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FRONT MATTER: DISCOVERY

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Applying systems-level approaches to elucidate regulatory function during mammalian hibernation*

Comment on: Pan P, Treat MD, van Breukelen F. A systems level approach to understanding transcriptional regulation by p53 during mammalian hibernation. J Exp Biol 2014; 217:2489–98; PMID: 25031456; http://dx.doi.org/10.1242/jeb.103614

When is a transcription factor not a transcription factor? The non-steady state of mammalian hibernation means that assumptions based on steady-state regulation may be erroneous. I will discuss how applying systems level approaches helped define p53s role in hibernation and its implications on elucidating the cellular processes of hibernation.

Rodent hibernators enter bouts of torpor wherein core body temperature (T_b) and metabolic rate are markedly reduced. T_b may approach that of ambient and in the case of some ground squirrels may be ≤ 0 °C. Concordant with marked depression of oxygen consumption (may be $\leq 1\%$ of active rates), there is global depression of transcription in the livers of hibernating ground squirrels.[1] There is a moderate depression of initiation of transcription but the low temperatures typical of torpor leads to an essential arrest of transcriptional elongation. More recently, other investigators noted that transcription factors move into the nucleus during hibernation. One might reasonably presume such movement is biologically important. After all, why would a transcription factor move into a nucleus if not to regulate transcription? Tempering that interpretation was our earlier finding of marked depression of transcription. If there was essentially no transcription then how could transcription factor function affect transcription? Perhaps, there was a small amount of transcription that was preferentially fostered by the transcription factor?

In the featured paper, we focused on an exemplary transcription factor, p53, to establish if there was bona fide function (Fig. 1).[2] P53 is regulated by at least 160 different mechanisms. We found 5 regulators to be consistent with an activation of p53 during winter (both torpid squirrels and those that were euthermic between bouts of torpor). Factors that should have increased or decreased with p53 activation did as much at either the mRNA or protein levels. Furthermore, p53 was increased in the nuclei of winter squirrels. We found a significant increase in the amount of p53 that was bound to DNA and that there was a moderate increase in the recruitment of RNA polymerase II during winter. However and most importantly, this seeming activation of p53 did not lead to expected changes of up and down-regulated target genes associated with p53 function in other systems. In other words, while p53 went through most of the steps one might expect of a functioning transcription factor, it appears that the depression of transcriptional elongation as a function of low T_b means little change in the concentrations of mRNAs of target genes.

An appropriate question to ask is why should a transcription factor go through most of the motions of function only to be ineffective at affecting transcription? One presumes such imprecise regulation of cellular function would be energetically costly. Many view the cell as a machine that functions with precision; efficient regulation leads to homeostasis with minimal energetic waste. Such a view minimizes a more exquisite reality of cellular function being akin to a poorly run factory. Rather than attempting the seemingly impossible approach

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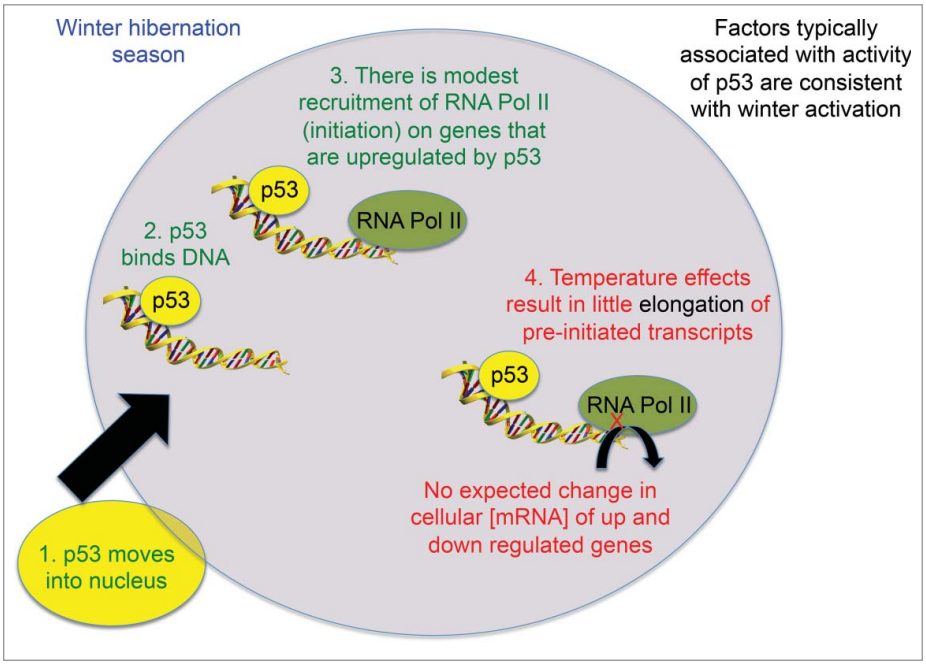

Figure 1. Cartoon depicting the role of p53 in regulating transcription during hibernation. Factors associated with p53 activation in other systems are consistent with an activation of p53 during winter. Immunohistochemistry and western blotting revealed p53 is increased in the nuclei of squirrel hepatocytes sampled in both the active and torpid states of winter. Chromatin immunoprecipitation assays demonstrated increased binding of p53 to target gene DNA during winter and nuclear run-on assays suggested moderate recruitment of RNA polymerase II. An earlier finding suggested that the cold temperatures typical of torpor depressed transcriptional elongation. Consistent with this finding, the seeming activation of p53 did not result in predicted changes in mRNA abundance for up or down regulated target genes.

of getting processes right in the first place i.e. an efficient assembly line, the beauty of cellular function is in the numerous processes that help rectify problems. For instance, protein folding in the endoplasmic recticulum oftentimes leads to misfolded proteins e.g. ~90% of the acetylcholine receptor proteins are misfolded following their production. Processes like chaperoning, endoplasmic recticulum associated degradation, and the unfolded protein response work in a tiered fashion and help ensure against an accumulation of misfolded proteins. In this context, would it be reasonable to suggest that hibernation with its variable T_bs could offer precise regulation of the numerous cellular processes that are not precisely regulated in the first place?

p53 metabolism joins a list of regulatory pathways wherein homeostasis was effectively placed on hold suggesting hibernation is not a steady state condition.[3] Many processes that are normally coordinated are decoupled e.g., the ubiquitylation of proteins is not very temperature sensitive whereas protein degradation is; the result is an accumulation of ubiquitylated proteins during hibernation.[4] Indeed, we speculate the energetic costs of reconciling such mismatches lead to the significant mortality associated with hibernation (as many as 20–50% of adult squirrels die each winter).

In some instances, there appears to be evolution toward more precise regulation e.g. ubiquitylation in squirrels is more thermally sensitive than rats. Perhaps the reason that there was not tremendous selective pressure to ensure more precise matching in all cellular processes is that the most effective way to slow down a machine is to break a machine. Hibernation, then, might represent a condition wherein processes are allowed to 'break.' The result is marked energetic savings. Such an idea also predicts the key processes that foster survival of hibernation are in the arousal process. Mechanisms need to repair the numerous systems that were adversely affected by torpor. In support of this concept, we found ground squirrels preferentially load ribosomes with internal ribosome entry site (IRES)- bearing mRNAs for the arousal process.[5] In other systems, transcripts bearing IRESes are known to preferentially encode for stress proteins including many of the proteins that are increased during hibernation. In other words, it appears that the implausibility of precise regulation during hibernation gave rise to a form of effective regulation that may not be very efficient. Identification of

more processes that help to reverse the effects of homeostatic mismatches may be critical to our understanding the processes of torpor.

Our original p53 paper demonstrates how assuming links between processes based on what occurs under steady-state conditions, may mislead investigators. While a systems-level approach is arguably burdensome, exploiting that approach toward understanding if those same links are maintained in the context of the non-steady state of hibernation might help separate the fundamentally key processes that allow and foster torpor use from events that are simply correlative.

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