Maintaining a memory by transcriptional autoregulation

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One of the key features of cellular differentiation programs is stability. Although differentiation is reversible in principle, many components of the gene batteries induced upon terminal differentiation are maintained throughout a cell's life. For example, muscle cells continuously express the myosin gene, and GABAergic neurons continuously express genes for GABA synthesis and transport. Maintaining gene expression patterns in the nervous system is a particular challenge given the non-renewing nature and therefore extensive life span of many neuronal cell types.

How are gene expression patterns maintained? Much recent discussion focused around the importance of chromatin modifications in maintaining the state of cellular gene expression programs. To achieve specificity, however, chromatin modifications need to be targeted to specific genes via DNA sequence-specific transcription factors. What is the nature of the DNA recognition programs that confer maintenance of the differentiated phenotype? I will highlight here a few core principles of how DNA-binding transcription factors mediate stability in gene expression patterns, illustrating these points with some paradigmatic analysis of neuronal gene expression programs in the nematode *Caenorhabditis elegans*, the genetic amenability of which has made it possible to rigorously examine and test concepts of gene regulation.

Terminal differentiation of many different neuron types in *C. elegans* is controlled by terminal selector transcription factors, which are expressed in a neuron-type specific manner and turned on about the time when neuroblasts terminally divide. They directly bind to specific motifs in the *cis*-regulatory control regions of terminal differentiation genes to activate their expression (Figure 1A). Terminal differentiation genes (also termed effector or realizator genes) are the nuts and bolts determinants of neuronal function and encode enzymes and transporters involved in neurotransmitter metabolism, ion channels that define a neuron's resting potential, and so on. These genes fail to be expressed upon removal of the terminal selectors and, consequently, the respective neuron type either remains in an undifferentiated state or switches its identity to that of another neuron type.

Terminal differentiation genes do not just have to be induced, however, they need to be maintained throughout the life of the neuron. This is ensured by the sustained presence of terminal selectors throughout the life of a neuron. If a terminal selector is genetically removed post-developmentally, a loss of terminal differentiation features is observed, rendering neurons dysfunctional. The sustained presence of terminal selectors is ensured through a simple logical device: transcriptional autoregulation of the terminal selector, mediated by the presence of binding sites of the terminal selector in its own *cis*-regulatory control region (Figure 1A). Consequently, removal of a terminal selector results in loss of expression of the terminal selector itself (Figure 1B).

Mutations in a terminal selector gene affect only sustained

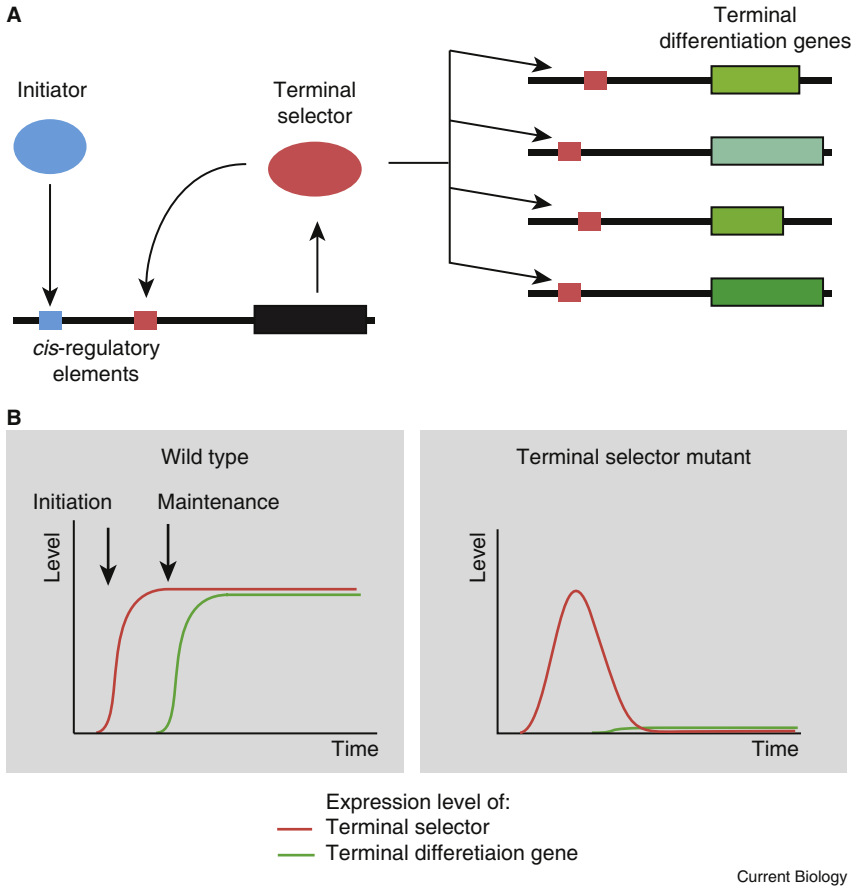

Figure 1. Principles of maintaining neuronal identity through autoregulation.
(A) Transcription factors ('terminal selectors') drive the expression of terminal differentiation genes (encoding 'nuts-and-bolts' neuronal identity determinants, such as ion channels, neurotransmitter synthesizing enzymes and so on) through a co-regulatory strategy that involves direct binding to *cis*-regulatory control regions of terminal differentiation genes. These transcription factors autoregulate their expression, therefore ensuring their maintained expression and regulation of downstream targets. Terminal selectors may act alone are be a combination of heterodimerizing transcription factors. Terminal selector gene expression is induced by initiation mechanisms that operate transiently to kick off the autoregulatory mechanism. (B) Levels of expression of terminal selector transcription factor and terminal differentiation genes as assessed by reporter genes that measure transcriptional regulation. Animals with mutations in a terminal selector will still show initiation of their own expression, but due to their dysfunction, will not be able to sustain their own expression and not induce downstream targets.

production of the selector itself — not initiation of its expression (Figure 1B). Initiation of terminal selector gene expression is, rather, triggered through transient regulatory inputs — signals and transcription factors that are only temporally present in a short window of time when a neuron is born. These transient inputs feed into the terminal selector locus via *cis*-regulatory elements that are separate from the autoregulatory elements that ensure maintenance after the initial 'kick off' phase (Figure 1A). The autoregulatory logic is thus a 'lock-in' or memory device that remembers a transient regulatory state. By current definition, a terminal selector therefore fulfills the function of a self-sustaining epigenetic trait that changes a cellular phenotype independent of alteration of genomic DNA sequence. As classic work has shown, transcriptional autoregulation and the resulting epigenetic maintenance and memory of a specific regulatory state is a deep evolutionary phenomenon, employed by systems as simple as bacteriophages.

As transcriptional events usually lead to a characteristic pattern of histone modifications at the transcribed locus, terminal selector autoregulation and regulation of downstream terminal differentiation genes also involve histone-modifying enzymes. Yet the key specificity determinant is the terminal selector transcription factor that selects target genes in a sequence-specific manner.

Further reading

Bertrand, V., and Hobert, O. (2009). Lineage programming : navigating through transient regulatory states via binary decisions. Curr. Opin. Genet. Dev. *20*, 362–368

Crews, S.T., and Pearson, J.C. (2009). Transcriptional autoregulation in development. Curr. Biol. *19*, R241–R246.

Hobert, O. (2008). Regulatory logic of neuronal diversity: terminal selector genes and selector motifs. Proc. Natl. Acad. Sci. USA *105*, 20067–20071.

Davidson, E.H. (2010) Emerging properties of animal gene regulatory networks. Nature *468*, 911-919.

Hobert, O., Carrera, I., and Stefanakis, N. (2010). The molecular and gene regulatory signature of a neuron. Trends Neurosci. *33*, 435-445.

Ptashne, M. (2007). On the use of the word 'epigenetic'. Curr. Biol. *17*, R233-R236.

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Scott's collections help reveal accelerating marine life growth in Antarctica

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Scott remains famous for coming second to Amundsen in the race for the South Pole and the fatalities on the journey back to base, but scientific effort on his expedition was never sacrificed and set many invaluable physical and biological baselines. Amongst these were collections of benthos, such as the bryozoan *Cellarinella nutti*, which records environmental information in tree-ring-like growth check lines. We measured the growth of *C. nutti* in the Ross Sea from museum and new collections and find no trend from 1890–1970 but a rapid increase from the 1990s to present. This reflects coincident increases in regional phytoplankton production. Thus, it is the first evidence that greater surface productivity is being sequestered to the seabed and thus of increasing polar carbon sinks.

A key consequence of rapid recent regional warming is the changing of parts of the planet from white (ice) to blue (sea) to green (phytoplankton blooms) [1,2]. While many positive feedbacks (e.g., decreased albedo) have been described, one significant negative feedback is a predicted [3] increase in carbon sequestered to the seabed. Evidence of increased sequestration can be indirectly provided by measuring growth of primary consumers but is hindered by the lack of early baselines and brevity of time series. The polar primary consumer *C. nutti* has been collected for more than a century thanks to Scott's refusal to give up science during the quest for the South Pole. Here we show that *C. nutti*, which forms annual growth bands (like tree-rings), provides the first evidence of recent rapid polar growth increase.

The erect, branching bryozoan *C. nutti*, like some other bryozoans, brachiopods, and molluscs, produces check lines marking the end of growth each year. The height and area or mass produced in annual sections of such animals varies with both age and the duration of food (phytoplankton) availability [4]. For colonies in which a growing branch tip or tips are intact and the date of collection known, each growth section can be back-measured and ascribed to a particular year. Growth data across years can be compared using anomalies (deviation from age-standardised averages). New initiatives, such as the Census of Antarctic Marine Life, have led to recent collections. The current study specimens were collected in 2008 by McMurdo and Scott base personnel as well as by the RV Tangaroa cruises of the National Water and Atmospheric Institute (NIWA) of New Zealand. Older specimens were from Scott's collections during the National Antarctic and British Antarctic expeditions (1901 and 1913), the Discovery expeditions (1936) and later research cruises of the 1950–1970s (held in the Natural History Museum London, British Antarctic Survey, National Museum of Natural History, Smithsonian Institution, Washington D.C., Virginia Museum of Natural History (US), NIWA and University of Otago (NZ)).

We examined 887 annual growth increments of *C. nutti* (Figure S1 in Supplemental Information, published with this article online) and found that the average annual growth of this species was 3.90 mm (± 0.05 SE) in height and 46.19 mm^2 (± 2.04 SE) in area between 1890 and 1970. This growth produces about 30 mg of calcium carbonate per specimen per year and is similar in quantity to values measured in *C. nutti* from the Weddell Sea in the 1980s and 1990s [5]. We then analysed the age-standardised anomalies and found no significant increase or decrease over time between 1890 and 1970. There was, however, more variability in the data from the 1950s and 1960s than in earlier years (1946–1972 = 0.09 SE compared with 1890–1936 = 0.04 SE). From the early 1990s to 2008 the mean growth of *C. nutti* increased (Figure 1) to twice (age means 1.1–3.8 times greater than) that during the rest of the century. This trend from the 1990s was highly significant (with $p < 0.001$ even without the last two years' data) and eight of the ten highest mean growth values from 1890 to 2008 occurred in the last decade. Pelagic data also suggest recent changes in