Chapter 18. Neuronal pathfinding, vesicle transport and adult-onset neurodegenerative disease.

Neuronal outgrowth deploys similar cytoskeletal remodelling mechanisms as in other cell types, with pioneering neurones able to respond to cues from the epithelial surfaces over which they grow [1] [2]. Unlike epithelial cells, neurones are not divided by an Ap/Ba membrane compartment, although a diffusion barrier is retained between the cell body and the initial neuronal segment [3]. Neuronal/epithelial contacts are stabilised at FA-like anchoring points, while the AJs themselves are restricted to the neuronal cell bodies [4]. Regulatory interactions may take place between adjacent cell bodies, but any interactions between the growth cone and nucleus will be limited by transmission delays. Such delays are consistent with the function of the Chinmo TF (Chronologically inappropriate morphogenesis) in neuronal pathfinding and differentiation, which is dependent on kinesin (Khc) motor function [5] [6]. By contrast to peripheral pathfinding neurones, the cell bodies in the CNS are organised in successive layers [7] [8] [9] [10]. In principle, re-iterated metachronal waves might allocate a unique fate to each neuronal cell body, as the extending neuronal tips navigate within a laminated, 3D volume. In particular, alternative splicing may generate multiple receptor variants from the *Dscam* TU [11], consistent with assembly of the spliceosome-complex being regulated by cell-cycle dependent mechanisms. Meanwhile, the precise spatio-temporal transcriptional regulation of long neuronal TUs may be co-ordinated by intron-delay [12] [13].

The extending growth cones of segmental neurones follow parasegmental, rather than segmental boundaries; which generates a frame-shift between muscle blocks and their innervating neurones, in both arthropods and vertebrates [14]. As neuronal outgrowth continues, rapid responses to epithelial patterning cues may be mediated through Golgi outposts, which can act as MTOCs [15] [16] [17]. By contrast, dendrite outgrowth is initiated from Golgi associated MTOCs within the neuronal cell body, giving antiparallel microtubule arrays with mixed plus-end orientations. Subsequent dendritic branch-points may be associated with ectopic Golgi, with the assembly of α/β-tubulin dimers nucleated from γ-tubulin rings [18]. In this system, reduction in the activity of minus-end directed Dynein motors results in a proximal shift of dendritic branch-points, with an increased proportion of antiparallel microtubules [19]. By contrast, during neuronal extension microtubule assembly is initiated from the cortical actin microfibril/microtubule boundary behind the extending growth cone. In general, microtubule extension is driven by α/β-Tubulin oligomers transported along pre-existing microtubules by Kinesin-2 (Klp64D); with uniform polarity being dependent on EB1 and APC [20]. Antiparallel microtubule filaments may assemble during the initial outgrowth of neurones; with minus-end directed filaments being selectively removed by Dynein motor assemblies. As a result, labile microtubule bundles are maintained, with plus-ends directed towards the axonal tips [6]. The antiparallel microtubule bundles in dendrites are stabilised by MAP2 cross-links, while the parallel bundles in neurones are crosslinked by Tau, reviewed in [3]. By implication, the accumulation of ectopic Tau seen during Alzheimer's disease progression may be consistent with an increase in mixed, antiparallel filaments, and reduced cargo-trafficking. In this context, the Shaggy kinase regulates motor protein activity and contributes to the progression of Alzheimer's disease [21] [22]. A similar mechanism may underly myoclonus epilepsy associated with reduced Pk^{sple} activity, with rate-limited vesicle transmission across the neuro-muscular junction [23] [24] [25]. $Pk^{sple/+}$ larvae show compromised anterior-grade axonal transport; with adult seizures that are ameliorated by reduction of the Kinesin motor protein (in $pk^{sple+/-}$ $kh^{+/-}$ double mutants). By contrast, heterozygous $pk^{pk/+}$ and $pk^{pk/sple}$ flies show no seizure phenotypes [25]. Pk co-localises with Kh on motile punctae, with altered run lengths, pause times and reversals in $pk^{sple/+}$ and $pk^{pk/+}$ larvae [25] [26]. An increased

proportion of antiparallel microtubules is also observed in *fz* mutant wing discs, with altered vesicle trafficking [28].

Summary:

Neuronal patterning mechanisms (and adult neuronal function) require the polarised transport and delivery of cytoskeletal sub-units, motor proteins, and other cargo components. Morphogenetic interactions between pioneering neurones and underlying epithelial surfaces are mediated through specialised junctional complexes. Dendrites extend from the neuronal cell body via antiparallel microtubule arrays, with anchored Golgi outposts at dendritic branch-points. By contrast, parallel microtubule arrays are assembled behind the neuronal growth cone. Any nuclear transcriptional responses will be limited by transmission delays and dependent on microtubule integrity during neuronal extension, which may contribute to morphogenetic patterning mechanisms. The CNS may be constructed in successive morphogenetic waves, generating a laminate, 3D architecture. Co-ordinated regulatory interactions may take place between adjacent cell bodies, while neuronal growth cones extend through successive neuronal laminae. Such neuronal patterning mechanisms would be particularly sensitive to microtubule integrity and motor-protein function, while reduced cargo transport may affect neuro-muscular transmission and contribute epilepsy and neurodegenerative diseases. Notably, LOF mutations that are recessive in other tissues may be associated with dominant, adult-onset, neurodegenerative diseases.

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