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Mitochondrial STAT3: Powering up a potent factor

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ABSTRACT

The JAK-STAT3 signaling pathway is engaged by many cytokines and growth factor stimuli to control diverse biological processes including proliferation, angiogenesis, survival, immune modulation, and metabolism. For over two decades it has been accepted that STAT3-dependent biology is due to its potency as a transcription factor capable of regulating the expression of many hundreds of genes. However, recent evidence of non-canonical and non-genomic activities of STAT3 has emerged. The most exciting of these activities is its capacity to translocate into the mitochondria where it regulates the activity of the electron transport chain and the opening of the mitochondrial permeability transition pore. These have broad consequences including cell survival and the production of reactive oxygen species and ATP in both normal tissue and under pathological conditions. Despite these fascinating observations there are many key unanswered questions about the mechanism of STAT mitochondrial activity.

1. Introduction

Inter- and intracellular communication is essential to the correct functioning of all organisms. This process is regulated by tightly controlled, complex signaling networks. One of the most potent families of signaling molecules are the *S*ignal *T*ransducer and *A*ctivator of *T*ranscription (STAT) proteins. There are 7 members of this family, STAT1, 2, 3, 4, 5a, 5b and 6. STAT3 is required to transmit signals from a diverse array of cytokines and growth factors (including IL-6 family, IL-10, EGF, PDGF and LIF) and is essential for life [1]. The prevailing dogma of STAT3 signaling is that it exists as a latent, un-phosphorylated monomer in the cytosol until cytokines or growth factors engage their cognate receptor. Ligand/receptor interaction causes a change in confirmation and concurrent activation of receptor associated Janus Kinase (JAK) proteins. Activated JAKs trans-phosphorylate each other and the cytoplasmic tail of the receptor on tyrosine residues that provides docking sites for STAT proteins to be recruited via their SH2 domain. Once recruited, STAT proteins are phosphorylated on a single carboxy-terminal tyrosine residue (Y705 in STAT3) by JAKs. This tyrosine phosphorylation alters the confirmation of STAT dimers, which translocate into the nucleus and bind to consensus DNA sequences (γ-activated sequence (GAS) TTCNNNGAA) and initiate transcription. This pathway is tightly regulated and transient. STAT proteins can be dephosphorylated by protein-tyrosine phosphatases or STAT signaling can be impeded by the Suppressors of Cytokine Signaling (SOCS) proteins. The *SOCS* genes are direct transcriptional targets of STATs and they either block STAT-receptor recruitment or degrade target proteins through an ubiquitin directed mechanism.

2. Non-canonical STAT3 activities

The importance of JAK-STAT signaling has been revealed by knockout mice and by humans with spontaneous mutations in receptors, kinases e.g., JAK (severe combined immunodeficiency [2] and myeloproliferative disease [3]) or in STAT3 itself (loss of function mutations in hyper IgE syndrome [4]). The diversity of STAT3-dependent biological responses has, until recently, been considered to be entirely dependent on its activity as a tyrosine phosphorylated transcription factor. However, recent evidence suggests that there is far more complexity.

Abbreviations: EGF, epidermal growth factor; ETC, Electron Transport Chain; GAS, γ-activated sequence; IκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; JAK, Janus Kinase; LIF, leukemia inhibitory factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; mPTP, mitochondrial permeability transition pore; PDGF, platelet-derived growth factor; SOCS, Suppressors of Cytokine Signaling; STAT, Signal Transducer and Activator of Transcription; TIM, Transporter Inner-Membrane; TOM, Transporter Outer Membrane.

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2.1. Transcriptional activity of unphosphorylated STAT3

Recombinant, unphosphorylated STAT3 binds to a DNA probe containing the STAT3 consensus binding site from the *c-fos* promoter, albeit with lower affinity than the Y705 phosphorylated form of STAT3 [5]. These data show that Y705 phosphorylation is dispensable for dimerization and DNA binding and possibly transcription of STAT3 target genes. Indeed, the reconstitution of STAT3 low or null cells with either wild-type or a STAT3 mutant incapable of phosphorylation on Y705 (Y705F) results in significant changes in the expression of similar numbers of genes. Interestingly, two thirds of these genes are unique to the specific phosphoform of STAT3 [6], suggesting that the transcriptional profile and likely the biological response will depend on the dominant STAT3 post-translational modification. These data imply that nuclear import of STAT3 is independent of Y705 phosphorylation and dimerization or that STAT3 can be chaperoned into the nucleus. Both are the case. Nuclear shuttling of wild type and Y705F mutant STAT3 are equivalent [7], suggesting that tyrosine phosphorylation has no impact on nuclear import of STAT3. Additionally, unphosphorylated STAT3 competes with IκB for binding to the p65 and p50 subunits of NF-κB. These STAT3-NF-κB complexes enter the nucleus and bind to promoters containing both GAS and NF-κB motifs (e.g. in the *RANTES* gene) [8]. The transcriptional activity of unphosphorylated STAT3 is influenced by acetylation on K685 [9], however it is not clear whether this is dependent on NF-κB.

The contribution of K685 acetylated STAT3 to its transcriptional activity raises the interesting issue of additional STAT3 post-translational modifications. STAT3 has now been reported to be tyrosine, serine and threonine phosphorylated [10], acetylated [11], alkylated [12], methylated [13], ubiquitinated [14] and glutathionylated [15]. However, it is important to note that all of these modifications were identified using STAT3 protein purified from entire cells - therefore no knowledge of compartment specific modifications and/or combinations of modifications that may be critical to STAT3 activity is available. Additionally, all of these modifications were exclusively tested for their capacity to modulate transcriptional activity and as such their influence on other non-nuclear activities of STAT3 remains unknown.

2.2. Mitochondrial STAT3

The linear signal transduction pathway described in Section 1 is too simplistic. Early evidence for alternate and non-nuclear activities of STAT3 came from the identification of higher order STAT protein complexes ranging between 200 kDa and 1MDa [16–18]. In addition STAT3 associates with other cytosolic structures including focal adhesions, microtubules, and the mitotic spindle, as well as with membrane elements, such as plasma membrane rafts [19], endo-lysosomes [20] and mitochondrial enriched fractions [21–23].

Recently STAT3 was shown to be present in a protease-resistant mitochondrial fraction of cardio-myocytes, pro-B cells and in Ras-transformed cell lines demonstrating its capacity to be imported into the mitochondria [21,22]. This pool of STAT3 supports the activity of the Electron Transport Chain (ETC) required for ATP production and the opening of the mitochondrial permeability transition (mPTP) pore. These mitochondrial activities of STAT3 have consequences both in normal tissue homeostasis (e.g., neurite outgrowth and cardiac function) and in pathologic conditions (e.g., tumor growth and tissue damage in response to ischemia/reperfusion injury) discussed in Section 2.2.3.

The study of the mitochondrial activities of STAT3 is an exciting new field that is likely to contribute much to our knowledge of how STAT3 regulates many biological processes both in normal and in diseased tissues. However, the discovery of this pool of STAT3 poses more questions than it answers including the mode of import and mechanism of action, both of which are addressed in Sections 2.2.1 and 2.2.2.

2.2.1. STAT3 mitochondrial import

Mitochondria are a critical organelle that are the major source of cellular ATP, but are also critical for the synthesis of ribose, lipid and protein necessary for cellular replication. They retain their own genome that encodes a mere 13 of the more than 1000 known mitochondrial proteins. Therefore, over 98% of mitochondrial proteins need to be imported following synthesis on cytosolic ribosomes highlighting the need for an efficient and accurate mechanism for targeting nuclear encoded proteins to the correct sub-mitochondrial compartment [24]. Mitochondrially directed proteins contain targeting motifs that are recognized by receptors of the Transporter Outer Membrane (TOM) complex. Most mitochondrial proteins carry an N-terminal pre-sequence that is recognized by TOM receptors TOM20 and TOM22 (Fig. 1). Following receptor binding proteins are shuttled through the TOM40 outer membrane pore and into the inner membrane space. However, nearly a third of imported mitochondrial proteins lack this pre-sequence [25] and are directed to the mitochondria by a cryptic internal targeting signal. STAT3 mitochondrial import is enhanced by PKC-dependent phosphorylation and interaction with the HSP70 and HSP90 chaperones [26]. This is consistent with TOM70 recognition and import, in which proteins are delivered to the import machinery by chaperones and shuttled through the

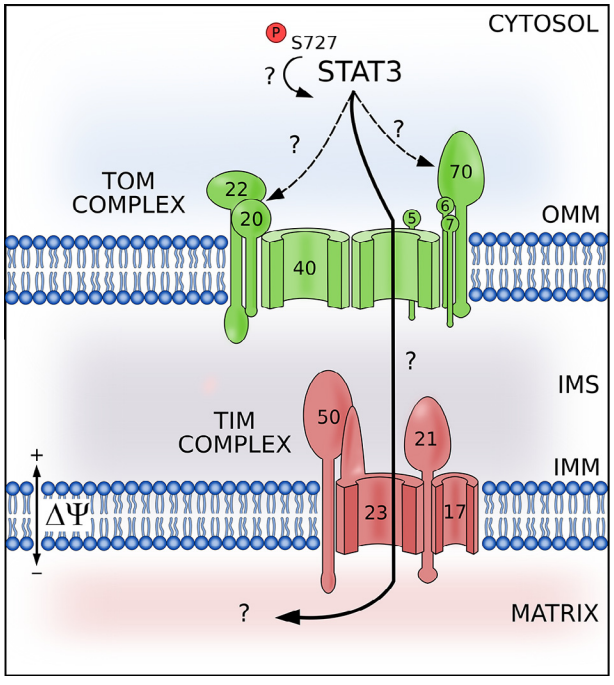

Fig. 1. Mitochondrial import of STAT3. Mitochondrial proteins encoded by the nuclear genome are imported into the mitochondria via two major mechanisms. Proteins with an N-terminal targeting signal are typically recognized by the TOM20 receptor on the mitochondrial outer membrane. In contrast proteins with an internal targeting signal are recognized by TOM70. Both classes of proteins are imported through a pore comprised of TOM40. There is some controversy about the requirement for STAT3 to be phosphorylated on S727 prior to mitochondrial import. Also whilst STAT3 can interact with TOM20, the amino terminus of STAT3 is not required for import. Therefore it is likely that STAT3 is imported via an internal targeting signal and TOM70 mediated import. Biochemical fractionation and functional studies imply that mitochondrial STAT3 is localizes to the mitochondrial inner membrane or matrix space, impling that it must also engage the TIM complex that is required for transit across the mitochondrial inner membrane. However at this stage no information about a STAT3-TIM interaction is available.

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