water indicating that the organic material in the non-vent water is more resistant, and represents the less-labile, carbon-rich fraction of the vent POM. The ratio of total lipid/POC also confirmed this conclusion. The lipid class compounds, one of the more labile forms of organic matter, make a larger contribution to the vent water organic carbon than to the non-vent water organic carbon.

The change in concentration of particulate organic matter at this site appears to be a very localized event. In this investigation all samples, including the non-vent sample of bottom water, were taken within an area that was actively emitting warm water and sustaining a large population of benthic organisms. The POC and PON levels measured within the warm waters and in the non-vent water at the vent site vary, in general, by at least a factor of 2–5, the higher values being in warm water. Observations from *Alvin* indicate that the vent organism at 21 °N live in or extremely near to a source of warm vent water, a feature common to other vent sites as well. Our analyses reflect this phenomenon and indicate that primary sources for organic carbon are localized in or near the warm vent water site[25].

The relative contribution to the POM by microbial versus animal carbon sources may be partially assessed by using our data on the organic compound source biomarkers. The wax esters, triacylglycerols, and sterols dominate the lipid fraction. The wax ester, triacyglycerol, and fatty alcohol compound classes that were present in the suspended particulate material are typical of marine zooplankton. Indeed, many amphipods and copepods were found in vent waters and collected in our *in situ* pump filters. The sterol distribution on the particulate material matches that of the *Riftia*. The microbial component of the lipid fraction cannot be completely resolved with our data set. Microbially produced lipids, such as hopanols and isoprenoid hydrocarbons, were not found on the filters. Fatty acids, and the more polar phospho- and glycolipids are in the process of being analysed, and these compound classes may contain a large fraction of microbial source markers. Another interesting feature of these analyses is that the typical markers for microbial transformation of sterols such as sterenes, stanones, or stanols, were not present within the limits of our detectability. Thus, we would conclude on the basis of this data set that the zooplankton and larger benthic organisms appear to be making the major contribution to the suspended particulate organic matter in the warm vent waters at 21 °N.

Further verification of both microbial and larger benthic organism contributions and analyses of other source markers will be needed to confirm these initial conclusions from the filtered vent water. Analyses of sediments, bacterial isolates[26], and larger organisms from 21° N are underway to ascertain more specifically the quantitative contributions of sources to the vent water organic matter.

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Experimental manipulation of a contact guidance system in amphibian gastrulation by mechanical tension

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Contact guidance[1,2] has been implied in various morphogenetic movements including neural crest cell migration[3], primordial germ cell migration[4] and guidance of axonal growth cone[5]. In urodele gastrulae, we reported[6] the presence of an aligned network of extracellular fibrils on the inside of the ectodermal layer and suggested that it guides the migration of the presumptive mesodermal cells from the blastopore towards the animal pole. We also reported[7] *in vitro* experiments in which the fibril network of the ectodermal layer was transferred onto the surface of a coverslip. Dissociated mesodermal cells attach to and locomote actively on such conditioned surfaces in an oriented fashion along the blastopore–animal pole axis (bp–ap axis) of the ectodermal layer that conditioned the surface. Recent reports[8,18] suggest that these fibrils contain fibronectin. We now report that the fibril network on the conditioned surface can be artificially aligned in any orientation by exerting mechanical tension on the ectodermal layer during the conditioning. Such prepared surfaces cause cell movements aligned along the tension axis, even when the tension axis is perpendicular to the natural axis of alignment along the bp–ap axis. These results suggest that the extracellular matrix fibrils aligned by the mechanical stress that arises in embryos during development can orient cell migration by the contact guidance, in a similar manner to that reported in the collagen gel and fibroblasts system[9,10].

We used *Ambystoma maculatum* eggs collected in North Carolina and Virginia. Developmental stages were determined according to Harrison stages[11]. Stage 10− represents very early gastrulae in which the mesodermal cells have not yet started their migration away from the blastopore. In more advanced early gastrulae (stage 10), cell migration is under way. Our previous study[7] showed that conditioning by stage 10 ectodermal layer causes substantial alignment of mesodermal cell movement, while the conditioning by a stage 10− ectodermal layer

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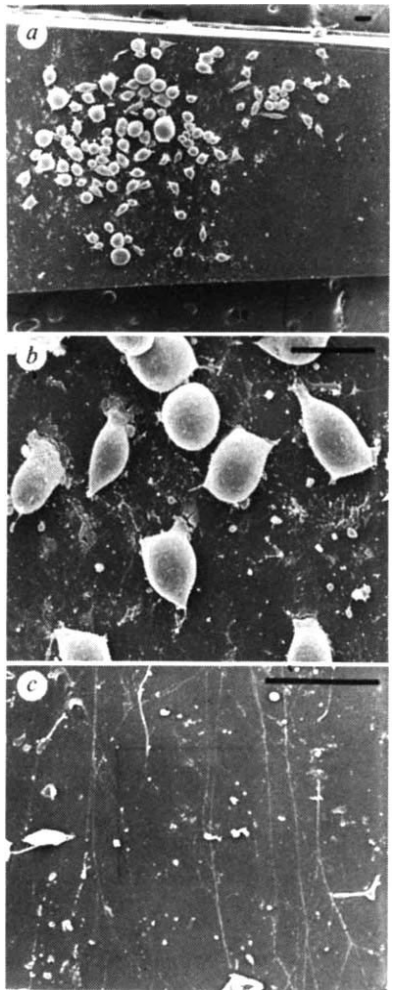

Fig. 1 Scanning electron micrographs of mesodermal cells attached to the conditioned coverslip strip. *a*, A low magnification view. Scale bar, 0.1 mm. *b*, Central part of *a* at higher magnification. Scale bar, 100 μm. *c*, Extracellular fibrils deposited on the coverslip surface. They are apparently aligned along the axis of tension (vertical axis). Scale bar, 10 μm. Strips were cut out from plastic coverslips for tissue culture (Thermanox coverslips, Miles) with a sharp steel blade. The composition of media used in this study is the same as used in the previous conditioning experiments[7]. The basic salt solution is a modified Stearns' solution (MSS)[15,16] buffered with 5 mM HEPES (Sigma). Mesodermal cells were dissociated in 0.02 M sodium citrate[17] in MSS lacking Ca^{2+} and Mg^{2+}. For conditioning of substrata with explants of the ectodermal layer, we used MSS with a *p*H of 8.0, a Ca^{2+} concentration of 110 μM, and containing 0.5% bovine serum albumin (BSA) (Sigma). This Ca^{2+} concentration is just high enough to prevent tissue dissociation and yet low enough to prevent curling of the ectodermal fragments, thus it allows the ectodermal fragments to adhere to the substratum and remain flat. The dissociated mesodermal cells were cultured in MSS *p*H 8.0, a Ca^{2+} concentration of 100 μM, and containing 0.5% BSA, which we have found to be optimal for cell locomotion.

gives only very weak alignment. This difference may be caused by the alignment of the fibril network by the expansion and stretching of the ectodermal layer along the bp–ap axis during gastrulation[12].

Rectangular pieces of the ectodermal cell layer were cut out from the whole blastocoelic roof of early gastrulae (stage 10−, or 10) using fine forceps and hair loops. The pieces were explanted on the coverslip strips (2 mm × 10 mm), which were set on top of two small fragments of glass capillary (outer diameter, 2 mm) like a bridge, so that the explant could be hung with both ends drooping free from the edges of the strip, thus producing constant mechanical tension perpendicular to the longer axis of the strip of coverslip. The inner surface of the ectodermal

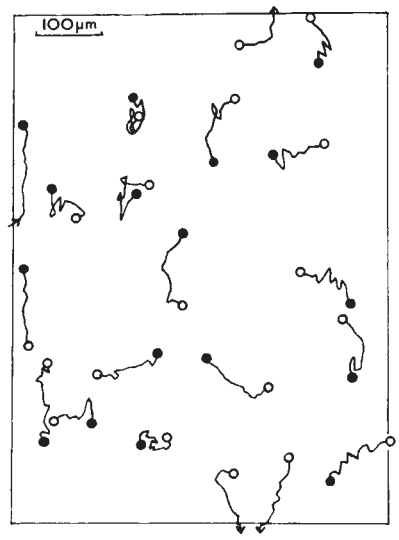

Fig. 2 Cell trails drawn from a time-lapse film of the tilted conditioning. ○, Starting points; ●, finishing points after 1 h. They are apparently aligned along the axis of tension (vertical axis). The overall *R* value calculated for all the cell trails here is +0.47. Time-lapse 16-mm films were taken with ×10 phase contrast lens at 8-s intervals. At the end of filming, the coverslip was fixed and processed for scanning electron microscopy[7]. We drew cell trails by projecting the time-lapse films and marking the centre of cell body at 4-min intervals[15].

layer adhered to the coverslip surface. The strip of coverslip had been set horizontally or tilted by approximately 30° by applying a small amount of Vaseline between the glass capillary and the strip. The tilting allowed the mechanical tension to be maintained throughout the explant during the conditioning. In the horizontal setting, both edges of the coverslip held the ectodermal layer tightly and prevented the tension from transmitting to the central part of the explant. On the other hand, with tilting, there was one free edge so that the tension and stretching generated by the weight of the explant could be transmitted across the entire sheet. Explants from stage 10 embryos were placed on the coverslip with their bp–ap axis parallel to the longer axis of the strip. Explants from stage 10− embryos were oriented randomly, because they have not yet developed their natural alignment along the bp–ap axis[7].

After 5 h of culture at room temperature (22–24 °C), explants were removed from the coverslip by flushing them away with a Pasteur pipette. The coverslip strips were rinsed with culture medium, and placed horizontally in a plastic Petri dish. They were seeded with dissociated mesodermal cells from middle gastrulae (stage 10+) using a Pasteur pipette. The cells attach to the conditioned surface and start to migrate actively within 30 min. The mesodermal cells show no attachment outside the conditioned area. Figure 1*a* shows a low magnification view of a coverslip strip and attached cells. The cells frequently have a leading end with a large lamellipodium and associated filopodia and a slender or rounded trailing end (Fig. 1*b*). The conditioned surface is covered with fragments of cell-surface structures such as filopodia and a network of extracellular fibrils that can be observed only at higher magnifications (Fig. 1*c*). The network was apparently aligned along the axis of tension (Fig. 1*c*). In fact, when scanning electron micrographs of 10 randomly selected areas from the tilted conditioning were analysed by the microcomputer system (see below), they gave positive *R* values; mean and standard deviation were $+0.74 \pm 0.52$.

Cell trails from the tilted conditioning showed an apparent alignment along the axis of tension (Fig. 2). No alignment was noticeable when we used the horizontal conditioning. We further analysed the cell trails by using an image-analysing system with a microcomputer (Apple II Plus Computer and Graphics Tablet)[7]. The computer programs[7] we used enable us to determine

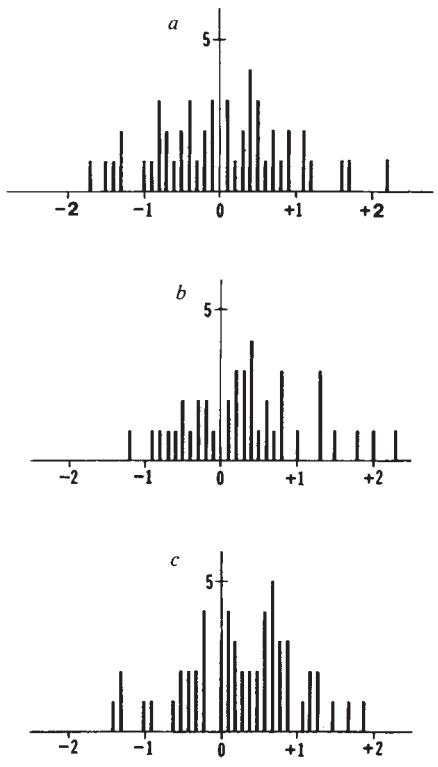

Fig. 3 Histograms showing distribution of cell trails according to their R values. Horizontal axis, R value; vertical axis, number of cell trails. *a*, Data from the horizontal conditioning, showing nearly normal distribution centred at zero (mean −0.01 and s.d. ±0.90, 52 cells). *b*, Tilted conditioning using the stage 10− ectodermal layer. The distribution is clearly shifted to the right (mean +0.36, s.d. ±0.78, 40 cells). The shift of mean is significant ($P < 0.005$). *c*, Tilted conditioning using the stage 10 ectodermal layer. The shift to the right (mean +0.29, s.d. ±0.73, 55 cells) is significant ($P < 0.005$).

whether the cell trails are aligned along the axis of the mechanical tension during the conditioning. Also, they were used to analyse alignment of the fibril network deposited on the conditioned surface. The programs calculate displacement (Δy and Δx) along the y-axis (axis of tension) and along the perpendicular x-axis in each short segment of the cell trail, and give sums of $|\Delta y|$ and $|\Delta x|$ separately for the whole cell trail. Then, it gives R values, $R = \log_2(\Sigma|\Delta y|/\Sigma|\Delta x|)$, as a parameter of the alignment; a zero value for non-aligned random movement, positive values of R for movement aligned along the axis of tension, and negative values for movement aligned perpendicular to the axis of tension. Figure 3 shows histograms of the distribution of cell trails according to their R values. When horizontal conditioning was used (Fig. 3*a*) we observed nearly normal distribution centred at zero, showing that the cell trails are not aligned. On the other hand, when we used tilted conditioning (Fig. 3*b*, *c*) we observed a distribution that was shifted to the right (positive values). The shift of the mean (+0.36 and +0.29) is larger than that of the natural alignment along the bp–ap axis in the stage 10 ectoderm conditioning (+0.22)[7]. Substratum conditioning using stage 10− ectodermal layers (Fig. 3*b*) gives stronger alignment than that using stage 10 ectoderm (Fig. 3*c*). The most likely explanation for this is that the stage 10 explant was oriented on the coverslip strip in such a way that its natural alignment along the bp–ap axis must be cancelled before any appearance of the alignment along the axis of tension.

Contact inhibition of cell movement is another mechanism that can direct cell migration from the blastopore with a high population of migratory cells towards the animal pole where the fibril network on the inner surface of the ectodermal layer offers an adequate substratum for cell attachment and locomotion. This possibility has been suggested[6,7,13,14], and the gastrula mesodermal cells indeed show contact paralysis against each other when cultured *in vitro*[13,15].

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Forskolin activation of adenylate cyclase *in vivo* stimulates nerve regeneration

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The previous demonstration of an increase and redistribution of adenylate cyclase activity in injured peripheral nerve[1] suggests that an increase in neuronal cyclic AMP concentration could play a role in peripheral nerve regeneration. We report our finding that accumulating adenylate cyclase activity was translated into a twofold increase in cyclic AMP concentration in the regenerating nerve stump, coincident with the initiation and elongation of regenerative nerve sprouts. We sought to magnify the role of cyclic AMP in regeneration by using forskolin, a robust activator of adenylate cyclase[2], to produce an additional increase in neuronal cyclic AMP *in situ*. Forskolin *in vitro* produced an approximately 40-fold greater elevation in neuronal cyclic AMP than an equimolar (10^{-5}) concentration of isoprenaline. Moreover, the elevated cyclic AMP concentration persisted for at least 60 min in the continued presence of forskolin. Daily injection of forskolin into the dorsal lymph sac of *Rana pipiens*, or delivery of forskolin through an implanted osmotic pump produced a sustained 40% increase in the rate of sensory nerve regeneration in freeze-lesioned sciatic nerves. We conclude that an increase in cyclic AMP concentration and, presumably, the activation of appropriate protein kinases stimulates regenerative nerve growth following trauma.

Adenylate cyclase, the enzyme responsible for catalysing the formation of cyclic AMP from ATP, undergoes anterograde, but not retrograde axonal transport in normal frog sciatic nerve. If the nerve is subjected to crush or transection, however, adenylate cyclase activity accumulates proximal to the site of injury and is incorporated in the retrograde transport system for return toward the cell body[1]. While these observations provide the basis for a stimulated production of cyclic AMP in regenerating nerve, increased production does not necessarily lead to increased concentration. A parallel elevation of phosphodiesterase activity, an enzyme which also undergoes axonal transport[3], could prevent accumulation of the cyclic nucleotide. We measured cyclic AMP concentration in specific regions of axotomized frog sciatic nerve at 2, 4 and 7 days after a freeze-

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