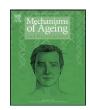
FISEVIER

Contents lists available at ScienceDirect

# Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev



# Commentary

# Paternal genome effects on aging: Evidence for a role of *Rasgrf1* in longevity determination?

João Pedro de Magalhães \*

Integrative Genomics of Ageing Group, Institute of Integrative Biology, University of Liverpool, Liverpool, UK

#### ARTICLE INFO

Article history:
Received 17 August 2010
Received in revised form 17 November 2010
Accepted 28 November 2010
Available online 21 December 2010

Keywords: Brain Epigenetics Genetics Imprinting Ras

#### ABSTRACT

A recent study by Kawahara and Kono (2010) reports that mice artificially produced with two sets of female genomes have an increased average lifespan of 28%. Moreover, these animals exhibit a smaller body size, a trait also observed in several other long-lived mouse models. One hypothesis is that alterations in the expression of paternally methylated imprinted genes are responsible for the life-extension of bi-maternal mice. Considering the similarities in postnatal growth retardation between mice with mutations in the *Rasgrf1* imprinted gene and bi-maternal mice, *Rasgrf1* is the most likely culprit for the low body weight and extended lifespan of bi-maternal mice. *Rasgrf1* is a neuronal guanine-nucleotide exchange factor that induces Ras signaling in a calcium-dependent manner and has been implicated in learning and memory. Like other long-lived mouse strains, *Rasgrf1* mutants are known to have low growth hormone and IGF-1 levels and the *Rasgrf1* yeast homolog *CDC25* had been previously associated with lifespan. Therefore, although the evidence is not conclusive, it does point towards the involvement of *Rasgrf1* in the regulation of longevity, hypothetically through a mechanism similar to that observed in other long-lived mice of low GH/IGF-1 signaling causing a low body weight and life-extension.

© 2010 Elsevier Ireland Ltd. All rights reserved.

A recent study by Kawahara and Kono (2010) reports that animals produced without a father are long-lived. Bi-maternal mice were generated by combining two haplotype genomes obtained from oocytes at two different developmental stages, namely, derived from newborn pups and adult mice (Kawahara et al., 2007). The longevity of these animals with two sets of female genomes was significantly different than that of female controls and average lifespan was increased by 28%. Although this result is impressive, the increase in maximum lifespan was less remarkable ( $\sim$ 5%), the number of animals used was modest (n = 13 for each cohort) impeding a detailed analysis of mortality patterns to estimate if aging was delayed or its onset postponed (e.g., see de Magalhaes et al., 2005), and the average lifespan of controls from the same B6D2F1 × C57BL/6 cross as the bi-maternal mice was lower than what other groups have observed for B6D2F1 and C57BL/6 mice (Forster et al., 2003). Nonetheless, the Kaplan-Meier survival curves clearly show an increased longevity in bi-maternal mice which, unsurprisingly, was highly statistically significant (Kawahara and Kono, 2010). In view of these results, Kawahara and

E-mail address: jp@senescence.info.

Kono suggest a detrimental effect on longevity by the paternal genome. Paternally methylated imprinted genes could contribute to longevity, though there are dozens of genes in which the paternal allele is expressed (http://www.mousebook.org/catalog.php?catalog=imprinting). Not surprisingly, previous work by Kawahara and Kono and colleagues characterizing bi-maternal mice show alterations in gene expression in a number of genes (Kawahara et al., 2007). Therefore, it is possible that alterations in specific genes, and maybe even in a single gene, are responsible for the life-extension observed in bi-maternal mice.

Although bi-maternal mice are apparently normal, they have a small body size with an adult body weight  $\sim\!\!35\%$  smaller than controls (Kawahara and Kono, 2010; Kawahara et al., 2007). A negative relationship between growth and longevity has been observed in mice and rats (Rollo, 2002), and a number of growth stunted mouse strains are long-lived, which could have a causal mechanistic relationship (de Magalhaes and Faragher, 2008). Therefore, it is likely that the small body size of bi-maternal mice is related to their long lifespan. In other words, it is plausible that if a single gene determines the small body size of bi-maternal mice that same gene may also determine the long lifespan of these animals.

In order to overcome imprinting mechanisms preventing mammalian parthenogenesis, one of the donor oocytes from the

<sup>\*</sup> Correspondence address: Biosciences Building, Crown Street, Liverpool L69 7ZB, UK. Tel.: +44 151 7954517; fax: +44 151 7954408.

bi-mutant mice was genetically modified in two regions on chromosomes 7 and 12. The deletions targeted differentially methylated regions Igf2-H19 and Dlk1-Gtl2 to restore gene expression regulation for these imprinted genes (Kawahara et al., 2007). Nonetheless, other genes expressed from the paternal genome could be differentially expressed in bi-maternal mice that cause them to be long-lived. Bi-maternal mice have a normal body weight during embryogenesis and at birth, but develop growth retardation postnatally. Paternally expressed genes involved in growth stimulation are normally implicated in fetal growth, however, limiting the number of candidates. The Rasgrf1 gene located on chromosome 9 is expressed from the paternal allele in neonates. Interestingly, mice lacking Rasgrf1 have a normal weight during embryogenesis and at birth but exhibit postnatal growth retardation (Drake et al., 2009; Itier et al., 1998), a phenotype remarkably similar to the patterns observed in bi-maternal mice (Kawahara and Kono, 2010; Kawahara et al., 2007). Previously, Rasgrf1 was shown to be considerably underexpressed in bimaternal mice (Kawahara et al., 2007), and thus Kawahara and Kono consider that Rasgrf1 is the most likely candidate for explaining the low adult body weight of bi-maternal mice (Kawahara and Kono, 2010; Kawahara et al., 2007). Therefore, one hypothesis is that Rasgrf1 is the main culprit for the lifeextension observed in bi-maternal mice.

Rasgrf1 is a guanine-nucleotide exchange factor, highly expressed in the central nervous system, that induces Ras signaling in a calcium-dependent manner and has been implicated in learning and memory (Brambilla et al., 1997; Drake et al., 2009). Interestingly, Rasgrf1 has been shown to regulate synthesis and release of growth hormone (GH). In addition to low GH levels, Rasgrf1 mutant mice - not surprisingly - have low IGF-1 levels and are 15-30% smaller than controls (Drake et al., 2009; Itier et al., 1998). There is ample evidence for an association between low GH/IGF-1 signaling and extended longevity in mouse models (Berryman et al., 2008; de Magalhaes and Faragher, 2008). Moreover, it is interesting to note that Rasgrf1is a homolog of yeast CDC25 which in turn has been associated with aging since mutations in CDC25 extend lifespan in yeast (Lin et al., 2000). Although it is possible that other genes contribute to the low body size and long lifespan of bi-maternal mice, Rasgrf1 is the most likely culprit. Further work is certainly necessary to establish this hypothesis - for example, it is not known at present whether bimaternal mice have altered levels of GH and/or IGF-1. Rasgrf1 mutants also exhibit long-term memory defects (Brambilla et al., 1997), but this has not been studied to date in bi-maternal

Whether *Rasgrf1* null mice are long-lived is unknown but, even if this locus is not responsible for the longevity of bi-maternal mice, it is clear that *Rasgrf1* merits further study in the context of aging and longevity. Interestingly, mice have been created whose *Rasgrf1* expression bypasses the need for methylation and therefore express *Rasgrf1* from both alleles (Yoon et al., 2005). These *Rasgrf1* biallelic mice have a roughly 3-fold increase in protein levels in the brain and exhibit opposite effects on behavior than the *Rasgrf1* null mice (Fasano et al., 2009). These animals are larger than controls and thus it seems that the amount of neonatal *Rasgrf1* expressed is a determinant of body size in adulthood, possibly through the GH/IGF-1 axis (Drake et al., 2009). Although longevity has not been

studied in these *Ragrf1*-overexpressing mice, the availability of several lines of *Rasgrf1* mutant mice means that the hypotheses proposed here are readily testable. In particular, the combination of *Rasgrf1* null mice with low body size and large biallelic animals could be a powerful new paradigm of mammalian aging.

Overall, the recent work of Kawahara and Kono (2010) reveals another important model for mammalian life-extension. If confirmed with a larger sample size by independent investigators, a nearly 30% increase in lifespan ranks among the most impressed observed in mammals to date (http://genomics.senescence.info/genes/search.php?organism=musculus). Although the evidence is not conclusive, it does point towards the involvement of *Rasgrf1* in the regulation of longevity, hypothetically through a mechanism similar to that observed in other long-lived involving the GH/IGF-1 axis causing a low body weight and life-extension. Clearly, *Rasgrf1* merits further study in the context of biogerontology.

### Acknowledgments

Many thanks to Riccardo Brambilla, Richard Miller and George Martin for comments and suggestions on previous drafts of the manuscript. The work in my lab is supported by the BBSRC, the Ellison Medical Foundation and a Marie Curie International Reintegration Grant within EC-FP7.

## References

- Berryman, D.E., Christiansen, J.S., Johannsson, G., Thorner, M.O., Kopchick, J.J., 2008. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. Growth Horm. IGF Res. 18, 455–471.
- Brambilla, R., Gnesutta, N., Minichiello, L., White, G., Roylance, A.J., Herron, C.E., Ramsey, M., Wolfer, D.P., Cestari, V., Rossi-Arnaud, C., Grant, S.G., Chapman, P.F., Lipp, H.P., Sturani, E., Klein, R., 1997. A role for the Ras signalling pathway in synaptic transmission and long-term memory. Nature 390, 281–286.
- de Magalhaes, J.P., Cabral, J.A., Magalhaes, D., 2005. The influence of genes on the aging process of mice: a statistical assessment of the genetics of aging. Genetics 169. 265–274.
- de Magalhaes, J.P., Faragher, R.G., 2008. Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity. Bioessays 30, 567–578.
- Drake, N.M., Park, Y.J., Shirali, A.S., Cleland, T.A., Soloway, P.D., 2009. Imprint switch mutations at Rasgrf1 support conflict hypothesis of imprinting and define a growth control mechanism upstream of IGF1. Mamm. Genome 20, 654–663.
- Fasano, S., D'Antoni, A., Orban, P.C., Valjent, E., Putignano, E., Vara, H., Pizzorusso, T., Giustetto, M., Yoon, B., Soloway, P., Maldonado, R., Caboche, J., Brambilla, R., 2009. Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular signal-regulated kinase (ERK) signaling in the striatum and long-term behavioral responses to cocaine. Biol. Psychiatry 66, 758–768.
- Forster, M.J., Morris, P., Sohal, R.S., 2003. Genotype and age influence the effect of caloric intake on mortality in mice. FASEB J. 17, 690–692.
- Itier, J.M., Tremp, G.L., Leonard, J.F., Multon, M.C., Ret, G., Schweighoffer, F., Tocque, B., Bluet-Pajot, M.T., Cormier, V., Dautry, F., 1998. Imprinted gene in postnatal growth role. Nature 393, 125–126.
- Kawahara, M., Kono, T., 2010. Longevity in mice without a father. Hum. Reprod. 25, 457-461.
- Kawahara, M., Wu, Q., Takahashi, N., Morita, S., Yamada, K., Ito, M., Ferguson-Smith, A.C., Kono, T., 2007. High-frequency generation of viable mice from engineered bi-maternal embryos. Nat. Biotechnol. 25, 1045–1050.
- Lin, S.J., Defossez, P.A., Guarente, L., 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 289, 2126–2128
- Rollo, C.D., 2002. Growth negatively impacts the life span of mammals. Evol. Dev. 4, 55–61.
- Yoon, B., Herman, H., Hu, B., Park, Y.J., Lindroth, A., Bell, A., West, A.G., Chang, Y., Stablewski, A., Piel, J.C., Loukinov, D.I., Lobanenkov, V.V., Soloway, P.D., 2005. Rasgrf1 imprinting is regulated by a CTCF-dependent methylation-sensitive enhancer blocker. Mol. Cell Biol. 25, 11184–11190.