PERSPECTIVES: SIGNAL TRANSDUCTION

Inositol Phosphates in the Nucleus

Tian H. Chi and Gerald R. Crabtree

Perhaps one of the most delightful characteristics of biology is that just when we think we know all about something, a new observation comes along that exposes our state of ignorance. For more than a decade, numerous reports have hinted that there are nuclear counterparts of inositol signaling pathways in the cytoplasm. For example, the conventional enzymes and inositol derivatives involved in the inositide cycle—lipid kinases, phospholipases, inositol phosphate kinases, inositol lipids, diacylglycerol, and inositol phosphates—are all found in the nucleus, and their activities or abundance seem causally linked to cell function (*1*–*3*). However, exactly what these molecules do in the nucleus is not clear. Two reports from York and colleagues, one of which appears on page 2026 of this issue, now present the unexpected finding that inositol signaling in the nucleus regulates gene expression. This discovery points to a new level of control over this critical process (*4*, *5*).

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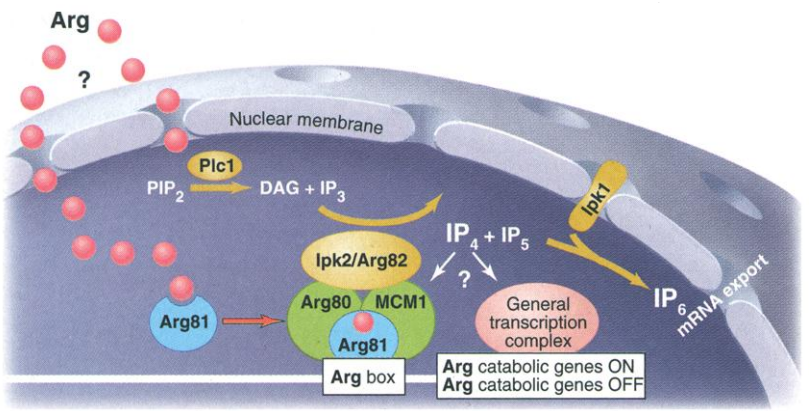
Exploiting the power of yeast genetics, York *et al.* conducted a genetic screen to identify yeast genes that contribute to the nuclear export of messenger RNA (mRNA) (*4*). Unexpectedly, they recovered three genes that together constitute an inositol signaling pathway. The three genes are: *PLC1* encoding phospholipase C [which cleaves the inositol lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3)]; *IPK1* encoding the inositol polyphosphate kinase Ipk1p [which converts inositol 1,3,4,5,6-pentakisphosphate (IP_5) to inositol hexakisphosphate (IP_6)], and an uncharacterized third gene, now designated *IPK2* [which encodes another inositol polyphosphate kinase Ipk2p that converts IP_3 to inositol 1,4,5,6-tetrakisphosphate (IP_4) and IP_5] (*4*). A mutation in any of these three genes (on certain genetic backgrounds) blocks mRNA export from the nucleus. Thus, IP_6, the end product of this metabolic pathway, is likely to be the effector molecule that regulates mRNA export. This molecule is known to modulate the trafficking of synaptic vesicles in mammalian cells through a direct interaction with synaptotagmin, the Ca^{2+}-sensitive vesicle protein that regulates both endo- and exocytosis. York *et al.* suggest that IP_6 may similarly facilitate mRNA export in yeast by binding to the export machinery of the nuclear pores. Consistent with this idea, a small amount of the nuclear yeast protein Ipk1p is strategically localized to the nuclear pores. Thus, a role for inositol phosphates in RNA export seems inescapable.

The more recent surprise, reported by York and colleagues in this issue (*5*), turned up when they cloned *IPK2*. This gene turns out to be identical to the previously characterized yeast gene *ARG82*, which encodes Arg82p, a pleiotropic kinase that regulates diverse processes including sporulation, mating, and the response to stress (*6*). However, Arg82p is best known for its involvement in arginine metabolism; it is an essential component of the ArgR-MCM1 transcription complex that activates or represses multiple genes involved in arginine production or breakdown (*7*, *8*) (see the figure). York and co-workers now show that this metabolic process requires the kinase activity of Ipk2p and the inositol phosphates it generates. Engineering mutations in *IPK2* to specifically remove this activity, or deleting *PLC1* to cut off the substrate supply for the kinase, effectively prevents the growth of yeast on media with arginine as the sole nitrogen source. The Ipk2p kinase appears to act at the level of transcription rather than mRNA export because *IPK1*, which makes IP_6, is not essential for arginine metabolism, and the mRNA level of at least one arginine-induced gene is down-regulated in mutants lacking Ipk2p (*9*). Although regulation of mRNA stability might be an alternative way in which this kinase could alter mRNA abundance, the association of Ipk2p with promoters suggests that it directly regulates transcription. The major effector in this case might be IP_4 because an *Ipk2* mutant that is selectively defective in making IP_5 retains the ability to support arginine metabolism. Preliminary data indicate that Ipk2p kinase activity is also required for other Ipk2p-dependent processes (*9*).

These data immediately raise questions about the mechanisms through which Ipk2p modulates gene expression. Messenguy and colleagues previously proposed that the sole function of Ipk2p in some (but not all) *IPK2*-dependent processes, including arginine metabolism, is to stabilize Mcm1p and Arg80p through direct physical interactions. Thus, the protein levels of Mcm1p and Arg80p are significantly reduced in yeast mutants that lack



Nuclear inositol phosphates control mRNA export and transcription. Arginine (Arg) either induces or represses genes involved in its metabolism. This effect is mediated by the ArgR-MCM1 transcription complex consisting of the Arg-specific transcription factors Arg80 and Arg81 and the global regulatory proteins MCM1 and Arg82/Ipk2. All of these are DNA binding proteins except for Arg82/Ipk2, which is a key kinase in the inositol signaling pathway. In the presence of arginine, these proteins cooperatively bind to the specific elements (Arg box) in the promoters of arginine-regulated genes (*7*). (This may be triggered by arginine-induced Arg81 isomerization) (*8*). The arrangement of the proteins with respect to each other reflects physical interactions that have been previously identified (*8*). Inositol signaling is required for induction of genes whose proteins catabolize arginine. The effect seems to be mediated by IP_4, but its mechanism of action is unknown, although binding of the ArgR-MCM1 transcription complex to gene promoters, or transcription initiation, or mRNA elongation might be affected. IP_6, especially that produced by Ipk1 localized at the nuclear pore, presumably contributes to the export of mRNA.

The authors are in the Department of Developmental Biology and Department of Pathology, Stanford University Medical School, Stanford, CA 94305, USA. E-mail: hf.grc@forsythe.stanford.edu

Ipk2p, and overexpression of either *MCM1* or *ARG80* rescues some of the phenotypes (*6, 8*). This argument is contradicted by the finding that yeast mutants lacking Plc1p or with defective Ipk2p kinase activity fail to use arginine and yet they contain normal amounts of Mcm1p and Arg80p (as measured by ArgR-MCM1 complex formation in vitro). This indicates that the ability of Ipk2p to stabilize Mcm1p or Arg80p is not sufficient for its regulation of transcription (*2*). A trivial resolution of this conflict is that deletion of Plc1p, or Ipk2p kinase mutations slightly decrease the abundance of Mcm1p and Arg80p so that they still bind to DNA in vitro but not in vivo, where chromatin interferes with DNA-protein interactions. A yet more interesting possibility is that Ipk2p may not only stabilize Mcm1p and Arg80p but may also facilitate their binding to chromatin. It should be noted that both effects may be bypassed by overexpression of *MCM1* or *ARG80*. This speculation is inspired by the observation that the inositol lipid PIP_2 (which regulates actin) and perhaps inositol phosphates as well, stimulate binding of the actin-containing, chromatin-remodeling BAF complex to chromatin in mammalian cells (*10*). Yeast probably have a similar actin-containing complex called ARI (*11*), which raises the possibility that inositol phosphates regulate chromatin remodeling to assist in the assembly of the ArgR-MCM1 transcription complex in vivo. Alternatively, IP_4 may directly bind to and regulate components of the general transcription machinery or the ArgR-MCM1 complex. This mechanism is reminiscent of an earlier observation that a low-activity form of human DNA polymerase α hydrolyzes PIP to IP, which in turn stimulates polymerase activity through a direct physical interaction (*12*).

Extracellular stimuli are capable of regulating the nuclear inositol signaling pathway. Insulin-like growth factor (IGF), when bound to its receptor in the plasma membrane, rapidly activates nuclear PLC-β1 in tissue culture, and an antisense RNA against PLC-β1 completely abolishes the mitogenic effect of IGF (*4*). In yeast, stress enhances IP_6 levels (*13*), which may increase the export of certain mRNAs that when translated into proteins counteract the stressful stimulus (*4*). External stimuli probably influence the nucleus in the same way that they affect the cytoplasm, that is, by controlling the localization or posttranslational modification of rate-limiting enzymes such as Plc1p. But there may well be unpredictable and exciting nuclear-specific variations on this theme.

Finally, the recent discoveries of York and co-workers also fuel speculation that other potential inositol lipid kinases such as FRAP/Tor may also regulate gene expression. Indeed, the yeast Tor proteins directly modulate transcription in response to multiple nutrient-sensitive signaling pathways, including the nitrogen discrimination pathway that also comes into play in arginine metabolism (*14*). Although current evidence indicates that Tor is a protein kinase, its putative lipid kinase activity is perhaps functionally important because the growth defect of certain Tor mutants can be corrected by overexpression of *PLC1* (*15*). It may be worthwhile investigating whether other members of the FRAP/Tor family, including the DNA repair proteins DNA-KC and ATM, regulate transcription. It is beginning to look as if the complexity of the inositol cycle in the cytoplasm is mirrored in the nucleus.

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PERSPECTIVES: EVOLUTIONARY BIOLOGY

Limbless Tetrapods and Snakes with Legs

Harry W. Greene and David Cundall

Evolution of an elongated body form and a reduction in limb size is observed in organisms as diverse as salamanders, weasels, and whales. Among the lizards, a serpentine (snakelike) body form has evolved dozens of times. Not surprisingly, the biomechanical, adaptive, and morphogenetic mechanisms that underlie this panoply of limbless and near-limbless tetrapods have long fascinated biologists (*1*, *2*). Now, some controversial and stunningly well-preserved ancient fossils from the Cretaceous sediments of Ein Yabrud in the Middle East are lifting the veil of mystery that surrounds the origins of snakes. In particular, the newest and best preserved Middle Eastern fossil, reported by Tchernov *et al.* (*3*) on page 2010 of this issue, seems set to lay to rest the notion that snakes started off life in the sea. The investigators place their fossil with its well-developed hindlimbs in a new taxon called *Haasiophis* and demonstrate that this Cretaceous serpent more closely resembles living terrestrial snakes, such as pythons and boas, than it does other extinct marine reptiles, such as mosasaurs. Their work underscores the need for precise nomenclature and explicit logic when attempting to infer the natural history of extinct organisms.

H. W. Greene is in the Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14850–2701, USA. E-mail: hwg5@cornell.edu D. Cundall is in the Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015–3190, USA. E-mail: dlc0@lehigh.edu

Although some pythons and other relatively primitive snakes have tiny, clawlike hindlimbs, used during courtship and in combat between males, limblessness has long been viewed as the essence of being a serpent (*2*). Recently, Caldwell, Lee, and their colleagues claimed that a Cretaceous fossil snake with obvious hindlimbs (also from Ein Yabrud) called *Pachyrhachis problematicus*, is the oldest known snake (*4–7*). They argue that mosasaurs (extinct marine lizards with limbs adapted as fins) represent "a crucial intermediate stage" in the evolution of modern snakes and that ancestral snakes had limbs and were aquatic. Their ideas contrast dramatically with the long-held view that snakes evolved from small, terrestrial lizards or even burrowing lizards by an increasing reduction in limb size (*2*, *8*).

Crown-clade snakes (suborder Serpentes) include the most recent common ancestor of blindsnakes (Scolecophidia) and other extant snakes (Alethinophidia), and all of its fossil and living descendants (see the figure, top of next page) (*8*). Caldwell and Lee (*4*) used several characteristics to classify *Pachyrhachis* as both "the most primitive snake" and the closest relative of animals traditionally called snakes (*5*). They showed their drawings and reconstructions of *Pachyrhachis* to a number of nonscientists, who use "snake" in its vernacular sense, and all identified *Pachyrhachis* as a snake rather than a lizard. Many people, however, apply the term "snake" to creatures as diverse as