linked formate dehydrogenase activity[18] and NAD- and glutathione-linked formaldehyde dehydrogenase activities[18] were detected in some samples but were variable.

Two major carbon assimilatory pathways exist in methane-oxidizers: the ribulose monophosphate pathway, found in type I strains; and the serine cycle, found in type II strains. The key enzyme for the first pathway, hexulose phosphate synthase, was detected at high levels in gill tissue (132–314 nmol per min per mg protein), in five samples from three mussels, using a coupled assay[19], but was not detected in mantle or foot tissue. Enzymes of the serine cycle, hydroxypyruvate reductase[20], and serine-glyoxylate aminotransferase[21] were either not detectable or were present at variable concentrations, but were never found together. The key enzyme of autotrophic assimilation, ribulose bisphosphate carboxylase, was not detectable in any sample, using two different assay methods[22,23] under a variety of assay conditions and methods of lysis.

The presence of enzyme activities unique to assimilatory and dissimilatory pathways of methylotrophic metabolism and the absence of detectable ribulose bisphosphate carboxylase strongly suggest that methylotrophic metabolism occurs in the gills of the seep mussels. The co-occurrence of type I methylotrophic enzyme activities, type I intracytoplasmic membrane-containing symbionts and low $\delta^{13}C$ values in mussel tissue also suggest that methane-oxidizers exist in symbiosis with these mussels, and probably provide a major source of nutrition. The identity of the other bacteria-like structures seen in the electron micrographs remains to be determined.

Although the distribution and occurrence of methylotrophic symbioses is not known, similar types of membranes have been observed in bacterial symbionts of one pogonophore[24], and low $\delta^{13}C$ values have been noted for these and other marine invertebrates[3,5,25]. Although further characterization is necessary to determine the nature of these associations, the benefits afforded both partners suggest that symbioses between invertebrates and methylotrophs may be widespread in nature.

Supported in part by the North Atlantic Treaty Organization under a grant awarded in 1985 to C.M.C., by ONR contract no. N00014-85-K-0061 and NOAA Sea Grant no. NA79AA-D-00091 with Harvard University to R.M. and by ONR grant no. N00014-85-K-0444 to M.E.L. We thank J. Hook, Captain Baker and the crew of the Atlantis II, Ralph Hollis and the Alvin Group for their assistance in obtaining specimens; R. D. Turner and B. Hecker for helpful discussion; and S. Houghton for assistance with TEM. We thank J. de Bont for MMO assays and Hans van Dijken for his enthusiasm, helpful advice and direction.

Note added in proof: Recently another species of symbiont-containing mussel from the Louisiana slope of the Gulf of Mexico has been shown to consume methane[26].

Received 10 July; accepted 4 November 1986.

1. Paull, C. K. *et al. Science* **226**, 965–967 (1984).
2. Cavanaugh, C. M. *Biol. Soc. Wash. Bull.* **6**, 373–388 (1985).
3. Paull, C. K., Jull, A. J. T., Toolin, L. J. & Linick, T. *Nature* **317**, 709–711 (1985).
4. Rau, G. H. & Hedges, J. I. *Science* **203**, 648–649 (1979).
5. Spiro, B., Greenwood, P. B., Southward, A. J. & Dando, P. R. *Mar. Ecol. Prog. Ser.* **28**, 233–240 (1986).
6. Claypool, G. E. & Kaplan, I. R. in *Natural Gases in Marine Sediments* (ed. Kaplan, I. R.) 99–139 (Plenum, New York, 1974).
7. Cavanaugh, C. M. *Nature* **302**, 58–61 (1983).
8. Coleman, A. W. *Limnol. Oceanogr.* **25**, 948–951 (1980).
9. Giere, O. *Zoomorphology* **105**, 296–301 (1985).
10. Dando, P. R., Southward, A. J., Southward, E. C., Terwilliger, N. B. & Terwilliger, R. C. *Mar. Ecol. Prog. Ser.* **23**, 85–98 (1985).
11. Fiala-Medioni, A. & Metivier, C. *Mar. Biol.* **90**, 215–222 (1986).
12. Remsen, C. C. *Int. Rev. Cytol.* **76**, 195–223 (1982).
13. Anthony, C. *The Biochemistry of Methylotrophs* (Academic, London, 1982).
14. Lowry, O. H., Rosebrough, N. J, Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
15. Lidstrom, M. E. & Somers, L. *Appl. environ. Microbiol.* **47**, 1255–1260 (1984).
16. Habets-Crutzen, A. Q. H., Brink, L. E. S., van Ginkel, C. G., de Bont, J. A. M. & Tramper, J. *Appl. Microbiol. Biotechnol.* **20**, 245–250 (1984).
17. Prior, S. D. & Dalton, H. *J. gen. Microbiol.* **131**, 155–163 (1985).
18. Weaver, C. A. & Lidstrom, M. E. *J. gen. Microbiol.* **131**, 2183–2197 (1985).
19. Levering, P. R., van Dijken, J. P., Veenhuis, M. & Harder, W. *Arch. Microbiol.* **129**, 72–80 (1981).
20. Large, P. J. & Quayle, J. R. *Biochem. J.* **87**, 386–395 (1963).
21. Blackmore, M. A. & Quayle, J. R. *Biochem. J.* **118**, 53–59 (1970).
22. Beudeker, R. F., Cannon, G. C., Kuenen, J. G. & Shively, J. M. *Arch. Microbiol.* **124**, 185–189 (1977).
23. Tabita, F. R., Caruso, P. & Whitman, W. *Analyt. Biochem.* **84**, 462–472 (1978).
24. Southward, A. J. *et al. Nature* **293**, 616–620 (1981).
25. Kulm, L. D. *et al. Science* **231**, 561–566 (1986).
26. Childress, J. J. *et al. Science* **233**, 1306–1308 (1986).

Peptides containing the cell-attachment recognition signal Arg-Gly-Asp prevent gastrulation in *Drosophila* embryos

Corinne Naidet*, Michel Sémériva*, Kenneth M. Yamada† & Jean Paul Thiery‡

* Laboratoire de Génétique et de Biologie Cellulaires, Faculté de Luminy, 70, route Léon Lachamp Case 907 13288 Marseille Cedex 9, France
† Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892, USA
‡ Institut d'Embryologie, 49 bis, Avenue de la Belle Gabrielle, 94130 Nogent-sur-Marne, France

It has recently been suggested that the Arg-Gly-Asp sequence (RGD) forms part of a widespread cell–extracellular matrix recognition system (see for example ref. 1). Analysis of the cell binding sites of vertebrate fibronectin[2,3] and other extracellular proteins that interact with cell surfaces implicate the same amino acid triplet[1]. Peptides containing this sequence inhibit certain developmental events such as cell–matrix adhesion or cellular migration *in vitro*[2–7] and *in vivo*[8]. The RGD-sequence is also part of the cellular recognition site of the aggregation protein discoidin I in *Dictyostelium*[9] suggesting that the RGD-recognition system could be universally used. In *Drosophila*, despite its advanced genetics, very little is known about the extracellular components that are involved in cell movements and morphogenesis. We report here that peptides containing the RGD-sequence prevent gastrulation of *Drosophila* embryos. The phenotypic effect is similar to that observed in the dorsal-group mutants: no ventral furrow is formed and the embryos lack dorsal–ventral polarity. The specificity of the inhibiting action suggests that the RGD-sequence may also be used by invertebrates to mediate cell-attachment phenomena.

Early *Drosophila* embryogenesis is initiated by 13 rapid and synchronous nuclear cleavages which occur within a syncytium. At the end of the 13th cycle, cellular membranes begin to form at the periphery of the embryo; their completion defines the cellular blastoderm[10]. Soon after, gastrulation starts with the invagination of mid-ventral blastoderm cells. An integrated series of complex movements follows, consisting of 4 other invaginations which produce the major rudiments of the endoderm[11]. Cellular adhesion is presumably of crucial importance in these different morphogenetic movements characterizing gastrulation.

Injections of synthetic RGD-peptides were therefore carried out at the syncytial blastoderm stage. RGD-containing peptides injected in the ventral periplasm of the embryos prevent gastrulation (Table 1). Our criterion for gastrulation was the appearance of the ventral furrow, which is readily observed by light microscopy. As embryos without a ventral furrow never hatch, we designate these defective embryos as non-viable or non-gastrulating. Control peptides lacking the RGD-sequence have no effect on the viability of embryos, even at concentrations 50 times higher than those effective with RGD-peptides, and are not more harmful than the injection medium alone. Also, Arg-Gly-Asp-Ser (RGDS) is as efficient as longer peptides suggesting that, as in cultured vertebrate cells[2,3], the active sequence contains the RGD-sequence. The specificity of this sequence is best demonstrated by the peptide Gly-Arg-Gly-Glu-Ser, where the

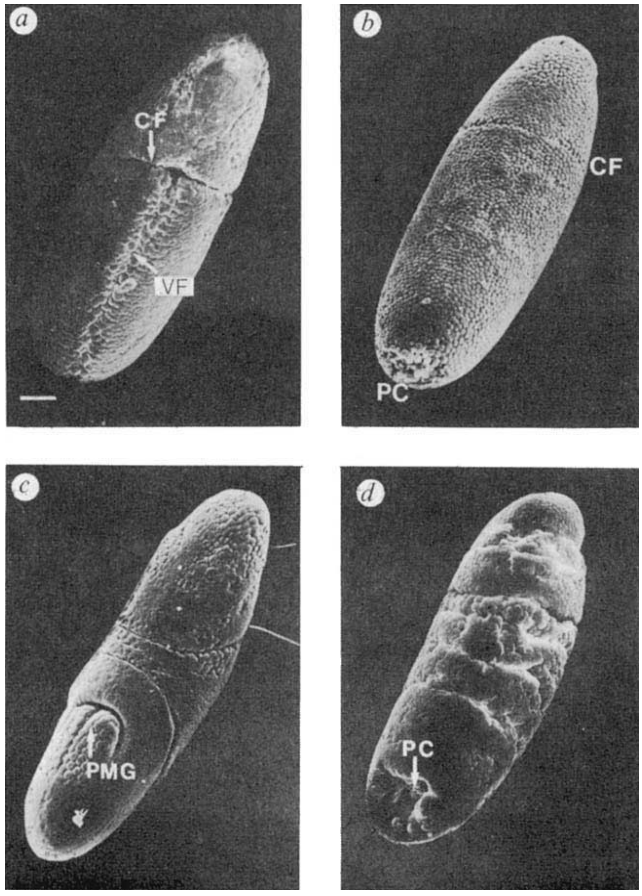

Fig. 1 Scanning electron microscopy of normal and arrested *Drosophila* embryos. *a*, Ventral view of a 3.5-h embryo injected with a control peptide showing the ventral furrow (VF) and the cephalic furrow (CF) which are both already visible at 3 h. *b*, Ventral view of an embryo injected with RGD-containing peptide. No ventral furrow is formed in an embryo of the same age as in *a*, but a fold (CF) which might be the cephalic furrow begins to appear at that time. *c*, Dorsal view of a 4.25-h embryo injected with a control peptide. The post-midgut rudiment (PMG) is moved anteriorly by the elongating germ band. *d*, Dorsal view of a 4.25-h embryo injected with RGD-containing peptide. This embryo is symmetrical in the dorsal-ventral axis. Its orientation can only be ascertained by the curvature which normally occurs on the ventral side of the embryo. None of the multiple folds visible at the surface of the embryo seem to correspond to normal invaginations seen in *c*. The distribution of the invaginations does not follow a well-established pattern. Polar cells (PC) are internalized at the posterior tip of the embryo instead of being carried along the dorsal surface by the migration of the posterior midgut rudiment. This abnormal location of the polar cells seems to be a constant feature of RGD-embryos. For scanning electron microscopy, the embryos were prepared according to Turner and Mahowald[23], after careful removal by toluene of the mineral oil used for microinjection. Following critical point drying, the embryos were coated with gold and examined with a JEOL JSM 35 scanning electron microscope at 15 kV; scale bar, 20 μm.

replacement of Asp by Glu is sufficient to abolish the inhibiting effect on gastrulation.

The peptides affecting gastrulation do not appear to be cytotoxic, as the incorporation of ^{3}H-thymidine or ^{3}H-uridine in treated embryos strictly follows the same kinetics as for untreated embryos, at least during the first six hours of development (data not shown).

The inhibiting concentrations of peptides correspond to doses even lower than those that block gastrulation in *Pleurodeles*[8]. If injected stock solutions are assumed to be uniformly diluted in the embryo, the concentration for optimal inhibition by Gly-Arg-Gly-Asp-Ser is 10 μM. As peptides probably do not diffuse freely inside the embryo, the effective concentration would probably be similar to those for cultured vertebrate cells (~50 μM for half-maximal inhibition of the spreading of BHK cells[3] and ~250 μM for NRK cells[2]).

The representative phenotype of embryos injected with RGD-containing peptides (RGD-embryos) is shown in Fig. 1. An apparently normal cellular blastoderm is formed at the proper time (2.75 h) in RGD-embryos. Embryos injected with a control peptide (C-embryos) start forming ventral and cephalic furrows within 5–10 min after the cellular blastoderm. As shown in Fig. 1*a*, these invaginations are completed 3.5 h after fertilization, and control embryos have initiated germ band extension on the dorsal side. For RGD-embryos, only one invagination becomes visible at that time in the anterior part (Fig. 1*b*) and may correspond to the cephalic furrow, even though it seems somewhat different from that normally observed. During this period, the embryos resemble a normal bastoderm that persists for an abnormally long time (45 min ± 15 min). About one hour later, instead of the characteristic invaginations of a control embryo (Fig. 1*c*), multiple disorganized folds can be observed in the ectoderm all around the circumference of the embryo (dorsal view in Fig. 1*d*). The polar cells do not migrate dorsally following the posterior midgut rudiment but instead are internalized directly at the posterior tip of the embryo (Fig. 1*d*).

Phenotypically, RGD-embryos behave as if they were symmetrical along the dorsal–ventral axis. Later in development, no muscular movements are observed and no internal organs are formed. These late effects are most probably a consequence of the lack of invagination of the mid-ventral blastoderm cells, which prevents the formation of mesoderm. This interpretation is supported by examination of semi-thin sections of 3.5-h RGD-embryos (data not shown). We have not exhaustively searched for intermediate phenotypes. However, when lower doses of the peptide Gly-Arg-Gly-Asp-Ser were injected in the ventral periplasm, only the proportion of viable embryos was increased. Defective embryos still had no ventral furrow, rather than an incomplete one.

The identity and the nature of the molecule(s) bearing the RGD-sequence in *Drosophila* remain to be established. By analogy with other well known systems, it is tempting to postulate a fibronectin-like role for the RGD-bearing molecule(s) in cell adhesion during the gastrulation step. Some basement membrane components have been identified in *Drosophila* embryos[12], but none of them is present at gastrulation and no fibronectin-like protein(s) has been identified. Our experiments to characterize such proteins have failed. This suggests that even though fibronectin has been well conserved during evolution, insect proteins may have preserved only the RGD-cell recognition signal sequence. Experiments are now in progress to isolate the RGD-bearing molecule(s).

The forces involved in the movements of gastrulation are not yet known, but several hypotheses have been proposed invoking some contractile system, change of cell shape[11] and direct involvement of cytoplasmic yolk bridges[13]. The inhibiting effect of RGD-peptides described here suggests that cell adhesion mediated by interaction with an extracellular matrix could in addition be implicated, at least as far as ventral furrow formation is concerned.

Finally, the phenotype of RGD-embryos was found to resemble closely that of the dorsal-group mutants. Ten loci have been identified for maternal effect mutations which fail to establish normal dorsal–ventral polarity[14,15]. As for at least two other zygotic mutants, they do not form the ventral furrow[16] and have consequently also been termed gastrulation defective[15]. Figure 2 shows scanning electron micrographs of one dorsal-group mutant, *snake*, at stages comparable to those depicted in Fig. 1. Strong similarities can be observed: no ventral furrow is formed; the appearance of the cephalic furrow is delayed compared with the wild-type embryo; multiple folds in the ectoderm

Table 1 Microinjection of synthetic peptides into early *Drosophila* embryos

	Initial peptide concentration (mg ml^{-1})	Injected embryos	Gastrulating embryos	Gastrulating embryos (%)
Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro (P1)	1	378	53	14
Cys-Glu-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr (P3)	10	372	328	90.6
Gly-Arg-Gly-Asp-Ser	0.2	72	9	12.5
Gly-Arg-Gly-Glu-Ser	0.2	85	78	91.70
Arg-Gly-Asp-Ser	1	51	4	7.8
Tyr-Ala-Gly-Gly	10	39	34	87.2
Injection medium (0.2 nl)	—	210	191	91

Staged embryos were obtained from Oregon R flies at 25 °C, manually dechorionated, placed on a microscope slide coated with Scotch-tape glue and desiccated for 5 min in a $CaCl_2$ atmosphere. They were covered with mineral oil at 18 °C and observed under an inverted light microscope. Embryos were microinjected with the aid of a De Fonbrune micromanipulator with a 1 μm glass needle. An average volume of 0.2 nl, corresponding to 2.5% of the total volume of the egg, was delivered to each embryo. Only one concentration of each peptide was used, except for the peptide Gly-Arg-Gly-Asp-Ser which has been also injected at lower concentrations (see text). P1 is a specific and highly conserved amino-acid sequence of vertebrate fibronectin which contains the cell-attachment recognition site[3]. P3 is another highly conserved sequence in the collagen-binding domain of fibronectin and is not related to the attachment of fibronectin to the cells. The injection medium was 17 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$ and 2.5 mM CaCl2 at *p*H 7.2. Injections were carried out in the ventral periplasm of embryos at stage 13 according to Zalokar and Erk[10] during the 12th cleavage cycle which lasts 13 min (ref. 22). Ventral furrow formation was monitored by light microscopy for 2 h after the onset of the cellular blastoderm. Underlined, RGD sequences.

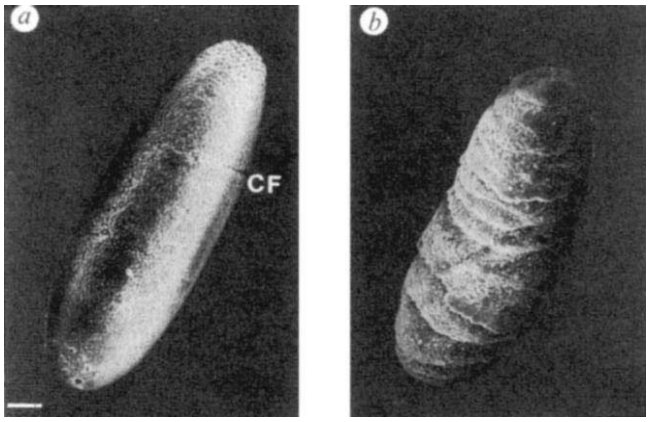

Fig. 2 Scanning electron microscopy of *snake* mutant. Experimental conditions as in Fig. 1. *Snake* embryos were collected from homozygous females carrying the strong allele *snk*^{073} (gift of Dr Nüsslein-Volhard). *a*, Ventral view of a 3.5-h *snake* embryo showing no ventral furrow. Cephalic furrow (CF) begins to appear at that time. *b*, Dorsal view of a 4.5-h *snake* embryo. As for the embryo shown in Fig. 1*d*, the folds extend around the whole circumference of the embryo. The orientation of the embryo in this photograph does not show the internalization of the polar cells, which occurs in a similar way as for RGD-embryos.

are produced and the polar cells stay at the posterior tip of the embryo. The analogy can be pursued further with cuticle preparations, which show the same general features as for the most severe alleles of the dorsal-group. RGD-embryos can secrete cuticle but only structures corresponding to dorsal cuticle can be distinguished (data not shown). As cuticle deposition is a late event that arises well after gastrulation, this suggests that at least some cells in the embryo have undergone an adequate differentiation programme. The absence of ventral cutile in the dorsal-type mutant has been shown to result from an alteration of the final differentiation fate of blastoderm cells[17]. Assuming that the same is true for RGD-treated embryos, our data suggest that the developmental programme of the ventral blastoderm cells can also be modified by affecting the cell-matrix interactions responsible for normal gastrulation.

Genetic analysis supports a picture of *Drosophila* gastrulation as a cascade of events: affecting any single step leads to the same ultimate morphological defects. Is one of the dorsal gene products directly involved in cell-matrix interaction? Direct involvement of a purely maternal gene product seems very unlikely. RGD-peptides are effective not only at gastrulation but also at other times in *Drosophila* embryonic development (C.N. and M.S., in preparation), suggesting instead the participation of a zygotic gene product. Molecules involved in cell-matrix recognition are often ubiquitous components that intervene in various morphogenetic processes[18] and at different times in development[19]. Thus, if specific gastrulation gene products are related to a cell-matrix recognition system, they are more likely to be regulators rather than actual components of this recognition system. Rapid progress in the molecular genetics of the dorsal genes[20,21], together with an identification of the RGD-bearing molecules, will probably help to understand better the control of the morphogenetic processes of gastrulation.

We thank Dr Ruth Griffin-Shea and Dr Danielle Gratecos for their help in the preparation of this manuscript. We are grateful to Dr Nüsslein-Volhard who provided us with the various dorsal mutants.

Received 30 June; accepted 20 November 1986.

1. Ruoslahti, E. & Pierschbacher, M. D. *Cell* **44**, 517-518 (1986).
2. Pierschbacher, M. D. & Ruoslahti, E. *Nature* **309**, 30-33 (1984).
3. Yamada, K. M. & Kennedy, D. W. *J. Cell Biol.* **99**, 29-36 (1984).
4. Suzuki, S., Oldberg, A., Hayman, E. G., Pierschbacher, M. D. & Ruoslahti, E. *EMBO J.* **4**, 2519-2524 (1985).
5. Gartner, T. K. & Bennett, J. S. *J. biol. Chem.* **260**, 11891-11894 (1985).
6. Plow, E. F., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G. A. & Ginsberg, M. H. *Proc. natn. Acad. Sci. U.S.A.* **82**, 8057-8061 (1985).
7. Haverstick, D. M., Cowan, J. F., Yamada, K. M. & Santoro, S. A. *Blood* **66**, 946-952 (1985).
8. Boucaut, J. C. *J. cell. Biol.* **99**, 1822-1830 (1984).
9. Springer, W. R., Cooper, D. N. W. & Barondes, S. H. *Cell* **39**, 557-564 (1984).
10. Zalokar, M. & Erk, I. *J. Microscopie biol. cell.* **25**, 97-106 (1976).
11. Turner, F. R. & Mahowald, A. P. *Devl Biol.* **57**, 403-416 (1977).
12. Fessler, J. H. in *The Role of Extracellular Matrix in Development*, 89-121 (Liss, New York, 1984).
13. Rickoll, W. L. & Counce, S. J. *Wilhelm Roux Arch. dev. Biol.* **188**, 163-177 (1980).
14. Anderson, K. V. & Nüsslein-Volhard, C. *Nature* **311**, 223-227 (1984).
15. Konrad, K. D. & Mahowald, A. P. in *Molecular Aspects in Development* (eds Malacinsky, G. M. & Klein, W. H.) 167-188 (Plenum, New York), 1984).
16. Anderson, K. V. & Nüsselein-Volhard, C. in *Pattern Formation: A Primer in Developmental Biology* (eds Malacinsky, G. & Bryant, S.) 10-20 (Macmillan, New York, 1983).
17. Nüsslein-Volhard, C. in *Determinants of Spatial Organization* (eds Subtelney, S. & Konigsberg, I. R.) 185-210 (Academic, New York, 1979).
18. Yamada, K. M. *A. Rev. Biochem.* **52**, 761-799 (1983).
19. Thiery, J. P. *A. Rev. Cell Biol.* **1**, 91-113 (1985).
20. Steward, R., McNally, F. & Schedl, P. *Nature* **311**, 262-265 (1984).
21. De Lotto, R. & Spierer, P. *Nature* **323**, 688-692 (1986).
22. Foe, V. E. & Alberts, B. M. *J. Cell Sci.* **61**, 31-70 (1983).
23. Turner, F. R. & Mahowald, A. P. *Devl. Biol.* **50**, 95-108 (1976).