CELL BIOLOGY

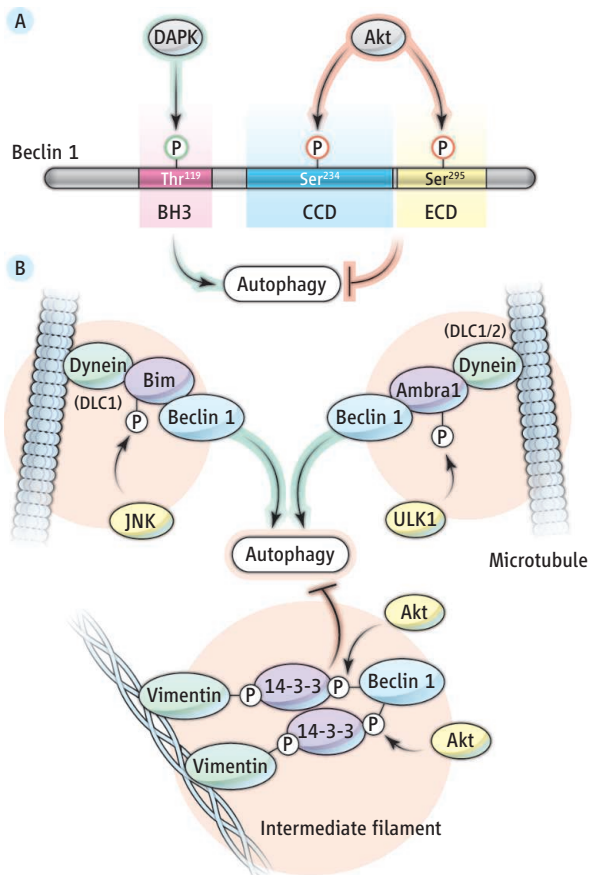
Promoting Tumorigenesis by Suppressing Autophagy

Tumorigenesis is controlled by the sequestration of an autophagy machinery component to the cytoskeleton.

Itay Koren and Adi Kimchi

Autophagy controls cellular homeostasis by degrading long-lived proteins, protein aggregates, and defective organelles. It also suppresses tumorigenesis by limiting inflammation, eliminating toxic unfolded proteins, and removing damaged mitochondria that produce reactive oxygen species (which damage DNA). Loss of these protective events could promote cancer initiation (*1*, *2*). Support for the tumor suppressive function of autophagy emerged from the findings that the gene encoding the essential autophagic protein Beclin 1 functions as a haplo-insufficient tumor suppressor in mice and humans (*3*–*6*). However, a comprehensive mechanistic view of how autophagy is turned off during tumor development and, more specifically, whether the tumor-suppressive activity of Beclin 1 results from its canonical autophagic function, was still missing. On page 956 in this issue, Wang *et al*. (*7*) establish a connection between Beclin 1 and the Akt signaling pathway, which controls a large spectrum of cellular functions associated with cancer ranging from cell proliferation and survival to angiogenesis and metabolism. The finding underscores the importance of autophagy in tumor suppression (*8*).

Akt can block autophagy by phosphorylating, and thereby inhibiting, two targets: the tuberous sclerosis complex 2 (TSC2) and the proline-rich Akt substrate of 40 kD (PRAS40) (*7*). Both targets block the protein mTOR; when active, mTOR inhibits autophagy. In a search for additional direct substrates of Akt that are part of the core autophagic machinery, Wang *et al*. identified Beclin 1 as a target of Akt. The authors show that endogenous Akt associates with Beclin 1 in cultured human epithelial cells and phosphorylates it on specific sites (Ser^{295} and Ser^{234}). Phosphorylation by Akt generated binding sites for the protein 14-3-3, which enhanced the binding of Beclin 1 to 14-3-3 and to the cytoskeletal protein vimentin. Sequestration of Beclin 1 to the cytoskeleton reduced its interaction with Vps34, a lipid kinase involved in autophagy nucleation. Consequently, Vps34 activity decreased, resulting in reduced autophagy. Notably, mutation of the Akt-specific phosphorylation sites in Beclin 1 that increased autophagy also suppressed different features of Akt-induced tumorigenesis such as anchorage-independent cell growth in culture and tumor growth in immunodeficient mice. These observations establish a link between the autophagic and the tumor-suppressive functions of Beclin 1, both of which are regulated through specific phosphorylation by Akt. To further strengthen these causative relationships, it will be necessary to examine whether the tumor-suppressive activity of the nonphosphorylated Beclin 1 mutant can be reversed by disrupting the autophagy pathway, for example, by simultaneously reducing the expression of downstream autophagic genes in this Akt-driven tumorigenesis system.



Phosphorylation and sequestration. (**A**) Phosphorylation of Beclin 1 with the indicated kinases either promotes or inhibits autophagy. (**B**) Beclin 1 is inactive when it associates with the dynein motor [dynein light chain 1/2 (DLC1/2)] and the microtubule cytoskeleton. Phosphorylation of Bim by JNK or of Ambra1 by ULK1, releases Beclin 1 and activates autophagy. Akt phosphorylates Beclin 1, allowing the adaptor protein 14-3-3 to link it to the cytoskeleton (vimentin and intermediate filaments). This blocks autophagy. P, phosphorylation; BH3, Bcl-2 homology 3 domain; CCD, coiled coil domain; ECD, evolutionarily conserved domain; JNK, c-Jun N-terminal kinase.

Two interesting paradigms on the function and mode of regulation of Beclin 1 are currently emerging. Beclin 1 activity appears to be tightly controlled, either negatively or positively, by signaling kinases with opposing functions in cells. It has been previously reported that DAP kinase (DAPK), a stress-induced kinase, phophorylates Beclin 1 within its BH3 domain (position Thr^{119}) and that this modification prevents binding of Beclin 1 to its inhibitor, Bcl-2/Bcl-x_L, thus promoting its autophagic activity (*9*). By contrast, the phosphorylation of Beclin 1 at distal sites by the cell growth–promoting kinase Akt suppresses the autophagic activity of Beclin 1 by controlling its intracellular localization (see the figure). Whereas Akt is a potent oncogene that is activated in tumors, the opposing kinase, DAPK, is a tumor suppressor subjected to loss or inactivation in many tumors (*10*), thus providing an additional correlative link between Beclin 1 activity and tumorigenesis. It would be interesting to screen human tumors that did not lose Beclin 1 or DAPK expression for mutations in these phosphorylation sites—either those that mimic Beclin 1 phosphorylation by Akt, or those that eliminate phosphorylation by DAPK.

Another emerging paradigm relates to sequestration of Beclin 1 to different cytoskeletal elements as a mechanism to control its func-

Department of Molecular Genetics. Weizmann Institute of Science, Rehovot 76100, Israel. E-mail: itay.koren@weizmann.ac.il; adi.kimchi@weizmann.ac.il

Published by AAAS

tion. Sequestration of Beclin 1 to microtubule elements was known to block autophagy (*11*, *12*). In one case, Bim, a BH3-only member of the Bcl-2 family, interacts with Beclin 1 and links Beclin 1 to the microtubule element, dynein light chain 1 (DLC1, also called LC8). The mislocalization of Beclin 1 to the dynein motor complex inhibits autophagy. A stimulus for autophagy was suggested to induce Bim phosphorylation, leading to the dissociation of Bim and Beclin 1 from DLC1 and autophagy induction (*11*). In another scenario, Beclin 1 is tethered to the microtubule cytoskeleton through an interaction with Ambra1, a protein that associates with DLC1/2. When the protein kinase ULK1 is activated at the initiation stage of autophagy, it phosphorylates Ambra1; Beclin 1 is subsequently released from the dynein motor and becomes available to promote autophagy (*12*). In these two examples, regulation takes place through phosphorylation of Beclin 1–interacting proteins, which leads to activation of Beclin 1 and autophagy. Wang *et al*. document a quite different mechanism in which Akt phosphorylates Beclin 1 itself, promoting Beclin 1 sequestration to the cytoskeleton and suppressing its function in autophagy.

Several modes of Beclin 1 regulation appear to operate through its binding to different cytoskeletal elements. The mechanism elucidated by Wang *et al*. may have important implications in tumor development.

References

1. D. Gozuacik, A. Kimchi, *Oncogene* **23**, 2891 (2004).
2. E. White, *Nat. Rev. Cancer* **12**, 401 (2012).
3. V. M. Aita *et al*., *Genomics* **59**, 59 (1999).
4. X. H. Liang *et al*., *Nature* **402**, 672 (1999).
5. X. Qu *et al*., *J. Clin. Invest.* **112**, 1809 (2003).
6. Z. Yue, S. Jin, C. Yang, A. J. Levine, N. Heintz, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15077 (2003).
7. B. D. Manning, L. C. Cantley, *Cell* **129**, 1261 (2007).
8. R. C. Wang *et al*., *Science* **338**, 956 (2012).
9. E. Zalckvar *et al*., *EMBO Rep.* **10**, 285 (2009).
10. D. Gozuacik, A. Kimchi, *Autophagy* **2**, 74 (2006).
11. S. Luo *et al*., *Mol. Cell* **47**, 359 (2012).
12. S. Di Bartolomeo *et al*., *J. Cell Biol.* **191**, 155 (2010).

10.1126/science.1230577

CHEMISTRY

Functional DNA Origami Devices

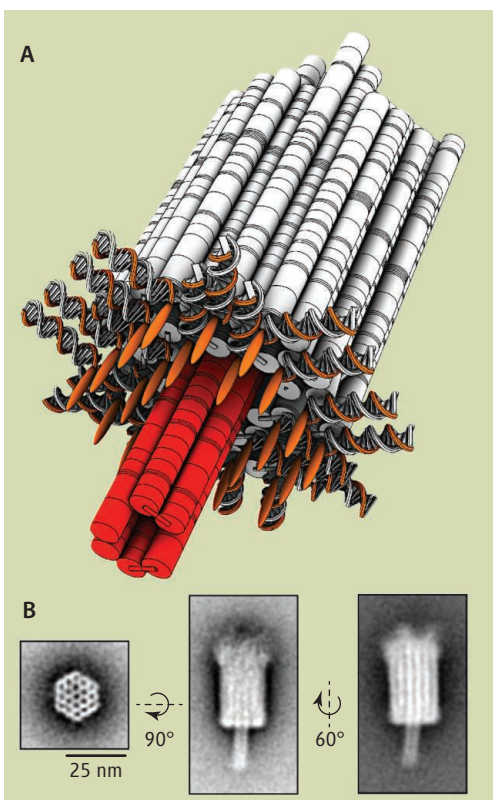
Advances in programmed DNA assembly are beginning to yield highly precise functional devices.

Michael S. Strano

DNA is central to the existence of all life on Earth and uniquely encodes the instructions for producing such life. But it has another set of remarkable properties shared by a much larger class of molecules, most of which are yet to be invented. These "chemically sequenced polymers" (CSPs) are built by adding distinct monomers one at a time and can be programmed in such a way as to assemble into arbitrarily complex and three-dimensional shapes. Nature is far ahead of us in the creation and utility of such molecules, but on page 932 of this issue, Langecker *et al*. (*1*) provide a glimpse of the structural precision and functionality they offer for programmed assembly.

When the monomer units of a CSP are nucleic acids, the engineering of such structures is called DNA origami (*2*, *3*) after the Japanese art of paper folding. Here, different parts of a long single-stranded DNA segment are brought and "stapled" together using smaller oligonucleotides that are programmed to hybridize at key locations in the structure. In this way, one can staple and fold the system into any three-dimensional shape.

CSPs are also used in protein folding and engineering, where amino acids are the sequencing elements; however, creation of programmable, functional devices with amino acids would require the ability to predict protein folding under different condition. The original attempts to create synthetic CSPs are simplistic by comparison, but the field of multiblock polymers (*4*) highlights how elegant order can be generated from just a handful of sequenced "bases" represented by the distinct polymer blocks. Scientists are only just beginning to understand self-assembly and the connections between these various materials (*5*).



Membrane docking channel from DNA origami. (**A**) Cartoon of the DNA origami channel reported by Langecker *et al*. The channel has two parts, an extramembrane mouth (gray/orange) and a hydrophobic stem (red) extending into the membrane and can control the transport of a range of molecules. (**B**) Channel structure in averaged negative stain TEM images (*1*).

To underscore the point, Langecker *et al*. report a stunning advance toward programmable structure and function. Using DNA origami, they have designed and synthesized a working ion channel that can spontaneously assemble into a lipid bilayer. The design is derived from α-hemolysin (a protein that is excreted by *Streptococcus* bacteria to perforate a target cell, causing it to leach iron through the resulting channel for the bacterium to consume) (*6*). In the resulting device (see the figure), one part of the DNA is folded into a hydrophobic barrel that inserts itself into a nearby lipid bilayer membrane. An extramembrane portion on top of the barrel forms the mouth of the channel.

The level of complexity and function of this device is remarkable, as the TEM images and electrochemical measurements confirm. Once immobilized in a target membrane, the ion channel demonstrates gating behavior when an electrical potential is placed across it. The observed stochastic fluctuations in ion current resemble those seen in many biological (*6*) and synthetic (*7*) ion channels. The channel can also transport and recognize

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. E-mail: strano@mit.edu

Promoting Tumorigenesis by Suppressing Autophagy

Itay Koren and Adi Kimchi

Science **338** (6109), 889-890.
DOI: 10.1126/science.1230577