

SRGAP2 and the gradual evolution of the modern human language faculty

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Abstract

In this article, we examine a new source of evidence that draws on data from archaic human genomes to support the hypothesis 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 molecular pathway has been shown to have an important role in the context of vocal learning. Though the relevance of the *SRGAP2* gene duplication in the emergence of some aspect of language has not gone completely 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 ancestral *SRGAP2A*, which in turn modulates the axon guidance function of the SLIT-ROBO molecular pathway. This, we claim, could have contributed to the establishment of 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.

Key words: 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 (Bickerton 2002; Mithen 2005; Chomsky 2010; Dediu and Levinson 2013). A recent special issue on the biology and evolution of language also reflects the disparity of competing positions (Fitch 2017).

When addressing this question, a part of the problem 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 (West-Eberhard 2003; Boeckx 2013). 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 (Fitch 2017). 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 (Pääbo 2014). A famous example of this was given by Krause et al. (2007), who found that *FOXP2*, a gene associated with language impairments and hampered orofacial movements (Lai et al. 2001), 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 (Enard et al. 2009; Schreiweis et al. 2014), 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 (Mendez et al. 2016). In this article, we focus on the evolutionary history of *SRGAP2*, which codes for the SLIT-ROBO Rho GTPase activating protein 2 (*SRGAP2*). We offer, on the basis of what we have learned from other species about vocal learning, another argument in support of the idea 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 (Chakraborty et al. 2015; Hillert 2015), we show how it has now 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 learn to reproduce communicative signals from conspecifics. 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) (Petkov and Jarvis 2012; Shen 2017). Among the pieces interlocked within 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 (Jarvis and Mello 2000; Jarvis 2004; Chakraborty et al. 2015). 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 (Jarvis 2004). Among them, all three learning avian species

exhibit several brain 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 the main focus 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 controls the syrinx in birds and the larynx in humans (Jarvis 2004; Feenders et al. 2008; Pfenning et al. 2014; Simonyan 2014; Chakraborty et al. 2015).

On the basis of such similarities, a motor theory of vocal learning has been proposed (Feenders et al. 2008), arguing that cerebral systems specialized for vocal learning in distantly related lineages are independent evolutions of a motor system inherited from their common ancestor. Analyses in gene expression (Feenders et al. 2008; Shimizu et al. 2010; Wang et al. 2010; Jarvis et al. 2013) certainly point in this direction, further supporting that the posterior pathway, which we will focus on next, must have emerged from a primitive motor system (Feenders et al. 2008; Fitch et al. 2010; Chakraborty et al. 2015). 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 that then projected to the brainstem MN in charge of the vocal organs (Fitch 2011; Chakraborty et al. 2015). Pathway duplication unfolds in a manner analogous to gene duplication—with a whole pathway duplicating and the duplicate taking on new function—and actually having gene duplication as one possible underlying mechanism (Chakraborty and Jarvis 2015).

Neuroanatomical research conducted with primates has identified homologous representations of the larynx in the motor cortex (LMC) both in human (Penfield and Boldrey 1937; Rödel et al. 2004) and in nonhuman primates, such as chimpanzees (*Pan troglodytes*) (Leyton and Sherrington 1917), rhesus monkeys (*Macaca mulatta*) (Sugar et al. 1948; Hast et al. 1974), and squirrel monkeys (*Saimiri sciureus*) (Hast and Milojkovic

1966; Hast et al. 1974). However, although the LMC connectivity network is broadly similar among primates tested, a robust cortico-laryngeal direct projection to the vocal MN in the brainstem has been found only in humans (Simonyan 2014; Belyk and Brown 2017).

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 nonvocal learning suboscine species. Indeed, as Liu et al. (2013) have shown, 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. 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 case in point that can serve as an example for such specializations can be found in parrots, known to be able to imitate vocalizations not only of conspecifics, but also sounds produced by other species. A study involving the three superfamilies of parrots (*Strigopoidea*, *Cacatuoidea*, and *Psittacoidea*) (Joseph et al. 2012) 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 (Chakraborty et al. 2015). Chakraborty and Jarvis (2015) 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 structures 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 (Boeckx 2017).

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

Although *SRGAP2* is highly conserved among mammals (Dennis et al. 2012) 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) (Sporny et al. 2017), it has given rise to three human-specific duplications, two of which underwent subsequent mutations. The sequence of events, identified by Dennis et al. (2012), 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 (Brunet et al. 2002; Vignaud et al. 2002; Brunet et al. 2005) and genetic analyses (Patterson et al. 2006): the first duplication took place 2.8–3.9 mya, when the promoter and first nine exons of the original gene—designated *SRGAP2A* to distinguish it from its derivatives—were duplicated from the locus 1q32.1 to 1q21.1, thus giving rise to the primitive *SRGAP2B* (*P-SRGAP2B*). A second duplication occurred 2.0–2.8 mya, when *P-SRGAP2B* was copied from 1q21.1 to 1p12, leading to the primitive *SRGAP2C* (*P-SRGAP2C*). In the aftermath of this event (Dennis et al. 2012; Sporny et al. 2017), the two primitive duplicated copies, *P-SRGAP2B* and *P-SRGAP2C*, accumulated nonsynonymous 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* within 1q21.1, thus giving rise to *SRGAP2D* (Dennis et al. 2012). Consistent with the timing of their appearances, all three human paralogs, *SRGAP2B*, *SRGAP2C*, and *SRGAP2D*, have been found also in the genomes of Neanderthals and Denisovans (Hillert 2015).

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 duplications contributed to these phenotypic changes (Buckner and Krienen 2013; Hillert 2015; Boeckx 2017). Thus, the time of the first duplication (*P-SRGAP2B*) matches 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

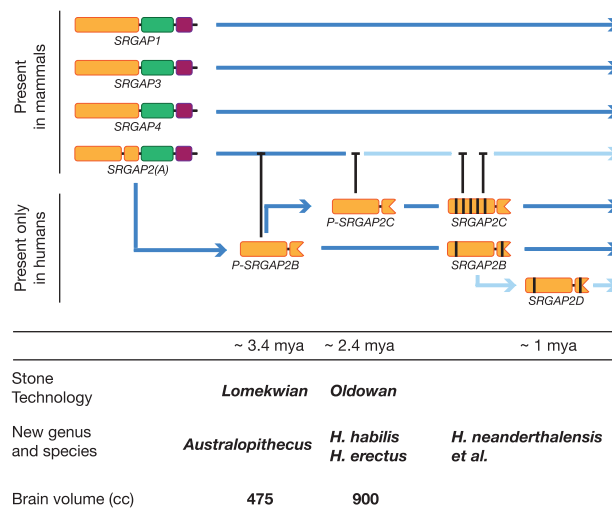


Figure 1. Evolutionary history of SRGAPs and chronological correspondence with human landmarks. On top, the colored figures represent each of the SRGAP genes. In orange, the F-BAR domains, with an F-BAR extension in the case of SRGAP2(A). The human duplicate copies are devoid of RhoGAP (green) and SH3 (violet) domains, but conserve the most part of the F-BARx domain. Darker arrows symbolize functional continuity of the gene; the reduced activity of SRGAP2(A) by SRGAP2C, along reduced activity of SRGAP2D, are represented by arrows in a lighter shade. 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 parallels the first (Lomekwian) and second (Oldowan) known generations of stone technology. The amino acid replacements that P-SRGAP2B and P-SRGAP2C underwent to reach their modern forms (two in SRGAP2B, five in SRGAP2C) are represented by black bars. Around ~1 mya, SRGAP2D emerged as a copy of SRGAP2B and carries the same two substitutions. The penultimate row in the figure gives account of the chronological correspondence between the duplication events that led to P-SRGAP2B and P-SRGAP2C, and the appearances of the genus *Australopithecus* and *Homo* (*H. habilis*; *H. erectus*), respectively; similarly, the appearance of *H. neanderthalensis*, likewise that of other sister *Homo* species, parallels the emergence of SRGAP2D. The last row depicts the differences between the estimated brain size of *Australopithecus* (475 cc) and those of *H. habilis* and *H. erectus* (900 cc).

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 *H. erectus*, of Neanderthals and of other sister species (Hillert 2015). In addition, the timing of the first and the second duplications, P-SRGAP2B (~3.4 mya) and P-SRGAP2C (~2.4 mya), shows a fairly close correspondence with the first and second generations of the use of stone tool technology, Lomekwian and Oldowan (Sporny et al. 2017).

In light of claims that total number of neocortical neurons is shown to be a better correlate of cognitive complexity than brain size *per se* (both absolute or relative) (Herculano-Houzel 2012; 2016), it is also 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 (Tiway 2016).

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 (Chakraborty et al. 2015). Building on the existing

literature on the functional effects of the relevant duplications, Chakraborty and Jarvis (2015) 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—*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 role, for reasons we discuss briefly in the next subsection.

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

As 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 pathways to eventually reach their synaptic targets in the brainstem through a process which requires the action of axon guidance genes (Dickson 2002).

In this regard, as alluded to in the quote from Chakraborty et al. (2015), studies conducted with birds from the three groups of species of avian vocal learners (Pfenning et al. 2014; Wang et al. 2015) 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 gene belonging to the *SLIT* family of repulsive axon guidance genes (Dickson 2002), shows a differential downregulation precisely in the songbird RA and in the analog regions in parrots (AAC) and hummingbirds (VA), that is, 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 nonvocal 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 (Wang et al. 2015). All in all, the particular pattern of

expression of *SLIT1* strongly suggests a functional relation between the downregulation of the axon guidance factor 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 (Pfenning et al. 2014).

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 (Brose et al. 1999; Dickson 2002; Long et al. 2004). Similarly to *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 (Wang et al. 2015).

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* (Pontus Aspenström 2008). The first three were uncovered in 2001 by Wong et al. (2001) 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 member of the Rho GTPase family, which has a well-documented role in the regulation of the cytoskeletal dynamics (Hall 1998). 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 it possible, therefore, for *SRGAP2* to interact with *ROBO1* downstream of an axon guidance cue, which 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 Wong et al. (2001), Yao et al. (2008) investigated the SRGAPs messenger RNA (mRNA) expression in rat brain, at various developmental stages and could find only a relative coincidence with the localized ROBO1 expression reported by other scholars (Marillat et al. 2002; Whitford et al. 2002). A subsequent study (Bacon et al. 2009) 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. Li et al. focused on the CC3 motif of ROBO1 that Wong et al. (2001) had found in interaction with the SH3 domain of SRGAP1, and then assessed their binding with the SH3 domains of SRGAP1, SRGAP2, and SRGAP3 (Li et al. 2006). The result was that most of the recreated peptides did not bind, and only one showed a feeble and transient interaction. Similarly, Okada et al. (2011) did not identify ROBO1 as a ligand for SRGAP2. (Below we return to these unsuccessful attempts, as a recent study (Guez-Haddad et al. 2015) provides 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 of cortical neurons in mice showed that the knockdown of SRGAP2 significantly decreased both dendritic and axonal branching, while, on the other hand, neurons with short hairpin (shRNA)-silenced expression of SRGAP2 migrated roughly 25% faster than the control group, thus showing an inhibitory effect (Guerrier et al. 2009). These results support the suggestion in Wong et al. (2001) (based on experiments on SRGAP1) that SRGAPs can regulate cell migration. A subsequent study (Charrier et al. 2012) 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 bi-ancestral SRGAP2 knockdown, viz. an increase in the rate of cell migration. In the knockdown condition, Charrier et al. (2012) 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 in comparison with the control group, with thinner and longer spines. Charrier et al. (2012) 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 (Benavides-Piccionne et al. 2002), and led to claims linking SRGAP2 duplication with this particular property of the human neocortex.

As a final remark on the function of SRGAPs, 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 (Fossati et al. 2016) in mouse cortical pyramidal neurons has shown that SRGAP2A increases the growth of inhibitory synapses and restricts their density. Curiously, in a way similar to the one mentioned earlier for dendritic spines, SRGAP2C antagonizes functions of SRGAP2A during synaptic development, prolonging their maturation period and increasing their final density.

As a result, SRGAP2 duplication has not figured prominently in the literature on the evolution of vocal learning, since to the best of our knowledge neotenic spines are not (yet) considered a central property of vocal learners. Other more established neural traits associated with vocal learning appear not to be directly connected with the role of SRGAP2. Nevertheless, in the following sections we show how the well-documented function of SRGAP2, namely its ability to regulate protrusions at the plasma membrane of the neuron (Guerrier et al. 2009; Coutinho-Budd et al. 2012; Dominik Fritz et al. 2015; Sporny et al. 2017), can be related to more canonical properties of vocal learning brain-readiness, specifically axon guidance.

2.3 SRGAP2 and axon guidance: an indirect link

In the process of axon guidance, 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 toward or away from the signaling source. These directional changes take place at the growth cone, a motile 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 (Dickson 2002; Dent and Gertler 2003). 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 (Dent and Gertler 2003).

In relation to filopodia and axon guidance, a recent study *in vivo* in mouse dorsal root ganglia cells (McConnell et al. 2016) has investigated the dynamics of the growth cone specifically during the axonal repulsion evoked through the SLIT-ROBO molecular pathway. Crucially for us, it has reached an unexpected conclusion: despite the classic view whereby 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 molecular 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' (2015) suggestion, and enabling us to provide novel support for the claim that vocal learning was established fairly early in our lineage.

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 (Sporny et al. 2017). 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 (Guerrier et al. 2009; Coutinho-Budd et al. 2012; Dominik Fritz et al. 2015). 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. (2009) showed that the overexpression of the *SRGAP2A* F-BAR in cortical neurons induced filopodia-like membrane protrusions, whereas Fritz et al. (2015) 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. (2015) note, one of them must be the upstream input that it receives, most likely from the SLIT-ROBO pathways. In fact, they show that the detected effect of *SRGAP2A* is elicited in the downstream of the *SLIT2-ROBO4* signaling. 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* (Guez-Haddad et al. 2015). As Guez-Haddad et al. (2015) point out, 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 (Sporny et al. 2017).

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 (~3.4 mya), thus being present in the primitive forms, *P-SRGAP2B* and *P-SRGAP2C*. As Sporny et al. (2017) have recently shown, *SRGAP2C* has the ability to heterodimerize with

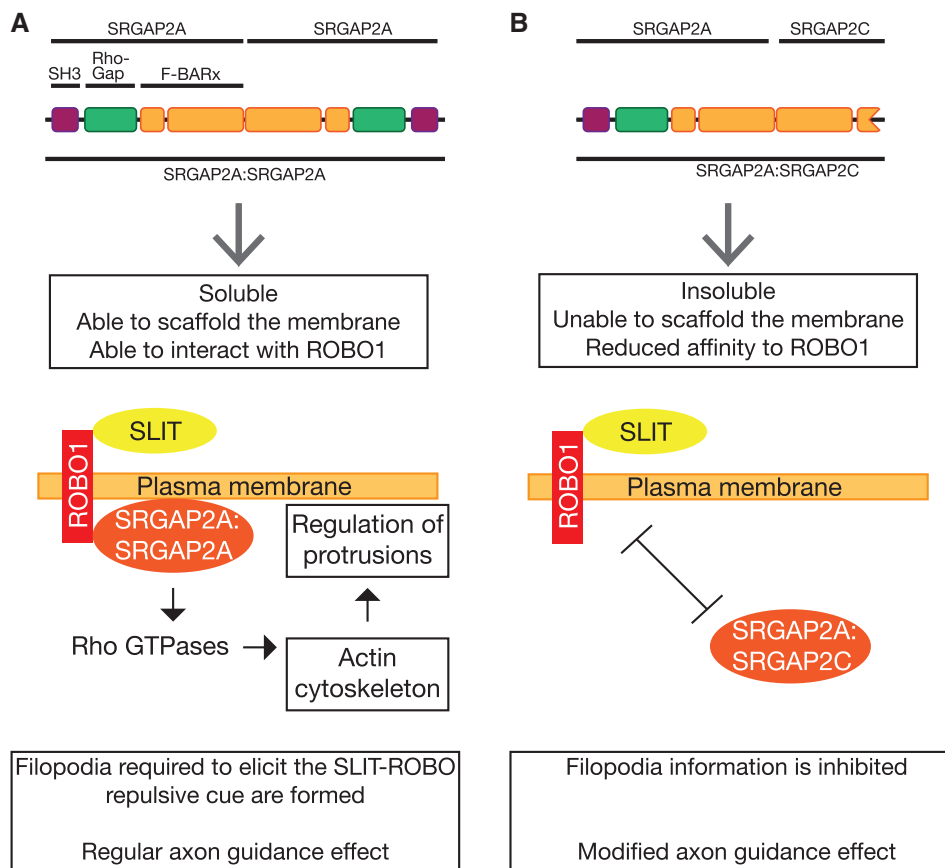


Figure 2. Proposed model for the implication of SRGAP2A and SRGAP2C in an axon guidance signaling pathway. (A) SRGAP2A molecules homodimerize through their F-BARx domains, thus forming soluble dimers. These dimers have a singular inverse geometry which allows them to colocalize at the membrane at sites 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 toward 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.

SRGAP2A, a property which was already present in the primal form *P-SRGAP2C*, which 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. (2017) reflecting that, when coexpressed with recreated *P-SRGAP2C* and with SRGAP2C in Sf9 cells, 60% and 40% 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 (Sporny et al. 2017).

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 (Bacon et al. 2009).

Also, in mice, Guerrier et al. (2009) 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. (2012) compare its expression with that of *SRGAP2C* and reach the conclusion that both are expressed in embryonic and adult human brain (though not always in exactly the same way). Various human brain expression databases we consulted 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.) The resources we consulted include: 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.gtportal.org>) 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 (Guerrier et al. 2009; Charrier et al. 2012; Fossati et al. 2016), 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 Chakraborty et al. (2015). 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 Guez-Haddad et al. (2015) and Sporny et al. (2017) and the link between filopodia and axon guidance made precise in McConnell et al. (2016) that we can adduce a greater degree of plausibility to the claim in Chakraborty et al. (2015) 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 predated 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 (Coutinho-Budd et al. 2012). 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 (Ip et al. 2011) carried out in human developing neocortical neurons has shown a relation between *ROBO1* and *SRGAP1*. Both genes were found to be co-expressed 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 Wang et al. (2015), *SLIT1* is a direct target of *FOXP2* (Vernes et al. 2007; Konopka et al. 2009). Although human *FOXP2* has been reported to modulate stronger upregulation of *SLIT1* than chimpanzee *FOXP2* (Konopka et al. 2009), which does not fit well with the relevant convergent downregulation of *SLIT1* in vocal learning birds found in Wang et al. (2015), *SLIT1* is among the *FOXP2* targets found to be significantly downregulated in response to *FOXP2* expression in Devanna et al. (2014). 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 Chen et al. (2016), (mouse) *Foxp2* controls synaptic wiring of corticostriatal circuits, critical for vocal learning, by opposing *Mef2c*, which itself suppresses corticostriatal synapse formation and striatal spinyogenesis. So, achieving a positive effect (establishment of a vocal learning circuit) by inhibiting inhibitors or suppressing the activity of suppressors, appears to have been a common strategy in the evolution of our lineage and our cognitive phenotype.

We still do not 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 not only evidence for a recent *H. sapiens*-specific partial selective sweep (Maricic et al. 2013; Mallick et al. 2016), but also evidence for another, much earlier sweep (Mallick et al. 2016; Supplementary 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 (Liu et al. 2013), core vocal learning circuit stage (Wang et al. 2015), shell vocal learning circuit stage (Chakraborty et al. 2015)).

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 is not enough 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.

Authors' contributions

C.B. formulated the hypothesis and directed the study. P.T.M., M.M., and C.B. reviewed the literature, and wrote the article.

Conflict of interest statement. None declared.

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