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Mechanisms of Ageing and Development 126 (2005) 17–21
mechanisms of ageing and development
www.elsevier.com/locate/mechagedev

Large-scale identification in yeast of conserved ageing genes

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Available online 21 October 2004

Abstract

Recent advances have suggested the existence of phylogenetically conserved pathways regulating ageing in eukaryotes. At least two of these ''public'' longevity-determining pathways appear to have been evolutionarily conserved from yeast through mammals. We have developed a high-throughput, genome-wide approach to identify a large fraction of the non-essential, single-gene deletion mutations that confer increased longevity in yeast. The identification and characterization of conserved genes that regulate the ageing process across eukaryotic species is likely to result in an improved understanding of the causes of human ageing and provide potential therapeutic targets for drug discovery.

Keywords: Yeast aging; Replicative life span; Genome-wide; Calorie restriction; Sir2

1. Conserved features of ageing among eukaryotes

Understanding the biology of ageing in mammals is a daunting task. The study of ageing in simple eukaryotes, such as yeast, worms, and flies has served as a useful method for reducing the complexity of the problem. Nevertheless, the extent to which ageing is conserved between mammals and these simpler models remains unclear. Fortunately, recent work has suggested a surprising overlap between the genetic determinants of longevity across a variety of eukaryotic species.

The Sir2 family of proteins, referred to collectively as Sirtuins (Buck et al., 2004), defines one example of a conserved regulatory factor controlling longevity. A role for Sirtuins in ageing was first uncovered by the finding that elevated dosage of Sir2 extends the replicative life span of yeast cells (Kaeberlein et al., 1999). Following up on this work, Tissenbaum and Guarente (2001) showed that overexpression of the orthologous protein, Sir-2.1, increases life span in *Caenorhabditis elegans*. This is particularly surprising, given that the mechanism by which longevity is enhanced through up-regulation of Sirtuins appears to be different between these two organisms (Tissenbaum and Guarente, 2002). In yeast, Sir2 enhances longevity by inhibiting the formation of toxic extrachromosomal rDNA circles (Kaeberlein et al., 1999). In worms, there is no evidence that rDNA circles cause ageing, or that rDNA circles are even present in old animals. In contrast, accumulating evidence suggests that Sir-2.1 exerts its anti-ageing activity by regulating the activity of the Daf-16 transcription factor, in a well-characterized insulin/IGF-1-like pathway (Hekimi and Guarente, 2003). The evolutionary mechanism by which Sir2 might have evolved to maintain its role as a regulator of ageing in organisms that appear to age in fundamentally different ways remains a matter of much speculation.

A second conserved ageing gene is represented by yeast *SCH9*, which codes for a protein with homology to *C. elegans* SGK-1, AKT-2, and AKT-1. Deletion of *SCH9* increases yeast replicative life span by 30–40% (Fabrizio et al., 2004) and increases chronological life span by nearly 100% (Fabrizio et al., 2001). In worms, decreased expression of SGK-1 by RNAi results in a 60% increase in longevity (Hertweck et al., 2004). Intriguingly, SGK-1, AKT-2, and AKT-1 are thought to function together in a complex to regulate worm longevity by phosphorylation of Daf-16. Thus, SGK-1 and Sir-2.1 may both act in a common

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0047-6374/$ – see front matter
doi:10.1016/j.mad.2004.09.013

pathway, upstream of Daf-16. In yeast, the genetic relationship between Sch9 and Sir2 has not yet been determined.

Perhaps, the best evidence suggesting that ageing processes might be conserved among eukaryotes is the observation that calorie restriction (CR) increases life span in a wide variety of organisms, including yeast (Lin et al., 2000), worms (Lakowski and Hekimi, 1998; Houthoofd et al., 2003), flies (Clancy et al., 2002; Mair et al., 2003), and rodents, and a number of others. Although, life span extension by CR in mammals was first reported more than 70 years ago (McCay et al., 1935), a mechanistic understanding of how CR slows ageing remains elusive. Simple eukaryotic models for CR, such as yeast, are likely to provide insight into this question.

It was initially reported that calorie restriction slows ageing of yeast cells by activation of Sir2 (Lin et al., 2000). This seemed an attractive model because it was already known that overexpression of Sir2 is sufficient to increase life span (Kaeberlein et al., 1999). Several reports suggested possible mechanisms by which CR might activate Sir2, including elevated NAD^+, decreased NADH, and decreased nicotinamide (Lin et al., 2002; Anderson et al., 2003a; Anderson et al., 2003b; Lin et al., 2004). Recent work, however, has demonstrated that Sir2 is not required for a majority of the life span extension associated with CR, and that combining CR with overexpression of SIR2 results in a nearly two-fold increase in longevity (Kaeberlein et al., 2004). Interestingly, however, these extremely long-lived strains still display normal mortality curves, and the pathways limiting longevity in these strains remain totally unknown. Thus, yeast replicative life span is regulated by at least two, and likely more, distinct pathways: one involving the Sir2 protein deacetylase, another responsive to CR, and the other(s) still undefined (Fig. 1).

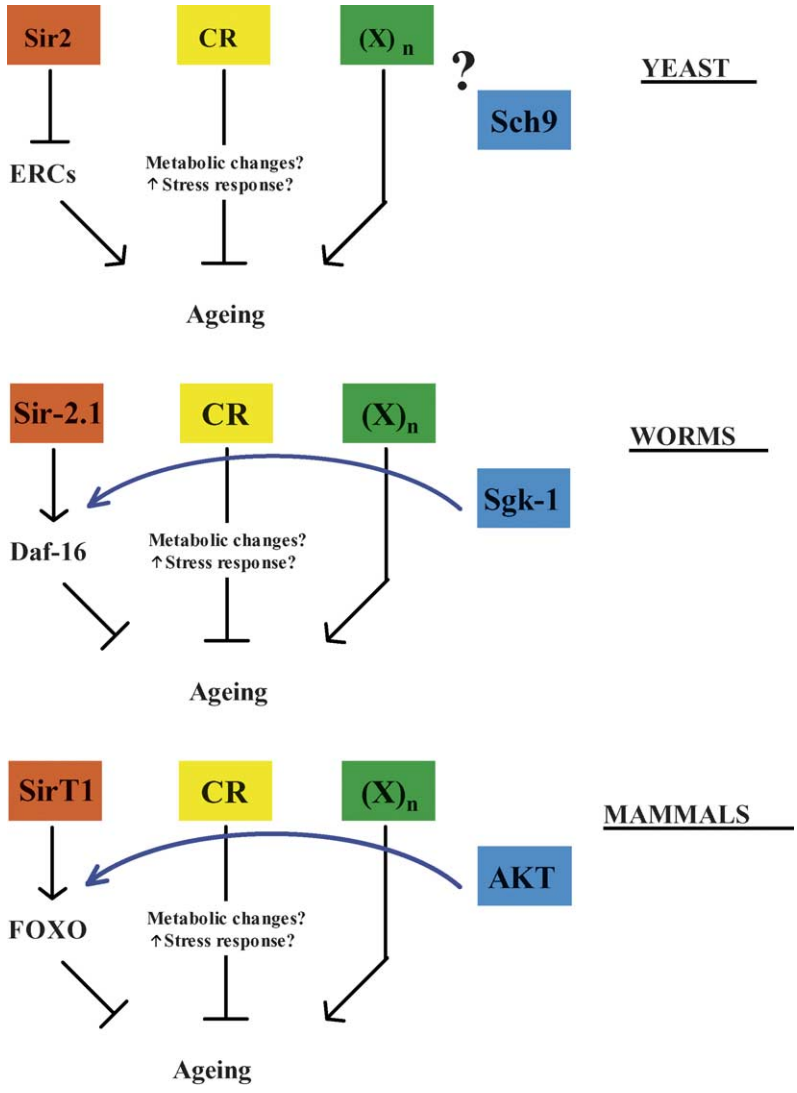

Fig. 1. Conserved pathways regulating ageing in eukaryotes. Life span extension by calorie restriction is a nearly universal phenomenon amongst eukaryotes. In simple eukaryotes, such as yeast and worms, Sir2 and Sch9 proteins also regulate longevity. Genetic analysis has placed Sir2 and CR in different pathways in both organisms, suggesting the possibility of multiple conserved pathways regulating eukaryotic ageing.

Interestingly, CR and Sir2 also appear to be genetically distinct in *C. elegans*. In contrast to Sir-2.1, life span extension by CR is independent of Daf-16 (Houthoofd et al., 2003) and is additive with longevity enhancing mutations in the insulin/IGF-1 receptor ortholog Daf-2 (Lakowski and Hekimi, 1998). This is strikingly similar to the case in yeast, where combining overexpression of Sir2 with CR results in an additive life span increase (Kaeberlein et al., 2004).

There is also accumulating evidence that both CR and Sirtuins are likely to be conserved longevity factors in mammals. The mammalian Sir2 ortholog, SirT1, has recently been identified as a regulator of the Daf-16 ortholog, Foxo3a, a putative downstream target of insulin and IGF-1 signaling (Brunet et al., 2004; Motta et al., 2004). While CR alters insulin/IGF-1 levels in mammals, it is also clear that a significant fraction of the longevity benefit associated with CR is independent of the insulin/IGF-1 pathway (Bartke et al., 2001), consistent with a two-pathway model in which CR and insulin/IGF-1 are at least partially independent.

It should be noted, however, that SirT1 has also been implicated in the regulation of a number of other proteins that may influence ageing including p53, FOXO4, Ku70, PPARγ and NFκB (Luo et al., 2001; Vaziri et al., 2001; Langley et al., 2002; Cohen et al., 2004; Picard et al., 2004; van der Horst et al., 2004; Yeung et al., 2004). Which of these activities, if any, link SirT1 to mammalian ageing remains to be determined. Not surprisingly, the genetic relationships between conserved longevity factors are likely more complex in mammals than in simpler eukaryotes.

2. The hunt for additional conserved ageing genes

Given that both CR and Sirtuins appear to have conserved effects on longevity in multiple eukaryotic species (Fig. 1), it seems likely that other conserved ageing factors exist. We have recently completed a comprehensive analysis of more than 40 genes reported to affect ageing in yeast when deleted. This study was carried out in the genetic background of the MATα yeast ORF deletion set (BY4742), a strain with a mean life span 20–50% longer than most reported lab strains. By using a long-lived strain, we feel confident that mutations resulting in increased life span are more likely to represent general ageing factors in yeast. Consistent with this surmise, of the five mutations observed to significantly increase life span in this study, three are genetic models of CR (*hxk2Δ, gpa2Δ, gpr1Δ*), one is in the Sir2 pathway *(fob1Δ),* and the fifth is deletion of *SCH9* (Kaeberlein et al., 2004. In addition, we have determined that overexpression of Sir2 increases life span in BY4742, as does CR by growth on low glucose (Kaeberlein et al., 2004). Thus, of the seven genetic and environmental interventions known to increase life span in this long-lived strain, six can be linked to enhanced longevity in higher eukaryotes and the seventh (*fob1Δ*) is linked to life span extension by Sir2.

The discovery that a majority of the life span benefit from CR is independent of Sir2 was unexpected. Several questions still remain: (1) Is CR completely Sir2-independent? (2) What is the molecular mechanism by which CR increases life span? (3) Are there additional, undiscovered, pathways regulating ageing? (4) How great is the conservation between ageing in simple eukaryotes and ageing in humans?

In order to attempt to answer these questions, we have initiated a project to determine the chronological and replicative ageing properties for >4500 single-gene deletion

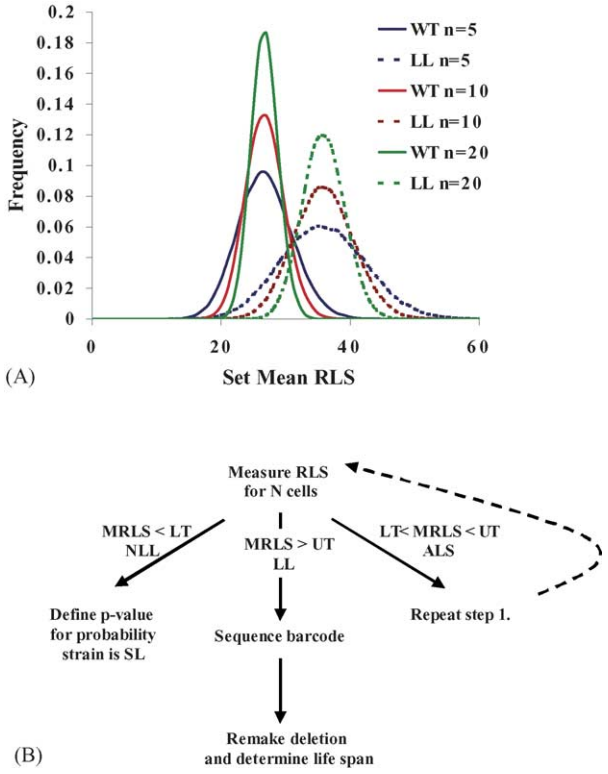

Fig. 2. A method for genome-wide analysis of replicative life span. (A) Data sets were created that contained either 250 experimentally determined wild-type mother cell life spans (not long-lived = NLL) or 250 long-lived (*fob1Δ, hxk2Δ, gpa2Δ*, or *gpa1Δ* = LL) mother cell life spans. From each set, 100,000 subsets were randomly selected and used to generate a probability distribution of subset mean replicative life span (RLS) for subsets of size $n = 5$, 10, and 20. In order to identify novel LL mutants from the ORF deletion collection, appropriate lower threshold (LT) and upper threshold (UT) subset mean RLS cutoffs were determined for $n = 5$, such that the probabilities of false negative errors (misclassifying a LL strain as NLL) are minimized while keeping the false positive (misclassifying a NLL strain as LL) less than 0.01. (B) Replicative life span is determined simultaneously for $N = 5$ mother cells each from approximately 100 different single-gene deletion mutants. Those strains that have a five-cell set mean RLS less than the LT are classified as NLL; those strains that have a five-cell set mean RLS greater than the LT but less than the UT are classified as ambiguous and subjected to another round of five-cell RLS analysis; strains that have a five-cell mean RLS greater than the UT are classified as LL and verified.

strains obtained from the MATα ORF deletion collection (Winzeler et al., 1999). We have recently developed a high-throughput method for replicative life span analysis that will facilitate this endeavor (Fig. 2). To date, approximately 10% of the non-essential ORFs (∼500 genes) have been assayed. From this analysis, we have determined that roughly 20% of non-essential single-gene mutations result in a significant reduction of life span ($p < .05$). Many of these mutations that shorten life span cause enhanced stochastic death and are unlikely to be relevant to the normal ageing process of yeast cells.

In contrast, mutations that increase life span, by definition, alter the ageing properties of the population, and are of fundamental interest. From our analysis, we have identified several previously unknown single-gene deletion mutations that significantly enhance mother cell life span. We are currently placing these genes into (or out of) known genetic pathways and characterizing the mechanism(s) by which life span is enhanced. In this way, we hope to discover novel pathways influencing longevity. We also expect many of the identified mutations to behave as genetic mimics of CR, perhaps giving insight into the molecular mechanisms by which CR slows ageing. Approximately, half of the newly identified yeast ageing genes have identifiable orthologs in worms, flies, and mammals. It will be of interest to discover which, if any, also have conserved roles as determinants of longevity in other ageing model systems and ultimately, humans.

3. Conclusion

The apparent conservation of two genetically distinct ageing pathways from yeast to mammals suggests the existence of, as yet undiscovered, orthologous gene families that regulate longevity across eukaryotes. Identification of these genes will lead to a better understanding of the molecular processes that cause ageing as well as the mechanisms by which some mutations and interventions, such as CR, confer increased life span in mammals. We have developed a method to rapidly identify a majority of the genes that increase life span when deleted in yeast. This information, combined with data generated in similar genome-wide RNAi screens in *C. elegans* (Dillin et al., 2002; Lee et al., 2003) is likely to lead to the identification of additional genes that regulate ageing in both yeast and worms, and possibly mammals.

Acknowledgements

We thank S. Fields, K. Kirkland and T. Powers for helpful discussion. Large-scale analysis of replicative ageing in yeast has been funded by grants to B.K.K. by the American Federation for Aging Research and the University of Washington Nathan Shock Center of Excellence for the Basic Biology of Aging. M.K. is supported by National Institutes of Health Training Grant P30 AG013280. B.K.K. is a Searle Scholar.

References

Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., Sinclair, D.A., 2003a. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. Nature 423, 181–185.

Anderson, R.M., Latorre-Esteves, M., Neves, A.R., Lavu, S., Medvedik, O., Taylor, C., Howitz, K.T., Santos, H., Sinclair, D.A., 2003b. Yeast lifespan extension by calorie restriction is independent of NAD fluctuation. Science 302, 2124–2126.

Bartke, A., Wright, J.C., Mattison, J.A., Ingram, D.K., Miller, R.A., Roth, G.S., 2001. Extending the lifespan of long-lived mice. Nature 414, 412.

Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., Hu, L.S., Cheng, H.L., Jedrychowski, M.P., Gygi, S.P., Sinclair, D.A., Alt, F.W., Greenberg, M.E., 2004. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303, 2011–2015.

Buck, S.W., Gallo, C.M., Smith, J.S., 2004. Diversity in the Sir2 family of protein deacetylases. J. Leukoc. Biol. 75, 939–950.

Clancy, D.J., Gems, D., Hafen, E., Leevers, S.J., Partridge, L., 2002. Dietary restriction in long-lived dwarf flies. Science 296, 319.

Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., Sinclair, D.A., 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305, 390–392.

Dillin, A., Hsu, A.L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., Kenyon, C., 2002. Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.

Fabrizio, P., Pletcher, S.D., Minois, N., Vaupel, J.W., Longo, V.D., 2004. Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in *Saccharomyces cerevisiae*. FEBS. Lett. 557, 136–142.

Fabrizio, P., Pozza, F., Pletcher, S.D., Gendron, C.M., Longo, V.D., 2001. Regulation of longevity and stress resistance by Sch9 in yeast. Science 292, 288–290.

Hekimi, S., Guarente, L., 2003. Genetics and the specificity of the aging process. Science 299, 1351–1354.

Hertweck, M., Gobel, C., Baumeister, R., 2004. *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev. Cell. 6, 577–588.

Houthoofd, K., Braeckman, B.P., Johnson, T.E., Vanfleteren, J.R., 2003. Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in *Caenorhabditis elegans*. Exp. Gerontol. 38, 947–954.

Kaeberlein, M., Kirkland, K.T., Fields, S., Kennedy, B.K., 2004. Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. 2, E296.

Kaeberlein, M., McVey, M., Guarente, L., 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. 13, 2570–2580.

Lakowski, B., Hekimi, S., 1998. The genetics of caloric restriction in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 95, 13091–13096.

Langley, E., Pearson, M., Faretta, M., Bauer, U.M., Frye, R.A., Minucci, S., Pelicci, P.G., Kouzarides, T., 2002. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. Embo. J. 21, 2383–2396.

Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J., Ruvkun, G., 2003. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat. Genet. 33, 40–48.

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.

Lin, S.J., Ford, E., Haigis, M., Liszt, G., Guarente, L., 2004. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev. 18, 12–16.

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A.P.D., Culotta, V.C., Fink, G., Guarente, L., 2002. Calorie restriction extends *Saccharomyces cerevisiae* life span by increasing respiration. Nature 418, 344–348.

Luo, J., Nikolaev, A.Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L., Gu, W., 2001. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 107, 137–148.

Mair, W., Goymer, P., Pletcher, S.D., Partridge, L., 2003. Demography of dietary restriction and death in *Drosophila*. Science 301, 1731–1733.

McCay, C.M., Crowell, M.F., Maynard, L.A., 1935. The effect of retarded growth upon the length of life and upon ultimate size. J. Nutr. 10, 63–79.

Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M., Guarente, L., 2004. Mammalian SIRT1 represses forkhead transcription factors. Cell 116, 551–563.

Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M.W., Guarente, L., 2004. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 429, 771–776.

Tissenbaum, H.A., Guarente, L., 2001. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. Nature 410, 227–230.

Tissenbaum, H.A., Guarente, L., 2002. Model organisms as a guide to mammalian aging. Dev. Cell. 2, 9–19.

van der Horst, A., Tertoolen, L.G., de Vries-Smits, L.M., Frye, R.A., Medema, R.H., Burgering, B.M., 2004. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J. Biol. Chem. 279, 28873–28879.

Vaziri, H., Dessain, S.K., Ng Eaton, E., Imai, S.I., Frye, R.A., Pandita, T.K., Guarente, L., Weinberg, R.A., 2001. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 107, 149–159.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El Bakkoury, M., Foury, F., Friend, S.H., Gentalen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Davis, R.W., et al., 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285, 901–906.

Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A., Mayo, M.W., 2004. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. Embo. J. 23, 2369–2380.