

Minireview

A passport to neurotransmitter identity

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Abstract

Comparison of a regulatory network that specifies dopaminergic neurons in *Caenorhabditis elegans* to the development of vertebrate dopamine systems in the mouse reveals a possible partial conservation of such a network.

The human brain is the most complex vertebrate ‘organ’, consisting of roughly 10-100 billion neurons each with a unique identity in terms of neurotransmitter phenotype, anatomical location and connections to other neurons. One of the quests in genome biology is to understand the principles by which the human genome with its limited number of genes generates such highly diverse and yet precisely connected sets of neurons. Addressing a similar issue in the much simpler nervous system of the nematode *Caenorhabditis elegans*, a recent paper in *Nature* by Flames and Hobert [1] has revealed a potentially conserved regulatory logic underlying the terminal differentiation of dopaminergic neurons - neurons that secrete the neurotransmitter dopamine.

Specification of neuronal neurotransmitter type

C. elegans has a well-defined nervous system of 302 neurons in which 118 neuronal types can be distinguished. Six pairs of neurons, each originating from four separate lineages, use dopamine as a neurotransmitter. Flames and Hobert’s starting point in delineating the mechanism by which these different neurons acquire the components for dopaminergic neurotransmission is the concept that the genes required in a functional pathway may be coordinately activated by a single or limited number of transcription factors acting on shared *cis*-regulatory elements. This basic concept has been discussed for more than 30 years using terms such as ‘realizator genes’ [2], ‘neuron-type selector genes’ [3] and ‘post-mitotic selector genes’ [4] to describe these putative sets of coordinately regulated genes. The idea has more recently been re-formulated by Hobert [5] using the terms ‘terminal selector genes’ (for the transcription factors involved), ‘terminal gene batteries’ (the genes making up the pathway, on which the transcription factors act), and ‘terminal selector motifs’ (the shared *cis*-elements). The

experimental investigation of this concept in the differentiation of dopaminergic neurons in *C. elegans* by Flames and Hobert [1] has proved extremely successful, revealing the regulatory codes for the dopamine pathway in this animal.

Using green fluorescent protein (GFP) reporters, Flames and Hobert dissected the *cis*-regulatory regions of genes operating in dopamine synthesis, release and re-uptake. Through systematic analysis of these regions they find that genes for tyrosine hydroxylase (TH, *cat-2*), GTP cyclohydrolase (GTPCH, *cat-4*), amino-acid decarboxylase (AADC, *bas-1*), the vesicular monoamine transporter (VMAT, *cat-1*), the dopamine transporter (DAT, *dat-1*), and also for two dopamine-associated ion channels (*asic-1* and *trp-4*), share a common element, dubbed the ‘DA motif’. This is a predicted binding site for transcription factors of the ETS family. By testing *C. elegans* mutants that lacked each of the ten ETS transcription factors found in this animal, they retrieved AST-1 as the factor responsible for acting on the DA motif in all types of dopaminergic neurons in *C. elegans* [1].

Loss- and gain-of-function studies defined *ast-1* as necessary and sufficient for the induction and maintenance of the dopaminergic identity of these neurons (Figure 1). In the *ast-1* loss-of-function mutant, the expression of all five dopamine-pathway genes was virtually lost, whereas ectopic induction of *ast-1* via transgenesis could induce *dat-1* and *cat-2*. The DA motif seems to function in *C. elegans* as a cell-lineage-independent genomic passport given to a set of genes that, when stamped by the ETS transcription factor AST-1, are permitted entrance to the terminal differentiation pathway in order to specify the dopaminergic identity of neurons.

The authors [1] then went on to test the conservation of this regulatory mechanism in the mouse, an organism with a more complex genome and nervous system, by testing the consequence of the knockout of the ETS transcription factor Etv1, the mouse ortholog of AST-1, which is expressed in dopaminergic neurons of the mouse olfactory bulb. The DA motif seems indeed to have a conserved function, as in this system Etv1 acts similarly to AST-1 in

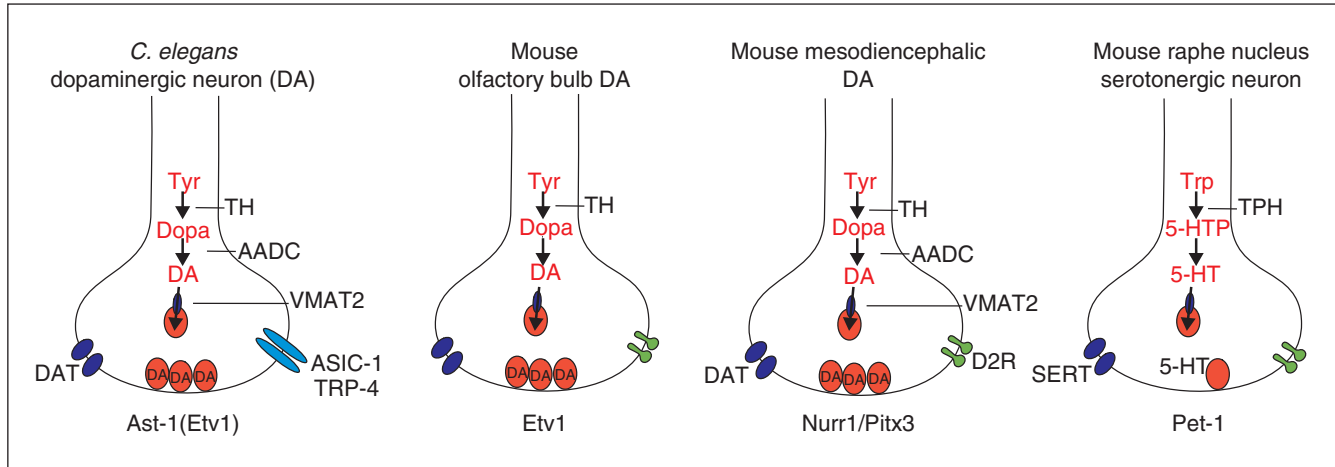


Figure 1

Neurotransmitter phenotypes and the master transcription factors that determine them. The essential transcription factors are shown under each neuron. The proteins whose genes are known to be regulated by the essential transcription factors are indicated. AADC, amino-acid decarboxylase; D2R, dopamine receptor D2; DAT, dopamine transporter; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; VMAT2, vesicular monoamine transporter. ASIC-1, TRP-4, DAT and SERT are membrane transport or channel proteins. The neurotransmitter-synthesis pathway is indicated in red inside each nerve terminal. Dopamine (DA) is synthesized from tyrosine (Tyr) via the intermediate Dopa. Serotonin (5-HT) is synthesized from tryptophan (Trp) via the intermediate 5-hydroxytryptophan (5-HTP).

regulating the gene for tyrosine hydroxylase. In the mouse, *Etv1* not only mediates specification of dopaminergic identity, but is also required for the proliferation and maintenance of bulbar dopaminergic neurons. However, this is only one of multiple dopamine systems in the vertebrate brain, and Flames and Hobert suggest that the others may express different ETS factors that fulfill the same role.

Specification of mouse mesodiencephalic dopaminergic neurons

Given the importance of AST-1 in defining the dopaminergic phenotype in *C. elegans*, Flames and Hobert speculate that the mouse ETS factor *Etv5*, which is expressed in mesodiencephalic dopaminergic (mdDA) neurons, may play an important role in defining the dopaminergic phenotype in vertebrate mdDA neurons. This neuronal group is essential for defining mood and movement control. However, there are other candidates for potential terminal selector genes for mdDA neurons. It is well established that *Nurr1* (an orphan nuclear hormone receptor) is an essential regulator of the mdDA neuronal phenotype through its activation of the genes *Th*, *Vmat2*, *Dat*, and *cRet* (which encodes a receptor tyrosine kinase) (for a review, see [6]). In addition, neuronal maintenance relies on *Nurr1* activity because mdDA neurons lacking *Nurr1* function are gradually lost, and this loss cannot be attributed to the loss of defined dopaminergic markers.

A second transcription factor with a well-established role in the terminal differentiation of mdDA neurons is the paired-like homeodomain transcription factor *Pitx3* (for a

review, see [6]). From knockout studies in mice it is clear that the development of substantia nigra (SNc) neurons, a subset of mdDA neurons, is severely compromised by a lack of *Pitx3* expression, as marked by the loss of *Th* expression [7]. The SNc dopaminergic neurons are the ones chiefly lost in Parkinson's disease. Recent results have shown that the specific dependence of the SNc neuronal phenotype on *Pitx3* is due to SNc-specific activation of the gene for aldehyde dehydrogenase 2 (*Ahd2*) by *Pitx3*. *Ahd2* activity locally generates the small signal molecule retinoic acid, whose signaling is crucial for the activation of *Th* and the terminal differentiation of SNc neurons [8]. As two different transcription factors are essential to drive *Th* expression within the mdDA, it is not clear which should be designated as the 'terminal selector gene' or whether both should be. In line with the latter idea, it has recently been established that *Nurr1* and *Pitx3* interact, and that they regulate histone deacetylase (HDAC) activity through release of the co-repressor *Smrt*, which in turn regulates activation of the dopamine pathway gene battery, including the genes for amino-acid decarboxylase (AADC) and the dopamine receptor D2 (D2R). This interaction is essential for the development of specific mdDA subsets, such as SNc. The initial finding that *Nurr1* regulates most of the dopaminergic gene battery has now been refined to suggest that *Pitx3* functions as an essential co-regulator in the *Nurr1* gene-activation complex (Figure 1). In conclusion, in the mammalian mdDA system it is very difficult to designate a single terminal selector gene for dopaminergic neurons, especially as other dopamine systems present in the

vertebrate central nervous system depend on different factors to drive their dopaminergic phenotype.

The ETS factor *Pet-1* and terminal differentiation of serotonergic neurons

In regard to other types of neurons, developing serotonergic neurons, which secrete the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin), express a related, but distinct, gene battery compared with dopaminergic neurons, and depend completely on the ETS transcription factor *Pet-1* for their development and differentiation [9-13]. Serotonergic neurons that survive *Pet-1* ablation are deficient in expression of the serotonin re-uptake transporter (*Sert*) and tryptophan hydroxylase (*Tph*) [11]. Analyses of promoter regions of *Sert* and *Tph* have shown consensus binding sites for ETS factors [10], suggesting that *Pet-1* might directly activate transcription of these genes in developing serotonergic neurons. The timing of *Pet-1* expression, the presence of binding sites for *Pet-1* on many genes of the serotonergic pathway and the *Pet-1*-dependent terminal differentiation of serotonergic neurons in the vertebrate central nervous system would mark *Pet-1* as a terminal selector gene. However, other results hint at an additional dependence on the transcription factors *Lmx1b* and *Nkx2.2* for the full activation of the serotonergic phenotype [9], indicating that a different level of complexity is involved in the vertebrate central nervous system.

As defined by Flames and Hobert [1,5], the concept of 'terminal selector genes' is an attractive way to define the role of master transcription factors in the development of specific neuronal populations (Figure 1). As they show, the 'DA motif' as the passport to coordinated gene activation during terminal differentiation of neuronal dopaminergic identity operates beautifully in *C. elegans*. Such a mechanism may equip invertebrates with the efficient means of creating functional pathways using a single master transcription factor. Vertebrate genomes seem to build on this principle, as illustrated by aminergic and glutamatergic neurons in the mouse brain, but with the increasing level of brain complexity the molecular programming becomes more complicated, involving additional and different transcription factors [4,6,12-15]. The findings of Flames and Hobert open a new window for control of passports to neuronal neurotransmitter identity. Let's see which borders in genome biology can be passed with it.

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