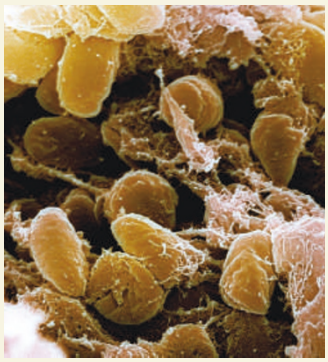
MICROBIOLOGY

Perspectives on plague

The bacterium *Yersinia pestis* (pictured) is notorious as the cause of bubonic plague. When it is breathed in, however, it also causes the rarer but deadlier pneumonic plague. The pathology of this disease in humans and animals is fairly well understood, but much less is known about the earliest stages. Wyndham W. Lathem and colleagues (*Proc. Natl Acad. Sci. USA* **102,** 17786–17791; 2005) have developed a mouse model of pneumonic plague that gives perspectives on these stages as experienced by the host and by the bacterium.

The team infected mice with *Y. pestis* through the nose, and the animals developed a disease that closely resembled pneumonic plague in humans. Bacterial numbers in the animals' lungs increased massively in the first 24 hours after infection. Yet when the authors studied the levels of inflammatory molecules normally produced during an immune response, there was little change during this period. So the bacteria must have a potent anti-inflammatory activity that allows them to become established before the host immune system detects them. After 48 hours, the levels of inflammatory molecules escalated, showing that the mouse immune response does eventually kick in; but it would seem to be too little, too late.

And what happens in *Y. pestis*? A microarray analysis showed that there is a change in the expression of about 10% of the bacterium's genes after it infects its host. Notably, many of these genes are associated with virulence, and in particular with the so-called type III secretion system. This system was already known as a potential means for the bacterium to subvert its host's immune system by altering the types and amounts of inflammatory molecules. That the expression of genes for the system



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is increased in the mice confirms this animal model as biologically valid. Moreover, a comparison with *in vitro* studies showed that the regulation of this system is more complex *in vivo* — suggesting that the model will provide greater insight into this devastating infection. **Helen Dell**

before five million years ago the rivers of the Punjab flowed eastwards as part of the Ganges system to feed the Bengal fan (Fig. 1).

This explanation of the former drainage of the Land of Five Rivers makes sense in the light of the sediments deposited at the foot of the Himalaya, known as the Siwalik Group sediments. These were deposited by ancient river systems that cut into the rising Himalayan mountains, and indeed it was previously suggested[7,8] that there was a continental-scale flip-flop of drainage between the Bengal and Indus sinks. The real value of these new results[1] is therefore not the idea that drainage diversion can occur on a continental scale, but that the isotopic data from the deep-sea Indus fan provide such striking support for that view.

We are now developing a more dynamic impression of the way in which sediment is routed from mountains to the sea. Such routing is strongly influenced at a relatively local scale by the emergence of new mountain ranges in response to continuing continental convergence. For example, the growth of the Salt Range of northern Pakistan may have triggered the diversion of the main tributaries of the ancient Indus to the Arabian Sea. But at the larger scale there are the subtle changes in regional floodplain slopes caused by the flexing of the Indian tectonic plate in response to the growth and erosion of the adjacent mountain belt, whose great mass acts downwards — like a swimmer on the end of a diving board. The longitudinal sediment-filled troughs produced by such flexural downbending[9], known as foreland basins, are particularly prone to major diversion of river systems flowing along the axis of the basin. Unlike steep rivers in tectonically uplifting mountain areas, which cut down into bedrock like cheese wires, low-gradient rivers in foreland basins are easily deflected. Continental-scale diversion might also result from the effects of climate change on river discharge, which may allow one river to dominate its neighbours and capture their drainage systems.

Clearly, investigators attempting to interpret the sedimentary record of the deep sea must be careful to disentangle the effects of climate change, variations in tectonically driven erosion, and continental-scale switches of river drainage. The use of a range of isotopic signatures in river and deep-sea sediments will help in this challenging undertaking. ■

Philip A. Allen is in the Department of Earth Science and Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, UK.
e-mail: philip.allen@imperial.ac.uk

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DEVELOPMENTAL BIOLOGY

A message to the back side

Wolfgang Driever

Vertebrate embryos from fish to mammals seem to use different routes to work out which way is up and which side is front. Yet a novel system involved in defining the dorsal side of fish might be conserved in mammals.

Vertebrates have many developmental processes in common; but so far, no unifying mechanism that specifies the dorsal–ventral (back-to-belly) axis in the vertebrate early embryo has been found. Egg cells, or oocytes, are in general roughly spherical and have only one axis: animal–vegetal, often characterized by the cell nucleus being in the 'animal' portion and away from the 'vegetal' yolk-rich pole. In amphibians and fish, after fertilization certain protein signals are physically transported from the vegetal region to the future dorsal side, contributing to the specification of dorsal. By contrast, the mechanisms of axis formation in mammals are not understood.

On page 1030 of this issue, Gore and colleagues[1] present evidence from zebrafish that *nodal* messenger RNAs, which encode the dorsal signal protein Nodal, are progressively localized to the cells that go on to form the dorsal side. Surprisingly, this dorsal localization also occurs if sequence elements from human *nodal* mRNA are used instead of the zebrafish ones, indicating an evolutionarily conserved mechanism.

The zebrafish version of Nodal is formally called Ndr1 (for Nodal-related 1) and, like the Nodal proteins found in all other vertebrates investigated, it is involved in specifying dorsal structures and two of the major embryonic

tissues, mesoderm and endoderm. This Nodal activity occurs at stages when the embryo has more than 1,000 cells and expresses its own (zygotic) genes[2,3], as opposed to relying on mRNA generated during oogenesis (that is, maternal mRNA). Gore and Sampath[4] previously showed that the distribution of maternal *ndr1* is uniform throughout oogenesis and up to fertilization. In the current work, Gore *et al.*[1] found that during the first three cell divisions of the zygote, maternal *ndr1* mRNA is localized to two of the eight cells of the embryo — the two cells that will later form the dorsal side of the embryo (Fig. 1). Furthermore, they injected fluorescent *ndr1* mRNA into the one-cell embryos and showed that its dorsal movement over the next cell cycles depends on the microtubules of the cytoskeleton, the cell's internal transport tracks.

Gore *et al.* demonstrated that maternal *ndr1* mRNA is involved in dorsal specification, because inhibiting the synthesis of protein from the maternal as well as the zygotic *ndr1* mRNAs leads to the formation of radially symmetric embryos that lack all dorsal structures . By contrast, in embryos in which just the zygotic *ndr1* had been mutated only some of the dorsal structures were lost. The authors have not analysed the fates of embryos mutant for both the maternal and zygotic *ndr1* contribution, which would provide the ultimate proof for an axis-specifying function of Ndr1.

The Wnt signalling pathway is the best-characterized pathway involved in dorsal specification. Activation of one of its components, β-catenin, is both necessary and sufficient to induce dorsal structures in vertebrates ranging from fish to birds[5]. To see whether β-catenin is involved in *ndr1* mRNA localization, Gore *et al.*[1] examined zebrafish with a mutation that eliminates maternal β-catenin from oogenesis onwards. They showed that labelled *ndr1* mRNA still moves to the same two cells, so this mechanism is independent of the Wnt pathway during dorsal specification. However, studies of the mutant also showed that during later development, β-catenin contributes to the expression of zygotic *ndr1* at the dorsal side of the embryo, which will make it difficult to separate out the two pathways. It is unclear what the targets of the localized maternal *ndr1* message may be. Nodal can induce its own expression[6], but maternal Nodal seems not to be sufficient to induce zygotic *ndr1* expression in the absence of β-catenin activity[7]. Perhaps Nodal signalling derived from maternal mRNA in dorsal cells, even though at low levels, contributes to a propensity to respond fully to other signalling pathways.

This is the first time that dynamic mRNA localization has been reported to be involved in specification of the vertebrate dorsal–ventral axis. Many invertebrates use mRNA localization to define dorsal–ventral or anterior–posterior axes — and are very creative with regard to mechanism. For example, embryos of the fruitfly *Drosophila*[8] use active mRNA transport involving local attachment to the cytoskeleton (the *bicoid* mRNA), as well as local mRNA synthesis after moving a nucleus (*gurken* mRNA) and local stabilization of mRNA (*nanos* mRNA).

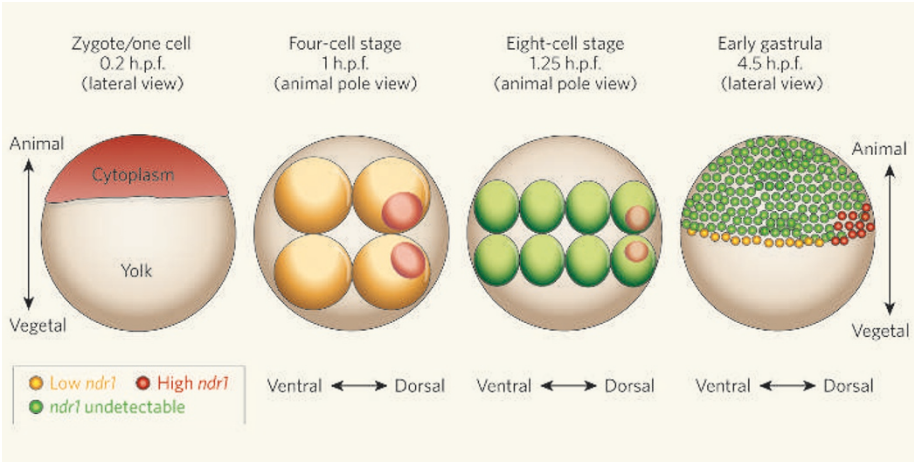

Figure 1 | Maternal mRNA that encodes Nodal moves to the dorsal side. Gore *et al.*[1] followed the progress of maternal as well as injected fluorescent *ndr1* messenger RNA through the first few cell divisions of the zebrafish embryo. At the eight-cell stage the mRNA is found only in the cells that will go on to form the dorsal side of the fish. (h.p.f., hours post-fertilization.)

Gore *et al.*[1] next examined a non-coding portion (the 3′ region) of the *nodal* mRNAs from eight vertebrate species, from fish to humans, because such regions mediate subcellular mRNA localization in other systems[8]. Three short elements in this region are evolutionarily conserved among the species. The authors showed that the *ndr1* 3′ elements are required for dorsal localization, and that both zebrafish and human 3′ elements can direct an mRNA to the dorsal side of zebrafish embryos.

Does this result mean that similar mechanisms of dorsal specification act in fish and mammals? If so, *nodal* mRNA distribution would have to be asymmetrical in the mouse zygote at the one-cell stage, and features of asymmetry must be expressed from the two-cell stage onwards. Such early asymmetry is fiercely debated, with several developmental biologists reporting early axis specification[9], whereas others conclude that the earliest polarity is only established three days later, once the cells have formed the blastocyst (32 cells and greater)[10]. Although *nodal* mRNA has not been detected at the blastocyst or earlier stages, it is present in embryonic stem cells and shortly after implantation[11]. Interestingly, there is a hypothesis that, at the level of developmental mechanisms, exactly this stage would be conceptually equivalent to the eggs of most non-mammalian species[10].

One can only speculate on how mechanisms localizing *nodal* mRNA within cells of a mammalian implantation-stage embryo might contribute to dorsal specification in a cellular context — proper cellular localization may control mRNA stability and efficiency of expression as well as transport of Nodal. Alternatively, Nodal at the zygote stage (previously undetected) and its asymmetric distribution may propagate through the positive and negative autoregulatory loops intrinsic to the Nodal signalling pathway, and slowly establish a bias between dorsal and ventral. Such an initially weak dorsal bias has been postulated to explain the regulatory nature of the early mammalian embryo[12]. Zygote-stage asymmetries in Nodal distribution might also contribute to asymmetries in intermediary targets rather than continuously maintaining Nodal expression. Such intermediaries could then contribute to dorsal specification by novel mechanisms.

The movement of *ndr1* mRNA in zebrafish zygotes is an exciting lead into potential unifying mechanisms of axis formation in vertebrate embryology. However, mechanisms of *nodal* mRNA localization need to be better understood, and demonstrated in mammals. Detecting Nodal protein distribution is a notoriously difficult task, but it must be done, or some reliable reporter found that can monitor slight differences in Nodal signalling. Finally, identifying targets of Nodal's early axis-determining activity could reveal whether there truly are conserved pathways of axis specification in vertebrates. ■

Wolfgang Driever is in the Developmental Biology Unit, Department of Biology, University of Freiburg, Hauptstrasse 1, D-79104 Freiburg, Germany.
e-mail: driever@biologie.uni-freiburg.de

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