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# 1 **Timing neurogenesis: a clock or an algorithm?**

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6

## 7 **Abstract**

8 Emerging evidence supports the existence of dedicated molecular mechanisms under  
9 evolutionary selection to control time during neurogenesis. Here, we briefly review these  
10 mechanisms and discuss a potentially useful conceptual framework inspired by Computer  
11 Science to think about how these biological mechanisms operate during brain development  
12 and evolution.

## 13 **Main text**

14 In a fundamental sense, time represents the continuous progression of existence and events. It  
15 unfolds moment by moment and permits a structured chronology where events occur in a  
16 specific sequence, not randomly or all at once. This framework allows events to be ordered  
17 from the past through the present into the future, which in turn helps us understand causal  
18 chains where causes precede effects.

19 In developmental biology, the concept of time is closely intertwined with the pace of  
20 embryonic development. This connection can be viewed in at least two ways: the specific  
21 sequence of events that occur in a particular chronological order as well as the total amount of  
22 time this sequence occupies. Both of these dimensions are found in one of the most studied  
23 and evolutionarily conserved biological mechanisms, neurogenesis, the generation of neurons  
24 from neural progenitor cells (NPCs). Not only are different neuronal subtypes generated at  
25 different time points in a specific order, but the total amount of time dedicated to  
26 neurogenesis is itself a species-feature of brain development. Intriguingly, while the  
27 sequential stages of brain development share ancestral blueprints, the temporal dynamics can

28 diverge considerably among species. The human brain, for example, undergoes a particularly  
29 extended period of neurogenesis compared to rodents and even other primates, allowing for a  
30 disproportional increase in the number of neurons, especially in the cerebral neocortex [1],  
31 [2]. Thus, there is a correlation between differences in timing and brain size, presumably  
32 intimately linked to differences in cognitive capabilities.

33 This review will discuss the temporal dynamics of neurogenesis and the factors that drive  
34 neural progenitor cells (NPCs) to their transition into post-mitotic neurons. We will explore a  
35 conceptual framework for understanding how and why changes in timing arose during  
36 evolution and what this framework predicts as possible future experimental avenues.

37 • **Time in cell state transitions and its control through intrinsic and extrinsic cues**

38 Classical analyses using immunohistochemistry and, more recently, single-cell  
39 transcriptomics have revealed that distinct cellular states are associated with specific gene  
40 expression patterns. Therefore, the acquisition of a specific cellular identity requires a  
41 remodeling of these gene regulatory networks (GRNs) that underlie cell state-specific  
42 expression patterns. Studies across species and neural tissue types, from fruit flies to human *in*  
43 *vitro* models, have shown that the commitment of a NPC to neurogenesis is influenced by  
44 internal and external cellular cues that act on its GRN to determine whether it will continue to  
45 self-renew as a progenitor or differentiate into a neuron. One of the causal molecular  
46 mechanisms of this dynamic equilibrium between the progenitor and neuron states is  
47 mediated by feedback regulation between the Notch signaling pathway and a class of basic  
48 Helix-Loop-Helix (bHLH) transcription factors called proneural proteins [3]. When the  
49 proneural protein is maintained and stabilized in a NPC, it will overcome the anti-  
50 differentiation signal of the Notch pathway and promote neurogenesis ([3]–[5]). Additionally,  
51 in the local environment of these NPCs, many secreted morphogens by specific brain regions  
52 and the NPCs themselves with their neuronal progeny will weigh on this balance. For  
53 example, WNT, FGF, and Shh, play a significant role in maintaining progenitors' self-  
54 renewal through different processes to repress proneural genes (reviewed in [6]). In other  
55 words, controlling the temporal dynamics of the expression and activity of transcription  
56 factors by intrinsic and extrinsic cues can significantly impact the timing of the switch from  
57 progenitor self-renewal to differentiation and, thus, the time available for neurogenesis. In the  
58 case of the particularly slow pace of human cortical neurogenesis, it has been shown that a  
59 human-specific variant of the Notch protein contributes to the developmental tempo by

60 intrinsically promoting increased levels of Notch activity in progenitors, presumably  
61 inhibiting proneural protein expression [7\*\*]. On the other hand, the conserved Amyloid  
62 Precursor Protein involved in Alzheimer's disease is required in human cortical NPCs to  
63 mediate extrinsic WNT signaling to delay the NPC to neuron transition, and this correlates  
64 with reduced expression of the proneural protein Neurogenin2 [8\*\*]. Finally, evidence shows  
65 that the rate of degradation of transcription factors involved in neurogenesis is slower in  
66 humans than in mouse NPCs [9\*\*]. There is also evidence that more stable or longer-lived  
67 proneural proteins correlate with [10] and can cause [11\*] an increase in neuronal production.  
68 Emerging evidence also causally implicates mitochondrial metabolism in the NPC-neuron  
69 fate decision [12]. Whether this is mechanistically linked to changes in transcription factor  
70 activity or stability remains unclear. Interestingly, however, stem cell programming into  
71 neurons by Neurogenin2 overexpression results in large-scale remodeling of cellular  
72 organelles and proteomes, suggesting that a link may also exist during normal neurogenesis  
73 [13\*].

74 Overall, these examples support the view that the temporal dynamics of neurogenesis is  
75 controlled mainly by cell-intrinsic mechanisms that appear to converge on regulating the  
76 expression and activity levels of transcription factors and that these mechanisms operate on  
77 different time scales in different species. However, this only pushes the question from why  
78 the phenotypes (i.e. neurogenesis) emerge on different time scales to why the intrinsic cellular  
79 mechanisms operate on different time scales. This question is particularly intriguing because  
80 the operant mechanisms discovered so far are highly conserved across eukaryotes. Although  
81 mitochondrial homeostasis and metabolism or protein turnover seem to be slower in humans  
82 than in mice, for example, there is no evidence that the highly conserved enzymes that carry  
83 out these reactions have intrinsically slower substrate processing rates. Finally, given that all  
84 these mechanisms appear to converge on the activity of transcription factors that are also  
85 themselves highly conserved, the conundrum seems even more perplexing. One way to think  
86 about the problem from a broader perspective is to shift from a mechanistic view to a  
87 theoretical view in order to provide a general framework into which the currently understood  
88 mechanisms would fit. This would guide future hypotheses that could be tested with  
89 mechanistic approaches. What might such a theoretical framework look like?

90 **Time from Computer Science to Biology**

91 Perhaps our human conception of time as something to be measured with clocks misleads our  
92 understanding of developmental time. In Computer Science, time is a measure of  
93 computational complexity. It describes the number of elementary operations an algorithm  
94 uses to process a set of input data. Interestingly, this measurement, known as time complexity,  
95 characterizes how the execution time of an algorithm increases as a function of the size of the  
96 input. The larger the input data set, the longer an algorithm will take to complete its  
97 processing.

98 To translate this concept to biology, one needs to begin by defining what constitutes an  
99 algorithm and what constitutes the source of data. We propose that an operationally useful  
100 way of thinking about this is to consider the sum of all cellular biological reactions as the  
101 algorithm. After all, during development, various proteins literally “process” input in the  
102 shape of other biomolecules or metabolites to produce a cell state transition as a phenotypic  
103 output. More importantly, the genome is the only source of biological data for which we have  
104 evidence supporting a causal role in generating evolutionary change. As we have argued  
105 above, and as decades of molecular genetic mechanistic studies have shown, the biochemistry  
106 of fundamental cellular processes, that is to say, the algorithm in this metaphor, is highly  
107 conserved. What is changing is the data in the shape of evolutionary innovations in the  
108 content of genomic information. A particularly striking example in the context of  
109 neurogenesis and neuronal differentiation comes from the transplantation of human cortical  
110 pyramidal neurons into the neonatal mouse brain, which develops much faster than the human  
111 brain. What these studies show is that the maturation of the human neurons in the mouse brain  
112 takes up to a year [14], [15] suggesting that the mechanisms underlying the developmental  
113 timing are cell intrinsic and encoded by the human genome. A last important angle to consider  
114 is the processing modality of the cellular algorithm. Most, if not all, cellular interaction  
115 networks are partially promiscuous. This is referred to as “many-to-many” interaction  
116 networks, and they are very prevalent in ligand-receptor and transcription factor dimerization  
117 networks, for example, including for key neurogenesis pathways such as WNT, Notch and the  
118 proneural proteins. It has recently been argued that such networks are both more robust and  
119 have greater computational power [16\*] than highly specific, one-to-one interaction networks,  
120 likely explaining why they have been favored by evolution. Furthermore, just like for an  
121 algorithm, several biological processes run in parallel but converge on a terminal step that  
122 induces the transition to the next state, such as proneural protein levels and activity in NPCs  
123 (see next paragraph). This terminal step acts as a coincidence detecting gatekeeper ensuring

124 that different aspects of cellular metabolism are all compatible with the impending state  
125 transition.

126 In summary, a potentially helpful way to think about how the temporal dynamics of biological  
127 processes operate is to assume that a highly conserved algorithm of flexible biochemical  
128 interactions processes a variable amount of data encoded by the genome. In this conception,  
129 the differences in developmental timing would arise principally from differences in the  
130 amount of information encoded by the genome.

131

132 • **Sources of differences in the amount of genomic information**

133 One important source of increase in genomic information during evolution is gene  
134 duplication, a major evolutionary driving force [17]. Interestingly, in *Drosophila*, where  
135 proneural genes were discovered, there are 58 bHLH genes whereas there are 39 in *C. elegans*  
136 and 125 in humans [18], [19]. Moreover, these transcription factors induce the expression of  
137 Notch ligands such as Delta and Serrate in flies. Here again, we observe an evolutionary  
138 change in the number of proteins. In flies, there are two ligands of Notch, Delta and Serrate,  
139 while in vertebrates there are 5 known ligands: Delta like DLL1, DLL3, DLL4 and the Jagged  
140 family composed of Jagged1 and Jagged2. In addition, the number of Notch receptors has  
141 been multiplied. In flies, there is a single Notch receptor, while in humans, there are 4  
142 (Notch1-4) [20]. These ligands and receptors are expressed in complex overlapping spatio-  
143 temporal patterns. Altogether, these duplications of many genes multiply the possible  
144 interactions between ligands and receptors. The increased complexity in these ligand-receptor  
145 interactions carrying both activation and inhibition signals can create delays in signal  
146 interpretation into a specific pattern of effector activation [21]. Furthermore, different ligands  
147 can transmit opposing patterning information [22] and thus their co-expression would also  
148 require more time for that information to be resolved into a fate decision. Finally, many  
149 molecular interactions, especially ligand-receptor interactions and gene activation by  
150 proneural proteins, are quantitative in that they depend on the absolute amount of active  
151 protein. Because levels of any biomolecule at any time are subject to variation due to both  
152 stochastic and random noise, the greater the number of proteins in an interaction network, the  
153 more noise in the network and, thus, the longer it may take for efficient signal transmission  
154 [23]. Having a single Notch receptor and a single ligand makes the signal transmission direct.

155 As discussed above, gene duplication in humans appears to have been taken to a new level  
156 with entire families of rapidly evolving genes dedicated to the attenuation of neurogenic  
157 signals, as in the example of Noch2NL [24\*\*].

158 Other potential sources of increase in information are gene regulatory elements, notably  
159 highly conserved enhancer/repressor sequences that have been subject to variation only in the  
160 human lineage called Human Accelerated Regions (HARs). Interestingly, more than half of  
161 these regions tested in vitro displayed enhancer activity, specifically in NPCs [25]–[27] such  
162 as HAR5 that enhances WNT signaling through FZD8 to promote NPCs self-renewal [28].  
163 Another source of quantitative regulation of protein levels is post-transcriptional control by  
164 noncoding RNAs, considered as another driver of brain evolution. They represent 10 to 15%  
165 of the human genome [29]. There is almost twice the number of miRNAs in humans than in  
166 mice and six times that in *Drosophila* [30]. For example, the miR-2115, a great ape-specific  
167 miRNA, promotes NPCs proliferation by targeting the ancient gene *ORC4* mutations which  
168 cause the Meier-Gorlin microcephalic syndrome [31\*], [32]. Furthermore, a primate lncRNA  
169 mediates Notch signaling during neuronal development by sequestering miRNA, promoting  
170 NPCs self-renewal [33]. The increase in the number of quantitative post-transcriptional  
171 inhibitors of neurogenic proteins would be expected to delay the accumulation of these  
172 proteins to functional levels and thus delay cell state transitions that depend on these proteins.

173 In summary, the combination of gene duplication and increase in the number of regulators  
174 increases both the total amount of information the cellular algorithm must process as well as  
175 the amount of noise in this information. The combined effect is to lengthen processing time  
176 and thus delay the time interval of cell state transitions during neurogenesis.

177 • **Time, robustness, and size**

178 Why would natural selection have favored these temporal delays over evolutionary time, in  
179 the primate and specifically human lineage for example? Needless to say, evolution does not  
180 have a plan to produce humans. It must, therefore, be that such a delay in neurogenesis  
181 confers a selective advantage for the fitness of the organism. One argument for which there is  
182 both experimental and theoretical evidence is that the increase in the number of genes, the  
183 promiscuity of protein interactions, and the stochastic noise in molecular processes all confer  
184 greater robustness on cell state transitions and developmental patterning [16\*], [23] in this  
185 scenario, the principal selection pressure is on robustness and the increase in neurogenic time

186 is an unintended but inevitable consequence; an exaptation [34] in the “Gouldian” sense,  
187 rather than an adaptation. However, it can also be argued that increasing the amount of time a  
188 neural progenitor remains in a progenitor state increases the number of neurons it can  
189 generate, and this, in turn, results in increasing the brain’s adaptative cognitive capabilities. In  
190 this scenario, the random accident of gene duplication, for example, gave rise to time delay,  
191 which gave rise to an increase in brain size with increased cognitive capabilities, and it is this  
192 that confers the selective advantage [35]. The truth most probably is that both views are  
193 correct. Exaptation and adaptation are synergetic, and both processes can be seen at work at  
194 organismal, cellular, and subcellular levels in evolutionary history [36].

195

## 196 **Conclusion**

197 In this review, we hypothesized that the amount, rather than the type, of information encoded  
198 by the genome controls developmental time, with a focus on neurogenesis and its mechanisms  
199 as an example. Using a concept borrowed from computer science, we suggest that  
200 fundamental cellular metabolism, the *type* of information in this metaphor, constitutes a  
201 conserved algorithm that processes information at a conserved pace. In contrast, the total  
202 amount of molecules and their interactions constitute the data that this algorithm must  
203 process, and the *amount* of this data dictates the time necessary for a developmental process  
204 like neurogenesis to be completed. The emerging evidence that conserved processes and  
205 genes can have different effects on neurogenesis in different species [8\*\*], [9\*\*] by altering  
206 time is testament to the key importance of temporal regulation in brain evolution. If the  
207 genomic changes that drive brain evolution have the main effect of altering developmental  
208 time, it can be reasonably argued that temporal patterning is the main feature of the genetic  
209 code, and it is related to the total amount of “data” encoded by the genome. To test this idea,  
210 future experiments and modeling efforts should be directed towards greater accuracy in the  
211 measurement of time in molecular processes at high resolution during cell state transitions and  
212 deciphering the causal effects of quantitative changes in protein levels on the timing, size and  
213 complexity of neurogenic processes.

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219 **Author contributions:**

220 J.P. and B.A.H conceived of and wrote the manuscript. J.P. came up with the idea of  
221 comparing biological processes to an algorithm.

222 **Competing interest:**

223 The authors declare no competing interests.

224

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