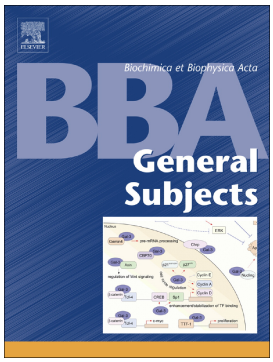

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FoxO4 activity is regulated by phosphorylation and the cellular environment during dehydration in the African clawed frog, *Xenopus laevis*

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Abstract

Background: The African clawed frog, *Xenopus laevis*, is capable of enduring seasonal bouts of severe dehydration stress resulting from transcriptional regulation that facilitates a pro-survival response. Previous studies have shown that dehydration increases antioxidant gene expression in this amphibian. As FoxO4 is known to regulate several antioxidant genes, we sought to understand how differential phosphorylation and environmental factors (urea, temperature) may contribute to its transcriptional regulation during dehydration exposure.

Methods: Immunoblotting was used to quantify relative amount of total FoxO4, of phosphorylated FoxO4, and of the factors in the Ras-Ral pathway that regulate FoxO4 activity in *X. laevis* skeletal muscle during dehydration. DNA-protein interaction (DPI)-ELISA was used to measure transcription factor-binding to their consensus sequences in the promoters of target genes. Environmental DPI-ELISA was used to assess the effect of the cellular environment on transcription factor binding.

Results: FoxO4 protein levels do not change during dehydration, but FoxO4-binding to DNA increases with higher dehydration. The Ras-Ral pathway does not appear to be involved in regulating FoxO4 during dehydration, but Akt-mediated FoxO4 phosphorylation at Ser-193 decreases during high dehydration exposure, which is indicative of increased FoxO4 activity. Further assessment indicated that FoxO4-DNA binding affinity is drastically affected by environmental changes in urea and temperature.

Conclusion: FoxO4 plays an important role during dehydration stress in *X. laevis*, and its activity could be regulated through Akt-mediated phosphorylation, and changes in temperature or urea.

General Significance: Dehydration triggers regulatory mechanisms of transcription by inducing differential phosphorylation and changes to urea in *X. laevis*.

Keywords

DPI-ELISA, Dehydration, *Xenopus laevis*, Western Blotting, Forkhead Box O, Myogenin

1. Introduction

Many amphibians enter a state of estivation in climates with dry seasons (high temperatures, and low food and water availability), and they do so by taking refuge underground to tolerate these dry conditions. These animals undergo both physiological and biochemical adaptations to reduce body water loss, and to survive using the energy reserves that were built up prior to feeding [1,2]. Anuran estivation