

provided that the reactive control layer is inactive. (6) Chaining through LTM sequences is achieved by biasing the LTM matching process.

The competition among LTM segments takes place on a quantity m where m_l^k of segment l of sequence k is defined as:

$$m_l^k = c_l^k t_l^k \quad (5)$$

where c_l^k is defined as $d(E, E_l^k)$ using the definition of d provided in equation (4). $t_l^k \in [0; 1]$, is a dynamic threshold that provides a probabilistic mechanism for chaining through a LTM sequence. In case segment l of sequence k wins the competition by virtue of having the lowest value of m , it will reduce t of segment $l + 1$, of k , t_{l+1}^k , to a fixed value $\beta, \beta \in [0; 1]$. t_l^k relaxes to its default value of 1 according to $t_l^k(t + 1) = \alpha_T + (1 - \alpha_T)t_l^k(t)$, $\alpha_T \in [0; 1]$. The LTM segment that wins the competition will dominate the behavioural output of the overall system when its m is below a fixed threshold.

In the present implementation, STM is a ring buffer with a fixed length of 25 segments, while the capacity of LTM is limited to 64 sequences.

Received 3 March; accepted 7 August 2003; doi:10.1038/nature02024.

- Olshausen, B. A. & Field, D. J. Emergence of simple-cell receptive field properties by learning a sparse code for natural images. *Nature* **381**, 607–609 (1996).
- Rao, R. & Ballard, D. Predictive coding in the visual cortex: a functional interpretation of some extra-classical receptive-field effects. *Nature Neurosci.* **2**, 79–87 (1999).
- Logothetis, N. & Sheinberg, D. Visual object recognition. *Annu. Rev. Neurosci.* **19**, 577–621 (1996).
- Goldstone, R. Perceptual learning. *Annu. Rev. Psychol.* **49**, 585–612 (1998).
- Mackintosh, N. *The Psychology of Animal Learning* (Academic, New York, 1974).
- Lavond, D. G., Kim, J. J. & Thompson, R. F. Mammalian brain substrates of aversive classical conditioning. *Annu. Rev. Psychol.* **44**, 317–342 (1993).
- Thorndike, E. Animal intelligence: an experimental study of the associative processes in animals. *Psychol. Rev. Ser. Monogr. Suppl.* **2**, 1–109 (1898).
- Sutton, R. S. & Barto, A. G. *Reinforcement Learning: An Introduction* (MIT Press, Cambridge, Massachusetts, 1998).
- Squire, L. & Kandel, E. *Memory: From Mind to Molecules* (Scientific American Library, New York, 1999).
- Verschure, P. F. M. J., Kröse, B. & Pfeifer, R. Distributed adaptive control: The self-organization of structured behavior. *Rob. Auton. Syst.* **9**, 181–196 (1992).
- Verschure, P. F. M. J. & Voegtlin, T. A bottom-up approach towards the acquisition, retention, and expression of sequential representations: Distributed adaptive control III. *Neural Netw.* **11**, 1531–1549 (1998).
- Verschure, P. F. M. J. & Pfeifer, R. in *From Animals to Animats: Proc. 2nd Int. Conf. Simul. Adapt. Behav. (Honolulu, Hawaii)* (eds Meyer, J. A., Roitblat, H. & Wilson, S.) 210–217 (MIT Press, Cambridge, Massachusetts, 1992).
- McFarland, D. & Bossert, T. *Intelligent Behavior in Animals and Robots* (MIT Press, Cambridge, Massachusetts, 1993).
- Clancey, W. *Situated Cognition: On Human Knowledge and Computer Representations* (Cambridge University Press, Cambridge, UK, 1996).
- Arkin, R. *Behavior-Based Robotics* (MIT Press, Cambridge, Massachusetts, 1998).
- Pfeifer, R. & Scheier, C. *Understanding Intelligence* (MIT Press, Cambridge, Massachusetts, 1999).
- Verschure, P. F. M. J. & Althaus, P. A real-world rational agent: Unifying old and new AI. *Cogn. Sci.* **27**, 561–590 (2003).
- Massaro, D. *Perceiving Talking Faces: From Speech Perception to a Behavioral Principle* (MIT Press, Cambridge, Massachusetts, 1997).
- Mondada, F., Franzi, E. & Jenne, P. *Experimental Robotics III: Proc. 3rd Int. Symp. Exp. Rob. (Kyoto, Japan, 28–30 October 1993)* 501–513 (Springer, Berlin, 1993).
- Tolman, E. Cognitive maps in rats and men. *Psychol. Rev.* **55**, 189–208 (1948).
- Bell, A. Levels and loops: the future of artificial intelligence and neuroscience. *Phil. Trans. R. Soc. Lond. B* **354**, 2013–2020 (1999).
- Sur, M. & Leamy, C. Development and plasticity of cortical areas and networks. *Nature Rev. Neurosci.* **2**, 251–261 (2001).
- Mehta, M., Barnes, C. & McNaughton, B. Experience-dependent, asymmetric expansion of hippocampal place fields. *Proc. Natl Acad. Sci. USA* **94**, 8918–8921 (1997).
- Houde, J., Nagarajan, S., Sekihara, K. & Merzenich, M. Modulation of the auditory cortex during speech: An MEG study. *J. Cogn. Neurosci.* **14**, 1125–1138 (2002).
- Rescorla, R. & Wagner, A. in *Classical Conditioning 2. Current Theory and Research* (eds Black, A. H. & Prokasy, W. E.) 64–99 (Appleton-Century-Crofts, New York, 1972).
- Schultz, W. & Dickinson, A. Neuronal coding of prediction errors. *Annu. Rev. Neurosci.* **23**, 473–500 (2000).
- Kalman, R. A new approach to linear filtering and prediction problems. *Trans. ASME J. Basic Eng.* **82**, 35–45 (1960).
- Sanchez-Montanes, M., Verschure, P. F. M. J. & König, P. Local and global gating of plasticity. *Neural Comput.* **12**, 519–529 (2000).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank A. Baumgartner and J. Manzollini for their support in performing the Markov analysis. Part of this research is supported by the Swiss National Science Foundation, the Volkswagen foundation and the Körber Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to P.F.M.J.V. (pfmjv@ini.phys.ethz.ch).

Regulation of neuroblast competence in *Drosophila*

Bret J. Pearson & Chris Q. Doe

Institutes of Neuroscience and Molecular Biology, Howard Hughes Medical Institute, University of Oregon 1254, Eugene, Oregon 97403, USA

Individual neural progenitors generate different cell types in a reproducible order in the retina^{1–3}, cerebral cortex^{4–6} and probably in the spinal cord⁷. It is unknown how neural progenitors change over time to generate different cell types. It has been proposed that progenitors undergo progressive restriction⁸ or transit through distinct competence states^{9,10}; however, the underlying molecular mechanisms remain unclear. Here we investigate neural progenitor competence and temporal identity using an *in vivo* genetic system—*Drosophila* neuroblasts—where the Hunchback transcription factor is necessary and sufficient to specify early-born cell types¹¹. We show that neuroblasts gradually lose competence to generate early-born fates in response to Hunchback, similar to progressive restriction models⁸, and that competence to acquire early-born fates is present in mitotic precursors but is lost in post-mitotic neurons. These results match those observed in vertebrate systems, and establish *Drosophila* neuroblasts as a model system for the molecular genetic analysis of neural progenitor competence and plasticity.

Despite substantial progress in vertebrates, we still know little about the molecular basis for how a single neural precursor sequentially generates different cell types. This is primarily due to the lack of an *in vivo* model system where a single neural progenitor can be studied at reproducible times during its lineage. The *Drosophila* embryonic central nervous system (CNS) lends itself well to the study of neural progenitor plasticity because neural progenitors (neuroblasts (NBs)) can be individually identified, each NB generates different cell types in a reproducible order, molecular markers exist for each of these cell types, intrinsic factors are known that confer different temporal identities, and gene expression can be readily manipulated at specific points within the NB lineage¹¹. NBs repeatedly divide in a stem-cell-like mode to ‘bud off’ a series of smaller daughter cells called ganglion mother cells (GMCs). Cell lineage studies show that every GMC has a unique identity based on its ‘birth’ order within the NB lineage, and generates a characteristic pair of neurons or glia. Recently, four transcription factors have been identified that are excellent candidates for specifying GMC temporal identity^{11,12}. NBs sequentially express the transcription factors Hunchback (Hb)→Krüppel→Pdm1→Castor, with GMCs inheriting the transcription-factor profile of the parental NB on their generation, which is then maintained in their own neuronal progeny (Fig. 1)¹¹. *hb* is both necessary and sufficient for specifying the first-born temporal identities in multiple NB lineages, even though first-born cells can be motor neurons, interneurons, or glia¹¹. Here we manipulate the timing and levels of Hb in a model NB lineage (NB7-1) to ask two fundamental questions: when does a NB lose competence to generate first-born neurons (immediately after Hb downregulation, progressively during its lineage, or never)? And when during neuronal differentiation is competence to generate first-born neurons lost (in NBs, GMCs, or post-mitotic neurons)?

To assay changes of temporal identity within a single lineage, we precisely define the birth order and sibling relationships of the early NB7-1 lineage. NB7-1 generates five motor neurons (U1–U5) and about 30 interneurons^{13,14}. The five U motor neurons have stereotyped positions in the CNS (Fig. 1a), express the Even-skipped (Eve) transcription factor (Fig. 2a), and innervate specific body wall

muscles^{11,13,15}. It has been proposed that U1–U5 develop from the first five GMCs in the lineage^{11,16}. Here we use a genetic method¹⁷ to induce permanent, positively marked cell clones at different points of the NB7-1 lineage. We find that the first five GMCs (GMC1–5) sequentially generate the U1–U5 motor neurons, and that each *Eve*⁺ motor neuron has an *Eve*⁻ sibling (Fig. 1b; Supplementary Movie S1).

To test whether NBs lose competence to make first-born fates as they progress through their cell lineages, we use *prospero-Gal4* to re-express high levels of Hb in the NB7-1 lineage just after the birth of the Hb-negative GMC3 (Fig. 2b, cartoon). This has no effect on the normal U1–U3 cell fates, as expected, but subsequently the NB generates an average of 6.3 ($n = 60$) extra U1 motor neurons based on molecular marker expression (Fig. 2b and Table 1, row 2). To more rigorously determine whether these neurons have a U1 identity, we assay the axon projections of single or small groups of the putative U1 neurons, and observe that the ectopic U1 motor neurons project out of the intersegmental nerve to dorsal body wall

muscles, consistent with the normal U1 neuron (Fig. 2d; see also Supplementary Movie S2). We conclude that NB7-1 shows plasticity: first-born U1 motor neurons can be induced even after downregulation of endogenous Hb.

Hb is required to activate and repress target genes in a concentration-dependent manner along the anterior–posterior body axis during segmentation¹⁸. To test the idea that different levels of Hb might have different functions in the CNS, we repeated the previous experiment (Fig. 2b) but with lower levels of Hb expressed in precisely the same temporal pattern (Fig. 2c). Whereas high levels of Hb generate extra U1 neurons (Fig. 2b and Table 1, row 2), low levels of Hb produce presumptive U2 neurons (Fig. 2c and Table 1, row 3; we call them ‘presumptive’ U2 neurons because we cannot distinguish them from U3 motor neurons without using Hb as a marker). We conclude that high levels of Hb can induce U1 fates, whereas lower levels can induce presumptive U2 fates. This may explain how Hb generates two distinct temporal identities in the NB7-1 lineage (U1 and U2).

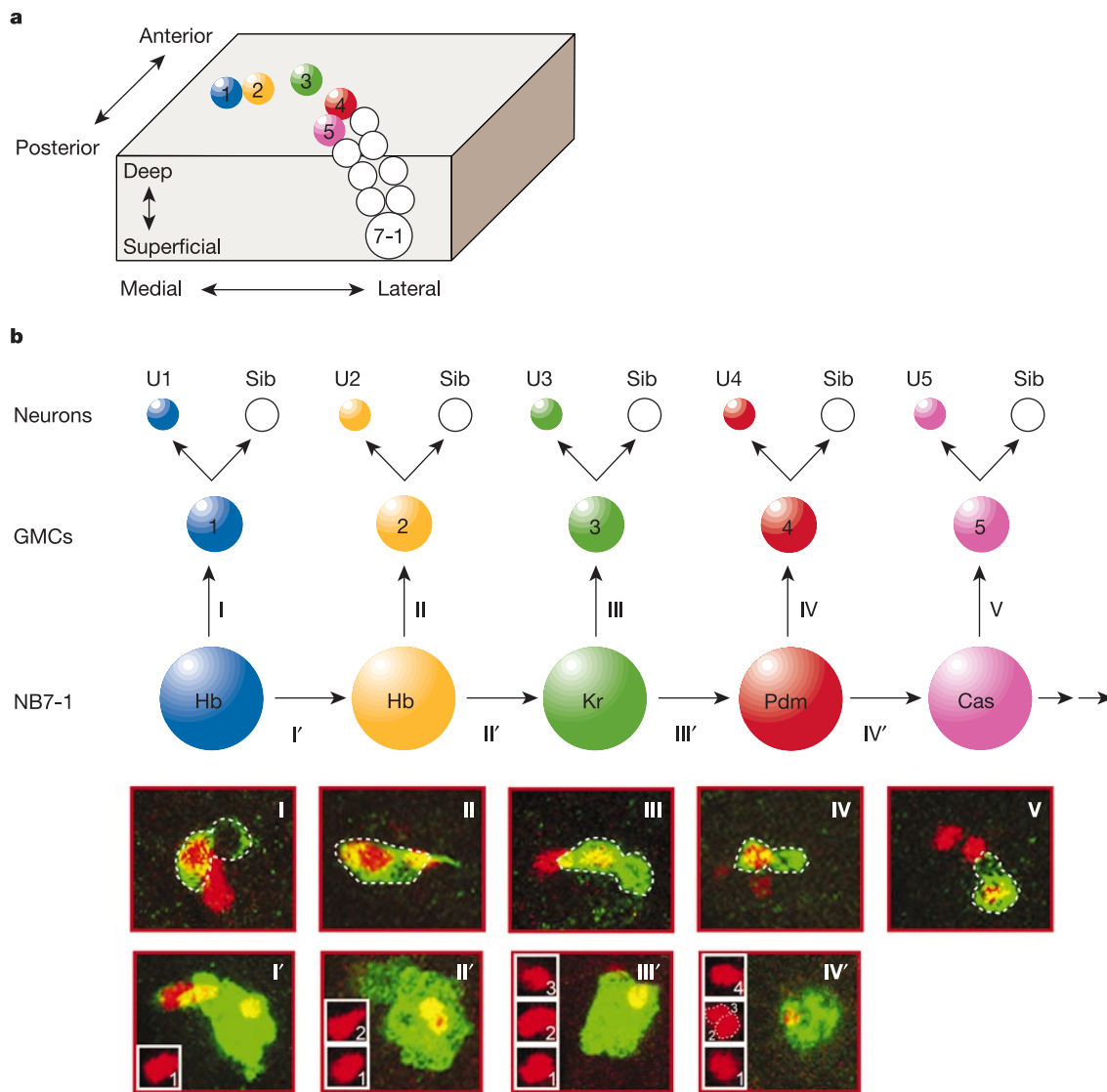


Figure 1 The early lineage of NB7-1. **a**, The *Eve*⁺ U1–U5 motor neurons (1–5) have stereotyped positions within the mature CNS¹⁴. One hemi-segment is shown. **b**, Top: Schematic of the early NB7-1 lineage, based on data shown below. *Eve*⁺ U motor neuron, coloured; *Eve*⁻ sibling cell, white. Bottom: Clonal analysis of the U1–U5 motor neurons (see Methods and Supplementary Movie 1 for details). Anterior, up; medial, left.

Clones induced in GMCs label two sibling neurons (*Tau*:: β gal, green; clone outlined) that always contain one *Eve*⁺ U motor neuron (red) and one *Eve*⁻ sibling cell (I–V). Clones induced in the NB label all progeny generated after the event (*Tau*:: β gal, green; clone outlined) (I'–IV'). Insets show *Tau*::lacZ-negative U1, U2, U3 or U4 motor neurons from the same hemi-segment.

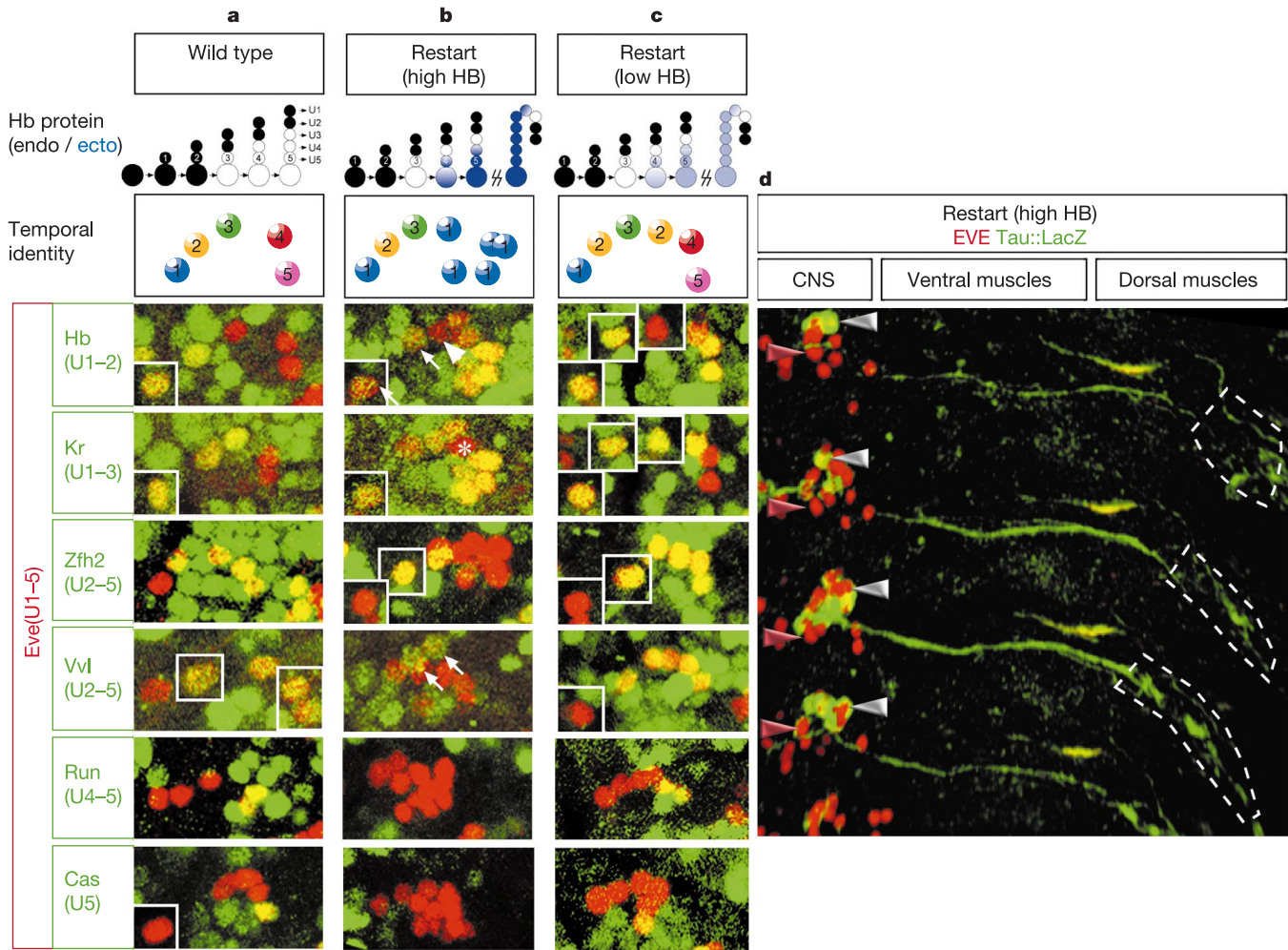


Figure 2 NB7-1 shows plasticity in response to Hunchback. In this figure and in Fig. 3, the top panels summarize Hb expression (endogenous Hb, black; ectopic Hb, blue), middle panels summarize U1–U5 temporal identity based on the data shown below, and bottom panels show the U1–U5 *Eve*⁺ neurons (red) stained for the indicated temporal identity markers (green). All panels show one hemi-segment of a stage-16 CNS (anterior, up; medial, left). **a**, Wild type. **b**, Re-expression of high levels of Hb in an older NB generates ectopic U1 neurons; the genotype is *+ / UAS-hb; prospero-Gal4/UAS-hb* at 23 °C. Ectopic Hb is not detected until after the birth of the Hb-negative U3 neuron (arrowhead), when it is observed in all later-born, lateral positioned U neurons (right side of panel). Asterisk indicates occasional Krüppel-negative U neuron of unknown identity. **c**, Re-expression of

low levels of Hb in an older NB generates ectopic presumptive U2 neurons; the genotype is *+ / +; prospero-Gal4/UAS-hb* at 18 °C. Hb staining was imaged at high gain to clearly document the Hb-positive neurons; the Hb levels are much lower than in **b**. **d**, Ectopic U1 motor neurons project to dorsal muscles, similar to endogenous U1 motor neurons. The U1–U5 marker *Eve* is red, whereas the β gal from an *eve[+ 3.5–4.3]tau::lacZ* transgene does not express β gal (red arrowheads), but the β gal-positive ectopic U1 motor neurons (silver arrowheads) project to dorsal muscles. The transgene also shows ectopic body wall staining (dotted lines). Five segments are shown.

Table 1 Quantification of *hb* misexpression phenotypes in the NB7-1 lineage

Expt	Genotype*	Ectopic Hb levels†	Ectopic Hb timing‡	Number <i>Eve</i> ⁺ U neurons§	U neuron temporal identity					Conclusion
					U1	U2	U3	U4	U5	
1	Wild type	None	NA	5 (100)	1	1	1	1	1	Wild type
2	<i>pros-gal4, 2 × UAS-hb</i> (23 °C)	High	U4 →	9.3 (60)	7.3	1	1	0	0	Old NB is competent; high Hb = U1 fate
3	<i>pros-gal4, 1 × USA-hb</i> (18 °C)	Low	U4 →	6.1 (20)	1	2.1	1	1	1	Old NB is competent; low Hb = U2 fate
4	<i>en-gal4, 2 × USA-hb</i>	High	NB/GMC	16.4 (20)	16.4	0	0	0	0	Mitotic precursors competent
5	<i>eve-gal4, 2 × USA-hb</i>	High	Neurons	5 (30)	1	1	1	1	1	Post-mitotic neurons not competent
6	Wild type with heat shock	None	NA	5 (38)	1	1	1	1	1	Control for heat shock
7	<i>hs-hb</i> (3.5–5.5 h)	Low	U1/2	7.6 (30)	2.3	2.3	1	1	1	NB competent to make early-born fates
8	<i>hs-hb</i> (6.0–8.0 h)	Low	U3/4	5.9 (29)	1	1.9	1	1	1	NB less competent to make early-born fates
9	<i>hs-hb</i> (8.5–10 h)	Low	U4/5	5.4 (22)	1	1.4	1	1	1	NB less competent to make early-born fates
10	<i>hs-hb</i> (10.5–24 h)	Low	INs	5 (27)	1	1	1	1	1	NB not competent; temporal identity fixed

NA, not applicable.

* Genotype and temperature of experiment where relevant.

† Levels of ectopic Hunchback in lineage (qualitative).

‡ Time of ectopic Hunchback in lineage (that is, U4 → indicates Hb expressed in NB7-1 as it generates GMC4/U4 and all subsequent progeny). INs, interneurons.

§ Number per hemi-segment. Numbers in parentheses indicate number of hemi-segments scored.

|| Average number of each cell type, including endogenous U1/U2 neurons, based on markers described in Fig. 2a.

The previous experiments induced Hb at a single point in the NB7-1 lineage, just after U3 is generated. To test the competence of NB7-1 at later points in its lineage, we use a heat-inducible *hsp70-hb* transgene¹⁸ to provide pulses of Hb at progressively later points in the lineage. When the Hb pulse is initiated early in the lineage, when U1 and U2 are normally generated, we detect several ectopic U1 and U2 neurons (Table 1, row 7). When Hb is provided later in the lineage, when U3 and U4 are normally generated, we observe just one ectopic presumptive U2 neuron (Table 1, row 8), and as Hb is

given progressively later, we observe a gradual decline in the production of presumptive U2 neurons, until no response to Hb is detected (Table 1, rows 9 and 10). Interestingly, an early pulse of Hb generates first-born U1 fates, but later pulses generate only presumptive U2 fates; perhaps only the early pulse, which combines induced and endogenous Hb levels, can achieve Hb levels sufficient to generate the U1 fate. We conclude that the NB shows a progressive restriction in its ability to generate early-born fates in response to Hb, consistent with models of progressive restriction proposed for vertebrate neural progenitors⁸.

We next address the question of when temporal identity is fixed during the process of neuronal differentiation. We begin by assaying NB and GMC competence, using *engrailed-Gal4* to limit Hb to NBs, GMCs and young neurons (Fig. 3a, 'early' panel), but not in mature post-mitotic neurons (Fig. 3a, 'late' panel), in the NB7-1 lineage. We observe a massive induction of U1 motor neurons, which stably maintain their U1 molecular marker profile through to the end of embryogenesis without detectable Hb protein (Fig. 3a and Table 1, row 4). To perform the converse experiment, we use *eve-Gal4* to express Hb specifically in post-mitotic U1–U5 motor neurons (Fig. 3b, cartoon). Despite high levels of Hb in these neurons, all U1–U5 cell fate markers remain wild type (Fig. 3b and Table 1, row 5). Similarly, *hsp70-hb*-induced expression in newly post-mitotic neurons has no effect on their temporal identity (Table 1, rows 9 and 10). Thus, transient Hb expression in mitotic progenitors can induce early-born U1 identity, but permanent Hb in post-mitotic neurons does not alter their temporal identity. We currently cannot distinguish whether competence is lost in GMCs or in newly post-mitotic neurons. We conclude that mitotic progenitors, but not mature post-mitotic neurons, are competent to establish early-born neuronal identity in response to Hb.

Our results lead to two major conclusions. First, NBs exhibit both plasticity and progressive restriction during their cell lineage. Older NBs are competent to make early-born neurons in response to Hb, but this competence progressively declines over time (Fig. 4a). Some NBs may lose competence even more rapidly than NB7-1, however, as NB1-1 and NB4-2 show a more limited response to ectopic Hb¹¹. Second, only mitotic progenitors are competent to respond to Hb, and transient Hb in these progenitors is sufficient to establish heritable early-born neuron identity (Fig. 4b). This latter result may reflect the normal function of Hb in the CNS, because Hb is absent from *Eve*⁺ neurons by larval stages, and thus it may normally act to maintain *Eve* expression by inducing a heritable gene expression cascade or by an epigenetic mechanism. Interestingly, transient Hb leads to epigenetic silencing of *HOX* gene expression during *Drosophila* segmentation¹⁹, and the Hb-related Ikaros protein is required to silence gene expression in mature B cells during mammalian haematopoiesis²⁰. In both systems, the Hb/Ikaros proteins physically interact with Mi-2-Polycomb complex proteins

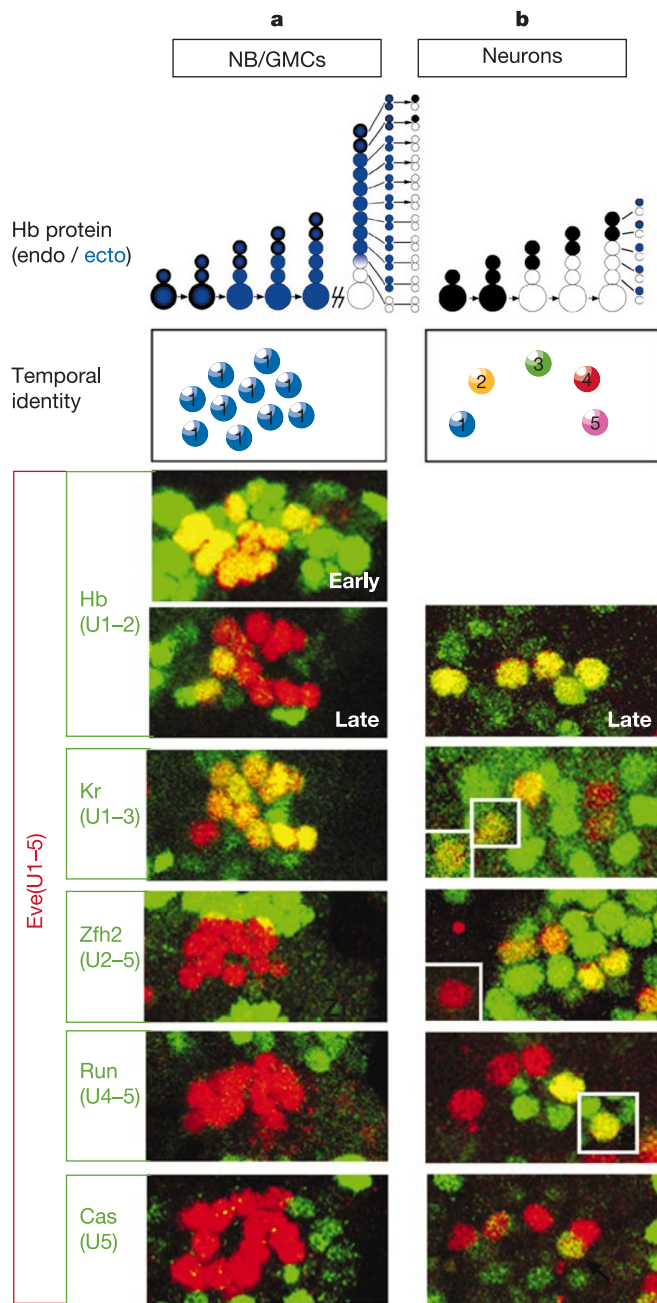


Figure 3 Mitotic progenitors but not post-mitotic neurons show competence to respond to Hunchback. **a**, Hb expression in NBs, GMCs and young neurons (early panel, stage 13 shown); the genotype is *engrailed-Gal4/UAS-hb*; +/*UAS-hb*. A large pool of mature U1 neurons maintain their temporal identity despite the absence of Hb protein (late panel, stage 17 shown). **b**, Hb expression in post-mitotic neurons; the genotype is *eve[+3.5-4.3]-Gal4/UAS-hb*; +/*UAS-hb*. Despite high Hb levels, all U1–U5 markers are expressed normally.

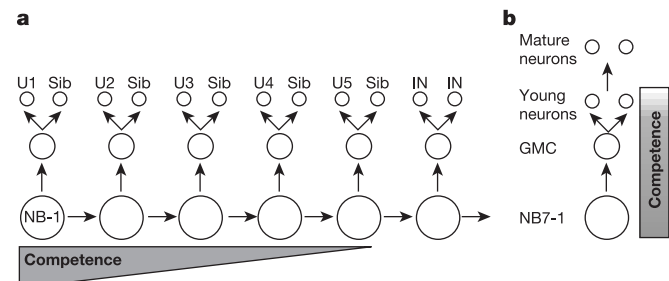


Figure 4 Summary of NB, GMC and neuronal competence to respond to Hb. **a**, NB7-1 shows progressive loss of competence to generate early-born neurons in response to Hb. **b**, The competence to generate early-born neurons in response to Hb is lost by the time a neuron becomes post mitotic.

to induce histone deacetylase-mediated gene silencing^{21,22}. This suggests an attractive model for temporal specification in the *Drosophila* CNS, in which Hb recruits Mi-2-Polycomb to silence genes conferring later-born temporal identity. The molecular basis of competence remains unclear, with one exception: we know that downregulation of Hb triggers progressive loss of competence, because maintaining continuous high Hb levels can indefinitely maintain competence¹¹. Is competence due to the presence of an unknown Hb target and cofactor, or the absence of a negative factor that suppresses early-born fates (such as Klumpfuß²³)? Does loss of competence in old NBs and in differentiating neurons occur by different mechanisms? Is there a distinct, later competence state for specifying subsequent Krüppel-positive temporal identities? *Drosophila* NBs now provide a model system for investigating the molecular nature of neural progenitor competence. □

Methods

We used the following fly stocks: (1) *hsp70-hb* (HB476.1 homozygous on chromosome III); (2) *yw; UAS-hb; UAS-hb*; (3) *yw; +; UAS-hb*; (4) *prospero-Gal4/prospero-Gal4* on chromosome III; (5) *prospero-Gal4/CyO, ftz-lacZ; eve-tau::lacZ/TM3, ftz-lacZ*; (6) *eve[+3.5-4.3]-Gal4/eve[+3.5-4.3]-Gal4* on chromosome II; (7) *engrailed-Gal4/engrailed-Gal4* on chromosome II; (8) *yw, hsp70-FLP* on chromosome X; and (9) *+/Act5c-FRT-stop-FRT-Tau::lacZ, CyO*.

For *hs-hb* experiments, embryos were collected for 1–2 h, aged to the indicated time, and subjected to three cycles of 30 min at 37 °C then 1 h at 22 °C, and allowed to develop to stage 16–17. This generates the maximum Hb level without affecting control embryos; Hb protein is detected throughout the CNS for 4 h after induction. Before heat shock, some embryos were fixed and stained with Eve/Hb for developmental staging. For *hs-FLP* experiments, embryos were subjected to heat shock for 22 min at 37 °C and aged to stage 16–17. Detailed methods available upon request.

All antibodies, staining procedures and imaging methods have been described previously¹¹, except for guinea-pig anti-Runt (1:500) and rat anti-Vvl (1:100) antibodies. All images were collected as confocal image stacks, processed in ImageJ (NIH), and shown as two-dimensional projections. U neurons are shown as insets in their approximate spatial position if they would be obscured in the projection.

Received 14 May; accepted 9 July 2003; doi:10.1038/nature01910.

1. Turner, D. L. & Cepko, C. L. A common progenitor for neurons and glia persists in rat retina late in development. *Nature* **328**, 131–136 (1987).
2. Perron, M., Kanekar, S., Vetter, M. L. & Harris, W. A. The genetic sequence of retinal development in the ciliary margin of the *Xenopus* eye. *Dev. Biol.* **199**, 185–200 (1998).
3. Cepko, C. L., Austin, C. P., Yang, X., Alexiades, M. & Ezzeddine, D. Cell fate determination in the vertebrate retina. *Proc. Natl Acad. Sci. USA* **93**, 589–595 (1996).
4. McConnell, S. K. The determination of neuronal fate in the cerebral cortex. *Trends Neurosci.* **12**, 342–349 (1989).
5. Angevine, J. B. Jr & Sidman, R. L. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192**, 766–768 (1961).
6. Qian, X. *et al.* Timing of CNS cell generation: a programmed sequence of neuron and glial cell production from isolated murine cortical stem cells. *Neuron* **28**, 69–80 (2000).
7. Zhou, Q. & Anderson, D. J. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell* **109**, 61–73 (2002).
8. Desai, A. R. & McConnell, S. K. Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development* **127**, 2863–2872 (2000).
9. Livesey, F. J. & Cepko, C. L. Vertebrate neural cell-fate determination: lessons from the retina. *Nature Rev. Neurosci.* **2**, 109–118 (2001).
10. Rapaport, D. H., Patheal, S. L. & Harris, W. A. Cellular competence plays a role in photoreceptor differentiation in the developing *Xenopus* retina. *J. Neurobiol.* **49**, 129–141 (2001).
11. Isshiki, T., Pearson, B., Holbrook, S. & Doe, C. Q. *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* **106**, 511–521 (2001).
12. Kambadur, R. *et al.* Regulation of POU genes by castor and hunchback establishes layered compartments in the *Drosophila* CNS. *Genes Dev.* **12**, 246–260 (1998).
13. Bossing, T., Udolph, G., Doe, C. Q. & Technau, G. M. The embryonic central nervous system lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. *Dev. Biol.* **179**, 41–64 (1996).
14. Schmid, A., Chiba, A. & Doe, C. Q. Clonal analysis of *Drosophila* embryonic neuroblasts: neural cell types, axon projections and muscle targets. *Development* **126**, 4653–4689 (1999).
15. Landgraf, M., Bossing, T., Technau, G. M. & Bate, M. The origin, location, and projections of the embryonic abdominal motoneurons of *Drosophila*. *J. Neurosci.* **17**, 9642–9655 (1997).
16. Skeath, J. B. & Doe, C. Q. Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* **125**, 1857–1865 (1998).
17. Buenzow, D. E. & Holmgren, R. Expression of the *Drosophila* gooseberry locus defines a subset of neuroblast lineages in the central nervous system. *Dev. Biol.* **170**, 338–349 (1995).
18. Struhl, G., Johnston, P. & Lawrence, P. A. Control of the *Drosophila* body pattern by the hunchback morphogen gradient. *Cell* **69**, 237–249 (1992).
19. Zhang, C. C. & Bienz, M. Segmental determination in *Drosophila* conferred by hunchback (hb), a repressor of the homeotic gene Ultrabithorax (Ubx). *Proc. Natl Acad. Sci. USA* **89**, 7511–7515 (1992).
20. Sabbatini, P. *et al.* Binding of Ikaros to the lambda5 promoter silences transcription through a mechanism that does not require heterochromatin formation. *EMBO J.* **20**, 2812–2822 (2001).

21. Kehle, J. *et al.* dMi-2, a hunchback-interacting protein that functions in polycomb repression. *Science* **282**, 1897–1900 (1998).
22. Kim, J. *et al.* Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. *Immunity* **10**, 345–355 (1999).
23. Yang, X., Bahri, S., Klein, T. & Chia, W. Klumpfuß, a putative *Drosophila* zinc finger transcription factor, acts to differentiate between the identities of two secondary precursor cells within one neuroblast lineage. *Genes Dev.* **11**, 1396–1408 (1997).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank J. Posakony, M. Fujioka and J. Jaynes for fly stocks; W. Odenwald and M. Lundell for antibodies; and R. Grosskortenhaus, M. Freeman, M. Rolls, T. Isshiki, M. Westerfield, T. Brody and W. Odenwald for comments. This work was funded by NIH and HHMI, where C.Q.D. is an Investigator.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to C.Q.D. (cdoe@uoneuro.uoregon.edu).

Basal body dysfunction is a likely cause of pleiotropic Bardet–Biedl syndrome

Stephen J. Ansley^{1*}, Jose L. Badano^{1*}, Oliver E. Blacque^{3*}, Josephine Hill⁴, Bethan E. Hoskins^{1,4}, Carmen C. Leitch¹, Jun Chul Kim³, Alison J. Ross⁴, Erica R. Eichers⁵, Tanya M. Teslovich¹, Allan K. Mah³, Robert C. Johnsen³, John C. Cavender⁷, Richard Alan Lewis^{5,6}, Michel R. Leroux³, Philip L. Beales⁴ & Nicholas Katsanis^{1,2}

¹Institute of Genetic Medicine, and ²Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland 21287, USA

³Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada

⁴Molecular Medicine Unit, Institute of Child Health, University College London, London WC1N 1EH, UK

⁵Departments of Molecular and Human Genetics, and ⁶Departments of Ophthalmology, Pediatrics, and Medicine, Baylor College of Medicine, Houston, Texas 77030, USA

⁷King Khaled Eye Hospital, Riyadh, 11462, Saudi Arabia

* These authors contributed equally to this work

Bardet–Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized primarily by retinal dystrophy, obesity, polydactyly, renal malformations and learning disabilities. Although five BBS genes have been cloned^{1–6}, the molecular basis of this syndrome remains elusive. Here we show that BBS is probably caused by a defect at the basal body of ciliated cells. We have cloned a new BBS gene, *BBS8*, which encodes a protein with a prokaryotic domain, *pilF*, involved in pilus formation and twitching motility. In one family, a homozygous null *BBS8* mutation leads to BBS with randomization of left–right body axis symmetry, a known defect of the nodal cilium. We have also found that *BBS8* localizes specifically to ciliated structures, such as the connecting cilium of the retina and columnar epithelial cells in the lung. In cells, *BBS8* localizes to centrosomes and basal bodies and interacts with PCMI, a protein probably involved in ciliogenesis. Finally, we demonstrate that all available *Caenorhabditis elegans* BBS homologues are expressed exclusively in ciliated neurons, and contain regulatory elements for RFX, a transcription factor that modulates the expression of genes associated with ciliogenesis and intraflagellar transport.

BBS exhibits substantial genetic heterogeneity and, although typically inherited in an autosomal recessive pattern, in some