Identification of Novel Genes Involved in the Plasticity of Pituitary Melanotropes in Amphibians

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Abstract

Melanotrope cells from the amphibian intermediate lobe are composed of two subpopulations that exhibit opposite secretory behavior: hypersecretory and hormone-storage hyposecretory melanotropes. Isolation of these subpopulations allowed a comparison of their gene expression profiles by differential display, leading to the identification of a number of genes differentially expressed in hypersecretory or hyposecretory melanotropes. Among them, we chose two (preferentially expressed in hyposecretory cells) of unknown function but structurally related to proteins involved in the secretory process: Rab18 and KIAA0555. We demonstrate that, upon activation of the regulated secretory pathway, Rab18 associates with secretory granules, inhibits their mobilization, and, consequently, reduces the secretory capacity of neuroendocrine cells. The other gene, KIAA0555, was predicted by *in silico* analysis to encode a protein with a long coiled-coil domain, a structural feature also shared by different proteins related to intracellular membrane traffic (i.e., golgins), and a hydrophobic C-terminal domain that could function as a transmembrane domain. A database search unveiled the existence of a KIAA0555 paralogue, KIAA4091, displaying a long coiled-coil region highly similar to that of KIAA0555 and an identical C-terminal transmembrane domain. Both KIAA0555 and KIAA4091 were found to be predominantly expressed in tissues containing cells with regulated secretory pathway, that is, endocrine and neural tissues. Moreover, when exogenously expressed in HEK293 cells, both proteins showed a yuxtanuclear distribution, which partially overlaps with that of a Golgi complex marker, thus suggesting a possible role of these two proteins in the control of the secretory process.

Introduction

The α-melanocyte stimulating hormone (α-MSH) produced by melanotrope cells of the amphibian intermediate lobe (IL) controls animal skin color adaptation to background color changes.1 Secretion of this hormone is regulated by environmental conditions through a complex multifactorial system comprising different stimulatory and inhibitory neurohormones that are produced by hypothalamic neurons, the terminals of which contact directly with melanotropes.[2,3] Fine tuning between the stimulatory and inhibitory inputs reaching the IL dictates the overall amount of α-MSH released by the gland at any given point of time. Notwithstanding this, when studied individually melanotrope cells, far from comprising a homogeneous population, present different behaviors in terms of their secretory response, both under basal conditions and upon reception of extracellular stimuli. In fact, owing to their different secretory granule pool size IL melanotropes can be split by density gradient separation techniques into two subpopulations that exhibit different phenotypes (i.e., cells that possess low intracellular α-MSH content display high basal secretion and a strong response to hypothalamic regulators versus cells that have high α-MSH content, low basal secretion, and respond only weakly to hypothalamic regulators).[4–7] Differences in the secretory status exhibited by melanotrope subtypes might well be dictated, in addition to the equilibrium of extracellular inputs reaching cells, by differences in intracellular components of the machinery that controls the sequential steps of the regulated secretory pathway (i.e., hormone synthesis and processing, sorting, packaging, transport, and release). Given the intricate nature of the secretory pathway, many of the intracellular components that govern this process remain undiscovered. In an attempt to identify new components involved in the acquisition and/or maintenance of a particular secretory status, we conducted a comparative analysis between the gene expression patterns of the two melanotrope subpopulations by differential display, which allowed us to identify several genes preferentially expressed either in hypersecretory or hyposecretory melanotropes (Table 1). Among them, we focused our effort in characterizing two genes preferentially expressed in the hyposecretory melanotropes that, given their sequence features, could be involved in the modulation of particular steps of the secretory pathway: Rab18 and a gene of unknow function referred to as KIAA0555. In this work, we review recent advances on the molecular and cellular characterization of these two gene products in relation to their possible role in the regulated secretory pathway of neuroendocrine cells.

Molecular and Cellular Characterization of RAB18in Neuroendocrine Cells

Rab18 belongs to a large family of proteins composed of more than 70 members in humans, namely the family of Ras-related, small, guanosine 5[r]-triphosphate (GTPase) proteins. Rab proteins are implicated in the regulation of specific steps of the secretory pathway, from vesicle budding from the donorcompartment to membrane fusion with the acceptor compartment, and they have been considered as intracellular membrane organizers,[8] although the specific function of many ofthem remain to be elucidated. In particular, Rab18 is a relatively unexplored member of the Rab GTPase protein family. Concerning its function, Rab18 was originally proposed to participate in apical

endocytosis in kidney epithelial cells because of its specific intracellular localization.[9] More recently, Rab18 has been found to localize around lipid droplets near specific membrane cisternae of the rough endoplasmic reticulum, suggesting that this protein may mediate interaction between lipiddroplet surface and the endoplasmic reticulum.[10,11] However, in neuroendocrine cells (i.e., rat pheochromocytome PC12 cells and mouse adrenocorticotropic hormone (ACTH)- producing AtT20 cells), our subcellular colocalization studies have demonstrated that Rab18 localizes diffusely in the cytosol under basal conditions. After induction of the regulated secretory pathway by either treating cells witha depolarizing pulse of K^+ (i.e., PC12 cells)or with the classical hypothalamic regulator of ACTH release, corticotropin-releasing factor (CRF) (i.e., AtT20 cells), Rab18 is specifically recruited to the membrane of a particular subset of secretory granules.[12] These resultsled us to propose that induction of secretion provokes Rab18 activation and its recruitment to these organelles. Thus, as depicted in Figure 1, under basal conditions Rab18 wouldlocalize as soluble in the cytosol in the inactive guanosine 5r-diphosphate (GDP)-boundconformation, whereas upon activation of the regulated secretory pathway, the subsequent exchange of GDP by GTP in Rab18 would lead to the interaction of the GTPase withthe surface of secretory granules, wherein it would exert its specific function. In support ofthis, a dominant-inactive GDP-bound Rab18 mutant was found to distribute completely diffuse in the cytosol and was unable to translocate to granules after stimulation, whereas adominant-active GTPase-deficient Rab18 mutant was predominantly associated with secretory granules even under nonstimulated conditions.[12] Therefore, alternation between the GDP-bound and the GTP-bound stateis a phenomenon that ultimately determinesthe activity of the Rab protein. In line with this and similar to that observed for Rab18, all mammalian Rabs investigated to date locate in their inactive GDP-bound states in the cytosol and in their active GTP-bound states they are recruited to specific target compartments.[13–15]

In the particular case of Rab18, tracking of the movement of secretory granules bearing theGTPase in its active state by time-lapse videomicroscopy revealed that association of Rab18 with secretory granules severely reduced their motility. Furthermore, overexpression analyseshave revealed that Rab18 inhibits the capacity of secretory cells to respond to extracellularstimuli, likely by impairing the transport of se-cretory granules to active zones of exocytosis at the plasma membrane.[12] Our findings using the tumor cell lines are in good agreementwith our observations that both Rab18 mRNAand protein content are higher in melanotropes exhibiting hyposecretory phenotype than in melanotrope cells displaying a hypersecretoryphenotype.[12] In addition, immunoelectron microscopy of amphibian melanotrope subpopulations revealed that hyposecretory melanotropes present twice as many Rab18-positive granules than hypersecretory melanotropes,[12] which is in accordance with the proposed inhibitory action of Rab18 on the secretory process and, in turn, suggests that the hyposecretory phenotype of melanotropes might be, at least in part, a result of their higher Rab18 content.

In summary, during the course of this studywe have provided novel morphological, molecular, and functional evidence that Rab18 participates as an inhibitory component of the secretory pathway in neuroendocrine cells. Thus, our results introduce this Rab protein as a new member of the intracellular machinery that controls the secretory pathway and thereby open new perspectives to better understand the cellular mechanisms

responsible for the maintenance of a particular secretory behavior.

In support of this hypothesis are our findings on Rab18 in human pituitary tumors causing acromegaly.[16] This syndrome is associated with high circulating levels of growth hormone (GH) as a result of not only a somatotrope hyperplasia[17] but also an alteredsecretion of GH from individual somatotrope cells.[16] By measuring Rab18 mRNA expression level and protein content in pituitary tissues from patients bearing acromegaly, we found that in all cases analyzed Rab18 expression and protein content were significantly lower than those in normal pituitary samples or in nonfunctioning pituitary adenomas from patients with normal or even reduced plasma GH levels.[16] Deficient Rab18 production in somatotropinoma cells may cause a loss of control in the molecular mechanisms responsible for retaining a particular population of secretory granules from being released upon an extracellular input and can, ultimately, determine a supraphysiological level of hormone secretion. Moreover, analysis of single-cell secretory activity in somatotropinoma cells overexpressingRab18 showed that these cells exhibited a secretory response to the classic hypothalamic regulator of pituitary GH secretion, GHRH, which was significantly reduced compared to that displayed by nontransfected or mock-transfectedcells.[16] To our knowledge these results providethe first evidence that defects in the intracellularmachinery that regulates GH secretion in somatotrope cells can be responsible for the supraphysiological levels of plasma GH observedin patients suffering from acromegaly and pavethe way for using Rab18 as a prognostic and/ortherapeutic target for handling this and, likely,other human diseases characterized by hormone hypersecretion.

Identification and Molecular Characterization of KIAA0555and Related Genes

The second cDNA identified by differential display from hormone-storage hyposecretory melanotropes showed high identity with a human cDNA of unknown function referred to as KIAA0555. Using a frog pituitary cDNA library, we cloned the frog full-length cDNA, which encodes a protein of 820 amino acids with an expected molecular weight of 96.5 kDa and a pI of 5.87.[18] Structural analysis of the amino acid sequence revealed the existence of seven regions of variable length displaying high probability to form coiled-coil structures, whichspan almost the entire sequence and define a long coiled-coil domain (Fig. 2). This domain isa key feature of, among others, specific proteins participating at different steps of the secretory process, including golgins (associated with theGolgi complex)[19]; the endosome-related proteins EEA1, rabaptin-5, and rabip4[20]; and the components of the cytomatrix at the presynaptic active zone CAST and liprin-α proteins.[21] Accordingly, it has been proposed that these proteins act as tethering factors either for the maintenance of organelle structure or for bringing transport vesicles close to the membrane of the target organelle prior to membrane fusion.[22]

In addition to the long coiled-coil domain,a 19-amino acid hydrophobic region was predicted at the C terminus of KIAA0555 protein, likely corresponding to a transmembrane domain (Fig. 2). Prediction of the membrane topology using bioinformatics (HMMTOP; transmembrane domain predictor algorithm at http://www.enzim.hu/hmmtop/index.html) indicated that the N-terminal bulk of thisputative transmembrane protein would beoriented towards the cytosol whereas the C terminus would be inserted in cellularmembranes.

Database search enabled the identification of frog KIAA0555 orthologues in the genomes ofall vertebrates, including human, mouse, and *Takifugu rubripes*, but not in invertebrates and revealed that KIAA0555 amino acid sequence and structural organization are highly con-served.[18] Likewise, *in silico* studies unveiled theexistence of a second human KIAA0555 iso- form, likely originating by alternative splicing,which lacks the transmembrane domain. Interestingly, both the long and the short KIAA0555 isoforms are expressed in the two melanotropecell subtypes, as determined by reverse transcriptase (RT)-PCR. As expected from differential display data, RT-PCR quantification confirmed that the long isoform was moreabundant in hyposecretory melanotropes than in hypersecretory cells; however, surprisingly,the expression levels of the isoform lacking the transmembrane domain were slightly higher inthe latter (Fig. 3A). Notwithstanding this, the expression of the short isoform was negligibleas compared to that of the long isoform in thetwo melanotrope cell subtypes.

Once we confirmed the presence of KIAA0555 in melanotropes, we next focused on elucidating whether the expression of this gene could be modified by factors regulating the secretory pathway in these cells—tobe more specific, the stimulator thyrotropin- releasing factor (TRH) and the inhibitor neuropeptide Y (NPY).[2] Thus, 24-h treatment of IL cell cultures with 10^{-7} mol/L TRH didnot alter KIAA0555 mRNA content whereas 10^{-7} mol/L NPY induced a twofold increase (Fig. 3B). Together these results indicate that expression of KIAA0555 is induced by receptor-mediated inhibition of hormone secretion, thussuggesting a role for this protein in conveying the information from negative extracellular inputs to the secretory pathway.

In addition to the KIAA0555 orthologues mentioned above, two paralogues of this gene were identified, which, like KIAA0555, are only present in vertebrates: the Marlin-1/Jamip1[23,24] and a gene of unknown function referred to as KIAA4091 (Fig. 2). Marlin-1/Jamip 1 is shorter than the protein codedby KIAA0555, lacks the putative transmembrane domain, and only contains four coiled-coil regions. On the other hand, KIAA4091 codes a protein containing eight coiled-coil re-gions, most of them highly similar to those ofKIAA0555, which span almost the entire se- quence. Likewise, the KIAA4091 amino acid sequence also contains a putative C-terminal transmembrane domain identical to that of KIAA0555.

We further characterized KIAA0555 as wellas its paralogue KIAA4091 by analyzing theirdistribution in frog, rat, and human tissues. RT-PCR analysis revealed a similar distribution pattern of both KIAA0555 and KIAA4091mRNAs; that is, they are both expressed in endocrine glands (e.g., pituitary, adrenal, testis) and especially in the central nervous system whereas they are absent or present in negligible amounts in peripheral organs.[18] The preferential expression of both KIAA0555 andKIAA4091 in tissues containing cells possessingthe regulated secretory pathway further sup- ports a role for these proteins in regulated secretion. Together, these observations combinedwith the particular structural characteristicsof KIAA0555 and KIAA4091 amino acid sequences have led us to refer to them as NECC1and NECC2 (neuroendocrine long coiled-coil protein 1 and 2), respectively.[18] Interestingly, the short isoform of NECC1 is exclusively expressed in endocrine cells but not in the brain, thus indicating that this gene is alternatively spliced in a tissue-specific manner and suggesting that the two isoforms could play, at least partially, distinct functions.[18]

In addition to examining the tissue distribution of NECC1 and NECC2, we also investigated their intracellular localization by using HEK293 cells transfected with

expressionvectors coding for the c-myc-tagged proteins. This revealed that both c-myc-NECC1 andc-myc-NECC2 accumulated in juxtanuclearstructures displaying tubulo-vesicular shape, likely corresponding to Golgi complex cisternae. In support of this, the exogenously expressed proteins exhibit partial colocalization with a Golgi complex marker [i.e., the ts045 variant of the vesicular stomatitis virus glycoprotein conjugated to green fluorescent protein (GFP) (VSVG-GFP ts045)]. These findings, together with the common structural organization of NECC proteins and golgins, strongly suggest that NECC1 and NECC2 mayact in the secretory pathway by controlling membrane fusion events at the Golgi complex and/or contributing to the maintenanceof Golgi structure, as has been proposed for the golgin family of long coiled-coil proteins.[19–22]

Concluding Remarks

Tight regulation of the secretory pathwayis critical for the correct functioning of the organism. Therefore, any malfunctioning inthe mechanisms regulating the multiple, sequential steps of the secretory pathway may result in generalized disruption of secretory homeostasis. Hence, further characterization of the molecular components that control the secretory pathway could provide essen- tial information to better understand this intricate intracellular process. With this aim in mind, we empirically addressed the problem using first the amphibian IL of the pituitary, more specifically α-MSH-producing melanotrope cells, because they represent an excel- lent biological model from which identification of genes related to the secretory process can be achieved with relative ease. The findings obtained in amphibians were next investigated on mammalian (neuro)endocrine cell lines, fur- thering our knowledge of these proteins inrelation to their intracellular localization and specific function within the secretory process.[12] Finally, these newly characterized components of the regulated secretory pathway could well represent putative indicators of the secretory activity of a particular gland under physiological and/or pathological situations. Therefore, we have recently undertaken the task of investigating their relation with human diseases that entail hypersecretory states, as is the case of Rab18 expression. We have demonstrated this expression to be inversely related to the pathological level of secretory activity of pituitary somatotrope cells from patients with acromegaly.[16] Overall we are gaining translational insight on components that belong to the intracellular machinery that controls the regulated secretory pathway, beginning with their isolation from amphibian neuroendocrine cell models, continuing with their cellular and molecular characterization in mammalian cell lines, and finishing with a crucial jump to understand their implication in human diseases.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Bagnara, J.T. & M.E. Hadley. 1973. *Chromatophores and Color Change: The Comparative Physiology of Animal Pigmentation*. Prentice Hall. Englewood Cliffs, NJ.
2. Tonon, M.C. *et al*. 1993. Multihormonal regulation of pituitary melanotrophs. *Ann. N. Y. Acad. Sci.* 680: 175–187.
3. Leenders, H.J. *et al*. 1993. Differential effects of co-existing dopamine, GABA and NPY on alpha-MSH secretion from melanotrope cells of Xenopus laevis. *Life Sci.* 52: 1969–1975.
4. Gonzalez de Aguilar, J.L. *et al*. 1994. Morphologi- cal and functional heterogeneity of frog melanotrope cells. *Neuroendocrinology* 59: 176–182.
5. Gonzalez de Aguilar, J.L. *et al*. 1997. Two frog melan- otrope cell subpopulations exhibiting distinct bio- chemical and physiological patterns in basal con- ditions and under thyrotropin-releasing hormone stimulation. *Endocrinology* 138: 970–977.
6. Gonzalez de Aguilar, J.L. *et al*. 1999. Differential effects of dopamine on two frog melanotrope cell subpopulations. *Endocrinology* 140: 159–164.
7. Vazquez-Martinez, R.M. *et al*. 1999. Analysis by mass spectrometry of POMC-derived peptides in amphib- ian melanotrope subpopulations. *Life Sci.* 64: 923– 930.
8. Zerial, M. & H. McBride. 2001. Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2: 107–117.
9. Lutcke, A., *et al*. 1994. Cloning and subcellular lo- calization of novel rab proteins reveals polarized and cell type-specific expression. *J. Cell Sci.* 107 (Pt 12): 3437–3448.
10. Martin, S., *et al*. 2005. Regulated localization of Rab18 to lipid droplets: effects of lipolytic stimula- tion and inhibition of lipid droplet catabolism. *J. Biol. Chem.* 280: 42325–42335.
11. Ozeki, S., *et al*. 2005. Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. *J. Cell Sci.* 118: 2601–2611.
12. Vazquez-Martinez, R., *et al*. 2007. Rab18 inhibits secretory activity in neuroendocrine cells by in- teracting with secretory granules. *Traffic* 8: 867– 882.
13. Pfeffer, S. & D. Aivazian. 2004. Targeting Rab GT-Pases to distinct membrane compartments. *Nat. Rev. Mol. Cell Biol.* 5: 886–896.
14. Pfeffer, S. 2005. A model for Rab GTPase localiza- tion. *Biochem. Soc. Trans.* 33: 627–630.
15. Seabra, M.C. & C. Wasmeier. 2004. Controlling the location and activation of Rab GTPases. *Curr. Opin. Cell Biol.* 16: 451–457.
16. Vazquez-Martinez, R. *et al*. 2008. Rab18 is reduced in pituitary tumors causing acromegaly and its over- expression reverts growth hormone hypersecretion. *J. Clin. Endocrinol. Metab.* 93: 2269–2276.
17. Melmed, S. *et al*. 2002. Guidelines for acromegaly management. *J. Clin. Endocrinol. Metab.* 87: 4054– 4058.
18. Cruz-Garcia, D. *et al*. 2007. Identification and characterization of two novel (neuro)endocrine long coiled-coil proteins. *FEBS Lett.* 581: 3149– 3156.
19. Barr, F.A. & B. Short. 2003. Golgins in the structure and dynamics of the Golgi apparatus. *Curr. Opin. Cell Biol.* 15: 405–413.
20. Hayakawa, A. *et al*. 2007. Evolutionarily conserved structural and functional roles of

the FYVE domain. *Biochem. Soc. Sym.* 74: 95–105.
21. Schoch, S. & E.D. Gundelfinger. 2006. Molecular organization of the presynaptic active zone. *Cell Tissue Res.* 326: 379–391.
22. Gillingham, A.K. & S. Munro. 2003. Long coiled- coil proteins and membrane traffic. *Biochim. Biophys. Acta* 1641: 71–85.
23. Steindler, C. *et al*. 2004. Jamip1 (marlin-1) de- fines a family of proteins interacting with janus ki- nases and microtubules. *J. Biol. Chem.* 279: 43168– 43177.
24. Couve, A. *et al*. 2004. Marlin-1, a novel RNA-binding protein associates with GABA receptors. *J. Biol. Chem.* 279: 13934–13943.
25. Ostermeier, C. & A.T. Brunger. 1999. Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of rabphilin-3A. *Cell* 96: 363– 374.

Figures and Tables

TABLE 1. Genes Preferentially Expressed Either in Melanotropes Exhibiting Hypersecretory Phenotypeor in those Displaying Hyposecretory Phenotype

	Gene	% Homology	Species	
				26.
Hypersecretory	*Apg3p*	87 (*NM_022488*)	*Homo sapiens*	E2-like enzyme
	Cyt b	84 (*AB036398*)	*Rana porosa*	Mitochondrial electron transport chain component
	Gpx4	75 (*NM_017165*)	*Rattus norvegicus*	Glutathione peroxidase
	S6	86 (*L19996*)	*Xenoupus laevis*	Ribosomal protein
	POMC	100 (*M62770*)	*Rana ridibunda*	Precursor protein
	SSAT	83 (*XM_131957*)	*Mus musculus*	N-acetyltransferase
	SEC23	86 (*NM_006363*)	*Homo sapiens*	COPII-coat protein
	PTP4A1	86 (*NM_003463*)	*Homo sapiens*	Tyrosine phosphatase
	TAX1bp1	80 (*BC110936.1*)	*Xenopus laevis*	Ubiquitin enzyme activator
	OGDH	88 (*BC061938.1*)	*Xenopus laevis*	Mitochondrial enzyme
	Cadherin 11	86 (*NM_009866.4*)	*Mus musculus*	Cell-adhesion molecule
	PHLDA2	89 (*BC165501.1*)	*Danio rerio*	Pleckstrin homology-like domain
Hyposecretory	*FBP*	84 (*NM_022488*)	*Mus musculus*	Fibroblast growth factor-binding protein
	XHp1	85 (*XM_167037*)	*Xenopus laevis*	Unknown
	VCP	82 (*BC0167037*)	*Homo sapiens*	Target of Akt signaling
	AC006081	88 (*NM_127571*)	*Arabidopsis thaliana*	Unknown
	XCTGF	85 (*XLU43524*)	*Xenopus laevis*	Connective tissue growth factor
	L36/BARD1	71 (*NP_115868*)	*Homo sapiens*	Mitochondrial ribosomal protein
	KIAA0555	88 (*AB011127*)	*Homo sapiens*	Unknown
	CGB	99 (*AF453421.1*)	*Rana ridibunda*	Neuroendocrine secretory protein
	RAB18	85 (*AF137372*)	*Homo sapiens*	Small GTPase protein

Assessed by differential display. Percent homology was calculated matching the sequence obtained by differential display with that corresponding to the Gene Bank Accession Number indicated between parentheses. Abbreviations: E2, ubiquitin-conjugating enzyme 2; COPII, coat-protein complex II; Akt, protein kinase B.

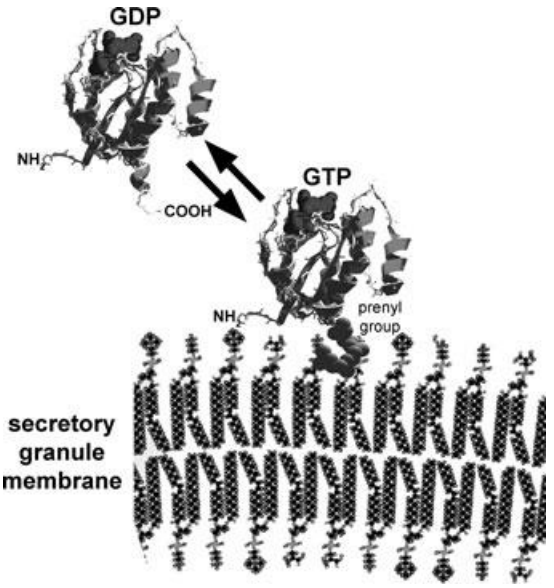

Figure 1. A model for Rab18 recruitment to se- cretory granule membranes. Rab18 structure has been modelled based on the X-ray crystallographyof Rab3A.[25] Rab18 in its guanosine 5[r]-diphosphate (GDP)-bound conformation is present in the cytosol.Upon activation of the secretory process, Rab18 would be activated by a specific GDP-to-guanosine 5[r]-triphosphate (GTP) exchange factor, converting the protein into its GTP-bound form and allowing the prenyl group located at the C-terminal domain of Rab18 to bind to the granule surface. GTP-boundRab18 would then be able to recruit yet unknowneffectors that would stabilize the GTPase on the se- cretory granule membrane.

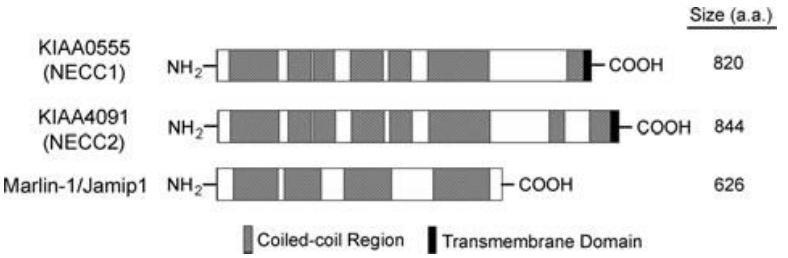

Figure 2. Schematic representations of KIAA0555 (NECC1), KIAA4091 (NECC2), and Marlin-1/Jamip1 showing their relative sizes and overall structures. Coiled-coil regions and transmembrane domains are represented by hatched and black boxes, respectively.

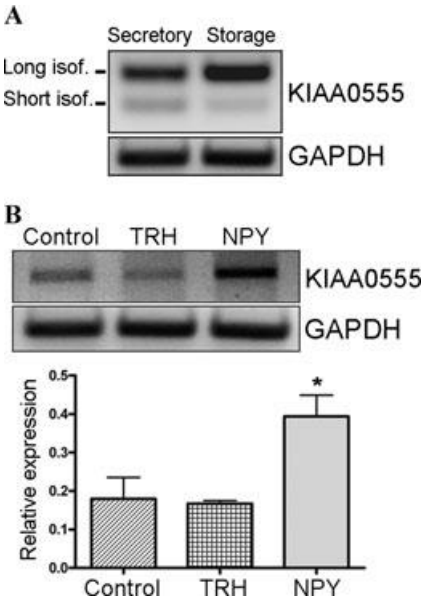

Figure 3. Regulation of KIAA0555 (NECC1) ex- pression in frog melanotrope cells. (A) Semiquantitative reverse transcriptase (RT)-PCR analysis of the longand short isoforms of KIAA0555 in total RNA extracts from secretory and storage melanotropes. (B) Semi- quantitative RT-PCR analysis of KIAA0555 mRNA frog melanotrope cell cultures incubated for 24 h in the absence or presence of 10^{-7} mol/L thyrotropin- releasing factor (TRH) or 10^{-7} mol/L neuropeptide Y (NPY). Amplification was carried out using a primer pair amplifying both KIAA0555 mRNA isoforms. Results are shown as the ratio to glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) mRNA lev-els from at least three independent experiments. Asterisk indicates $P < 0.05$. In both analyses, GAPDH amplification was used as the internalcontrol.