

Developmental biology

BMP signalling specifies the pyloric sphincter

Sphincters are muscular valves that form at the boundaries between organs of the gut; for example, the pyloric sphincter forms at the junction of the small intestine and stomach. We show here that signalling by bone morphogenetic protein (BMP) from the avian small intestine induces the cells of the adjacent gizzard (the equivalent of the stomach in the chicken) primordium to form a sphincter. This finding and related studies of the role of Hox genes in the specification of the iliocaecal sphincter¹ provide insights into the processes by which new cell fates are specified at the borders between distinct embryological domains.

To investigate the inductive signalling involved in the development of the chicken gut, we examined the expression pattern of several members of the BMP family, including BMP-2, BMP-4, BMP-5 and BMP-7, and compared them with the expression domains of the known BMP receptors². BMP-4 is the only member of the family to be expressed in the early chicken gut, appearing in the mesoderm of the small intestine from embryonic day 2.5 (E2.5) (Fig. 1a,b, and data not shown).

Several BMP receptors are expressed in the intestinal mesoderm in a position to mediate BMP-4 signalling in this portion of the gut (data not shown). However, the type I receptor, *BMPR1B*^{3,4}, is specifically expressed in the gizzard mesoderm from E2.5 (Fig. 1c), despite the fact that neither BMP-4 nor any other related BMP family member we examined (data not shown) are expressed in the early gizzard mesoderm.

One possible explanation is that the expression of this receptor in the developing gizzard reflects a role in transducing BMP signals originating from adjacent regions of the gut, such as the developing small intestine where BMP-4 is expressed, thereby mediating the patterning of structures at the border of the gizzard and the small intestine.

The pyloric sphincter forms at the junction of the gizzard and the small intestine, and acts as a gate to regulate the passage of ingested food from the stomach to the small intestine. The sphincter differs from the rest of the gizzard with regard to the mesodermal and endodermal layers⁵.

While searching for a molecular marker for the pyloric sphincter, we examined the expression pattern of *Nkx2.5*, a homeo-domain-containing transcription factor that is expressed in the midgut⁶. We found that *Nkx2.5* is a specific marker for the mesoderm of the pyloric sphincter in the chick embryo (Fig. 1e,f). In the early stages, it is expressed adjacent to the area where BMP-4 is expressed, and overlaps with the expression pattern of *BMPR1B* in the posterior of its domain (Fig. 1b–d).

To investigate the role of BMP-4 in the formation of the pyloric sphincter, we injected a retrovirus containing the *mBMP-4* complementary DNA⁷ into stage-10 chick embryos⁸. Ectopic expression of *Nkx2.5* was seen in the gizzard, but not in the small intestine, of injected embryos, even though both organs were infected with the retrovirus (Fig. 1i,j). Sections through infected gizzards showed that *Nkx2.5*, like the endogenous domain, is limited to the mesodermal layer (Fig. 1g,h). BMP signalling is therefore sufficient to activate *Nkx2.5* in the gizzard primordium.

We tested whether BMP signalling is necessary for endogenous *Nkx2.5* activation in the developing sphincter by injecting a virus containing the secreted BMP antagonist Noggin⁹ into chick embryos ($n=12$). *Nkx2.5* was downregulated at the border of the gizzard and the small intestine in injected embryos (Fig. 1k), indicating that BMP signalling is required for *Nkx2.5* expression in the gut. Noggin is a specific antagonist of BMP-2, -4 and -7 (ref. 10), of which only BMP-4 is expressed in this region of the gut, indicating that the endogenous signal for *Nkx2.5* induction is BMP-4 from the small intestine. Noggin misexpression also altered development in other regions of the gut (data not shown), reflecting different roles of BMP signalling in gut patterning².

To test the morphological consequence of altering BMP signalling during the establishment of the pyloric sphincter, we allowed BMP4-infected embryos to develop to E9 (Noggin-infected embryos did not survive long enough to allow morphological analysis of guts where BMP signalling was disrupted). In addition to its role during sphincter specification, prolonged BMP expression interferes with muscle development and affects the rates of mesodermal proliferation and apoptosis² (data not shown). We therefore limited our morphological analysis to the endoderm.

Although the gizzard endoderm has long, thin villi that are completely covered by a thick layer of a protective keratin-like substance⁷ (Fig. 1l), the pyloric sphincter endoderm has short villi, each of which is thin at the base and bulbous at the tip (Fig. 1m). Instead of the long, keratin-covered villi characteristic of the gizzard, the endoderm of BMP4-infected gizzards had short villi with thin bases and bulbous extensions,

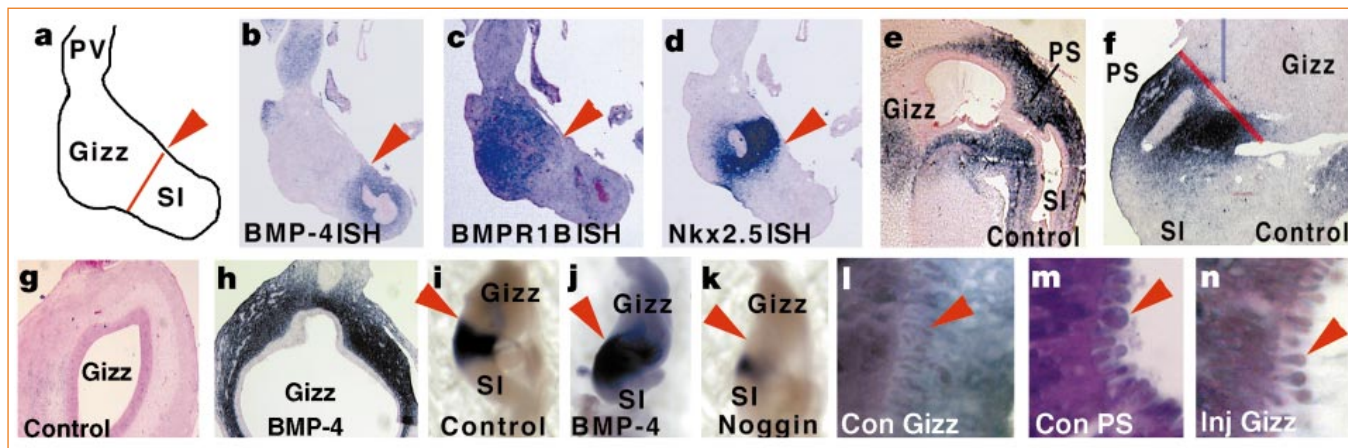


Figure 1 BMP signalling is necessary and sufficient to specify a pyloric sphincter. **a**, Diagram of the regions of the embryonic gut. **b–d**, Section *in situ* hybridization (ISH)² of an E4.5 gut with adjacent sections stained by using riboprobes for BMP-4 (**b**), *BMPR1B* (**c**) or *Nkx2.5* (**d**). Red arrowheads point to the posterior border of expression of *Nkx2.5* and *BMPR1B* as well as the anterior border of BMP-4 expression. **e**, Section ISH of an E9.5 chicken gut using an *Nkx2.5* riboprobe. **f–h**, Section ISH of E9 gizzard sections hybridized with an *Nkx2.5* riboprobe on a control gut (**f, g**), or an embryo injected with RCAS-BMP-4 (ref. 7) (**h**) showing ectopic *Nkx2.5* expression in the gizzard. Red line in **f** demarcates the anterior boundary of *Nkx2.5* expression in wild-type embryos; in **g** and **f**, the blue line shows the plane of sectioning. **i–k**, Whole-mount ISH¹¹ using *Nkx2.5* as a riboprobe on an E4.5 control gut (**i**), an E4.5 embryo injected with RCAS-BMP-4 showing ectopic *Nkx2.5* in the gizzard (**j**), or an E4.5 *Noggin*⁹-injected embryo showing a decrease in *Nkx2.5* expression at the pyloric sphincter (**k**). The red arrowheads indicate the anterior border of *Nkx2.5* expression in a control gut. **l–n**, Sections through an E9 embryo showing morphology of the endoderm of either the gizzard (**l**), pyloric sphincter (**m**) or the gizzard (**n**) of an embryo injected with BMP-4 with villi resembling that of the pyloric sphincter. Red arrowheads point to villi at the tips of endodermal cells. Gizz, gizzard; SI, small intestine; ISH, *in situ* hybridization; PS, pyloric sphincter; PV, proventricularis; inj, injected; con, control.

devoid of overlying keratin (Fig. 1n), resembling those of the pyloric sphincter.

Our results indicate that BMP-4 from the intestinal mesoderm acts on the adjacent region of the gizzard to induce the expression of *Nkx2.5* and to regulate the formation of the pyloric sphincter. BMP signalling is therefore important in the regionalization of the gut, specifying the sphincter at its proper location to act as a gate between the gizzard and intestine.

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- Zakany, J. & Duboule, D. *Nature* **401**, 761–762 (1999).
- Roberts, D. J., Smith, D. M., Goff, D. & Tabin, C. J. *Development* **125**, 2791–2801 (1998).
- Zou, H., Wieser, R., Massagué, J. & Niswander, L. *Genes Dev.* **11**, 2191–2203 (1997).
- Natsumi, T. *et al.* *J. Biol. Chem.* **272**, 11535–11540 (1997).
- Romanoff, A. L. *The Avian Embryo* (Macmillan, New York, 1960).
- Buchberger, A. *et al.* *Mech. Dev.* **56**, 151–163 (1996).
- Duprez, D. *et al.* *Mech. Dev.* **57**, 145–157 (1996).
- Hamburger, V. & Hamilton, H. L. *J. Morphol.* **88**, 49–92 (1951).
- Capdevila, J. & Johnson, R. L. *Dev. Biol.* **197**, 205–217 (1997).
- Zimmerman, L. B., Dejesus-Escobar, J. M. & Harland, R. M. *Cell* **96**, 599–606 (1996).
- Riddle, R. D., Johnson, R. L., Laufer, E. & Tabin, C. *Cell* **75**, 1401–1416 (1993).

Chronometry

Effect of the 1999 solar eclipse on atomic clocks

Solar eclipses have been reported to have a strange influence on the behaviour of atomic clocks¹ and pendulums^{2,3}, which has been attributed to some unknown feature of gravity⁴. Here we correct this idea after being unable to detect any anomalous changes in the relative rates of three types of atomic clock, based on the ground-state hyperfine transitions of hydrogen, rubidium and caesium, during the solar eclipse of 11 August 1999 over central Europe.

The reported effects of solar eclipses on atomic clocks and the movement of pendulums have been mixed. A study of the relative rates of rubidium and caesium atomic clocks during four partial solar eclipses between 1987 and 1992 (ref. 1) recorded an accumulated time difference between two caesium clocks and two rubidium clocks located in the same laboratory of 468 ns and 65 μ s, respectively. In these experiments, the change in the clock rates occurred not only during the eclipse, but also up to one day before and after the maximum of the eclipse.

Peculiar movements of a Foucault pendulum were reported at the time of the onset of solar eclipses in 1954 and 1959 (ref. 2). A variation in the oscillation period of torsion pendulums allegedly occurred

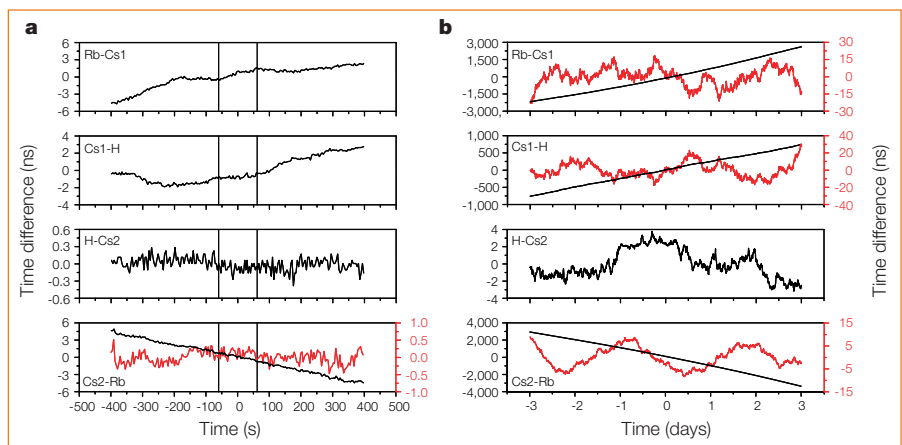


Figure 1 Time differences in four atomic clocks during the solar eclipse. Rb-Cs1, Cs1-H, H-Cs2, Cs2-Rb are the time differences between two caesium clocks, a rubidium clock and a hydrogen maser. **a**, Time period of 800 s around the eclipse. Vertical lines mark the beginning and end of the total eclipse; time 0 indicates the maximum of the eclipse. **b**, Time period of 6 days. Some plots show a smooth drift because the clocks are not perfect. Red curves are obtained by removing this drift by fitting a low-order polynomial.

during the onset of an eclipse³, although this was never confirmed^{5–7}. In addition, there has been a report of a highly significant variation in the gravitational field during the onset of a solar eclipse⁸.

Although general relativity predicts that gravity can influence clock rates, it does not explain why clocks in the same place should be affected differently. It is not clear why this effect should occur when the Sun is eclipsed by the Moon, and not just when the Moon is new and the Sun and Moon are almost aligned, unless gravity is shielded. But then, why does it not occur every day, given that the Sun is shielded by the Earth once every revolution⁹? A change in the value of the fine-structure constant during the eclipse might explain the changes in clock rate.

To try and make sense of these observations, we repeated the experiments of Zhou *et al.*¹ by comparing four atomic clocks during the 1999 total solar eclipse over central Europe. The clocks, based on ground-state hyperfine splitting of different species of atom, were placed in an air-conditioned room in a basement at Wessling in Germany (48° 05' 03" N, 11° 16' 40" E, 620 m above sea level). Their frequency depends differently, with known coefficients, on the value of the fine-structure constant¹⁰. A computer recorded their time difference every 4 seconds from 3 August to 23 August.

To exclude the influence of any spurious effects, such as variations in the background magnetic field (resulting from changes in the ionosphere, for example), we recorded variations in temperature, power lines and barometric pressure. We detected increased magnetic-field fluctuations of up to ± 0.1 microtesla in the vertical direction during working hours from Monday to Friday as a result of human activity. We recorded the external light intensity to verify synchronization of the data with the eclipse. The time axis was constantly calibrated by a

time signal broadcast by the Physikalisch-Technische Bundesanstalt in Braunschweig and zeroed at 10:37:46 universal time, the maximum of the eclipse. No significant changes in these parameters seemed to be connected with the eclipse.

The relative time differences of four atomic clocks (one rubidium and two caesium clocks and one hydrogen maser) are shown in Fig. 1 for time intervals of 800 seconds and 6 days. Phase drifts are visible in some cases, depending on the type of clock and the duration of the observation (black curves), indicating that the clocks are not perfectly stable in their relative rates. To reveal possible effects of the eclipse, we fitted these drifts with polynomials of the order of less than four to obtain the residue shown in red. To enable comparison with the results of Zhou *et al.*¹, who observed changed clock rates for 8 hours in one case and 3 days in another, we plotted our data over a period of 6 days.

We can set an upper limit, independent of the type of clock, of ± 6 ns for a non-regular time difference accumulated over 800 seconds, and ± 20 ns accumulated over 6 days. It could be argued that some features might have been suppressed by the way the data were handled, so the complete data set is available at <http://www.mpg.de/~haensch/eclipse/eclipse.html>

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- Zhou, S. W., Huang, B. J. & Ren, Z. M. *Nuovo Cimento C* **18**, 223–236 (1995).
- Allais, M. *Aero/Space Eng.* **9**, 46–55 (1959).
- Saxl, E. J. & Allen, M. *Phys. Rev. D* **3**, 823–825 (1971).
- Eckhard, D. H. *Phys. Rev. D* **42**, 2144–2145 (1990).