COMMENTARY

STAT5 isoforms: controversies and clarifications

Haydeé L. RAMOS, John J. O'SHEA and Wendy T. WATFORD¹

Molecular Immunology and Inflammation Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892, U.S.A.

STAT (signal transducer and activator of transcription) family transcription factors are critical regulators of the development and differentiation of many cell types. STAT isoforms are generated by alternative splicing, but have also been suggested to be generated post-transcriptionally. In this issue of the *Biochemical Journal*, Schuster and colleagues have identified cathepsin G as the protease that cleaves full-length STAT5 (STAT5 α) to generate a C-terminally truncated form in immature myeloid cells. However, the authors argue that this proteolytically generated isoform does

not occur naturally *in vivo*; rather, it is artificially generated by cathepsin G during the preparation of cell extracts. This new evidence calls into question the physiological significance of this putative isoform and forces the general re-examination of proteolytically generated STAT isoforms.

Key words: cathepsin G, Janus kinase (JAK), proteolysis, signal transducer and activator of transcription 5 (STAT5), transcription factor.

One of the principal modes of intracellular signalling used by cytokines, interferons and colony-stimulating factors is the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathway. Activation of the receptor-associated JAKs results in the phosphorylation of latent cytosolic transcription factors called STATs. Activation induces nuclear accumulation, DNA binding and induction of genes that control cell proliferation, differentiation, development and survival [1,2]. STAT5a and STAT5b are encoded by two closely linked genes and are activated by growth hormone, prolactin, interleukins and colony-stimulating factors. Consequently, they are important for stature, mammary gland development and haemopoiesis [3,4]. STAT5 also has important immunoregulatory functions [3].

Structurally, STATs are proteins of 750–850 amino acids with conserved domains that contribute to the transduction of ligand-specific signals [5,6]. The central DNA-binding domain is conserved among the STAT family members and mediates the binding to consensus sites within gene regulatory elements, whereas the N-terminal domain is involved in homo- and heterotypic interactions among the STATs. The TAD (transcriptional activation domain) is located in the C-terminal region, which is the most variable region among the STATs and may be critical for transcriptional activation.

Two major STAT isoforms have been recognized from the early days of the field: the full-length transcription factor (the so-called α -isoform) and C-terminally truncated isoforms that lack the transactivation domain (β -isoforms) [7,8]. These β -isoforms have been identified for STATs 1, 3, 4, 5a and 5b, and are generally thought to be produced by alternative splicing [9]. There is some controversy as to whether β -isoforms act as dominant-negative STATs due to the lack of a TAD. STAT5 β has been suggested to suppress expression of genes normally regulated by full-length STAT5 α [10,11]. However, elegant work in this area comes from the analysis of gene-targeted mice unable to synthesize the STAT3 β isoform. These mice exhibited an altered pattern of STAT3-responsive gene expression, suggesting a role for the STAT3 β isoform in controlling systemic inflammation [12]. Similarly, other work has argued that the STAT3 β isoform is not a true dominant-negative factor, as it retains the ability to activate a number of STAT3 target genes [13]. The STAT4 β

isoform has also been shown to induce many IL-12 (interleukin 12)-responsive genes in common with STAT4 α , in addition to a unique subset of genes [14].

Is alternative splicing the only way that STAT isoforms arise? Other STAT products can be seen on immunoblots, some of which have been argued to be due to proteolysis. A case in point is STAT5, the topic of the study by Schuster et al. [15] in this issue of the *Biochemical Journal*. Work from several groups has pointed to the existence of additional STAT5 isoforms that could not be explained by alternative splicing. This led to the notion that STAT isoforms could also be generated by proteolysis [16,17]. Similarly to the alternatively spliced isoform, proteolytically cleaved STAT5 lacks a TAD and is therefore thought to negatively regulate transcription [16,18,19].

However, there has been some controversy over whether cleaved isoforms occur *in vivo* [20,21]. The existence of this cleaved STAT5 isoform *in vivo* has been inferred, in part, on the basis of its apparent controlled regulation. For example, prevalence of this isoform has been reported to be regulated by the maturation state of the cell [22]. Cytokine stimulation also induces the appearance of STAT5 proteolytic isoforms [23]. Piquing interest further is that proteolytically cleaved isoforms have been associated with leukaemia [24,25]. Indeed, in some cases, the expression of such isoforms has been suggested to correlate with disease progression [24]. These findings have intensified interest in the regulation and roles of these isoforms in disease pathogenesis [26,27].

If STATs are proteolytically cleaved, then what is the protease responsible and how is it regulated? Through biochemical analysis, Lee et al. [23] partially characterized the predominant STAT5 protease in myeloid cells, and found a protein of approx. 25 kDa, but whose identity remained unknown. The present study by Schuster et al. [15], appearing in this issue of the *Biochemical Journal*, finally sheds some light on the identity of the protease, and, more importantly, the mechanism by which STAT5 isoforms are generated; however, the answer is not likely to please everyone. The authors purified cathepsin G as the protease that cleaves full-length STAT5 into the C-terminally truncated forms. However, they conclude that this happens as a result of sample preparation *in vitro* and argue that it has no *in vivo* cellular correlate.

¹ To whom correspondence should be addressed (email watfordw@mail.nih.gov).

The initial clue that this phenomenon was occurring *in vitro* was that the prevalence of the STAT5 proteolytically cleaved isoform in immature myeloid cells (cell line 32D) correlated with the method of cell lysate preparation. The authors subsequently used liquid chromatography and MS to identify a precursor of murine cathepsin G, a serine protease usually found in the azurophilic granules of myeloid cells. They went on to show that purified cathepsin G cleaves STAT5 to yield the expected products. Furthermore, a selective inhibitor prevented the appearance of proteolytically cleaved STAT5, leading the authors to conclude that this putative isoform is actually an artifact owing to cell lysate preparation and that this phenomenon does not occur *in vivo* in intact myeloid cells.

Having challenged the physiological relevance of STAT5 proteolytic cleavage products *in vivo*, the present findings make it necessary to carefully revisit previous conclusions arguing for correlation between STAT5 isoforms with malignant transformation. If proteolytic STAT5 products are indeed artifactual, then what can explain the body of data arguing for its correlation with malignant transformation? Since it has been shown that cathepsin G is overproduced by AML (acute myeloblastic leukaemia) cell lines, one possibility is that enhanced STAT5 cleavage is simply due to elevated levels of cathepsin G at the time of lysis [28]. Similarly, cytokine stimulation may regulate the abundance or activation state of the protease and may explain the apparent cytokine-induced regulation of the cleaved isoform. Moreover, it is also possible that cathepsin G may not be the only protease that might contribute to STAT5 proteolysis, particularly in other cell types. For instance, calpain has also been reported to cleave STAT3 and STAT5 in platelets [29]; clearly, the burden is on investigators to carefully exclude the possibility that the generation of any proposed isoforms is artifactual. This caveat appears to be pertinent for other STATs. Shelburne et al. [21] demonstrated that a STAT6 truncated isoform described in mast cells was derived by proteolysis during sample preparation and has no cellular correlate.

In summary, proteolytic generation of STAT isoforms was first noted nearly a decade ago. However, the protease responsible and the physiological significance have been elusive. While clinical data are provocative, the present study raises a troubling concern over the interpretation of previous data. It will be incumbent upon future studies to confirm or refute the occurrence of natural STAT5 cleavage products *in vivo*; clearly, extra effort will be required to prove that putative STAT isoforms are not artifactual owing to *in vitro* proteolysis.

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