Commentary

Conserved role of Ras-GEFs in promoting aging: from yeast to mice

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Commentary on: *Borrás C et al. RasGrf1 deficiency delays aging in mice. Aging. 2011; 3: 262-276*
Received: *4/24/11; Accepted: 4/26/11; Published: 4/26/11*
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In a new article published in the journal Aging, Borras et al. report that Ras-GRF1 homozigous deletion increases both median and maximum longevity in laboratory mice [1]. The human Ras, superfamily now counts more than 150 different proteins subdivided into five different protein families: Ras, Rho, Rab, Arf and Ran. These protein families regulate many cellular processes [2] including cellular differentiation and proliferation (Ras), cell citoskeleton organization and cell shape (Rho), intracellular protein trafficking (Rab, Arf) and nucleo-citoplasmic transport (Ran).

Ras protein, the founding member of the small GTP binding protein superfamily, is found mutated in 30% of all human tumors and, in mammals, consists of three genes and four gene products (N-Ras, H-Ras, K-Ras4A and KRas4B). Many of these proteins are ubiquitously expressed but regulated by a multitude of specific Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). In fact, even though this protein superfamily has the endogenous capability to hydrolyze the bound GTP, GEFs and GAPs, respectively catalyze the activating and the inactivating reactions [3]. It is interesting to note that, although Ras-GRF1 (one of the mammalian GEFs) shows only partial homology to the yeast CDC25 (one of the two yeast GEFs) and mammalian and yeast Ras proteins have limited functional homology, both exchange factors are regulated by the PKA serine/threonine kinase [4, 5] suggesting the existence of conserved Ras-dependent signaling networks. Both RasGRFs were first discovered for their ability to exchange the nucleotide bound to Ras proteins [6, 7] but these multidomain proteins can have additional activities. Other than the REM and CDC25 domain, capable of exchanging the Ras-bound GDP, full length RasGRFs contain, in fact, a PH domain that can interact with the NGF receptor TrkA [31] and an IQ domain capable of calmodulin binding and responsible for calcium modulation [8; 9]. It also contains a second PH-DH domain that is capable of binding to membrane bound PI(4,5)P2, microtubules [10], phosphatidic acid, Rho and Rac GTPase [11, 12, 5] and spinophilin, a scaffold protein that interact with actin filaments and p70 S6 kinase [13]. It is therefore possible that the RasGRF1 -/- mouse phenotype may be due to the impairment of GTP binding proteins other than Ras or to inhibition of other signaling cascades. However, it must be noted that Ras-GRF1 signaling is required for normal beta cell development and glucose homeostasis and that isolated islet from GRF1 knockout fail to activate Akt and Erk [14] suggesting a major role of RasGRF1 in Ras activation in this cell type. A clear but much reduced effect of the same knockout can be also seen in the amount of activated Erk protein in isolated retina [15].

Ras-GRF1 was previously implicated in beta cell langherans islet development, glucose homeostasis [14], learning and memory impairment and retinal defects [16, 17, 18]. More recently [19] Ras-GRF1 has been invoked as a possible explanation for the longevity observed in mice obtained without paternal contribution [20]. These mice generated using two sets of female genomes display increased average longevity and reduced body weight. Since the RasGRF1 locus is imprinted in female gametogenesis, leaving the whole protein production to the paternal allele, it was argued that bi-maternal condition is functionally equivalent to

the RasGRF1 deletion [19].

Ras-GRF1 is normally expressed in brain (hypothalamus and hippocampus), pancreatic cells and skeletal muscle [21]. Messenger length and composition are quite heterogeneous because the Ras-GRF1 locus is heavily affected by alternative splicing. This results in a variety of mRNA isoforms that show tissue and developmental specific expression. Consequently, protein isoforms range from a 140 KDa full-length protein expressed in brain and in pancreatic islet to the smallest 20kDa isoform Ras-GRFβ, expressed in mouse pancreas [21]. These isoforms share only some of the functional domains raising the possibility that different isoforms may perform different tasks.

In their study, Borras et al. [1] find increased average and maximal Lifespan in *RasGrf1-/-* mice. Survival curves revealed a marked increase (20%) in the average lifespan of *RasGrf1-/-* male mice (mean values WT: 100.5±4.2 weeks and RasGrf1-/-: 120.7±4.7 weeks; median WT: 104 weeks; RasGrf1-/-: 124 weeks) and mice with *RasGrf1-/-* showed better motor coordination than controls. At the molecular level they find: a) Increased Expression of the 16S rRNA in *RasGrf1-/-* mice. 16S rRNA is one of the mitochondrial rRNA and mitochondrial gene expression and function has been demonstrated to positively correlate with longevity in organisms from yeast to human [40, 41, 42, 43] b) Increased expression of SIRT1 in *RasGrf1-/-* mice. Sirtuins play a role in a variety of diseases [44], but their importance in mammalian lifespan is not clear [45]. c) Maintained in vivo glucose uptake in aged *RasGrf1-/-* mice.

Reduced glucose uptake is associated with aging [39]. They analyzed the in vivo glucose uptake in young and old animals showing higher uptake levels in *RasGRF1-/-* mice with respect to the wild type of the same age, d) RasGRF1-/- mice displayed reduced IGF1 activity and blood metabolomic analysis showed clear similarities with calorie-restricted animals. IGF1 activity has a consistent effect on the aging process [23], suggesting that the downstream Ras pathway may play a central role in this process in mammals as it does in yeast [23]. Higher glycogen content in mice depleted of the RasGRF1 was also observed. Notably, glycogen and trehalose content positively correlates with stress resistance and Ras/AC/PKA depletion in yeast while constitutively activated Ras/AC/PKA pathway reduces both carbohydrate content [22]. Borras et al. suggest the longevity phenotype observed in mice with homozygous Ras-GRF1 deletion may be the outcome of decreased Ras activity.

In yeast the Ras, Tor and Sch9 signaling pathways are partly responsible for integrating nutrient inputs into cell growth, division and aging [23]. Impairment of the Ras/AC/PKA pathway increases stress resistance and longevity [24, 25, 26] through different downstream effectors whose roles are functionally conserved in higher eukaryotes including mammals [23]. Mechanistically, downregulation of Ras/AC/PKA in yeast provokes relocalization/activation of the transcription factors Msn2/4 [28], activation of the transcription factors Gis1 through Rim15, [29, 26, 28, 30] and Hsf1 activation [29]. All these factors enhance cellular protection systems activating stress response genes such as SODs, catalase, HSPs, autophagy and probably many others. [31, 23, 27, 28].

Orthologs of genes that function downstream of Ras in yeast have also been implicated in longevity extension in mice. Deletion of the mouse adenylate cyclase type 5 and the consequently PKA downregulation extends lifespan, stress resistance and mediates upregulation of SODs [32] and mice lacking PKA RII or Cβ subunits are protected from age-related deleterious changes such as weight gain, hypertrophic liver and cardiac dysfunction and enlargement [33, 34]. In addition, serum from a cohort of individuals with Growth Hormone Receptor (GHR) mutations, showing very low cancer and diabetes incidence, inhibited the expression of N-Ras, TOR and PKA when added to cultured primary human epithelial cells [35].

It is therefore tempting to propose that the pro-longevity effect of the homozygous Ras-GRF1 deletion observed by Borras is due to a reduced activity of the Ras or an analogous pathway and to consider that the increased longevity observed may be evidence for the conserved role of the Ras pathway in the aging process in organisms ranging from yeast to mammals [49]. However, the presence of more isoforms of the Ras-GRF1 coupled with the loose ligand specificity of Ras-GRF1 that is capable to bind Ras, Rac, Rho GTPase, microtubules, PI(4,5)P2 and fosfatidic acid indicates that additional studies are necessary to determine how altered Ras-GRF1 signaling promotes aging in mammals.

In summary, the identification of a pro-aging role of Ras in aging therapy is an important and welcomed discovery especially considering that drug companies have identified a number of compounds capable to inhibit GEFs such as Brefeldin A (large ArfGef), SecinH3 (small ArfGef) and NSC23766 (RacGef) [36, 37, 38]. These examples demonstrate that pharmacological GEF inhibition may be a feasible strategy to extend longevity. In particular, the possibility to obtain GEF inhibition,

stabilizing the interaction between the G protein and its GEF, an approach described as "interfacial inhibition" [36], is very interesting because the compound doesn't need to compete with the natural substrate and hence inhibition may be obtained even by low affinity compounds. Other interfacial inhibitors are already in clinical practice. Rapamycin for example, which inhibits mTOR signaling, has been shown to extend the life span of mice [46, 47, 48].

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