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The remembrance of the things past: Conserved signalling pathways link protozoa to mammalian nervous system

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ABSTRACT

The aim of the present article is to analyse the evolutionary links between protozoa and neuronal and neurosecretory cells. To this effect we employ functional and topological data available for ciliates, in particular for *Paramecium*. Of note, much less data are available for choanoflagellates, the progenitors of metazoans, which currently are in the focus of metazoan genomic data mining. Key molecular players are found from the base to the highest levels of eukaryote evolution, including neurones and neurosecretory cells. Several common fundamental mechanisms, such as SNARE proteins and assembly of exocytosis sites, GTPases, Ca^{2+}-sensors, voltage-gated Ca^{2+}-influx channels and their inhibition by the forming Ca^{2+}/calmodulin complex are conserved, albeit with different subcellular channel localisation, from protozoans to man. Similarly, Ca^{2+}-release channels represented by $InsP_3$ receptors and putative precursors of ryanodine receptors, which all emerged in protozoa, serve for focal intracellular Ca^{2+} signalling from ciliates to mammalian neuronal cells, eventually in conjunction with store-operated Ca^{2+}-influx. Restriction of Ca^{2+} signals by high capacity/low affinity Ca^{2+}-binding proteins is maintained throughout the evolutionary tree although the proteins involved differ between the taxa. Phosphatase 2B/calcineurin appears to be involved in signalling and in membrane recycling throughout evolution. Most impressive example of evolutionary conservation is the sub-second dynamics of exocytosis-endocytosis coupling in *Paramecium* cells, with similar kinetics in neuronal and neurosecretory systems. Numerous cell surface receptors and channels that emerge in protozoa operate in the human nervous system, whereas a variety of cell adhesion molecules are newly "invented" during evolution, enabled by an increase in gene numbers, alternative splice forms and transcription factors. Thereby, important regulatory and signalling molecules are retained as a protozoan heritage.

1. Introduction

The fundamental parallels in behaviour of unicellular organisms and metazoans have been advocated at the beginning of 20 s century by Jennings [1] in his remarkable book "*Behavior of the Lower Organisms*". On a molecular level several cardinal signalling cascades critical for the nerve cells function emerged in protozoa, where they provided for excitation and behavioural response. In the present narrative we shall extend this reasoning further to recognise essential similarities and divergences, as we shall present pivotal proteins and protein-based mechanisms that are conserved from protozoa up to humans.

Mammalian neurones and neuroendocrine cells share the following properties: (i) They possess an electrically excitable cell membrane. (ii) Generation of cytosolic Ca^{2+} signals relies upon a complement of voltage-gated and other plasmalemmal Ca^{2+} channels and intracellular Ca^{2+} release channels (CRCs; represented by ryanodine receptors, RyRs, and $InsP_3$ receptors, $InsP_3Rs$). (iii) Cytosolic Ca^{2+}-binding proteins (CaBP) localise Ca^{2+} signals, while Ca^{2+}-ATPases/pumps, together with cation exchangers ascertain homeostatic recovery of $[Ca^{2+}]_i$. (iv) Transmitter vesicles are delivered to the cell surface via microtubular rails. (v) Membrane proteins for targeted delivery and docking at the cell membrane include GTPases, SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors), H^+-ATPase and actin. (vi) A Ca^{2+}-sensitive fusogenic protein, synaptotagmin, mediates excoytotic transmitter release and membrane fusions. (vii) Ca^{2+}-dependent cascades provide for internalisation and recycling of the membranes of emptied vesicle. Thus interneuronal communications, as well as integrative processes that occur in pre- and post-

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synaptic compartments are regulated by ionized Ca^{2+} [2–4].

In this review we consider proteins and protein-based mechanisms in neuronal and neuroendocrine cells, which have originated in protozoa. We discuss peripheral neurones and neuromuscular junction, neuroendocrine cells, as well as neurones of the central nervous system. We also examine fundamental aspects of exocytosis of clear or dense core-secretory vesicles. Despite their widely different structure and molecular endowment, clear and dense vesicles share many similarities at the organellar and molecular level. For instance, both types of vesicles operate in nociceptive neurones [4,5]), which release glutamate and peptides of different molecular weight [6]. Similarly, neuroendocrine cells, such as adrenal medullary cells, contain large dense core vesicles ("chromaffin granules") for release of catecholamines [7], together with clear vesicles for release of acetylcholine [8]. Neuropeptides, e.g. those delivered from the hypothalamus to the pituitary gland are frequently packaged in large dense core vesicles [5]. Since comparison of anatomical features does not appear feasible we rather concentrate on examples which have proved easily accessible for cell biological investigation. We present arguments which allow to trace evolutionary origins of key proteins and protein-based mechanisms in the different neuronal and neurosecretory cells to protozoa.

Choanoflagellates and their close relatives, the filastereans, are closest to the evolutionary roots of metazoans (Fig. 1 and [9]). Several insights into the early evolution of molecular components of neuronal cells are derived from data mining of choanoflagellate databases, although functional data are still rather limited. In contrast, ciliates provide considerable information about complexity, function and intracellular localisation of a variety of proteins relevant for Ca^{2+} signalling, vesicle trafficking and exocytosis [10–13]. Experimental analysis of ciliates is based on electrophysiology, cell fractionation, light and electron microscopy, gene silencing etc. Therefore, the current survey is contemplated not only to complement studies focusing on functions predicted for choanoflagellates based on sequencing data, but to elaborate on aspects known already in considerable detail from other protozoans, notably ciliates, and in part also from the myxamoeba *Dictyostlium*, Ca^{2+}-binding proteins (CaBPs) being an example [14]. On this background, we may attempt to trace some characteristics to protozoa. The emphasis will be on two genera of ciliated protozoa, such as *Paramecium* and *Tetrahymena,* notably *P. tetraurelia* and *T. thermophila*, for which substantial data are available.

Choanoflagellates, together with myxamoebae, and ciliates (ciliophora) are respective representatives of two main evolutionary lineages, monokonts and bikonts. Despite some significant differences between the two lineages, there are also remarkable similarities [12,15]; for example, Ca^{2+} as a key regulatory molecule for vesicle trafficking and mechanisms of exocytosis/endocytosis are conserved from early eukaryotes onwards [15,16].

Progressing through the phylogeny we find key players in different structural and functional context, as evolution is driven not only by duplication and recombination of a common toolkit, but also by relocalisation and re-functionalisation of proteins [17,18]. Proteins engaged in Ca^{2+} regulation and signalling seemingly evolved more dramatically than many other cell components [19]; some examples of such proteins in protozoa are summarised in Table 1. Some other key proteins, such as SNAREs, are present in comparable basic forms and numbers in *P. tetraurelia* (disregarding "ohnologs" from recent whole genome duplications [20]) and in mammals [21,22].

2. Similarities and differences between ciliates, neurones and neuroendocrine cells

2.1. Biogenesis and transport of secretory organelles

The molecular machinery required for formation and release of clear vesicles and dense core-vesicles is essentially the same: SNAREs are needed, as are GTPases and a Ca^{2+} sensor protein, synaptotagmin, though in different isoforms [23,24]. Dense core-secretory organelles are called chromaffin granules/vesicles in neurosecretory chromaffin cells (Fig. 2A), trichocysts in *Paramecium* (Fig. 2B and C) and mucocysts in *Tetrahymena*. The trichocysts originate in part in the Golgi complex and subsequently are transported by saltatory movement along microtubules, emanating from ciliary basalbodies [25,26] to the cell membrane for stimulated exocytosis. There are more forms of dense core-secretory organelles in protozoa, with different (ultra)structure, cargo

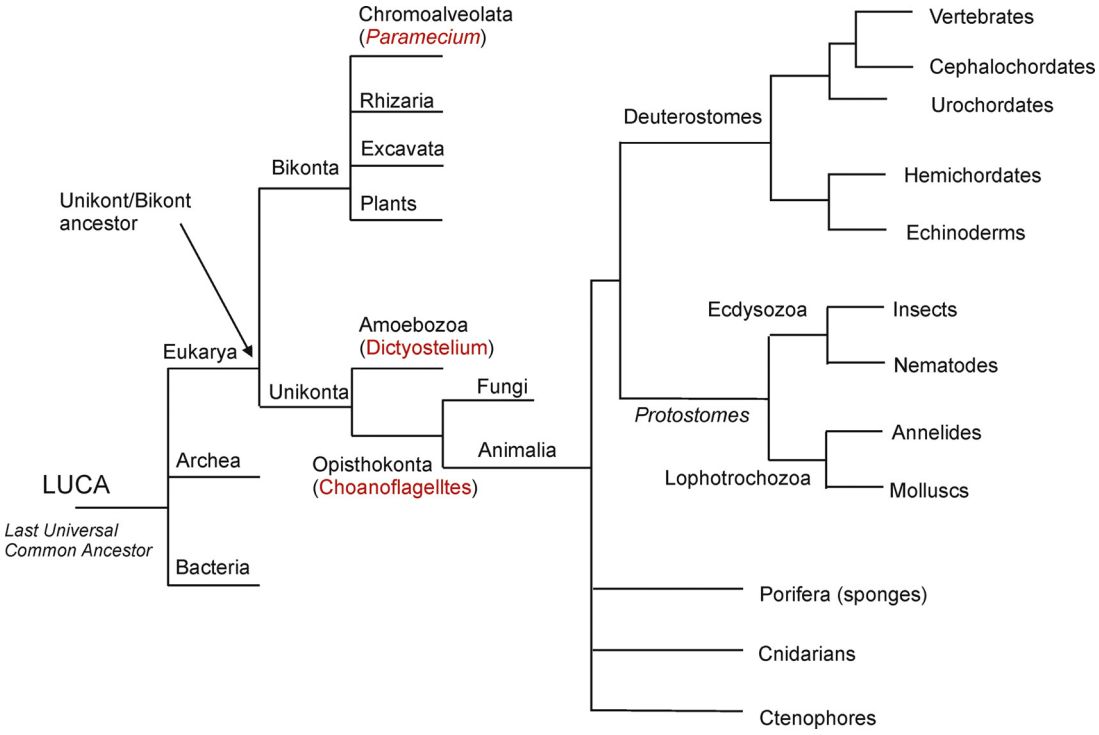

Fig. 1. The tree of life.