Seminars in Cell & Developmental Biology 23 (2012) 827–833

Contents lists available at SciVerse ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Structures of YAP protein domains reveal promising targets for development of new cancer drugs

Marius Sudol[a,b,*], Denis C. Shields[c], Amjad Farooq[d,e]

[a] Laboratory of Signal Transduction and Proteomic Profiling, Weis Center for Research, Geisinger Clinic, Danville, PA 17821, USA
[b] Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA
[c] UCD Complex and Adaptive Systems Laboratory, UCD Conway Institute and School of Medicine and Medical Sciences, University College Dublin, Dublin 4, Ireland
[d] Department of Biochemistry and Molecular Biology, Leonard Miller School of Medicine, University of Miami, Miami, FL 33136, USA
[e] Braman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, Leonard Miller School of Medicine, University of Miami, Miami, FL 33136, USA

ARTICLE INFO

Article history:
Available online 17 May 2012

Keywords:
TEAD transcription factor
WW domain
PDZ domain
Nuclear localization
Digitoxin
Dobutamine

ABSTRACT

YAP (Yes-associated protein) is a potent oncogene and a major effector of the mammalian Hippo tumor suppressor pathway. In this review, our emphasis is on the structural basis of how YAP recognizes its various cellular partners. In particular, we discuss the role of LATS kinase and AMOTL1 junction protein, two key cellular partners of YAP that bind to its WW domain, in mediating cytoplasmic localization of YAP and thereby playing a key role in the regulation of its transcriptional activity. Importantly, the crystal structure of an amino-terminal domain of YAP in complex with the carboxy-terminal domain of TEAD transcription factor was only recently solved at atomic resolution, while the structure of WW domain of YAP in complex with a peptide containing the PPxY motif has been available for more than a decade. We discuss how such structural information may be exploited for the rational development of novel anti-cancer therapeutics harboring greater efficacy coupled with low toxicity. We also embark on a brief discussion of how recent in silico studies led to identification of the cardiac glycoside digitoxin as a potential modulator of WW domain–ligand interactions. Conversely, dobutamine was identified in a screen of known drugs as a compound that promotes cytoplasmic localization of YAP, thereby resulting in growth suppressing activity. Finally, we discuss how a recent study on the dynamics of WW domain folding on a biologically critical time scale may provide a tool to generate repertoires of WW domain variants for regulation of the Hippo pathway toward desired, non-oncogenic outputs.

Contents

1. Introduction

YAP is a transcriptional co-activator and a major effector of the mammalian Hippo tumor suppressor pathway [1]. Upon activation of the Hippo pathway by cell-to-cell contacts, YAP becomes phosphorylated at various S residues, including S127, by a concerted action of two upstream kinases, MST and LATS [2]. The pS127 and the flanking residues in turn serve as a docking site for 14-3-3 proteins and the resulting interaction is primarily responsible for the cytoplasmic localization of YAP in response to activation of Hippo

Abbreviations: A, alanine; AMOTL1, angiomotin-like 1; EMT, epithelial-to-mesenchymal transition; LATS, large tumor suppressor; MST, mammalian ste20-like protein kinase; PDZ domain, Psd-95 (post synaptic density protein), DlgA (Drosophila disc large tumor suppressor) and ZO1 (zonula occludens-1 protein); P, proline; S, serine; TAZ, transcriptional co-activator with PDZ-binding motif, also known as WWTR1, WW-domain-containing transcription regulator 1; TEAD factor, TEA domain-containing transcription factor, WW domain, Tryptophan–Tryptophan domain; Y, tyrosine; YAP, Yes kinase-associated protein; ZO, zona occludens.

* Corresponding author at: Laboratory of Signal Transduction and Proteomic Profiling, Weis Center for Research, Geisinger Clinic, Danville, PA 17821, USA.
Tel.: +1 570 271 6677.
E-mail address: msudol1@geisinger.edu (M. Sudol).

1084-9521/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcdb.2012.05.002

pathway [3]. Within the cytoplasm, YAP mediates pro-apoptotic signals. However, phosphorylation of S residues other than S127 is believed to lead to ubiquitination and proteosomal degradation of YAP, thereby down-regulating pro-apoptotic signaling through YAP [2]. Remarkably, the absence of phosphorylation of YAP at S127 serves as a signal for its translocation to the cell nucleus where it forms a complex with members of the TEAD family of transcription factors that drive transcription of growth-promoting and anti-apoptotic genes [1,4].

Importantly, YAP is a *bona fide* oncogene. The amplification or over-expression of the YAP gene was demonstrated in human cancers of various organs, and YAP over-expression in mammalian cells was shown to elicit a plethora of oncogenic parameters [5,6]. The nuclear localization of YAP in tumor biopsies correlates with poor prognosis for cancer patients [1,2].

As mentioned in previous chapters of this issue, the Hippo tumor suppressor pathway was originally delineated in Drosophila by genetic screening approaches [7]. YAP is the mammalian ortholog of Drosophila Yki, and MST kinase is the mammalian ortholog of the Drosophila Hippo kinase from which the name of the pathway was derived.

Several structural studies have provided valuable insight into the details of YAP signaling via complexes with partner proteins. In particular, the crystal structures of YAP in complex with TEAD4 and the WW domain of YAP with its PPxY sequence-containing ligands were solved and will be discussed in detail [8,9]. We will shed light on how the cardiac glycoside digitoxin [10] may serve as a lead compound for the development of drugs that could antagonize the oncogenic activity of YAP in cells and animal models, and ultimately be of use in managing cancer in clinics. We will also briefly address PDZ domains that recognize the tail sequence of YAP protein and act as mediators of important regulatory complexes with YAP oncogene, representing potential targets for developing anti-cancer drugs [11].

2. Modular structure of YAP1 and YAP2 isoforms

There are two major isoforms of YAP that are derived by differential splicing [12–14]. These are YAP1, containing one WW domain and YAP2, containing two WW domains (Fig. 1). Actually, this very difference between the two YAP isoforms observed in the process of characterization of various cDNA clones of YAP led to identification of the WW domain as a signaling module [13–15]. Today, we know that there are more than 2 isoforms of YAP (at least 8), which are generated by differential splicing of short exons located within the transcriptional activation domain of YAP.

The modular structure of both YAP1 and YAP2 contains at the amino terminal region a TEAD factor-binding domain that is located between amino acids 47 and 154 [4]. The first WW domain is located between amino acids 174 and 204, and the second WW domain that is present only in YAP2 is located between amino acids 233 and 263. Both YAP1 and YAP2 also contain an SH3-binding motif [12], and a transcriptional activation domain, the latter located at the carboxy-terminal half of the protein [14]. A PDZ-binding motif composed of 5 terminal amino acids, -FLTWL, critical for nuclear translocation and binding to PDZ domains of ZO-1 and ZO-2 proteins, is located on the very carboxy-terminal end of YAP1 and YAP2 proteins [11].

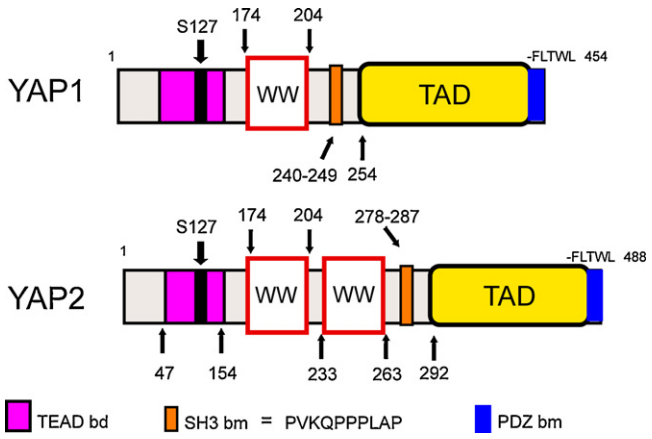

Fig. 1. Modular structures of the two major isoforms of YAP protein. TEAD binding domain (bd), WW domains, SH3 domain-binding motif (bm) transcriptional activation domain (TAD), and PDZ domain-binding motif (bm) are demarcated on the scheme of structures. See text for more details.

3. YAP–TEAD complex as a primary target of drugs

The complex between YAP and TEAD proteins is necessary for growth promoting activity of YAP oncogene [12], therefore it is a subject of intense structure–function analyses. Two groups have solved crystal structures of YAP and TEAD complexes at ~3 Å resolution, revealing interesting molecular details of the binding interface [8,9]. Two α-helices (α1 and α2) of YAP, along with the connecting hydrophobic linker, sequester TEAD in a manner akin to a pair of forceps (Fig. 2). Indeed, three major interaction sites that accommodate the clip are clearly discernible in the TEAD protein in this complex: site 1, where the α1 helix fits in a rather large groove of TEAD surface, site 2 that accommodates the linker sequence, and site 3 into which the α2 helix of YAP fits snugly [8]. The relatively high resolution of both structures allowed for unequivocal identification of specific residues that make hydrogen bonds, van der Waals contacts and hydrophobic interactions between TEAD and YAP. Notably, the structures provided the rationale for the Y421H disease-causative mutation present in TEAD1, responsible for Sveinsson's chorioretinal atrophy syndrome [8,16]. The Y421 residue is located in a specific portion of the carboxyterminal-region of TEAD1, which was mapped as the region required for physical and functional interaction with YAP. From the structure, one could deduce that the replacement of Y421 with H eliminated a hydrogen bond with a neighboring S and affected a hydrophobic contact with closely located F, both of these neighboring residues present in YAP. From this rather significant change in molecular contacts, one could predict that the complex between YAP and TEAD1 (one of the four members of the TEAD family of transcription factors) would be affected. Indeed, the prediction was well confirmed by earlier biochemical results showing that the Sveinsson's Y421H mutation abrogated the TEAD1 complex with YAP [17].

As is evident from the structure itself, and also from the biochemical interrogations of various point mutants of the interface residues in terms of the stability of the complex, it seems that all three sites of interaction act in concert to mediate the YAP–TEAD complex [8,9]. It is important to stress that the deletion of the short linker sequence in YAP results in a diminished interaction between YAP and TEAD [9]. This conformation closely resembles