



Minireview

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# Prothymosin $\alpha$ , a protein implicated in the proliferation and survival of lymphocytes

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## Article Info

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## ABSTRACT

Prothymosin  $\alpha$  (ProT $\alpha$ ) is a 109-11 amino acid protein widely distributed in mammalian tissues and particularly abundant in lymphoid cells. Genomic and proteomic studies led to consider ProT $\alpha$  as a multifunctional protein implicated in nuclear and cytoplasmic functions. The nuclear function of ProT $\alpha$  is related to chromatin activity through its interaction with core histones and proteins involved in chromatin remodelling, whereas, processes related to the phosphorylation, the proteolytic processing to generate Thymosin  $\alpha$ 1, and the role as anti-apoptotic factor of ProT $\alpha$ , are linked to its cytoplasmic location. Affinity chromatography and co-immunoprecipitation experiments have demonstrated novel interactions of ProT $\alpha$  with acidic proteins such as SET, ANP32A, and ANP32B in the cytoplasm of proliferating lymphocytes. The stabilization of these interactions by chemical cross-linking with formaldehyde shows that they are formed through associations in six acidic complexes which correspond to selective interactions of SET and ANP32 proteins with ProT $\alpha$ . These ProT $\alpha$ -complexes also include cytoplasmic proteins implicated in membrane remodelling and in mitochondrial activity. In conclusion, these novel protein interactions of ProT $\alpha$  observed in proliferation activity and apoptosis studies, suggest that they might be related to mechanisms involved in the proliferation activity and the apoptotic control of lymphocytes.

## Introduction

In 1966 White and Goldstein's group intended to identify protein factors which could be implicated in the biological function of the thymus and thus isolated a mixture of polypeptides named thymosin<sup>1</sup>. The best known thymosin preparation is a calf thymus extract named Thymosin Fraction 5 (TF5). The TF5 contains about 30 different heat-stable polypeptides showing immune regulatory properties in several assay systems<sup>2</sup> *in vitro* and *in vivo*. The first purified, sequenced and most characterized component of TF5 is the Thymosin  $\alpha$ 1 (T $\alpha$ 1), a polypeptide comprised of 28 amino acids. T $\alpha$ 1 is 10-1000 times more active than TF5 in different immune assays<sup>3</sup>. Thymosin  $\alpha$ 11 (T $\alpha$ 11), another component of TF5 structurally related to T $\alpha$ 1, was later isolated and sequenced<sup>4</sup>. The structure of T $\alpha$ 11 corresponds to the sequence of T $\alpha$ 1 plus seven additional amino acid residues at its C-terminus. T $\alpha$ 11 is about four times less abundant than T $\alpha$ 1 in TF5, however, it shows similar activity to T $\alpha$ 1 in some *in vivo* assays<sup>5</sup>.

The strategy to characterize a precursor of the  $\alpha$ -thymosins in the thymic cells from translation products of calf thymus mRNAs led our group to isolate a protein which included T $\alpha$ 1 in its sequence<sup>6,7</sup>. This protein, isolated from rat thymus was later sequenced and named Prothymosin  $\alpha$  (ProT $\alpha$ )<sup>8</sup>. The sequence of ProT $\alpha$  contains a T $\alpha$ 1 structure located at its first 28 amino acid residues and a T $\alpha$ 11 structure located at its 35 amino acid residues. The primary structure of ProT $\alpha$  obtained from other mammalian tissues demonstrates that ProT $\alpha$  is an acidic protein with a highly conserved sequence containing between 109 and 111 amino acids. Figure 1a shows the sequence of human ProT $\alpha$ <sup>9</sup>, which includes a central

acidic region (residues 40-82) with glutamic and aspartic residues and other structural characteristics.

Later studies on ProT $\alpha$  gene expression end the theory of an exclusive role of the  $\alpha$ -thymosins in the thymus immune function, as deduced from its generalized presence in different mammalian tissues<sup>10,11</sup>. The functional ProT $\alpha$  gene (PTMA) is located on chromosome 2 in humans<sup>12</sup>. A significant feature of ProT $\alpha$  is its high level of gene expression in proliferating cells<sup>13,14</sup>, on the range of core histones, especially in lymphoid tissues<sup>11</sup>. In Figure 1b are summarized the concentrations of ProT $\alpha$  in different rat tissues.

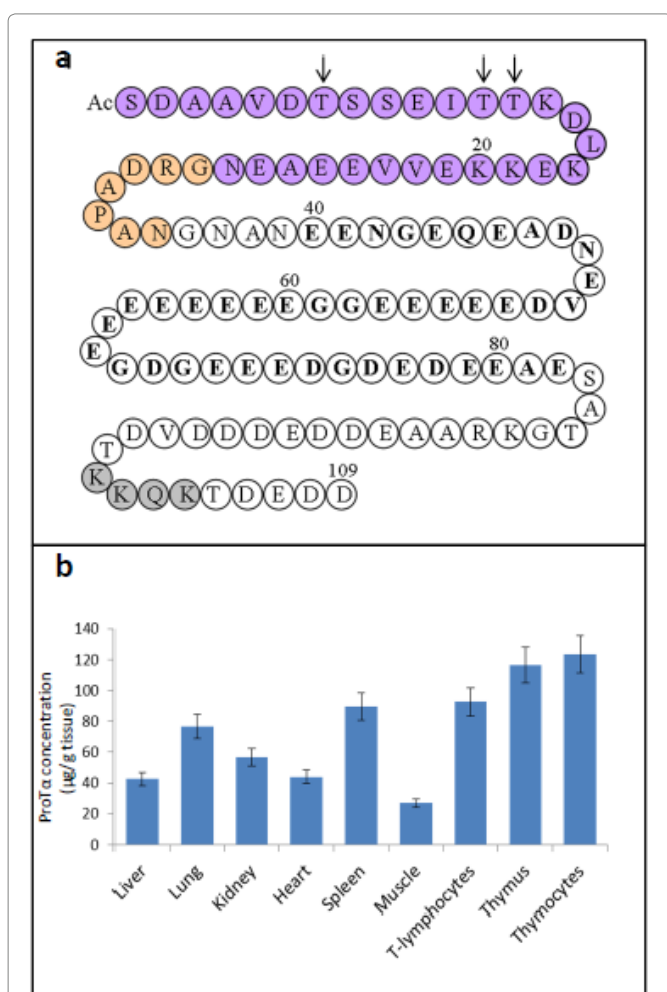
### The biological function of ProT $\alpha$

Further research on the intracellular evolution of ProT $\alpha$  provides important clues on its possible biological role. Characteristics of its gene structure and expression show the absence of a signal peptide<sup>15</sup>. Similarly, co-translational acetylation of its N-terminal serine residue<sup>16</sup> indicates that ProT $\alpha$  should have an intracellular site of action or at least that it can be secreted by a non-conventional mechanism. Studies on the subcellular distribution of ProT $\alpha$  demonstrate a preferential nuclear location in proliferating cells<sup>17,18</sup> which is in agreement with the presence of a nuclear localization signal at the C-terminus of its sequence<sup>19</sup>. However, the prominent cytoplasmic presence of ProT $\alpha$  in cell lysates demonstrates a special ability of this protein to leak from the nucleus during the cell extraction process<sup>15,20</sup>.

The characterization of the components present in a nuclear-protein complex isolated by affinity to ProT $\alpha$ <sup>20,21</sup> has served towards the elucidation of the putative mechanisms by which ProT $\alpha$  might be involved in the nuclear activity. This nuclear-protein complex is comprised of core histones, acetyltransferases of histone H3 and H4, the methyltransferase of histone H3, as well as transcription factors and other proteins implicated in chromatin remodelling<sup>22</sup>. Its presence in this complex supports a nuclear role of ProT $\alpha$  related with the chromatin activity.

In addition to nuclear activity, studies on the cytoplasmic evolution of ProT $\alpha$  also contributed to deepen the knowledge of its biological function. In this sense, data revealing the natural processing of ProT $\alpha$  to generate T $\alpha$ 1 and T $\alpha$ 11, and those showing the cytoplasmic phosphorylation of ProT $\alpha$  are especially relevant.

The lysosomal asparaginyl endopeptidase, mammalian legumain, is responsible for the processing of ProT $\alpha$  *in vivo* to generate T $\alpha$ 1 and T $\alpha$ 11, demonstrating the natural origin of these polypeptides<sup>23</sup>. This research also demonstrates that T $\alpha$ 1 is the main product in the cytoplasm, showing a concentration similar to that of ProT $\alpha$ . Although potential immunotherapeutic effects of T $\alpha$ 1 have been recently



**Figure 1. a) Human Prothymosin  $\alpha$  amino acid sequence (1-109)<sup>9</sup>.** AcS indicates acetylated N-terminal serine derived from the generalized co-translational processing which undergoes ProT $\alpha$ <sup>16</sup>. T $\alpha$ <sub>1</sub> sequence is indicated in purple circles. T $\alpha$ <sub>11</sub> sequence comprises the T $\alpha$ <sub>1</sub> sequence plus an additional 7 residues at its C-terminus (indicated in orange circles). Other structural characteristics of the ProT $\alpha$  sequence (referred in the text) are also indicated: arrows mark the phosphorylation sites of ProT $\alpha$  in normal (Thr7 and Thr12 or 13) and carcinogenic (only Thr7) lymphocytes; acidic region is indicated in bold type and nuclear localization signal is shown in grey circles. **b) Concentrations of ProT $\alpha$  determined in extracts of different rat tissues<sup>11</sup>.**

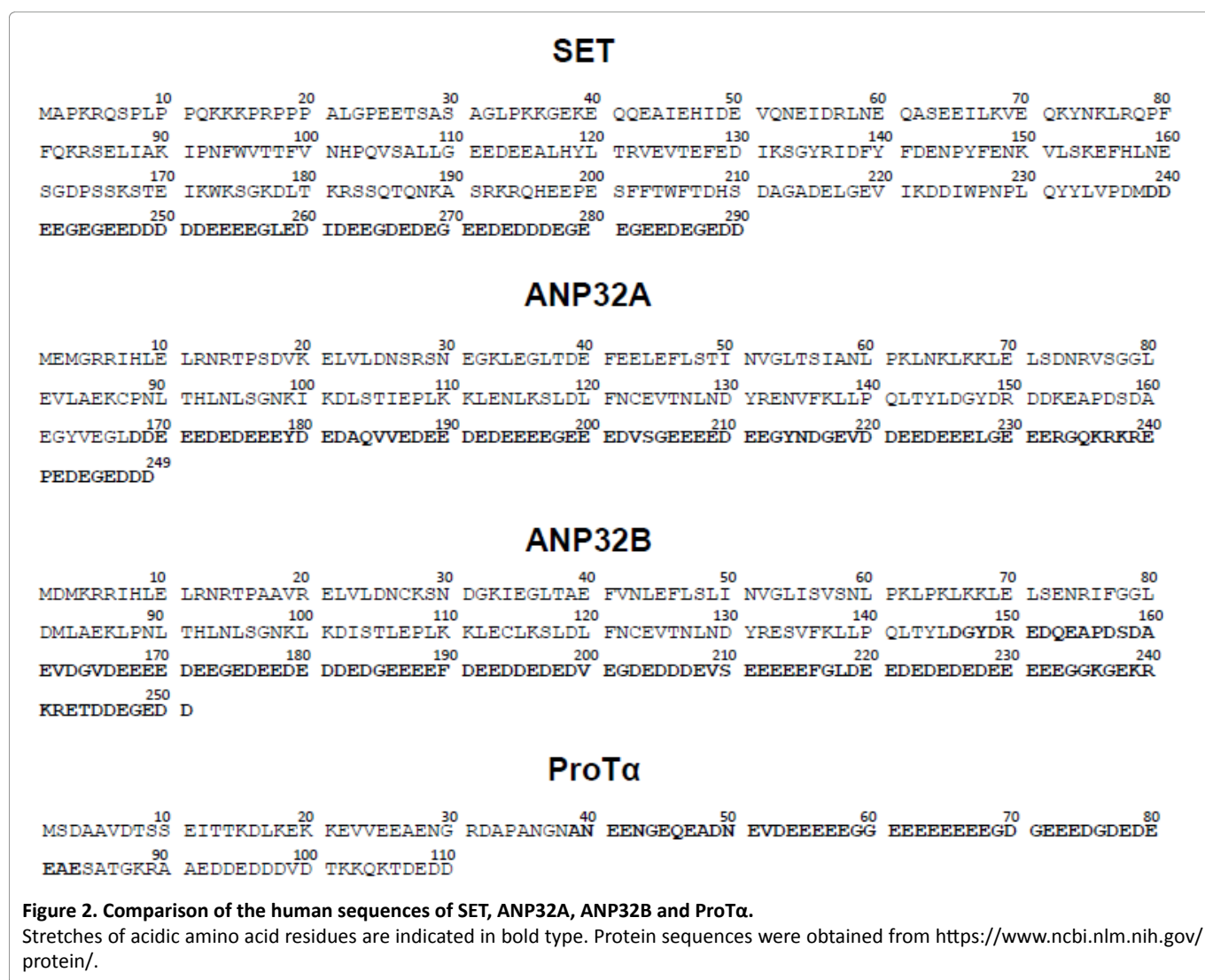
reported<sup>24</sup>, the mechanism of the biological role of this polypeptide remains elusive.

Cytoplasmic phosphorylation of ProTα in threonine residues is dependent on the proliferation activity<sup>25,26</sup> and the carcinogenic transformation of the cells. Thus, residues Thr12 or Thr13 are phosphorylated in addition to Thr7 in normal lymphocytes while in carcinogenic lymphocytes Thr7 is the only residue phosphorylated<sup>27</sup>. The M2 isoenzyme of the pyruvate kinase is the enzyme responsible for the phosphorylation of ProTα that shows this novel dual function<sup>28</sup>.

A cytoplasmic function of ProTα as an anti-apoptotic factor has also been reported. ProTα seems to be related to the control of the apoptosome activity counteracting the pro-apoptotic action of the protein ANP32A (also known as PHAPI, pp32 or I1PP2A) to promote the caspase-3 activation<sup>29</sup>. Experiments using cells transformed with ProTα mutated to prevent its phosphorylation, indicate that phosphorylation of ProTα might be required for its anti-apoptotic activity<sup>18</sup>. This role of ProTα in the apoptotic

control is shared by other acidic proteins like SET<sup>30</sup> (also named TAF-I, PHAPII or I2PP2A) and ANP32B<sup>31</sup> (also named PHAPI2, and APRIL). These proteins, and ANP32A, are nucleus-cytoplasmic shuttling proteins with a long stretch of acidic amino acids. They have a remarkable structural and functional homology with ProTα and a common ability to interact with histones and to influence the chromatin activity. In Figure 2 are shown their sequences.

Although implication of SET in the apoptotic control is well characterized, the mechanisms by which ProTα and the ANP32 proteins are implicated in this process remain elusive. Data from our laboratory, recently published in the Archives of Biochemistry and Biophysics<sup>35</sup>, reveal interactions of ProTα with SET, ANP32A, ANP32B and other cytoplasmic and mitochondrial proteins that shed some light on the mechanisms of a cytoplasmic action of ProTα and their targets. According to the actual data on the cellular site of action of ProTα, reports showing extracellular effects of this protein should be questioned, at least until the mechanisms for its cellular secretion can be demonstrated.



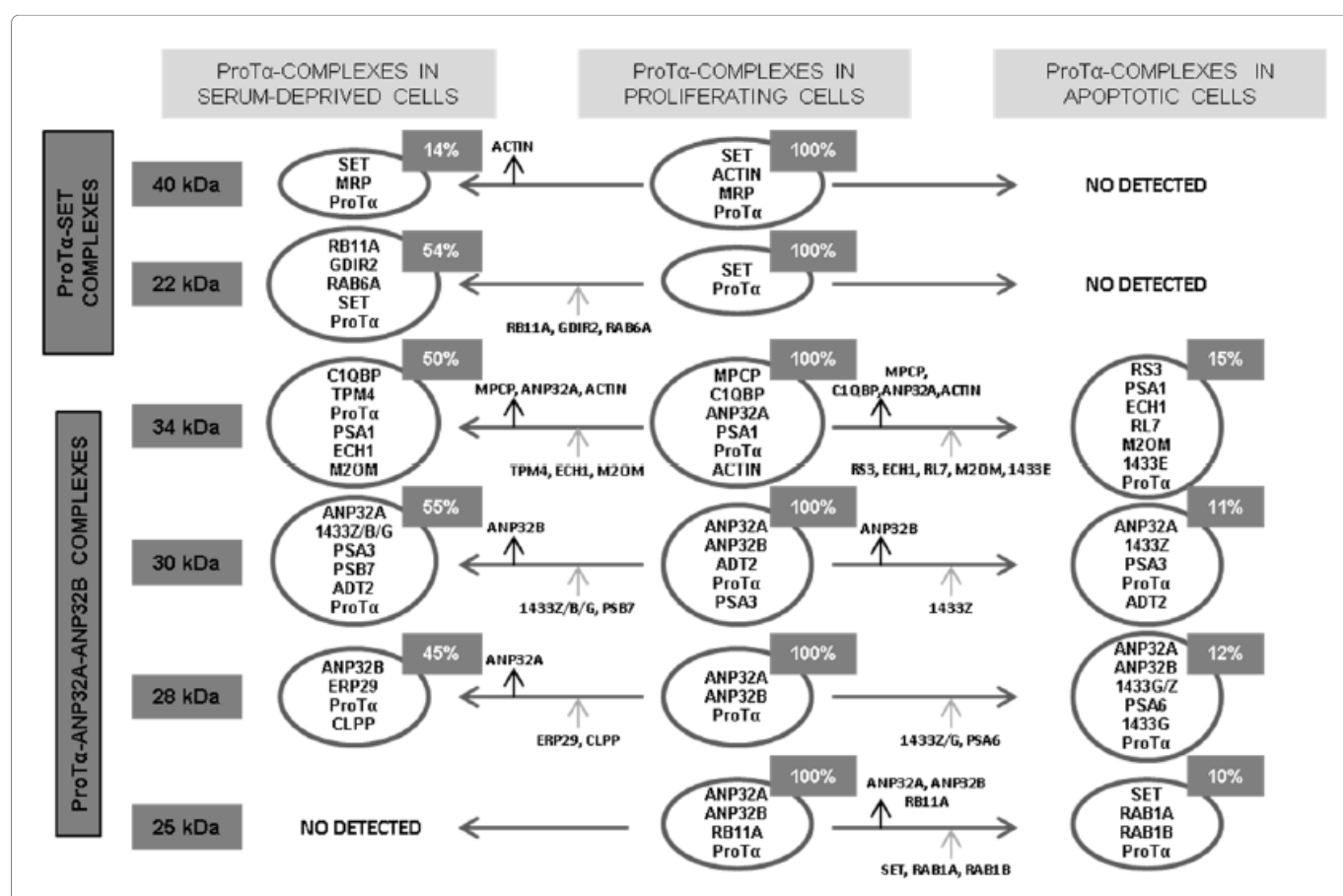
**Figure 2. Comparison of the human sequences of SET, ANP32A, ANP32B and ProTα.** Stretches of acidic amino acid residues are indicated in bold type. Protein sequences were obtained from <https://www.ncbi.nlm.nih.gov/protein/>.

### The interactions of ProTα with SET, ANP32A and ANP32B in lymphocytes

Affinity chromatography and co-immunoprecipitation experiments performed in subcellular fractions of transformed human T-lymphocytes (Jurkat cells) show a novel ability of ProTα to interact *in vivo* with ANP32A and ANP32B proteins in the cytoplasm of these cells. These results also confirm the ability of ProTα to interact with SET<sup>36</sup>.

Further experiments with Jurkat cells previously treated with formaldehyde to stabilize possible interactions by chemical cross-linking, lead to isolating six acidic protein complexes ranging from 25 to 45 kDa. These results confirm the ability of ProTα to interact with SET and ANP32 proteins.

The composition of the ProTα-complexes (see Figure 3) denoted a different association of ProTα with SET (complexes 40 kDa and 22 kDa), ANP32A and ANP32B (complexes 30, 28 and 25 kDa), and ANP32A (complex 34 kDa). Other components in complexes of 40, 34, 30 and 25 kDa, are multifunctional proteins which would relate them with cytoplasmic functions such as: proteolytic processing, membrane trafficking, mitochondrial permeability and cell proliferation. The characterized interactions of ProTα with SET and ANP32 proteins reveal a significant capacity of these acidic proteins to associate. This property has been already stated in complexes in which interactions between SET and ANP32 proteins are involved in the apoptotic control<sup>30</sup> or in the regulation of histone acetylation<sup>37</sup>.



**Figure 3. Scheme summarizes the composition of the ProTα-immunoreactive complexes isolated from proliferating Jurkat cells, as well as their evolution when proliferating activity is reduced by serum deprivation or apoptosis induced by staurosporine.**

Arrows indicate addition (grey arrows) or loss (black arrows) of proteins in the diverse complexes from proliferating cells when proliferation is reduced or apoptosis induced. Relative abundance of each complex is indicated in the upper insets. **Acronyms of proteins other than ProTα, SET, ANP32A, ANP32B and β-actin present in the different complexes:** **40 kDa:** MRP (MARCKS-related protein); **22 kDa:** RB11A (Ras-related protein Rab-11A), GDIR2 (Rho GDP-dissociation inhibitor 2), RAB6A (Ras-related protein Rab-6A); **34 kDa:** MPCP (Phosphate carrier protein, mitochondrial), C1QBP (Complement component 1Q subcomponent-binding protein), PSA1 (Proteasome subunit alpha type-1), TPM4 (Tropomyosin alpha-4 chain), ECH1 (Delta(3,5)-delta(2,4)dienoyl-CoA isomerase, mitochondrial), M2OM (Mitochondrial 2-oxoglutarate/malate carrier protein), RS3 (40S ribosomal protein S3), RL7 (60S ribosomal protein L7), 1433 E (14-3-3 protein epsilon); **30 kDa:** ADT2 (ADP/ATP translocase 2), PSA3 (Proteasome subunit alpha type-3), PSB7 (Proteasome subunit beta type-7), 1433Z/B/G (14-3-3 protein zeta-delta/beta-alpha/gamma); **28 kDa:** ERP29 (Endoplasmic reticulum resident protein 29), CLPP (Putative ATP-dependent Clp protease proteolytic subunit), PSA6 (Proteasome subunit alpha type-6), 1433G/Z; **25 kDa:** RB11A (Ras-related protein Rab-11A), RAB1A/1B (Ras-related proteins Rab-1A and Rab-1B).



Quantification in the affinity chromatography and co-immunoprecipitation experiments of ProTα, SET and ANP32A and ANP32B proteins, as well as in the isolated complexes, indicate that only a small part of the cellular content of these proteins (about 10%) is implicated in the characterized interactions. This is in agreement with the low cytoplasmic concentration of these proteins found in the immunofluorescence analysis of proliferating lymphocytes<sup>35</sup>. However, data from the evaluation of the subcellular concentrations of ProTα, SET, ANP32 proteins in extracts from Jurkat cells show a surprising preponderant cytoplasmic presence of these proteins<sup>35</sup>. This effect might be due to their nuclear leaking produced during the homogenization procedure, as previously demonstrated with ProTα<sup>20</sup>.

The loss of interaction of ProTα with its targets is observed when cell proliferation of the Jurkat cells is reduced by serum deprivation or apoptosis induced by staurosporine. These effects seem to indicate that these interactions are related to cell proliferation and survival. In Figure 3 is shown the composition of the ProTα-complexes and their evolution in serum-deprived and apoptotic cells.

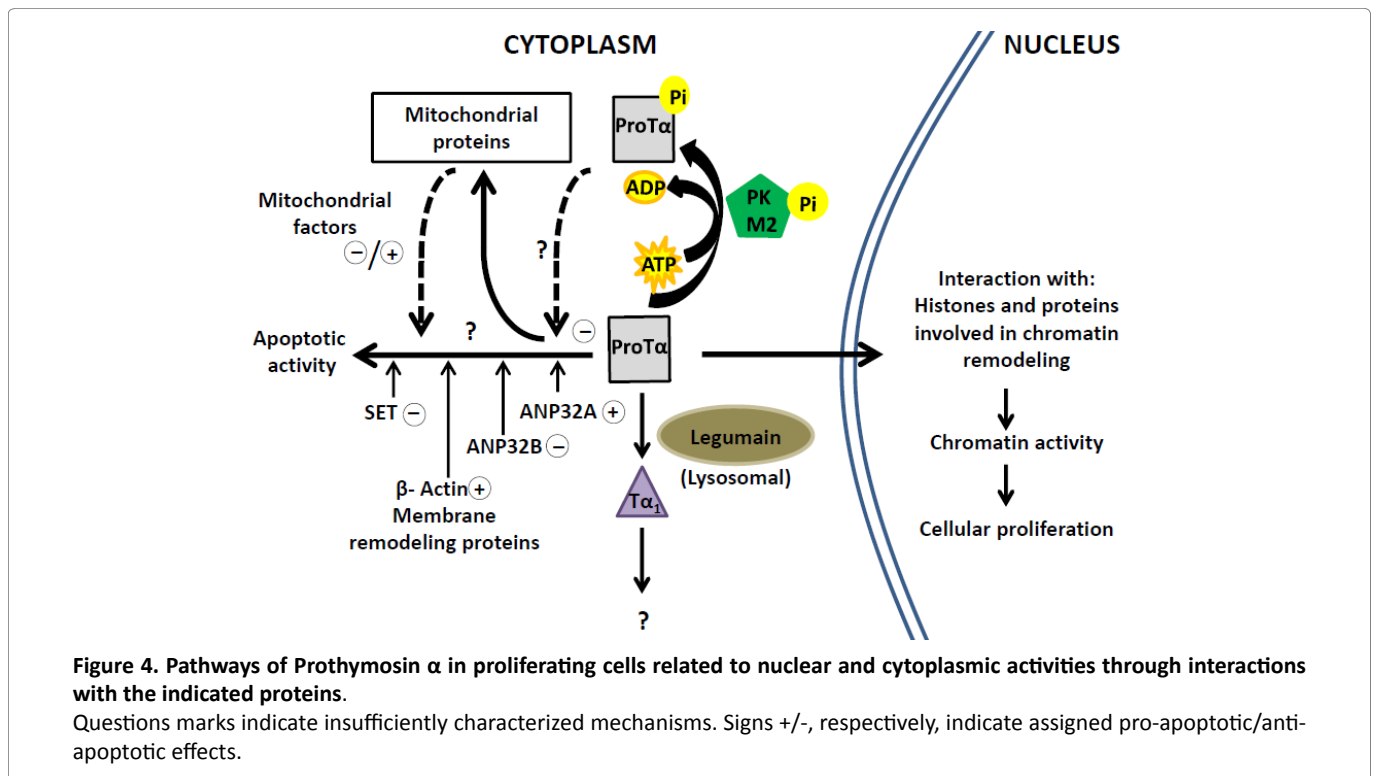
The cytoplasmic functions attributed to the proteins interacting with ProTα, like ANP32A<sup>29</sup> and β-actin<sup>38</sup> leads to the hypothesis that ProTα might exert its anti-apoptotic effect counteracting the pro-apoptotic effect described for these proteins. On the contrary, and considering the anti-apoptotic activity of ANP32B<sup>31</sup>, the interactions with ProTα might reflect its role as a collaborative factor. In

addition, interactions of ProTα with SET influencing cell survival might be related to different mechanisms of those attributed to SET as the anti-apoptotic factor through inhibition of the nuclease NM23-H1<sup>30</sup>. On the other hand, interactions of ProTα with ANP32A/ANP32B in complexes 34 and 30 kDa, seem to be involved in mechanisms in which the mitochondrial activity is implicated.

### Concluding remarks

Novel interactions described for ProTα provide clues for the understanding of the cytoplasmic activity of ProTα and its targets. The biological functions attributed to proteins interacting with ProTα, lead to hypothesize that the ProTα-complexes are implicated in cell survival by preventing the progress of apoptotic pathways in proliferating lymphocytes. This ability of ProTα supports its role as a multitasker protein with differentiated nuclear and cytoplasmic functions. The scheme in Figure 4 summarizes the cellular processes involving ProTα according to currently available data.

Most of the cellular ProTα migrates to the nucleus of proliferating cells to affect the chromatin activity. Cytoplasmic ProTα undergoes a proteolytic processing to generate Tα1 and a small part of the ProTα pool (about 10%) is phosphorylated by M2-PK concomitantly with cell proliferation. A similar amount of ProTα is involved in the interactions with SET, ANP32A, ANP32B and other cytoplasmic and mitochondrial proteins. On this regard, it should be mentioned the possibility that phosphorylated ProTα could be implicated in such protein interactions as



deduced from the analysis of levels of effector caspases in cells transfected with ProT $\alpha$ <sup>18</sup>. However, we failed to detect ProT $\alpha$ -phosphorylated peptides in the structural analysis of the ProT $\alpha$ -complexes. This question should be addressed in future research to elucidate the functional mechanisms of ProT $\alpha$  and its targets.

## Disclosure

The authors have declared that no conflict of interest exists.

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