New Roles for Old Characters: An Educational Primer for Use with "Vps Factors Are Required for Efficient Transcription Elongation in Budding Yeast"

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SUMMARY An article from Alan Hinnebusch's laboratory in the March 2013 issue of *GENETICS* establishes an exciting new link between proteins with well-established roles in the endomembrane system and the process of transcription elongation. This Primer article provides tools needed for students to fully appreciate, analyze, and critically evaluate the experiments and interpretations of Gaur *et al.* (2013). The primer includes detailed descriptions of techniques used in the study, such as the chromatin immunoprecipitation assay and assays for transcription elongation, and it provides a framework to facilitate an understanding of how a combination of genetic, biochemical, and cell microscopy experimental approaches were used by the authors to converge on a single major conclusion. Suggestions for using this Primer article in an undergraduate or graduate-level course in conjunction with the original article to promote student learning are also presented.

Related article in *GENETICS*: Gaur, N. A., J. Hasek, D. G. Brickner, H. Qiu, F. Zhang *et al.*, 2013 Vps factors are required for efficient transcription elongation in budding yeast. Genetics **193:** 829–851.

Background

The findings reported by Gaur *et al.* (2013) in the article entitled "Vps factors are required for efficient transcription elongation in budding yeast" in the March 2013 issue of *GENETICS* provide a wonderful example of the unexpected complexities that can govern basic biological processes and also remind us of the importance of "thinking outside the box" when considering mechanisms that could account for certain experimental observations. The major contribution of this work is the discovery that some proteins with well-established roles in controlling protein trafficking within the endomembrane system of eukaryotic cells also appear to enhance transcription elongation in a manner involving their physical proximity to nuclear pores and transcribed chromatin. For readers to be able to thoroughly analyze this work, brief overviews of the endomembrane system and the process of transcription, as well as a discussion of previous work by the Hinnebusch laboratory, are warranted.

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Endomembrane system

A subpopulation of the proteins synthesized within eukaryotic cells enters the endomembrane system using the endoplasmic reticulum (ER) as the entry point. Members of this large group of proteins include soluble and membrane-bound proteins that carry out their functions in the ER or in a subsequent component of the endomembrane system—such as the Golgi complex, plasma membrane, or lysosomes—as well as proteins destined for secretion outside the cell. Of particular relevance to this work are the later portions of the endomembrane system, the compartments of the system referred to as late endosomes, or multivesicular bodies (MVBs). The outer membranes of these compartments are populated with proteins delivered by transport vesicles originating from either the late Golgi or the early endosomes (compartments that contain proteins internalized from the plasma membrane through endocytosis). Many of the proteins localized to the outer membranes of MVBs are selectively incorporated into vesicles that bud toward the interior of the MVBs, thus resulting in organelles that contain vesicles within them (hence the origin of the name for these compartments). Subsequent fusion events between the MVBs and lysosomes result in the delivery of these vesicles into the

lumen of the lysosomes, normally resulting in either protein degradation (in the case of endocytosed proteins) or protein activation (in the case of resident lysosomal proteins that have traversed the endomembrane pathway) (Katzmann *et al.* 2002; Bowers and Stevens 2005). Surprisingly, Gaur *et al.* (2013) find that some of the proteins involved in MVB formation also have roles in the regulation of transcription.

Gene transcription process

The process of gene expression refers to the collective set of steps by which information encoded in DNA is used to produce a molecule (RNA or protein) that carries out one or more functions within a cell. In transcription, DNA is used as a template for the production of an RNA molecule, which, in the case of protein-encoding genes, will subsequently be used to synthesize the corresponding protein. Transcription can be divided into three distinct phases: initiation, elongation, and termination. During initiation, transcription activators bound to their cognate-binding sites on DNA [known as enhancers or upstream activating sequence (UAS) elements in yeast] stimulate the formation of the pre-initiation complex (PIC), which contains general transcription factors and RNA polymerase II (Pol II), at gene promoters. During elongation, Pol II and associated factors travel across transcription units and promote the synthesis and processing of RNA molecules. During termination, Pol II, the fully synthesized RNA molecule, and other factors dissociate from the DNA. When considering the process of transcription, it is important to keep in mind that eukaryotic DNA is in the form of chromatin [a compacted structure that includes the nucleosome—itself composed of 147 bp of DNA wrapped around a histone octamer—as its fundamental unit (Luger *et al.* 1997)] and that the modulation of chromatin structure is an inherent part of transcription (Kaplan 2012; Rando and Winston 2012). Previous work by the Hinnebusch laboratory established a role for vesicular trafficking components in regulating transcription initiation at specific genes (Zhang *et al.* 2008). In the Gaur *et al.* (2013) article, they further report that vesicular trafficking components appear to have additional roles in controlling transcription elongation.

Previous findings from the Hinnebusch laboratory

The original insight that revealed a functional connection between components of the endomembrane system and the regulation of transcription is presented in an article published by Alan Hinnebusch and colleagues in 2008 (Zhang *et al.* 2008). The Hinnebusch laboratory has a long-standing interest in understanding the regulatory mechanisms controlling amino acid biosynthesis in eukaryotic cells using the budding yeast *Saccharomyces cerevisiae* as the model system (more on the advantages of yeast as a model system below). Of central relevance to the present discussion is the notion that, upon amino acid starvation in yeast, the transcription activator Gcn4 is recruited to the UAS elements of biosynthetic genes and stimulates PIC assembly, thereby promoting expression of these genes and subsequent synthesis of amino acids.

In their previous work (Zhang *et al.* 2008), Hinnebusch and colleagues identified new factors involved in the regulation of this pathway. To do so, they took advantage of a preexisting library of yeast cells in which all nonessential genes had been deleted individually and asked which of these deletion strains are sensitive to sulfometuron, a drug that simulates amino acid starvation. The logic here is that if a gene is required for the biosynthesis of amino acids, cells that lack it should be unable to grow in conditions in which one or more amino acids are limiting. Among the genes that they identified in this genetic screen were many ***v**acuolar **p**rotein **s**orting* (*vps*) genes that encode proteins involved in the formation of the MVBs of the endomembrane system. But how can the absence of certain Vps proteins affect the amino acid biosynthetic process? Subsequent experiments in the same study showed that MVB defects impair expression of Gcn4-regulated genes by somehow crippling the ability of Gcn4 to promote assembly of the PIC at these genes, thus unveiling the unexpected link between the endomembrane system and transcription regulation. Based on their findings, the authors proposed a model in which defective MVBs trigger a pathway from the cytoplasm to the nucleus that dampens expression of biosynthetic genes by preventing Gcn4-promoted PIC assembly (Zhang *et al.* 2008). Such a model would make sense in the context of cell physiology because during hard times—that is, when cells are experiencing problems with MVB function—they would not want to spend energy producing amino acids for protein synthesis.

Several puzzling observations made by the Hinnebusch lab provided hints that mutations in specific Vps proteins could also affect other aspects of transcription. First, the authors found that impairment of Gcn4-dependent transcription due to loss of certain Vps proteins was more pronounced in the context of a gene known to be particularly sensitive to defects in transcription elongation (the bacterial *lacZ* gene—more on this later). Second, they determined that the ability of other transcription factors to activate transcription was also impaired in the absence of certain Vps proteins, albeit to a lesser degree. These findings indicated that some Vps proteins have other roles in transcription regulation in addition to controlling the ability of Gcn4 to stimulate PIC formation, including a possible positive role in regulating transcription elongation.

The model system

S. cerevisiae is a unicellular eukaryotic organism that serves as one of the most popular experimental systems in the scientific research community. Because of the high degree of evolutionary conservation in basic biological processes between yeast and higher eukaryotic cells (including human cells), advances in our understanding of yeast cell biology and genetics has had, and will continue to have, a great impact on our understanding of eukaryotic cell biology in general. In addition, certain aspects of the yeast system make it a very attractive and powerful experimental organism. These aspects include the ability of yeast cells to grow in both the haploid and the diploid states (thus facilitating

genetic analyses), access to large amounts of both cells and cell material, and the availability of highly sophisticated and powerful genetic engineering approaches. The basic nature of the questions addressed in this study and their general relevance to eukaryotic biology make yeast an ideal system for this work.

Unpacking the Work

The big question and experimental strategy

Thus the stage is set for the big question addressed in the paper by Gaur *et al.* (2013): Are Vps factors involved in positively regulating transcription elongation? The approach used by the authors to address this question was first to determine if loss of these factors confers defects consistent with impaired transcription elongation [figures 1–4 of Gaur *et al.* (2013) relate to these experiments]. Once Gaur *et al.* (2013) were able to establish that this was indeed the case, they then went on to address the molecular mechanism underlying Vps function during transcription elongation [figures 5–10 and the supporting information in Gaur *et al*. (2013) relate to these experiments]. The results from all these experiments support the authors' hypothesis and allow them to formulate a possible model for how Vps proteins participate in the transcription elongation process.

Specific research questions

Note that most of the experiments done by Gaur *et al.* (2013) focused on the Vps15 and Vps34 proteins, since their previous work showed that mutations in these proteins confer the most dramatic effects on Gcn4-dependent transcription (Zhang *et al.* 2008). Thus, we will examine several aspects of the experiments in Gaur *et al.* (2013) from the prospective of these two factors, keeping in mind that the effect of other Vps proteins were also assayed in some of the studies. Vps15 and Vps34 are members of the so-called class D proteins of the endomembrane pathway, which have roles in promoting fusion between transport vesicles originating from the late Golgi and the MVBs (Bowers and Stevens 2005); it is therefore not surprising that mutations in these proteins prevent proper formation of MVBs. The molecular function of Vps34 is to modify the phosphorylated versions of phosphatidylinositol (PI), a normal component of cellular membranes, by adding an additional phosphate group to generate PI(3)P moieties. Readers should keep this modification in mind as possible models for Vps34 participation in transcription elongation are considered. Vps15 interacts with Vps34 and is required for its activity.

Let us consider the main questions that the authors asked to determine if Vps proteins have a positive role in transcription elongation:

The drugs mycophenolic acid (MPA) and 6-azauracil (6-AU) reduce levels of intracellular nucleotides, and cells defective for some well-established transcription elongation factors have been shown to be sensitive to them. Are cells lacking functional Vps15 or Vps34 also sensitive to these drugs?

Previous work has shown that the efficiency of transcription through either long transcribed units or transcribed units rich in GC nucleotides (such as the bacterial *lacZ* gene) is particularly sensitive to conditions that impair transcription elongation (Chavez *et al.* 2001; Morillo-Huesca *et al.* 2006). Are cells lacking functional Vps15 or Vps34 impaired for transcription through these types of transcribed units?

Picture a scenario in which a gene that is being actively transcribed is abruptly shut down. One would expect that the occupancy of Pol II across this gene would quickly dissipate as the pre-existing Pol II complexes on the gene continue to travel downstream and eventually fall off at the end of the gene and no additional Pol II complexes are recruited to the gene promoter. It might be further expected that the rate of Pol II loss across such a gene might be slowed in cells in which a factor that stimulates Pol II movement across genes is defective. Does the lack of either Vps15 or Vps34 cause a decreased rate of Pol II loss in this type of scenario?

Consider the observation made by Alan Hinnebusch and colleagues in their previous work (Zhang *et al.* 2008) regarding an impairment of transcription activation by transcription activators other than Gcn4 (such as Gal4) as a result of loss of Vps proteins. As indicated earlier, the effects of Vps mutations on transcription initiation by Gcn4 is through prevention of Gcn4-promoted PIC formation. Does this mechanism account for the reduced transcription activity of Gal4 in Vps mutants? If not, could a defect in transcription elongation account for the inability of Gal4-regulated genes to be fully active in the context of Vps mutations?

Determine for yourself whether the results from the experiments addressing the questions above are supportive of a role for Vps15 and Vps34 in positively affecting the transcription elongation process. If so, do these experiments address the mechanisms of their action(s)? Can these results be used to establish whether Vps15 and Vps34 facilitate transcription elongation through a direct or an indirect mechanism? As you read the Gaur *et al.* (2013) article further, you will find that the authors have designed additional experiments to tackle some of these issues directly by asking the following set of questions:

Previous work by others had pointed to a possible functional interaction between Vps15/Vps34 and Epl1, a component of the histone acetyl transferase complex NuA4 (Lin *et al.* 2008), which had previously been implicated in the transcription elongation process (Ginsburg *et al.* 2009). Might Vps15 and Vps34 in some way be required for recruitment of NuA4 to transcribed genes?

The *GAL1* gene is known to be recruited to the nuclear periphery through interactions with the nuclear pores upon transcriptional activation (Ahmed and Brickner 2007). Since the absence of Vps15 and Vps34 appears to affect

the transcription elongation processes at various genes driven by the *GAL1* promoter (including the *GAL1* gene itself), might these Vps proteins normally play a role in stimulating the association of the *GAL1* gene with the nuclear periphery? Could the Vps proteins also be involved in regulating the nuclear localization of *INO1*, another gene known to associate with the nuclear periphery following transcription activation?

To address the question of direct *vs.* indirect mechanisms of action: if the Vps proteins regulate the transcription elongation process through a direct mechanism, one might expect them to be physically localized at the genes that they regulate, and, more specifically, enriched across transcribed units *vs.* the promoter regions. Can the Vps proteins be detected in association with transcribed regions of genes?

As Pol II travels across genes during the elongation phase of transcription, the phosphorylation state of the carboxy terminal domain (CTD) of Rpb1—the largest subunit of Pol II—changes, and this phenomenon is at least in part responsible for directing the recruitment of a variety of RNA-modifying enzymes and transcription elongation factors to genes (Buratowski 2009). If the Vps proteins do in fact physically associate with transcribed units of genes, is their ability to do so dependent on Pol II and the phosphorylation state of its CTD?

Finally, if the VPS proteins physically interact with the genes that they regulate, they will be found in the cell nucleus in addition to their well-known locations within the endomembrane system. Using standard cell biology techniques, can the Vps proteins be found within cell nuclei? If so, are they predominantly found at a *specific* subnuclear location? Based on the nuclear location, what insight can be gained concerning Vps protein function during transcription elongation?

Tools in the molecular biologist's toolbox used to address the specific questions

To fully appreciate how the authors addressed the specific questions presented in the preceding section—as well as to be able to critically evaluate the associated results—readers of this work need to understand the experimental techniques used. Whereas some of these techniques should be familiar to the budding geneticist or be sufficiently simple to be easily grasped without additional instruction, others are more complex and warrant a brief discussion.

Gene length-dependent accumulation of mRNA assay: This assay, developed by Sebastián Chávez and colleagues, allows investigators to determine whether specific gene mutations confer defects in the elongation phase of transcription (Morillo-Huesca *et al.* 2006). Because this technique specifically assays for transcription elongation defects—and not for transcription initiation defects—it has become a powerful tool for the identification and characterization of new transcription elongation factors.

In a version of this assay, wild-type cells and mutant cells [*e.g.*, cells deleted for the *VPS15* gene—*vps15*Δ cells—used by Gaur *et al.* (2013)] are transformed with either a plasmid harboring the *PHO5* gene under the control of the promoter of the *GAL1* gene (we will call this the "short" gene) or a plasmid with the same configuration but that also includes a DNA sequence ~3000 bp in length (or 3 kb) from the fungal *LAC4* gene downstream from the *PHO5* coding region (the "long" gene). Both the short and the long genes encode the same protein, Pho5, but Pol II must travel much farther before disengaging from the DNA template of the long gene compared to that of the short gene. In each case, the efficiency of transcription elongation is measured indirectly through the use of a simple enzymatic assay specific for the Pho5 protein. The data are presented as the ratio of Pho5 activity derived from the long gene to that derived from the short gene [known as the gene length-dependent accumulation of mRNA (GLAM) ratio]. Armed with this information, students can critically evaluate the data obtained from the GLAM assays [in figures 2 and 3 of Gaur *et al.* (2013)] to determine for themselves whether the results are consistent with the conclusions of the article.

Visualization of specific chromosomal regions within cells: The location of an entity in its natural environment, including within cells, can provide important clues to its function. Thus, a variety of techniques have been developed to visualize the location of chromosomal regions, proteins, and other cellular components. To determine the nuclear location of the *GAL1* gene in both wild-type and *vps* mutant cells, Gaur *et al.* (2013) used (i) a version of the *GAL1* gene that had been engineered to contain an array of DNA-binding sites for the bacterial Lac repressor protein (the same protein familiar to all genetics undergraduate students and made famous by Jacques Monod and François Jacob in their work with the Lac operon) downstream from its transcribed sequence and (ii) a hybrid gene expressing a chimeric protein consisting of the Lac repressor DNA-binding domain and green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. When the modified *GAL1* gene and the gene expressing the Lac repressor/GFP hybrid proteins are present within the same cell—be it wild type or mutant—the hybrid proteins will concentrate at the Lac repressor binding sites near the *GAL1* gene and can be visualized directly in live cells or by employing standard immunofluorescence microscopy techniques using antibodies against the GFP protein. The location of the fluorescent signals is indicative of the nuclear location (*e.g.*, associated with the nuclear membrane or not) of the chromosomal region that includes the *GAL1* gene. The authors used this approach to study the effects resulting from the absence of the Vps15 and Vps34 proteins on *GAL1* nuclear localization.

Chromatin immunoprecipitation: Molecular geneticists are interested in determining whether certain proteins physically associate with specific regions of chromosomes. Establishing such interactions can provide important insights into the function

and the site of action of the proteins under investigation. The chromatin immunoprecipitation (ChIP) technique provides a powerful experimental platform to identify and quantify protein:DNA interactions and was utilized extensively by Gaur *et al.* (2013) to better characterize the role of the Vps proteins in regulating transcription elongation.

In a standard ChIP assay performed in yeast, logarithmically growing cells are incubated for ~20 min in the presence of the cross-linking agent formaldehyde, thereby essentially "freezing" proteins in the place in which they are found at the time of addition of the cross-linking agent. Because formaldehyde can generate covalent bonds between different proteins as well as between proteins and nucleic acids, proteins associated with specific sites across chromosomes will also be cross-linked to each other and to the underlying DNA sequence. Following formaldehyde incubation, the cells are lysed, and the chromatin is obtained and fragmented (either by sonication or through other means) to an average size of ~200–500 bp. Next, small samples of the sheared chromatin are aliquoted into two separate tubes: in one tube [the immunoprecipitation (IP) tube], chromatin is incubated in the presence of antibodies (themselves coupled to magnetic beads) that specifically recognize a protein of interest, and in the other tube (the Input tube), no antibody is added. The material in the IP tube is then allowed to incubate for a few hours to allow the antibodies to find and bind to the protein of interest, ultimately resulting in complexes composed of the antibodies (and associated magnetic beads), the protein of interest, and any material that is associated with the protein of interest (*i.e.*, the chromosomal fragments bound to it). These complexes are collected using a magnet—in essence "precipitating" them through the use of antibodies (in this case coupled to the magnetic beads), hence the term "immunoprecipitation"—and the antibodies/protein of interest/chromatin fragments are eventually eluted off the beads.

The resulting IP tube and the original Input tube are then subjected to a common series of manipulations. These include steps to reverse the cross-links and to degrade all proteins in the samples, thus resulting in the two tubes containing only DNA molecules: the Input tube containing DNA fragments representative of the entire genome and the IP tube containing a population of DNA fragments enriched for those that had been bound by the protein of interest. The two populations of DNA fragments are then compared to each other—normally using either standard quantitative PCR methods or quantitative real-time PCR technology—to determine the relative amount of a specific region of DNA (defined by the PCR primers used in the experiment) present in the IP tube with respect to the Input tube. The resulting values, normally expressed as percentage of IP (%-IP), are directly related to the level of occupancy of the protein of interest in that specific location of the genome: the higher the %-IP value, the higher the level of occupancy. It is often convenient to obtain a baseline %-IP value for a region of the genome that is thought not to be occupied by the protein of interest that can be used to normalize the data.

Armed with this knowledge about ChIP assays, let us now consider the data from the ChIP experiments shown in figure 5B of Gaur *et al.* (2013) as an example. In these experiments, the protein of interest is Epl1-myc (a component of NuA4), and the authors sought to determine its level of occupancy across two regions of the *GAL1* open reading frame (5′ ORF and 3′ ORF in figure 5A) either in the absence of galactose (un-inducing conditions) or in the presence of galactose (inducing conditions) in wild-type, *vps15*Δ, and *vps34*Δ cells. The ChIP experiments were carried out essentially as described above, and the authors used an intergenic region (*i.e.*, a region between two genes and therefore not transcribed) on chromosome V as control for normalization. The "occupancy" values on the *y*-axis reflect the ratio of binding of Epl1-myc to either the *GAL1* 5′ ORF or 3′ ORF to the baseline binding seen at the control region on chromosome V. Thus, when considering the level of binding of Epl1-myc at the *GAL1* 3′ ORF in wild-type cells under un-inducing conditions, one can see that there is essentially no specific binding at this location since the ratio here is very close to 1. Conversely, significant binding—approximately four times above the baseline level—is seen at the same location when the same cells are grown in inducing conditions. The other ChIP experiments in this work follow the same logic, except that different proteins and different genomic locations are assayed in the context of different genetic backgrounds and growth conditions.

Suggestions for Classroom Use

The content of this Primer article should facilitate an in-depth analysis and critical evaluation of the studies presented by Gaur *et al.* in the March 2013 issue of *GENETICS*. Instructors can ask students to begin their analysis of this Primer article by first reading up to, but not including, the *Tools in the molecular biologist's toolbox used to address the specific questions* section and then moving on to the Gaur *et al.* (2013) article itself, referring back to this Primer article as needed for any clarifications and using the *Tools in the molecular biologist's toolbox used to address the specific questions* section as they encounter the specific techniques covered in this section in the Gaur *et al.* (2013) article. This initial exercise could be completed by the students outside the classroom, possibly in the context of small study groups, and then could be followed by one or two classroom sessions dedicated to addressing any topics with which the students may be having difficulties. Finally, the classroom conversation could turn to the topics and questions listed below (grouped as *Detailed questions* and *Big picture topics and questions*) to stimulate further class discussion and to ensure that all students fully grasp both the fine details of the work as well as the big picture implications.

Detailed questions

1. Vps34 is a kinase: What is the target of this kinase and what is one of the critical residues of Vps34 required for this activity? Where do you suppose the potential targets

for this kinase can be found in cells? How might cells ensure that, of all the possible targets for Vps34, only the appropriate ones are actually modified by this kinase?
2. When testing the effects of deleting certain *VPS* genes on certain phenotypes or processes, the authors often included yeast strains carrying the *VPS* gene deletion (*e.g.*, *vps15*Δ cells) that also harbored a plasmid with the wild-type version of the same gene [*e.g.*, *vps15*Δ/*VPS15* in figure 2B of Gaur *et al.* (2013)]. What was the purpose of using such strains in these experiments?
3. Consider the GLAM data shown in figure 2B of Gaur *et al.* (2013): What type of carbon source do you suppose was included in the media used for growing the cells for these experiments? Predict the results that would have been obtained if another commonly used carbon source was included instead.
4. What were the original data that suggested a functional interaction between Vps15/Vps34 and the NuA4 complex? In your deliberations, consider the fact that the NuA4 components Epl1 and Esa1 are essential.
5. For several experiments, the authors employed functional tagged versions of proteins. Describe what it means for proteins to be tagged and what the use of these proteins was in the context of these experiments. In addition, what do the authors mean by "functional," and how do you suppose they were able to establish that this was in fact the case for the proteins they used?
6. The behavior of the Spt4 protein or the consequences of removal of the gene encoding it were analyzed in some of the experiments described in this article. Considering that the article focuses on the Vps proteins, why do you think that the authors decided to include these analyses? Can you predict the results that might have been obtained if the authors included Spt4-myc in addition to Epl1-myc and Yng2-myc in the experiments presented in figure 5 of Gaur *et al.* (2013)?
7. As discussed in the article, upon activation the *GAL1* and *INO1* genes are recruited to the nuclear periphery through interactions with the nuclear pores. Can you propose a reason for why such localization would be part of the gene expression mechanism for these genes? Would you expect this localization phenomenon to be unique to *GAL1* and *INO1*, or could it be more widespread?
8. The authors showed some level of physical interaction between certain transcribed genes and protein synthesis initiation factors. Why do you suppose that these factors were tested in the first place? Discuss possible interpretations of these data.

Big picture topics and questions

1. Scientists need to read broadly to take advantage of knowledge gained in seemingly disparate areas of biology in their own research. List at least five pieces of information unrelated to Vps proteins that the authors used to design and interpret their experiments. For example, they needed to know about the effect of the drugs MPA and 6-AU to use them to stress the yeast cells.
2. The authors put forth an attractive model for how Vps15 and Vps34 could participate in stimulating transcription elongation through a direct mechanism. Draw a cartoon representation of the model that includes the nuclear envelope, a nuclear pore, the site of action of Vps15 and Vps34, the outcome of Vps34's enzymatic activity, NuA4, an actively transcribed gene, and the corresponding RNA product.
3. Is the model that you illustrated above consistent with the author's finding that the absence of Vps34 does not cause impairment of transcription elongation in the biochemical assay as described in the *Discussion* of Gaur *et al.* (2013)?
4. How do the other Vps factors that the authors identified as having roles in transcription elongation fit within the context of the model that you illustrated? Consider both their physical association with transcribed genes and their stimulatory role during transcription elongation.
5. How do previous studies using other experimental systems support various aspects of the model that you illustrated?
6. What type of experiments would you propose that could provide additional support of the model you illustrated?
7. Can you propose an alternative model that would be consistent with the data reported in this work? How would you design an experiment that would test your alternative model?

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