Binding of the Cancer-Related TCTP Protein to Mcl-1/Bcl-xL Requires and Triggers an Order-to-Disorder Transition to Make its BH3 Region Accessible

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The small ~20 kDa TCTP protein acts in key cellular functions such as apoptosis, transcription or protein synthesis and favors cell growth and survival, proliferation, and malignant transformation. High TCTP levels are found in many tumor cells and since silencing/targeting TCTP leads to less malignant phenotype, it is an established target in several cancers with on-going clinical trials[1]. Despite advanced therapeutic applications, the molecular basis of TCTP biology is still poorly understood. TCTP pro-survival properties partially result from the potentialization of Bcl-xL and Mcl-1 anti-apoptotic properties and in a negative feedback regulatory loop with p53[2,3] (Fig 1A). TCTP contains a ~20 aa BH3-like motif that adopts an α-helix when bound at the BH3 binding groove of Bcl-xL but this BH3-like sequence has anti-apoptotic instead of the pro-apoptotic properties as observed for most other BH3-only proteins. Surprisingly, this BH3 region (blue) is solvent-protected and forms two solvent-protected β-strands in the free TCTP (Fig 1B), suggesting a major conformational rearrangement in TCTP upon Bcl-xL binding. Furthermore, TCTP also interact with Mcl-1 through a yet unknown manner.

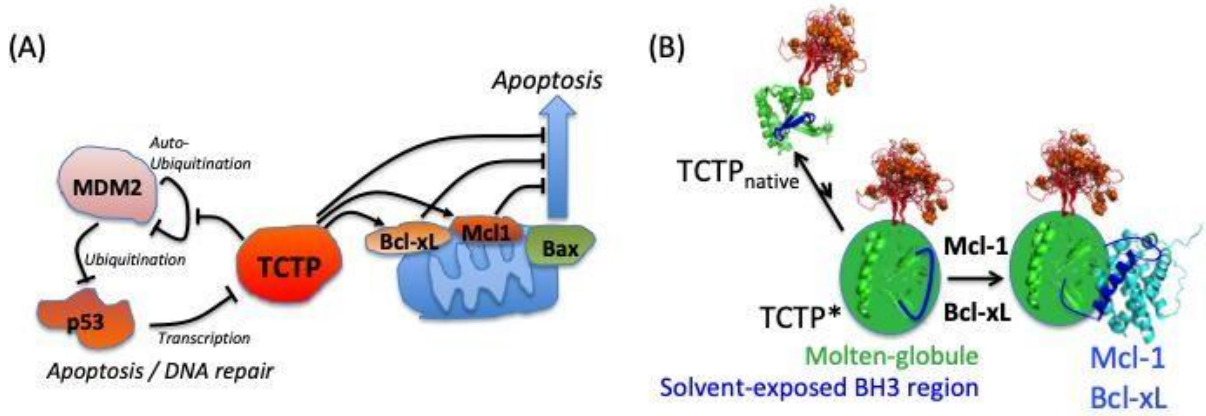

Figure 1: (A) TCTP interacts with Bcl-xL and Mcl-1 to increase their anti-apoptotic function. TCTP also increases p53 degradation by inhibiting the auto-ubiquitination of the p53 E3-ligase MDM2. (B) Model describing TCTP and Bcl-xL/Mcl-1 complex formation involving the on-pathway TCTP* intermediate state. The BH3 region (shown in blue) can adopt β-, random coil, or α-helical conformations in the free TCTP, TCTP* and in TCTP in complex with Bcl-xL/Mcl-1.

In this work, we addressed the binding mechanism of TCTP to Bcl-xL and Mcl-1 by a combination of NMR, SAXS, fluorescence, circular dichroism, and limited proteolysis techniques. We show that the unpinning of the BH3 region is required prior to its interaction with Bcl-xL/Mcl-1 partners, which results in a destabilization of the TCTP core region (in green) into a molten globule

[1] Amson, R., Auclair, C., Andre, F., Karp, J., and Telerman, A. (2017), *Results Probl Cell Differ 64*, 283-290.
[2] Amson, R., Pece, S., Lespagnol, A., Vyas, R., Mazzarol, G., Tosoni, D., Colaluca, I., Viale, G., Rodrigues-Ferreira, S., Wynendaele, J., Chaloin, O., Hoebeke, J., Marine, J. C., Di Fiore, P. P., and Telerman, A. (2012), *Nature medicine 18*, 91-99.
[3] Assrir, N., Malard, F., and Lescop, E. (2017), *Results Probl Cell Differ 64*, 9-46.

(MG) state characterized by preserved secondary structure elements and a compact destabilized hydrophobic core (Fig 1B). The newly identified disordered TCTP* state is the on-pathway binding intermediate that is lowly populated in absence of binding partner but stabilized by high-pressure (1500 bars) or by (rather low) urea concentration while preserving its binding capacity. We further showed that the TCTP BH3 sequence binds the classical BH3 binding groove of Mcl-1 with rather low affinity and in a presumably helical conformation, through a highly dynamic protein-protein interface. We further identified that the atypical aspartate residue D16 at the position h1, usually occupied by an isoleucine in more classical BH3 sequences, is responsible for the decreased affinity to Mcl-1 and for the increased interface dynamics, which could be related to the unexpected anti-apoptotic behavior of TCTP. Taken together, this work illuminates how protein order-to-disorder transition may control functional protein-protein interaction with important cellular fate and controlling such transition opens new avenue in therapeutic applications.