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PLANT CELL, Nov 2012; 24: 4670 - 4686.

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Glycobiology, Apr 2013; 23: 426 - 437.

**ORIGINAL MANUSCRIPT:**
**Wnt5a promotes human colon cancer cell migration and invasion but does not augment intestinal tumorigenesis in *Apc*1638N mice**
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**Carcinogenesis,** Jul 2013; 10.1093/carcin/bgt215.





# Wnt5a promotes human colon cancer cell migration and invasion but does not augment intestinal tumorigenesis in *Apc*1638N mice

**Elvira R.M. Bakker[1], Asha Mooppilmadham Das[2], Werner Helvensteijn[1], Patrick F. Franken[3], Sigrid Swagemakers[4], Martin A. van der Valk[5], Timo L.M. ten Hagen[2], Ernst J. Kuipers[1,6], Wendy van Veelen[1] and Ron Smits[1,*]**

[1] Department of Gastroenterology and Hepatology,

[2] Department of Surgery,

[3] Department of Experimental Pathology and

[4] Department of Bioinformatics, Erasmus MC University Medical Center Rotterdam, 3015 CE Rotterdam, The Netherlands,

[5] Department of Experimental Animal Pathology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands and

[6] Department of Internal Medicine, Erasmus MC University Medical Center Rotterdam, 3015 CE Rotterdam, The Netherlands Correspondence: *To whom correspondence should be addressed. Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. Tel: +31 10 7035944; Fax: +31 10 7032793; Email: m.j.m.smits@erasmusmc.nl

Whereas aberrant activation of canonical Wnt/β-catenin signaling underlies the majority of colorectal cancer cases, the contribution of non-canonical Wnt signaling is unclear. As enhanced expression of the most extensively studied non-canonical Wnt ligand WNT5A is observed in various diseases including colon cancer, WNT5A is gaining attention nowadays. Numerous *in vitro* studies suggest modulating capacities of WNT5A on proliferation, differentiation, migration and invasion, affecting tumor and non-mutant cells. However, a possible contribution of WNT5A to colorectal cancer remains to be elucidated. We have analyzed WNT5A expression in colorectal cancer profiling data sets, altered WNT5A expression in colon cancer cells and used our inducible Wnt5a transgenic mouse model to gain more insight into the role of WNT5A in intestinal cancer. We observed that increased *WNT5A* expression is associated with poor prognosis of colorectal cancer patients. WNT5A knockdown in human colon cancer cells caused reduced directional migration, deregulated focal adhesion site formation and reduced invasion, whereas Wnt5a administration promoted the directional migration of colon cancer cells. Despite these observed protumorigenic activities of WNT5A, the induction of Wnt5a expression in intestinal tumors of *Apc*1638N mice was not sufficient to augment malignancy or metastasis by itself. In conclusion, WNT5A promotes adhesion sites to form in a focal fashion and promotes the directional migration and invasion of colon cancer cells. Although these activities appear insufficient by themselves to augment malignancy or metastasis in *Apc*1638N mice, they might explain the poor colon cancer prognosis associated with enhanced *WNT5A* expression.

**Abbreviations:** APC, adenomatous polyposis coli • DMEM, Dulbecco's modified Eagle's medium • FAK, focal adhesion kinase • FBS, fetal bovine serum • PBS, phosphate-buffered saline • p/s, penicillin/streptomycin • shRNA, short hairpin RNA.

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**CELL BIOLOGY:**
**Nitric Oxide Modifies Global Histone Methylation by Inhibiting Jumonji C Domain-containing Demethylases**
Jason R. Hickok, Divya Vasudevan, Divya Vasudevan, William E. Antholine, William E. Antholine, Douglas D. Thomas, and Douglas D. Thomas
**J. Biol. Chem.,** May 2013; 288: 16004 - 16015.



**Background:** The methylation status of histone tails is a balance

between methylation and demethylation.
**Results:** Nitric oxide inhibits lysine demethylase 3A and alters cellular histone methylation patterns.
**Conclusion:** Nitric oxide can significantly modify the epigenetic landscape.
**Significance:** These results establish nitric oxide as a physiological epigenetic regulator acting through a nonclassical cell signaling mechanism.

**Nitric Oxide Modifies Global Histone Methylation by Inhibiting Jumonji C Domain-containing Demethylases**[*◆]

**Jason R. Hickok[‡], Divya Vasudevan[‡], William E. Antholine[§], and Douglas D. Thomas[‡1]**

From the [‡]Departments of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, Illinois 60612 and the [§]Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

## ABSTRACT

Methylation of lysine residues on histone tails is an important epigenetic modification that is dynamically regulated through the combined effects of methyltransferases and demethylases. The Jumonji C domain Fe(II) α-ketoglutarate family of proteins performs the majority of histone demethylation. We demonstrate that nitric oxide (•NO) directly inhibits the activity of the demethylase KDM3A by forming a nitrosyliron complex in the catalytic pocket. Exposing cells to either chemical or cellular sources of •NO resulted in a significant increase in dimethyl Lys-9 on histone 3 (H3K9me2), the preferred substrate for KDM3A. G9a, the primary methyltransferase acting on H3K9me2, was down-regulated in response to •NO, and changes in methylation state could not be accounted for by methylation in general. Furthermore, cellular iron sequestration via dinitrosyliron complex formation correlated with increased methylation. The mRNA of several histone demethylases and methyltransferases was also differentially regulated in response to •NO. Taken together, these data reveal three novel and distinct mechanisms whereby •NO can affect histone methylation as follows: direct inhibition of Jumonji C demethylase activity, reduction in iron cofactor availability, and regulation of expression of methyl-modifying enzymes. This model of •NO as an epigenetic modulator provides a novel explanation for nonclassical gene regulation by •NO.

**Key Words:** Cell Biology • Epigenetics • Histones • Iron • Nitric Oxide • Demethylases • Methyltransferases
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Correspondence: [1] To whom correspondence should be addressed: Dept. of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St. (MC 781), Chicago, IL 60612-7231. Tel.: 312-996-6156; Fax: 312-996-7107; E-mail: ddthomas@uic.edu.



**Oesophagus**
Original article

## microRNA-145 in Barrett's oesophagus: regulating BMP4 signalling via GATA6

**Jantine W P M van Baal[1], Romy E Verbeek[1], Pauline Bus[1], Matteo Fassan[2], Rhonda F Souza[3], Massimo Rugge[2], Fiebo J W ten Kate[4], Frank P Vleggaar[1] and Peter D Siersema[1]**

[1] Department of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, The Netherlands
[2] Department of Medical Diagnostic Sciences and Special Therapies, Surgical Pathology and Cytopathology Unit, University of Padova, Padova, Italy
[3] Department of Medicine, UT Southwestern Medical Center, Dallas, Texas, USA
[4] Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands

Correspondence: [Correspondence to] Jantine W P M van Baal, Department of Gastroenterology and Hepatology, F02.816, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA Utrecht, The Netherlands; j.w.p.m.vanbaal-2@umcutrecht.nl

Objective: Barrett's oesophagus (BE) is a metaplastic condition of the distal oesophagus which predisposes to oesophageal adenocarcinoma (EAC). It has been suggested that microRNAs (miRNAs) are involved in the process of development of BE and EAC; however, few functional miRNA data are available. The aim of the study was to perform a tissue-specific miRNA profile and, based on this, to examine the function of miRNA-145 in the oesophagus.
Design: miRNA expression profiling using microarray analysis in EAC, BE and normal squamous epithelium of the oesophagus (SQ) was performed and validated using real-time PCR in samples from 15 patients and in situ hybridisation in samples from 10 patients. The proliferative effect of miRNA-145 precursor transfection in the SQ (HET-1A) and BE cell line (BAR-T) was measured. Downstream targets of miRNA-145 were determined by analysing mRNA and protein expression from miRNA-145 transfected cells.
Results: Three unique miRNA expression profiles were found in tissue from EAC, BE and SQ, which showed that miRNA-145 was upregulated in BE compared with EAC and SQ. Overexpression of miRNA-145 in HET-1A and BAR-T cells reduced cell proliferation and inhibited GATA6, BMP4 and SOX9 mRNA expression. Furthermore, altered BMP4 signalling was observed in vitro on miRNA-145 overexpression. These effects were blocked when cells were co-transfected with a miRNA-145 specific inhibitor. Additionally, BMP4 incubation of HET-1A cells altered miRNA-145 and GATA6 expression over time.
Conclusion: These results imply that miRNA-145 indirectly targets BMP4 via GATA6 and is potentially involved in the development of BE.
**Key Words:** Barrett's oesophagus • microRNA profiling • microRNA-145 • bone morphogenetic protein 4 • cell signalling • Barrett's metaplasia • Barrett's carcinoma • Barrett's oesophagus • gene expression • Barrett's carcinoma • Barrett's oesophagus • carcinogenesis • molecular pathology • molecular oncology • molecular carcinogenesis • gastric adenocarcinoma • gastric metaplasia • gastrointestinal pathology • pre-malignancy—GI tract • helicobacter pylori —damage • hepatitis • endoscopy • colorectal carcinoma • quality of life • oesophageal cancer • palliation of oesophageal cancer • oesophageal disease • brachytherapy • stents





**Biological microdosimetry based on radiation cytotoxicity data**

**B. R. Scott[1,*], J. Hutt[1], Y. Lin[1], M. T. Padilla[1], K. M. Gott[1] and C. A. Potter[2]**

[1] Lovelace Respiratory Research Institute, Albuquerque, NM, USA

[2] Sandia National Laboratories, Organization 00545, Albuquerque, NM, USA

Correspondence: [*] Corresponding author. bscott@lrri.org

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Researchers in the field of radiation microdosimetry have attempted to explain the relative biological effectiveness (RBE) of different ionising photon radiation sources on the basis of the singly stochastic, microdose metric lineal energy *y*, which only addresses physical stochasticity related to energy ($\varepsilon$) deposition via single events in the critical targets (cell nuclei assumed here). Biological stochasticity related to variable nuclei geometries and cell orientations (relative to the incoming radiation) is usually not addressed. Here a doubly stochastic microdose metric, the *single-event hit size q* ($=\varepsilon/T$), is introduced which allows the track length *T* to be stochastic. The new metric is used in a plausible model of metabolic-activity-based *in vitro* cytotoxicity of low-dose ionising photon radiation. The cytotoxicity model has parameters $E\{q\}$ (*average single-event hit size with q assumed to be exponentially distributed*) and $E\{\alpha\}$, which is the average value of the cellular response parameter $\alpha$. $E\{\alpha\}$ is referred to as the *biological signature* and it is independent of *q*. Only $E\{q\}$ is needed for determination of RBE. The model is used to obtain *biological-microdosimetry-based q spectra* for 320-kV X-rays and $^{137}$Cs gamma rays and the related RBE for cytotoxicity. The spectra are similar to published lineal energy *y* spectra for 200-kV X-rays and $^{60}$Co gamma rays for 1-μm biological targets.

**BIOENERGETICS:**

**Asymmetric Dimethylarginine Induces Endothelial Nitric-oxide Synthase Mitochondrial Redistribution through the Nitration-mediated Activation of Akt1**

Ruslan Rafikov, Olga Rafikova, Saurabh Aggarwal, Christine Gross, Xutong Sun, Julin Desai, David Fulton, and Stephen M. Black

**J. Biol. Chem.,** Mar 2013; 288: 6212 - 6226.



**Background:** Asymmetric dimethylarginine (ADMA) can induce endothelial nitric-oxide synthase (eNOS) redistribution from the plasma membrane to the mitochondria.

**Results:** AMDA induces nitration of Akt1 at Tyr$^{350}$ within the client-binding domain, increasing its activation and enhancing eNOS phosphorylation.

**Conclusion:** Under physiologic conditions, Akt1-mediated redistribution of eNOS to the mitochondria enhances mitochondrial coupling.

**Significance:** Reducing Akt1 nitration may reduce the deleterious effects of Akt1 signaling in various pathologies.

## Asymmetric Dimethylarginine Induces Endothelial Nitric-oxide Synthase Mitochondrial Redistribution through the Nitration-mediated Activation of Akt1[*]

**Ruslan Rafikov, Olga Rafikova, Saurabh Aggarwal, Christine Gross, Xutong Sun, Julin Desai, David Fulton, and Stephen M. Black[1]**

From the Pulmonary Disease Program, Vascular Biology Center, Georgia Health Sciences University, Augusta, Georgia 30912

### ABSTRACT

We have recently demonstrated that asymmetric dimethylarginine (ADMA) induces the translocation of endothelial nitric-oxide synthase (eNOS) to the mitochondrion via a mechanism that requires protein nitration. Thus, the goal of this study was elucidate how eNOS redistributes to mitochondria and to identify the nitrated protein responsible for this event. Our data indicate that exposure of pulmonary arterial endothelial cells to ADMA enhanced eNOS phosphorylation at the Akt1-dependent phosphorylation sites Ser$^{617}$ and Ser$^{1179}$. Mutation of these serine residues to alanine (S617A and S1179A) inhibited nitration-mediated eNOS translocation to the mitochondria, whereas the phosphormimic mutations (S617D and S1179D) exhibited increased mitochondrial redistribution in the absence of ADMA. The overexpression of a dominant-negative Akt1 also attenuated ADMA-mediated eNOS mitochondrial translocation. Furthermore, ADMA enhanced Akt1 nitration and increased its activity. Mass spectrometry identified a single nitration site in Akt1 located at the tyrosine residue (Tyr$^{350}$) located within the client-binding domain. Replacement of Tyr$^{350}$ with phenylalanine abolished peroxynitrite-mediated eNOS translocation to mitochondria. We also found that in the absence of ADMA, eNOS translocation decreased mitochondrial oxygen consumption and superoxide production without altering cellular ATP level. This suggests that under physiologic conditions, eNOS translocation enhances mitochondria coupling. In conclusion, we have identified a new mechanism by which eNOS translocation to mitochondria is regulated by the phosphorylation of eNOS at Ser$^{617}$ and Ser$^{1179}$ by Akt1 and that this is enhanced when Akt1 becomes nitrated at Tyr$^{350}$.

**Key Words:** Akt • Bioenergetics • Endothelium • Mitochondria • Nitric-oxide Synthase

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Correspondence: [1] To whom correspondence should be addressed: 1459 Laney Walker Blvd., CB 3211-B, Augusta, GA 30912. Tel.: 706-721-7860; Fax: 706-721-9799; E-mail: SBLACK@gru.edu.

**ENZYMOLOGY:**

**Crystal Structure of Reduced MsAcg, a Putative Nitroreductase from *Mycobacterium smegmatis* and a Close Homologue of *Mycobacterium tuberculosis* Acg**

François-Xavier Chauviac, Martin Bommer, Martin Bommer, Jun Yan, Jun Yan, Gary Parkin, Gary Parkin, Tina Daviter, Tina Daviter, Philip Lowden, Philip Lowden, Emma L. Raven, Emma L. Raven, Konstantinos Thalassinos, Konstantinos Thalassinos, Nicholas H. Keep, and Nicholas H. Keep

**J. Biol. Chem.,** Dec 2012; 287: 44372 - 44383.



**Background:** Acg proteins are up-regulated during dormancy in tuberculosis.

**Results:** Acg proteins bind flavin mononucleotide like nitroreductases but with the active site closed by a lid. They are not reduced by NADPH or NADH.

**Conclusion:** Acg proteins may have evolved from active nitroreductases to sequester FMN instead.

**Significance:** Turning off a flavin-dependent pathway may be important in tuberculosis dormancy.

## Crystal Structure of Reduced MsAcg, a Putative Nitroreductase from *Mycobacterium smegmatis* and a Close Homologue of *Mycobacterium tuberculosis* Acg

François-Xavier Chauviac[‡1], Martin Bommer[§¶], Jun Yan[‡‖], Gary Parkin[**], Tina Daviter[#], Philip Lowden[‡], Emma L. Raven[**], Konstantinos Thalassinos[‡‖], and Nicholas H. Keep[‡2]

From the [‡]Crystallography, Institute for Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London, WC1E 7HX, United Kingdom,

[§]Institut für Biologie, Strukturbiologie/Biochemie, Humboldt-Universität zu Berlin, 10115 Berlin, Germany,

[¶]Macromolecular Crystallography Group, Institute of Soft Matter and Functional Materials, Helmholtz-Zentrum Berlin für Materialien und Energie, Albert-Einstein-Strasse 15, 12489 Berlin, Germany,

[‖]Institute of Structural and Molecular Biology, Division of Biosciences, Darwin Building, University College London, Gower Street, London, WC1E 6BT, United Kingdom,

[**]Department of Chemistry, University of Leicester, Leicester, LE1 7RH, United Kingdom, and

[#]Biophysics Centre, Institute for Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London, WC1E 7HX, United Kingdom

## ABSTRACT

This paper presents the structure of MsAcg (MSMEG_5246), a *Mycobacterium smegmatis* homologue of *Mycobacterium tuberculosis* Acg (Rv2032) in its reduced form at 1.6 Å resolution using x-ray crystallography. Rv2032 is one of the most induced genes under the hypoxic model of tuberculosis dormancy. The Acg family turns out to be unusual flavin mononucleotide (FMN)-binding proteins that have probably arisen by gene duplication and fusion from a classical homodimeric nitroreductase such that the monomeric protein resembles a classical nitroreductase dimer but with one active site deleted and the other active site covered by a unique lid. The FMN cofactor is not reduced by either NADH or NADPH, but the chemically reduced enzyme is capable of reduction of nitro substrates, albeit at no kinetic advantage over free FMN. The reduced enzyme is rapidly oxidized by oxygen but without any evidence for a radical state commonly seen in oxygen-sensitive nitroreductases. The presence of the unique lid domain, the lack of reduction by NAD(P)H, and the slow rate of reaction of the chemically reduced protein raises a possible alternative function of Acg proteins in FMN storage or sequestration from other biochemical pathways as part of the bacteria's adaptation to a dormancy state.

**Key Words:** Enzyme Structure • Flavin • Mycobacteria • Mycobacterium tuberculosis • Protein Evolution • Protein Structure • X-ray Crystallography
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## FOOTNOTES

[1] Sponsored by a Bloomsbury Colleges Studentship. Present address: Virology Dept., Structural Virology Unit, Institut Pasteur, 28 rue du docteur Roux, 75724 Paris cedex 15, France.

Correspondence: [2] To whom correspondence should be addressed: Crystallography, Institute for Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London, WC1E 7HX, UK. Tel.: 4420-7631-6852; Fax: 4420-7631-6803; E-mail: n.keep@mail.cryst.bbk.ac.uk.



RESEARCH ARTICLES

## A Galactoglycerolipid Lipase Is Required for Triacylglycerol Accumulation and Survival Following Nitrogen Deprivation in *Chlamydomonas reinhardtii*[C],[W]

Xiaobo Li[a,b], Eric R. Moellering[a,c,1], Bensheng Liu[c], Cassandra Johnny[c], Marie Fedewa[c], Barbara B. Sears[b], Min-Hao Kuo[c] and Christoph Benning[c,2]

[a] Department of Energy–Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

[b] Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824

[c] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824

Correspondence: [2] Address correspondence to benning@msu.edu.

Following N deprivation, microalgae accumulate triacylglycerols (TAGs). To gain mechanistic insights into this phenomenon, we identified mutants with reduced TAG content following N deprivation in the model alga *Chlamydomonas reinhardtii*. In one of the mutants, the disruption of a galactoglycerolipid lipase-encoding gene, designated *PLASTID GALACTOGLYCEROLIPID DEGRADATION1* (*PGD1*), was responsible for the primary phenotype: reduced TAG content, altered TAG composition, and reduced galactoglycerolipid turnover. The recombinant PGD1 protein, which was purified from *Escherichia coli* extracts, hydrolyzed monogalactosyldiacylglycerol into its lyso-lipid derivative. In vivo pulse-chase labeling identified galactoglycerolipid pools as a major source of fatty acids esterified in TAGs following N deprivation. Moreover, the fatty acid flux from plastid lipids to TAG was decreased in the *pgd1* mutant. Apparently, de novo–synthesized fatty acids in *Chlamydomonas reinhardtii* are, at least partially, first incorporated into plastid lipids before they enter TAG synthesis. As a secondary effect, the *pgd1* mutant exhibited a loss of viability following N deprivation, which could be avoided by blocking photosynthetic electron transport. Thus, the *pgd1* mutant provides evidence for an important biological function of TAG synthesis following N deprivation, namely, relieving a detrimental overreduction of the photosynthetic electron transport chain.

**Abbreviations:** TAG, triacylglycerol • PtdCho, phosphatidylcholine • DGTS, diacylglycerol-*N,N,N*,-trimethylhomoserine • ACP, acyl carrier protein • ER, endoplasmic reticulum • TLC, thin layer chromatography • PtdEtn, phosphatidylethanolamine • MGDG, monogalactosyldiacylglycerol • DGDG, digalactosyldiacylglycerol • PtdGro, phosphatidylglycerol • SQDG, sulfoquinovosyldiacylglycerol • ROS, reactive oxygen species • DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea • TBARS, thiobarbituric acid reactive substances • TAP, Tris-acetate-phosphate



## Octamerization is essential for enzymatic function of human UDP-glucose pyrophosphorylase

Jana Führing[2], Sebastian Damerow[2,5], Roman Fedorov[3,4], Julia Schneider[2], Anja-Katharina Münster-Kühnel[2] and Rita Gerardy-Schahn[1,2]

[2] Institute for Cellular Chemistry

[3] Research Division for Structure Analysis

[4] Institute for Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

Correspondence: [1] To whom correspondence should be addressed: Tel: +49 511 532 9801; Fax: +49 511 532 8801; e-mail: gerardy-schahn.rita@mh-hannover.de

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Uridine diphosphate-glucose pyrophosphorylase (UGP) occupies a central position in carbohydrate metabolism in all kingdoms of life, since its product uridine diphosphate-glucose (UDP-glucose) is essential in a number of anabolic and catabolic pathways and is a precursor for other sugar nucleotides. Its significance as a virulence factor in protists and bacteria has given momentum to the search for species-specific inhibitors. These attempts are, however, hampered by high structural conservation of the active site architecture. A feature that discriminates UGPs of different species is the quaternary organization. While UGPs in protists are monomers, di- and tetrameric forms exist in bacteria, and crystal structures obtained for the enzyme from yeast and human identified octameric UGPs. These octamers are formed by contacts between highly conserved amino acids in the C-terminal β-helix. Still under debate is the question whether octamerization is required for the functionality of the human enzyme. Here, we used single amino acid replacements in the C-terminal β-helix to interrogate the impact of highly conserved residues on octamer formation and functional activity of human UGP (hUGP). Replacements were guided by the sequence of *Arabidopsis thaliana* UGP, known to be active as a monomer. Correlating the data obtained in blue native PAGE, size exclusion chromatography and enzymatic activity testing, we prove that the octamer is the active enzyme form. This new insight into structure–function relationships in hUGP does not only improve the understanding of the catalysis of this important enzyme, but in addition broadens the basis for studies aimed at designing drugs that selectively inhibit UGPs from pathogens.

**Key Words:** functional oligomerization • Leloir pathway • nucleotide sugar metabolism • UDP-glucose pyrophosphorylase

[5] Present address: Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, Scotland, UK.