and biochemical methods (*8*), the viruses obtained from individual backcross lines involving either inducible strain could be compared.

A total of 55 cell lines was established from individual $N \times (N \times C58)F_1$ backcross embryos. Cultures of each were treated with IdU (20 μg/ml) for 24 hours, and supernatants were assayed for C-type virus by both the reverse transcriptase assay and the XC plaque assay at weekly intervals for up to 1 month after treatment. Induction of virus was detected with 51 out of 55 lines. Each of the four $N \times (N \times C58)F_1$ backcross lines, which had remained virus-negative, were subsequently treated again in three separate experiments. However, in each case, they remained noninducible. In Table 1, the percentage of virus-inducible backcross $N \times (N \times C58)F_1$ lines (92.7 percent) is compared with the percentages expected for two, three, or four loci. The results fit most closely with the percentage expected for three or four independently segregating dominant alleles for virus inducibility in C58 cells.

Proof that virus inducibility loci represent viral structural rather than regulatory information would first require the demonstration that all virus isolates at one locus were identical and yet distinct from those activated at some other locus. Thus, the biologic properties of viruses activated from cell lines of 8 individual $N \times (N \times B)F_1$ backcrosses, containing the BALB/c inducibility locus (*8*), were compared with viruses activated at loci for induction in 25 individual $N \times (N \times C58)F_1$ backcross lines. These viruses were also compared with isolates obtained from parental as well as $(N \times B)F_1$ and $(N \times C58)F_1$ hybrid lines. The amount of murine leukemia virus gs antigen was determined for each virus preparation. Since there is a uniform amount of gs protein per virion, the total gs protein in each virus stock provided a measure of the number of physical particles. Each virus was also assayed for its reverse transcriptase activity. These biochemical and immunologic measurements provided a means of standardizing the different viruses prior to tests of their biologic activity.

As can be seen from the results summarized in Table 2, the biologic properties of each $N \times (N \times B)F_1$–activated virus were indistinguishable from those of virus induced from cells of the parental B strain. Each formed very small XC plaques; as determined by both the XC plaque assay and by measurement of virion-associated reverse transcriptase in tissue culture fluids of infected cell cultures, each was around 100- to 200-fold less infectious than a standard wild-type strain. In contrast, each of 25 isolates from activatable $N \times (N \times C58)F_1$ embryo lines formed large XC plaques and, like the virus activated from C58 parental cells, were almost as infectious per nanogram of viral gs protein as the standard wild-type strain. Thus, the biologic properties of each of several viruses activated from backcross embryo cells containing an induction locus of the B strain, while indistinguishable from each other, were quite different from those of viruses obtained from backcross lines containing virus induction loci of the C58 strain.

The foregoing results indicate that the inducibility loci detected may represent C-type viral structural information and that regulatory factors necessary for their activation are either present in all cell strains or closely linked to the viral structural loci. Alternative possibilities are that each class of endogenous virus is present in an integrated state within cells of all mouse strains or that the endogenous viruses of a particular strain exist in multiple extrachromosomal copies. The loci observed could then be regulatory genes that allow virus activation. The possibility of extrachromosomal virus location appears to be excluded by recent biochemical evidence indicating that mouse embryo cells contain murine leukemia virus-specific DNA which is covalently linked to host cell sequences in the high molecular weight fraction of cellular DNA (*13*).

The multiple (more than two) loci for murine leukemia virus detected in cells of the C58 strain are more than have been observed in genetic studies with other mouse strains (*5*, *6*). The very infectious nature of the endogenous viruses of the C58 cell undoubtedly contributes to the ease of their detection. It is possible, therefore, that in other mouse strains, there may be additional alleles representing viruses with altered or defective properties, which cannot be detected by available methods. Whether the large number of biologically highly active endogenous viruses in the C58 strain can be causally linked to the very high leukemia incidence observed in that mouse strain remains to be determined.

JOHN R. STEPHENSON
STUART A. AARONSON
Viral Carcinogenesis Branch,
National Cancer Institute,
Bethesda, Maryland 20014

References

1. L. Gross, *Oncogenic Viruses* (Pergamon, New York, ed. 2, 1970).
2. ———, *Acta Haematol.* **10**, 18 (1953); *Cancer Res.* **18**, 371 (1958); H. S. Kaplan and M. B. Brown, *J. Nat. Cancer Inst.* **13**, 185 (1952).
3. S. A. Aaronson, J. W. Hartley, G. J. Todaro, *Proc. Nat. Acad. Sci. U.S.A.* **64**, 87 (1969).
4. D. R. Lowy, W. P. Rowe, N. Teich, J. W. Hartley, *Science* **174**, 155 (1971); S. A. Aaronson, G. J. Todaro, E. M. Scolnick, *ibid.*, p. 157.
5. B. A. Taylor, H. Meier, D. D. Myers, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 3190 (1970); W. P. Rowe, *J. Exp. Med.* **136**, 1272 (1972).
6. J. R. Stephenson and S. A. Aaronson, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2798 (1972).
7. W. P. Rowe, J. W. Hartley, T. Bremmer, *Science* **178**, 860 (1972).
8. J. R. Stephenson and S. A. Aaronson, *J. Exp. Med.* **136**, 175 (1972).
9. W. P. Rowe, W. E. Pugh, J. R. Hartley, *Virology* **42**, 1136 (1970).
10. J. L. Jainchill, S. A. Aaronson, G. J. Todaro, *J. Virol.* **4**, 459 (1969).
11. J. R. Stephenson, R. K. Reynolds, S. A. Aaronson, *Virology* **148**, 749 (1972).
12. E. M. Scolnick, W. P. Parks, D. M. Livingston, *J. Immunol.* **109**, 570 (1970).
13. L. Gelb, J. Milstein, M. Martin, S. Aaronson, in preparation.

12 December 1972; revised 9 February 1973 ■

Fordilla troyensis Barrande: The Oldest Known Pelecypod

Abstract. *Specimens of the small bivalved animal* Fordilla troyensis *Barrande from New York State show that this fossil is the oldest known pelecypod mollusk and not a conchostracan arthropod. This finding extends the range of the class Pelecypoda backward in time from the Early Ordovician (about 495 million years ago) to the Early Cambrian (about 540 to 570 million years ago). The morphology of* Fordilla troyensis *suggests that it lived infaunally and that it was ancestral to the pelecypod subclasses Heteroconchia and Isofilibranchia.*

Fordilla troyensis Barrande (*1*) is a small bivalved invertebrate best known from Lower Cambrian rocks of New York State; it also occurs in rocks of the same age in Newfoundland, Greenland, and perhaps England, Denmark, and Portugal. Although in recent years there has been general agreement that this species is a crustacean and not a pelecypod, there was a long debate as to its zoological placement. Opinion was divided as to whether *Fordilla* is a pelecypod (*1–4*), possibly a pelecypod (*5*, *6*), or a bivalved conchostracan crustacean (*7*).

The Early Cambrian age of *Fordilla*

troyensis is widely agreed upon. Lochman (*5*, p. 1351) has documented its range as late Early Cambrian in New York State, and Palmer and Taylor (*8*) have concurred. The material studied by Poulsen (*3*) is from the Bastion Formation of Greenland, which is placed in the *Olenellus* Zone (Lower Cambrian) by Cowie (*9*, p. 34). Poulsen (*4*, p. 2) regarded *Fordilla troyensis* as a guide fossil to rocks of Early Cambrian age.

We have examined more than 300 specimens of *Fordilla troyensis* from the major museums of North America, as well as the five New York type specimens studied by Barrande, which are at the National Museum, Prague, Czechoslovakia. Shell morphology, especially previously undocumented muscle insertion areas, show that this species is a pelecypod mollusk. *Fordilla* is bivalved; both right and left valves occur in the same collection, although to date no articulated specimens have been found (Fig. 1). Growth is by additive deposition of shell material from an enveloping mantle with growth lines recurved toward the beak of each valve (Fig. 1C). Most important for proving that it is a pelecypod are internal molds that have typical pelecypod muscle markings consisting of anterior and posterior adductor muscle insertions connected by a pallial line (Fig. 1, A and B; Fig. 2). Above the adductor insertions are small pedal retractor muscle insertions (Fig. 1, A and B; Fig. 2), and there are one or two pairs of small umbonal muscle insertions which may represent pedal or visceral muscles (Fig. 2).

The musculature of *Fordilla* is unusual in that the posterior part of the pallial line is expanded and consists of several bundles of muscle insertions which give it a moniliform appearance (Figs. 1A and 2). The function of this enlarged part of the pallial line is uncertain; it may represent the insertions of expanded radial mantle muscles which acted as siphonal retractors. Bundled siphonal retractors which do not form a pallial sinus occur in such pelecypods as *Panomya ampla* Dall (*10*). This part of the pallial line may have functioned as a ventral adductor similar to that of some pholads (*11*), or it may represent enlarged mantle muscles extending from the inner layer of the mantle to the shell, as in *Euciroa* (*12*). The function of these muscles in *Euciroa* is uncertain, but by contraction they could have caused a pulsation of the mantle to create water currents.

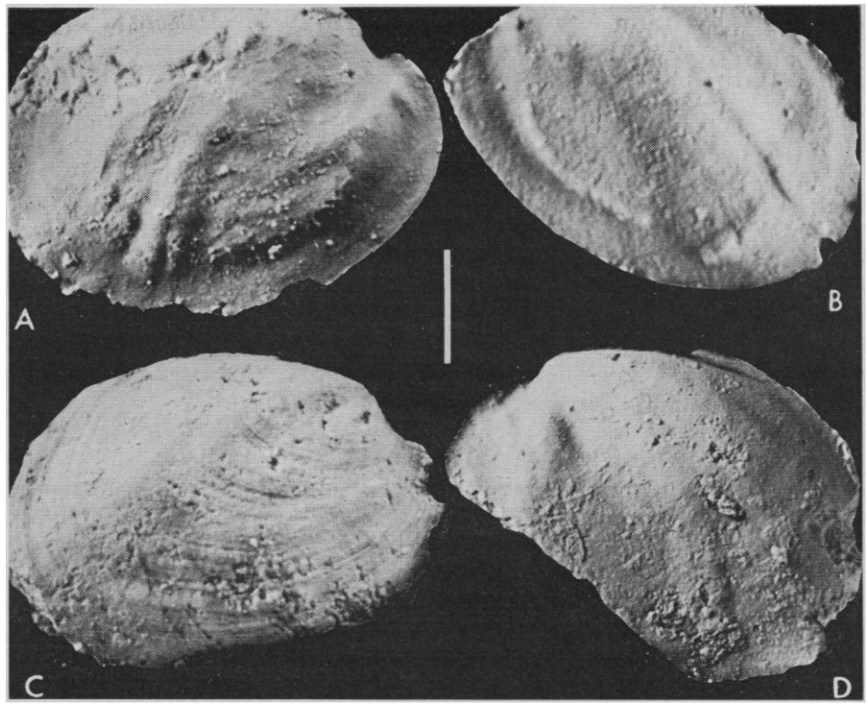

Fig. 1. (A and B) Right and left valve internal molds showing adductor, pedal, and pallial muscle insertion areas. (C) Right valve exterior showing growth lines. (D) Incomplete left valve internal mold, preserving an impression of the posterior part of the hinge showing that there were no posterior teeth. All the photographs are of *Fordilla troyensis*; (B) and (D) were made from latex replicas of two of Barrande's specimens. Bar equals 1 mm.

It is not postulated that *Fordilla* is directly related to any of the much younger forms that show hypertrophy of the pallial line; rather, these younger forms show that pelecypods have the ability to enlarge the muscles that form the pallial line. *Fordilla* shows that this ability was present from an early date.

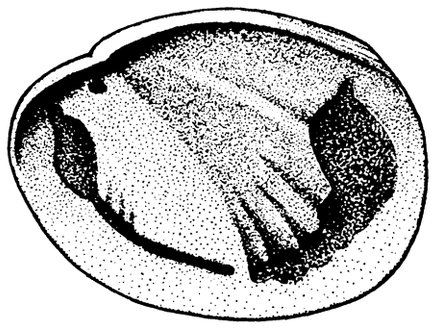

Fig. 2. Composite drawing of the interior of a right valve of *Fordilla troyensis*, showing the muscle insertion areas of the anterior and posterior adductors and pedal retractors, the pallial line, and one umbonal muscle. The stippled parts of the hinge indicate parts that have been seen and are known to lack teeth; the unstippled part of the hinge is not observable on any of the specimens seen by us. Bar equals 1 mm.

Fordilla resembles modern pelecypods adapted for infaunal to semi-infaunal life. It is laterally compressed and has a well-developed anterior end with attendant musculature. In epi-infaunal pelecypods, the anterior end and its musculature are usually severely reduced or entirely lost. Thus, the oldest known pelecypod as well as most Early Ordovician pelecypods (*13*) show adaptations to an infaunal mode of life; it does not seem likely that pelecypods could have been primitively epifaunal as suggested by Valentine and Gertman (*14*).

The Early Cambrian age of *Fordilla troyensis* shows that the known fossil record of the pelecypods began 540 to 570 million years ago (*15*) near the base of the known fossil record of animals with hard parts. Previously the oldest undoubted pelecypods were regarded as Early Ordovician (Tremadocian) in age—about 495 million years old (*13*, *15*). *Lamellodonta*, a possible Middle Cambrian pelecypod, was described and figured by Vogel (*16*); however, the zoological placement of this form remains uncertain (*13*).

Although the full hinge of *Fordilla* is not known, there are no anterior or

posterior lateral teeth (Figs. 1D and 2); it is not known whether cardinal teeth were present. The posterior hinge area is concave and nearly vertical; there is no sign of nymphs or ligamental grooves, which suggests that the ligament was a simple structure consisting of inner and outer layers connecting the two valves. The outer layer would connect the valve edges along the dorsal midline; below this and attached to the concave hinge area would be the inner part of the ligament.

The shape of *Fordilla troyensis* suggests that it is allied to the Ordovician actinodontoid heteroconchs or the Isofilibranchia; the known musculature is consistent with assignment to either group. Possibly the actinodontoids and isofilibranchs may be related to each other through *Fordilla*. Because of the lack of anterior and posterior lateral teeth it seems unlikely that *Fordilla* can be treated as a direct ancestor of the palaeotaxodonts or the pteriomorphs of the Ordovician. The former group has taxodont dentition which extends along the length of the hinge, and the latter group usually has prominent posterior lateral teeth. The remaining subclass of Ordovician pelecypods, the anomalodesmatans, is composed of elongate burrowing forms of quite different shape from *Fordilla*. Tentatively, it is suggested that *Fordilla* gave rise to the Actinodontoida and the Isofilibranchia and that the Palaeotaxodonta, Pteriomorphia, and Anomalodesmata arose from the Isofilibranchia or the Actinodontoida (*13*).

JOHN POJETA, JR.
U.S. Geological Survey,
Washington, D.C. 20242
BRUCE RUNNEGAR
University of New England,
Armidale, New South Wales, Australia
JIRI KRIZ
Central Geological Survey,
Prague, Czechoslovakia

References and Notes

1. J. Barrande, *Système Silurien du centre de la Bohême* (Bellmann, Paris, 1881), vol. 6, plate 361.
2. C. D. Walcott, *U.S. Geol. Surv. Bull. No. 30* (1886), p. 123.
3. C. Poulsen, *Medd. Groenland* **87**, 16 (1932).
4. ———, *Mat. Fys. Medd.* **36**, 2, 15 (1967).
5. C. Lochman, *Geol. Soc. Amer. Bull.* **67**, 1331 (1956).
6. T. Kobayashi, *J. Fac. Sci. Univ. Tokyo Sect. 2* **9**, 123, 183 (1954).
7. E. O. Ulrich and R. S. Bassler, *Proc. U.S. Nat. Mus.* **78**, 97 (1931); H. W. Shimer and R. R. Shrock, *Index Fossils of North America* (Wiley, New York, 1944), p. 660; P. E. Raymond, *Bull. Mus. Comp. Zool. Harvard Univ.* **96**, 304 (1946).
8. A. R. Palmer and M. E. Taylor, personal communications.
9. J. W. Cowie, *Medd. Groenland* **164**, 34 (1961).
10. C. M. Yonge, *Malacologia* **11**, 20 (1971).
11. R. D. Turner, in *Treatise on Invertebrate Paleontology*, R. C. Moore *et al.*, Eds. (Geological Society of America and Univ. of Kansas Press, Lawrence, 1969), part N, p. 704.
12. B. Runnegar, in preparation.
13. J. Pojeta, Jr., *U.S. Geol. Surv. Prof. Pap. No. 695* (1971), p. 46.
14. J. W. Valentine and R. L. Gertman, *Geol. Soc. Amer. Abstr. Programs* **4**, 696 (1972).
15. J. W. Cowie, *Quart. J. Geol. Soc. London* **120**, 255 (1964).
16. K. Vogel, *Akad. Wiss. Lit. Mainz Abh. Math. Naturwiss. Kl. 4* (1962), p. 197.

17. J.P. publishes with the permission of the director, U.S. Geological Survey; B.R.'s contribution was prepared while on study leave from the University of New England and was supported by a grant from the Smithsonian Institution; J.K.'s contribution was prepared during the tenure of a Smithsonian Institution postdoctoral fellowship. Figured specimens are on deposit at the U.S. National Museum, Washington, D.C. (Fig. 1, A, B, and D), or the New York State Museum, Albany (Fig. 1C).

20 February 1973

Macrophage Nonimmunologic Recognition: Target Cell Factors Related to Contact Inhibition

Abstract. *Activated mouse macrophages were not cytotoxic to contact-inhibited nontumorigenic 3T3 fibroblasts, but caused marked destruction to non-contact-inhibited, tumorigenic 3T12 and simian virus 40–transformed fibroblasts. Nonimmunologic recognition and destruction of target cells by activated macrophages is independent of altered morphology, abnormal karyotype, and ability for continuous multiplication in vitro—all characteristics of 3T3 fibroblasts. A modification of the target cell surface that results in a high in vitro saturation density, agglutinability by plant lectins, and tumorigenicity appears to evoke a cytotoxic response by activated macrophages.*

Activated macrophages recognize and nonspecifically destroy in vitro target cells with abnormal growth properties by a nonphagocytic mechanism (*1*, *2*). The present results suggest the abnormal growth characteristic important in nonimmunologic macrophage recognition as a target cell surface membrane modification associated with loss of contact inhibition. The important role of the surface membrane in control of cell behavior is indicated by the in vitro phenomenon of contact inhibition of mitosis (*3*) and movement (*4*). Loss of contact inhibition at confluency in tissue culture reflects a fundamental change in sensitivity to short-range cellular growth control signals and can be correlated with tumorigenicity (*5*). The selective recognition and response by activated macrophages to target cells that have lost contact inhibition could be the in vitro correlate of a host defense mechanism directed against cell surface changes associated with neoplastic growth.

In order to show the importance of surface factors in the nonimmunologic cytotoxicity reaction between activated macrophages and target cells, the following cell lines were evaluated: BALB/c 3T3 fibroblasts (a contact-inhibited nontumorigenic cell line), BALB/c 3T12 fibroblasts (a non-contact-inhibited, tumorigenic cell line from the same original pool of mouse cells as the BALB/c 3T3 line), and simian virus 40–transformed BALB/c 3T3 fibroblasts (SV-3T3 cells—a non-contact-inhibited tumorigenic cell line) (*6*). The results show that 3T3 fibroblasts were not destroyed while 3T12 and SV-3T3 fibroblasts were destroyed by syngeneic and allogeneic activated macrophages. This suggests that surface alterations related to loss of contact inhibition, a property of the 3T12 and SV-3T3 lines, is an important factor in target cell recognition and destruction. Abnormal characteristics of the 3T3 fibroblast line that appear not to play a role in nonimmunologic recognition and destruction by activated macrophages include (i) loss of normal cell morphology, (ii) hypotetraploid karyotype, (iii) maximum growth in vitro to confluency from a very low inoculum, and (iv) continuous multiplication in vitro (*6*). In addition, activated macrophages were not cytotoxic to secondary cultures of mouse kidney cells (MKC) but were cytotoxic to EMT-6 mouse mammary adenocarcinoma cells, suggesting a similar mechanism of recognition of contact-inhibited epithelial cells as for contact-inhibited fibroblasts.

Immunologic activation in vivo of macrophages was produced by infecting 6-week-old C3H/He female mice with the C_{56} strain of *Toxoplasma gondii* as previously described (*2*) or by infecting 6-week-old female C3H/He or BALB/c mice with the Paris strain of Bacillus Calmette-Guérin (BCG). BCG [0.2 mg (wet weight) in