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Letter

The role of chromogranin A and the control of secretory granule genesis and maturation

Taeyoon Kim[1], Jung-Hwa Tao-Cheng[2], Lee E. Eiden[3] and Y. Peng Loh[1]

[1]Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[2]EM facility, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, MD 20892, USA
[3]Laboratory of Cellular and Molecular Regulation, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA

In a recent Opinion article in *Trends in Endocrinology and Metabolism* [1], Day and Gorr review recent work in the chromogranin field, including our own work on chromogranin A (CgA) and our proposal that CgA is an 'on/off' switch, acting at a post-transcriptional level, for the biogenesis of neuroendocrine large dense-core secretory granules [2]. They raise the question of whether CgA plays a role as a universal master gene, 'on/off' switch, or assembly factor in the control of dense-core secretory granule biogenesis in endocrine and neuroendocrine cells. They argue that CgA is unlikely to act as such a switch only at the transcriptional level. We agree: our data show that suppression of CgA synthesis in PC12 cells causes an abolition of large dense-core secretory granule proteins, but not the mRNAs that encode them, clearly pointing to a post-transcriptional component of the action of CgA in granule biogenesis. Day and Gorr also point out that secretory granules exist in cells that do not synthesize CgA. These exceptions are particularly interesting. As pointed out by us previously [2], mast cells use a non-protein substance, heparin, to enable mast cell granule formation, and lactotrophs contain prolactin rather than CgA in their secretory granule. Our definition of an 'on/off' switch for granulogenesis is a factor that regulates and is required for initiation of granule formation at the level of the trans-Golgi network (TGN). According to this definition, molecules other than CgA, such as heparin [3], could function as 'on/off' switches for granule biogenesis, depending on cell type. Based on the fact that most endocrine and neuroendocrine cells [4–6], as well as some exocrine cells [7], synthesize CgA, and our data implicating CgA in large dense-core granule biogenesis in PC12 and 6T3 cells [2], CgA is likely to be the most widely used granulogenic factor in neuroendocrine cells.

What of experiments in which overexpression of CgA does not reconstitute large dense-core secretory granule formation [8], or in which the spontaneous loss of regulated secretion in a PC12 variant cell cannot be rescued by exogenous CgA expression [9]? Tellingly, forced expression of CgA completely reconstitutes granule formation and function in PC12 cells in which endogenous CgA suppression first leads to loss of granulogenesis and regulated secretion [2], and forced expression of CgA in non-neuroendocrine cells likewise results in the formation of significant numbers of CgA containing large dense-core granule-like vesicles, albeit less abundant than the granule complement of normal endocrine cells [2]. Thus, in a cell with all other components predisposing to granulogenesis (PC12 cells), CgA is the post-translational 'switch' that turns it on, whilst in nonendocrine cells (e.g. fibroblasts), CgA might well act only as an assembly factor, because structures or organelles formed in the CgA-expressing fibroblasts (CV-1 cells) are most likely not *bona fide* secretory granules, in the sense that they contain prohormones and have prohormone processing capability, and so on. PC12 variant cells that have also permanently lost neurosecretion competence [9–12] cannot be expected to recover simply by overexpressing CgA [9], unless it can induce the expression of all the necessary genes, thus acting as a 'master' gene. However, CgA has never been proposed as such a gene. By contrast, PC12 and 6T3 cells have not permanently lost their synthesis of regulated secretory pathway proteins upon downregulation of CgA synthesis and thus exhibit reversible competence for neurosecretion upon restoration of CgA synthesis. Thus, our data from PC12 and 6T3 cells support the hypothesis that CgA not only acts as an assembly factor, but also controls large dense-core granule biogenesis through regulating the stability and hence availability of proteins required for functional secretory granule formation. This

Corresponding author: Y. Peng Loh (ypl@codon.nih.gov).

is supported by the intriguing evidence that, in the absence of CgA, but not CgB, these granule proteins are rapidly degraded, in either the lysosomal and/or a pre-lysosomal compartment [2]. Clearly, more work is necessary to provide further evidence for this hypothesis, including defining the mechanism by which CgA regulates secretory granule protein stability.

Overall, our observations, and those cited by Day and Gorr, argue strongly for the cooperation of multiple factors in granulogenesis in the TGN, and in the immature and mature secretory granules. In fact, multiple roles for CgA itself in the process of granulogenesis, including both aggregation and condensation (collectively, 'assembly') as well as upstream events can be envisaged. What makes the notion of the 'switching' on and off of granulogenesis driven by the regulated transcription of the CgA gene and expression of the CgA protein so compelling? It reconciles *in vivo* observations that up- and down-regulation of CgA protein levels, by various hormonal maneuvers *in vivo*, leads to up- and down-regulation, respectively, in the abundance of large dense-core secretory granules in neuroendocrine cells [13].

Although we certainly have not ruled out the involvement of other molecules in regulating large dense-core secretory granule biogenesis, perhaps through triggering gene transcription, CgA seems to be a key player in this process in endocrine and neuroendocrine cells, beyond just acting as an assembly factor. Regardless of where CgA is finally located in the molecular cascade leading from the transcriptome to the secretory granule, establishment of the function of CgA as a granulogenic factor has begun an important new phase of research into the molecular mechanisms of regulated secretion in neurons, neuroendocrine, endocrine and exocrine cells.

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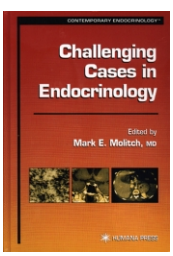
Book Review

Challenging cases made easy: you don't have to be brilliant, just systematic

Challenging Cases in Endocrinology edited by Mark E. Molitch. Humana Press, 2002. US$145.00 (xi + 421 pages) ISBN 0 896 03914 5

Barbara A. Cooper

Malcolm Grow Medical Center, 89th MDG/SGOMI, Andrews Air Force Base, MD 20762, USA



Molitch writes in the preface that *Challenging Cases in Endocrinology* is for the practicing endocrinologist. However, I disagree with that limitation. As an internist stationed at a military hospital near Washington, DC, I found this book incredibly intriguing. A large percentage of my patients have endocrine disorders, ranging from diabetes mellitus and adrenal insufficiency to reproductive and sexuality disorders. It can become quite easy to order lab work and start initial work-ups for patients who present with obvious signs and symptoms of endocrine pathology. However, what should be done when the case presentation is not that straightforward? Because my patients are busy (and often concerned with insurance issues), I have found it increasingly difficult to get them referred to an endocrinologist. I found this book helpful because it reinforced the importance of using a systematic thought process and work-up to diagnose even the most challenging case.

So, how is this book structured? To anyone who is used to reading the classical case history followed by a case

Corresponding author: B.A. Cooper (barbara.cooper@mgmc.af.mil).