

No effect of RDGS peptides

SIR — Peptides containing the sequence Arg-Gly-Asp-Ser (RDGS), which, in vertebrates, inhibit the binding of fibronectin and other ligands to their receptors¹, have been reported by Naidet *et al.*² to interfere with gastrulation or the establishment of the dorsoventral axis in *Drosophila*. But the known *Drosophila* integrins are not required for gastrulation or dorsoventral axis formation³. In view of the facts that one of the genes responsible for organizing the dorsoventral axis in the *Drosophila* embryo encodes a serine protease with the sequence RGDS in its active site⁴, and that dorsoventral axis formation can be inhibited by the injection of protease inhibitors⁵, we attempted to repeat the experiments in which RGDS-peptides are injected into *Drosophila* embryos to test whether they act in the same way as protease inhibitors.

We found that the peptide GRGDSP had no effect on *Drosophila* gastrulation or dorsoventral axis formation, even when we used peptide solutions with concentrations up to 100 mg ml⁻¹. These solutions were simultaneously tested and shown to be active (at 1 mg ml⁻¹) in a *Xenopus* cell culture system where they inhibited the adhesion and spreading of mesodermal cells on fibronectin. The peptide was injected at different times and into different parts of the embryo, testing in each case at least 200 embryos. Most injected embryos developed normally through gastrulation.

We tested various aspects of these embryos' development. They formed a normal ventral furrow and extended their germ band normally during gastrulation. Neither of these processes occurs in genetically or experimentally dorsalized embryos. We fixed injected embryos at early gastrulation stages and stained them with antibodies against the twist protein as an assay for changes of the dorsoventral fate map. These embryos were indistinguishable from wild-type embryos. Finally, we prepared cuticles from injected embryos that had been left to complete embryogenesis. We found no signs of defective gastrulation or of dorsalization in these cuticles. Neither when protease inhibitors are effective (nuclear division cycle 6–10), nor at the time RGDS peptides were reported to be active (cycle 12–13) did the peptide affect development. It also made no difference whether the peptide

was injected into the periplasm (the cytoplasm near the egg surface) or the perivitelline space (the liquid-filled space surrounding the egg, in which the protease is thought to act, and in which ligands for cell surface receptors would be expected to be found).

Thus the dorsoventral system and gastrulation are not inhibitable by RGDS. Indeed, as we were unable to observe

any effects of RGDS peptides (with the exception of minor distortions at the anterior and posterior poles of the embryo), the effects observed by Naidet *et al.* might have to be ascribed to something other than RGDS peptides.

MARIA LEPTIN

BARBARA GRUNEWALD

DAVID STEIN

Max-Planck-Institut für
Entwicklungsbiologie,
Spemannstr. 35,
7400 Tübingen, Germany

Protein engineering in haemoglobin

SIR — The paper by Komiyama *et al.*¹ on the structural and functional significance of the D helix in human adult haemoglobin (HbA) has important evolutionary implications, particularly concerning assembly of proteins into tetrameric structures.

In HbA, O₂ binding at the haems is modulated by functional interactions with heterotropic ligands such as protons, inorganic and organic ions. Temperature also has a large effect on the O₂ affinity of HbA so that heat absorption and release can be considered a physiologically relevant modulating factor, similar to heterotropic ligands. Many interesting adaptation mechanisms involving the interplay of temperature with heterotropic ligands may be missed when experiments are performed under only one set of conditions and a single temperature.

Human fetal haemoglobin (HbF) displays at 20 °C a lower affinity for O₂ than HbA in the absence of organic phosphates². The physiologically important reverse situation is achieved at 37 °C upon addition of 2,3-diphosphoglycerate (DPG), whose effect on HbF is related to some amino-acid substitutions present in γ chains³. However, the difference in O₂ affinity observed at 37 °C is not solely due to the different modulation power of DPG with respect to HbA and HbF. In fact, when different experimental conditions are taken into account, new aspects linked to the interplay of temperature and organic phosphates are revealed. The effect of DPG on HbF renders almost identical the O₂ affinity of the haemoglobins at 20 °C, abolishing the difference observed in the absence of the effector. On going from 20 to 37 °C, by virtue of the lower overall heat of oxygenation (ΔH) displayed by HbF when in the presence of DGP ($\Delta H = -5.5$ kcal mol⁻¹ of O₂ for HbF and -8.7 kcal mol⁻¹ HbA at pH 7.4 and corrected for the heat contribution of O₂ in solution), HbA shows a lower O₂ affinity, as should be the case if O₂ has to be transferred from maternal to fetal blood.

We would like to comment on the

experimental data reported by Komiyama *et al.* in the light of this striking example. These authors make the important contribution that the presence or the absence of D helix does not affect assembly at α and β chains into cooperative tetramers, implying that this assembly and the loss of the D helix in α subunits could have been independent events in haemoglobin evolution. But as far as the functional properties are concerned, the experiments reported by Komiyama *et al.* show only that the haemoglobin molecule does not need the D helix on the β subunits under the conditions used (which unfortunately are not indicated). Hence we still do not know if the absence of the D helix induces some important alterations. At this stage it is too early to say that "the role of the D helix may simply be to link the C and E helices" or that "it is remarkable that the loss or addition of the D helix has such a small effect on O₂ binding properties and stability". A detailed functional characterization taking into account the whole network of functional interactions is needed.

B. GIARDINA, R. SCATENA
M. E. CLEMENTI, L. CERRONI,
S. G. CONDÒ

Department of Experimental Medicine
and Biochemical Sciences,
University of Rome "Tor Vergata",
00173 Rome, Italy

M. NUUTINEN

Department of Pediatrics,
University of Oulu, Oulu, Finland

O. BRIX

Zoological Laboratory,
University of Bergen, Bergen, Norway

NAGAI *ET AL.* REPLY — Giardina *et al.* discuss an interesting instance of the effect of temperature on the oxygen affinity of two haemoglobins, HbA and HbF. Because HbF has a lower oxygen affinity than HbA at 20 °C, but a higher oxygen affinity at 37 °C in the presence of 2, 3 diphosphoglycerate, Giardina *et al.* suggest that our conclusions¹ regarding the functional role of the D helix from experiments carried out at only one temperature were premature. We would

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3. Leptin, M., Bogaert, T., Lehmann, R. & Wilcox, M. *Cell* **56**, 401–8 (1989).

4. Chasen, R. & Anderson, K. V. *Cell* **56**, 391–400 (1989).

5. Stein, D. & Nüsslein-Volhard, C. *Cell* **68**, 429–440 (1992).