A phantom of the ocean

Theodore J. Smayda

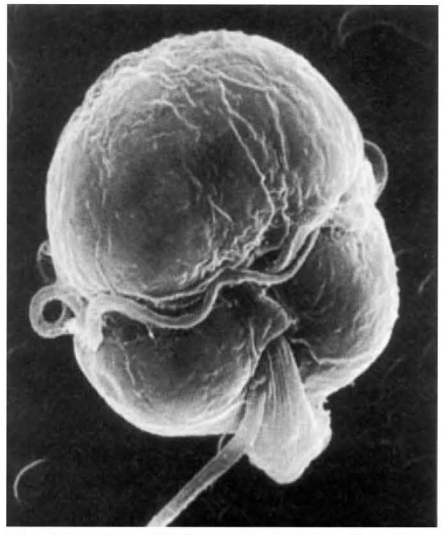
A CHARMING explanation of so-called 'red tides' is to be found in the rich folklore of the sea: the characteristic phosphorescence (bioluminescence) and associated surface discolorations are, it is said, produced by an enormous fish, The Devil of the Waters, which inhabits the seabed, spitting fire to destroy its prey, and thus reddening the waters[1]. Well, this fictitious monster would have met its match in the marine David described by Burkholder and her colleagues on page 407 of this issue[2]. They report the discovery of a microscopic, unicellular organism which, at one stage in its remarkable life cycle, secretes a powerful toxin which kills fish; most unusually, the organism uses dying fish to its nutritional advantage.

The organism concerned is a photosynthetic dinoflagellate, and it lurks dormant on the seabed until live fish approach. Then, sometimes within minutes, it excysts, releasing a motile, vegetative stage which swims, grows and swarms within the water column. There it secretes a potent, water-soluble neurotoxin which causes fish death, sometimes on a massive scale — in one episode, about a million menhaden (*Brevoortia* sp.) were killed. Several hours later, the dinoflagellate cells encyst, sink to the bottom sediments and await a renewed, ichthyo-stimulated foray.

The authors appropriately liken this stunningly rapid and ephemeral sequence of lethal appearance and disappearance to that of a phantom. The chemical nature of the neurotoxin remains to be elucidated, as does the broader distribution of the dinoflagellate. But although it is known only from two estuaries in the United States, the authors predict that it is widespread and has been responsible for many of the enigmatic fish kills which occur in coastal regions.

Red tides (which may in fact be red, green, yellow or brown) are of course one manifestation of blooms of phytoplankton, population explosions undergone by dinoflagellates, diatoms and several other groups of photosynthetic microalgae. Such blooms have underpinned marine foodwebs for at least three billion years, but the red-tide blooms of nutritionally inadequate or toxic species have less benign effects. That has been known for a long time. What is new is an apparent increase in bloom frequency and spread of noxious species; the occurrence of toxicity in species that had been thought to be harmless; and the growing number of reports of catastrophic mass deaths of marine mammals, including whales[3], and of fish and invertebrates[4].

Nor can the public at large stand aloof from these phenomena. In the best known of the toxic bloom cases, paralytic shellfish poisons' (PSP) toxicity, shellfish feeding upon certain dinoflagellates accumulate the neurotoxic saxitoxin and its analogues without harming themselves. But when the shellfish are eaten by humans, serious illness or death can occur: a recent PSP outbreak



The "phantom-like" dinoflagellate described by Burkholder *et al.*[2]. The toxic vegetative cells extend a tongue-like organelle, the peduncle (shorter structure, bottom), which attaches to and sucks the contents from fragments of fish tissue.

in Guatemala resulted in 26 deaths and 187 cases of illness[5]. Likewise, reports of fish deaths caused by red-tide outbreaks are not new as such[4,6,7]. But we are seeing the emergence of hitherto unsuspected toxins and toxic syndromes, such as the 1987 discovery of the amnesic shellfish poisoning in Cardigan Bay, Nova Scotia, caused by the neuroexcitatory amino acid, domoic acid[8]. Before then, toxin production by diatoms, a major component of the phytoplankton, was unknown. This toxin also suddenly appeared in Monterey Bay, California, in 1991, where it caused the death of pelicans feeding on anchovy which had in turn accumulated domoic acid from its diatom prey[9].

Another recent development, underlining how easily the phytoplankton can spring surprises on us, was the report of a previously undescribed mode of feeding (termed dasmotrophy) by a toxic, photosynthetic phytoplankter on other phytoplankton[10]. This toxic flagellate, also lethal to fish, invertebrates and macroalgae, is thought to produce membrane-puncturing compounds which allow it to extract nutrients from its prey.

It is against this background that the paper of Burkholder *et al.* must be viewed. With the possible exception of dasmotrophy, phytoplankton toxins have not been reported to have a nutritional role; rather, it is thought that they may serve as chemical deterrents against predation, even protecting shellfish which have sequestered them during feeding[11]. By contrast, the dinoflagellate described by Burkholder *et al.* does derive nutritional gain during its kill — it digests flecks of sloughed-off fish tissue to which it attaches by means of a peduncle. Another difference between the biology of this dinoflagellate and that of other toxin producers is in the surprising influence of phosphorus. The addition of phosphate, unlike nitrate or ammonia, stimulated the growth of gametes, whereas it is limitation of this nutrient that has been implicated in toxigenesis in other species[12].

Burkholder *et al.* point out that the apparent correlation between increasing nutrient enrichment of global coastal waters and increased incidences of harmful algal blooms is probably not a factor in blooms of the phantom dinoflagellate. But a sobering consequence of their discovery is that it further complicates resolution of the matter of whether harmful algal blooms in the sea are actually increasing, or whether the increase is due to improved monitoring of such events. However that may be, the public are subject to periodic alarms over the safety of seafood, and fisheries and aquaculture enterprises suffer considerable economic loss.

Several big questions need to be resolved. Are toxic blooms in general being triggered largely by increased nut-

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rient loadings in coastal waters, or by fisheries and aquacultural activities, or are they manifestations of long-term cyclical trends? Are they perhaps a worrying consequence of global deterioration of the marine environment? The next chance for all concerned to put their heads together and discuss these issues will be in October 1993, when the sixth international conference on toxic blooms takes place. We cannot hope to have the answers by then, but we can hope for more data to go on. □

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TRANSCRIPTIONAL REGULATION

A closer look at E2F

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ADENOVIRUS, a mammalian DNA tumour virus, has been a key player in investigations of fundamental cellular control mechanisms, as the virus must subvert the cell's machinery for transcription and replication for its own purposes. In 1986, studies of transcription control mediated by the adenovirus protein E1A identified a cellular DNA-binding protein, termed E2F, that recognizes key regulatory elements in the viral E2 gene promoter[1]. Further experiments defined the role of the factor in the regulation of transcription of the viral gene, but the significance of this cellular protein beyond the realm of adenovirus was unclear.

Things have changed over the past year, and dramatically — E2F has been thrust into the midst of cellular proliferation control mechanisms that are attacked by viral oncoproteins. This intense activity has now reached a crescendo with the cloning of the gene encoding E2F, as reported by Helin *et al.*[2] and Kaelin *et al.*[3] in the latest issue of *Cell*. A protein which had previously been studied as a band in a gel retardation assay is now ready for a closer look.

Oncogenic action

The recent developments of the E2F story actually began in 1988, involving studies of the oncogenic action of E1A, when it was realized that one of the cellular proteins originally identified as an E1A-binding protein, and thus a candidate target for E1A action, was the product of the retinoblastoma (Rb) gene[4]. It soon emerged that each of the DNA tumour viruses, including adenovirus, SV40 and human papillomavirus, encode proteins that bind the Rb protein. Clearly, these otherwise distinct viruses had a common activity, presumably essential for viral replication, that intersected with the action of a cellular tumour suppressor protein. But what was the activity and what function of the Rb protein was altered?

Analysis of the effect of viral E1A on the activity of E2F provided important clues. In adenovirus-infected cells, E2F is complexed to a 19K product of the viral E4 gene, an interaction that allows E2F to bind in a cooperative fashion to the E2 promoter. It turns out that the formation of the E2F–E4 complex requires the assistance of the E1A gene product because, in most cell types, E2F is complexed with cellular proteins that prevent its interaction with the E4 protein. An *in vitro* assay demonstrated that E1A could release E2F from these cellular protein complexes, making it available to bind to the E4 protein and hence the E2 gene promoter[5].

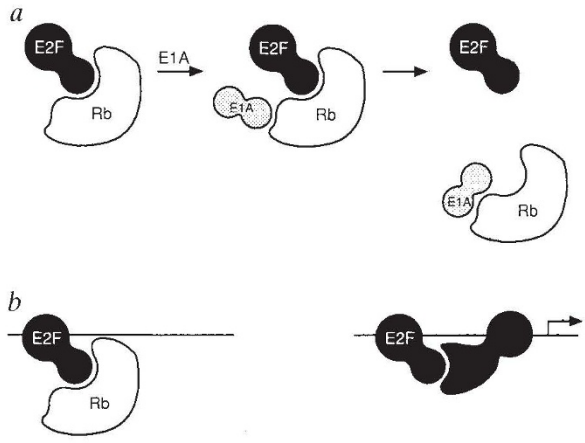
When it became clear that the same E1A domains were needed for the dissociation of these E2F complexes as were required for E1A binding to proteins such as Rb, a unifying mechanism became evident (see figure). That is, if Rb were one of the proteins associated with E2F, then the process of E1A-mediated dissociation of the complex might transfer Rb to E1A. Analysis of the E2F complexes for the presence of the Rb protein showed that this was indeed the case[6–8]. An independent approach demonstrated that complexes of Rb with cellular proteins possess sequence-specific DNA-binding activity specific for the E2F recognition sequence[9]. Thus, two approaches reached the same conclusion — E2F and Rb are cellular partners.

It was the ability of the Rb protein to interact specifically with E2F that has led to the cloning of a complementary DNA encoding E2F. Expression libraries initially probed with labelled Rb protein yielded several clones[10], but none of them turned out to be E2F. Additional screening, however, brought to light clones termed *RBP3* by Helin *et al.*[2] and *RBAP-1* by Kaelin *et al.*[3], which encode identical proteins with properties suggestive of E2F. The molecular weight of the encoded protein is close to that previously reported for E2F and the expressed protein exhibits the appropriate E2F DNA-binding properties. Antibodies specific for the cloned protein immunoprecipitate E2F activity and detect a protein of appropriate molecular weight in an enriched preparation of E2F. Moreover, the cloned protein interacts with the adenovirus E4 protein, as well as with the Rb protein, but not with mutant versions of these proteins. Finally, expression of the cDNA in transfected cells stimulated E2F-dependent transcription of a reporter gene.

Strong evidence

Taken together, then, the data in the two papers provide strong evidence that the clone does indeed encode E2F. But one question to be resolved is whether it encodes all of the E2F activity in the cell. Attempts to abolish or alter the mobility of the family of E2F complexes that are detected in cell extracts met with only partial success. Although technical difficulties could be the explanation, there may be more to come.

What does the sequence of the E2F clone tell us about its function? Although comparison with known sequences offers no evidence that E2F has been previously cloned, or that it is a close relative of a known protein, a 102-amino-acid segment essential for DNA binding contains a basic helix–loop–helix motif. It is not yet known if this motif is essential for the DNA-binding capacity of E2F, but it does raise the possibility that E2F functions as a protein dimer, or even as a heterodimer, possibly changing the DNA-binding specificity and thus generating an entirely



a, The E2F–Rb complex is depicted as an association of Rb with the carboxy-terminal domain of E2F. As a consequence of E1A action, E2F is displaced from the complex, leaving E1A bound to Rb. The intermediate involving an association of E1A with the E2F–Rb complex is speculative. *b*, The interaction of E2F with a promoter and the possible influence of Rb on the contacts normally made with other components of the transcriptional apparatus.

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