

Asymmetric Numb Distribution is Critical for Asymmetric Cell Division of Mouse cerebral cortical stem cells and neuroblasts

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Background

- Few cells make many different types of cells.
 - E10 mice only have single layer of proliferating cells
- Understand the mechanisms that allow diversification
 - Understand normal neural development
 - Repair damaged nervous system

Invertebrate model

- Numb causes asymmetric divisions in both PNS and CNS
 - Loss of Numb function produces identical daughter cells
- However Numb does not confer a specific fate
- Fate lineage trees of mice cells are similar to those of *Drosophila*
 - Evolutionarily conserved mechanism

Vertebrate Models

- Numb homologue found in chicken, rat, mouse, human
 - Mouse Numb can rescue fly Numb mutant
- Contradictory findings
 - Over expression *in vivo* in chick CNS cause progenitor proliferation
 - Over expression *in vitro* leads to increased neuronal production
 - Multiple roles of numb during development?
- Asymmetric Numb distributions are found, but does this lead to different cell fates?

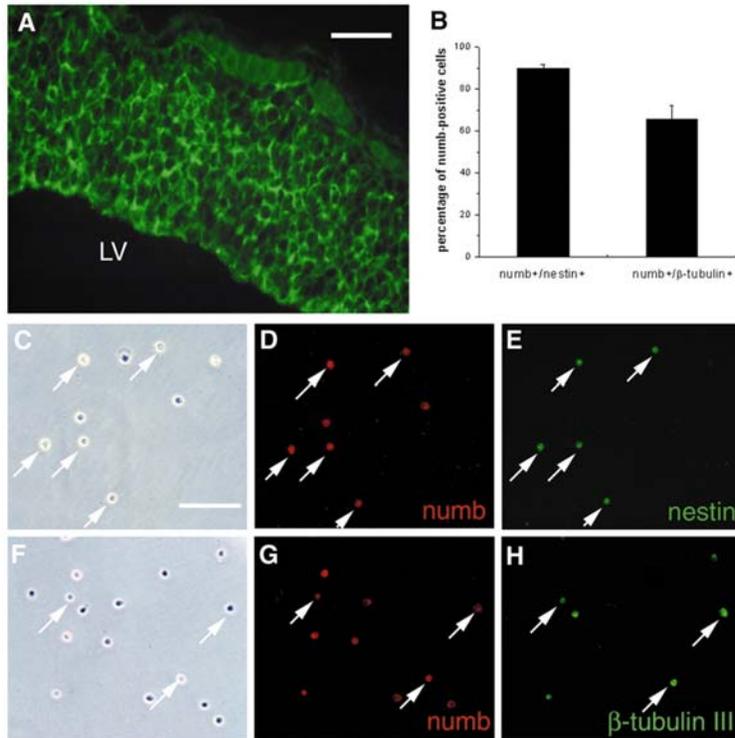
Methods

- Mouse embryos E10-E14
- Used low density cultures to watch cell division
- Video Microscopy
- Used immunohistochemistry to show Numb, Nestin, Beta-Tubulin-III, LeX
- Morphological measurements
 - process number, lengths, branch points, etc.

Results

- Asymmetric Numb distribution is necessary for asymmetric cell division
- Numb distribution changes at different developmental stages
- Numb levels influence morphology of symmetrical cell pairs

Numb expression

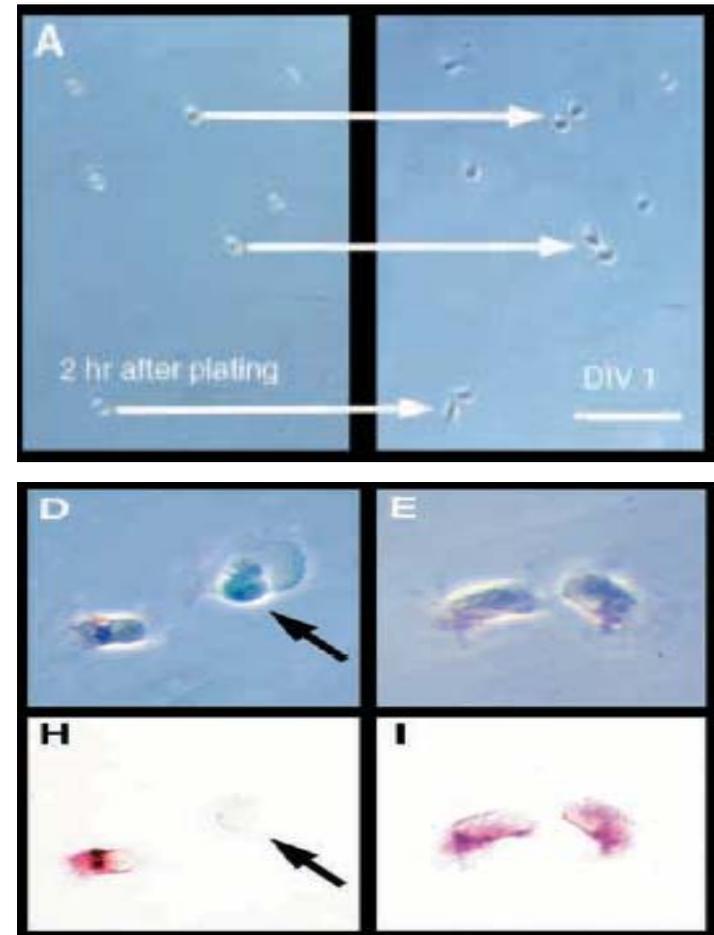


- Numb is expressed throughout cortical neuroepithelium
- Numb is expressed in cells with:
 - Nestin 90%
 - LeX 83%
 - Beta-tubulin-III⁺ 65%

Source: Shen, Q., W. Zhong, Y. N. Jan, and S. Temple. "Asymmetric Numb Distribution is Critical for Asymmetric Cell Division of Mouse Cerebral Cortical Stem Cells and Neuroblasts." *Development* 129 (2002): 4843 - 4853. Courtesy of The Company of Biologists. Used with permission.

Numb Distribution

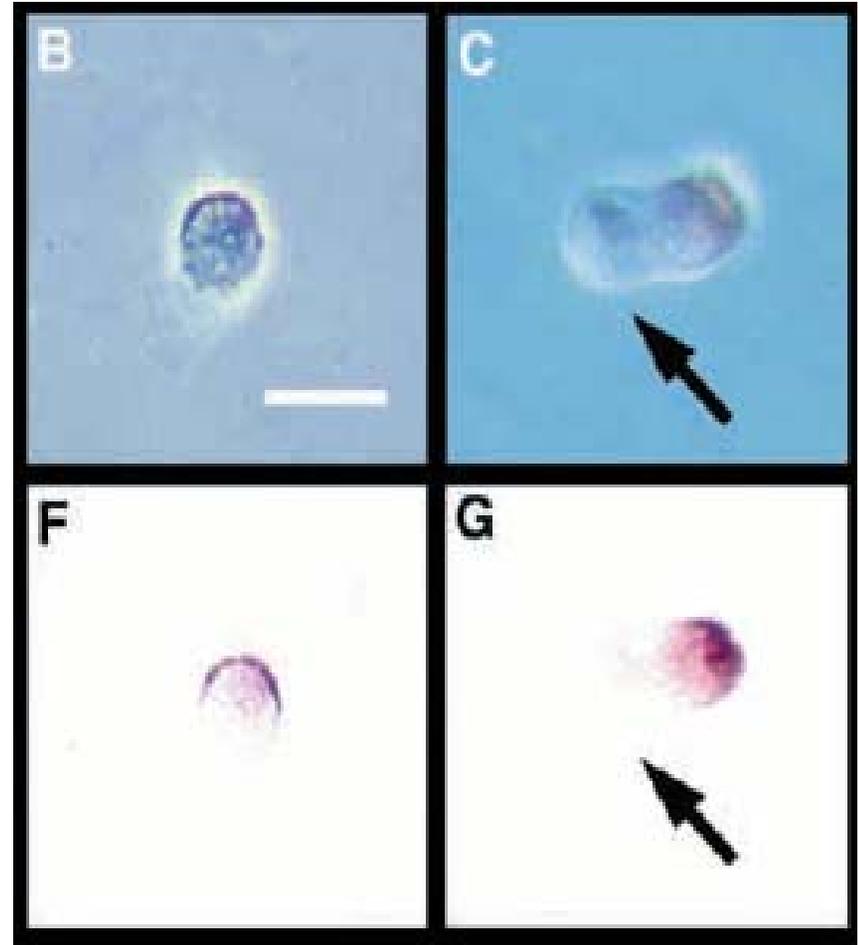
- Some pairs Numb only expressed in one cell (Asymmetric)
- Other pairs both show Numb (symmetric)
- Symmetric cells show similar Numb amounts



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Numb Distribution

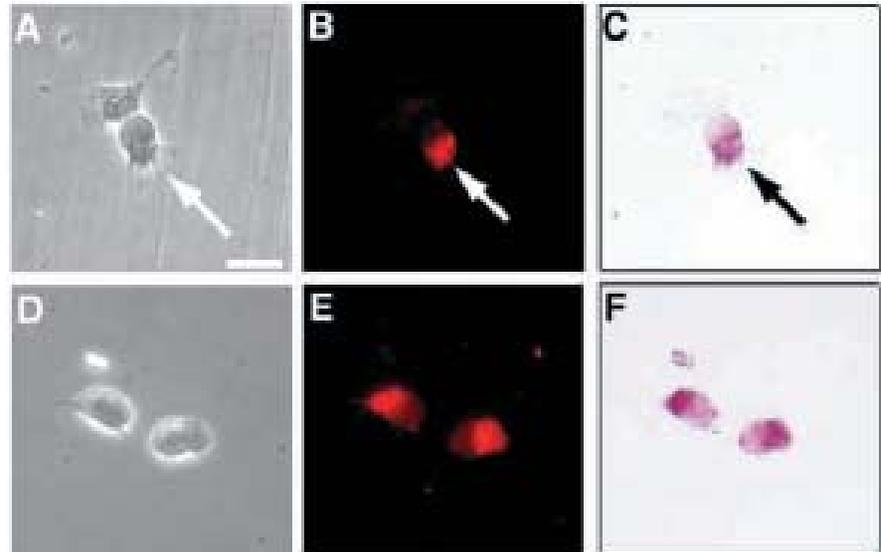
- 3 options
 - Asymmetric segregation
 - De novo synthesis
 - Degradation
- Since its seen only in one cell so closely after mitosis, likely to be segregation



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Asymmetric Numb and Cell Divisions

- Progenitor/Neuron (P/N) asymmetric cell division
- P/N pairs show asymmetric Numb levels (top row)
- N/N pairs show symmetric Numb levels (bottom row)



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Fewer P/N pairs in Numb knockout

- Numb knockout mice die ~E11.5
- E10 progenitor pairs have impaired asymmetric division

Table 3. *m-numb* mutation reduces the number of cortical asymmetric P/N divisions

	Asymmetric P/N	Symmetric	
	pairs 		
Wild-type (839 pairs)	15.73%	37.55%	46.72%
Mutant (624 pairs)	8.17%**	37.98%	53.85%*

 β -tubulin III⁺ daughter cell.

** $P < 0.001$, * $P < 0.01$, χ^2 test.

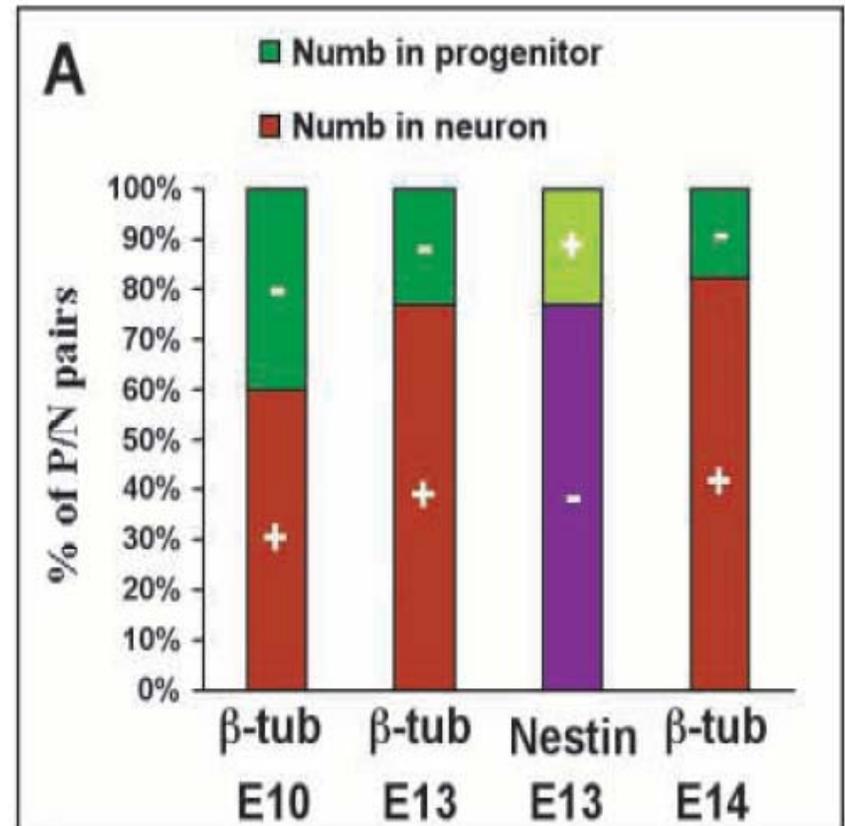
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Results

- Asymmetric Numb distribution is necessary for Asymmetric cell division
- **Numb distribution changes at different stages**
- Numb levels influence morphology of symmetrical cell pairs

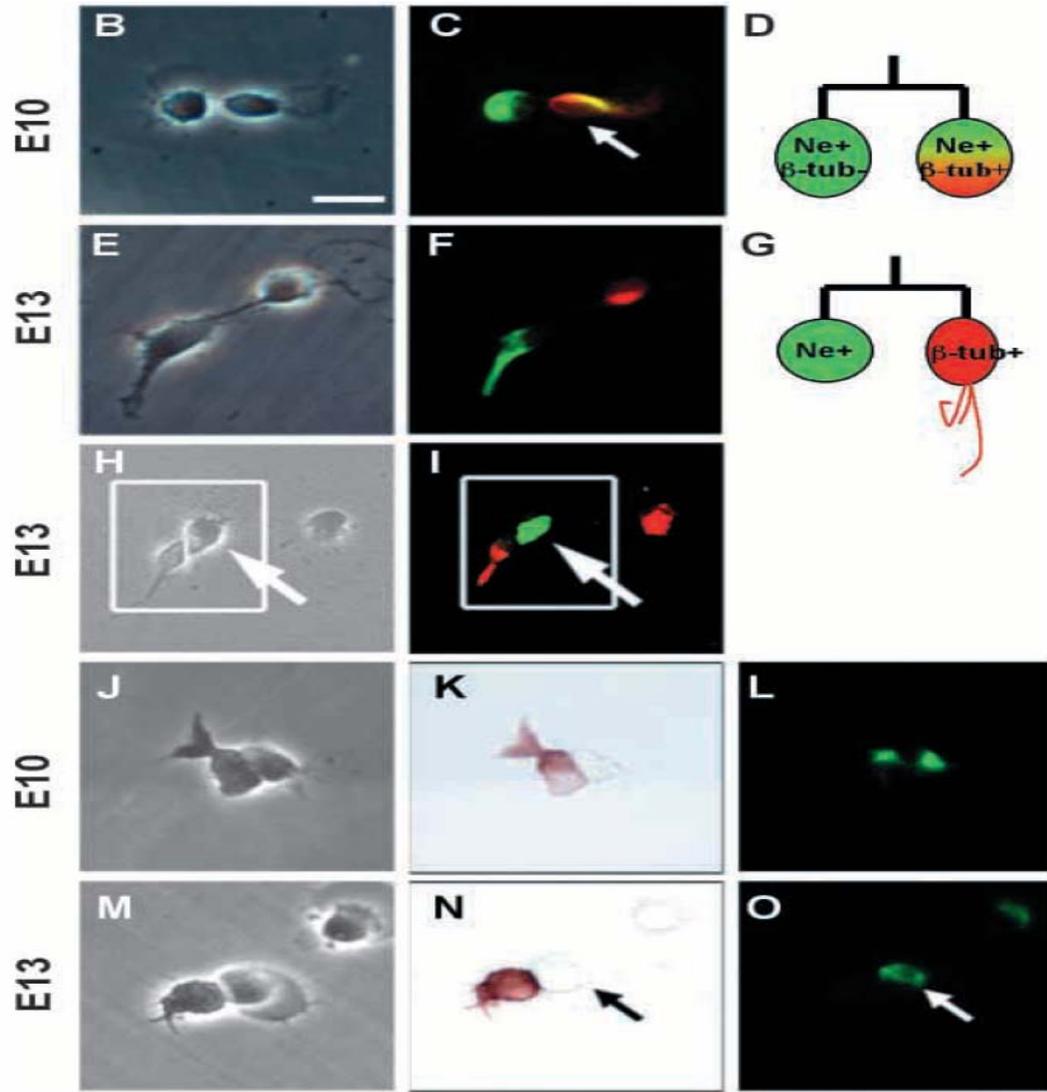
Developmental change in Numb Distribution

- E10 cells
 - Both P/N daughters are Nestin+
 - Asymmetric Numb goes to either P or N
- E13
 - Nestin+ only in P cell
 - Numb mostly in the N cell



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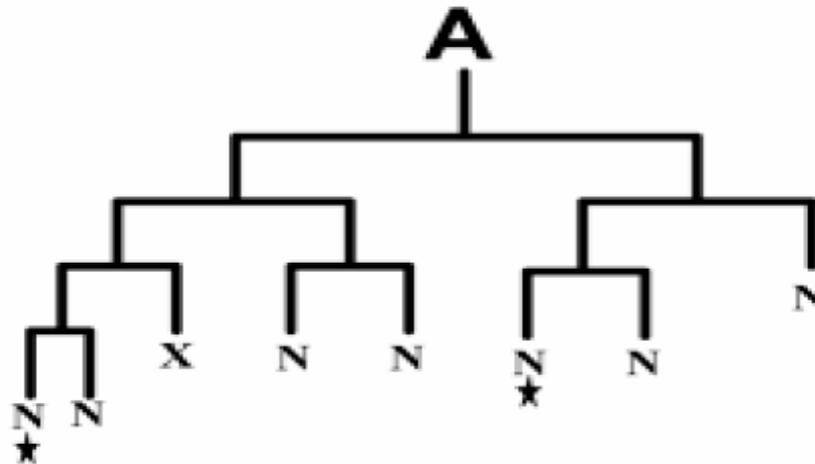
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Results

- Asymmetric Numb distribution is necessary for Asymmetric cell division
- Numb distribution changes at different stages
- **Numb levels influence morphology of symmetrical cell pairs**

Numb influences Morphology

- Numb is expressed in N/N pairs both symmetrically (80%) and asymmetrically (20%)



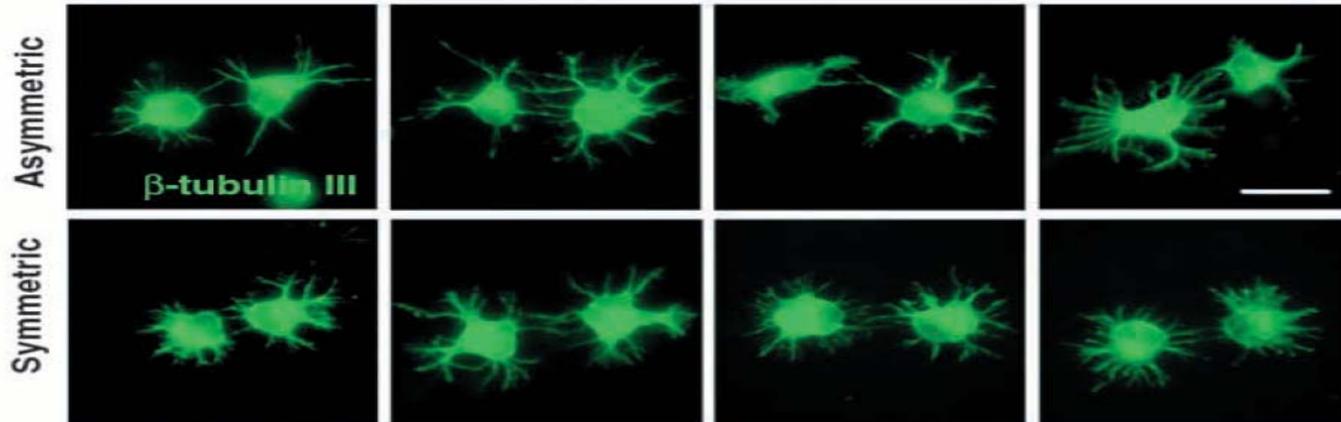
- Does this asymmetry affect morphology?

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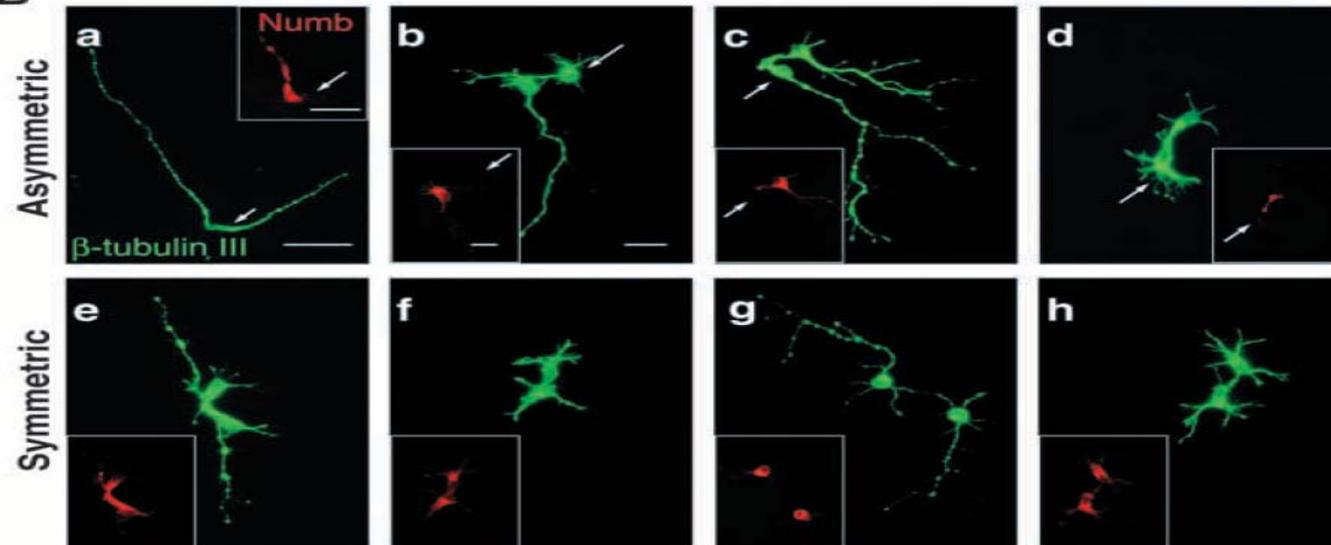
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Yes it does.

A



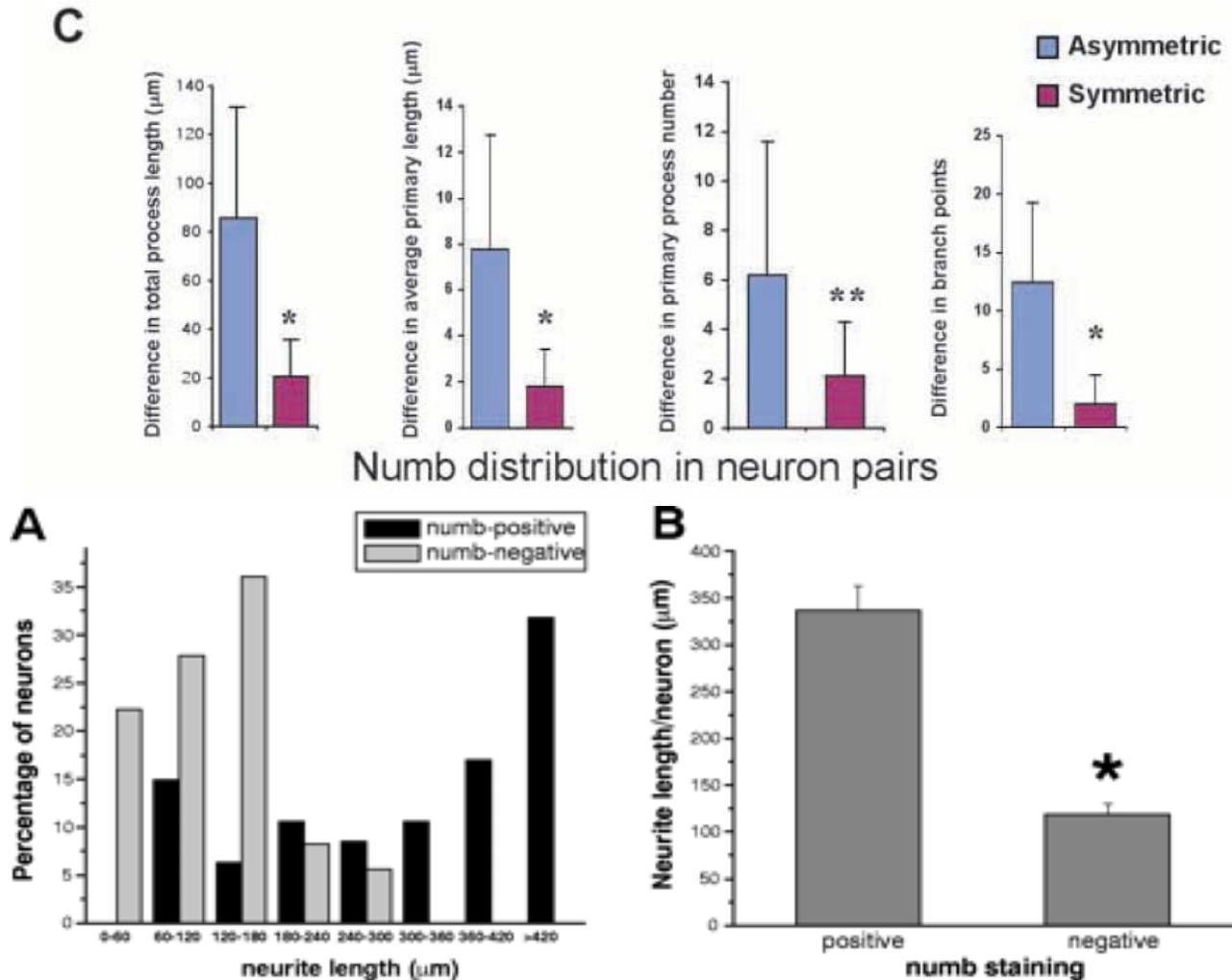
B



Source: Shen, Q., W. Zhong, Y. N. Jan, and S. Temple. "Asymmetric Numb Distribution is Critical for Asymmetric Cell Division of Mouse Cerebral Cortical Stem Cells and Neuroblasts." *Development* 129 (2002): 4843 - 4853.

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Quantitative Morphology



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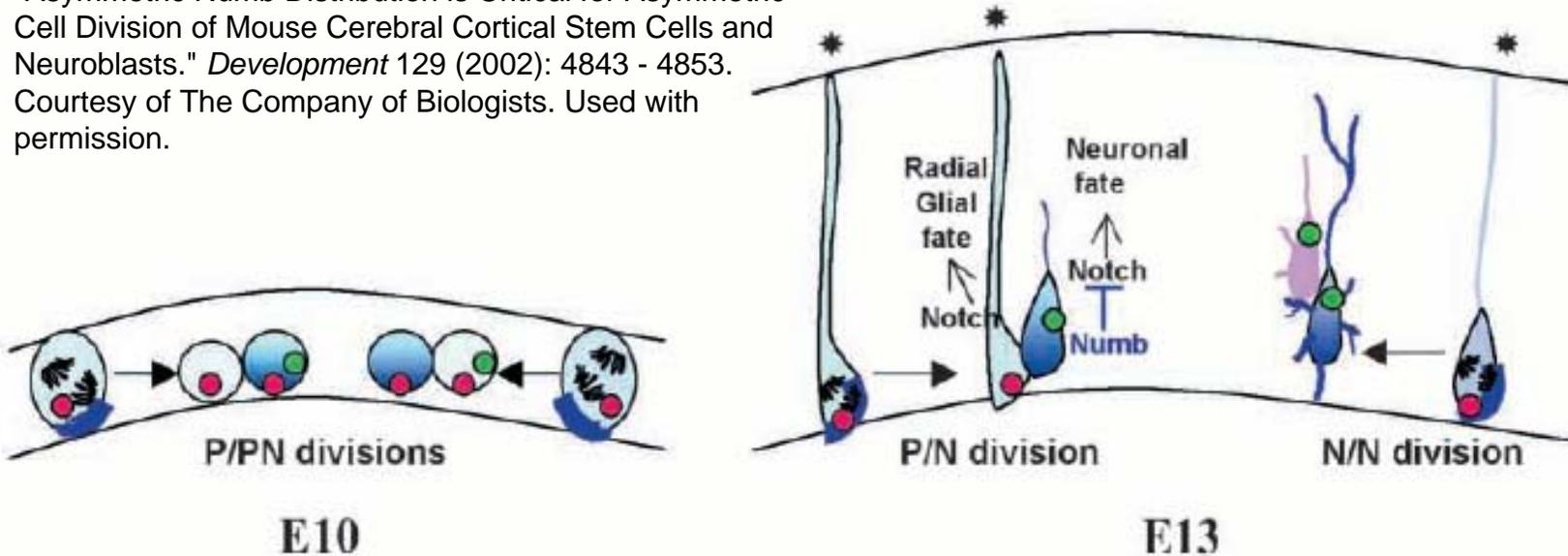
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Recap

- Numb can be symmetrically or asymmetrically distributed
 - Distribution is necessary
- At different times during growth Numb has different effects
 - E10 Numb goes to either P or N cell
 - E13-14 Numb chooses N cell
- Morphology is affected by Numb distributions

Numb Model

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- β -tubulin III
- Nestin
- Numb

- P=progenitor
- N=neuron
- PN=neuronal progenitor
- *= radial glial progenitor

Fig. 8. Model of Numb function during cortical development. β differences between sister cells at different stages of development generates different Nestin⁺ progenitor cells. At E13 progenitor divisions (not shown) Numb generates asymmetric β may function by inhibiting Notch activity in some or all of these during E13 P/N divisions Numb may inhibit Notch activity in a cell to make it differentiate into a neuron. At N/N divisions, Numb daughter to generate a different neuron type.