greater than 500 pg of p24 antigen per milliliter in all cases. Plasma concentrations of p24 antigen as low as 5 to 10 pg per milliliter can be detected in this assay.
14. Antibodies to HIV-1 were assessed by diluting the pooled plasma samples from HIV-1–positive hu-PBL-SCID mice tenfold and applying the samples to Dupont immunoblot strips.
15. In situ hybridization of hu-PBL-SCID mice with the Blur-8 probe [P. L. Deininger, D. J. Jolly, C. M. Rubin, T. Friedmann, C. W. Schmid, *J. Mol. Biol.* **151**, 17 (1981)] for human *Alu* sequences showed a concentration of labeled cells in the peripheral area of the periarteriolar "lymphoid" sheath (a misnomer in SCID mice, in which these areas are normally devoid of lymphocytes) and fewer labeled cells scattered through the red pulp. Examination of these tissues by hematoxylin and eosin staining revealed the presence of lymphoid cells in the same areas beginning about 4 weeks after human PBL injection into SCID mice. Thus, small numbers of human lymphoid cells migrate from the peritoneal cavity to the spleen at some interval after injection.
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17. All inbred strains of mice, including the BALB/c allotype congenic C.B-17 strain in which the *scid* mutation occurred, contain endogenous murine leukemia proviral sequences in their genome. Endogenous xenotropic viruses can be recovered from human grafts recovered from athymic *nude* mice (*16*), but xenotropic virus recoveries from SCID and hu-PBL-SCID mice were similarly low (D. E. Mosier *et al.*, unpublished observations). We have attempted to isolate HIV-1_{IIIB} from infected hu-PBL-SCID mice by culture with human foreskin fibroblasts in the presence or absence of soluble CD4. No virus was isolated from such cultures, nor could infectious HIV-1 be rescued by the subsequent addition of T lymphoblasts. Starting with the same cell-free supernatant from spleens of infected mice, we were easily able to isolate virus by culture with human blasts stimulated with PHA and IL-2, and this isolation was blocked by soluble CD4 (D. E. Mosier *et al.*, in preparation).
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The Assimilation of Elements Ingested by Marine Copepods

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The efficiency with which a variety of ingested elements (Ag, Am, C, Cd, P, S, Se, and Zn) were assimilated in marine calanoid copepods fed uniformly radiolabeled diatoms ranged from 0.9% for Am to 97.1% for Se. Assimilation efficiencies were directly related to the cytoplasmic content of the diatoms. This relation indicates that the animals obtained nearly all their nutrition from this source. The results suggest that these zooplankton, which have short gut residence times, have developed a gut lining and digestive strategy that provides for assimilation of only soluble material. Because the fraction of total cellular protein in the cytoplasm of the diatoms increased markedly with culture age, copepods feeding on senescent cells should obtain more protein than those feeding on rapidly dividing cells. Elements that are appreciably incorporated into algal cytoplasm and assimilated in zooplankton should be recycled in surface waters and have longer oceanic residence times than elements bound to cell surfaces.

THE PACKAGING OF ELEMENTS INTO rapidly sinking particles in the ocean is predominantly controlled by biological processes, including the production of zooplankton fecal pellets, which concentrates and encapsulates unassimilated elements associated with small suspended food particles (phytoplankton, microzooplankton) (*1*). Elements that are largely unassimilated in organisms are therefore likely to have short residence times in surface waters, whereas those that are assimilated should be recycled with organic matter (*2*). To better understand the cycling and vertical distribution of elements in the ocean, we have conducted a series of experiments to measure the efficiency with which marine copepods, important components of many zooplankton communities, assimilate a variety of essential and nonessential elements ingested in their food.

Experiments were conducted with monospecific assemblages of *Acartia tonsa, Acartia hudsonica*, or *Temora longicornis*. These calanoid copepods were collected with a 63-μm mesh according to availability from coastal waters off Long Island, New York, just before the experiments were performed. Adults were separated by pipette from other particulates and were fed uniformly radiolabeled diatoms, *Thalassiosira pseudonana* (clone 3H). The diatoms were grown axenically in modified f/2 medium prepared with sterile filtered seawater (*3*) to which the radionuclides ^{110m}Ag, ^{241}Am, ^{14}C, ^{109}Cd, ^{32}P, ^{35}S, ^{75}Se, and ^{65}Zn were added in trace amounts, individually or in pairs (*4*). Cells were taken from cultures after at least 3 days (≥six generations) of exposure to the radiotracers. We fed both diatoms that were actively growing (log-phase cells) and senescent (stationary-phase) cells to the copepods. Feeding suspensions were 200 ml and contained 1×10^5 cells per milliliter (2.2 mg of dry weight or 6.1×10^9 μm^3 $liter^{-1}$) and 20 to 47 animals. Immediately before the feedings, some of the labeled diatoms were lysed by resuspension in deionized water (pH 8.0) and fractionated by centrifugation to yield three pelletized fractions (pellets 1, 2, and 3) and a final supernatant fraction (*5*), each of which was assayed for radioactivity.

Assimilation efficiencies of ingested elements in the copepods were measured in two ways. For C and Se, a radiotracer ratio method was followed in which ^{51}Cr was used as an inert tracer of bulk ingested material in conjunction with ^{14}C (*6*), and ^{241}Am was used as an inert tracer in conjunction with ^{75}Se (*7*). For the other elements, we calculated assimilation efficiencies by dividing the amount of radiotracer retained by the animals after gut evacuation (*8*) by the amount ingested. We monitored the grazing activity of the animals by detecting changes in cell density with the use of in vivo chlorophyll a fluorescence (*9*). The radioactivity per diatom cell (*10*) stayed essentially constant during the 6-hour feedings. The gamma-emitting isotopes were measured with a Pharmacia-Wallac LKB gamma counter equipped with a well-type NaI(Tl) crystal; the beta emitters (^{14}C, ^{32}P, and ^{35}S) were measured with an LKB Rack Beta liquid scintillation counter (*11*).

The cellular fractionation of the radiotracers in the diatoms (Table 1) varied with the element; for example, only 10.2% of the total cellular ^{75}Se and 93.0% of the total cellular ^{241}Am were contained in pellet 1. Generally, the proportions of the nonessential elements (Ag, Am, and Cd) in the cytoplasmic fractions (that is, in pellets 2

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supernatant) were lower than those of the essential elements. Protein concentrations were determined (*12*) in the four cellular fractions of cells in log and stationary phase that had full and reduced nutrient enrichments (Table 2). Although the total protein per cell in complete f/2 medium did not change markedly with culture age (6.03 pg per cell in log phase, 5.99 pg per cell in late log phase, 5.38 pg per cell in stationary phase), the protein distribution changed with culture age, such that the cytoplasm of each stationary-phase cell had 1.94 times as much protein as that of each log-phase cell (Table 2). In complete f/2 medium, the fraction of total cellular protein in pellet 1 decreased from 72.2% at 3 days in log phase to 39.4% at 7 days in stationary phase; stationary-phase cells cultured with reduced concentrations of trace metals had 37.3% of their protein in this fraction. The higher content of cytoplasmic protein observed in stationary-phase cells was accompanied by an increase in cytoplasmic Zn and S (Table 1). [Sulfur content varied directly with the amount of protein in the subcellular fractions (slope = 1.01 ± 0.085) (7)]. Thus, the cellular fractionation of at least some elements in these diatoms can clearly vary with the physiological state of the cells.

Overall, 77% of the proteins in *T. pseudonana* cells had molecular weights of >300,000 g mol^{-1} [Table 3 (*13*)]. The proteins in pellet 1 had molecular weights almost entirely >300,000 g mol^{-1}, whereas the proteins in the cytoplasm were significantly smaller (Table 3).

The assimilation efficiencies of the ingested elements in the copepods were 0.9% for ^{241}Am, 17.4% for ^{110m}Ag, 26.8% for ^{65}Zn in log-phase cells and 47.3% in stationary-phase cells, 30.4% for ^{109}Cd, 49.4% for ^{35}S in log-phase cells and 64.3% in stationary-phase cells, 71.9% for ^{32}P, 84.4% for ^{14}C, and 97.1% for ^{75}Se. The assimilation efficiencies were directly related to the fractions of each radiotracer in the diatom cytoplasm (Fig. 1). Four replicate experiments with ^{75}Se, conducted with different batches of animals and diatoms, gave a coefficient of variation for the assimilation efficiency determination of 1.5% (7). No significant deviations from this line were observed for any of the copepod species or elements examined. The intercept of the line in Fig. 1 is not significantly different from 0 and the slope is close to 1. This relation suggests that digestion in copepods and possibly other zooplankton proceeds by breaking open the ingested cells and assimilating soluble cellular material from the algal cytoplasm.

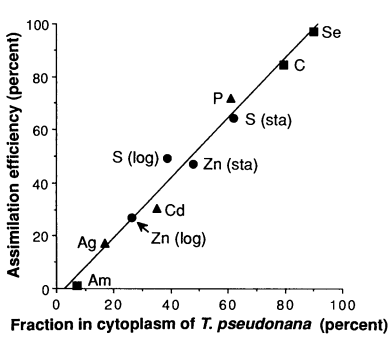

Fig. 1. Assimilation efficiency (in percent) of ingested elements in copepods fed *Thalassiosira pseudonana* as a function of the cytoplasmic fraction (percent of total cell content) of those elements in the diatoms. (■) *Acartia tonsa*, (●) *Acartia hudsonica*, (▲) *Temora longicornis*. $y = (1.131 \pm 0.061)\ x - (3.503 \pm 4.929)$. The grazing rates varied with each batch of animals; mean rates (milligrams of algal dry weight per gram dry weight of animal per hour) were 39 ± 19 for *Acartia* spp. and 5.3 ± 3.5 for *T. longicornis*; sta, stationary growth phase; log, logarithmic growth phase.

The short gut residence time of ingested food in these animals (<30 min) appears to be insufficient to allow for breakdown and assimilation of the larger insoluble macromolecules associated with algal cell walls and membranes. Moreover, gut transit time in copepods is not appreciably affected by the cell density of algal suspensions over a range of 0.25 to 2.02 mg of dry weight per liter (*14*); these data suggest that comparable results would be obtained with different experimental feeding conditions. Microscopic and physiological studies have shown that peritrophic membranes enveloping crustacean (including copepod) fecal pellets can function as a filter, selectively retaining particulate matter with diameters of >10 nm and permitting the exchange of more soluble material (*15*). The extent to which this simple model of a "liquid" digestion strategy applies to other marine invertebrates remains to be examined.

Table 1. Percentage distribution of elements in different subcellular fractions of *T. pseudonana*. For P, Ag, and Am, only pellet 1 and supernatant from pellet 1 were analyzed. Growth phase is also indicated.

Element	Phase	Pellet 1	Pellet 2	Pellet 3	Supernatant
Se	Stationary	10.2	1.7	4.3	83.8
C	Stationary	20.4	4.9	14.6	60.1
P	Log	39.1			60.9
S	Log	61.2	7.8	2.0	29.0
S	Stationary	37.9	9.3	13.2	39.6
Zn	Log	73.7	2.7	1.8	21.8
Zn	Stationary	52.1	5.8	3.2	38.9
Cd	Log	65.1	8.1	2.8	24.0
Ag	Log	83.3			16.7
Am	Stationary	93.0			7.0

Table 2. Protein distribution in subcellular fractions of *Thalassiosira pseudonana* cells, in picograms. Values are expressed on a per cell basis. Numbers in parentheses are percent of total. Log-linear cell division rates (μ) are given as divisions per day at each sample time. Reduced nutrient medium contained f/2 nutrient additions except for trace metals (f/50), with no Cu, Zn, or EDTA. Lt log, late log; sta, stationary.

μ	Phase	Cells analyzed	Pellet 1	Pellet 2	Pellet 3	Supernatant	Total
				Complete f/2			
2.45	Log	1.23×10^9	4.35 (72.2)	0.41 (6.7)	0.34 (5.7)	0.93 (15.4)	6.03 (100)
1.52	Lt log	6.40×10^8	3.22 (53.8)	0.58 (9.7)	0.48 (7.9)	1.71 (28.6)	5.99 (100)
0	Sta	5.48×10^8	2.12 (39.4)	0.46 (8.5)	0.80 (14.8)	2.00 (37.3)	5.38 (100)
				Reduced nutrients			
0	Sta	5.79×10^7	3.44 (37.3)*		2.49 (27.0)	3.30 (35.7)	9.23 (100)

*Protein data for pellets 1 and 2 combined.

Table 3. Size distribution of proteins within subcellular fractions of log-phase *T. pseudonana* cells in picograms. Values are expressed on a per cell basis. Values in parentheses are percentages of total protein within each fraction; MW, molecular weight.

MW g mol^{-1}	Pellet 1 (pg)	Pellet 2 (pg)	Pellet 3 (pg)	Supernatant (pg)	Whole cell (pg)
>300,000	4.05 (92.9)	0.20 (48.8)	0.14 (40.0)	0.27 (29.0)	4.66 (77.3)
50,000 to 300,000	0.05 (1.1)	0.03 (7.3)	0 (0)	0.19 (20.4)	0.26 (4.3)
1,000 to 50,000	0.06 (1.4)	0 (0)	0 (0)	0.11 (11.8)	0.17 (2.8)
<1,000	0.20 (4.6)	0.18 (43.9)	0.21 (60.0)	0.36 (38.7)	0.94 (15.6)
Total	4.35	0.41	0.34	0.93	6.03

The data further suggest that a copepod would assimilate almost twice as much protein by ingesting a stationary-phase cell as it would by ingesting a log-phase cell. Significantly higher assimilation efficiencies for Zn and S were observed in animals feeding on stationary-phase cells than on actively dividing cells, in close agreement with the relative quantities of these elements and protein in the cytoplasm of the cells (Fig. 1 and Tables 1 and 2). Copepods grazing on algal cells toward the end of a bloom could therefore be expected to assimilate more protein and protein-associated elements than those grazing on equal amounts of midbloom phytoplankton cells.

The class B and borderline metals (Ag, Cd, and Zn), which have greater affinities for S than N than O (*16*), showed greater penetration into the algal cytoplasm and therefore greater assimilation in grazers than did class A metals [Am (*10*) and our data; Th and Pu (*17, 18*)], which have greater affinities for O than N than S (*16*). The assimilation efficiencies that we measured for animals that ate stationary-phase diatoms are virtually identical to those measured for Cd (29.6%), Zn (48.4%), and Am (4.5%) in a separate study in which the copepod *Anomalocera patersoni* was fed stationary-phase *Isochrysis galbana* cells (*18*). The similar results suggest that our conclusions apply to other copepods feeding on nondiatom phytoplankton cells. Those elements that penetrate into the cytoplasm of phytoplankton and are assimilated by zooplankton thus enter the organic cycle in the sea, and through recycling, have longer oceanic residence times (*2*), consistent with the inverse relations observed between element residence times and enrichment factors in zooplankton fecal pellets (*19*). The results also explain why zooplankton fecal pellets are more enriched in particle-reactive nonessential elements than are the animals that produced them or the algal food from which they were produced (*1*).

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4. ^{110m}Ag was taken from a solution in 0.1*N* HNO_3 (added to cultures to give 74 kBq $liter^{-1}$, 21 nM); ^{241}Am was in 3*N* HNO_3 (in different experiments, a range of 18.5 to 37 kBq l^{-1}, 0.6 to 1.2 nM, was used); ^{14}C was in $NaH^{14}CO_3$ in distilled water (98.8 kBq $liter^{-1}$); ^{109}Cd was added in 0.1*N* HCl (296 kBq $liter^{-1}$, 9.3 nM); ^{32}P was in the form $NaH_2{}^{32}PO_4$ in distilled water (148 kBq $liter^{-1}$, 4 nM); ^{35}S was in the form $Na_2{}^{35}SO_4$ in distilled water (3.18 MBq $liter^{-1}$); ^{75}Se was in 0.5*N* HCl (in different experiments, a range of 37 to 660 kBq $liter^{-1}$, 0.136 to 2.42 nM selenite, was used); ^{65}Zn was in 0.1*N* HCl (296 kBq $liter^{-1}$, 0.18 nM). All acids were Ultrex. Radiotracers were added in microliter amounts; the pH of the cultures was adjusted to 8.0.
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Senescence of Nickel-Transformed Cells by an X Chromosome: Possible Epigenetic Control

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Transfer of a normal Chinese hamster X chromosome (carried in a mouse A9 donor cell line) to a nickel-transformed Chinese hamster cell line with an Xq chromosome deletion resulted in senescense of these previously immortal cells. At early passages of the A9/CX donor cells, the hamster X chromosome was highly active, inducing senescence in 100% of the colonies obtained after its transfer into the nickel-transformed cells. However, senescence was reduced to 50% when Chinese hamster X chromosomes were transferred from later passage A9 cells. Full senescing activity of the intact hamster X chromosome was restored by treatment of the donor mouse cells with 5-azacytidine, which induced demethylation of DNA. These results suggest that a senescence gene or genes, which may be located on the Chinese hamster X chromosome, can be regulated by DNA methylation, and that escape from senescence and possibly loss of tumor suppressor gene activity can occur by epigenetic mechanisms.

NEOPLASTIC TRANSFORMATION IS known to be a multistep process (*1*). One class of genes implicated in this process are tumor suppressor genes that in normal cells are thought to suppress the expression of transformation, malignancy, or metastatic progression and must either be lost, inactivated, or mutated in tu-