Cluedo in the cell: Enzyme location controls enzyme activity

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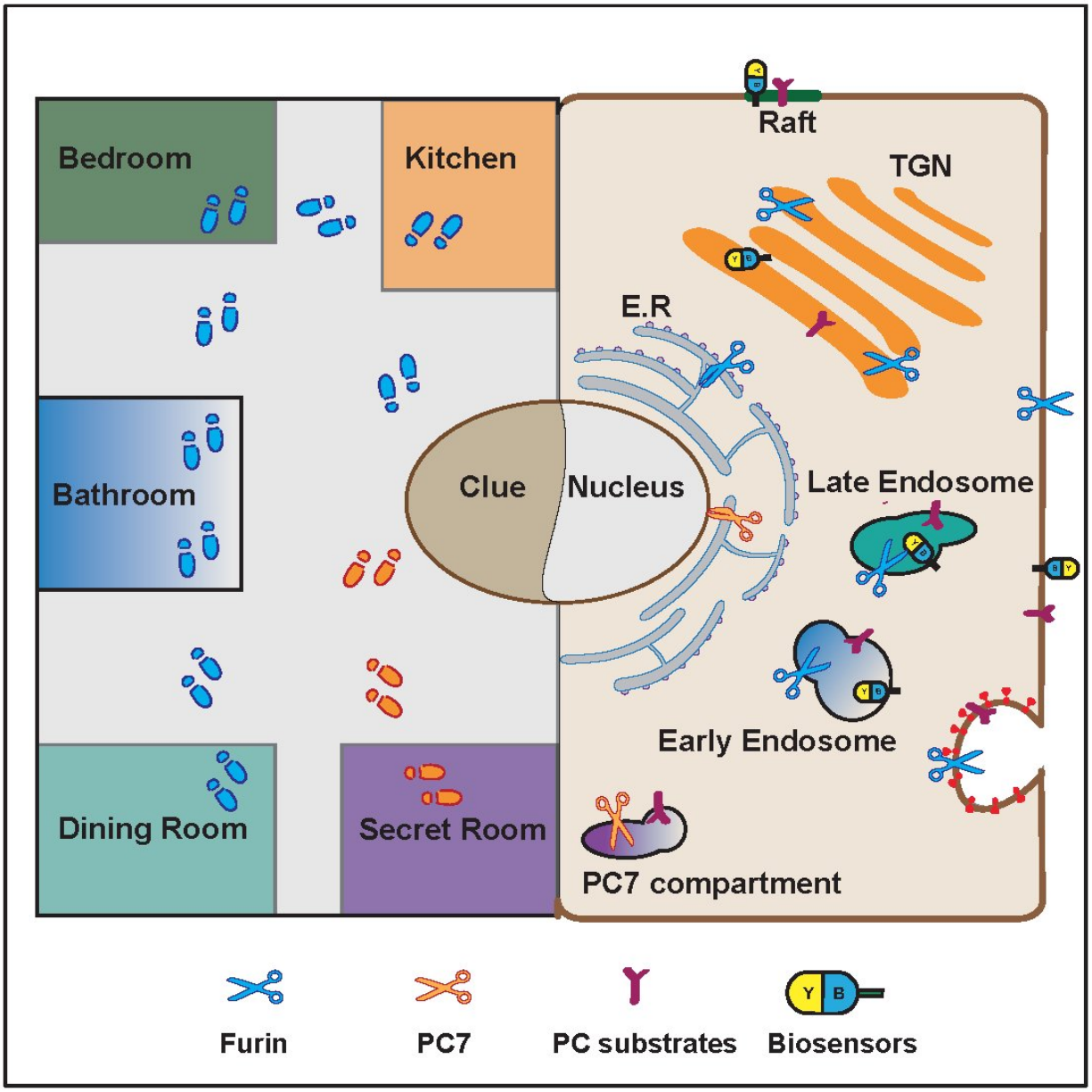

Illustration showing the 'Cluedo' game of proprotein convertase activity. Credit: D.B. Constam/EPFL

Most proteins in the cell are not produced "ready to go". Instead, they are first synthesized with chains of amino acids that block their activity until they are removed by enzymes called "proprotein convertases" (PCs). This family of enzymes plays significant but very different roles in various cancers, and regulating the activity of PCs could help develop cancer treatments. But PCs overlap in terms of activity, meaning that two or more of these enzymes can process the same protein. This overlap makes it very difficult to distinguish and map out the functional profile of each PC.

One important aspect of a PC's life is its trafficking in secretory vesicles to the cell surface, and its internalization in endosomes that mediate recycling to the so-called trans-Golgi network, a trafficking hub where secretory vesicles and endosomes exchange protein cargo, and where PC's have been thought to perform most of their protein-activating work.

The lab of Daniel Constam at ISREC (EPFL) has now developed targeted biosensors that can image specific PCs on a subcellular level, thereby overcoming the problems of overlap. The biosensors are based on an original sensor molecule developed by Constam in 2010 called CLIP (Cell-Linked Indicator of Proteolysis), to image where a cell's convertase enzymes are active in living cells.

With the CLIP biosensors, the researchers were able to successfully track the activity of the "prototypical" PC, furin, at subcellular resolution. Furin's trafficking has been shown to influence its activity by regulating which proteins it can access and activate; in other words, furin activity depends on where it is enriched inside the cell.

Using a PC inhibitor, the researchers found that when inside endosomes, furin is ten times less inhibited but enriched more than three times compared to the trans-Golgi network. Another PC that resists this inhibitor (PC7) was active in distinct vesicles and it only reached the trans-Golgi network, the endosomes and the cell surface when it was over-expressed.

"We are particularly interested in one of the substrates, Activin-A, because of its newly discovered immunosuppressive role in melanoma," says Daniel Constam. "We now found that different 'rooms' cleave it in a stepwise fashion, leaving a mark of where it has been, presumably to regulate where it should go next and with whom to interact."

The researchers then turned their attention to the amino acid sequence of the PCs they were studying. They found that a "PLC motif" (a sequence of three amino acids, Proline, Leucine, and Cysteine) in the cytosolic tail of PC7 was specifically required for its recycling in the trans-Golgi network and for rescuing proActivin-A cleavage in furin-depleted melanoma cells, but was relatively dispensable for PC7's activity inside endosomes.

"Our study provides a proof-in-principle that compartment-specific biosensors can be used to gain insight into the regulation of PC trafficking and to map the tropism of PC-specific inhibitors," says the study's first author, Pierpaolo Ginefra. "It's like a game of Cluedo: we want to know in which of the room(s) in a cell a given substrate is cleaved and by which PC."

More information: Pierpaolo Ginefra, Bruno G.H. Filippi, Prudence Donovan, Sylvain Bessonnard, Daniel B. Constam. Novel compartment-specific biosensors reveal a complementary subcellular distribution of bioactive Furin and PC7. *Cell Reports* 27 February 2018. DOI: 10.1016/j.celrep.2018.02.005

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