 1. Cell cycle progression is controlled by the L:D transition (Wide bar represents dark; narrow represents light phase).

There are numerous examples of diurnal phasing of cell division among protists as well as metazoans and higher plants (for reviews see Edmunds, Cell Cycle Clocks, Dekker, New York, 1984). However, the mechanisms by which the diurnal cycle entrains the cell cycle remain elusive in all systems examined. Since many metabolic processes in plants are dependent on photosynthetic activity, we first investigated the role of photosynthesis in regulating cell cycle progression in G. toxicus. To determine if the signal permitting cell cycle progression at the L:D transition is coupled to the cessation of photosynthesis, cells at the time of the normal L:D transition were either transferred to dark or maintained in the light, in the absence or presence of the photosystem II inhibitor dichlorodimethylurea (DCMU; 10 μg/ml). Cells transferred to dark entered mitosis in 6 h, as observed previously. By contrast, cells which did not receive the L:D signal did not enter mitosis, regardless of whether photosynthesis continued or was inhibited with DCMU (Figure 2). (DCMU did cause a partial decline in the number dark treated of cells progressing into mitosis, reflecting a toxic effect of long term exposure to DCMU). These results indicate that the signal permitting cell cycle progression through the L:D transition is independent of photosynthesis.

In plants, two signal transduction pathways have been identified which relay light dependent signals to intracellular targets to elicit cellular responses (for review, Short and Briggs, Annu. Rev. Plant Physiol. Mol. Biol 45: 143-71, 1994). One pathway is sensitive to red light (mediated by phytochromes) and one sensitive to blue light (mediated by cryptochromes). We therefore investigated the involvement of red light and blue light in transduction of light dependent signals which may promote cell cycle progression at the L:D transition (Figure 3). At the time of the normal L:D transition, cells were either transferred to dark, maintained in white light, or were exposed to blue light only (Roscolux Filter No. 19) or red light only (Roscolux Filter No. 36). Mitotic index was then determined 6 h later. Cells maintained in light, which did not receive the L:D transition, did not enter mitosis 6 h after the normal time of L:D (or at any time during that diurnal cycle, data not shown). Cells exposed to blue light only (cessation of red light) similarly did not proceed to mitosis. By contrast, cells exposed to red light (cessation of blue light) proceeded into mitosis as if they were in the dark. This suggests that a blue light dependent signal transduction pathway is involved in cell cycle progression at the L:D transition.

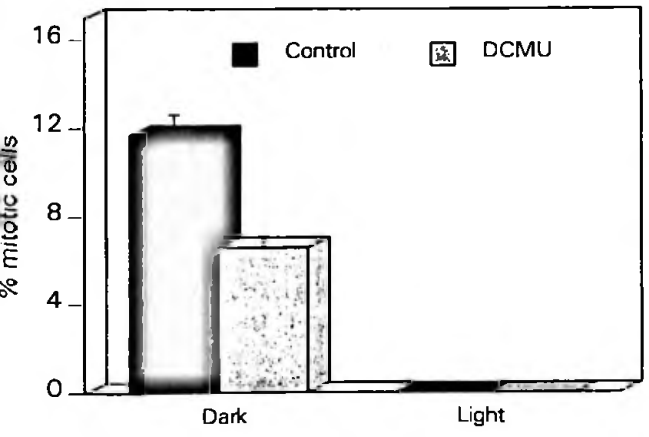

Figure 2. Effect of photosynthesis inhibitor, DCMU, on progression through the L:D transition.

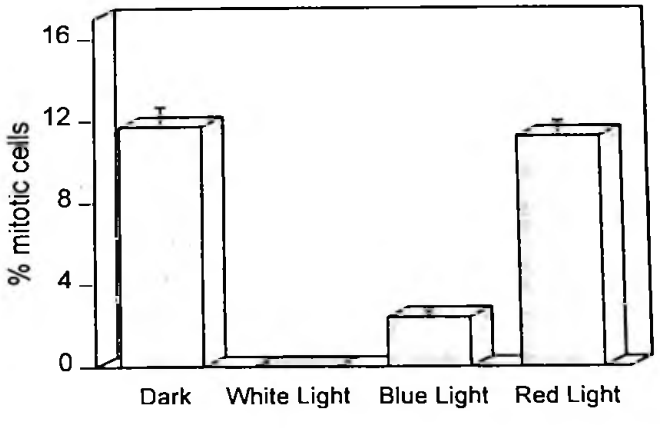

Figure 3. Involvement of red and blue light in cell cycle progression through the L:D transition.

The eukaryotic cell cycle is regulated by two classes of proteins, the cyclins and the cyclin dependent kinases, which together control both the initiation of DNA synthesis and the entry into mitosis (Murray and Kirschner, Science 246:614-21, 1989). We have recently demonstrated the presence of a cdc2-like kinase in G. toxicus, which is activated concurrent with the onset of mitosis, indicating that dinoflagellates possess a similar cell cycle machinery as that present in higher eukaryotes. Cell cycle progression is generally regulated at two control points, the G1/S phase transition and the G2/M phase transition, which coincide with the activation of cyclin dependent kinase complexes. We therefore sought to determine if the L:D transition regulates progression of the G. toxicus cell cycle at G1/S or G2/M. G. toxicus cells were treated at different times prior to mitosis with the S-phase inhibitor, aphidicolin (10µg/ml) and permitted to continue until the normal time of mitosis (22 h after onset of light). The number of mitotic cells present in aphidicolin treated cultures (black bars) at 22 h was then determined relative to untreated controls (grey bar). Aphidicolin added as late as 3 h after the onset of dark (denoted by dark bar on the X-axis) prevented entry into mitosis. This suggests that S-phase is not completed until after the onset of dark. Therefore, the L:D transition most likely controls cell cycle progression prior to the G2/M transition, possibly at the onset of S-phase.

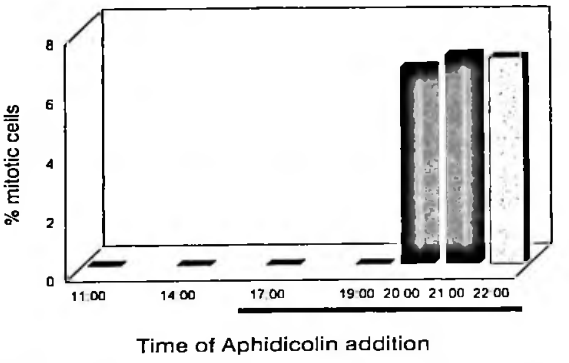

Figure 4. Effect of S-phase inhibitor, aphidicolin, on the percent of cells in mitosis at 22:00 h after onset of light (black bars), relative to untreated controls (grey bar). L:D transition (dark denoted by black horizontal bar) occurred at 16:00 h.

We are currently investigating components of the signal transduction pathway that relays the cessation of blue light to the cell cycle engine. If S-phase entry coincides with the L:D transition, then our results suggest that blue light provides an inhibitory signal which prevents progression of the cell into the cell division cycle. At the G1/S checkpoint in yeast and metazoans, the cell assesses that adequate nutrients are available and that adequate cell size has been attained prior to committing to the energy expensive processes of DNA replication and cell division. Negative regulators of growth (e.g., growth inhibitory polypeptides) have been demonstrated to act at this checkpoint. In G. toxicus, blue light may provide an inhibitory signal which prevents the cell from committing to replication during the light phase of the diurnal cycle.

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