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COMMENTARY

Heterotrimeric G-proteins and Development

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ABSTRACT. Heterotrimeric G-proteins are well-known transducers of signaling from a populous class of heptihelical, membrane receptors to a smaller group of effector molecules that includes adenylylcyclases, cyclic GMP phosphodiesterases, phospholipases (type Cβ), and various ion channels. Dramatic changes in specific G-protein subunits that coincide with commitment to highly-specialized cell types suggest a key role for these extrinsic membrane proteins in cell differentiation and development. Through analysis of the effects of gain-of-function and loss-of-function mutants, it has been possible to explore this new dimension in G-protein biology, intimately linking specific G-proteins to development. G-protein subunits are shown to be important molecular switches in the complex biological processes controlling both cellular differentiation and early development. *Copyright © 1996 Elsevier Science Inc.*, BIOCHEM PHARMACOL 53;1:1–4, 1997.

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Heterotrimeric G-proteins† are composed of three subunits, α, β, and γ (1:1:1), with sizes ranging from 39,000 to 80,000 for the >20 α-subunits, ~35,000 for 6 β-subunits, and ~8,000 for >9 γ-subunits [1]. Easily dissociable into an α-subunit and a β/γ complex upon GTP binding by the α-subunit, both products of dissociation can alter the activities of specific effectors, e.g. α-subunit regulation of cyclic GMP phosphodiesterase in vision and β/γ complex regulation of various ion channels [2]. These G-proteins are responsible for signal propagation from a populous class of heptihelical, membrane receptors (estimated at >1000 members) to the smaller group of effector molecules including adenylylcyclase, phospholipases, and ion channels [3]. GTP hydrolysis, intrinsic to the α-subunits, terminates the activation by GTP binding; the β/γ complex re-combines with the GDP-bound α-subunit that now must await a new round of the GDP/GTP exchange catalyzed by G-protein-linked receptors, which permits GTP re-binding, α- and β/γ subunit dissociation, and effector activation again.

G-proteins, providing critical points of regulation in complex pathways, were adopted as substrates targeted by bacterial toxins, which take advantage of these pathways to ensure widespread infection. Thus, the spread of cholera is virtually ensured by the ability of its toxin to activate the stimulatory G-protein for adenylylcyclase and provoke transluminal water movement to the bowel, spreading the infection to the environment [3]. So too, their central role in controlling critical biological processes makes G-proteins valuable points of regulation in cell growth, differentiation, and development. Although lacking many details that await further study, a Gestalt constructed with recent data from a variety of experimental models provides a compelling and provocative case for a central role for G-proteins in development.

Early insight into possible roles of G-proteins in growth and development was revealed by study of the effects of nerve growth factor, (NGF) on human PC12 pheochromocytoma cells [4]. The α-subunit of the G-protein, termed G_o was localized in high abundance to growth cones of developing neurites in cells treated with NGF. G_o is highly abundant in nervous tissue, and its localization to growth cones of neurites and regulation by GAP-43 suggested a critical role of G_o in the development of nervous tissue. Furthermore, blocking the expression of G_o in the PC12 cells caused a collapse of the nerve growth cones [5]. Although unclear at the time of these studies, G-proteins appear to play a role in growth factor action, mitogenesis, and differentiation. It will be of great interest to probe the role of specific G-protein subunits in the developing nervous system of transgenic mice in which the genes encoding these important molecules are disrupted by homologous recombination, i.e. "knocked-out."

Adipogenesis provides a dramatic model for the study of differentiation, as embryonic stem cells "turn on" an array of genes that impart to them the capacity to synthesize and store triglyceride. The NIH 3T3-L1 cell line has proven invaluable as a model for adipogenesis in which either high concentrations of insulin or the simultaneous addition of dexamethasone and methylisobutylxanthine induce a 7- to 10-day progression of stem cells to lipid-laden adipocytes

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† *Abbreviations:* G-protein, heterotrimeric GTP-binding regulatory proteins; MAP Kinase, mitogen-activated protein kinase; KO, knock-out of a gene by homologous recombination; TKO, technical knock-out of a target gene by RNA antisense to the gene mRNA; NGF, nerve growth factor.

[6]. In these cells, analysis of G-protein complement revealed a startling decline in the α-subunit of the G-protein, *stimulatory* with respect to adenylylcyclase, termed G_s [7]. $G_{s\alpha}$ expression was shown to repress the progression of 3T3-L1 cells to adipocytes; oligodeoxynucleotides antisense, but not sense or missense, to $G_{s\alpha}$ accelerated the ability of inducers to promote progression from >7 to <3.5 days. Oligodeoxynucleotides antisense to $G_{s\alpha}$ alone were capable of inducing adipogenesis, mimicking the decline in $G_{s\alpha}$ promoted by classical inducers [7]. Stabilizing $G_{s\alpha}$ levels by use of an expression vector blocked the ability of inducers to promote adipogenesis. It is important to note that these changes in $G_{s\alpha}$ regulate adipogenesis independently of adenylylcyclase activation and cyclic AMP levels [8], reflecting a role of a G-protein subunit not related to its known effector output.

The F9 teratocarcinoma stem cells provide a unique approach to the study of early mouse development [9]. The well-known morphogen retinoic acid induces these stem cell cultures to primitive endoderm, highlighted by the expression of the protease tissue-plasminogen activator, a hallmark of the primitive endoderm phenotype. Unlike adipogenesis in which $G_{s\alpha}$ plays a dominant role as a repressor, in the F9 cells the α-subunit of the G-protein, *inhibitory* with respect to adenylylcyclase, termed $G_{i\alpha 2}$, plays a critical role in blocking progression to primitive endoderm [10]. For these cells, the role of $G_{i\alpha 2}$ was probed by a different technique, stable infection with a retrovirus harboring an expression vector that produced RNA antisense to $G_{i\alpha 2}$ [10]. Mimicking the sharp decline in $G_{i\alpha 2}$ that retinoic acid provokes, antisense RNA (loss-of-function) promoted progression of the stem cells to primitive endoderm, whereas over-expression of the wild-type or constitutively active Q205L mutants of $G_{i\alpha 2}$ (gain-of-function) blocked progression in response to the morphogen [11]. Expression of RNA antisense to other G-protein subunits failed to induce progression. Recently, it has been shown that $G_{i\alpha 2}$ exerts this influence on stem cell progression via phospholipase C, which, like adenylylcyclase, is suppressed by $G_{i\alpha 2}$ [12, 13]. In addition, the linkage of $G_{i\alpha 2}$ via phospholipase C has been shown to extend to the mitogen-activated MAPK regulatory network [13].

Other linkages between cell growth and G-proteins have been illuminated. Expression of constitutively active $G_{i\alpha 2}$ induces neoplastic transformation of Rat-1 cells [14, 15]. Mutations of $G_{i\alpha 2}$ have been identified in several human endocrine tumors, including adrenal cortex and ovary [16, 17]. $G_{i\alpha 2}$ has been shown to translocate to the nucleus upon growth factor-induced cell division [18] and microinjection of antibodies to $G_{i\alpha 2}$ has been shown to block the ability of the G-protein-linked receptor for thrombin to stimulate DNA synthesis [19]. These provocative data prompted the analysis of $G_{i\alpha 2}$ function *in vivo* using two complementary, but distinct approaches. Use of conditional, tissue-specific promoters for expression of antisense RNA is in its infancy, but already has proven successful for the "technical knockout (TKO)" of $G_{i\alpha 2}$ in two major sites, liver and adipose tissue [20]. Mice deficient of $G_{i\alpha 2}$ in these target tissues display a runted phenotype and blunting of the inhibitory adenylylcyclase pathway [20]. More recently, it has been shown that these mice display frank insulin resistance, with respect to anti-lipolytic, hexose transport and glycogen synthase activities [21], providing a linkage between the G-protein-linked and tyrosine kinase-linked, growth factor-activated pathways. Via homologous recombination, $G_{i\alpha 2}$-deficient knock-out mice were created [22], displaying many of the features reported earlier with the conditional TKO mice [20]. In addition, loss of $G_{i\alpha 2}$ was associated with ulcerative colitis and adenocarcinoma of the colon [22]. Thus, G-proteins display the ability to regulate the MAPK regulatory network at a variety of upstream and downstream points, rich with crosstalk to those elements shared with the growth factor-sensitive pathways [23, 24].

Several major questions evolve from our current yet largely incomplete understanding of G-proteins as critical elements influencing growth, differentiation, and development, namely, how do they work in this regard, how is their expression controlled, and what role, if any, do they play in human development? Clearly, some effects of G-proteins in development are exerted through well-known effectors, explaining the potential of $G_{i\alpha 2}$ and $G_{s\alpha}$ as proto-oncogenes in cells for which cyclic AMP is mitogenic [16, 17]. In the F9 teratocarcinoma cells, $G_{i\alpha 2}$ represses phospholipase C and the morphogen-induced decline in $G_{i\alpha 2}$ de-represses its activity, yielding inositol phosphates involved in calcium mobilization and diacylglycerol which activates protein kinase C and, thereby, MAPK [13]. Other effectors for G-proteins are more poorly understood. The control of adipogenesis by $G_{s\alpha}$ is a good example, for it seems likely that some effector(s) that can be regulated by the $G_{i\alpha 2}/G_{s\alpha}$ axis, other than adenylylcyclase, is (are) operating. Although speculation, we hypothesize the existence of G-protein-sensitive tyrosine kinase(s) linking G-proteins with downstream effectors involved in mitogenesis, differentiation, and perhaps, apoptosis (Table 1). Changes in the level of phosphotyrosine content of the 3T3-L1 cells occur throughout progression to adipocytes and are sensitive to the $G_{i\alpha 2}/G_{s\alpha}$ axis, but not to either elevation or suppression of cyclic AMP [8]. Further support for this hypothesis was obtained through recent studies in which several non-receptor tyrosine kinases were revealed to be obligatory for G-protein-coupled activation of MAPK [25]. Although gathered from a variety of cell lines that may not be representative of all, or even most, cells, these data support a testable hypothesis which, if validated, would provide a new paradigm in cell signaling, highlighting the interplay between G-proteins and tyrosine kinases.

How are levels of G-protein subunits controlled in cells? Although many studies have illuminated the phenomenology of the changes in G-protein expression, few molecular details have emerged. Clearly, G-protein levels are subject to translational and post-translational mechanisms of regulation. Much further work will be required to describe in

TABLE 1. Schematic for G-protein-mediated control of the MAPK regulatory network: Hypothesis for the existence of non-receptor tyrosine kinase(s) sensitive to G-protein subunits

	G-protein-linked receptor	Growth factor & receptor
Ligand	Hormones, autocoids, neurotransmitters	Growth factors
Receptor	G-protein-linked receptor heptihelical, membrane-bound	Growth factor receptor with intrinsic *tyrosine kinase* membrane-bound
Elements downstream	Heterotrimeric G-*proteins*	SHC/GRB2<Sos<*ras*
"Hypothesized"	Non-receptor *tyrosine kinases*	
MAPKKK	MEKK	Raf-1
MAPKK	MEK	MEK
MAPK	MAPK	MAPK
	Transcription factors	
	Mitogenesis, differentiation, apoptosis	

See the text for details. Abbreviations: MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPK kinase kinase; and MEK, mitogen and extracellular receptor-activated protein kinase, a member of the MAPKK family.

precise terms, for example, how retinoic acid stimulates a rapid and profound loss of $G_{i\alpha 2}$ in F9 cells, or how inducers of adipogenesis stimulate the decline in $G_{s\alpha}$.

Based upon the foregoing discussion of G-proteins and their roles in regulation, one would predict the occurrence of human disease in which G-protein biology is perturbed. Infectious diseases, such as whooping cough and cholera, provide graphic examples of pathophysiologies in which bacterial infection targets G-proteins in humans. Endocrine tumors arise from the activation of the proto-oncogenes for $G_{i\alpha 2}$ and $G_{s\alpha}$ (16, 17]. Genetic mutations of $G_{s\alpha}$ are associated with pseudohypoparathyroidism, Albrights hereditary osteodystrophy (AHO), and McCune-Albright syndrome (MAS) [26]. Patients suffering from either AHO or MAS display sometimes profound alterations in normal development. Not surprising, mutations in the receptors that are coupled to G-proteins also are responsible for a variety of human diseases [27, 28]. As we progress in our understanding of G-protein biology in development, as well as in our experience with conditional, targeted expression of G-protein subunits and RNA antisense to them, we can begin not only to address the molecular features of these human diseases, but also to develop new therapeutic strategies via gene transfer. Future studies will undoubtedly reveal new facets of G-protein function as well as a new set of problems perhaps more challenging than those with which we are confronted today.

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