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EDITORIAL

Deciphering the core instructions of neuronal differentiation

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The amazing field of Developmental Neuroscience has provided fascinating insights into mechanisms regulating the generation of a huge array of different neuron populations. The discovery of a large set of growth factors influencing cellular differentiation choices and transcription factors regulating expression of genes specific to progenitor and neuron populations has been instrumental. The analysis has failed, however, to establish a clear idea on the acquisition of the common structural as well as information-propagating and -processing neuronal features during neurogenesis. Moreover, it is not resolved how this is coordinated with the diversification of the distinct neuron populations.

Focusing attention on the early developmental expression of shared molecular players involved in formation of the neuronal cytoskeleton, generation of action potentials and regulation of neurotransmitter release has begun to change the field. Together with the recognition of several layers in the coordination of gene expression extending from (1) modification of nuclear and chromatin organization to (2) action of transcription regulator complexes at enhancer and promoter sequences to (3) posttranscriptional regulation by powerful non-coding RNA/protein networks, a comprehensive picture of the route from neural progenitor to neuron takes shape.

Fueled by the observation that a limited set of regulators from each of these layers has the potential to drive neuronal differentiation in stem cells or even from unrelated cell types such as fibroblasts, the finding of developmental expression patterns of genes coding for neuronal cytoskeletal and synaptic proteins conserved across vertebrate neuron populations prompts the question for a shared generic differentiation program. Involvement of some of those regulators and expression of certain target genes in sensory receptor and endocrine cell differentiation may define a group of related signal-communicating cells and further refine the core of a neuronal differentiation program. Comparison to invertebrate neural development demonstrating related functions of orthologous regulators will decipher the evolutionary core instructions on 'how to build a neuron'. The consequences of such a basic comprehension of generic neuronal differentiation for stem cell-derived tissue replacement approaches, as well as the neuro-pathological and neuro-oncological clinic, are of particular interest.

The exciting search for the mechanisms governing neuronal development has received additional momentum by the goal to predictably tailor programming and reprogramming routines for cell and tissue replacement techniques. Both basic and applied approaches have uncovered or employed, respectively, regulatory schemes related to the diversification of the enormous variety of neuron classes. In order to generate a specific type of neuron in vitro this procedure has already proven successful. Yet Ninkovic and Götz (2014, this issue) discuss the question whether application of a common principle of neuronal differentiation could benefit therapeutic goals and how this could figure molecularly. For the basic understanding of neuronal development, the characterization of this still hypothetical common principle would complement the anatomical and physiological foundations of the 'neuron theory'.

The powerful term 'neuron', giving name to an entire scientific discipline, refers to essential shared features within a huge variety of information-propagating cells of distinct but highly divergent, morphology. As Brunet and Ghysen (1999) pointed out, there may be no generic neuron. Yet the question remains whether there is a significant component of the developmental program shared between all neuron classes and different from other cell types.

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The title line 'How to make and how to define a neuron' (Yang et al., 2011) entails several key aspects relevant to these considerations. The first concerns the critical issue of unambiguous identification of a cell as being a neuron. On the other hand, it is currently not clear to which extent procedures to program neurons in vitro do and need to resemble in vivo development (Amamoto and Arlotta 2014).

Cell morphology as well as the expression of certain markers, mostly molecules associated with the neuronal cytoskeleton, provide histological and molecular evidence for a neuronal phenotype in vivo and in vitro. Many of the molecular markers (Sarnat 2014, this issue) are not entirely neuron-specific, particularly during early embryogenesis (Ernsberger 2012, for review). Usually, their detection needs to be complemented by a morphological feature, the presence of one or more neuritic processes. Mechanisms involved in neurite initiation are central to neuronal differentiation (Sainath and Gallo 2014, this issue), yet answers to the question to which extent they rely on transcription of new gene sets, posttranscriptional or posttranslational alterations are surprisingly incomplete.

Electrical activity and regulated transmitter release provide physiological evidence for neuronal function and become increasingly appreciated in the analysis of cell type reprogramming. One problem with the corresponding molecular analysis is the large number of protein classes and isoforms involved in both processes. Determination of the proteome for synapses and synaptic compartments (Laßek et al. 2014, this issue) provide a remarkable detailed picture and emphasize the dimension of the challenge to understand, at the molecular level, not only synaptic function but also development.

An important concern comes with the expression of proteins involved in both functional contexts, electrical activity and mediator release by vesicle fusion, not only in neurons but also endocrine or sensory receptor cells (Thiel et al. 2014; Fritzsch et al. 2014; Ernsberger 2014, this issue). The similarities in transcriptome and proteome of neurons, sensory receptor cells and certain classes of endocrine cells reflect in the vast overlap of transcription factors regulating their development (Huber, 2014; Raft and Groves 2014; Ernsberger 2014, this issue). This is the case for SRY (sex determining region Y)-box 2 (Sox) as well as basic helix-loop-helix (bHLH) proteins.

Sox proteins are of outstanding interest for their crucial involvement in neuronal and glial development (Reiprich and Wegner 2014, this issue) as well as sensory receptor cell (Raft and Groves 2014; Fritzsch et al. 2014, this issue) and endocrine cell differentiation (Huber, 2014; Ernsberger 2014, this issue). The importance of SoxB proteins in vertebrate neural development and their association with neurogenic regions in invertebrates prompts the question whether they could be the trigger of a universal mechanism of generic neuronal differentiation and how this relates to development of related cell types. The regulatory cascades of *Drosophila* neurogenesis (see Kang and Reichert 2014, this issue) with only a few remaining neurons in *Drosophila* carrying combined mutations for the two *Sox B* genes *Dichaete* and *SoxNeuro* demonstrate that the majority but not all of the insect neurons depend on a SoxB trigger for development. DNA binding studies in *Drosophila* and mice demonstrate targeting of proneural *bHLH* genes among other transcriptional regulators. Apart from developmental regulator genes, Sox proteins bind to terminal differentiation genes. In mouse, SoxB and SoxC protein binding is detected at the neuronal *ß-III tubulin* gene in chromatin environments reflecting different transcriptional activity states. Also, the *Snap25* gene, expressed in neurons and sensory receptor cells, as well as in endocrine cells derived from endoderm and ectoderm, is targeted by both groups of Sox proteins. Yet the functional implication of such sites is not resolved.

bHLH protein usage also overlaps largely between neuron, sensory receptor and endocrine cell development (Raft and Groves 2014; Fritzsch et al. 2014; Huber 2014; Ernsberger 2014, this issue). The binding to genes involved in morphological and functional development is less well characterized, however. Yet, their importance in regulation of cell cycle regulators and the transition from progenitor proliferation to neuronal differentiation receives enhanced interest with the recognition of cell cycle-regulated dynamics of abundance (Kageyama et al. 2014, this issue) and activity by protein phosphorylation (Hardwick et al. 2014, this issue).

In addition to transcriptional and posttranslational processes, the importance of posttranscriptional regulation by microRNAs in the transition from neural stem cell or progenitor to neuron is being intensely studied. let-7 and miR-200-modulated networks involve bHLH and Sox proteins (Trümbach and Prakash 2014; Rehfeld et al. 2014, this issue). miR-9 and miR-124 affect different key regulators of neuronal development (Stappert et al. 2014; Abernathy and Yoo 2014, this issue). miR-124 targets a range of effectors including the neuron-restrictive silencer factor (NRSF/REST), implicated in neuronal development and endocrine function (Thiel et al. 2014; Stappert et al. 2014, this issue) and the polypyrimidine tract binding proteins 1 (PTBP1) and consequentially PTBP2, RNA-binding proteins and splicing regulators involved in neuronal differentiation (Yano et al. 2014; Abernathy and Yoo 2014, this issue). miR-9 is able to modulate bHLH oscillations and neurogenesis via regulation of Hes family members (Kageyama et al. 2014; Stappert et al. 2014, this issue). Notably, the microRNA equipment of neurons and sensory cells overlaps considerably. Yet, microRNAs in endocrine cells show significant differences (Huber 2014; Ernsberger 2014, this issue). Their role in the divergence between neuronal, sensory and endocrine lineages deserves further attention.

The tightly interwoven regulatory cascade by Sox and bHLH proteins complemented by microRNAs provides a regulatory scaffold for the formation of neural networks of different levels of complexity. The stunning intricacy of the cerebral cortex, brain stem and spinal cord are achieved by an interplay of stem cell and progenitor proliferation, migration, differentiation composed of generic neuronal and subtype-specifc components in the neuronal branch and the segregation of the glial branch. This involves a diversity of progenitor types in the developing cortex (Laguesse, 2014, this issue) or spinal cord (Agius 2014, this issue) with distinct proliferation and differentiation patterns. Their regulation by growth factor signaling is of key importance. Members of the wingless-type MMTV integration site family (Wnt), bone morphogenetic protein family (Bmp) and the hedgehog-related sonic hedgehog (shh) are crucial players (Agius 2014, this issue). The pleiotropic factors affect not only proliferation but also various aspects of differentiation (Inestrosa and Varela-Nallar 2014, this issue). How generic and subtypic-specific neuronal differentiation are integrated at this level is one of the least understood problems concerning our question.

The interface of growth factor signaling and the matching between proliferation and differentiation is not only a key issue in neurogenesis but also in tumor formation. The putative diversion of neural progenitors to tumor stem cells by deregulated growth factor signaling cascades and transcription factor action relates to all effectors mentioned above and beyond (Swartling et al. 2014, this issue). The therapeutic potential of inducing neuronal differentiation in brain tumors warrants careful considerations given the expanding knowledge of the coordination between proliferation and differentiation of neural progenitors.

The appreciation of growth factor action and transcription factor networks in neuronal differentiations established in the 1980s and 1990s became critically extended on the one hand by the recognition of the role of chromatin modification, acting in a sense pre- and possibly co-transcriptional and on the other hand by the unexpected discovery of posttranscriptional modulation by microRNAs, both gaining momentum in the 2000s. These two regulatory levels converge to provide concepts of generic neuronal differentiation centered around the interaction of NRSF/REST with miR-9 and miR-124 as well as the cross-regulation of miR-9 and Hes family members (Abernathy and Yoo 2014; Stappert et al. 2014, this issue).

The resulting networks have been shown to directly regulate a number of but not all, genes required for the neuronal core features of axonal projection, electrical activity and neurotransmission (Ernsberger 2012, for review). The recognition of Sox protein binding to a large numbers of genes involved in these functions provides major progress (Reiprich and Wegner 2014; Ernsberger 2014, this issue). SoxB and SoxC protein binding in chromatin environments reflecting different transcriptional activity states in stem cells, progenitors and differentiated neurons points to the importance of histone modification for transcriptional regulation during developmental realization of the differentiated state. The Polycomb Repressive Complexes (Corley and Kroll 2014, this issue) and Jumonji family histone demethylases (Fueyo et al. 2014, this issue) are central players. In addition, long non-coding RNAs interacting among others with Polycomb Repressive Complexes establish an emerging layer of regulation whose significance in neural and neuronal development awaits analysis (Corley and Kroll 2014, this issue).

The characterization of enhancer and promoter regions at genes coding for neuronal cytoskeletal and synaptic proteins is critically required to understand not only the transcriptional control of neuronal differentiation but also of the divergence among neuronal, sensory receptor and endocrine cell lineages. The retina, inner ear and sympathoadrenal system will be crucial model systems to study the key mechanisms in vertebrates. To what extent a Sox protein-based cascade operates in invertebrates and how the alternatives may look are important steps in the search for a putative universal mechanism of generic neuronal differentation.

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