Sargasso Sea for hatchling turtles, so the adaptive responses of hatchlings do not necessarily require a global map; a list of intensity–inclination pairs and the appropriate heading for each would be sufficient. For older turtles with their ability to home accurately, however, this would seem to have matured into a conventional map based on magnetic cues. It would be wonderful to know for sure that newts, lobsters, and older turtles can home accurately after virtual displacements of longitude only. The other challenge for the future is to learn whether homers and migrators in fact use gradients, and to discover how the magnetic cues are processed to create this illusion of magic.

References

1. Schmidt-Koenig, K. (1979). Avian Orientation and Navigation (New York: Academic Press).
2. Gould, J.L. (2008). Animal navigation: the longitude problem. Curr. Biol. *18*, R214–R216.
3. Putnam, N.F., Endres, C.S., Lohmann, C.M.F., and Lohmann, K.J. (2011). Longitude perception and bicoordinate magnetic maps in sea turtles. Curr. Biol. *21*, 463–466.
4. Wiltschko, R., and Wiltschko, W. (1985). Pigeon homing: change in navigational strategy during ontogeny. Anim. Behav. *33*, 583–590.
5. Chernetsov, N., Kishkinev, D., and Mouritsen, H. (2008). A long-distance avian migrant compensates for longitudinal displacement during spring migration. Curr. Biol. *18*, 188–190.
6. Thorup, K., Bisson, I.A., Bowlin, M.S., Holland, R.A., Wingfield, J.C., Ramenofsky, M., and Wikelski, M. (2007). Evidence for a navigational map stretching across the continental U.S. in a migratory songbird. Proc. Natl. Acad. Sci. USA *104*, 18115–181159.
7. Wallraff, H. (2005). Animal Navigation: Pigeon Homing as a Paradigm (Berlin: Springer Verlag).
8. Jorge, P.E., Marques, A.E., and Philips, J.B. (2009). Activational rather than navigational effects of odors on pigeon homing. Curr. Biol. *19*, 650–654.
9. Gould, J.L., and Gould, C.G. (2011, in press). Animal Navigation. (Princeton: Princeton University Press).
10. Gould, J.L. (1980). The case for magnetic sensitivity in birds and bees (such as it is). Am. Sci. *68*, 256–267.
11. Gould, J.L. (1980). The map sense of pigeons. Nature *296*, 205–211.
12. Freake, M.J., Muheim, R., and Phillips, J.B. (2006). Magnetic maps in animals: a theory comes of age? Q. Rev. Biol. *81*, 327–347.
13. Lohmann, K.J., Lohmann, C.M.F., and Putman, N.F. (2007). Magnetic maps in animals: nature's GPS. J. Exp. Biol. *210*, 3697–3705.
14. Phillips, J.B., Freake, M.J., Fischer, J.H., and Borland, S.C. (2002). Behavioral titration of a magnetic map coordinate. J. Comp. Physiol. A *188*, 157–160.
15. Schmidt-Koenig, K., and Walcott, C. (1978). Tracks of pigeons homing with frosted lenses. Anim. Behav. *26*, 480–486.
16. Boles, L.C., and Lohmann, K.J. (2003). True navigation and magnetic maps in spiny lobsters. Nature *421*, 60–63.
17. Lohmann, K.J., and Lohmann, C.M.F. (1996). Detection of magnetic field intensity by sea turtles. Nature *380*, 59–61.
18. Lohmann, K.J., and Lohmann, C.M.F. (1994). Detection of magnetic inclination angle by sea turtles: A possible mechanism for determining latitude. J. Exp. Biol. *194*, 23–32.
19. Lohmann, K.J., Cain, S.D., Dodge, S.A., and Lohmann, C.M.F. (2001). Regional magnetic fields as navigational markers for sea turtles. Science *294*, 364–366.
20. Lohmann, K.J., Lohmann, C.M.F., Ehrhart, L.M., Bagley, D.A., and Swing, K. (2004). Geomagnetic map used in sea turtle navigation. Nature *428*, 909.

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003, USA.
E-mail: gould@princeton.edu

DOI: 10.1016/j.cub.2011.01.063

Autophagy: Regulation by Energy Sensing

Autophagy is inhibited by the mTOR signaling pathway, which is stimulated by increased amino acid levels. When cellular energy production is compromised, AMP-activated protein kinase is activated, mTOR is inhibited and autophagy is stimulated. Two recent studies have shed light on the molecular mechanism by which AMPK controls autophagic flux.

Alfred J. Meijer[1,*] and Patrice Codogno[2]

Autophagy is responsible for the turnover of long-lived proteins and organelles that are either damaged (e.g. mitochondria) or functionally redundant and is crucial for the maintenance of cellular homeostasis in all eukaryotic cells. The primary role of autophagy is to protect cells against stress. For example, during starvation, when nutrients fall short, autophagy is activated in order to produce oxidizable substrates and other compounds (e.g. amino acids) that are essential for cell survival. Deregulation of autophagy is involved in aging and in many pathologies such as neurodegeneration, cancer, cardiomyopathy, liver disease, gastrointestinal disorders, and diabetes [1,2]. Two recent studies, published in *Science* [3] and in *Nature Cell Biology* [4], have provided insight into the mechanism by which autophagy is stimulated when cellular energy production declines.

During autophagy, part of the cytoplasm containing the material to be degraded becomes surrounded by a double membrane, resulting in the formation of an autophagosome. The outer autophagosomal membrane then fuses with a lysosome, the inner autophagosomal membrane vesicle is released into the lysosomal interior and this vesicle with its sequestered macromolecular material becomes degraded. The degradation products are returned to the cytosol for reutilization in metabolism, completing the autophagic process. The formation of the autophagosome, a process in which at least 18 different Atg (autophagy-related) proteins participate, is the rate-controlling step in autophagy. Among these proteins are the protein kinases Unc-51-like kinases 1 or 2 (Ulk1 or Ulk2, respectively — the mammalian homologs of the yeast Atg1), components of a multi-protein complex that also contains Atg13, Atg101 and FIP200 and which is involved in the initiation of autophagosome formation [2].

Autophagy is tightly controlled by the mammalian target of rapamycin (mTOR)-dependent signal transduction pathway, which responds to growth factors and changes in amino acid levels [1] (Figure 1). Amino acids inhibit autophagy by activating the protein kinase mTOR within the mTORC1 complex. The molecular target of mTOR in the autophagic machinery is Ulk1, which becomes inhibited by mTOR-mediated phosphorylation, although the exact phosphorylation sites had not been described [2,5–7]. Amino acids signal to mTOR through the Rag proteins in the mTORC1 complex [8,9], while insulin activates mTOR through the G protein Rheb, which is also part of this complex [10] (Figure 1). Inhibition of mTOR by amino-acid depletion or by treatment with the mTOR inhibitor rapamycin stimulates autophagy [1].

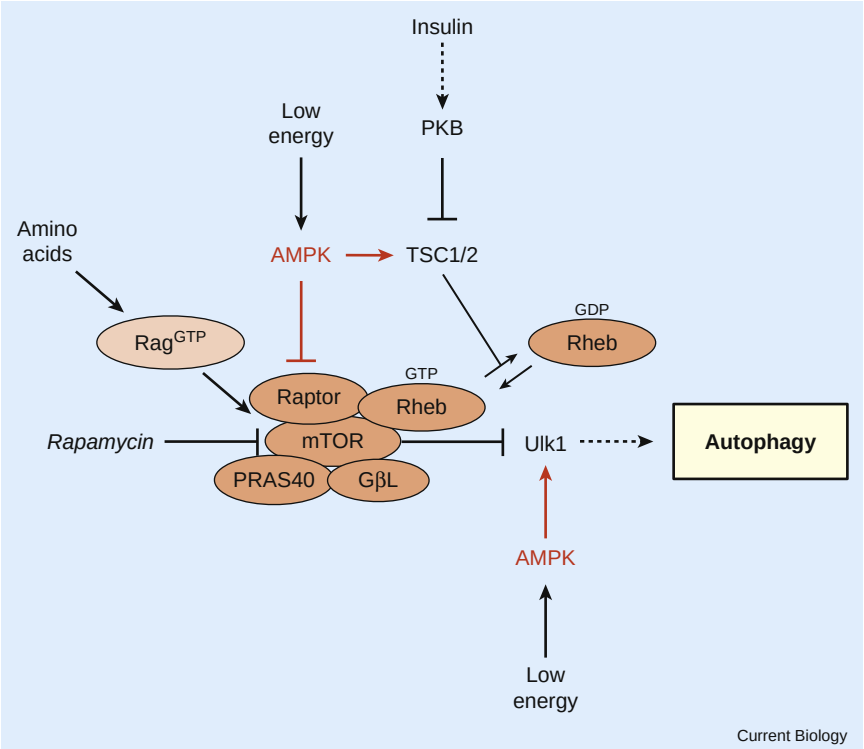

Figure 1. Stimulation of autophagy by AMPK.

mTOR is part of the mTORC1 complex, which also contains Raptor, Rheb, PRAS40 and GβL. AMPK stimulates Ulk1 and autophagy by coordinated inhibition of mTOR through phosphorylation of TSC2 and Raptor, and phosphorylation of Ulk1. mTOR, activated by amino acids and insulin, phosphorylates Ulk1 at a different site from the site phosphorylated by AMPK, prevents association of Ulk1 and AMPK, and inhibits Ulk1 activity and autophagy. Abbreviations: PKB, protein kinase B; Rag, Ras-related small GTP-binding protein; Raptor, regulatory associated protein of mTOR; Rheb, Ras homolog enriched in brain; mTOR, mammalian target of rapamycin; TSC, tuberous sclerosis complex; Ulk1, Unc-51-like kinase 1.

Autophagy can also be activated by impairment of cellular ATP production. Decreased ATP production stimulates AMP-activated protein kinase (AMPK), which, in line with its role in switching on catabolic pathways [11], not only stimulates but is also essential for autophagy under energy-deprived conditions [1,12,13]. Stimulation of AMPK inactivates mTOR [14] through AMPK-mediated phosphorylation of both TSC2 [15] and Raptor [16] and results in association of AMPK with Ulk1 [17] (Figure 1). Now, following up on these data, the recent studies by Egan *et al.* [3] and Kim *et al.* [4] have shown that AMPK increases autophagy not only indirectly through inactivation of mTOR but also directly through phosphorylation of Ulk1.

Egan *et al.* [3] used a two-part screen to identify conserved substrates of AMPK. First, they searched eukaryotic databases for proteins containing a conserved AMPK substrate motif. Second, because *in vivo* substrates of AMPK not only conform to this motif but also bind to the phospho-binding protein 14-3-3 [16,17], the authors screened for proteins that bound to recombinant 14-3-3 in wild-type but not AMPK-deficient cells under conditions of energy stress in which AMPK is activated. Using this approach, Egan *et al.* [3] identified Ulk1 and Ulk2 as potential substrates of AMPK. Genetic analysis in various mammalian cells, including liver cells, and also in *Caenorhabditis elegans*, confirmed the requirement of AMPK and Ulk1 (which is more important than Ulk2 in the control of autophagy) for autophagy. Using tandem mass spectrometry on epitope-tagged Ulk1 isolated from control cells and from cells in which AMPK activity was increased either by pharmacological inhibition of the mitochondrial respiratory chain, by specific pharmacological activation of AMPK or by expression of a constitutively active form of the enzyme, combined with *in vitro* kinase experiments, the authors were able to identify four potential AMPK phosphorylation sites in Ulk1 — Ser^{467}, Ser^{555},Thr^{574} and Ser^{637}. Of these, phosphorylation of Ser^{467} and Ser^{555} by AMPK appeared to be required for autophagy induction. Cells expressing a non-phosphorylatable Ulk1 mutant (in which all four phosphorylation sites were replaced by alanine) were defective in induction of autophagy in response to starvation, as indicated by the aberrant accumulation of the protein adaptor p62, which binds ubiquitinated cargo destined for autophagic breakdown, and of abnormal mitochondria. Autophagy in Ulk1-deficient cells could only be restored by wild-type Ulk1, but not by the non-phosphorylatable mutant. Likewise, Ulk1-deficient cells died upon starvation, and this phenotype could be rescued by expression of wild-type Ulk1, but not the mutant.

In the study by Kim *et al.* [4], also carried out with mammalian cells, systematic Ulk1 deletion and mutation experiments were performed and the accumulation of LC3-II, the lipidated form of the protein LC3 (the homolog of yeast Atg8), following lysosomal inhibition, an established measure of autophagic flux, was examined. The authors used glucose depletion to stimulate AMPK and autophagy and, in combination with *in vitro* phosphorylation experiments, identified Ser^{317} and Ser^{777} as the major AMPK phosphorylation sites of Ulk1, which they found to be essential for its activity. Interestingly, in the presence of nutrient excess, activation of mTOR was found to prevent Ulk1 activation through phosphorylation of Ulk1 at Ser^{757}, which disrupted the interaction of Ulk1 with AMPK. In other words, phosphorylation of Ulk1 by AMPK and mTOR has opposing effects on its activity and thus on autophagy. This coordinated phosphorylation of Ulk1 by mTORC1 and AMPK provides an exquisite mechanism for nutrient signal integration to ensure proper adjustment of autophagic flux in response to metabolic requirements [4] (Figure 1).

It is important to stress that, in contrast to glucose depletion, amino-acid starvation (or rapamycin administration) stimulated autophagy by an AMPK-independent mechanism, did not increase Ser^{317} and Ser^{377} phosphorylation of Ulk1, did not activate AMPK, and was also observed

in AMPK-deficient cells. Clearly, activation of Ulk1 by amino-acid starvation proceeds by a different mechanism than activation of Ulkl by energy depletion [4].

The studies by Egan *et al.* [3] and by Kim *et al.* [4] clearly indicate that AMPK can directly phosphorylate Ulk1 and in this way provides a mechanism for the activation of autophagy when cellular energy production becomes compromised. Surprisingly, the two studies [3,4] are not in agreement with regard to the position of the AMPK phosphorylation sites in Ulk1. One can only guess for the reasons underlying these differences. A possible explanation is that accumulation of mitochondria [3] reflects a specific form of autophagy, mitophagy, rather than non-specific bulk autophagy, as measured by LC3-II accumulation [4], and that regulation of these two modes of autophagy requires different phosphorylation sites on Ulk1 [18].

Phosphorylation of Ulk1 by AMPK occurs in combination with the activation of autophagy via inhibition of mTOR activity through phosphorylation of TSC2 and Raptor (Figure 1). The fact that AMPK acts at multiple levels to stimulate flux through the autophagic pathway resembles the mechanism by which another protein kinase, cAMP-dependent protein kinase (PKA), affects the flux through metabolic pathways, e.g. its coordinated inhibition of hepatic glycolysis through simultaneous phosphorylation of phosphofructokinase-2 and of pyruvate kinase [19]. This inhibition of glycolysis, combined with the stimulatory effect of PKA on hepatic glycogen breakdown and on autophagy to provide amino acids as gluconeogenic substrates, ensures the maximal output of glucose in response to a rise in glucagon levels during starvation in mammals. mTOR-mediated signaling is inhibited by glucagon [1] and this may be sufficient for the induction of autophagy. However, it is likely that, in analogy with AMPK, Ulk1 is also a substrate for PKA in mammalian cells. In yeast cells, Atg1 is, indeed, a PKA substrate, although in this case this leads to inhibition rather than stimulation of autophagy [20]. In conclusion, the data of Egan *et al.* [3] and of Kim *et al.* [4] on AMPK-mediated phosphorylation of Ulk1 provide a fascinating mechanism responsible for the initiation of autophagy when cellular ATP production falls. This work also nicely extends early studies on the involvement of AMPK in the control of autophagy [12,13].

References

1. Meijer, A.J., and Codogno, P. (2009). Autophagy: regulation and role in disease. Crit. Rev. Clin. Lab Sci. *46*, 210–240.
2. Chen, Y., and Klionsky, D.J. (2011). The regulation of autophagy - unanswered questions. J. Cell Sci. *124*, 161–170.
3. Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., *et al.* (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science *331*, 456–461.
4. Kim, J., Kundu, M., Viollet, B., and Guan, K.L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat. Cell Biol. *13*, 132–141.
5. Ganley, I.G., Lam, D.H., Wang, J., Ding, X., Chen, S., and Jiang, X. (2009). ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J. Biol. Chem. *284*, 12297–12305.
6. Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., *et al.* (2009). Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol. Biol. Cell *20*, 1981–1991.
7. Jung, C.H., Jun, C.B., Ro, S.H., Kim, Y.M., Otto, N.M., Cao, J., Kundu, M., and Kim, D.H. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol. Biol. Cell *20*, 1992–2003.
8. Sancak, Y., Peterson, T.R., Shaul, Y.D., Lindquist, R.A., Thoreen, C.C., Bar-Peled, L., and Sabatini, D.M. (2008). The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science *320*, 1496–1501.
9. Kim, E., Goraksha-Hicks, P., Li, L., Neufeld, T.P., and Guan, K.L. (2008). Regulation of TORC1 by Rag GTPases in nutrient response. Nat. Cell Biol. *10*, 935–945.
10. Dennis, M.D., Baum, J.I., Kimball, S.R., and Jefferson, L.S. (2011). Mechanisms involved in the coordinate regulation of the mammalian target of rapamycin complex 1 (mTORC1) by insulin and amino acids. J. Biol. Chem. doi: 10.1074/jbc.M110.209171, epub ahead of print.
11. Hardie, D.G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat. Rev. Mol. Cell Biol. *8*, 774–785.
12. Meley, D., Bauvy, C., Houben-Weerts, J.H., Dubbelhuis, P.F., Helmond, M.T., Codogno, P., and Meijer, A.J. (2006). AMP-activated protein kinase and the regulation of autophagic proteolysis. J. Biol. Chem. *281*, 34870–34879.
13. Matsui, Y., Takagi, H., Qu, X., Abdellatif, M., Sakoda, H., Asano, T., Levine, B., and Sadoshima, J. (2007). Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ. Res. *100*, 914–922.
14. Meijer, A.J., and Dubbelhuis, P.F. (2004). Amino acid signalling and the integration of metabolism. Biochem. Biophys. Res. Commun. *313*, 397–403.
15. Corradetti, M.N., Inoki, K., Bardeesy, N., DePinho, R.A., and Guan, K.L. (2004). Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes Dev. *18*, 1533–1538.
16. Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., and Shaw, R.J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell *30*, 214–226.
17. Lee, J.W., Park, S., Takahashi, Y., and Wang, H.G. (2010). The association of AMPK with ULK1 regulates autophagy. PLoS One *5*, e15394.
18. Zhao, M., and Klionsky, D.J. (2011). AMPK-dependent phosphorylation of ULK1 induces autophagy. Cell Metab. *13*, 119–120.
19. Rider, M.H., Bertrand, L., Vertommen, D., Michels, P.A., Rousseau, G.G., and Hue, L. (2004). 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: head-to-head with a bifunctional enzyme that controls glycolysis. Biochem. J. *381*, 561–579.
20. Stephan, J.S., Yeh, Y.Y., Ramachandran, V., Deminoff, S.J., and Herman, P.K. (2009). The Tor and PKA signaling pathways independently target the Atg1/Atg13 protein kinase complex to control autophagy. Proc. Natl. Acad. Sci. USA *106*, 17049–17054.

[1]Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands. [2]INSERM U756, Université Paris-Sud 11, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France.
*E-mail: a.j.meijer@amc.uva.nl

DOI: 10.1016/j.cub.2011.02.007

Multisensory Integration: What You See Is Where You Hear

Recent studies of multisensory integration compel a redefinition of fundamental sensory processes, including, but not limited to, how visual inputs influence the localization of sounds and suppression of their echoes.

Micah M. Murray[1,2,3,4] and Lucas Spierer[2]

Imagine yourself sleeping in your dark bedroom with its lofty vaulted ceilings and spartan decor. This bedroom is clean and serene; at least, until the alarm clock jolts you awake. Keeping your eyes firmly closed, you frantically reach out to find the 'snooze' button with the hope of a few more minutes of torpor. A deconstruction of this vignette into its component neurobiological processes would