J Physiol 0.0 (2023) pp 1–15

The Journal of Physiology

TOPICAL REVIEW

The fallacy of functional nomenclature in the kingdom of biological multifunctionality: physiological and evolutionary considerations on ion channels

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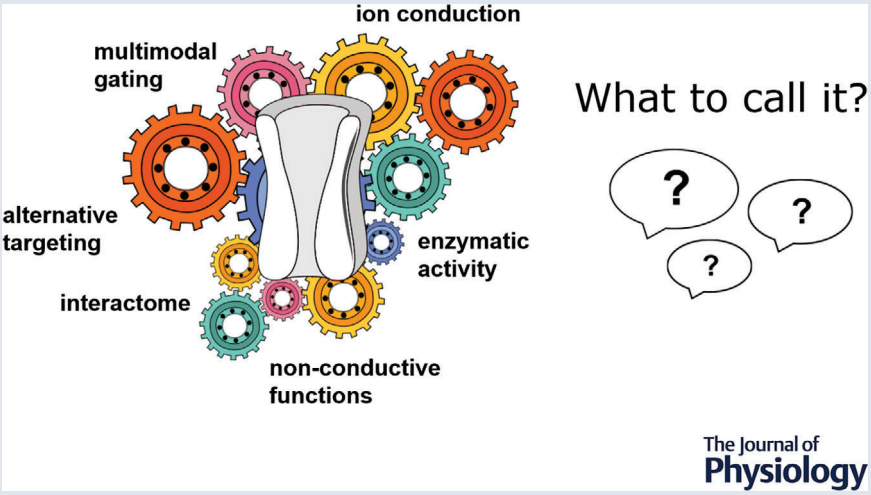
Handling Editors: Laura Bennet & Peying Fong

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP284422#support-information-section).



Abstract Living organisms are multiscale complex systems that have evolved high degrees of multifunctionality and redundancy in the structure–function relationship. A number of factors, only in part determined genetically, affect the jobs of proteins. The overall structural organization confers unique molecular properties that provide the potential to perform a pattern of activities, some of which are co-opted by specific environments. The variety of multifunctional proteins is expanding, but most cases are handled individually and according to the still dominant 'one structure–one function' approach, which relies on the attribution of canonical names typically referring to the first task identified for a given protein. The present topical review focuses on the multifunctionality of ion channels as a paradigmatic example. Mounting evidence reports the ability of many ion channels (including members of voltage-dependent, ligand-gated and

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DOI: 10.1113/JP284422

transient receptor potential families) to exert biological effects independently of their ion conductivity. 'Functionally based' nomenclature (the practice of naming a protein or family of proteins based on a single purpose) is a conceptual bias for three main reasons: (i) it increases the amount of ambiguity, deceiving our understanding of the multiple contributions of biomolecules that is the heart of the complexity; (ii) it is in stark contrast to protein evolution dynamics, largely based on multidomain arrangement; and (iii) it overlooks the crucial role played by the microenvironment in adjusting the actions of cell structures and in tuning protein isoform diversity to accomplish adaptational requirements. Biological information in protein physiology is distributed among different entwined layers working as the primary 'locus' of natural selection and of evolutionary constraints.

(Received 11 May 2023; accepted after revision 24 July 2023; first published online 4 August 2023)
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Abstract figure legend Different features underlying the multifunctional nature of ion channels.

Introduction

The functional roles of proteins depend on several factors. The most obvious and intuitive relies on their overall structure, which is defined by the amino acid composition and spatial conformation and also, in most cases, the assembly of several subunits to form a multimeric complex. The ultimate organization confers some unique features in terms of molecular dynamics that provide the potential (in Aristotelian thought) to perform specific actions. Nevertheless, the tasks engaged by an active biomolecule are established by the environmental milieux in which it is placed to work. A nice example comes from biophysics. Ion channel activity explicitly requires the presence of an ion-conducting pore, in addition to a domain for the detection of selective stimuli that tune its gating; the resulting electrical and possible chemical (calcium-dependent) signals transduce biological information to mediate cellular responses.

According to the 'one structure–one function' perspective focused only on a single canonical purpose, the occurrence of a mislocalization or a structural alteration that disrupts the expected function should eventually yield a disease. The conceptual landscape changes radically, however, if we view the problem in the perspective of the biological complexity marked by multitasking.

Pleiotropy, multidomain proteins, promiscuity and moonlighting are all terms dealing with the ability of a protein to fulfil two or more roles (Espinosa-Cantú et al., 2020). These terms are widely used in the literature, although there is a significant lack of consensus on their connotation, with frequent overlapping. 'Pleiotropy' refers to conditions where a single genetic factor contributes to different cellular, physiological or organismic traits; it can result from several molecular actions, each of which has an influence on a separate biological process, or from a single action that participates in a variety of biological processes. 'Multidomain proteins', which usually arise from gene fusion, contain domains with independent evolutionary history, structure or function (Ekman et al., 2005). Protein 'promiscuity', also referred to as 'broad specificity' or 'poly-specificity', suggests multiple catalytic activities or, in the case of non-enzymatic events, many bindings (Copley, 2020). 'Promiscuous' interactions are very common in the crowded extra- and intra-cellular milieux. They can perturb protein activity at the molecular level, but as long as they do not compromise organismal fitness, they will not be removed by natural selection. Although many promiscuous effects are physiologically irrelevant, they can provide a vast reservoir of potential utilities, acting as the starting point for evolution of new tasks (Copley, 2020). Finally, when a polypeptide performs two or more actions that are not the product of gene fusion, splice variants or multiple proteolytic fragments, it is known as moonlighting (Espinosa-Cantú et al., 2015, 2020).

All these facets of protein multifunctionality are increasingly being recognized and investigated in detail. However, rather than being based on a collective strategy, most of the evidence is handled case by case; moreover, a significant delay in our knowledge is likely to be caused by the still prevalent 'one gene–one function' logic (Beadle & Tatum, 1941; Chapple et al., 2015). Alternative jobs, which are frequently discovered by chance, are arbitrarily demoted to secondary status and referred to as 'non-canonical', because proteins are still primarily studied in terms of their known 'canonical' purpose (Chapple et al., 2015).

From an evolutionary standpoint, multifunctional proteins can increase the functional repertoire and overall complexity in organisms with small genomes, such as viruses, early metazoans and symbionts; alternatively, they

can be used to coordinate the crosstalk and integration between different biochemical pathways of the intricate metabolic and regulatory systems usually associated with larger genomes (Espinosa-Cantú et al., 2020; Faust et al., 2017). On the contrary, pleiotropy is often thought of as an evolutionary constraint based on the idea that, as functional complexity is added to a polypeptide sequence, it becomes increasingly challenging to introduce substitutions and new roles without perturbing existing ones (Espinosa-Cantú et al., 2020). Yet, other models hint that pleiotropy might have a positive impact on the development of novel utilities (Harman et al., 2020). Moreover, experimental and computational approaches converge on the idea that multifunctional genes undergo a slower evolutionary rate because mutations that enhance one function frequently have negative impacts on the others (Pritykin et al., 2015; Salathé et al., 2006).

A significant amount of specialized literature is devoted to a few archetypal instances of protein multifunctionality, which are widely explored from the molecular, cellular and evolutionary perspectives; they include crystallins and uncoupling proteins, in addition to several hormones, growth factors, receptors and enzymes (Freeman et al., 2000; Gaudry & Jastroch, 2019; Hass & Barnstable, 2021; Legendre & Davesne, 2020; Mahata & Corti, 2019; Mahata et al., 1997; Pasqua et al., 2017; Piatigorsky, 2006; Rial & Zardoya, 2009; Rieger et al., 2021; Slingsby et al., 2013). Here, we extend the discussion to cover the vast biophysical subject of ion channels that mediate passive ion fluxes through biological membranes. They offer an engaging context for debating the concepts of redundancy and multifunctionality for a number of reasons. Their long evolutionary history, which originated in the very first prokaryotic forms of life and expanded into distinct structural and functional classes during metazoan evolution, resulted in a combination of conserved traits and the creation of a great diversity. In addition, their modular organization underwent recombinations and remodelling of protein domains, which sometimes originated from evolutionarily distant protein families (Anderson & Greenberg, 2001; Tikhonov & Zhorov, 2018; Coyote-Maestas et al., 2021; Himmel & Cox, 2020; Jaiteh et al., 2016; Moran et al., 2015; Okamura et al., 2005).

According to the conventional picture, ion channels are 'pores' that convert an array of microenvironmental stimuli into electrical currents and membrane potential changes. Even standing in the comfort zone of such a canonical view, the same ion-conducting activity can be involved multiple biological processes. Remarkable examples of multifunctionality in its various forms are provided by transient receptor potential (TRP) proteins (Cao, 2020), which are well known to mediate transmembrane fluxes of cations, including calcium (Ca^{2+}). When TRP proteins are expressed on the plasma membrane (PM) and form ion pores with well-defined biophysical properties, they trigger changes in cytosolic Ca^{2+} associated with a broad variety of downstream effects and biological events (Fig. 1). By the way, intriguingly, the name for TRP refers to the original experiments performed in *Drosophila* compound eyes, where their role was demonstrated in the onset of the receptor potential, a membrane-associated electrical signal requiring TRP activity as ion channels, triggered by light and responsible for phototransduction (Montell, 2021). The TRP proteins are currently recognized to be expressed in all tissues throughout the animal kingdom and to contribute to a wide range of cellular processes, including proliferation, differentiation and migration. Of course, their name is meaningless outside of the sensory systems from which they were first isolated and cloned (Damann et al., 2008).

The multimodal ability of a single TRP protein complex to sense and respond to diverse environmental stresses is very ancient, being evident already for *Saccharomyces cerevisiae* vacuolar chemo- and osmo-activated TRP yeast-1 (TRPY1) (Ahmed et al., 2022; Amini et al., 2021), making them a fascinating object of interest for cell biology, physiology and evolutionary fields (Fig. 1). To take one of the many examples, TRP vanilloid 1 (TRPV1) identifies a protein with different alternative names, 'vanilloid receptor' for its activation upon chemical binding with vanilloids, such as capsaicin, and 'heat receptor' to designate its involvement in thermosensation; in addition, in the alternative name, OSM-9-like TRP channel 1 (OTRPC1), 'osm' refers to the homologue gene osm-9 identified in a genetic screen for *Caenorhabditis elegans* mutants with a defective response to osmotic shock (osmolarity) and odorants (Colbert et al., 1997; Ranade et al., 2015; Zhang et al., 2021). TRP ankyrin 1 (TRPA1) is another polymodal channel sensitive to tissue damage, noxious cold, endogenous compounds released by oxidative reactions, and to the pro-inflammatory peptide bradykinin via phospholipase C signalling (Bandell et al., 2004).

Multimodality is not restricted to TRP proteins. Acid-sensing ion channels (ASICs) are a group of proton-gated cation-permeable channels that belong to the family of the degenerin (Deg)/epithelial sodium channel (ENaC) group (Hanukoglu, 2017). Interestingly, although they are canonically recognized as pH sensors activated by a drop in extracellular pH below 7.0, ASIC1–ASIC4 also participate in the regulation of vertebrate mechanosensitivity. ASIC1 is widely distributed in the visceral sensory ganglia and contributes to oesophageal and colonic afferent mechanotransduction and to urothelium and bladder compliance sensation (Corrow et al., 2010; Page et al., 2005; Yoshiyama et al., 2020). ASIC2 acts as a cardiovascular baroreceptor and is broadly distributed in the somatosensory system, while ASIC3 is functional in dorsal

root ganglia (García-Añoveros et al., 2001; Lin et al., 2016; Lu et al., 2009; Ruan et al., 2021; Wei et al., 2022).

Finally, the ENaC is a major contributor to the control of extracellular sodium homeostasis and displays multiple gating, being sensitive to extracellular sodium and proteases, mechanical forces and oxidative stress (Hanukoglu, 2017; Ilatovskaya et al., 2013; Kizer et al., 1997; Mobasheri et al., 2005).

Unfortunately, it is very challenging to classify multimodal gating unambiguously as pleiotropy, multidomain, promiscuity or moonlighting forms of multifunctionality owing to the limited understanding of the structure–function relationship in the intrinsic membrane protein complexes.

Non-conductive activities of ion channels

The multitasking potential of ion channels goes far beyond the multimodality of pore regulation. It is now increasingly recognized that they are able to exert biological effects regardless of their ion conductivity (Kaczmarek, 2006; Lee et al., 2014; Vrenken et al., 2016); evidence is reported for members of voltage-dependent (mainly K_v and Ca_v), ligand-gated (glutamatergic, purinergic and nicotinic receptors) and the polymodal TRP channel families (Fig. 1).

A first example is provided by potassium (K^+) channels. Cell adhesion is mediated by the recruitment of several proteins that form large complexes called adhesomes; these molecular platforms are composed of, among other things, integrin receptors, growth factors, cytoskeletal elements and a variety of ion channels (Ca^{2+} channels and related Ca^{2+} signalling, proton fluxes and K^+ channels) acting as structural and functional hubs (Becchetti et al., 2017, 2019). Their relationships are reciprocal; K^+ channels, for instance, are activated by integrin engagement but, in turn, regulate the expression of integrin receptors and other adhesion molecules. In cancer cells, the interaction of β1 integrins with the

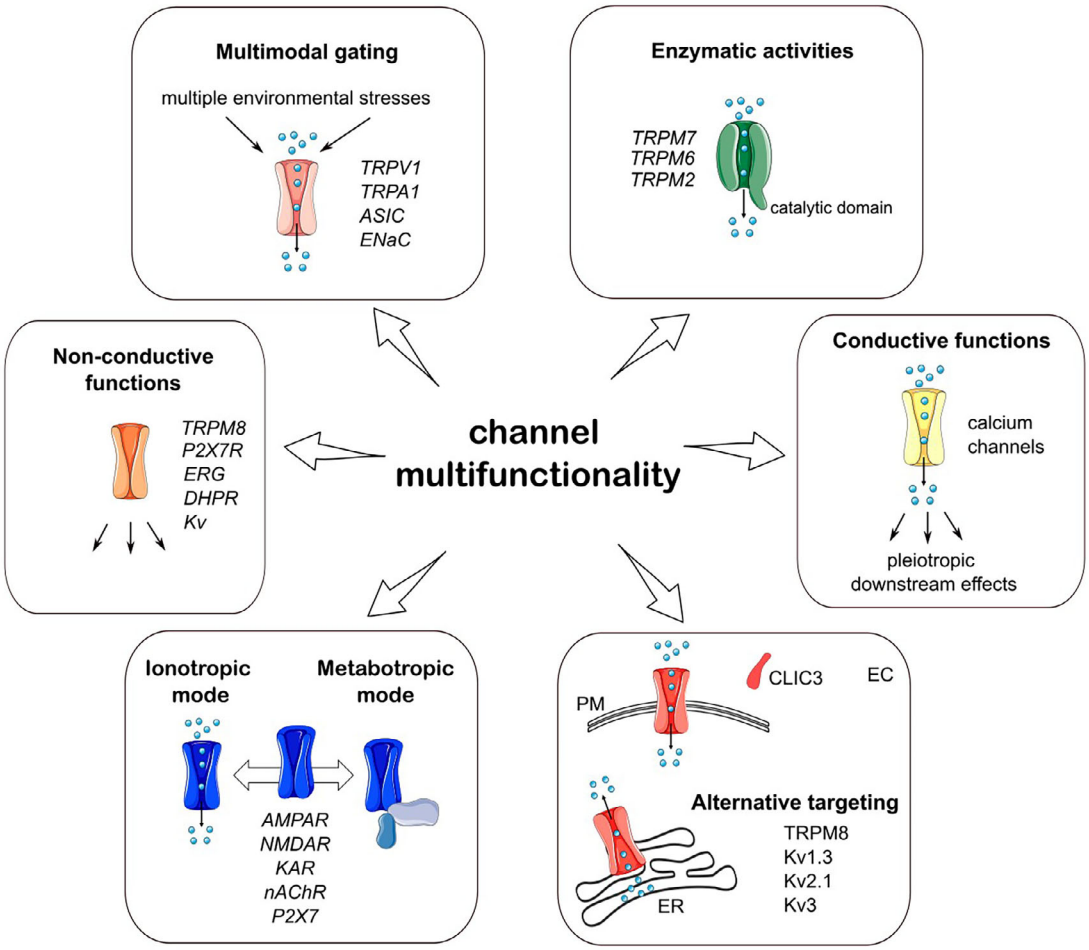

Figure 1. Scheme summarizing the main ion channel multifunctional modalities
The examples are discussed in the text. Abbreviations: EC, extracellular medium; ER, endoplasmic reticulum; and PM, plasma membrane.

human ether-a-go-go-related gene 1 K^+ channel (hERG1 or $K_v11.1$) was found to stimulate distinct signalling pathways based on the conformational state of hERG1 and affect different aspects of tumour progression (Becchetti et al., 2017, 2019). In particular, $\beta 1$ integrin-dependent cell signalling leading to the downstream focal adhesion kinase (FAK) autophosphorylation is modulated by hERG1 activation and requires normal current flow through the ion channel, whereas the membrane macromolecular complex recruits hERG1 channels in the non-conducting states (Becchetti et al., 2017). According to Arcangeli and colleagues, an intriguing explanation for the dual nature of ion channels has to do with their capacity to provide flexibility of adhesion-related signals. By quickly propagating throughout cells, electrical signals allow coordinated cell responses, such as contraction or exocytosis, within milliseconds. Moreover, ionic currents dissipate electrochemical gradients leading to an amplification of local chemical signals; this feature strongly increases the signal-to-noise ratio but makes electrical signals energetically expensive. In contrast, protein–protein interactions optimize specificity of signalling pathways. Additionally, they use less energy, making them better suited to control of long-term biological activities (Becchetti et al., 2019).

The case of K_v channels does not seem to be an isolated exception (Lee et al., 2014). Indeed, evolution exploited a similar strategy to accomplish skeletal muscle contraction by the use of the prototypical voltage-dependent PM Ca^{2+} channels $Ca_v1.1$; they are also 'neutrally' called dihydropiridine receptors (DHPRs) according to their pharmacological sensitivity to dihydropiridine (Di Biase & Franzini-Armstrong, 2005; Mackrill & Shiels, 2020; Rios & Brum, 1987). Unlike the cardiac one, which has a distinct subunit composition, the skeletal muscle DHPR complex is physically connected to ryanodine receptors (RyRs) situated in the sarcoplasmic reticulum membranes; this interaction is directly responsible for calcium release and the subsequent mechanical event of contraction. Depolarization that propagates along the PM triggers a conformational change in DHPRs owing to their voltage sensors; this event, rather than the incoming Ca^{2+} ions, initiates skeletal muscle contraction by tuning RyR activity via a direct coupling in a unique coordination between two separate membrane systems within the same cell (Di Biase & Franzini-Armstrong, 2005; Mackrill & Shiels, 2020; Rios & Brum, 1987). Accordingly, skeletal muscle contraction is retained in the absence of extracellular Ca^{2+}, showing that the Ca^{2+} entry via DHPR is not necessary for the initiation of the process (Armstrong et al., 1972; Flucher & Tuluc, 2017). Interestingly, the physiological role of the more conventional job of $Ca_v1.1$ as a Ca^{2+} channel in this context is still a matter of debate, and it has been suggested that some form of activity-dependent Ca^{2+} entry, termed excitation-coupled Ca^{2+} entry, might contribute (Cho et al., 2017). Hence, nomenclature should somehow reflect the dual nature of the same protein ($Ca_v1.1$); the pore-forming, alpha-1S subunit of the voltage-gated calcium channel (protein $Ca_v1.1$ and gene *CACNA1S*) gives rise to L-type Ca^{2+} currents, on the one hand, and acts, on the other, as an interactor for RyRs.

Multifunctionality is reported for another Ca^{2+} channel belonging to the same family, $Ca_v1.4$, which is the predominant Ca_v channel in rod and cone photoreceptors (Maddox et al., 2020). Several mutations in the related human gene cause vision impairment, and knockout mice lack synaptic responses of photoreceptors. This evidence was related originally to a requirement for $Ca_v1.4$ as a canonical pore-forming structure that mediates Ca^{2+} fluxes and the consequent presynaptic glutamate release. However, the physical absence of the presynaptic $Ca_v1.4$ in knockout mice led to the identification of an alternative, non-conductive role in the molecular assembly of rod synapses. Presynaptic Ca_v channels serve both as organizers of synaptic building blocks acting as scaffolding proteins and as sources of Ca^{2+} ions in the first step of the visual transduction (Maddox et al., 2020).

Also, voltage-dependent sodium (Na_v) channels play roles unrelated to their conductivity (Marchal & Remme, 2022; Rivaud et al., 2020). The cardiac $Na_v1.5$ is responsible for the fast initial upstroke of the action potential and, as such, is a crucial determinant of cardiomyocyte excitability and electrical impulse conduction through the myocardium. Most of the human diseases associated with $Na_v1.5$ dysfunction are clearly the consequence of channel defects leading to conduction slowing, repolarization abnormalities and consequent arrhythmias. However, some $Na_v1.5$-related disorders, in particular structural abnormalities, cannot be explained directly or entirely by a defective $Na_v1.5$ expression or biophysics; indeed, mutations can also lead to the development of cardiac fibrosis, dilatation and hypertrophy, which cannot readily be ascribable to a simple electrical anomaly. Interestingly, these different disease entities can co-exist with electrical defects (Rivaud et al., 2020). The non-conductive purposes of $Na_v1.5$ could rely on its interaction with a variety of other proteins involved in cytoskeletal anchoring, signal transduction and cell adhesion, including dystrophin, laminin, integrins and extracellular matrix components. Although it is clear that this interactome modulates channel function, it is not yet known whether the reciprocal relationship is also true, as shown for the aforementioned $K_v11.1$ (Becchetti et al., 2017, 2019). The broad disease spectrum associated with $Na_v1.5$ mutations, far beyond cardiac tissue and including several tumours, centres around the multifunctionality of the protein complex, whose contribution in a physiological context deserves further investigation.

The ability to perform multiple jobs is also reported for the 'ligand-gated ion channels', a term referring to the

large family of ion channels that are gated by extracellular ligands (Anderson & Greenberg, 2001; Tikhonov & Zhorov, 2018; Jaiteh et al., 2016); nonetheless, the same proteins are defined as 'ionotropic receptors' to emphasize their intrinsic ability to act as receptors which, upon ligand binding, directly promote membrane currents. These are two annotations (receptors/ion channels) for the same biological structures of paramount relevance, because they include widespread and conserved membrane transducers for the bioactivity of neurotransmitters such as acetylcholine, glutamate, γ-aminobutyric acid (GABA) and others, in addition to purines, including extracellular ATP (P2X purinergic receptors). This ambiguity alone represents a first non-trivial pitfall for cell physiologists and raises evolutionary considerations about which function appeared first and the co-evolution of distinct tasks.

A higher level of complexity can now be recognized thanks to recent developments. Glutamate-sensitive cation channels in postsynaptic membranes are triggered by an extracellular agonist and thus called 'ligand-gated' receptors. However, as previously mentioned, they are also canonically annotated as ionotropic, based on their intrinsic conductivity, unlike the metabotropic counterparts lacking this feature. In recent years, such a dichotomy has become blurred (Valbuena & Lerma, 2016); although α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), *N*-methyl-D-aspartate receptors (NMDARs) and kainate receptors (KARs) are referred to as ionotropic receptors, they can also signal via non-canonical metabotropic pathways (Pressey & Woodin, 2021; Nabavi et al., 2013; Rozas et al., 2003; Wang et al., 1997; Fig. 1). In particular, the awareness that KARs have the ability to signal using either a canonical (ionotropic) route or a non-canonical (metabotropic) route has shed light on the peculiar fact that, despite being abundantly expressed throughout the brain, there are very few recordings of synaptic KAR currents reported in the literature (Pressey & Woodin, 2021). Another prominent example are the nicotinic receptors (nAChRs) that belong to the Cys-loop family of ligand-gated ion channels (Changeux, 2012). They are membrane-spanning homo- or hetero-pentamers that, upon binding of agonists, form cationic pores permeable to Na^+, K^+ and Ca^{2+}. In addition to their canonical ionotropic functions, flux-independent, metabotropic pathways have been proposed in nervous and immune systems (Kabbani & Nichols, 2018; Richter & Grau, 2023; Valbuena & Lerma, 2016; Zakrzewicz et al., 2017; Zhong et al., 2013).

Similar metabotropic behaviour has been described for P2X7 purinergic receptors, the products of a highly polymorphic gene giving rise to several splice variants (P2X7A–P2X7J) (Pegoraro et al., 2021). The 'conventional' forms include two-transmembrane domains organized in trimeric complexes and usually regarded as Ca^{2+}-permeable ionotropic receptors sensitive to high extracellular ATP levels; however, a number of intracellular signalling pathways are not related to Ca^{2+} fluxes even in the presence of Ca^{2+} conductivity (Amstrup & Novak, 2003; Kopp et al., 2019; Morioka et al., 2008; Scarpellino et al., 2019; Scarpellino, Genova, et al. 2022; Scarpellino, Munaron, et al., 2022; Shabbir et al., 2008). Furthermore, some truncated isoforms called 'non-functional' (nfP2X7) do not express the prototypical biophysical properties of the pore or, as in the case of P2X7J, completely lose the conductivity (Pegoraro et al., 2021). P2X7J is expressed preferentially in epithelial cancer cells and lacks the entire intracellular carboxy terminus, the second transmembrane domain and the distal third of the extracellular loop. It fails to form pores and interacts with the wild-type P2X7R, possibly acting as a dominant negative on P2X7A (Feng et al., 2006). This example might well represent a paradigm for the regulation of ion channels by their truncated isoforms; the same process is reported for other proteins, including voltage-dependent K^+, Na^+ and H^+ channels, with functional outcomes that deserve further investigation (Gong et al., 2018; Hondares et al., 2014; Kerr et al., 2008; Serna et al., 2023; Thalhammer et al., 2020; Tomita et al., 2003; Ventura et al., 2020).

Some members of the melastatin-like TRP group (TRPM) are particularly fascinating for their multidomain organization; they are referred to as 'chanzymes', based on the unique dual nature of channels and enzymes (Fig. 1). TRPM7, TRPM6 and TRPM2 mediate Ca^{2+} currents and are characterized by peculiar cytosolic C-terminal domains with catalytic properties (Y. Huang et al., 2020). Beyond their role in the regulation of intracellular Ca^{2+} and Mg^{2+} homeostasis, TRPM7 and the highly homologous TRPM6 are known for their unique serine/threonine α-type protein kinase activity (Duan et al., 2018; Luongo et al., 2018). Intriguingly, the truncated carboxyl terminus, which harbours the kinase ability, localizes to the nucleus, where it modulates gene expression through histone phosphorylation (Koch, 2014). In contrast, TRPM2 exhibits a specific C-terminal nudix-type motif 9 (NUDT9)-homology (NUDT9-H) domain, which cleaves ADPR into AMP and ribose-5-phosphate (Tóth et al., 2014). The operational coupling and the potential interplay between the pore-forming and enzymatic domains is still poorly understood and debated, although experimental data seem to support their independence (Matsushita et al., 2005; Tóth et al., 2014). Again, it is unknown how the two started to co-exist and which function emerged first during evolution.

The environmental factor

The environment in which cellular components are put to act is one of the causative factors affecting their 'effective', real performance. At the molecular level, we refer to the microenvironment as the local context, the collection of biomolecules in the immediate vicinity along with the solvent, each with its own unique mechanical and physicochemical characteristics, such as ion composition, pH and viscosity.

In PM ion channels, the pore domain is directly responsible for the generation of electrical and/or chemical responses to external chemical, electrical, mechanical or thermal perturbations. Undeniably, in order to be activated and fulfil their canonical task, they must be located on the PM. An alternative targeting could convey them where the stimulus is not available; in this case, even if it retains its pore-forming ability, the 'ion channel' would cause an entirely unrelated downstream impact or, perhaps more intriguingly, it might fail to produce net currents, not necessarily as a result of inner structural changes or gene alteration, but rather as an effect of misplacement. This occurs when the complex enters intracellular membranes [say, endoplasmic reticulum (ER), Golgi or vesicles] that are exposed to other environments; thus, electrochemical gradients (the driving force) and/or pore gating are altered. It is worth noting that this does not necessarily imply a functional lack *senso strictu*, because the pore structure is unaffected. Hence, it is not surprising to find this phenomenon as a contributor to cell physiology and, eventually, to overt pathologies. The loss of the pore-related canonical activity is equivalent to a loss of function from a 'one structure–one function' perspective. However, if we consider the potential multitasking of biological systems, we cannot rule out a scenario in which alternative targeting, rather than mistargeting, with its negative connotation, entails the (potential) assumption of other roles. Such new 'non-canonical' functions are increasingly being observed, not only in diseases but also in physiology (Fig. 1). Of course, the use of the term 'non-canonical' is a direct and consistent consequence of the original viewpoint whereby only one action, usually the originally discovered action, was assigned to the protein.

Similar to the aforementioned P2X7, TRP channel regulation is highly influenced by alternative splicing processes, which can result in some 'non-functional' short variants that operate as dominant-negative mutants against the full-length channel. These small isoforms, in particular, have little to do with the standard full-length pore-forming structure, but instead represent proteins that affect its tetramerization, stability and function. TRPV1 (Wang et al., 2004), shorter TRPM1 products (Zhiqi et al., 2004), s-TRPV2 (Nagasawa et al., 2007) and several short TRPM8 splice variants, such as s-TRPM8 (Bidaux et al., 2012), might all contribute to the formation of non-functional heteromers by preventing their activity and/or translocation to the PM.

The picture is complicated further by the existence of other variants, in addition to small non-functional ones, that retain the pore structure but exhibit different properties in terms of activity, protein–protein interaction and/or subcellular localization (Stamm et al., 2005). A very interesting example is provided by the human TRPM8. Full-length TRPM8 is expressed mainly on the PM, where it performs its main action as a Ca^{2+}-permeable channel, influencing a number of intracellular signalling pathways. Recently, a short isoform characterized by an unconventional structure, with four instead of six transmembrane domains, was found to be localized, in part, in mitochondria-associated ER membranes, where it mediates the mitochondrial uptake of Ca^{2+} released from the ER (Bidaux et al., 2018). Additionally, a short isoform is expressed selectively on the endothelial ER, revealing a job that is entirely unrelated to Ca^{2+} dynamics and traceable as an inhibitor of the small GTPase Rap1A, resulting in a reduction of cell motility (Genova et al., 2017). The same evidence has been reported in prostate and other epithelial cancer cells (Chinigò et al., 2022), suggesting that TRPM8 'ectopic' expression might provide new functions owing to a change in the interactomic neighbourhood. The close and bidirectional interplay between TRP channels and small GTPases is noteworthy; the former affects the activity of small GTPases through Ca^{2+}-dependent or -independent pathways; the latter, in turn, impact on TRP channel tasks by controlling their intracellular trafficking and their gating (Chinigò et al., 2020). Intriguingly, the expression of TRPM8 isoforms with distinct prostate localizations is strongly correlated with the degree of cell differentiation and reliance on androgen regulation, suggesting that the two variants might derive from an alternative TRPM8 gene promoter with different sensitivity to the hormone (Bidaux et al., 2007). TRPV6 also appears to be under androgenic regulation in the prostate, supporting the idea that the cellular milieu might influence both the expression and the localization of ion channels and, consequently, the 'canonical' or 'non-canonical' functions they might exert (Lehen'kyi et al., 2007).

Analogous non-conductive actions of the voltage-gated potassium channels $K_v2.1$, $K_v1.3$ and K_v3 have been reported, which are often associated with particular locations within the PM in both physiological and altered conditions (Deardorff et al., 2021; Johnson et al., 2019; Kaczmarek, 2006; Styles et al., 2021; Wu et al., 2021). $K_v2.1$ provides delayed rectifying currents in rat hippocampus, but its neuronal membrane distribution pattern is unusual; while one component is freely diffusive on the PM, as predicted by the generalized Singer and

Nicolson fluid mosaic model, another subpopulation selectively clusters on the soma, dendrites and axonal initial segment. This restricted targeting is attributable to the formation of ER/PM junctions that contain hundreds of non-conductive $K_v2.1$ channels (Fox et al., 2013). The relevance of ER/PM connections, already discussed in the classical context of skeletal muscle contraction, is widespread, because they contribute to the crosstalk between cell organelles, regulate ion fluxes and play a role in lipid transfer and in endo- and exocytosis (Johnson et al., 2019). Another K^+ 'channel', $K_v1.3$, modulates cellular respiration through a non-conductive route to generate reactive oxygen species that drive proliferation (Styles et al., 2021). Finally, a surprising task that is unrelated to potassium conductance has recently been reported for K_v3 proteins, known to control neurotransmitter release by repolarizing action potentials. In mouse nerve terminals, $K_v3.3$ organizes the presynaptic F-actin cytoskeleton to facilitate endocytosis and vesicle mobilization, which maintains synaptic transmission during repetitive firing. Errors in such non-conductive purposes might be involved in several neurological diseases (Wu et al., 2021).

A final and somewhat extreme example is the 'chloride intracellular channel protein 3' (CLIC3), which, surprisingly, is found as an abundant constituent of the secretome released by cancer-associated fibroblasts, stromal components taking part in tumour invasion and angiogenesis (Hernandez-Fernaud et al., 2017). Secreted CLIC3 promotes endothelial invasiveness to prompt angiogenesis and enhances cancer progression; it acts as a glutathione-dependent oxidoreductase that reduces transglutaminase-2 (TGM2) and tunes TGM2 binding to its cofactors (Hernandez-Fernaud et al., 2017). Needless to say, CLIC3 is a largely misleading designation for a protein that can no longer act as a membrane pore owing to its membrane-free targeting.

As clearly depicted in several examples discussed thus far, the environment matters. In addition to its direct impact on protein functions through the specification of a particular set of 'working conditions' (including pH, temperature, hypoxia and the interactome), the complex interplay with genomic dynamics tunes the structural features and variety of forms encoded by the same gene. The abundance and functional relevance of protein isoforms are gaining more attention; a recent deep human proteome sequencing identified a million unique peptides from 17,717 protein groups, revealing a new and largely unexplored dimension of protein variation (Sinitcyn et al., 2023). This expansion of transcriptomic and proteomic diversity is the setting that enhances phenotypic plasticity, which is the ability to trigger a rapid plastic response to counteract acute environmental stresses. RNA processing, including alternative splicing and polyadenylation, is crucial for the generation of multiple mRNA isoforms and is widespread across all higher eukaryotes, being engaged in cell differentiation, organ development and disease (Huang & Zhan, 2021; Zhu et al., 2018). Nearly 95% of multi-exon mammalian genes undergo alternative splicing and >70% express polyadenylated isoforms (Zhang et al., 2020). Intriguingly, phenotypic flexibility is of paramount relevance from a functional perspective, both in physiological events and in human disorders; however, whether and how it impacts evolution is controversial. Based on the seminal work by Waddington (1953), the so-called 'plasticity-first' hypothesis (also known as plasticity-driven evolution) postulates that plastic behaviour often introduces phenotypic variants that can improve organismal fitness under abrupt environmental fluctuations, thus enabling evolutionary rescue by 'buying time' for future adaptation to occur through genetic accommodation (Levis & Pfennig, 2019). This is a major issue for a new evolutionary model whereby functional, genetic and environmental components crosstalk in an intricate network.

Gene ontology, towards a solution?

Deeply connected to this topic, gene ontology (GO) still deserves a separate discussion. Since the 2000s, under the influence of nascent high-throughput omics technologies, it has become necessary to set up a new systematics to organize the current knowledge about genes and gene products in such a way that it could be managed and understood by both humans and machines within the bioinformatics context. For this purpose, GO has been one of the most appreciated answers, providing a modular, extensible, multiscale, hierarchical and species-agnostic framework for describing biological systems (Ashburner et al., 2000; Carbon et al., 2009). GO defines three independent ontologies to provide three respective representations of all the cellular components, molecular functions and biological processes across the multiplicity of living species. Each cellular component/molecular function/biological process is referred to as a class or term and is represented by a node in the respective ontology graph. These three biological levels of increasing complexity are structures made up of thousands of nodes each, which can be used eventually to annotate genes with one or more functions, simply by tagging gene names (or, better, gene symbols) to one or more GO terms, based on the current and evolving evidence about their roles. Within the GO system, the following assumptions are made: (i) annotations represent the normal (physiological) jobs of the gene products; (ii) a gene product can be annotated at zero or more terms from each ontology; and (iii) if a gene product is unannotated, its role is still unknown. Given that the association of genes with GO terms is, in general, a many-to-many relationship, both redundancy and multifunctionality can be accounted

for easily; a single GO term can be associated with many different genes having the same function and, most relevant for this commentary, a single gene can be annotated in more than one functional class. For example, the aforementioned chanzyme TRPM7 is currently annotated under the following terms of the molecular function ontology: protein serine/threonine kinase activity (accession GO:0004674), calcium channel activity (accession GO:0005262) and ATP binding (accession GO:0005524) (Fig. 2). Notably, none of these terms is labelled as the principal or canonical task. In the case of multiple annotations, GO does not provide any ranking of 'functional relevance'. However, multiple annotated functions can be trusted differently, because each GO annotation also includes an evidence code describing the nature of the evidence supporting the annotation (experimental, phylogenetic, computational etc.).

It is worth noting that GO deals only with gene functions and not with gene nomenclature. Other consortia (such as HUGO, HGNC and UniProt) are in charge of choosing, modifying and recommending the official nomenclature for genes and their products.

However, despite all the best efforts, it would still be impossible to find exhaustive functional names for all genes. In fact, the legacy of historical nomenclature is often a heavy burden that cannot easily be left behind. Instead of searching for the most appropriate and inclusive names, functional ontologies take conventional gene names as such (as symbols) and go ahead, building abstract functional classes to be used as annotations for genes. Ultimately, the redundancy and the multitasking of many genes or gene products are among the main reasons behind the birth and the success of the GO project. Without an ontology, we would need as many terms as

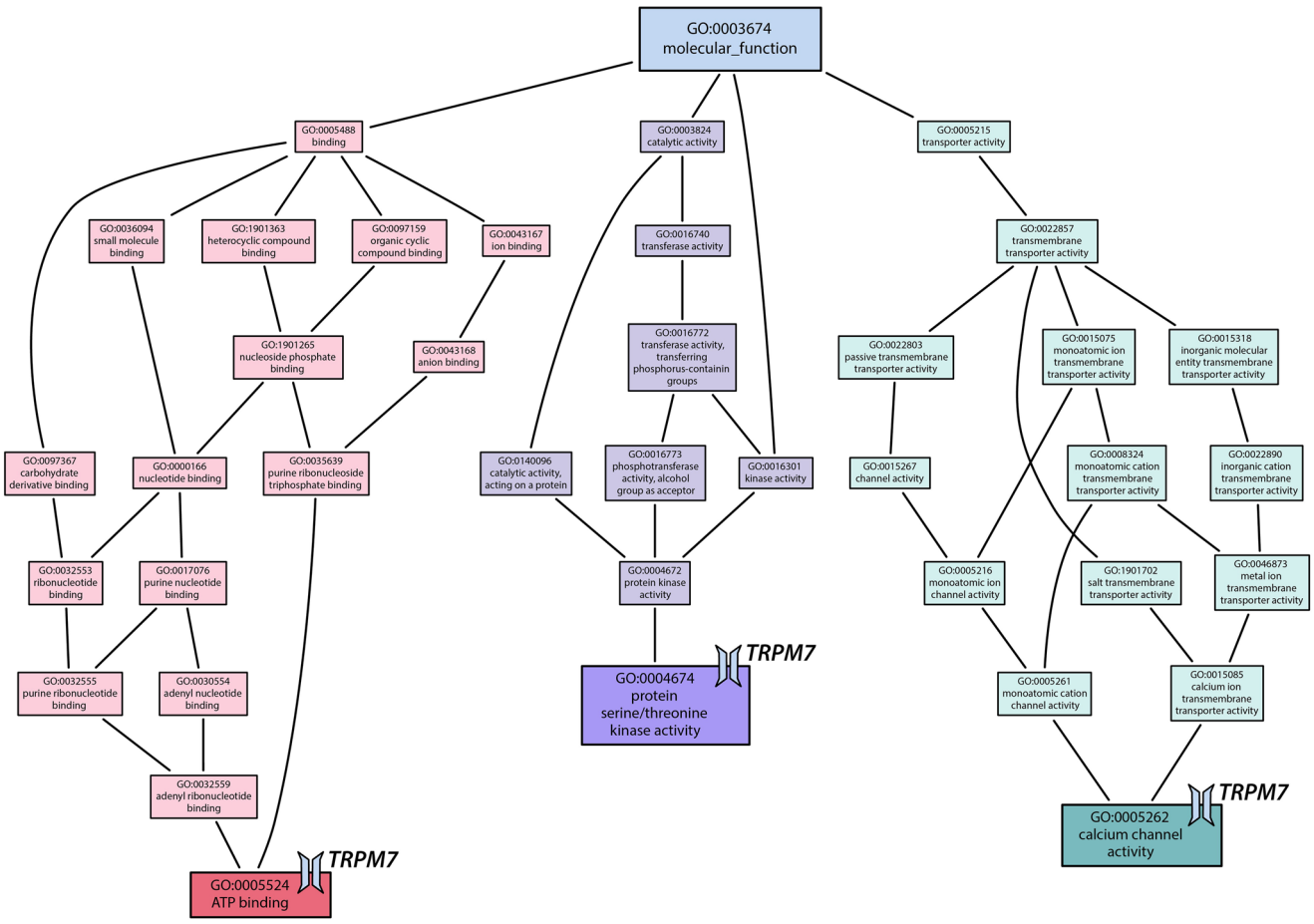

Figure 2. Graphical representation of the gene ontology direct acyclic graph of the deepest (i.e. most specific) molecular function terms to which TRPM7 is currently annotated
The same gene product is associated with three unrelated terms to account for the multitasking nature of this protein (ion channel and enzyme): the ATP binding (GO:0005524) sub-DAG in red; the protein serine/threonine kinase activity (GO:0004674) sub-DAG in violet; and the calcium channel activity (GO:0005262) sub-DAG in teal green. At the same time, these three gene ontology terms are associated with many other gene symbols (to date, 1510, 460 and 129 human gene products, respectively), making it possible to address both multifunctionality and functional redundancy. Abbreviation: DAG, direct acyclic graph.

there are genes (for all species!) in order to describe what we know about their roles. In contrast, ontology provides a controlled vocabulary of classes and internal relationships that allows us to enunciate shared and multiple gene functions simply by combining a small set of terms.

The GO database is easily accessible through the AmiGO 2 web browser (http://amigo.geneontology.org/amigo/landing), the official tool supported by the GO Consortium for retrieving information about terms and annotations (Carbon et al., 2009). Such a system might represent an effective alternative for cellular biologists to get rid of the problem of gene nomenclature that is misleading, legacy, inconsistent or lacking multifunctionality, always acknowledging that GO can, at most, reflect the currently available knowledge about gene functions, and it is not free from errors, inaccuracies or omissions. However, and more importantly, GO is also a highly dynamic entity, because the GO Consortium regularly curates and releases new versions of the GO knowledgebase, containing changes to both the annotations and the underlying structure of GO terms, on a regular basis, also integrating the contributions from the research community.

Conclusion

The assignment of names often referring to a single function is a convenient solution and a prevalent way of action for our brain, which detects, locates and categorizes everything it describes. It is a way to bring order and provide apparently reliable knowledge. However, at the same time, this habit does not do justice to the complexity of physiology and biological evolution, which are characterized (among other things) by pervasive multitasking and redundancy.

Although history remembers Jean-Baptiste de Lamark, the father of modern evolutionary thought, for his outstanding contribution to the redefinition of animal taxonomy (vertebrates and invertebrates), the French scientist critically and clearly explained the boundaries of classifications in the life sciences in the first chapter of his Philosophie Zoologique (1809), significantly entitled 'On artificial devices in dealing with the productions of nature' (translation by Hugh Elliot):

> Throughout nature, wherever man strives to acquire knowledge he finds himself under the necessity of using special methods, 1st, to bring order among the infinitely numerous and varied objects which he has before him; 2nd, to distinguish, without danger of confusion, among this immense multitude of objects, either groups of those in which he is interested, or particular individuals among them; 3rd, to pass on to his fellows all that he has learnt, seen and thought on the subject. Now the methods which he uses for this purpose are what we call the artificial devices in natural science—devices which we must beware of confusing with the laws and acts of nature herself.
>
> Instead of deceiving ourselves into confusing our works with hers, we should recognize that classes, orders, families, genera and nomenclatures are weapons of our own invention. We could not do without them, but we must use them with discretion…these groupings, of which several have been so happily drawn up by naturalists, are altogether artificial (Lamarck, 1914).

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Additional information

Competing interests

None declared.

Author contributions

L.M.: literature analysis, writing and original draft preparation, general revision; G.C: literature analysis, writing, general revision; G.S.: literature analysis, writing; F.A.R.: literature analysis, writing, general revision. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

None.

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Keywords

cellular physiology, evolutionary physiology, functional names, ion channels, multifunctionality

Supporting information

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