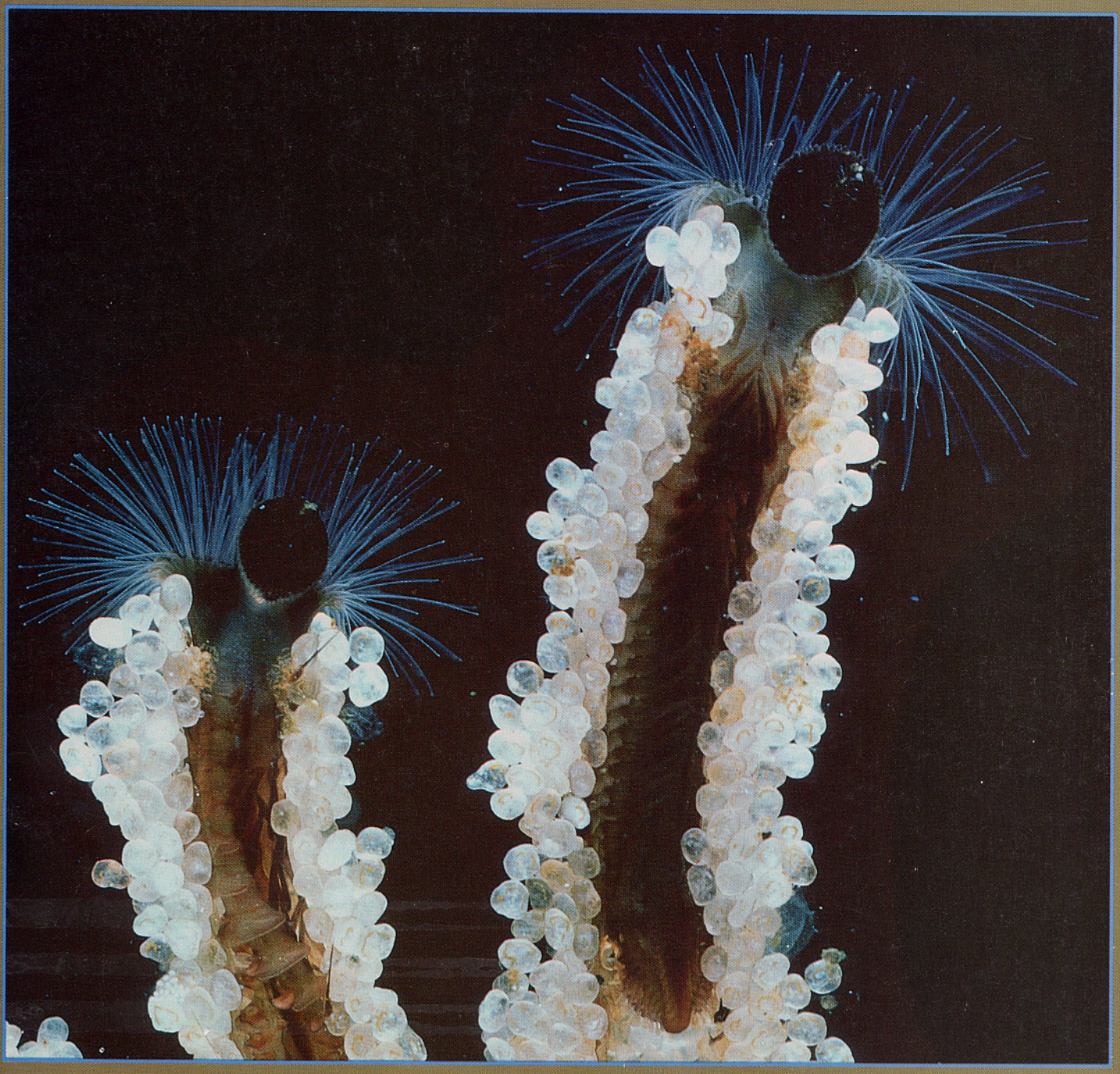
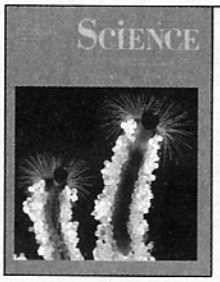
American Association for the Advancement of Science

SCIENCE

25 January 1991 $6.00
Vol. 251 ■ Pages 349–488





COVER Adult reef-building marine polychaetes (*Phragmatopoma lapidosa californica*) that have built sand tubes against a pane of glass. Planktonic larvae of these worms settle when they contact adult tube sand, a response that is facilitated by chemical and hydrodynamic processes. See page 421. [Photograph by Joseph R. Pawlik, University of North Carolina at Wilmington]

Hydrodynamic Facilitation of Gregarious Settlement of a Reef-Building Tube Worm

JOSEPH R. PAWLIK,* CHERYL ANN BUTMAN, VICTORIA R. STARCZAK

Experiments testing the effects of hydrodynamic processes and chemical cues on substrate selection were conducted with larvae of the marine tube worm *Phragmatopoma lapidosa californica*. In flume experiments, larvae were presented an array of sand treatments, including two substrates previously shown to induce metamorphosis in this species, under fast and slow flow regimes. Larvae preferentially metamorphosed on the inductive substrates in both flows. Delivery to the array was higher in fast flow because larvae tumbled along the bottom, whereas in slow flow, larvae were observed swimming in the water column. Thus, in addition to chemical cues, behavioral responses to flow conditions may play an important role in larval recruitment to the benthos.

HABITAT SELECTION OCCURS FOR many benthic marine invertebrates when their planktonic larvae settle and metamorphose into benthic adults. Initiation of settlement presumably is a response to chemical cues associated with preferred adult habitats (*1*). An understanding of the interaction between the relevant chemical and physical processes has been impeded, however, because the naturally occurring compounds that induce larval settlement have eluded isolation and identification and because experimental assessments of larval substrate selection have been conducted in still water rather than under realistic flow conditions. Active selection of substrates at the time of settlement has only recently been shown to occur under realistic flow conditions (*2*).

Gregarious settlement is common among marine invertebrates, including barnacles, mussels, and oysters, and is thought to be mediated by chemical signals originating from conspecific adults (*1, 3*). The larvae of some sabellariid polychaetes settle preferentially upon contact with the cemented sand tubes of adult conspecifics to form large aggregations (*4, 5*). Worm reefs are known from many parts of the world; they support a diverse community of invertebrates and fishes and are important in preventing coastal erosion in some areas (*5*). Settlement of *Phragmatopoma lapidosa californica*, a reef-forming sabellariid from the eastern Pacific, can be induced by free fatty acids (FFAs) isolated from the cemented sand matrix of adult tubes (*6*). In a series of single-substrate, still-water experiments, settlement in response to FFAs was shown to depend on the length, conformation, and functionality of the inducer molecules (*7*). Furthermore, this response was highly species-specific, thus far restricted to the genus *Phragmatopoma* within the polychaete family Sabellariidae (*6, 8*); for example, larvae of *Sabellaria alveolata*, a gregarious species from European waters, do not respond to FFAs, and these compounds are an order of magnitude less abundant in the sand of their tubes (*9*). Yet it has been suggested that FFAs do not occur in sufficient quantities in the tube sand of *P. l. californica* to induce settlement under natural conditions and alternative cues have been proposed (*10*).

We conducted multiple choice experiments in two flume flows to determine the relative importance of chemical cues, physical processes, and larval behavior in affecting settlement of *P. l. californica*. Experiments were conducted in a racetrack-design, paddle-wheel-driven, recirculating, laboratory flume (6 m long straightaway, 50 by 30 cm deep channel, filled to 10 cm depth, 1000 liter volume) (*11*). Laboratory-reared, competent larvae (*12*) were added to the flume, which contained a 21 by 21 cm sediment array set into the flume bottom. The array contained five replicate squares (4 by 4 by 0.3 cm deep) of five treatments arranged in a Latin-square design (*13*). Polystyrene spheres, with sinking speeds within the range of downward swimming speeds of *P. l. californica* larvae, were also added as larval mimics (*14*). Larvae and spheres (2000 of each) were added to the flume at upstream distances calculated to maximize the probability that they would encounter the array on their first transit down the straightaway (*15*).

Replicate settlement experiments were conducted in two steady, unidirectional, smooth-turbulent flows with surface velocities of approximately 15 cm/s (fast) and 5 cm/s (slow). The boundary shear velocities (u_*) were 0.81 ±0.13 and 0.34 ±0.10 cm/s for fast and slow flows, respectively, as determined from velocity-profile data collected with a laser-Doppler velocimeter (*16*). These flows simulated typical tidal flows of

Table 1. Results of statistical analyses of the 2-hour (fast flow) and 6-hour (slow flow) experiments. The experiments were conducted as a split plots design with flow effect as the whole plot, the three batches of larvae as replications of the whole plot, and sediment treatment and row and column effects as subplots in a Latin-square design (*13*). The ANOVA model for the analysis was $y = \mu$ + flow + batch + flow × batch + treatment + flow × treatment + row + flow × row + column + flow × column + batch × treatment + batch × row + batch × column + flow × batch × treatment + flow × batch × row + flow × batch × column + error. All interaction terms not in the model were assumed to be nonsignificant. The ANOVA model was used to test total animals, metamorphosed juveniles, and spheres separately, and the analysis assumed no interaction among them. Data for juveniles and spheres were $\log(x + 1)$ transformed to homogenize the variances. The results for the batch effect and the two-way batch interactions are not presented because batch was considered a random factor in the ANOVA and is not relevant to this study (*24*). The results of Tukey's multiple comparisons tests for total animals and metamorphosed juveniles are presented in Tables 2 and 3, respectively. For spheres, comparisons tests revealed no significant differences in mean sphere distribution between rows in slow flow. In fast flow, significantly more spheres occurred in row 1 than in rows 2 and 3, and significantly more were in rows 2 and 3 than in rows 4 and 5 ($P \leq 0.05$).

Source of variation	df	*F* values		
		Total animals	Juveniles	Spheres
Flow	1	22.75*	14.17	0.17
Treatment	4	3.39	161.42***	1.33
Row	4	7.40**	19.52***	23.56***
Column	4	10.30**	2.24	1.94
Flow × treatment	4	2.08	7.66**	0.98
Flow × row	4	4.89*	5.74*	26.78***
Flow × column	4	4.96*	0.70	0.66
Flow × batch × treatment	8	2.41*	1.61	1.11
Flow × batch × row	8	3.74***	0.91	0.31
Flow × batch × column	8	0.76	0.95	1.62
Error	72			

*$P \leq 0.05$. **$P \leq 0.01$. ***$P \leq 0.001$.

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shallow, subtidal environments (*17*) where *P. l. californica* forms massive reefs (*18*). This species also builds large aggregations in the intertidal, where oscillatory flows with much higher u_* predominate (*19*). We chose to test the simpler, subtidal, steady-flow case first. The flume flow regimes were chosen such that $u_* < u_{*crit}$ (shear velocity required to initiate particle motion) for spheres in slow flow, and $u_{*crit} < u_* < u_{*susp}$ (shear velocity for suspended-load transport) in fast flow. Thus, after falling to the bottom in fast flow, spheres rolled or saltated along the bottom as they were transported around the flume, a condition described as bedload transport.

Larvae were presented with a choice of quartzite sand treated in five ways (*20*): (i) sand that had been incorporated into the tubes of adult worms maintained in laboratory aquaria (tube sand), (ii) sand that had been kept in shallow dishes in the same aquaria as (i) and that had presumably developed the same organic-microbial film (filmed sand), (iii) sand treated with palmitoleic acid, an inductive FFA isolated from adult tube sand (16:1), (iv) sand treated with palmitic acid, a noninductive FFA (16:0), and (v) untreated sand (clean). Preliminary experiments were run for 20 (± 1) hours with concentrations of FFAs at 1000 μg per gram of sand, and there were two replicate experiments per flow. Subsequent experiments were shorter in length and controlled for the total distance traveled by the recirculating water-mass at the two surface flow velocities: 2 hours for fast flow and 6 hours for slow flow (*21*). The FFAs were assayed at 100 μg/g for these shorter term experiments, and there were three replicate experiments per flow. At the end of each experiment, the contents of each treatment square were placed in separate jars containing preservative solutions that permitted separation of metamorphosed juveniles from unmetamorphosed larvae (*22*). Replication was sufficient for meaningful statistical analyses of the shorter, but not the longer term experiments (that is, because of a power problem when $n = 2$ replicates). Statistics are reported only for the shorter term distance-corrected experiments (Tables 1 to 3), and results of the longer term experiments are discussed qualitatively.

Larvae of *P. l. californica* consistently metamorphosed in greater numbers on conspecific tube sand and sand treated with palmitoleic acid (16:1) than on the other three treatments in both flow regimes and for all experimental durations (Fig. 1 and Tables 1 to 3). The treatment effect was statistically significant for juveniles but not for total animals (unmetamorphosed larvae plus metamorphosed juveniles) (Table 1), suggesting that different processes control larval delivery to the array and the subse-

Table 2. Tukey's multiple comparisons of mean total animals (larvae + metamorphosed juveniles) carried out to test which treatments, rows, or columns differed when these effects were statistically significant (Table 1) (*13*). Because the batch × flow × column interaction was not significant, the multiple comparisons test for column was performed for each flow condition separately, using the average of each column from each batch ($n = 3$). This average value was used to compute the means for the column comparisons. For each comparison, means that do not share a superscript (a, b, or c) are significantly different from one another ($P \leq 0.05$). Rows were oriented perpendicular to the flow direction, with row 1 at the leading edge (upstream) and 5 at the trailing edge (downstream) of the 5 by 5 array. Columns 1 through 5 were arranged left to right with row 1 at the bottom. T = tube sand, F = filmed, C = clean.

Flow	Batch	Total animals				
		Treatment				
		T	16:1	16:0	F	C
Fast	1	56.2^{a}	34.8^{ab}	41.0^{ab}	22.4^{bc}	28.0^{c}
Slow	3	6.0^{ab}	10.2^{a}	5.8^{ab}	2.8^{b}	6.8^{ab}
		Row				
		1	2	3	4	5
Fast	1	58.4^{a}	48.2^{ab}	33.0^{bc}	22.6^{c}	20.2^{c}
Fast	2	78.8^{a}	24.0^{b}	14.8^{b}	15.4^{b}	11.8^{b}
Fast	3	30.0^{a}	14.6^{b}	14.6^{b}	14.4^{b}	7.6^{b}
		Column				
		5	4	1	2	3
Fast		36.0^{a}	33.0^{a}	24.9^{ab}	24.0^{ab}	17.6^{b}

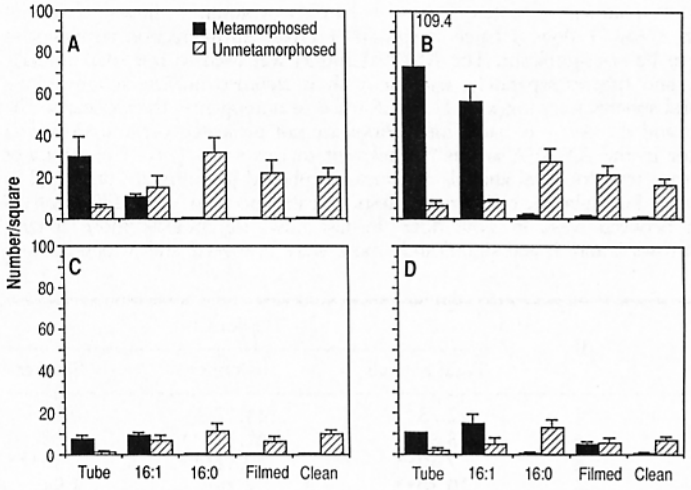

Fig. 1 (**left**). Mean number and standard error of unmetamorphosed larvae and metamorphosed juveniles in each treatment (five squares per treatment per array) for experiments conducted under the following conditions: (**A**) fast flow, $u_* = 0.81$ cm/s, 2 hours, $n = 3$; (**B**) fast flow, 20 ± 1 hour, $n = 2$; (**C**) slow flow, $u_* = 0.34$ cm/s, 6 hours, $n = 3$; and (**D**) slow flow, 20 ± 1 hour, $n = 2$. Mean percentages of animals added to the flume that were collected in the array at the end of the experiments were: (A) 34%, (B) 53.2%, (C) 13.5%, and (D) 16.4%. Treatments were Tube, sand that had been incorporated into the tubes of adult worms; 16:1, sand treated with palmitoleic acid, an inductive FFA; 16:0, sand treated with palmitic acid, a noninductive FFA; Filmed, sand that had been exposed to seawater and presumably developed the same organic-microbial film as tube sand; and Clean, untreated sand.

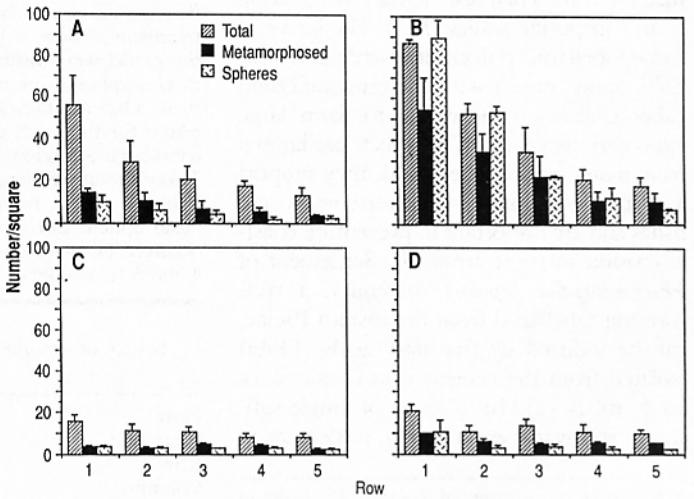

Fig. 2 (**right**). Mean number and standard error of total animals, metamorphosed juveniles, and spheres in each row (five squares) of the array for conditions described in Fig. 1. Rows were oriented perpendicular to the flow with row 1 at the leading edge (upstream) and 5 at the trailing edge (downstream) of the 5 by 5 array.

quent metamorphic response. The number of larvae metamorphosing in inductive treatments was much higher in fast than in slow flow, because significantly more larvae reached the array in fast flow (Fig. 1 and Table 1).

The distribution of total animals in the rows of the array closely resembled the distribution of spheres in both flows (Fig. 2). In fast flow, the sphere distribution was as expected for bedload transport, showing a pronounced upstream-downstream gradient with highest abundances in the row at the leading edge of the array. In slow flow, however, many fewer animals and spheres reached the array; the row effect was not significant for total animals and was only marginally significant for juveniles (Tables 2 and 3).

The ultimate distribution of juveniles in the fast and slow flow arrays likely resulted from a combination of both passive (physical) and active (behavioral) processes. After larvae were added to the flume in fast flow, they were observed to swim straight down to the bottom, where they then tumbled and bounced along the bed. The qualitative agreement between the number of spheres and total animals present in the array in fast flow suggests that larvae, after reaching the bottom, were delivered to the array like passive particles by bedload transport. As they moved along the bottom, larvae first encountered treatment squares in the leading row of the array. If the first square encountered contained an inductive treatment, the larvae metamorphosed. Otherwise, they moved out of the treatment, were carried by the flow to other treatments downstream, or repeated the process on subsequent passes across the array. Fast flow experiments conducted for 20 hours allowed ample time for larvae to leave noninductive treatments and recirculate until they encountered inductive treatments and metamorphosed; hence, there were much higher percentages of juveniles in inductive treatments in the 20-hour as compared to the 2-hour experiments (Fig. 1).

In slow flow, spheres entered the array only as they fell through the water on their first transit down the straightaway because $u_* < u_{*crit}$. If larvae missed the array on the first transit, however, they could swim up into the flow to be carried around again. The water traveled the same total distance in the 2-hour fast and 6-hour slow flow experiments, and thus, it is surprising that significantly fewer larvae were collected in the slow flow arrays. During slow flow experiments, many larvae were observed near the water surface or swimming in meandering spirals in the water column, quite unlike their behavior in fast flow. Larvae may actively avoid settling in slow flow. Such behavior may have adaptive significance, because adult *P. l. californica* are suspension feeders that require currents to bring both food and grains of sand for tube building. Inasmuch as this species also lives in the intertidal zone, we are currently investigating larval responses to higher flow velocities and oscillatory flows more characteristic of an intertidal habitat.

Table 3. Tukey's multiple comparisons of mean metamorphosed juveniles carried out to test which treatments or rows differed when these effects were significant (Table 1) (*13*). Tests were performed with the average of each treatment or row for each batch (n = 3). See legend to Table 2.

Flow	Metamorphosed juveniles				
	Treatment				
	T	16:1	16:0	F	C
Fast	29.9^a	10.5^b	0.9^c	0.5^c	0.3^c
Slow	7.3^a	9.5^a	0.5^b	0.3^b	0.4^b
	Row				
	1	2	3	4	5
Fast	14.5^a	10.9^b	7.1^b	5.9^b	3.8^b
Slow	3.4^{ab}	3.0^{ab}	5.0^a	4.1^{ab}	2.5^b

In this study, the effects of hydrodynamic processes and chemical cues on larval settlement were tested simultaneously under controlled conditions. Recent models of benthic invertebrate recruitment have disregarded the importance of behavior, treating larvae as passive particles (*23*). Our results suggest, however, that larval responses to both physical and chemical factors may greatly alter recruitment patterns. Although physical processes are largely responsible for the delivery of larvae to potential habitats, larval behavior in response to flow conditions may facilitate substrate contact. Our results suggest the intriguing possibility that larvae respond first to proper flow conditions, causing them to remain suspended or to swim to the bottom and begin substrate explorations, and then to chemical cues that induce metamorphosis.

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11. Brief descriptions of the flume can be found in (*2*); a more detailed description is provided in L. S. Mullineaux and C. A. Butman [*Mar. Biol.*, in press].
12. Larval culture techniques have been described previously (*6–8*). For the 20-hour experiments, replicates were conducted with one batch of larvae, but for the 2- and 6-hour experiments, replicates involved three batches of larvae (Table 1).
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14. Downward swimming speed (range of 0.2 to 0.5 cm/s; motion analysis of video tapes) is about twice the gravitational sinking speed (range 0.07 to 0.16 cm/s) [C. A. Butman *et al.*, *Ophelia* **29**, 43 (1988)]. The plastic spheres (DVB Microspheres; density, 1.05 g/cm^3, Duke Scientific) were 390 ± 20 μm in diameter for the 20-hour experiments and 480 ± 26 μm in diameter for the 2- and 6-hour experiments. The spheres sink at mean speeds of 0.22 ± 0.01 and 0.34 ± 0.04 cm/s (20°C, 30 per mil seawater, Stokes' equation). Spheres of both sizes behaved similarly in the two flows tested.
15. Upstream distance = (water depth/sinking speed) × (surface flow speed). For the 20-hour experiments, spheres were added at two distances, using the end points in the calculated range of sinking speeds, and larvae were added at three distances, using an average gravitational sinking speed (0.10 cm/s) and the lower (0.20 cm/s) and upper (0.50 cm/s) limits to the range of downward swimming speed. For the 2- and 6-hour experiments, spheres were added at one point, based on their mean sinking speed, and larvae were added at one point based on a downward swimming speed of 0.34 cm/s. Addition location appeared to have little effect on the outcome of these and other experiments (*2, 24*).
16. Boundary shear velocity was calculated by the "profile method" described in T. F. Gross and A. R. M. Nowell [*Cont. Shelf Res.* **2**, 109 (1983)]; r^2 = 0.9811 (n = 7) for fast flow and 0.9560 (n = 6) for slow flow.
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19. See examples given in M. W. Denny [*Biology and the Mechanics of Wave-Swept Environments* (Princeton Univ. Press, Princeton, NJ, 1988)].
20. Ottawa cement testing standard sand, 20–30 mesh (Fisher Scientific). Prior to use in experiments, sand was baked clean at 550°C for 6 hours.
21. The potential confounding factor of time was tested in 2- and 6-hour experiments in still water using the same three batches of larvae used in the flow experiments and the same experimental design with time

instead of flow as a whole plot factor. There was no significant ($P > 0.05$) time effect or time by treatment interaction for total animals or metamorphosed larvae.
22. Larvae exposed to 3% formalin or 90% ethanol in seawater spread their provisional chaetae and were readily discernable (after staining with rose bengal) from juveniles that had lost their chaetae during metamorphosis.
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24. See C. A. Butman, J. R. Pawlik, V. R. Starczak (in preparation) for additional ANOVA tables.
25. We thank G. Bachelet, R. J. Chapman, C. H. Clifford, C. M. Fuller, R. F. Petrecca, and P. V. R. Snelgrove for assistance with the experiments. D. Manker and T. Molinski helped collect worm reef. J. P. Grassle and L. S. Mullineaux provided helpful comments on an early draft of the manuscript. Supported by NSF grant OCE88-12651 to C.A.B. and J. P. Grassle, ONR grant N00014-89-J-1112 to C.A.B. and L. S. Mullineaux, and a Killam Memorial Postdoctoral Fellowship to J.R.P. Use of the facilities at Friday Harbor Laboratories, University of Washington, and the Coastal Research Center, Woods Hole Oceanographic Institution, are kindly acknowledged. Contribution 7466 from the Woods Hole Oceanographic Institution.

27 August 1990; accepted 7 December 1990

Participation of the TATA Factor in Transcription of the Yeast U6 Gene by RNA Polymerase C

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Fractionation of transcription extracts has led to the identification of multiple transcription factors specific for each form of nuclear RNA polymerase. Accurate transcription in vitro of the yeast U6 RNA gene by RNA polymerase C requires at least two factors. One of them was physically and functionally indistinguishable from transcription factor IID (TFIID or BTF1), a pivotal component of polymerase B transcription complexes, which binds to the TATA element. Purified yeast TFIID (yIID) or bacterial extracts that contained recombinant yIID were equally competent to direct specific transcription of the U6 gene by RNA polymerase C. The results suggest the formation of a hybrid transcription machinery, which may imply an evolutionary relation between class B and class C transcription factors.

THE MAJOR CLASSES OF EUKARYOTIC genes that are transcribed by RNA polymerase C (pol C), the tRNA and 5*S* RNA genes, have intragenic control regions (*1*). However, some class C genes are controlled by regulatory elements that lie upstream of the structural gene (*2*) and that are similar to those found in class B genes (*3, 4*). These include the 7SK gene and the U6 gene, the latter of which encodes a small nuclear RNA required for splicing of precursor RNA. That transcription of these genes is carried out by pol C is inferred on the basis of a number of convincing, albeit indirect, criteria, including a characteristic α-amanitin sensitivity and the presence of a typical pol C thymidine (T)–rich termination signal (*2*). The upstream elements of the U6 and 7SK genes include a sequence about 30 bp upstream of the start site of transcription that is similar to the pol B TATA box. This TATA sequence is required for efficient transcription by pol C of *Xenopus*, human and plant U6 RNA genes (*5–7*), and the human 7SK gene (*3*).

The U6 gene from *Saccharomyces cerevisiae* is transcribed by pol C both in vitro and in vivo (*8*). The template used in these previous studies [pTaq6 plasmid DNA (*9*)] harbors the whole SNR6 coding region (−120 to +125) with the T-rich termination site and 120 bp of upstream flanking sequence that contains a consensus TATA element at −30. This template contains the minimum sequence information necessary for directing accurate transcription in a reconstituted in vitro system that contains purified pol C and partially purified transcription factor IIIB (TFIIIB) (*8*). The other class C transcription factors, TFIIIA and Tau (TFIIIC), are not required for transcription with this template (*8*). Size fractionation by gel filtration of a TFIIIB preparation in high salt buffer (*10*) resolved two components that, on their own, did not support U6 RNA synthesis by purified pol C (Fig. 1, lanes 1 to 3). However, U6 transcription could be fully restored when both fractions were combined (lanes 4 and 5), as shown by the production of the full-length U6 RNA transcript [114 nucleotides (nt)]. Therefore, at least two distinct factors, eluted in peak 1 (fractions 55 to 59) and peak 2 (fractions 63 to 68), participated in U6 RNA synthesis (Fig. 1B). A nuclease activity that cleaves the 114-nt transcript and generates a 90-nt RNA species was found to partly overlap with peak 2 on the Superose column (Fig. 1B) (*11*). The larger component in peak 1 (~130 kD) (Fig. 1B) had TFIIIB activity, as monitored by transcription of the $tRNA_3^{glu}$ gene in the presence of Tau and purified pol C (90-nt transcript) (Fig. 1A, lanes 6 and 7). The smaller component (~30 kD) (Fig. 1B, peak 2) did not correspond to any known pol C transcription factor.

A TATA box at position −30 is present in all U6 genes in vertebrates and yeast, and has been shown by mutagenesis to be important for transcription of vertebrate genes (*5, 6*). Therefore, we performed a transcription-competition experiment to explore the possibility that the small component was a U6-specific TATA binding factor. Oligonu-

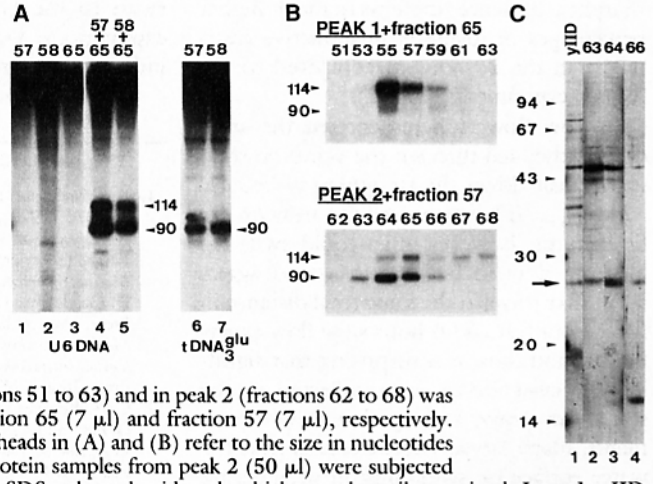

Fig. 1. Two factors required for in vitro transcription of the U6 gene. (**A**) Fractions from the Superose 12 column (*10*) were assayed for transcription activity with the U6 gene (U6 DNA, lanes 1 to 5) or the $tRNA_3^{glu}$ gene (lanes 6 and 7). Transcription mixtures (*10*) received 7-μl aliquots of different column fractions as indicated. Transcripts were analyzed on polyacrylamide-urea gels and revealed by autoradiography. (**B**) Factor activity in peak 1 (fractions 51 to 63) and in peak 2 (fractions 62 to 68) was assayed in the presence of fraction 65 (7 μl) and fraction 57 (7 μl), respectively. The numbers next to the arrowheads in (A) and (B) refer to the size in nucleotides of the RNA transcripts. (**C**) Protein samples from peak 2 (50 μl) were subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel, which was then silver-stained. Lane 1, yIID (G-3000-SW fraction) (*13*); lanes 2 to 4, peak 2 fractions 63, 64, and 66, respectively. The arrow indicates the 27-kD yIID polypeptide. The arrowheads indicate molecular size markers in kilodaltons.

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