Signaling networks in aging

Eric L. Greer[1,2] and Anne Brunet[1,2,3,*]

[1]Department of Genetics, 300 Pasteur Drive, [2]Cancer Biology Program, and [3]Neurosciences Program, Stanford University, Stanford, CA 94305, USA

*Author for correspondence
(e-mail: anne.brunet@stanford.edu)

Journal of Cell Science 121, 407-412
Published by The Company of Biologists 2008
doi:10.1242/jcs.021519

Aging – long considered to be solely the result of wear and tear – is in fact regulated by specific genetic pathways. Simple changes in the environment (e.g. dietary restriction) can drastically extend lifespan, suggesting that several of these genetic pathways control longevity in response to changes in the surroundings. Here we summarize the key signaling modules identified so far that regulate aging and longevity.

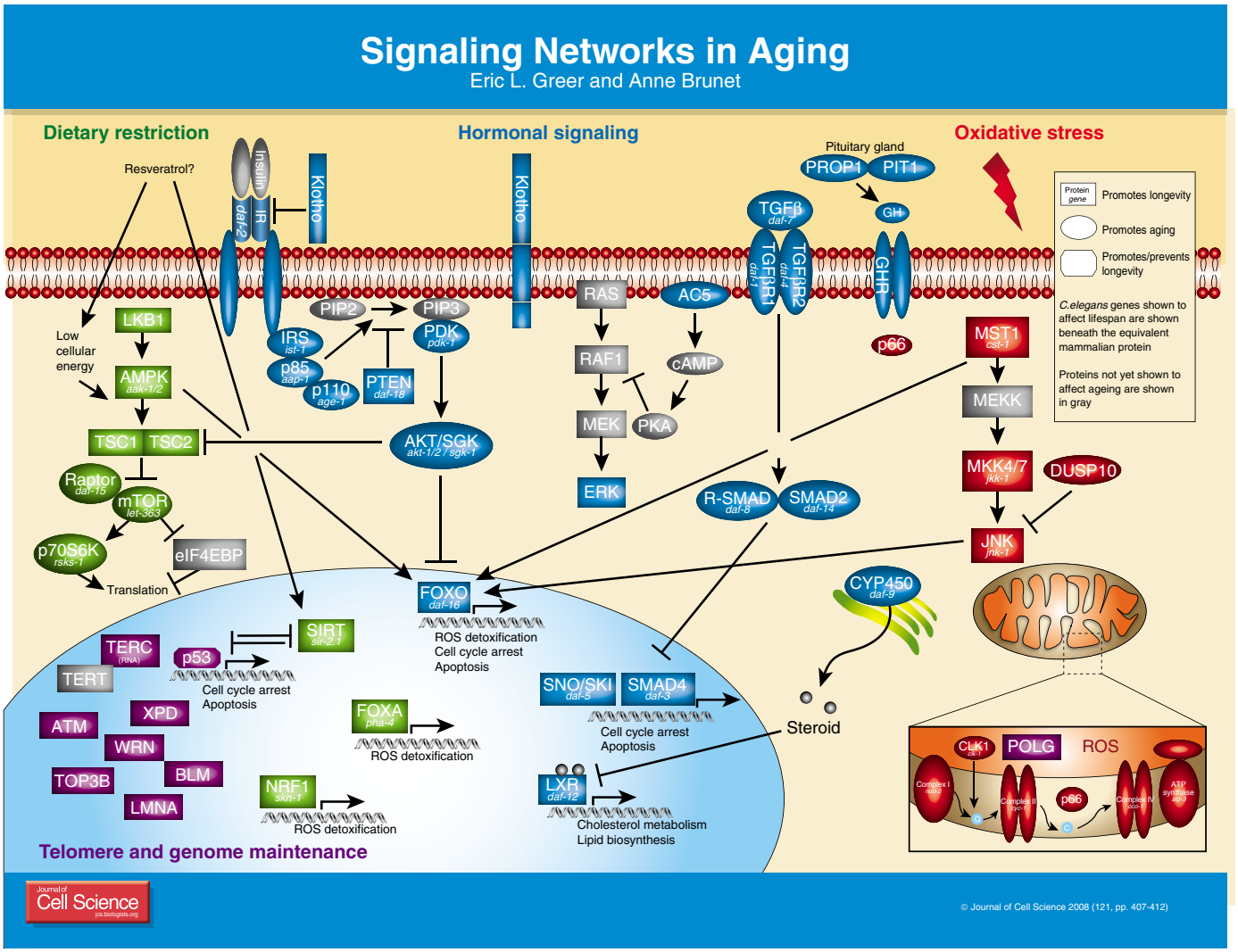
Hormonal signaling

Insulin and insulin-like signaling

The first example of a specific pathway controlling longevity came from studies of *Caenorhabditis elegans*. Mutations that reduce the activity of the insulin receptor DAF-2 (Kenyon et al., 1993; Kimura et al., 1997) or the phosphoinositide 3-kinase (PI3K) AGE-1 (Friedman and Johnson, 1988; Morris et al., 1996) extend lifespan in adult worms by more than 100%. The insulin receptor mediates its effects via the PI3K-AKT/SGK signaling pathway, which culminates in the negative regulation of the Forkhead transcription factor FOXO/DAF-16 (Brunet et al., 1999; Kops et al., 1999; Lin et al., 1997; Ogg et al., 1997). Insulin signaling regulates aging in a conserved manner, from worms to mammals. In *Drosophila melanogaster*, mutations in the insulin receptor extend female lifespan by ~85% and mutation of the insulin-receptor substrate (IRS) Chico causes an ~48% increase in lifespan (Clancy et al., 2001; Tatar et al., 2001). Overexpression of *foxo* in *Drosophila* extends lifespan by ~15-52% (Giannakou et al., 2004; Hwangbo et al., 2004). In mice, animals that lack one allele of the insulin-like growth factor 1 (IGF1) receptor gene show a 26% increase in mean lifespan (Holzenberger et al., 2003). The insulin signaling pathway is important in a variety of tissues to extend lifespan. Mutation of the insulin receptor in adipose tissue increases mouse lifespan by 18% (Bluher et al., 2003), whereas a brain-specific IRS2 knockout extends mouse lifespan by ~18% (Taguchi et al., 2007).

Growth hormone signaling

In mice, the Snell and Ames dwarf mutations, which are in the genes encoding



(See poster insert)

the pituitary transcription factors PIT1 (POU1F1) and PROP1, respectively, extend lifespan by 42-67% (Brown-Borg et al., 1996; Flurkey et al., 2001). The extension in longevity in both mouse models is likely to be due to defects in the ability of the pituitary gland to secrete growth hormone (GH), because mice that have a null mutation in the GH receptor ($GHR^{-/-}$) also display an ~21-40% increase in lifespan (Coschigano et al., 2003), whereas transgenic mice that overexpress GH live significantly shorter than wild-type mice (Wolf et al., 1993). Interestingly, the Ames dwarf mice and the $GHR^{-/-}$ mice have reduced levels of circulating IGF1, fasting insulin and glucose (Brown-Borg et al., 1996; Coschigano et al., 2003), raising the possibility that the increased longevity of these mice is mediated by insulin/IGF1 signaling.

Klotho

Disruption of the expression of klotho, a cell-surface protein whose extracellular domain can act as a circulating hormone (Shiraki-Iida et al., 1998), accelerates aging in mice (Kuro-o et al., 1997). Conversely, overexpression of klotho in mice leads to an ~19-31% lifespan extension in one strain of mice (Kurosu et al., 2005). The precise mechanisms by which klotho extends lifespan are still under investigation, but klotho has been found to repress insulin/IGF1 signaling (Kurosu et al., 2005) and to regulate phosphate and calcium homeostasis (Imura et al., 2007) by affecting fibroblast growth factor 23 (FGF23) (Urakawa et al., 2006) and the Na^+/K^+-ATPase (Imura et al., 2007).

AC5

Mice lacking type 5 adenylyl cyclase (AC5) have an ~32% increase in lifespan compared with wild-type littermates (Yan et al., 2007). AC5 probably transduces signals emanating from a hormonal seven-transmembrane-domain receptor, although the identity of this receptor is unknown. The increase in lifespan in AC5-deficient mice correlates with decreased levels of circulating GH, increased resistance to oxidative stress and increased Raf-MEK-ERK signaling (Yan et al., 2007). The chronological lifespan of yeast expressing a mutant form of adenylyl cyclase (CYR1) or yeast overexpressing the MAP kinase ERK2 is also increased, suggesting that the relevance of this pathway to longevity is conserved throughout evolution (Fabrizio et al., 2001; Yan et al., 2007).

TGFβ

In adult worms, mutations in TGFβ (*daf-7*) or in the TGFβ receptors DAF-1 (TGFβR1) and DAF-4 (TGFβR2) extend worm lifespan by 18-120% (Shaw et al., 2007). TGFβ signaling is mediated by two SMAD transcription factors, DAF-8 and DAF-14, which inhibit the action of another SMAD transcription factor, DAF-3 (SMAD3) (Shaw et al., 2007). DAF-3, together with its co-activator DAF-5, upregulates genes involved in cell cycle arrest and apoptosis, a large number of which are also regulated by the FOXO transcription factor DAF-16 (Shaw et al., 2007). Thus, the TGFβ and the insulin pathways might regulate lifespan by acting on similar subsets of genes.

Steroid signaling

In worms, the loss-of-function mutation of a cytochrome P450 (*daf-9*), a predicted steroidogenic hydroxylase, extends lifespan in a manner that is dependent on DAF-12 (Gerisch et al., 2001; Jia et al., 2002), a nuclear hormone receptor with closest homology to LXRα (liver X receptor alpha) in mammals. DAF-9 might regulate the synthesis of a steroid ligand that inhibits the receptor DAF-12 (Gerisch et al., 2001; Jia et al., 2002). In line with this prediction, the steroid dafachronic acid is a ligand for DAF-12 that shortens the lifespan of *daf-9*-mutant worms (Gerisch et al., 2007). Conversely, another steroid, pregnenolone, extends worm lifespan in a DAF-12-dependent manner (Broue et al., 2007). In flies, the steroid termed juvenile hormone has been found to reverse the lifespan extension caused by mutation of the insulin-like receptor (Tatar et al., 2001). In mammals, the effects of steroids or steroid receptors on overall lifespan have not been directly examined, but the steroid dehydroepiandorosterone sulfate (DHEA-S) has been found to be associated with increased longevity in primates and humans (Roth et al., 2002).

Hormonal signaling pathways are extremely potent regulators of lifespan, perhaps because they coordinate the longevity of several key organs by acting in a systemic manner.

Nutrient sensing and signaling

The most efficient environmental intervention to delay aging is dietary restriction (DR) – restriction of food intake without malnutrition. Dissecting the mechanisms underlying DR-induced longevity has allowed the identification of novel signaling pathways that regulate aging.

Sirtuin deacetylases

The Sirtuin family of NAD-dependent protein deacetylases was identified early on as a key regulator of replicative lifespan in yeast (Kaeberlein et al., 1999; Kennedy et al., 1995). The role of Sirtuins in lifespan is conserved in metazoans. An increased number of copies of *sir-2.1*, a worm ortholog of yeast *SIR2*, extends worm lifespan by 15-50% (Tissenbaum and Guarente, 2001) and expression of *Drosophila Sir2* extends fly lifespan by 18-29% (Rogina and Helfand, 2004). Importantly, Sirtuin proteins mediate the beneficial effects of DR on lifespan and behavior in yeast, worms, flies and mice (Chen et al., 2005; Lin et al., 2000; Rogina and Helfand, 2004; Wang and Tissenbaum, 2006). Note that Sir2 is necessary for increased lifespan induced by some, but not all, methods of DR in yeast (Kaeberlein et al., 2004; Lin et al., 2002). In mammals, there are seven Sirtuin proteins. The role of mammalian Sirtuin proteins in longevity has not yet been entirely described, but three pieces of recent evidence support a conserved role for Sirtuins in mammalian lifespan. First, $Sirt6^{-/-}$ mice display signs of accelerated aging (Mostoslavsky et al., 2006). Second, a polymorphism in the human *SIRT3* gene has been correlated with increased survival in centenarians (Rose et al., 2003). Third, Sirtuin proteins are one of the targets of the polyphenol compound resveratrol, which extends lifespan of invertebrates and obese mice (Baur et al., 2006; Viswanathan et al., 2005; Wood et al., 2004).

The Sirtuin pathway intersects with the insulin/IGF1 pathway. *sir-2.1* lifespan extension in worms is dependent on FOXO (Tissenbaum and Guarente, 2001) and SIRT1 interacts with and directly deacetylates FOXO in mammalian cells (Brunet et al., 2004; Daitoku et al., 2004; Frescas et al., 2005; Motta et al., 2004; Van Der Horst et al., 2004; Yang et al., 2005).

AMPK

AMP-activated protein kinase (AMPK) is an energy sensor that is activated in response to low energy levels. AMPK overexpression extends lifespan in worms by ~13% (Apfeld et al., 2004; Greer et al., 2007a). AMPK is necessary for worm lifespan extension by one DR method in *C.*

elegans (Greer et al., 2007a), but not another (Curtis et al., 2006). The role of AMPK in mammalian longevity is not clear yet, but compounds that activate AMPK have been proposed to act as DR mimetics and can extend mouse lifespan (Anisimov et al., 2005; Ingram et al., 2004). AMPK is also activated by resveratrol (Baur et al., 2006; Dasgupta and Milbrandt, 2007; Zang et al., 2006). AMPK acts in part via FOXO transcription factors to extend lifespan in worms (Greer et al., 2007a). In mammalian cells, AMPK directly phosphorylates FOXO, which suggests cross-talk between the AMPK and the insulin/IGF1 pathways (Greer et al., 2007b). AMPK activation in mammalian cells is known to result in the inhibition of target of rapamycin (TOR), a protein kinase that regulates protein translation (Inoki et al., 2003); so, part of the effects of AMPK on longevity could also be mediated by TOR (see below).

TOR and translation signaling

Mutation of TOR in worms extends lifespan by 150% (Hansen et al., 2007; Henderson et al., 2006; Vellai et al., 2003). In addition, mutation of raptor (DAF-15), a protein that forms a regulatory complex with TOR, extends worm lifespan by ~30% (Jia et al., 2004). Raptor transcription is regulated by FOXO/DAF-16 (Jia et al., 2004), highlighting the intersection of the insulin receptor (IR) and the TOR pathways. TOR regulates translation through activation of p70S6K and inhibition of the translation repressor eIF4EBP. Recent studies have confirmed the importance of regulation of translation in longevity. Knocking down three translational regulators, eIF4G, eIF4E and eIF2B homologs, or p70S6K (RSKS-1) in *C. elegans* extends worm lifespan (Hansen et al., 2007; Henderson et al., 2006; Pan et al., 2007; Syntichaki et al., 2007). Similarly, in flies, modulation of translation by a dominant-negative form of TOR or its downstream target S6K extends lifespan (Kapahi et al., 2004).

FOXA/PHA-4

FOXA/PHA-4, another transcription factor of the Forkhead family, plays a central role in the extension of longevity induced by DR in worms (Panowski et al., 2007). FOXA/PHA-4 mediates the increase in lifespan of *eat-2*, a mutation that causes a decreased eating rate in *C. elegans* and is used to mimic DR (Avery, 1993; Lakowski and Hekimi, 1998). FOXA/PHA-4 also mediates the entire lifespan extension caused by another DR method, in which food is restricted in liquid medium (Panowski et al., 2007). FOXA/PHA-4 promotes worm longevity by upregulating a set of superoxide dismutase genes (*sod-1*, *sod-2*, *sod-4* and *sod-5*), whereas FOXO/DAF-16 induces a different set of superoxide dismutase genes (*sod-1*, *sod-3* and *sod-5*) (Panowski et al., 2007).

NRF1/SKN-1

DR-induced longevity is also mediated by a member of the family of bZIP transcription factors called SKN-1 in worms (NRF1 in mammals). A mutation in *skn-1* decreases average lifespan in *C. elegans* (An and Blackwell, 2003). Expression of SKN-1 in two sensory neurons in worms is necessary for lifespan extension by DR where food is restricted in liquid medium (Bishop and Guarente, 2007), which suggests that SKN-1 acts to sense DR in these neurons. Interestingly, SKN-1 has been proposed to act by increasing the respiration rate in worms (Bishop and Guarente, 2007).

Mitochondria and ROS signaling

Mitochondria have been proposed to act as central organelles in the regulation of organismal aging (Wallace, 2005), because they control cellular energy levels, reactive oxygen species (ROS) production/detoxification and apoptosis, all of which are crucially important in determining lifespan.

ETC components, Clk-1 and p66^{shc}

One of the first pieces of evidence that components of the electron-transport chain (ETC) in mitochondria directly control lifespan came from genetic studies in *C. elegans*. Loss-of-function mutations in *clk-1*, which encodes a protein required for the biosynthesis of ubiquinone (coenzyme Q), an essential cofactor in the ETC, extend worm lifespan by 7-41% (Lakowski and Hekimi, 1996). The extension caused by the *clk-1* mutation appears to be independent of insulin signaling (Lakowski and Hekimi, 1996) but might act in the same pathway as DR, because the *clk-1* mutation does not further extend the lifespan of *eat-2*-mutant worms (Lakowski and Hekimi, 1998). Interestingly, mice that lack one allele of the *Clk1* gene live 15-31% longer than wild-type mice (Liu et al., 2005), which suggests that ubiquinone plays a conserved role in lifespan regulation.

Mutation of the iron sulfur protein (ISP-1) of the mitochondrial complex III increases worm lifespan by 68-77% (Feng et al., 2001). RNAi directed against several other components of the ETC (*nuo-2*, NADH/ubiquinone oxidoreductase; *cyc-1*, cytochrome *c* reductase; and *cco-1*, cytochrome *c* oxidase), as well as against mitochondrial ATP synthase (*atp-3*), extends worm lifespan (Dillin et al., 2002; Lee et al., 2003). This suggests that reducing the energy production and/or reducing the production of ROS associated with electron transfer is crucial for lifespan extension.

In mice, the deletion of the gene encoding p66^{shc} causes an ~28% increase in mouse lifespan compared with wild-type littermates (Migliaccio et al., 1999). Intriguingly, p66^{shc} is present within the mitochondrial intermembrane space and oxidizes cytochrome *c*, thereby generating ROS (Giorgio et al., 2005; Pinton et al., 2007).

Stress-induced protein kinases: JNK and MST-1

The activation of JNK, a MAP kinase family member activated by oxidative stress, extends longevity in worms and flies (Oh et al., 2005; Wang et al., 2003). The JNK pathway intersects with the insulin pathway. In *C. elegans,* overexpression of JNK leads to a FOXO-dependent increase in worm lifespan of ~23-37% (Oh et al., 2005). In flies, FOXO is necessary for the lifespan extension of mutants in which JNK is activated (Wang et al., 2005).

The expression of another oxidative-stress-induced protein kinase (MST-1) extends worm lifespan by 7-18% in a FOXO-dependent manner (Lehtinen et al., 2006). Both JNK and MST-1 directly phosphorylate FOXO in worms and mammals and antagonize the effect of insulin on FOXO by promoting FOXO nuclear localization (Lehtinen et al., 2006; Oh et al., 2005).

Genome surveillance pathways

DNA repair and telomere maintenance

Mutations in a number of DNA repair genes (*XPD*, *ATM*, *WRN*, *BLM*, *TOP3B* and *POLG*) cause premature aging, suggesting that repair of nuclear and mitochondrial DNA lesions is crucial for the normal lifespan of an organism. This subject is comprehensively reviewed

elsewhere (Lombard et al., 2005). Interestingly, mutations in humans in some of these DNA repair genes (*XPD*, *ATM*, *BLM* and *WRN*) and in a gene encoding a protein involved in nuclear architecture (lamin A) are responsible for pathologies known as progeroid syndromes, in which patients display signs of premature aging (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003; Navarro et al., 2006). Telomere maintenance is also crucial for a normal lifespan. Telomere synthesis requires both telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC). Mice lacking TERC ($mTR^{-/-}$) display signs of accelerated aging at the sixth generation (Rudolph et al., 1999). The fact that rapid aging is only observed in later generations of telomerase-deficient mice suggests that it is the telomere length or accumulation of DNA damage, rather than the absence of the enzyme, that causes premature aging. Although it is clear that intact DNA repair and telomere maintenance pathways are necessary for normal lifespan, an important issue that remains to be addressed is whether the overexpression of genes involved in DNA-repair or telomere-maintenance pathways would be sufficient to extend lifespan.

Tumor suppressors and antagonistic pleiotropy

Tumor suppressors are likely to help promote longevity by preventing cancer. Intriguingly, a few examples have indicated that tumor suppression might occur at the expense of longevity, illustrating the 'antagonistic pleiotropy' theory of aging. For example, a mouse mutant that has constitutively activated p53 develops fewer tumors but also shows signs of rapid aging (Tyner et al., 2002). Mice overexpressing p44, a truncated activating version of p53, also have shortened lifespans (Maier et al., 2004). Moreover, expression of a dominant-negative form of p53 in flies extends lifespan by 32-58% (Bauer et al., 2005). A potential explanation for these findings is that the cellular responses triggered by activated p53 (cellular senescence) act as potent barriers against cancerous lesions but also have deleterious roles in tissues (Campisi, 2005). 'Antagonistic pleiotropy' is not seen in all mouse models expressing tumor suppressors. For example, transgenic mice that express extra copies of p53 exhibit tumor resistance but age normally (Garcia-Cao et al., 2002; Matheu et al., 2007). Transgenic mice that express both p53 and the tumor suppressor $p19^{Arf}$ even display a 16% extension in lifespan (Garcia-Cao et al., 2002; Matheu et al., 2007). Similarly, the tumor suppressor $p16^{Ink4a}$ causes cellular senescence, but does not shorten overall lifespan, when overexpressed in mice (Matheu et al., 2004). These findings indicate that, under some circumstances, tumor-suppressor genes can prevent cancer and extend lifespan.

Perspectives

Genetic and environmental manipulations have revealed that aging is regulated by specific signaling pathways. However, whether these signaling pathways exert their effects in all tissues or regulate aging in specific 'master' tissues that then affect aging systemically or are the rate-limiting organs for longevity remains to be determined for most pathways. Genome-wide RNAi screens in *C. elegans* have identified numerous genes that affect lifespan (Chen et al., 2007; Curran and Ruvkun, 2007; Hamilton et al., 2005; Hansen et al., 2005; Lee et al., 2003). Whether additional signaling modules regulating lifespan in other species exist will be interesting to determine. The identification of aging-signaling pathways has expanded the number of potential targets for small molecules that could stimulate longevity pathways or inhibit aging pathways. Compounds that affect aging could help prevent a wide range of diseases that are age-dependent, including cancer and neurodegenerative diseases.

References

An, J. H. and Blackwell, T. K. (2003). SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. *Genes Dev.* **17**, 1882-1893.

Anisimov, V. N., Berstein, L. M., Egormin, P. A., Piskunova, T. S., Popovich, I. G., Zabezhinski, M. A., Kovalenko, I. G., Poroshina, T. E., Semenchenko, A. V., Provinciali, M. et al. (2005). Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp. Gerontol.* **40**, 685-693.

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P. S. and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. *Genes Dev.* **18**, 3004-3009.

Avery, L. (1993). The genetics of feeding in Caenorhabditis elegans. *Genetics* **133**, 897-917.

Bauer, J. H., Poon, P. C., Glatt-Deeley, H., Abrams, J. M. and Helfand, S. L. (2005). Neuronal expression of p53 dominant-negative proteins in adult Drosophila melanogaster extends life span. *Curr. Biol.* **15**, 2063-2068.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K. et al. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337-342.

Bishop, N. A. and Guarente, L. (2007). Two neurons mediate diet-restriction-induced longevity in C. elegans. *Nature* **447**, 545-549.

Bluher, M., Kahn, B. B. and Kahn, C. R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299**, 572-574.

Broue, F., Liere, P., Kenyon, C. and Baulieu, E. E. (2007). A steroid hormone that extends the lifespan of Caenorhabditis elegans. *Aging Cell* **6**, 87-94.

Brown-Borg, H. M., Borg, K. E., Meliska, C. J. and Bartke, A. (1996). Dwarf mice and the ageing process. *Nature* **384**, 33.

Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J. and Greenberg, M. E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857-868.

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. et al. (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**, 2011-2015.

Campisi, J. (2005). Aging, tumor suppression and cancer: high wire-act! *Mech Ageing Dev.* **126**, 51-58.

Chen, D., Steele, A. D., Lindquist, S. and Guarente, L. (2005). Increase in activity during calorie restriction requires Sirt1. *Science* **310**, 1641.

Chen, D., Pan, K. Z., Palter, J. E. and Kapahi, P. (2007). Longevity determined by developmental arrest genes in Caenorhabditis elegans. *Aging Cell* **6**, 525-533.

Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., Leevers, S. J. and Partridge, L. (2001). Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292**, 104-106.

Coschigano, K. T., Holland, A. N., Riders, M. E., List, E. O., Flyvbjerg, A. and Kopchick, J. J. (2003). Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. *Endocrinology* **144**, 3799-3810.

Curran, S. P. and Ruvkun, G. (2007). Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet.* **3**, e56.

Curtis, R., O'Connor, G. and DiStefano, P. S. (2006). Aging networks in Caenorhabditis elegans: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways. *Aging Cell* **5**, 119-126.

Daitoku, H., Hatta, M., Matsuzaki, H., Aratani, S., Ohshima, T., Miyagishi, M., Nakajima, T. and Fukamizu, A. (2004). Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc. Natl. Acad. Sci. USA* **101**, 10042-10047.

Dasgupta, B. and Milbrandt, J. (2007). Resveratrol stimulates AMP kinase activity in neurons. *Proc. Natl. Acad. Sci. USA* **104**, 7217-7222.

De Sandre-Giovannoli, A., Bernard, R., Cau, P., Navarro, C., Amiel, J., Boccaccio, I., Lyonnet, S., Stewart, C. L., Munnich, A., Le Merrer, M. et al. (2003). Lamin a truncation in Hutchinson-Gilford progeria. *Science* **300**, 2055.

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

Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., Erdos, M. R., Robbins, C. M., Moses, T. Y., Berglund, P. et al. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* **423**, 293-298.

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

Feng, J., Bussiere, F. and Hekimi, S. (2001). Mitochondrial electron transport is a key determinant of life span in Caenorhabditis elegans. *Dev. Cell* **1**, 633-644.

Flurkey, K., Papaconstantinou, J., Miller, R. A. and Harrison, D. E. (2001). Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl. Acad. Sci. USA* **98**, 6736-6741.

Frescas, D., Valenti, L. and Accili, D. (2005). Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. *J. Biol. Chem.* **280**, 20589-20595.

Friedman, D. B. and Johnson, T. E. (1988). A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75-86.
Garcia-Cao, I., Garcia-Cao, M., Martin-Caballero, J., Criado, L. M., Klatt, P., Flores, J. M., Weill, J. C., Blasco, M. A. and Serrano, M. (2002). "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO J.* **21**, 6225-6235.
Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V. and Antebi, A. (2001). A hormonal signaling pathway influencing C. elegans metabolism, reproductive development, and life span. *Dev. Cell* **1**, 841-851.
Gerisch, B., Rottiers, V., Li, D., Motola, D. L., Cummins, C. L., Lehrach, H., Mangelsdorf, D. J. and Antebi, A. (2007). A bile acid-like steroid modulates Caenorhabditis elegans lifespan through nuclear receptor signaling. *Proc. Natl. Acad. Sci. USA* **104**, 5014-5019.
Giannakou, M. E., Goss, M., Junger, M. A., Hafen, E., Leevers, S. J. and Partridge, L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. *Science* **305**, 361.
Giorgio, M., Migliaccio, E., Orsini, F., Paolucci, D., Moroni, M., Contursi, C., Pelliccia, G., Luzi, L., Minucci, S., Marcaccio, M. et al. (2005). Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **122**, 221-233.
Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., Gygi, S. P. and Brunet, A. (2007a). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. *Curr. Biol.* **17**, 1646-1656.
Greer, E. L., Oskoui, P. R., Banko, M. R., Maniar, J. M., Gygi, M. P., Gygi, S. P. and Brunet, A. (2007b). The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J. Biol. Chem.* **282**, 30107-30119.
Hamilton, B., Dong, Y., Shindo, M., Liu, W., Odell, I., Ruvkun, G. and Lee, S. S. (2005). A systematic RNAi screen for longevity genes in C. elegans. *Genes Dev.* **19**, 1544-1555.
Hansen, M., Hsu, A. L., Dillin, A. and Kenyon, C. (2005). New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a Caenorhabditis elegans genomic RNAi screen. *PLoS Genet.* **1**, 119-128.
Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S. J. and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell* **6**, 95-110.
Henderson, S. T., Bonafe, M. and Johnson, T. E. (2006). daf-16 protects the nematode Caenorhabditis elegans during food deprivation. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 444-460.
Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Even, P. C., Cervera, P. and Le Bouc, Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**, 182-187.
Hwangbo, D. S., Gersham, B., Tu, M. P., Palmer, M. and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**, 562-566.
Imura, A., Tsuji, Y., Murata, M., Maeda, R., Kubota, K., Iwano, A., Obuse, C., Togashi, K., Tominaga, M., Kita, N. et al. (2007). alpha-Klotho as a regulator of calcium homeostasis. *Science* **316**, 1615-1618.
Ingram, D. K., Anson, R. M., de Cabo, R., Mamczarz, J., Zhu, M., Mattison, J., Lane, M. A. and Roth, G. S. (2004). Development of calorie restriction mimetics as a prolongevity strategy. *Ann. N. Y. Acad. Sci.* **1019**, 412-423.
Inoki, K., Zhu, T. and Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577-590.
Jia, K., Albert, P. S. and Riddle, D. L. (2002). DAF-9, a cytochrome P450 regulating C. elegans larval development and adult longevity. *Development* **129**, 221-231.
Jia, K., Chen, D. and Riddle, D. L. (2004). The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. *Development* **131**, 3897-3906.
Kaeberlein, M., McVey, M. and 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.
Kaeberlein, M., Kirkland, K. T., Fields, S. and Kennedy, B. K. (2004). Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* **2**, E296.
Kapahi, P., Zid, B. M., Harper, T., Koslover, D., Sapin, V. and Benzer, S. (2004). Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. *Curr. Biol.* **14**, 885-890.
Kennedy, B. K., Austriaco, N. R., Jr, Zhang, J. and Guarente, L. (1995). Mutation in the silencing gene SIR4 can delay aging in S. cerevisiae. *Cell* **80**, 485-496.
Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. *Nature* **366**, 461-464.
Kimura, K. D., Tissenbaum, H. A., Liu, Y. and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* **277**, 942-946.
Kops, G. J., de Ruiter, N. D., De Vries-Smits, A. M., Powell, D. R., Bos, J. L. and Burgering, B. M. (1999). Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* **398**, 630-634.
Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E. et al. (1997). Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* **390**, 45-51.
Kurosu, H., Yamamoto, M., Clark, J. D., Pastor, J. V., Nandi, A., Gurnani, P., McGuinness, O. P., Chikuda, H., Yamaguchi, M., Kawaguchi, H. et al. (2005). Suppression of aging in mice by the hormone Klotho. *Science* **309**, 1829-1833.
Lakowski, B. and Hekimi, S. (1996). Determination of life-span in Caenorhabditis elegans by four clock genes. *Science* **272**, 1010-1013.
Lakowski, B. and Hekimi, S. (1998). The genetics of caloric restriction in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **95**, 13091-13096.
Lee, S. S., Lee, R. Y., Fraser, A. G., Kamath, R. S., Ahringer, J. and Ruvkun, G. (2003). A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. *Nat. Genet.* **33**, 40-48.
Lehtinen, M. K., Yuan, Z., Boag, P. R., Yang, Y., Villen, J., Becker, E. B., DiBacco, S., de la Iglesia, N., Gygi, S., Blackwell, T. K. et al. (2006). A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* **125**, 987-1001.
Lin, K., Dorman, J. B., Rodan, A. and Kenyon, C. (1997). daf-16: an HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. *Science* **278**, 1319-1322.
Lin, S. J., Defossez, P. A. and 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., Kaeberlein, M., Andalis, A. A., Sturtz, L. A., Defossez, P. A., Culotta, V. C., Fink, G. R. and Guarente, L. (2002). Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. *Nature* **418**, 344-348.
Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E. and Hekimi, S. (2005). Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. *Genes Dev.* **19**, 2424-2434.
Lombard, D. B., Chua, K. F., Mostoslavsky, R., Franco, S., Gostissa, M. and Alt, F. W. (2005). DNA repair, genome stability, and aging. *Cell* **120**, 497-512.
Maier, B., Gluba, W., Bernier, B., Turner, T., Mohammad, K., Guise, T., Sutherland, A., Thorner, M. and Scrable, H. (2004). Modulation of mammalian life span by the short isoform of p53. *Genes Dev.* **18**, 306-319.
Matheu, A., Pantoja, C., Efeyan, A., Criado, L. M., Martin-Caballero, J., Flores, J. M., Klatt, P. and Serrano, M. (2004). Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging. *Genes Dev.* **18**, 2736-2746.
Matheu, A., Maraver, A., Klatt, P., Flores, I., Garcia-Cao, I., Borras, C., Flores, J. M., Vina, J., Blasco, M. A. and Serrano, M. (2007). Delayed ageing through damage protection by the Arf/p53 pathway. *Nature* **448**, 375-379.
Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P. P., Lanfrancone, L. and Pelicci, P. G. (1999). The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* **402**, 309-313.
Morris, J. Z., Tissenbaum, H. A. and Ruvkun, G. (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. *Nature* **382**, 536-539.
Mostoslavsky, R., Chua, K. F., Lombard, D. B., Pang, W. W., Fischer, M. R., Gellon, L., Liu, P., Mostoslavsky, G., Franco, S., Murphy, M. M. et al. (2006). Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124**, 315-329.
Motta, M. C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M. and Guarente, L. (2004). Mammalian SIRT1 represses forkhead transcription factors. *Cell* **116**, 551-563.
Navarro, C. L., Cau, P. and Levy, N. (2006). Molecular bases of progeroid syndromes. *Hum. Mol. Genet.* **15** Spec. No 2, R151-R161.
Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A. and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature* **389**, 994-999.
Oh, S. W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R. J. and Tissenbaum, H. A. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc. Natl. Acad. Sci. USA* **102**, 4494-4499.
Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J. and Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. *Aging Cell* **6**, 111-119.
Panowski, S. H., Wolff, S., Aguilaniu, H., Durieux, J. and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. *Nature* **447**, 550-555.
Pinton, P., Rimessi, A., Marchi, S., Orsini, F., Migliaccio, E., Giorgio, M., Contursi, C., Minucci, S., Mantovani, F., Wieckowski, M. R. et al. (2007). Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* **315**, 659-663.
Rogina, B. and Helfand, S. L. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc. Natl. Acad. Sci. USA* **101**, 15998-16003.
Rose, G., Dato, S., Altomare, K., Bellizzi, D., Garasto, S., Greco, V., Passarino, G., Feraco, E., Mari, V., Barbi, C. et al. (2003). Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. *Exp. Gerontol.* **38**, 1065-1070.
Roth, G. S., Lane, M. A., Ingram, D. K., Mattison, J. A., Elahi, D., Tobin, J. D., Muller, D. and Metter, E. J. (2002). Biomarkers of caloric restriction may predict longevity in humans. *Science* **297**, 811.
Rudolph, K. L., Chang, S., Lee, H. W., Blasco, M., Gottlieb, G. J., Greider, C. and DePinho, R. A. (1999). Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* **96**, 701-712.
Shaw, W. M., Luo, S., Landis, J., Ashraf, J. and Murphy, C. T. (2007). The C. elegans TGF-beta dauer pathway regulates longevity via insulin signaling. *Curr. Biol.* **17**, 1635-1645.
Shiraki-Iida, T., Aizawa, H., Matsumura, Y., Sekine, S., Iida, A., Anazawa, H., Nagai, R., Kuro-o, M. and Nabeshima, Y. (1998). Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein. *FEBS Lett.* **424**, 6-10.
Syntichaki, P., Troulinaki, K. and Tavernarakis, N. (2007). eIF4E function in somatic cells modulates ageing in Caenorhabditis elegans. *Nature* **445**, 922-926.
Taguchi, A., Wartschow, L. M. and White, M. F. (2007). Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **317**, 369-372.
Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, C. M. and Garofalo, R. S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107-110.
Tissenbaum, H. A. and Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* **410**, 227-230.
Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G.,

Cooper, B., Brayton, C. et al. (2002). p53 mutant mice that display early ageing-associated phenotypes. *Nature* **415**, 45-53.
Urakawa, I., Yamazaki, Y., Shimada, T., Iijima, K., Hasegawa, H., Okawa, K., Fujita, T., Fukumoto, S. and Yamashita, T. (2006). Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* **444**, 770-774.
Van Der Horst, A., Tertoolen, L. G., De Vries-Smits, L. M., Frye, R. A., Medema, R. H. and Burgering, B. M. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2SIRT1. *J. Biol. Chem.* **279**, 28873-28879.
Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A. L., Orosz, L. and Muller, F. (2003). Genetics: influence of TOR kinase on lifespan in C. elegans. *Nature* **426**, 620.
Viswanathan, M., Kim, S. K., Berdichevsky, A. and Guarente, L. (2005). A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span. *Dev. Cell* **9**, 605-615.
Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* **39**, 359-407.
Wang, M. C., Bohmann, D. and Jasper, H. (2003). JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. *Dev. Cell* **5**, 811-816.
Wang, M. C., Bohmann, D. and Jasper, H. (2005). JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* **121**, 115-125.
Wang, Y. and Tissenbaum, H. A. (2006). Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. *Mech. Ageing Dev.* **127**, 48-56.
Wolf, E., Kahnt, E., Ehrlein, J., Hermanns, W., Brem, G. and Wanke, R. (1993). Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: lessons from transgenic animal models. *Mech. Ageing Dev.* **68**, 71-87.
Wood, J. G., Rogina, B., Lavu, S., Howitz, K., Helfand, S. L., Tatar, M. and Sinclair, D. (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430**, 686-689.
Yan, L., Vatner, D. E., O'Connor, J, P., Ivessa, A., Ge, H., Chen, W., Hirotani, S., Ishikawa, Y., Sadoshima, J. and Vatner, S. F. (2007). Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* **130**, 247-258.
Yang, Y., Hou, H., Haller, E. M., Nicosia, S. V. and Bai, W. (2005). Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation. *EMBO J.* **24**, 1021-1032.
Zang, M., Xu, S., Maitland-Toolan, K. A., Zuccollo, A., Hou, X., Jiang, B., Wierzbicki, M., Verbeuren, T. J. and Cohen, R. A. (2006). Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes* **55**, 2180-2191.

Cell Science at a Glance on the Web
Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.

Commentaries

JCS Commentaries highlight and critically discuss recent exciting work that will interest those working in cell biology, molecular biology, genetics and related disciplines. These short reviews are commissioned from leading figures in the field and are subject to rigorous peer-review and in-house editorial appraisal. Each issue of the journal usually contains at least two Commentaries. JCS thus provides readers with more than 50 Commentaries over the year, which cover the complete spectrum of cell science. The following are just some of the Commentaries that will appear in JCS over the coming months:

Integrin-dependent phagocytosis *Emmanuelle Caron*

Cell polarity and cancer *Valeri Vasioukhin*

Cell-surface and mitotic-spindle RHAMM/HMMR *Eva A. Turley*

Adherens junctions and VE-cadherin in vascular permeability *Elisabetta Dejana*

Filopodia *Richard Cheney*

FTSZ and chloroplast division *Peter Beech*

Electric fields in wound healing and cell migration *Colin D. McCaig*

TCF-directed transcriptional complexes *Katherine Jones*

PI4P and PI4P-binding proteins in the Golgi *Antonella De Matteis*

Golgi fragmentation *Jennifer Lippincott-Schwartz*

Intra-Golgi transport *Catherine Jackson*

Although we discourage submission of unsolicited Commentaries to the journal, ideas for future articles – in the form of a short proposal and some key references – are welcome and should be sent to the Executive Editor at the address below.

Journal of Cell Science, Bidder Building, 140 Cowley Rd, Cambridge, CB4 0DL, UK
E-mail: jcs@biologists.com
Website: http://jcs.biologists.org