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is dynamically modulated. If Cdk5 activity is critical for synaptic plasticity, one expects changes during the process of learning and cyclin E-Cdk5 interactions may represent such a regulatory node.

Returning to the cell cycle, Cdk5 may function as a cell-cycle suppressor in postmitotic neurons and drive differentiation (Zhang et al., 2008). It is therefore of importance to track, through development, the association of cyclin E with Cdk5. While Odajima, Wills, and colleagues (2011) have attempted this in their proteomic analysis, greater spatiotemporal resolution would be necessary to address this question. Stress conditions, including pathological stresses, are known to drive neurons into the cell cycle and subsequent neuronal death. It is possible that cell-cycle re-entry may involve modulation of the interaction between cyclin E and Cdk5.

It appears that a group of core cell-cycle regulators, cyclin E being the latest to be identified, have evolved to function in a considerably different cellular milieu. Sequestration of Prospero in *Drosophila* (Berger et al., 2010) and atypical Cdks, such as Cdk5 (as shown here), are interesting examples involving cell-cycle-independent functions of cyclin E. Thus, cyclins appear to have extended their posttranslational regulatory functions beyond the cell-cycle. Future work should provide interesting insights into evolutionary logic of these "dual specificity" proteins, but it appears proliferation and differentiation may form a continuum with the same players employed parsimoniously in multiple contexts.

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The Hormone of Love Attracts a Partner for Life

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Neurovascular integration during embryonic development is essential for adult physiology. In this issue of *Developmental Cell*, Gutnick et al. (2011) report that hypothalamic neurons secrete oxytocin as a guidance cue for endothelial cells to establish their vascular supply—a prerequisite for neuroendocrine secretion from the neurohypophysis in adult life.

The nervous and vascular systems are functionally different but form networks that are often in close association and communication. This is exemplified by the vascularization of axon bundles or, conversely, by the innervation of muscular vessels with the autonomic nerves that control vascular tone. Much research effort has been directed at identifying the molecules that guide these interactions (reviewed by Segura et al., 2009). For example, it has been shown that nerve-cell-derived VEGF promotes the arterial differentiation of blood vessels that are coaligned with peripheral nerves (Mukouyama et al., 2005) and that endothelial cells of the external carotid artery guide sympathetic axons by releasing chemoattractive endothelins (Makita et al., 2008). In contrast, the molecular and cellular mechanisms that establish the neurovascular interface of neuroendocrine organs such as the posterior pituitary have not been identified. In this issue of *Developmental Cell,* Gil Levkowitz and colleagues demonstrate how hypothalamic axons regulate endothelial morphogenesis to induce the neurovascular congruence that provides the anatomical basis for neuroendocrine secretion from the posterior part of the pituitary (Gutnick et al., 2011) (Figure 1).

Magnocellular neurons in the hypothalamus extend axons to interface with fenestrated capillaries in the posterior pituitary, the neurohypophysis, to release the neuropeptides oxytocin and vasopressin into the bloodstream (reviewed by Burbach et al., 2001). Oxytocin, a word that in Greek means "quick labor," is often referred to as the hormone of love for its multiple roles in sexual

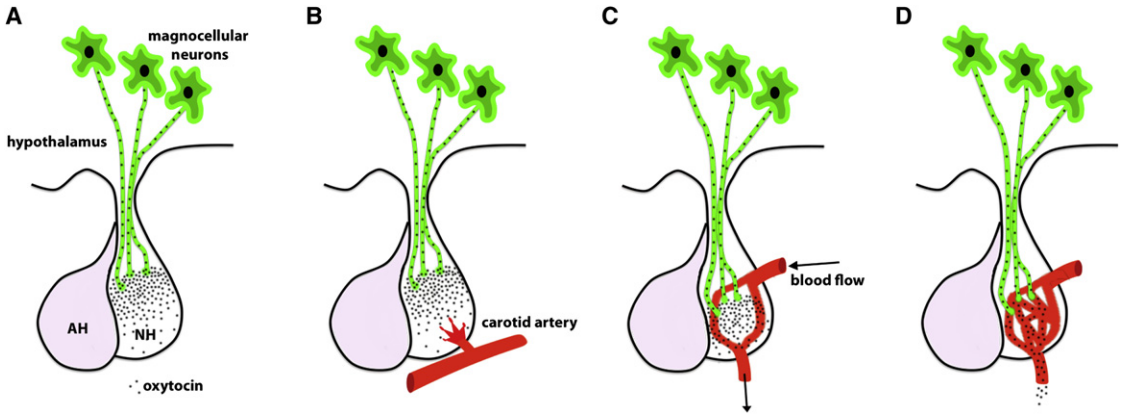

Figure 1. Model for the Development of the Neurovascular Interface in the Neurohypophysis

(A–C) Schematic representation of vascular development in the zebrafish neurohypophysis. (A) The first axons of hypothalamic magnocellular neurons (green) reach the embryonic posterior pituitary 24 hr after fertilization, before its vascularization. The neurons release oxytocin into the neurohypophysis. (B) Oxytocin stimulates the sprouting of blood vessels (red) from the carotid artery into the neurohypophysis over the next day. (C) A proper neurohypophyseal vascular network has formed by 72 hr.

(D) Schematic representation of the neurovascular interface in the adult neurohypophysis, where oxytocin is released into a network of fenestrated capillaries. The following abbreviations are used: AH, adenohyphophysis; NH, neurohypophysis.

reproduction, maternal bonding, and pair bonding, although some neural functions are considered independent of its secretion into the blood stream (reviewed by Neumann, 2008). Whereas previous studies identified the oxytocin-secreting magnocellular fibers with classical histochemical methods, Gil Levkowitz and colleagues took advantage of genetically modified live zebrafish to image fluorescently labeled neurons and blood vessels (Gutnick et al., 2011). Thus, they created transgenic fish that expressed the green fluorescent protein EGFP in the progenitors of magnocellular neurons to trace hypothalamic axons as they innervated the neurohypophysis, which was vascularised by endothelial cells expressing the red fluorescent protein dsRed. The authors also generated fish in which the oxytocinergic population of the magnocellular neurons was labeled with EGFP and the endothelial cells with red fluorescent mCherry protein to demonstrate axonal contact with pituitary vessels at both developmental and adult stages.

After establishing the time course of neurovascular interactions in the neurohypophysis by imaging their transgenic zebrafish (Figures 1A–1C), the authors used two complementary genetic approaches to determine the relationship of oxytocin-secreting axons and pituitary blood vessels during their development. They first asked if the dependence of adult axons on fenestrated endothelium for neuropeptide secretion was predated by a reliance of developing axons on neurohypophyseal vascularization. However, they found that axons projected normally in zebrafish *cloche* mutants, which lack the head vasculature. This finding was consistent with their observation that hypothalamic axons arrived in the neurohypophysis before blood vessels (Figure 1A). They next performed the reciprocal experiment by investigating if the neurohypophyseal vasculature required oxytocinergic axons to develop by ablating the precursors of magnocellular neurons. Strikingly, loss of the magnocellular axons led to hypotrophic blood vessels that did not form an appropriate vascular loop in the neurohypophyseal area where neurosecretory axons normally terminate.

Prompted by previous observations that oxytocin stimulates the migration and sprouting of human endothelial cells in vitro and that endothelial cells can express the oxytocin receptor in situ (e.g., Cassoni et al., 2006; Thibonnier et al., 1999), Levkowitz and colleagues further investigated if oxytocin stimulated blood vessel growth in the zebrafish neurophypophysis. The depletion of oxytocin or its receptor through morpholinos induced an aberrant vascular phenotype similar to that caused by ablating magnocellular axons. These results support a role for oxytocin in attracting the blood vessels that are necessary for its secretion (Figures 1B and 1C). Importantly, knocking down vasopressin, another neuropeptide secreted from the neurophypophysis, did not obviously impair the formation of neurohypophyseal vessels, and knocking down oxytocin did not affect carotid artery development. These experiments elegantly demonstrated specificity of the oxytocin axis for neurovascular copatterning in the posterior pituitary.

By identifying oxytocin as a key signaling cue for the establishment of the neurovascular interface in the pituitary, Levkowitz and colleagues have elucidated a molecular pathway in which a neurohormone directly affects endothelial morphogenesis. However, as is often the case for studies that are direction setting, their findings also raise several new questions. First, it will be important to investigate why neurohypophyseal vessels are dependent on oxytocin, whereas surrounding head vessels are not, and how this difference relates to the expression of the oxytocin receptor on different types of blood vessels in situ. Second, the previously described ability of oxytocin to stimulate the migration and sprouting of human endothelial cells needs to be reconciled with the vascular defects of oxytocin-deficient zebrafish, in which blood vessels sprout and migrate into the neurohypophyseal area but consisted of abnormally few endothelial cells. This type of vascular morphology could be better explained by reduced endothelial cell proliferation or excessive apoptosis rather than impaired migration. Finally, it

will be interesting to investigate whether oxytocin is also required for the subsequent vascular specialization in the pituitary, where the endothelium needs to become fenestrated instead of forming a blood brain barrier (Figure 1D) (reviewed by Burbach et al., 2001). Such studies will likely be facilitated by the beautiful double-transgenic zebrafish line that Levkowitz and colleagues created to covisualize oxytocin-secreting axons and blood vessels from development into adulthood (Gutnick et al., 2011).

From a physiological point of view, it appears sensible that a neurohormone such as oxytocin should function as a vascular guidance cue to promote the growth of blood vessels that will ultimately help its release into the circulation. A different type of neurovascular relationship promotes the function of the anterior pituitary of mammals, where the gonodotropin-releasing hormone GnRH induces the release of the gonadotropins LH and FSH into the circulation. In this system, the vascular growth factor VEGF promotes the survival of GnRH-secreting neurons during their migration from the nasal placode to the hypothalamus, independently of blood vessels (Cariboni et al., 2011). Taken together with the new study by Levkowitz and colleagues, it appears that the development of the hypothalamic hormonal response system relies on neurovascular congruence at different levels. Because the general function of the neurohypophysis and the GnRH neuron system are conserved in vertebrates, findings made in one model organism are likely to be relevant for other vertebrates. Hence, using optically transparent transgenic zebrafish embryos may help to elucidate how vascular growth factors promote the development of the GnRH neuronal system, while the oxytocin-deficient mouse (Nishimori et al., 1996) should enable further research into neuropeptide-mediated vascular patterning in the mammalian neurohypophysis.

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Long, Saturated Chains: Tasty Domains for Kinases of Insulin Resistance

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The mechanistic basis of how cells respond to increased fatty acids (FAs) is murky but potentially involves receptor-mediated activation or inhibition by different FA classes. Holzer et al. (2011) recently propose in *Cell* that expansion of intracellular membrane microdomains induced by saturated FA recruit and activate c-Src for JNK activation.

In this era of unprecedented caloric excess, we face increased incidences of obesity, metabolic syndrome, and diabetes mellitus; natural selection has left us ill equipped for unrestricted food. The first adverse sign is insulin resistance—decreased glucose transport into cells that is matched by an increase in serum insulin at the cost of elevated blood insulin, free fatty acids (FAs), and inflammatory mediators to maintain blood glucose homeostasis. Although the insulin receptor signaling cascade is redundant, with one insulin receptor substrate compensating for the loss of the other's function, c-Jun n-terminal kinase family members 1 and 2 (JNK, aka stress-activated protein kinases, a subset of mitogen-activated protein kinases), when activated, act as intracellular mediators of insulin resistance by disrupting both arms of this cascade. The Randle hypothesis links increased free FA to insulin resistance and proposes that FA compete