Mini-review

Neuronal and glial markers of the central nervous system

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Summary. This brief review evaluates the expression of cell-specific markers on differentiated neural cells and, where necessary, on their developing precursors. Within these limitations only the commonly used markers are discussed and those deemed unequivocal are only briefly appraised.
Key words. Neurons; glia; cell markers; central nervous system.

The neuroepithelial cells of the neural tube give rise to the neurons, astrocytes, oligodendrocytes and ependymal cells that largely constitute the central nervous system (CNS)[20]. In the early 1970s neurobiologists concentrated their efforts in trying to establish a battery of neural cell-specific markers which would serve studies of lineage and functional identification both in vivo and in vitro. Unfortunately the veracity of all markers has not stood the test of time. This has been particularly evident in developmental studies. Thus this brief overview will appraise the expression of cell-specific markers on differentiated neural cells and, where necessary, on their developing precursors. Within these limitations only the commonly used markers will be discussed and those deemed unequivocal only briefly covered.

Antibodies to intermediate filament proteins have been extensively used for cell identification. Neurons may be characterised by their associated neurofilament protein, both in vitro and in vivo[15,55]. Astrocytes also contain characteristic intermediate filaments, called glial filaments, which are largely polymers of the glial fibrillary acidic protein (GFAP)[2]. Therefore these cells can be readily identified in tissue sections and cultures of CNS by immunohistochemical techniques using anti-GFAP antibodies[2,29,41]. Two different types of $GFAP^+$ astrocytes can be distinguished in cultures of developing rat optic nerves on the basis of morphology, growth characteristics and labeling with ligands that bind to polysialogangliosides such as tetanus toxin and the monoclonal antibody A2B5[42,43]. These ligands have been reported to bind to the gangliosides GD1b and GT1 and GQ, respectively[10,56] and were originally thought to bind only to neurons[7,33]. Type 1 astrocytes have a fibroblastoid morphology and do not bind tetanus toxin or A2B5 antibody. Type 2 astrocytes have a process-bearing morphology, resembling neurons or oligodendrocytes, and bind tetanus toxin and A2B5 antibody. Whereas type 1 astrocytes are found in cultures of white matter and grey matter, type 2 astrocytes are seen in significant numbers only in cultures of white matter and probably correspond to fibrous (fibrillary) astrocytes[31].

The two types of astrocytes develop in vitro from two different precursor cells. Type 2 astrocytes develop from a bipotential $A2B5^+$, $GFAP^-$ progenitor cell which can differentiate in a developmental sequence into type 2 astrocytes ($A2B5^+$, $GFAP^+$) and oligodendrocytes ($A2B5^-$, $GFAP^-$)[32,42]. Such oligodendrocytes are also positive for the oligodendrocyte specific marker galactocerebroside[40,42]. Glutamine synthetase is also specific for fibrous and protoplasmic astrocytes[37], as well as for retinal Muller cells[45], and is not found in oligodendrocytes or CNS neurons[42]. To date markers for Schwann cells are poorly defined and include antisera generated against chemically-induced neural tumours[6] and primary Schwann cell cultures[22]. CNPase[39] and antisera to the peripheral myelin basic proteins (P_1 and P_2) and glycoprotein (P_0) can be used once myelination has been initiated[34] and as such are useful indicators of this process.

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase; EC 4.2.1.11), which catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate, has also been extensively used in neural cell identification. Enolases are dimeric molecules derived from three immunologically distinct subunits, designated α, β and γ, coded by separate genetic loci[12,38,52]. Three homodimeric forms purified from different tissues have been characterised. The $\beta\beta$ form is present in relatively high concentrations in muscle[44] and has not so far been detected in nervous tissue. The $\alpha\alpha$ homodimer has been shown to be strictly localised in the astrocytes of the CNS and has been designated non-neuronal enolase (NNE)[23,46]. In contrast the $\gamma\gamma$ form is specifically localised within neurons[24,46,49] and cells of the diffuse neuroendocrine system, formerly designated the amine precursor uptake and decarboxylation (APUD) system[47]. This isozyme is termed neuron-specific enolase (NSE) and is homologous to the 14-3-2 protein isolated from bovine brain[3,35]. A further brain form has been described representing a hybrid between the forms $\alpha\alpha$ and $\gamma\gamma$ termed enolase $\alpha\gamma$[25]. Although antisera to $\alpha\alpha$ and $\gamma\gamma$ crossreact with $\alpha\gamma$ in vitro, immunohistochemical studies indicate it to be artefactual or present below the threshold of detection.

Biochemical and morphological analyses using NSE antisera have shown the appearance of NSE in CNS tissues closely parallels neuronal development and maturation and as such serves as a useful marker for studying the differentiation of the CNS[26,28,54]. This increase in NSE observed during development represents a shift in the

production of the α-subunit NNE to the γ-subunit NSE in the neuronal precursor population [48, 53].

The S-100 protein, originally isolated by Moore [35], was previously considered to be specific to the nervous system. It is composed of three forms – S-100a_0, S-100a and S-100b which are dimers with the subunit composition of αα, αβ and ββ, respectively [17–19]. Recent studies have demonstrated the α-subunit to be present in much higher levels in heart and skeletal muscles than in the CNS [21]. In muscle it is present as S-100a_0 whereas in the CNS the predominant forms are S-100a and S-100b [18, 21]. Within the CNS the exclusively glial localisation of S-100 is still controversial and several reports indicate it to be present in neurons [11, 13, 27]. These observations are consistent with the proposal that it is released in a soluble form from glial cells [50] and can bind to a receptor in the synaptic membrane with high affinity [8]. Although the functional role of S-100 remains unclear, structural studies have suggested these proteins bind calcium and are members of a protein superfamily comprising parvalbumin, troponin-C, myosin light chains and calmodulin which have divergently evolved from a common ancestor [18].

The neural cell adhesion molecule (N-CAM) is composed of three immunologically related cell surface glycoproteins of 180–200 kilodalton (kD), 135–140 kD and 115–120 kD molecular weight [1, 51] which are believed to be intimately involved in the early structuring of the CNS [9, 30]. N-CAM is related, if not identical, to the D2-protein and brain-surface protein 2 (BSP-2) [36]. D2 (N-CAM) was originally believed to be neuron-specific as judged by immunofluorescence [29] and immunoelectrophoretic [4, 5] techniques, however more recent studies have indicated the 135–140 kD and 115–129 kD components to be present on astroglia [14, 36, 51] in low amounts [16].

In conclusion, advances in understanding the structure and role of cell-specific markers have greatly increased their usefulness in that they will now allow functional aspects of the brain to be studied in its developmental, differentiated and diseased states. However, as a corollary, great caution must be exercised in the interpretation of changes in the expression of such markers.

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Short Communications

A cryptic intermediate in the evolution of chameleon tongue projection

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Summary. An incipient form of tongue projection occurs in *Phrynocephalus helioscopus*, a generalized agamid lizard. We argue that this condition represents a functional intermediate between typical lingual prehension and chamaeleontid tongue projection, and that tongue projection evolved in chameleons by augmentation of ancestral mechanisms still operating in related, generalized lizards.
Key words. Lizard; Chamaeleontidae; Agamidae; tongue; feeding; evolution.

Chamaeleontid lizards can project their tongues as much as one and a half times their body length. Air[1], blood[2], and inertia[3] were proposed alternatively as the agents of lingual projection. Cuvier correctly surmised that muscle contraction imparts the necessary force to the tongue[4], although his particular interpretation was flawed. We owe our present conception of this mechanism largely to the anatomical inferences of Brücke[5] and subsequent investigators[6–9].

Despite the attention to and controversy surrounding the mechanism of chameleon tongue projection, the evolution of this singular adaptive complex has been curiously neglected. Our ignorance probably stems from two factors: first, lingual projection is fundamentally a discontinuous process; the tongue is either projected or it is not. In an engineering sense, an intermediate form of tongue projection is difficult to envision and has never been reported. Second, variation in tongue morphology and function among even generalized lizards has remained largely unexplored[10]. In particular, we lack data on the role of the tongue in feeding, especially its use in prey capture.

In this paper we adduce new functional data demonstrating that an intermediate form of tongue projection exists among extant taxa. We analyze these data in the context of recent phylogenetic hypotheses of squamate relationships and conclude that this functional intermediate represents the retention of an ancestral state linking generalized lizards to chameleons in the evolution of lingual projection.

Materials and methods. Phylogenetic conclusions are based on three recent cladistic studies that have established the monophyly of Squamata and the relationships of Iguania,