SRGAP2 and the gradual evolution of the modern human language faculty

Maties Marí[1], Pedro Tiago Martins[1], and Cedric Boeckx[1,2,3,*]

[1] *Section of General Linguistics, Universitat de Barcelona*
[2] *Universitat de Barcelona Institute for Complex Systems*
[3] *ICREA*
[*] Corresponding author: `cedric.boeckx@ub.edu`

Abstract

In this paper we argue that vocal learning in *Homo* preceded the emergence of Anatomically Modern Humans. We build our claim on the evolutionary history of the SLIT-ROBO GTPase 2 gene (SRGAP2). The SLIT-ROBO pathway has been shown to have an important role in the context of vocal learning. Though the influence of particularly SRGAP2 in the emergence of this aspect of language has not gone unnoticed, recent results now allow us to articulate a mechanistic hypothesis of its role in the context of axon guidance. Specifically, SRGAP2C, a duplication of SRGAP2 crucially also found in Neanderthals and Denisovans, but not in extant mammals, inhibits the "original" *SRGAP2A*, which in turn modulates the axon guidance function of SLIT-ROBO. This, we claim, could have played a role in achieving the critical cortico-laryngeal connection of the vocal learning circuit. Our conclusions support the idea that complex vocal learning could already have been part of the arsenal of some of our extinct ancestors.

Keywords: SRGAP2, vocal learning, language evolution, FOXP2, birdsong

1 Introduction

There has been much controversy among scholars regarding when the faculty of language arose in the evolutionary history of our species. Proposals put forward in the last decades cover a range of dates as large as 100,000-500,000 years ago [1, 2, 3, 4]. A recent special issue on the biology and evolution of language also reflects the disparity of competing positions [5].

Part of the problem when addressing this question lies in the fact that many researchers continue to see the language faculty as a homogeneous organic object. But we believe that it is far more promising, from a biological point of view, to see our linguistic competence as a complex mosaic formed by a species-specific ('novel') combination of several inherited and phylogenetically heterogeneous traits, tinkered with along traditional Darwinian lines [6, 7]. We expect many of these pieces of the language mosaic to be fairly straightforwardly recognized in other species (homologies), whereas other pieces may have less transparent roots [8]. Inasmuch as the appearance and development of these various traits is directly related to genetic factors, a crucial source of evidence for tracing the phylogenetic history of language, and ultimately timing its emergence, comes from

the study of the genetic material remaining in fossils of ancient organisms. Progress in paleogenetics has dramatically changed the testability of some evolutionary scenarios [9]. A famous example of this was given in 2007 by Krause et al. [10], who found that *FOXP2*, a gene associated with language impairments and hampered orofacial movements [11], has the same two unique mutations in both Neanderthals and humans, critically missing in our closest extant great ape relatives. To the extent that these two mutations contributed to the establishment of some aspects of our brain's language-readiness [12, 13], Krause et al.'s discovery strongly suggests that aspects of our language faculty had evolved prior to the divergence of the two lineages, some 600,000 years ago [14]. In this paper we focus on the evolutionary history of *SRGAP2*, which codes for the SLIT-ROBO GTPase activating protein 2 (SRGAP2). We argue, on the basis of what we have learned from other species about vocal learning, that vocal learning was established in *Homo* before the emergence of anatomically modern humans. While the link between *SRGAP2* duplication and language evolution has been mooted before [15, 16], we show how it has become possible to provide a mechanistic articulation of this link, making the hypothesis fully testable.

1.1 Vocal learning in birds: a mirror for human language evolution

Vocal learning is the ability to acquire and produce vocalizations by imitating communicative acoustic patterns produced in the environment, prototypically by members of the same species. Such an ability is displayed in a limited number of lineages phylogenetically scattered across some groups of mammals (bats, elephants, cetaceans, pinnipeds, and humans) and birds (songbirds, parrots, and hummingbirds) [17]. Among the pieces interlocked in the language mosaic, we have decided to focus on vocal learning here because it is the best understood to date in light of the recent literature [18, 19, 15]. As such, it provides the best testing grounds for evolutionary scenarios concerning some important aspects of human language.

The vocal learning literature, especially the line of research pursued by Erich Jarvis and colleagues, already offers interesting scenarios to test. Let us briefly sketch them here, as they will play an important role in the background of the next sections. Vocal learning birds and humans share a number of forebrain structures specialized in song and speech control, respectively [19]. Among them, all three learning avian species exhibit seven cerebral and various thalamic vocal nuclei that are distributed in two pathways: the anterior, or vocal learning pathway, which is mainly specialized in vocal imitation and malleability, and the posterior, or vocal production pathway, which associates with the intentional production of learned vocalizations. Within this posterior pathway, which will be focus of major interest in the following sections, oscines, parrots, and hummingbirds present three analogous motor regions in the cortex, namely the robust nucleus of the arcopallium (RA), the central nucleus of the anterior arcopallium (AAC), and the vocal nucleus of the arcopallium (VA), respectively, which are in turn analogous to the laryngeal motor cortex (LMC) in humans. In both learning birds and humans, this nucleus makes a direct projection to the brainstem motor neurons (MN) that control the syrinx in birds and the larynx in humans [20, 15, 21, 19].

On the basis of such similarities, a motor theory of vocal learning has been proposed [21], arguing that cerebral systems specialized for vocal learning in distantly related lineages are independent evolutions of a motor system inherited from their common ancestor. Experiments in gene expression [21, 22, 23, 24] certainly point in this direction, further supporting that the posterior pathway, which we focus on next, must have emerged from a primitive motor system [15, 21, 25]. Since several forebrain motor learning pathways with sensory input appear to be formed during early development by successive duplications, thereafter projecting to various brainstem or spinal cord

neurons associated with different muscle groups, it has been proposed that the posterior connection appeared similarly as one further duplication which then projected to the brainstem MN in charge of the vocal organs [26, 15].

There are reasons to believe that the posterior pathway develops gradually, as it is present at a very rudimentary level in the brain of a non-vocal-learning suboscine species. Indeed, as Liu et al. have shown [27], the eastern phoebe (*Sayornis phoebe*), closely related to songbirds, possesses a specialized forebrain region that seems homologous to the RA in oscines. This region presents descending projections to the brainstem respiratory nucleus and has a singing-associated function. In this regard, eastern phoebes present a long period (8-9 months) of song plasticity before its crystallization. Symptomatically, this circuitry seems to be a proto-form of what we find in vocal learning oscines, though not developed enough for vocal learning brain-readiness inasmuch as, unlike in songbirds, there is no direct projection from the arcopallial RA-like nucleus to the tracheosyringeal neurons.

Once this critical neural pathway is established, it is quite likely to undergo further elaborations, giving rise to more complex forms of vocal learning. A symptomatic case that can serve as an example for such specializations can be found in parrots, known to be able to imitate vocalizations of conspecifics, but also sounds produced by other species. A study involving the three superfamilies of parrots (*Strigopoidea*, *Cacatuoidea*, and *Psittacoidea*) [28] has revealed an internal subdivision in their song cortical nuclei, wherein a core region shows different gene expression from the surrounding shell area, while both exhibit in turn different expression from the surrounding motor cortical region. Interestingly, the posterior connection to the brainstem MN associated with the syrinx, along with other connections with different forebrain vocal regions, is projected exclusively from the core region and not from the shell [15]. Chakraborty and Jarvis [15] suggest that the core region in the parrot AAC evolved convergently in all three avian vocal learning species via duplication from the surrounding motor regions, and subsequently the shell area was developed in parrots, allowing for their more complex vocal proficiency.

As we just saw, critical neural stuctures such as the posterior pathway, taken as a reference point for the origin of the vocal learning capacity, likely emerge in proto-form, and, once present, can be subject to further elaboration, under the influence of several factors. We believe that the same could be true for the emergence of language in our lineage [29].

1.2 The *SRGAP2* gene suite and the timing of critical evolutionary steps in Homo

Although *SRGAP2* is highly conserved among mammals [30] and has remained unchanged at least in the last 6 million years of our evolution (its F-BARx domain is identical in humans, chimpanzees, bonobos, and orangutans) [31], it has given rise to three human-specific duplications, two of which underwent subsequent adaptive mutations. The sequence of events, identified by Dennis et al. [30], illustrated in Fig. 1, happened as follows (the chronological ranges have been calculated assuming the timing of divergence between chimpanzee and human lineages within a span of 5-7 million years ago (mya), based on fossil records [32, 33, 34] and genetic analyses [35]): The first duplication took place 2.8–3.9 mya, when the promoter and first nine exons of the original gene —which we will hereafter call *SRGAP2A* to distinguish it from its mutations— were copied from the locus 1q32.1 to 1q21.1, thus originating the primitive *SRGAP2B* (*P-SRGAP2B*). A second duplication occurred 2.0–2.8 mya, when *P-SRGAP2B* was copied from 1q21.1 to 1p12, thus giving rise to the primitive *SRGAP2C* (*P-SRGAP2B*). In the aftermath of this event [30, 31], the two primitive duplicated

copies, *P-SRGAP2B* and *P-SRGAP2C*, accumulated non-synonymous mutations which resulted in the contemporary *SRGAP2B* and *SRGAP2C* forms, carrying five (R73H, R108W, R205C, R235H, R250Q) and two (R79C, V366L) aminoacid replacements, respectively. Finally, the third and last duplication, which occurred 0.4-1.3 mya, copied the modern *SRGAP2B* from 1p12 to 1q21.1, thus giving rise to *SRGAP2D* [30]. Consistently with the timing of its appearance, all three human paralogs, *SRGAP2B*, *SRGAP2C*, and *SRGAP2D*, have been found also in the genomes of Neanderthals and Denisovans [36].

Importantly, the timing of the *SRGAP2* duplications appears to correspond fairly closely to some landmarks in our lineage in terms of brain size and use of stone tools in the transition from *Australopithecus* to *Homo*, raising the possibility that the relevant duplication contributed to these phenotypic changes [37, 16, 29]. Thus, the time of the first duplication (*P-SRGAP2B*) parallels the appearance of *Australopithecus*, which had an average brain size of ca. 475 cc, similar to that of genus *Pan*. The second duplication span (*P-SRGAPC*) corresponds to the appearance of *Homo habilis* and *Homo erectus*, having an average brain size of ca. 900 cc. Finally, the last duplication (*SRGAP2D*) is associated with the emergence of late *Homo erectus*, of Neanderthals and of other sister species [36]. In addition, the timing of the first and the second duplications, *P-SRGAP2B* ($\sim$3.4 mya) and *P-SRGAP2C* ($\sim$2.4 mya), shows a fairly close correspondence with the first and second generations of the use of stone tool technology, Lomekwian and Oldowan [31].

In light of [38, 39], where number of neocortical neuron is shown to be a better correlate of cognitive complexity than brain size per se (both absolute or relative), it is interesting to point out that the evolutionary rate of the *SRGAP2* gene has been claimed to positively correlate with an increase in the number of cortical neurons in mammals [40]

Not surprisingly, several authors suggested that *SRGAP2* duplications may underlie some of the changes that led to human cognition. The most explicit suggestion along these lines that we are aware of was made in [15]. Building on the existing literature on the functional effects of the relevant duplications, Chakraborty and Jarvis [41] write:

> The duplicated copies act as competitive inhibitors to slow cortical dendritic development of already existing brain pathways, which in turn allow greater neural plasticity into adulthood. *SRGAP2* modulates activity of the ROBO axon guidance receptors, which are in turn activated by the SLIT family of protein ligands to modulate axonal/dendritic migration and branching in various brain regions. Intriguingly, the *SLIT1* ligand is uniquely downregulated in the song production nucleus RA analogue of vocal learning birds (songbird RA, parrot AAC and hummingbird VA) and the analogous human LMC, which would mean that there could be a synergistic effect of the duplicated *SRGAP2* GTPase and lower *SLIT1* levels in the duplicated vocal motor pathways in humans." [references omitted]

We find this suggestion very insightful, and what follows is meant to provide support for it. Doing so requires spelling out some of the assumptions and findings that are alluded to in this quote. We turn to this next.

2 *SRGAP2* genes, filopodia, and axon guidance

The first thing to point out in the context of Chakraborty and Jarvis' suggestion is that the existing literature on *SRGAP2* does not immediately support it. Despite their names (*SRGAP* genes —

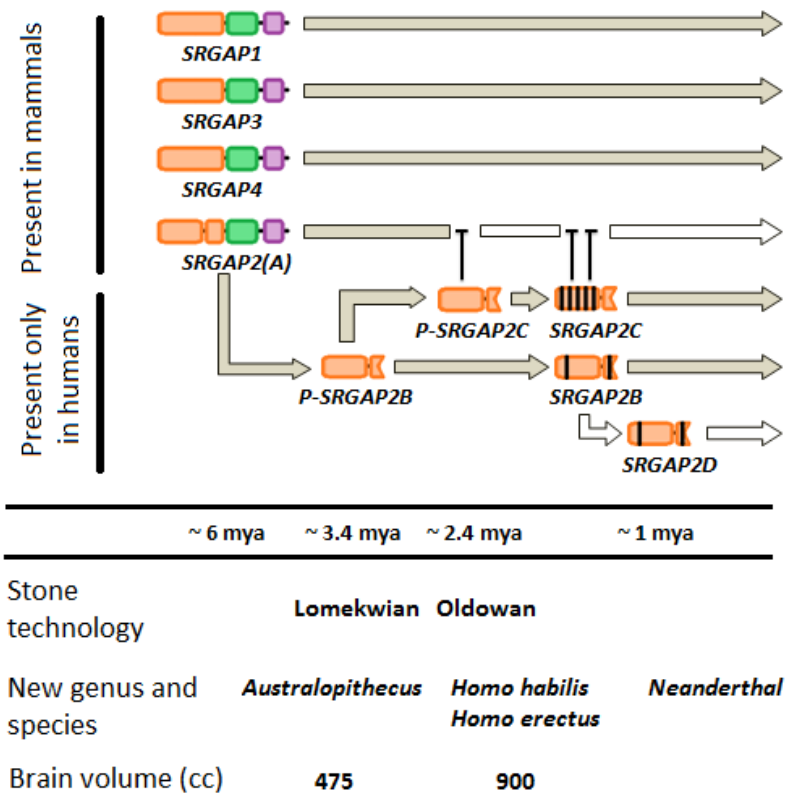

Figure 1: Evolutionary history of SRGAPs and chronological coincidences with human landmarks. On top, the colored figures represent each of the SRGAP genes/proteins. In orange, the F-BAR domains, with an F-BAR extension in the case of SRGAP2(A). The human duplicated copies are devoid of RhoGAP (green) and SH3 (violet) domains, but conserve the most part of the F-BARx domain. Grey arrows symbolize functional continuity of the gene; the functional deactivation of SRGAP2(A) by SRGAP2C, along with the non-functionality of SRGAP2D, are represented by color white in arrows. The dates in the central horizontal fringe correspond to the emergence of the primitive form of SRGAP2B (P-SRGAP2B; ∼3.4 mya) and SRGAP2C (P-SRGAP2C; ∼2.4 mya), which parallel the first (Lomekwian) and second (Oldowan) known generations of stone technology. The aminoacid replacements that P-SRGAP2B and P-SRGAP2C underwent to reach their modern forms (two in SRGAP2B, five in SRGAP2C) are represented by black bars. Emerged ∼2.4 mya, SRGAP2D is a copy of SRGAP2B and carries the same two substitutions. The penultimate row in the figure gives account of the chronological coincidences between the duplication spans that originated P-SRGAP2B and P-SRGAP2C, and the appearances of the genus Australopithecus and Homo (H. habilis; H. erectus), respectively; similarly, the appearance of Neanderthals, likewise that of other sister Homo species, parallels the emergence of SRGAP2D. At the last row, the differences between the estimated brain size of Australopithecus (475 cc) and those of Homo habilis and Homo erectus (900 cc).

SLIT-ROBO GTPase activating protein coding genes), the nature of the interactions between *SLIT* genes, *ROBO* genes, and *SRGAP* genes does not always go in the desired direction for vocal learning, by which we mean the axon guidance direction, for reasons we discuss briefly in the next subsection.

2.1 *SLIT* and *ROBO* axon guidance genes and the vocal learning posterior pathway

As it has been said above, a direct neural projection from a cortical/pallial motor nucleus and the brainstem MN controlling the larynx/syrinx appears to be a key component in the evolution of the vocal learning ability. To form this structure during the early development of the brain, the axonal extensions of the neurons in the cortical region must be sent and guided along intricate pathways to eventually reach their synaptic targets in the brainstem through a process which requires the action of axon guidance genes [42].

In this regard, as alluded to in the quote from [15], studies conducted with birds from the three groups of species of avian vocal learners [43, 20], have shown that axon guidance genes of the SLIT-ROBO families present a convergent differential regulation in the pallial motor nucleus of the learning species.

Summarizing briefly these results, we can say that *SLIT1*, a secreted molecule whose encoding gene belongs to the *SLIT* family of repulsive axon guidance genes [42], shows a differential downregulation precisely in the songbird RA and in the analog regions in parrots (AAC) and hummingbirds (VA), i.e. the arcopallial nuclei making the direct projection to the brainstem MN. The expression of *SLIT1* in these nuclei is remarkably low compared to the surrounding arcopallium. More precisely, in the case of the parrot AAC, which has a subdivision between core and shell we had already expounded, the downregulation of *SLIT1* occurs only in the core region, which is the one sending the projection to the brainstem MN. In contrast, no such regulation of *SLIT1* was observed either in the arcopallium of non vocal learning birds tested (quails and ring doves) or in a recently discovered putative LMC of mice, thus highlighting the specificity of this expression pattern to vocal learning lineages [43]. All in all, the particular pattern of expression of *SLIT1* strongly suggests a functional relation between the downregulation of the axon guidance protein and the formation of the neural projection from the cortical nucleus to the brainstem MN in charge for the syrinx, a relation which would be consistent with the similar downregulation of *SLIT1* that has been found in the human LMC [20].

ROBO1 belongs to the *Roundabout* (*ROBO*) family of axon guidance genes, whose encoded proteins act as receptors of SLIT ligands to transduce the repulsive cue into the intracellular domain [44, 42, 45]. As SLIT1, ROBO1 also shows a differential expression in relation to the posterior pathway: upregulated in the parrot AAC core and in the hummingbird VA, compared to the surrounding arcopallium, whereas in the songbird RA it is downregulated. Despite the divergence in songbirds with respect to the other two groups, ROBO1 has been observed to be temporarily upregulated in male zebra finches (endowed with a higher capacity for song compared to females) between posthatch days 35 and 65, a period deemed critical for vocal learning [43].

2.2 SRGAPs, SLITs, and ROBOs

In mammals, the *SRGAP* family of genes consists of four members: *SRGAP1*, *SRGAP2*, *SRGAP3*, and the distantly related *SRGAP4* [46]. The three first were uncovered in 2001 by Wong et al. [47] in a yeast two-hybrid experiment in which the SRGAPs were found to interact with the C-terminal

region of rat *ROBO1*. After their identification, the researchers further analyzed, through different in vitro experiments in human embryonic kidney (HEK) cells, various aspects of the interaction between *SRGAP1* and *ROBO1*, including the effect of extracellular SLIT2 in such binding. Among other results, they found that extracellular SLIT2 upregulated ROBO1-SRGAP1 binding in a dose-dependent manner, thus leading to the inactivation of CDC42, a protein of the Rho GTPase family, which has a well-documented role in the regulation of the cytoskeletal dynamics [48]. In the light of these findings, the authors proposed that the newly discovered SRGAPs are intracellular effectors in the downstream of a SLIT-ROBO signaling pathway and play a role in the guidance function of SLITs. This approach would make possible, therefore, that SRGAP2 proteins, by interacting with *ROBO1* in the downstream of an axon guidance cue, are part of the mechanism leading to the constitution of the aforementioned posterior pathway.

However, and disappointingly for our purposes, subsequent research did not provide support for this initial proposal concerning ROBO1-SRGAP2 binding. Building on the suggestion in [47], [49] investigated the SRGAPs mRNA expression in rat brain, at various developmental stages and could find only a relative coincidence with the localized expression of ROBO1 reported by other scholars [50, 51]. A subsequent study [52] on SRGAPs expression in several embryonic and postnatal stages noted similarities of SRGAP2 pattern with that of ROBO2, but did not report any interaction with ROBO1. [53] focused on the CC3 motif of *ROBO1* that [47] had found in interaction with the SH3 domain of *SRGAP1*, and then assessed their binding with the SH3 domains of *SRGAP1*, *SRGAP2*, and *SRGAP3*. The result was that most of the recreated peptides did not bind, and only one showed a feeble and transient interaction. Similarly, [54] did not identify *ROBO1* as a ligand for *SRGAP2*. (Below we return to these unsuccessful attempts, as [55] provide a possible reason for these results.)

On a more positive note, SRGAPs, and specifically *SRGAP2* on which we focus here, have been reported to serve various functions regarding cortical development at early stages. First, SRGAP2 has been shown to regulate axon-dendrite morphogenesis and neuronal migration through its ability to induce protrusions at the plasma membrane. A study [56] ex vivo in mice cortical neurons showed that the knockdown of *SRGAP2* significantly decreased both dendritic and axonal branching, while, on the other hand, neurons with shRNA-silenced expression of *SRGAP2* migrated roughly 25 per cent faster than the control group, thus showing an inhibitory effect. These results support the suggestion in [47] (based on experiments on SRGAP1) that SRGAPs can regulate cell migration. A subsequent study [57] showed the same effects in vivo, and demonstrated, in addition, that the expression of SRGAP2C in mouse cortical neurons had a similar effect to that caused by SRGAP2 knockdown, viz. an increase in the rate of cell migration. In the knockdown condition, [57] added another function of *SRGAP2* to those already established: it promotes the maturation of the dendritic spines and limits their density. Indeed, an experiment in vivo carried out with heterozygous SRGAP2-knockout mice revealed a substantially higher density of dendritic spines by comparison with the control group, with thinner and longer spines. [57] also found that the expression of SRGAP2C in mouse pyramidal neurons inhibited the function of *SRGAP2A* and extended the period of development of the spines (spinal "neoteny"), thus evoking an increase in their number per unit area and in their length. Interestingly, this last trait is considered characteristic of the human neocortex [58], and led to claims linking SRGAP2 duplication with this particular property of the human neocortex.

As a final remark on the function of SRGAP, we report their ability to co-regulate the ratio between excitatory and inhibitory synapses at their early development to reach the correct equilibrium at the mature stage. A recent in vivo study [59] in mouse cortical pyramidal neurons has shown

that *SRGAP2A* increases the growth of inhibitory synapses and restricts their density. Curiously, in a similar way to that we mentioned earlier for the dendritic spines, *SRGAP2C* antagonizes the function of *SRGAP2A* during the synaptic development, thus extending the period of maturation and increasing the final density.

As a result, SRGAP2 duplication has not figured prominently in the literature on language evolution, since to the best of our knowledge neotenous spines are not (yet) considered a central property of vocal learners. Other more established neural traits associated with vocal learning appear not be directly connected with the role of *SRGAP2*. Nevertheless, we endeavor to show how the well-documented function of *SRGAP2*, namely its ability to regulate protrusions at the plasma membrane of the neuron [56, 60, 61, 31] can be related to more canonical properties of vocal learning-ready brains, specifically axon guidance.

2.3 SRGAP2 and axon guidance: an indirect link

Axon guidance is a process whereby a neuronal projection, the axon, extends from the cell body and is guided to the proper synaptic targets in order to establish the correct neural connectivity [42]. In this process, a series of secreted proteins, such as the SLIT family, act as extracellular biochemical guiding effectors by evoking a signaling cascade that ultimately changes the cytoskeletal dynamics of the axon and directs its outgrowth either towards or away from the signaling source. These directional changes take place at the growth cone, a motil structure located at the distal end of the axon which is endowed with two types of F-actin–based structures: filopodia, which are narrow cylindrical protrusions based in unbranched parallel bundles of actin filaments (F-actin) formed by Ena/VASP and formin proteins, and lamellipodia, sheet-like protrusions based in a network of branched actin which is formed by the Arp2/3 complex. Axon guidance can be understood as a directed, recurrent process of enlargement and maturation of the growth cone, starting with the formation and extension of filopodia and lamellipodia at its leading edge, through the polymerization of actin filaments, followed by the flow of filopodia along the sides of the growth cone. The final step of the process is their eventual retraction at the base of the growth cone caused by the depolymerization of the F-actin. This last retraction allows the membrane to contract, thus forming a cylindrical consolidated axon shaft [62, 42]. Although the mechanisms whereby axons manage to find the correct pathways across the nervous system remain to be fully characterized, the two actin-supported structures that are characteristic of the axon growth cone, filopodia and lamellipodia, are considered to play a crucial role [62].

In relation with filopodia and axon guidance, a recent study in vivo in mouse dorsal root ganglia cells [63] has investigated the dynamics of the growth cone specifically during the axonal repulsion evoked through the SLIT-ROBO pathway. Crucially for us, it has reached an unexpected conclusion: despite the classic view by which a repulsive signal entails actin depolymerization at the side of the growth cone facing the guidance source, the amino-terminal fragment of SLIT2 that contains the domain responsible for binding to ROBO1 and ROBO2 induced the formation and elongation of actin-based filopodia at the axon growth cone via SLIT-ROBO pathway. Importantly, these SLIT-induced filopodia, which are longer and elongate distinctively toward the sources of the repulsive cue, are indispensable to elicit the guiding signal in the downstream of SLIT-ROBO. We think that these results are essential to understand how *SRGAP2A*, and perhaps some of its human-specific paralogs, can be related to axon guidance (see Fig. 2), thus supporting Chakraborty and Jarvis [41] 's suggestion, and enabling us to provide novel support for the claim that vocal learning was established fairly early in our lineage.

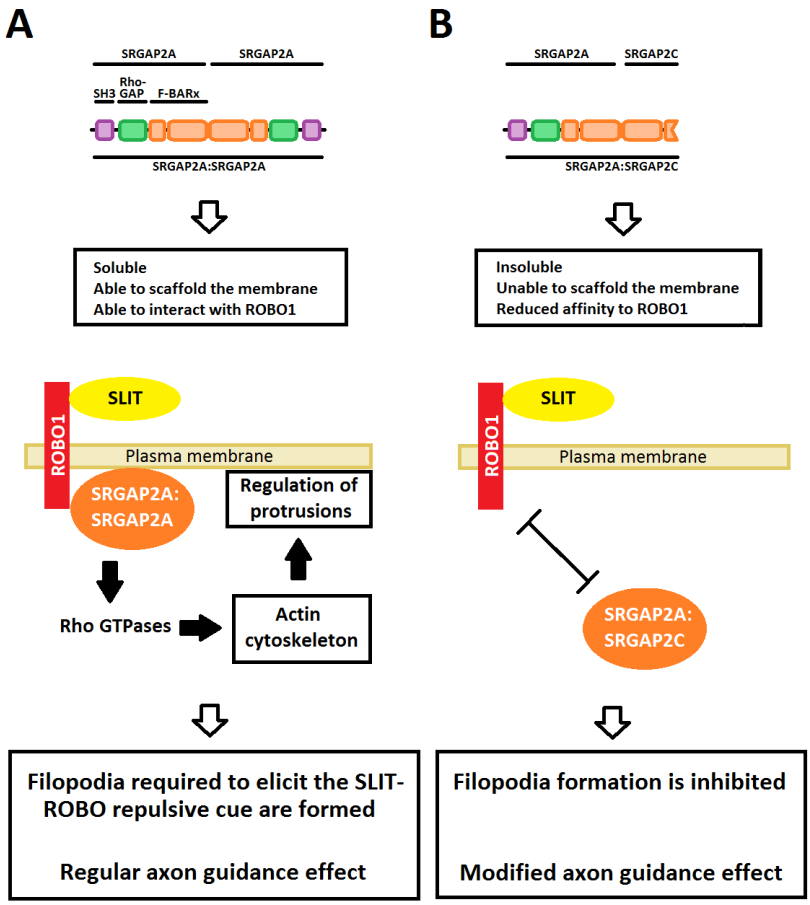

Figure 2: Proposed model for the implication of SRGAP2A and SRGAP2C in an axon guidance signaling pathway. A: SRGAP2A molecules homodimerize through their F-BARx domains, thus forming soluble dimers. These dimmers have a singular invers geometry which allows them to colocalize the membrane at sites of protrusions. Once in place, these molecules are able to transduce a SLIT-ROBO axon guidance cue by interacting with Rho GTPases through their RhoGAP domains, thus regulating the actin cytoskeleton and scaffolding protrusions. The chain of interactions leads to the constitution of filopodia which extend towards the sources of SLIT. These filopodia are crucial to elicit the repulsive axon guidance cue. B: SRGAP2C heterodimerizes with SRGAP2A. The resulting molecule is insoluble, unable to scaffold the membrane, and has a limited affinity for ROBO1. Thus, SRGAP2C inactivates SRGAP2A's ability to regulate filopodia, ultimately resulting in a modified effect in axon guidance.

2.4 SRGAP2A and SRGAP2C

SRGAP2A has a singular threefold composition: an F-BAR domain, which has an amino-terminal extension; a RhoGAP domain, and an SH3 domain [31]. Remarkably, the extended F-BARx domain allows the protein to explore the geometry of the membrane and to bind selectively to bulging sites or protrusions [60, 56, 61]. Once in place, *SRGAP2A* can regulate the dynamics of the actin-based cytoskeleton through its RhoGAP domain, thus evoking different effects in these protrusions. As examples of this, Guerrier et al. [56] showed that the overexpression of the *SRGAP2A* F-BAR in cortical neurons induced filopodia-like membrane protrusions, whereas Fritz et al. [61] have shown that it evoked a retraction of the membrane protrusions in a cell-cell overlap context by inactivating local pools of Rac1 and CDC42 which, in turn, caused a breakdown of the actin-supported cytoskeleton and the subsequent retraction. There may be several factors conditioning the specific result of the protrusion regulation that *SRGAP2A* evokes, but, as Fritz et al. note [61], one of them must be the upstream input that it receives, most likely from SLIT-ROBO. In fact, they show that the detected effect of *SRGAP2A* is elicited in the downstream of the SLIT2-ROBO4 signaling pathway. It is in the context of binding axon guidance molecules that the SH3 domain has shown to be indispensable, although not exclusive, since all three domains (F-BARx, Rho-GAP, and SH3) have been proven to exert a cooperative participation in binding ROBO1 [55]. As Guez-Haddad et al. point out [55], this must be the reason why previous attempts to attest a significant interaction between *ROBO1* and the isolated SH3 domain of *SRGAP2A* (summarized above) had failed. Summing up then, the particular threefold composition of *SRGAP2A* endows it with the ability to regulate membrane protrusions likely in the downstream of the axon guidance SLIT-ROBO pathway.

SRGAP2A molecules are homodimers in solution. Prototypically, F-BAR domains form anti-parallel dimers that bind the plasma membrane through their concave N-surface, thus associating with membrane invaginations. However, the *SRGAP2A* homodimerization is not only mediated by the F-BAR domain, as typically could be expected, but rather by a large interface that includes the F-BAR, its Fx extensions, the RhoGAP, and the SH3 domains. This particular cooperative dimerization, which additionally increases the ability of the dimer to bind the membrane, evokes an inverted, convex N-surface that associates with protrusions instead of invaginations. The potential of *SRGAP2A* to regulate membrane protrusions likely depends on this particular form of homodimerization [31].

The duplicated copy *SRGAP2C* consists of a truncated form of *SRGAP2A* containing nearly all of the F-BARx with three modifications, two of which occurred in the first duplication event (around 3.4 mya), thus being present in the primitive forms *P-SRGAP2B* and *P-SRGAP2C*. As Sporny et al. [31] have recently shown, *SRGAP2C* has the ability to heterodimerize with *SRGAP2A*, a property which was already present in the primal form *P-SRGAP2C* appeared 2.4 mya. Crucially, unlike *SRGAP2A* homodimers, SRGAP2A:SRGAP2C heterodimers are insoluble, thus being unable to reach the proper sites in the plasma membrane and consequently being rendered inactive. An experimental quantification of the effect of *P-SRGAP2C* and *SRGAP2C* in compromising *SRGAP2A* solubility has been carried out by Sporny et al., reflecting that, when coexpressed with recreated P-SRGAP2C and with SRGAP2C in Sf9 cells, 60 and 40 per cent of *SRGAP2A* respectively were insoluble. In light of these data, it is clear that *SRGAP2C* acts as an inhibitor of SRGAP2A by cancelling its ability to bind to the membrane and regulate protrusions. Relevantly, this capacity of *SRGAP2C* to form stable heterodimers with SRGAP2A and its consequent efficiency at antagonizing the original gene was evolutionarily refined over the mutagenesis phase which took place after the duplication event (about 2.4 mya). In addition, but independently from their insolubility, the

SRGAP2A:SRGAP2C heterodimers present a significantly reduced ability to bind ROBO1 [31].

SRGAP2A mRNA has been shown to be expressed in different regions of the central nervous system at early developmental stages. It was found to be expressed at embryonic and postnatal days in many tissues in mice, including the dorsal and ventral thalamus, the ventrolateral thalamic nucleus, the superior and inferior colliculi, the cerebellum, and the spinal cord [52]. Also in mice, Guerrier et al. [56] detected that it follows an increasing pattern of expression during early development in the cortex, reaching its maximum level at postnatal day 1 (P1), then stabilizing until P15, and gradually decreasing although still being expressed in adult stages. Charrier et al. [57] compare its expression with that of *SRGAP2C* and reach the conclusion that both are expressed in embryonic and adult human brain (thoughnot always in exactly the same way). Various human brain expression databases we consulted[1] generally agree that SRGAPs are expressed in frontal parts of the neocortex early in development. (Data on *SRGAP2C* specifically tend to be too sparse to draw any firm conclusion at this point.)

3 Concluding remarks

SRGAP2C may have had other functional consequences [57, 56, 59], but we have provided evidence that mechanistically we can expect SRGAP2C to have had an effect on the SLIT-ROBO axon guidance pathway, and (no doubt together with other genetic changes) may have contributed to the establishment of a critical aspect of the vocal learning circuit, as first suggested in [15]. We have shown that until very recently studies focusing on *SRGAP2* failed to provide evidence in this direction. It is only thanks to the results in [55, 31] and the link between filopodia and axon guidance made precise in [63] that we can adduce a greater degree of plausibility to the claim in [15] that *SRGAP2* duplications may have contributed to the emergence of aspects of our language faculty (a claim made at a time when the relevant results we rely on had not yet been obtained). Since paleogenomic work has shown that the relevant mutation that led to this effect is not specific to *Homo sapiens*, we are led to conclude that core ingredients of the vocal learning pathway pre-dated the emergence of our species.

In a certain sense, *SRGAP2C* acts like the member of the SRGAP family that most closely interacts with *ROBO1*: *SRGAP1*. Unlike *SRGAP2A*, which as we saw, induces filopodia-like membrane protrusions, SRGAP1's F-BAR domain prevents filopodia [60]. By inhibiting the ability of *SRGAP2A* to induce filopodia, SRGAP2C makes *SRGAP2* function like *SRGAP1*. In light of this, it is noteworthy that a gene expression study [64] carried out in human developing neocortical neurons has shown a relation between *ROBO1* and *SRGAP1*. Both genes were found to be coexpressed in human corticospinal axons at various fetal periods during the formation of the corticospinal tract, which is the main descending sensorimotor projection, an elaboration of which could have given rise to the critical connection of the posterior vocal learning circuit.

As pointed out in [43], *SLIT1* is a direct target of *FOXP2* [65, 66]. Although human *FOXP2* has been reported to modulate stronger upregulation of *SLIT1* than chimpanzee *FOXP2* [66], which does not fit well with the relevant convergent downregulation of *SLIT1* in vocal learning birds found in [43], *SLIT1* is among the *FOXP2* targets found to be significantly downregulated in response to

[1] Brainspan (`http://www.brainspan.org`), Human Brain Transcriptome (`http://hbatlas.org`), Bgee (`http://bgee.org`), Proteomics DB (`https://proteomicsdb.org`), Human Protein Atlas (`http://www.proteinatlas.org`), Gene Enrichment Profiler (`http://xavierlab2.mgh.harvard.edu/EnrichmentProfiler/index.html`), and GTex (`http://www.gtexportal.org`).

FOXP2 expression in [67]. So, there could be another synergistic effect here between the effect of FOXP2 on SLIT1 and the action of *SRGAP2C* on the SLIT-ROBO pathway.

Incidentally, just like *SRGAP2C* works its effect on the SLIT-ROBO pathway by inhibiting an inhibitor (in this case, *SRGAP2A*), *FOXP2* also appears to work its effects by inhibiting inhibitors, such as *MEF2C*. As reported in [68], (mouse) *Foxp2* controls synaptic wiring of corticostriatal circuits, critical for vocal learning, by opposing *Mef2c*, which itself suppresses corticostriatal synapse formation and striatal spinogenesis. So, achieving a positive effect (establishment of a vocal learning circuit) by inhibiting inhibitors or suppressing the activity of suppressors, appear to have been a common strategy in the evolution of our lineage and our cognitive phenotype.

We still don't know exactly when the relevant *FOXP2* mutations emerged in our lineage, so we cannot know for sure if the emergence of modern *SRGAP2C* coincided with the two *FOXP2* mutations thought to be critical for vocal learning. Evidence for a selective sweep associated with *FOXP2* yields ambiguous results (assuming that the relevant mutations were the actual selection targets): there is evidence for a recent, *H. sapiens* specific partial selective sweep [69, 70], but also evidence for another, much earlier sweep [70, Suppl Table S12.1].

It remains to be seen if these sweeps correspond to landmarks in the establishment of the human vocal learning circuit, possibly corresponding to the stages that can be derived from the work on vocal learning birds (e.g., suboscine/proto-vocal-learning stage [27], core vocal learning circuit stage [43], shelll vocal learning circuit stage [15]).

Though modest, we think that our contribution is of a kind that is necessary to make claims about when components of our language faculty mosaic emerged. It won't do to simply identify changes on potentially relevant genes. It is necessary to show that the changes have functional effects of the right kind. We hope to have taken a small step in this direction.

Author contributions statement

CB formulated the hypothesis and directed the study. MM, PTM, and CB reviewed the literature, and wrote the article. The authors declare no conflict of interest.

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