Then the birds were given access to both trays, while observed by individual A, or individual B, or a control individual who had seen food stored in one or other tray *but not by the subject itself*. The jay's cache protection strategies were adjusted to the particular individual who had watched them store: when individual A was present, they multiply re-cached food in tray A, and so on. Critically, when a jay that had not watched them store food was present, they did not use the multiple re-caching strategy — even though that bird had seen food stashed in one of the trays, so was presumably eyeing the tray just as keenly. Scrub jays are able to keep track of who has seen their caching done, and where.

Does this mean that scrub jays, like humans, possess 'theory of mind' [10] — that they know what other individuals know, and work out what to do to wrong-foot them? Some scientists would prefer to construct instead an elaborate explanation in terms of behavioural predispositions and specific learning mechanisms. This — as the researchers themselves note — is certainly feasible. However, the dichotomy of mentalism/behaviourism is an inherently unhelpful one (see [11], a Primer on this topic in a recent issue of *Current Biology*). Theory of mind is not itself an explanation: it is a convenient label for a range of interesting cognitive capacities that *need* explanation; moreover, mentalist accounts are hard to disentangle from the phenomenology of verbal thought and pictorial imagery. Conversely, explanations that depend on putting forward an array of ad hoc, special-purpose learning mechanisms are unlikely to lead to a broader understanding of the evolution of cognition. What is needed is to go beyond wrangles over whether an animal is human-like or not in some way, whether the attribute is 'intelligence' or 'theory of mind', and instead to build up a well specified cognitive model that describes their competence. With the rapidly growing understanding of corvid cognition it should not be long before researchers are able to do this, for a kind of cognition that evolved quite independently from our own, in a taxon that has not shared an ancestor with us for 280 million years.

References

1. Mitchell, R., and Hamm, M. (1997). The interpretation of animal psychology: anthropomorphism or behavior reading? Behaviour *134*, 173–204.
2. Zuberbuhler, K. (2000). Causal cognition in a non-human primate: field playback experiments with Diana monkeys. Cognition *76*, 195–207.
3. Janmaat, K.R.L., Byrne, R.W., and Zuberbuhler, K. (2006). Primates take weather into account when searching for fruits. Curr. Biol. *16*, 1232–1237.
4. Rizzolatti, G., Fadiga, L., Fogassi, L., and Gallese, V. (1996). Premotor cortex and the recognition of motor actions. Brain Res. *3*, 131–141.
5. Gallese, V., and Goldman, A. (1998). Mirror neurons and the simulation theory of mind-reading. Trends Cogn. Sci. *2*, 493–501.
6. Balda, R.P., and Kamil, A.C. (1992). Long-term spatial memory in Clark's nutcracker, Nucifraga columbiana. Anim. Behav. *44*, 761–769.
7. Clayton, N.S., and Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. Nature *395*, 272–278.
8. Emery, N.J., and Clayton, N.S. (2004). The mentality of crows: Convergent evolution of intelligence in corvids and apes. Science *306*, 1903–1907.
9. Dally, J.M., Emery, N.J., and Clayton, N.S. (2006). Food-caching western scrub-jays keep track of who was watching when. Science *312*, 1662–1665.
10. Frith, C., and Frith, U. (2005). Theory of mind. Curr. Biol. *15*, R644–R646.
11. Byrne, R.W., and Bates, L.A. (2006). Why are animals cognitive? Curr. Biol. *16*, R445–R448.

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DOI: 10.1016/j.cub.2006.08.009

Liver Specification: A New Role for Wnts in Liver Development

Secreted Wnt proteins control a diverse array of developmental decisions. A recent analysis of the zebrafish mutant *prometheus* points to a previously unknown role for Wnts during liver specification.

Zoë D. Burke, Shifaan Thowfeequ and David Tosh

The endoderm generates many important tissues, including the lung, liver, intestine and pancreas. The development of endodermal organs occurs by a coordinated sequence of events. For example, liver development involves establishment of competence for liver formation, followed by liver specification, hepatic bud formation, growth and differentiation [1,2]. While we know quite a lot about the molecular cues that control competence and differentiation, much less is known about the factors regulating liver specification. In the last decade, close genetic analysis and studies using embryonic tissue explants have provided valuable information on the molecules and pathways involved in early mouse liver development. Tissue interactions and mesodermal signalling are hallmarks of endodermal patterning. In the mouse, members of the forkhead box A (FoxA) and GATA families of transcription factors initially enable the ventral foregut endodermal cells to enter a stage of competence; this allows cells to respond to inductive mesodermal signals converging on a common endodermal domain along the primitive gut tube [3–5].

Fibroblast growth factor (FGF) signals, emanating from the cardiac mesoderm, have been shown to be important orchestrators of hepatogenesis at multiple and distinct stages. While FGF1 and FGF2 can induce the expression of hepatic genes in the specified endoderm, FGF8 is thought to play a role in liver

outgrowth and cell differentiation [6]. Bone morphogenetic protein (BMP) signals from the septum transversum mesenchyme work co-ordinately with FGFs to initiate a programme of hepatic gene expression [7] (Figure 1). These signals subsequently establish a nascent hepatoblast population (bipotential precursors) from which the two functional epithelial cells of the liver, hepatocytes and bile duct cells, are derived. An array of liver-enriched transcription factors, including CCAAT/enhancer binding proteins, hepatocyte nuclear factors and Fox proteins, regulate the cell fate choice of hepatoblasts and act to further promote their differentiation towards a hepatocyte or ductal phenotype [4].

In zebrafish, the tissue interactions and signalling molecules involved in the early stages of liver formation are less well documented than in mouse. The earliest hepatocyte precursors in zebrafish are detected at the level of the first somite; at this time, the cardiac mesoderm is located at the level of the midbrain–hindbrain boundary [2], while in the mouse, the cardiac mesoderm and foregut endoderm lie in close proximity [6]. In zebrafish, cells of the cardiac and hepatic lineages arise from the same region of the embryo much earlier than in the mouse, but interactions between these two cell lineages may be conserved [8]. Alternatively, the factors mediating hepatic differentiation may be conserved between mouse and zebrafish but might originate from different tissues [9].

Ober *et al.* [10] have recently reported the first genetic evidence for the involvement of the Wnt signalling pathway in zebrafish liver development. The Wnt signalling pathway plays a central role in many aspects of embryonic development as well as in the maintenance of adult stem cells and in tumour progression in humans [11,12]. Wnts are secreted glycoproteins, homologous to Wingless in *Drosophila*, that signal via at least two receptors; Frizzled, a seven-pass transmembrane domain-containing serpentine

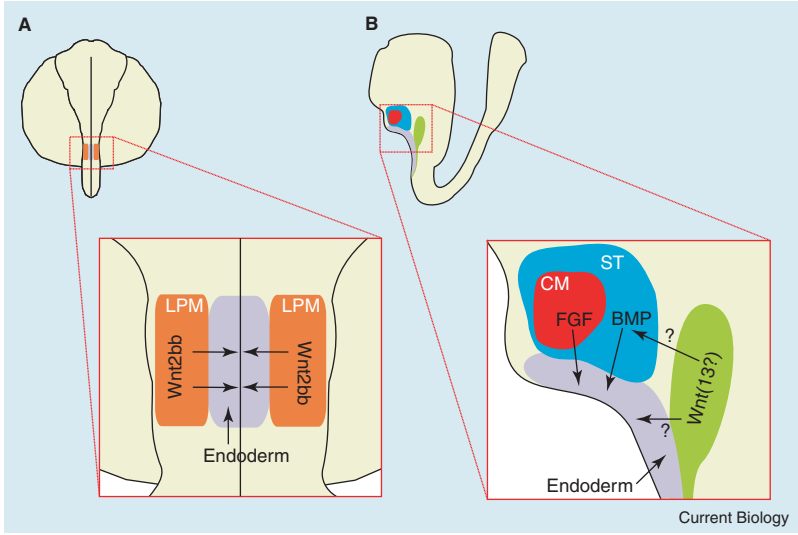

Figure 1. Schematic models of liver development in zebrafish and mouse.
(A) In zebrafish embryos (18 hpf), the lateral plate mesoderm (LPM) associated with the liver-forming region of endodermal epithelium emits a Wnt signal. (B) In mouse, FGF and BMP signals emanating from the cardiac mesenchyme (CM) and septum transversum (ST) may be involved in mouse liver specification from the ventral foregut endoderm. The mouse homologue of the zebrafish Wn2bb, Wnt13, is expressed in 8.5d embryos under the heart on both sides of the foregut involution. Wnts may regulate liver specification either directly or indirectly via BMP.

protein, and the low-density-lipoprotein-related protein (LRP) receptor. Recent *in vitro* studies have shown that, in addition to promoting liver growth and hepatocyte maturation, Wnts are required for the specification of the bile duct lineage in mouse liver [13].

Unfortunately the early embryonic lethality of mice carrying null mutations for β-catenin [14], a downstream component of the intracellular Wnt signal transduction pathway, has precluded investigations to determine the role of Wnt signalling during early specification of the hepatic endoderm. The data presented by Ober *et al.* [10] highlight the importance of generating a conditional knockout mouse model to determine the role of Wnt signalling in liver specification in a model system that, to date, has dominated the field of early liver development.

Ober *et al.* [10] generated the *prometheus* (*prt*) mutant using a forward genetics approach involving N-ethyl-N-nitrosourea (ENU) mutagenesis of the transgenic zebrafish line *Tg(gutGFP)*^{s854}. In this line, green fluorescent protein (GFP) is used as a reporter and is expressed throughout the developing endoderm [2]. The *prt* mutants exhibit defective hepatic fate specification and the liver is either absent or strongly reduced. This is accompanied by reduced or no expression of the early hepatoblast markers *hhex* and *prox1*. These genes normally mark the early phase of liver formation. While *prt* mutants completely lack hepatic tissue at early developmental stages — 28 hours post fertilisation (hpf) — some mutants do develop a liver at later stages (50 hpf) and can progress into adulthood. However, the authors demonstrate the absence of the hepatic differentiation markers *ceruloplasmin* and *selenoproteinPb* from mutants at 52 hpf and it is not clear why some embryos survive into adulthood despite the lack of a differentiated liver during development.

Positional cloning revealed that the *prt* gene encodes a novel Wnt2b homologue, named Wnt2bb. This is an interesting result, as it may be the first example of genetic evidence of a role for Wnt signalling in liver formation. A specific time window (16–25 hpf) during which Wnt signalling can mediate hepatic specification through the

canonical Wnt pathway was revealed using an additional transgenic zebrafish line, one expressing a GFP-tagged, dominant negative form of the downstream transcription factor T-cell factor (TCF) from a heat shock promoter to inhibit the Wnt/β-catenin pathway. Furthermore, the time-frame of liver specification determined by inhibiting β-catenin coincides with the temporal bilateral expression profile of *wnt2bb* in the lateral plate mesoderm, and is independent of any endodermal signals. Grafting of wild-type labelled cells into the lateral plate mesoderm rescued the prt phenotype and showed that the prt gene is essential to mediate mesodermal–endodermal crosstalk during liver specification. Combined, these data provide strong evidence for a precise and essential role for Wnt signalling in specification of the zebrafish liver.

Although a role for Wnt signalling in determining cell fate and differentiation of many tissue types during development has been well documented [11], there is little evidence in the literature for a role in liver specification. In fact, expression of the *secreted frizzled-related protein 5 gene*, which encodes a Wnt inhibitor, in the foregut endoderm in mouse [15] suggests that inhibition of the Wnt pathway may be required during hepatic specification. Therefore, the finding that Wnt2bb can mediate liver specification in zebrafish in such a spatiotemporal manner is intriguing. Furthermore, the expression pattern of Wnt2bb in zebrafish closely resembles that reported for the mouse orthologue [16], Wnt13, perhaps advocating a yet unknown role for Wnt signalling in mammalian species.

It is possible that precisely executed waves of inhibition and activation of Wnt signalling may mediate the various stages of endoderm patterning, such as liver development, or that convergence of other pathways known to operate during this time, such as BMP signalling, may act to enhance the Wnt pathway, as BMP2 enhances Wnt2b expression in keratinocytes [17]. Interestingly, FGF8 and BMP4 are essential for hepatic induction and differentiation and both are known targets of Wnt signalling [18,19]. Whether these factors act downstream of, or in a feedback loop with, an earlier temporal specifying Wnt signal in the mouse liver remain to be elucidated.

References

1. Zhao, R., and Duncan, S.A. (2005). Embryonic development of the liver. Hepatology *41*, 956–965.
2. Field, H.A., Ober, E.A., Roeser, T., and Stainier, D.Y.R. (2003). Formation of the digestive system in zebrafish. I. Liver morphogenesis. Dev. Biol. *253*, 279–290.
3. Lee, C.S., Friedman, J.R., Fulmer, J.T., and Kaestner, K.H. (2005). The initiation of liver development is dependent on Foxa transcription factors. Nature *435*, 943–947.
4. Costa, R.H., Klanichenko, V.V., Holterman, A.-X.L., and Wang, X. (2003). Transcription factors in liver development, differentiation, and regeneration. Hepatology *38*, 1331–1347.
5. Holtsinger, A., and Evans, T. (2005). Gata4 regulates the formation of multiple organs. Development *132*, 4005–4014.
6. Jung, J., Zheng, M., Goldfarb, M., and Zaret, K.S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science *284*, 1998–2003.
7. Rossi, J.M., Dunn, N.R., Hogan, B.L.M., and Zaret, K.S. (2001). Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. Genes Dev. *15*, 1998–2009.
8. Warga, R.M., and Nüsslein-Volhard, C. (1999). Origin and development of the zebrafish endoderm. Development *126*, 827–838.
9. Stainier, D.Y. (2001). Zebrafish genetics and vertebrate heart formation. Nat. Rev. Genet. *2*, 39–48.
10. Ober, E.A., Verkade, H., Field, H.A., and Stainier, D.Y. (2006). Mesodermal Wnt2b signalling positively regulates liver specification. Nature, Jun 21; [Epub ahead of print].
11. Cadigan, K.M., and Nusse, R. (1997). Wnt signalling: a common theme in animal development. Genes Dev. *11*, 3286–3305.
12. Pinto, D., and Clevers, H. (2005). Wnt, stem cells and cancer in the intestine. Biol. Cell. *97*, 185–196.
13. Monga, S.P.S., Monga, H.K., Tan, X., Mulé, K., Pediaditakis, P., and Michaelopouos, G.K. (2003). β-catenin antisense studies in embryonic liver cultures: Role in proliferation, apoptosis and lineage specification. Gastroenterology *124*, 202–216.
14. Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K., and Kemler, R. (1995). Lack of beta-catenin affects mouse development at gastrulation. Development *121*, 3529–3537.
15. Finley, K.R., Tennessen, J., and Shawlot, W. (2003). The mouse secreted frizzled-related protein 5 gene is expressed in the anterior visceral endoderm and foregut endoderm during early postimplantation development. Gene Exp. Patt. *3*, 681–684.
16. Zakin, L.D., Mazan, S., Maury, M., Martin, N., Guenet, J.L., and Brulet, P. (1998). Structure and expression of Wnt13, a novel mouse Wnt 2 related gene. Mech Dev. *73*, 107–116.
17. Yang, L., Yamasaki, K., Shirakata, Y., Dai, X., Tokumaru, S., Yahata, Y., Tohyama, M., Hanakawa, Y., Sayama, K., and Hashimoto, K. (2006). Bone morphogenetic protein-2 modulates Wnt and frizzled expression and enhances the canonical pathway of Wnt signaling in normal keratinocytes. J. Derm. Sci. *42*, 111–119.
18. Kawakami, Y., Capedevila, J., Buscher, D., Itoh, T., Rodrigeuz Esteban, C., and Izpisua Belmonte, J.C. (2001). Wnt signals control FGF-dependent limb initiation and AER induction in the chick embryo. Cell *104*, 891–900.
19. Weigo, S., Guttentag, S., Wang, Z., Andl, T., Ballard, P., Lu, M.M., Piccolo, S., Birchmeier, W., Whitsett, J.A., Millar, S., *et al*. (2005). Wnt/β-catenin signalling acts upstream of N-myc, BMP4, and FGF signalling to regulate proximal-distal patterning in the lung. Dev. Biol. *283*, 226–239.

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DOI: 10.1016/j.cub.2006.08.011

Endosymbiosis: Double-Take on Plastid Origins

Plastids — the light-harvesting machines of plant and algal cells — evolved from cyanobacteria inside a eukaryotic host more than a billion years ago. New data reveal that a mysterious unicellular alga acquired its photosynthetic apparatus much more recently than other eukaryotes, affording a second look at the primary endosymbiotic origin of plastids.

John M. Archibald

> *"I call this experiment 'replaying life's tape.' You press the rewind button and, making sure you thoroughly erase everything that actually happened, let the tape run again and see if the repetition looks at all like the original."*
>
> Stephen J. Gould (*Wonderful Life*)

In his famous treatise on the Cambrian fossils of British Columbia's Burgess Shale,