Dopaminergic signaling is predominantly mediated through altered downstream signaling mechanisms that control protein phosphorylation. Though the majority of studies in this field have focused on the activity of protein kinases, a significant body of research has also investigated the role of protein phosphatases. In particular, the serine/threonine protein phosphatase PP2A is known to dephosphorylate several targets within dopamine-regulated signaling pathways. One of these is DARPP-32, a mediator of psychostimulant action through regulation of its phosphorylation sites (Figure 1a) (Swennington et al. 2005; Walaa et al. 2011).

PP2A is a heterotrimer composed of a catalytic C-subunit, a regulatory A-subunit, and a regulatory B-subunit. The regulatory B-subunit allows for substrate selectivity, with at least four identified distinct subtypes of B-subunits (Lambrecht et al. 2013). The striatin family, comprised of striatin-3, and zinedin, are members of the B” family of B-subunits and are of interest due to their recently demonstrated capability in non-neuronal systems to organize large signaling complexes. These complexes have been implicated in a variety of cellular processes in non-mammalian model systems, including endocytosis, cell function stability, and Golgi assembly, and are referred to as STRIPAK: striatin-interacting proteinase and kinase complexes (Figure 1b) (Hwang and Pallas 2014). However, nothing is known of striatin or STRIPAK function within the striatum, despite the fact that striatin-1 is highly enriched within this brain region. Through the use of the in vitro immunoprecipitation and affinity purification to interrogate the PP2A-striatin interacosome and through in vitro assays measuring dephosphorylation of endogenous PP2A substrates, we have accumulated evidence identifying DARPP-32 as a substrate of PP2A-striatin. Due to the role of DARPP-32 as a mediator of the action of drugs of abuse, this data may also implicate striatin and the STRIPAK complex as potential targets of these drugs.

**RESULTS**

Figure 1: DARPP-32 and the structure of the STRIPAK complex.

![A schematic of the structure of DARPP-32 and phosphorylation sites relevant to dopaminergic signaling.](image)

**MATERIALS AND METHODS**

**Protein culture**

Following determination of striatal subunits and PP2A binding partners through mass spectrometry, these results were verified using antibodies specific to identified binding partners in primary striatal cultures. Striatal cultures were harvested at 18, 28, and 56 days post-confluence and lysed in 0.25 M sucrose, 10 mM Hepes, and 1 mM EDTA containing protease inhibitors and phosphatase inhibitors. Immunoprecipitations were performed from striatal lysates using both PP2A-specific and the PP2A-subunit-specific antibodies. Immunoprecipitation product was then examined by Western blotting for detection of DARPP-32 and PP2A-B, respectively. To determine if the PP2A substrates identified through mass spectrometry were also impaired in primary neuronal cultures, primary striatal cultures were also harvested at 18, 28, and 56 days post-confluence and lysed in 0.25 M sucrose, 10 mM Hepes, and 1 mM EDTA containing protease inhibitors and phosphatase inhibitors. Immunoprecipitations were performed from striatal lysates using both PP2A-specific and the PP2A-subunit-specific antibodies. Immunoprecipitation product was then analyzed by Western blotting for detection of DARPP-32 and PP2A-B, respectively.

**REFERENCES**


For a detailed list of references, please consult the original article. *Neuropharmacology* 30, 589-592 (2000).