High-resolution mapping of the protein interaction network for the human transcription machinery and affinity purification of novel RNA polymerase II-associated chaperone

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Numerous reports have described how RNA polymerase II (RNAPII) enacts transcription of protein-coding genes and how this enzyme is regulated by a variety of cues, but, paradoxically, little is known about the assembly of this multisubunit machine or how it travels from the site of its synthesis (the cytoplasm) to the site of its activity (the nucleus). Tandem affinity purification (TAP) of human RNAPII subunits and associated factors have led to the identification of a novel chaperone complex that combines prefoldins and prefoldin-like proteins, the human ortholog of the yeast R2TP complex, and some other factors, including RPB5, a common subunit of all 3 nuclear RNAPs. In this case, the presence of RPB5 does not appear to be a target by which this chaperone interacts with RNAPII, but rather a bona fide component of this complex. Expression of GFP-tagged subunits and immunofluorescence microscopy localized the complex primarily to the cytoplasm. Surprisingly, knockdown of one component of this complex, RPAP3, led to mislocalization of RNAPII to the cytoplasm. Experiments are ongoing to assess the involvement of this chaperone in RNAPII assembly or transport.

Expression of heat shock proteins and heat shock factor1 in response to dehydration in *Xenopus laevis*

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The highly permeable skin of amphibians typically forces them to live in wet habitats. However, some species experience seasonally dry environments and need strategies to survive when water is scarce. The African clawed frog, *Xenopus laevis*, is native to South Africa and lives in ponds that dry out during the dry season. When this occurs, frogs dig underground and enter a resting state called estivation. We hypothesized that, under desiccating conditions, *X. laevis* cells respond by increasing the production of molecular chaperones that protect/stabilize other macromolecules during stress. Western blotting was used to quantify a specific class of chaperone, heat shock proteins (HSPs), in 6 organs of control vs. dehydrated *X. laevis*. Levels of HSP73, HSP70, HSP60, HSP40, and HSP10 were assessed and show widespread upregulation of selected proteins during dehydration. Organ-specific analysis of changes in the expression of the heat shock transcription factor 1, which regulates HSP expression, are also presented.