tions, we observed a decrease in IP_7 cellular concentrations in response to phosphate starvation (A.S., unpublished data), suggesting that more characterization of this response may be necessary.

Lee et al. convincingly provide a link between Vip1-mediated inositol pyrophosphate signaling and phosphate metabolism. However, previous reports have also linked Kcs1 and its IP6K family members to phosphate metabolism, suggesting that both 5PP-IP_5 and 4/6PP-IP_5 have roles in these processes. Indeed, the first clue to a relationship between inositol pyrophosphate signaling and phosphate metabolism came from the discovery that PiUS, a stimulator of inorganic phosphate uptake, was in fact IP6K2 (Bennett et al., 2006). Then it was shown that inorganic phosphate uptake in yeast lacking Kcs1 is less efficient than in wild-type cells. Yeast lacking Kcs1 also have increased expression of the PHO phosphate response genes and reduced intracellular polyphosphate levels (Auesukaree et al., 2005; Bennett et al., 2006). Moreover, yeast lacking Kcs1 constitutively express the acid phosphatase PHO5, which is activated by Pho4, indicating that the IP_7 synthesized by Kcs1 also influences the PHO transcriptional pathway (Auesukaree et al., 2005). These observations may reflect different aspects of IP_7 function in phosphate sensing. It is possible that 4/6PP-IP_5 mainly regulates the PHO pathway by protein binding, whereas 5PP-IP_5 primarily functions as a phosphate donor.

The two new studies raise several exciting possibilities that could advance our understanding of inositol pyrophosphates and their roles in many different aspects of cell signaling. Certainly, the notion that two structurally different IP_7 molecules can have distinct cellular roles is intriguing and will no doubt guide the way to more pioneering work. In the past decade, a large number of inositol pyrophosphate species, not only IP_7 and IP_8, have been discovered as well as several inositol pyrophosphate synthases and phosphatases. Further studies into these fascinating signaling molecules may result in the discovery of other species, perhaps even pyrophosphorylated inositol lipids.

REFERENCES

Auesukaree, C., Tochio, H., Shirakawa, M., Kaneko, Y., and Harashima, S. (2005). J. Biol. Chem. *280*, 25127–25133.

Bennett, M., Onnebo, S.M., Azevedo, C., and Saiardi, A. (2006). Cell. Mol. Life Sci. *63*, 552–564.

Huang, C.F., Voglmaier, S.M., Bembenek, M.E., Saiardi, A., and Snyder, S.H. (1998). Biochemistry *37*, 14998–15004.

Lee, Y.S., Mulugu, S., York, J.D., and O'Shea, E.K. (2007). Science *316*, 109–112.

Menniti, F.S., Miller, R.N., Putney, J.W., Jr., and Shears, S.B. (1993). J. Biol. Chem. *268*, 3850–3856.

Mulugu, S., Bai, W., Fridy, P.C., Bastidas, R.J., Otto, J.C., Dollins, D.E., Haystead, T.A., Ribeiro, A.A., and York, J.D. (2007). Science *316*, 106–109.

Saiardi, A., Erdjument-Bromage, H., Snowman, A.M., Tempst, P., and Snyder, S.H. (1999). Curr. Biol. *9*, 1323–1326.

Saiardi, A., Bhandari, R., Resnick, A.C., Snowman, A.M., and Snyder, S.H. (2004). Science *306*, 2101–2105.

Stephens, L., Radenberg, T., Thiel, U., Vogel, G., Khoo, K.H., Dell, A., Jackson, T.R., Hawkins, P.T., and Mayr, G.W. (1993). J. Biol. Chem. *268*, 4009–4015.

York, S.J., Armbruster, B.N., Greenwell, P., Petes, T.D., and York, J.D. (2005). J. Biol. Chem. *280*, 4264–4269.

Guiding Ligands to Nuclear Receptors

Liliane Michalik[1] and Walter Wahli[1,*]
[1]Center for Integrative Genomics, National Research Center Frontiers in Genetics, University of Lausanne, Switzerland
*Correspondence: walter.wahli@unil.ch
DOI 10.1016/j.cell.2007.05.001

Retinoic acid—the active metabolite of vitamin A—influences biological processes by activating the retinoic acid receptor (RAR). In this issue, Schug et al. (2007) demonstrate that retinoic acid also activates the peroxisome proliferator-activated receptor β/δ (PPARβ/δ). Remarkably, retinoic acid signaling through RAR or PPARβ/δ—which depends on cytoplasmic retinoic acid transporters—commits the cell to opposite fates, apoptosis or survival, respectively.

The beneficial effects of vitamin A might have been recognized first by the ancient Egyptians, who treated eye disease with raw liver. The fact that they recognized a connection between eye problems and the liver, which is the richest source of dietary vitamin A, is astonishing. Today, we know that vitamin A (retinol) and its biologically active derivatives, the retinoids (the most potent of which is all-*trans*-retinoic acid), regulate key processes such as inhibition of cell proliferation, differentiation, apoptosis, shaping of the embryo,

and organogenesis. Vitamin A deficiency causes congenital malformations of the eye, heart, gonads, and lungs. In both youths and adults, lack of vitamin A impairs growth, vision, reproduction, and homeostasis of several organs (Mark et al., 2006). Active metabolites of vitamin A can prevent and cure a majority of these defects. In addition, retinoids have anticancer properties, as illustrated by their efficacy in the treatment of promyelocytic leukemia.

A long-standing question has been how a simple molecule like all-*trans*-retinoic acid (RA) exerts pleiotropic actions. In an elegant study presented in this issue, Noy and colleagues (Schug et al., 2007) provide a new and unexpected answer to this question. These authors reveal that RA can signal through two nuclear hormone receptors, the retinoic acid receptor (RAR) and the peroxisome proliferator-activated receptor β/δ (PPARβ/δ), leading to opposite outcomes for the cells expressing these receptors: apoptosis and survival, respectively (Figure 1).

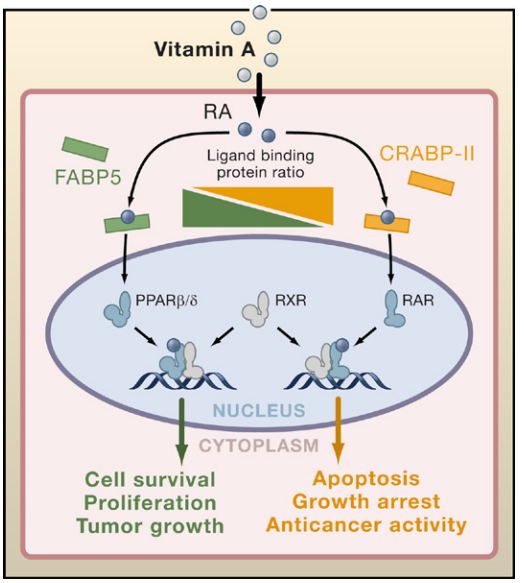

Figure 1. Retinoic Acid Activates RAR and PPARβ/δ
Vitamin A is internalized by the cell and metabolized into its active derivative, retinoic acid (RA). In the aqueous intracellular milieu, RA is transported by the retinoid-binding protein CRABP-II, or by the fatty-acid-binding protein FABP5, depending on the ratio of FABP5 to CRABP-II. In a cell type expressing high CRABP-II and low FABP5, RA activates the retinoic acid receptor (RAR), whereas in the presence of the reverse ratio (low CRABP-II and high FABP5), RA activates the peroxisome proliferator-activated receptor β/δ (PPAR). This leads to opposite cellular outcomes: either apoptosis, growth arrest, and anticancer activity or survival, proliferation, and tumor growth, respectively. In both situations, retinoid X receptor (RXR) is the indispensable dimerization partner of the nuclear receptor involved.

To date, only one type of nuclear receptor, RAR (α, β, and γ), was thought to bind to both RA and 9-*cis*-retinoic acid (Germain et al., 2006). RARs form heterodimers with retinoid X receptors (RXRs) α, β, or γ, which only bind to 9-*cis*-retinoic acid. RAR-RXR heterodimers regulate gene expression through molecular mechanisms involving a large number of coregulators (Glass and Rosenfeld, 2000). The interplay of these actors establishes a gradient of gene activity ranging from repression to full activation. As each component of this machinery is itself under specific transcriptional and posttranscriptional control, a diversity of responses is possible.

During their transport in the aqueous intracellular milieu, many hydrophobic ligands including retinoids are solubilized and stabilized by binding to proteins that participate in mediating their biological activities. Two such proteins, CRABP-I and CRABP-II, have a high affinity for RA. CRABP-I is thought to dampen the cellular responses to RA by promoting its degradation, whereas CRABP-II delivers RA to RAR. This activity is mediated by a protein-protein interaction between CRABP-II and RAR, resulting in a direct transport of the ligand to the receptor (Dong et al., 1999).

Both RA-binding proteins belong to an evolutionarily conserved family, which also includes nine fatty acid-binding proteins (FABPs) (Chmurzynska, 2006). Some of the latter are known to deliver ligands to the PPARs. For instance, specific interactions with keratinocyte FABP (FABP5) and adipocyte FABP (FABP4) selectively enhance the activity of PPARβ/δ and PPARγ, respectively. Interestingly, these FABPs relocate to the nucleus when bound to ligands that are selective for the PPAR isotype they activate. Thus, these FABPs control the transcriptional activities of their own ligands, which they transport to cognate PPARs in the nucleus (Tan et al., 2002). Interestingly, PPARs belong to the same type 2 class of receptors as RARs in the nuclear receptor superfamily (Michalik et al., 2006). Together, these findings suggest a coevolution of the fatty-acid- and retinoid-binding protein families in parallel with the RAR and PPAR families, which enabled the emergence of a system for directing a ligand to the appropriate receptor. An interesting question is whether the two associated systems, CRABPs-RAR and FABPs-PPARs, have completely tight modes of action or if some promiscuity remains at the expense of specificity but in favor of an increased diversity in responses.

The work of Schug et al. (2007) provides a first answer to this interrogation. RA usually displays anticarcinogenic, proapoptotic activity. The authors were intrigued by an effect of RA in promoting cell survival in some organs, such as skin, in an RAR-independent manner (Chapellier et al., 2002) and in a way that is reminiscent of PPARβ/δ activity (Di-Poi et al., 2002). They hypothesized that RA can control a repertoire of cellular responses that involves either RAR or PPARβ/δ. They found that—depending on the ratio of FABP5 to CRABP-II—RA activates RAR or PPARβ/δ. In mammary carcinoma MCF-7 cells and in keratinocytes, which express a low and high FABP5 to CRABP-II ratio, respectively, RA activates RAR in the former and PPARβ/δ in the latter. Thus, the surprising finding is that when the FABP5 to CRABP-II ratio is high, RA serves as a physiological ligand for PPARβ/δ. This directed transport of RA to PPARβ/δ broadens the spectrum of physiological regu-

lation of the receptor's activity in an unexpected way, and it sheds new light on RA as an antiapoptotic agent and on PPARβ/δ as an RA receptor. This raises numerous new questions. Does RA exert antiapoptotic, proproliferative effects in tumors other than the mammary cancer model used by Schug et al. (2007)? Are some developmental processes, which are controlled by RA, dependent on directed transport of RA to PPARβ/δ? Can the switch from proliferation to differentiation observed in organ development be a consequence of ligand preference for one receptor over the other? PPARβ/δ, in addition to cell-survival functions, also participates in metabolic regulation under the control of fatty-acid ligands. Does RA participate in these regulations under specific conditions?

In fact, the key issue raised by the work of Schug et al. (2007) concerns the importance of directed ligand transport in nuclear receptor activation and ligand-dependent crosstalk between different receptor types. Breaking barriers between receptor categories by this mechanism may not be unique to RAR and PPARβ/δ. Promiscuity induced by directed ligand transport may participate significantly in the astonishing pleiotropic effects of key members of the nuclear receptor superfamily.

REFERENCES

Chapellier, B., Mark, M., Messaddeq, N., Calleja, C., Warot, X., Brocard, J., Gerard, C., Li, M., Metzger, D., Ghyselinck, N.B., and Chambon, P. (2002). EMBO J. *21*, 3402–3413.

Chmurzynska, A. (2006). J. Appl. Genet. *47*, 39–48.

Di-Poi, N., Tan, N.S., Michalik, L., Wahli, W., and Desvergne, B. (2002). Mol. Cell *10*, 721–733.

Dong, D., Ruuska, S.E., Levinthal, D.J., and Noy, N. (1999). J. Biol. Chem. *274*, 23695–23698.

Germain, P., Chambon, P., Eichele, G., Evans, R.M., Lazar, M.A., Leid, M., De Lera, A.R., Lotan, R., Mangelsdorf, D.J., and Gronemeyer, H. (2006). Pharmacol. Rev. *58*, 712–725.

Glass, C.K., and Rosenfeld, M.G. (2000). Genes Dev. *14*, 121–141.

Mark, M., Ghyselinck, N.B., and Chambon, P. (2006). Annu. Rev. Pharmacol. Toxicol. *46*, 451–480.

Michalik, L., Auwerx, J., Berger, J.P., Chatterjee, V.K., Glass, C.K., Gonzalez, F.J., Grimaldi, P.A., Kadowaki, T., Lazar, M.A., O'Rahilly, S., et al. (2006). Pharmacol. Rev. *58*, 726–741.

Schug, T.T., Berry, D.C., Shaw, N.S., Travis, S.N., and Noy, N. (2007). Cell, this issue.

Tan, N.S., Shaw, N.S., Vinckenbosch, N., Liu, P., Yasmin, R., Desvergne, B., Wahli, W., and Noy, N. (2002). Mol. Cell. Biol. *22*, 5114–5127.

DegrAAAded into Silence

Elizabeth H. Bayne,[1] Sharon A. White,[1] and Robin C. Allshire[1,*]

[1]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Kings Buildings, Michael Swann Building, Mayfield Road, Edinburgh, EH9 3JR, UK

*Correspondence: robin.allshire@ed.ac.uk

DOI 10.1016/j.cell.2007.05.004

In fission yeast, RNA interference (RNAi)-dependent heterochromatin formation silences transgenes inserted at centromeres. In this issue, Bühler et al. (2007) demonstrate that the RNAi machinery directly targets transgene transcripts. Furthermore, they link transgene silencing to a protein complex resembling the TRAMP complex of budding yeast, which promotes transcript degradation via the exosome. Thus, RNAi-independent transcript degradation may also contribute to heterochromatin gene silencing.

The packaging of chromosomal DNA into heterochromatin is important for cellular processes such as regulation of gene expression and accurate chromosome segregation. In the fission yeast, *Schizosaccharomyces pombe*, heterochromatin is found at the mating-type locus, telomeres, and centromeres. Regions of heterochromatin are generally associated with transcriptional repression, and consistent with this finding, marker genes inserted into fission yeast heterochromatin are silenced.

Heterochromatin assembly involves an ordered series of events in which lysine 9 on histone H3 becomes methylated by the histone methyltransferase Clr4 (equivalent to metazoan Suv39), creating a binding site for chromodomain proteins such as Swi6, Chp1, and Chp2 (HP1-related proteins). RNAi is required to establish and maintain heterochromatin at centromeres but is dispensable for maintenance of heterochromatin at the mating-type locus (Grewal and Jia, 2007). In mutants of the RNAi pathway centromeric small interfering (si)RNA production is defective and homologous centromeric repeat transcripts accumulate. This has led to a model whereby siRNAs generated from centromere transcripts are required to target chromatin-modifying machinery to the centromere, resulting in tran-