CALMODULIN-DEPENDENT NUCLEAR IMPORT PATHWAY OF THE TESTIS-DETERMINING FACTOR SRY

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Modulation of the nuclear entry of transcription factors (TFs) and chromatin components is a means by which eukaryotic cells can regulate gene expression in response to extracellular signals and the cell cycle during differentiation and development. TFs and chromatin components access the nucleus through nuclear localisation sequences (NLSs), which mediate interaction with components of the cellular nuclear import machinery, such as members of the importin superfamily. The Ca^{2+}-binding protein calmodulin (CaM) has previously been shown to bind at or near NLSs in several nuclear-localising proteins that have important roles in testis development including the Y chromosome-encoded HMG-domain-carrying chromatin remodelling factor SRY, and related factor SOX9, both of which are key regulators of gonadal development. SRY function in the nucleus of somatic cells of the fetal gonad, in particular, is essential for development of a testis in males. Here we present new findings implicating a role for CaM in modulating SRY nuclear accumulation, whereby treatment of transfected cells with CaM antagonists significantly reduces nuclear accumulation of green fluorescent protein (GFP)-fusion proteins encoding either full length SRY or the SRY HMG domain alone. An *in vitro* nuclear transport assay using bacterially expressed fluorescent proteins showed similar results, with native gel electrophoresis/fluorimaging and fluorescence polarisation assays, indicating direct binding of CaM to the SRY HMG domain in Ca^{2+}-dependent fashion. Since clinical mutations resulting in sex reversal occur within SRY's CaM-binding NLS, these results may shed new insight into CaM-dependent pathways of nuclear protein import, and how this may relate to testis development.

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