NOTCH IN MEMORY FORMATION

Notch is a cell surface receptor that mediates intercellular communications through physical contact. It is well known for its roles in the regulation of a wide array of biological processes, in animals from hydra to humans. Much data in the field relates to its roles in development and cell differentiation but there is significant evidence that Notch also plays critical roles in numerous processes through physical contact. It is well known for its roles one of the non-canonical signaling mechanisms (Heitzler, 2010; Guruharsha et al., 2012).

Two studies in Drosophila adults that used conditional Notch mutants and inducible transgenes clearly demonstrate a role for Notch in memory formation (Ge et al., 2004; Presente et al., 2004). These studies used the olfaction-based, Pavlovian paradigm and showed that Notch is required for LTM but not learning. Amazingly, when the full length Notch protein (NFull) was expressed before training, a single training was sufficient to form significant memory instead of 10 required in control flies (Ge et al., 2004). Similar experiments with Suppressor of Hairless (Su(H)) in the Drosophila homolog of RBP-j, show that it is also important for LTM (Song et al., 2009). This report shows that LTM is specifically blocked in Su(H) mutants and the expression of the wild-type Su(H) protein in mushroom bodies, the key brain region for Drosophila LTM, is sufficient to rescue the memory defect. Interestingly, the study also showed that over-expression of Su(H) protein in the wild-type background caused LTM defects (Song et al., 2009). Another study has identified the homotypic cell adhesion molecule Kloston as functioning downstream of Notch in LTM (Matsumo et al., 2009), but it is not clear whether it is regulated by NICD. Thus, data from both mice and Drosophila raise doubts about the involvement of canonical Notch signaling in LTM. The confounding data relate to RBP-j/Su(H) knockout and over-expression.

The relationship between Notch and RBP-j/Su(H) is not simple. Su(H) knockout in Drosophila results in the loss of not only NICD but also NFull expression (Kold et al., 1998; Lebecourtiis and Schweisguth, 1998; Wesley and Mok, 2003). The Notch receptors that are stable in the absence of Su(H) are the naturally produced, truncated Notch receptors lacking the carboxyl terminal ubiquitination, transcription activation domain, and PEST sequences (Wesley and Mok, 2003). On the other hand, over-expression of...
Su(H) results in increased nuclear localization of NICD that is in the background (Kidd et al., 1998). Similar relationships between RBB-j and the full length Notch1 protein and NICD can be found in mammals as well (Schloot et al., 1998; Sato et al., 2012). In addition, Notch and Su(H) display a stoichiometric relationship that appears to determine whether Su(H), NICD, or both are retained in the cytoplasm or translocated to the nucleus (Ghosh et al., 1996; Kidd et al., 1998). A further complicating matter is that NICD expression from a transgene in the wild-type background suppresses the cell surface expression of NFull produced from the endogenous Notch gene, possibly due to titration of Su(H) (Bardot et al., 2005). Incidentally, this observation also implies that transgenic expression of NICD in the wild-type background while reproducing bona fide functions of endogenously produced NICD could also manifest additional effects linked to the loss of non-canonical NFull functions at the cell surface. Thus, manipulation of RBB-j/Su(H) may not be the best way to determine whether the canonical or a non-canonical Notch signaling activity is involved in a process. Since a vast amount of data from worms to humans indicates that transgenic expression of NICD reproduces functions that are based on canonical Notch signaling, the best approach could be to use NICD for determining if canonical signaling is involved and explore other non-canonical Notch mechanisms if it is not.

**NON-CANONICAL NOTCH SIGNALING MECHANISMS IN DROSOPHILA DEVELOPMENT**

Since much information on Notch function in LTM formation is from *Drosophila*, we will restrict ourselves to this model organism.

A non-canonical Notch mechanism is known to function during the development of a class of adult sensory bristles called microchaetae. The development of these bristles is suppressed by a collection of Notch alleles (called mcd alleles) with mutations that delete the carboxy-terminal portion of the Notch protein (thereby deleting the transcriptional activation domain and the PEST sequences). mcd alleles signal through a poorly understood signaling mechanism that interfaces with the Wingless/Wnt pathway (Ramain et al., 2001). However, as this signaling persists in the Su(H) knock out background it is not clear whether it is based on NFull or the naturally produced truncated Notch receptor. However, as there is very little evidence that AB kinase promotes LTM, we do not discuss this Notch activity any further.

We recently discovered another non-canonical Notch function that is involved in dorsal closure and dorso-ventral axis formation in embryos. Dorsal closure is a zipper-like process driven by F-actin dynamics that remodels and mobilizes lateral epithelial cells to close the dorsal “hole” being created by the apoptosing extra-embryonic amnioserosal cells (Harden, 2002). Notch involvement in dorsal closure was reported previously but the underlying signaling mechanism and its target were obscure (Zecchini et al., 1999). The dorso-ventral axis is established by the opposing gradients of Toll/Dorsal and Dpp signaling. Dorsal is the *Drosophila* homolog of NFκB and Dpp is the *Drosophila* homolog of TGFβ/Bone Morphogenetic Protein. The newly discovered non-canonical Notch activity was found to up-regulate the level of F-actin and promote the formation of the longitudinal F-actin cables during dorsal closure (Wesley et al., 2011). During dorso-ventral axis formation, it was found to up-regulate the level of a phosphorylated form of Cactus, the *Drosophila* homolog of IκB, that is a negative regulator of Toll/Dorsal (NFκB) signaling (Tremmel et al., 2013).

Some important features of the new non-canonical Notch signaling are identified (Wesley et al., 2011; Tremmel et al., 2013). This signaling is based on NFull, is activated soon after ligand binding, and involves the activation of Pκc98E, a *Drosophila* homolog of the novel isoform of protein kinase C (PKC). Treatment of Notch expressing cells with diacyl glycerol (DAG) analog elicits the same response as ligand treatment. DAG analog treatment is known to result in plasma membrane localization and activation of Pκc (Akiba et al., 2002). As activated NFull, Pκc, Cactus, and F-actin exhibit significant overlap in their expression at the cell surface (Wesley et al., 2011, Tremmel et al., 2013), it is possible that NFull activation promotes interactions among these proteins. This possibility is supported by the information that (1) Cactus was initially isolated in a yeast two-hybrid system where activated PKC, Cactus, and F-actin interact (Prekeris et al., 1992), (2) a mammalian homolog of *Drosophila* Pκc98E associates with F-actin during neuronal differentiation (Prekeris et al., 1996; Zaidman et al., 2002), and Pκc98E contains a domain similar to the Notch ankyrin repeats (Tremmel et al., 2013). Apparently, the non-canonical NFull-Pκc signaling competes with canonical Notch signaling for NFull: suppression of Pκc98E expression while reproducing mutant phenotypes related to the loss of NFull-Pκc activity also results in mutant phenotypes related to increased canonical Notch signaling. Finally, our studies show that the *Drosophila* embryo can be divided into distinct zones based on whether the canonical Notch signaling is up-regulated (e.g., ventral region) or the non-canonical NFull-Pκc signaling is up-regulated (e.g., lateral regions, Wesley et al., 2011; Tremmel et al., 2013). Since Notch activities are importantly regulated at the levels of trafficking and recycling to the cell surface (Reichardt and Knoblich, 2013), it is possible that some of these regulations are involved in modulating the relative levels of Notch signaling activities at the cell surface and in the nucleus.
NFULL-PKC ACTIVITY IN MEMORY FORMATION IN
Drosophila ADULTS

Cyclic-AMP response element binding protein (CREB) is a tran-
scription factor that plays pivotal roles in intrinsic and synaptic
plasticity during LTM formation (Yin et al., 1995; Benito and
Barco, 2010; Chen et al., 2012; Hirano et al., 2013). CREB over-
expression prior to olfaction-based training was also found to
reduce from 10 to 1 the number of training required for form-
ing LTM (Yin et al., 1995; Tubon et al., 2013). We studied Notch
and CREB together in memory formation in adult flies using
temperature-sensitive conditional and inducible alleles and trans-
genes. We found that NFULL-PKC activity up-regulates the level
of a hyper-phosphorylated form of CREB (hyper-PO4 CREB; 
Zhang et al., 2013). Remarkably, the experimental details either
in adult flies or in cultured cells were similar to the regulation
of P-Cactus. Incidentally, Cactus and CREB share functionally related
phosphorylation sites (Taylor et al., 2000). Hyper-PO4 CREB is
cyttoplasmic (just as P-Cactus) and one of the residues phospho-
rylated is Serine 231. This Serine is equivalent to Serine 133 in
mammalian CREB, the phosphorylation of which is shown to be
important for LTM in mammals (Gonzalez and Montminy, 1989;
Silva et al., 1998).

We also found an intriguing feature: a single pulse of Notch
activity triggers an ultradian oscillation of hyper-PO4 CREB level
that is linked to accumulation of nuclear CREB isoform. Wild-
type flies also show robust hyper-PO4 CREB oscillation during
daylight and after olfaction-based training for memory formation.
These observations raise the possibility that the frequency and
the amplitude of hyper-PO4 CREB ultradian oscillation are used
for repeating the strength of the initial LTM-forming stimulus.
Such repetition might be useful for memory consolidation and for
identifying the LTM-forming stimulus. It could be also used to
store information as wave tracks in the brain that differ in their
ability for persistence or reactivation, akin to the way amplitude
and frequency of electromagnetic waves are used to convey, store,
and retrieve information.

NFULL-PKC activity up-regulates not only hyper-PO4 CREB but also
F-actin in the adult brains, with a much higher level accumu-
lating in the mushroom bodies and antennal lobes (Figure 1).
Mushroom bodies are the primary centers for LTM formation
and Notch and CREB functions are required there (Presente et al.,
2004; Vu et al., 2006; Hirano et al., 2013). Antennal lobe is also shown
to require Notch function, in olfaction stimulation (Lieber et al.,
2011). An increase in F-actin level has been reported in associa-
tion with forgetting in Drosophila (Davis, 2010; Shuai et al., 2010).
However, the forgetting mechanism appears to be independent of
cyclic-AMP and CREB pathways. Thus, the Notch-mediated up-
regulation of F-actin might be involved in a different F-actin
process that promotes LTM formation. That a single pathway
could regulate CREB and F-actin could be significant since F-actin
dynamics are known to play diverse roles in neuronal functions,
from modification of synapses to molecular transport.

POSSIBLE FOR CROSSTALK WITH WINGLESS/Wnt SIGNALING

The Wingless/Wnt pathway and the Notch pathway often func-
tion in the same contexts, in development (Andersen et al., 2012)
and adults (Inestrosa and Arenas, 2010; Nichos, 2012).

The target of Wingless/Wnt signaling is the transcriptional activity
of Armadillo/β-Catenin in the nucleus. In the absence of this
signaling, Armadillo/β-Catenin is targeted for degradation by
Shaggy/GSK3 kinase. When Wingless/Wnt binds its receptor com-
plex composed of Frizzled and Lrp/Arrow, the intracellular protein
Disheveled is activated, which in turn blocks the Shaggy/GSK3
activity. As a consequence, Armadillo/β-Catenin is stabilized and
translocated to the nucleus for activation of target genes. A few
weeks ago, an exciting finding was reported: suppression of Wing-
less, Armadillo/β-Catenin, or Arrow expression in the mushroom
bodies suppresses LTM formation in adult flies (Tan et al., 2013).
An earlier study has shown that Disheveled can bind to the
Notch intracellular domain and inhibit canonical Notch signal-
ing (Axelrod et al., 1996). Taken together, an interesting possibility
arises. A stimulus for LTM formation results in the activation of
Wingless/Wnt and Delta ligands. These ligands activate Frizzled/
Arrow and NFULL-PKC activities, respectively, that synergistically
block Shaggy/GSK3 kinase and NICD activities to promote the
LTM-related nuclear activities of Armadillo/β-Catenin and CREB
(Figure 2). Blocking NICD activity might be important as our
data from embryos suggest that this activity would suppress the
expression of genes whose functions are promoted by NFULL-
PKC activity (Wesley et al., 2011). The Wingless/Wnt pathway
is known to regulate many cytoskeletal remodeling processes
during development (Payre et al., 1999; Delfon et al., 2003; Chanut-
Delalande et al., 2006). Thus, the simultaneous activation of this
pathway and the Notch-PKC activity might be important for inte-
grating signaling with F-actin-based processes during memory
formation.

FUTURE DIRECTIONS

One of the challenging questions is determining the cellular
contexts for NFULL-PKC and Wingless/Wnt activities in LTM
formation. Do they function in response to neuron-neuron com-
munication or neuron-glia communication? Do they function in
the same cells? If not, how do they both promote LTM formation?
This information would provide clues to the spatio-temporal configurations underlying LTM formation, as the two activities could regulate F-actin, hyper-P04 CREB, and Armadillo/F-catenin both spatially and temporally.

The more challenging question is how the ultradian oscillation of hyper-P04 CREB is generated. We have some evidence from embryos and cultured cells that suggest the involvement of a self-sustaining mechanism. Immediately following NFull activation, when the PKC-dependent activity is high, P-Cactus and F-actin levels are high. These levels diminish over time, coincident with the accumulation of NICD (Wesley et al., 2011; Tremmel et al., 2013). Thus, it is possible that a single pulse of Notch activation generates the two Notch activities in a time sequence that leads to ultradian oscillation of hyper-P04 CREB (Figure 2B). During vertebrate somitogenesis, both Notch and Wingless/Wnt activities are reported to manifest ultradian oscillation (Jensen et al., 2010; Kageyama et al., 2010). It would be fascinating to find out if Wingless/Wnt activity also oscillates during LTM formation. If so, finding out whether it is in phase or out of phase with hyper-P04 CREB oscillation would provide important clues for identifying the parameters controlling the oscillations. A mathematical analysis of memory formation may become possible.

IMPLICATIONS FOR DEMENTIA

The functions of the Notch, PKC, and CREB genes are disrupted in many neurodegenerative diseases, including Alzheimer’s disease (AD; Wang et al., 1994; Pakaski et al., 2002; Costa et al., 2003; Selkoe and Kopan, 2003; Pugazhenthi et al., 2011). One study has reported that NICD production is dramatically increased in the brains of AD patients (Berezovska et al., 1998). Furthermore, PKC and CREB activities are down regulated in AD animal models and the activation of a novel isofrom of PKC or an increase in CREB phosphorylation is shown to significantly improve their cognitive function (Coog et al., 2004; Hongpaisan et al., 2011). Thus, there is a good chance that a disruption of mammalian non-canonical Notch-PKC-like activity is involved in dementia. That such an activity could also regulate F-actin is significant because studies in mice show that F-actin up regulation is important for memory formation and triggers the translocation of cytoskeleton-associated protein Arc/Arg3.1 into synapses (Lamprecht, 2011; Liu et al., 2012).

Interestingly, Arc/Arg3.1 is required for proteolytic processing of Notch and synaptic plasticity (Alberi et al., 2011), which is consistent with our perspective that the sequence of activation of non-canonical and canonical Notch signaling might be important for LTM formation. Constitutive over-expression of either one of these activities might interfere with LTM formation. Since there is also evidence suggesting that the loss of Wingless/Wnt signaling is involved in AD (Boonen et al., 2009; Lucas et al., 2003; Jackson et al., 2002; Sofola et al., 2010), understanding how the non-canonical NFull-PKC signaling, the canonical Notch signaling, and Wingless/Wnt signaling function together in LTM formation might help us understand memory formation and memory loss upon neurodegeneration. It might also help us understand memory decline with age. One interesting possibility could be that the ultradian oscillation of hyper-P04 CREB becomes more variable, due to either loss of synchrony between the oscillations in different cell types or deterioration in the coupling between the mechanisms controlling the frequency and the periodicity of the oscillation.

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