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Diel nucleic acid synthesis and particulate DNA concentrations: Conflicts with division rate estimates by DNA accumulation[1]

Abstract—The diel rate of microbial nucleic acid synthesis and the concentration of particulate DNA were measured at various locations in the oligotrophic Pacific Ocean. Net synthesis of RNA and DNA was observed over 24-h periods at all locations investigated, but the actual rate sometimes varied with time of day. It is concluded that whenever possible, daily rates of synthesis should be determined from a series of consecutive incubations conducted over a complete 24-h period. In addition, nonreplicating DNA comprised 75–90% of the total particulate DNA at the locations investigated. These results appear to preclude the use of light-dependent DNA accumulation as a measure of phytoplankton growth rate in the oligotrophic oceans.

Nucleic acid synthesis rates measured with [^{3}H]adenine have been used to quantify microbial production and cell division in various marine environments (Karl 1979; Karl et al. 1981*b*; Karl and Winn 1984; Winn and Karl 1984*a*). In recent field investigations, Karl and Winn (1984) and Winn and Karl (1984*a*) multiplied average hourly rates of DNA synthesis, measured over incubation periods of 6–12 h, by 24 to approximate daily rates of synthesis. This calculation assumes that DNA is synthesized at a relatively constant rate over 24-h periods, at least in the oligotrophic ocean. These extrapolations appeared to be justified because previous studies had shown that rates of nucleic acid synthesis in mixed populations of phytoplankton and bacteria were equivalent in light and dark incubations (Karl et al. 1981*b*). However, Falkowski and Owens (1982), in describing a new method to estimate phytoplankton growth rate, presented data indicating that particulate DNA (p-DNA) concentrations increase only in the light. Although Falkowski and Owens conducted their experiments in continental shelf waters, their data might be interpreted as evidence that the assumption of a relatively constant diel rate of nucleic acid synthesis in more oligotrophic environments is inappropriate. In view of the data presented by Falkowski and Owens we have conducted several experiments using an algal culture and natural microbial assemblages to test the hypothesis that rates of microbial nucleic acid synthesis are independent of time of day in the oligotrophic ocean. The results of these experiments are summarized herein. The data presented by Falkowski and Owens also indicate that little or no "detrital" DNA was present in the seawaters they investigated. We include a discussion of our field data which lead us to conclude that significant quantities of nonreplicating p-DNA exist in the oligotrophic Pacific Ocean.

The term "nonreplicating DNA" as used here refers to DNA that cannot be accounted for from measurements of microbial biomass or activity. This DNA may be associated with dead, dormant, or debilitated cells or may be adsorbed onto nonliving particulate materials. The p-DNA fraction that cannot be accounted for by the presence of living biomass has been termed detrital DNA (Holm-Hansen 1969*a*; Falkowski and Owens 1982). However, we prefer to use the term nonreplicating DNA, since some of this DNA may be associated with viable but nongrowing (i.e. dormant) cells and therefore may not be truly detrital.

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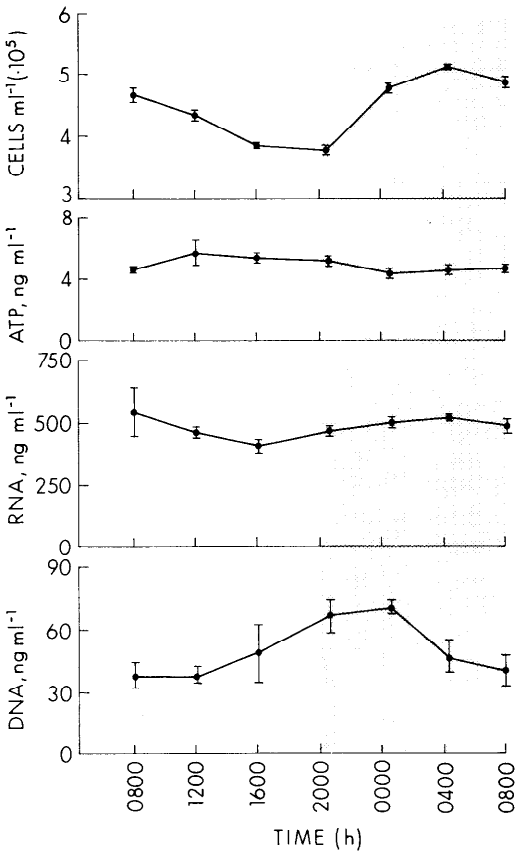


Fig. 1. Cell number and the concentration of ATP, RNA, and DNA in *Pavlova lutheri* cyclostat culture. Dilution rate 0.5 d^{-1}. Error bars are ±1 SD unit (n = 3).

pedition was made possible through an invitation from R. W. Eppley. A review by J. Paul has improved the presentation and interpretation of these data.

We began this investigation by growing the marine chrysophyte *Pavlova lutheri* in a cyclostat on a 12 : 12 light-dark cycle. The culture was maintained on modified FCRG medium (Eppley et al. 1967) containing 50 μM NO_3^- and 5 μM PO_4^{3-} at 22°C and illuminated with "cool-white" fluorescent lights. The medium was labeled with $^{32}PO_4^{3-}$ at a known specific radioactivity, and the steady state concentrations of RNA and DNA were determined from the $^{32}PO_4^{3-}$ activity in the purified RNA and DNA fractions. RNA and DNA were selectively hydrolyzed by the Schmidt-Thannhauser procedure and radiochemical concentrations quantified by liquid scintillation counting (Winn and Karl 1984*b*). Rates of nucleic acid synthesis in the light and in the dark portions of the 12 : 12 L/D cycle were measured using [^{3}H]adenine (Karl et al. 1981*a*; Karl 1981). Bacteria were present as contaminants in the culture, but direct microscopy revealed that they comprised <2% of the biomass within the culture vessel. Under these conditions the impact of bacterial metabolism on the rate of nucleic acid synthesis measurements was considered negligible.

Algal cell number increased in the beginning of the dark period and then decreased in the light. The concentrations of ATP and RNA showed little variation over the diel light cycle, whereas the concentration of DNA peaked in the dark (Fig. 1). The kinetics of [^{3}H]adenine incorporation into ATP, RNA, and DNA were similar during 4-h time-course experiments conducted in both the light (1200–1600 hours) and the dark (0000–0400), and the measured rates of DNA synthesis were 19 pg DNA ml^{-1} min^{-1} in the light and 24 in the dark.

Cyclic oscillation in the rate of cell division in response to diel light cycles is a common phenomenon in marine planktonic algae, and in general DNA synthesis occurs just before cell division (Chisholm 1981; Puiseux-Dao 1981). The data presented in Fig. 1 show that the major portion of DNA synthesis occurs near the end of the light period. However, the [^{3}H]adenine measurements reveal that DNA was synthesized both in the light and in the dark, indicating that cell division was not completely synchronized in this culture. Division patterns similar to those shown in Fig. 1 have been observed in *P. lutheri* cultures (Nelson and Brand 1979). Furthermore, in their study of 13 algal species, Nelson and Brand showed that, of the 22 algal clones displaying cyclic oscillations in division rate, about 50% divided more rapidly in the dark than in the light. These results, coupled with the nucleic acid synthesis patterns that we observed for *P. lutheri* (Fig. 1), would lead one to suspect that nucleic acids are synthesized by phytoplankton populations over 24-h periods in natural microbial assemblages.

We next measured the rates of RNA and

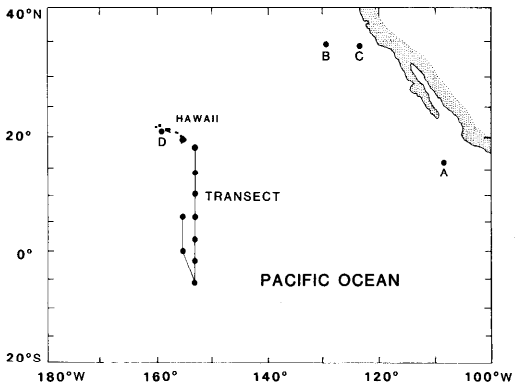


Fig. 2. Pacific Ocean station and transect locations. Station A—15°43′N, 107°20′W; station B—34°45′N, 129°50′W; station C—34°40′N, 123°50′W; station D—21°26′N, 158°15′W.

DNA synthesis over 24-h periods and the concentrations of ATP and DNA at various Pacific Ocean locations (Fig. 2). Water samples were prefiltered through 102-μm Nitex mesh to remove larger particles, and nucleic acid synthesis rates were determined using [^{3}H]adenine (Karl et al. 1981*a*; Karl 1981). Particulate materials were collected on Whatman GF/F glass-fiber filters with a median pore size, as defined by Sheldon (1972), of 0.4 μm. DNA concentrations were determined fluorometrically using DAPI (Falkowski and Owens 1982) and ATP determined as described above. Incubations were conducted in acid-washed polycarbonate bottles either on deck or in situ. Time-course experiments were conducted during on-deck incubations, and synthesis rates were calculated by dividing the label incorporated into RNA and DNA by the integral specific activity of the ATP precursor pool (Winn and Karl 1984*b*). Constant temperature for on-deck incubations was maintained with circulating surface seawater, and in situ light quality and quantity were simulated with combinations of shade cloth and blue Plexiglas (Rohm and Haas No. 2069).

RNA and DNA synthesis rates were measured on deck at midday and midnight at station A. Both RNA and DNA were synthesized in the light and in the dark; however, rates measured in the light period were usually higher than those measured in the dark (Table 1). Representative time-courses from this experiment are shown in Fig. 3A and B, and the results of a simultaneous 24-h time-course experiment in Fig. 3C. The rate of adenine incorporation into nucleic acids in the 24-h time-course (Fig. 3C) decreased with time of incubation, but it is evident that RNA and DNA were synthesized over the entire 24-h period. It is not clear whether the reduction in the rate of nucleic acid synthesis during the 24-h incubation was due to a change in the in situ rates of synthesis or whether this was an artifact of the long incubation.

Table 1. Diel nucleic acid synthesis rates at station A.

Depth (m)	Incubation interval	ATP (ng liter^{-1})	RNA* (pmol liter^{-1} h^{-1})	DNA* (pmol liter^{-1} h^{-1})
5	0800–1600	126	591	108
	2000–0400	137	629	80
50	0800–1600	152	333	117
	2000–0400	236	265	39
100	0800–1600	32	73	17
	2000–0400	37	70	8

* Nucleic acid synthesis rates were determined from time-course incubations conducted on deck.

To evaluate whether the above results are a general phenomenon in the oligotrophic oceanic environment, we repeated these experiments at station B. At this location, rates of synthesis were measured in situ at three intervals over a 24-h period. The results (Table 2) show that RNA and DNA were synthesized at about equal rates in both the light and dark portions of the diel cycle. These results again indicate that microbial RNA and DNA synthesis occurs continuously over 24-h periods.

As an independent verification of the [^{3}H]adenine results, we also performed a set of experiments using $^{32}PO_4^{3-}$ as a metabolic tracer. Phosphate incorporation into nucleic acids was monitored during a series of 4-h time-course incubations conducted over 24 h at station C. The absolute rate of nucleic acid synthesis cannot be calculated from $^{32}PO_4^{3-}$ incorporation unless the specific activity of the direct precursor to incorporation of $^{32}PO_4^{3-}$ into nucleic acids is determined independently (Karl and Bossard 1985). However, the results do show that both RNA and DNA were synthesized over the entire 24-h period, since $^{32}PO_4^{3-}$

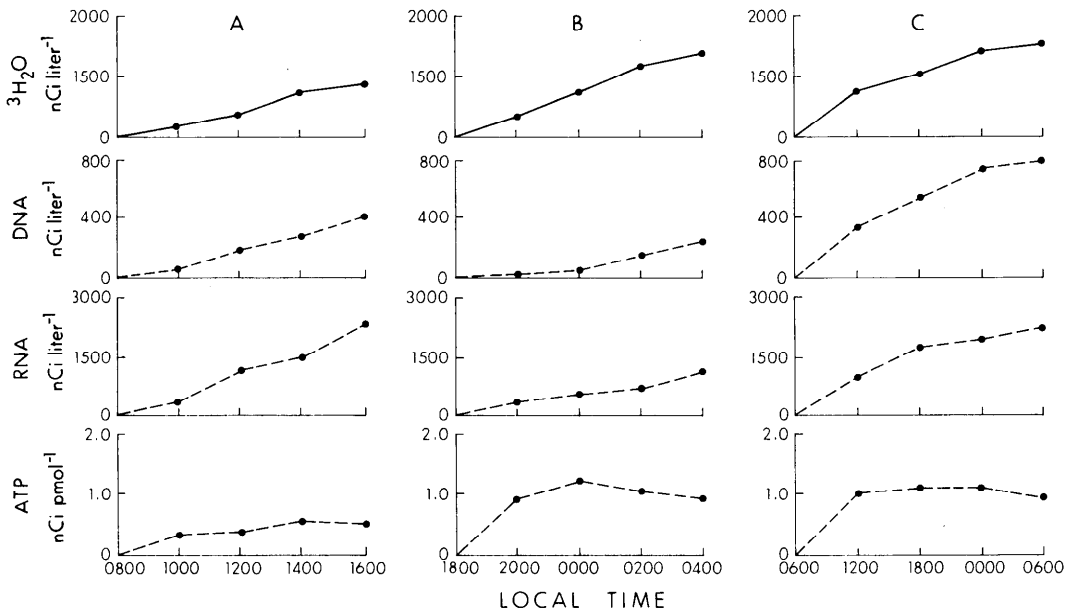


Fig. 3. Time-course of RNA and DNA labeling and ATP specific activity in surface samples at station A. A, B—Representative day and night time-courses; C—concurrent 24-h time-course.

was incorporated into both macromolecules at all times (Table 3).

Falkowski and Owens (1982) have shown that DNA accumulates in incubation bottles kept in the light, but not in those kept in the dark. Their data imply that at the locations they investigated DNA was synthesized by both autotrophic and heterotrophic populations only in the light. An alternative hypothesis might be that, in contrast to autotrophs, heterotrophs synthesized DNA in both the light and the dark, but that the contribution of heterotrophs to the overall rate of DNA synthesis was negligible. The data presented here show that DNA is synthesized over 24-h periods in the oligotrophic Pacific Ocean. Our results might therefore be interpreted as a direct contradiction to those of Falkowski and Owens. However, the data are not directly comparable for two reasons. First, Falkowski and Owens did their experiments in relatively nutrient-rich shelf waters whereas ours were in the oligotrophic ocean. It is thus possible that the differences in the two sets of results are entirely due to environmental differences. Second, Falkowski and Owens measured the accumulation of

Table 2. Diel nucleic acid synthesis rates at station B.

Depth (m)	Incubation interval	ATP (ng liter^{-1})	RNA* (pmol liter^{-1} h^{-1})	DNA* (pmol liter^{-1} h^{-1})
20	0530–1415	48.1	48.4	7.0
	1255–2145	50.1	33.3	4.8
	2020–0615	61.0	33.9	6.5
80	0530–1415	59.8	49.2	4.9
	1255–2145	63.7	58.9	5.0
	2020–0615	67.0	48.1	4.8
100	0530–1415	35.3	22.4	3.0
	1255–2145	31.8	32.6	3.4
	2020–0615	46.5	22.2	3.0

* Nucleic acid synthesis rates were calculated from end-point determinations. Incubations were conducted in situ.

Table 3. Phosphate incorporation into nucleic acids at station C.

Incubation interval	RNA* (nCi liter^{-1} h^{-1})	DNA* (nCi liter^{-1} h^{-1})
0640–1030	20.7	1.3
1145–1545	17.1	0.72
1845–2215	11.2	0.27
0000–0200	11.0	0.31
0715–1130	7.7	0.44

* Incorporation rates determined for surface seawater from time-course incubations conducted on deck.

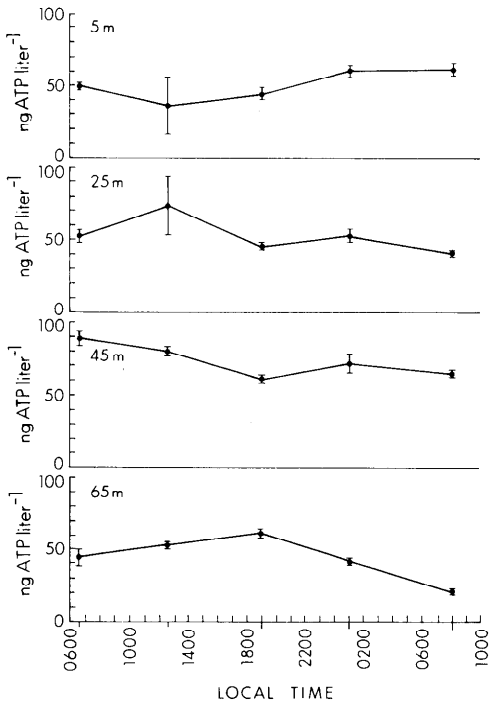


Fig. 4. ATP concentrations in the water column at station A. Error bars are ±1 SD unit (n = 3).

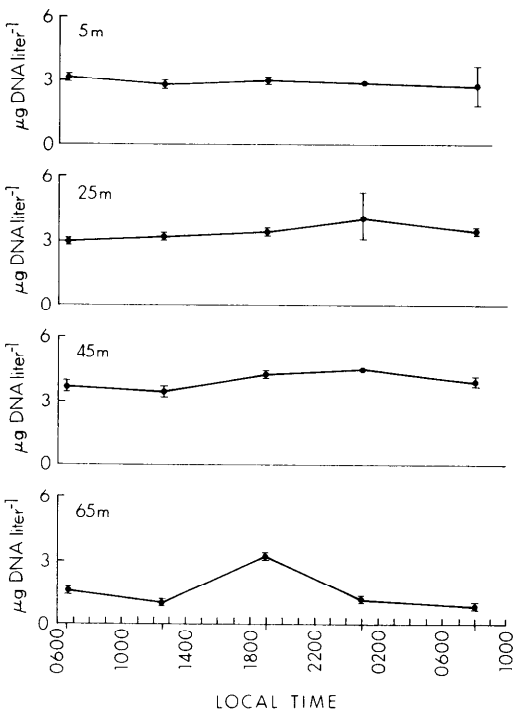


Fig. 5. As Fig. 4, but of DNA concentrations.

DNA whereas we measured the rate of DNA synthesis. Consequently it is also possible that DNA is synthesized in both the light and the dark, but, for some as yet unexplained reason, accumulates only in the light.

Falkowski and Owens (1982) also concluded that little or no detrital p-DNA was present in seawater at their study locations, since their data gave the best fit to an exponential increase equation when it was assumed that no detrital DNA was present. The conclusion that little or no detrital p-DNA was present in seawater is in agreement with the results of a study in other nutrient-rich waters by Dortch et al. (1983), but in conflict with earlier work indicating that significant quantities of detrital p-DNA are present in seawater (Holm-Hansen et al. 1968; Holm-Hansen 1969*a*; Sutcliffe et al. 1970; Karl and Winn 1984).

We have measured concentrations of ATP and DNA in a variety of oceanic marine environments and, as a result, conclude that large quantities of nonreplicating p-DNA do seem to exist in these waters. At station A, concentrations of ATP and DNA measured at several depths over a 24-h period remained relatively constant with time of day (Figs. 4 and 5). This observation might be used to argue that DNA is synthesized at a constant rate over 24-h periods, since daily fluctuations in DNA concentrations do not occur. However, the data reveal that the concentrations of p-DNA are so large that any increase in DNA concentration due to phytoplankton growth would be impossible to detect with current methodologies. This is true even if we make the assumption that the microbial biomass at these depths is comprised exclusively of phytoplankton. For example, assuming a C:ATP ratio of 250 (Karl 1980), the concentration of ATP at 25 m (about 50 ng liter^{-1}: Fig. 4) indicates that at most about 12.5 μg of living phytoplankton C liter^{-1} is present at this depth. Since DNA represents about 2% by weight of total C in phytoplankton (Holm-Hansen 1969*b*; Dortch et al. 1983) and in growing bacteria (Mandelstam and McQuillen 1976), there should be a maximum of about 0.25

Table 4. Living and total p-DNA on a transect in the equatorial Pacific Ocean.

Lat	Depth (m)	ATP (μg m^{-3})	p-DNA (mg m^{-3})	Living DNA* (mg m^{-3})	p-DNA / Living DNA
18°N	30	25.8	1.20	0.16	7.5
	80	23.2	1.50	0.14	10.7
14°N	30	35.7	1.32	0.22	6.0
	80	39.7	0.75	0.25	3.0
10°N	30	38.8	1.17	0.24	5.0
	80	62.9	1.32	0.25	3.4
6°N	30	52.6	1.81	0.33	5.5
	80	72.1	2.03	0.45	4.5
2°N	30	59.0	1.48	0.37	4.0
	80	68.7	1.45	0.43	3.4
2°S	30	47.5	1.74	0.30	5.8
	80	47.4	1.48	0.30	5.0
6°S	30	68.5	2.38	0.43	5.5
	80	78.6	2.06	0.49	4.2
0°	30	61.1	1.87	0.38	4.9
	80	50.6	1.40	0.32	4.4
6°S	30	44.9	1.65	0.28	5.9
	80	45.9	1.25	0.31	4.0

* Living DNA estimated from ATP as described in text.

μg of phytoplankton DNA liter^{-1} at this depth. This value is about an order of magnitude less than the total p-DNA concentration actually measured (Fig. 5). Clearly, even if we make the assumption that all of the living carbon in this environment is comprised of phytoplankton, an impossibly high rate of phytoplankton production would be required to yield a measurable increase in total DNA concentration during a 12–24-h incubation period. Similar calculations, with similar results, can be made for each depth.

We also measured concentrations of ATP and DNA at two depths on a transect across the equatorial Pacific Ocean (Fig. 2). The ratio of total to living DNA, estimated as above, varied from about 4–10 in these waters (Table 4). This suggests that 10–25% of total p-DNA is associated with growing microbial cells and that an even smaller proportion is associated with phytoplankton.

We have also estimated the proportion of nonreplicating p-DNA, using the relationship between the specific radioactivities of the cellular ATP pool and of d-AMP in DNA and an independently derived estimate of microbial growth rate. These data were collected during the PRPOOS expedition, where specific growth rates, estimated by a variety of techniques, ranged from 1 to 2 d^{-1} (Laws et al. 1984). At two locations, represented by station D, 24-h [^{3}H]adenine time-course experiments were conducted along with measurements of growth rate and concentrations of DNA and ATP. The specific activity of d-AMP in DNA was determined by dividing the radiochemical concentration in DNA by the concentration of chemical DNA (this calculation assumes that d-AMP averages 25 mol% in DNA), and the specific activity of ATP was determined as described previously (Karl et al. 1981*a*). The contribution of nonreplicating p-DNA can be estimated from these experiments from the relationship between the specific activity of the ATP precursor and the specific activity of d-AMP contained in DNA. The thrust of this argument is that the specific activity of d-AMP in DNA is equal to half the specific activity of the ATP pool after an incubation period equivalent to one generation time. This would be true if all members of the microbial population were growing at about equal rates, the population size were relatively constant over the incubation period, and all of the p-DNA were contained in growing cells. Given these assumptions, the relationship between the specific activities of d-AMP in DNA and ATP derived from the [^{3}H]adenine experiments can be described by

$$D_s/A_s = 1 - \exp[\mu(t)] \qquad (1)$$

where μ is the specific growth rate, t is time, and D_s and A_s are the specific activities of d-AMP in DNA and in ATP. The difference between the observed specific activities and the theoretical values can then be used to estimate the proportion of the total p-DNA that is not replicating.

A representative time-course of the relationship between the specific activities of ATP and d-AMP in DNA is shown in Fig. 6. Using Eq. 1, the data in Fig. 6 (i.e. an average ATP specific activity over 24 h of 2.7 nCi pmol^{-1}; DNA specific activity at 24 h of 0.39 nCi pmol^{-1}), and the specific growth rate estimates of 1 and 2 d^{-1}, we estimate that nonreplicating DNA comprises between 77 and 83% of the total DNA in these waters. Similar results, with non-

replicating DNA comprising 90–92% of the total p-DNA, were derived from an identical experiment conducted nearby (data not shown). The estimates of the proportion of nonreplicating DNA in oligotrophic seawaters derived from this approach are independent of, and in good agreement with, those derived from ATP : DNA concentration ratios.

An obvious question that arises from our estimates of the quantity of nonreplicating DNA in the oligotrophic ocean is, how does one account for this large DNA component? Although our results do not allow us to determine the nature of this DNA, we can examine three hypotheses that might be advanced to account for our data. The first is that our assumed C : ATP ratio is in error and yields an underestimate of living microbial biomass. However, a C : ATP ratio of 250 : 1 has been shown in various studies to provide a reliable estimate of living microbial carbon in natural environments (*see* Karl 1980). Laws et al. (1984) presented a comparison of direct microscopic measurements and ATP concentrations in the oligotrophic waters around Hawaii which demonstrates that multiplying ATP by 250 yields a fairly consistent estimate of total living carbon in the plankton. Furthermore, an underestimate of living microbial carbon by ATP would account only for the discrepancies observed between the concentrations of ATP and DNA. An underestimate of living biomass would not account for the equally large discrepancy between the quantity of DNA present in these environments and that which is metabolically active as determined by our ATP and DNA specific activity determinations.

A second hypothesis is that there are large numbers of living cells in the oligotrophic oceans that are enriched in DNA relative to ATP. Fuhrman and Azam (1982) have presented data that can be interpreted as evidence that the very small (<0.6 μm) bacterioplankton have DNA : C ratios >10%. However, Fuhrman and Azam pointed out that it is difficult to rule out the presence of DNA absorbed to particulate material in this size fraction. In addition, Paul and Carlson (1984) have shown that the majority of the p-DNA in the oligotrophic ocean

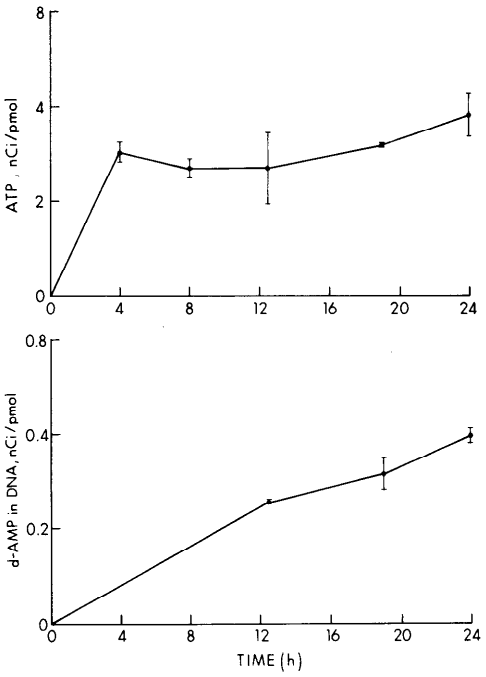


Fig. 6. Time-course specific activities of ATP and DNA during a [^{3}H]adenine experiment at station D. DNA specific activity was determined by dividing the radiochemical concentration in DNA by the total particulate DNA present in the sample. Error bars represent results of duplicate determinations.

is associated with the size fraction that is <1 μm.

A third hypothesis is that large quantities of DNA are contained in dead cells or adsorbed to particulate material in these environments. Further work is needed before definitive conclusions can be reached, but our results are consistent either with the hypothesis that the nonreplicating DNA in the oligotrophic ocean is contained in living bacterioplankton with high DNA : ATP ratios or with the hypothesis that this DNA is truly detrital (i.e. contained in dead cells or adsorbed to particulate material). Our comparison of the specific activities of ATP and DNA during [^{3}H]adenine incubations does indicate, however, that if the excess p-DNA in the oligotrophic waters around Hawaii is associated with living bacterioplankton with high DNA : ATP ratios, then these cells are not actively synthesizing DNA and may be metabolically inactive.

We would like to make a final point in

connection with the high concentration of p-DNA in the oligotrophic ocean. Dawes and Large (1970), Naheed et al. (1980), and Amy et al. (1983) have all shown that starved bacteria contain ATP as long as they are viable. In addition, Amy et al. showed that the concentration of ATP per viable cell is at least as high in starved marine bacteria as it is in growing cells. These data indicate that if the high concentrations of p-DNA in the oligotrophic ocean are due to the presence of living bacterioplankton with high DNA : ATP ratios, then these bacteria are enriched in DNA relative to carbon rather than depleted in ATP.

We have demonstrated that nucleic acids are synthesized over 24-h periods in natural microbial assemblages in the oligotrophic Pacific Ocean. This conclusion is derived from the measurement of absolute rates of nucleic acid synthesis using [^{3}H]adenine and from the incorporation of $^{32}PO_4^{3-}$ into RNA and DNA. We have also found that DNA is synthesized in at least a portion of both the light and dark periods by the marine phytoplankter, *P. lutheri,* grown in cyclostat culture on a 12 : 12 light-dark cycle. Although one might interpret our data as a direct contradiction of the observations made by Falkowski and Owens (1982), the locations we have investigated are relatively oligotrophic compared to the station locations occupied by Falkowski and Owens, and we have not ruled out the possibility that DNA is synthesized continuously over the complete diel cycle but, for some as yet unexplained reason, accumulates only in the light. In addition to demonstrating that RNA and DNA are made over 24-h periods in the oligotrophic Pacific Ocean, our data have also shown that DNA synthesis rates can vary with time of day, at least at certain locations. We therefore recommend that whenever possible, daily rates of synthesis be estimated from a series of rate measurements made over a complete 24-h period. We have adopted this protocol in all of our field studies. Finally, simultaneous determinations of ATP and DNA, and the relationship between the specific activity of ATP and d-AMP in DNA during [^{3}H]adenine experiments, have led us to conclude that there are large quantities of nonreplicating DNA in the oligotrophic Pacific Ocean. We conclude, that in these waters, about 75–90% of the total DNA is associated with dead or dormant cells or is absorbed to particulate material.

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