# SOCS1: a host factor required for HIV-1 Gag trafficking

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## Summary

Evaluation of — Ryo A, Tsurutani N, Ohba K *et al.*: SOCS1 is an inducible host factor during HIV-1 infection and regulates the intracellular trafficking and stability of HIV-1 Gag. *Proc. Natl Acad. Sci. USA* 105, 294-299 (2008).

Gag is an essential HIV protein that is responsible for assembling infectious viral particles. The journey of Gag from synthesis to virion formation is an attractive target for anti-HIV therapy. While a lot is known about the cellular factors required for virion assembly and budding, relatively little is known about factors required for Gag trafficking. Ryo and colleagues provide the first evidence of such a factor, the suppressor of cytokine signaling 1 (SOCS1). They demonstrated that SOCS1 is important for Gag stability and plays a critical role in its trafficking within the cell. Without SOCS1 Gag accumulates in perinuclear bodies and is degraded by lysosomes. The reviewed paper thus highlights a new and novel target for stopping the production of HIV, a target that belongs to the relatively invariant host rather than the rapidly evolving virus.

### Keywords

Gag, SOCS1, trafficking, protein stability, host factors, immunotherapy, bevirimat

## Introduction

The suppressor of cytokine signaling 1 (SOCS1) protein was recently reported as an essential cofactor for HIV-1 Gag stability and trafficking [23]. Gag has become a hot topic in HIV research in recent years and is seen as a novel target for developing antiretrovirals. Indeed the Gag-targeting drug bevirimat/PA-457 is now past phase I/II clinical trials and in phase IIb trials [25]. Bevirimat binds to Gag in the immature virion and blocks the final protease-mediated cleavage event required to mature the virion, thereby rendering HIV noninfectious. Much, however, is still unknown about where Gag traffics and with what cellular factors it interacts with on its itinerary from synthesis to virion incorporation. The work by Ryo and colleagues adds an important piece to this puzzle and their paper is the first to provide a detailed molecular picture of a cellular factor involved in Gag trafficking.

Gag is an essential polyprotein whose role is to form and direct the assembly of virus particles [22]. Gag is cleaved into its constituent proteins [matrix (MA), capsid (CA), spacer peptide 1 (SP1), nucleocapsid (NC), spacer peptide 2 (SP2) and p6] by the HIV protease following virion budding and maturation, but remains a polyprotein during virion assembly within the cell. Several reports demonstrate that Gag needs to interact with numerous cellular proteins on its way to or at the budding membrane [15]. Most of these proteins are associated with the endosomal sorting system of the cell and leads to the controversial hypothesis that late endosomes or multivesicular bodies are sites of virion assembly in addition to the plasma membrane [18, 19, 6, 27].

SOCS1, by contrast, is not associated with the endosomal sorting system. SOCS1 and other members of the SOCS family play an important role in modulating cytokine-induced intracellular signaling pathways [5]. *SOCS1* expression is upregulated when various cytokines bind their cognate receptors and initiate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling cascade. In a negative feedback loop, SOCS1 suppresses the signaling cascade by various mechanisms including inhibiting the kinase activity of JAKs and redirecting JAK2 for proteasomal degradation. *In vivo* studies in mice suggest that SOCS1 acts as a brake to counter the potentially toxic consequences of cytokine activation [8].

## Summary of Methods and Results

Thus the finding by Ryo and colleagues that HIV-1 can induce *SOCS1* expression is very novel [23]. The authors noticed *SOCS1* in gene array studies of cellular genes upregulated by HIV-1 infection. They confirmed the upregulation of *SOCS1* transcription in both a T cell line and healthy donor peripheral blood mononuclear cells (PBMCs) infected *in vitro* with HIV-1. Co-transfection of an adherent cell line with HIV-1 proviral DNA (pNL4–3) and a *SOCS1* expression plasmid showed a dose-dependent increase in virion production and intracellular Gag levels. These increases depended on the SH2 and SOCS box domains of SOCS1. Electron microscopy and replication kinetics data suggested that SOCS1 was promoting the production of infectious virions rather than noninfectious virus-like particles.

The team confirmed that SOCS1 interacts with Gag in a series of *in vitro* experiments. They found that only the SH2 domain of SOCS1 (most likely amino acids 100 – 107) was required for binding to recombinant Gag. Both the MA and NC domains of Gag can independently bind SOCS1 *in vitro*, but heterologous domain substitution experiments implied that functional interaction is with MA. Confocal microscopy experiments of cells overexpressing SOCS1 and a Gag-green fluorescent protein fusion (Gag-GFP) implied the two proteins may also interact within the cell. However, more convincing tests of intracellular interaction could have been used, such as fluorescence resonance energy transfer (FRET) [2].

One of the functional consequences of Gag–SOCS1 interaction is an increase in Gag stability. Pulse-chase experiments with pNL4–3 showed that SOCS1 extended the protein half-life of intracellular Gag and caused CA to appear earlier in the culture supernatant. In an attempt to visualise this by microscopy, confocal experiments were performed in cells co-transfected with pNL4–3 and SOCS1 plasmid. The authors used an anti-CA antibody to detect Gag but did not visualise SOCS1. They claimed SOCS1 increased Gag levels in the cytoplasm and at the plasma membrane, but the data presented in the paper are not strong. Confocal experiments of cycloheximide-treated cells transfected with Gag-GFP instead of pNL4–3 did reveal an increase of Gag-GFP at the plasma membrane when SOCS1 was overexpressed. The authors quantitated the amount of Gag-GFP at the plasma membrane but how they did this is unclear and whether they accounted for changes in Gag-GFP stability is unknown.

The final series of experiments explored the effects of knocking down endogenous SOCS1 using RNA interference (RNAi). Suppressing SOCS1 expression resulted in reduced intracellular Gag levels and decreased virion production, corroborating the earlier overexpression experiments. Knockdown also significantly reduced HIV-1 replication in both the Jurkat T cell line and $CD4^+$ T cells isolated from healthy donor PBMCs. Confocal experiments revealed prominent perinuclear accumulations of Gag-GFP when SOCS1 expression was suppressed. These accumulations overlapped with lysozyme, a marker for lysosomes. Treatment of SOCS1-depleted cells with ammonium chloride, which prevents lysosome acidification, increased Gag-GFP signals by western blot. So it appears that SOCS1 increases Gag stability by preventing its destruction in lysosomes, thereby promoting virion production and viral infectivity.

## Discussion

Overall the data presented by Ryo and colleagues clearly demonstrate that 1) HIV-1 can induce SOC1 expression, 2) SOCS1 promotes infectious virion production, and 3) SOCS1 achieves this by increasing Gag stability in the cell. What is unclear is the extent of SOCS1's involvement in Gag trafficking. As a result the paper raises many important questions. Does SOCS1 foster proper trafficking of Gag or does it indirectly enable it by increasing Gag's half-life? The perinuclear accumulation of Gag-GFP in the absence of SOCS1 expression coincides with observations made by Perlman and Resh, who described newly synthesised Gag-GFP transiently accumulating around the cell nucleus before trafficking to the plasma membrane [19]. Are these perinuclear aggregates a normal destination for Gag, for example to pick up viral and cellular RNA for virion incorporation [20, 17]? Is SOCS1's role

then to redirect Gag from these aggregates to sites of virion assembly before Gag gets degraded by lysosomes?

Ryo and colleagues demonstrated that the SOCS box domain of SOCS1 is crucial for its effect on virion production. SOCS1 indirectly interacts with E3 ubiquitin ligases via its SOCS box, thereby promoting the ubiquitination of JAK2 during the regulation of cytokine signaling [5]. Could SOCS1 also direct the ubiquitination of Gag? Evidence suggests that Gag ubiquitination is important for virion assembly and viral infectivity [11, 10]. Ubiquitination is thought to allow Gag to interact with and co-opt members of the endosomal sorting system (the ESCRT complexes [14]) for the purposes of virion production [9, 7, 21]. The *trans*-Golgi network-associated protein SH3RF1 (hPOSH) is the only E3 ubiquitin ligase known to directly interact with Gag, but it's unlikely to ubiquitinate Gag itself [1]. Thus it will be important to determine if SOCS1 can promote Gag ubiquitination, thereby cementing a key role of SOCS1 in Gag trafficking as well as Gag stability.

## Future Perspective

Ryo and colleagues end their paper by suggesting SOCS1 as a therapeutic target. Indeed this was proposed in 2006 (albeit from a different perspective) when Song and coworkers used RNAi against SOCS1 to enhance anti-HIV responses in mice [26]. In that study murine dendritic cells (DCs) were transduced with lentivirus expressing a small interfering RNA (siRNA) against SOCS1. These SOCS1-suppressed DCs were loaded with HIV envelope (Env) protein before being injected into mice. Song and colleagues reported enhanced T- and B-cell-mediated immune responses against Env following DC immunisation. This enhancement lasted at least 6 months post-immunisation and also worked when the SOCS1 siRNA was formulated as part of a DNA vaccine strategy. Thus silencing SOCS1 in certain immune cells appears to enhance broad anti-HIV immunity *in vivo*.

One of the concerns of this vaccine approach, even one designed to perturb Gag trafficking, is the long-term effects of SOCS1 suppression on the immune system [12]. Song and colleagues did not observe any obvious signs of pathology 7 months after the mice were immunised with the SOCS1-suppressed DCs [26], but the same may not be the case in humans. SOCS1 is required for curtailing interferon-mediated inflammation [4], reducing DC-mediated autoimmunity [13] and also appears to play an anti-cancer role [5]. So any vaccine utilising SOCS1 suppression will need to monitor for signs and symptoms of these pathologies. It has also been shown *in vitro* that types I and III interferon can increase HIV-1 uptake and replication in PBMCs owing to upregulated expression of CD4, CCR5 and CXCR4 receptors [24]. SOCS1 regulates the signaling of both these types of interferon [3, 8], so it remains to be seen if SOCS1 silencing will increase HIV-1 uptake *in vivo*. Finally, using cytokines as adjuvants in anti-HIV therapy has been suggested [16]. Combining cytokine adjuvants with SOCS1 suppression may further enhance anti-HIV responses, but it may also exacerbate the problems associated with dysregulated cytokine signaling.

Notwithstanding the above, viewing Gag trafficking and cytokine signaling pathways as drug targets is different from current antiviral strategies and offers exciting prospects for future HIV therapies. The experimental drug bevirimat demonstrates that Gag is a viable option for antiretroviral therapy [25], and modulating the host immune response rather than targeting HIV directly, if done safely, may

circumvent some of the issues of viral resistance. Of course better understanding Gag trafficking will enlighten even more viral and cellular targets for intervention. The paper by Ryo and colleagues is therefore an important first step in this enlightenment.

## Executive Summary

### Introduction

- HIV-1 Gag is an essential polyprotein required for virion assembly.
- Research into Gag trafficking is very active as it is seen as a novel area for developing anti-HIV therapies.
- The paper reviewed herein describes the suppressor of cytokine signaling 1 (SOCS1) as an essential cofactor for Gag stability and trafficking.
- SOCS1 is a cellular protein normally involved in regulating cytokine-induced signaling pathways. It does so to prevent toxic side effects of excessive cytokine signaling during immune activation.

### Summary of Methods and Results

- HIV-1 infection induced *SOCS1* expression in peripheral blood mononuclear cells.
- Overexpressing *SOCS1* in cells increased the production of infectious virus particles and increased intracellular Gag levels.
- Functional interaction was mapped between the SH2 domain of SOCS1 and the matrix domain of Gag.
- SOCS1 increased the half-life of Gag within the cell, apparently by preventing Gag degradation by lysosomes.
- *SOCS1* overexpression lead to more Gag at the plasma membrane, whereas RNAi-mediated suppression of SOCS1 caused Gag to accumulate in perinuclear aggregates.

### Discussion

- SOCS1 is important for Gag stability, but it's unclear how SOCS1 directs proper Gag trafficking.
- Further experiments are needed to elucidate the nature of the perinuclear aggregates. (*E.g.*, are they a normal destination for Gag? Does SOCS1 then redirect Gag to sites of virion assembly?)
- It will be important to determine if SOCS1 can direct the ubiquitination of Gag, as it does for other proteins. Gag ubiquitination plays an essential role in virion assembly.

**Future Perspectives**

- SOCS1 has been proposed as a target for anti-HIV therapy.
- Others have shown that SOCS1-suppressed dendritic cells may be used as an immunotherapeutic, leading to broad anti-HIV immunity in mice.
- Concerns exist as to the safety of SOCS1 suppression. SOCS1 regulates inflammation, suppresses autoimmunity and prevents certain types of cancer.
- Nevertheless, SOCS1 and Gag trafficking are exciting targets for future HIV therapies. Better understanding of Gag trafficking and interacting factors is therefore needed.

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## Reference Annotations

### References of Considerable Interest

- [5]: Good introduction to SOCS1 and its role in immunity.
- [15]: A current overview of Gag trafficking in the cell and the host factors it interacts with.
- [26]: A vaccine strategy based on silencing SOCS1 expression in dendritic cells.

### References of Interest

- [10]: Evidence that Gag ubiquitination is required for proper virion assembly.
- [14]: Review of the ESCRT complexes, many of whose components interact with Gag.
- [20]: Evidence that Gag and viral RNA colocalise at perinuclear sites.
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