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The Promises of the Novel Connection Between HEXIM and Hedgehog Signalling

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Commentary

Despite the profusion of molecular data describing the biochemical interactions between HEXIM and its partners, very little is known about its physiological function. In recent works, we provided *in vivo* data showing that HEXIM is a key controller of Hedgehog signalling during wing morphogenesis. In this commentary, we discuss the known biological processes controlled by HEXIM in the light of our new results.

Biological Processes

HEXIM (Hexamethylene bis-acetamide inducible protein 1) is an evolutionary conserved protein factor found in a wide range of species ranging from protostomes to deuterostomes. It is involved in various regulatory processes (transcriptional regulation, p53 pathway), as well as a number of human pathologies (cardiopathies, cancers, inflammation) [1]. Not surprisingly, it has been the focus of numerous mechanistic studies and a potential target of anti-cancer strategies [2,3]. The point of this commentary is to highlight that despite our good understanding of the molecular mechanisms related to HEXIM biology, little is still known about its physiological and developmental output. Therefore any novel insight about HEXIM physiological function is both of fundamental and applied (medical) relevance.

In term of molecular mechanism, HEXIM was first found to participate to RNA pol II transcriptional regulation by controlling elongation, where it acts like a sink that sequesters away the pro-elongation factor P-TEFb located at active promoters. By doing so, HEXIM prevents the catalytic subunit of the P-TEFb kinase (CDK9) to phosphorylate the carboxy-terminal domain (CTD) of RNA pol II, which can not switch to a productive elongation state. Typically, RNA pol II pauses between 40 and 50 nucleotides downstream the transcriptional start site, resulting in a paradoxal functional state called "transcriptional pause", where gene transcription already started but without the production of full length RNAs. Disrupting HEXIM and P-TEFb physical interactions releases free P-TEFb and the stalled transcription can resume very quickly. This nice mechanism allows a fast transcriptional response as well as a strong transcriptional repression mechanism depending to the context [4]. Therefore, and somewhat counter intuitively, the transcriptional regulation of these genes may not be dependent on the complex recruitment of a cohort of very specific transcription factors, and the hunt for specific transcription factors responsible for their very fast transcriptional response may be inconclusive. Historically, transcriptional pause was first described for heat shock response [5], but during the last decade, it has been also found at many other genes. Importantly, the pause mechanism may be of crucial importance for the coordinated expression of many developmentally control genes [4]. Unfortunately, little is still known about the cellular mechanisms involved and the gap between the molecular choregraphy at transcription and developmental processes needs to be filled in.

More recently, a second molecular function has been devoted to HEXIM, and it is also linked to tumor suppressor activity. HEXIM stabilizes p53 through direct protein-protein interaction and prevents HDM2 (human double minute-2 protein)-mediated ubiquitin ligation and its subsequent degradation at the proteasome [1]. The resulting increase of p53 biological activity is linked to cell cycle arrest and apoptosis, ultimately leading to a strong reduction of tumor progression.

Molecular Interactions

At the molecular level, HEXIM interacts with key factors of the p53 pathway [2]: the basic region located in the C-terminal domain of HEXIM is ubiquitinated by HMD2, which competes with p53 and thus prevents ubiquitination and degradation. This acts together with the protective effect of HEXIM-p53 direct interaction (see above). HEXIM also interacts with NPM, which favors its ubiquitination and degradation at the proteasome, thus regulating its intracellular levels (Figure 1A).

In the cell, HEXIM often belongs to a large ribonucleoprotein complex that regulates the biological activity of P-TEFb (Figure 1A). The composition and stoechiometry of the components of this complex is well described [1], where protein factors (MEPCE, LARP7) assemble around an RNA scaffold (7SK snRNA). The 7SK is by far the most abundant snRNA in the nucleus, and although it is not clear whether its biological function is limited to a structural role, this nonetheless highlights the biological importance of this complex. This 'small' complex can bind and sequester away the P-TEFb kinase, thus forming a 'large' and catalytically inactive complex. This is mediated through direct contact between Cyclin T1 and the highly conserved motif PYNT of HEXIM [1]. This regulatory mechanism is at the heart of the control of transcription elongation and the "pause" mechanism. This is of wide interest because it has been involved in various cancers, several pathologies (cardiomyopathies, HIV expression) and developmental processes.

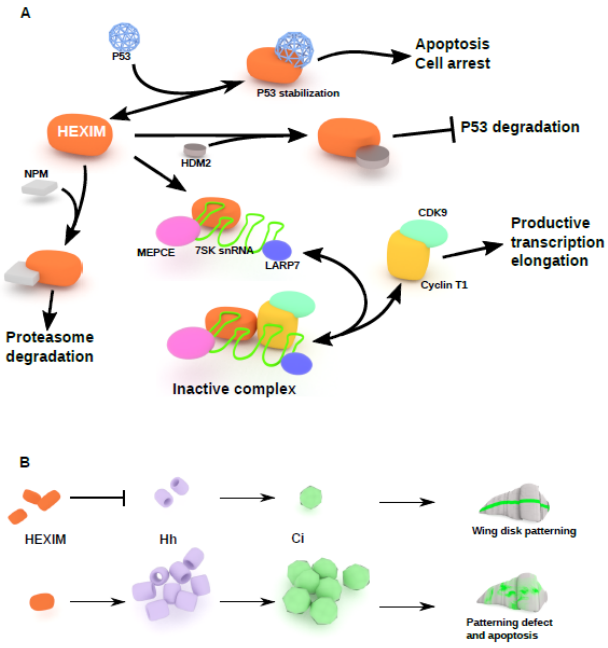

Figure 1: HEXIM, a central regulator of various molecular processes. A. HIXEM stabilizes p53 either through direct interactions, or though interactions with HDM2. The increased p53 activity activates apoptosis and cell cycle arrest, thus (potentially) reducing tumor growth. HEXIM also regulates transcription elongation by sequestring the P--TEFb kinase, and preventing phosphorylation of the RNA Pol II, stalled in a non--productive complex. HEXIM is thus an inhibitor of RNA polymerase II processivity. When bound by NPM, HEXIM is degraded at the proteasome. B. Developmental function of HEXIM during wing disc development. In wild--type wing disc, HEXIM regulates Hh pathway, which signals to the Ci transcription factor defines a specific wing disc pattern. In the HEXIM knockdown mutant, Hh is strongly induced and is expressed at additional ectopic disc compartments. This leads to altered patterns, developmental defects and apoptosis. It is not clear whether the p53 or the P--TEFb pathway (or both) is involved.

Physiological and Developmental Importance of HEXIM

Unfortunately, despite exquisite molecular and mechanistic details, the physiological relevance of HEXIM is still poorly known. In a first paper [6], we showed that HEXIM is an essential gene for Drosophila development, since full knockdown at early developmental stages is embryonic lethal. Furthermore, in any tissue tested, HEXIM knockdown systematically leads to strong development defects (head, legs, wings) with a (almost) complete ablation of organs. We also characterized the small and large P-TEFb complexes, thus showing that the architecture of molecular complexes is evolutionary conserved. This is not a trivial result. We then proposed that the phenotype of HEXIM knockdown mutants is a consequence of misregulated P-TEFb activity, although the precise mechanism was not clear.

Several alternative hypothesis could account for these phenotypes: mutants might suffer from genome wide loss of control of gene transcription, thus leading cells to engage in apoptosis pathways; or knockdown results in the mis-expression of a limited number of genes, that would hinder proper morphogenetic patterns and profoundly affect organ development.

We addressed this point in our more recent paper [7]. We showed that organ failures to develop in HEXIM knockdown mutants was not a trivial consequence of the death of precursor cells. They instead result from strong alterations of morphogenetic patterns. Strikingly, wing imaginal discs of HEXIM knockdown mutants were actually larger than wild-type, and not reduced in size. We also showed that HEXIM knockdown increases ectopic expression of Hedgehog (Hh) and of its terminal transcriptional effector, Cubitus interuptus (Ci) [8], thus giving rise to a strong patterning defect (Figure 1B). The resulting signalling defect induces the apoptosis of proliferative cells, which in turn triggers the compensatory proliferation of neighboring cells. This apoptosis-induced cells proliferation mechanism (AIP) was highly effective and accounts for the larger disc size of mutants. Continuous reduction of HEXIM expression sustained inappropriate patterning ultimately leading to failure of organ formation and growth. It is important to note that AIP maintains tissue homeostasis in both differentiating and proliferative cells, although the underlying mechanisms are different. Indeed, proliferative wing and eyes cells activate AIP through JUNK (Jun N-terminal kinase) pathway and p53, whereas differentiated cells induce AIP by sensing elevated levels of Hh [9]. Thus, in HEXIM knockdown mutant, ectopic Hh expression is not a response to apoptosis, it is more a causative agent. At the molecular level, we showed that both Ci mRNA and protein levels were increased in HEXIM knockdown mutants, and that Ci is a genetic suppressor of HEXIM. Strikingly, the wing phenotype of double HEXIM Ci knockdown mutants is almost wild-type, with only a missing anterior crossvein and an altered vein 3. This unambiguously shows that in wing disc, HEXIM is not a master controller of the genome expression as a whole, but rather a regulator of a more limited number of genes. Although we did not address this in more details, these facts clearly point to Ci as a potential direct target of HEXIM. This is the first time that a single physiological target gene of HEXIM has been proposed *in vivo*.

HEXIM and Cancers

Numerous links between HEXIM and cancers have been reported. Historically, HEXIM was first shown to be involved in breast cancer [10]. More than half of these tumors over-express ERα (oestrogens receptors α). HEXIM physically interacts with ERα, and HEXIM competes for binding to Cyclin T, and thus interferes with the signal transduction of oestrogen-activated ERα to the transcription [11]. In patients treated with the oestrogen antagonist Tamoxifen (Nolvadex), HEXIM expression level is anti-correlated with tumor progression: HEXIM expression is lower in breast cancer cells and HEXIM overexpression inhibit cell proliferation. Furthermore, the high recurrency of tumor in patient exposed to Tamoxifen is associated with lower expression of HEXIM [11]. HEXIM is also involved in angiogenesis, by activating the ubiquitination of HIF-1α and the induction of VEGF (Vascular endothelial growth factor) expression. When HEXIM expression level is decreased, invasion of cells is activated whereas its overexpression suppressed metastasis and angiogenesis. Importantly, this role in angiogenesis is P-TEFb-independent. As noted above (section "Molecular interactions"),

HEXIM also interacts with several components of the p53 pathway in a P-TEFb-independent manner [1], which points to a possible molecular mechanism.

A better description of the molecular mechanisms involved (and thus potential targets) would surely help the design/use of anti-cancer drugs. A few drugs are already available: P-TEFb can be inhibited by Flavopiridol, DRB or other CDK9 inhibitors, while p53 can be activated by Doxorubicin, etoposide and nutlin-3 [3].

On the HEXIM – Hh Signalling Connection

The interaction we found between HEXIM and Hh signalling is a first mechanistic link that may have broad implications. Indeed, in addition to its well established role during embryonic development, Hh signalling is also crucial to maintain homeostasis and repair of adult organs. Depending on the context, Hh can either have a mitogenic function that control cell proliferation, or a morphogenic action that establishes the shape of organs. For example, Hh is involved in liver organogenesis but also in adult liver regeneration after partial hepatectomy (HP) [12], by acting on liver progenitor cell populations to regenerate a pool of hepatocytes and ductular cells. HEXIM action in liver physiology and pathology is presently not addressed and surely deserves some attention. This connection is broad and not limited to physiological regulations. For example, by inducing high level of cell proliferation, Hh signalling also fuels different type of cancers, such as basal cell carcinoma (BCC) or medulloblastoma. More recently, it has been shown that altered levels of Hh renders neighboring cells resistant to apoptosis and create a micro-environment prone to tumorigenesis [13]. Altogether, these data show that the connection between HEXIM and Hh signalling may have a wide field of applications both for human physiology and pathology.

Conclusion

In summary, our work establishes for the first time a functional link between HEXIM and the Hh pathway, and provides a molecular cascade underpining the organ developmental failure. To date, the details of the molecular mechanisms are not fully known, and it is not clear (yet) whether in this context, HEXIM acts on P-TEFb or p53. As a side note, during the course of this work, we noticed a partial rescue of the HEXIM-phenotype, in the differentiating eye cells of a p53 null background (unpublished observation, Uguen). This preliminary data would suggest that p53 may be a suppressor of HEXIM, that could possibly be explained by the inhibition of apoptosis in absence of p53.

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